Maria Rita Silva Alexandre

Exploring short-snout seahorse (*Hippocampus hippocampus*) feeding ecology, habitat variation and their relationship

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Mestrado em Aquacultura e Pescas
(Especialidade em Pescas)

Trabalho efetuado sob a orientação de: Dr.Jorge Palma (Centro de Ciências Marinhas, Universidade do Algarve)

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Declaração de autoria

Exploring short-snout seahorse (*Hippocampus hippocampus*) feeding ecology, habitat variation and their relationship

Declaro ser autora deste trabalho, que é original e inédito. Autores e trabalhos consultadosestão devidamente citados no texto e constam da listagem de referências incluída.

(Maria Rita, 29.09.2024)

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RESUMO

Os cavalos-marinhos (Género *Hippocampus*) são espécies únicas com uma morfologia característica e distinta das demais espécies de peixes ósseos. Em virtude disso, possuem fraca mobilidade, facto que implica uma maior fidelidade a habitats preferenciais como áreas costeiras rasas, incluindo lagoas costeiras, zonas de recife e estuários. A sua alimentação tem por base diferentes grupos de crustáceos, desde os copépodes nas primeiras fases de vida até pequenas espécies de camarões, misidáceos, e anfípodes entre outros, na fase de vida adulta. Globalmente, as diferentes espécies de cavalos-marinhos sofrem impactos antropogénicos incluindo a destruição dos seus habitats, captura como espécies acessórias (*by-catch*) e captura direta motivada principalmente pela sua utilização na medicina tradicional chinesa e como espécies ornamentais. Em face disto, todas as espécies de cavalos-marinhos (*Hippocampus* spp.) estão atualmente incluídas no Anexo II da Convenção sobre o Comércio Internacional de Espécies da Fauna e Flora Selvagens Ameaçadas de Extinção (CITES) e na Lista Vermelha de Espécies Ameaçadas da IUCN. Para além disso, as duas espécies europeias de cavalos-marinhos, o *Hippocampus guttulatus* (cavalo-marinho de focinho comprido) e *H. hippocampus* (cavalo-marinho de focinho curto) estão abrangidas pela Convenção de Berna e pela Convenção de Barcelona para a Proteção do Ambiente Marinho e da Região Costeira do Mediterrâneo. Presentes em águas nacionais, a sua ocorrência em maior abundância foi historicamente referenciada na Ria Formosa, sendo este um ecossistema costeiro complexo que oferece uma variedade de habitats, quer para o *H. guttulatus* quer para o *H. hippocampus*. A região é composta por uma série de ilhas-barreira, canais, extensos sapais, áreas de salinas e vastas pradarias marinhas, destacando-se as formações de *Zostera noltii* e *Cymodocea nodosa*. A presença destes diversos habitats proporciona diferentes níveis de complexidade estrutural na zona subtidal, criando oportunidades para os cavalos-marinhos se associarem a habitats preferenciais. Nas áreas de pradarias marinhas, estas espécies encontram abrigo, uma preferência que pode ser atribuída á sua morfologia e comportamento singulares. Estas características únicas permitem aos cavalos-marinhos explorar eficazmente esses habitats em busca de alimento, proteção e condições de reprodução. Além disso, a preferência do *H. hippocampus* por habitats menos densamente vegetados pode ser uma estratégia adaptativa para evitar predadores e competidores. No entanto, pode torná-los mais vulneráveis a ameaças antropogénicas, como a degradação do habitat e a pesca excessiva, sendo assim então essencial entender a relação entre a ecologia alimentar do *H. hippocampus* e a variação do habitat, para desenvolver estratégias de conservação eficazes para este peixe. As estruturas preferidas desta espécie incluem ervas marinhas e algas, que oferecem abrigo contra predadores e potenciam a ocorrência de fontes de alimento. A disponibilidade de presas que compõem a dieta natural do *H. hippocampus*, pode variar de acordo com a estrutura ecológica do habitat, influenciando diretamente o seu comportamento alimentar. É importante notar que a diversidade de presas disponíveis pode ser um indicador de saúde geral do ecossistema, e uma diminuição na variedade de presas pode sinalizar um ecossistema em declínio. Assim, as alterações ambientais induzidas pela ação humana têm um impacto significativo na qualidade dos habitats, geralmente resultando na sua degradação e numa redução da sua complexidade estrutural. Estas alterações podem incluir a destruição física do habitat, a introdução de espécies invasoras, a poluição da água e a alteração dos padrões de salinidade e temperatura. Cada uma dessas alterações pode ter efeitos profundos na capacidade do *H. hippocampus* comprometendo a sua sobrevivência. Por isso, é essencial entender a relação destas espécies com os seus habitats preferenciais, incluindo a relação de dependência trófica, e como a sua variação pode afetar a sua ocorrência e bem-estar. Por exemplo, se um tipo específico de presa se torna menos abundante devido á degradação do habitat, o *H. hippocampus* pode ser forçado a se adaptar a um novo tipo de presa ou a procurar um novo habitat, resultando daiimplicações significativas para a sobrevivência das populações. Neste contexto, este estudo procurou determinar o comportamento alimentar do *H. hippocampus* em termos de consumo diário de alimento e de preferência alimentar em função dos diferentes habitats de ocorrência e da sua complexidade estrutural. Para isso, foram recriados em cativeiro, três diferentes tipos de habitat de ocorrência da espécie (erva marinha *C. nodosa,* sedimento misto composto por elementos inertes e alga *Codium* spp., e de alga *C. prolifera*), cada um com a sua densidade de ocorrência padrão em ambiente natural (densidade normal) e com uma redução de 50% na sua densidade estrutural (densidade baixa). Em cada habitat, foram fornecidos individualmente, e posteriormente em simultâneo, três tipos de presas representativas da alimentação do *H. hippocampus* no meio selvagem: anfípodes (*Gammarus* spp.), misidáceos (*Diamysis lagunaris*) e camarões (*Palaemon varians*). Durante o estudo, foram observados 48 adultos de *H. hippocampus*, tendo sido alocados quatro indivíduos em cada um dos 12 tanques de observação. Quando fornecido individualmente na primeira experiência este estudo, cada tipo de presa foi fornecido *ad libitum* para avaliar o consumo diário de alimento sob diferentes condicionantes ambientais. Quando fornecido conjuntamente na segunda experiência, cada tipo de presa foi disponibilizado na quantidade média anteriormente observada, para avaliar uma eventual preferência alimentar do *H. hippocampus* em função da disponibilidade de presas e do tipo e complexidade estrutural de cada habitat. A informação recolhida permitiu avaliar o consumo diário de alimento e a dependência da sua abundância e disponibilidade em ambiente natural, bem como identificar eventuais variações de preferência alimentar em função do habitat de ocorrência e da sua complexidade. Além disso, foi possível observar como essas preferências alimentares podem mudar em resposta a alterações no habitat, proporcionando uma visão mais dinâmica da ecologia alimentar do cavalo-marinho de focinho curto. Por exemplo, em habitats de baixa densidade, os cavalos-marinhos demonstraram uma maior preferência por misidáceos, especialmente em áreas com menor complexidade estrutural, como é o caso do habitat de *C. prolifera*. Em conjunto, esta informação é de especial relevância pois destaca a importância de considerar a ecologia alimentar na gestão de habitats e na elaboração de estratégias de conservação de espécies marinhas. Compreender as preferências alimentares do *H. hippocampus* e como elas estão ligadas á estrutura do habitat pode auxiliar na identificação de áreas prioritárias para a conservação e desenvolver medidas de gestão que apoiam a saúde e a resiliência das populações desta espécie.

Termos-chave: cavalos-marinhos, *Hippocampus hippocampus*; regime alimentar; preferência alimentar, dependência trófica, estrutura ambiental

ABSTRACT

Seahorses (Genus *Hippocampus*) are unique bony fish with poor mobility, primarily inhabiting shallow coastal areas. Their diet consists of various crustaceans, from copepods to small shrimp. Due to anthropogenic impacts, all seahorse species are listed in CITES Appendix II and the IUCN Red List. European species, *H. guttulatus* and *H. hippocampus*, are also protected by regional conventions. The Ria Formosa, a complex coastal ecosystem, offers diverse habitats for both species, including seagrass beds that provide shelter and food sources. However, human-induced environmental changes can degrade these habitats, compromising seahorse populations. This study aimed to determine the feeding behavior of *H. hippocampus* in different habitats and structural complexities. Three habitat types were recreated in captivity: *Cymodocea nodosa* seagrass, mixed sediment with *Codium* spp. algae, and *Caulerpa prolifera*, each with normal and low-density variations. Three prey types representative of *H. hippocampus'* wild diet were provided individually on a first trial, and then simultaneously on a second trial: amphipods (*Gammarus* spp.), mysids (*Diamysis lagunaris*), and shrimp (*Palaemon varians*). The study involved 48 adult *H. hippocampus* individuals, with four allocated to each of the 12 observation tanks, observing their daily food consumption and preferences under various environmental conditions. Results showed variations in prey preferences based on habitat type and complexity. For example, in low-density habitats, seahorses preferred mysids, especially in simpler environments like *C. prolifera*. This research highlights the importance of integrating feeding ecology into habitat management and conservation strategies to support the health and resilience of *H. hippocampus* populations. Understanding the relationship between seahorses and their preferred habitats is crucial for preserving these important indicators of marine ecosystem health.

Keywords: seahorses, *Hippocampus hippocampus*, diet, prey preference, trophic dependence, environmental structure

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Abbreviations

ANOVA: Analysis of Variance

CF: Condition Factor

CITES: Convention on International Trade in Endangered Species of Wild Fauna and Flora

FAI: Food Availability Index

IUCN: International Union for Conservation of Nature

MPAs: Marine Protected Areas

Spp.: Species (plural)

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1. Introduction

1.1 Species biology, ecological insights, and geographic range

Seahorses (genus *Hippocampus*), along with pipefishes and seadragons, belong to the family *Syngnathidae*. A distinctive characteristic of this family is the fusion of their jaws, which are fused into a tube-like structure. The name "*Syngnathidae*" reflects this feature, derived from the Ancient Greek words "*syn*" (meaning "together") and "*gnathos*" (meaning "jaw).

The genus *Hippocampus* is characterized by elongated torsal structures, a prehensile tail, and a cranial configuration that bears a striking resemblance to that of a horse (*Figure 1.1*). Alongside, the species of this genus bear a remarkable ability to alter their coloration, thereby seamlessly blending into their surroundings (Bell & Vincent, 2024). Such adaptability renders them inconspicuous to both potential threats and potential prey, often mirroring the colors and textures of the seagrass or coral environments they inhabit (Bell & Vincent, 2024).

Within this genus, the short-snout seahorse (*H. hippocampus*) exhibits distinctive morphological attributes. As its name suggests, it possesses a notably short snout, typically less than one-third of its head length, which is slightly upward-bent (Lourie et al., 2004). This species showcases the genus' outstanding capability for camouflage, as observed by Bell & Vincent (2024).

Furthermore, it is pertinent to underscore the extensive geographical distribution of the species, encompassing regions across Europe and North Africa, including the Atlantic Ocean, Mediterranean Sea, and Black Sea (Gristina et al., 2021). This wide distribution highlights the species' adaptability to various marine environments within its range.

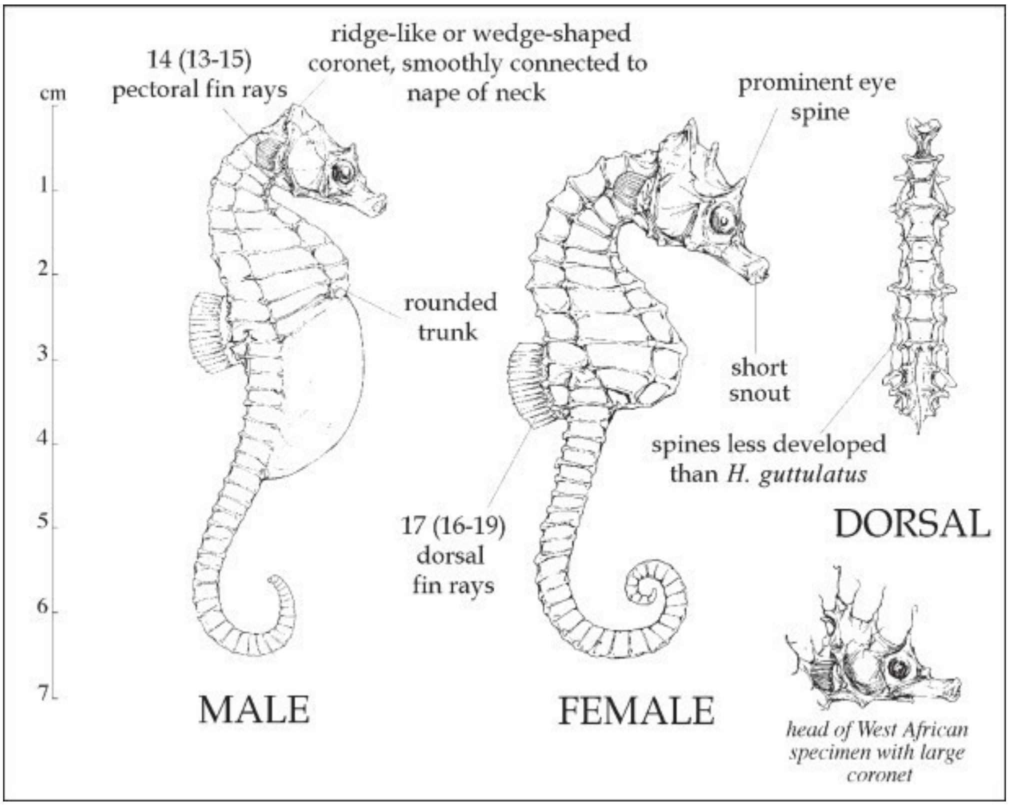


Figure 1.1 Morphology of the short-snout seahorse (*H. hippocampus*) male (on the left side) and female (on the rightside) (adapted from Lourie,S.,A., et al., 2004)

1.2 Reproductive biology

Seahorses showcase a fascinating and distinctive reproductive system characterized by a shared reproductive role where both males and females assume tasks. However, the responsibility for receiving, protecting, and nourishing the developing embryos within a specialized brood pouch belongs to the males (Stölting & Wilson, 2007). *H. hippocampus*, much like its seahorse counterparts, participates in intricate courtship rituals before transferring the eggs to the male's brood pouch for gestation (Qin et al., 2017), yielding offspring ranging from 123 and 350 (with an average around 173 ± 71.1) (Silva, 2022).

