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Seahorse (Hippocampinae) population fluctuations in the Ria Formosa Lagoon, south Portugal

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Comparisons of three sets of surveys in the Ria Formosa Lagoon, Portugal, over a 13 year period (2001–2002, 2008–2009 and 2010–2013) revealed significant population fluctuations in at least one of the two seahorse (Hippocampinae) species living there, and that those fluctuations were potentially associated with habitat changes in the lagoon. After a significant decline between the first two survey periods (2001–2002 v. 2008–2009), long-snouted seahorse *Hippocampus guttulatus* populations increased significantly between 2008–2009 surveys and new 2010–2013 surveys. There were no significant differences in *H. guttulatus* populations between the 2001–2002 and 2010–2013 surveys. In contrast, there were no significant differences in short-snouted seahorse *Hippocampus hippocampus* densities among the 16 sites surveyed throughout the three sampling periods, although the ability to detect any change was hampered by the low densities of this species in all time periods. Fluctuations in *H. guttulatus* densities were positively correlated with the percentage of holdfast coverage, but with none of the other environmental variables tested. These results highlight the importance of holdfast availability in maintaining stable seahorse populations. While population fluctuations are certainly more promising than a consistent downward decline, such extreme fluctuations observed for seahorses in the Ria Formosa Lagoon could still leave these two species vulnerable to any additional stressors, particularly during low density periods.

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Key words: habitat changes; Hippocampinae; *Hippocampus guttulatus*; *Hippocampus hippocampus*; long-term survey.

INTRODUCTION

Determining sources of variability in population abundance and identifying factors causing population fluctuations are crucial questions in ecology (Hunter & Gibbs, 2009). In the past century, much attention has been focused on population biology and the reasons why populations fluctuate (Moran, 1953; May, 1974; Beddington & May, 1977; Kareiva, 1987). These questions remain relevant, particularly in reference to the management and conservation of exploited and threatened species (Clark, 2010).

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In marine ecosystems, populations are not only influenced by a variety of anthropogenic stressors but can also fluctuate naturally due to the temporal heterogeneity of marine environments (Kolasa *et al.*, 1991). Baillie *et al.* (2004) reported that most marine extinctions could be attributed to overexploitation (55%) followed by habitat loss and degradation (37%). The effects of habitat loss, however, are difficult to separate from other effects, as they are commonly one of the multiple stressors. For example, human-induced habitat loss has been linked to coastal development with associated pollution, leading to sediment loading and added nutrient inputs (Short & Wyllie-Echeverria, 1996). To understand the relative vulnerability of marine organisms to such multiple anthropogenic stressors, the effects of natural environmental heterogeneity on population variability must be addressed first. Studies of unexploited species and protected areas that link spatial and temporal population variability with changes in environmental and biological factors can help to disentangle the effects of such natural and anthropogenic effects (Martin-Smith & Vincent, 2005).

Seahorses (Hippocampinae) have life-history characteristics that could make their populations particularly vulnerable to the combined pressure resulting from their naturally shifting coastal habitats and anthropogenic activity in those habitats. Typically, seahorses have a sparse distribution, low mobility, small home ranges, low fecundity, lengthy parental care and mate fidelity, which could render them vulnerable to overfishing and habitat damage (Foster & Vincent, 2004). Most seahorse species use their prehensile tail as a means to grasp different holdfasts (*e.g.* sponges, coral, seagrass, mangrove branches and even artificial structures), thus relying on some degree of habitat structure (Foster & Vincent, 2004; Harasti *et al.*, 2010; Hellyer *et al.*, 2011). Although these fishes are described as sedentary, they can move at least 150 m in a single day (Caldwell & Vincent, 2013) and can have home ranges of up to 400 m² (Garrick-Maidment *et al.*, 2011). These movements are probably influenced by habitat specificity and presence of other seahorses, adjusting their location depending on holdfast and food availability, density, fishing activities and weather conditions throughout the year (Correia *et al.*, 2014). Most seahorses are currently listed by IUCN as data deficient or in one of the categories indicating some degree of threat (IUCN, 2014), so information is needed to properly assess their threat status. Considering that changes in abundance is one of the metrics used by IUCN to assess threat status, long-term monitoring is valuable as a conservation and management tool.

