



## Can artificial holdfast units work as a habitat restoration tool for long-snouted seahorse (*Hippocampus guttulatus* Cuvier)?



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### ABSTRACT

The recent decline of the seahorse populations in the Ria Formosa lagoon could indicate the presence of a stressful factor due to habitat loss. Artificial structures have been successfully used as a recovery tool to cope with habitat degradation in many countries but none for this seahorse species (*Hippocampus guttulatus*). Four different artificial holdfasts (S1–S4) were tested in laboratory for seahorse preference under different conditions and different holdfast densities. Seahorses, both juveniles and adults, preferred the holdfast S4, consisting of a “*Codium*-like” polyethylene nautical rope, even when submitted to different water flows. Preferred holdfast density was 156 holdfast·m<sup>-2</sup>, and most of seahorses were observed grasping at the base of these structures (0–10 cm in height). This study provides preliminary data and promising results on an approach to designing artificial holdfasts for seahorses in low complexity damaged or depleted areas. The use of these structures may contribute to the settlement of seahorse populations, thus broadening their potential habitat as part of a wider restoration strategy.

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### 1. Introduction

Typically, seahorses have a sparse distribution, low mobility, small home ranges, low fecundity, lengthy parental care and mate fidelity, rendering them vulnerable to overfishing and habitat damage (Foster and Vincent, 2004). Most seahorse species use their prehensile tail as a means to grasp different holdfasts, from sponges to coral, seagrass, mangrove branches and even artificial structures (Foster and Vincent, 2004; Harasti et al., 2010; Hellyer et al., 2011). Although some seahorse species prefer a particular holdfast type (Rosa et al., 2007), others like *Hippocampus guttulatus* exhibit no obvious preference although occurring in seagrass dominated habitat (Curtis and Vincent, 2005).

The long snouted seahorse, *H. guttulatus*, is a European species, which occurs in the Ria Formosa lagoon, South Portugal, along with the short-snouted seahorse *Hippocampus hippocampus*. The greatest population size recorded for this species, throughout its range, was recorded in the early 2000's (Curtis and Vincent, 2005), however recent field data showed a significant decrease in seahorse populations within this lagoon (94% and 73% for *H. guttulatus* and *H. hippocampus* respectively) (Caldwell and Vincent, 2012). Although the causes for such declines remain unknown, human related activities (fisheries, including illegal fishing, anchoring and dredging) and natural changes in the Ria's dynamics (e.g. silting events and shifting currents), may be the main

causes for an overall habitat loss (Curtis et al., 2007). In the Ria Formosa, some natural *Zostera noltii* beds have been replaced by clam farms (Guimarães, Cunha, Nzinga, Marques, 2012), harbors, industries and coastal constructions, or dredged to open and maintain navigation channels, such as the opening of a new inlet in Fuseta island and channel dredging that are destroying vast areas of this species (Cunha, Assis, Serrão, 2013). These anthropogenic activities and natural events are known to alter the seahorses' habitat conditions and reduce the amount of natural holdfast available, essential for seahorse settlement (2010, Correia pers. orbs.). Although *H. guttulatus* is not exclusively found in seagrass beds, this type of habitat is one of the most important as for feeding and protection of this species (Curtis and Vincent, 2005). Considering that the lack of holdfast availability may explain the disappearance of some populations within the Ria (Curtis et al., 2007), the use of artificial holdfasts could help increase the habitat complexity that would encourage seahorse population settlement and potentially reduce the declines.

Increased habitat complexity has been recognized to have a positive influence on the diversity and abundance of marine organisms (Silvertown, 2004). Structural complexity can be provided by plants in ecosystems by density and form (Silvertown, 2004). Diversity and abundance of marine species is usually greater in seagrass beds than in non-vegetated habitats (Edgar et al., 1994; Hutchings et al., 1991; Kirkman et al., 1991; Lee et al., 2001; Orth, 1992), as seagrass increases the amount of physical structures usable as living space, promotes the number of microhabitats in sediment deposition and stabilization, acts as a food resource and provides protection from predators (Lee et al.,

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2001). Many species of the Syngnathidae family, which include seahorses, pipefish and seadragons, have been found in higher densities inside seagrass beds rather than in non-vegetated areas (Bell et al., 2003; Diaz-Ruiz et al., 2000; Kendrick and Hyndes, 2005; Teixeira and Musick, 1995). Seagrasses are globally threatened marine habitats (Shepherd et al., 1989; Short and Wyllie-Echeverria, 1996; Spalding et al., 2003) that have been degraded primarily by human activities (Bell et al., 2002; Lee et al., 2001; Shahbudin et al., 2011) with a marked decline along some European coasts; i.e., *Zostera marina* in the Wadden Sea (Wolff, 2000) and *Posidonia oceanica* in the Corsican coast (Pasqualini et al., 1998, 1999), with previous areas of 8000 km<sup>2</sup> and 1400 km of seagrass beds, respectively.

