



ECOSYSTEMS

Environmental characterization of home range of Antillean manatees (*Trichechus manatus*) released in northeastern Brazil

SEBASTIÃO S. DOS SANTOS, IARA S. MEDEIROS, ISIS C. DE ALMEIDA, VANESSA A. REBELO, ALLAN O.B. CARVALHO, RAFAEL MENEZES, MIRIAM MARMONTEL & JOÃO CARLOS G. BORGES

Abstract: The Antillean manatee occurs discontinuously from the state of Amapá to the state of Alagoas on the coast of Brazil. There is also evidence of reintroduced manatees using the coasts of Sergipe and Bahia, with a preference for calm shallow waters. This study characterized the home range areas of six rehabilitated manatees released in northeastern Brazil. The activities were conducted in the states of Paraíba, Sergipe, and Bahia. Type of environment, substrate, depth, aquatic vegetation, physicochemical variables of the water, presence of solid waste, human settlements, and watercraft were considered to characterize the areas. The results showed a manatee preference for sheltered areas. Resources were available in larger quantities in the dry season, and a reduction in the availability of food items was found over the years. High overlap was found in the multivariate space of the individuals in terms of the characteristics of the habitats. The estuary of the Paraíba River and the coastal area of Cabedelo Beach in Paraíba showed the greatest amount of solid waste, human settlements, and watercraft. Released manatees exhibited a preference for sites shallower than two meters, with food resources and fresh water availability.

Key words: Distribution, environmental degradation, food items, habitat characteristics, sheltered areas, sources of fresh water.

INTRODUCTION

Populations of the Antillean manatee (*Trichechus manatus*) in Brazil are distributed discontinuously from the state of Amapá to the state of Alagoas (Luna et al. 2008a, Lima et al. 2011). Manatees that have been rescued, rehabilitated, and returned to the wild have been using the coasts of Sergipe and Bahia (Santos et al. 2022).

The presence of manatees depends on the ecological needs of the species, such as food resources, sources of fresh water, protected refuge areas for resting, and warm, shallow water (Castelblanco-Martínez et al. 2012, Lima &

Passavante 2013, Normande et al. 2016). Several factors restrict the use of a site by manatees, such as human activities that cause environmental degradation (Lima et al. 2011), the presence of watercraft (Borges et al. 2007, Mercadillo-Elguero et al. 2014), and fishing activities (Silva et al. 2011). Such characteristics and factors influence the adaptation of rehabilitated and released manatees to life in the wild, and therefore can indicate the success of reintroductions (Lima et al. 2007).

In Brazil, manatees rescued as stranded calves, rehabilitated and subsequently released (with satellite and VHF telemetry systems),

presented home ranges between 2.56 and 42.07 km²; all fidelity sites (areas used most frequently) were within protected areas (Santos et al. 2022). These animals used estuarine ecosystems frequently, and also the transition area between estuaries and marine (mixed) and the marine ecosystem (Normande et al. 2016).

Knowledge on characteristics of preferred habitats assists in defining priority areas for a species. Therefore, this study aimed to characterize the areas used by Antillean manatees released in northeastern Brazil.

MATERIALS AND METHODS

Study area

This study was conducted in the states of Paraíba, Sergipe, and Bahia, which are situated in

northeast Brazil (Figure 1). The areas used by six rescued, rehabilitated, released, and monitored manatees (two males and four females) were identified and characterized.

In Paraíba, manatees mainly use the northern coast of the state, where the climate is warm and humid, with a mean annual temperature ranging from 22°C to 25°C (Francisco & Santos 2017). The estuaries of the Mamanguape River and the Paraíba do Norte River are located along this coastline. The estuary of the Mamanguape River, second largest in the state, is located in the municipality of Rio Tinto (A.E.B. Alencar, unpublished data); the estuary of the Paraíba do Norte River, where the Cabedelo Port is located, with considerable motorboat traffic, is located between the municipalities of Cabedelo and Lucena (Marcelino et al. 2005).

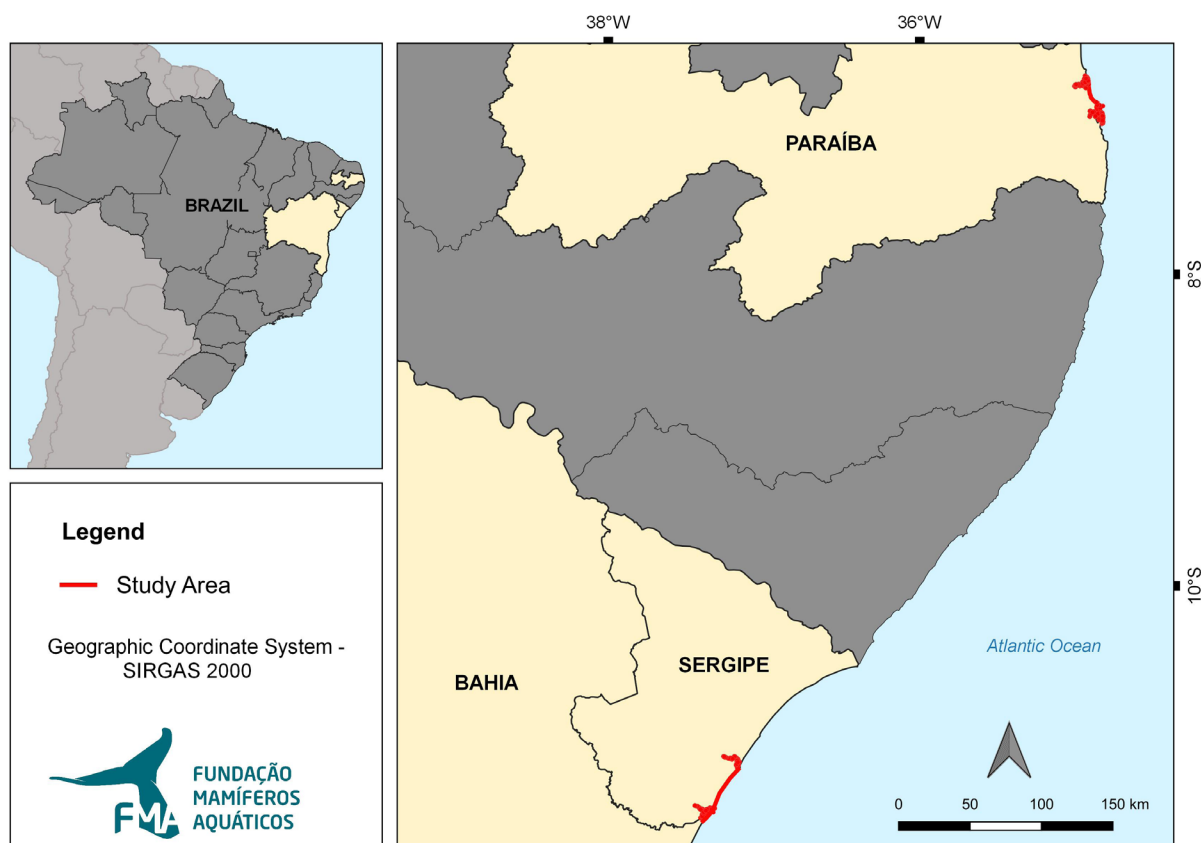


Figure 1. Study area, showing the location of the three states (Paraíba, Sergipe, and Bahia) used in the characterization of home ranges of Antillean manatees.

