Does the endangered Knysna seahorse, *Hippocampus capensis,* have a preference for aquatic vegetation type, cover or height?

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The Knysna seahorse, *Hippocampus capensis,* is an endangered teleost confined to three South African estuaries. Its abundance within these systems is low and distributions are patchy. Consequently, monitoring population sizes is labour-intensive. The aim of this study was to establish if Knynsa seahorses are associated with specific regions within the estuaries, on which conservation efforts could concentrate. The relationship of Knysna seahorses with aquatic vegetation was analysed in the Knysna Estuary (the largest of the three estuarine systems inhabited by *H. capensis*) to determine whether this species shows a preference for a particular plant species, vegetation density or vegetation height. Seahorses were associated with five dominant aquatic plants: *Zostera capensis, Caulerpa filiformis, Codium extricatum, Halophila ovalis* and *Ruppia cirrhosa.* Together, these comprised 96% of the submerged objects with which seahorses were associated. The relative abundance of plant species changed along the estuary, but seahorses were present throughout the system, except at the estuary mouth, which was characterized by low plant densities and strong currents. No significant difference was found between the proportion of plant species present in a particular region of the estuary and the proportion of plants that seahorses used as holdfasts. However, when *Z. capensis* and *C. filiformis* were present at the same sites, adult seahorses preferred *C. filiformis* as holdfast. Adult seahorse density (individuals/m²) was significantly correlated with percentage vegetation cover and with holdfast length, but juvenile seahorse density was not. Nonetheless, significantly more adult and juvenile seahorses were found at sites characterized by high vegetation cover (>75%) than at sites with lower cover. Our results indicate that although there is some evidence that Knynsa seahorses prefer certain plant species over others, they are likely to be encountered anywhere in the estuary where aquatic plants are present. Conservation efforts in the Knysna Estuary should thus concentrate on such vegetated areas, which comprise approximately 11% of the total submerged surface area.

Key words: Knysna seahorse, *Zostera capensis, Caulerpa filiformis, Codium extricatum, Halophila ovalis, Ruppia cirrhosa,* holdfast preference, conservation.

INTRODUCTION

Seahorses (Syngnathidae: *Hippocampus*) are teleost fishes characterized by the absence of a caudal fin, the head at a right angle to the trunk, a brood pouch sealed along the midline, a raised dorsal fin base, and a prehensile tail (Fritzsche 1980). Their muscular prehensile tail serves as an anchor and is used to grasp 'holdfasts', e.g. seagrass stems, coral heads, or other suitable objects (Lourie *et al.* 1999). This behaviour prevents seahorses from being washed away by tidal currents (Whitfield 1995). Seahorses rely on camouflage as means of predator avoidance and to ambush prey, and they can change colouration and grow skin filaments to match their surroundings (Foster & Vincent 2004). The appearance of some species (most notably the Indo-Pacific pygmy seahorses *H. bargibanti* and *H. denise*) matches the colouration and shape of their holdfasts in remarkable detail (Kuiter 2000, Lourie & Randall 2003).

The estuarine Knysna seahorse, *Hippocampus capensis* Boulenger, 1900, was the first seahorse species to be listed as endangered on the IUCN Red Data List (Hilton-Taylor 2000). Populations of *H. capensis* exist in only three South African estuaries (Lockyear *et al.* 2006). Construction developments and associated anthropogenic impacts

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necessitated investigations into the species' habitat requirements to evaluate management of these estuaries.

Lockyear *et al.* (2006) showed that seahorses occurred along the entire length of the Knysna Estuary, except in the marine-dominated mouth area. Seahorse density was estimated at only 0.0114 individuals/m^2 and distributions were patchy. We thus investigated if this species has a preference for certain areas of the estuary and if this may be related to the types of available holdfasts.

