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Population structure of the seahorse *Hippocampus reidi* in two Brazilian estuaries

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Abstract

This study aimed to evaluate and compare seasonal variations of *Hippocampus reidi* population structures in two Brazilian coastal areas. Samples were collected from the rocky shores of Urca and Duas Irmãs Island by freediving along fixed transects. All seahorses observed in the study were recorded, measured, and identified by sex and reproductive stage. Seahorse behavior and fish depth were also registered. All individuals were photo-identified to estimate recapture rates. Holdfast used by seahorses was also recorded. Results showed low densities at both sites; sex ratio and juvenile/adult proportion did not appear to vary with season. The seahorse height differed between the two areas but did not vary with season nor sex. Seahorses in both sites were found in shallow waters (i.e. up to 4 meters), and the depth did not vary by season nor sex. Males were more frequently recaptured than females in both areas. All seahorses were reproductively active year-round, peaking at rainy periods in both areas. The seahorse population structure and seasonality appeared similar. These data provide useful baseline data for future population assessments. **Descriptors:** Habitats; *Hippocampus Reidi*, Population structure, Seasonality.

INTRODUCTION

Estuaries provide many ecological functions for inhabiting organisms such as nurseries, refuges, foraging sources, migration areas, and reproduction sites (Gibson, 1994; Koutsikopoulos et al., 1989; Miller, et al., 1985; Vasconcelos et al., 2011). These ecological functions are extremely important, mainly to estuarine fish, as some

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species rely on these environments throughout their whole life cycle.

Teleost fish in temperate estuaries, where environmental variation is broader and more seasonal, generally show seasonal patterns of growth, reproduction, abundance, and density (Blaber, 2000; Elliott and Hemingway, 2002). Consequently, fish species can adapt to reach desirable reproduction and recruitment conditions, with adjustments in populations such as density, sex ratio, juvenile abundance, residence time, and height class curve. Although water parameters may drive biological responses, biotic trends may also play an important role for the species (Power, 2000; Power

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et al., 2000, 2002; Power and Attrill, 2002, 2003). Food and habitat availability, competition, and predation can also be important to explain and predict the temporal and spatial patterns of estuarine organisms (Ogburn-Matthews and Allen, 1993; Lankford and Targett, 1994; Barry et al., 1996). The relationship between the biota distribution with biotic and abiotic factors in estuaries have received considerable attention (Akin et al., 2005; Kotta et al., 2015; Conroy et al., 2017; Newcomer et al., 2018; Grimaldo et al., 2020). These studies focused on fish population and community patterns in this ecosystem and are one of the most dominant groups (Blaber and Blaber, 1980; Marshall and Elliott, 1998; Whitfield, 1999; Akin et al., 2005; Grimaldo et al., 2020).

Seahorses are coastal fish which inhabit shallow water in tropical and temperate areas (Lourie et al., 1999). Of the 46 seahorse species worldwide, seven are found in estuaries (Correia et al., 2018; Correia et al., 2015; Lourie et al., 1999; Harasti et al., 2012), of which Hippocampus capensis is exclusively estuarine (Claassens et al., 2020). Species of temperate estuaries, such as Hippocampus guttulatus, have an intense response to seasonal variation of environmental conditions, particularly temperature (Correia et al., 2018), as do Hippocampus reidi in tropical estuaries, reproducing mostly in the dry windy season (Mai and Velasco, 2012). In Brazil, three species are commonly found in ecosystems near rocky reefs, mangroves, and coral reefs (Rosa et al., 2007; Freret-Meurer and Andreata, 2008; Mai and Rosa, 2009; Freret-Meurer et al., 2018a, b). The longsnout seahorse Hippocampus reidi Ginsburg, 1933 is the most common along the Brazilian coast and is currently rated Vulnerable in the Brazilian red list of the Brazilian Ministry of Environment (ICMBIO, 2018) and Near Threatened globally (Oliveira and Pollom, 2017).

Tracking and understanding seahorse population structure dynamics, especially in regional estuaries, is extremely important. This can help evaluate the effect of environmental changes on population and even help develop regional conservation estuary policy. These organisms generally exhibit patchy distribution, low mobility, and small home ranges (Foster and Vincent 2004; Caldwell and Vincent 2012), leaving their populations vulnerable to decline or even eradication.

