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Do longsnout seahorses *Hippocampus reidi* (Syngnathiformes: Syngnathidae) have a holdfast preference?

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Coastal systems encompass a range of ecotones that are important for fish species, providing diverse micro-habitats and grounds for foraging, protection from predation, reproduction and areas for recruitment. However, most of these systems face major threats from human activities. Considering the increasing levels of human disturbance in coastal ecosystems, understanding fish-habitat associations may provide important insights into patterns of species occurrence and distribution in human-impacted systems, which can support the development of effective conservation and management measures. In this context, we investigated the relationship between seahorses (*Hippocampus reidi*) and both habitat complexity and different holdfast species, to determine possible variation between locations and among seasons. Data were obtained from the rocky reefs of Guanabara (Urca Beach) and Sepetiba (Duas Irmãs Island) bays, in southeastern Brazil. Seahorses were counted, and the holdfast being used by each individual was recorded and identified to species or assigned to a morphofunctional group. The beaches differed in holdfast composition and morphofunctional groups, yet sharing some morphofunctional groups. Seahorses were more frequently associated with branching holdfasts at both sites, and also with foliaceous and massive. Association with algae were particularly found in Urca, while the coral *Carijoa riisei* in Duas Irmãs Island.

Keywords: Estuary, Habitat, Morphofunctional groups, Reef fish, Rocky reefs.

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Os sistemas costeiros abrangem uma variedade de ecótonos que são importantes para as espécies de peixes, fornecendo diversos micro-habitats e áreas para alimentação, proteção contra predação, reprodução e áreas para recrutamento. No entanto, a maioria desses sistemas enfrenta grandes ameaças de atividades humanas. Considerando os níveis crescentes de perturbação humana nos ecossistemas costeiros, a compreensão das associações peixe-habitat pode fornecer informações importantes sobre os padrões de ocorrência e distribuição de espécies em sistemas afetados pelo homem, que podem apoiar o desenvolvimento de medidas eficazes de conservação e gestão. Neste contexto, investigamos a relação entre os cavalos-marinhos (*Hippocampus reidi*) e a complexidade do habitat e diferentes espécies de fixação, para determinar a possível variação entre locais e entre estações do ano. Os dados foram obtidos nos recifes rochosos das baías de Guanabara (Praia da Urca) e Sepetiba (Ilha das Duas Irmãs), no sudeste do Brasil, por mergulho livre ao longo de transectos fixos. Os cavalos-marinhos foram contados, e o substrato usado por cada indivíduo foi registrado e identificado como espécie ou atribuído a um grupo morfofuncional. As praias diferiram quanto à composição e grupos morfofuncionais, embora compartilhando alguns grupos morfofuncionais. Os cavalos-marinhos foram mais frequentemente associados a substratos arborescentes/ramificados em ambos os locais, a foliáceos e maciços. A associação com algas foi particularmente encontrada na Urca, enquanto o coral *Carijoa riisei* na Ilha de Duas Irmãs.

Palavras-chave: Costão rochoso, Estuário, Grupo morfofuncional, Habitat, Peixe recifal.

INTRODUCTION

Coastal areas encompass several different types of ecotones, such as mangroves and estuaries, which provide numerous ecological and economic services to humans, including production of food and recreation. Coastal environments also tend to support a considerable biological diversity of marine, freshwater, and brackish water species through the provision of feeding resources, breedings grounds, and refuges from predators (Barletta *et al*., 2010; Barbier *et al*., 2011). Despite their biological richness, estuaries are among the ecotones most vulnerable to the effects of human impacts (Elliott, Whitfield, 2011; Whitfield *et al*., 2012). The geographical position and high natural resources availability of those systems have historically fostered human occupation and urbanization, leading to the progressive degradation of their conditions due to pollution, habitat destruction and fisheries (Wilkie, Fortuna, 2003; Wilkinson, 2004; Lotze *et al*., 2006; Gibson *et al*., 2007). Estuaries are influenced by both marine and freshwater systems, which creates a gradient of environmental conditions, in particular, salinity (Chaves *et al*., 2018), which tends to drive the distribution of species within the estuaries, selecting organisms based on their associations with specific aquatic conditions (Potter *et al*., 2015).

Fish are a major component of the biodiversity of estuaries, which they are attracted to due to their high productivity, temperature, and the availability of refuges (Dolbeth *et al*., 2008; Kerr *et al*., 2010). They may colonize estuarine systems permanently or during specific stages of their life cycles, either to seek protection against predation (Hindell *et al*., 2000; Laegdsgaard, Johnson, 2001), recruitment, breeding sites, and food resources (Kwak, Klumpp, 2004; Whitfield, 2017). Seahorses typically use estuarine habitats for reproduction, refuge and feeding (Foster, Vincent, 2004; Freret-Meurer *et al*., 2018; Fernández *et al*., 2022). As seahorses depend on holdfasts to anchor themselves (Lourie *et al*., 1999) during courtship (Faleiro *et al*., 2008) and foraging (Curtis, Vincent, 2005), and also when resting (Lourie *et al*., 1999) or sheltering (Claassens, 2016), the structure of the benthic habitats is an important determinant of the presence of these fish. It is still unclear; however, which factors may determine the selection of holdfasts by the seahorses, and whether the choice of holdfast is related to any specific architectural or morphofunctional traits. A better understanding of this phenomenon would be fundamental to the definition of the ecological requirements of the seahorses, and the most effective management measures for the habitats they occupy.

