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# Population structure of the seahorse *Hippocampus reidi* (Syngnathiformes: Syngnathidae) in a Brazilian semi-arid estuary

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The longsnout seahorse (*Hippocampus reidi*) is a vulnerable species found along most of the Brazilian coastline, such as semi-arid estuaries with strong rainfall seasonality, hypersalinity, and low depth. To evaluate the population structure of H. reidi over time, we monitored the seahorse population in the Pacoti estuary (Brazil) for one year, based on 248 registered specimens. Salinity, water transparency, sex, pregnancy, body height, and holdfast use were registered. Mixed linear models revealed that sampling month, salinity, and transparency had no influence on population density in the lower zone of the estuary. Pregnant individuals were more frequent in the dry season and at higher salinities. Mean body height (12.7 cm) increased in the dry season. Bright colors were predominant. The seahorses employed nine types of holdfasts, most often mangrove roots, and were found to reproduce throughout the year, peaking in the dry season. Salinity and transparency did not impact population density. In Brazilian semi-arid estuaries, the longsnout seahorse is strongly associated with mangrove vegetation, used as holdfast. Therefore, the conservation of seahorse populations depends on the conservation of the local mangrove forests.

Keywords: Coastal ecosystem, Distribution, Exploitation, Mangrove, Population density.



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O cavalo-marinho Hippocampus reidi é uma espécie vulnerável encontrada ao longo da maior parte da costa brasileira, incluindo estuários semiáridos com forte sazonalidade de chuvas, hipersalinidade e baixa profundidade. Para avaliar a estrutura populacional do H. reidi ao longo do tempo, monitoramos a população de cavalos-marinhos no estuário do Pacoti (Brasil) por um ano, com base em 248 espécimes registrados. Registramos a salinidade, a transparência da água, o sexo, a gravidez, a altura do corpo e o uso de substratos de fixação. Modelos lineares mistos revelaram que o mês de coleta, a salinidade e a transparência não tiveram influência na densidade populacional na zona inferior do estuário. Indivíduos grávidos eram mais frequentes na estação seca e em salinidades mais altas. A altura média do corpo (12,7 cm) aumentou na estação seca. Cores vibrantes foram predominantes. Os cavalos-marinhos utilizaram nove tipos de substratos de fixação, com maior frequência em raízes de mangue, e foram encontrados se reproduzindo ao longo do ano, com pico na estação seca. Em estuários semiáridos brasileiros, o cavalo-marinho H. reidi está fortemente associado à vegetação de mangue, utilizada como substrato de fixação. Portanto, a conservação das populações de cavalos-marinhos depende da preservação dos manguezais.

Palavras-chave: Densidade populacional, Distribuição, Ecossistema costeiro, Explotação, Manguezal.

## **INTRODUCTION**

Seahorses are bony fishes of the family Syngnathidae, classified in a single genus, *Hippocampus* Rafinesque, 1810. Most species are monogamous and populations are characterized by low density and mobility (Foster, Vincent, 2004; Vincent *et al.*, 2005; Curtis, Vincent, 2006) and considered sedentary (Caldwell, Vincent, 2013; Gristina *et al.*, 2017). Their limited mobility prevents them from traveling long distances or resisting the drag of water currents, especially in the juvenile stage (Qin *et al.*, 2014). Adults only travel larger distances when exposed to major disturbances (Caldwell, Vincent, 2013). In other words, seahorses are strongly habitat-dependent. Adverse anthropic impacts on aquatic ecosystems, such as habitat destruction and pollution, greatly increases the vulnerability and risk of extinction of seahorses (Vincent *et al.*, 2011; Pollom *et al.*, 2021).

