



# Evaluation of Population Parameters of Seahorses in Areas with and without Tourism in Federal Marine Protected Areas in Northeast Brazil

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**ABSTRACT** – In Brazil, there is the tourist attraction of interacting with seahorses, which occurs through raft rides, during which the animals are captured and displayed in glass containers to be photographed and filmed by tourists. Two federal protected marine areas where this activity takes place were monitored, Jericoacoara National Park (monitored area of 600 m<sup>2</sup>), in the state of Ceará, and Environmental Protection Area (EPA) Delta do Parnaíba (monitored area of 547 m<sup>2</sup>), in the state of Piauí. Between 2011 and 2015, in both PMAs, the active search for animals was carried out by snorkeling during low tide, and the only species found was *Hippocampus reidi*, with 1286 sightings of seahorses in the National Park and 602 in the EPA. Results such as the mean height at formation of the male brood pouch (11.35 and 11.52 cm at EPA and National Park, respectively), size at first sexual maturity (14.2 and 14.7 cm, respectively) and reproductive period throughout the year were very similar between the populations of the two protected areas. All sites in both PMAs suffered a significant reduction in seahorse population during the study, except for the site P2 in EPA where the seahorse numbers remained stable throughout the study.

**Keywords:** Endangered species; *Hippocampus reidi*; protected area; sustainability.

## Avaliação dos Parâmetros Populacionais de Cavalos-marinhos em Áreas com e sem Turismo em Áreas Marinhas Protegidas Federais no Nordeste do Brasil

**RESUMO** – No Brasil, existe a atração turística de interagir com cavalos-marinhos, que ocorre por meio de passeios de jangada, durante os quais os animais são capturados e expostos em recipientes de vidro para serem fotografados e filmados pelos turistas. Foram monitoradas duas unidades de conservação marinhas federais onde essa atividade ocorre, o Parque Nacional de Jericoacoara (área monitorada de 600 m<sup>2</sup>), no estado do Ceará, e a Área de Proteção Ambiental (APA) Delta do Parnaíba (área monitorada de 547 m<sup>2</sup>), no estado do Piauí. Entre 2011 e 2015, em ambas as áreas protegidas, a busca ativa de animais foi realizada por snorkeling durante a maré baixa, e a única espécie encontrada foi *Hippocampus reidi*, com 1286 avistamentos no Parque Nacional e 602 na APA. Resultados como a altura média na formação da bolsa incubadora do macho (11,35 e 11,52 cm na APA e Parque Nacional, respectivamente), o tamanho na primeira maturidade sexual (14,2 e 14,7 cm, respectivamente) e o período reprodutivo ao longo do ano foram muito semelhantes entre as populações das duas áreas marinhas protegidas. Todos os sítios em ambas áreas protegidas sofreram uma redução significativa na população de cavalos-marinhos durante o estudo, exceto o sítio P2, na APA, onde os números de cavalos-marinhos permaneceram estáveis ao longo do estudo.

**Palavras-chave:** Área protegida; espécie ameaçada; *Hippocampus reidi*; sustentabilidade.



## Evaluación de los Parámetros de la Población de Caballitos de Mar en Áreas con y sin Turismo en Unidades Federales de Conservación en el Noreste de Brasil

**RESUMEN** – En Brasil, existe el atractivo de los turistas interactuaren con los caballitos de mar durante los paseos en canoa, en los que estos animales son capturados y expuestos en recipientes de vidrio para ser fotografiados y filmados. En este estudio fueron analizados parámetros de población de los caballitos del mar en dos Unidades Federales de Conservación Marina en Brasil donde ocurre esta actividad: el Parque Nacional Jericoacoara, en Ceará (área analizada = 600 m<sup>2</sup>), y el Área de Protección Ambiental (APA) Delta do Parnaíba, en Piauí (área analizada = 547 m<sup>2</sup>). Entre 2011 y 2015 se realizó una búsqueda activa de caballitos de mar en ambas AMP durante la bajamar mediante buceo con esnórquel. La única especie encontrada fue *Hippocampus reidi*, con 1286 avistamientos en el Parque Nacional y 602 en la APA. Fueron observados resultados similares en las dos áreas protegidas en algunos parámetros investigados, como la altura media de la bolsa de cría de los machos (APA = 11,35 y Parque Nacional = 11,52 cm), la altura de los individuos en la primera madurez sexual (APA = 14,2 y Parque Nacional = 14,7 cm) y el período reproductivo durante el año. Todos los locales en ambas áreas protegidas tuvieron una reducción significativa en la población de caballitos de mar durante el estudio, excepto un local en el APA en el cual el número de caballitos de mar se mantuvo estable durante todo el estudio.

**Palabras clave:** Área protegida; especie en peligro; *Hippocampus reidi*; sostenibilidad.

### Introduction

Tourist activities for interaction with wild fauna have been growing all over the world. These interactions range from the observation of animals in captivity to touching activities, feeding, and swimming and diving with animals in their natural environment (Fernandez et al., 2009; Nakamura & Nishida, 2009; González-Pérez & Cubero-Pardo 2010; Bernardon & Nassar 2012; Van der Duim et al., 2014; Vidal et al., 2017). Observation of aquatic animals alone moves millions of dollars and attracts more than nine million people annually in approximately 120 countries (WAP, 2020).

In Brazil, the tourist attraction of interacting with seahorses through rafts or canoes has been taking place since 1995 in the municipality of Ipojuca, state of Pernambuco (Silveira et al., 2011). With the publicity of these activities at this location, tourist interactions with seahorses also began to be popular at the National Park of Jericoacoara (NP), state of Ceará, the Environmental Protection Area Delta do Parnaíba (EPA), in Piauí. In all these places, the activity is carried out by members of the local communities, most of them from artisanal fishing and who found in this tourist activity a way to make a living or complement the family income.

The management of protected areas where tourist interactions with seahorses are developed belongs to the Chico Mendes Institute for Biodiversity Conservation (ICMBio), agency of the

Ministry of the Environment of Brazil, as they are federal protected areas. Only in Pernambuco does the activity take place in a municipal area managed by the city of Ipojuca. In all these protected areas, the activity consists in taking groups of tourists on tours embarked through the mangrove areas, where tour operators capture seahorses, either from the boats themselves or through free diving, placing them in glass containers (Figure 1), so that they are observed and photographed or filmed by tourists (Silveira et al., 2011; Martins et al., 2022). After a few minutes, the seahorses are returned to the water.

