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POPULATION STUDY OF THE SEAHORSE Hippocampus reidi GINSBURG, 1933 (TELEOSTEI: SYNGNATHIDAE) IN TWO ESTUARIES OF NORTHEASTERN BRAZIL

Estudo populacional do cavalo-marinho *Hippocampus reidi* Ginsburg, 1933 (Teleostei: Syngnathidae) em dois estuários do nordeste do Brasil

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ABSTRACT

Seahorses, classified as bony fishes within the genus *Hippocampus*, possess biological characteristics that make them particularly susceptible to overexploitation. Intense commercial demand, coupled with habitat degradation, has contributed significantly to population declines. Given the global threats faced by these organisms and their status as a "flagship species," ecological studies focused on seahorses are of critical importance. This study aimed to assess the spatial and temporal distribution of *Hippocampus reidi* abundance in the Pacoti and Malcozinhado Rivers, as well as to document other ecological characteristics of the species. Transects covering an area of 100 m^2 were established along the riverbanks, with each river divided into two sampling stations at increasing distances from the sea. The mean density of *H. reidi* was 0.008 individuals/m^2. The Pacoti River exhibited significantly higher mean densities compared to the Malcozinhado River ($p = 0.019$). No significant differences in density were observed between the two stations within the Pacoti River, whereas a marked difference was noted between stations in the Malcozinhado River. Although both rivers exhibited a decreasing trend in densities over the study period (Periods 1 and 2), statistical testing of these trends was not conducted due to the lack of standardization in data collection methods between the two periods. No seasonal variation in abundance was detected. The seahorses in the Pacoti River were significantly larger than those from the Malcozinhado River ($p = 0.027$). The sex ratio was 1:1, but males exhibited a greater mean size than females ($p = 0.013$). Black and brown color morphs were more prevalent than other colorations. Additionally, seahorses showed a preference for anchoring to the roots of *Laguncularia racemosa* as holdfast structures. This study highlights that environmental variations between estuaries—such as the extent of mangrove coverage, discharge volumes, and susceptibility to anthropogenic impacts—can influence the abundance and ecological patterns of *H. reidi*. Effective conservation and management of this threatened species will depend on a comprehensive understanding of its ecological requirements and responses to environmental factors.

Keywords: Population structure, bioecology, habitat conservation.

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RESUMO

Os cavalos-marinhos, classificados como peixes ósseos do gênero Hippocampus*, possuem características biológicas que os tornam extremamente vulneráveis à sobreexploração. O intenso comércio aliado à degradação de habitats contribui significativamente para o declínio de suas populações. Considerando o nível de ameaça a esses animais em escala global e sua relevância como "espécie bandeira", estudos sobre sua ecologia são de extrema importância. O objetivo deste estudo foi determinar a distribuição espacial e temporal da abundância de* Hippocampus reidi *nos rios Pacoti e Malcozinhado, registrando também outros aspectos ecológicos da espécie. Transectos com área de 100 m² foram realizados ao longo das margens dos rios. Cada rio foi dividido em duas estações de amostragem situadas a distâncias crescentes do mar. A densidade média observada foi de 0,008 indivíduos/m². O Rio Pacoti apresentou uma densidade média significativamente maior em comparação ao Rio Malcozinhado (p = 0,019). Não houve diferença significativa entre as duas estações no Rio Pacoti, enquanto no Rio Malcozinhado foi notada uma diferença evidente. Embora ambos os rios tenham apresentado tendência decrescente nas densidades ao longo do período de estudo (períodos 1 e 2), essas tendências não foram testadas estatisticamente, porque os métodos de coleta de dados não foram padronizados entre estes dois períodos. Não foi detectada variação sazonal na abundância. Os indivíduos do Rio Pacoti foram significativamente maiores do que os do Rio Malcozinhado (p = 0,027). A proporção entre os sexos foi de 1:1, mas os machos apresentaram um tamanho médio maior que as fêmeas (p = 0,013). Cavalos-marinhos de coloração preta e marrom foram mais abundantes do que os de outras cores. Além disso, os indivíduos demonstraram preferência por se fixarem nas raízes de* Laguncularia racemosa *como estrutura de ancoragem. Este estudo evidenciou que diferenças ambientais entre os estuários—como a cobertura de manguezais, o volume de descarga e a vulnerabilidade a impactos antropogênicos—podem influenciar a abundância e os padrões ecológicos de* H. reidi*. O manejo adequado dessa espécie ameaçada depende fortemente de um conhecimento aprofundado sobre sua ecologia e suas respostas aos fatores ambientais.*

***Palavras-chave**: Estrutura populacional, bioecologia, conservação de habitat.*

INTRODUCTION

Seahorses inhabit shallow tropical and temperate waters, typically in habitats such as seagrass beds, estuaries, mangroves, and reef environments. The coastal nature of these habitats increases their vulnerability to anthropogenic impacts, including mangrove deforestation, pollutant discharge into estuaries and adjacent reef areas, and sediment deposition, leading to the degradation of seagrass beds. The destruction of these environments represents a major risk factor for seahorse populations. Additionally, intense commercial exploitation significantly contributes to population declines, with millions of individuals being extracted from their natural habitats annually (Vincent, 1995b; Lourie, Vincent & Hall, 1999). Members of the genus *Hippocampus*, which includes all seahorse species, exhibit unique biological traits such as prolonged parental care, monogamy during breeding seasons, low fecundity, limited mobility, restricted home ranges, and sparse distribution (Vincent, 1995a; Lourie, Vincent & Hall, 1999; Foster & Vincent, 2004). These characteristics amplify their vulnerability to habitat degradation and overexploitation (Vincent, 1995c; Lourie, Vincent & Hall, 1999).