In a study on ecological issues relevant to the conservation of *H. capensis,* Bell *et al.* (2003) found 86% of seahorses sampled in the Knysna Estuary in areas with less than 20% vegetation cover, and 76% of them were observed to be grasping the seagrass *Zostera capensis.* If areas characterized by low densities of seagrass are indeed a preferred habitat type of *H. capensis* (as postulated by Bell *et al.* 2003), then such areas should be placed under protection. Also, in order to monitor population densities, sampling should be done specifically in such areas. In the present study, we tested the results of the previous study using a data set approximately seven times as large as that generated by Bell *et al.* (2003), and analysed the data in more depth to determine if *H. capensis* in the Knysna Estuary shows a preference for certain plant species, vegetation cover, and vegetation height.

MATERIALS & METHODS

Sampling procedure

Between January and December 2001, 159 sites in the Knysna Estuary were searched for seahorses. Each site comprised between two and four transect lines (583 transects in total). Transects were 30 m in length and were searched for seahorses by two scuba divers within 1 m on either side of the transect line. An estimate of percentage vegetation cover was made every 5 m along the transect line. Sampling of seahorses in the Knysna Estuary is difficult, because many areas are characterized by dense vegetation and poor visibility. To improve sampling success in areas where vegetation was too dense to search visually (approximately >60% cover), a pushnet was used to collect the vegetation, which was searched for seahorses on a boat or in shallow water. This pushnet consisted of a 1 m^2 metal frame that served as an opening and was held in a vertical position, a bag 1.5 m in length with a 5 mm mesh size, and a 20 cm rake structure attached underneath the metal frame, which was pushed into the substratum below the roots to dislodge the vegetation without harming the seahorses (Lockyear *et al.* 2006).

The type and height of a seahorse's holdfast, and the approximate percentage cover of each aquatic plant species within a radius of 1 m of the seahorse, were recorded. It was also recorded whether the seahorse was an adult or a juvenile. It is difficult to distinguish between juvenile and female seahorses, as secondary sexual characteristics are not obvious. Adult males were recognizable by the presence of a brood pouch and were recorded at a standard length (Lourie 2003) of 4 cm or larger. Individuals without a brood pouch were recorded as adult females if their standard length was greater than 4 cm, and as juveniles if it was less than 4 cm. As the sex ratio of Knysna seahorses is even (Bell *et al.* 2003) and the species is at least temporarily monogamous (Foster & Vincent 2004), data from adult males and females were pooled. Observations from adult and juvenile seahorses were analysed separately to determine whether the two groups have different habitat preferences. It was not feasible to estimate vegetation cover by counting individual plants at each site and measuring how much surface area their roots covered. Thus, each of the two divers made an independent visual estimate of percentage vegetation cover, and the mean of the two estimates was used for statistical analyses. In some analyses, vegetation cover data were categorized as 0 (no vegetation), 1 (>0–25% vegetation cover), 2 (>25–50% cover), 3 (>50–75% cover) and 4 (>75–100% cover).