Coastal regions with high concentrations of human settlements are impacted by a wide array of anthropic stressors, including fishing, pollution, navigation, habitat destruction, eutrophication, and the introduction of competitive species. These factors are particularly adverse to vulnerable species like seahorses (Pollom *et al.*, 2021). Because of their 'charisma' (Vincent *et al.*, 2011), seahorses are also coveted by aquariophiles and are in many locations subject to unsustainable commercial practices (Vincent *et al.*, 2011; Koning, Hoeksema, 2021). In addition, seahorses may be harmed by accidental capture (Vaidyanathan *et al.*, 2021) and have long been exploited for souvenirs, charms and cult objects (Alves, Rosa, 2006; Law, 2021; Pereira *et al.*, 2021; Loiola *et al.*, 2022). The international seahorse trade is regulated by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), which provides a list of all the species of the genus *Hippocampus* in their appendix II (CITES, 2023). The Convention has been successful at reducing the commercial pressure on natural populations, especially trading of live specimens (Foster *et al.*, 2022), but studies have shown that unregistered and clandestine international trade, especially of dried seahorses, remain common in many countries (Foster *et al.*, 2019). Specimens exported for aquarium use are still captured in the wild (Koning, Hoeksema, 2021), regardless of regulations to the contrary, leading to the depletion of natural stocks (Vaidyanathan, Vincent, 2021).

In Brazil, seahorses have been on the National List of Endangered Species since 2014, pursuant to Ordinance #445 of 17 Dec 2014 issued by the Ministério do Meio Ambiente (MMA, 2014). The ban covers the capture, transportation, storage and sales of specimens from natural populations. More recently, the list of endangered seahorses was updated through Ordinance #148 of 7 June 2022 issued by the Ministério do Meio Ambiente (MMA, 2022) and now includes three species considered vulnerable: *Hippocampus reidi* Ginsburg, 1933, *H. erectus* Perry, 1810, and *H. patagonicus* Piacentino & Luzzatto, 2004. These species are also listed as vulnerable in the Livro Vermelho da Fauna Brasileira Ameaçada de Extinção (Di Dario *et al.*, 2018).

Despite these mechanisms of protection, Brazil is a major supplier of live seahorses for the aquarium trade (Rhyne *et al.*, 2012, 2017; Gurjão, Lotufo, 2018; Koning, Hoeksema, 2021). Currently, exported specimens are claimed to be bred in captivity (Foster *et al.*, 2022), but information on the regional and national seahorse trade is scant and unreliable (Koning, Hoeksema, 2021). Among Brazilian states, Ceará is one of the largest exporters of seahorses for the aquarium trade (Monteiro-Neto *et al.*, 2003; Gurjão *et al.*, 2018). The only seahorse species confirmed to occur in Ceará is *H. reidi*, one of the most widely traded species in the world (Foster *et al.*, 2016). This scenario alerts us regarding the urgency of carrying out natural history studies to understand better ways to support conservation efforts.

Ceará is inserted in the northeastern coastline of Brazil, in a large semi-arid region extending from Maranhão to Rio Grande do Norte (Soares *et al.*, 2021). The rainfall pattern in this region is mainly determined by the Intertropical Convergence Zone (ITCZ), resulting in a well-demarcated and often intense rainy season in the first semester. The reduced river flow associated with high temperatures and high rates of evaporation decreases the freshwater flow in estuaries, which tend to be shallow and seasonally hypersaline (Schettini *et al.*, 2017). Thus, environmental factors may be expected to vary significantly between the rainy season and the dry season (Montagna *et al.*, 2018), with higher salinity and greater water transparency in the latter due to the reduced inflow of freshwater and organic matter of continental origin.

Salinity is considered the second-most important determinant of marine life (Tyberghein *et al.*, 2012) as it regulates the distribution of organisms according to osmotic pressure. It is particularly important in estuaries where it is the main regulator of biological population density (Schettini *et al.*, 2017). Seahorses are euryhaline animals with glomerular kidneys (Martinez, 2017) allowing them to tolerate salinities between nine and 37 (Jiaxin *et al.*, 1990), although adult specimens of *H. reidi* have been observed at salinities around five (Silveira, 2005). Seahorses tolerate slow changes in salinity well (Tseng *et al.*, 2020), but abrupt changes in salinity following the sudden discharge of

masses of freshwater into the estuary can lead to a spike in mortality (Hora *et al.*, 2016) and upset the temporal distribution of seahorses throughout the ecosystem. In addition, juveniles have a smaller osmotic regulation capacity and so are more likely to be affected by osmotic stress (Hora *et al.*, 2016; Tseng *et al.*, 2020). Although seahorses thrive best at salinities near their isosmotic point (Hora *et al.*, 2016) of 11.68 (well below the average estuarine environment), reproduction peaks when the salinity is most stable, and the osmotic stress is smallest (usually during the dry season).