Morphofunctional traits approaches, for benthic organisms, are essentially the description of similarity in the morphology or traits and functionality among species (Littler, Littler, 1980). Through the characterization of organisms, traits enable the establishment of a connection between individuals and their environment, aiding in the elucidation of mechanisms that underlie species coexistence (Garnier *et al*., 2016; Kunstler *et al*., 2016). Identifying and applying this methodology to any organism is relatively straightforward, resulting in reduced handling time and costs across studies (Veiga *et al*., 2013), which deviates from the traditional approach of taxonomically classifying the species. This morphofuntional group (MFG) application has contributed to advancements in ecological studies, enhancing our understanding of latitudinal gradients (Gaspar *et al*., 2017). Additionally, it has played a role in improving monitoring programs (Pagliosa *et al*., 2012) and environmental impact studies (Orfanidis *et al*., 2011; Martins *et al*., 2013). However, MFGs come with certain limitations, including a lack of a clear mechanism for species classification (Phillips *et al*., 1997).

The MFG approach is commonly used in study of aquatic ecosystems to discriminate macroalgal (Vadas, Steneck, 1988; Lirman, Biber, 2000; Vanderklift, Lavery, 2000; Konar, Iken, 2009), coral reef (Alvarez-Filip *et al*., 2009, 2011), and rocky reef assemblages (Voerman *et al*., 2017). The benthic approach used in marine ecosystems usually relates the morphological habitat created by benthic species and its ecological function to the assemblage (Norton *et al*., 1982), as well as it may also be a predictor to habitat selection (Philips *et al*., 1997). As some seahorses typically inhabit reefs and use benthic organisms as holdfast, a morphofunctional approach may provide the most effective analytical tool for the evaluation of the association of these fish with specific types of holdfasts. This approach can also help assess the abundance and diversity of their crustacean and gastropod prey (Warfe, Barmuta, 2004), which may mediate their occurrence.

In this context, understanding the relative importance of the different benthic species that are used by seahorses as holdfasts, and the architecture and ecological functions of these species, may provide important insights. These insights can contribute to the development of effective measures for the conservation and management of seahorse populations. It's noteworthy the genus *Hippocampus* Rafinisque, 1810 are listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), which monitors and regulates international trade (Foster *et al*., 2022). Additionally, they are included in the list of threatened species in Brazilian fauna (Ordinance #148 of June 7, 2022), classified as Vulnerable (VU).

In this sense, the present study aimed to investigate the relationship between the abundance and density of longsnout seahorse *Hippocampus reidi* (Ginburg, 1933) and habitat selection according to species, as well as morphofunctional traits in two tropical estuaries (Guanabara Bay and Sepetiba Bay), testing whether it has preference for a given type of holdfast (species) or habitat (morphofunctional group), and whether this preference varied between sites and seasons.

MATERIAL AND METHODS

Study area. Data were collected on rocky reefs at two locations along the Rio de Janeiro coast: Urca beach (22°56'33"S 43°09'27"W), Guanabara Bay, and Duas Irmãs island, Sepetiba Bay (22°56'38"S 43°57'46"W; Fig. 1). The rocky reef at Urca beach (U) is located near the entrance channel to Guanabara Bay and is considered a touristic site with considerable human influence, with 1.6 beach users per 100 m^2 on average (Franco *et al*., 2016). A number of different types of holdfasts are available for seahorses in this area, including tunicates, hydrozoans, and seaweed (Sola, Paiva, 2001), as well as artificial holdfast (Sola, Paiva, 2001), such as ropes and solid objects, including plastic debris (Franco *et al*., 2016). According to Fernández *et al*. (2022), Rodrigues *et al*. (2020), and Seixas *et al*. (2016), the water salinity from Urca beach varies between 29 to 35 (g L−1), dependending mostly on the rainy period. Duas Irmãs island (DI) is located near the coast of Sepetiba Bay and is accessible only by boats. It is surrounded by rocky reefs dominated by macroalgae, tunicate, corals and poriferans (Széchy *et al*., 2005; Fernández *et al*., 2022). The salinity can vary between 22 to 35 g L−1, also depending on the rainy periods (Fernández *et al*., 2022).

Data were obtained during monthly surveys conducted by freediving, between February 2018 and January 2019, for the collection of records on seahorse density and habitat (holdfast) use. The dive sites were selected based on information collected previously indicating the presence of seahorses in the area. During each dive, four fixed transects (marked by visual reference points) of 20 m x 4 m (320 m^2) were surveyed, following the approach of Freret-Meurer *et al*. (2018). Over the 12-month study period, this resulted in 48 transects per site (n = 96), six transect per month and area. The seahorses encountered along each transect were counted, and the characteristics of the holdfast were recorded when the seahorse was found anchored to a benthic organism or types of other holdfast (as in Fernández *et al*., 2022). Concurrently with the surveys, we conducted monthly sampling of three random quadrats (50 cm x 50 cm) at a maximum depth of 7 m (Freret-Meurer *et al*., 2018). This was done to evaluate the availability of holdfasts for seahorse anchoring, aligning the data with the percent cover in the rocky reefs (n = 36 quadrats per site). The cover of benthic organisms within each quadrat was estimated visually in the field, with all the holdfasts within a quadrant equaling 100% (McKenzie *et al*., 2001). Each quadrat was subdivided into one hundred squares to