In Sergipe and Bahia, the areas were situated in the estuary of the Vaza Barris River, of approximately 20 km, and the Piauí-Fundo-Real estuarine complex. The Piauí River is 132 km long and flows into the ocean between the municipalities of Estância (Sergipe) and Jandaíra (Bahia) after the confluence with the Fundo River on the left margin and Real River on the right margin (Carvalho & Fontes 2006). The climate of the region is megathermal humid to sub-humid, with a mean annual temperature generally above 26°C (J.A.C. Fontes, unpublished data).

The database of the Aquatic Mammal Foundation was used to identify environmental sampling points. The database contains the geographic coordinates of six released manatees

monitored through telemetry (VHF and satellite) from January 2017 to December 2019, with a total of 2,717 days. The locations of each animal were plotted using the QGIS 2.18 program, and density maps of the areas used were created. This spatialization assisted in the definition of sites for environmental characterization (Figure 2). A total of 178 points were defined throughout the home ranges of the six manatees (Table I).

Characterization of areas used by released manatees

After the identification of the main sites used by the manatees, each area was characterized considering the following aspects: a) type of environment (sheltered or open); b) type of substrate (sandy, clay, or clay-sandy); c) depth

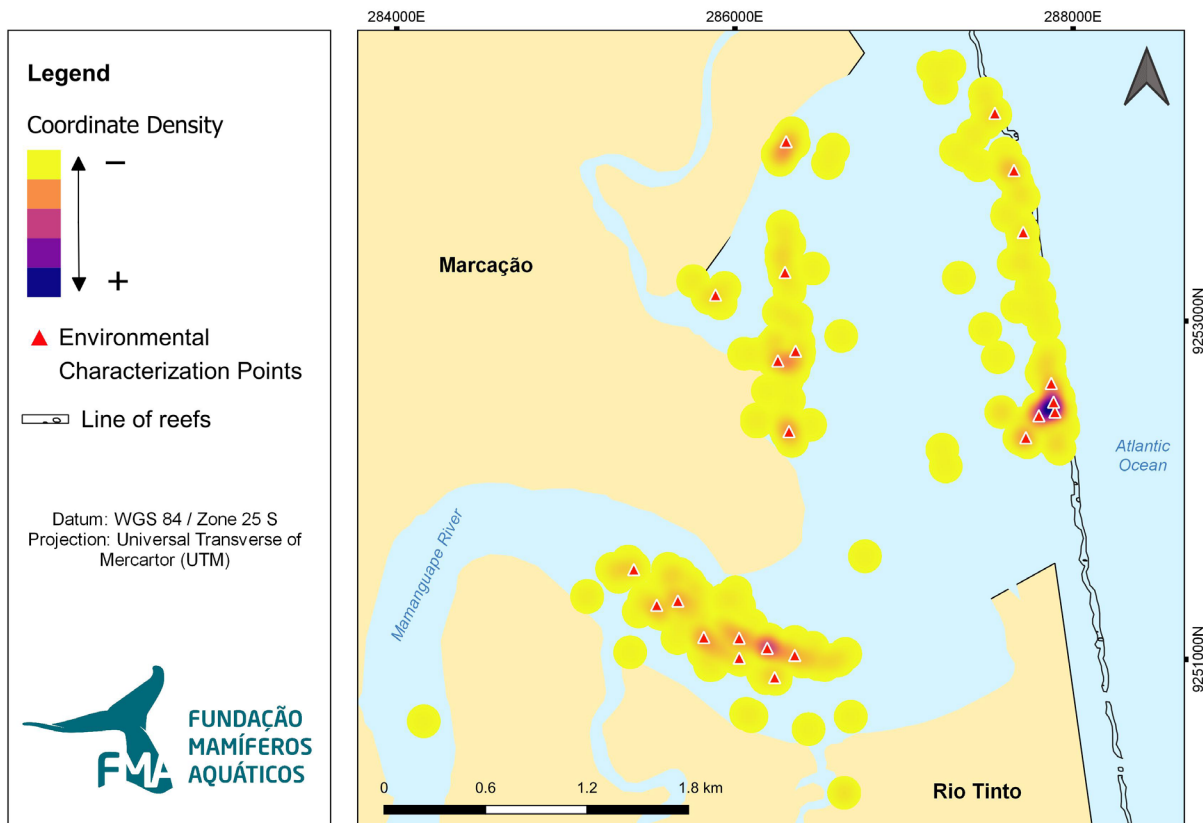


Figure 2. Spatialization of geographic coordinates and selection of environmental characterization points within the home range of manatee “Yara” in Mamanguape River, Paraíba.

Table I. Number of points for environmental characterization within areas used by six Antillean manatees in the states of Paraíba, Sergipe, and Bahia.

Animal	N° of monitoring days	N° of environmental characterization points
Astro	799	26
Mel	1.319	50
Puã	603	43
Tita	174	10
Yara	117	23
Zelinha	993	26

of site (Class 1: 0 to 1.50 m; Class 2: 1.51 to 3.00 m; Class 3: > 3 m); d) presence of food items; e) physicochemical variables (pH, conductivity, dissolved oxygen, salinity, and temperature); f) presence of solid waste; g) human settlements (presence/absence of residences or commercial establishments); h) watercraft (present/absent).

For the classification of the type of environment, sites within bays, estuaries, or those protected by reef barriers were identified as “sheltered”. The sites exposed to the action of the sea were classified as “open” (Lima et al. 2011).

