

## Monthly population density and structure patterns of an endangered seahorse *Hippocampus capensis*: a comparison between natural and artificial habitats

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This study investigated aspects of the population ecology of the endangered Knysna seahorse *Hippocampus capensis* within different habitat types. High densities of *H. capensis* were found within artificial Reno mattress habitat, within the Knysna Estuary, South Africa. Monthly surveys at three sites were conducted from October 2015 to August 2016 to compare population densities of *H. capensis* in this artificial habitat with natural eel grass *Zostera capensis* habitat. *Hippocampus capensis* densities varied significantly across all sites and highest population densities were consistently observed within the Reno mattress habitat. *Hippocampus capensis* were also found to be significantly larger within the Reno mattress habitat and pooled data showed that males were significantly larger than females. The overall sex ratio for all three sites was female biased, although this varied across seasons at two sites. The findings suggest that artificial Reno mattresses provide novel habitat for this endangered species and consideration should be given to the usefulness of these structures in future conservation actions.

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Key words: conservation; *Hippocampus capensis*; Knysna; population ecology; seagrass; seahorses.

### INTRODUCTION

Successful conservation depends on empirical knowledge of species-specific population variables such as abundance, geographical range, distribution and habitat requirements (McLean *et al.*, 2016). Changes in these characteristics and the reasons behind such changes are central to ecology, specifically, the nature of the relationship between abundance and distribution (Brown, 1984; Guisan & Thuiller, 2005). In marine environments, population variability can be caused by various natural and anthropogenic factors (Sissenwine, 1984; McCain *et al.*, 2016) and it is crucial that these are understood for management and conservation.

Gaps in life-history information are common for many marine species, especially small to medium-sized fish (Foster & Vincent, 2004). For example, 42.5% (updated list since original submission) of seahorse *Hippocampus* Rafinesque 1810 species listed on the IUCN Red Data List of Threatened Species are classified as Data Deficient ([www.iucnredlist.org](http://www.iucnredlist.org)). According to Lourie *et al.* (2016), 'population surveys and

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on-going monitoring are urgently required to accurately ascertain and effectively improve the conservation status of all seahorse species'.

Population density information, however, does exist for some seahorse species: *Hippocampus guttulatus* Cuvier 1829 (Curtis & Vincent, 2005; Correia *et al.*, 2015a; Gristina *et al.*, 2015; Woodall *et al.*, 2015); *Hippocampus hippocampus* (L. 1758) (Curtis & Vincent, 2005; Correia *et al.*, 2015a; Gristina *et al.*, 2015; Otero-Ferrer *et al.*, 2015); *Hippocampus reidi* Ginsburg 1933 (Dias & Rosa, 2003; Freret-Meurer & Andreato, 2008); *Hippocampus whitei* Bleeker 1855 (Harasti *et al.*, 2012; Harasti, 2016); *Hippocampus capensis* Boulenger 1900 (Bell *et al.*, 2003; Lockyear *et al.*, 2006); *Hippocampus comes* Cantor 1849 (Perante *et al.*, 2002; Marcus *et al.*, 2007); *Hippocampus zosterae* Jordan & Gilbert 1882 (Mason-Jones *et al.*, 2010); *Hippocampus abdominalis* Lesson 1827 (Martin-Smith & Vincent, 2005); *Hippocampus breviceps* Peters 1869 (Moreau & Vincent, 2004). Many of these studies only presented a snapshot of the status of a population (Bell *et al.*, 2003; Curtis & Vincent, 2005; Lockyear *et al.*, 2006; Gristina *et al.*, 2015), which could lead to misleading conclusions. For example, numbers of *H. guttulatus* and *H. hippocampus* within the Ria Formosa, Portugal, decreased by *c.* 90% between 2001 and 2009 (Curtis & Vincent, 2005; Caldwell & Vincent, 2012), then started to increase by 2013 (Correia *et al.*, 2015a). In Tasmania, population numbers of *H. abdominalis* fluctuated dramatically from year to year (Martin-Smith & Vincent, 2005). Whilst short-term population density estimates can provide population base-line information, it is only through longer-term monitoring that underlying processes responsible for population variability can be understood.

Seahorses are commonly found in shallow coastal and estuarine habitats where they are vulnerable to environmental variation. For example, the decrease in *H. whitei* populations in Australia was directly linked to the loss of essential habitats (Harasti, 2016). Habitat alteration, degradation and loss have been identified as major threats to seahorses (Vincent *et al.*, 2011) and specifically to the endangered Knysna seahorse *H. capensis* (Whitfield, 1995). How seahorses adapt to habitat alterations and additions, if at all, is an important characteristic that must be understood to ensure their successful future conservation (Vincent *et al.*, 2011). Habitat use is also an important aspect in seahorse population assessments (Curtis *et al.*, 2004), because seahorse abundance, population density and distribution change across different habitat types (Curtis & Vincent, 2005; Harasti *et al.*, 2014a; Otero-Ferrer *et al.*, 2015). Habitat-use data are also helpful to understand possible reasons for seahorse population variability and many studies have focussed on population density and distribution within different habitat types (Curtis & Vincent, 2005; Gristina *et al.*, 2015; Otero-Ferrer *et al.*, 2015).

