ASPECTS OF THE POPULATION ECOLOGY, HABITAT USE AND BEHAVIOUR OF THE ENDANGERED KNYSNA SEAHORSE (*HIPPOCAMPUS CAPENSIS* BOULENGER, 1900) IN A RESIDENTIAL MARINA ESTATE, KNYSNA, SOUTH AFRICA: IMPLICATIONS FOR CONSERVATION

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Aspects of the population ecology, habitat use and behaviour of the endangered Knysna seahorse (*Hippocampus capensis* Boulenger, 1900) in a residential marina estate, Knysna, South Africa: implications for conservation.

Abstract

The Knysna seahorse *Hippocampus capensis* is South Africa's only endemic seahorse species, and is found in only three adjacent estuaries along the southern coast. The conservation of this endangered species is important on a national and international level. This study presents the first research on this species within the Knysna estuary since 2001 and specifically focuses on aspects of its ecology within a residential marina estate (Thesen Islands Marina). The physico-chemical and habitat features of the marina were described and the population ecology, habitat use, and behaviour of the Knysna seahorse were investigated.

Physico-chemical conditions within the western section of the marina, characterised by high water current velocities, were similar to that of the adjacent estuary. The eastern section of the marina was characterised by lower water current velocities and higher turbidity. Four major habitat types were identified within the marina canals: (I) artificial Reno mattress (wire baskets filled with rocks); (II) *Codium tenue* beds; (III) mixed vegetation on sediment; and (IV) barren canal floor. Seahorse densities within the marina were significantly higher compared to densities found historically within the estuary. Highest seahorse densities were specifically found within the artificial Reno mattress structures and within the western section of the marina. Seahorse density varied spatially and temporally and the type of habitat was an important predictor for seahorse occurrence. An experimental investigation found that *H. capensis* chooses artificial Reno mattress habitat over *Zostera capensis* when given a choice. GoPro cameras were used successfully to investigate daytime seahorse behaviour within the Reno mattress habitat. Seahorses were more active during the morning, spent most of their time (> 80 %) feeding, and morning courting behaviour for this species were confirmed. However, during the summer holiday period (mid-December to mid-January) few seahorses were observed on camera, which suggests that the increase in motor boat activity and the related increase in noise had a negative effect on *H. capensis* feeding and courting behaviour. The marina development, and in particular the Reno mattresses, created a new habitat for this endangered species within the Knysna estuary. In addition to the protection and restoration of natural habitats in which *H. capensis* is found, the conservation potential of artificial structures such as Reno mattresses should be realised.

Table of contents

Chapter 1: General Introduction

Chapter 2: Seahorse habitat within a residential marina estate

2.2.5 Suitability of identified habitat types as seahorse habitat

2.3 Results

2.3.3 Suitability of identified habitat types as seahorse habitat

Chapter 3: Population density, distribution, and structure of *Hippocampus capensis* within a residential marina estate

3.2 Materials and methods

3.3 Results

Chapter 4: An endangered seahorse selectively chooses an artificial structure

4.2 Materials and methods

Chapter 5: Insights into the behaviour of *Hippocampus capensis* within a residential marina estate using GoPro cameras

5.2 Materials and methods

Chapter 6: General discussion, conservation recommendations and future research

Appendix A

Appendix B

Chapter 7: References

List of figures

Chapter 1: General Introduction

Chapter 2: Seahorse habitat within a residential marina estate

Chapter 3: Population density, distribution and structure of *Hippocampus capensis* within a residential marina estate

Chapter 4: An endangered seahorse selectively chooses an artificial structure

Chapter 5: Insights into the behaviour of *Hippocampus capensis* within a residential marina estate using GoPro cameras

List of tables

Chapter 1: General Introduction

Chapter 2: Seahorse habitat within a residential marina estate

Chapter 3: Population density, distribution and structure of *Hippocampus capensis* within a residential marina estate

Chapter 4: An endangered seahorse selectively chooses an artificial structure

Chapter 5: Insights into the behaviour of *Hippocampus capensis* within a residential marina estate using GoPro cameras

Chapter 6: General discussion, conservation recommendations and future research

Preface

Findings from Chapters 2 and 3 have been published in Estuarine, Coastal and Shelf Science (Claassens, 2016) and a copy of the paper is presented in Appendix B.

Findings from Chapter 4 have been submitted to the Journal of Experimental Marine Biology and Ecology and is under review: Claassens, L., Booth, A.J., Hodgson, A.N., An endangered seahorse species selectively chooses an artificial habitat.

Findings from chapter 5 have been submitted to the Journal of Zoology: Claassens, L., Hodgson, A.N. Gaining insights into *in situ* behaviour of an endangered seahorse using action cameras.

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Declaration

This thesis is the result of the author's own work, except where acknowledged or specifically stated in the text. It has not been submitted for any other degree or examination at any other university or academic institution.

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"The subject may appear an insignificant one, but we shall see that it possesses some interest; and the maxim 'de minimis lex non curat' (the law is not concerned with trifles) does not apply to science"

Charles Darwin - The formation of vegetable mould, through the actions of worms, with observations on their habits (1881)

Chapter 1

General Introduction

1.1 Horses of the sea

Syngnathidae Bonaparte, 1831 are unique teleost fish characterised by male pregnancy and specialised morphology (Fritzsche, 1980; Berglund *et al.*, 1986). This enigmatic group was recognised by Linnaeus and described in his tenth edition of *Systema Naturae* in 1735 (see Lourie *et al.*, 2016). What is in a name? The words “syn” and “gnathus”, Greek for together and jaws respectively, describe the tube-like fused jaws inherent to all syngnathids. Syngnathidae include two subfamilies: seahorses (Hippocampinae) which consist of one genus (*Hippocampus* Rafinesque, 1810) (Teske *et al.*, 2004; Wilson and Orr, 2011) and pipefish (Syngnathinae Bonaparte, 1831) (WoRMS, accessed August 2016).