Seahorses are predators that ambush small crustaceans and larvae floating in the water column. To do so, they bring their long snout within striking range and suck in the prey (Foster, Vincent, 2004). Turbidity would therefore seem to compromise foraging and, in fact, the relationship between foraging and luminosity has been evaluated in *ex situ* studies on *H. reidi* (Felício *et al.*, 2006) and other species (*e.g.*, *Hippocampus trimaculatus* Leach, 1814 by Sheng *et al.*, 2006; *H. barbouri* Jordan & Richardson, 1908 by Er *et al.*, 2020). On the other hand, few studies have evaluated the influence of turbidity *in situ*. One example is a study by Claassens, Hodgson (2018) which failed to detect a significant effect of turbidity on the distribution of *Hippocampus capensis* Boulenger, 1900, in coastal waters, including estuaries. A more systematic evaluation of the influence of water transparency on population structure would help clarify the ecology of seahorses in tropical semi-arid estuaries.

Research on how *H. reidi* populations along the semi-arid coast of Brazil are temporally affected by changes in environmental factors (*e.g.*, prolonged dry or rainy seasons altering salinity and water transparency) can shed light on seahorse population structure and thus help manage populations in tropical estuaries. The main purpose of this study was to evaluate the patterns of *H. reidi* population structure in a Brazilian semi-arid estuary, with emphasis on temporal variations, contrasting the impact of the abiotic variables associated with the dry season and the rainy season. We expected temporal variations in environmental factors to influence population structure (higher salinity and greater water transparency associated with higher densities of *H. reidi*) and we expected reproduction to occur throughout the year, with a peak in the dry season (greater proportion of pregnant males).

## MATERIAL AND METHODS

**Study area.** The study was carried out in the Pacoti River estuary on the coast of Ceará, a semi-arid region in Northeastern Brazil. The mean salinity of the estuary was 37.7 (range: 37.3–38.7), the mean temperature was 29.2°C (range: 28.1–31.3) (Schettini *et al.*, 2017) and the mean annual rainfall in the region was 1584 mm (INMET, 2023). The estuary receives a large inflow of freshwater during the rainy season, from January to June, whereas very little rain falls in the dry season, between July and December (Molisani *et al.*, 2006). With an average depth of ~3 m, the estuary is characterized as shallow, with well-mixed waters (Schettini *et al.*, 2017).

The mangrove forest along the Pacoti River includes the species *Rhizophora mangle* L. (red mangrove), *Avicennia germinans* L. and *Avicennia schaueriana* Stapf & Leechm. ex Moldenke (black mangrove), *Laguncularia racemosa* (L.) C. F. Gaertn. (white mangrove), and *Conocarpus erectus* L. (button mangrove) (Gorayeb *et al.*, 2004). In the zone affected

by the tides, about 158 ha is covered by mangrove (SEMACE, 2010). The species *L. racemosa* and *R. mangle* are predominant in the lower part of the estuary where the present study was conducted.

The first kilometer upstream from the river mouth was divided into 11 sampling areas (Fig. 1), consisting on non-linear transects of variable length (range: 65-611 m) and 1 m width. The area of potential occupation by *H. reidi* in the Pacoti River estuary was defined based on the availability of habitats and knowledge of the local ecology (Loiola *et al.*, 2022). Earlier studies (Rosa *et al.*, 2007; Osório, 2008; Silva, 2018) sampled seahorses further upstream, but hydromorphological changes appear to have reduced the occurrence of *H. reidi* in the upper zone, as reported by Silva (2018) who attributed the low seahorse density upstream to silting. Large dams have been built in the Pacoti River, reducing the flow of freshwater which would otherwise have expelled sediments more effectively, thereby altering silting rates and estuarine morphology (Lacerda *et al.*, 2007). Based on these reports and the experience of local fishermen, we focused our sampling efforts on the lower zone of the estuary, where the population of seahorses was concentrated.

