



Effects of artificial holdfast units on seahorse density in the Ria Formosa lagoon, Portugal



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ABSTRACT

A significant decline in the seahorse populations in the Ria Formosa has been recently reported and holdfast availability suggested as a particularly important variable that influences the abundance of the long-snouted seahorse (*Hippocampus guttulatus*) and the short-snouted seahorse (*H. hippocampus*). In order to test the usefulness of artificial holdfast units (AHU) to enhance habitat recovery, several of these structures were deployed at four different locations with distinct environmental characteristics and surveyed for seahorse abundance during a 6 month period. All AHU were colonized by seahorses within a month after deployment, reaching a maximum density of 13.1 seahorse m⁻² at one of the sites. Results suggest that these AHU have the potential to aggregate seahorses in damaged habitats but have limited effect when placed close to natural high complexity habitats. The results from this experiment provide useful guidance in the use of artificial structures to improve degraded seahorse habitats in other similar situations, as part of management plans for seahorse population recovery.

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1. Introduction

Worldwide, seahorse populations are threatened mostly due to degradation of their habitats, incidental capture in fishing gear (by-catch), and over-exploitation for use in the aquarium trade, curiosities and traditional medicines (Vincent, 1996). Seahorses are particularly vulnerable to population decline because of their distinctive life history, behavior and ecology: they provide lengthy and vital parental care for small broods, exhibit low mobility and site-fidelity (Foster and Vincent, 2004).

In addition, seahorses inhabit shallow, coastal areas worldwide, where anthropogenic disturbances tend to be most frequent and severe (Bell et al., 2003). Due to their peculiar morphology, seahorse use their prehensile tail to grasp different holdfasts, from sponges to coral, seagrass, mangroves and even artificial structures (Foster and Vincent, 2004; Harasti et al., 2010; Hellyer et al., 2011) as their life history is fully dependent of this behavior. Although some seahorse species prefer particular holdfast types (Rosa et al., 2007), others like *Hippocampus guttulatus* exhibit no obvious preference, grasping both natural and artificial structures (Curtis and Vincent, 2005).

The long-snouted seahorse (*H. guttulatus*) and the short-snouted seahorse (*H. hippocampus*) are sympatric species and the only two that live in the Northeast Atlantic (Pardo et al., 2007), including the Ria Formosa lagoon, South Portugal. Although the highest population abundances of these species have been recorded in the Ria Formosa in

the early 2000s (Curtis and Vincent, 2005), recent findings by Caldwell and Vincent (2012) showed a significant decrease in those populations within this lagoon: 94% for *H. guttulatus* and 73% for *H. hippocampus*, respectively. The causes for such decline are currently under investigation (Correia, 2015), but human related activities (e.g. fisheries, including illegal fishing, anchoring/mooring and dredging) and natural changes in the Ria's dynamics (e.g. silting events and shifting currents) seem to be the main causes for an overall habitat loss (Curtis et al., 2007). The Ria Formosa sustains numerous human related activities including clam farming (Guimarães et al., 2012), harbors, shipyards, coastal constructions and episodic dredging activity (to open and maintain navigation channels), which combined are responsible for the destruction of vast areas of potential seahorse habitat (Cunha et al., 2013). The combination of these anthropogenic activities and natural events are known to alter the seahorses' habitat conditions and reduce the amount of natural holdfasts available, essential for seahorse settlement (2010, Correia pers. obs.). Furthermore, there are potential climate change effects due to a consistent documented increase in the average water temperature in the lagoon and its associated effects (e.g. dissolved oxygen), a factor that is also known to have a negative impact on seagrass beds (Cunha et al., 2013), thus reducing holdfast availability. Therefore, as the lack of holdfast availability may explain the disappearance and/or desegregation of some populations within the Ria (Curtis et al., 2007), the use of artificial holdfasts is a potential tool to increase habitat complexity, thus encouraging seahorse population settlement and mitigating some of the causes of their decline.