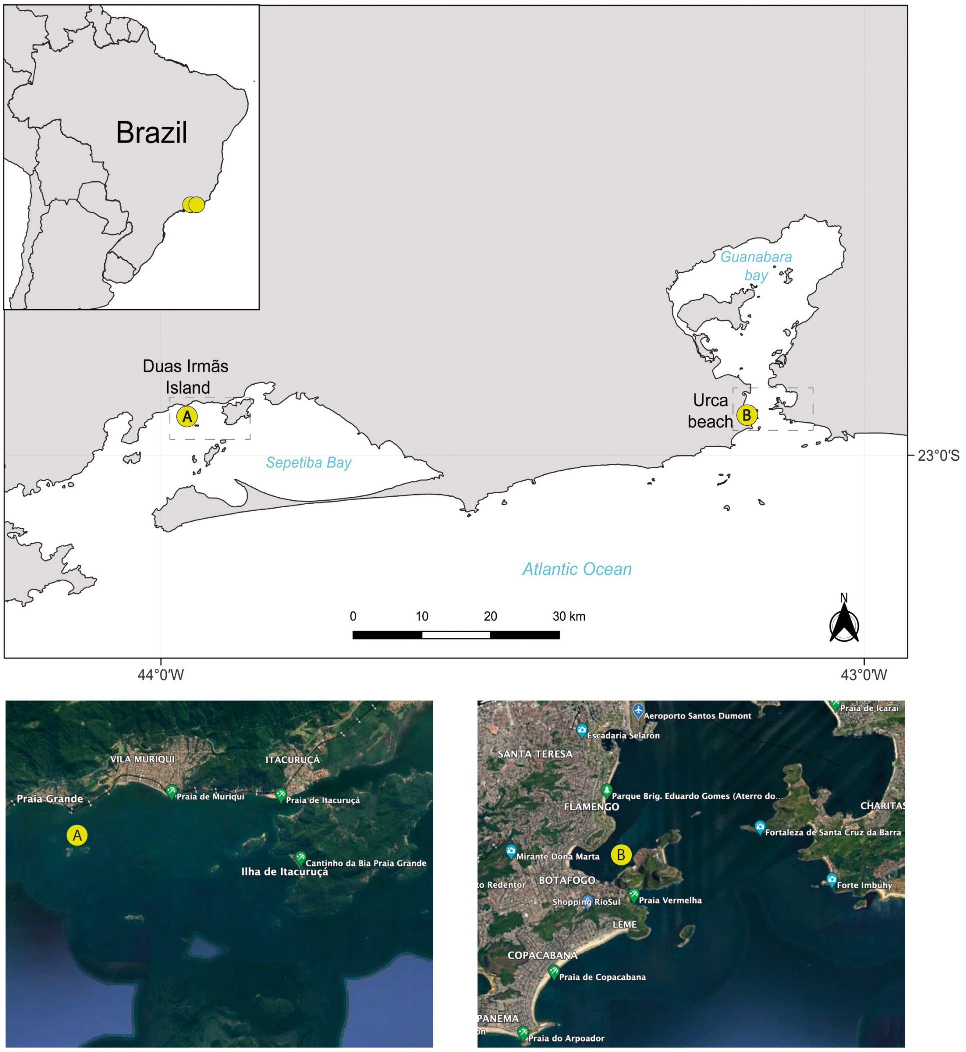

FIGURE 1 | Location of the two study sites at which *Hippocampus reidi* was surveyed in 2018–2019 within two coastal bays in Rio de Janeiro, southeastern Brazil: A = Duas Irmãs island in Sepetiba bay; B = Urca beach in Guanabara Bay.

ensure the accuracy of the count. We counted the most prevalent organism within each 5 cm square, and rare species found within the quadrat were also considered, following the method outlined by Krebs (1999).

The benthic microhabitats observed within each quadrat, including holdfasts which seahorses were anchored, were identified to species level and classified according to their morphofunctional groups. The holdfast species were identified in the field following Joly (1967), Muricy, Hajdu (2006), and Wynne (2011). Tree branches, wood, and leaves were classified as allochthonous holdfasts. Human materials, such as parts of boat parts, rope, and plastic, were identified as artificial holdfasts, when detected within the quadrats or when a seahorse was anchored. The holdfasts were also classified in morphofunctional groups (Tab. **S1**), as filamentous, articulated limestone, cylindrical-corticated, foliose, massive, branching and encrusting, according to Littler *et al.* (1983), Steneck, Dethier (1994), Boury-Esnault, Rutzler (1997), Bell, Barnes (2001), and Reyes-Bonilla (2004). Bivalves and echinoderms were classified as massive. Further information on the seahorse's density in the study areas is available in Fernández *et al*. (2022). The temperature and salinity of the water were measured at the water surface at each site during each monthly survey with a mercury thermometer and a refractometer, respectively. Monthly rainfall data (in mm) were obtained from the Instituto Nacional de Meteorologia (INMET) to determine the dry (April to September) and rainy (October to March) seasons as previously classified by Fernandez *et al*. (2022). All the seahorses were identified individually by photo identification (see Freret-Meurer *et al*., 2013), to avoid recounting an individual during the surveys.