Interactive tourism with seahorses can be a pleasant and educational experience for tourists, helping to conserve animals and generate income for the families involved. As the natives involved in the tourist exploitation of seahorses are trained and informed about the need to conserve these animals (not only because their livelihood depends on seahorses but because they are endangered species), they become potential partners (Martins et al., 2022). Acting as inspectors, they protect seahorses from ornamental fish fishermen and seahorse collectors for other purposes, such as handicrafts and sympathies. However, even as a partner, it is necessary to monitor this activity through the environmental agency, because when not done properly and monitored, it can represent a real danger to these animals, which are already at risk of extinction. The three species

of seahorses (*Hippocampus reidi*, *H. erectus* and *H. patagonicus*) occurring in Brazil are included in the Red List of the International Union for the Conservation of Nature (IUCN, 2017) and

in the official Brazilian list of fauna species threatened with extinction (MMA, 2014), placed in the vulnerable category (VU), only *H. reidi* is considered “near threatened” (NT) in the IUCN Red List.



Figure 1 – Tourist activity with seahorse interaction in Jericoacoara National Park, Brazil.

Accordingly, the monitoring of seahorse populations in areas exploited for tourism is extremely important for the management of this resource, which already suffers greatly from undesirable practices, such as the incidental capture of seahorses during shrimp or fish trawling, intentional capture to supply the souvenir trade and popular medicine, and the intense trade in live individuals for ornamental purposes, which is largely based on specimens of *H. reidi* captured in North and Northeast Brazil (Rosa et al., 2010; Neto, 2011; Silveira et al., 2018). In this sense, this article evaluates and compares the population parameters of seahorses in two protected areas in northeastern Brazil, where these animals are under tourist exploitation. The results can contribute to the development of public policies aimed at organizing tourist interactions with seahorses in and outside Brazilian protected areas.

## Methods

### Study area

The study was carried out at EPA Delta do Parnaíba and at Jericoacoara National Park, both federal protected areas located on the Brazilian northeast coast.

EPA Delta do Parnaíba, created by federal decree in 1996, has an area of 307,590.51 ha covering the entire coast of the state of Piauí, advancing into some of the neighboring states of Ceará and Maranhão. The Parnaíba region has a tropical climate with rain in the summer and dry season in the winter. The climate classification is Aw according to Köppen and Geiger (Kottek et al., 2006). It has an average annual temperature of 27.4°C and average annual rainfall of 1283 mm, with September being the driest and warmest

month (2 mm and 28.2°C) and March the rainiest and coolest month (304 mm and 26.7°C) (Climate-Data.org, 2020).

The EPA's objective is to guarantee the protection of the deltas of the Parnaíba, Timonha and Ubatuba rivers, with their fauna, flora and dune complex, to improve the quality of life of the resident populations through guidance and discipline of economic activities, and to promote ecological tourism and environmental education. EPA is part of the "Rota das Emoções" or "Route of Excitement," a tourist itinerary that connects this protected areas and Jericoacoara National Park and Lençóis Maranhenses, covering 14 municipalities in the states of Ceará, Piauí and Maranhão.

Tourist interactions with seahorses in EPA Delta do Parnaíba take place in the estuary of the Cardoso and Camurupim Rivers, near Vila de Barra Grande, municipality of Cajueiro da Praia, in Piauí. The basin of the Cardoso and Camurupim Rivers extends in the south-north direction, with incidence of anastomosed channels in flooded areas with a predominance of mangroves (Sousa, 2019).

Jericoacoara National Park, created by federal decree in 2002, has an area of 8,863.03 ha and is situated in the state of Ceará. The Jericoacoara region has a tropical climate, according to Köppen and Geiger (Kottek et al., 2006), and is classified as Aw. It has an average annual temperature of 27.8°C and an average annual rainfall of 1178 mm, with August being the driest month (1 mm), November the warmest month (28.6°C) and March being the rainiest month also with the lowest temperature (323 mm and 27°C) (Climate-Data.org, 2020).

The basic objective of Jericoacoara National Park is the preservation of natural ecosystems of great ecological relevance and scenic beauty, enabling scientific research and the development of environmental education and interpretation activities, recreation in contact with nature and ecological tourism. Tourism in the village of Jericoacoara started in the late 70s of the 20th century and in 2019 Jericoacoara National Park was the third most visited park in the country, with 1,322,883 visitors (ICMBio, 2020). Tourist

interactions with seahorses in Jericoacoara National Park take place in the estuary of the Guriú River, in stretches of mangroves located near the village of Mangue Seco, municipality of Jijoca de Jericoacoara.

### Data collection and analysis

In the estuary of the Cardoso and Camurupim Rivers, in EPA Delta do Parnaíba, two study sites were defined: Site 1 (P1) – Ilha do cavalo-marinho (Seahorse Island) (2°54'39.7"N and 41°25'41.0"W), where there is the tour for interaction with seahorses, and Site 2 (P2) – Camboa do Cavalo (2°55'13.8"N and 41°25'40.3"W), where there are no tourist activities with these animals. At these sites, transects were established with areas of 216 m<sup>2</sup> (108 x 2 m) and 331 m<sup>2</sup> (165.5 x 2 m), respectively (Figure 2). The sites were separated by a distance of approximately 1.4 km and the choice of these sites was based on information provided by local drivers, according to Mai & Rosa (2009) and by EPA Delta do Parnaíba managers, who cited the continuous occurrence of seahorses in those places and the aforementioned tourist tour. Data were collected from April 2014 to December 2015, three days a month, totaling 54 days of collection over 18 months (each day, only one point was investigated). All data were obtained at low tide and recorded on a PVC sheet. The same researcher worked at the two sites with the support of local guides and their vessels.

In the Guriú River estuary, Jericoacoara National Park, three study sites were defined: Sites R and S, located in areas where interactions with seahorses occur, and Site S4, in an area where tourist activities do not occur with these animals (Figure 2). Three transects were established at Site R (2°50'19.41" N and 40°34'31.38" W), another three transects at Site S (2°50'32.10" N and 40°34'57.38" W), and one at Site S4 (2°50'48.45" N and 40°35'10.59" W). All transects were established with an area of 100 m<sup>2</sup> (100 m x 1 m) along the banks of the river, with a distance of 100 m between the transects of the same site and a minimum distance of 600 m between the transects of different sites. In both protected areas (EPA and National Park), the predominant mangrove trees were *Rhizophora mangle*.



Figure 2 – Study areas in Jericoacoara National Park/CE (+) and EPA Delta do Parnaíba/PI (+).

