



Predation success of declining long-snouted seahorse (*Hippocampus guttulatus*) population under habitat shifts

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ABSTRACT

Seahorse populations are facing significant declines worldwide, often associated to illegal trade and habitat loss or fragmentation. Emergent habitat shifts towards invasive seaweeds, facilitated by global warming, can also be at play, having still unknown effects on seahorse ecology. Particularly, indirect impacts on seahorse populations via altered predation success remains largely unexplored. Here, we conducted mesocosm experiments to investigate differences in the prey capture success of the long-snouted seahorse (*Hippocampus guttulatus*) among native habitats (i.e. seagrass meadows and unvegetated sediments) and non-native *Caulerpa prolifera* meadows when offered different preys (amphipods and shrimps), at low or high prey availability, and under low or high habitat complexity. Great plasticity of *H. guttulatus* to all habitats was revealed, as habitat type did not significantly affect the capture success. Instead, capture success depends on prey identity and availability regardless of the habitat type, as well as on habitat complexity. Seahorse capture success was higher under high prey availability, as well as when predating on amphipods that were offered together with shrimps. High habitat complexity of both vegetated habitats enhanced by double the capture success, whereas an opposite effect of similar magnitude was found for more complex unvegetated habitats. This indicates that vegetation complexity, regardless their native or invasive nature, reduces prey awareness to predator, whereas small structures in unvegetated habitats provide refuge for preys but not seahorse camouflage. We found no evidence that shifts from native habitats to invasive *C. prolifera* negatively impact *H. guttulatus* capture success, but further research on other aspects of its feeding ecology is still necessary.

1. Introduction

Habitat shifts are major threats to terrestrial and marine biodiversity and to the survival of many species (Pimm and Raven, 2000; Powers and Jetz, 2019; Sala et al., 2000). These events can modify the habitat structural complexity and the environmental context in which other species interact (Bartlett et al., 2016; Ewers and Didham, 2006; Tylianakis et al., 2007). Particularly, the loss or fragmentation of highly complex habitats such as seagrass meadows and their shift towards less complex unvegetated habitats, with reduced habitable structures, are known to alter macroinvertebrate communities, as well as the fish communities that predate on them (McCloskey and Unsworth, 2015; Reed and Hovel, 2006). On the other hand, shifts towards habitats dominated by invaders have been increasingly reported in temperate systems (Holmer et al., 2009; Tuya et al., 2013; Williams and Smith, 2007), as global warming facilitates the invasion of tropical seaweeds

(Atkinson et al., 2020; Brodie et al., 2014; Straub et al., 2019). By rapidly changing the native habitat structural complexity to monospecific mats, invasive seaweeds can also alter the native faunal communities (Gallardo et al., 2016).

Seahorses inhabit shallow coastal areas worldwide, with temperate species predominantly inhabiting seagrass meadows (Foster and Vincent, 2004). They have sparse distribution, low mobility, small home ranges, low fecundity, lengthy parental care and mate fidelity (Foster and Vincent, 2004). Such unique traits make them extremely prone to population declines and vulnerable to extinctions, particularly when facing illegal fishing and trade for the Traditional Chinese Medicine (Vincent, 1996), as well as habitat degradation and loss (Harasti, 2016). They are currently included in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES; Morton et al., 2022) and in the 2023 IUCN red list of Threatened Species where, out of 42 seahorse species listed, 12 are Vulnerable, 2 Endangered, 10 Least

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Concern, 17 Data Deficient and 1 Near Threatened (IUCN, 2024). Both European seahorse species, the long-snouted seahorse (*Hippocampus guttulatus*) and the short-snouted seahorse (*Hippocampus hippocampus*), are considered Data Deficient (IUCN, 2024 red list), except in the Mediterranean Sea, where they are considered Near Threatened (Pollom, 2016a, 2016b).

In the Ria Formosa lagoon (southern Portugal), seahorses have long been iconic species, particularly after Curtis and Vincent (2005) unraveled that this system contained the world largest population of the long-snouted seahorse. Unfortunately, such population suffered a 90 % decline after 20 years of drastic fluctuations (Caldwell and Vincent, 2012; Correia, 2022; Correia et al., 2015). Reports of illegal fishing in the lagoon targeting the seahorse trade, as reviewed by Correia (2022), have been identified as the most plausible cause for this decline. In 2001–2002, the long-snouted seahorse was reported as associated to more complex habitats of the lagoon, namely habitats with higher cover of seagrasses, seaweeds and sessile invertebrates (Curtis and Vincent, 2005). In addition to the illegal fishing and trade, the species decline was also suggested as related to a reduced coverage of fixing structures or holdfasts on which seahorses grasp their prehensile tails (Correia et al., 2015). More recently, an unprecedented and aggressive spread of the green seaweed *Caulerpa prolifera*, caused the takeover of the subtidal unvegetated bottoms, outcompeting native seagrass meadows in shallower subtidal areas. *C. prolifera* spread is having large impacts on the support of biodiversity and nursery services by the lagoon, also causing a reduction in tanaid preys for seahorses when native unvegetated habitats are colonized by *C. prolifera* (Parreira et al., 2021). However, indirect consequences on seahorse populations dynamics via altered presence or abundance of target preys, as well as modified seahorse predation success, remain largely unknown. Overall, *C. prolifera* spread together with the broad decline of seagrass meadows in the lagoon, are raising major concerns regarding the persistence of the already declining seahorse populations subjected to habitat loss and shifts (Correia, 2022; Parreira et al., 2021).