The location and extension of the transects were based on the availability of habitats with anchoring structures: we selected areas with at least two microhabitats, mostly mangrove roots and sand, along with other bottoms (*e.g.*, seaweed, seagrass, rocks), avoiding areas with highly homogenous microhabitats (*e.g.*, large uninterrupted tracts of sand). Priority was given to the availability of habitats rather than to transect size (as in many previous studies), taking into account that the expected association between biological diversity and area may be an artifact of the number of locally available habitats (Rabelo *et al.*, 2017).

**Sampling.** Seahorse specimens were registered and abiotic data was collected during low tide (0.3–0.6 m) once a month between December 2017 and November 2018. Each month, the sampling sites were chosen at random and the greatest possible number of sites were covered within the time window allowed by the low tide (Tab. **S1**). Specimens of *H. reidi* were located visually from above or, if the water was deep enough, by skin diving, as proposed by Sabino (1999).

*Predictors of population structure*. Salinity and water transparency (cm) were measured with a refractometer and a Secchi disk, respectively, for each sampling site and at each sampling event. The rainy season and dry season were considered to extend from January to June, and from July to December, respectively, based on monthly rainfall indices retrieved from the National Institute of Meteorology, covering the period 1991–2020 (INMET, 2023b).

*Descriptors of population structure.* We determined the sex of each individual based on the presence of a brood pouch (present = male; absent = female) (Lourie *et al.*, 2004). Males with swollen brood pouch were considered pregnant. In addition, the height of the smallest individual with a brood pouch was used as cut-off to segregate adults from juveniles (Baum, Vincent, 2005).

The species were identified based on external morphology, using the guide of Project Seahorse (Lourie *et al.*, 2004). The individuals were photographed and measured as proposed by Lourie *et al.* (2004), with the height corresponding to the distance between the tip of the coronet to the tip of the uncurled tail. The predominant color of each

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individual was registered without taking into account stripes, blotches, and spots commonly displayed by individuals (Lourie *et al.*, 2004). The holdfast (*e.g.*, mangrove roots, seagrass, sand, etc.) employed at the moment of sampling was registered.

**Statistical analyses.** The collected data were analyzed with the R programming language (R Development Core Team, 2023). Population density was estimated for each event (*e.g.*, sample month) and expressed as the ratio between the number of individuals observed in each transect and the area of that transect. We initially verified the existence of significant differences in density, water transparency, and salinity between the dry season and the rainy season using the generalized least squares (GLS) test (Zuur *et al.*, 2009).



**FIGURE 1** | Geographic location of the Pacoti River estuary, Ceará, Brazil (**A**, **B**), indicating *Hippocampus reidi* sampling locations (**A** to **K**) (**C**).

The sex ratio was determined by comparing proportions and analyzing the difference between the expected and actual frequency for males and females, using the chi-squared test ( $\chi^2$ ) (R Development Core Team, 2023). The proportion of pregnant males each month was expressed as the ratio between pregnant males and the total number of males.

We then evaluated the effect of the sampling month, salinity, and water transparency on population density and mean body height using a linear mixed model (LMM) and the *lme4* package (Bates *et al.*, 2015). The effect of the sampling month, salinity, and water transparency on the proportion of pregnant males was analyzed with generalized linear mixed models (GLMM), again using the *lme4* package. In our models, the sampling area was considered a random variable in view of the varying size of the transects in order to control for the effect of spatial autocorrelation. To select the models, we started out by creating a null model which was then contrasted with models fitted with predictors using the function *anova()* (Zuur *et al.*, 2009). Marginal and conditional R<sup>2</sup> values were calculated using the *performance* package (Lüdecke *et al.*, 2021).