There are some studies on *H. reidi* populations in Brazil (Dias and Rosa, 2003; Rosa et al., 2005; Rosa et al., 2007; Mai and Velasco, 2012; Aylesworth et al., 2015; Freret-Meurer et al., 2018a, b). These focused on the environment and certain aspects of population structure. However, none of them hypothesized on seasonal population variation, extremely important in estuaries due to their dynamics.

The present work seeks to investigate the effect of seasonal estuarine trends (salinity and temperature) on the longsnout seahorse *H. reidi* population structure in two estuaries in the state of Rio de Janeiro, Brazil. We expected to find (1) similar overall water physical and chemical factors in these two areas; (2) a difference in environmental conditions in accordance with dry and rainy seasons; and (3) similar habitats and seasonal structures among seahorse populations.

METHODS

This study was conducted with the permission of the Chico Mendes Institute for Biodiversity Conservation (ICMBio) (permit number 44409-3). Data collection was conducted at two coastal rocky shore locations in Rio de Janeiro: Urca beach (U) (22°56'33" S - 43°09'27" W), located on the west side of Guanabara Bay, and Duas Irmãs island (DI), located in Sepetiba Bay (22°56'38" S, 43°57'46" W; Figure 1). The two factors that helped determine these locations were the presence of *H. reidi* and water visibility, crucial for data accuracy.

Urca beach is located in Guanabara Bay, which has an area of 380km² (Kjerfve et al., 1997). The bay is 84% shallow water (up to 10 m in depth), though some areas reach 40 m in depth, as recorded by the central channel (Figueiredo et al., 2014). Mean rainfall during the rainy and dry seasons varies between 3 mm – 85 mm and 80 mm – 110mm, respectively (INMET, 1992). Urca beach is at the entrance channel of the bay and is considered a tourist site. It has a rocky reef composed of a diverse range of holdfast (Sola and Paiva, 2001). Seahorse populations had previously been recorded at the site.



Figure 1. Location of the two surveyed sites for *Hippocampus reidi* during 2018-2019 in Rio de Janeiro coastal bays - a) Guanabara bay (Urca beach) and b) Sepetiba bay (Duas Irmás island).

Duas Irmas island is Sepetiba bay, which has a surface area of approximately 305 km² and depth ranging between 8.6 meters and 30 meters (Fonseca, 1978). Annual rainfall values range between 1,000mm and 2,100 mm (Barbieri and Kronemberger 1994). The two beaches are surrounded by rocky shores. Like Urca Beach, it is also a tourist site. Duas Irmãs island was chosen due to previous bibliographic research about local benthic characteristics, which led us to believe that it could offer seahorse habitats as it is surrounded by rocky reefs with seaweed, bryozoans, and porifera (Széchy et al., 2005).

Local seahorse population was monitored monthly through snorkeling between February 2018 and January 2019. During dives, four fixed 20 m x 4 m (total of 320 m²) transects were conducted, for an effective search time of 90 minutes per dive along the rocky shores (Freret-Meurer et al., 2018a). Maximum survey site depth was 4 cm in both areas. A total of 96 transects were performed, and 36 diving hours were spent on surveys. Temperature and salinity were measured monthly with a mercury thermometer and a refractometer, respectively, on the water surface. Monthly cumulative rainfall data were drawn from the National Institute of Meteorology (INMET) to categorize seasons into rainy (October to March) and dry (April to September) periods for further seasonal analyses.

Seahorse individuals were counted during transects for abundance and density estimates. Further, seahorse sex was determined by the presence of brood pouches (males) or their absence (females) (Lourie, Vincent and Hall, 1999).

All seahorses were photographed using a Nikon AW300 for individual identification and further recapture analysis (Freret-Meurer et al., 2013), using I3S Classic version 4.02 software. The Constancy Index (CI) (adapted from Dajoz, 1983) was calculated to categorize as follows for each recaptured individual: rare (CI < 25%), visitors (25% < CI < 50%), and constant (CI > 50%) (Freret-Meurer and Andreata, 2008). A Jolly-Seber stochastic model for open populations (Jolly, 1965;

Seber, 1982) was used to estimate population size, assuming the same capture probability for marked and unmarked animals. The model formula was computed using MS Excel.

Height and depth of occurrence were also measured underwater. Seahorse height was measured as a straight line from the coronet top to the prehensile tail end (Lourie, 2003). Individuals with a brood pouch and cloaca smaller than 56 mm were considered juveniles (Freret-Meurer et al., 2018a).