Increased habitat complexity has been recognized as beneficial to the biodiversity and abundance of marine organisms (Silvertown,

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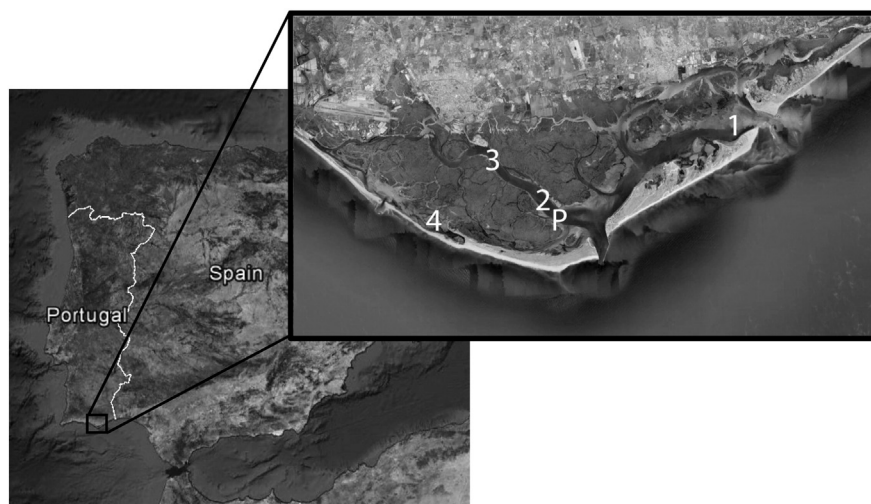


Fig. 1. Sites location in the Ria Formosa lagoon. AHU Prototype (P), Site 1 (1), Site 2 (2), Site 3 (3) and Site 4 (4). All AHU and Control sites were distant of more than 200 m apart.

2004). In many countries, artificial structures have been used as a method to replace damaged natural ecosystems providing a habitat for various marine organisms, nursery grounds 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 (Correia et al., 2013; Fernandez et al., 2009; Hellyer et al., 2011; Sirota and Hovel, 2006) and nylon ribbons (Lee et al., 2001) to Dorken Advance Engineer Rubber (Shahbudin et al., 2011).

This study aimed to test the effect of using artificial holdfast units (AHU) under natural conditions on habitat use and density of seahorses in the Ria Formosa lagoon.

2. Materials and methods

2.1. Site description

This study was conducted at eight locations (Fig. 1) in the Ria Formosa lagoon, South Portugal (36°59'N, 7°51'W). Four of these locations were chosen for AHU deployment as they were previously surveyed using underwater visual census (UVC) methodology (Correia, 2015) and their low seahorse abundance (<0.02 seahorse m^{-2}) and limited holdfast coverage was confirmed. In close vicinity to each of these locations, four other locations were chosen to be surveyed as control locations. Caldwell and Vincent (2013) referred a maximum of 150 m movement for *H. guttulatus* in the Ria Formosa. So, under this premise, each of the four AHU deployment sites and their respective control sites were deployed over 200 m from each other to prevent home range overlap. Three of these control locations were the same that were previously surveyed in Correia (2015). Each selected site had comparable characteristics regarding habitat complexity, depth, hydrodynamics and human impact (Table 1), whereas Site 3 had no seahorse populations in a 100 m^2 radius. Habitat complexity was considered as low if bottom coverage, i.e., number of holdfasts available, was under two holdfasts m^{-2} ; medium for 2 to 10 holdfasts m^{-2} ; and high for

more than 10 holdfasts m^{-2} . Holdfast distribution was considered as patchy when there was a distance between holdfasts in more than a 3-m radius. At each sampling, human related activities were recorded to determine their impact in each site. Sites were considered as highly impacted by human activities when two or more activities were observed in every sampling occasion, i.e., fisheries, boat traffic and anchoring; medium impact when at least two of those activities were observed in at least half of the sampling occasions; and low impact when less than two activities were observed in less than half of the sampling occasions.

2.2. Artificial holdfast units

The design of the artificial holdfast units (AHU) used in this study was an output of a previous experiment (Correia et al., 2013), where different holdfast materials, holdfast size and density were tested under controlled conditions. Each AHU was composed by a metal grid covered with polypropylene plastic base measuring 100 × 100 cm (with 10 × 10 cm grid gaps) where holdfasts were attached. The holdfasts, made of 1.6 cm Ø polyethylene nautical rope and measuring 40 cm long, were evenly distributed in the AHU, at a density of 100 holdfast m^{-2} .