The geographical distribution of *H. capensis* is restricted to the Knysna, Swartvlei and Keurbooms estuaries (Bell *et al.*, 2003; Lockyear *et al.*, 2006) on the south coast of South Africa. Three population assessments have been done for *H. capensis* in the Knysna Estuary (Bell *et al.*, 2003), the last completed in 2001 (Lockyear *et al.*, 2006). A more recent population assessment of *H. capensis* was limited in extent and focussed on seahorse populations in the waterways of Thesen Islands Marina (Claassens, 2016) (Fig. 1), an artificial water body (terminology from EC, 2003) located in the lower reach of the estuary. These assessments provided a baseline of the population density and distribution of this species within the Knysna Estuary. In the earlier studies (Bell *et al.*, 2003; Lockyear *et al.*, 2006) habitat specific population density was not taken

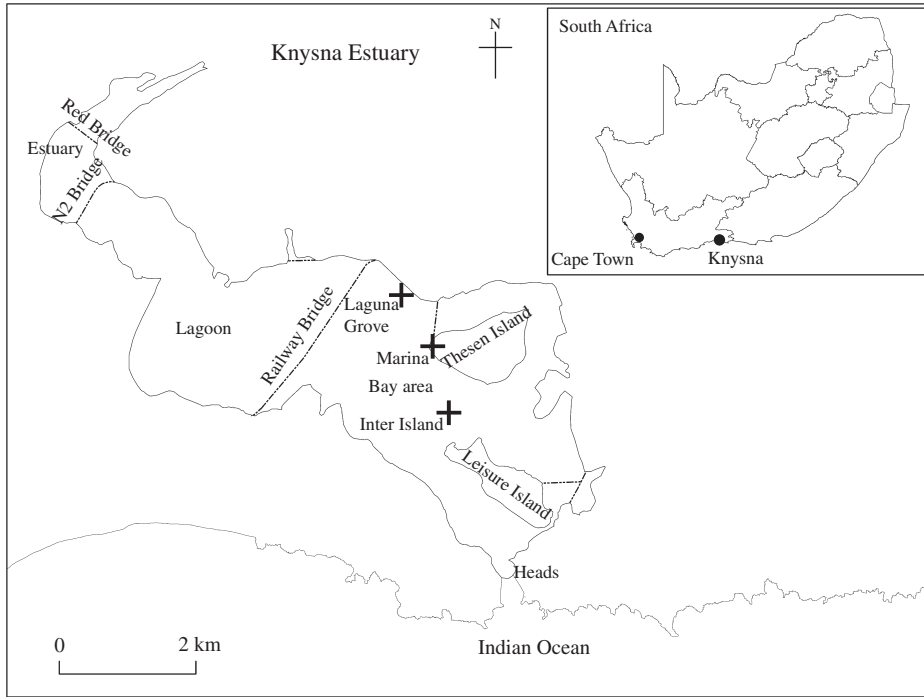


FIG. 1. Location of Knysna in the Western Cape Province, South Africa and the layout of the Knysna Estuary. The bay regime stretches from the Heads to the Railway Bridge; the lagoon regime from the Railway Bridge to the N2 Bridge, and the estuary regime from the N2 Bridge up to the Knysna River (Largier *et al.*, 2000). The Ashmead Channel lies on the eastern side of Thesen Island (Switzer, 2003).

into consideration, although Teske *et al.* (2007) concluded that *H. capensis* is likely to occur throughout the system where vegetation is available.

The more recent population survey of *H. capensis* by Claassens (2016), found high seahorse densities (mean  $\pm$  s.d. of  $0.13 \pm 0.01$  individuals  $m^{-2}$ ) within artificial Reno mattresses (wire boxes filled with stones; [www.maccaferri.com](http://www.maccaferri.com)) [Fig. 2(a), (b)] located within Thesen Islands marina. In addition, significantly higher seahorse population densities were found on the Reno mattress habitat compared with natural vegetation in the marina (Claassens, 2016). This study aimed to gain a better understanding of the habitat value provided to *H. capensis* by artificial Reno mattresses in the Knysna Estuary. This aim was achieved by: comparing *H. capensis* densities between natural and artificial habitats; investigating the density patterns of *H. capensis* within natural and artificial habitats on a monthly basis; investigating population structure, size differences and holdfast use of *H. capensis* within natural and artificial habitats.

## MATERIALS AND METHODS

### STUDY SITE

The Knysna Estuary [Fig. 1; subtidal area of 1000 ha (Bell *et al.*, 2003)] is an estuarine bay (Whitfield, 1992), situated on the southern Cape coast of South Africa and is considered