We used the scan method to assess seahorse behavior (Altmann, 1974), which was addressed upon seahorse discovery. Behavior was classified as Foraging, Swimming, Courting, and Resting (Freret-Meurer et al., 2018b). To establish breeding peaks, the reproductive stage was determined according to Lourie (2003), where for males: 0 =just given birth, pouch flabby; 1 = pouch empty, pouch flat; 2 = pregnant, pouch rounded; 3 = about to give birth, pouch extremely rounded and shiny, and for females: 0 = eggs just given away, belly sunken; 1 = no mature eggs, belly flat; 2 = bearing mature eggs, belly slightly raised; 3 = hydrated eggs, belly distended.

We recorded the holdfasts where seahorses were anchored. Holdfasts were identified in the field, but samples were collected and taken to the laboratory for further analysis when identification difficulties were encountered, according to Brusca et al. (2018), Muricy and Hajdu (2006), and Joly (1967). The holdfasts were classified at the species levels or higher taxonomic groups. The allochthonous holdfast was classified as a holdfast from other ecosystems and artificial as anthropogenic holdfasts.

The differences in sex ratio and the juvenile/ adult proportion between seasons for each area were analyzed using the Chi-Squared test. Seasonal variations of seahorse height and depth of occurrence were checked by paired Student's T-test in GraphPad InStat 3.0, and a Kolmogorov-Smirnov test was used to confirm normal distribution for heights and anchorage depth. Student's tests were also used to calculate the difference between sex among these two measurements. In addition, the frequency of occurrence (FO) was calculated to infer the association with the holdfast, and a Shannon-diversity index was used to evaluate the holdfast association diversity.

Two-way permutational analysis of variance (PERMANOVA; Anderson, 2005) was applied to test for density differences, precipitation, temperature, and salinity between beaches (Urca Beach and Duas Irmãs Island) and periods (dry and rainy) using CANOCO 5.0 software. A mixed model was used to deal with spatial pseudoreplication (Anderson et al., 2008), where the rainy and dry periods were used as fixed and orthogonal factors and sampling period as a random factor. The Bray-Curtis distance was used in PERMANOVA, and data was permuted 9,999 times per analysis.

RESULTS

Precipitation was significantly higher at Urca Beach than at Duas Irmãs (F = 18.236, p = 0.0004), even when comparing the rainy and dry seasons at both sites (F = 24.1049, p = 0.0001). However, there was no significant interaction between these factors (F = 0.558, p = 0.476). For both sites, temperature was significantly higher during the rainy season (F = 4.134, p = 0.027), and salinity was higher during the dry season (F = 5.067, p = 0.038), with no significant difference between beaches (F = 1.468, p = 0.275; F = 1.892, p = 0.179, respectively) or interaction between beach and period (F = 1.164, p = 0.332; F = 0.286, p = 0.591, respectively) (Figure 2).

A total of 66 seahorses were found at Urca Beach and 52 at Duas Irmãs Island, resulting in an average monthly abundance of 6 ± 1 individuals/ month and 4 ± 2 individuals/month respectively. The mean seahorse density was higher at Urca Beach than at Duas Irmãs Island (Table 1 and Figure 3).

Sex ratio at Urca Beach was 1:1 (Figure 4a), and adult/juvenile proportion was 8:1 (Figure 5a). At Duas Irmãs Island, sex ratio was 3:2 (Figure 4b), and 50 adults were recorded for two juveniles; 25:1 (Figure 5b). There was no significant difference in sex ratio between seasons at either area when analyzed separately ($\chi 2_{urca} = 0.02$, p = 0.8; $\chi 2_{Duas irmãs} = 0.0$, p = 1.0), nor for juvenile/adult proportion ($\chi 2_{urca} = 0.06$, p = 0.7; $\chi 2_{Duas irmãs} = 0.0$, p = 1.0). The sex ratio and juvenile/adult proportion



Figure 2. Boxplots season (dry and rainy seasons) values of: A. monthly cumulative precipitation in mm; B. water temperature in °C; and C. salinity in psu. Rhombus represent season mean values and circles represent mean area values. Black dots are outliers.

Table 1. Results of *Hippocampus reidi* abundance (n total of individuals), density (ind.m⁻²; mean \pm SD), height (mm; mean \pm SD) and depth (m; mean \pm SD) at Urca beach and Duas Irmãs island. F=female, M=male, J=juveniles, D=dry and R=rainy.