Extreme fluctuations have been documented in populations of at least six seahorse species, but the causes of such fluctuations have been more difficult to discern: *Hippocampus abdominalis* Lesson 1827 (Martin-Smith & Vincent, 2005), *Hippocampus reidi* Ginsburg 1933 (Freret-Meurer & Andreatta, 2008), *Hippocampus zosterae* Jordan & Gilbert 1882 (Masonjones *et al.*, 2010), *Hippocampus hippocampus* (L. 1758) (Caldwell & Vincent, 2012), *Hippocampus guttulatus* Cuvier 1829 (Caldwell & Vincent, 2012) and *Hippocampus whitei* Bleeker 1855 (Harasti *et al.*, 2014). Among the variety of hypothesized causes for the fluctuations in these seahorse species were human-related activities, including direct effects (coastal construction and exploitation) and indirect effects (changes in water quality) (Masonjones *et al.*, 2010), natural fluctuations due to recruitment failure (Martin-Smith & Vincent, 2005), changes in predator abundance (Harasti *et al.*, 2014) and changes in holdfast availability (Rosa *et al.*, 2007). With the exception of *H. whitei*, in which fluctuations were associated with predator abundances in New South Wales, Australia (Harasti *et al.*, 2014), none of the other documented population declines were linked with any obvious cause

(Martin-Smith & Vincent, 2005; Freret-Meurer & Andreatta, 2008; Masonjones *et al.*, 2010; Caldwell & Vincent, 2012)

The two sympatric seahorse species that inhabit the Ria Formosa Lagoon (the long-snouted seahorse *H. guttulatus* and the short-snouted seahorse *H. hippocampus*) have been monitored since the early 2000s (Curtis & Vincent, 2005; Caldwell & Vincent, 2012; Correia *et al.*, 2014). During 2001–2002, seahorse populations in the lagoon were found at higher densities than anywhere else recorded for those species (Curtis & Vincent, 2005). During 2008–2009, surveys showed that these populations had declined by 94% for *H. guttulatus* and 73% for *H. hippocampus* (Caldwell & Vincent, 2012). The causes for such declines remain unknown but could be linked to human-related activities (including illegal fishing, anchoring and dredging) and natural changes in the Ria's dynamics (*e.g.* silting events and shifting currents) leading to overall habitat loss (Curtis *et al.*, 2007).

Estuaries, such as the Ria Formosa Lagoon (south Portugal), are naturally variable ecosystems that have been focal points for human settlement and marine resource use throughout history, exposing them to extended anthropogenic effects. Such continuous overexploitation, habitat transformation and pollution have masked the overall magnitude of habitat degradation and biodiversity loss throughout the world's estuaries (Jackson *et al.*, 2001; Pandolfi *et al.*, 2003; Lotze & Milewski, 2004; Lotze *et al.*, 2005). The Ria Formosa Lagoon is a highly productive ecosystem that sustains a wide variety of commercial species with high economic value [*e.g.* sparid species such as *Sparus aurata* L. 1758, *Diplodus sargus* (L. 1758) and *Diplodus vulgaris* (Geoffroy Saint-Hilaire 1817); Moronidae such as *Dicentrarchus labrax* (L. 1758) and several flatfish (Pleuronectiformes) species] (Ribeiro *et al.*, 2006). Although the Ria Formosa is a semi-protected lagoon where only some fishing methods are allowed, the fishing gears that target such commercially important species can still have direct (by-catch) and indirect (habitat degradation) effects on non-targeted species. In addition, there has also been an increase in reports of illegal fishing in the Ria Formosa (bottom trawling and beach seine fisheries) in addition to legal fishing activities (trap and line fisheries) (Erzini *et al.*, 2002). Southern Portugal is also a renowned tourist area with many associated activities including aquatic sports, boat traffic and boat anchoring that could affect the lagoon's habitats. Seagrass beds have been particularly affected, with many natural *Zostera noltii* beds being replaced by clam farms (Guimarães *et al.*, 2012), and other seagrass beds destroyed by coastal construction and dredging to open and maintain navigation channels (Cunha *et al.*, 2014).