Data collection took place from May 2011 to December 2013, and from January to December 2015, once a week (each day, only one point was investigated), totaling 140 days of collection over 44 months.

For data collection at both protected areas, scuba diving was not necessary, since the local depth was low, and the observations were always made at low tide. The seahorses were located through active searches carried out by snorkeling; on many occasions, the visualization was done from above the vessel so that the researcher could enter the water. The researcher traversed the area of the transect, being accompanied by the vessel and its driver on all routes. The fish were captured manually for data collection that allowed the calculation of the following parameters.

- Height of the seahorse (linear measurement from the top of the head to the tip of the stretched tail (Lourie et al., 2004) was obtained with a plastic ruler.
- Mean height at formation of the male brood pouch was determined with a logistic regression. In this regression, the presence of a pouch was coded with a binary variable where 0 represented an individual with an incomplete pouch and 1 an individual with a fully-formed pouch (adapted from Silveira et al., 2020a). A significance level of 5% was

adopted. Mean height at formation of the male brood pouch was determined using all young males (from the smallest to the largest male with the brood pouch in formation, this size range also contains the first fully formed brood pouches).

- Height at first sexual maturity ( $H_m$ ), when 50% of the population is reproductive (Vazzoler, 1996), it was carried out through the relative frequency of pregnant males by height classes, with the following logistic function being fitted:

$$F = \frac{A}{1 + e^{-r(H_t - H_m)}} ; A \leq 1$$

where:

- F** is the frequency of pregnant males by length class.
- A** is asymptote of the logistic curve for the mean frequency of pregnant males.
- r** is a parameter regarding the rate of change of immature-mature state.
- $H_t$**  is the height of the body.
- $H_m$**  is size at first sexual maturity.

Fitting was done with the non-linear regression routine of program R, version 3.5.0,

using the Levenberg-Marquardt algorithm. The same height at first sexual maturity was assumed for males and females (Silveira, 2005; Silveira et al., 2016).

The density (ind/m<sup>2</sup>) was estimated by dividing the number of seahorses sighted at each study site by the respective area, always considering a monthly average of sighting for each site, avoiding overestimating the sample number.

A trend analysis was performed, using linear regression for each sampling site to see if there was a decrease or increase in density or if it was stationary between the study sites (Moretton & Toloj, 2006).

To characterize the population structure, the relative frequency of males, females and juveniles (with undetermined sex) was used. The juvenile (undetermined sex) has the same phenotype as a female, so if the mean height at brood pouch formation is not known, there is a risk of classifying future males as females, overestimating the sample of females and creating a female bias in the population. In this work, the criterion that separates the juvenile (undetermined sex) is the brood pouch formation event, from which we can classify males, females and juveniles, regardless of sexual maturity, just to structure the population, taking into consideration the following.

- Juveniles = animals without a brood pouch and with height up to the “mean height at brood pouch formation (X)”.
- Females = animals without a brood pouch and with “height greater than the mean height of brood pouch formation (from X, 1)”.
- Males = animals with brood pouch at any height.

Analysis of variance and the Tukey test were used to compare the proportion of males, females and juveniles over the years. For the comparison of sex ratio, the binomial test was used. The software used for statistical analysis was R, version 3.5.0 (The R Core Team, 2021), and the level of significance adopted in all hypothesis tests was 5%.

The salinity of the water was measured with an analog refractometer and the temperature with a digital thermometer. The correlation analysis was performed considering the number of animals

observed per day, with the salinity and temperature means for the same day. Abundance per site and the sum of all sites were considered. The analysis was performed using Pearson's correlation coefficient.

## Results

### Population density

During the data collection period, 602 sightings of *H. reidi* seahorses were recorded, involving the two study sites in EPA Delta do Parnaíba/Piauí. This was the only seahorse species recorded during the study.

#### *Site 1 – Ilha do Cavalo-marinho (Seahorse Island), place with tourist interactions*

This site proved to be the most populous ( $p = 0.0390$ ), with a mean of 258.5 seahorses out of a total of 406 (P1 + P2). During 2014, the density was  $0.087 \pm 0.047$  ind/m<sup>2</sup>, and in 2015, it was  $0.054 \pm 0.056$  ind/m<sup>2</sup>, showing a significant decrease in sightings ( $p=0.0426$ ). Two clear patterns were noted, separated by an irregularity in the series that illustrates the temporal evolution of density at this site (Figure 3A). From April 2014 to February 2015, density tended to be lower. Although there were some fluctuations in the period, we found a decrease rate of 15.33% per month (Table 1).

After that period, there was an irregularity in the series, with an abrupt increase in density, followed by a very rapid decline. As there were only three months in which this occurred, it was not possible to say that there was an increasing or decreasing trend. Soon after, from June to December 2015, there was a steady increase in the series, without fluctuations, at a rate of 41.28% per month (Table 1).

#### *Site 2 – Camboa do Cavalo-marinho, a place without tourist interactions*

In 2014, the site without apparent influence of interaction tourism showed a density of  $0.026 \pm 0.007$  ind/m<sup>2</sup>, while in 2015 the density was  $0.028 \pm 0.013$  ind/m<sup>2</sup>. However, this increase was not statistically significant ( $p = 0.3245$ ), and the population structure showed stability (Figure 4). The series was identified as stationary; that is, no

growth or decrease pattern was observed during this period (Figure 3B, Table 1).

In Jericoacoara National Park, Ceará, during the data collection period, 1286 seahorse sightings were recorded involving the two research sites (R and S). The initial experimental design of the experiment intended to have at least one location (S4) without human interference. However, as on the start of the observation period, touristic activity was noticed in the area, this location was excluded from further observation.

Site R was the most populous in all time series, starting in 2011 with an average density of

$0.168 \pm 0.08 \text{ ind/m}^2$ , and at the end of the survey (2015,) it showed a density of  $0.096 \pm 0.05 \text{ ind/m}^2$ . Ponto S showed an initial density of  $0.142 \pm 0.09$  in 2011 and  $0.071 \pm 0.03 \text{ ind/m}^2$  at the end of the study (Table 1).

The evolution of the density of seahorses at these sites was considered in two time series, with the 2011 to 2013 series showing a decrease in the density of animals at both sites (R and S), at a rate of 2.44 and 8.26% per month, respectively. After this drop in animal density, stabilization was observed during 2015 (Figure 3, Table 1).

Table 1 – Trend analysis in population growth of *H. reidi* in EPA Delta do Parnaíba, Piauí (Sites P1 and P2) and Jericoacoara National Park, Ceará (Sites R and S). Significant differences are indicated by P-values in bold.