Several seahorse species are listed in the 2023 IUCN Red List of Threatened Species under the "Vulnerable" category (Pollom *et al.*, 2021; IUCN, 2023). Three species are recorded in Brazil: *Hippocampus reidi*, *H. erectus*, and *H. patagonicus* (Silveira *et al.*, 2014), with some studies conducted on these species in their natural habitats in the state of Ceará (Silva, 2018; Loiola *et al.*, 2022; Valentim *et al.*, 2023). In the estuaries of Ceará, only *H. reidi* has been documented (Silva, 2018; Loiola *et al.*, 2022; Valentim *et al.*, 2023). *Hippocampus reidi* is currently classified as "Near Threatened" according to the IUCN Red List criteria (2023).

According to Ordinance MMA 148/2022, the three seahorse species occurring in Brazil are listed as "Vulnerable" (VU). At present, the import and export of seahorses are regulated by IBAMA Ordinance 102/2022, and the export of wild-caught seahorses is prohibited due to their threatened status (MMA Ordinance 445/2014; MMA Ordinance 148/2022).

In this context, the objective of this study was to investigate the population parameters of *Hippocampus reidi* in two estuaries in northeastern Brazil. Specifically, we analyzed: i) the abundance of *H. reidi* in two estuaries characterized by distinct mangrove coverage and hydrographic patterns; ii) the spatial distribution of *H. reidi* along an increasing distance gradient from the sea within each estuary; iii) potential seasonal variations in abundance between the dry and rainy seasons; and iv) additional ecological aspects of the species. The findings aim to inform and support current and future management strategies for the conservation of *H. reidi* in the estuaries of northeastern Brazil.

MATERIAL AND METHODS

Data collection was conducted in two distinct periods: the first from 2002 to 2003 and the second from 2006 to 2007, in the estuaries of the Pacoti and Malcozinhado rivers, located in the state of Ceará, Brazil. These estuaries exhibit notable differences in hydrography, precipitation, drainage basin characteristics, mangrove areas, and vulnerability to environmental impacts (Molisani *et al.*, 2006) (Tables 1 and 2). For each estuary, two sampling stations were established, each spanning 1 km along the riverbanks, positioned at increasing distances from the river mouth. Station 1 was located near the mouth, while Station 2 was situated 2 km upstream from Station 1 (Figures 1 and 2).

In the Pacoti River, 80 transects were conducted during the first period and 56 during the second. In the Malcozinhado River, 19 transects were carried out in the first period and 56 in the second. During the second period, 28 transects were conducted at each station in both estuaries. Each transect covered a linear distance of 50 meters along the riverbanks, extending one meter on either side, resulting in a sampled area of 100 m^2 per transect. Observations of individuals were conducted through underwater surveys or surface observations, depending on water visibility and depth (Rosa, 2007; Bell *et al.*, 2003).

For all individuals observed, the following data were recorded on a waterproof clipboard: the number of individuals per transect, collection station, date of sighting (day/month/year), season (dry or rainy), height, life stage, sex, pregnancy status, presence or absence of dermal filaments, coloration, support substrate, salinity, depth of occurrence, distance between individuals, group formation, and activity status (sedentary or swimming).

A t-test with a significance level of 2,5% was employed to analyze the spatio-temporal distribution of seahorses across the study locations, considering a Bonferroni correction. Data were transformed into density, expressed as the average number of individuals per m^2 for each transect. The analyses included comparisons of: (1) seahorse densities between the two estuaries, (2) density gradients along the distance from the river mouth (across different stations within the Pacoti estuary), and (3) densities between rainy and dry seasons. The rainy season was defined as occurring from February to June, while the dry season spanned from July to January. Seasonal classification was based on rainfall data for 2006 and 2007 from the "Coast of Fortaleza" macroregion, accessed through the (Ceará, 2024). For these density comparisons, only data from 2006 to 2007 were considered. Additionally, a t-test with a 5% significance level was applied to compare the mean heights of individuals based on sex, estuary, collection period, and climatic season.

The χ^2 test, with a significance level of 5%, was employed for the following comparisons: (1) to assess whether there were significant differences in the sex ratio between the two estuaries, and (2) to compare the number of pregnant and non-pregnant females between collection stations 1 and 2 in the Pacoti River estuary, based on data from 2006 and 2007.