Seahorse species have a prehensile tail used to grasp natural holdfasts, as well as excellent camouflage capabilities used to capture preys and avoid predators (review by Foster and Vincent, 2004). They have a powerful suction feeding mechanism (Avidan et al., 2023), used not only to engulf small crustaceans such as copepods and amphipods, but also to dismantle larger preys, such as caridean shrimps and pagurids (Ape et al., 2019; Felício et al., 2006; Woods, 2002). Most seahorse species are diurnal predators, but still able to locate prey at low light intensities (Felício et al., 2006; James and Heck, 1994). Seahorses are ambush predators, which may actively select seagrass habitats that provide them camouflage, thus reducing prey awareness and improving their predation success (Flynn and Ritz, 1999). In artificial seagrass habitats, firstly *Hippocampus erectus* and later *Hippocampus reidi*, have been experimentally reported to use a “sit-and-wait” foraging strategy, while actively pursue their prey in areas with little to no vegetation (Felício et al., 2006; James and Heck, 1994). Despite such general knowledge and such few studies examining seahorse predation success under reduced density and loss of artificial seagrass, the specific foraging behavior and predation success of *H. guttulatus* remains largely unknown. Furthermore, the effect of habitat shifts towards unvegetated sediments on seahorse predation success with different levels of structural complexity or towards habitats dominated by an invader seaweed remains largely unexplored. This knowledge is particularly relevant for guiding successful management practices and conservation actions to prevent the collapse of declining *H. guttulatus* populations.

Here, we experimentally investigated the effects of habitat shifts on the predation success of the seahorse *H. guttulatus*. Specifically, we investigated the independent and interactive effect of habitat type with the effect of three factors that are intrinsically related to habitat shifts: prey identity, prey availability and habitat complexity. By considering the habitat shifts currently occurring at Ria Formosa, namely the invasion by *C. prolifera* and subsequent decrease of both native unvegetated

and seagrass habitats, we particularly elucidate the potential consequences of invaders on endangered seahorse populations of this lagoon.

2. Materials and methods

2.1. Specimens' collection

In this study, we considered the three subtidal habitat types currently present at Ria Formosa lagoon (Faro, Portugal): the native subtidal seagrass meadows (‘Seagrass’: *Cymodocea nodosa* and *Zostera marina*), the native unvegetated sediments (‘Sediment’) and - the invasive *C. prolifera* meadows (‘Caulerpa’). Seagrasses were collected at the water receiving tank of the Aquaculture Pilot Station of Olhão (EPP0), to avoid damaging natural meadows, whereas *C. prolifera* fronds with rhizoids, as well as oyster shells and pebbles for the unvegetated habitat, were collected near the Ramalhete Research Station of the Centre of Marine Sciences (CCMAR).

A total of 60 adult *H. guttulatus* (30 males and 30 females) were carefully hand collected by scuba divers from the lagoon, on June 28th of 2023. They were transported by boat to the Ramalhete station, where the experiments took place. All fish were adult individuals of similar size to avoid potential feeding variation associated to seahorse ontogeny, with mean length (L) of 16.4 ± 1.5 cm ($L_{\text{males}} = 16.8 \pm 1.4$ cm and $L_{\text{females}} = 16 \pm 1.4$ cm) and mean weight of 9.6 ± 2.9 g (male weight = 11.1 ± 2.8 g and female weight = 8.1 ± 2.1 g) (see biometrics in Fig. S1). To minimize stress, seahorse total length was measured at the beginning of the experiments according to Pritchard (1993) by summing the seahorse height (distance between the coronet and the tip of the tail) to the head length.

Two prey types of contrasting size and accessibility to predators were selected: amphipods (*Cymadusa filosa* and *Gammarus insensibilis* mix at ca. 50/50) and caridean Atlantic ditch shrimps (*Palaemon varians*). Both prey are commonly predated by *H. guttulatus* (Ape et al., 2019; Valladares et al., 2017) and by other seahorse species (Castro et al., 2008; Woods, 2002), as well as regularly found in all three habitats covered in this study (Parreira et al., 2021). Preys were collected near the Ramalhete station and carefully sorted by species and size (1–1.5 cm for amphipods and 2.5–2.9 cm for shrimps) to ensure experimental consistency.

2.2. Conditions for seahorse maintenance

Prior to the experiments, fish were kept for one week in a stock tank of 250 L (100 cm length, 60 cm width, and 54 cm high) with artificial holdfasts as described by Correia et al. (2013) and daily fed *ad libitum* with live mysids (*Mesopodopsis slabberi*). Experiments were conducted in an outdoor flow-through mesocosm system with 48 circular experimental tanks (treatment and control) of 26 L (26 cm in diameter and 45.5 cm in height), all with the same amount of sediment (3 Kg). We chose circular experimental tanks with enough height to allow vegetation to extend within the water column providing the tridimensional structure that form seahorse habitats, and to allow seahorse mobility in all directions. Seahorse fitness (i.e. lack of stress reflected by a normal behavior) and the suitability of experimental tanks and conditions were tested prior to the experiments. Such preliminary tests revealed signs of stress (i.e. high opercular beat, constant movement and no feeding) when *H. guttulatus* individuals were isolated, whereas a normal behavior was observed after a few hours of acclimation to experimental condition when they were in pairs (1 male and 1 female).

Stock and experimental tanks were kept in the same semi-closed circulation system receiving seawater from a reservoir tank, which was fed with seawater from the lagoon that passed by two mechanical filter systems (sand and cotton bags) to prevent the input of small organisms. Dissolved oxygen was kept at saturation levels using aeration in the reservoir, stock and experimental tanks. Seawater temperature (mean \pm standard deviation: 21.0 ± 1.5 °C) and salinity (38.3 ± 0.3) in

the stock and experimental tanks were regularly monitored using a multiparametric sonde (VWR® pHenomenal® OX 4110 H). Seawater temperature was controlled using a water refrigerator (I-Komfort RC 700–25 m³), since fish are sensitive to large thermal oscillations due to water residence time in the station’s tubing. To prevent prey escape from stock and experimental tanks, a 500 µm mesh size outflow filter and a 1 cm mesh size top cover were used.

2.3. Experimental design

Experiments were conducted in July 2023 during four weeks, running one 24h experiment per week. Prior to each experiment, fish were starved for 24h, of which 12h were in the stock tank and 12h were acclimation in the treatment tanks. For each 24h experiment, the introduction of prey in the experimental tanks marked the beginning of the experiment and the removal of fish back to the stock tank determined the end. After each 24h experiment, fish were allowed to remain undisturbed in the stock tank for the remaining 5 days of the week until the start of the next experiment. Four mesocosm experiments (Fig. 1) were set up to examine the effect of habitat type in interaction with three independent factors (i.e. prey identity, prey availability, and habitat complexity) on the prey capture success by *H. guttulatus*. Each independent factor was investigated in a separate experiment with two levels per factor, as detailed in the corresponding sections below.