Through repeated monitoring of seahorse populations in the Ria Formosa Lagoon, this study aimed to (1) determine whether the apparent past decline in seahorse population densities has continued since previous surveys and (2) assess whether population changes within the lagoon are associated with any measured environmental variables. Although previous monitoring indicates a decline in seahorse densities between 2001 and 2002 and 2008 and 2009 within the Ria Formosa Lagoon, it is unclear whether that decline is still occurring or if the populations are recovering. Updating the information through a new seahorse population census could help clarify this question. Furthermore, although previous efforts have been unable to identify environmental factors associated with past declines in the Ria Formosa, this may have been due to the low temporal resolution of past surveys (*i.e.* comparing only two time periods). Collecting similar environmental data to that collected in past seahorse surveys in this third monitoring effort (*i.e.* depth, temperature and habitat cover) could provide the additional

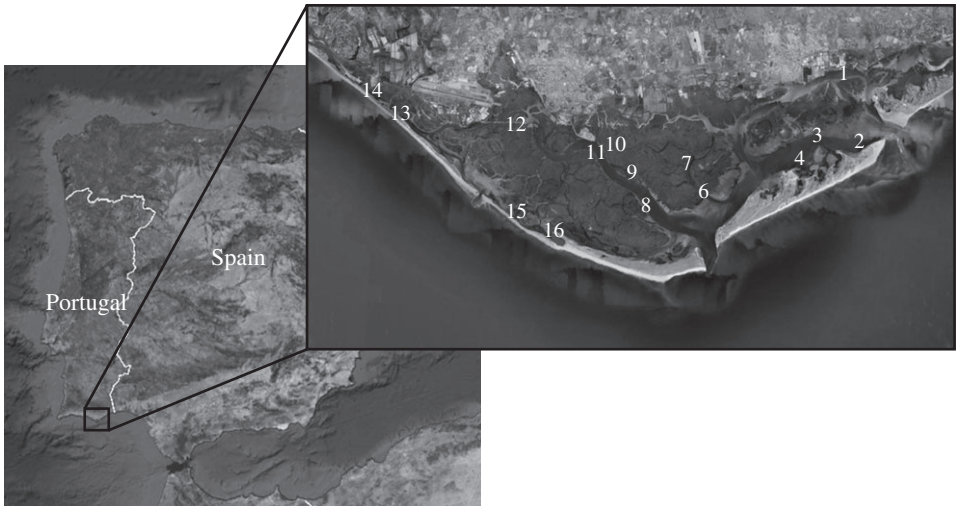


FIG. 1. Location of the 16 sites surveyed for Hippocampinidae during 2001–2002, 2008–2009 and 2010–2013.

temporal resolution needed to identify potential environmental drivers of seahorse population changes in the Ria Formosa.

MATERIALS AND METHODS

UNDERWATER VISUAL CENSUS SURVEYS

In 2010, 2012 and 2013, underwater visual census (UVC) surveys for seahorses were conducted at 16 sites (Fig. 1) that had been previously surveyed in the Ria Formosa Lagoon, south Portugal (36° 59' N; 7° 51' W). The UVC method used was the same as described by Curtis & Vincent (2005) and Caldwell & Vincent (2012). Surveys were performed at high slack tides, when there was less turbidity and weaker currents. A GPS unit was used to determine the locations of each study area and the same bearing was taken while laying each transect so that the same area could be consistently covered on each sampling occasion. As in the previous surveys, when a seahorse was found, its species and sex were recorded along with three environmental variables comparable to previous surveys: water depth and water temperature at depth (recorded using Suunto Mosquito dive computer; www.suunto.com), and the percentage cover of holdfasts (*i.e.* the percentage of the benthos within a 1 m² quadrat centred around the seahorse that was covered by either living or non-living material that could be grasped by a seahorse's tail) were recorded.