Site	Period	Result	Rate (%)	95%CI	P-value
P1	Apr/2014 to Feb/2015	Decreasing	15.33	[3.04; 27.62]	<b>0.0433</b>
	Jun/2015 to Dec/2015	Increasing	41.28	[25.31; 57.25]	<b>0.0159</b>
P2	May/2014 to Feb/2015	Stationary	-	-	0.9009
R	2011-2013	Decreasing	2.43	[0.35; 4.52]	<b>0.0077</b>
	2015	Stationary	-	-	0.3660
S	2011-2013	Decreasing	8.26	[5.12; 11.40]	<b>0.0001</b>
	2015	Stationary	-	-	0.4200

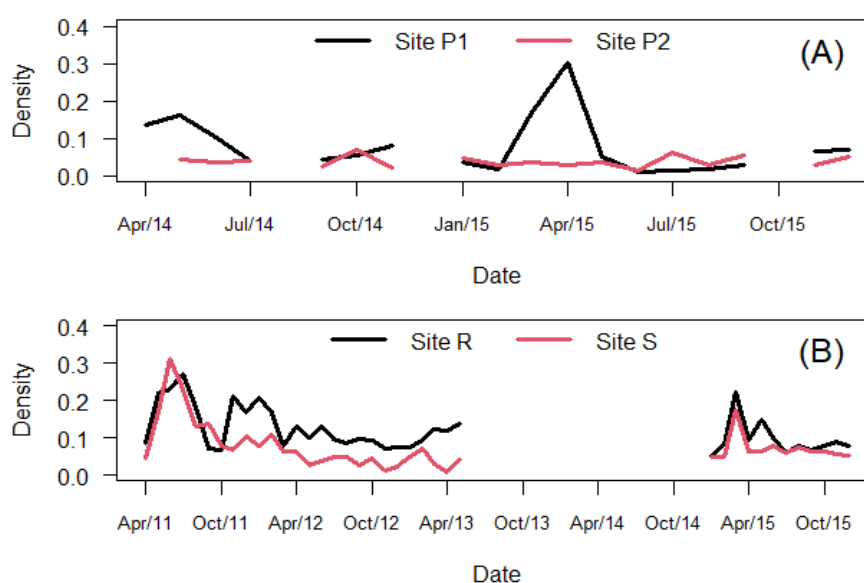


Figure 3 – Temporal representation of the density of *H. reidi* in (A) EPA Delta do Parnaíba, Piauí and (B) Jericoacoara National Park, Ceará.

### Population structure and sex ratio

In the EPA Delta do Parnaíba, Site 1 – Ilha do Cavalo-marinho, location with tourist interactions, showed an unstructured population with significantly more males (M) than females (F) in 2014 ( $p < 0.001$ ), a phenomenon that occurred again in 2015 ( $p = 0.003$ ). The analysis by season, considering the entire study period, revealed the same discrepancy in sex ratio (Table 2).

The number of juveniles (J) showed a mean relative frequency of 42.3% in 2014, reaching a maximum of 69% in May and minimum of representativeness in the months of July and November with 23.5%. In 2015, juveniles showed a mean number of sightings of 52.5%, reaching a maximum of 84% in March and April 2015 and 0% in July of the same year (Figure 4).

#### Site 2 - Camboa do Cavalo-marinho, a place without tourist interactions

In P2, the proportion between males and females resulted in a p-value of 0.2678 in 2014 and 0.2478 in 2015 (Table 2). In other words, in both years there was no difference in sex ratio, as expected for monogamous fish, such as seahorses.

The mean monthly occurrence of juveniles at this site was 15.74% in 2014, with extremes between zero in the months of May and November and 50% in the month of September. In 2015, sightings of juveniles were 20.33%, with their extremes varying between zero (April and June) and 41.6% (March) (Figure 4). At this site, more pregnant males were recorded, and the analysis of seahorses according to height class revealed that the largest animals inhabited P2, with some classes present only at this site, while juveniles were present in large quantities at P1 (Figure 5).

In Jericoacoara National Park, the temporal and season analysis (considering the entire study period) showed that both sites had a stable population structure, with no significant difference between the number of males and females, with the exception of 2012, where a difference was observed in sex ratio only for Site S, where the proportion of females (57.37%) was significantly higher than that of males (42.63%) ( $p$ -value = 0.023), Table 2. The proportion of juveniles ranged from  $8.5 \pm 6.17\%$  to  $12.44 \pm 18.95\%$  for Site R and from  $7.3 \pm 2.85\%$  to  $13.42 \pm 11.08\%$  for Site S (Table 5).

Table 2 – Sex ratio of *H. reidi* in EPA Delta do Parnaíba, Piauí and Jericoacoara National Park, Ceará. Significant differences are indicated by P-values in bold.

Year/Season	Males (%)	Females (%)	P-value	Males (%)	Females (%)	P-value
<b>EPA</b>	<b>Site 1</b>			<b>Site 2</b>		
2014	88 (76.52)	27 (23.48)	<b>&lt;0.001</b>	38 (57.58)	28 (42.42)	0.2678
2015	39 (70.91)	16 (29.09)	<b>0.003</b>	-	-	-
Spring	23 (82.14)	5 (17.86)	<b>0.001</b>	17 (62.96)	10 (37.04)	0.2478
Summer	14 (93.33)	1 (6.67)	<b>&lt;0.001</b>	-	-	-
Fall	61 (72.62)	23 (27.38)	<b>&lt;0.001</b>	11 (55.00)	9 (45.00)	0.8238
Winter	29 (67.44)	14 (32.56)	<b>0.032</b>	10 (52.63)	9 (47.37)	1.0000
<b>National Park</b>	<b>Site R</b>			<b>Site S</b>		
2011	348 (50.51)	341 (49.49)	0.819	301 (49.10)	312 (50.90)	0.6867
2012	343 (47.31)	382 (52.69)	0.158	107 (42.63)	144 (57.37)	<b>0.0238</b>
2013	144 (48.00)	156 (52.00)	0.525	48 (48.48)	51 (51.52)	0.8419
2015	273 (46.99)	308 (53.01)	0.158	223 (51.50)	210 (48.50)	0.5643
Spring	194 (48.38)	207 (51.62)	0.549	227 (48.82)	238 (51.18)	0.6434
Summer	273 (46.35)	316 (53.65)	0.083	228 (50.00)	228 (50.00)	1.0000
Fall	354 (48.49)	376 (51.51)	0.437	93 (46.04)	109 (53.96)	0.2916
Winter	287 (49.91)	288 (50.09)	1.000	131 (47.99)	142 (52.01)	0.5457