The habitat types were recreated using the mean density of vegetation (i.e. ‘Caulerpa’ and ‘Seagrass’ habitats) or of non-living structural

components (i.e. ‘Sediment’ habitat) measured in Ria Formosa (Table S1). In the case of the habitat complexity experiment we used half and double of such mean natural densities. Vegetation density, expressed as the number of seagrass shoots (i.e. *C. nodosa* and *Z. marina*) or of *C. prolifera* fronds per square meter, was measured in three replicate samples per site and in three replicate sites per habitat type (n = 9). Each sample consisted in a fine mesh bag (<0.5 mm) affixed to a flexible 30 cm diameter hoop following the method by Martínez-Crego et al. (2021). Three unvegetated sites were also visited to estimate the mean density of the main non-living structural constituents identified. This resulted in 15 *C. nodosa* shoots and 6 *Z. marina* shoots per tank for the ‘Seagrass’ habitat, 67 *C. prolifera* fronds per tank for the ‘Caulerpa’ habitat and in 4 pebbles and 4 oyster shells per tank for the ‘Sediment’ habitat.

Prey amount offered in the experiments was determined by seahorse daily food requirements, expressed as the percentage of the mean fish bodyweight, following a commonly used procedure as reported elsewhere (e.g. Costa et al., 2023; Palma et al., 2011). To relate the prey count to biomass, prior to the experiments, prey individuals already sorted by sizes (see section above), were measured and weighted to obtain the weight-number of prey relationship for each prey type. A reference value of 4% the average fish fresh bodyweight (resulting in 8 amphipods or in 3 shrimps) was chosen for daily consumption (i.e. prey identity and habitat complexity experiments). In the case of the prey availability experiment we used half and double of such reference value (2 and 9 shrimps for low and high availability, respectively).

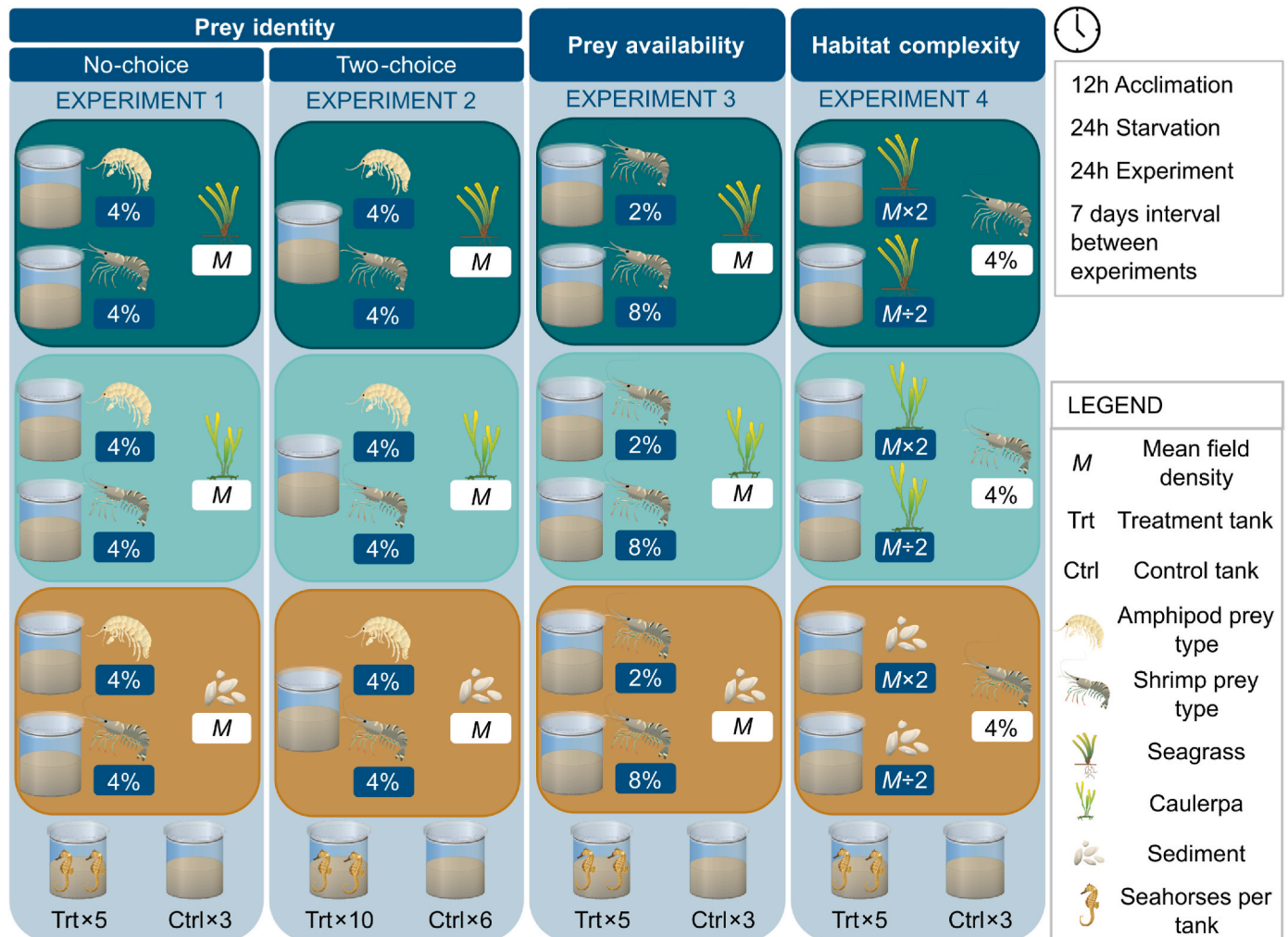


Fig. 1. Schematic representation of the experimental design.