Categorized as 'bright' or 'dull', coloring was expressed in relative frequencies. Orange, yellow, and red colors were considered 'bright' and were easily spotted in the environment. Brown, black, green, white, and grey were considered 'dull' and provided better camouflage. Using the chi-squared test ( $\chi^2$ ) (R Development Core Team, 2023), we tested for possible correlations between coloring and season. Finally, the relative frequency of holdfast use was determined.

## RESULTS

**Predictors of population structure.** At the sampling locations, the mean salinity was 25.9 (SD = 5.62) (Fig. 2A) while the mean water transparency was 0.62 m (SD = 0.24 m) (Fig. 2B). These two parameters varied significantly between the dry season and the rainy season (salinity:  $\chi^2$  = 2686.70, df = 1; p <0.01; water transparency:  $\chi^2$  = 1.84, df = 1; p <0.01). Salinity was higher during the former, and water transparency was greater during the latter (Tab. 1).

**TABLE 1** | Study variables potentially predictive of *Hippocampus reidi* population structure (salinity and water transparency) in the lower zone of the Pacoti River estuary (mean ± standard deviation, and range). Comparison of the dry season and the rainy season. Significant differences were determined with the generalized least squares (GLS) method.

	Salinity	Transparency water
Rainy season	22.35 ± 6.27; 10 - 31	0.71 m ± 0.22 m; 0.23 m - 1.35 m
Dry season	28.96 ± 2.22; 23 - 32	0.54 m ± 0.22 m; 0.11 m - 0.95 m
Difference between dry and rainy seasons	L.Ratio = 132.26; p < 0.0001	L.Ratio = 39.04; p < 0.0001

**Descriptors of population structure.** In total, 248 specimens of *H. reidi* were registered in the 11 transects. Population density varied throughout the year, from 0.004 to 0.104 ind.m<sup>-2</sup>, with a mean density of 0.044 ind.m<sup>-2</sup> (SD = 0.026). Density values varied significantly between the dry season and the rainy season (L.ratio = 7.206; p < 0.01).

Contrary to our expectations, seahorse population density was not correlated with sampling month, salinity, or water transparency in the lower zone of the Pacoti River estuary ( $\chi^2 = 21.112$ , df = 13, p = 0.07; conditional R<sup>2</sup> = 0.062, marginal R<sup>2</sup> = 0.000) (Fig. 2C; S2).

Our sample included 114 males and 116 females, indicating a sex ratio of 1:1 for the population ( $\chi^2 = 0.017$ , df = 1, p = 0.89) (Tab. 2), and 67 of the males were classified as pregnant. The proportion of pregnant males each month varied from 0% to 100% (Tab. 2), but pregnant males were more likely to be observed in the dry season and at sites with higher salinity ( $\chi^2 = 33.673$ , df = 11, p < 0.01; conditional R<sup>2</sup> < 0.01, marginal R<sup>2</sup> = 0.929) (Fig. 2D; Fig. 3).

The mean height of the observed specimens was 12.7 cm (SD = 2.02 cm; range: 5.5-17 cm). The smallest seahorse with a brood pouch measured 10 cm. Using this size as cut-off between adults and juveniles, 18 of the specimens observed during the complete study period were classified as juveniles. A strong correlation was seen between sampling month and body height ( $\chi^2$  = 42.749; df = 11; p < 0.01; conditional R<sup>2</sup> = 0.168; marginal R<sup>2</sup> = 0.154) since the captured specimens were slightly larger in the dry season (13.2 cm, SD = 2.05) than in the rainy season (12.1 cm, SD = 1.84) (Fig. 2E).

**TABLE 2** I Number of males, females and juveniles of *Hippocampus reidi* registered in the lower zone of the Pacoti River estuary, along with sex ratios and the proportion of pregnant males.  $X^2$ , df and p are parameters of the chi-squared test ( $\chi^2$ ).