Statistical analyses. The data on seahorse density, holdfast utilization, and water conditions (temperature and salinity), available in Fernández *et al*. (2022), were analyzed to examine their potential relationship with the availability of holdfasts. The percentage cover of each holdfast species was calculated by summing their abundance in the three quadrats sampled per site, multiplying the total by 100, and then dividing by 300 (the overall percentage of quadrats surveyed). This method enables us to determine the total percentage cover of each type of holdfast within each study area (McKenzie *et al*., 2001).

An ANOVA test was performed to find a number of quadrats that would show an invariability in the benthic organism community. The selectivity of *H. reidi* for benthic species and morphofunctional groups as holdfasts was analyzed using Ivlev's Electivity Index (IVLEV) and Strauss' linear selection Index (L). The Ivlev's index was calculated as $IEI = (a - b)/ (a + b)$, where a = the percentage of the seahorses using a given specie/morphofunctional group and b = the habitat area as a percentage of the total available specie/morphofunctional group (Jacobs, 1974). The value of IEI varies from -1.0 to $+1.0$, where positive values indicate preferred (*i.e.*, suitable) of habitats, negative values indicate the avoidance (*i.e.*, unsuitable) of habitats, and zero indicates no preference (Ivlev, 1961). The formula for Strauss' linear selection index is $L = ri-pi$, where ri = the proportion of the holdfast use and pi = the proportion of the holdfast available in the environment (Strauss, 1979). The diversity of the holdfast species and morphofunctional groups was also calculated using the Shannon-Wiener diversity index (H') (Shannon, 1948) with the formula: $H' = -\sum pi * \ln pi$, where $pi = ni/N$, N = abundance of organisms; *ni* = abundance of organisms of the species *i*; ln = neperian base logarithm (e).

Spearman correlations coefficients were used to assess the relationship between the density of seahorses and diversity of both holdfast species and morphofunctional groups, with the formula: $r=1-6\sum(n-1)$, where *r* is the coefficient and *n* is the number of points in the data set. For each point (*x*,), the square of the difference in the ranks of the two coordinates is represented by *d*, and the sum of each of these squares is represented by the expression *d*.

A non-metric multidimensional scaling (NMDS) analysis was used to evaluate the two study areas (Urca and Duas Irmãs): seasons (rainy and dry), occurrence of both benthic holdfast species (arcsine square-root transformed data) and classes of habitat morphofunctional groups (arcsine square-root transformed data). The NMDS was based on the Jaccard distance measure. The NMDS is an ordination method based on ranked distances, which arranges samples in low-dimensional space reflecting the similarity of the ranking among the different groups (Clarke, 1993). The adequacy of the NMDS was assessed based on stress values, where values of less than 0.2 are considered adequate for evaluation, whereas values of over 0.2 require examination at higher levels to avoid misinterpretation (Clarke, Warwick, 2001).

A Principal Component Analysis (PCA) was used to ordinate the variation in the availability of the different holdfast morphofunctional groups (arcsine square root transformed) between the two study sites, using function *rda* in the *vegan* package (Oksanen *et al*., 2016). The PCA is an ordination ordinate approach that preserves the Euclidean distance among sites in the form of eigenvectors (Borcard *et al*., 2011). The number of significant axes was estimated based on the broken-stick criterion, available in the function PCAsignificance function of the BiodiversityR package (Kindt, 2018). The broken-stick criterion provides an accurate estimate of the dimensionality of the data, by retaining only the components of the PCA that have eigenvalues greater than those given by a null model (Jackson, 1993). A distance-based Permutational Multivariable Analysis of Variance (PERMANOVA) was used to assess the variation among sites, periods, and the coverage of species and morphofunctional groups, as well as, for the relationship between *H. reidi* (abundance and density) and abiotic variables (temperature, salinity, and rainfall). We also tested the relationship the between seahorse density and the morphofunctional groups with the greatest eigenvectors values in the PCA (*i.e*., branching, cylindrical corticated, foliose, limestone, and massive groups) to better evaluate the differences between the two sites (regarded as a random factor with two levels) using the *adonis2* function in the *vegan* package. The PERMANOVA was based on Bray-Curtis dissimilarity measures and 1,000 permutations of the residuals (Anderson, 2001). All the statistical analyses were run in the R software, v. 4.2.1 (R Development Core Team 2022).

RESULTS

A total of 66 seahorses were detected at Urca beach, with a monthly mean of 6 ± 1 individuals/month, while 52 individuals were found at Duas Irmãs Island (mean = 4 ± 2 individuals/month). The ANOVA test showed no differences between the quadrats at each site, indicating that the three random quadrats were sufficient to perform the aim present in this paper ($F_{urca} = 0.32$, $p = 0.73$; $F_{duas\ irmãs} = 1.31$, $p = 0.46$).

Turf algae were the most organisms recorded at both sites (U = 33%, DI = 22%; Tab. **S2**). The seahorses were also observed frequently associated to the ascidian *Styela plicata* (Leseuer, 1823) at Urca beach (19%), and the octocoral *Carijoa riisei* (Duchassaing and Michelotti 1860) at Duas Irmãs island (48%) (Tab. **S3**). Regarding morphofunctional groups, filamentous was the most available at both sites (U = 35%; DI = 29%; Tab. **S4**). The group used most by the seahorses was the branching type (U = 42% and DI = 48%; Tab. **S5**).