Prior to the deployment of the AHU in the selected locations, one single prototype was placed in an area closely located to Site 2 (Table 1) in order to obtain background information before starting the experiment. This prototype, consisting of 4 AHU, was displayed in a square shape, summing a total area of 4 m^2 . The prototype was deployed in early July 2012 and surveyed for 6 months on a monthly basis. In each monthly survey, the number of seahorses, species, size and overall status of the AHU were recorded.

The prototype proved its reliability under natural conditions, so later on, three identical replicate units (3 × 4 m^2), were placed 10 m apart at a same depth (Fig. 2) in each of the four selected locations (Table 1). Each AHU was firmly attached to the bottom substrate with plastic covered metal pins to avoid being dragged by water currents or fishing gears. These AHU were deployed in February 2013 and then surveyed on a monthly basis over a 6-month period (March–August 2013).

Table 1
Description of each surveyed site regarding depth (meters), hydrodynamics (water flow), habitat complexity in the vicinities (holdfast availability), holdfast distribution, and human impact.

Location	Depth	Hydrodynamics	Habitat complexity	Human impact
Site 1	5–6 m	Medium (0.5–1 $m.s^{-1}$)	High	High (fisheries and boat anchoring)
Site 2	3–4 m	High (0.3–0.7 $m.s^{-1}$)	Low	Medium (occasional fisheries)
Site 3	3–4 m	High (0.1–0.5 $m.s^{-1}$)	Low	Low (occasional human activity)
Site 4	2–3 m	Low (0.1–0.5 $m.s^{-1}$)	High	Low (occasional human activity)

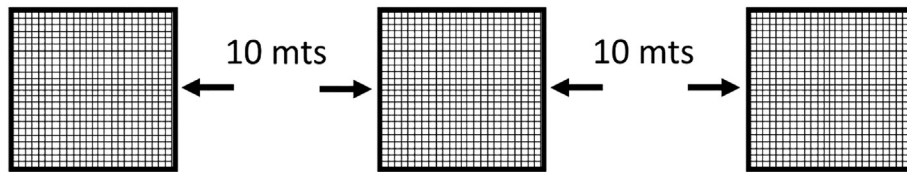


Fig. 2. Schematic of the AHU deployed at each Site (AHU1 to 4).

Considering those four selected locations' habitat, four control sites were chosen with similar habitat characteristics, located more than 200 m apart from the respective AHU to avoid home range overlap. The same survey methodology was used in the control areas, but with no AHU deployed. During each survey, seahorses found in the AHU and control sites were identified for species, counted and sexed. All *H. guttulatus* found were photographed for individual identification purposes (Correia et al., 2014). Photo-identification will determine the individual use of the AHU deployed. For accuracy and replicability, a GPS unit was used to determine the exact location of the replicate AHU in each study area.

2.3. Statistical analysis

In the prototype trial, seahorse species and gender differences were compared using a Student t-test (Zar, 1999).

As for the AHU deployed in the four different locations, seahorse density was tested for statistical differences using a one-way ANOVA. Tukey's post hoc test was used to identify whether there were differences in preference within each replicate group. In all test procedures, data were 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. First trial—AHU prototype

One month after deployment (July), seahorses were observed on the AHU prototype at a density of 3.5 seahorse m^{-2} . The lowest seahorse density was observed in August (2.3 seahorse m^{-2}), when the AHU were covered by bryozoan *Zoobotryon verticillatum*. From September onwards, the bryozoan decreased its area of occupancy and completely disappeared in October 2012 again leading to a new increase in seahorse occupancy reaching a peak of abundance during December at a density of 9.5 seahorse m^{-2} (Fig. 3).

Both seahorse species were observed on the AHU prototype. Maximum density of *H. hippocampus* was recorded in August (1.5

seahorse m^{-2}), while highest *H. guttulatus* density was observed in December 2012 (8.75 seahorse m^{-2}). During the 6 month survey period, the abundance of *H. guttulatus* was significantly higher than the *H. hippocampus* ($P = 0.03$). No significant differences in gender were found for *H. guttulatus* and *H. hippocampus* ($P = 0.61$ and $P = 0.80$, respectively).