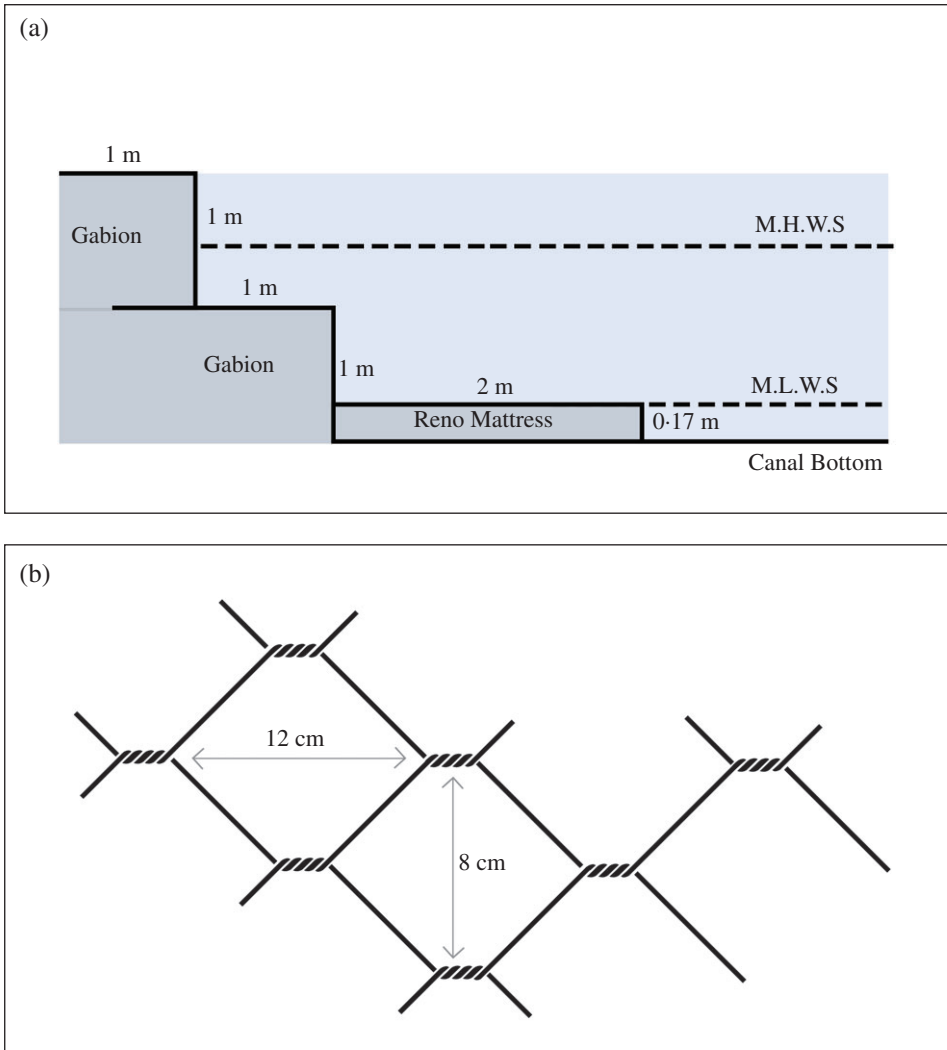


FIG. 2. Thesen Islands Marina canal wall design, illustrating the (a) vertical gabion walls, which rests upon a 2 m wide, horizontal Reno mattress; (b) Reno mattresses and gabions are made out of double twisted, PVC coated wire boxes filled with stones.

the most important estuary in South Africa in terms of conservation (Turpie *et al.*, 2002). In a recent review of *Zostera capensis* within South Africa, it was estimated that the Knysna Estuary contains *c.* 240 ha of eelgrass (Adams, 2016). Thesen Islands Marina (34° 02' 47.16" S; 23° 03' 18.84" E) was completed in 2005 and added 25 ha of artificial canals to the Knysna Estuary. The marina canals, excavated to 1.75 m below mean sea level, have sides made out of gabions (wire boxes filled with rocks; Maccaferri, 2005) and lined with Reno mattresses [flat gabion boxes positioned horizontally (Maccaferri, 1972); Fig. 2(a), (b)] (Claassens, 2016; Claassens *et al.*, 2018). Four major habitat types have been identified within the marina canals: artificial Reno mattress; the seaweed *Codium tenue*; mixed vegetation (*Zostera capensis*, *Caulerpa filiformis*, *Asparagopsis taxiformis*) on sediment; barren canal floor (Claassens, 2016).

## POPULATION ASSESSMENT

Monthly *H. capensis* population density surveys were conducted from October 2015 to August 2016 at one site within Reno mattress habitat in Thesen Islands Marina and two sites within *Zostera capensis* beds in the bay regime (terminology after Largier *et al.*, 2000) of the Knysna Estuary (Fig. 1). The marina site was chosen based on high *H. capensis* densities found during initial population surveys conducted by Claassens (2016) and is located on the western side of the marina development. The 2 m wide wire Reno mattresses located on the sides of the marina canals [Fig. 2(a), (b)] was the only habitat type surveyed within the marina.

Outside the marina within the estuary, two *Z. capensis* sites were chosen based on the relatively high *H. capensis* densities found during the 2001 population assessment by Lockyear *et al.* (2006): Laguna Grove (0.22 individuals m<sup>-2</sup>) and an Inter Island site (0.19 individuals m<sup>-2</sup>) (Fig. 1). The Inter Island site is located between Leisure Island and Thesen Island in the bay regime of the estuary. The area is covered by *Z. capensis* beds of varying density and water depth ranges from 1.0 to 2.7 m across the site during high tide. This area is affected by bait digging activity and large areas of *Z. capensis* beds are damaged in the process. Laguna Grove is located on the northern banks of the bay regime of the estuary. A pier located on the eastern side of the site is a popular fishing spot and litter is a common sight. Depth ranges from less than 1.0 to 3.6 m across the site during high tide. Vegetation cover is patchy across the site and includes *Z. capensis*, *A. taxiformis* and *C. filliformis* beds. An exploratory assessment in September 2015 confirmed the availability of suitable *H. capensis* habitat at these two sites as well as the presence of *H. capensis*.

Visual scuba survey transects, as per Curtis *et al.* (2004), were used for all population density surveys. Within the marina, each transect sample consisted of a 10 m long transect line with a 0.5 m search width on either side (10 m<sup>2</sup>). A similar method was used at the two *Z. capensis* sites with the following adjustments: a 30 m transect line, marked in 10 m intervals, with a 1 m search width was used (30 m<sup>2</sup>). The dominant vegetation type and percentage cover per 10 m<sup>2</sup> section were recorded in all sites. Sampling was done during high tide.