Area				Ur	ca beach							Duas Ir	mãs islar	nd		
	Abund		Density		Height		Depth of		Abund.		Density		Height		Depth of	
	(n)		(ind.m- ²)		(mm)		occurrence (m)		(n)		(ind.m ⁻²)		(mm)		occurrence (m)	
Period	D	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R
F	17	12	0.009	0.006	104.9	82.4	0.92	1.16	11	10	0.005	0.005	89	87.8	1.34	0.86
			±	±	±	±	±	±			±	±	±	±	±	±
			0.007	0.006	20.7	24.6	0.32	0.18			0.002	0.002	17.77	17.8	0.47	0.21
М	16	14	0.008	0.007	121.3	122.4	0.87	1.17	15	14	0.007	0.007	96.27	92.6	1.23	0.93
			±	±	±	±	±	±			±	±	±	±	±	±
			0.002	0.005	12.7	19.9	0.28	0.24			0.003	0.004	10.60	18.6	0.55	0.32
J	3	4	0.002	0.002	47.3	50.7	0.57	1.12	1	1	0.0005	0.0005	52	40	1.6	0.6
			±	±	±	±	±	±			±	±				
			0.003	0.002	1.5	4.1	0.06	0.47			0.001	0.001				
Total	36	30	0.02	0.02	96.9	107.4	0.86	1.16	27	25	0.01	0.01	91.7	88.6	1.29	0.89
			±	±	±	±	±	±			±	±	±	±	±	±
			0.01	0.002	33.2	25.8	0.30	0.24			0.003	0.01	16.1	20.4	0.50	0.28



Figure 3. Mean density (ind.m⁻²) and standard deviation of the seahorses *H. reidi* at Urca beach and Duas Irmãs island from February 2018 to January 2019.





Figure 4. H. reidi sex ratio (%) (female x male) at at Duas Irmãs island and Urca beach.



Figure 5. Hippocampus reidi proportion of juveniles and adults (%) at Duas Irmãs island and Urca beach.

did not differ in either area (χ 2 =0.3, *p* = 0.6; χ 2 = 1.0, *p* =0.3, respectively).

At both sites, males were more frequently resighted than females. At Urca Beach, re-sights were 7% for females and 17% for males. At Duas Irmãs Island, results were 24% for females, 31% for males, and 50% for juveniles. Annual re-sight rates were 11% at Urca Beach and 29% at Duas Irmãs Island. One female was re-sighted six times at Urca Beach, while one male was re-sighted five times at Duas Irmãs Island. The other individuals were re-sighted less than four times in each area. Per the Constancy Index, individuals from Urca Beach were considered rare (n=4) and visitors (n=3). Those from Duas Irmãs Island were deemed rare (n=11) and visitors (n=3). There were no resident seahorses in either area.

The Jolly-Seber model suggested a possible population of 236 individuals at Urca Beach and 140 at Duas Irmãs Island.

No seasonal difference was noted for seahorse height at either area ($t_{\text{urea}} = 1.64$, p = 0.11; $t_{\text{buas}} = 0.48$, p = 0.63) (Table 1, Figure 6a and 6b). However, at Urca Beach males were larger than females (t= 4.84, p= 0.001), in contrast with Duas Irmãs Island (t=1.33, p= 0.19). Individuals from Urca Beach were significantly larger than at Duas Irmãs Island (t = 2.65, p= 0.009).

Seahorse depths differed between periods at both sites ($t_{IIrca} = 3.54, p = 0.001; t_{Duas irmãs} = 2.90, p$ = 0.005), with greater depth recorded during rainy periods at Urca Beach and dry period at Duas Irmãs Island (Table 1). Depth showed a different pattern by sex and study site. Both females and males were found significantly deeper during the rainy season at Urca Beach (t= 2.6, p= 0.02; t= 2.0, p= 0.08, respectively), while only females showed a significant difference between periods (t = 2.3, p= 0.04) at Duas Irmãs Island, where males recorded t = 1.5 and p= 0.1. Despite the difference between periods, there was no significant difference in depth of occurrence between male and females in either area (t_{Urca} = 0.06, p = 0.90; t_{Duas} $_{imas}$ = 0.20, p = 0.90) (Table1). The mean depths were 1.52 m \pm 0.02 m and 2.27 m \pm 0.04 m at Urca Beach and Duas Irmãs Island, respectively.