In many countries, artificial seagrass has been used as a method to replace the damaged natural seagrass ecosystem providing marine habitat for various marine organisms, nursery ground for juveniles, and habitat and protection for small fishes (Kenyon et al., 1999; Lee et al., 2001; Shahbudin et al., 2011; Sogard, 1989; Sogard and Able, 1994). Different materials have been used to build these structures, from polypropylene/polyethylene and nylon ribbons to Dorken Advance Engineer Rubber (Fernandez et al., 2009; Hellyer et al., 2011; Lee et al., 2001; Shahbudin et al., 2011; Sirota and Hovel, 2006).

This study tested the holdfast preference for *H. guttulatus*, under controlled conditions, comparing different artificial structures that mimic the most important recorded natural holdfasts for this species, such as *Codium* spp. and seagrass (*Z. noltii*, *Z. marina* and *Cymodocea nodosa*). The results from this experiment will be used to inform guidelines for trialing artificial holdfast units in focal areas within the Ria Formosa lagoon as a mechanism to help the recovery of declining seahorse populations.

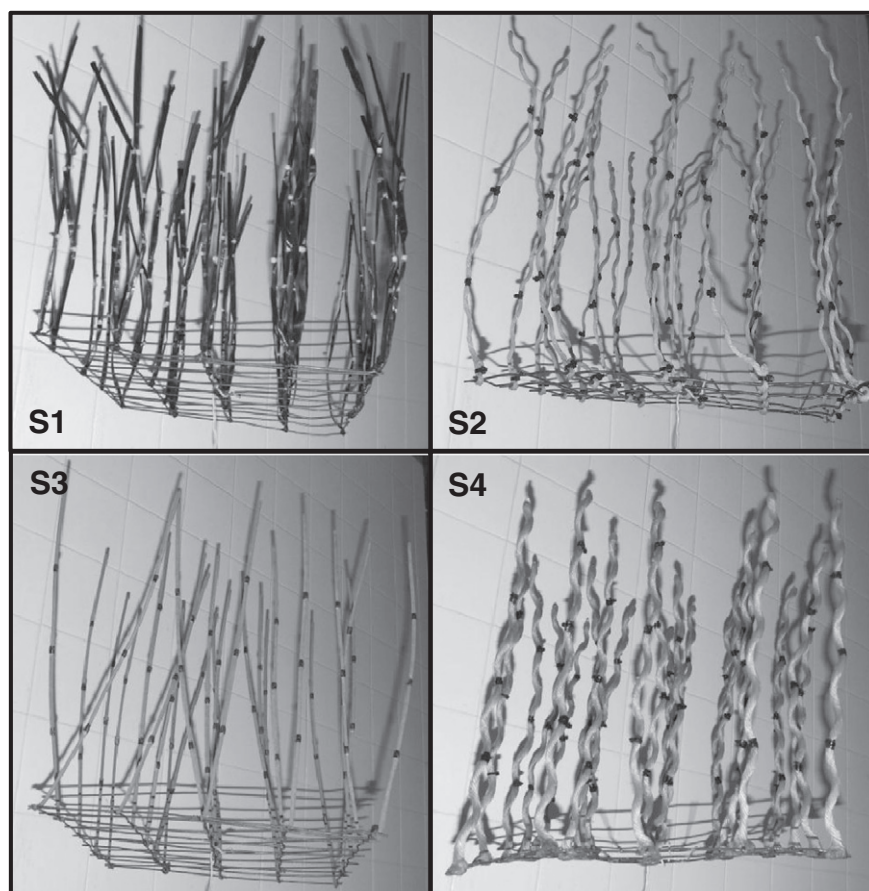
## 2. Materials and methods

### 2.1. Artificial holdfast units

Four different holdfast types were tested and assembled in a common metal structure coated with plastic, or Artificial Holdfast Unit (AHU) (Fig. 1). Each AHU measured 40 × 40 cm with a 10 × 10 cm grid. The different holdfast materials tested were i) 0.5 cm “seagrass-like” black polyethylene plastic strips (S1); ii) 0.5 cm Ø polyethylene nautical green rope (S2); iii) 0.6 cm Ø “*Codium*-like” rigid plastic strings (S3); and iv) 1.6 cm Ø “*Codium*-like” polyethylene nautical rope (S4). Each holdfast type measured 40 cm long and was marked every 10 cm with a black cable tie. These markings were used to be able to record the preferential location of seahorses in each holdfast.