Ivlev's Electivity Index (IEI) and Strauss' index (L) showed that seahorses at Urca preferred allochthonous and artificial holdfasts, the bryozoan *Amathia vercilata* (Harvey 1833), the ascidian *Botrylloides nigrum* Herdman, 1886, *Clavelina oblonga* Herdman, 1880, *Styela plicata*, rocks, the macroalga *Codium* sp., polychaete tubes, the poriferan *Hymeniacidon heliophila* (Wilson, 1911) (Tab. 1) and the cylindrical-chordate, massive, and branching morphofunctional groups (Tab. 2). At Duas Irmãs, the preference was for the cnidarian *Carijoa riisei* and the alga *Acanthophora* sp. (Tab. 1), while the branching holdfasts were the preferred morphofunctional group (Tab. 2).

The Shannon-Wiener index (H') diversity of holdfast species at Urca beach was 2.21 and at Duas Irmãs Island was 2.10 and, the diversity of the morphofunctional groups at Urca was 1.69 and at Duas Irmãs island was 1.54 (Fig. 2). The diversity of holdfast species and morphofunctional groups did not correlate significantly with the density of seahorses at Urca beach (rspecies = 0.05; *p*species = 0.87; rmorphofunctional groups = 0.27; *p*morphofunctional groups = 0.39) netheir at Duas irmãs ($r_{species} = 0.47$; $p_{species} = 0.13$; $r_{morphofunctional\ groups} = 0.12$; $p_{morphofunctional\ groups} = 0.72$).

TABLE 1 | Electivity Index (Ivlev) and Strauss' Linear Selection Index for holdfast species associated with seahorses at Urca beach and Duas Irmãs island. Values > 0 (seahorses preferred the holdfast). Preference values are in bold type.

Species	Urca beach IVLEV	Urca Strauss	Duas Irmãs island IVLEV	Duas Irmãs island Strauss
Acanthophora sp.	-		**0.23**	**1.43**
Allochthonous holdfast	**0.97**	**12.71**	-	-
Amathia vercilata (Harvey, 1833)	**0.49**	**3.17**	-0.08	-0.68
Artificial holdfast	**1**	**6.45**		
Botrylloides nigrum (Herdman, 1886)	**0.05**	**0.14**	-	-
Carijoa riisei (Duchassaing & Michelotti, 1860)	-	-	**1**	**48.08**
Clavelina oblonga (Herdman, 1880)	**0.30**	**1.48**	-	-
Codium sp.	**0.95**	**12.60**	-	-
Hymeniacidon heliophila (Wilson, 1911)	**0**	-0.02	-	-
Hypnea sp.	-	-	-0.05	-1.44
Limestone seaweed	-	-	-0.52	-12.54
Mycale sp.	-0.27	-1.22	-	-
Plocamium sp.	-	-	-0.53	-13.09
Polychaeta	**0.93**	**4.67**	-	-
Rocks	**0.22**	**2.89**	-	-
Styela plicata (Leseuer, 1823)	**0.91**	**18.46**	-	-
Turf algae	-0.30	-15.29	-0.06	-2.60

TABLE 2 | Electivity Index (Ivlev) and Strauss' Linear Selection Index for holdfast morphofunctional groups associated with seahorses at Urca beach and Duas Irmãs island. Values > 0 (seahorses preferred the holdfast). Preference values are in bold type.

Morphofuncional group	Urca beach IVLEV	Urca beach Strauss	Duas Irmãs island IVLEV	Duas Irmãs island Strauss
Branching/Arborescent	**0.72**	**33.19**	**0.98**	**47.52**
Articulated calcareous	-	-	-0.20	-6.73
Cylindrical-chordate	**0.95**	**10.69**	-0.58	-15.87
Filamentous	-0.22	-12.69	-0.03	-1.85
Incrusting	-0.22	-5.03		-19.06
Massive	**0.03**	**1.25**	-	

The NMDS (Fig. 3) revealed differences in the coverage of benthic species between sites, with a stress of 0.17 (*adonis2* F = 9.71, p = 0.001). While the morphofunctional groups overlapped between the sites, the benthic coverage was also significantly different, but apparently the two sites also shared a varied number of groups, with stress = 0.12 (*adonis2* F = 11.69, p = 0.001). Neither holdfast species (*adonis2* F = 1.07; p = 0.4) nor the morphofunctional groups (*adonis2* F= 0.66; p = 0.5) varied significantly between seasons.