Hippocampus spp. have specific synapomorphic morphological characteristics which set them apart from other Syngnathidae which include: a prehensile tail, absence of a caudal fin, a head positioned at a right angle to the body, a brood-pouch sealed along the mid line, and a raised dorsal fin base (see Foster and Vincent, 2004). Seahorses range in size from a height of 20 mm (*H. denise* Lourie & Randall, 2003) (Lourie and Randall, 2003) to 300 mm (*H. abdominalis* Lesson, 1827) (Francis, 1998). The body is covered with bony plates rather than scales (see Foster and Vincent, 2004). Seahorses can change colour and such changes are usually associated with mating and breeding (Vincent, 1995; Vincent and Sadler, 1995; Moreau and Vincent, 2004).

Phylogenetic evidence suggests that seahorses shared a common ancestor with the pygmy pipehorse genus *Idiotropiscis* Whitley, 1947*,* 25–28 million years ago (mya) (Teske and Beheregaray, 2009), while an age of 13 million years for *Hippocampus* was estimated from fossil records (Zalohar *et al.*, 2009). Seahorse fossils have been found in Italy (Sorbini, 1988) from the late Pliocene (3 mya), and Slovenia (Zalohar *et al.*, 2009) from the middle Miocene (13 mya), the latter including fossils of two new, and the only extinct, seahorse species (*H. sarmaticus and H. slovenicus*) (Zalohar *et al.*, 2009). Two opposing views of the evolutionary origin of *Hippocampus* spp. exist. According to Teske *et al.* (2004) the genus has an Australian origin owing to the fact that *H. breviceps* Peters, 1869 and *H. abdominalis*, both at the most basal position in the main clade according to molecular data, are Australian. Three potential sister genera of *Hippocampus* (*Amphelikturus* Parr, 1930*; Acentronura* Kaup, 1853; and *Idiotropiscis* Whitley, 1947) are also found within Australian waters which supports an Australian origin (Teske *et al.*, 2004). Subsequent to the origin of the genus, the main clade split off into three major lineages, two of which remained in Indo-Pacific waters and the third dispersed on a global scale (Teske *et al.*, 2004). By contrast, Casey *et al.* (2004) found no evidence of an Indo-Pacific origin and argued that the high seahorse diversity found within this area is rather owing to different speciation events such as tectonic plate movements and sea level variability that occurred during the Pleistocene and Miocene. An Atlantic origin is supported by the fact that most species of the closely related genus *Syngnathus* Linnaeus, 1758 are associated with an Atlantic biome (Casey *et al.*, 2004). Two distinct seahorse groups were also recognized by Casey *et al.* (2004): the first refers to species found solely in the Indo-Pacific and the second, named the Kuda Complex, refers to species with morphological features similar to *H. kuda* Bleeker, 1852.

Regardless of origin, seahorses are found circum-globally (see Foster and Vincent, 2004). Seahorse life histories and means of dispersal make founder dispersal an important genetic divergence mode (Teske *et al.*, 2007a). The highest diversity of seahorses is found in the Indo-Pacific region (see Foster and Vincent, 2004) and 41 species are currently officially recognized (Lourie *et al.*, 2016). However, seahorse taxonomy remains problematic owing to historical misidentification and high morphological diversity (Lourie *et al.*, 2016).

Most seahorse species are found in shallow coastal and estuarine environments (see Foster and Vincent, 2004). Exceptions to this include *H. kelloggi* Jordan & Snyder, 1901 recorded at depths of 90 m (Choo and Liew, 2003) and *H. minotaur* Gomon, 1997 found in 100 m deep trawls off Australia (Gomon, 1997). Seahorses predominantly inhabit seagrasses (temperate regions) and coral reefs (tropics) (see Foster and Vincent, 2004). According to Lourie *et al.* (2016), *Hippocampus capensis* Boulenger, 1900 (the Knysna seahorse) is the only known true estuarine seahorse species. However, *H. whitei* Bleeker, 1855 has also been found to be exclusively estuarine in eastern Australia (Harasti *et al.*, 2012). Furthermore, a number of other species can occur in estuaries (*H. reidi* Ginsburg, 1933 – Rosa *et al.*, 2007; *H. zosterae* Jordan & Gilbert, 1882 – Mason-Jones *et al.*, 2010).

The absence of caudal and pelvic fins leaves the seahorse with only one dorsal fin, used for forward propulsion, and two small pectoral fins along the head for stabilisation and steering, and consequently seahorses are slow swimmers. To prevent unwanted dispersal by strong currents, seahorses depend on holdfasts to secure themselves. Most species are exclusively found in habitats that provide holdfasts of some type - although the density and type of holdfast vary. For example, *H. hippocampus* Linnaeus, 1758 is associated with sparsely vegetated areas (Curtis and Vincent, 2005), while *H. bargibanti* Whitley, 1970 is exclusively associated with *Muricella* spp. gorgonian corals (Smith *et al.*, 2012). Crypsis is predominantly used as protection against predators (Foster and Vincent, 2004).