### 2.2. Experimental design

Seahorses were placed and maintained in an 1800 litre fiberglass raceway tank (4.5 m × 1 m × 0.4 m), assembled in an open flow-through system. The tank was divided by a polyethylene mesh panel (1 cm Ø) in 3 equal sections (latter used as replicates) measuring 1.5 m × 1 m × 0.4 m. Light, water-flow and aeration were constant throughout the experiment and identical in each section. Water was filtered through a UV light with a temperature and salinity averaging 16.5 ± 0.5 °C and 36 ± 1‰, respectively. Water quality parameters were monitored every 3 days and were stable throughout the experiment, with ammonia values always below detectable levels, nitrate <0.3 mg L<sup>-1</sup> and nitrite <12.5 mg L<sup>-1</sup>. In each experiment, a total of 30 adult (15 males and 15 females) captive bred seahorses, *H. guttulatus*



**Fig. 1.** Different holdfast types used to assess seahorse preference: S1 – 0.5 cm “seagrass-like” black plastic strips (S1); S2 – 0.5 cm Ø nautical green rope, S3 – 0.6 cm Ø “*Codium*-like” rigid plastic strings (S3) and S4 – 1.6 cm Ø “*Codium*-like” bendable nautical rope.

(F<sub>2</sub>) were used and randomly distributed in each of the three replicates in a 1:1 gender ratio (5 males and 5 females per replicate). Each seahorse was tagged with a soft braided fishing line necklace with a specific bead color for individual recognition. After the termination of the observation period, the adults were removed from the tank and a new experiment was done with 30 juvenile seahorses (10 per replicate) using the same methodology as for the adults. Juveniles used in this experiment came from the same brood and aged 4 months old with an average length of  $12 \pm 1.4$  cm. This size class was selected as it is the most common size class of juveniles observed in the wild in the Ria Formosa. Juveniles were not divided by gender as individual sex was not distinguishable. Juveniles were then tested for holdfast preference using the same methodology as for adult seahorses.

All seahorses were sampled at the start and at the end of the experiment and the weight and height were recorded. In order to minimize handling and stress during sampling, as an alternative to the measuring protocol proposed by *Lourie et al. (1999)*, a simplified protocol was used. Instead of the three measurements proposed by *Lourie et al. (1999)* (the sum of head, trunk and tail lengths) seahorses were measured by the sum of the head length and total height (from the top of the coronet until the tip of the tail). Seahorse averaged  $17.9 \pm 3.1$  cm and  $17.1 \pm 0.9$  g; and  $9.5 \pm 0.4$  cm and  $2.4 \pm 0.3$  g for adult and juveniles, respectively. There were no significant differences in seahorse length or wet weight between treatments, within or between sexes, or replicates at the start of the experiment (ANOVA,  $P > 0.05$ ).

During the 2 days acclimation period and throughout the experiments, adult and juvenile seahorses were fed *ad libitum* with a mix of frozen shrimp (*Palaemonetes varians*) and live mysids (*Mesopodopsis slabberi*). After the beginning of the experiments, seahorses were only fed *ad libitum* once a day and after the observation period to avoid feeding effects on seahorse behavior, as most feeding activity was observed during the first hour after the feed was provided. Each replicate tank was siphoned on a daily basis, after the observation period and prior to feeding to remove uneaten feed, feces and other detritus.

At the end of the experiments, the necklace tags were removed and seahorses were returned to their grow-out tanks. No injuries, disease outbreaks or mortalities were recorded during the course of this study.

### 2.3. Holdfast preference

#### 2.3.1. Holdfast type and location

One of each holdfast type (S1 to S4) was placed in each replicate section, equidistant (20 cm) from each other. During the 2 days acclimation period, 10 seahorses were released into each replicate tank and allowed to freely choose the holdfast to grasp. Location, individual identification, gender and holdfast preference were recorded for each individual on a 60 min interval, 6 h per day (from 09:00–12:00 and 14:00–17:00) for 10 days, completing a total of 800 single observations per replicate.

#### 2.3.2. Holdfast density

Considering the results obtained in the previous experiment addressing the holdfast type preference, the holdfast type used in this experiment (S4) was the one that yielded the best results in the previous experiment. In order to assess seahorse holdfast density preference, 3 AHU of different densities were used per 3 replicates. Holdfast density was 9, 25 and 41 holdfasts per AHU (56, 156 and 256 holdfast·m<sup>-2</sup>). Again, the preference for holdfast density for adults and juveniles was recorded every 60 min, 6 h per day (from 09:00–12:00 and 14:00–17:00) during 10 days, completing a total of 800 single observations per replicate.