Table 1 – Characteristics of the Pacoti and Malcozinhado River Estuaries

	Pacoti	Malcozinhado
Drainage Basin	141 Km^2	279 Km^2
Mangrove Area	2,69 Km^2	0,37 Km^2
River Flow	19 – 1 m^3/s	2 - <1 m^3/s
Volume	518.502 m^3	80.520 m^3
Tidal Prism	476.011 m^3	46.764 m^3
Precipitation	1140 – 247 mm	914 a 127 mm

Table 2 - Classification of the Pacoti and Malcozinhado River Estuaries based on indices and categories defined by ZEE (2006). DCP = Nutrient Dissolution Potential; EXP = Nutrient Export Potential; VI = Estuary Vulnerability Index; CS = Carrying Capacity

Estuarie	DCP	EXP	VI	CS
Pacoti	Moderate	Moderate	Moderate	Moderate-Low
Malcozinhado	Low	Low	High	Low

Figure 1 – Pacoti River Estuary showing collection stations

Figure 2 – Malcozinhado River Estuary showing collection stations

RESULTS

Spatial Variation in Abundance

During 2006 and 2007, a total of 93 individuals were observed across 112 transects (56 in each river), with 69 individuals recorded in the Pacoti River and 24 in the Malcozinhado River. The overall mean density across both estuaries was 0.008 individuals/m^2. The Pacoti River estuary exhibited a significantly higher mean density (0.012 individuals/m^2) compared to the Malcozinhado River estuary (0.004 individuals/m^2, $p = 0.019$). Notably, this difference in mean density between the two rivers was primarily attributed to the absence of seahorses at Station 2 in the Malcozinhado River. In contrast, no significant difference was detected between the mean densities at Station 1 of the two estuaries ($p = 0.816$).

In the Pacoti River estuary, the mean densities were identical between Station 1 ($n = 34$) and Station 2 ($n = 35$). Conversely, in the Malcozinhado River estuary, marked differences were observed between the two stations. At Station 1, 24 individuals were recorded, whereas no seahorses were observed at Station 2. Due to this absence, statistical comparison of mean densities between the two stations in the Malcozinhado River was not feasible (Table 3).

Table 3 - Comparison of seahorse densities (individuals/m^2) in the estuaries of the Pacoti River (total n = 69; station 1 n = 34; station 2 n = 35) and the Malcozinhado River (total n = 24; station 1 n = 24; station 2 n = 0)

Estuaries		Mean (min - max)	Standard Error	Mode	Median
Pacoti	**Station 1 (n = 34)**	0,012 (0 – 0.07)	0,,005	0	0.005
	Station 2 (n = 35)	0,012 (0 – 0.06)	0,005	0	0.005
	Total (n = 69)	0,012 (0 – 0.035)	0,003	0,010	0,010
Malcozinhado	**Station 1 (n = 24)**	0,011 (0 – 0.035)	0,003	0	0,07
	Station 2 (n = 00)	0 (0 – 0)	-	-	-
	Total (n = 24)	0,004 (0 – 0.017)	0,001	0	0,002
Pacoti and Malcozinhado	**Total** (n = 93)	0,008	0,002	0,008	0,006

Temporal variations

Inter-annual variations in abundance

A total of 238 seahorses were observed in the Pacoti River, with 169 individuals recorded during period 1 (2002-2003) and 69 individuals in period 2 (2006-2007). A decrease in density was noted in the second period. In the Malcozinhado River, 86 individuals were observed, 62 of which were recorded in period 1 (2003) and 24 in period 2 (2006-2007). Similar to the Pacoti River, a reduction in seahorse density was observed (Table 4). Statistical comparison of these periods was not possible due to the lack of standardization in data collection.

Table 4 - Densities (individuals / m^2) of *H. reidi* in two collects periods in the Pacoti River (n total = 238; n Period 1 = 169; n Period 2 = 69) and in the Malcozinhado River (Total number of transects (n)= 86; n Period 1 = 62; n Period 2 = 24)

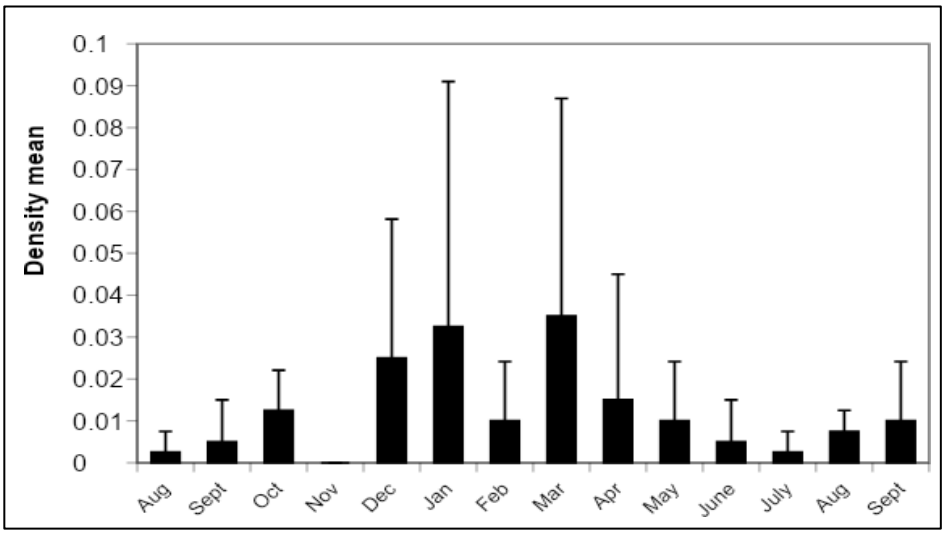
Periods		Mean (min - max)	Standard Error	Mode	Median
Pacoti	**Period 1** (2002-2003)	0.017 (0 – 0.05)	0.004	0	0.013
	Period 2 (2006-2007)	0.012 (0 – 0.03)	0.003	0.01	0.010
	Total (2002-2003 and 2006-2007)	0.015 (0 – 0.05)	0.003	0.01	0.010
Malcozinhado	**Period 1** (2002-2003)	0.026 (0 – 0,07)	0.015	0	0.007

