

Influence of sex and home range on released Antillean manatees' behavior in Brazil

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Abstract

The Antillean manatee is a threatened aquatic mammal subspecies which inhabits the northern and northeastern Brazilian coasts and estuarine areas. In this study we analyzed behavior data from rehabilitated and released manatees, to evaluate if sex and home range affect behavior richness and activity budget. Behavioral data from 29 manatees (15 females and 14 males)

Keywords:

activity budget, aquatic mammal, home range, richness, Sirenia

of northeastern Brazil was analyzed from 2006 to 2018. The animals were monitored by trained staff, who followed the animals using satellite and VHF telemetry systems. Six behavior states and 17 behavior events were recorded. The data was analyzed using GLM, ANOVA, and regression statistical tests. Behavior richness varied from three to 14 and activity budget indicated that individuals spent more time engaged in behaviors such as moving slowly and feeding in the bottom of the water body, but neither behavior richness nor activity budget were affected by sex. Behavior richness related to home range varied from five to 14 behaviors. Behavior richness and the activity budget for two social behaviors events - touch and interaction with wild manatees - were positively influenced by home range size, highlighting the importance of the use of space on behavior expression in manatees.

Introduction

The Antillean manatee (*Trichechus manatus manatus* Linnaeus, 1758) is an aquatic mammal from the Sirenia order which inhabits mangroves, estuaries, and coastal waters on the northern and northeastern Brazilian coast (Lima et al., 2011; Normande et al., 2016; dos Santos et al., 2022). Herbivores, manatees feed on macrophytes, seagrasses, and marine algae and access fresh water from the rain, underground wells in the seafloor, and rivers (Domning & Magor, 1978; Favero et al., 2020; dos Santos et al., 2022).

ARTICLE INFO

Manuscript type: Article

Article History

Received: 31 May 2023

Received in revised form: 23 August 2023

Accepted: 23 August 2023

Available online: 27 February 2024

Handling Editors: Carolina Loch, Rodrigo Amaral

Citation:

do Val, H. G. P., Attademo, F. L. N., Normande, I. C., Borges, J. C. G., Costa, A. F., de Alencar, A. E. B., Melo, L. I. S., Paiva-Jr, L. H. P., & Luna, F. O. (2024). Influence of sex and home range on released Antillean manatees' behavior in Brazil. *Latin American Journal of Aquatic Mammals*, 19(1), 7-14. <https://doi.org/10.5597/lajam00325>

During the reproductive season, pregnant females search for calm environments, far from the ocean tides, to give birth, such as estuaries and freshwater bodies (Lima et al., 2005). Due to the increasing anthropization of the coast and improper pollutant disposal, most Brazilian estuaries are no longer suitable or available for females to nurse their calves, leaving them with no other choice than giving birth at sea, which increases the rate of calf strandings (Balensiefer et al., 2017; Medeiros et al., 2021). The low reproductive rate, along with nursing habitat loss and overhunting, led *T. manatus manatus* populations to a fast decline over the decades, being now considered one of the most threatened aquatic mammal species of Brazil (Parente et al., 2004; Meirelles, 2008; Luna et al., 2012; Meirelles et al., 2022). In 1994, the increasing fragmentation and population reduction led the Brazilian government to establish a manatee rehabilitation program, the *Projeto Peixe-boi* (Lima et al., 2007). With the main purpose of linking isolated manatee populations on the northeastern region, reducing the bottleneck effect and recolonizing historical areas of occurrence throughout the north and northeastern coast, the initiative consists of rescuing, rehabilitating and releasing stranded calves into the wild (Luna et al., 2012).