Suction feeding, a common feature in most teleost fish (Muller and Osse, 1984), is used by seahorses (Branch, 1966; Van Wassenbergh *et al.*, 2013). The prey animal is sucked up via the widening of the mouth to create a buccal cavity volume increase with a related inflowing current (Branch 1966; Bergert and Wainwright, 1997; Van Wassenbergh *et al.*, 2013). What sets seahorses and other syngnathids apart, is the tubular snout and small terminal mouth (Branch, 1966; Bergert and Wainright, 1997). The size of the prey animal to be consumed and the size and shape of the snout play an important part in feeding, as prey needs to fit through the small mouth (Tipton and Bell, 1988; Teixeira and Musick, 2001). Larger seahorses have been noted to feed on larger prey (Woods, 2002; Castro *et al.*, 2008; Yip *et al.*, 2015), and ontogenetic changes in diet can occur as juveniles tend to be able to only eat smaller animals (Teixeira and Musick, 2001; Castro *et al.*, 2008; Yip *et al.*, 2015). Most common prey consumed by seahorses include isopods, amphipods, copepods and other small slow moving crustaceans (Tipton and Bell, 1988; Teixeira and Musick, 2001; Woods, 2002; Kendrick and Hyndes, 2005; Castro *et al.*, 2011; Yip *et al.*, 2015). High numbers of nematodes were also found in the gut of *H. reidi* in Brazil (Castro *et al.*, 2011). Most seahorses are generalist feeders and will consume any prey animal able to fit through the small mouth opening, although, Yip *et al.* (2015) concluded that *H. spinosissimus* Weber, 1913 and *H. trimaculatus* Leach, 1814 are specialised predators that target slow-moving, epibenthic, hyperbenthic, and canopy dwelling crustaceans. Most seahorses are considered to be 'sit and wait' predators (see Foster and Vincent, 2004); however, hunting behaviour has been noted for *H. erectus* Perry, 1810 in habitats devoid of holdfast structures (James and Heck, 1994), and for *H. reidi (*Felicio *et al.*, 2006). Seahorses are visual predators and a decrease in feeding has been recorded in at least one species (*H. reidi* - Felicio *et al.*, 2006) owing to a decrease in light intensity.

Male parental care is common in many fish species (Blumer, 1982). Male pregnancy, as found in the Syngnathidae, is however a unique feature of this family (Herald, 1959; Wilson *et al.*, 2001). Historically, the structure of the characteristic male brood pouch was used in classification. George Duncker (1870 - 1953) divided syngnathids into two major groups based on the location of the brood pouch: Gastrophari for abdominal pouches and Uruphori for tail pouches (see Wilson and Orr, 2011). Seahorses diverged into trunk brooding with an enclosed brood pouch while pipefish were classified into monophyletic lineages according to the method of pouch closure e.g. inverted, semi-inverted, overlapping, and everted (see Wilson and Orr, 2011). It was concluded that the fully enclosed brood pouch of seahorses developed from pipefish ancestors with an everted pouch (Herald, 1959). All syngnathids undergo male pregnancy where the female transfers mature, yolk-rich eggs into the specialised brooding structure of the male (Breder and Rosen, 1966; Wilson *et al.*, 2003). The parent osmoregulates, aerates, protects and nourishes the eggs within the brood pouch, for the duration of the pregnancy (Linton and Soloff, 1964; Stölting and Wilson, 2007). In a recent study, Van Look *et al.* (2007) found that fertilization in *H. kuda* takes place outside of the brood pouch and suggests that spermatozoa are ejaculated into a mixture of ovarian fluid and eggs during the mating process, where after the mixture is transferred into the brood pouch. Many seahorse species are monogamous within at least a single breeding season (*H. fuscus* Ruppell, 1838 - Vincent, 1995; *H. zosterae* – Mason-Jones and Lewis, 1996) and monogamous behaviour in *H. whitei* was recorded for three breeding seasons (Harasti *et al.*, 2012). It is suggested that monogamy promotes increased reproductive efficiency which leads to larger broods and reduces time spent on courtship and finding a suitable mate (see Scales, 2010). Furthermore, the male has high paternal confidence and the absence of sperm competition is suggested to be responsible for the reduced sperm production in seahorses - the lowest found in any fish species (Van Look *et al.*, 2007). Promiscuous behaviour and polygamy have been found in some seahorse species. For example, a wild population of *H. breviceps* in Australia, showed no monogamous social behaviour (Moreau and Vincent, 2004). *Hippocampus subelongatus* populations in western Australia were found to be both monogamous and polygamous. Males that switched mates over a breeding season travelled further and had longer inter-brood periods compared to monogamous males (Kvarnemo *et al.*, 2000). Polygamy could be a consequence of higher population densities and a higher rate of temporal movement (see Foster and Vincent, 2004). Breeding seasons for seahorses are variable and can be influenced by light, temperature and food availability (see Foster and Vincent, 2004). Many different breeding triggers have been observed which range from day-light length (*H. zosterae*) to the phase of the moon (*H. guttulatus* Cuvier, 1829) (see Foster and Vincent, 2004). Some seahorse species (e.g. *H. trimaculatus*) breed throughout the year and peaks have been found to be related to the rainy season (e.g. *H. comes* Cantor, 1849) (Perante *et al.*, 2002).