Year	Month	Juvenile	Male	Female	$X^2$	df	р	Sex ratio	Proportion of pregnant male
2017	December	2	9	4	1.923	1	0.17	1:1	0.75
2018	January	3	6	1	3.571	1	0.06	1:1	0.00
	February	1	7	6	0.077	1	0.78	1:1	0.17
	March	2	9	14	1.087	1	0.30	1:1	0.00
	April	3	7	6	0.077	1	0.78	1:1	0.33
	Мау	1	18	11	1.690	1	0.19	1:1	0.73
	June	1	9	9	0.000	1	1.00	1:1	0.30
	July	1	15	22	1.324	1	0.25	1:1	0.64
	August	0	13	11	0.167	1	0.68	1:1	1.00
	September	2	11	12	0.043	1	0.83	1:1	1.00
	October	1	6	11	1.471	1	0.23	1:1	0.73
	November	1	6	7	0.077	1	0.78	1:1	0.71
Number of seahorses		18	114	116					
Total proportion		0.07	0.46	0.47					

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**FIGURE 2** I Temporal variation of environmental variables: (A) salinity and (B) water transparency (cm), and *Hippocampus reidi* population variables: (C) population density (ind.m<sup>-2</sup>), (D) proportion of pregnant males (Y = pregnant male record, N = non-pregnant male record) and (E) individual height (cm), in the Pacoti River estuary, Ceará, Brazil, between December 2017 and November 2018. The months of the rainy season are highlighted in blue.

Eight color patterns were observed in the seahorse population of the Pacoti River estuary. Bright colors were predominant (Fig. 4A), with orange as the most common (44%), followed by brown, yellow, and red. Black, green, white, and grey were less common. The proportion of bright-colored specimens varied between the seasons ( $\chi^2$  = 3.931, df = 1; p = 0.04), with bright colors being slightly more common in the rainy season (Fig. S3).

Seahorses were recorded using nine different substrates as holdfasts (Fig. 4B), the most common being white mangrove roots (*L. racemosa*; 39.9%), followed by sand (22.6%) and red mangrove roots (*R. mangle*; 17.3%). Less commonly used for anchoring were fallen trunks and branches, seagrass (*Halodule wrightii* Asch.), seaweeds, a native oyster species (*Crassostrea* sp. Sacco, 1897) and rocks. A few specimens were seen employing artificial holdfasts, such as abandoned fishing nets and ropes, and a few were observed while swimming in the water column.



**FIGURE 3** | Spatial variation in the proportion of pregnant males of *Hippocampus reidi* along the salinity gradient in the Pacoti River estuary, Ceará, Brazil, between December 2017 and November 2018. Y = pregnant male record, N = non-pregnant male record.



**FIGURE 4** | Proportion of color patterns (**A**) and holdfast use (**B**) of *Hippocampus reidi* in the Pacoti River estuary, Ceará, Brazil, between December 2017 and November 2018.

## DISCUSSION

Our results show that the seahorse population density in the lower zone of the Pacoti River estuary was not influenced by salinity or water transparency. The significant variations observed in these parameters throughout the 1-year study period could not explain the density variation, suggesting the population structure of *H. reidi* over time is independent of the evaluated abiotic factors. On the other hand, the reproductive behavior was influenced by seasonal variation.

The mean population density was higher in this study than in earlier surveys of the Pacoti River estuary by Osório (2008;  $0.012 \text{ ind.m}^{-2}$ ) and Silva (2018;  $0.005 \text{ ind.m}^{-2}$ ), possibly due to (i) differences in sampling methodology and/or (ii) differences in the location of the transects. Osório (2008) and Silva (2018) used fixed 100 m<sup>2</sup> transects to survey the population of *H. reidi* but, as pointed out by Foster, Vincent (2004), fixed transects tend to yield lower densities than focal grids. Considering our methodology of selecting areas based on the potential occupation of *H. reidi*, we expected to find higher densities compared to surveys based on fixed transects. As for the location of the transects along the estuary, Silva (2018) reported that no specimens were observed at the sampling sites farthest from the river mouth, and that the low overall density of *H. reidi* may be explained by the absence of substrates for anchoring, such as mangrove roots, seaweeds and seagrass, due to the growing silting of some of the sampling sites. This silting and destruction of habitats and anchoring structures was also reported by local fishermen during pilot samplings (GAV, 2018, pers. obs.), indicating that some of the areas evaluated by Osório (2008) may be devoid of seahorses today.