1.3 Conservation significance

Hippocampus species confronts a range of threats stemming from habitat destruction, overexploitation for traditional Chinese medicine, and the demands of the aquarium trade (Rosa et al., 2012). Declines in populations have sparked conservation concerns, prompting initiatives like public awareness and the establishment of marine protected areas to safeguard seahorse habitats (Vincent et al., 2011). Despite taxonomic uncertainties, conservation strategies targeting seahorses may inadvertently benefit a broader array of species reliant on shared habitats, such as seagrass beds (Shokri et al., 2008). Recent studies underscore the importance of understanding key factors like habitat availability and environmental conditions in preserving seahorse populations (Correia et al., 2018). In this context, the Ria Formosa estuary emerges as a vital habitat for a wide range of marine fauna, prominently featuring the long-snouted and short-snouted seahorses (Correia, 2015). These seahorse species exhibited a notable abundance in the early part of the 21st century within the Ria Formosa. However, a marked decrease in their populations since that period has raised significant concerns (Correia, 2015), leading to intensified efforts towards their preservation and protection.

1.4 Feeding ecology of the short-snout seahorse

Recent studies underscore the importance of habitat availability and environmental conditions in preserving seahorse populations, including the species *H. hippocampus* (Correia et al., 2018). These seahorses while on the first stages of life primarily subsist on plankton, but young adults and adults subsist on tiny crustaceans. They are also known for their impressive ambush predation, capitalizing on their exceptional camouflage to capture readily available prey (Trewhella & Hatcher, 2017). Their feeding habits, encompassing feeding rates and selectivity, are influenced by environmental factors and age (Sheng et al., 2006). Adult seahorses need to consume approximately 30 to 50 mysids daily (Garrick-Maidment, 1997), while juveniles seahorses exhibit voracious appetites, consuming around 3,000 food items daily (Wallis, 2004). The dietary needs of *H. hippocampus* are shaped by factors such as food source availability, water temperature, and predator presence (Silveira et al., 2022). They primarily feed on small crustaceans like mysids, amphipods, and decapod larvae (Yip et al., 2014), employing a sit-and-wait predation strategy and utilizing their snouts with remarkable swiftness to suction their prey (Wassenbergh et al., 2011).

Seahorses, being carnivorous, rely heavily on a protein-rich diet from crustaceans for their growth and metabolic functions (Yip et al., 2014). They obtain essential vitamins and minerals, such as calcium, from their prey, which are crucial for maintaining their bony structure and buoyancy (Chang, 2000). In captivity, it is critical to emulate their natural prey to ensure their health and successful reproduction. Live foods like brine shrimp and mysids are typically used to ensure proper nutrition and breeding program success (Palma et al., 2012). However, studies on *H. hippocampus* diet have shown that *Artemia* alone is not an optimal food source, especially in early life stages. Palma et al. (2023) found that the use of *Artemia* is conditional on the prior use of other natural prey, at least in the initial life phases. The research demonstrated that a diet incorporating copepods yielded better results in terms of growth and survival compared to diets solely based on enriched *Artemia*. A recent study has shown that a live prey diet, particularly mysid shrimp, has a significant positive impact on the spawning quality of *H. hippocampus* (Otero-Ferrer et al., 2012). These findings align with the established importance of live prey, such as mysid shrimp, for gonad development in short-snout seahorses (*H. hippocampus*) (Otero-Ferrer et al., 2012).

1.5 Research and knowledge gaps

Despite the limited published data on the two European seahorse species, the existing information predominantly focuses on *H. guttulatus*. This results in a significant gap in our understanding of *H. hippocampus*, underscoring the urgent need for dedicated studies to address this disparity. In fact, the marine community science project iSeahorse has demonstrated invaluable potential in collecting data for understudied species, including *H. hippocampus* (Camins et al., 2023). Early iSeahorse data has already contributed to the first full set of IUCN Red List assessments for seahorses, completed between 2014 and 2017, and continues to provide new information on various parameters such as geographic distribution and habitat, for many species of seahorses (Camins et al., 2023).

Seahorses continue to captivate scientists, particularly due to their feeding habits that demand a specialized diet for optimal health. However, our understanding of their feeding behaviors and nutritional requirements remains incomplete, highlighting the ongoing need for further research (Cohen et al., 2016). Cohen et al. (2016) outline’s several areas where our understanding of seahorse ecology remains incomplete, including specific nutritional requirements, dietary diversity, feeding behaviors, cultivation methods, the influence of habitat changes, and interactions with prey.

Addressing these knowledge gaps will contribute to a more comprehensive understanding of seahorse nutrition, ultimately playing a vital role in their conservation and overall well-being.

2. Objectives

The main objectives of this work were to evaluate and characterize the feeding behavior of

H.hippocampus across various habitats and to ascertain the impact of habitat structural complexityon food consumption and prey preference. This study focuses on three naturally occurring habitats within the Ria Formosa lagoon: seagrass meadows, a sediment habitat consisting of inert elements such as stones and shells combined with algae, and a habitat dominated by *Caulerpa prolifera*. These habitats were replicated in a controlled environment at two structuraldensities: normal and low.

In the first trial, representative species from each of the three main feeding groups in the seahorse diet (amphipods, mysids, and shrimp) were provided individually to assess daily food consumption over a five-day period for each type of live feed, taking into account the prey's habitat density.

In a second trial, conducted as a follow-up to the initial experiment, all three prey types were offered simultaneously. The amount of each prey was based on the previously observed consumption rates, ensuring that each type was fully available throughout the trial. Seahorses' consumption was monitored over a five-day period, providing insights into their dietary preferences and feeding behaviors in a more diverse food environment.

In addition, the information obtained from *ex situ* experiments was compared with data collected from the same three observed habitats in the wild with the aim of detailing species composition and abundance. This approach provided a more comprehensive understanding of the diet of *H. hippocampus* and its reliance on specific habitats. The additional data collection helped to further clarify the ecological dynamics of seahorses and contributed to the development of more effective conservation strategies.

3. Material and methods

3.1 Study location and environmental context

The experimental work was conducted at the Ramalhete Marine Station of Center of Marine Sciences (CCMAR), University of Algarve, Faro, Portugal. To study the seahorses' feeding behavior in relation to different habitat conditions, three distinct natural environmentswere replicated within each observation tank. The seagrass habitat, composed exclusively of *Cymodocea nodosa*, and the sediment habitat, which included a specific number of small rocks, shells, and algae *Codium* spp., were recreated. Both habitats are recognized as naturally occurring habitats for *H. hippocampus* in the Ria Formosa lagoon. On its turn, a third habitat, the *Caulerpa prolifera* (algae) habitat was recreated to assess the *H. hippocampus* feeding behavior in response to this new habitat type imposed by the rapid spread of this algae in the Ria Formosa lagoon. All three habitats were replicated at both their normal and low structural densities. The normal habitat density was defined as the average bottom coverage observed in the Ria Formosa for each habitat type, which was set at 60% coverage (Palma, unpublished data). For low structural density, the bottom coverage of each habitat was reduced by 50%, resulting in a final coverage of 30%. To evaluate the daily feed consumption, three different live feeds were used: amphipods (*Gammarus* spp.), mysid shrimp (*Diamysis lagunaris*), and shrimp (*Palaemon varians*).

3.2 Experimental approach and procedures

3.2.1 First trial – Isolated feed evaluation in diverse habitats

a) Seagrass *Cymodocea nodosa* habitat

The first habitat replicated in this study was the *C. nodosa* seagrass habitat. This was accomplished using twelve 40-liter glass tanks, arranged in a flow-through system. Throughout the experiment, environmental parameters were maintained constant across all tanks, including dissolved oxygen levels of 6.2±0.2 mg/l, a 12-hour light/12-hour dark photoperiod, and a temperature of 20.5±1°C. A sand substrate (2.5 cm height) was placed in all tanks. Six of the tanks were set at normal density (as defined above) using 36 seagrass shoots each, and the remaining six at low-density, containing 18 shoots each (*Figure 2*). Four seahorses (two males and two females) were assigned to each tank, and all seahorses (n=48) were observed at both seagrass habitat densities and exposed to three types of live feed: amphipods, mysids, and shrimp.

Each trial, for both habitat density and live feed type, was conducted over a five-day period. At the beginning of each trial, the seahorses were measured for length and weight to calculate the fish biomass in each tank. For the feeding routines, the amphipods were provided at ≈5% of the body weight (% BW d^{-1}) of each of the four seahorses, previously weighed before the beginning of the experiments. Mysids were initially provided at 5% BW d^{-1}. However, due to the higher consumption of this prey, *ad libitum* conditions were only met when the amount was increased to 10% BW d^{-1}. This adjusted amount was provided for the remainder of the trials. Shrimp feed was also provided at 5%BW d^{-1}.

Every morning, uneaten prey was carefully collected from each tank, weighed, and subtracted from the amount provided on the previous day. Fresh feed was then supplied, and this routine was repeated daily. This rigorous methodology allowed for a detailed examination of the feeding behavior of *H. hippocampus* across various habitat densities and under different feeding conditions.