3.2. Second trial—artificial holdfast units at different locations

All AHU were occupied by seahorses 1 month after deployment in each of the four locations. The maximum abundance was reached in May in all sites and then a constant decrease was observed until the last survey in August (Fig. 4). The highest seahorse density was observed 3 months after deployment in May at Site 2 (13.1 seahorse m^{-2}). In Site 4, all AHU replicates became progressively buried under sand due to continuous silting event and in July, the AHU were no longer visible so no further surveys were made in this site from that point onwards. Similar to the prototype trial, in Site 1 to 3, AHU became covered in the bryozoan *Zoobotryon verticillatum*. This was first observed 5 months after deployment in July and remained until the end of the study in August 2013.

H. guttulatus was significantly more abundant than *H. hippocampus* ($P < 0.05$) in all sites and in all survey events. No gender differences ($P > 0.05$) were found for both species in Site 1 and Site 4 at all survey events (Fig. 5), nonetheless, in Site 2 and Site 3, significant gender differences ($P < 0.05$) were found during June 2013.

Seahorse density was significantly different between Site 4 and Sites 2 and 3 ($P < 0.05$) (Table 2).

3.3. Photo-identification

A total of 709 photographs of first and re-sighted *H. guttulatus* were shot in all sites (86 in Site AHU1, 428 in Site AHU2, 166 in Site AHU3 and 29 in Site AHU4). Recapture percentage, i.e., percentage of *H. guttulatus* re-sighted at least once in a sampling period, varied from a minimum of 5.5% in Site 1 to a maximum of 16.0% in Site 2. The total number of first sight individuals found during the sampling period was 81 for Site 1, 373 for Site 2 and 147 for Site 3 (Fig. 6). At Site 4, a major silting event was observed 3 months after AHU deployment and after 5 months all replicates were buried, so monitoring stopped from that point onwards. Overall repeat sightings accounted for 23.5% of the total observed seahorses.

4. Discussion

In many countries, the use of artificial structures has been a recognized approach to compensate for dwindling natural ecosystems and has been used as an alternative habitat for various marine organisms (Kenyon et al., 1999; Lee et al., 2001; Shahbudin et al., 2011; Sogard and Able, 1994). In particular, artificial seagrass units (ASUs) have been proven to be a valid replacement, even if temporary, while the natural habitat recovers (Bell et al., 1985; Sogard, 1989). These structures have also proven adequate to provide a suitable habitat for small fishes, increase prey density, promote nursery grounds for juveniles, and to provide 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).

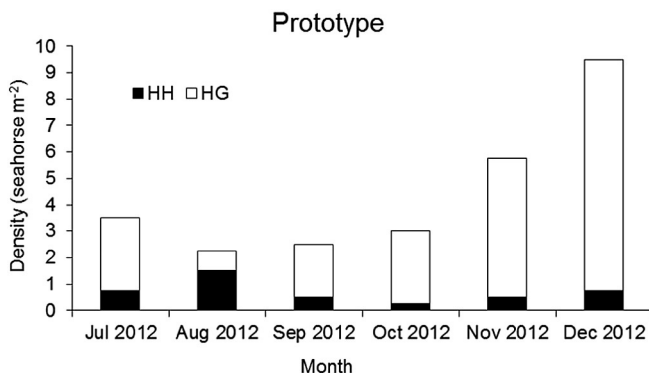


Fig. 3. Density (seahorse m^{-2}) for *H. hippocampus* (HH) and *H. guttulatus* (HG) in the AHU Prototype at the different survey events.