1.2 Threats and conservation

Hippocampus spp. are vulnerable to anthropogenic impacts as they generally occur in shallow, coastal systems and have a number of characteristics (sparse distribution, low mobility, small home-ranges, and low fecundity) which leave them susceptible to habitat destruction and overexploitation (see Foster and Vincent, 2004 and Scales, 2010). The three main anthropogenic threats to seahorses include: Overexploitation in target fisheries, incidental take using non-selective gear (by-catch), and threats from habitat degradation and loss (Vincent *et al.*, 2011; Harasti, 2016).

Seahorses are harvested for Traditional Chinese Medicine, the aquarium trade and curiosities (McPherson and Vincent, 2004; Vincent *et al.*, 2011), and in 1995 it was estimated that 20 million individual seahorses entered the international market

(Vincent, 1996). The greatest percentage of harvested seahorses (95 %) is used for Traditional Chinese Medicine (see Vincent *et al.*, 2011). As much as 95 % of seahorses used in trade come from shrimp trawl by-catch (see Vincent *et al.*, 2011). These seahorse by-catches can either be used to generate a secondary income (seahorse trade), discarded, or used in low-grade fishery by-products such as fishmeal. Individual seahorse by-catch per vessel is generally low (Meeuwig *et al.*, 2006) but the cumulative impact can be devastating with 2.2 million (Giles *et al.*, 2005) animals caught per annum in Vietnam in the late 1990s. In a recent review, Lawson *et al.* (2017) estimated an annual seahorse by-catch rate of 37 million animals from 21 countries assessed. It is especially subsistence fishermen in developing countries that turn to seahorse harvesting to make a living (McPherson and Vincent, 2004). The impacts from fishing are numerous: destruction of monogamous seahorse pairs that can lead to a reduction in production, impact on population structure, and the destruction of habitats (see Vincent *et al.*, 2011).

Available data on the effects and impacts from habitat change and loss on seahorse populations are poor (see Vincent *et al.*, 2011) and only one study specifically quantifies the direct impact from habitat loss on seahorse populations (Harasti, 2016). Impacts on seahorse habitat can include physical alterations and/or loss or the deterioration of habitat quality via pollutants, eutrophication, noise or global warming (see Vincent *et al.*, 2011). Decreasing seahorse populations have been linked to habitat loss (Marcus *et al.*, 2007) and the decline of an *H. whitei* population in Australia was directly attributed to loss of essential marine habitats (Harasti, 2016). Some seahorse species are, however, able to acclimate to habitat changes (*H. guttulatus* - Curtis and Vincent, 2005; Gristina *et al.*, 2015; *H. whitei* - Clynick *et al.*, 2008b), which emphasizes the need for more research on the adaptive ability of seahorses to habitat change. Seahorse conservation can only be successful through a holistic approach which takes overexploitation in target fisheries, overexploitation through by-catch, and threats from habitat degradation and loss into consideration (see Vincent *et al.*, 2011).

Thirty-Three *Hippocampus* spp. are listed on the IUCN Red List of Threatened Species (www.IUCNredlist.org accessed on 15 April 2017) and seahorse trade is controlled by the Convention on International Trade in Endangered Species (CITES) (*Hippocampus* was listed under Appendix 2 of CITES in 2004) (see Vincent *et al.*, 2011). Twenty of the IUCN listed species are Data Deficient, eleven are Vulnerable, one Endangered (*H. capensis*) and one of Least Concern (www.IUCNredlist.org accessed on 15 April 2017). Information on life histories of seahorses is a crucial aspect in successful conservation and Data Deficient species research is needed. The need for: “empirical data, longer-term monitoring of wild seahorse populations and fisheries documentation and port surveys” is emphasized by Vincent *et al.* (2011).

1.3 Seahorse of the Cape

A dearth of knowledge exists on the status of seahorse species found within the coastal and estuarine waters of South Africa, although there is some information on *H. capensis*. Four seahorse species have been recorded along the South

African coast (only valid species according to Lourie *et al.* (2016) were used) (Table 1.1).

Table 1.1. Summary of seahorse species found along the southern African coast.

Species	Location	Notes	Reference
H. capensis	Knysna, Swartvlei and Keurbooms Estuaries		Smith and Heemstra, 1988 Bell *et al.*, 2003 Lockyear *et al.*, 2006 Lourie *et al.*, 2004
H. histrix	Vicinity of Durban		Smith and Heemstra, 1988 Lourie *et al.*, 2004
H. kuda	Mossel Bay to Mozambique	*H. fuscus* and *H. borboniensis* (recorded in South African waters in Lourie *et al.*, 2004) were synonymized with *H. kuda* in Lourie *et al.* (2016)	Smith and Heemstra, 1988
H. camelopardalis	Inhambane to Durban	According to Lourie *et al.* (2004) *H. camelopardalis* can be found as far as Cape Town	Smith and Heemstra, 1988 Lourie *et al.*, 2004

Hippocampus capensis is South Africa's only endemic seahorse species and currently this estuarine seahorse is found exclusively in the Knysna, Swartvlei and Keurbooms estuaries (Lockyear *et al.*, 2006), all in close proximity to each other on the south coast of the Western Cape of South Africa. Anecdotal records suggest that *H. capensis* was found in other estuaries close to Knysna including the Klein Brak (Bell *et al.*, 2003), Breede, Duiwenshok and Goukou (Lockyear *et al.*, 2006) estuaries (Fig. 1.1). Seahorse populations were, however, only found within the Keurbooms (Lockyear *et al.*, 2006), Knysna and Swartvlei (Bell *et al.*, 2003; Lockyear *et al.*, 2006) estuaries during 2000 and 2001–2003 population assessments.