Five replicate treatment tanks and three replicate control tanks were used per factor level within each habitat type. Tanks were distributed using a random block design, in which two replicate treatments and one control were arranged in each block to maximize similarity in their experimental conditions. Seahorse individuals were distributed in the treatment tanks including 1 male and 1 female per treatment tank x 5 replicate treatment tanks x 3 habitat types x 2 independent factor levels, summing a total of 60 seahorse individuals. The control tanks contained no seahorses, allowing to account for non-predatory changes in prey numbers during the experiment (e.g. mortality, cannibalism). At the end of each experiment, after fish removal, all remaining preys were counted and identified by carefully disassembling every tank and meticulously inspecting all structural components (i.e. seagrass leaves, rhizomes and roots; seaweed fronds and rhizoids; or pebbles and shells).

2.3.1. Prey identity

To investigate the effect of habitat type (fixed factor with three levels: 'Seagrass', 'Caulerpa' and 'Sediment') in interaction with prey identity (two levels: amphipods and shrimps) on prey capture success by *H. guttulatus*, we conducted sequential (i) no-choice assays (i.e. seahorses were offered each type of prey in separate tanks) and (ii) two-choice assays (i.e. seahorses were offered a choice between the two preys in the same tank). Both preys were offered in equal amount (i.e. 4 % of the average fish fresh bodyweight as a reference for daily consumption) under mean densities of habitat structural elements (see Table S1). In the two-choice assays, since both preys were offered together in the same tank, the total number of replicate treatment tanks was 10 instead 5.

Prey capture success (CSp) was calculated per prey type and replicate treatment tank as the number of offered preys that were consumed corrected for non-predatory changes in prey numbers during the experiment in the corresponding control, as represented by Equation (1).

$$CSp = (Pi \times (Cf / Ci)) - Pf \quad (1)$$

Where Pi and Pf are the initial and final number of preys in the treatments and Ci and Cf are the initial and final number of preys in the corresponding control (i.e. control and treatment tanks within the same block), respectively (adapted from Martínez-Crego et al., 2015). Since in this experiment the initial number of preys offered was different for shrimps and amphipods, prey capture success (CSp) was expressed as proportion (PCSp) by dividing CSp by the initial preys offered corrected using the control. Thus, a proportion equal to 1 indicates that 100 % of the preys that were initially offered were captured, with slight deviations to absolute zero or 1 values due to control corrections.

2.3.2. Prey availability

To examine the effect of habitat types (fixed factor) in interaction with prey availability (fixed factor with two levels: low and high) on prey capture success by *H. guttulatus*, we conducted another experiment under mean natural densities of habitat structural elements (see Table S1) using shrimps as a single prey. Shrimps were selected based on the results of the previous prey identity experiment, which revealed a higher variation between habitats in the capture success of shrimps than of amphipods. The high and low levels of prey availability were double (8 % fish bodyweight) and half (2 % bodyweight) the above-mentioned daily reference value, respectively.

The effect of prey availability was estimated as the Enhancement ratio between the prey capture success under high (CSp_{High}) and low (CSp_{Low}) availability of preys (adapted from Erzini et al., 2022), as represented by Equation (2).

$$Enhancement\ ratio = CSp_{High} / CSp_{Low} \quad (2)$$

An Enhancement ratio >1 indicates that the capture success was enhanced when the prey availability was high, while a ratio <1 means

that the capture success decreased under high prey availability. An enhancement ratio = 1 means no difference in the capture success at high and low prey availability levels.

2.3.3. Habitat structural complexity

The last experiment investigates the effect of habitat types (fixed factor) in interaction with habitat complexity (fixed factor with two levels: low and high) on shrimp capture success by *H. guttulatus*. Shrimps were offered as the daily reference value of 4 % fish bodyweight. High and low levels of habitat structural complexity were double and half the mean natural density at each habitat type, respectively (Table S1). The effect of habitat complexity was calculated using the Enhancement ratio between prey capture success under high and low habitat complexity, as described above for prey availability.

2.4. Statistical analysis

In the two-choice assays of the prey identity experiment, since both preys were offered together in the same tank, the two levels of the factor prey identity were dependent (i.e. the consumption or capture success of one prey was dependent on the capture success of the other prey within each replicate tank). Then, for such assays, differences in the proportion of prey capture success between both preys were examined using paired t-tests. Differences between habitats were examined for each prey separately using unpaired Welch-tests, since prey were offered in independent tanks per habitat and data did not meet homogeneity of variances. All data met the parametric assumptions of normality (Shapiro-Wilk test).

In the no-choice assays of the prey identity experiment, since both preys were offered in separate tanks, the two levels of the factor prey identity ('amphipod' and 'shrimp') were independent, as also occurred in the two other experiments with the levels of prey availability and of habitat complexity. Then, univariate permutational analysis of variance (PERMANOVA) were conducted to individually analyze data from each experiment. Since all data met parametric assumptions of normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test) of the residuals, PERMANOVA tests were performed using Euclidean distances from untransformed data and used 9999 unrestricted permutations of raw data. Two-way PERMANOVA tests were used to explore differences in the response variable (PCSp or CSp) between habitats (fixed factor with three levels: 'Caulerpa', 'Seagrass' and 'Sediment') and between the other fixed factor with two levels independently considered in the experimental design (namely prey identity in no-choice assays: shrimp and amphipod, prey availability: low and high, or habitat complexity: low and high). One-way PERMANOVA tests examining differences between habitats were used for enhancement ratios. When a significant effect or interaction was detected, pairwise comparisons were performed. When the number of possible permutations was low (<100 , following Anderson et al., 2008) in any PERMANOVA test or pairwise comparison, the Monte Carlo p-value was considered instead of the permutation p-value.

A 95 % confidence interval (significant p-values <0.05) was considered in all experiments. PERMANOVA tests were performed using Primer v6 with Permanova add on, while all other data analyses and all plots were performed using R Statistical Software (v4.3.2; R Core Team, 2024).