Despite considerable fluctuations between the dry season and the rainy season, salinity was not an explanatory factor of seahorse population density in the lower zone of the Pacoti River estuary. This rather unexpected finding may be due to the high tolerance to salinity of *H. reidi*, but it should be pointed out that current knowledge of the relationship between seahorse population density and salinity is based mainly on experimental studies, which have generally failed to investigate the tolerance of seahorses to temporal variations in salinity. As for water transparency, our results are supported by Claassens, Hodgson (2018) who found turbidity to have no measurable influence on the distribution of *H. capensis* in South African estuaries. Thus, while luminosity plays an important role in prey visualization and therefore in seahorse foraging behavior (Felício *et al.*, 2006), in the lower zone of estuaries, where the water column is relatively transparent, it seems to have no impact on population structure.

Some other studies conducted in Brazilian semi-arid estuaries have identified environmental variables influencing the population density of seahorses, most especially the availability of habitats (Rosa *et al.*, 2007; Aylesworth *et al.*, 2015). Aylesworth *et al.* (2015) also found smaller depth and higher temperature to be associated with greater density in a northeast Brazilian estuary. In a shallow estuary like Pacoti, especially in the lower zone, depth and temperature are not expected to vary throughout the year enough to impact seahorse population density, matching the pattern of other estuaries in the semi-arid region (Pinto, 2023). It should be kept in mind that much of our knowledge of the natural history and ecology of seahorses is based on spatial distribution data, not temporal variation, something that may explain the discrepancy between our findings and the literature. Studies evaluating temporal variations of these parameters and their possible correlations with seahorse populations in such ecosystems should therefore be encouraged.

The sex ratio observed in this study (1:1) is common for seahorse populations, most likely due to their monogamous behavior in each reproductive season (Foster, Vincent, 2004). Monogamy tends to increase reproductive success rates in fishes that, like seahorses, occur in low density and have low mobility (Vincent, Sadler, 1995). An equitable sex ratio is suggestive of a healthy population not affected by sex-specific impacts or pressure.

The presence of pregnant males in almost all the sampling months (exceptions were January and March) indicates that reproduction occurs throughout the whole year, as reported by Silveira (2005), and corroborates the claim that seahorses have longer productive periods in the tropics than in temperate regions (Foster, Vincent, 2004).

Pregnant males were more frequent in June and July, as salinity increased and stabilized around 30, meaning the newborn were exposed to less osmotic stress. In contrast, Osório (2008) observed more pregnant males in the first semester, coinciding with the rainy season. The timing and duration of the reproductive season are influenced by the availability of food and environmental variables affecting the development and growth of the fry (Vincent, Giles, 2003). On average, salinities were higher in Osório (2008) than in the present study, suggesting that seahorse populations were exposed to less osmotic stress that year, despite the rainy season. In Ceará, the dry season coincides with windier weather (INMET, 2023a), a factor pointed out by Mai, Velasco (2012), increasing the potential for dispersal of the planktonic fry of *H. reidi*.

The registered seahorses were on average larger in the dry season than in the rainy season, contradicting Osório (2008) who registered no seasonal difference in size for the same estuary. Adult seahorses have a greater capacity for osmoregulation; thus, the increased frequency of large seahorses in the dry season may not be related to fluctuations in salinity but to other environmental factors not evaluated in this study.