#### 2.3.3. Hydrodynamic effect

**2.3.3.1. Holdfast type.** In order to test the effect of water current on holdfast preference, two power pumps Eheim®159GPH (600 L·h<sup>-1</sup> max. capacity) were placed in the tank. Prior to the introduction of seahorses in the experiment tank, current flow was measured and adjusted to

$1 \text{ m}\cdot\text{s}^{-1}$  and directed at the AHU. This value is equivalent to the average maximum current flow recorded in the Ria Formosa lagoon (*Pacheco et al., 2010*). A pairwise comparison between holdfast types (S1 to S4) was conducted. Each day, a combination of 2 different holdfast types was tested during 2 h 30 min and preference recorded in a 15 min interval. At the beginning of each observational period, 10 seahorses were placed in the tank while the pumps were working to assess immediate holdfast preference, as the water flow used in this experiment is greater than the long term swimming capability of seahorses, as observed in the wild and in laboratory. As the water flow used in this experiment mimic the maximum water flow occurring under natural conditions, this experiment allowed to observe the seahorse holdfast preference behavior when exposed to more severe hydrodynamic conditions. The duration of this experiment was aimed to simulate the tidal peaks that naturally occur in the wild. This experiment lasted for three consecutive days (one replicate per day), and each replicate was tested for preference with an overall 330 single observations per combination.

**2.3.3.2. Holdfast density.** Two AHU's (S4) of the 2 different densities that yielded the best results in the previous experiment (*Section 2.3.2* – 156 and 256 holdfast·m<sup>-2</sup>) were placed in the tank, equally distant to the water pumps. Tank setup, data collection and experimental period were the same as described above (*Section 2.3.3.1*).

**2.3.3.3. Water flow.** This experiment is aimed to test the effect of current on the ability of a seahorse to grasp a holdfast. Seahorses were placed in a separate section of the raceway prior to the beginning of the experiment. Considering the results obtained in *Sections 2.3.1* and *2.3.2*, three AHU of the preferred holdfast type (S4) and density (156 holdfast·m<sup>-2</sup>) were used and placed inside the experimental tank, parallel to the water pump. Each AHU was placed in parallel and equally distant to each other, at the same time that a submersible water pump Eheim® 159GPH (600 L·h<sup>-1</sup> max. capacity) created a water flow of 1, 0.3 and 0.1 m·s<sup>-1</sup>, respectively, when passing through the structures. Before the start of the experiment, seahorses were moved to the main experiment section with the water flow and to the left to freely choose an AHU. Observations were made twice a day for 2 h 30 min each (10:00–12:30 and 14:00–17:30), in a 15 min interval. The experiment lasted 2 days completing 440 single observations.

### 2.4. Statistical analysis

Seahorse holdfast type preference, density and water current were compared using a one-way ANOVA. Tukey's post-hoc test was used to identify whether there were differences in preference within each replicate group. Gender preference was tested using a two-way ANOVA. In all test procedures, data was analyzed for normality and homogeneity, and whenever one of these requisites was not present, alternative non-parametric tests were used (*Zar, 1999*). All statistical analysis was performed for a significance level of 0.05, using Statistica 6.0 software (StatSoft Inc. Data).

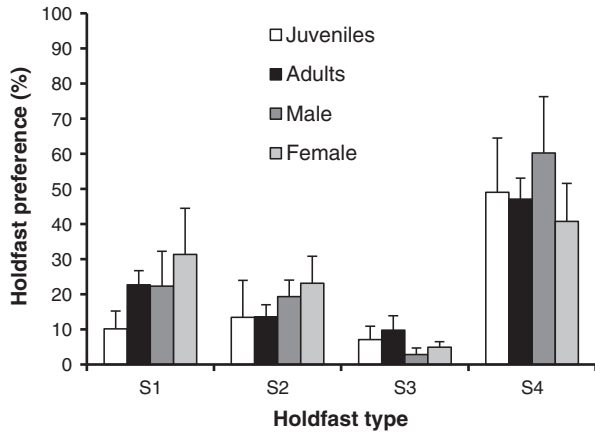
## 3. Results

### 3.1. Holdfast preference

#### 3.1.1. Holdfast type and location

Using one-way ANOVA, both juvenile and adult seahorses showed a significant preference to grasp to holdfast S4 (*Fig. 2*), compared to the other three holdfast types ( $F = 35.29$ ,  $P < 0.001$ ;  $F = 111.6$ ,  $P < 0.001$  for juveniles and adults, respectively). No statistical difference was observed in preference between genders ( $F = 0.21$ ,  $P = 0.653$ ) with both males and females preferring the S4 holdfast.