CHANGES IN SPECIES DENSITY AMONG SURVEY PERIODS

To assess whether seahorse densities had changed over time, species densities recorded in this study were compared with data collected by Curtis & Vincent (2005) and Caldwell & Vincent (2012). Seahorse densities (number of individuals m⁻²), rather than abundances, were compared for each species at each site to account for differences in surveyed areas per site in each time period, as described in the study of Caldwell & Vincent (2012). These densities were compared for all 16 sites during the three time periods. Since species densities were non-normal and transformations for normality were unsuccessful, densities were compared among the three time periods using a non-parametric Friedman test to account for repeated measures at each of the 16 sites. When Friedman tests were significant, Wilcoxon signed rank tests were used to

identify which of the three time periods differed, using a Bonferroni corrected α value of 0.0167 to account for multiple comparisons (*i.e.* 0.05/3). R statistical software was used for both the Friedman test and Wilcoxon *post-hoc* tests (`friedman.test` and `Wilcox.test` functions in the stats R package; R Core Team; www.r-project.org).

ENVIRONMENTAL CORRELATES OF SPECIES DENSITY CHANGES

To identify whether changes in seahorse densities were associated with any changes in their environment, the differences in the two species densities between successive surveys were compared with differences in the three environmental variables collected during those same survey periods (*i.e.* depth, temperature and per cent holdfast cover). The differences in seahorse densities and environmental conditions were calculated between the latest survey period and the middle survey period (2010–2013 *v.* 2008–2009) and between the middle survey period and the earliest survey period (2008–2009 *v.* 2001–2002), resulting in two sets of differences (*i.e.* changes in seahorse density and environment) for each of the 16 sites.

Changes in density were compared with changes in environment using generalized linear mixed effect model (GLMM), fitted to the data using a forward stepwise process informed by Spearman rank correlations. Before fitting GLMMs, non-parametric Spearman rank correlations were used to compare changes in individual environmental correlates with changes in seahorse densities and to identify any co-linearity among the environmental variables (using `cor.test` function in the R stats package; R Core Team). A non-parametric test was used as the data were non-normal and transformations for normality were unsuccessful. Results of the Spearman rank correlations were then used to inform model selection. Starting with the variable with the highest significance (*i.e.* lowest P value), GLMMs were created in a forward stepwise process, adding variables with successively lower significance. GLMMs were used instead of generalized linear models (GLM) to include the effect of site as a random factor. Each GLMM used a Gaussian distribution with a log-link function within the `lmer` function of the R Core Team package `lme4` (Bates *et al.*, 2014). Successive GLMMs were compared with previous models using the 'ANOVA' function of the stats R package (R Core Team) to test for differences in the amount of deviance explained (Zuur *et al.*, 2009). Variables and their interactions were only retained if their addition explained significantly more deviance according to a χ^2 test.

RESULTS

The most recent surveys of seahorse densities in the Ria Formosa Lagoon (2010–2013) revealed significant increases in *H. guttulatus* densities since the previous 2008–2009 surveys, but no significant changes in *H. hippocampus* densities among the 16 sites surveyed (Fig. 2). Densities of *H. guttulatus* fluctuated between the three surveys with high densities in 2001–2002, low densities in 2008–2009 and high densities again in 2010–2013 (Table I). According to a Friedman test, there were significant differences in *H. guttulatus* densities among the three survey periods (Friedman test, $F_r = 20.3$, d.f. = 2, $P < 0.001$). *Post hoc* tests further revealed that the differences in *H. guttulatus* densities were due to a significant decrease between the earliest survey and the middle survey (2001–2002 *v.* 2008–2009; Wilcoxon signed rank test, $W = 120$, d.f. = 15, $P < 0.01$), followed by a significant increase between the middle survey and the most recent survey (2008–2009 *v.* 2010–2013; Wilcoxon signed rank test, $W = -85$, d.f. = 15, $P < 0.01$), with no difference between the earliest survey and the most recent survey after applying a Bonferroni correction (2001–2002 *v.* 2010–2013; $P < 0.05$). Although mean densities of *H. hippocampus* appeared to follow a similar fluctuating trend (Table I), and a Friedman test indicated that there were significant differences in *H. hippocampus* densities ($P < 0.05$), none of the *post hoc* tests were