2.5. Foraging behavior observations

To avoid disturbing the fish during the experiments, a separate tank per habitat was assembled under low structural complexity conditions for video footage of *H. guttulatus* and prey behavior, after the experiments. Low structural complexity was chosen to provide higher visibility of seahorses and prey. Six seahorses from the stock were used in video footages after the regular acclimation period (i.e. 1 male and 1 female per tank x 1 tank per habitat type x 3 habitat types). Preys offered in

separate recordings were (i) 10 amphipods and (ii) 6 shrimps. The behavior of *H. guttulatus* and of prey was recorded for 10-min intervals using a stationary underwater video camera (GoPro® HERO4) placed on the top border of a single tank per habitat, facing downwards. The foraging behavior of each seahorse within each tank, as well as the prey response to seahorse attack, was registered per attack according to the ethogram in Table S5 (adapted from James and Heck, 1994). Due to the lack of replication, behavior recordings allow detailed observations but not further statistical data analysis or significance interpretation.

Ethical statement

Seahorses, crustaceans (amphipods and shrimps), and seagrasses were collected in accordance with the permits issued by the Portuguese ICNF- Instituto da Conservação da Natureza e das Florestas (licenses n° 738/2023/CAPT and n°15/2023/DET, 110/2023/CAPT, and 109/2023/CAPT, respectively). Scuba diving in the Ria Formosa Natural Park was approved by the national maritime authorities (permit, Order/10/CPFARO/2023).

All experiments were performed in accordance with ARRIVE guidelines, with directives 86/609/EU and 2010/63/EU, and Portuguese legislation from the DGAV- Direção Geral de Alimentação e Veterinária for the use of animals in experiments (Law n° 113/2013). CCMAR facilities are certified to house and conduct experiments with live animals (Group-C licenses approved by DGAV). All researchers conducting fish manipulation during the experiments were professionally trained following the guidelines of DGAV and FELASA (Federation for Laboratory Animal Sciences Associations) according to the Portuguese legislation (Article 13 of the Law 113/2013).

3. Results

3.1. Prey identity

Results of the prey identity experiment showed no significant differences in the capture success proportion by *H. guttulatus* of amphipods (0.59 ± 0.25) and shrimps (0.64 ± 0.41) when offered separately in no-choice assays. When both preys were offered together in two-choice assays, we found significant differences in the capture success between prey types, with a proportion of amphipod capture success (0.89 ± 0.12) on average twice the shrimp proportion (0.44 ± 0.36) (Fig. 2, Tables S2–S4). Both assays, showed no significant differences in the proportion of capture success of any prey type between habitats under the experimental conditions, namely mean levels of structural complexity and prey availability.

3.2. Prey availability

Results of the prey availability experiment showed enhanced capture success by *H. guttulatus* under high shrimp availability in all three habitats (Enhancement ratio >1) under the experimental conditions of mean field structural complexity, with no significant differences between habitats (Fig. 3, Table S3). This was further confirmed by a significantly higher capture success under high availability of shrimps than under low availability regardless the habitat, with on average 3.6-fold times higher capture success under high than under low shrimp availability (Fig. S2, Table S2).

3.3. Habitat structural complexity

Results of the habitat complexity experiment revealed an enhanced capture success of shrimps at higher structural complexities in both vegetated habitats ('Caulerpa' and 'Seagrass'; with ER > 1 and nearly double mean capture success at high structural complexity than at low

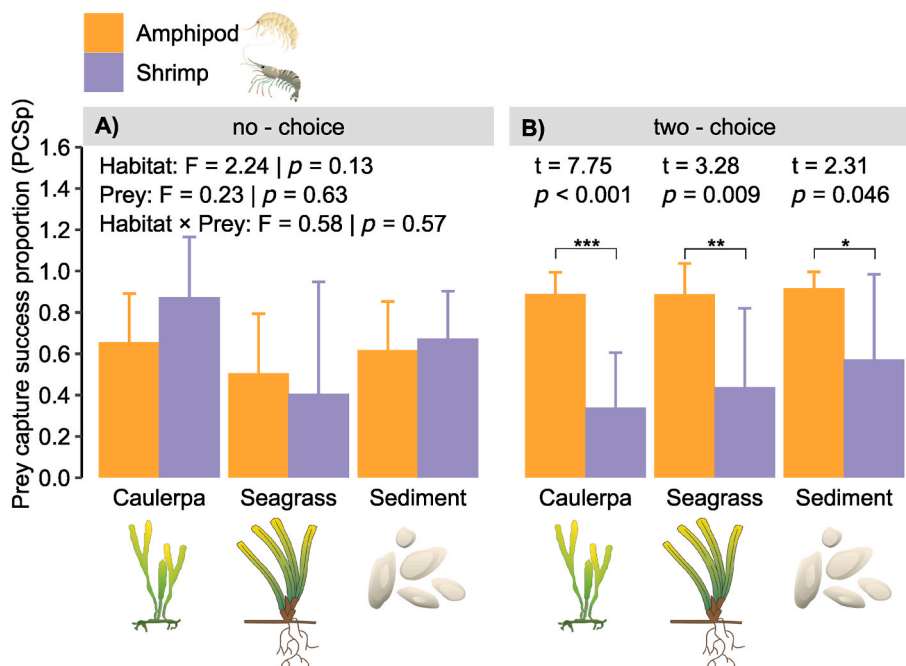


Fig. 2. Results of the prey identity experiment. Capture success proportion (mean \pm standard deviation) of amphipods and shrimps by *Hippocampus guttulatus* on 'Caulerpa', 'Seagrass' and 'Sediment' habitats in no-choice (A) and two-choice (B) assays. Preys were offered in equal biomasses (with a total of 4 % of the fish fresh bodyweight as a reference for daily consumption) and under mean field habitat complexity level. Proportion capture success equal to 1 indicates that 100 % of the preys that were initially offered were captured, with slight deviations from absolute zero or 1 values due to autogenic control corrections. Results from PERMANOVA test and paired t-tests are shown within plot A and B, respectively, where * denotes significant differences for $p < 0.05$, ** for $p < 0.01$ and *** for $p < 0.001$. The number of assays included in each test was $n = 5$ for no-choice assays (A) and $n = 10$ for two-choice assays (B).