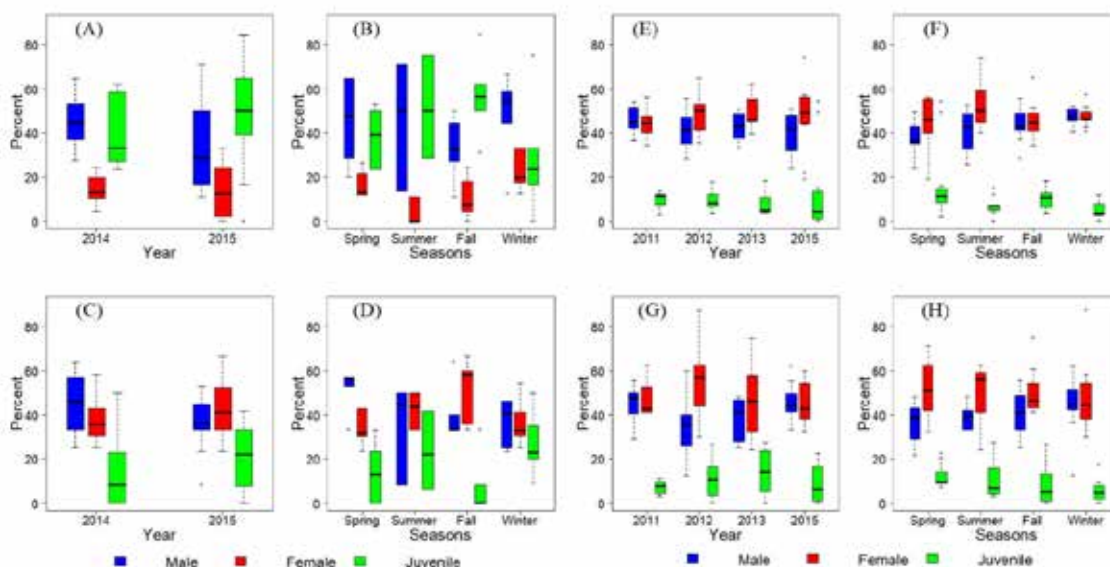


Figure 4 – Relative annual and seasonal frequency of males, females and juveniles of *H. reidi* at P1 (A, B) and P2 (C, D) of EPA Delta do Parnaíba, Piauí. Relative annual frequency and season for males, females and juveniles of *Hippocampus reidi* at Site R (E, F) and Site S (G, H) of Jericoacoara National Park, Ceará.

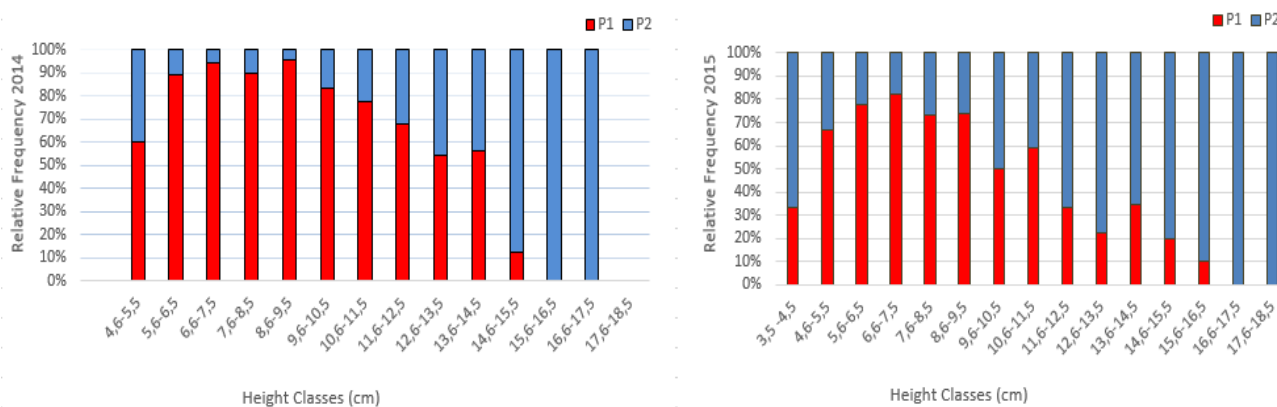


Figure 5 – Relative frequency of *H. reidi* analyzed according to height class and site (1 and 2) in EPA Delta do Parnaíba, Piauí.

**Jericoacoara National Park x EPA Delta do Parnaíba: juveniles**

2 of EPA with Sites R and S of the National Park (Table 3).

The mean number of juveniles at each site in the two states showed a similarity between Site

Table 3 – Relative frequency of juveniles in the population of *Hippocampus reidi* in Jericoacoara National Park, Ceará, and EPA Delta do Parnaíba, Piauí.

Year	Site R (CE)	Site S (CE)	Site 1 (PI)	Site 2 (PI)
2011	9.09±3.71	7.30±2.85	–	–
2012	9.77±4.33	10.51±8.11	–	–
2013	8.50±6.17	13.42±11.08	–	–
2014	–	–	42.30±16.9	15.74±18.98
2015	12.44±18.95	9.09±8.40	52.50±27.5	20.33±14.51



The percentage analysis of juveniles by season showed a difference between Sites R and S only in the spring, whereas at Sites 1 and 2, this difference occurred in the spring, summer and fall. The analysis of the four sites in the two states

showed a similar structure between Site 2 of EPA and Sites R and S of the National Park, separating Site 1 of EPA and showing its particular condition (Table 4).

Table 4 – Comparison between the relative frequency and percentage of juveniles at Sites 1 and 2 of EPA Delta do Parnaíba and Sites R and S of Jericoacoara National Park. The superscript letters (A, B and C) represent the pairwise comparison with the binomial test: the same letters join similar sites.