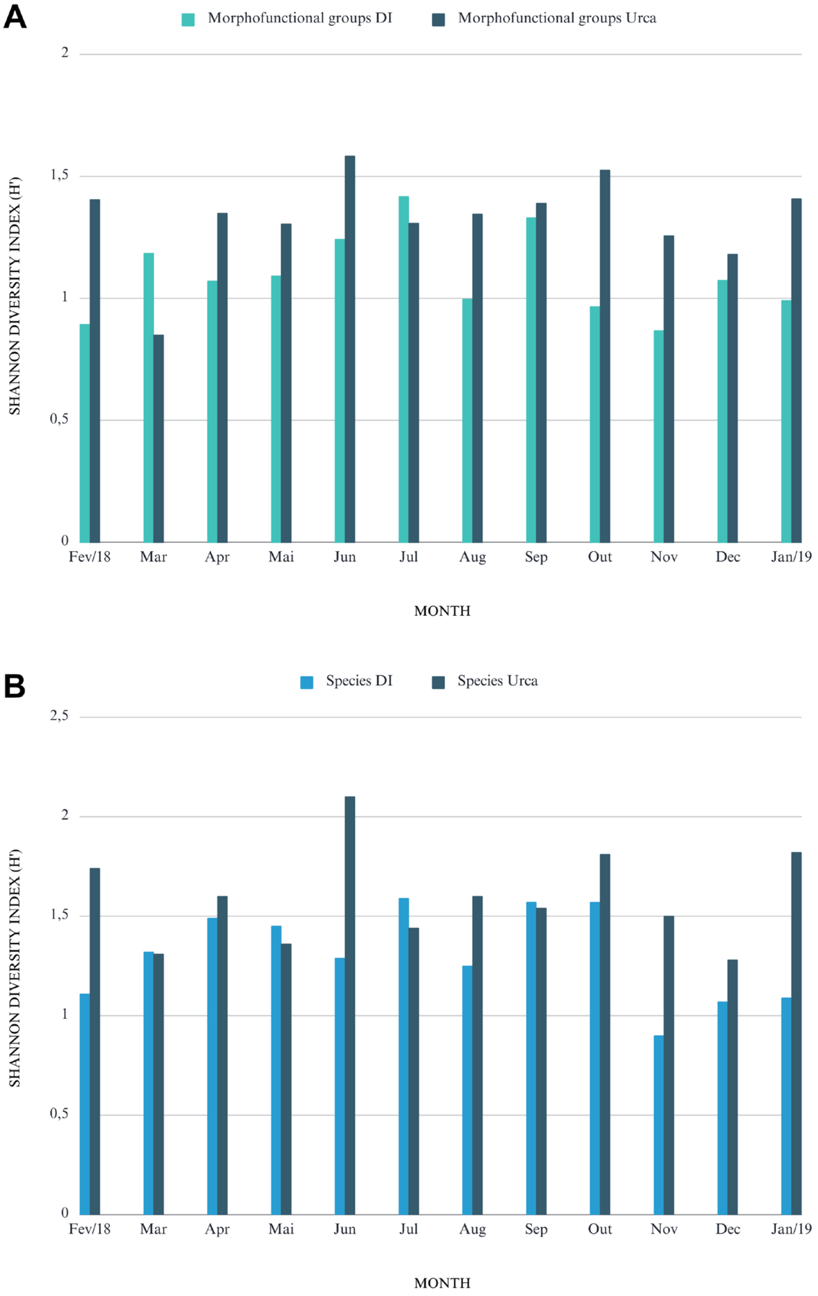

FIGURE 2 | Shannon-Wiener indices for the diversity of species and morphofunctional groups recorded at Urca beach (**A**) and Duas Irmãs Island (**B**) between February 2018 and January 2019.

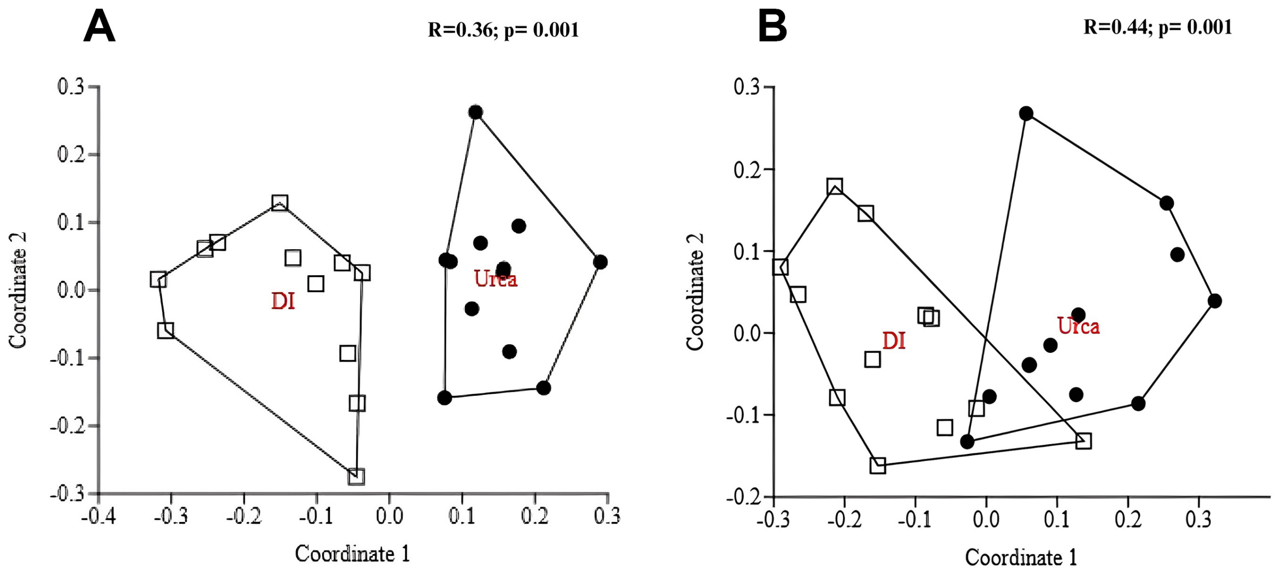

FIGURE 3 | Two-dimensional ordination plots of Non-Metric Multidimensional Scaling (NMDS) for the availability of holdfast species availability (**A**) and morphofunctional habitat groups (**B**). The circles correspond to Urca beach and the squares to the Duas Irmãs island.