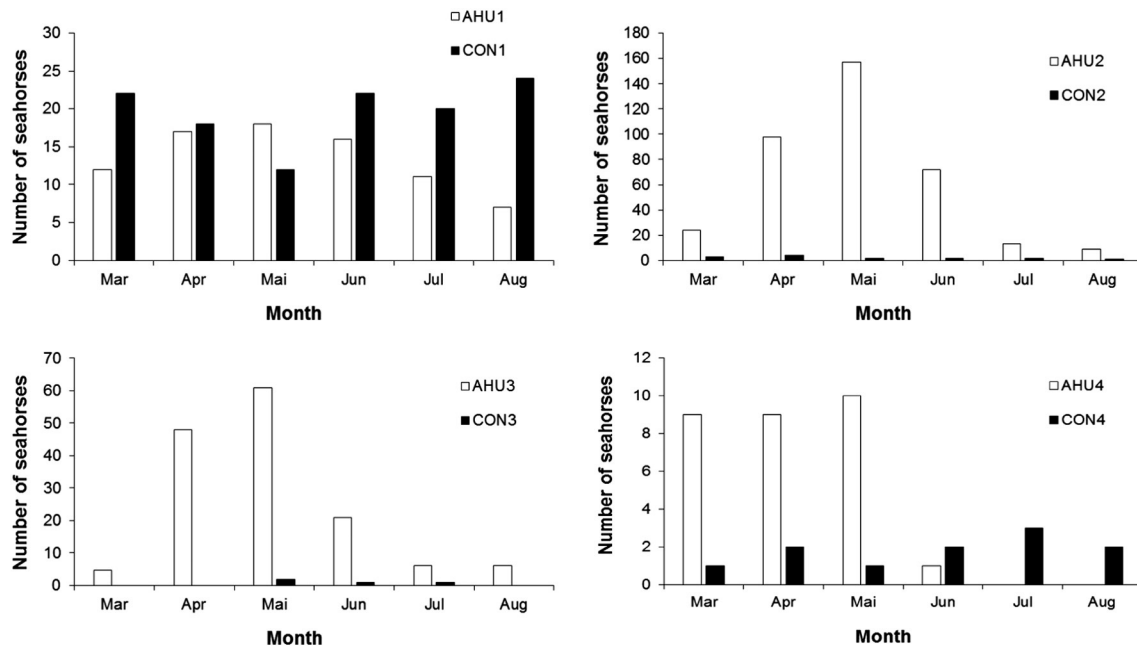


Fig. 4. Overall number of monthly sighted seahorses on the AHU and Control sites (CON#) located at Sites 1 to 4.

The use of artificial structures to rehabilitate damaged areas such as 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 used to reestablish fish populations 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). On the contrary, many authors consider that they act as fish aggregation devices rather than increasing overall abundance (Grossman et al., 1997; Pickering and Whitmarsh, 1997) and in the case of sedentary fish such as seahorses, these assemblages in a non-protected area might render them more vulnerable to adverse human related activities or natural events. Although these arguments may be true, the use of these structures has the potential to provide a long-term beneficial effect on the recovery of seahorse populations, particularly as one component of a wider rehabilitation and management plan. The use of these artificial structures may provide an improved habitat for seahorses, promoting the settlement of individuals that will provide more opportunity for reproduction and protection from predators. In the case of the Ria Formosa, seahorse aggregation on AHU should not affect their collection from the wild as there are no reports of ongoing fisheries that specifically target seahorses. However, such aggregations may render them vulnerable to damaging activities such as illegal bottom trawling and dredging.

Seahorses' cryptic behavior, poor swimming ability and their dependence on using their prehensile tail to grip holdfasts, makes them vulnerable to habitat loss or degradation (Curtis et al., 2007) as they are equally dependent upon holdfast structures for hunting and predator avoidance (Curtis and Vincent, 2005; 2006; Foster and Vincent, 2004). Furthermore, habitat degradation may limit migration 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 thus contributing to a significant long-term population decrease. Caldwell and Vincent (2012) reported a 94% decrease in *H. guttulatus* abundance and a 73% decrease in *H. hippocampus* abundance over the course of the past 10 years in this same study area. The causes for this drastic reduction are currently under study, but it became evident that the diminished holdfast availability ranks high as a factor to consider (Correia,

2015). In the Ria Formosa lagoon, the seahorses' dependence for holdfasts is also influenced by the fact that the Ria is highly hydrodynamic, where the average maximum current speed can go up to 1.25 m.s^{-1} (Pacheco et al., 2010), a constraint that enhances the need for stable holdfasts in order to prevent them to be swept away from their preferred habitats (Curtis and Vincent, 2005). Previous studies suggest that when exposed to different water flows, *H. guttulatus* not only prefers current speed below 1.0 m.s^{-1} (Correia et al., 2013) but also prefers more stable holdfasts which they grasp close to the base to minimize instability.