The type of substrate was determined through dives at the sites using visual and tactile methods (IBGE 2007). In the field, the substrate was evaluated based on the sensation of rubbing a sample of moist substrate between the fingers. The predominance of sand transmits a sensation of friction, whereas clay transmits a sensation of plasticity and tackiness. Samples with equal portions of clay and sand were classified as clay-sandy substrates (IBGE 2007).

The depth of the sampling sites was measured with a Secchi disk and measuring tape. The presence of food items was determined through either free-diving or scuba diving, following straight-line transects from a central

point in four directions (north, south, east, and west) with 10 m radius. Aquatic vegetation was collected and photographed for subsequent identification (SILVA IB, unpublished data). These data were collected during the dry (September to February) and rainy (March to August) seasons (INMET 2010) from 2017 to 2019.

The Kruskal-Wallis test was used to determine differences in the presence of food items (response variable) between years (2017 to 2019). The Mann-Whitney test was used to verify the presence of food items between the dry and rainy seasons. When differences were found, Dunn’s post hoc test was used to identify heterogeneous groups.

During the field monitoring of the Mamanguape River estuary, a multi-parameter probe (AKSO, model AK 88) was employed at sites used by the manatees to collect data on pH, salinity, temperature, conductivity, and dissolved oxygen. These data were collected when the animal was first sighted and after each hour of monitoring. Principal component analysis (PCA) was used with log-transformed data ($\log x+1$) to reduce the multi-variability of the data and identify possible similarities regarding the use of habitats by the individuals.

The presence of solid waste was determined through dives, traveling a 10 m radius in four directions. Items were collected and identified in the following categories: plastic, rubber, glass, nylon, and others. Human occupation was identified visually and recorded based on the type of building, such as houses, hunting/fishing communities, restaurants, buildings, etc. The presence of watercraft was recorded considering boats in ports and those sailing around the evaluated sites.

RESULTS

Type of environment

All points of environmental characterization for the six manatees were located in sheltered environments. In Paraíba, these points were distributed in three areas. The first was the estuary of the Mamanguape River, where a sandbank on the right margin of the mouth reduces the action of currents from the coast into the estuary; a reef barrier situated immediately offshore of this sandbank further diminishes the action of the currents and waves. Manatees did not use the areas past these reefs. The second area was the coastal zone in Cabedelo, where reefs provide an environment of calm, shallow waters. The third area was the estuary of the Paraíba do Norte River, where the manatees used one of its tributaries approximately 7 km upstream from the coast, with calm waters and no waves.

In Sergipe and Bahia, the estuary of the Vaza Barris River and the Piauí-Fundo-Real estuarine complex have similar characteristics, with sandbars at the mouth providing greater protection from current and wave action. All characterization points were upstream in the main river or one of its tributaries, with calm waters and no waves.

Type of substrate

Sandy substrate was found at 53% of the points evaluated, clay-sandy at 33.3%, and clay at 13.7%. The distribution of substrates was similar in points in Paraíba, with a greater incidence of sandy substrates along the beachline and near the mouth. Clay substrates were located higher upstream in the river and tributaries, with interspersed points containing clay-sandy substrates (Figure 3a and b). In the Piauí-Fundo-Real estuarine complex, only two points had clay substrates, located near the mouth. Sandy

substrates prevailed on the left margin and clay-sandy substrates on the right (Figure 3c). In the estuary of the Vaza Barris River, no clay substrates were found at the sites used by the manatees; a small area of sandy soil was found near the mouth, and clay-sandy soil prevailed at all other points (Figure 3d).

Depth

The depth at the characterization points ranged from 0.0 to 5.2 meters. Class 1 (0.0 – 1.50 m) was the most representative, accounting for 72% of the points, followed by Class 2 (1.51 – 3.00 m, 25.5%), and Class 3 (> 3 m, 2.5%) of the areas used by the manatees.

In Paraíba, the Mamanguape and Paraíba rivers estuaries exhibited shallower points near the mouth and deeper points used by the manatees up river or in one of the tributaries (Figure 4a and b). In the Piauí-Real-Fundo estuarine complex, both near-the-mouth and upstream areas used were shallow (Figure 4c). The estuary of the Vaza Barris River had shallow points at both the mouth and more upstream, but deeper points between these two sites (Figure 4d).

Presence of food items

Thirty-four species of marine algae were identified in the home ranges of the six animals monitored: 15 of green, 13 of red, and six species of brown algae. Four species of seagrass and five freshwater macrophytes were also identified (Table II).

In the estuary of the Mamanguape River, food items were distributed mainly near the mouth, with only seagrasses found in the more upstream points of the river. Green and red algae, and seagrasses were found in the estuary of the Paraíba River and in Cabedelo Beach; brown algae were found only in the area of the coast in Cabedelo Beach, and there were no records of