The first two axes of the Principal Component Analysis (eigenvalues = 3.45 and 1.43) jointly explained 81.4% of the variance of the morphofunctional groups and were significant, based on the broken-stick criterion (Fig. 4). The first axis explained 57.6% of the variance and separated the two sites based on their local availability of morphofunctional groups. Samples from Duas Irmãs island were mainly related to a greater occurrence of foliose, branching and massive. By contrast, the Urca beach was mostly related to cylindrical corticated, encrusting, and limestone. The filamentous group was more associated with PCA 2 (23.9%) and was more associated with variations within each system rather than between systems.

The distance-based PERMANOVA indicated that the density of *H. reidi* was related to the presence of branching holdfasts (*adonis2* function: F= 3.34; p = 0.049). Relations with the frequency of foliaceous (*adonis2* function: F= 8.88; p = 0.007) and massive (*adonis2* function: F = 3.82; p = 0.049) holdfasts, although, they were found almost exclusively at Urca beach. However, the abiotic variables did not indicate relation with seahorse densities (*adonis2* function: F = 2.18; p = 0.17).

Precipitation exhibited a statistically significant increase at Urca Beach compared to Duas Irmãs (F = 18.236, p = 0.0004), a pattern consistent across both the rainy and dry seasons for both sites (F = 24.1049, p = 0.0001). Nevertheless, there was no noteworthy interaction between these variables (F = 0.558, p = 0.476). In terms of temperature, both locations experienced a significant elevation during the rainy season (F = 4.134, p = 0.027), while salinity showed a higher concentration in the dry season (F = 5.067, p = 0.038). Notably, no substantial differences were observed between the two beaches for temperature (F = 1.468, p = 0.275) or salinity (F = 1.892, p = 0.179). Similarly, there was no significant interaction between beach and period for these variables (F = 1.164, p = 0.332; F = 0.286, p = 0.591, respectively).

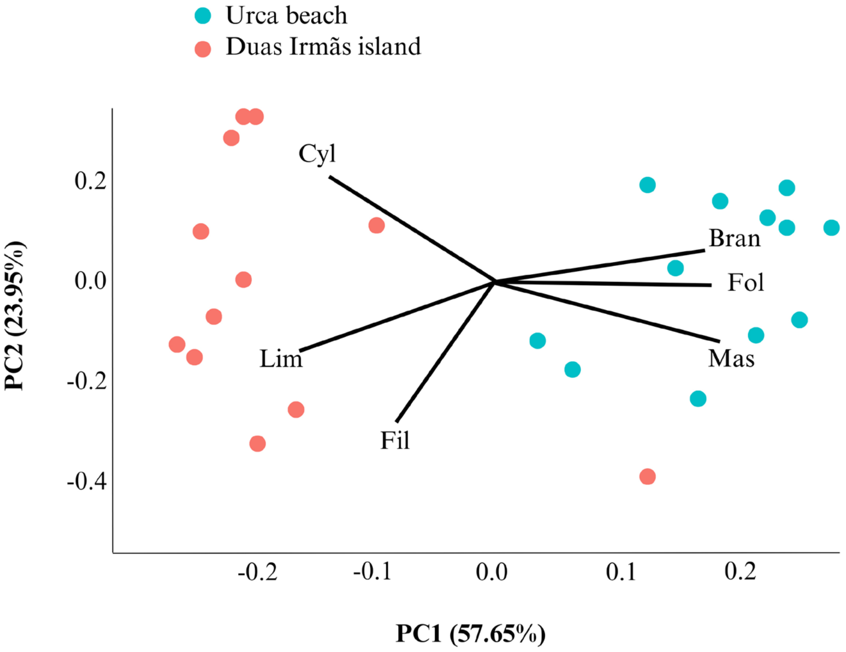

FIGURE 4 | Plot of the Principal Components Analysis (PCA) of the morphofunctional groups at the two sites. All the variables were centered and standardized. Fil = Filamentous, Mas = Massive, Cyl = Cylindriccorticated, Fol = Foliose, Bran = Branching/Arborescent and Lim = Articulated limestone.

DISCUSSION

This present study is the first to describe the preference of *H. reidi* for specific morphofunctional groups during their selection of holdfast. In particular, the results indicated that this seahorse prefers physically complex over simple microhabitats. The seahorses preferred vertical structures as anchorages, especially filamentous and branching organisms, such as macroalgae and cnidarian species. The holdfast species and morphofuncional groups did not correlate with the seahorse density at either site. However, branching holdfasts and seahorses densities were correlated significantly at both Urca beach and Duas Irmãs island.

The decline in seahorse populations has been attributed to alterations in habitat availability (Correia *et al.*, 2015; Harasti, 2016; Correia, 2022). Additionally, there is a positive correlation between habitat availability and the density of *Hippocampus guttulatus* Cuvier, 1829, irrespective of the specific nature of the available habitat (Correia *et al.*, 2015; Correia, 2022). While habitat availability appears to be correlated with seahorses, our findings did not demonstrate a direct relationship between the density of *H. reidi* and the holdfast species. The present study also demonstrated the importance of the branching holdfasts for *H. reidi* at both study sites, although holdfast diversity did not have an influence on the seahorse population.