Regardless of the holdfast type, seahorses were mostly located near the base of the holdfast (0–10 cm;  $P < 0.05$ ), and only occasionally on higher sections (> 10 cm; *Fig. 3*).



**Fig. 2.** Holdfast preference (%) for juveniles and adult seahorses comparing four different holdfast types (S1–S4). Preference by sex is detailed for adult seahorses. Vertical bars represent standard deviation.

During the course of this experiment, courtship behavior was observed and no mortality occurred.

Even when submitted to a current effect, both juvenile and adult *H. guttulatus* showed a preference for S4 holdfast. Significant differences were found, using one-way ANOVA, for both groups tested ( $F = 14.05$ ,  $P < 0.001$ ;  $F = 15.39$ ,  $P < 0.001$ , respectively). The preference for S4 holdfast was significantly higher when compared with all other holdfasts tested for both juvenile and adult seahorses (Table 1).

**3.1.2. Holdfast density**

In this experiment the S4 holdfast type was used, as seahorses had shown the strongest preference for it in the previous experiment. Statistical differences were found, using one-way ANOVA, in holdfast density

**Table 1**

Holdfast preference (%) for different holdfast types (S1–S4), at a water flow of  $1 \text{ m} \cdot \text{s}^{-1}$ , for juvenile and adult *H. guttulatus*. Tukey Multiple comparison results are shown in each section. Significant differences are indicated with \*.

		Holdfast type			
		S1	S2	S3	S4
Juveniles		$18.5 \pm 7.6\%$	$22.9 \pm 8.5\%$	$18.9 \pm 8.6\%$	$39.7 \pm 7.3\%$
Tukey	S1	–	1.672	0.157	7.953*
	S2	1.672	–	1.515	6.282*
	S3	0.157	1.515	–	7.796*
	S4	7.953*	6.282*	7.796*	–
Adults		$18.7 \pm 13.9\%$	$16.7 \pm 13.7\%$	$17.8 \pm 8.6\%$	$46.8 \pm 6.0\%$
Tukey	S1	–	0.533	0.214	7.585*
	S2	0.533	–	0.319	8.117*
	S3	0.214	0.319	–	7.799*
	S4	7.585*	8.117*	7.799*	–

preferences ( $F = 119.90$ ,  $P < 0.001$ ;  $F = 94.46$ ,  $P < 0.001$ , for juveniles and adults, respectively). Both juvenile and adult *H. guttulatus* showed preference for medium and high holdfast densities when compared to the lowest density (Fig. 4). Using Tukey’s multiple comparison test, no significant differences were found between medium and high densities ( $q = 0.961$ ,  $P > 0.05$ ;  $q = 2.983$ ,  $P > 0.05$ , for juveniles and adults, respectively), while significant differences were found between low density and the latter (low vs medium:  $q = 17.290$ ,  $P < 0.05$ ;  $q = 17.300$ ,  $P < 0.05$  for juveniles and adults, respectively; low vs high:  $q = 16.330$ ,  $P < 0.05$ ;  $q = 20.280$ ,  $P < 0.05$  for juveniles and adults, respectively).

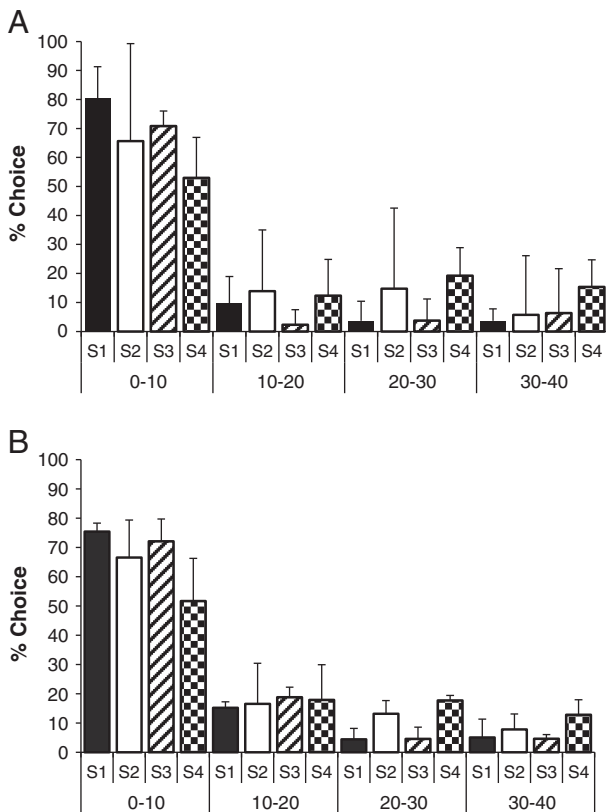
When submitted to a current effect, both juvenile and adult *H. guttulatus* showed a preference for S4 holdfast at medium density ( $156 \text{ holdfast} \cdot \text{m}^{-2}$ ) with occupancy of  $69.4 \pm 14.1\%$  for adults and  $63.7 \pm 6.9\%$  for juveniles. Significant differences were found for both groups tested ( $P < 0.001$ ).