Despite their relatively higher abundance, *H. guttulatus* has been reported to favor more complex habitats (Correia, 2015) when compared to *H. hippocampus*, using both biological and artificial holdfasts (Curtis and Vincent, 2005). *H. guttulatus* is the most abundant seahorse species in the Ria Formosa, with the established population to be one order of magnitude greater than *H. hippocampus* (Curtis and Vincent, 2005). This fact was confirmed in this study as the *H. guttulatus* was found at higher densities in all AHU and in all surveyed sites. In fact, the presence of *H. hippocampus* on the AHU was almost negligible, considering all structures deployed. This could be attributed to the fact that *H. hippocampus* are not attracted to these structures due to their lower demand for complex habitats, generally occur at lower abundance, or, more concerning, if these low numbers are an expression of the continuous population decrease first reported by Caldwell and Vincent (2012). In addition, in this study, *H. hippocampus* has been reported up to 20 times less abundant than the other sympatric species (Correia, 2015).

Seahorses benefit from macroalgae *Codium* sp. (Correia, pers. obs.) as holdfasts, as the size of this seaweed provide not only more stable holdfast than other macroalgae and seagrasses but can also help decrease bottom currents, thus creating a beneficial habitat for small prey and the species that depend on them. The previous results obtained by Correia et al. (2013) showed that the best compromise for artificial holdfasts are those that mimic *Codium* sp. Those findings conducted *ex situ*, are now confirmed by the present study performed in natural conditions, as these structures were readily colonized by seahorses at higher densities than the surrounding areas of less holdfast availability. In addition, it was also observed during the surveys that all AHU were colonized by many other fish species, including other syngnathids (pipefishes), different species of gobies, wrasses and sparids, invertebrates (including