Sampling areas

To account for changes in physico-chemical and biological conditions along the length of the Knysna Estuary, the system was divided into six sampling areas (A–F, Fig. 1), which can be assigned to one of the three estuarine regimes proposed by Largier et al. (2000): Area A represents the marine-dominated 'bay regime', characterized by high salinity (>34), strong currents and sparse aquatic vegetation, areas B–E corresponded to the 'lagoonal regime' of lower salinity (30–34), weak currents and extensive beds of aquatic vegetation, and area F represents the 'estuarine regime' characterized by low salinity (<30) and high turbidity. The lagoonal regime was subdivided into four areas
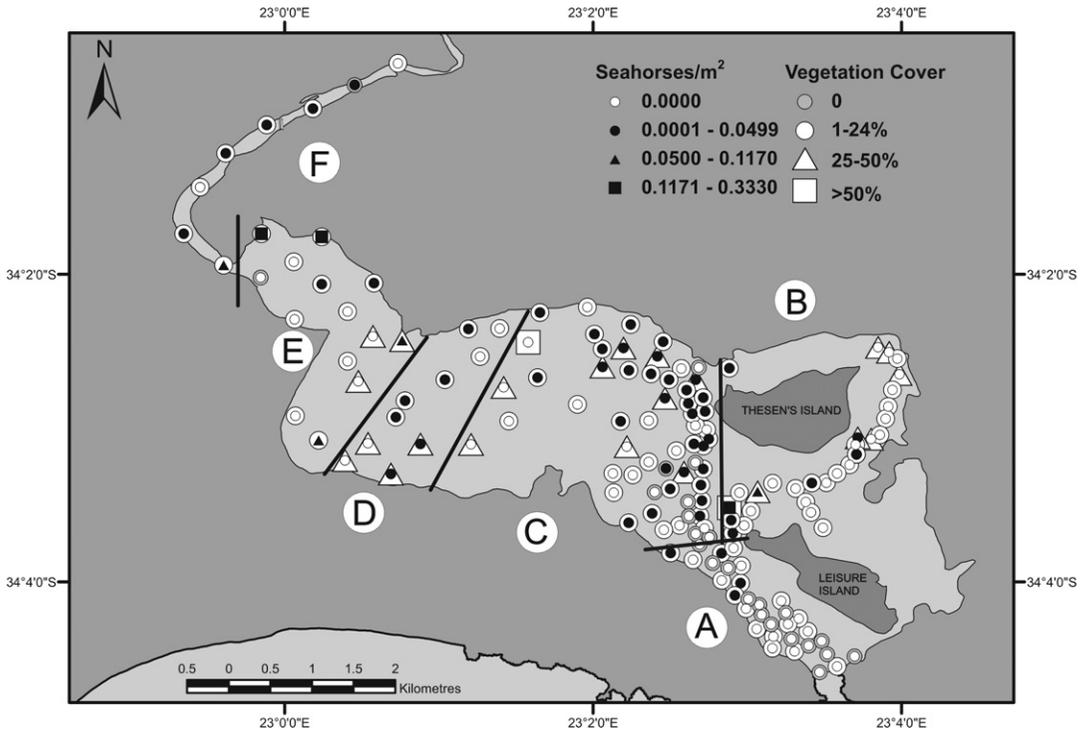
Fig. 1. Map of the Knysna Estuary showing seahorse density and percentage plant cover at each sampling site within six sampling areas (A–F).

based on geographic or biological data (B: area around Thesen's Island; C: upper distribution limit of the spiny starfish, *Marthasterias glacialis;* D: *Codium extricatum* as the dominant plant; E: upper distribution limit of the shaggy seahare, *Bursatella leachi*).

Data analysis

Relative proportions of holdfasts within the individual sampling areas were plotted, and statistical analyses were performed to test (a) if seahorses showed a preference for a particular plant species, and (b) if they showed a preference for a particular vegetation cover or height.

a) Preferences for plant species

Most seahorses were associated with plant holdfasts rather than sessile invertebrates or inanimate objects (see Results). The question whether Knysna seahorses showed a preference for a particular plant species was investigated using the following two approaches: First, χ^2-tests as implemented in the program PopTools (Hood 2005) were used to determine whether there was a significant difference between the proportional frequency of a particular plant species being used as a holdfast, and the proportional abundance of that species within each sampling area and within the entire Knysna Estuary. To eliminate zeros, 0.0001 was added to each value. Only sites at which seahorses were recorded were included in the analysis. Data for adults and juveniles were tested separately. To test if adults and juveniles preferred different holdfasts, a χ^2 analysis was performed on data for the whole estuary.