**3.1.3. Water flow**

Juveniles showed significantly lower tolerance to stronger currents, whereas adults resisted to higher currents. Juveniles preferred grasping to the S4 holdfast ( $46.9 \pm 17.4\%$ ) at lowest water current, whereas adults preferred stronger water flows ( $31.8 \pm 16.3\%$  and  $39.7 \pm 15.7\%$  for  $1 \text{ m} \cdot \text{s}^{-1}$  vs  $0.1 \text{ m} \cdot \text{s}^{-1}$ , respectively) (Fig. 5). Statistical differences (KW test) in both experiments ( $P < 0.05$ ) and Dunn’s multiple comparison test showed significant differences ( $P < 0.05$ ) between  $1 \text{ m} \cdot \text{s}^{-1}$  vs  $0.1 \text{ m} \cdot \text{s}^{-1}$  for juveniles and  $0.3 \text{ m} \cdot \text{s}^{-1}$  vs  $0.1 \text{ m} \cdot \text{s}^{-1}$  for adults.

**4. Discussion**

*H. guttulatus* is the most abundant seahorse species in the Ria Formosa and, when compared to the sympatric species (*H. hippocampus*), it has shown a more drastic reduction in numbers in the past 10 years (94% and 73%, respectively) (Caldwell and Vincent, 2012). As *H. guttulatus* favors more complex habitats than *H. hippocampus*, and was naturally found using both biological and artificial holdfasts (Curtis and Vincent, 2005), this species was used to test the potential of artificial holdfast units (AHUs) in this study. The AHUs designed for this study were based on the natural preferences recorded for this species. As *H. guttulatus* was associated with seagrass beds (Curtis and Vincent, 2005), the S1 holdfast type was designed to simulate the seagrass leaves as they were of the same shape, size and thickness. Curtis and Vincent (2005) reported that seahorses were found on seagrass beds (58.6%) and on macroalgae (20.8%) considering the total covered area. In addition, and according to our own observations in the wild, this species has been observed grasping on macro-algae, particularly *Codium* spp. Therefore, the S2 to S4 holdfasts aimed to mimic different thickness and hardness of *Codium*-like holdfasts, each providing different structural behavior when placed in a hydrodynamic environment. S3 holdfasts were tested as an artificial structure that would



**Fig. 3.** Location preference (cm) for *H. guttulatus* adults (A) and juveniles (B) in each holdfast type tested (S1–S4). Vertical bars represent standard deviation.

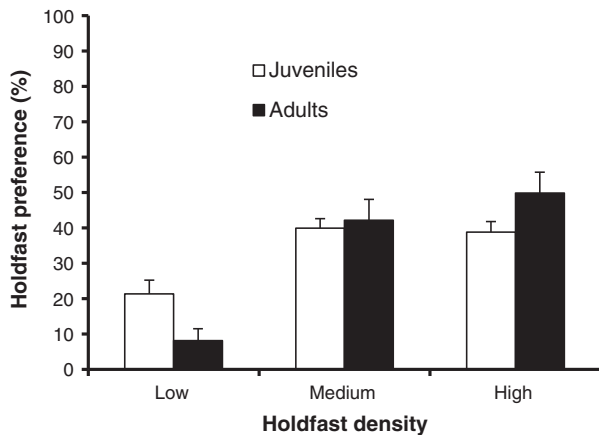


Fig. 4. Preference (%) for holdfast S4 densities (56, 156 and 256 holdfast·m<sup>-2</sup>), for juvenile and adult *H. guttulatus*. Vertical bars represent standard deviation.