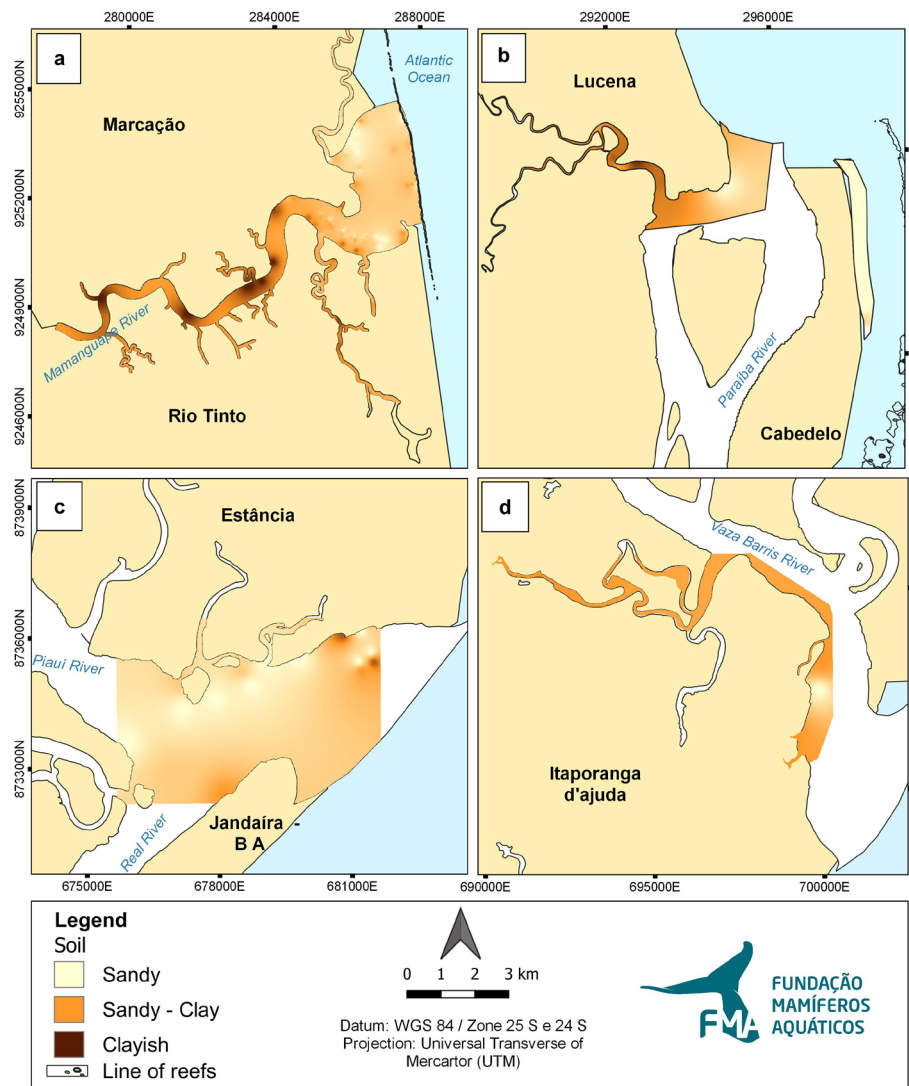


Figure 3. Types of substrate in areas used by Antillean manatees in the states of Paraíba, Sergipe, and Bahia. a = estuary of Mamanguape River, b = estuary of Paraíba River and Cabedelo Beach, c = Piauí-Real-Fundo estuarine complex, d = estuary of Vaza Barris River.

freshwater macrophytes. The Piauí-Real-Fundo estuarine complex had green and red algae, and seagrasses, whereas only seagrasses were found in the estuary of the Vaza Barris River (Figure 5).

Temporal differences were found regarding food items (Kruskal-Wallis $H_{(2)} = 6.69, p = 0.03$), particularly between 2017 and 2019. A gradual reduction in the presence of food items was found throughout the sampling period (Figure 6). Analyzing heterogeneity in the percentage of food items between the dry and rainy seasons, the dry season had significantly greater presence of food items (Mann-Whitney $U = 59, p = 0.04$) (Figure 7).

Physicochemical variables

The physicochemical variables of water in the manatees’ areas had similar minimum-maximum ranges (Table III). The first principal components explained 74% of the total variation in the data, with negative scores of Principal Component 1 (PC1) (52.77%) explaining pH, conductivity, and salinity, whereas negative scores of PC2 (21.28%) were related to temperature. The dissolved oxygen was poorly explained by both axes (Table IV). Generally, the PCA showed high overlap in the multivariate space of the individuals regarding the characteristics of the habitats. However, manatee “Tita” was positioned away

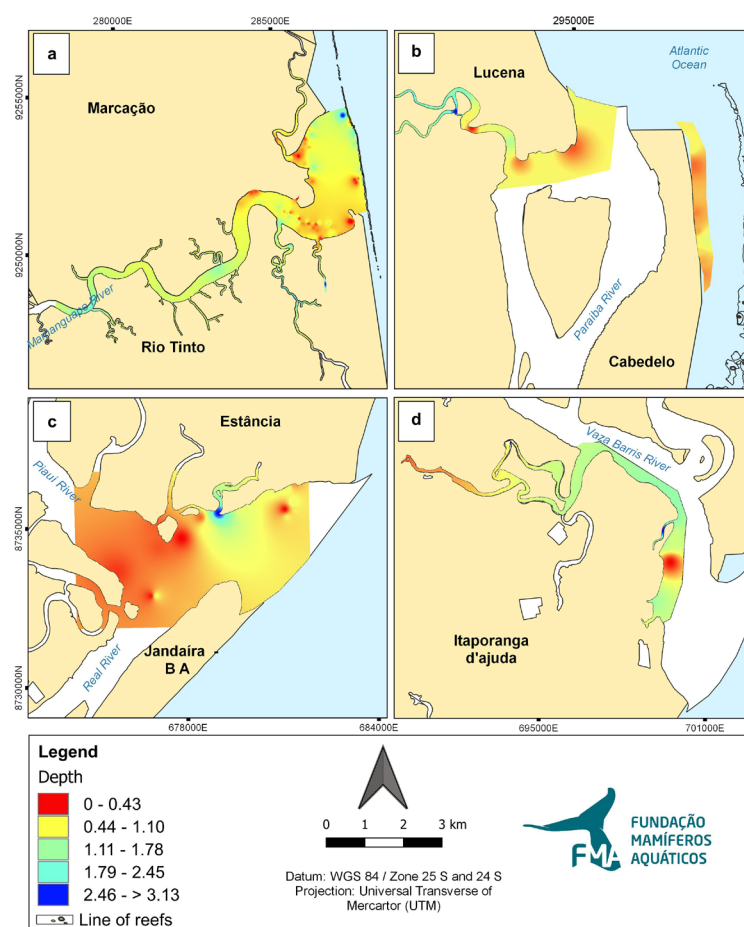


Figure 4. Depth of areas used by Antillean manatees in the states of Paraíba, Sergipe, and Bahia. a = estuary of Mamanguape River, b = estuary of Paraíba River and Cabedelo Beach, c = Piauí-Real-Fundo estuarine complex, d = Estuary of Vaza Barris River.

from the other manatees because of the greater range of variation (Figure 8).

The pH ranged from 6.3 to 8.8, with lower values upstream. Salinity ranged from 0.3 to 36.9 ppm, with lower values found at the sites used to obtain freshwater. The water temperature ranged from 22.4 to 33.1°C. Conductivity ranged from 10.8 to 59.5 $\mu\text{S}/\text{cm}$, with the lowest values upstream, increasing progressively toward the mouth. Dissolved oxygen ranged from 0.4 to 12.2 mg/l, with similar values both upstream and near the mouth (Figure 9a, b, c and d).