Such rehabilitation and release programs have been facilitating several studies on manatees' ecology and biology, including use of space and distribution (Lima et al., 2012; Normande et al., 2016), health (Vergara-Parente et al., 2003; Gomes et al., 2007; Silva et al., 2009; Anzolin et al., 2012; Mendonça et al., 2019) and behavior (Araújo & Marcondes, 2003; Gomes et al., 2008; Umeed et al., 2018, 2022; Lucchini et al., 2023). Attademo et al. (2020) compiled the behaviors from *T. manatus manatus* and *T. inunguis*, including feeding, movement, resting, parental care, agonistic, sociability and clinical afflictions, which is used to guide behavior data sampling from captive and wild manatees in Brazil. Animal behavior can be described as every act performed by an animal in response to biological or ecological cues and includes how it interacts with conspecifics and organisms from other species (Alcock, 2011). Behavior is also an important tool for measuring animal welfare by evaluating the expression of natural or abnormal repetitive behaviors, also known as stereotyped behaviors (Melfi, 2009; Vicino & Miller, 2015). Studies suggest that animal welfare could be evaluated by the use of behavioral diversity indexes, such as behavior evenness, behavior richness, behavior variety and activity budgets (Miller et al., 2011, 2020, 2021; Antonenko et al., 2017; Hacker et al., 2018). Therefore, the present study aims (i) to verify if sex affects manatee's behavioral richness and activity budget and (ii) to evaluate if a larger home range size would influence the variety of expressed behavior events and/or the expression of some specific behavior due to the larger use of space and access to different types of resources.

Material and Methods

1. Subjects' data

The data referred to monitoring files from 29 manatees, 15 females and 14 males, with tracking data ranging from 2006 to 2018. Of the 29 animals, 25 stranded as calves and four were born in captivity (Ariel, Tamunha, Sereno and Xiquinho) at *Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos* CMA

(National Center for Research and Conservation of Aquatic Mammals), located at Itamaracá Island on the northern coast of the state of Pernambuco, Brazil. The animals had been in captivity from 2.5 to 12 years before release (Table 1).

2. Behavioral data

After rehabilitation, manatees were tagged with VHF, ARGOS or GPS devices (Reid et al., 1991; Normande et al., 2016) and released into the wild. The animals were monitored by three trained teams, one at each release site (Paripueira, Tatuamunha River, and Mamanguape River Estuary), who followed them and observed their behavior from the coast and, less frequently, in small boats. Animals were recognized firstly by tracking devices and later by the presence of acquired marks (scars and/or identification marks made at the end of the caudal fin - called 'cookie'), morphological characteristics (some animals have particularities in their bodies) and characteristic behaviors. The sampling data followed the focal animal sampling method (Bosholn & Anciães, 2017) conducted at different times of the day (morning/ afternoon), following the VHF signal of each individual, during variable monitoring time effort. Given that the teams responsible for monitoring the animals were animal keepers, and the data was collected continually as long as the staff could see the individual, there is no information regarding time blocks for behavior sampling.

3. Home range data

Accounting for the home range data, the present study made use of the data by Normande et al. (2016) using the Kernel Density Estimates - KDE method with 95% of area use for 19 manatees: 11 males and eight females, monitored from 2008 to 2013.

4. Data analysis

We used two of the most commonly used diversity indexes (Miller et al., 2020), the activity budget and the behavioral richness, to evaluate how behavioral traits were affected by variables such as sex, captive and rehabilitation time, and home range size. The activity budget was calculated for each observed behavior event (see Table 2) and represented the percentage of time in which the animal was engaged in that behavior, providing information regarding the time spent by animals in expressing each behavior and if there is a more or less dominant behavior (Miller et al., 2020). Moreover, the behavioral richness corresponds to the number of distinct behavior events registered for each monitored animal.

For the analyses regarding home range size, both activity budget and behavioral richness were calculated to fit precisely the same time scale of Normande's study, from 2008 to 2013. Considering that Normande et al. (2016) had already tested the effect of sex and release site on home range size and did not find any relationship between those variables, we chose to not replicate these analyses here.

Given the different amount of time in which each animal was monitored, we performed a General Linear Mixed Model test (GLMM) to evaluate the effect of sex on behavioral richness controlling for the random effect of sampling effort. Thus, we built a model with behavior richness as the response variable, sex as the predictor variable, and sample effort as a categorical variable of three levels (low: one to three years; medium: four to six years; and high: seven to nine years). Then, we tested the

Table 1. Summary data on the 29 Antillean manatees (*Trichechus manatus manatus*) released in northeastern Brazilian coast between 2006 and 2018 indicating their identity, rehabilitation period and behavioral aspects.