provide stiffer holdfasts that could be potentially suitable for seahorse holdfast, considering the Ria Formosa's hydrodynamics. Although it was expected that *H. guttulatus* would prefer the S1 holdfast, as it mimics the seagrass bed, this study showed that this species showed significant preference for the S4 holdfast type. Teske et al. (2007) reported that *Codium* spp. was the preferred holdfast for *Hippocampus capensis*, compared to all other available holdfasts, even the more abundant *Zostera* sp. These authors also suggested the fact that more seahorses were found grasping seagrass might be due to its higher availability and not because it was the preferred holdfast. In addition, when comparing the four different holdfast types in strong current situation (1 m·s<sup>-1</sup>), there was again a clear preference for the S4 holdfast type. This may be due to the fact that the S4 holdfast provided a more stable structure for seahorses, which enables them to camouflage and hunt for prey more effectively, at the same time that it grants them protection against stronger currents. This will probably allow broadening of their habitat, allowing the *H. guttulatus* population settlement in areas that no longer have conditions for seagrass beds or other natural structure to exist. Similarly, Clynick (2008) reported that the use of swimming pool nets granted a good habitat for seahorses in Sydney Harbor, a highly human impacted area. The fact that seahorses were found grasping all the tested holdfast, emphasizes the holdfast versatility reported for *H. guttulatus* in the Ria Formosa, as they grasp different holdfasts like seagrass blades, macroalgae, tunicates, bryozoans, polychaete tubes, sea urchins, and artificial structures (Curtis and Vincent, 2005). There were no individual or sex related differences between holdfast

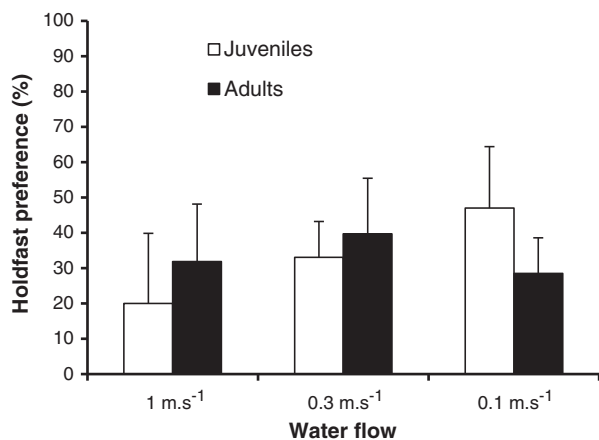


Fig. 5. Holdfast preference (%) under different water flows, using S4 holdfast at 156 holdfast·m<sup>-2</sup>, for juvenile and adult *H. guttulatus*. Vertical bars represent standard deviation.

preferences, demonstrating a clear preference for one particular AHU type from this study (holdfast S4).

As for holdfast density, seahorses showed preference for densities of 156 and 256 holdfasts·m<sup>-2</sup> (S4 holdfast type). Seahorses have been found in seagrass beds (*C. nodosa*) with a mean leaf height ranging from 11.1 to 34.2 cm and mean shoot density ranging from 233.3 to 848.3 shoots·m<sup>-2</sup> (Curtis and Vincent, 2005), which support our results. Higher densities were not tested as no significant differences were found between both medium and high densities (156 and 256 holdfasts·m<sup>-2</sup>). Prey density usually increases with increasing habitat complexity, resulting in higher predator feeding success at intermediate complexities (Canion and Heck, 2009; Crowder and Cooper, 1982). According to James and Heck (1994), even in high structure densities (up to 3032 seagrass blades·m<sup>-2</sup>) predation success is not significantly affected by sedentary predation, in contrast with active predation. Curtis and Vincent (2005) found that the abundance of *H. guttulatus* is positively and significantly correlated with the percentage cover of vegetation and immobile benthic invertebrates, which is supported by the findings in this study.

Stressed by the highly hydrodynamic nature of the Ria Formosa lagoon, where average maximum current speed can go up to 1.25 m·s<sup>-1</sup> (Pacheco et al., 2010) and their low mobility, *H. guttulatus* requires holdfasts in order to prevent them to be dragged away from their preferred habitats (Curtis and Vincent, 2005). In this study, as holdfast preference could be dependent on current speed and results obtained in a static environment could be misleading, the water flow experiments were aimed to replicate the natural conditions thus testing the effectiveness of the different AHU's and ultimately their viability in a future deployment in focal sites. In the case of current preference, adult seahorses preferred the 0.3 m·s<sup>-1</sup> water flow, even when lower current was available. This might indicate the natural preference for hydrodynamic environments, which may be explained by their feeding and cryptic behavior (Curtis and Vincent, 2005; Foster and Vincent, 2004). Juveniles occupied the holdfast set at lower current speed (0.1 m·s<sup>-1</sup>) which may be due to their inability to cope with strong water flows. This might be indicative that juveniles prefer sheltered and low current conditions in their natural habitat. Nevertheless, these assumptions require an *in situ* confirmation through underwater visual census surveys (UVC), monitoring several sites within the Ria with different hydrodynamics and thus assessing juvenile habitat preference. In fact, the need for holdfasts in a hydrodynamic environment indicates that one of the possible causes for seahorse decline in the Ria Formosa reported by Caldwell and Vincent (2012) could be in fact due to habitat loss or degradation. Therefore, it seems to be important to continuously monitor habitat changes and seahorse population trends during an extended period of time.