Second, a 'holdfast preference index' was calculated to determine if seahorses selected a particular plant species as holdfast over other plant species present at the same sites. The estimated percentage cover of the plant used as a holdfast at a particular site was divided by total vegetation cover at the same site, and the result was subtracted from 1. Thus, by dividing percentage cover by total cover, the index becomes independent of plant density. If the plant used as a holdfast is the only plant present at the site, then the index is 0. If there is more than one plant species, and if the plant used as a holdfast is rare (e.g. 1% of total

plant cover) at a site is otherwise dominated by one or more other plants (99% of total plant cover), then the index is 0.99. The index is thus high if a particular plant is preferred as a holdfast over other plants and low if seahorses hold on to any plant species present at that site. To test if seahorses preferred a particular plant species, indices for each plant species were calculated using only data from sites at which more than one plant species was present. Significant differences in the magnitude of the index between plant species were determined by estimating 95% confidence intervals of the means by generating 10 000 bootstrap replications of each plant species' data-set.

b) Preference for vegetation cover and height

Relationships between seahorse density and categorized plant cover, as well as seahorse density and plant height, were established using Spearman rank correlations as implemented in STATISTICA version 7 (StatSoft, Inc. 2004). In this case, it was not taken into account with which plant species the seahorses were associated. In addition, the percentage of the total number of seahorses at each plant cover category was estimated. Significance was determined by calculating 95% confidence intervals of the means of 10 000 bootstrap replications as described above. A χ^2 analysis was performed to test whether the ratios of adult and juvenile seahorses in each of the vegetation cover categories differed.

RESULTS

Seahorses were found at 64 out of the 159 sampling sites (Fig. 1). Aquatic vegetation was present at all sites where seahorses were found, but no seahorses were found at 76 sites that had vegetation cover. The approximate cover of submerged vegetation in the Knysna Estuary was estimated at 11.3%. Seahorses were associated with five plant species: *Zostera capensis, Caulerpa filiformis, Codium extricatum, Halophila ovalis* and *Ruppia cirrhosa* (hereafter referred to by their genus names). *Zostera* and *Ruppia* are seagrasses with long, narrow leaves. *Caulerpa* and *Halophila* are comparatively low in height and grow by means of creeping rhizomes. *Codium* is characterized by spongy branches and formed dense clumps in some areas of the estuary. Several other aquatic plant taxa were recorded, for example, *Enteromorpha intestinalis, Ulva* sp. and various rhodophytes, but these were not used as holdfasts. In addi-

tion to aquatic plants, seahorses were associated with *Pyura stolonifera* (a large ascidian) and with sponges.

Preference for plant species

Zostera was the most frequently used holdfast of both adult and juvenile seahorses (47% and 61% of the total number of holdfasts used, respectively; Fig. 2) followed by *Caulerpa* (30% and 13%, respectively) and *Codium* (13% for both). Comparatively few seahorses were associated with *Halophila* (4% and 5%) and *Ruppia* (5% and 6%). The relative proportion of plants used as holdfasts changed along the length of the estuary (Fig. 2). *Zostera* was the most commonly used holdfast in the lower reaches, *Caulerpa* became more abundant in the middle reaches, *Codium* was the most commonly used holdfast in area D, and areas E and F were characterized by a comparatively high proportion of *Ruppia.* No significant difference was found between the proportion of plants being used as holdfasts, and the proportional abundance of these plants at sites at which seahorses were recorded (all sampling areas combined, Table 1). The difference in the proportions of holdfasts used by adults and juveniles was not significant ($\chi^2 = 9.1$, $P = 0.058$).

'Holdfast preference indices' for each plant species were calculated using only adult seahorse data, because only seven of the sites at which juveniles were found had more than one plant species. The mean holdfast preference index calculated based on percentage plant cover was lowest for *Zostera*, higher for *Caulerpa*, and highest for *Codium* (Fig. 3). The indices for *Zostera* and *Caulerpa* were based on 33 and 27 samples, respectively. Samples sizes for *Codium, Halophila* and *Ruppia* were comparatively low (3, 5 and 2, respectively) and for that reason, their holdfast preference indices cannot be considered reliable.