Solid waste

The presence of solid waste was recorded at 44% of the characterization points throughout the three years of the study. The most common type of solid waste was plastic. In the estuary of

the Mamanguape River, solid waste was found at points near the mouth and on both banks of the river. Plastics were found at 80 points, nylon at ten, and rubber and metal at one point each. In the estuary of the Paraíba River, solid waste was present at more upstream points and on the left bank of the river; plastics were found at seven points, nylon at three, and rubber and metal at only one point. In Cabedelo Beach, nylon was found at only one point, whereas plastics were found at all others, to the north of the beach. In the Piauí-Real-Fundo estuarine complex, solid waste was found near the mouth, with Styrofoam found at one point and plastic found at all other points. In the estuary of the Vaza Barris River, plastic was found at points close to the mouth and upstream of one tributary (Figure 10).

Table II. Food items found in areas used by Antillean manatees in the states of Paraíba, Sergipe, and Bahia, 2017-2019.

GREEN-ALGAE	RED-ALGAE	BROWN-ALGAE	MARINE-ANGIOSPERMS	FRESHWATER-MACROPHYTE
<i>Caulerpa</i> sp.	<i>Amansia</i> sp.	<i>Dictyota</i> sp.	<i>Halodule wrightii</i>	<i>Sesuvium portulacastrum</i>
<i>Caulerpa cupressoides</i>	<i>Amansia multifida</i>	<i>Dictyopteris delicatula</i>	<i>Halophila baillonis</i>	<i>Blutaparon portucaloides</i>
<i>Caulerpa prolifera</i>	<i>Cryptonemia crenulata</i>	<i>Lobophora variegata</i>	<i>Halophila decipiens</i>	<i>Cyperus compressus</i>
<i>Caulerpa mexicana</i>	<i>Corallina officinalis</i>	<i>Lobophora</i> sp.	Mangrove leaves	<i>Bulbostylis</i> cf. <i>hirtella</i>
<i>Caulerpa sertularioides</i>	Rhodomelaceae	<i>Sargassum</i> sp.		Poacea
<i>Ulva</i> sp.	<i>Gracilaria</i> sp.	<i>Padina gymnospora</i>		
<i>Ulva lactuca</i>	<i>Gracilaria domingensis</i>			
<i>Ulva fasciata</i>	<i>Cryptonemia</i> sp.			
<i>Bryopsis</i> sp.	<i>Gelidium</i> sp.			
<i>Centroceras</i> sp.	<i>Gelidialla acerosa</i>			
<i>Derbesia</i> sp.	<i>Hypnea</i> sp.			
<i>Halimeda</i> sp.	<i>Hypnea musciformis</i>)			
<i>Penecillus</i> sp.	<i>Laurencia</i> sp.			
<i>Penecillus capitatus</i>				
<i>Udotea</i> sp.				

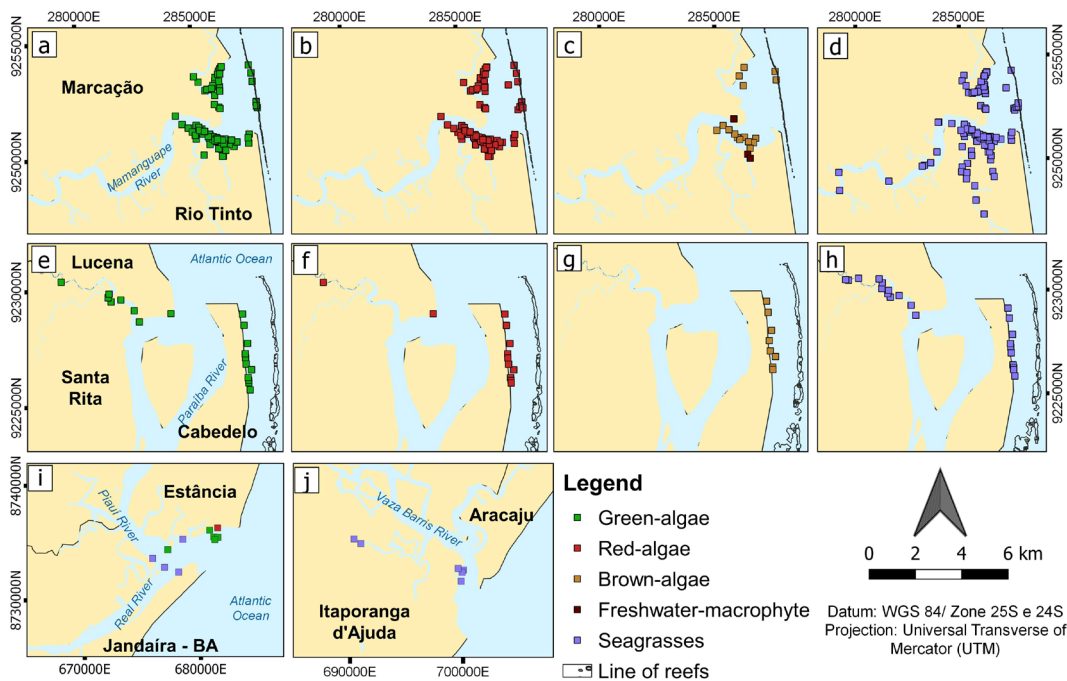


Figure 5. Distribution of potential food items for Antillean manatee. a, b, c, and d = estuary of the Mamanguape River, e, f, g and h = estuary of the Paraíba River and Cabedelo, i = Piauí-Real-Fundo estuarine complex, j = estuary of Vaza Barris River.

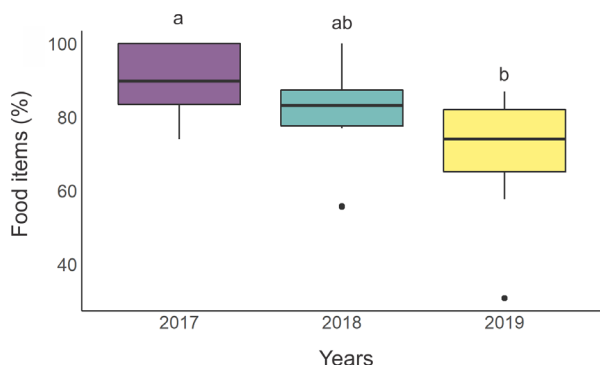


Figure 6. Percentage of presence of food items in areas of use of Antillean manatee from 2017 to 2019. a and b represent different groups. Colors represent different levels of categorical variables. Boxplots represented by median.