Seagrass destruction within the Ria Formosa and in other North Atlantic Ocean or Mediterranean Sea locations (Cunha et al., 2005) could result in a reduction of areas of *H. guttulatus* habitat, and hence local population sizes. Habitat degradation is one of the greatest threats to seahorse populations at low densities in a lagoon environment (Foster and Vincent, 2004; Harasti et al., 2010; Vincent et al., 2011). Allee effects (tendency for population to decline numerically when it gets below a certain threshold size or density) might be of important relevance in an environment with high patchiness and low population density (Vincent et al., 2011). The seahorse's cryptic behavior, poor swimming ability and dependence on using the prehensile tail to grip holdfast, renders them particularly dependent upon holdfast structures for hunting and predator avoidance (Curtis and Vincent, 2005, 2006; Foster and Vincent, 2004). In many countries, artificial seagrass has been recognized as an alternative method to cope with dwindling natural seagrass ecosystems and is becoming widely used as an alternative marine habitat for various marine organisms (Kenyon et al., 1999; Lee et al., 2001; Shahbudin et al., 2011; Sogard and Able, 1994). Artificial seagrass units (ASUs) have been used to investigate whether seagrass density (Bell et al., 1987) or distance from natural seagrass (Sogard, 1989) affected

settling fish and decapods and found that, although artificial seagrass has lower faunal assemblage capability when comparing to natural seagrass, they have proven to be a valid replacement, even if temporarily while the natural habitat recovers. Similarly, ASUs have been used to quantify spatial settlement patterns in enclosed embayment (Jenkins and Sutherland, 1997; Jenkins et al., 1996). These structures have also been shown to provide a suitable habitat for small fishes, increase prey density, promote nursery grounds for juveniles, and predatory protection for small fishes, thus playing a useful role in maintaining balance in marine environments when the natural habitat has been degraded or destroyed (Shahbudin et al., 2011).

The use of artificial structures to rehabilitate damaged areas including coral reefs, saltmarshes or other coastal areas, is still a subject of debate (Fernandez et al., 2009; Hauser et al., 2006; Moberg et al., 2011; Sirota and Hovel, 2006; Vega Fernández et al., 2009). Artificial structures have been referred to as tools for fish population reestablishment as they provide shelter and feeding grounds for many communities (Ambrose and Anderson, 1990; Bohnsack et al., 1994; Charbonnel et al., 2002; Claudet and Pelletier, 2004; Zalmon et al., 2002). Nevertheless, they may just act as an aggregation device for fish rather than increasing overall abundance (Grossman et al., 1997; Pickering and Whitmarsh, 1997). Although this argument may be true for more mobile species (Charbonnel et al., 2002; Santos and Monteiro, 1997), the use of these structures has the potential to provide a long term beneficial effect on more sedentary species like seahorses.

Like other seahorse species, *H. guttulatus* have low mobility and a small home range (Caldwell et al., 2011; Curtis and Vincent, 2005; Foster and Vincent, 2004). These characteristics render the seahorses vulnerable to human and natural disturbances which ultimately may be responsible to habitat loss or degradation (Curtis et al., 2007). In fact, habitat degradation may limit immigration and diminish the re-pairing of eventual widowers or of a disrupted couple (Foster and Vincent, 2004; Vincent and Sadler, 1995; Vincent et al., 2005). Habitat patchiness will result in a sparse distribution of seahorses and therefore may decrease the opportunities for sexual interaction and so contributing to a significant long term population decrease. The use of these artificial structures may provide both seahorse species (*H. guttulatus* and *H. hippocampus*) an improved habitat, promoting the populations settlement and ultimately maximizing both individual and sexual interaction. This study provided the first step in establishing how AHUs might be used as a management tool for enhancing declining seahorse populations in the Ria Formosa lagoon.

This study provides preliminary data and promising results on an approach to designing artificial holdfasts for seahorses in low complexity damaged or depleted areas. The use of these structures may contribute to the settlement of seahorse populations, thus broadening their potential habitat as part of a wider restoration strategy. In order to assess the viability of the use of these structures as habitat enrichment for local seahorse populations, more in depth *in situ* trials will be required with surveys of seahorse populations before and after deployment.

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