To compare *H. capensis* population densities between Reno mattress habitat within Thesen Islands Marina and the two estuary sites, a standard search effort unit had to be established because the habitat types differed between sites (Reno mattress within the marina and mixed vegetation in the estuary). This was done by recording the time spent searching for *H. capensis* along the transect [active search time = total search time per transect – (average handling time per *H. capensis* × total number of *H. capensis* found in transect)]. The average handling time (which included taking measurements, photographing the specimen *etc.*) was estimated to be 1 min per *H. capensis*. This approach allowed a *H. capensis* population density per unit time per unit area (search effort unit) to be established which was used to compare *H. capensis* densities spatially and temporally.

The height of each observed *H. capensis*, to the nearest 0.5 cm, was recorded, by measuring the vertical distance from the tip of the head to the tip of the outstretched tail (as per Lourie, 2003). During the measurement, the animal was held against a ruler with its head at a right angle to the body and the tail stretched out. *Hippocampus capensis* sex, brood pouch status for males and holdfast used by each specimen found were recorded. A distinction was made between a full brood pouch and an empty brood pouch (Curtis & Vincent, 2005). To ensure consistency with previous *H. capensis* surveys, animals larger than 4 cm with no brood pouch were considered to be females and an individual that showed no sexual differentiation and was smaller than 4 cm was considered to be juvenile, as established by Lockyear *et al.* (2006).

The coordinates of the starting point of each transect, chosen arbitrarily and taken with a hand-held Garmin GPSmap 62 ([www.garmin.com](http://www.garmin.com)), were logged. Surface water temperature using a thermometer and turbidity (NTU) using a Eutech TN-100 bench-top turbidity meter ([www.eutechinst.com](http://www.eutechinst.com)) were measured at each site.

Seven transects (210 m<sup>2</sup>) at each estuary site (Laguna Grove and Inter Island) and 10 transects (200 m<sup>2</sup>) within Reno mattress habitat in the marina were surveyed on a monthly basis from October 2015 to August 2016 (11 month period). To determine whether monthly variation in *H. capensis* population densities persisted on a longer-term basis, data from months were pooled and assessed seasonally (austral summer: December, January, February; autumn: March, April, May; winter: June, July, August; spring: October and November). Animals were not tagged and

the home-range of *H. capensis* is not known, therefore, the possibility exists that animals were resampled during repeated surveys.

## STATISTICAL ANALYSIS

The statistical programme R ([www.r-project.org](http://www.r-project.org)) was used in all analyses. The `ddply` function of the `Plyr`-package was used for all basic descriptive statistics (Wickham, 2011) and the `ggplot` package was used to create all graphs (Wickham, 2009).

The distribution of *H. capensis* was found to be over-dispersed (variance > mean) and followed a negative binomial distribution. A negative binomial generalized linear model (GLM) was used to assess *H. capensis* population differences spatially and temporally. Overall significance was tested using ANOVA. The `Car` (Fox & Weisberg, 2010) and `Mass` (Venables & Ripley, 2002) packages were used for generalized linear model analyses. Sex ratios were assessed with a  $\chi^2$ -test. ANOVA was used to compare temperature and turbidity spatially. Height differences between males and females and *H. capensis* height variability were assessed using ANOVA. Significant results from all ANOVA analyses were further assessed using a Tukey *post hoc* test. The assumptions of normality and equal variance, tested using Shapiro–Wilk test and Bartlett’s test respectively, were met ( $P > 0.05$ ).

## RESULTS

Both the marina and Laguna Grove sample sites were significantly more turbid ( $F = 18.7$ , D.F. = 2,  $P < 0.01$ ) and warmer (ANOVA,  $F_{2,359} = 5.2$ ,  $P < 0.01$ ) compared with the Inter Island site. The mean ( $\pm$ S.D.) turbidity and temperature data for all three sites are presented in Table I.

*Hippocampus capensis* population densities differed significantly (negative binomial GLM,  $F_{2,358} = 25.1$ ,  $P < 0.01$ ; Fig. 3) across all three sites and the highest population densities were consistently found in Reno mattress habitat within the marina. *Hippocampus capensis* population densities were lowest at the Inter Island site where no *H. capensis* were found in 87% of transects surveyed. *Hippocampus capensis* population densities varied significantly (negative binomial GLM,  $F_{10,358} = 3.5$ ,  $P < 0.01$ ) across months (Fig. 3). The highest *H. capensis* population density (mean  $\pm$  S.D.) in Reno mattress habitat in the marina was recorded in October 2015 ( $1.80 \pm 0.48$  individuals per search effort), with a marked decrease in population density during January and February 2016. *Hippocampus capensis* population density (mean  $\pm$  S.D.) at Laguna Grove was highest in November 2015 ( $1.21 \pm 0.33$  individuals per search effort). Densities at this site showed a continuous, gradual decrease from April 2016 onwards. Monthly *H. capensis* densities remained low at the Inter-island site across the entire sample period (Fig. 3) and data from this site were excluded from pooled analyses. Seasonal *H. capensis* population densities varied significantly (negative binomial GLM,

TABLE I. Surface water temperatures and turbidity (NTU) (mean  $\pm$  S.D.) for all sites sampled for *Hippocampus capensis* from October 2015 to August 2016

Site	Temperature (°C)	Turbidity (NTU)
Marina	$18.0 \pm 3.5$	$1.4 \pm 0.8$
Laguna Grove	$17.9 \pm 2.6$	$1.6 \pm 0.6$
Inter Island	$16.7 \pm 2.4$	$0.9 \pm 0.5$