Period 2 (2006-2007)	0.012 (0 – 0.03)	0.006	0,01	0.010
Total (2002-2003 and 2006-2007)	0.019 (0 – 0.07)	0.008	0	0.009

Intra-annual variations in abundance

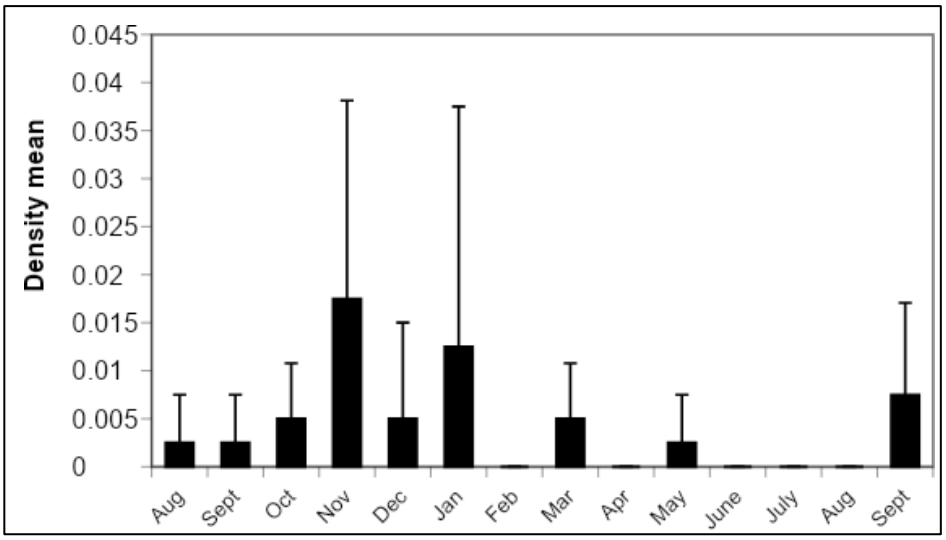
The density of seahorses in the estuaries exhibited notable variation over the 14 months of the survey, as anticipated. This variation was evident in both the Pacoti and Malcozinhado Rivers (Figures 3 and 4). In the Pacoti River, abundance began to increase from August to September, peaked in March, and subsequently declined, reaching a minimum in July (Figure 3). The rise in seahorse density in the Pacoti River corresponded with the onset of the dry season, reaching its peak during the first three months of the rainy season.

Figure 3 – Temporal variation in the mean density of *Hippocampus reidi* during the 2006 – 2007 in the Pacoti River



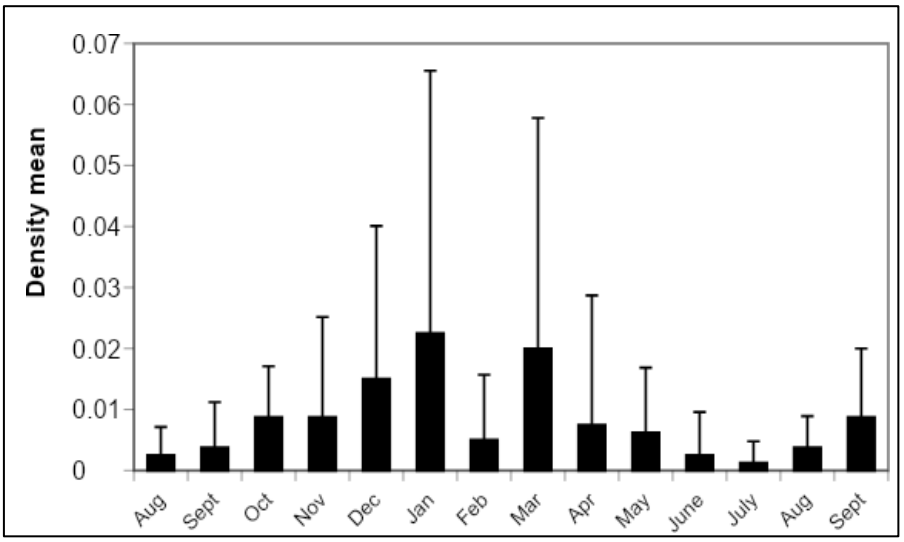
In the Malcozinhado River, abundance began to increase in October, peaked in November, and subsequently decreased from December onwards, continuing throughout the rainy season (Figure 4).