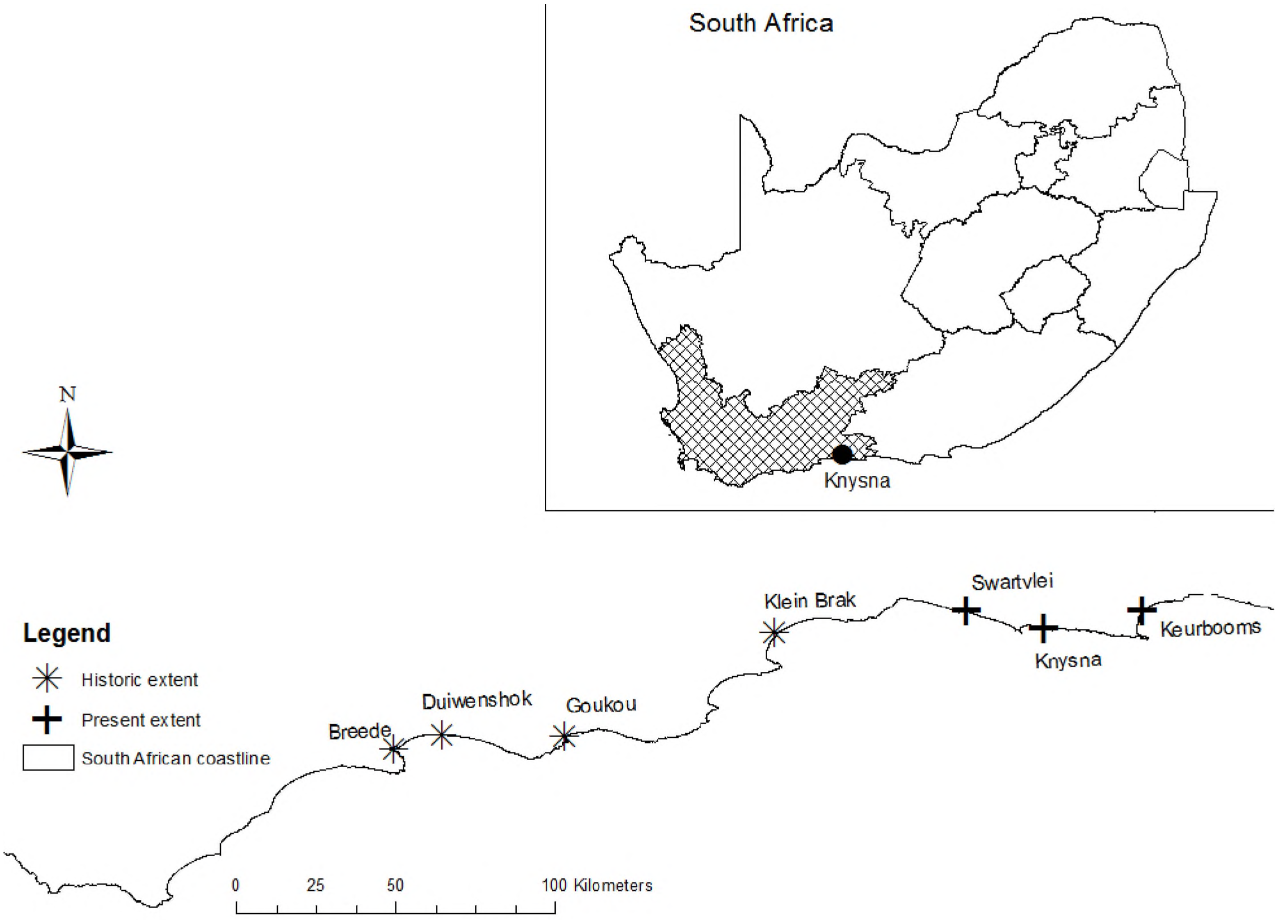


Figure 1.1. The location of the current and historic extent of *H. capensis* along the southern coast of South Africa. Hatched area in insert shows the Western Cape province of South Africa and the town of Knysna.

The Knysna seahorse was first described by Boulenger in 1900 (Fig. 1.2). The type specimen was found in Knysna's harbour during low tide - a scenario still relevant to this day (albeit in a somewhat modified harbour). The description of *H. capensis* by Boulenger has not been revised to date: "ten to eleven body rings with thirty-two to thirty-seven tail rings, a dorsal fin with seventeen rays which covers three rings, and a very short snout" (Boulenger, 1900; Lourie *et al.*, 2004). Boulenger suggested that *H. capensis* is very similar to *H. antiquorum* (a synonym previously used for *H. hippocampus* (Bailly, 2015)), an opinion shared by Lourie *et al.* (2004).