Most seahorses were found in stages 0, 2, and 3, representing reproductive activity year-round,



Figure 6. Length classes (mm) of the seahorses *H. reidi* according to rainy and dry seasons (a) and length class (mm) on Urca beach (b); Length classes (mm) of the seahorses *H. reidi* according to rainy and dry seasons (c) and length class (mm) on Duas Irmãs island (d).

peaking during rainy periods in both areas. Both populations exhibited different behaviors, such as courting, swimming, foraging, and resting. Courting and swimming were observed only at Urca Beach (5%, 2% respectively). Foraging was observed in only 2% of seahorses, and the most observed behavior were resting (>90%) in both areas.

Seahorses at Urca Beach were associated with the ascidian *Styela plicata* (Lesueur, 1823) and seaweed *Codium* sp. (Stackhouse, 1797) (FO = 13%), but juveniles were only found anchored to turf seaweed and the bryozoa *Amathia verticillata* (Lamouroux, 1812). Seahorses were most frequently detected anchored to seaweed, Ascidiacea, and allochthonous holdfast (plant branch) (Figure 7a).

On the other hand, seahorses from Duas Irmas Island were frequently anchored to the cnidarian *Carijoa riisei* (Duchassaing and Michelotti, 1860) (FO = 48%) and to the turf seaweed (FO = 19%). However, juveniles were only recorded on *C. riisei* and on seaweed *Acanthophora* sp. (J.V.Lamouroux, 1813). Cnidaria and seaweed were the two taxonomic groups most used at Duas Irmãs Island. Urca Beach had a higher holdfast association diversity (H' = 2.38) than Duas Irmãs Island (H'=1.52) (Figure 7b). The holdfast species where seahorses were found are listed in the Supplementary Materials (Table S1 and Table S2).

DISCUSSION

This study highlights a pattern in the population structure of both seahorse populations associated with rocky reefs in different bays within Rio de Janeiro State, suggesting stability in the yearround population structure predictions. In fact, the stability of population parameters of fish tends to disagree with the well-marked dry and rainy seasons (Castillo-Rivera, 2013, Qin et al., 2017), as observed in the present study. Both study sites were characterized by low precipitation, low temperature, and high salinity during the dry season, as well as high precipitation, high temperature, and lower salinity during the rainy season.

Estuarine fish tend to be broadly tolerant to physical and chemical factors of water, especially temperature and salinity, as documented



Figure 7. Number of *H. reidi* anchored on holdfast taxonomic group at Urca beach (a) and Duas Irmās island (b). Gray - juveniles; Black - males and White - females.

by various authors (Allen and Horn, 1975; Hoff and Ibara, 1977; Weinstein, Weiss and Walters, 1980; Loneragan et al., 1987; Peterson and Ross, 1991; Marshall and Elliot, 1998; Araujo, Bailey and Williams, 1999). These serve as prime environmental factors which directly and indirectly affect species association patterns in these ecosystems (Horn and Allen, 1978; Monaco, Lowery and Emmett, 1992; Emmett et al., 2000). Similarly reported in the study of *Hippocampus mohnickey*, which seasonal variations in temperature drives migrations in and out of the estuary (Qin et al., 2017).

In our study, neither salinity nor temperature differed statistically between areas, which could explain the similar pattern in the seahorse population structure. However, they did vary between seasons, which could influence migration and changes in population structure. Still, no significant changes were recorded in our study, corroborating Schwarz et al. (2021) and Mai and Velasco (2012), who studied H. reidi in an estuary in northeastern Brazil. In addition, rainfall plays an important role in estuaries (Mendoza et al., 2002), as does mediating fish abundance seasonality, where higher abundances were often recorded during rainy periods (Castillo- Rivera, 2013). In the present study, seahorse abundance and density did not appear to be affected by rainfall. The same was noted for salinity and temperature variations. A possible explanation is that H. reidi occurs in a variety of environments along the Brazilian coast, including mangroves (Mai and Velasco, 2012), open water rocky shores (Freret-Meurer and Oliveira, 2012; Freret-Meurer et al., 2018b), and hypersaline areas (Rosa et al., 2007). In addition, according to Tseng et al. (2020), high temperatures (>30°C) had a direct effect on the survival of this seahorse species, and they can survive within a salinity range of 15-34 g/kg, suggesting high tolerance to physical and chemical water factors.