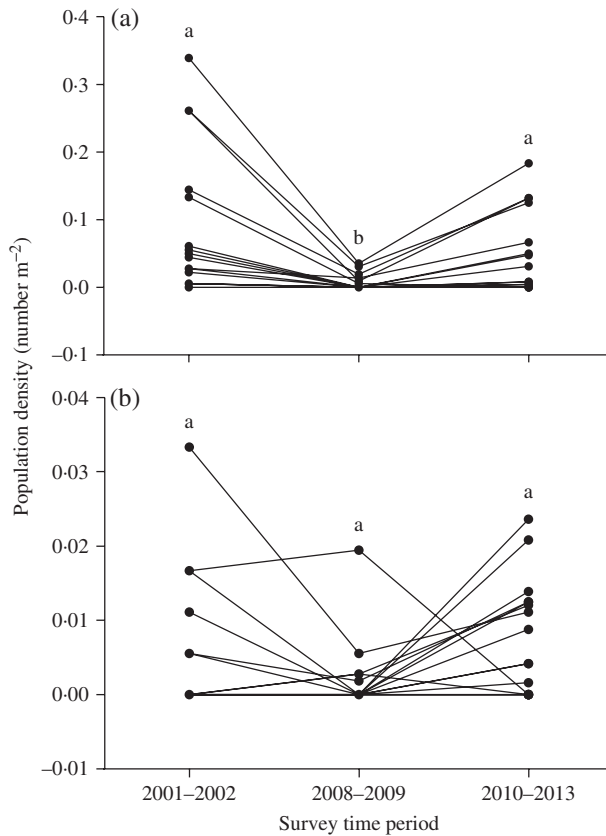


FIG. 2. Comparisons of (a) *Hippocampus guttulatus* and (b) *Hippocampus hippocampus* densities found during three underwater visual census survey periods of 16 sites (●) within the Ria Formosa Lagoon. Sites and surveys with the same lower-case letter are not significantly different (*i.e.* Wilcoxon signed rank *post hoc* test > 0.05).

significant after applying the Bonferroni correction (Wilcoxon signed rank tests; 2001–2002 v. 2008–2009: $W = 24$, d.f. = 15, $P > 0.05$; 2008–2009 v. 2010–2013: $W = -65$, d.f. = 15, $P < 0.05$; 2001–2002 v. 2010–2013: $W = -23$, d.f. = 15, $P > 0.05$), suggesting that none of the changes in *H. hippocampus* densities were significant. Lower overall densities of *H. hippocampus* compared with *H. guttulatus* make it harder to detect change in the former species. Even if overall densities of *H. hippocampus* did not change significantly, it appeared that their populations were expanding to new sites, as appreciably more of the surveyed sites appeared to be occupied by *H. hippocampus* during 2010–2013 (69%) compared with 2001–2002 (38%) or 2008–2009 (31%) (Table I).

Changes in *H. guttulatus* densities were associated with changes in holdfast availability over time but there were no significant relationships with any of the other environmental variables or between *H. hippocampus* densities and any of the environmental variables measured (Fig. 3). Correlations among all five variables (*i.e.* changes in the three environmental variables and the two seahorse species densities) were only significant for two relationships: (1) changes in *H. guttulatus* density were

TABLE I. Population densities (mean and maximum) and per cent occupancy of two seahorse species in 16 sites surveyed in three time periods in the Ria Formosa Lagoon

	<i>Hippocampus guttulatus</i>			<i>Hippocampus hippocampus</i>		
	2001–02	2008–09	2010–13	2001–02	2008–09	2010–13
Mean density ($n\ m^{-2}$)	0.090	0.007	0.053	0.007	0.002	0.008
Occupancy (%)	93.75	37.5	75.0	37.5	31.25	68.75
Maximum density ($n\ m^{-2}$)	0.339	0.035	0.183	0.033	0.019	0.024

positively correlated with changes in holdfast coverage (Spearman rank correlation, $r = 0.573$, $P < 0.001$) and (2) densities of the two seahorse species were positively correlated (Spearman rank correlation, $r = 0.5264$, $P < 0.01$). According to χ^2 tests comparing GLMMs with and without the addition of further environmental variables, none of the more complex models explained more of the deviance in *H. guttulatus* density changes than the model with percentage holdfast cover alone (χ^2 tests; all $\chi^2 < 1.97$, each d.f. = 1, all $P > 0.05$). For *H. hippocampus*, none of the environmental variables explained a significant amount of the deviance in density changes compared with the null GLMM including only the random effect of site (χ^2 tests; all $\chi^2 < 0.172$, each d.f. = 1, all $P > 0.05$).