Name	Sex	Time in rehabilitation (years)	Time in adaptation (days)	Sampling effort	Behavioral richness
Aira	F	6.2	259	high	12
Aldo	M	2.6	0	medium	14
Arani	M	7.4	204	high	14
Ariel	F	3.2	220	low	6
Artur	M	6.5	509	low	11
Astro	M	3.7	70	low	5
Clara	F	4.6	239	medium	12
Cristal	F	6.3	NA	low	3
Guga	M	3.7	181	low	7
Iara	F	4.5	7	medium	11
Joana	F	4.4	91	low	11
Lua	F	3.8	70	high	12
Luna	F	3.2	192	high	14
Mel	F	5.1	NA	medium	12
Natalia	F	3.4	341	low	9
Nina	F	2.8	7	low	11
Potiguar	M	6.2	259	low	9
Puã	M	5.4	759	medium	14
Sereno	M	9.2	575	low	5
Tamunha	M	2.7	236	medium	14
Telinha	F	5.5	51	medium	10
Tico	M	8.4	950	medium	14
Tinga	M	8.0	447	medium	14
Tita	F	7.4	646	low	13
Tuca	F	3.0	7	medium	12
Tupã	M	14.0	105	low	11
Xiquinho	M	3.2	373	low	9
Xuxu	M	9.9	10	low	5
Zelinha	F	5.9	343	medium	12

effect of sex (predictor variable) on the activity budget for each observed behavior event (response variable), performing a two-way Analysis of Variance test (ANOVA); and the effect of time in rehabilitation and on acclimatization facilities on behavioral richness using a Multiple Regression.

Regarding home range, we tested the effect of home range size as a predictor variable on both behavior richness and the activity budget for each behavior event (response variables) using Linear Regressions. All statistical analyses were performed in *R Studio* statistical computing software (R Core Team, 2021), version 2021.9.0, using $p < 0.05$ for significant values.

Results

The behavior richness calculated for all subjects ranged from three to 14 behavior events, as presented in Table 1 and the activity budget for all observed behavior events in each behavior state is presented in Fig. 1.

The only behavior observed for all animals was 'moving slowly', while the least observed was 'feeding in shallow waters', expressed

only by five animals. Within the feeding state, 'feeding at depth' was the most observed behavior event, and the expression of this behavior was most of the time associated with the presence of a food resource, being: seagrass (67.87%), marine algae (5.03%), and mangrove vegetation (8.44%). Courtship behavior was observed in six males and eight females, with maximum and minimum values of activity budget for females, 11.1% and 0.1% (mean = 0.72 ± 2.11), respectively. The courtship behavior was recorded for eight females: Aira, Ariel, Cristal, Iara, Mel, Nina, Tita, and Zelinha from 2007 to 2013. Agonistic behavior was more observed in males (five) than in females (two), with maximum and minimum values of activity budget of 9.6% and 0.1% for males (mean = 0.37 ± 1.77). As observed for behavior richness, sex did not influence the expression of any specific behavior event (ANOVA, $p > 0.05$ for all 14 behavior events), suggesting that animals tend to spend a similar amount of time in each behavior event, regardless of their sex.

Our analysis demonstrated that neither sex (ANOVA, $F(1,27) = 0.22$, $p = 0.63$), rehabilitation time (ANOVA, $F(1,24) = 0.11$, $p = 0.73$) or adaptation time in captivity (ANOVA, $F(1,24) = 1.76$, $p = 0.19$) seemed to influence the animals' observed behavioral

Table 2. Released Antillean manatees' (*Trichechus manatus manatus*) ethogram with each behavior state, its behaviors events and respective descriptions.