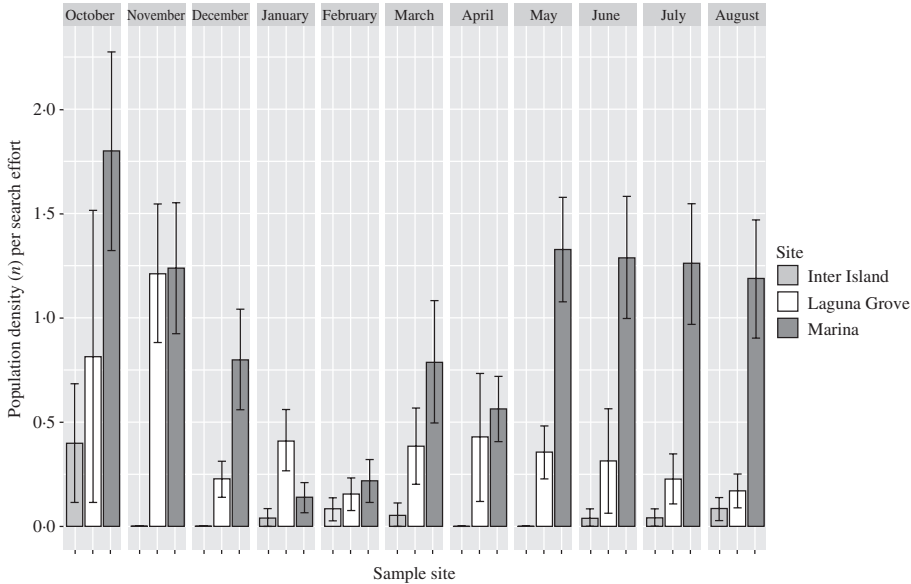


FIG. 3. Monthly (mean  $\pm$  S.E.) *Hippocampus capensis* population densities per search effort at the three sample sites recorded from October 2015 to August 2016.

$F_{3,365} = 9.3$ ,  $P < 0.01$ ; Fig. 4). Population densities within the Reno mattress habitat were stable, except for a significant population density decrease recorded in summer. Population densities were significantly higher during spring at Laguna Grove.

*Hippocampus capensis* height ranged from 4.0 to 10.5 cm across all sites, but were significantly larger (ANOVA,  $F_{2,645} = 27.6$ ,  $P < 0.01$ ) within the Reno mattress habitat (Fig. 5). Monthly *H. capensis* height varied significantly ( $F_{10,514} = 7.5$ ,  $P < 0.01$ ) only at the marina site, with significantly larger animals observed from October to February. The smallest *H. capensis* (4 cm) were observed at the Reno mattress and Inter Island sites, whilst the largest *H. capensis* (10.5 cm) was noted at the Reno mattress site. Pooled data across all sites showed that male *H. capensis* were significantly larger than females (ANOVA,  $F_{1,645} = 13.6$ ,  $P < 0.01$ ) and the height differences between males and females were significantly more pronounced within the marina only (Fig. 6).

Pooled data for the entire population (all sites) showed an overall female biased sex ratio ( $\chi^2 = 27.6$ , D.F. = 1,  $P < 0.01$ ; Table II). A seasonal change in sex ratio within Reno mattress habitat in Thesen Islands Marina and at Laguna Grove were noted (Table III). Because of the limited number of *H. capensis* found at the Inter Island site, seasonal sex ratios for this site could not be established.

Holdfast cover, estimated as percentage cover 10 m<sup>-2</sup> transect at all three sites, consisted of *Z. capensis*, *C. filiformis*, *Halophila ovalis*, *U. lactuca*, *Polysiphonia* sp. and wire. At Laguna Grove 68% and 5% of the transects were covered with *Z. capensis* and *C. filiformis*, respectively (the remaining 27% consisted of barren sand and mud). The majority of transects at the Inter Island site (93%) were covered by *Z. capensis*, the remainder (1% and 5%) was covered by *Polysiphonia* sp. and *U. lactuca*, respectively. Transects within Reno mattress habitat were covered with wire (90%), *H. ovalis* (5%) and *Z. capensis* (5%). The type of holdfast used by *H. capensis* varied across sites (Table IV).

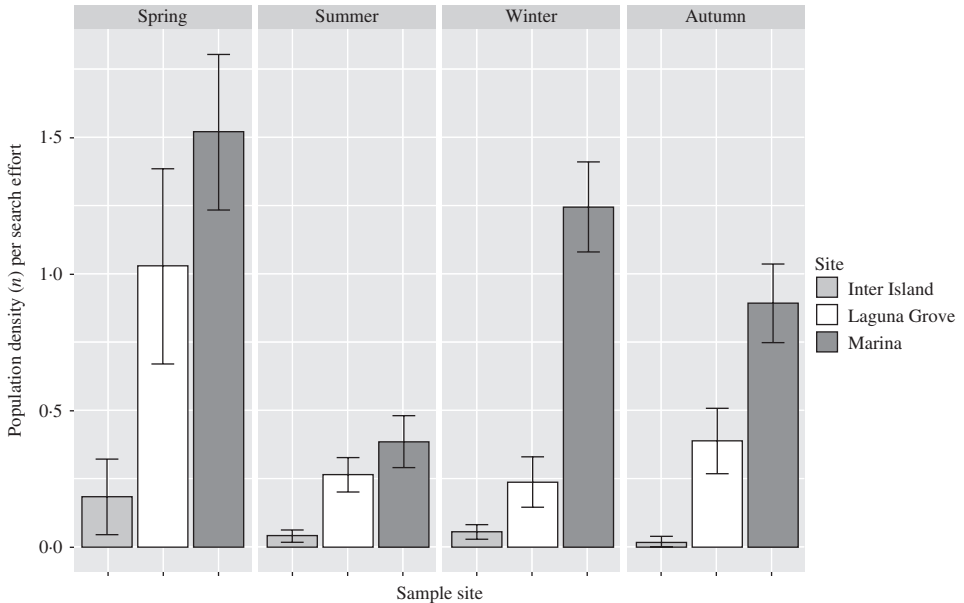


FIG. 4. Seasonal (mean  $\pm$  S.E.) *Hippocampus capensis* population densities per search effort at the three sample sites recorded from October 2015 to August 2016.