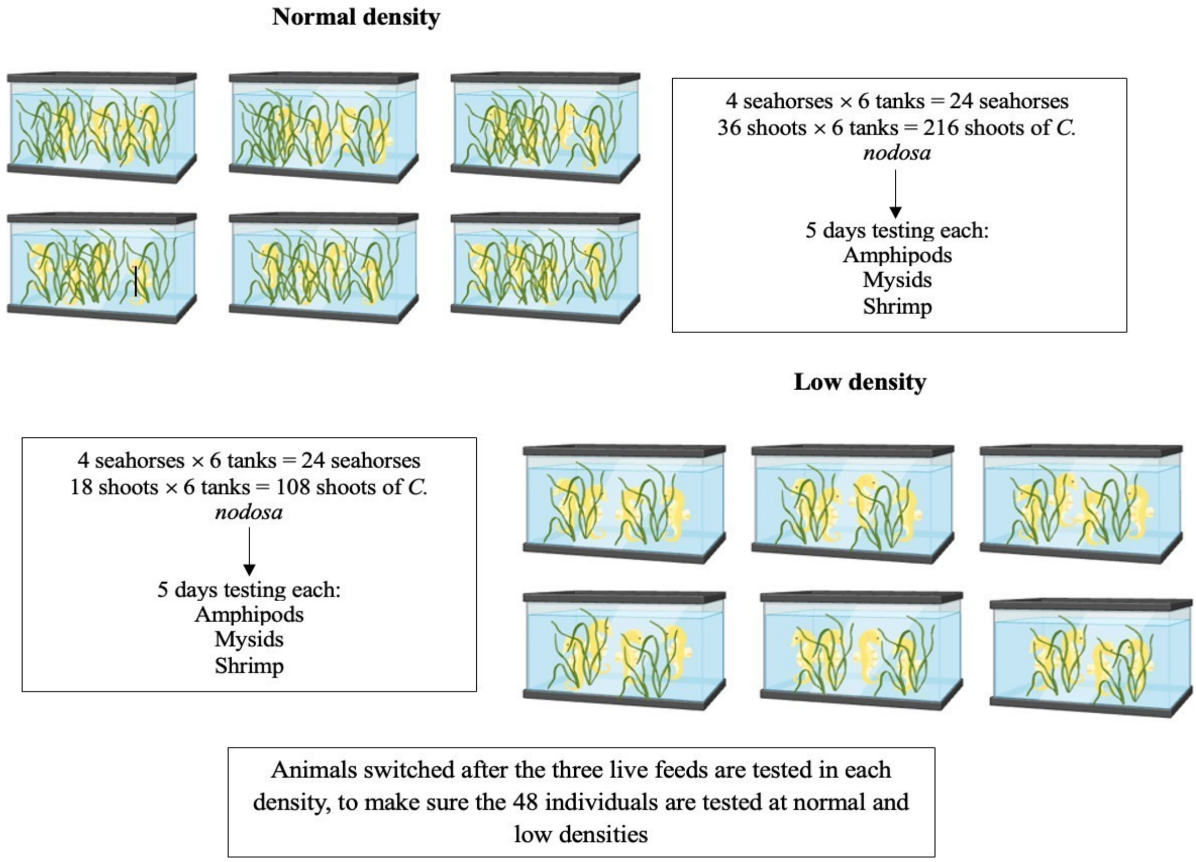
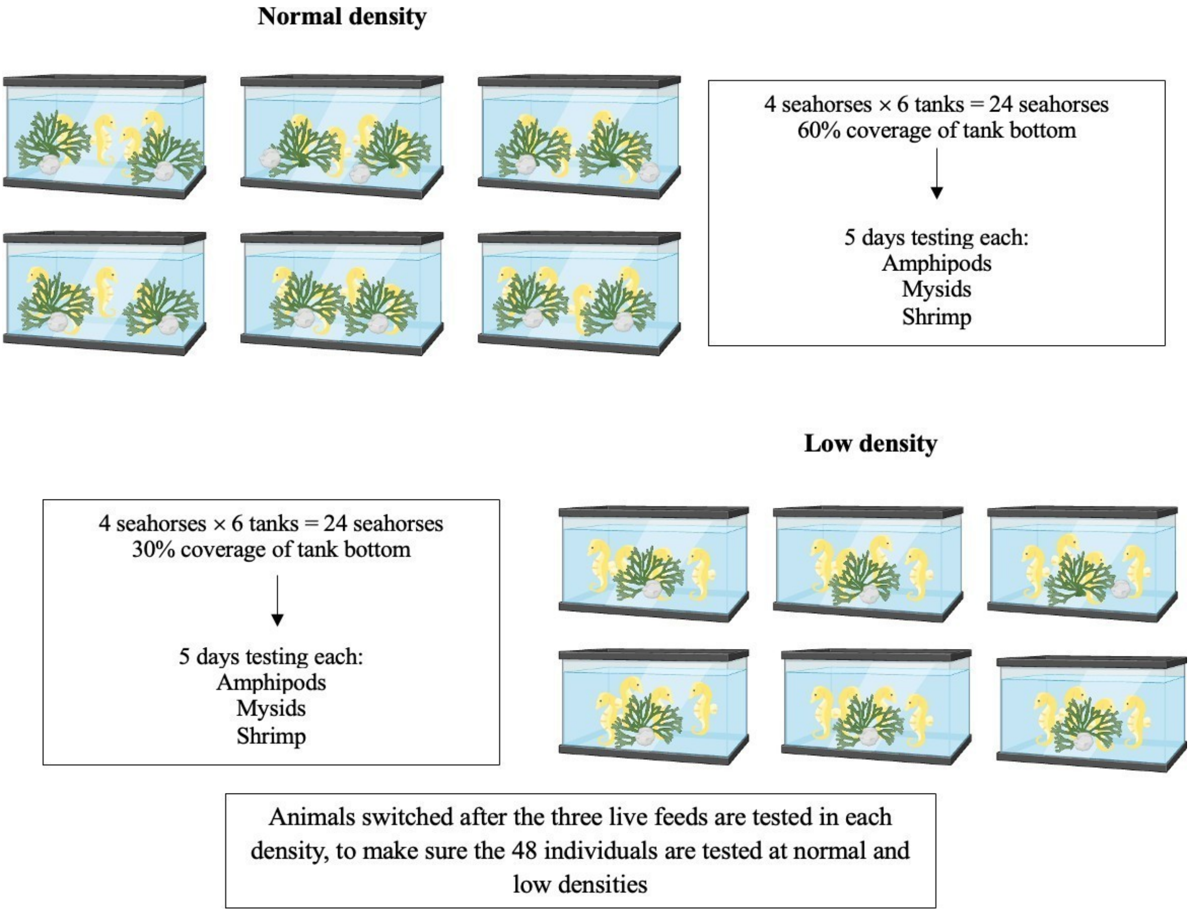


Figure 3.1 *C. nodosa* habitat representation, normal density (top left) and low-density (bottom right)

b) Sediment with *Codium* spp. habitat

The second habitat replicated in this study was the sediment habitat with the algae *Codium* spp. The tank assemblage was the same as described above, with the only variation being the habitat. The sediment habitat was composed of various elements such as shells, rocks, and the *Codium* spp. algae. The low-density habitat had 50% fewer structural elements compared to the normal density habitat.

The feed consumption of each of the tested feeds followed the same protocol as described above. This consistent methodology allows for a fair comparison of the seahorses' feeding behavior across different habitats and conditions.



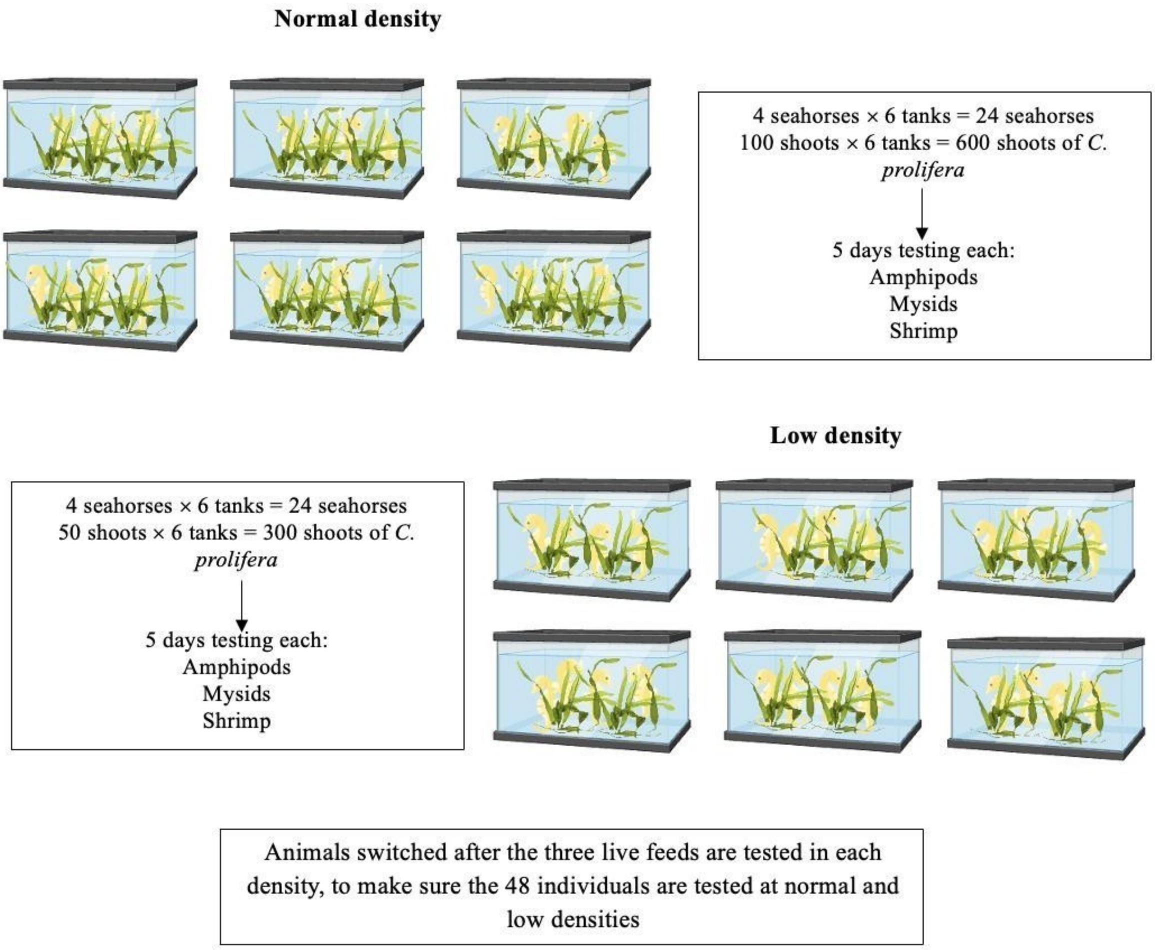
Animals switched after the three live feeds are tested in each density, to make sure the 48 individuals are tested at normal and low densities

Figure 3.2 Sediment with *Codium* spp. habitat representation, normal density (top left) andlow density (bottom right)

c) *Caulerpa prolifera* habitat

The last habitat replicated in this study was the *C. prolifera* habitat. This was achieved using the same experimental design described above. The normal habitat density was established by placing 100 shoots of *Caulerpa prolifera* in each tank, while the low-density habitat setup contained 50 shoots of *C. prolifera* per tank.

Again, the feed consumption of each of the tested feeds followed the same protocol as described above.



4 seahorses × 6 tanks = 24 seahorses
100 shoots × 6 tanks = 600 shoots of *C. prolifera*

5 days testing each:
Amphipods
Mysids
Shrimp

4 seahorses × 6 tanks = 24 seahorses
50 shoots × 6 tanks = 300 shoots of *C. prolifera*

5 days testing each:
Amphipods
Mysids
Shrimp

Animals switched after the three live feeds are tested in each density, to make sure the 48 individuals are tested at normal and low densities

Figure 3.3 *C. prolifera* habitat representation, normal density (top left) and low density(bottom right)

3.2.2 Second trial – Multi-feed assessment across habitats

The second trial of the study aimed to evaluate the feeding behavior and assess any potential prey preference of *H. hippocampus* when exposed to a combination of all three types of live feeds across different habitats. The experimental design mirrored that of the first trial, as the experimental conditions (tank and habitat setup) and environmental parameters remained consistent with those in the first trial. The daily feeding protocol was also unchanged, except that the amount of each feed offered corresponded to the daily food consumption (in %BW day^{-1}) observed in the first trial. This ensured that any of the three feeds could individually meet the species' daily consumption needs, thus allowing an assessment of feed preference when all three were offered together.

3.2.3 Analysis of habitat samples from the Ria Formosa

In parallel with the *ex-situ* experiments, samples from three distinct habitats in the Ria Formosa – seagrass (*C. nodosa*), sediment, and the algae *C. prolifera* habitat – were collected and analyzed. The objective was to understand the composition of these habitats in terms of biological components, and their influence on the feeding behavior of the seahorse *H. hippocampus*.

Sample collection

Three locations were selected for sample collection in the Ria Formosa, corresponding to each of the chosen habitats: seagrass (location: each of the chosen habitats: seagrass (location: 37° 0'13.23"N - 7°49'6.29"W), sediment (location: 37° 0'17.52"N - 7°48'40.23"W), and *C. prolifera* (location: 37° 0'21.87"N - 7°48'46.46"W). In each location, one sample was collected by dragging over a 50-meter distance, covering the benthic and supra-benthic sections, along with three replicated samples from the benthic and adjacent surface sediment sections. For the drag collection, a net was used, measuring 1 meter in width and 50 cm in height, with a mesh size of 150 microns at the bottom and 1 mm on the sides and top. The net was 1.5 meters long, with a removable mesh bag at the terminal section with 150-micron mesh.

At each location, a 50-meter transect tape was laid during a dive to guide the direction of sample collection. After laying the transect line, the drag was conducted along the line but 1 meter away to avoid interference in the sample composition caused by the prior placement of the transect tape. The net was operated by two divers holding the sides and dragging it along the designated distance, keeping it in contact with the seafloor. After the drag, the collected sample was immediately brought on board and kept in suitable transportation conditions.

Subsequently, three replicated samples were collected on the opposite side of the transect line from the previous collection. Each of the three replicated samples was collected at 15, 30, and 45 meters from the transect line. For these samples, mesh bags (one per sample) with a width and height of 50 cm and a 1 mm mesh were used. In each replicated location, the net was placed over the sediment, pressed evenly to a sediment depth of 2.5 cm, then closed with a wire to collect both the sediment section and the surface layer. Once collected, the samples were brought on board, placed in individual bags, and kept at a suitable transportation conditions. In the laboratory, samples collected by drag were immediately frozen for later observation, while the replicated samples were first processed to remove the inorganic components using sieves with progressively smaller mesh sizes to prevent the loss of organic material. After this, the samples were frozen for later observation.

Laboratory analysis

Once the samples were collected, they were analyzed in the laboratory. To ensure precise analysis of the biological components present in each sample, the samples were gradually thawed in batches. This approach allowed for a thorough examination of the biological material from the three habitats (seagrass (*C. nodosa*), sediment, and *C. prolifera*). By carefully managing the thawing process, any potential degradation of biological matter was minimized, ensuring the integrity and accuracy of the subsequent analyses and species identification.

After the triage, all the collected biological matter was sorted by species, weight, stored in small vials and preserved in a 70% alcohol solution, allowing later identification of initially unknown species.

Physiological condition assessment

In addition to analyzing the species composition of each habitat, we also assessed the physiological condition of *H. hippocampus* specimens from the experiment. This was done by calculating the Condition Factor (CF) using the following formula:

$$CF = \frac{wet\ weight\ (g)}{height^3 (cm)} \times 100$$

The CF values were calculated individually for each seahorse observed during the experimental trials in the three habitats, and later expressed as mean±s.d.