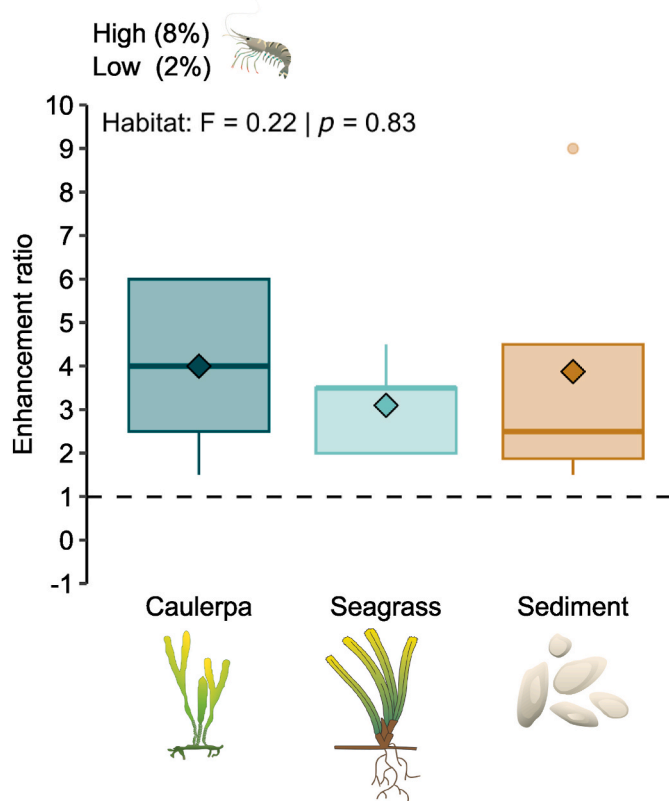


Fig. 3. Results of the prey availability experiment. Enhancement ratios of shrimp capture success by *Hippocampus guttulatus* on 'Caulerpa', 'Seagrass' and 'Sediment' habitats when preys were offered at high (8 % fish fresh bodyweight) and low (2 % fish bodyweight) availability levels (n = 5 replicates), under a mean field habitat complexity level. The horizontal dashed line (ratio = 1) indicates no difference in the capture success at high and low prey availabilities, while enhancement ratios >1 indicate an enhanced capture success under higher prey availability. Diamonds represent the mean Enhancement ratio in the box plot. Results from PERMANOVA test are shown within the plot.

complexity). Ratios in 'Caulerpa' and 'Seagrass' habitats were significantly higher than the ratio in the 'Sediment' habitat, where a lessened capture success at high levels of habitat complexity (with ER < 1 and nearly half capture success at high structural complexity) was detected (Fig. 4, Table S3). When values of prey capture success used to calculate the ratios were compared, we found a significant Habitat × Complexity interaction, with a significantly higher capture success (double on average) in 'Caulerpa' (2.40 ± 0.89) than in the 'Sediment' habitat (1.20 ± 1.30) under high structural complexities. We observed the same tendency (albeit slightly not significant; $p = 0.059$) of nearly twice capture success in the 'Seagrass' habitat (2.20 ± 0.84) than in 'Sediment' (1.20 ± 1.30) under high complexity (Fig. S3, Tables S2 and S3).

3.4. Foraging behavior observations

In the visualization of seahorse and prey behavior within the footages conducted under low levels of structural habitat complexity, we observed that the active pursuit and the "sit-and-wait" foraging strategies were adopted by *H. guttulatus* in all three habitats. The dominance of one strategy or another in each habitat (or the lack of dominance) was dependent on the prey identity. Namely, the two seahorse individuals observed in each habitat tank more frequently adopted the active pursuit strategy when attacking shrimps in 'Caulerpa' (i.e. 10 out of the 12 total attacks recorded; Video S1) and 'Sediment' (i.e. 6 out of 9; Video S2) habitats, while mainly adopted the "sit-and-wait" strategy when

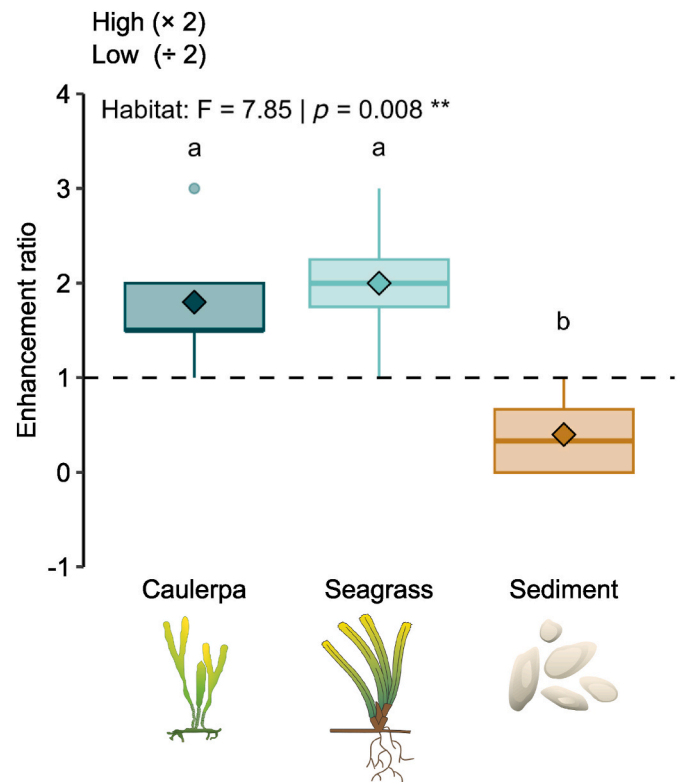


Fig. 4. Results of the habitat complexity experiment. Enhancement ratios of shrimp capture success by *Hippocampus guttulatus* on 'Caulerpa', 'Seagrass' and 'Sediment' habitats under high (i.e. double the mean field value) and low (i.e. double the mean field value) structural complexity of the habitat (n = 5 replicates). Reference biomass of preys (shrimps: 4 % of the fish bodyweight) were offered. The horizontal dashed line (ratio = 1) indicates no difference in the capture success at high and low habitat complexities, while ratios >1 indicate an enhanced and ratios <1 indicate a lessened capture success at high levels of habitat complexity compared to low levels. Diamonds represent the mean Enhancement ratio in the box plot. Results from PERMANOVA test are shown within the plot, where ** denotes significant differences for $p < 0.01$. Lower case letters denote significant differences from pairwise comparisons.