Behavior state	Behavior events and description
Feeding	At depth, ascending with a mouth full of food, exposing tail or back when diving. Breathing gap from 1 to 3 minutes In shallow waters: foraging with submersed body, visible from out of the water At the surface: eating leaves in the surface of the water, exposing the snout
Resting	In the deep, breathing gap from 4 to 6 minutes In shallow waters: still but with submersed body, visible from out of the water At the surface: moving with the back exposed in the surface
Drinking water	Drinking water (not specified) At the surface: exposing the mouth, far from the coast, from plants or rain From the river bank: exposing the mouth, close to the coast
Social	Social (not specified) Interacting with wild manatees Agonistic behavior: hitting other manatee with tail/head, or pushing them Touching conspecifics Courtship: males approach females from the back or from below, with or without penis exposition
Movement	Moving slowly: exploring, with the tracking device at the surface Traveling fast in one specific direction
Playing alone	Spinning around itself, touching itself

richness. The behavior richness calculated for all subjects ranged from three to 14 behaviors.

From the 29 manatees of the present study, 11 males and eight females had home range sizes provided by Normande et al. (2016), from 2008 to 2013. The home range size varied from 4.24 km² to 28.63 km² (mean = 10.88 ± 7.64). The behavioral richness recorded from the same time scale of the home range data ranged also from five to 14 behaviors (mean = 10.89 ± 2.82). Home range sizes have positive effects on behavioral richness (ANOVA, $F(1,17) = 5.34$; $p = 0.01$) and on the activity budget of two social behaviors: interacting with wild manatees (ANOVA, $F(1,17) = 15.34$; $p = 0.001$) and social behavior of touch (ANOVA, $F(1,17) = 6.99$; $p = 0.01$). These results indicate that animals with larger use of space expressed a wider variety of behaviors and engaged more in social behaviors with conspecifics than animals that had smaller home range sizes.

Discussion

Our results indicate that manatees' behavior richness, provided by the 12 years of releases and monitoring, had a wide variation, and that neither behavior richness nor the activity budget was influenced by time in rehabilitation/ adaptation, sampling effort or sex. However, regarding the manatees' movement ecology, home range was demonstrated to have a positive impact on both behavior richness and the activity budget of two social behaviors.

Behavior diversity is a measure that comprises the number of expressed behaviors and the frequency of each behavior and could be employed to evaluate animal welfare (Delfour et al., 2021). According to Miller et al. (2016), a high behavior diversity would indicate that the environment is meeting the animal's behavioral

needs, suggesting a good welfare condition; while a low diversity would indicate a lethargic or stereotyped animal (Mason & Latham, 2004). Analyses with behavior diversity were previously performed in aquatic mammals such as bottlenose dolphins (Delfour et al., 2021) and wild beluga whales (O'Corry-Crowe et al., 2020). Regarding manatees, Mercadillo-Elguero et al. (2014) performed a study with manatees in semi-captivity environments, and observed 24 behavior states and 19 behavior events. Although there was a reasonable difference in the observation of behavior states, compared to the present study (of only six states), the number of behavior events was similar between the investigations (16 in the present study). However, there are not yet studies approaching behavior diversity in free-ranging manatees to compare our data with.

Regarding behavior richness, as sampling effort and both time in rehabilitation or acclimatization facilities were not considered relevant factors, we can hypothesize that the lower behavior richness found for some animals may be due to the anthropic influence. Medina (2008) observed that *T. m. manatus* has higher sociability traits than the Florida manatee (*T. m. latirostris*), especially those in captivity. This could help to explain the occasional interactions between humans and released manatees on the northeastern Brazilian coast. For example, the male Xuxu had low behavior richness, and this can be explained by his general behavior and high interaction frequency with humans on some of the most popular beaches along the Brazilian coast (unpub. data; MacIntosh et al., 2011).

In this study we did not observe any correlation between behavior richness and manatees' sex. To the best of our knowledge there are no studies investigating this correlation in manatees, however, Delfour et al. (2021) observed differences between sexes in bottlenose dolphins. Nevertheless, some behaviors were more observed in females or males, such as courtship and