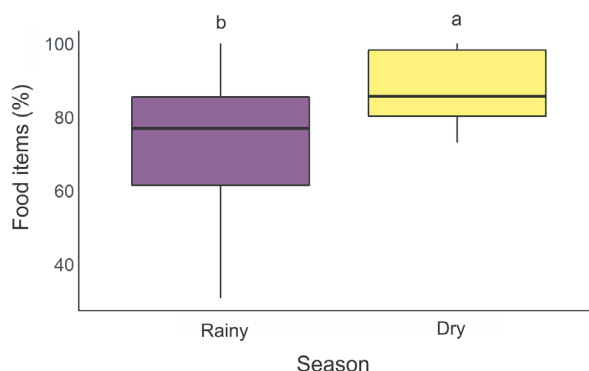


Figure 7. Presence of food items in areas of use of Antillean manatee in dry and rainy season from 2017 and 2019, in the states of Paraíba, Sergipe, and Bahia. a and b represent different groups. Colors represent different levels of categorical variables. Boxplots represented by median.

Table III. Minimum-maximum range of physicochemical variables analyzed in water of the Mamanguape River estuary in Paraíba, 2017-2019. Local used by five Antillean manatees.

Animal	pH		Salinity		Temperature		Conductivity		Dissolved oxygen	
	Min	Max.	Min	Max.	Min	Max.	Min	Max.	Min	Max.
Mel	7,0	8,2	0,3	36,9	25,2	31,1	25,0	59,5	0,7	10,8
Yara	7,3	8,8	24,6	36,2	25,9	30,3	46,6	58,6	3,0	8,7
Tita	6,3	7,7	5,4	36,9	27,2	33,1	10,8	54,1	0,4	4,5
Zelinha	6,7	8,3	1,1	36,8	22,4	31,6	23,1	58,6	0,4	9,5
Puã	6,7	8,3	1,7	36,8	28,1	31,2	30,2	57,5	3,8	12,2

pH = potential of hydrogen; Min = minimum; Max = maximum.

Table IV. Explanation of each variable by axis according to principal component analysis (PCA) to identify possible similarities regarding the use of habitats by Antillean manatees.

Variable	PC1	PC2
pH	-0.432	0.284
Conductivity	-0.579	-0.100
Dissolved oxygen	-0.351	0.146
Salinity	-0.591	-0.111
Temperature	-0.053	-0.935

PC1= principal components of axis 1; PC2= principal components of axis 2; pH = potential of hydrogen.

Human settlements

Human settlements were found at 29% of the characterization points. On the Mamanguape River, settlements were more common on the left bank and near the mouth. On the Paraíba River, settlements were more common at points upstream of a tributary used by the manatees and all characterization points on beaches in the municipality of Cabedelo. In the Piauí-Real-Fundo estuarine complex, five points had settlements – four on the left and one on the right bank. On the Vaza Barris River, settlements were found at two characterization points – one near the mouth and the other approximately 14 km upstream of a tributary (Figure 10).

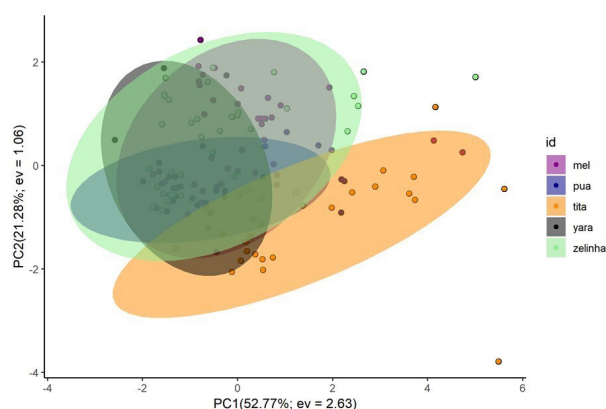


Figure 8. PCA using pH, conductivity, dissolved oxygen, salinity, and temperature in areas of use of Antillean manatees (Mel, Puã, Tita, Yara, and Zelinha) in the estuary of Mamanguape River. Id = individuals.

Watercraft

Watercraft were found at 38.8% of the points evaluated. On the Mamanguape River, a greater presence of boats was found on the margins near the mouth, and diminished upstream. On the Paraíba River, boats were recorded at six points – one near the mouth and five upstream. Watercraft were present at all points in Cabedelo beach area).

In the Piauí-Real-Fundo estuarine complex, boats were more common at the characterization points on the left margin near the mouth. Two points on the Vaza Barris River had boats – one located near the mouth and another upstream (Figure 10).

DISCUSSION

All environmental characterization points were located within estuaries or in locations protected by reef barriers. The preference of manatees for these sites is linked to the shelter offered in these environments (Normande et al. 2016). Additionally, it is common to find food items and shallow, calm, warm waters in these areas (Lima et al. 2011). Another factor that favors the use of estuaries by manatees is the importance

of these environments to the reproduction of the species since these sheltered sites serve as nurseries where females offer parental care to their offspring (Luna et al. 2008b).

A sandy substrate was found at 53% of the characterization points. This likely occurred because the points most used by the manatees were located near the mouth of the rivers or in beach areas (Neves & Neves 2010). Clay substrate was found less frequently and at more upstream points on rivers or tributaries. Many of these locations were used to obtain freshwater or as corridors to reach this resource, as also observed for the Antillean manatee in Mexico (Castelblanco-Martínez et al. 2013).

Most of the areas used by the manatees were no deeper than 1.5 meters, demonstrating a preference for shallow areas. Similar observations were described by Paludo & Langguth (2002) in the state of Rio Grande do Norte. Manatees can use sites with minimal depth, sufficient to enable passage (Flamm et al. 2005); however, during fast movements, they prefer greater depths (Edwards et al. 2016).

Manatees use and spend more time in areas with greater availability of food resources (Olivera-Gomez & Mellink 2005, Jiménez 2005, Castelblanco-Martínez et al. 2013, Lima et al. 2012), paramount to their presence or permanence at sites. For example, the number of manatees in the Caribbean was reduced in a large part of their distribution area due to a reduction in available foraging areas (Castelblanco-Martínez et al. 2011). The indication of a reduction in food items in this study serves as a warning against depletion of such resources in manatee areas of distribution in Brazil.