The specimens of *H. reidi* observed for this study in the Pacoti River estuary were on average larger than the seahorses previously observed in Ceará (Rosa *et al.*, 2007; Osório, 2008; Silva, 2018). In fact, the seahorses observed in Ceará by Osório (2008) and Rosa *et al.* (2007) are on average smaller than the seahorses observed in other Brazilian states (Rosa *et al.*, 2007; Carmo *et al.*, 2022). The mean height of the seahorses registered in Pacoti for our study is closer to the mean height found in other states and is also compatible with the findings of Silva (2018) for the same estuary, indicating an improvement in the condition of the local seahorse population, possibly as a consequence of the ban on seahorse fisheries and trade in Brazil through MMA directive #445 of 17 Dec 2014 (MMA, 2014). Since large specimens are the most marketable (Vincent *et al.*, 2011), the increase in mean body height of the registered specimens would seem to reflect a reduction in fishing pressure.

The population of seahorses in the Pacoti River estuary currently displays a predominantly orange coloring, contrasting with the black and brown coloring reported in earlier studies (Osório, 2008). Bright-colored specimens are better priced in the aquarium trade (Rosa *et al.*, 2005, 2011; Loiola *et al.*, 2022;) and would seem to be poorly camouflaged in the sandy or muddy environments near the river mouth. It is reasonable to assume that the reduced fishing pressure has allowed more bright-colored specimens to remain in the environment; on the other hand, seahorses are known for

their crypsis (Lourie *et al.*, 2004). Some have argued that a bright, disruptive coloring with blotches and spots might confound predators by obscuring the body outline, but the bright-colored specimens of *H. reidi* observed by Duarte *et al.* (2019) made no perceptible effort to blend in with the background. In any case, the color patterns observed in our study suggest that the fishing pressure on *H. reidi* in the lower zone of the Pacoti River estuary has decreased.

The seahorses in our study area displayed a preference for white mangrove roots (*L. racemosa*). Being poor swimmers, seahorses rely heavily on holdfasts like roots and trunks to which they anchor using their prehensile tail. The population density of *H. reidi* has been shown to be directly associated with habitat complexity (Aylesworth *et al.*, 2015), which translates into ample availability of protective holdfasts, and a microhabitat with mangrove roots and seagrass is considered more complex than a sand bottom (Whitfield, 2017). Over half the seahorses observed in our study (57.2%) used the roots of *L. racemosa* and *R. mangle* as holdfast, matching the findings of other studies from Northeastern Brazil (Aylesworth *et al.*, 2015). In short, the seahorses observed in our study displayed a preference for complex holdfasts, and seahorse occurrence appeared to be associated with mangrove vegetation. Therefore, the preservation of the local mangrove forest is essential for the maintenance of *H. reidi* populations in this ecosystem (Ternes *et al.*, 2023, 2016).

Our results show that the population density of *H. reidi* in the lower zone of Brazilian semi-arid estuaries can fluctuate over time independently of annual variations in salinity and water transparency, likely due to environmental factors not evaluated in this study. The registered seahorses reproduced throughout the year, peaking in the dry season, and pregnant males were more likely to be observed under higher salinity, possibly as an adaptive strategy to protect juveniles from osmotic stress. The occurrence of *H. reidi* in local estuaries is positively associated with the presence of mangrove vegetation, highlighting the vulnerability of seahorses to mangrove degradation. Thus, to conserve local seahorse populations, mangrove forests should be protected, the existing ban on fishing and trade should be maintained, and the ecosystem should be safeguarded against water pollution.

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Gabriela Alves Valentim: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Visualization, Writing-original draft, Writing-review and editing.

Leonardo Mesquita Pinto: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing-review and editing. Ronaldo César Gurgel-Lourenço: Conceptualization, Data curation, Formal analysis, Investigation, Software, Supervision, Visualization, Writing-review and editing.

Carlos Alberto de Sousa Rodrigues-Filho: Conceptualization, Formal analysis, Investigation,

Methodology, Software, Validation, Visualization, Writing-review and editing.

Jorge Iván Sánchez-Botero: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing-review and editing.

#### ETHICAL STATEMENT

Field data were collected under license #56416 issued by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio/SISBIO). The license allows for *in situ* record taking without harming or removing specimens from their natural habitats.

#### **COMPETING INTERESTS**

The author declares no competing interests.

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# Neotropical Ichthyology





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