Holdfast selection by seahorses is highly correlated to the availability of habitats (Curtis, Vincent, 2006; Rosa *et al.*, 2007). In Urca, benthic turf algae were more abundant during the current study period, contrasting with the findings of Taouil, Yoneshigue-Valentin (2002), who did not report the presence of this algae in the area. Chlorophyta and Rhodophyta were abundant in 2002. The present study showed that *H. reidi* preferred using turf algae and *Codium* sp., as reported by Rosa *et al*. (2007) and contrasts with the *H. reidi* population of northern Brazil, where these seahorses are often found in association with mangrove roots, ascidian and the Chlorophyta *Caulerpa racemosa* (Forsskål) J. Agardh, 1873, in the mangrove ecosystem (Dias, Rosa, 2003). These data highlight the importance of the availability of macroalgae and turf algae for *H. reidi,* probably because of the availability of refuges and phytal fauna for feeding. Moreover, they could utilize these types of structures due to their higher abundance in the area noted in this study. Seahorses at Urca beach have also been found in allochthonous holdfasts, as reported in other studies (Dias, Rosa, 2003; Curtis *et al*., 2004; Rosa *et al*., 2007; Clynick, 2008; Correia *et al*., 2015; Claassens *et al*., 2018; Simpson *et al*., 2020; Fernández *et al*., 2022). The use of these holdfasts suggests that the seahorses are also capable of using other types of holdfasts and may thus adapt to natural or anthropogenic changes in benthic habitats (Clynick, 2008).

The longsnout seahorse (*H. reidi*) at Duas Irmãs island preferentially selected the octocoral *C. riisei*, also observed by Freret-Meurer *et al*. (2018) at Guaiba Island. This cnidarian was not recorded in our benthic cover samples because it typically grows on vertical structures and holdfast, which were not sampled by the quadrats, possibly leading to an underestimate of the availability of *C. riisei.* Pádua *et al*. (2022) identified this octocoral as an ecosystem engineer in coastal reefs, where it may act as a refuge and hold diverse associated fauna, which may be attractive to seahorses. The predation tends to decrease in more complex habitats for fish (Choat, 1982), although habitat complexity in itself may not influence the foraging success of Syngnathidae species (Curtis, Vincent, 2005). Seahorses are small, vertically oriented ambush predators, which may nevertheless be favored by physically complex habitats.

In the present study, the morphofunctional groups selected by the seahorses were the branching and filamentous holdfasts, which are physically more complex and vertically oriented, as reported for *H. gutullatus* at Italy (Lazic *et al*., 2018). Vertically anchorage points can facilitate the camouflage of cryptic fish; as seahorses (Foster, Vincent, 2004). There may also be a relationship with behavior, as in the case of *Hippocampus erectus* (Perry, 1810), which prefers vertical holdfasts as a support for foraging (James, Heck, 1994), a phenomenon that requires attention in future studies.

Holdfast architecture may also be associated with seahorse abundance, where the more complex (3-D structures and shapes) the habitat, the greater the abundance of some seahorses' species (Gristina *et al*., 2014, 2017), a pattern also observed in the present study, specifically, the correlation of branching holdfasts with seahorses density. Also, in disturbed habitats, are expected low biomass of microhabitats and a predominance of foliose and filamentous species, which showed in both areas in this study (Murray *et al*., 2006).

Specific studies on the coverage of benthic species provides important data on the availability of holdfast and preference of the seahorses (Curtis, Vincent 2005; Ape *et al*., 2019). Nevertheless, evaluating the capability of morphofunctional groups as indicators for seahorse occurrence and abundance has demonstrated its utility. This is attributed to its enhanced insight into structural complexity, surpassing the mere assessment of habitat composition. The overlap observed in morphofunctional groups implies that structures with akin morphological and complexity features may exhibit comparable ecological roles or functions, irrespective of their species distinctions. This suggests that the morphofunctional approach holds value in scenarios where prioritizing an understanding of ecological roles takes precedence over intricate taxonomic differentiation. The present study also highlights the importance of surveys of habitat loss and the management, for seahorse conservation. Caldeira *et al*. (2017) reported a reduction of the biomass of the macroalgae community in Sepetiba bay, which may be affecting the physiognomy of the community, and this process is being repeated all around the world (Airoldi *et al*., 2008).

From this perspective, the seahorse population at Urca beach and Duas Irmãs island tend to select holdfasts with a vertical profile, as filamentous and branching. While the seahorses at Sepetiba bay preferred using the octoral *C. riisei* and allochthonous holdfasts at Guanabara Bay. The morphofunctional approach could determine seahorse's preference for holdfast and was able to split this preference among different regions, primarily to assist in microhabitat evaluations. Also, the morphofunctional groups approach appears to offer a valuable alternative, especially when taxonomic resolution is difficult. It may not replace the species-focused approach entirely, but it provides a complementary perspective that can be particularly useful in certain contexts, such as ecological studies or conservation management where fine-scale taxonomic information might be elusive. The choice between the two approaches should be guided by the specific objectives of the research and the practical constraints involved.

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AUTHORS’ CONTRIBUTION

Tatiane do Carmo Fernández: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing-original draft, Writing-review and editing.

Natalie Villar Freret-Meurer: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Supervision, Visualization, Writing-review and editing.

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Luciano Neves dos Santos: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing-original draft.

ETHICAL STATEMENT

This study was performed with a permission issued by the Instituto Chico Mendes de Conservação em Biodiversidade (ICMBio) through environmental license number 44409–3.

COMPETING INTERESTS

The author declares no competing interests.

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