Figure 4 - Temporal variation in the mean density of *Hippocampus reidi* during 2006-2007 in the Malcozinhado River



Considering the density data from both estuaries in 2006 and 2007, it was observed that the abundance of *Hippocampus reidi* began to increase in August, peaked in January, and then declined, reaching a minimum in July. Abundance increased again in August of the following year, indicating a cyclical pattern (Figure 5). However, in February, abundance was notably reduced compared to both the preceding and subsequent months.

Figure 5 - Temporal variation in the mean density of *Hippocampus reidi* during 2006-2007 in the two estuaries



Although an increase and decrease in the density of *H. reidi* was observed during a one-year interval, there was no significant difference between the abundances of the rainy season (February to June) and the dry season (July to January). In the Pacoti River in the years 2006-2007 ($p = 0.101$); in Rio Malcozinhado in the years 2006-2007 ($p = 0.576$); taking into account the two estuaries, in the period 2006-2007 ($p = 0.270$) (Table 5).

Table 5 - Abundance of *Hippocampus reidi* (individuals/m^2) during the rainy season (February to May) and dry season (June to January) for the years 2006 and 2007

Stations of Collect	Seasons	Mean (min - max)	Mode	Median
Pacoti	Rainy	0.018 (0.005 – 0.035)	0.010	0.012
	Dry	0.008 (0 – 0.,025)	0.002	0.006
Malcozinhado	Rainy	0.003 (0 – 0.012)	0	0.001
	Dry	0.005 (0 – 0.017)	0	0.003
Pacoti and Malcozinhado	Rainy	0.011 (0.002 – 0.022)	0.020	0.007
	Dry	0.006 (0.001 – 0.015)	0.009	0.006

Ecological aspects

The data used in this topic refers to 93 individuals sighted in the Pacoti River and the Malcozinhado River, during the years 2006 and 2007.

Salinities observed ranged from 0 to 50, with a mean of 32.78. In the Pacoti River, the mean salinity was 32.43, with station 1 having a mean of 34.82 and station 2 a mean of 30.03, exhibiting variations from 0 to 41. The Malcozinhado River exhibited higher salinities, with a mean of 33.14, including a mean of 33.57 at station 1 and 32.71 at station 2, with variations

ranging from 1 to 50. The salinity variation in the Malcozinhado River was notably greater, likely due to lower river flow.

The mean depth at which individuals were observed was 24.19 cm, ranging from the surface to 2.5 m. In the Pacoti River, the mean depth was slightly higher than the overall mean for the two estuaries, at 24.81 cm, with depths also ranging from the surface to 2.5 m. In the Malcozinhado River, the mean depth was slightly lower, at 22.42 cm, with depths ranging from the surface to 70 cm. Overall, depth variations were similar between the two estuaries.

The mean distance between observed individuals was 6.07 m, ranging from 0 m (indicating pair formation) to 36 m, within transects of 50 m. In the Pacoti River, the mean distance was 6.33 m, with a range from 0 m to 36 m. In contrast, the Malcozinhado River exhibited a shorter mean distance of 4.95 m, ranging from 0 m to 18 m. This indicates that individuals in the Malcozinhado River were generally closer together. Notably, 37.6% of individuals were observed at a maximum distance of 5 m from one another.

The majority of individuals (61%) were observed within a distance of up to 5 m. A total of 22% were located between 5 and 10 m. At distances of 15 to 20 m, 3.38% of individuals were found, while both the 25 to 30 m and 35 to 40 m ranges accounted for 1.69% of the individuals each. No individuals were observed within the distance intervals of 10 to 15 m, 20 to 25 m, 30 to 35 m, 40 to 45 m, or 45 to 50 m.

Males exhibited a greater mean height compared to females, with means of 10.36 cm and 8.98 cm, respectively, a difference that was statistically significant ($p = 0.013$) (Table 6). The smallest non-pregnant male measured 5 cm, while the largest measured 14.5 cm. Among pregnant males, heights ranged from 9 cm to 14.5 cm.

Body height ranged from 2 cm to 14.5 cm, with a mean of 9.36 cm. A significant difference was observed in the body heights of individuals between the Pacoti and Malcozinhado Rivers ($p = 0.027$). The mean height of individuals in the Pacoti River was 9.75 cm, compared to 8.22 cm in the Malcozinhado River (Table 7).

In the Pacoti River, no significant difference was observed in the mean height of individuals between the two data collection stations ($p = 0.084$), with mean heights of 10.22 cm at station 1 and 9.00 cm at station 2 (Table 7). This suggests either a lack of zonation between individuals at different life stages or that the distance between the two stations is insufficient to detect such differences. Similarly, no significant difference was found in the size of individuals between the rainy and dry seasons in either estuary ($p = 0.087$), with mean heights of 9.85 cm during the rainy season and 8.77 cm during the dry season (Table 8).