DISCUSSION

Seahorse population monitoring in the Ria Formosa identified a fluctuation in *H. guttulatus* densities within a decade. Although there were dramatic decreases in densities of *H. guttulatus* (94%) and *H. hippocampus* (73%) between 2001–2002 and

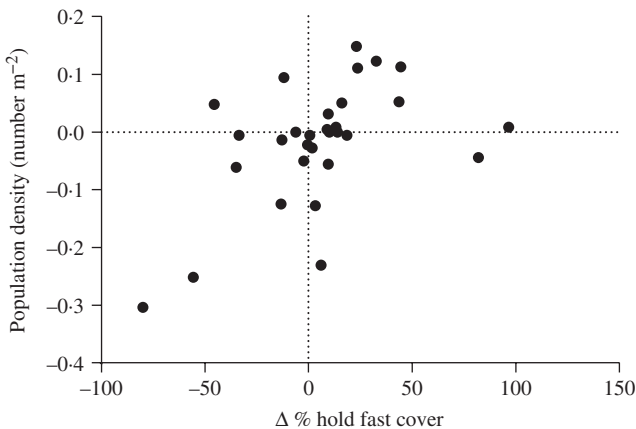


FIG. 3. Changes in *Hippocampus guttulatus* densities associated with changes in the percentage cover of holdfasts between three successive underwater visual census survey periods at 16 sites within the Ria Formosa Lagoon.

2008–2009 (Caldwell & Vincent, 2012), there have been subsequent increases for *H. hippocampus*. Although mean densities of *H. hippocampus* also appeared to follow a similar upward trend to *H. guttulatus* since their previous decline, these changes were not significant, perhaps due to their low overall densities in all time periods. One promising trend for *H. hippocampus* though, is an apparent increase in the number of sites occupied, perhaps indicating expansion of those populations to new sites within the lagoon. While such upward trends suggest that these populations are less vulnerable than if there had been a sustained decline over time, extreme fluctuations of >90% could still leave these populations vulnerable to any additional stressors affecting their populations during low density periods (Mace *et al.*, 2008).

Similar temporal fluctuations to that found for *H. guttulatus* in the Ria Formosa have been recorded for other fish species but most of those focused on species of commercial importance and many were unable to identify the causes of the fluctuations (Beare *et al.*, 2004; Daufresne & Renault, 2006; Costa *et al.*, 2012; Mariani *et al.*, 2013). The causes of such fluctuations are generally hard to determine as many variables have to be taken into consideration but exploitation, habitat loss and degradation and climate change have all been reported as potential candidates, each of which could ultimately lead to local extinction (Dulvy *et al.*, 2003).

Unlike previous documented seahorse fluctuations that were not found associated with changes in the environment (Martin-Smith & Vincent, 2005; Freret-Meurer & Andreatta, 2008; Masonjones *et al.*, 2010; Harasti *et al.*, 2014), the *H. guttulatus* population fluctuations in the Ria Formosa were found to be associated with changes in at least one habitat variable: the availability of holdfasts. There was a positive relationship between the availability of holdfasts (natural or artificial) and densities of *H. guttulatus* over time. Initial decreases in *H. guttulatus* densities were associated with declines in holdfast availability and subsequent density increases were associated with increased holdfast availability. These temporal results are consistent with previous spatial results, with *H. guttulatus* having a preference for highly complex habitat types throughout the Ria Formosa Lagoon (Curtis & Vincent, 2005). Holdfasts may be particularly important for seahorses in the Ria Formosa Lagoon given that it is a highly hydrodynamic system, where average maximum current speed can reach 1.25 m s^{-1} (Pacheco *et al.*, 2010). As seahorses are generally poor swimmers, they may need such holdfasts to prevent them from being dragged away from preferred habitats (Curtis & Vincent, 2005).