This reduction in food availability over the years was even greater during the rainy season. Seasonality in the availability of these resources has been reported in previous studies. Marine algae biomass was greater in

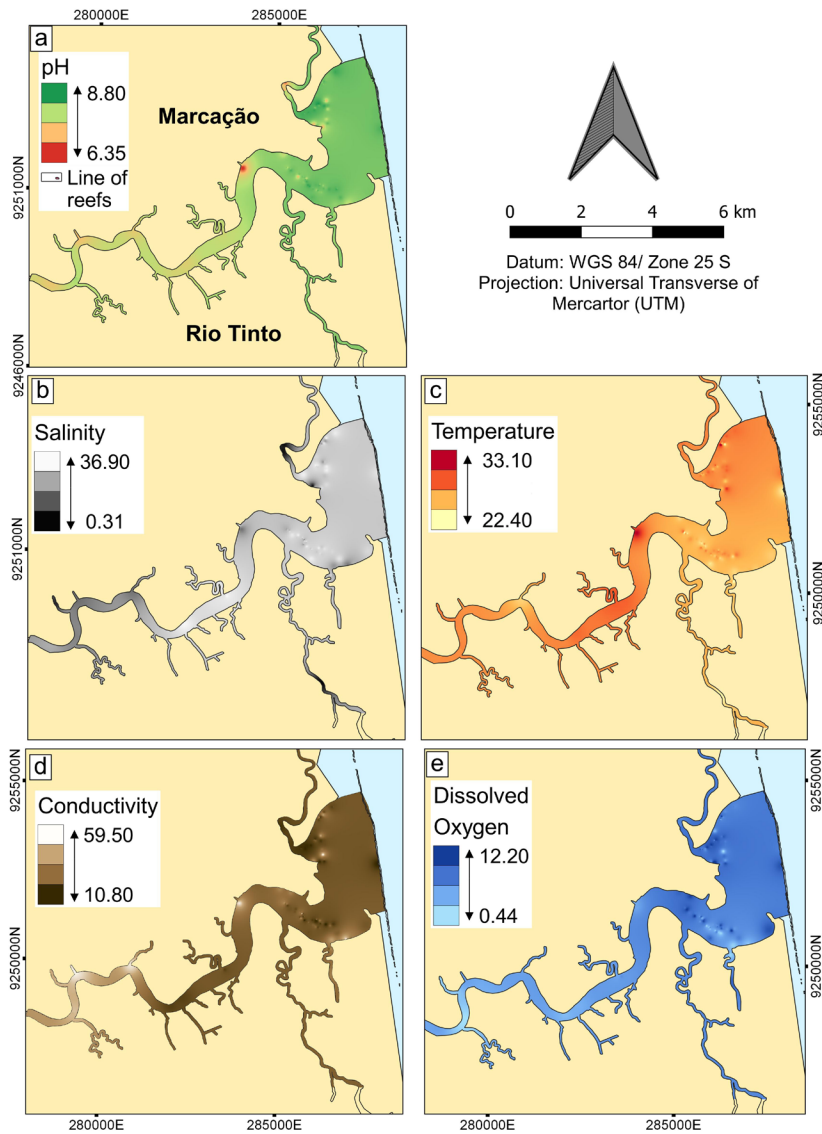


Figure 9. Water variables in the estuary of Mamanguape River. a = pH, b = salinity, c = temperature, d = conductivity, e = dissolved oxygen.

Tamandaré (northern coast of Pernambuco state) during the dry season (Short et al. 2006), whereas the species richness of macroalgae was greater during the rainy season in the same state (Vasconcelos et al. 2013). The reduction in food items at the characterization points in this study is influenced by the coastal dynamics of particular estuaries (influenced by the intensity of the currents), with the deposition of sediment on seaweed beds (AQUASIS 2016). Additionally, a greater river discharge in the rainy season leads to greater turbidity in estuaries, hence

a reduction in light penetration (J.A.C. Falcão, unpublished data).

The PCA revealed that physicochemical variables of the water at the sites used by the manatees were similar, with a slight difference regarding the areas used by the individual “Tita”, which had a larger range of variation. This difference may be linked to the fact that this individual used more upstream areas in the Mamanguape River, compared to the other individuals. Temperature seems not to be a limiting factor for manatee use at the studied sites. In Florida (USA), manatees perform