Inflated brood pouches (indicative of breeding) were noted from October 2015 to March 2016 at Laguna Grove and the marina sites only. Breeding activity was highest during October, November, December and January when  $>80\%$  of males were observed with an inflated brood pouch.

## DISCUSSION

This study provides a first look at monthly population density and structure patterns of *H. capensis* within both artificial and natural habitats in the Knysna Estuary. The densities of *H. capensis* within the artificial Reno mattress habitat in Thesen Islands Marina were consistently and significantly higher compared with two *Z. capensis* sites within the bay regime of the Knysna Estuary. Furthermore, the population densities of *H. capensis* within Reno mattress habitat, with an overall mean ( $\pm$ S.E.) population density of  $0.26 \pm 0.02$  individuals  $m^{-2}$ , exceeded those recorded by Bell *et al.* (2003;  $0.0089$  individuals  $m^{-2}$ ) and Lockyear *et al.* (2006;  $0.0114$  individuals  $m^{-2}$ ). The population densities recorded at the Inter Island and Laguna sites, with a mean ( $\pm$ S.E.) density of  $0.01 \pm 0.002$  to  $0.06 \pm 0.01$  individuals  $m^{-2}$ , respectively, are comparable with those found in the previous studies.

The higher *H. capensis* population densities consistently found in the marina is perhaps not surprising, as artificial habitats and structures can increase seahorse population densities (Harasti *et al.*, 2010; Correia *et al.*, 2015b), particularly in areas devoid of natural habitat (Correia *et al.*, 2015b). Seahorse species found in shallow waters tend to occur in habitats that are protected against adverse environmental effects, such as storms, and which provide suitable habitat in terms of holdfasts (Choo & Liew, 2003).



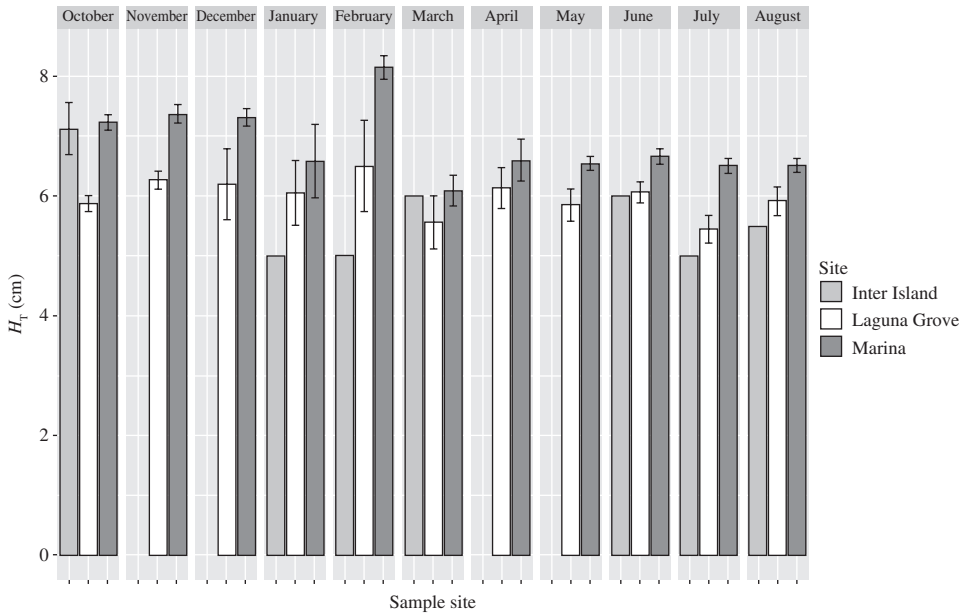


FIG. 5. Monthly *Hippocampus capensis* total height ( $H_T$ ; mean  $\pm$  s.e.) for all sites recorded from October 2015 to August 2016.

Because *H. capensis* is found in very shallow waters (<30 cm) and 67% of the marina canals are covered by some type of holdfast (Claassens, 2016), the marina provides suitable habitat for this species.

Two sympatric *Hippocampus* species in the Ria Formosa, *H. guttulatus* and *H. hippocampus*, were associated with highly complex and less complex habitat types, respectively, and finding a specific species depended on the characteristics of the available habitat (Curtis & Vincent, 2005). The distribution of *H. reidi* in Brazil was found to be determined by water depth, water temperature and the availability of holdfasts (Aylesworth *et al.*, 2015). *Hippocampus capensis* was found in a number of different habitat types, which confirms the result from Teske *et al.* (2007) that this species will occur wherever suitable vegetation (or holdfasts) are available. The artificial and natural habitat types utilized by *H. capensis* and investigated in this study, varied significantly in terms of temperature and turbidity, but these physico-chemical features did not seem to affect the distribution of *H. capensis*, although optimal physico-chemical conditions for the occurrence of *H. capensis* were not assessed.