Table 6 - Mean, mode, and median body length of individuals observed, categorized by sex. Total sample size: 79; males: 40; females: 39

Sex	Mean (min - max)	Mode (cm)	Median (cm)
Male (n= 40)	10.36 (5 – 14.5)	10	10.5
Female (n = 39)	8.98 (5,3 – 14)	8	9
Total (n = 79)	9.36 (2 – 14.5)	10	9.65

Table 7 - Mean, mode, and median body length of individuals observed, categorized by estuary and collection station. Pacoti River: station 1 (n = 33) and station 2 (n = 32); Malcozinhado River: station 1 (n = 24) and station 2 (n = 0)

Collect Stations		Mean (min - max)	Mode	Median
Pacoti	Station 1 (n=33)	10.22 (3 – 14.5)	9.5	10.1
	Station 2 (n=32)	9 (2 – 14.5)	10	9
	Total (n=65)	9.75 (2 – 14.5)	10	10
Malcozinhado	Station 1 (n= 24)	8.22 (2.5 – 13.2)	8	7.75
	Station 1 (n= 0)	_	_	_
	Total (n= 24)	8.22 (2,5 – 13.2)	8	7.75

Table 8 - Mean, mode, and median body length of individuals observed, categorized by season: rainy (n = 50) and dry (n = 42)

Collect Stations	Mean (min - max)	Mode	Median
Rainy (n = 50)	9.85 (2 – 14.5)	10	10
Dry (n = 42)	8.77 (2,5 – 14.5)	5.5	8.35
Total (n = 92)	9.36 (2 – 14.5)	10	9.65

Considering all the individuals in the research, the sex ratio was 1:1, where 47% of the individuals were females, 45% males and 8% with undetermined sex. The Malcozinhado River had a significantly higher proportion of females than the Pacoti River ($p = 0.016$).

No evidence of zonation based on sex was observed between the two collection stations in the Pacoti River, as the sex ratio at both sites was approximately 1:1. At station 1, the sex ratio was 1.46 males per female, while at station 2, it was 1.06 males per female.

A total of 23 pregnant individuals were found, representing 54.8% of the males sighted. Pregnant males were seen during the dry and rainy seasons, 9 months of the year. Only in the months of November, February and July were no pregnant males found. The months in which a greater number of pregnant males were found were the months of January (21,73%), March (17,39%) and May (17,39%), in the months of April (8,69%) and June (8,69%). In the remaining 6 months of observation (August and September of 2006 and 2007, October and December of 2006), only one seahorse was observed in each of these months, representing 4.34% per month of the total number of seahorses. Pregnant males showed no preference for collection stations ($p = 0.862$).

The base colors that the animals presented were: yellow, gray, orange, brown, black, green, and red. Black (34%) and brown (30%) individuals were the most abundant, followed by red and yellow with 13%, respectively, with green (6%), orange (3%) and gray (1%) the least abundant.

The base colors mentioned showed variations. Individuals with brown coloring were the most heterogeneous, presenting seven varieties of color patterns, followed by the red base color, with three. The basic colors yellow, orange, black and green presented two color variations, respectively (Table 9).

Table 9 - Color variation of the different base colors of *H. reidi* seen in the Pacoti and Malcozinhado Rivers

Base colors	**Staining variations of the different base colors of *H. reidi***
Yellow	Totally yellow
	Yellow with black spots
Gray	Gray
Orange	Totally Orange
	Orange and brown
Brown	Totally brown
	Brown with black spots
	Brown covered with slime
	Brown with white spots
	Brown with orange body part
	Brown with greenish body part
	Brown with red body part
Black	Totally black
	Black with white spots
Green	Totally green
	Green with yellowish body part
Red	Totally red
	Red with gray spots
	Red with black spots

A greater number of females showed “vibrant” colors (yellow, orange, red) than males. Females had 25 individuals with “non-vibrant” colors and 18 with “vibrant” colors, while males had 36 individuals with “non-vibrant” colors and only 6 with “vibrant” colors.

There is a strong relationship between the presence of dermal filaments and the life stage of the individual, so that such appendages were more present in juvenile individuals ($p_9 <$

0.0001). The animals were mainly found attached to some type of support substrate on the riverbanks, using their prehensile tail. A total of 85 individuals were trapped and only 8 individuals were swimming.

The preferred support substrate was *Laguncularia racemosa* (L.) C. F. Gaertn. root (36%), followed by fallen branches on the river bank (15%), *Rhizophora mangle* L. root (14%), seaweed (10%), dead tree root (9%), *Avicennia* sp. root (6%), mud (6%), oyster (3%) and nylon thread (1%). It was observed that the root of *L. racemosa* was the support substrate preferred by the individuals, regardless of the presence of filaments (Figure 10). It was also observed that this substrate was preferred by individuals of four colorations, including the most abundant ones.

DISCUSSION

When comparing the average densities of the Pacoti and Malcozinhado rivers with the values found by Rosa et. al. (2007) for the Brazilian coast, the values were lower than the Brazilian average, of 0.026 individuals/m^2, lying between the minimum values (0.0023 individuals/m^2 in Penha - SC) and maximum (0.066 individuals/m^2 in Tubarão – RN). The density of the two estuaries together (0.008 individuals/m^2) was similar to the minimum found by Dias & Rosa (2003) in the State of Rio Grande do Norte, Northeast Brazil, which ranged from 0.006 to 0.51 individuals/m^2. Long-term monitoring aimed at observing the behavior of the seahorse population is useful for identifying fluctuations in population density over time. This monitoring is very important to know if a population decline exists even after the prohibition of collecting these fish after export of seahorses extracted from the wild is prohibited because they are considered threatened (MMA Ordinance 445/2014 and MMA Ordinance 148/2022).