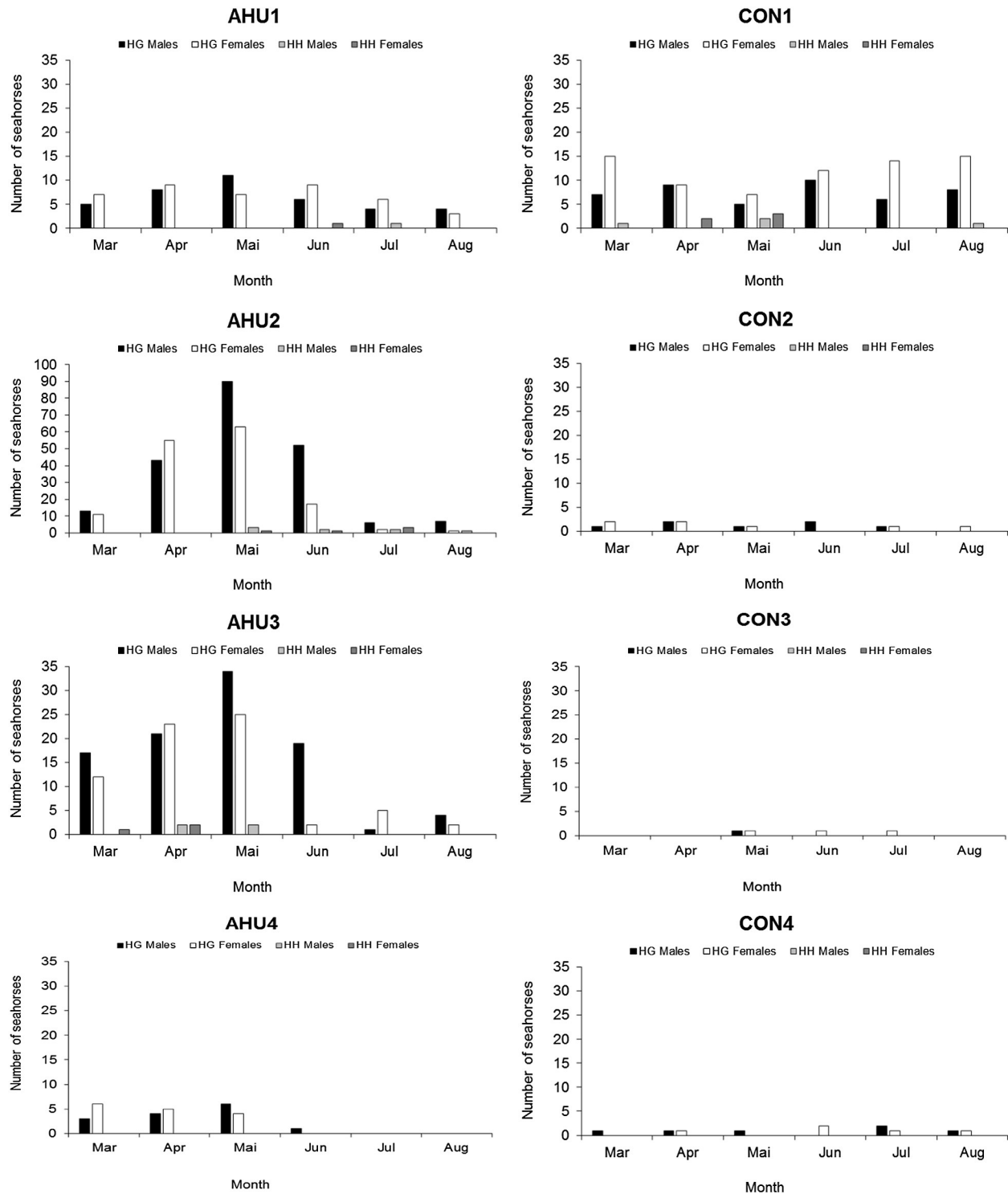


Fig. 5. Total number of monthly sighted seahorses by gender and species on the AHUs located at Sites AHU1 to 4.

Table 2

Tukey's multiple comparison test results (*P* values) after comparing seahorse density between sites. Statistical differences are represented by (*).

	Site 1	Site 2	Site 3	Site 4
Site 1		0.18	0.70	0.18
Site 2	0.18		0.17	0.01 *
Site 3	0.70	0.17		<0.01 *
Site 4	0.18	0.01 *	<0.01 *	

cephalopods) and crustaceans, including small Caridae shrimps, mysids and amphipods that constitute the base of seahorse diet. Therefore, the use of these AHU generates the potential to recreate complex habitats which benefit not only seahorses but also a number of other species. It was also observed that the artificial materials used to build the AHU maintain their effectiveness for at least 6 months, a time period long enough to generate the potential to modify the deployed area, creating the conditions for seahorse settlement.