Preference for vegetation cover or height

Density of adult seahorses (individuals/m^2) was positively correlated with vegetation cover and holdfast height ($n = 56$, $R = 0.39$, $P < 0.01$ and $n = 56$, $R = 0.22$, $P < 0.05$, respectively). These relationships were not significant for juvenile seahorses ($n = 18$, $R = 0.10$, $P = 0.70$ and $n = 18$, $R = 0.22$, $P = 0.38$). The percentage of seahorses present in areas characterized by very high vegetation cover (category 4: >75% cover) was higher than the percentage of seahorses present in other categories (Fig. 4). Data from adult and juvenile seahorses
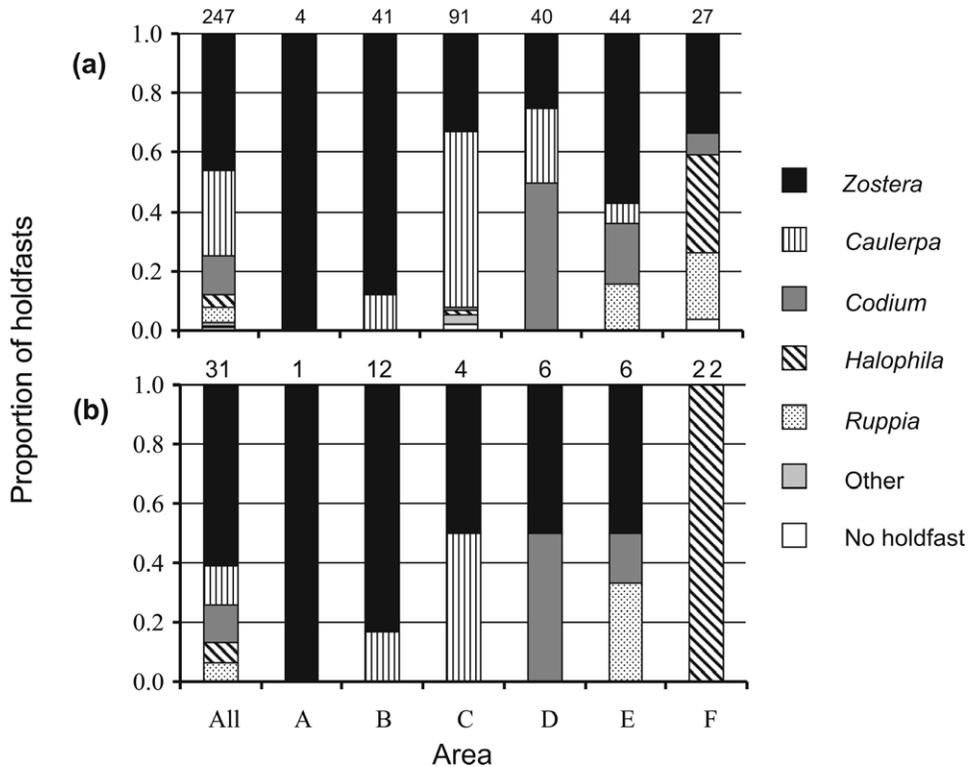
Fig. 2. Proportion of holdfasts used by (**a**) adult seahorses and (**b**) juvenile seahorses in the six sampling areas within the Knynsa Estuary and in the whole estuary. Sample sizes are shown above each column.

were combined because their ratios in the five vegetation cover categories did not differ (χ^2 = 4.38, P = 0.36).

DISCUSSION

The plant species used as holdfasts by Knysna seahorses changed with increasing distance from the estuary mouth. However, as no significant difference was found between the proportion of plants used as holdfasts by both adult and juvenile seahorses, and the proportion of plants present at sites where seahorses were found, we suggest that Knysna seahorses may select as holdfast any plant species present at a particular site. Most of the other seahorse species studied to date may also be associated with a variety of holdfasts. For example,

Table 1. Results of χ^2-tests of proportional mean abundance of aquatic plant species present within the six sampling areas in the Knynsa Estuary (based on categorized data) and the proportion of these that were used as holdfasts by Knynsa seahorses; (**a**) adult seahorses; (**b**) juvenile seahorses. The number of seahorses used to calculate proportions within sampling areas and within the whole estuary are given by *n*.