hunting amphipods in the 'Seagrass' (i.e. 7 out of 9; Video S3) and 'Sediment' (i.e. 6 out of 7; Video S4) habitats (Table S6). Regardless of the prey identity, the total number of attacks was higher in the 'Caulerpa' habitat (26 total attacks) than in the two other habitats (14 and 16 total attacks for 'Seagrass' and 'Sediment', respectively), whereas the attacks resulting in prey escape (missed attacks; Video S1) were more frequent in both vegetated habitats (21 out of 26 missed attacks for 'Caulerpa' and 9 out of 14 missed attacks for 'Seagrass') than in 'Sediment' (4 missed attacks out of 16). Interval between prey attacks ranged from 0 to 8.2 min, with seahorse conducting between 2 and 9 total attacks. In such intervals, independently of prey or habitat, seahorses predominantly conducted passive search. We observed no clear differences in seahorse behavior when separately considered the male and female individuals per tank (Table S7), therefore, observations are detailed regardless of the seahorse sex. Regarding prey behavior, amphipods were always unaware of the seahorse approach (Video S3, 4 and 5), whereas shrimp more frequently showed a "flight" or "freeze" response (Video S1, 2 and 6) showing awareness of the predator (Table S6). Shrimp preys were mostly dismantled before being ingested one piece at a time (Video S2), while amphipod preys were mostly suctioned entire (Video S4 and 5) regardless the habitat (Table S6).

4. Discussion

Our results reveal that *H. guttulatus* can predate successfully over a

wide range of habitats and preys, with such success depending on prey identity and availability, and on the habitat structural complexity, but being independent of the habitat type. Particularly relevant is the seahorse ability to exploit dense vegetation to successfully capture preys, regardless the native or invasive nature of the vegetation. To our knowledge, this is the first report on the lack of negative effect of invasive seaweeds on seahorses. Our finding agrees with previous studies reporting no effect of invasive seaweeds (Rosenblatt et al., 2022) and invasive freshwater macrophytes (Carniatto et al., 2020; Kovalenko et al., 2009) versus native vegetation on the capture success of macro-invertebrate prey by other predatory fish. We found that denser meadows of the invasive seaweed *C. prolifera* enhanced the seahorse predation success at a similar magnitude than denser seagrass habitats. The capture success of preys by *H. guttulatus* was, on average, double in more structurally dense vegetated habitats formed by both, the native seagrass or invasive seaweed, than under less dense vegetation. This contrasts with the decline in predation success by increased vegetation (seaweeds and seagrass) density, commonly reported for both, active and ambush fish predating on invertebrates (Coull and Wells, 1983; Heck and Thoman, 1981; Reynolds et al., 2018) or on smaller fish (Anderson, 2001; Gotceitas and Colgan, 1989; Savino and Stein, 1982). In contrast, our results reinforce the traditional view suggesting that high vegetation structural complexity favors seahorse ambush behavior by reducing prey awareness of predator presence (Flynn and Ritz, 1999; Foster and Vincent, 2004 and references therein).

Interestingly, the prey capture success in unvegetated habitats was enhanced to nearly double under low density of pebbles and shells compared to high density. A likely explanation is that the small structural elements in unvegetated habitats provide no seahorse camouflage to avoid detection by prey. In contrast to the vegetated habitats, the lack of camouflage in unvegetated habitats likely makes the amount of refuge for prey the determining factor for seahorse prey capture success. Our results highlights the key role of structural complexity within unvegetated habitats in mediating predator-prey interactions and, consequently, predator capture success; an aspect largely disregarded in previous studies on fish predation. Such studies commonly examine the effect of habitat complexity using unvegetated habitats as a control for the lack of vegetation, without considering their own complexity levels (e.g. Canion and Heck, 2009; Mattila et al., 2008). Indeed, we found no previous study like ours for other predatory fish (i.e. considering contrasting levels of unvegetated habitat's complexity), but only for a predatory amphipod (Alexander et al., 2012).

Whilst seahorses are widely known to inhabit habitats formed by native seaweeds (see review by Foster and Vincent, 2004 and references therein; and Ape et al., 2019; Vivas et al., 2024 specifically for *H. guttulatus*), previous studies on seahorse predation success are scarce and limited to artificial seagrass. Results by such studies are not uniform, indicating either no change in the capture of shrimp by *H. erectus* (James and Heck, 1994) or an increase in the capture of swarming mysids by *H. abdominalis* (Flynn and Ritz, 1999) in response to increased density of artificial seagrass (i.e. no vegetation, low, medium and high densities). In our study, the capture success of shrimps by *H. guttulatus* was, on average, double under high than low seagrass density levels. In James and Heck (1994), even though the same prey type and similar high and low seagrass density levels were considered, the use of artificial mimics might have discouraged the natural animal behavior, resulting in a different outcome. Despite the use of different prey types and live rather than artificial seagrass, our results agree with Flynn and Ritz (1999), which reported an increased capture success of mysids from low to medium-high densities of vegetation. However, the authors related this result to the splitting of mysid swarms into two groups at low seagrass density and many small groups at medium and high seagrass densities, whereas in our study, larger prey that do not form swarms were used. In our study, the use of living seagrass instead of artificial mimics might, for instance, have influenced the behavior, mobility and accessibility of seahorse preys which feed on living or necrotic parts of the seagrass

(absent in artificial seagrass). This is the case of the prey offered in the experiment examining the effect of habitat complexity, the shrimp *P. varians*, which is primarily a detritivore (Anderson, 1985).