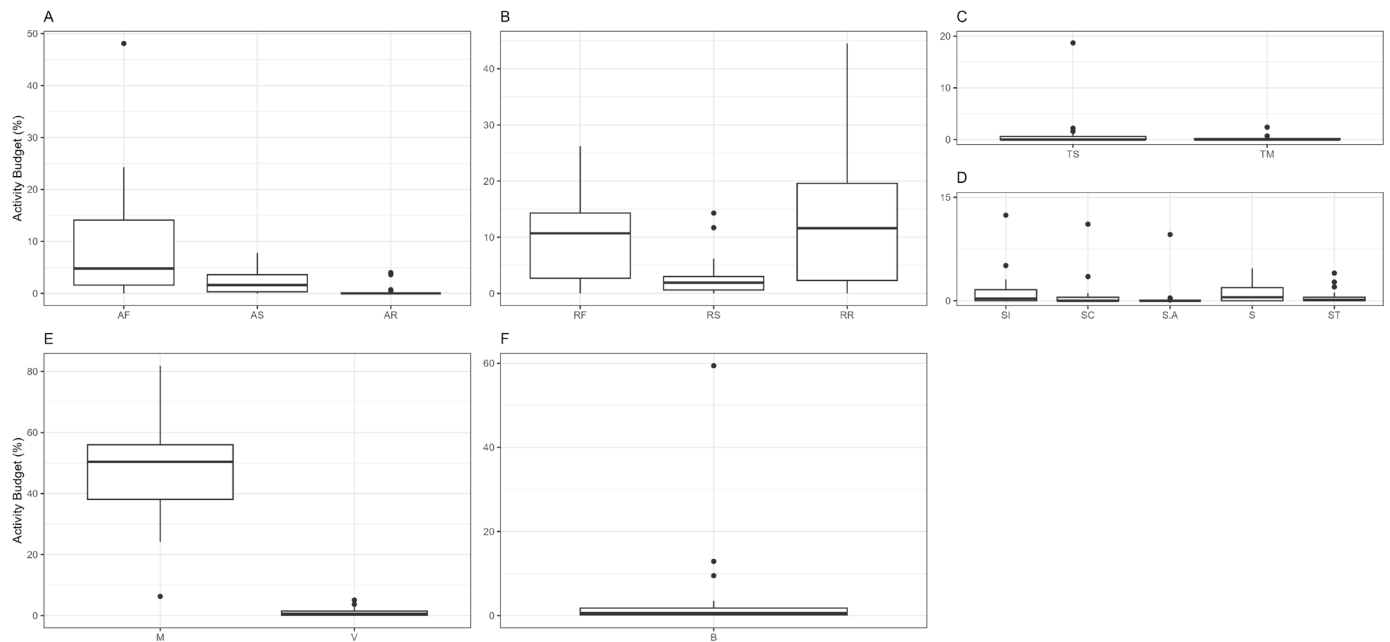


Figure 1. Boxplot of the six behavior states and their behavior events. A) feeding (AF: at depth, AS: at the surface, AR: in shallow waters). B) resting (RF: at depth, RS: at the surface, RR: in shallow waters). C) drinking water (TS: at the surface, TM: from the riverbank). D) social (SI: interacting with wild manatees, SC: courtship, SA: antagonist behavior, S: unspecified, ST: touching conspecific). E) movement (M: moving slowly, V: traveling fast). F) self-maintenance.

agonistic behavior, respectively. According to Hartman (1979), agonistic and sexual behaviors are most frequently expressed by males as a result of sexual maturity and competition for females, which agrees with our findings for the agonistic behavior, although not statistically. Of the nine females which expressed courtship behavior during the post-release monitoring, just one, Lua, had a recorded parturition in the same year in which the behavior was observed, in 2012 (Attademo et al., 2022). Of all four social behavior states, agonistic was the less frequently observed. Manatees are described to possess a non-aggressive temperament (Harms-Tuohy & Tuohy, 2017), and as agonistic behaviors are usually related to competition for females or resources (Hartman, 1979), our results suggest that the released manatees could either be well distributed in their habitat or that the area provides enough resource to the individuals, reducing dispute among them.