The long-snout seahorse exhibited low density and patchy distribution, a pattern similar to other species (Foster and Vincent, 2004, Rosa et al., 2007, Sigueira et al., 2017, Freret-Meurer et al., 2018a). The density of longsnout seahorse H. reidi varied along the Brazilian coast between 0.0023 to 0.066 fish/m2 (Rosa et al., 2007), lower than recorded in the present study. However, though low density is a group characteristic of seahorse populations, some studies have reported higher densities, such as Freret-Meurer et al., 2018a (0.21 ± 0.11 ind. m⁻²) and Schwarz et al., 2021 (0 to 0.32 fish/ m2). Low densities are often found for several other seahorse species, such as Hippocampus hippocampus in Ria Formosa lagoon and on Chalkidiki Peninsula (ranging between 0.05 ind.m⁻² and 0.015 ind.m⁻²), in the North Aegean Sea (Correia et al., 2015; Correia et al., 2020). Mean densities of 0.0089 m⁻² are observed for Hippocampus capensis in South Africa (Bell et al., 2003).

In addition to density, another population attribute analyzed was sex ratio, which was found not to change seasonally. Our finding corroborated other studies, such as for *H. comes* in the Philippines (Perante et al., 2002) and *H. reidi* in Rio de Janeiro (Freret-Meurer et al., 2018a). Seasonal changes in this attribute have been reported for *Hippocampus zostera* (Strawn, 1958) and *Hippocampus abdominalis* (Martin-Smith and Vincent, 2005), suggesting that sex ratios can vary according to seahorse species. It is also important to consider that females tend to have wider home ranges (Freret-Meurer and Andreata, 2008), which could explain the skew for larger male recapture rates in this study.

The adult proportion was greater than juveniles in this study, with no seasonal variation. The absence of seasonality was expected due to the year-round reproduction of the species (Freret-Meurer et al., 2018a). Low juvenile proportions in relation to adults were also documented for the same and other seahorse species, such as *H. guttulatus* (Correia et al., 2015), *H. capensis* (Lockyear et al., 2006), *and H. reidi* (Freret-Meurer et al., 2018).

The *H. reidi* height was consistent with the range generally found for the species (Lourie et al., 2004; Rosa et al., 2007; Aylesworth et al., 2015; Freret-Meurer et al., 2018a), but diverged between beaches; seahorses were larget at Urca Beach than at Duas Irmãs Island. The seahorse *H. reidi* height can vary along the Brazilian coast, such as in the Northeast (Rosa et al., 2007; Silveira, 2011) and on coastal rocky reefs from Rio de Janeiro (Freret-Meurer et al., 2018b), where the species is larger than observed in our study.

In the present study, H. reidi occurred in shallow areas, most likely due to the maximum depth of 4 meters recorded at the surveyed rocky shores. The longsnout seahorse has been found at 10 cm (Rosa et al., 2002) and 55 m (Vari, 1982). Further, it may appear in different setting types between sites, as reported by Freret-Meurer et al. (2018a), who found *H. reidi* in shallow waters (1.2m) and in an estuary rocky reef with maximum depth of 3m. This is in contrast with Oliveira and Freret-Meurer (2012), who reported larger abundances in deeper waters (5 m) in Arraial do Cabo, where the rocky bottom depth was 9 m; the occurrence depth of seahorses may also be related to the depth of the rocky shore. In addition to the results found in this study, shallow waters could provide better refuge and shelter (Wallace and Van Der Elst, 1975; Blaber, 1985) from possible predators that are more commonly found in deeper waters (Whitfield and Blaber, 1978).

Seahorse depth of occurrence differed between periods in both areas. This could be due to holdfast availability, which changes between periods at Duas Irmãs Island (Amado Filho et al., 2003) and Urca Beach (Breves-Ramos et al., 2005). There is evidence that holdfast availability and seahorse depth of occurrence could be correlated, as seen for *H. whitei* which aggregate in seagrass beds in shallow areas (Manning et al., 2018). Manning et al. (2018) also suggest that this correlation could offer spatial differences in feeding and reproductive opportunities.

The seahorses exhibited reproductive stages throughout the year with peaks during rainy periods, suggesting these populations are capable of reproducing all year. Our findings contrast with certain temperate species such as *H. guttulatus* and *H. hippocampus*, which tend to reproduce during the summer, responding to environmental conditions (Correia et al., 2018); this suggests *H. reidi* tolerance to water physical and chemical variables. Other behaviors were also observed, mainly resting, which is characteristic of Syngnathidae (Lourie, Vincent and Hall, 1999; Freret-Meurer, Andreata and Alves, 2012). This behavior is characterized by the seahorse anchored with their bodies close to the holdfast, as also described for *H. capensis* (Bell et al., 2003) and *H. abdominalis* (Martin-Smith and Vincent, 2005).