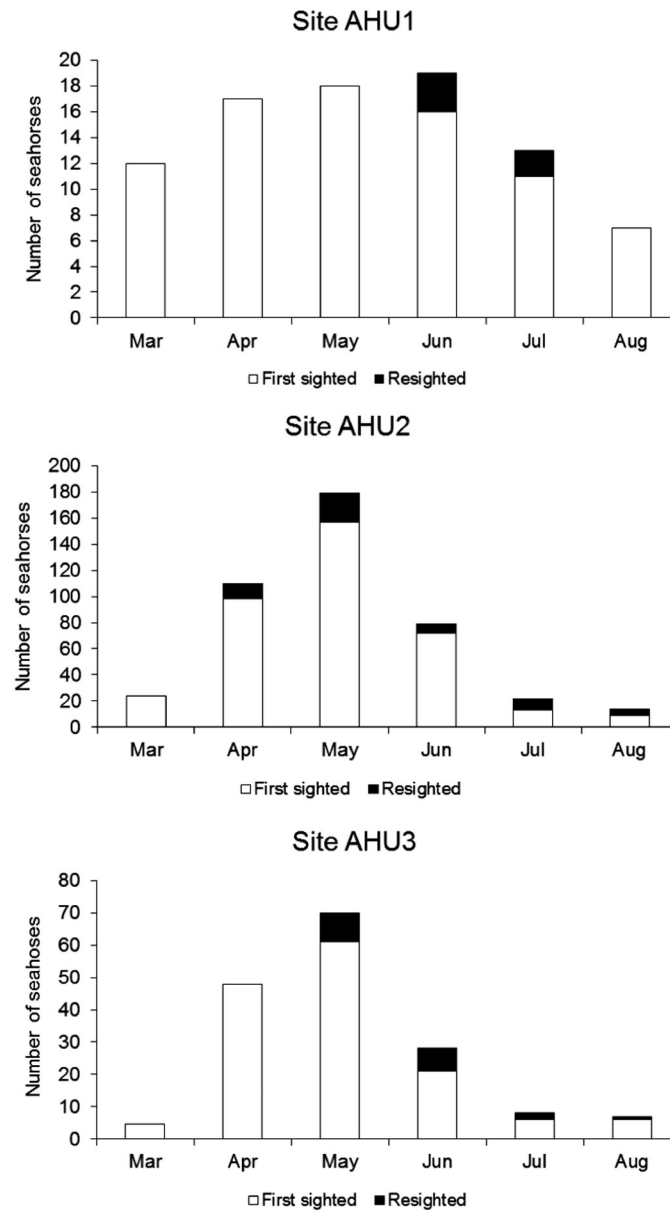


Fig. 6. Resight history of *H. guttulatus* (□ – first sighted and ■ – re-sighted) for Sites 1 to 3 on a monthly basis throughout the experiment.

In this study, the highest seahorse densities were observed in AHU deployed in the vicinities of the locations with low habitat complexity (Site 2) and barren area (Site 3). This might indicate that the AHU work as an aggregation device and provide a viable habitat for seahorses. While assemblages of seahorses in a non-protected area may render them more vulnerable to adverse human related or natural events, this aggregation might also contribute to an increase in opportunities for sexual interaction in depleted populations and so to help to support population recovery. In fact, the artificial structures used have the ability to provide habitat for seahorses at high densities of at least up to 13.1 seahorse m^{-2} . This density is far greater than the 0.51 seahorse m^{-2} reported by Curtis and Vincent (2005) and the 0.04 seahorse m^{-2} reported by Caldwell and Vincent (2012). The high densities observed on the AHU also confirms that the patchy distribution of seahorses, in particular *H. guttulatus* (Curtis and Vincent, 2005), might be due to holdfast availability. In fact, seahorses were observed at lower densities on the AHU deployed close to high complexity habitats (Site AHU1). In this case, seahorses seem to maintain their preference for the natural available holdfasts. Overall, these results suggest that these AHU have the potential to aggregate seahorses in damaged

habitats, but when in presence of natural complex habitats, seahorses tend to remain in their natural habitats. Sites with remaining natural complex habitats are unlikely to be part of a habitat or species recovery plan where AHU might be considered as a management option, while our findings here suggest that degraded sites might benefit from AHU to aid recovery as part of a management plan.

Seasonal variation in seahorse densities was observed in all AHU during the course of the experiment. Trends of decline were observed between the months of May until August in both trials in consecutive years. This reflects the same patterns observed in the monitoring of seahorses in the Ria Formosa over the course of a year (Correia, 2015). In addition, bryozoan (*Zoobotryon verticillatum*) thrived and covered the entire AHU area, from August to October 2012 for the prototype and from June to August 2013, in all sites. This event might be also responsible for the seahorse density decrease observed at that time, but again, the seasonal increase of this bryozoan has been recorded in previous years (author's observation). Bryozoan progressively covered the entire AHU, making it difficult for seahorses to grasp the AHU holdfast and so reducing the effectiveness of those artificial structures.

Based on the photo-identification used on *H. guttulatus* seahorses, only a low percentage of repeat sightings were reported when compared with those reported by Correia et al. (2014) at sites without AHU, i.e., 23% versus 38%. Additionally, the area occupied by the AHU was within the home range of 20 m² reported by Curtis and Vincent (2006) and is far smaller than the maximum distance moved by *H. guttulatus* of 150 m reported by Caldwell and Vincent (2013). These facts might suggest that, although the artificial structures provide alternative habitat for seahorses, these fish might prefer other nearby areas with more suitable characteristics, using the AHU as a temporary dwelling area. This information can be checked either by using molecular markers, as microsatellites, in order to understand where the seahorses are moving from and eventually recruiting (either nearby areas or longer distances), or by short-term tagging studies (Caldwell and Vincent, 2013).

In sum, the AHU design and methodology used in this study proved to be useful tools for habitat enrichment, especially in areas with low holdfast availability. These structures could be used to recover damaged habitats, even temporarily, contributing to the improvement of seahorse populations in similar scenarios. Our results suggest that holdfast availability is an important factor to consider and influences the seahorse density in the Ria Formosa lagoon. The results from this experiment can be foreseen as a future guideline for the use of artificial structures to improve degraded and impacted habitats within the Ria Formosa and even in other similar situations, as part of a management plan.

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