3.2.4 FAI (Food Availability Index)

In order to evaluate the Condition Factor (CF) calculated from the seahorses in the experimental tanks, we developed a Food Availability Index (FAI) (FAI = B/A, where B is total biomass of suitable prey items (g) and A is total sampled area ($m^{2)}$) based on the prey samples collected from each habitat in the Ria Formosa, selecting only the preys that short-snout seahorse would feed from.

According to this FAI, it was also calculated the percentage of contribution of each group by using the following formula:

$$Contribution\ (\%) = \frac{FAI\ of\ a\ specific\ group}{Total\ FAI\ of\ that\ group\ across\ all\ three\ habitats} \times 100$$

3.2.5 Statistical analysis

GraphPad Prism version 10.3.1 software was used for the statistical analysis of the study, to dissect the complex interplay between prey density, diversity, and habitat characteristics.
After confirming normality and homoscedasticity, variance analyses were performed. ANOVA (Analysis of Variance) test was applied to assess differences among multiple groups. To pinpoint specific group differences, we followed up with Tukey's post hoc test. This allowed to identify which pairs of groups significantly differed from each other ($p<0.05$). Additionally, t-tests were performed to further compare specific pairs of groups where relevant, ensuring a comprehensive assessment of significant differences ($p<0.05$).

4. Results

4.1 Isolated feed consumption in diverse habitats

Seagrass habitat

Under normal density conditions, mysid consumption was significantly higher ($p<0.05$) at 7.2% BW day^{-1}, compared to amphipod and shrimp consumption at 4.9% and 4.7% BW day^{-1}, respectively (*Figure 4.1a*). In low-density conditions, however, no significant differences ($p>0.05$) were observed among the three feed types. Mysid consumption decreased to 5.2% BW day^{-1}, while amphipod consumption remained similar to normal density at 4.8% BW day^{-1}, and shrimp consumption increased slightly to 5.5% BW day^{-1} (*Figure 4.1b*). Notably, amphipod and shrimp consumption rates did not differ significantly from each other in either density condition ($p>0.05$).

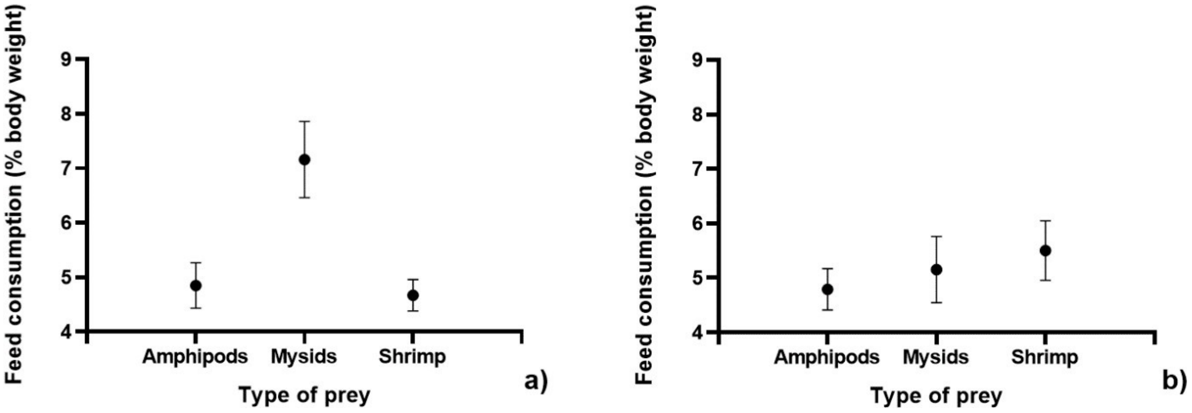


Figure 4.1 a) Feed consumption of amphipods (*Gammarus* spp.), mysids (*Diamysis lagunaris*) and shrimp (*Palaemon varians*) in the seagrass habitat under normal density conditions and b) under low-density conditions.

<u>Sediment with *Codium* spp. habitat</u>

In this habitat, mysid consumption was significantly higher ($p < 0.05$) compared to both amphipod and shrimp consumption under both normal and low-density conditions. Specifically, mysid consumption was 7% BW day^{-1} in the normal density condition, compared to 2.9% for amphipods and 4.3 % BW day^{-1} for shrimp (*Figure 4.2a*). In the low-density condition, mysid consumption was 9.4% BW day^{-1}, while amphipod and shrimp consumption were 3.9% and 4.7% BW day^{-1}, respectively (*Figure 4.2b*). No significant differences ($p > 0.05$) were observed between amphipod and shrimp consumption rates.

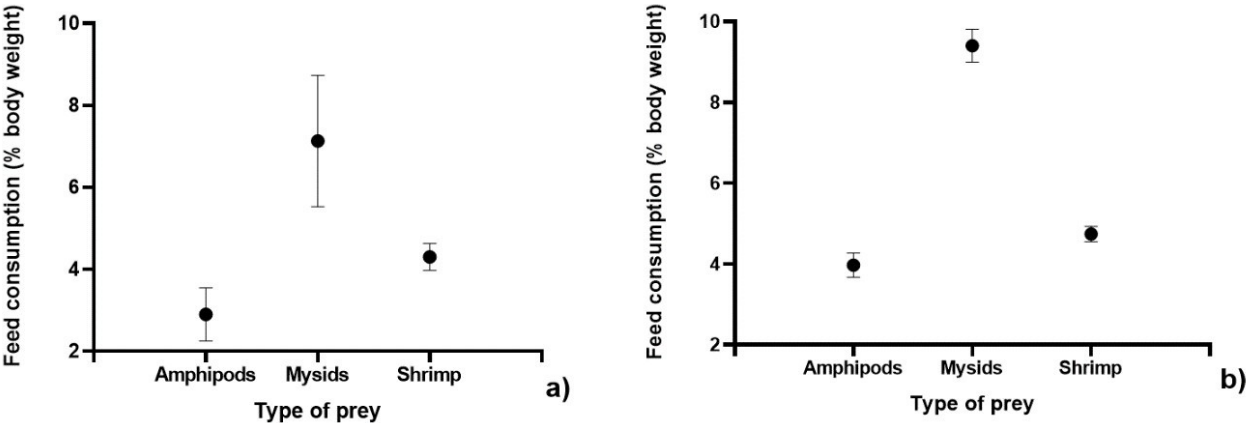


Figure 4.2 a) Feed consumption of amphipods, mysids, and shrimp in the sediment with *Codium*spp. habitat under normal density conditions and b) under low-density conditions

Caulerpa prolifera habitat

Like in the sediment habitat, in this trial, mysid consumption was significantly higher ($p < 0.05$) compared to both amphipod and shrimp consumption under both normal and low-density conditions. Specifically, in the normal density condition, mysid consumption was the highest at 7.4% BW day^{-1}, compared to 4.6% BW day^{-1} for amphipods and 4.2% BW day^{-1} for shrimp (*Figure 4.3a*). Under low-density conditions, mysid consumption further increased to 9.5% BW day^{-1}, while amphipod and shrimp consumption remained lower at 4.6% and 4.3% BW day^{-1}, respectively (*Figure 4.3b*). No significant differences ($p>0.05$) were observed between amphipod and shrimp consumption rates in both densities.

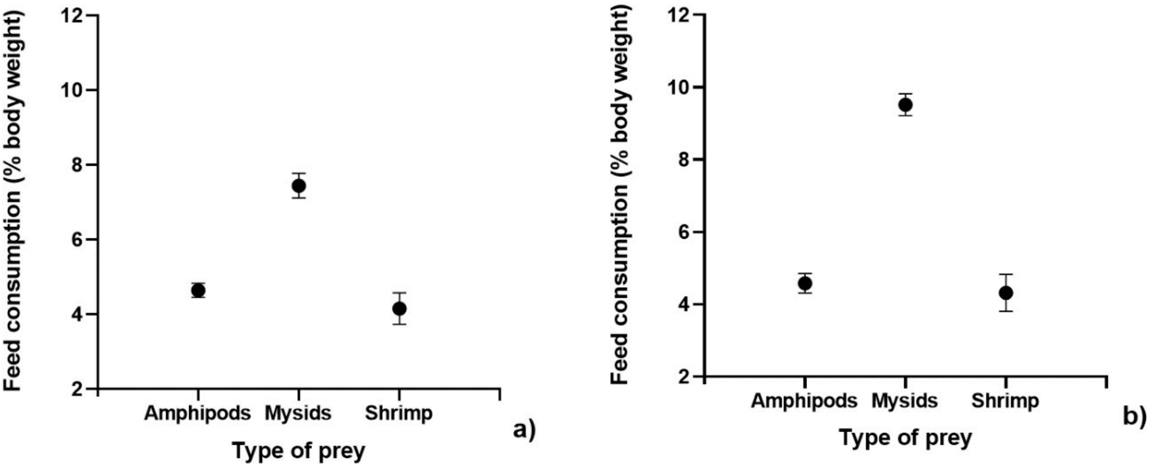


Figure 4.3 a) Feed consumption of amphipods, mysids and shrimp in the *C. prolifera* habitat under normal density conditions and b) under low-density conditions

4.2 Comparative analysis of the three live feeds given simultaneously across the habitats

4.2.1 Simultaneous feed consumption of the three prey items in diverse habitats

Seagrass habitat

When all three prey types were offered simultaneously in the normal seagrass habitat density, mysid consumption was significantly higher ($p<0.05$) compared to both amphipods and shrimp. Mysids were consumed at a rate of 9.2% of body weight per day (BW day^{-1}), while amphipods and shrimp had lower consumption rates of 3.4% and 3.7% BW day^{-1}, respectively (*Figure 4.4a*). No significant difference ($p>0.05$) was found between the consumption of amphipods and shrimp.

Under low seagrass density conditions, significant differences ($p<0.05$) were observed in the consumption rates of the three prey types. Mysid consumption was 7.3% BW day^{-1}, while amphipods and shrimp were consumed at rates of 4% and 3.4% BW day^{-1}, respectively (*Figure 4.4b*).

Additionally, the pairwise comparison of the consumption rates for each prey type across the two habitat densities revealed significant differences ($p<0.05$) for all prey types (amphipods, mysids, and shrimp).

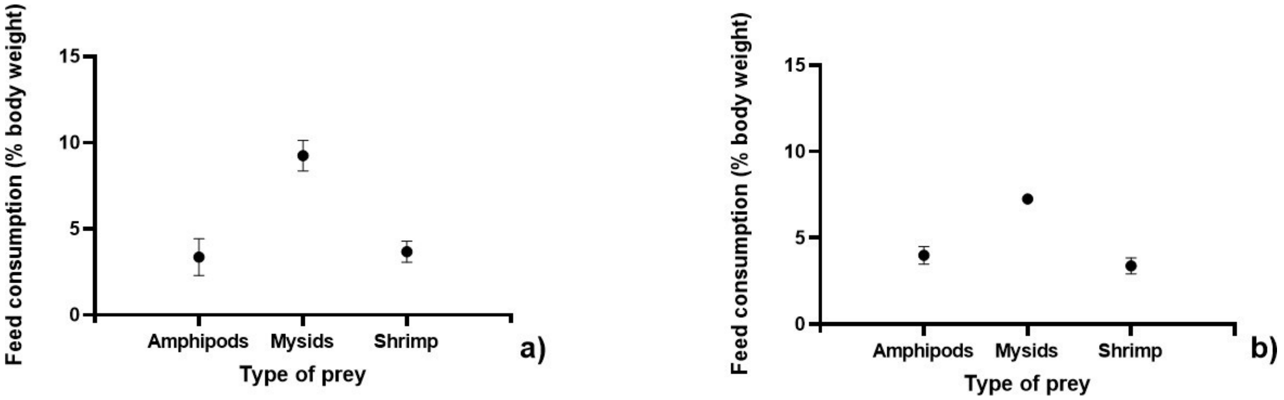


Figure 4.4 a) Feed consumption of amphipods, mysids and shrimp simultaneously in the seagrass habitat under normal density conditions and b) under low-density conditions

Sediment with *Codium* spp. habitat

In the sediment habitat, significant differences ($p<0.05$) were observed between the consumption rate of the three prey types when offered simultaneously at both densities.

At normal habitat density (*Figure 4.5a*), mysids were consumed at 6.8% BW day^{-1}, while amphipods and shrimp showed lower consumption rates of 2.2% and 4% BW day^{-1}, respectively. In low-density conditions (*Figure 4.5b*), mysid consumption increased to 8% BW day^{-1}, while amphipods and shrimp were consumed at 2.4% and 2.9% BW day^{-1}, respectively. In the pairwise comparison of the consumption rates for each prey type across the two habitat densities significant differences ($p<0.05$) were observed for all prey types (amphipods, mysids, and shrimp).