A marked decrease in *H. capensis* population densities at Laguna Grove and within the Reno mattress habitat in the marina was observed during January and February 2016, part of the breeding season of *H. capensis* (Whitfield, 1995). An opposite pattern was noticed in Sydney Harbour, Australia, where *H. whitei* abundance increased on a swim net during October and November 2007. This increase was attributed to the breeding season of this species with movement of *H. capensis* from adjacent seagrass beds to the artificial aggregation area (Harasti *et al.*, 2012). A trade-off between protection and being visible to a prospective mate is commonly found in *H. capensis* (Kleiber *et al.*, 2011). One would therefore expect that surveys during a breeding season would result in higher *H. capensis* observations and thus population densities. The

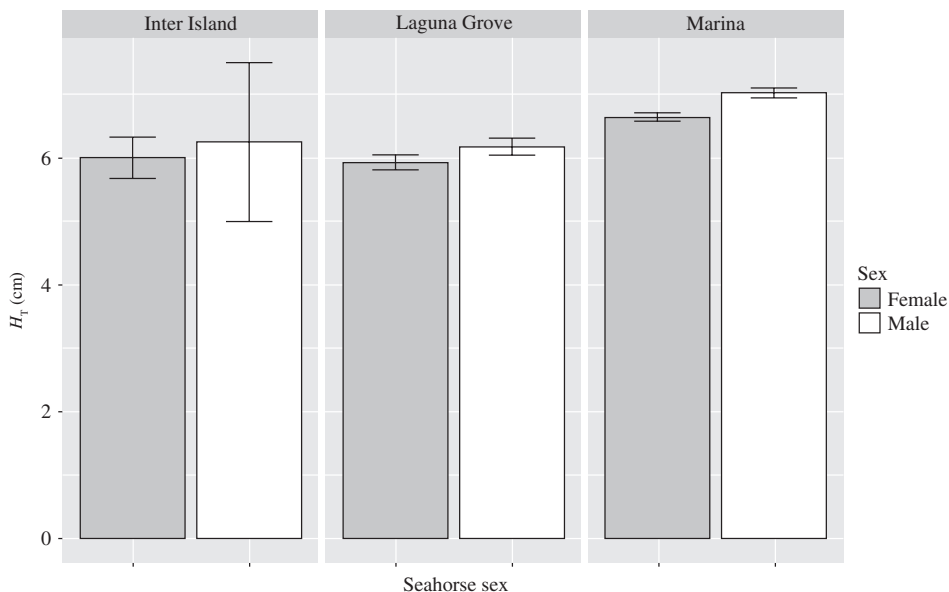


FIG. 6. Comparison of total height ( $H_T$ ; mean  $\pm$  s.e.) between male and female *Hippocampus capensis* across all sites sampled from October 2015 to August 2016.

decrease in population density of *H. capensis* during the summer suggests that some external factor might be involved. For example, the occupancy of houses at Thesen Islands Marina increases from 30% to 100% during the December and January holiday season (L. Claassens, pers. obs.) and this results in a marked increase in boat use within the canals and the estuary. When exposed to loud noise, *H. erectus* exhibited physiological, chronic stress responses with reduced mass and body condition (Anderson *et al.*, 2011) and it is possible that the increase in boat noise during the summer holiday period played a role in decreased population densities (Claassens & Hodgson, 2018). Further research on the potential effect of increased noise on *H. capensis* caused by boat activity is required. Some syngnathid species however, undergo seasonal migration to deeper water (*e.g.* *H. zosterae* and *Scovelli* populations in Tampa Bay, FL, U.S.A., showed some evidence of migrating out of shallow environments during the dry season [Mason-Jones *et al.*, 2010] and a *H. whitei* population in Port Jackson, Australia, also migrated to deeper water after the breeding season (Vincent *et al.*, 2005]) and this might be another explanation for the variability in *H. capensis*

TABLE II. *Hippocampus capensis* sex ratios for each sampling site, and all sites pooled, surveyed from October 2015 to August 2016

Site	Male/female ( $n$ )	$\chi^2$	D.F.	$P$	Sex ratio bias
Marina	224/309	13.6	1	<0.01	Female
Laguna Grove	40/84	15.6	1	<0.01	Female
Inter Island	2/10	5.3	1	<0.05	Female
All sites combined	263/398	27.6	1	<0.01	Female

TABLE III. Seasonal changes in the sex ratio of *Hippocampus capensis* within Thesen Islands Marina (Reno mattress habitat) and Laguna Grove (eel grass habitat)

Site	Season	Male/female (n)	$\chi^2$	D.F.	P	Sex ratio bias
Marina	Spring	60/83	3.7	1	<0.05	Female
	Summer	24/30	0.7	1	>0.05	Equal
	Autumn	54/74	3.1	1	>0.05	Equal
	Winter	86/122	6.2	1	<0.01	Female
Laguna Grove	Spring	21/29	1.3	1	>0.05	Equal
	Summer	6/11	1.5	1	>0.05	Equal
	Autumn	8/25	8.8	1	<0.01	Female
	Winter	5/19	8.2	1	<0.01	Female

TABLE IV. Holdfasts used by *Hippocampus capensis* within all sites surveyed from October 2015 to August 2016, recorded as percentage of *H. capensis* using a specific holdfast

Site	Wire (%)	Crevice (%)	<i>Zostera</i> (%)	<i>Asparagopsis</i> (%)	Other (%)
Marina	73	5	2	13	7
Laguna Grove	0	0	84	4	12
Inter Island	0	0	62	0	38

population densities across months. In addition, an increase in predators were found to play a role in decreasing population numbers of *H. whitei* in a marine protected area in Australia (Harasti *et al.*, 2014b) and the decrease in *H. capensis* numbers during the summer may also have resulted from a possible increase in predators.