The great amount of time spent by manatees on exploratory movements found in this study can be explained by the species' biological and ecological life story traits. Manatees have sensitive vibrissae in their mouth which capture environmental information in the water current, through low-frequency and pressure waves, making exploratory movements a necessary behavior to self-locate and to find feeding areas (Hartman, 1979; Ramirez-Jimenez et al., 2017). A similar result was found by Lucchini et al. (2021) for captive and semi-captive Antillean manatees, whose exploratory behaviors corresponded to 70% of the animals' tactile activity budget and self-maintenance was the less represented behavior. Regarding the effect of sex on behavior expression, according to Lucchini et al. (2021), although females were observed to express a wider range of tactile behaviors (social, self-maintenance, and exploring) only two social behaviors were related to sex. However, the authors suggest that it could have been a result of the high density of females in the pool, which is also supported by da Silva (1996) and Kendall et al. (2014).

The prevalence of seagrasses as a food resource reasserts the findings from Hartman (1971) that manatees feed predominantly on submerged vegetation rather than on floating vegetation, and isotope analysis has pointed out that Florida manatees consume food resources mainly from marine environments (Lefebvre et al., 2000; Reich & Worthy, 2006). Apart from habitat loss, human activity also poses some major threats to manatees' feeding areas due to changes in water quality, leading to the decrease or even elimination of seagrass beds in manatees' occurrence areas (Smith, 1993). Thus, our results emphasize the importance of seagrass beds conservation throughout the species occurrence area, as it is an important marine resource for manatees (Lefebvre et al., 2000). Regarding water consumption, it is known that Brazil presents several aquifers along its coast, with some covering the areas in which the manatees were released and monitored (CPRM, 2001). Therefore, the fact that no behavior of drinking water from freshwater sources at the sea floor was recorded, must not be interpreted as the absence of this water resource in the area, but rather as the difficulty to observe this behavior in the wild, due to the water turbidity.

Home range is an animal ecology and behavior concept that can be described as how animals perceive the environment they are inserted in, and how they will use it to access resources and improve fitness (Mitchell & Powell, 2012; Spencer, 2012). The home range and behavior analyses presented in this study demonstrated that animals that move long distances not only have higher behavior richness (number of recorded behaviors), but also interact more with wild manatees and engage more in touch behaviors with conspecifics. Our results are supported by Normande et al. (2016), who observed mating behavior between wild and wide-range released manatees, thus indicating that home range might have an impact on the interaction probability between native and released manatees through the connection between sub-populations. The social behavior of touch was observed in

sites far from the release area, such as the Persinunga River at the border of Pernambuco and Alagoas states. Therefore, our findings indicate that released manatees that moved far from the released area and that animals with a larger home range may have greater chances to encounter and interact with released or wild conspecifics than short range animals.

Our results emphasize the importance of the release of individuals which were rescued and had gone through a rehabilitating process, highlighting their ability to return to their natural habitat and interact with wild conspecifics. Animals' releases provide unique data on animal behavior and dispersion, and can add important information to the knowledge of wild individuals, especially in cryptic species. Here we provided insights that indicate that the expression of specific behaviors, especially the ones related to intra-specific sociability, can be affected by an individual's home range size, increasing the chances of reproduction between released and wild manatees, increasing variability within wild populations, and therefore, contributing to manatees' conservation.

Acknowledgments

We would like to acknowledge all the rehabilitation and field staff, who provided support in data sampling throughout the study period, as well as Instituto Chico Mendes para Conservação da Biodiversidade and Fundação Mamíferos Aquáticos managers who provided the necessary means for manatees tracking. Helena Val and Alexandra Costa thank the GEF-Mar Project through a fellowship grant. Coordination of Superior Level Staff Improvement (CAPES) provided the Master's scholarship to Lucas Inácio dos Santos Melo and the Brazilian National Council for Scientific and Technological Development (CNPq) provided the International Sandwich Doctorate scholarship granted to Iran Campello Normande. The Fundação Mamíferos Aquáticos also thank Petrobras through the Petrobras Socioenvironmental Program, and the National Program for the Conservation of the Antillean Manatee developed by the Fundação Mamíferos Aquáticos and ICMBio/CMA and sponsored by the Fundação Boticário de Proteção à Natureza. The NGI ICMBio Costa dos Corais thanks Fundação SOS Mata Atlântica and Fundação Toyota do Brasil. Finally, we would also like to thank Cássia Farias for the support throughout the data processing.

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