Season of year	Site 1 (%)	Site 2 (%)	Site R (%)	Site S (%)	P-value
Winter	14 (32.6) <sup>A</sup>	18 (26.1) <sup>A</sup>	48 (6.5) <sup>B</sup>	46 (7.5) <sup>B</sup>	<0.001
Fall	134 (60.6) <sup>A</sup>	5 (9.6) <sup>B</sup>	88 (11.1) <sup>B</sup>	46 (11.4) <sup>B</sup>	<0.001
Spring	36 (42.9) <sup>A</sup>	10 (17.9) <sup>B</sup>	59 (13.3) <sup>B</sup>	26 (8.7) <sup>C</sup>	<0.001
Summer	65 (67.7) <sup>A</sup>	8 (21.6) <sup>B</sup>	64 (12.4) <sup>B</sup>	29 (13.1) <sup>B</sup>	<0.001

### Mean height at brood pouch formation and mean height at first sexual maturity

In EPA Delta do Parnaíba, the regression results showed that, on average, the animal with a pouch still in formation had a height of 9.56 cm (95%CI 9.35; 9.78). For animals with already formed pouches, the average height was 1.97 cm greater (Table 5). That is, the mean height of the male of *H. reidi* with brood pouch fully formed was 11.53 cm (95%CI of 11.32; 11.75). The mean height at first sexual maturity was found to

be  $14.19 \pm 0.46$  cm (95%CI 13.09; 15.29). In Jericoacoara National Park, the regression result showed that, on average, the animal with a pouch still in formation had a height of 10.93 cm (95%CI [10.52; 11.33]). For animals with a formed pouch, the average height grows 0.59 cm in relation to the previous group. That is, the animals with a brood pouch already formed had an average height of 11.52 cm, with 95%CI in the interval [11.04; 12.00] (Table 5). The mean height at first sexual maturation was found to be  $14.69 \pm 0.53$  cm, 95%CI [13.42; 15.96] (Table 6).

Table 5 – Regression of young males of *Hippocampus reidi* to calculate the mean height at brood pouch formation at EPA Delta do Parnaíba, Piauí and in Jericoacoara National Park, Ceará. Significant differences are indicated by P-values in bold.

Variable	Parameter	Standard error	95% confidence interval	p-value
<b>EPA</b>				
Intercept	9.56	0.11	[9.35; 9.78]	<b>&lt;0.0001</b>
Brood pouch	1.97	0.18	[1.60; 2.33]	<b>&lt;0.0001</b>
<b>National Park</b>				
Intercept	10.99	0.29	[10.59; 11.40]	<b>&lt;0.0001</b>
Brood pouch	0.51	0.24	[0.03; 0.99]	<b>0.0370</b>

Table 6 – Setting using nonlinear regression - Levenberg-Marquard algorithm.  $H_m$ , mean height at first sex maturity for *Hippocampus reidi* in EPA Delta do Parnaíba, Piauí and in PARNA of Jericoacoara, Ceará. Significant differences are indicated by P-values in bold.

Parameter	Mean	Standard Deviation	95% confidence interval		P-value
			Lower	Upper	
<b>EPA</b>					
A	1.00	0.09	0.79	1.00	<b>0.0000</b>
r	0.67	0.16	0.28	1.06	<b>0.0049</b>
$H_m$	14.20	0.46	13.09	15.29	<b>0.0000</b>
<b>National Park</b>					
A	1.12	0.11	0.86	1.38	<b>0.0000</b>
r	0.56	0.12	0.28	0.84	<b>0.0022</b>
$H_m$	14.69	0.54	13.43	15.97	<b>0.0000</b>

### Reproductive period and male pregnancy

In the EPA Delta do Parnaíba, the reproductive period occurred throughout the year, without an evident reproductive peak, with 50% (or more) of the population in reproductive activity in the months of March, August, September and December (Fig. 6).

Of the 60 sightings of pregnant seahorses, 22% were at P1, while 78% of pregnant animals were seen at P2 (p=0.000). Within each site, the relative frequency of pregnant males was 9% in P1 and 53% in P2. There was no correlation between the abundance of pregnant males and the dry or rainy seasons (p= 0.6949).

In Jericoacoara National Park, the reproductive period occurred throughout the year (Fig. 6), without a defined reproductive peak, but there was a correlation between the relative frequency of pregnant males and the dry period, which corresponds to the second half of the year (p = 0.0012). The pregnant males were distributed at Sites R and S in similar quantities (89 at Site R and 78 at Site S). The smallest pregnant male (11.8 cm tall) was seen at Site R, while the largest (19.1 cm tall) was seen at Site S, where 92% of pregnant males over 17 cm height were also seen (n = 13).

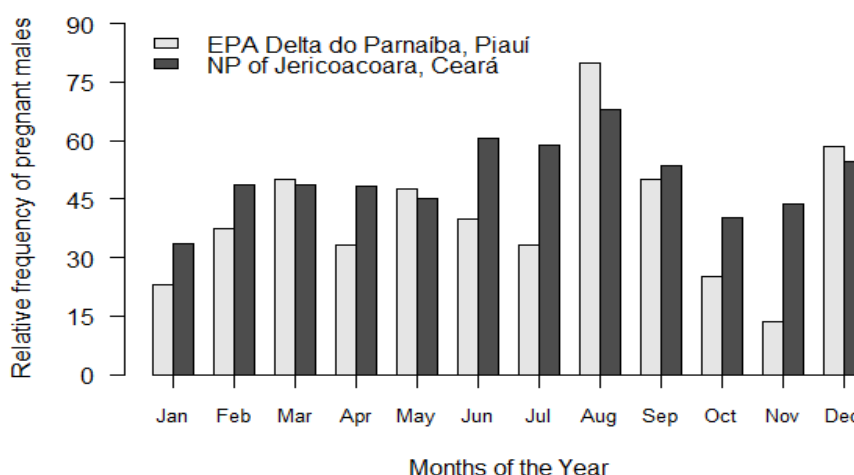


Figure 6 – Relative frequency of pregnant males over the months of the year in the EPA Delta do Parnaíba/Piauí, and Jericoacoara National Park/Ceará.

## Environmental parameters

The salinity and water temperature were quite similar at the two sites of each protected area with the lowest salinities recorded in the fall (Table 7). Salinity ranged from 33.88 to 39.78

and temperature from 27.89 to 28.30°C. Of these parameters, only temperature showed a negative correlation with the abundance of seahorses; that is, a greater abundance of seahorses was found in places with lower water temperature ( $p = 0.006$ ).

Table 7 – Mean salinity and water temperature at sites P1 and P2 along the seasons in EPA Delta do Parnaíba, Piauí, and at Sites R and S along the seasons in Jericoacoara National Park, Ceará.