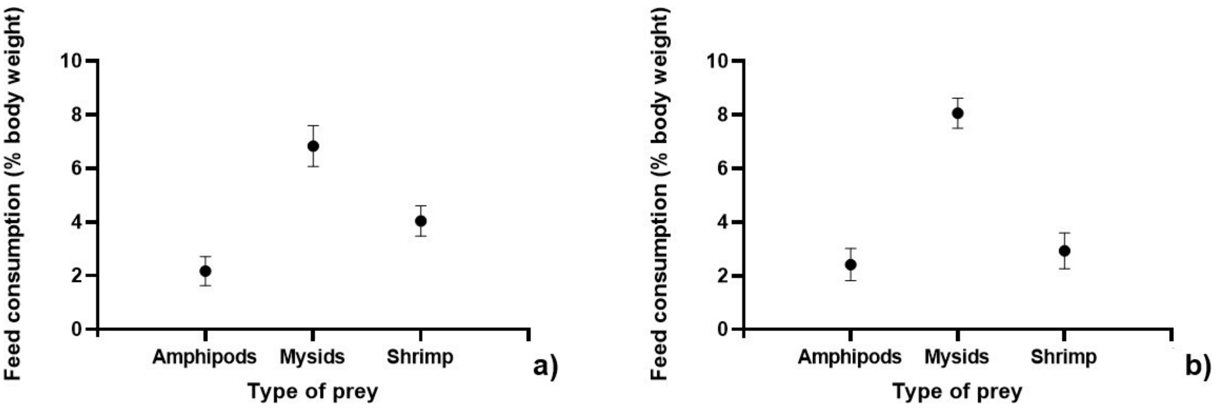


Figure 4.5 a) Feed consumption of amphipods, mysids and shrimp simultaneously in the sediment with *Codium* spp. habitat under normal density conditions and b) under low-density conditions

<u>*C. prolifera* habitat</u>

When presented simultaneously in the normal *C. prolifera* habitat density, mysid consumption was significantly higher ($p<0.05$) compared to both amphipods and shrimp. Mysids were consumed at a rate of 7.8% of body weight per day (BW day^{-1}), while amphipods and shrimp had lower consumption rates of 2.9% and 3.2% BW day^{-1}, respectively (*Figure 4.6a*). No significant difference ($p>0.05$) was found between the consumption of amphipods and shrimp. Under low seagrass density conditions, significant differences ($p<0.05$) were observed in the consumption rates of the three prey types. Mysid consumption was 9% BW day^{-1}, while amphipods and shrimp were consumed at rates of 2.4% and 1.5% BW day^{-1}, respectively (*Figure 4.6b*).

Finally, in the pairwise comparison of the consumption rates for each prey type across the two habitat densities significant differences ($p<0.05$) between all prey types (amphipods, mysids, and shrimp) were observed.

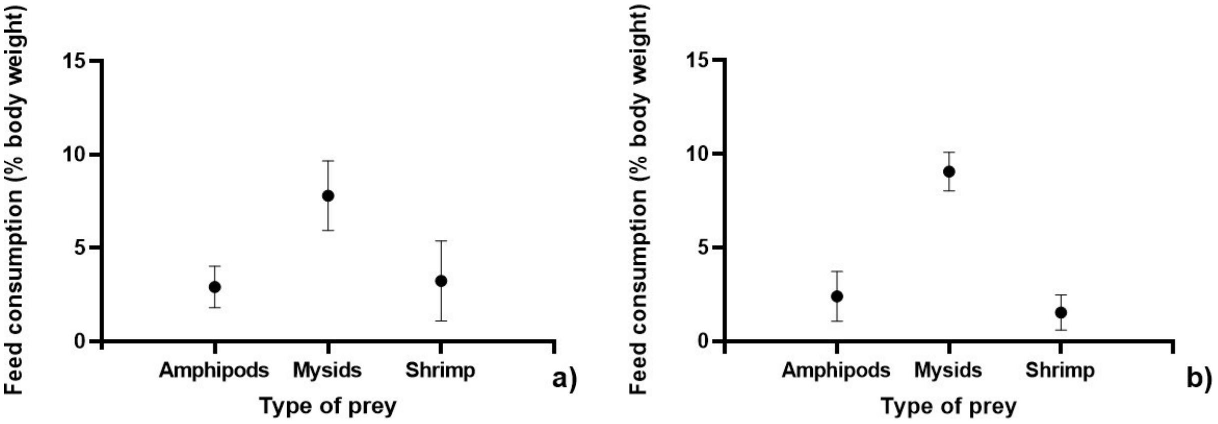


Figure 4.6 a) Feed consumption of amphipods, mysids and shrimp simultaneously in the *C. prolifera* habitat under normal density conditions and b) under low-density conditions

4.2.2 Overall food intake with multi-feed assessment across different habitats

<u>Seagrass habitat</u>

When analyzing the contribution of each prey item to the total feed consumption, it was observed that at normal seagrass density, mysids made up 56.8% of the total feed consumed, while at low density, they constituted 49.6% of the seahorses' diet (*Figure 4.7*). This was significantly higher (p<0.05) than the contribution of amphipods and shrimp to the diet in both conditions. Amphipods had the lowest consumption, comprising 20.7% of the total intake at normal density but showing a significant increase (p<0.05) to 27.3% at low density. Shrimp consumption remained relatively stable, accounting for 22.6% and 23.1% of the total intake at normal and low densities, respectively, with no significant differences (p>0.05) between the two densities (*Figure 4.7*).

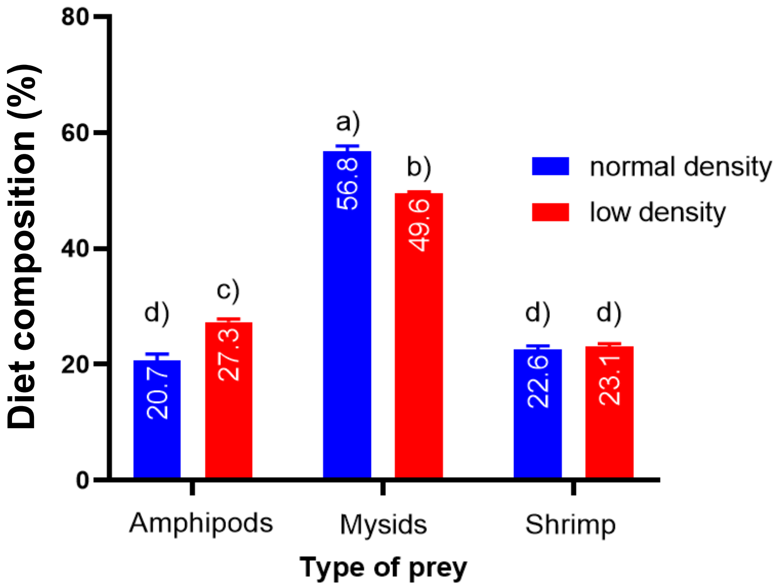


Figure 4.7 Feed consumption of the three live feeds (amphipods, mysids and shrimp) at normal and low-density conditions in the seagrass habitat. Column values with different superscripts are significantly different (p<0.05)

Sediment with *Codium* spp. habitat

In the sediment habitat, mysids were again the preferred prey across both density conditions. At normal density, mysids made up 52.4% of the total feed consumed, while at low density, they accounted for 60.2% of the seahorses' diet (*Figure 4.8*). These contributions were significantly higher (p<0.05) than those of amphipods and shrimp under both conditions. Amphipods had the lowest consumption, comprising 16.6% of the total intake at normal density and 18% at low density, with no significant difference (p>0.05) between the two (Figure 4.8). Shrimp consumption, however, varied significantly (p<0.05) between density conditions, making up 31% of the total feed intake at normal density and 21.9% at low density (*Figure 4.8*).

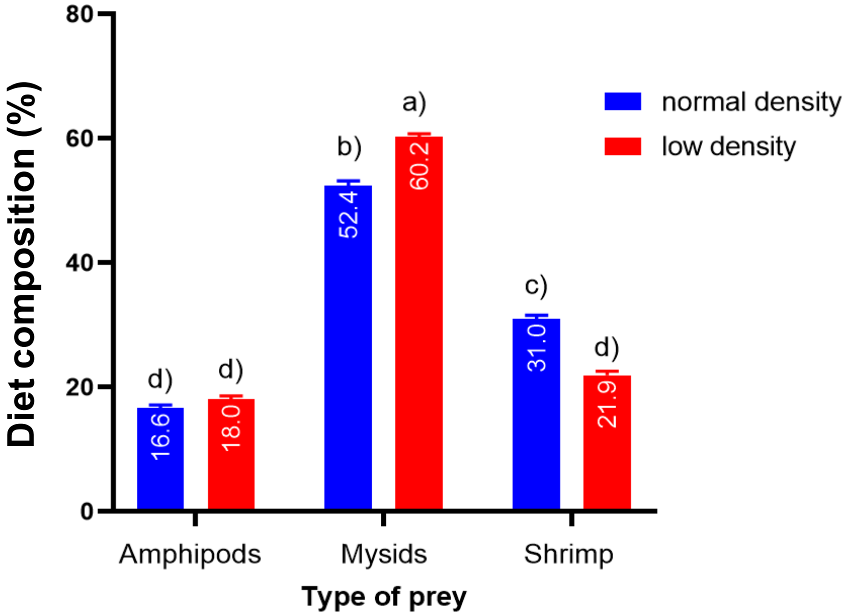


Figure 4.8 Feed consumption of the three live feeds (amphipods, mysids and shrimp) at normal and low-density conditions in the sediment with *Codium* spp. habitat. Column values with different superscripts are significantly different (p<0.05)

C. prolifera habitat

In the *C. prolifera* habitat, mysids were again the preferred prey, as observed in the previously tested habitats, comprising 55.9% of the total feed intake at normal density. At low density, mysids made up 69.7% of the seahorses' diet (*Figure 4.9*), a significantly higher proportion compared to the other two prey types ($p < 0.05$). Amphipods were consumed at relatively low levels, accounting for 20.9% of the total intake at normal density, with a slight decrease to 18.5% at low density. Shrimp consumption varied significantly between density conditions, comprising 23.2% of the diet at normal density and decreasing to 11.8% at low density ($p < 0.05$) (*Figure 4.9*).

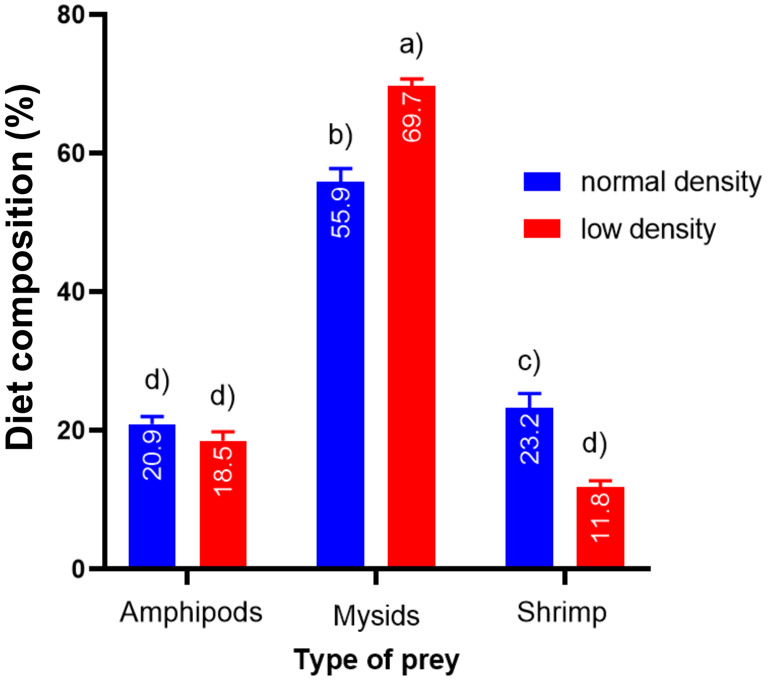


Figure 4.9 Feed consumption of the three live feeds (amphipods, mysids and shrimp) at normal and low-density conditions in the *C. prolifera* habitat. Column values with different superscripts are significantly different ($p<0.05$)

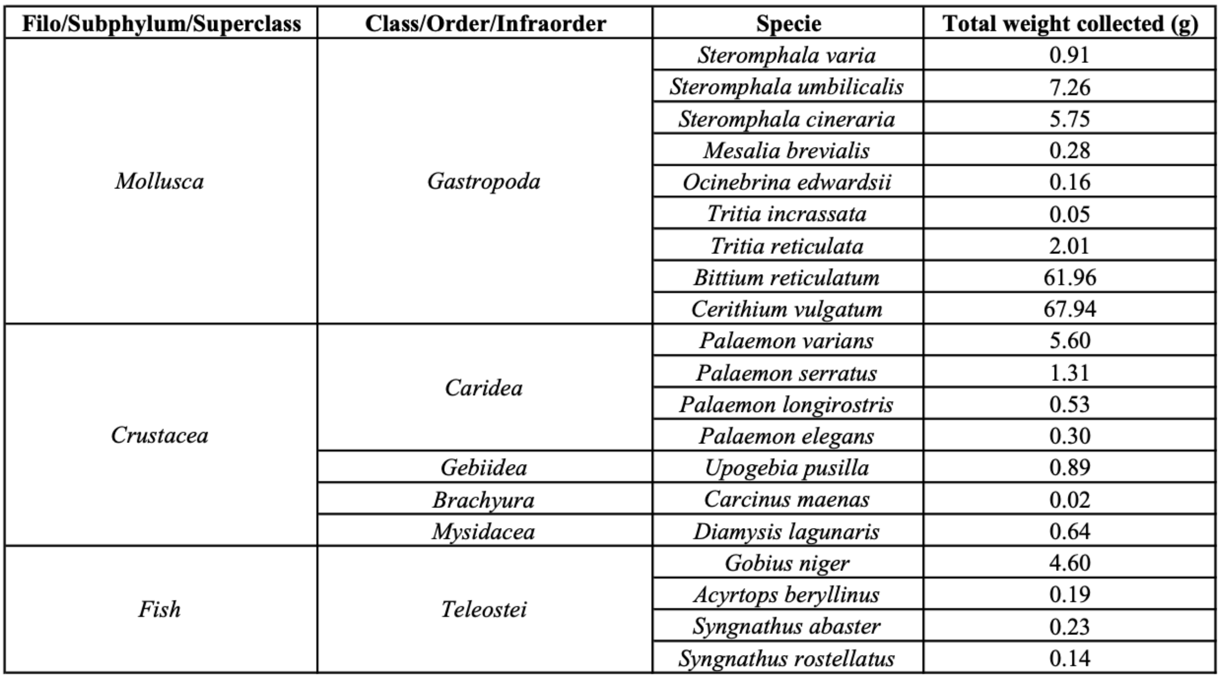
4.3 Comparative analysis of habitat samples from the Ria Formosa

Species identification

In the seagrass habitat, 20 species were identified: 9 species of mollusks, 7 species of crustaceans, and 4 species of fish (Table 4.1), representing 45%, 35%, and 20% of the species observed, respectively, and contributing 91%, 5.8%, and 3.2% of the total biomass in the collected samples.