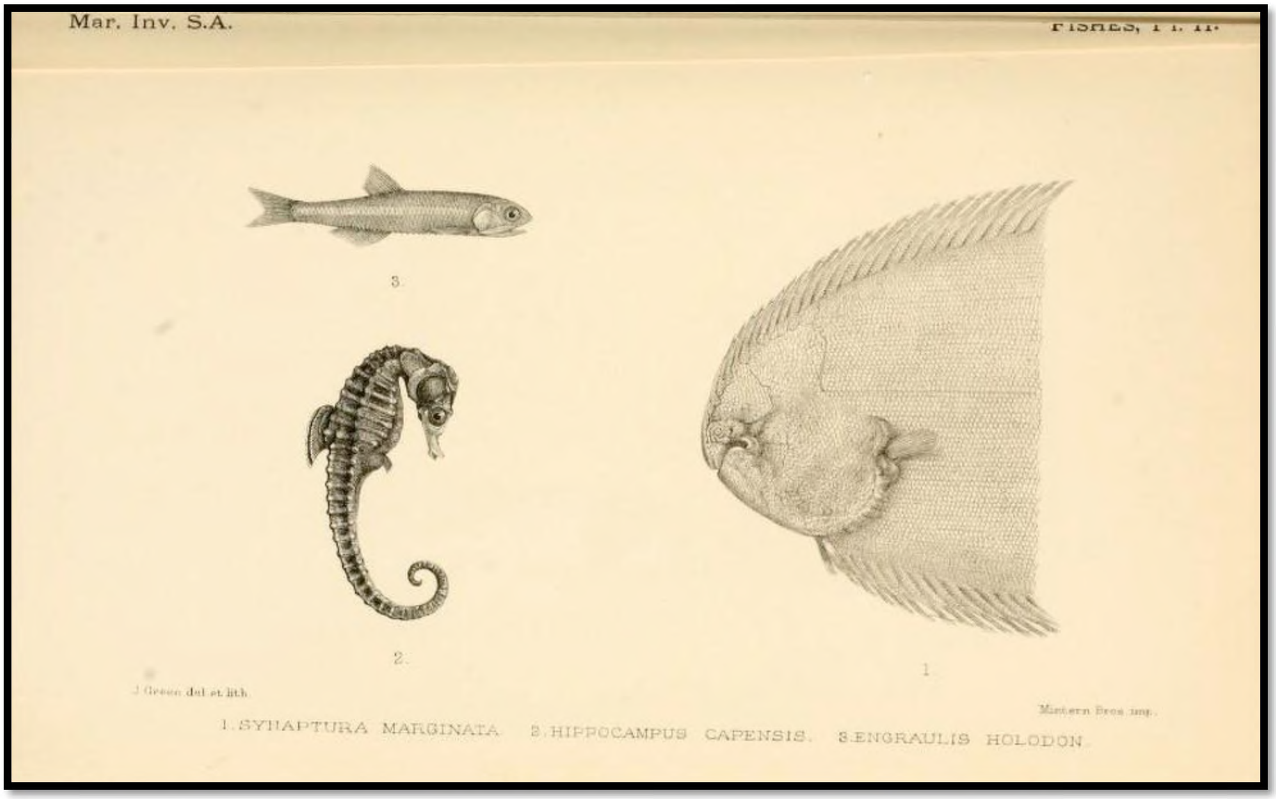


Figure 1.2. A copy of *H. capensis* type specimen drawing by Boulenger (1900).

The Knysna seahorse is part of the larger *Hippocampus kuda* clade (Lourie *et al.*, 2016), together with *H. fuscus, H. borboniensis* Duméril, 1870*, H. algiricus* Kaup, 1856, and *H. reidi.* In the most recent taxonomic review of seahorses it was decided to synonymize *H. borboniensis* and *H. fuscus* owing to a lack of distinguishable morphological, genetic, or geographic differences from *H. kuda* proper (from Southeast Asia) (Lourie *et al.*, 2016). *Hippocampus reidi* and *H.*

algiricus were retained owing to adequate morphological differences and wide geographic separation. *Hippocampus capensis* was also retained as a separate species owing to: “its noticeably and consistently smaller size, ecological considerations (it appears to be one of the most brackish-water tolerant seahorses and has only been found in estuaries), and conservation status” (Lourie *et al.*, 2016).

The genetic age of the Knysna seahorse is estimated to be between 46 000 and 186 000 years old (Teske *et al.*, 2003) and that a founder event took place during the Pleistocene. Oceanic conditions during this time were, however, much colder than today and it is unlikely that tropical or sub-tropical seahorses, which gave rise to *H. capensis*, could survive this far south, which suggests the founder event probably occurred during the Eemian interglacial period, 127 000 – 122 000 years ago, a short warmer phase within the Pleistocene (Teske *et al.*, 2003).

The founder event is believed to have taken place within the Knysna estuary owing to the high haplotypic diversity found in this population (Teske *et al.*, 2003). The large size, permanently open mouth and three distinct hydrographical regimes (bay, lagoon and estuary - Largier *et al.*, 2000) within the Knysna estuary would have presented an optimal transitional environment between marine and estuarine (Teske *et al.*, 2003). The Knysna seahorse is perfectly adapted to estuarine conditions with a salinity tolerance range of 1 to 59 (Riley, 1986). The extant Knysna seahorse populations found in the Keurbooms and Swartvlei estuaries (Lockyear *et al.*, 2006) could be explained by a historic vicariance event or dispersal of juvenile seahorses through ocean currents and related recruitment events (Whitfield, 1989; Teske *et al.*, 2003).