The density found by Valentim (2023) was higher than the density found in this work, but we do not attribute it to a populational increase in *H. reidi* over 15 years. Instead, we attribute the difference to the collection method, since in that research the collection sites were chosen according to the location of the availability of supporting substrate for seahorses, while in our research the locations where the transects were extended were random within each collection station.

The population density of seahorses of the species *H. reidi* observed in a decade after data collection for this research, with the same data collection protocol, was 0.005 ind./m^2 (Silva, 2018). Density much lower than population density, during the same months of collection of the present research, which was 0.0125 in./m^2. When compared the two population densities, it was seen that there was a statistically significant decrease significant ($p = 0.0073$). These results provide evidence that the seahorse population has been decreasing over the years.

Another interesting result found by Silva (2018), following the same data collection protocol and establishment of collection station locations, was that at station 2 (upper portion of the river) of the Pacoti River no seahorse individuals were found. This result, in addition to pointing to a reduction in the area occupied by *H. reidi* in the Pacoti River, also leads us to assume that the Malcozinhado River estuary was already in the same process of reducing the area occupied by *H. reidi* a decade ago. This observation points to a decrease in the area occupied by *H. reidi* in the state of Ceará, probably due to siltation of its habitats. We emphasize that there was no increase in the density of *H. reidi* in the lower region of the river, as the density of the lowest station of the river decreased over the years (Silva, 2018).

The discrepancy between seahorse densities in the two estuaries may be related to the difference in their mangrove areas, river flow, river volume, tidal prism and precipitation. Another factor may be their different Vulnerability Indexes (VI) and Support Capabilities (SC) (Molisani *et al.*, 2006). Seahorses from the Pacoti River were captured for commercialization for 10 years until the final date of data collection for this study, and their commercialization was prohibited 7 years later, but the abundance observed in this river is greater than that observed in the Malcozinhado River. This suggests that environmental factors other than collection may influence the conservation of *H. reidi* populations. The fact that Silva (2018) observed a lower density of *H. reidi*, when researching following the same protocol as this work, after a decade, and that even collection for commercial purposes for aquarists is prohibited, reinforces the statement that the environmental impacts on the Ecosystem Mangroves are currently the biggest threat to *H. reidi* in Northeast Brazil.

In 2002, the construction of a dam with a capacity of 11,291,000 m^3 was concluded (SIRH/CE, 2007) on the Malcozinhado River, although this river is inserted in a geoenvironmental context that makes it very vulnerable to some environmental impacts, due to its water renewal is very limited at ebb tide. This impact may have a negative influence on the seahorse population. Another approach would be needed to test such a hypothesis.

Direct observations at station 2 of the Malcozinhado river effectively indicated that water renewal is limited, as the flow is greatly reduced, in several areas the depth is shallow, there is a large accumulation of sediments and organic matter, in addition to presenting many sediments in suspension in much of the region. Some of the characteristics mentioned characterize eutrophic environments, according to Carneiro, Pegorini & Andreoli (2003). The lower portion of the Malcozinhado River was classified as oligotrophic/mesotrophic, while Pacoti was classified as oligotrophic (Molisani *et al.*, 2006). The eutrophication of the Malcozinhado River may be a determining factor in the lower density of seahorses found in this estuary, as this environmental condition may impact foraging and the meeting of partners, due to the greater turbidity of the water; and dissolved oxygen would be insufficient to meet the physiological demand of individuals.

Low visibility due to the large amount of suspended material can make *H. reidi* procession difficult at the second collection station on the Malcozinhado River. Turbid waters can make it difficult for *H. erectus* to find a mate (Teixeira, 2001). We hypothesize that low visibility may have contributed to the lack of sightings of seahorses at station 2 on the Malcozinhado river.

These results demonstrate that the environmental characteristics of estuaries are important drivers of the population parameters of *H. reidi* in their habitats. We found that such characteristics influence both the population size and the distribution of animals in a gradient of distance from the sea, along the river. The monitoring carried out in this research involved a short period of time, but a decrease in the density of *H. reidi* was already observed in both estuaries. In Derwenty (Tasmania, Australia) a decline in seahorse abundance was observed during the years 2001-2004, even in the absence of seahorse collection (Martin-Smith & Vincent, 2005). As they did not observe changes in the physical-chemical conditions of the water over the years, the authors attributed this decline to the presence of invasive species, diseases or reproductive limit.

The decline in density observed in the two estuaries of Ceará could have been greater if it were not for the publication of IBAMA Ordinance 56/2004, which considerably limited the collection of seahorses. Ceará, being one of the largest exporters of marine ornamental fish in Brazil (Monteiro-Neto *et al.*, 2003), had a great impact on seahorse populations in the estuaries where collections took place, with the export of 5,000 specimens of each species previously permitted by an ornamental fish exporter, per year.