Amphipods were preferentially captured by *H. guttulatus* when offered together with shrimps, regardless of the habitat type. The preference for amphipod preys agrees with previous studies on the same seahorse species (Gurkan et al., 2011; Kitsos et al., 2008) and also on *H. abdominalis* (Woods, 2002), which showed smaller crustaceans, and particularly amphipods and mysids, as most abundant preys in the gut content. The higher capture success of amphipods compared to shrimps is likely related to prey size, accessibility and evasiveness during seahorse attack. For instance, we found that whilst amphipods were mostly engulfed entire, the larger shrimp preys were firstly dismantled into pieces before ingestion. Also, footage observations showed contrasting prey behavior under the seahorse approach, i.e, amphipods were unaware of the predator and lacked a response, whilst shrimps frequently "flighted" away or "froze" when cornered by the predator. The capture success of amphipods by *H. guttulatus* was, on average, almost 90 % when they were offered together with shrimps, while it was 59 % when offered as a single prey. This result points to an unexplored interaction between both preys, with shrimps facilitating the accessibility of amphipods to the seahorse. The amphipod *Cymadusa filosa* is a herbivorous tube-building amphipod with low mobility that intensively use living seagrass for feeding and/or shelter construction (Martínez-Crego et al., 2014), also using *C. prolifera* rhizoids and sediment for nesting (personal observations during tank inspections in our experiments). *Gammarus insensibilis* has relatively higher mobility (without nesting) and is known to selectively feed on small amounts necrotic seagrass tissues (Martínez-Crego et al., 2014). The disturbance by shrimps of both contrasting amphipod behaviors, and most likely of *C. filosa* nesting, resulted in the increased amphipod accessibility to predators with almost all amphipods being captured (90 %) when they were offered together with shrimp preys.

When habitat complexity was at the mean structural density found in Ria Formosa, the habitat type did not affect *H. guttulatus* choice of prey or the capture success of shrimps nor amphipods. Our results agree with the generalized diet and trophic flexibility described by Ape et al. (2019) for *H. guttulatus* inhabiting two different seaweed-dominated habitats and one unvegetated habitat. Such trophic flexibility is likely related to seahorses versatility in their foraging strategies, which allow them to successfully attack and feed even on highly evasive preys. Our footage observations revealed that *H. guttulatus* adopts the same "sit-and-wait" foraging strategy and an active pursuit originally described for *H. erectus* (James and Heck, 1994) and later for *H. reidi* (Felício et al., 2006). The above-mentioned studies reported that both, *H. erectus* and *H. reidi*, adopt a "sit-and-wait" strategy on vegetated habitats and an active pursuit on unvegetated habitats. However, in our study, under low habitat complexity, seahorses often adopt contrasting strategies for the same prey in different habitats. Our footages showed that *H. guttulatus* use more frequently the "sit-and-wait" strategy when hunting amphipods in the native seagrass and unvegetated habitats, while adopted an active pursuit when hunting shrimps in *C. prolifera* and unvegetated habitats. This may suggest a habitat structural interference with prey behavior in determining the fish predation strategy, which agrees with a previous experiment by Savino and Stein (1989). The above-mentioned study revealed that the freshwater largemouth bass (*Micropterus salmoides*) adopted a "sit-and-wait" strategy when hunting elusive minnows regardless the density of artificial plants, while it switched from an active pursuit to a "sit-and-wait" strategy with increased plant density when hunting bluegills that use artificial plants as refuge.

Regarding prey availability, we found a 4-fold enhanced prey capture success by *H. guttulatus* under high than under low shrimp availability. Despite such great success improvement under high prey availability, our results also showed that, even with a prey availability as low as 2 % of bodyweight, *H. guttulatus* was still able to successfully capture, on average, 77 % of the preys offered in all habitats. Overall,

our results demonstrated that adult *H. guttulatus* are successful predators, well adapted not only to different habitats and preys with contrasting evasive behavior, but also to prey scarcity scenarios. This success is likely related to seahorse morphological adaptations that improve the biomechanics of prey capture, such as the 'horse shape' of the head that allow increasing the strike distance (Van Wassenbergh et al., 2011), while minimize the hydrodynamic disturbance of the surrounding water during predatory attack, bringing the snout close to even highly evasive prey without being detected (Gemmell et al., 2013).

In brief, *H. guttulatus* predate successfully over a wide range of habitats and preys using versatile foraging strategies. They take advantage of the dense structural complexity of vegetated habitats, both the native seagrass and the invasive seaweed, but are also successful in less complex unvegetated habitats where preys use structural elements for hiding. This may explain why *H. guttulatus* is able to inhabit the two native habitats of Ria Formosa (i.e. seagrass meadows and unvegetated sediment) as reported by Curtis and Vincent (2005), as well as the invasive *C. prolifera* habitat as found by Parreira et al. (2021) and Correia (2022). Indeed, Parreira et al. (2021), found that *H. guttulatus* was more abundant in *C. prolifera* meadows than in the two native habitats, and Correia (2022) reported this seaweed as the most frequent holdfast. Our results show that dense vegetation of the invader *C. prolifera* enhanced the seahorse predation success at a similar magnitude than denser seagrass habitats, suggesting no negative effects of *C. prolifera* on the predation success of *H. guttulatus* in Ria Formosa lagoon. However, it is of the outmost importance to interpret such results carefully when extracting conclusions for guiding seahorse conservation, since the aggressive spread of *C. prolifera* may affect other aspects of the seahorse foraging ecology than prey capture success, such as the disruption of prey communities or the competition for preys with other predators. For instance, Parreira et al. (2021) found negative implications of *C. prolifera* invasion on the overall biodiversity of the lagoon, including the invertebrate communities that constitute seahorse preys. Further investigation regarding the potential impact of invaders on aspects other than seahorse predation success is needed for a full understanding on the invasion implications. Our study reveals that the prey identity and prey availability are determining factors in the predation success of *H. guttulatus*. Thus, any factor impacting the abundance of prey communities, such as seaweed invasions, must be understood. On the other hand, our findings support the seahorse association with seagrass habitats commonly reported elsewhere (Foster and Vincent, 2004). Dense seagrass vegetation enhanced by 2-fold the predation success by *H. guttulatus*, reinforcing the extensive benefits of seagrass conservation and restoration reported elsewhere (e.g. Orth et al., 2020), by specifically highlighting their value for seahorse populations.

CRediT authorship contribution statement

Filipe Parreira: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jorge Palma:** Writing – review & editing, Resources. **Rui Santos:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Begoña Martínez-Crego:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2025.107247>.

Data availability

Data will be made available on request.

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