Commonly noted holdfasts to which *H. reidi* often anchor include seaweed, which agrees with similar studies for the species (Dias and Rosa, 2003; Rosa et al., 2005, 2007; Aylesworth et al., 2015). In addition, seahorses have also been found in allochthonous and artificial holdfasts, as observed in other studies (Dias and Rosa, 2003; Curtis et al., 2004; Rosa et al., 2007; Clynick, 2007; Correia et al., 2015; Simpson et al., 2019; Claassens and Harasti, 2020; Simpson et al., 2020a, 2020b). The use of artificial holdfasts suggests they may adapt to coastal changes (Clynick, 2007), or that *H. reidi* choose these holdfasts for a behavior not fully understood.

Urca Beach exhibited a higher diversity of anchorage points, but also a higher seahorse abundance, which could have influenced the results. Urca Beach is also generally calm and protected from wave exposure, whereas Duas Irmãs Island is more exposed and experiences higher hydrodynamic conditions during certain periods. This could influence the occurrence of certain holdfasts. In both sites, seahorses were frequently found on different seaweed species, suggesting that future approaches applying morphofunctional groups for testing holdfast use could be of interest.

Both population sizes seemed small compared to other seahorse populations, as was reported for *H. guttulatus* in Thau lagoon (Riquet et al., 2019) with an estimate of 2,742 individuals. In addition, a recent study reported an estimated population of 28,763 individuals for the Knysna seahorse *Hippocampus capensis* in Africa (Mkare et al., 2021). The most similar data to our study was reported for *Hippocampus whitei* in Port Stephens, Australia, with 213 individuals (Harasti et al., 2012). Our data should be carefully considered for estimated population size due to the small study area. They represent local population data and should be used with caution as a baseline for estimated seahorse population size in both bays.

Population structures of *H. reidi* in both study sites resemble each other in aspects such as height, depth of occurrence, and holdfast use. Moreover, the factors analyzed do not seem related to their density nor population structure. This species is found mostly in resting behavior and during reproductive periods throughout the year, with peaks during rainy periods. The low recapture rates could indicate that these areas work as a passage area, maybe to inner bay regions. These seasonal movements had already been reported for Hippocampus mohnikei Bleeker, 1853 on the Chinese coast, moving to the inner bay during summer (Qin et al., 2017). Also supporting these facts, Faro and Bonecker (2000) recorded seahorse fryes in the innermost part of Guanabara Bay (Rio de Janeiro), indicating a possible reproduction or nursery area. Seaweed was the most used holdfast for anchorage, highlighting the significance of the phytal ecosystems for seahorses.

Our results indicate that both bays shelter a population of the longsnout seahorse *H. reidi* with a stable structure year-round, despite a low abundance; these require further study and protection.

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AUTHOR CONTRIBUTIONS

- T.F.C.: Conceptualization; Investigation; Writing original draft; Writing review & editing.
- L.N.S.: Methodology; Software; Formal Analysis; Writing editing.

- A.B.A.: Writing original draft; Writing review & editing.
- N.V.F-M: Resources; Conceptualization; Investigation; Writ-

ing - original draft; Writing - review & editing.

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SUPPLEMENTARY MATERIALS

Table S1. Holdfast species use by seahorse *Hippocampusreidi* at Duas Irmãs island.

Holdfast use	Group
Acanthophora sp.	Seaweed
Amathia verticillata (Lamouroux, 1812)	Seaweed
Carijoa riisei	Cnidaria
Coralline seaweed	Seaweed
Hypena sp.	Seaweed
Plocamium sp.	Seaweed
Turf seaweed	Seaweed

Table S2. Holdfast species use by seahorse *Hippocampusreidi* at Urca beach.

Holdfast use	Group
Amathia verticillata (Lamouroux, 1812)	Seaweed
Botrylloides nigrum (Herdman, 1886)	Ascidiacea
<i>Clavelina oblonga</i> (Herdman, 1880)	Ascidiacea
<i>Codium</i> sp.	Seaweed
Hymeniacidon heliophila (Parker, 1910)	Porifera
Polychaeta	Polychaeta
Porifera	Porifera
<i>Styela plicata</i> (Lesueur, 1823)	Ascidiacea
Turf seaweed	Seaweed