Season	Salinity	Temperature	Salinity	Temperature
<b>EPA</b>	<b>Site 1</b>		<b>Site 2</b>	
Spring	28.40±2.21	27.60±0.61	32.03±2.02	29.38±0.49
Summer	30.00	29.00	31.76±0.66	29.88±0.33
Fall	25.00	27.80±0.40	24.11±1.40	29.23±0.66
Winter	28.27±2.10	27.9±1.30	28.27±1.71	28.30±0.97
<b>National Park</b>	<b>Site R</b>		<b>Site S</b>	
Spring	39.48±0.99	27.89±0.68	38.71±1.64	27.96±0.54
Summer	39.39±1.04	28.07±0.35	39.78±0.63	27.98±0.26
Fall	33.88±5.32	28.04±0.78	35.31±5.01	28.30±0.50
Winter	38.78±1.79	28.10±0.45	37.94±2.20	28.03±0.39

## Discussion

### Jericoacoara National Park vs EPA Delta do Parnaíba

*Mean height at brood pouch formation and mean height at first sexual maturity*

The formation of the brood pouch of the seahorse males varies with species and living conditions, whether in nature or in captivity (Wilson & Vincent, 2000; Silveira & Silva, 2016) because of their need for varied live food and their vulnerability to disease. We here report a pilot study on rearing broods from males of three species (*H. fuscus*, *H. barbouri*, and *H. kuda*, but mainly with the temperature factor that influences the whole physiology of living beings (Randall et al., 2011). Thus, the formation of this organ and other very particular events, such as height/age at first sexual maturity must be estimated for the species under local environmental or cultivation conditions

(Wilson & Vincent, 2000; Teixeira & Musick, 2001; Foster & Vincent, 2004; Silveira, 2005; Mai & Velasco, 2011; Silveira et al., 2016; Kawaguchi et al., 2017). In this work, the estimate for the mean height at brood pouch formation carried out in the two Brazilian states, whose data were collected by different researchers, obtained similar results: 11.53 cm in height in Piauí state (n=112) and 11.50 cm height in Ceará state (n = 103). With the same level of similarity (note that both estimates are within the same confidence interval), the mean height at first sexual maturity for Piauí was 14.2 cm (95%CI 13.09; 15.29), and for Ceará, it was 14.7 cm (95%CI (13.42; 15.96), suggesting great similarity between populations of *H. reidi*, since the research sites in Piauí are separated from the sites in Ceará by only 100 km along the coastline and without a physical barrier. The genetic data of the two populations show similarity between each other, indicating interchange of individuals (Martin Montes, personal communication).

### *Density, sex ratio and population structure in EPA Delta do Parnaíba, Piauí*

Between the two sites studied within EPA Delta do Parnaíba, Site 1 proved to be the most populous, despite supporting tourist interaction activities with seahorses. At this location, we obtained a significant decrease in the number of sightings driven by a reduction rate of 15.33% per month. Still in 2015, we detected an abrupt increase in density followed by an abrupt decay, revealing instability in the local population. Growth then resumed steadily at a rate of 41.28% over the last five months of the study. This population instability suggested by the decrease in density was corroborated by the structure and sex ratio found in the population, which had more males than females in 2014 and 2015, both annually and by season. Furthermore, the average number of juveniles (individuals with undetermined sex) had an increase over 40% in the two study years. These results indicate that there is a strong pressure acting on the population of Site 1, disrupting the population of seahorses in that location.

Although Site 2 showed lower population density in the two study years, this parameter remained stationary, with no significant increase or decrease. This stability in population density was accompanied by a sex ratio of 1:1, expected for monogamous fish, where the number between males and females did not show a significant annual difference, nor by season. In relation to juveniles (undetermined sex), this percentage was below 20.3% in the two years studied.

In research also carried out in the region of Barra Grande, the density of seahorses obtained by Mai & Rosa (2009) for Site 1, known by tourist drivers as “Ilha do cavalo-marinho”, was 0.062 ind/m<sup>2</sup>, data corresponding to a 12-month sampling between 2006 and 2007. Considering the different sampling methods and the elapsed time, the density estimated by the cited authors was an intermediate value compared to that found in our study, and we believe it represents the population well at that site. Site 2, known as “Camboa do cavalo-marinho”, was also analyzed by Mai & Rosa (2009) who found a density of 0.047 ind/m<sup>2</sup>, a higher number than that found in our study, both densities taken as evidence of past data for the region, also suggesting a decrease in sightings over the years. Unfortunately, the data on sex ratio and population structure of Mai & Rosa

(2009) were analyzed in general, encompassing all sites of that study (five in all), so that no difference was detected in the sex ratio of the population; however, the relative frequency of juveniles (undetermined sex) seemed to tend toward an increase. The relative frequency of juveniles found in 2006/2007 (31.5%) for the five sites of Mai & Rosa (2009) are replaced in this work by averages of 42.3% in 2014 and 52.5% in 2015, with extreme variations from 0 to 84% of juveniles at Site P1, while Site P2 maintained relative stability. Thus, as the population density of adult seahorses declines, the relative frequency of juveniles increases (Mai & Rosa, 2009; Silveira et al., 2017).

But what would an excess of juveniles in the seahorse population mean? How can we say that it is an excess? According to several studies carried out with species of the family Syngnathidae, the population structure of seahorses is often represented by a 1:1 sex ratio, similar size between sexes and sexual dimorphism, and proportionally with few juveniles (Perante et al., 2002; Bell et al., 2003; Moreau & Vincent, 2004; Silveira, 2005; Curtis & Vincent, 2006; Gristina et al., 2015; Pastor Gutiérrez et al., 2017) *Hippocampus capensis* Boulenger 1900, is a rare example of a marine fish listed as Endangered by the IUCN because of its limited range and *habitat* vulnerability. It is restricted to four estuaries on the southern coast of South Africa. This study reports on its biology in the Knysna and Swartvlei estuaries, both of which are experiencing heavy coastal development. We found that *H. capensis* was distributed heterogeneously throughout the Knysna Estuary, with a mean density of 0.0089 m<sup>-2</sup> and an estimated total population of 89 000 seahorses (95% confidence interval: 30 000-148 000).

Site 2, in addition to showing relative stability with stationary population density, even sex ratio, similar size between sexes and proportionally few juveniles, harbored the largest number of pregnant males (76%) and the largest sizes of seahorses (height >18.0 cm a height class present only at this site). All comparisons between Sites 1 and 2 were statistically significant, revealing a great difference between the two sites. The population instability found at Site 1 may be related to a series of anthropic factors, among them, the lack of order and regulation of tourist interactions with seahorses, the intentional capture of seahorses to supply the still existing illegal trade of these animals, and

the environmental degradation of the mangrove swamp due to strong real estate expansion and potentially impacting developments, such as shrimp farming.