The rainy period was identified between the months of February to June; and the dry season from July to January (Ceará, 2024), observing rainfall data from the years 2006 and 2007 for the Macroregion "Coast of Fortaleza". We verify that at the beginning of this season there is a growth in the density of *H. reidi*, this increase remains until the first three rainy months (January to March) and decreases in the second quarter of the rainy season. Probably these animals begin their complex courtship process during the dry period, taking advantage of the higher salinity and better visibility, extending only until the beginning of the rains (January to March). Such a hypothesis is corroborated by the fact that, in the months of the first semester, a greater number of pregnant males. Pregnancy comprises about two weeks (Rosa, Dias & Baum, 2002), with juveniles possibly benefited from greater abundance of food in the rainy season when released, since at this time a greater amount of nutrients is concentrated in estuaries and in the coastal zone. There was no seasonality of *H. reidi*, demonstrating that the estuaries in question allow the presence of this species throughout the year, probably due to the control of river flow by dams. This process provides salinity levels that allow the presence of seahorses for most of the year, regardless of the season (Molisani *et al.*, 2006). A three-year time interval showed no change in the population size of this species. However, the comparison between the collection years 2002 to 2003 and 2005 and 2006 cannot be statistically verified due to the lack of standardization in data collection from the two collection seasons.

The sex ratio found in our research was approximately 1:1, a result that corresponds to other research carried out later (Silva, 2018; Valentim *et al.*, 2023). This sex ratio is an indication that the collection of seahorses for trade has actually ceased, as the activity focused on female individuals (Osório, 2005).

We found that the *H. reidi* population may have some degree of dependence on *L. racemosa* roots as holdfast in the two estuaries studied. This dependence corroborates what was observed in two subsequent studies carried out in the Pacoti River estuary (Silva, 2018; Valentim *et al.*, 2023). This repeated result of dependence on the pneumatophores *H. reidi* and *L. racemosa* in the Pacoti River estuary station to an urgent need for conservation of mangrove vegetation.

Individuals were found mostly solitary, as verified by Rosa, Dias & Baum (2002), Foster & Vincent (2004), Rosa *et al.* (2007). Only 20 individuals were seen in pairs (the groups were limited to two individuals) which corroborates the statement by Foster & Vincent (2004) that *H. reidi* groups are mainly pairs. The groups were observed in November and December 2006 and January, February and March 2007. Among the groups found, only four were composed of pairs of male and female, these were observed in November 2006 (two pairs) and March 2007 (two pairs).

Body height ranged from 2 to 14.5 cm, with a mean of 9.36 cm; similar mean height for the state of Ceará was found by Rosa et. al. 2005b who found a mean height of 9.17 cm. The superior body height in males, in the *H. reidi* species, is mentioned in the literature (Foster & Vincent, 2004; Rosa *et. al.* 2005b; Rosa *et al.*, 2007). In the case of pregnant males, the smallest found was 9 cm and the largest 14.5 cm, corroborating the size at first maturation for *H. reidi*, which is 8 cm (Foster & Vincent, 2004; Rosa *et. al.* 2005b).

The Malcozinhado River had a significantly higher proportion of females than the Pacoti River. Collections of seahorses as ornamental fish were more intense among females, as pregnant male are not collected (Osório, 2005). Taking into account that there is no collection of seahorses in the Malcozinhado River and that this activity has existed in the Pacoti River for approximately ten years, including during the data collection for this research, such activity could be influencing the sex ratio in this river. However, more intense monitoring is needed to prove this hypothesis and safer sampling.

The presence of pregnant animals in almost every month indicates that the reproductive season of *H. reidi* lasts almost the entire year, corroborating other studies (Rosa; Dias & Baum, 2002; Foster & Vincent, 2004; Rosa *et. al.*, 2007; Valentim *et al.*, 2023)

A greater number of pregnant individuals was found in the rainy season. The gestation of *H. reidi* lasts approximately two weeks (Rosa; Dias & Baum, 2002), the juveniles released in this season of the year would have greater access to food, since there is a greater supply of nutrients in the coastal zone of the coast of Ceará, favoring the increase in zooplankton density.

Pregnant male did not show a preference for collection station ($p = 0.862$). This result leads us to assume that the osmotic stress of different collection times, despite the increasing distance from the sea, is not enough to compromise the osmoregulation of juveniles by males during gestation.

A greater number of females presented “vibrant” colors (yellow, orange, red) than males. Females presented 25 individuals with “non-vibrant” colors and 18 with “vibrant” colors, while males presented 36 individuals with “non-vibrant” colors and only 6 with “vibrant” colors. If this situation is true, the fact that the trade in seahorses as ornamental fish was only directed at individuals with red, yellow or orange coloring, at the time of the research, (Osório, 2005) may have caused changes in the sex ratio of the seahorse population. *H. reidi* in these estuaries. This hypothesis supports the importance of MMA Ordinance 445/2014 and MMA Ordinance 148/2022.

The distinct environmental characteristics of estuaries, coupled with their varying vulnerabilities and capacities to withstand environmental impacts, suggest that these factors significantly influence both population size and the distribution of organisms along a riverine gradient, extending from the sea inland. Based on these findings, the study highlights mangrove conservation as a critical strategy for safeguarding *Hippocampus reidi* populations, emphasizing the need to prioritize the protection of these habitats in conservation efforts for the species.

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