The most abundant species, *Cerithium vulgatum* and *Bittium reticulatum*, indicate a high presence of these gastropods in the seagrass beds, though their abundance may not directly reflect the diet of seahorses. More relevant to the diet of *H. hippocampus* are the crustaceans, with *Palaemon varians* being the most abundant species. Caridean shrimp comprised 83.3% of the total crustacean biomass, while *Gebiidae*, *Brachyura*, and *Mysidacea* contributed 9.6%, 0.2%, and 6.9%, respectively.

Table 4.1 Species composition in seagrass habitat samples



Filo/Subphylum/Superclass	Class/Order/Infraorder	Specie	Total weight collected (g)
Mollusca	*Gastropoda*	*Steromphala varia*	0.91
		Steromphala umbilicalis	7.26
		Steromphala cineraria	5.75
		Mesalia brevialis	0.28
		Ocinebrina edwardsii	0.16
		Tritia incrassata	0.05
		Tritia reticulata	2.01
		Bittium reticulatum	61.96
		Cerithium vulgatum	67.94
Crustacea	*Caridea*	*Palaemon varians*	5.60
		Palaemon serratus	1.31
		Palaemon longirostris	0.53
		Palaemon elegans	0.30
	Gebiidea	*Upogebia pusilla*	0.89
	Brachyura	*Carcinus maenas*	0.02
	Mysidacea	*Diamysis lagunaris*	0.64
Fish	*Teleostei*	*Gobius niger*	4.60
		Acyrtops beryllinus	0.19
		Syngnathus abaster	0.23
		Syngnathus rostellatus	0.14

In the sediment habitat, 30 species were identified: 16 species of molluscs (13 *Gastropoda*, 2 *Polyplachophora*, 1 *Cephalopoda*), 8 species of crustaceans, 3 species of fish, 1 species of echinoderm, and 2 species of annelids (Table 4.2), representing 53.3%, 26. 7%, 10%, 3.3%, and 6. 7% of the species observed, respectively, and contributing 97.11%, 2.65%, 0.10%, 0.001%, and 0.14% of the total biomass in the collected samples.

The most abundant species were *C. vulgatum* and *Mesalia brevialis*, indicating a high presence of these gastropods in the sediment habitat. Molluscs dominated the biomass, with gastropods alone accounting for 96.89% of the total biomass collected. However, their abundance may not directly reflect the diet of seahorses.

Again and more relevant to the diet of *H. hippocampus*, within the crustaceans, *Diogenes pugilator* (*Anomura*) was the most abundant species, comprising 46.3% of the total crustacean biomass. The distribution of crustacean biomass was as follows*:* *Anomura* 46.3%, *Brachyura* 32.7%, *Amphipoda* 14.2%, *Gebiidae* 4.1%, *Caridea* 2.4%, and *Mysidacea* 0.4%.

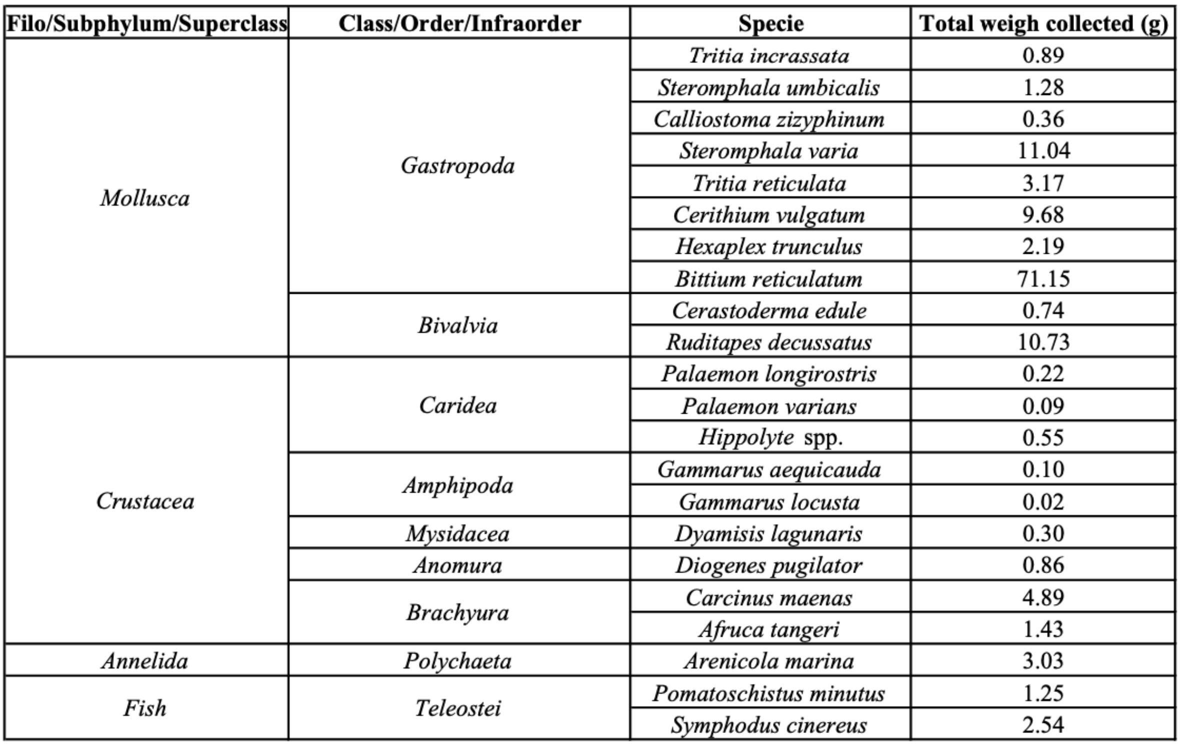
Table 4.2 Species composition in sediment with *Codium* spp. habitat samples

Filo/Subphylum/Superclass	**Class/Order/Infraorder**	**Specie**	**Total weight collected (g)**
Mollusca	*Gastropoda*	*Mesalia brevialis*	132.2
		Tritia reticulata	39.13
		Steromphala varia	5.63
		Cerithium vulgatum	280.2
		Tritia incrassata	3.70
		Hexaplex trunculus	16.44
		Conus ventricosus	1.69
		Calliostoma zizyphinum	1.85
		Tritia neritea	1.08
		Bittium reticulatum	116.7
		Steromphala umbilicalis	88.55
		Ruditapes decussatus	9.19
		Polititapes aureus	0.93
	Polyplachophora	*Rhyssoplax olivacea*	0.39
		Rhyssoplax corallina	0.06
	Cephalopoda	*Sepia officinalis*	0.28
Crustacea	*Brachyura*	*Pachygrapsus marmoratus*	0.20
		Carcinus maenas	6.04
	Mysidacea	*Dyamisis lagunaris*	0.07
	Caridea	*Hippolyte* spp.	0.40
		Palaemon longirostris	0.05
	Amphipoda	*Gammarus aequicauda*	2.70
	Anomura	*Diogenes pugilator*	8.82
	Gebiidae	*Upogebia pusilla*	0.78
Echinodermata	*Asteroidea*	*Hymenodiscus coronata*	0.01
Annelida	*Polychaeta*	*Ophelia bicornis*	0.81
		Arenicola marina	0.17
Fish	*Teleostei*	*Pomatoschistus minutus*	0.54
		Atherina presbyter	0.08
		Syngnathus abaster	0.11

In the *C. prolifera* habitat, 22 species were identified: 10 species of molluscs, 9 species of crustaceans, 1 species of annelid, and 2 species of fish (Table 4.3), representing 45.5%, 40.9%, 4.6%, and 9.1% of the species observed, respectively, and contributing 87.9%, 6.7%, 2.4%, and 3% of the total biomass in the collected samples.

The most abundant species, *B. reticulatum* and *Ruditapes decussatus*, indicate a high presence of these molluscs in the *C. prolifera* habitat, though their abundance may not directly reflect the diet of the short-snout seahorses. As for the crustaceans, *Carcinus maenas* was the most abundant species and within this group, *Brachyura* comprised 74.7% of the total crustacean biomass, while *Caridea*, *Amphipoda*, *Mysidacea*, and *Anomura* contributed 10.2%, 1.4%, 3.6%, and 10.2%, respectively.

Table 4.3 Species composition in *C. prolifera* habitat samples



Filo/Subphylum/Superclass	**Class/Order/Infraorder**	**Specie**	**Total weigh collected (g)**
Mollusca	*Gastropoda*	*Tritia incrassata*	0.89
		Steromphala umbicalis	1.28
		Calliostoma zizyphinum	0.36
		Steromphala varia	11.04
		Tritia reticulata	3.17
		Cerithium vulgatum	9.68
		Hexaplex trunculus	2.19
		Bittium reticulatum	71.15
	Bivalvia	*Cerastoderma edule*	0.74
		Ruditapes decussatus	10.73
Crustacea	*Caridea*	*Palaemon longirostris*	0.22
		Palaemon varians	0.09
		Hippolyte spp.	0.55
	Amphipoda	*Gammarus aequicauda*	0.10
		Gammarus locusta	0.02
	Mysidacea	*Dyamisis lagunaris*	0.30
	Anomura	*Diogenes pugilator*	0.86
	Brachyura	*Carcinus maenas*	4.89
		Afruca tangeri	1.43
Annelida	*Polychaeta*	*Arenicola marina*	3.03
Fish	*Teleostei*	*Pomatoschistus minutus*	1.25
		Symphodus cinereus	2.54

Physiological condition assessment

The condition factor (CF) of the fish varied slightly across the different habitats tested during the trials. In the first trial, where different prey were provided individually, the CF ranged from 0.19% to 0.23% (*Table 4.4*). In the second trial, where different prey were offered in combination, the CFranged from 0.18% to 0.20% (*Table 4.5*).

Table 4.4 Condition Factor (CF) of *H. hippocampus* in different habitats (seagrass, sediment with *Codium* spp., and *C. prolifera*) with single feed types (amphipods, mysids, and shrimp)

Habitat	Type of feed	CF (Condition Factor) (%)
Seagrass	Amphipods	0.23
	Mysids	0.21
	Shrimp	0.2
Sediment	Amphipods	0.22
	Mysids	0.19
	Shrimp	0.2
Caulerpa	Amphipods	0.22
	Mysids	0.2
	Shrimp	0.2

Table 4.5 Condition Factor (CF) of *H. hippocampus* in different habitats (seagrass, sediment with *Codium* spp., and *C. prolifera*) with three feeds simultaneously (amphipods, mysids, and shrimp)

Habitat	Type of feed	CF (Condition Factor) (%)
Seagrass	A, M, S	0.18
Sediment	A, M, S	0.2
Caulerpa	A, M, S	0.19

Our analysis revealed that the seagrass habitat had the highest FAI at 0.165 g/m^2, followed by the sediment habitat at 0.063 g/m^2, and *C. prolifera* at 0.025 g/m^2 (*Table 4.6*). These values suggest varying levels of food availability across habitats, which likely influence the condition of wild seahorses in the Ria Formosa. The seagrass habitat appears to offer significantly more abundant food resources compared to the other two habitats, with over twice the FAI of the sediment and more than six times that of the *C. prolifera*.

In terms of total *Crustacean* biomass, the sediment habitat had the highest FAI at 0.376 g/m^2, while seagrass followed with an FAI of 0.183 g/m^2, and *C. prolifera* was the lowest at 0.167 g/m^2 (*Table 4.6*).

Among the crustacean groups, *Caridea* had the highest FAI in seagrass environment (*Table 4.6*), at 0.153 g/m^2, with *C. prolifera* showing a lower value of 0.017 g/m^2, followed by the lowest FAI in the *Caridea*, on the sediment habitat, with 0.009 g/m^2.