#### *Density, sex ratio and population structure in Jericoacoara National Park, Ceará*

From 2011 to 2013, there was a significant decrease in density at both sites, with rates of 2.44 and 8.26% per month for Sites R and S, respectively. In 2014, it was not possible to carry out monitoring in this area and in 2015, when we returned, we found a significantly smaller population, but with apparent stability. This stability at both sites was accompanied by a population structure where the sex ratio was balanced (1:1) and the mean relative frequency of juveniles remained low, around 8.5 to 12% for Site R and 7 to 13% for Site S.

Population densities of several species of seahorses in natural environments have varied as follows: 0.00047 to 0.51 individuals/m<sup>2</sup> (Curtis & Vincent, 2005; Silveira, 2005; Rosa et al., 2007; Mai & Velasco, 2011; Pastor Gutiérrez et al., 2017; Woodall et al., 2018; Freret-Meurer et al., 2018). In the present work, there was a clear decrease in natural stocks in the two areas surveyed. There is also a consensus among the aforementioned authors that seahorse populations are declining in various parts of the world due to numerous factors such as incidental fishing, commercial fishing directed at seahorses, environmental degradation resulting from real estate and tourist developments, environmental contamination resulting from untreated or improperly disposed effluents, pesticides and uncontrolled tourist exploration activities with seahorses (Foster & Vincent, 2004; Silveira, 2005; Baum & Vincent, 2005; Rosa et al., 2007; Osório, 2008; Silveira et al., 2018). In addition, conservation actions for seahorses adopted in each member country of the International Convention on Trade in Endangered Species of Wild Fauna and Flora (CITES) may be being minimized by the insufficiency of information declared in the CITES database (Foster et al., 2016).

Although seahorse population declines occurred in both research areas in our study, the most critical results were at Site 1 in EPA Delta do Parnaíba, where significant disruption, decline in population and excess of juveniles were

found. This scenario deserves an interpretation that supports the design and implementation of appropriate management decisions for the conservation of seahorses, maintenance of tourist attractions and generation of income for those directly involved with the activity. However, it is a fact that the results revealed the impact of uncontrolled tourist exploitation of these animals. However, we must consider one other issue in particular which will be addressed below.

#### *Fishing directed at seahorses*

While conducting studies in both marine protected areas, tours drivers commented that there was a collection of seahorses for trade in the past, but that “now there is no more”.

Brazil declared to CITES that it exported 57,843 seahorses to various destinations between 1997 and 2018, mainly the species *H. reidi* (CITES, 2020). Worldwide, *H. reidi* is one of the most exported seahorse species as ornamental (live) fish, having been recorded in volumes between 8100–53,000 individuals during the period of 2004 to 2011. These numbers represent the animals that were obtained from nature, while the animals declared as bred in captivity accounted for 8000–50,000 individuals in the same period (Foster et al., 2016).

The city of Fortaleza in the state of Ceará is recognized as the largest exporter of ornamental marine fish in the Northeast region of Brazil, and seahorses are among the most commercialized ornamentals (Monteiro-Neto et al., 2003). In addition, Ceará also developed animal collection and acts as a receiving site for seahorses from neighboring states for export (Monteiro-Neto et al., 2003; Gasparini et al., 2005).

The population decline observed in this work in both marine protected areas is possibly related to unorganized management, but there is a different pressure on Site P1 of the EPA Delta do Parnaíba. This site, which is located in a more peripheral area of the mangrove and close to the beach, may represent a gateway for collectors of seahorses, which would explain why only the population of this site was out of balance, with more males than females and many juveniles. The fact of having more males than females may mean that collectors prefer females, thinking of saving males that could generate more individuals for

future collection. However, this may be a simplified view of the problem; Site P1 showed only 9% of its pregnant males over the 18 months of study and this did not seem to make sense, as *H. reidi* reproduces throughout the year in Piauí (Mai & Velasco, 2011; Silveira et al., 2017).

Pregnant seahorses are highly targeted for trade because they cost more, including CITES records the Brazilian export of fingerlings from the wild (CITES, 2020), which implies targeted fishing on pregnant males. The fact that Site P1 did not present any animals  $\geq 18.0$  cm in height and only 10 individuals between 16.0 and 17.6 cm, also suggesting fishing of the largest sizes, which get the best prices on the market for live or dried animals (Vincent, 1996; Silveira, 2005; Rosa et al., 2006). This all leads us to think about the possibility that the seahorses present in Site P1, in addition to suffering the pressure caused by interaction tourism, were for a long time exposed to a possible collection pressure, which led to the marked population disruption detected here.

Assuming that seahorses were collected for trade, we can also suppose that the seahorse population at this site was originally much larger than the current one, since it would add to the population found in this work the pregnant males and the females supposedly collected, in such a way that the ideal sex ratio was reached (1:1); also, the relative frequency of juveniles would drop to the levels seen at Site P2 or as in Jericoacoara National Park.

Among the changes induced by fishing is the break in the distribution by size/age classes, negatively affecting the life history of the species, and maybe even shifting the size at first maturity and maximum size observed. Fishing increases population fluctuations towards young and small individuals and with faster maturation, a situation that quickly and continuously destabilizes the population, even after fishing has ceased, highlighting a situation of reduction in fish resilience (Perante et al., 2002; Hutchings & Reynolds, 2004; Sharpe & Hendry, 2009; Kuparinen et al., 2016) although clearly necessary for population recovery, are often insufficient. Persistence and recovery are also influenced by life history, habitat alteration, changes to species assemblages, genetic responses to exploitation, and reductions in population growth attributable to the Allee effect, also known as depensation. Heightened extinction risks were highlighted recently when a Canadian

population of Atlantic cod (*Gadus morhua*). In this study, due to the short monitoring period in Piauí, it was not possible to assess whether there was any displacement in size at first sexual maturity, but the break in the distribution by height class, the apparent excess of juveniles, and the reduction in the maximum size observed were evident, making fishing the best explanation for the critical situation found at Site P1 of EPA Delta do Parnaíba, or the main factor impacting that population. Site P2 of the EPA, without interaction tourism, remained stable throughout the study period, and for R and S Sites in Jericoacoara, which showed decline but maintained a relatively stable population structure, we believe that a set of factors, such as the pressure of interaction tourism, the presence of shrimp farming activities and even fishing, were responsible for the population scenario we found. However, these hypotheses should be viewed with caution since we did not obtain conclusive results in this regard, and the possible influences of anthropic factors on the population structure of the species need to be tested in specific studies.

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