There have been overall habitat declines and degradation in the Ria Formosa Lagoon that could explain the initial decline in seahorse population densities, but not the subsequent increased densities. Seagrass beds have been declining throughout the lagoon over the past 20 years, with an overall 75% reduction in the distribution of *Z. noltii* (Cunha *et al.*, 2014). Considering that species in this study have been reported to favour seagrass as preferred habitat (Curtis & Vincent, 2005), this decrease in seagrass abundance should have a negative effect on seahorse populations, particularly for *H. guttulatus*, and could be responsible for the decline in their population densities between the 2001–2002 and 2008–2009 surveys (Ribeiro *et al.*, 2006). It is possible that seahorses in the lagoon may have shifted to new habitat types where their preferred seagrass habitats have disappeared, which could explain the upward trend in both *H. guttulatus* populations and holdfast availability. Human-related activities (*e.g.* fisheries including illegal fishing, anchoring and dredging) and natural changes in the lagoon's dynamics (*e.g.* silting events and shifting currents), appear to be the main causes for an overall habitat loss in the lagoon (Curtis *et al.*, 2007). The Ria Formosa sustains a clam

farming industry (Guimarães *et al.*, 2012), harbours, 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.*, 2014). There has also been an increase in reports of illegal fishing in the Ria Formosa (bottom trawling and beach seine fisheries) in addition to legal fishing activities (trap and line fisheries) (Erzini *et al.*, 2002). The combination of these anthropogenic activities and natural events are known to alter the seahorses' habitat conditions and reduce the available amount of natural holdfasts, essential for seahorse settlement (M. Correia, pers. obs.). These same activities have also introduced a variety of artificial habitat structures (*e.g.* mooring lines, lost traps and detritus). *Hippocampus guttulatus* is known to use a variety of holdfasts, both natural and artificial (Curtis & Vincent, 2005), and in this most recent survey the benthos was dominated by shells, tunicates, tube-dwelling polychaetes (*Sabella* sp.) and artificial holdfasts, whereas in 2001–2002 it was dominated by seagrasses and macroalgae (Curtis & Vincent, 2005). Microhabitat data were not available for each of the survey periods to test this hypothesis, but as their preferred seagrass habitats have disappeared in the lagoon, seahorses could be shifting to these newly available habitats.

If fluctuations are being driven by changes in holdfast availability, artificial habitats, such as artificial seagrass, could be used as a temporary measure to stabilize populations and thus make them less vulnerable to additional stressors. Artificial seagrass has been used in many countries as a method to replace damaged natural seagrass ecosystems thus providing marine habitat for various marine organisms, nursery grounds for juveniles and protective structures for small fishes (Sogard, 1989; Sogard & Able, 1994; Kenyon *et al.*, 1999; Lee *et al.*, 2001; Shahbudin *et al.*, 2011). Artificial holdfasts were also recently tested as a potential habitat restoration tool for *H. guttulatus*, first in captivity (Correia *et al.*, 2013) and then *in situ* (Correia, 2015), with promising results. Although these structures have proven their usefulness to promote seahorse settlement, they should be mainly considered for heavily degraded habitats and as temporary mitigation (Correia, 2015), with a longer-term goal of restoring natural habitats to these areas. As seahorses have life-history traits that make them particularly vulnerable, they may need such temporary measures to protect them from additional effects during low density periods.

There are a variety of additional influences that were not explored in this study that could also contribute to the observed seahorse population variability in the Ria Formosa, including changes in predators and food availability. According to Shelton & Mangel (2011), species interactions, such as predator–prey interactions, can be the cause of population fluctuation. In fact, Harasti *et al.* (2014) found a significant negative correlation between seahorse abundance and predator abundance. Although there are only two documented predators of *H. guttulatus* and *H. hippocampus* [*i.e.* the common octopus *Octopus vulgaris* and the loggerhead sea turtle *Caretta caretta* (Kleiber *et al.*, 2011)], cuttlefish *Sepia officinalis* have also been observed feeding on these seahorses (M. Correia, pers. obs.). Octopus and cuttlefish were commonly seen in 2008–2009 and 2010–2013 surveys, but these predators tend to be opportunistic feeders (Kleiber *et al.*, 2011). A decrease in prey abundance might drive these predators to feed on seahorses, but considering that the Ria Formosa is a nursery ground for several other potential prey species, this seems unlikely. Fluctuations in seahorse populations could also be related to changes in food availability, with direct effects on both adult and juvenile survival and indirect effects on brood size and quality through changes in

adult condition. The role of these additional influences on these populations could be the subject of future studies.

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