The Knysna seahorse has a maximum recorded standard length of 12.0 cm (Whitfield, 1995) and is found in depths of 0.5 m to 20.0 m (Whitfield, 1995). This smooth seahorse has a small coronet as a juvenile which is lost when maturity is reached. This species is generally mottled brown with green, black and white spots, but can range in colour from yellow (especially during the breeding season) to black (Bell *et al.*, 2003; Lourie *et al.*, 2004). Epiphytic growths on the skin are common (Bell *et al.*, 2003). Sexual maturity is reached within a year at a standard length of 6.5 cm (Whitfield, 1995). Breeding takes place from late September to early April when water temperatures rise above 20 °C (Whitfield, 1995; Lourie *et al.*, 2004) and a gestation period of two to four weeks (dependent on water temperature), has been recorded (Grange and Cretchley, 1995; Whitfield, 1995; Lockyear *et al.*, 1997; Lourie *et al.*, 2004). Both Grange and Cretchley (1995) and Lockyear *et al.* (1997) concluded that an increase in water temperature is the principle environmental cue that governs reproduction. Courting commences when a male and female pair off and the male indicates his intention to mate by grasping the female with his tail. Between 7 and 95 juveniles are produced per incubation (Lockyear *et al.*, 1997). In an *ex situ* study, Fourie, (1997) found no sexual dimorphism in seahorse length and males acted as suitor with a preference shown for larger females.

Seahorse trade in South Africa is limited (McPherson and Vincent, 2004) and trade in *H. capensis* deemed insignificant, although one specimen was found in a 2012 survey of Traditional Chinese Medicine markets in Taiwan (N = 58) (Chang *et al.*, 2013). South Africa is a signatory of CITES and bound by this agreement. *Hippocampus capensis* is protected under the National Environmental Management: Biodiversity Act, 2004 and listed as an endangered and protected species in Government Notice 389 of 2013 (publication of lists of species that are threatened or protected, activities that are prohibited and exemption from restriction). All three estuaries in which *H. capensis* is found, fall under protected status (Knysna and Swartvlei estuaries fall under the protection of SANParks and Keurbooms estuary under CapeNature).

1.4 Rationale and thesis structure

The last research on the Knysna seahorse population within the Knysna estuary took place in 2001 (see Lockyear *et al.*, 2006) and some major changes have since occurred in this system in terms of estuarine habitat quantity and quality. Of particular interest (and concern) with regards to the seahorse population within the Knysna estuary, was the development of Thesen Islands Marina (90.6 ha), a residential marina estate with 25 ha of canals. Since the completion of the marina in 2005, anecdotal accounts suggested that seahorses could be found in this new habitat, but the required research to confirm this remained absent. An initial aim at the outset of this project was to evaluate the anecdotal claims and assess the status of *H. capensis* populations – particularly in the light of new developments in the estuary. Did these developments have any impact on the density and distribution of this species?

Development and the transformation and loss of habitat are major threats to estuaries and estuarine inhabitants worldwide. This is particularly relevant to the Knysna seahorse because one of the reasons for its endangered status on the IUCN Red List of Threatened Species is the loss and damage of seagrass habitat used by this species (Czembor and Bell, 2012). Even though the Knysna estuary falls under the jurisdiction of SANParks and the Knysna seahorse is protected under the National Environmental Management: Biodiversity Act of 2004, actions taken to conserve this species are minimal. One of the reasons behind this lack of conservation action could be ascribed to the poor understanding of the behaviour, population dynamics and ecology of this species. What has happened to the Knysna seahorse population within the Knysna estuary over the past fifteen years?

The overarching objective of the Knysna Seahorse Status (KySS) project, established in 2014, is to ensure the effective conservation of the endangered Knysna seahorse. This objective will be achieved by:

- A population assessment of *H. capensis* – specifically focused on seahorse populations found within Thesen Islands Marina,
- An investigation of the adaptability of this species, if any, to anthropogenic additions to its natural environment and,
- An assessment of the habitat requirements of this species.

A logical first step in this investigation was to explore and describe the habitats and environmental conditions found within the marina canals, detailed in Chapter 2.

Once a detailed understanding of the new environment was established, the occurrence of *H. capensis* within the marina canals could be investigated. Focus was placed on the density and distribution of seahorses within identified habitat types across seasons. The larger habitat role of the marina within the estuary was explored through monthly seahorse surveys within and outside the marina development. These findings are presented in Chapter 3.

The observations made during the seahorse population assessment exposed the need to further investigate the habitat choices made by *H. capensis* within the marina estate – with a particular focus on the choice made between natural and artificial habitat types. The findings of this investigation are presented in Chapter 4.

Not only did the marina environment present a ‘natural’ scenario to investigate the role of estuarine developments in the provision of habitat (and the reaction of *H. capensis* to such developments), but it also provided a perfect quasi-natural environment (wild aquarium) to study the behaviour of this seahorse species *in situ* using cameras. The behavioural study, detailed in Chapter 5, aimed to quantify and document diel behavioural activities of *H. capensis* across time and attempted to quantify the behavioural effects of external impacts such as boat activity.

Chapter 6 presents a synthesis and discussion of the findings from chapters 2 to 5 with an overall conclusion of the findings and outcomes of the study with a focus on the international relevance of the research. Conservation recommendations are made and future research needs discussed.

Chapter 2

Seahorse habitat within a residential marina estate

2.1 Introduction

The world's coastal areas are under pressure from population growth and it is this anthropogenic onslaught in the form of exploitation, habitat degradation, pollution, and uncontrolled development that creates the biggest threat to the world's coastal and estuarine environments (Franco *et al.*, 2011; Waltham and Connolly, 2011; Wetzel *et al.*, 2014; Wilson *et al.*, 2015). One of the most pertinent threats to these systems is the inevitable change from a natural system to one wrought with artificial structures brought on by harbour, marina and, flood control development (Clynick, 2008a; Chapman and Underwood, 2011; Wetzel *et al.*, 2014; Dafforn *et al.*, 2015a; Wilson *et al.*, 2015). It is particularly estuaries that carry the brunt of anthropogenic modifications (Rivero *et al.*, 2013), and habitat loss is an important ecological impact which results from estuarine developments worldwide (Elliott and Whitfield, 2011). For example, the modification of sub-tidal habitats can alter the biodiversity, distribution, trophic interactions, community assemblages, and the quality of nursery habitats in an estuary (Dugan *et al.*, 2011).