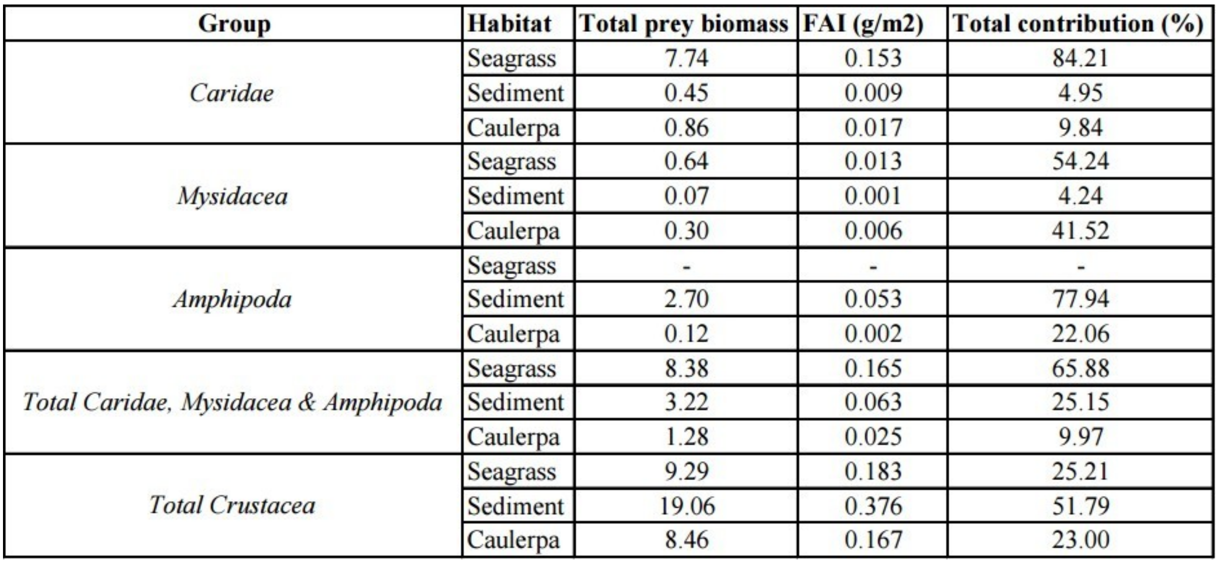
Mysidacea showed low FAIs across all habitats (*Table 4.6*), with values of 0.001 g/m^2 in the sediment habitat, 0.013 g/m^2 in the seagrass, and 0.006 g/m^2 in the *C. prolifera*.

Amphipoda contributed substantially to the sediment habitat with a value of 0.053 g/m^2, while the *C. prolifera* habitat only showed a FAI of 0.002 g/m^2. In the seagrass, no amphipods were registered (*Table 4.6*).

Finally, considering the total prey availability (*Table 4.6*), the analysis showed that the seagrass habitat accounted for 65.88% of the total prey availability, followed by the sediment habitat with 25.15%, and *C. prolifera* with 9.97%. In terms of the *Crustacea*, the sediment habitat contributed the most (51.8%), followed by the seagrass (25.2%), and *C. prolifera* (23%). For the *Caridea* group, the seagrass habitat had the highest contribution (84.2%), while sediment and *C. prolifera* contributed much less, at 5% and 9.85%, respectively. *Mysidacea*, although havinglow FAIs across all habitats, contributed 54.22% of their total biomass in seagrass, 4.25% in sediment, and 41.55% in *C. prolifera*. *Amphipoda* were most abundant in the sediment habitat,representing 77.95% of its total, with lower contributions in seagrass (12%) and *C. prolifera* (10.05%).

The sediment habitat stands out as a key area for overall prey biomass, particularly for *Amphipoda*, while seagrass plays a crucial role in supporting the highest abundance of *Caridea*.

Table 4.6 Prey biomass and percentage contribution of *Caridea, Mysidacea*, *Amphipoda*, and total Crustacea based on FAI (g/m^2)



Group	Habitat	Total prey biomass	FAI (g/m2)	Total contribution (%)
Caridae	Seagrass	7.74	0.153	84.21
	Sediment	0.45	0.009	4.95
	Caulerpa	0.86	0.017	9.84
Mysidacea	Seagrass	0.64	0.013	54.24
	Sediment	0.07	0.001	4.24
	Caulerpa	0.30	0.006	41.52
Amphipoda	Seagrass	-	-	-
	Sediment	2.70	0.053	77.94
	Caulerpa	0.12	0.002	22.06
Total Caridae, Mysidacea & Amphipoda	Seagrass	8.38	0.165	65.88
	Sediment	3.22	0.063	25.15
	Caulerpa	1.28	0.025	9.97
Total Crustacea	Seagrass	9.29	0.183	25.21
	Sediment	19.06	0.376	51.79
	Caulerpa	8.46	0.167	23.00

5. Discussion

The feeding behavior and dietary preferences of seahorses are known to vary significantly across different habitats, influenced by factors such as structural complexity and prey availability. More structurally complex habitats, such as seagrass meadows, can provide increased shelter for predators, allowing them to ambush prey more effectively, while simpler habitats may expose prey, affecting their availability and consumption patterns. Understanding how these habitat characteristics influence feeding strategies is critical forinterpreting species-specific dietary behaviors. These variations play a crucial role in shapingthe ecological dynamics of seahorse populations.

Generally, seahorse species have been observed to consume a variety of small crustaceans, with preferences often reported for mysids, amphipods, and small shrimp (Yip et al., 2014) across different species and regions. Building upon this broader context, our study focused specifically on *H. hippocampus*, providing a comprehensive understanding of its feeding behavior across different habitats. The findings from this study reveal significant insights into daily food consumption and prey preference dynamics, demonstrating that *H. hippocampus* exhibits varying levels of prey consumption depending on both the habitat and prey type, with consistent patterns in the preference for mysids over amphipods and shrimp in most conditions.

The results from the first trial of the study when isolated feed consumption across different structural habitats was evaluated, showed that mysid consumption was consistently higher compared to amphipods and shrimp, indicating a clear preference for this prey type. This pattern was particularly evident in the seagrass habitat under normal density conditions, where mysid consumption significantly exceeded that of both amphipods and shrimp. Under those density conditions, the complexity of seagrass offers seahorse structural cover, allowing them to ambush prey unnoticed (Manning et al., 2019). In the sediment habitat, for instance, mysid consumption appeared to increase under low-density conditions, suggesting that seahorses may intensify their feeding on preferred prey benefiting from lower habitat complexity which, in this case, may imply a bigger exposure of this specific prey.

Amphipods also play a vital role in the diet of *H. hippocampus*, as noted in the first trial.These organisms are widely distributed in marine environments and serve as an essentialsource of nutrition due to their high protein content and availability. Their small size andshape makes them easily accessible prey for seahorses (Storero & González, 2008), whichhave adapted their feeding strategies to capture such agile organisms. As noted by Woods (2002) in his analysis of *H. abdominalis*, the specific types of crustaceans consumed canreflect both the seahorse's predation technique and the habitat where the prey is found. Building on this, the results from this first trial showed that amphipods were consistentlyconsumed at lower rates compared to mysids across all habitat types and density conditions, though their consumption rates remained relatively stable, particularly in theseagrass habitat.

These findings are consistent with previous observations done by Bell & Harmelin-Vivien (1983). Although their study did not focus on the *Syngnathidae* family, the presence of amphipods in two fish species (*Coris Julius* and *Serranus cabrilla*) collected in the Gulf of Marseille (Mediterranean Sea) was noted, highlighting the role of amphipods in their diet. Considering the importance of amphipods in seahorse diets observed in the wild, their usehas been extended to captive feeding regimes. The consumption of amphipods by seahorses, as observed in this study, corroborates the findings of Kitsos et al. (2008), andalso Kwak et al. (2008), who observed that *Hippocampus mohnikei*, a species inhabiting eelgrass (*Zostera marina*) beds, also relies heavily on amphipods as a dietary component. Although amphipods were consumed at lower rates in this first trial, their presence in seahorses' diet is significant. This dietary inclusion, even at reduced levels, suggests that amphipods could be a valuable component in captive feeding programs. These observations bridge the gap between natural feeding behaviors and the development of effective captive diets for seahorses. Building on this foundation, recent research has further emphasized the importance of amphipods in seahorse diets. Vargas-Abúndez et al.(2018) observed high feeding activity of *H. erectus* when presented with frozen amphipods. Ina subsequent study, Vargas-Abúndez et al. (2021) demonstrated that juvenile H. erectus fed with frozen wild-captured amphipods (only or combined with *Artemia*) showed improved growth compared to those fed solely on enriched *Artemia*.

The analysis revealed significant differences in both the quantity of feed consumed and prey preferences among seahorses across the studied habitats. Specifically, seahorses in low-density conditions on the *C. prolifera* habitat exhibited a higher consumption rate of mysids compared to those in *C. nodosa*. The structural differences between these habitats likely play a significant role, as *C. prolifera* may provide a more open or accessible environment that facilitates the seahorses' ambush predation strategy, allowing them to capture prey more efficiently (Foster & Vincent, 2004). The feeding preferences observed in *H. hippocampus* align closely with the high energetic needs commonly found in *H. erectus* (Qin et al., 2018), as seahorses have specific metabolic requirements that dictate their prey choices.

In the second trial of the study, when the three prey types were offered simultaneously, mysid consumption remained the highest. However, a notable increase in overall food consumption was observed, suggesting that *H. hippocampus* is capable of diversifying its prey intake when a range of prey is available. For instance, in the seagrass habitat, significant differences in consumption were observed among the prey types. Mysids consistently dominated the diet, with a significantly higher consumption rate ($p<0.05$) compared to amphipods and shrimp under both normal and low-density conditions. At normal density, mysidconsumption was notably higher, reaching up to 9.2% BWd^{-1}, while at low-density, it decreasedslightly to 7.3% BWd^{-1}. Amphipods and shrimp were consumed at significantly lower rates, and their consumption patterns were less pronounced, especially under low-density conditions.The analysis of food intake (%) across different habitats revealed significant variations in consumption patterns, providing valuable insights into the feeding behavior and preferences ofthe *H. hippocampus*. Mysids, like in the feed consumption analysis, emerged as the dominant prey item across all the studied habitats, consistently comprising the majority of the diet (for example, in the seagrass habitat, mysids accounted for 56.8% of the diet). This preference for mysids was further accentuated under low-density conditions, particularly in the *C. prolifera* habitat where their consumption reached notably high levels (69.7%).

Despite this overall preference for mysids, variations in prey selection are evident when considering different environmental factors and habitat structures. For instance, while Kitsos et al. (2008) focal area was on the Aegean Sea, our research explores the varied environment within the Ria Formosa, offering a more intricate view of *H. hippocampus* feeding habits within this varied ecosystem.

Our findings also reveal that the short-snout seahorse adjusts its consumption rates based on prey density, with significant changes observed between normal and low-density conditions. For example, while mysids are consistently preferred across all habitats, except for the seagrass, their consumption increases significantly when the habitat density is lower. This indicates that *H. hippocampus* may prioritize certain types of prey when they are less available, potentially as a strategy to maximize energy intake.

The adaptability of *H. hippocampus* extends beyond just prey selection. Studies indicate that seahorses can modify their foraging behavior based on local prey abundance, which may enhance their foraging efficiency (Valladares et al., 2017). This ability to respond dynamically to variations in prey availability is crucial for their survival, particularly in fluctuating environments where food resources can be unpredictable. Such behavioral flexibility not only contributes to individual fitness but also plays a vital role in maintaining stable populations within diverse ecosystems.

Furthermore, the observations from this study highlight the critical role of habitat structure in influencing prey selection. The consistent patterns of prey consumption across different habitats, despite variations in prey availability, suggest that *H. hippocampus* can effectively exploit available resources, adjusting its feeding behavior to optimize survival in diverse environments (Spatafora et al., 2023).

This ability to modify its feeding behavior in response to local environmental conditions and prey availability may be a key factor in the survival of *H. hippocampus* across various marine ecosystems, potentially explaining its broad distribution despite specific habitat preferences.

Moreover, this study underscores the species' capacity to adapt its diet according to the availability of prey in specific habitats, suggesting that *H. hippocampus* may exhibit greater feeding flexibility than previously understood. The data demonstrate consistent feeding behaviors across various habitats, with a clear preference for certain types depending on environmental conditions.

The additional data gathered from wild habitats in the Ria Formosa has deepened our understanding of short-snout seahorse ecology. By pinpointing specific prey preferences in their natural environment, this research offers a more nuanced view of the ecological role that *H. hippocampus* plays within these ecosystems.

Interestingly, the consumption patterns of amphipods and shrimp varied across habitats and density levels. In the seagrass habitat, amphipod intake showed a moderate increase under low-density conditions, while shrimp consumption remained relatively stable.