		Sampling areas						
		All	A	B	C	D	E	F
(**a**)	χ^2	5.5	18.2	1.3	9.1	3.1	5.8	10.8
	P	ns	**	n.s.	n.s.	n.s.	n.s.	*
	n	247	4	41	91	40	44	27
(**b**)	χ^2	3.6	40.0	1.4	0.00	18.6	4.5	0.00
	P	n.s.	**	n.s.	n.s.	**	n.s.	n.s.
	n	31	1	12	4	6	6	2

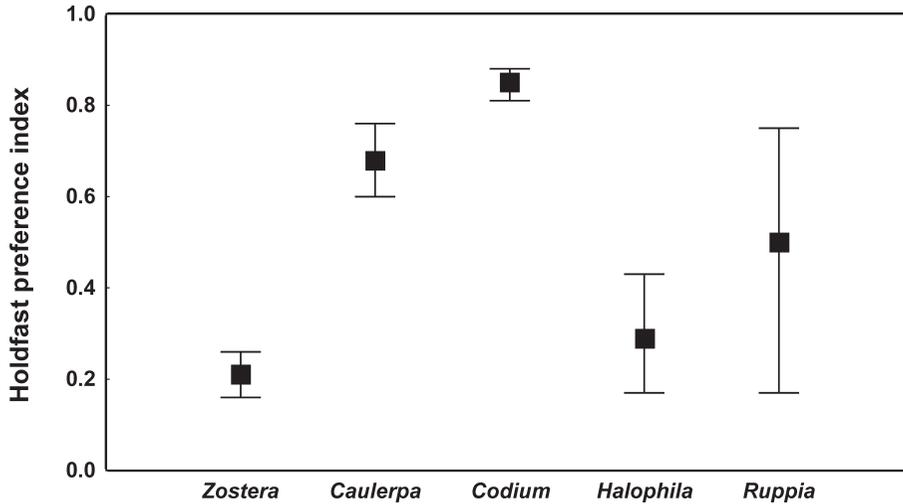n.s. = not significant, *P < 0.05, **P < 0.01.

Fig. 3. Holdfast preference indices of five aquatic plant species used as holdfasts by adult Knysna seahorses. Whiskers are 95% confidence intervals based on 10 000 bootstrap replications.

the Australian species *Hippocampus whitei* Bleeker, 1855, was most abundant in *Posidonia australis* seagrass meadows, but it may also use artificial structures, such as shark nets, as holdfasts (Vincent *et al.* 2005). *Hippocampus comes* Cantor, 1850, in the Philippines was associated with a variety of submerged objects (corals, sponges and mangrove twigs) and showed no holdfast preference (Perante *et al.* 2002). Moreau & Vincent (2004) found *H. breviceps* Peters, 1869, in vegetation that

included the seaweeds *Sargassum* sp., *Cystophora moniliformis*, *Caulocystis* sp. and *Laurencia majuscule*, and the seagrasses *Zostera* sp. and *Posidonia* sp. This species seemed to prefer *Sargassum* spp. and *L. majuscule* as holdfasts, and was never found on seagrass (Moreau, pers. comm.). The European species *H. guttulatus* and *H. hippocampus* were associated with a variety of holdfasts such as seagrass blades, macroalgae, tunicates, bryozoans, polychaete tubes, sea urchins, and artificial struc-
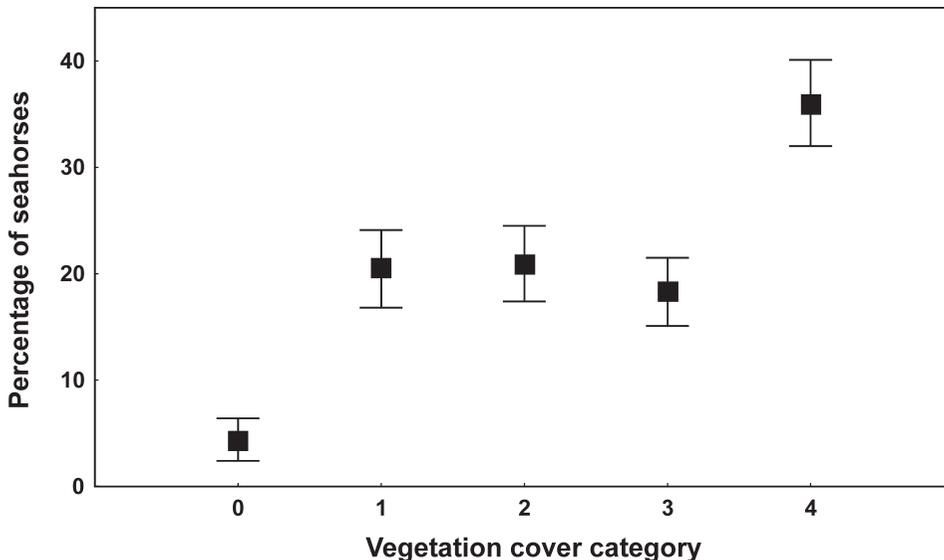