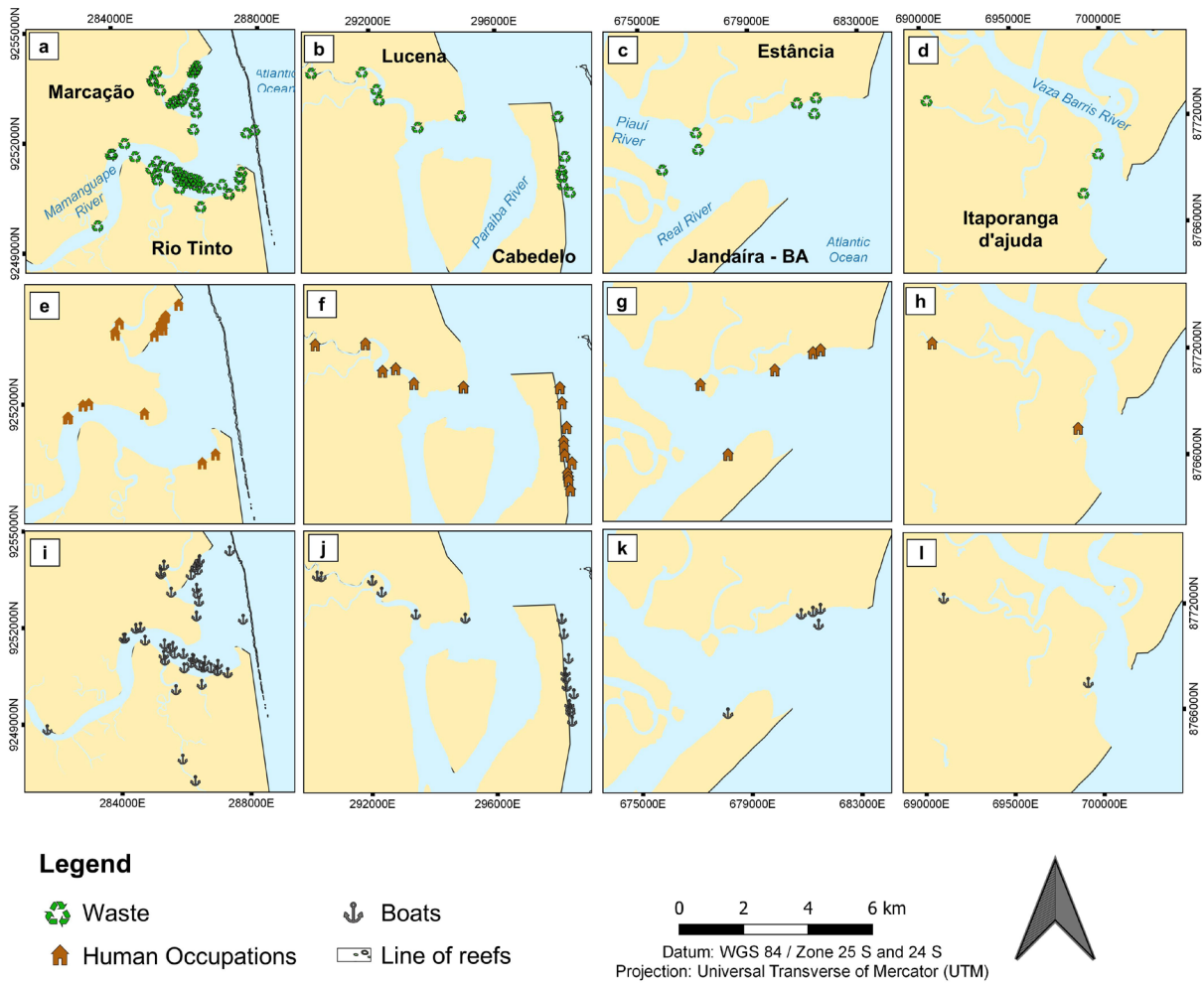


Figure 10. Presence of solid waste at characterization points. (a) estuary of Mamanguape River, (b) Paraíba River and Cabedelo Beach, (c) Piauí-Real-Fundo estuarine complex, (d) Vaza Barris River, (e) Presence of human settlements at environmental characterization points in estuary of Mamanguape River, (f) Paraíba River and Cabedelo Beach, (g) Piauí-Real-Fundo estuarine complex, (h) Vaza Barris River, (i) Presence of watercraft at environmental characterization points in estuary of Mamanguape River, (j) Paraíba River and Cabedelo Beach, (k) Piauí-Real-Fundo estuarine complex and (l) Vaza Barris River.

movements in search of refuges when the temperature reaches around 19°C (Reynolds et al. 2009). In the areas of distribution of the species in Brazil, especially in the northeast region, the annual variation in the temperature of coastal waters is minimal, with values higher than 24°C (Luna et al. 2008a), which is considered favorable to the occurrence of manatees (Lima et al. 2011).

A considerable variation in salinity was found. According to Castelblanco-Martínez et al. (2012), salinity varies with the seasonality

of rainfall. Considered a euryhaline species (AQUASIS 2016), it is common to find manatees in a wide range of salinity (Spiegelberger & Ganslosser 2005, Castelblanco-Martínez et al. 2012, Gonzalez-Socoloske et al. 2015). The lowest salinity values found in the areas studied were at upstream points used by manatees to obtain freshwater in the Mamanguape River (0.3 to 5.47 ppt). The pH data show that manatees in the wild visit environments with parameters different from those established in Normative

Instruction N° 03, of February 8, 2002 (IBAMA 2002), for manatees in captivity of 7.2 to 8.4.

Plastic was the most common type of solid waste found at characterization points. Besides causing environmental pollution, plastic adhered to marine algae can be ingested accidentally by manatees and cause obstructive processes in the gastrointestinal tract, with cases of death of reintroduced individuals reported (Attademo et al. 2015). Human settlements were recorded at 29% of the characterization points in this study. The planning of urban buildings is a factor that merits attention, as the occupation of coastal areas can exert an influence on the use of the environment by manatees (Morales-Vela et al. 2000, Flamm et al. 2005).

The movements of watercraft can also play an important role in the areas used by manatees, as reported in Costa Rica (Smethurst & Nietschmann 1999, Jiménez 2005), United States (Edwards et al. 2016, Reynolds et al. 2009), and Mexico (Morales-Vela et al. 2003, Rodas-Trejo et al. 2008). Besides the disturbances caused by the noise of the motors (Smethurst & Nietschmann 1999) and the release of oil residue (Bonde et al. 2004), there is risk of collisions between boats and manatees (Edwards et al. 2016). Such events have increased over the years in Brazil, with records in the states of Sergipe, Alagoas, Pernambuco, Paraíba, and Rio Grande do Norte, causing diverse forms of physical trauma to the affected animals (Borges et al. 2007, 2018).

In this study, the released Antillean manatees demonstrated a preference for shallow sites located within estuaries, classified as sheltered locations with food resources and sources of freshwater.

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SEBASTIÃO S. DOS SANTOS^{1,2}

<https://orcid.org/0000-0003-0194-2975>

IARA S. MEDEIROS^{1,2}

<https://orcid.org/0000-0002-7467-5970>

ISIS C. DE ALMEIDA^{1,2}

<https://orcid.org/0000-0002-3120-6568>

VANESSA A. REBELO^{1,2}

<https://orcid.org/0000-0001-7139-7189>

ALLAN O.B. CARVALHO²

<https://orcid.org/0000-0002-8274-2416>

RAFAEL MENEZES³

<https://orcid.org/0000-0003-2378-3805>

MIRIAM MARMONTEL⁴

<https://orcid.org/0000-0003-3747-9548>

JOÃO CARLOS G. BORGES^{1,2}

<https://orcid.org/0000-0002-0033-6781>

¹Programa de Pós-Graduação em Ecologia e Monitoramento Ambiental/PPGEMA, Universidade Federal da Paraíba/UFPB, Av. Santa Elisabete, 160, 58297-000 Rio Tinto, PB, Brazil

²Fundação Mamíferos Aquáticos/FMA, Estrada de Matapuã, 411, Anexo Chácara Anjo Gabriel, Mosqueiro, 49100-000 São Cristóvão, SE, Brazil

³Programa de Pós-Graduação em Ciências Biológicas (Zoologia), Universidade Federal da Paraíba/UFPB, Jardim Universitário, s/n, Campus I, Cidade Universitária, 58051-900 João Pessoa, PB, Brazil

⁴Instituto de Desenvolvimento Sustentável Mamirauá, Rua Estrada do Bexiga, 2584, 69553-225 Tefé, AM, Brazil

Correspondence to: **João Carlos Gomes Borges**

E-mail: jcgborges@hotmail.com

Author contributions

Sebastião S. dos Santos contributed substantially to the concept, study design, data collection, analysis and manuscript preparation; Isis Chagas de Almeida, Vanessa Araujo Rebelo and Allan O. B. Carvalho contributed to data collection and manuscript writing; Iara dos Santos Medeiros and Rafael Menezes to the analysis of results and manuscript writing; Miriam Marmontel contributed to the concept, analysis and manuscript preparation; and João Carlos Gomes Borges contributed to the concept, study design, analysis, manuscript preparation and supervision.