Historic sex ratios for *H. capensis* vary between male bias during a transect survey and equal in a focal grid study in 2000 (Bell *et al.*, 2003), to being equal in a later transect survey in 2001 (Lockyear *et al.*, 2006). Our findings of an overall female-biased sex ratio and changes in the sex ratio of *H. capensis* across seasons are unusual as most other *Hippocampus* spp. have an equal sex ratio [*H. breviceps* (Moreau & Vincent, 2004); *H. comes* (Perante *et al.*, 2002); *H. reidi* (Rosa *et al.*, 2007); *Hippocampus bargibanti* Whitley 1970 and *Hippocampus denise* Lourie & Randall 2003 (Smith *et al.*, 2012)]. Female-biased sex ratios have, however, been noted for *H. abdominalis* in the Derwent Estuary, Tasmania (Martin-Smith & Vincent, 2005); *H. hippocampus* in the Macaronesian Islands, specifically in artificial habitats (Otero-Ferrer *et al.*, 2015); in *H. erectus* in Chesapeake Bay, MD, U.S.A. (Teixeira & Musick, 2001); and Kvarnemo *et al.* (2007) found a wild population of *Hippocampus subelongatus* castelnau 1873 to be female biased with stronger sexual selection on females, a contradiction to the normal male sexual selection found in monogamous species (Vincent, 1994a, b; Naud *et al.*, 2009). Sexual selection in *H. capensis* is unknown, but Grange & Cretchley (1995) did find synchronicity between a pair of seahorses, *ex situ*, which suggest monogamy and thus male sexual selection is probable (Naud *et al.*, 2009). One explanation for the skewed sex ratio found in *H. capensis* is the possibility of a higher mortality rate of a particular sex, which would suggest that *H. capensis* males, which are larger, are more visible to potential predators compared with females. Male *Hippocampus*

spp. in general are especially more visible during the breeding season owing to courting and competitive behaviour (Rosenqvist & Berglund, 2011). Skewed sex ratios in monogamous species can have a marked effect on the reproductive success of excess individuals (Kvarnemo *et al.*, 2007) and further research is needed to determine if *H. capensis* is in fact monogamous and if so, what effect the observed sex ratios might have on the reproductive success of this species.

Males were found to be significantly larger (total height) than females across all seasons and sites. Longer males that had longer tails and shorter heads than females were also found by Bell *et al.* (2003). Longer tails in male seahorses are quite common and are thought to enable males with a large caudal pouch to still be able to grasp a holdfast, or to be advantageous during tail-wrestling (Vincent, 1990). Significant differences between the overall size of male and female *Hippocampus* spp. are, however, not common (Vincent & Giles, 2003; Kvarnemo *et al.*, 2007; Otero-Ferrer *et al.*, 2015). *Hippocampus capensis* within the Reno Mattress habitat were significantly larger than *H. capensis* in the *Z. capensis* sites. Larger *H. capensis*, both male and female, have been observed to produce more offspring (Vincent & Giles, 2003; Rosenqvist & Berglund, 2011), although reproductive success is not necessarily linked to size. Even though *H. capensis* within the Reno mattress habitat can theoretically produce more offspring, the reproductive success of this population is not known which warrants further research.

The most recent IUCN assessment of *H. capensis* (Pollom, 2017) states that this species occupies an area of only 27.0 km<sup>2</sup>. The author of the assessment concedes that this estimate is probably too liberal, particularly when one considers that only 2.4 km<sup>2</sup> of the Knysna Estuary's subtidal area (the largest system occupied by *H. capensis*) is covered by vegetation (Adams, 2016). In addition, a recent threat to subtidal vegetation (in particular *Z. capensis*) within the bay regime of the Knysna Estuary is an extensive and lengthy *Ulva lactuca* bloom (Allanson *et al.*, 2016). *Ulva* spp. have been found to displace saltmarsh vegetation (Watson *et al.*, 2015) and *Z. capensis* (Human *et al.*, 2016) and the available natural habitat within the Knysna Estuary is most likely decreasing.

The habitat found in Thesen Islands Marina provides additional habitat for *H. capensis* and supports higher densities of *H. capensis*. A recent study also found that *H. capensis* were more likely to move towards or remain on a Reno mattress structure compared to *Z. capensis* (Claassens *et al.*, 2018), which suggests that this habitat type has some positive feature (food, holdfasts, protection) preferred by *H. capensis*. Artificial structures have the potential to provide much needed habitat to threatened aquatic species (Guerra-Garcia *et al.*, 2004; Perkol-Finkel *et al.*, 2012; Wetzel *et al.*, 2014), but this does not necessarily equate to an overall benefit for the system (or even the species in question), particularly if these artificial habitats displace natural aquatic habitats (Waltham & Connolly, 2013; Wetzel *et al.*, 2014). The Reno mattresses in Thesen Islands Marina did not displace any natural aquatic habitat and thus provide a net increase in habitable environments for *H. capensis* in the Knysna Estuary. Future research should investigate if Reno mattress habitat equates to an increase in population biomass, as opposed to merely functioning as a habitat sink (Bohnsack, 1989; Grossman *et al.*, 1997; Pickering & Whitmarsh, 1997). It is however, suggested that where it is not possible to restore natural habitat, or in the event of new coastal and estuarine developments, these structures be considered as a tool to provide additional habitat.

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