Fig. 4. Percentage of Knysna seahorses (adults and juveniles combined) associated with one of five vegetation cover categories: 0 = no vegetation; 1 = 1–25% cover; 2 = 26–50% cover; 3 = 51–75% cover; 4 = 76–100% cover. Whiskers are 95% confidence intervals based on 10 000 bootstrap replications.

tures such as ropes, nets and bricks. Their preferences differed in that *H. guttulatus* grasped all holdfasts with equal probability, whereas *H. hippocampus* avoided both fauna and flora that formed large colonies or tracts of dense vegetation (Curtis & Vincent 2005).

Adult Knysna seahorses showed a preference for *Caulerpa* over *Zostera* at sites where both plants were present. The preference for *Caulerpa* (a species of low density and low height) and high vegetation cover may be an indication that adult seahorses compromise between the need to hide from predators (occurring in close proximity to the dense vegetation cover afforded by the long-bladed *Zostera*) and the need to be detected by a potential mate (using a plant as holdfast at which they are more exposed). This hypothesis could be further explored by comparing holdfast preference of adult seahorses during the breeding season with that seen during the remainder of the year.

Although most seahorses were associated with *Zostera,* this seagrass species may not be a preferred holdfast under conditions where there is a choice, as indicated by its comparatively low 'holdfast preference index'. The high occurrence of seahorses associated with *Zostera* may be explained by the fact that this seagrass is the most abundant plant species in the Knysna Estuary (Grindley 1985). The significant but weak correlation of adult seahorse abundance and vegetation height may also not be an indication of seahorse preference, because of the higher abundance of tall plants (*Zostera, Codium* and *Ruppia*; mean height 33, 42 and 61 cm, respectively) as compared to short plants (*Caulerpa* and *Halophila;* mean height 16 and 3 cm, respectively) in the Knysna Estuary. Frequencies, particularly of *Halophila* and *Ruppia,* were comparatively low, and their suitability as holdfasts could thus not be determined with a high degree of confidence.

Although there was some indication that adult Knysna seahorses selected *Caulerpa* over *Zostera* when both plants were present, seahorse density was not greater in sampling area C (where *Caulerpa* was particularly common) than in most of the other sampling areas (Lockyear *et al.* 2006). An exception was the mouth area of the estuary (area A), which is mostly devoid of aquatic vegetation. These results suggest that Knysna seahorses are likely to be encountered anywhere in the estuary where aquatic plants are present. As submerged vegetation covers only about 11% of the estuary, it is important that a sufficient amount of this habitat

be protected. In addition to discouraging boat use and other recreational activities in areas with aquatic vegetation, several locations in the estuary could be declared protected areas. Particularly areas where subtidal vegetation cover is continuous and where seahorses were found at adjacent sampling sites could be placed under protection. Suitable areas include the area northwest of Leisure Island, the area west of Thesen's Island, and the shoreline of area F (Fig. 1). However, additional surveys are required to determine whether these areas are continually inhabited by seahorses, or whether changes in vegetation cover (e.g. as a consequence of freshwater floods) may result in changes in seahorse distributions within the Knysna Estuary.

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