Since mysids were consumed in higher proportions, seahorses may have shifted their feeding focus to this more abundant or preferred prey, reducing their intake of shrimp. As sedentary ambush predators, seahorses, like other sedentary species, are more dependent on local environmental conditions for successful feeding (Öckinger et al., 2009).In contrast, both the sediment with *Codium* spp. and *C. prolifera* habitats exhibited a different trend. Here, shrimp consumption decreased markedly under low-density conditions, while mysid intake increased. This shift in diet composition could be attributed to several factors, including changes in prey availability, altered foraging strategies, or differences in the energy efficiency of capturing and consuming different prey types.

The preference for mysids among *H. hippocampus* is likely due to their high nutritional content, making them a more efficient food source than alternatives like amphipods or shrimp (Otero-Ferrer et al., 2012). This preference becomes even more critical in low-prey environments, where seahorses must optimize energy intake while minimizing the effort spent on hunting, since they can easily capture the mysid shrimp and use them as an energy source (Segade et al., 2015). Recognizing these dietary needs is key to developing effective conservation strategies, ensuring the growth and reproductive success of seahorse populations.

In contrast with the *C. prolifera* previously mentioned, the *C. nodosa* habitat presents a different structural environment for seahorses. This seagrass species typically forms dense meadows with long, narrow leaves that can reach up around 30cm in length (Cancemi et al., 2002). Likewise, the complex seagrass structure recreated in the tanks may offer more shelter and camouflage opportunities for the seahorses, but it could also influence the distribution and behavior of mysids, possibly making them less accessible to the seahorses. This interaction is supported by the findings of Yamada et al. (2012), who demonstrated that seagrass vegetation plays a crucial role in habitat partitioning among closely related *Neomysis* species. In their study, different *Neomysis* species showed distinct preferences for various zones within the seagrass bed, such as the canopy, mid-water, and near-bottom areas. This vertical stratification and species-specific habitat use within seagrass ecosystems suggest that the mysids species *D. lagunaris* observed in this study may adopt similar behaviors, potentially altering their availability as prey items for the short-snout seahorse. The seagrass structure might provide mysids with refuges from predation, allowing them to occupy specific niches within the vegetation that are less accessible to seahorses. Consequently, and as tested, a normal seagrass density could create a more complex predator-prey dynamic, where the benefits of increased shelter for seahorses might be partially offset by the reduced accessibility of their mysid prey. These structural characteristics of *C. nodosa* could explain the observed lower consumption rates of mysids when compared to the more open *C. prolifera* habitat (both at the same low-density conditions) and *C. nodosa* at low-density.

Additionally, the variations in the density across habitats (normal and low densities), also influenced feeding behavior. For instance, in more dense habitats, seahorses showed lower feed consumption rates, except for mysids in the seagrass habitat, which can also be explained by increased competition for food resources (Foster and Vincent, 2004). In higher density environments, seahorses may spend more time engaged in social interactions or competitive behaviors, reducing the time available for foraging (Masonjones et al., 2019). Furthermore, if the four individuals per tank (two males and two females), were clustered in a specific region, this could lead to localized depletion of prey items, forcing them to spend more energy searching for food, ultimately resulting in lower overall consumption rates in the normal density habitats since this *Syngnathidae* specie has an immobile or saltatory foraging behavior (Kendrick and Hyndes, 2005).

The dietary preferences of the short-snout seahorse are not unique among seahorses, with similar patterns observed across other *Hippocampus* species. For instance, Foster & Vincent (2004) report that crustaceans, particularly amphipods, are commonly consumed by various other species. Their research on *H. kuda* and *H. abdominalis* demonstrates a shared preference for highly mobile crustaceans, reinforcing the notion that prey availability and prey mobility are critical factors influencing seahorse diets. The similarity in prey choice across seahorse species suggests that these *Syngnathidae* have evolved specific feeding adaptations suited to their ambush predator strategy. However, the flexibility in prey choice observed in *H. hippocampus* across different habitats highlights its ability to adapt its feeding behavior to the specific environmental conditions, which may confer a survival advantage over more specialized species.

The observed dietary plasticity across habitats and density levels highlights the species' adaptability to varying environmental conditions. This flexibility in feeding behavior could be a key factor in the species' ability to thrive in diverse aquatic ecosystems. Furthermore, these findings underscore the complex interplay between habitat structure, prey availability, and population density in shaping the feeding ecology of aquatic organisms.

Regarding food preference, the study highlights that mysids are consistently favored by *H. hippocampus* across all habitats. This preference is evident from the consistently higher consumption rates of mysids compared to amphipods. Specifically, mysid consumption increases significantly in low-density habitats, likely due to the lower structural complexity of these environments. In such conditions, prey like mysids are more exposed to predators, as they have less capacity to evade predation (Ritz et al., 1997), compared to shrimp and amphipods, which exhibit better avoidance behaviors (Main, 1987; Pennuto & Keppler, 2007).

Given that mysids are a preferred prey item, and their consumption rates are higher in all habitat types, identifying and protecting habitats with the highest abundance of mysids is crucial. Our data suggests that habitats with greater mysid populations should be prioritized for conservation efforts. For example, despite providing food amounts in the experimental tanks that were calculated to be equivalent to several square meters of wild habitat in the Ria Formosa, the resulting condition factors (CF) of captive seahorses did not necessarily reflect what might be expected based solely on the food abundance index (FAI) measured in the wild. While the seagrass habitat in the Ria Formosa had the highest recorded FAI (0.165 g/m^2), seahorsesin tanks mimicking this habitat showed CF values ranging from 0.18% to 0.23%. These CF values are comparable to those reported by Woods (2005) for the species *H. abdominalis*, which found CF values of 0.19% and 0.21% for healthy seahorses incaptivity. However, considering the significantly lower wild FAI in the *C. prolifera* habitat(0.025 g/m^2), it is unlikely that seahorses in their natural environment would achieve identical CF values. The disparity between experimental and wild conditions suggests thatseahorses in their natural habitats, particularly in areas with lower prey abundance, mayface greater challenges in maintaining optimal condition factors.

This discrepancy may be attributed to the composition of available prey species within these habitats. In the seagrass habitat samples analyzed, 20 species were identified, including 9 species of mollusks, 7 of crustaceans, and 4 of fish. Among these, crustaceans—particularly *P. varians*—were critical to the seahorse diet, with Caridean shrimp comprising a significant portion of the total crustacean biomass. In the sediment habitat samples, although 30 species were identified, only crustaceans were relevant to the short-snout seahorse diet, accounting for a substantial percentage of total crustacean biomass. Similarly, in the *C. prolifera* habitat samples, 22 species were recorded, with crustaceans being particularly significant; *Brachyura* made up a large proportion of the crustacean biomass. These results suggest that while food abundance is a crucial factor influencing seahorse condition in the wild, other elements may also play a role. The physical condition of an animal largely depends on food consumption, but factors such as habitat quality, environmental stressors, predation pressure, and reproductive state can also affect energy allocation and overall condition.

The study underscores the critical need to protect and restore habitats that support preferred prey species of the short-snout seahorse but also maintain a diverse and abundant prey community. Our findings reveal that when presented with a variety of preyoptions, seahorses increased their overall food consumption, demonstrating their ability to use multiple prey types beyond their primary preference. This dietary flexibility highlights the importance of preserving habitats that harbor a higher biomass anddiversity of potential prey species. Such habitats can better ensure that seahorses have access to sufficient nutritional resources to meet their energetic needs, even when their preferred prey might be less abundant. Therefore, conservation efforts should focus on maintaining ecosystem complexity that supports a rich prey community, rather than solely emphasizing habitats with a single preferred prey species.

The invasive algae *C. prolifera*, introduces additional complexity, as its colonization of unvegetated soft bottoms represents a significant ecological shift with potential long-term consequences for trophic interactions within the ecosystem (Parreira et al., 2021). The impact of this invasive algae on *H. hippocampus* feeding behavior is likely multifaceted. While our study showed that seahorses in low-density *C. prolifera* habitats exhibited higher consumption rates of mysids compared to those in *C. nodosa*, this observation reveals a more nuanced ecological dynamic. Low-density *C. prolifera* habitats share similarities with sediment and seagrass habitats, creating an environment that seahorses can effectively use for foraging. This contrasts with normal density *C. prolifera* habitats, where the increased structural complexity may limit food consumption. These findings suggest that habitat structure plays a crucial role in determining seahorse foraging success, with moderately complex environments potentially offering an optimal balance between prey availability and accessibility.

The reduction in some prey populations due to *C. prolifera* invasion could force the short-snout seahorse in the wild to alter their prey preferences, potentially leading to increased reliance on a specific prey item (Parreira et al., 2021).

The invasion of this algae also presents a significant conservation challenge. While seahorses may show some adaptability in their feeding behavior, the overall reduction in prey diversity and abundance due to anoxic sediment created by *C. prolifera*, could have long-term negative impacts on seahorse populations (Parreira et al., 2021).

Moreover, the ongoing challenges posed by climate change, including ocean acidification and rising sea temperatures, are likely to intensify these disruptions in prey availability and the overall quality of habitats. As noted in a review by Aurélio et al. (2013), increasing temperatures due to environmental warming could impact the physiology and behavior of temperate seahorses (in the case of that review, *H. guttulatus*). The rising temperatures affect seahorse metabolic rates and potentially their survival, as these environmental pressures not only threaten to reduce the availability of prey but may also degrade the structural complexity of habitats crucial for *H. hippocampus*, which is vital for their survival and reproductive success.

In comparison to other prior research, particularly the study by Kitsos et al. (2008) already mentioned previously, some parallels are evident. Both studies highlight the importance of crustaceans, especially amphipods and mysids, in the diet of *H. hippocampus* and the influence of habitat structure on seahorse behavior and prey selection is apparent in both studies, with habitat-specific patterns observed.

Grasping these ecological interactions is essential for the successful management and conservation of seahorse populations. The study underscores the importance of protecting not just the species but also the ecosystems they rely on. This aligns with the findings of Correia et al. (2015), who observed significant population fluctuations of seahorses in the Ria Formosa lagoon, highlighting the need for comprehensive ecosystem-based conservation approaches. The findings carry significant implications for seahorse conservation, specifically for the short-snout specie. Habitat-specific feeding behaviors observed suggest that conservation strategies must prioritize the preservation of habitats that support the seahorses' preferred prey species. Moreover, since certain habitats are found to be more favorable for sustaining seahorse populations, focused protection of these areas could improve conservation outcomes, especially as environmental changes and habitat degradation continue to pose threats. This is supported by the work of Caldwell and Vincent (2012), who reported a significant decline in seahorse populations in the Ria Formosa, with a 94% decrease in *H. guttulatus* and a 73% in *H. hippocampus,* highlighting the critical link between habitat quality and seahorse survival. Several conservation strategies can be drawn from the results of this study. These include safeguarding the critical habitats identified in the study, establishing Marine Protected Areas (MPAs) that correspond to seahorses' prey preferences and restoring degraded habitats like seagrass beds and regions with high sedimentation (Correia et al., 2015). Such efforts would help maintain the prey diversity essential for thriving seahorse populations (Caldwell and Vincent, 2012).

It is crucial to recognize the limitations of this study, particularly the potential environmental factors to influence feeding behavior. These elements may restrict the extent to which the findings can be applied to broader seahorse populations. Future research should aim to expand the study to cover different seasons and additional locations, which would help validate and enhance the understanding of *H. hippocampus* feeding ecology.

Furthermore, long-term monitoring could offer valuable insights into how shifts in habitat conditions affect seahorse diets over time.

6. Conclusion

The *H. hippocampus* feeding behavior is greatly influenced by its habitat, which also affects the prey preferences, intake rates, and feeding strategies. The feeding habits of seahorses vary significantly depending on the habitat, and the availability of prey and structural traits are important determinants of these changes. For instance, the dense structure of seagrass (*C. nodosa*) environments made it difficult for seahorses to access prey, which resulted in lower mysid consumption rates than in the more open habitat of *C. prolifera*, where seahorses showed greater mysid consumption rates.

The results also highlight how prey density affects feeding behavior. In all habitats, seahorses' preference for mysids remains constant, but their consumption dramatically increases in lower-density areas, indicating an adaptive approach to optimize energy intake when food is scarcer. Furthermore, lower feeding rates were caused by greater competition in denser environments, highlighting the significance of habitat quality and food availability in sustaining seahorse populations.

The study also emphasizes the effects of the invasive algae *C. prolifera*, which increases mysid intake in low-density environments but presents long-term ecological hazards since it reduces prey availability and degrades habitat. This invasion highlights the need for habitat restoration efforts to maintain prey diversity and habitat quality and complicates the feeding dynamics of seahorses.

These discoveries have important ramifications for conservation plans, especially in areas where invasive species and habitat deterioration endanger seahorse populations, such as the Ria Formosa lagoon. Prey-rich settings and seagrass meadows are examples of essential habitats that must be preserved for the *H. hippocampus* species to thrive. Mitigating the decline of these endangered species requires the development of Marine Protected Areas (MPAs) and the restoration of damaged habitats.

In summary, the feeding ecology of *H. hippocampus* is mostly dependent on habitat structure and prey availability, and our study demonstrates how adaptable the species is to a variety of environmental circumstances. Understanding these ecological interconnections is crucial for creating successful conservation plans. Future studies should concentrate on long-term observation in various locations and seasons to gain a fuller understanding of how environmental changes impact seahorse survival and feeding habits.

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