Of concern within estuaries and low-lying coastal areas (Harvey and Stocker, 2015) is the growing trend in the development of marina residential estates, which covered a global area of 270 km^2 in 2011 (Waltham and Connolly, 2011). A marina estate is defined as: "a development with 50 % of its edges appearing straight or unnaturally smooth and greater than 50 % of its perimeter utilised for residential living" (Waltham and Connolly, 2011). According to Harvey and Stocker (2015), marina estates share three common elements: (I) used for residential purposes; (II) located on artificial waterways made up of interconnected canals to maximise waterside area; and (III) a connection with a natural water resource to enable flushing of the system.

Marina estates can be developed by the alteration of natural wetland, saltmarsh or mangrove areas (heavily modified water body), or by the development of canals and the related aquatic habitat in a terrestrial environment located beyond natural tidal levels (artificial water body) (European Commission, 2003; Waltham and Connolly, 2011). The habitat created by the latter development type is new additional aquatic habitat previously unavailable to aquatic biota. The global extent of marina estates together with the changes to the natural environment brought on by such developments make them deserving of attention from ecologists to understand the ecological role they may play (Waltham and Connolly, 2011; Rivero *et al.*, 2013).

Marinas are commonly built in sheltered areas characterised by calm water (Rivero *et al.*, 2013), and canals within marinas are typically deep, straight and blind-ending which usually contradicts the natural, physical character of the adjacent system (Davies and Stewart, 1984; Maxted *et al.*, 1997; Rivero *et al.*, 2013). It is these features that can lead to poor water quality within marinas which may include: decreased flushing and higher turbidity, temperature and pH, and suspended solids with elevated lead and copper concentrations (Rivero *et al.*, 2013). Features common to marinas such as anti-fouling paint and the maintenance of jetties, can also contribute to poor water quality (Rivero *et al.*, 2013).

Not only can marina developments have an impact on the physico-chemical features of an estuary, but also on biological compositions (Rivero *et al.*, 2013). Urban infrastructure supports different epibiota and associated assemblages when compared to natural habitats, which means that these structures do not function as natural habitat surrogates (Connell and Glasby, 1999; Burt *et al.*, 2009; Bulleri and Chapman, 2010). Existing environmental features (hard bottomed or soft bottomed) of a system should be taken into consideration in assessments to understand the mechanisms which regulate the patterns of abundance and distribution of organisms (Bulleri, 2005). One universal conclusion is thus not viable. The role of artificial structures in these systems is of particular concern as they can have various adverse effects e.g. act as a 'reservoir' or corridor for marine aliens (Arenas *et al.*, 2006; Mineur *et al.*, 2012). According to Dafforn *et al.* (2015b) marine infrastructure includes: i) basic recreational infrastructure (marinas, pilings, pontoons, boat ramps); ii) coastal and foreshore defence structures (seawalls, groynes, breakwaters); iii) offshore energy installations (gas and oil extraction, wind farms); and iv) fisheries infrastructure (artificial reefs, offshore aquaculture facilities). Impacts (positive and negative) to marine systems from these artificial structures include: i) direct physical disturbances; ii) addition of artificial habitat; iii) indirect physical disturbances; and iv) noise and light pollution (Dafforn *et al.*, 2015a).

Artificial structures associated with marinas, however, do attract fish (Clynick, 2008a), and increases in the abundance and diversity of sub-tidal biota have been recorded (Connell and Glasby, 1999). Sessile invertebrate growth on artificial structures can provide complex habitats preferred by some fish species (Clynick, 2008a) owing to increased shelter or protection and higher food availability (Hellyer *et al.*, 2011). A marina development in the Kromme Estuary on the south coast of South Africa increased plankton, benthic macro-invertebrate, and fish habitats (Baird *et al.*, 1981), and Rivero *et al.* (2013) found increased recruitment within a marina on the south-eastern coast of Australia of taxa with short-lived larval stages as opposed to those taxa with long-lived larval stages. It is important to include water body type (artificial vs heavily modified) when one considers the positive and/or negative effect of a development. For example, lakes built on terrestrial land provide novel new fish habitats (Waltham and Connolly, 2013) while lakes built on natural aquatic habitats would probably have a poor net outcome (Waltham and Connolly, 2013). A direct comparison to assess this has not yet been made.

The Knysna estuary (Fig. 2.1) is located on the southern coast of South Africa (34° 4'56.19"S, 23° 3'34.85"E) and is regarded as the most important estuary in South Africa in terms of biodiversity (Turpie *et al.*, 2002). This estuary is under pressure, even though it falls within the boundaries of the Garden Route National Park, owing to development pressures which result from a growing population and thriving tourism economy, all of which place strain on the natural habitats found in the estuary. The Knysna estuary has the greatest potential to ensure the longer-term survival of *Hippocampus capensis* owing to the relatively large population found in this system and subdued impacts from freshwater floods (Teske *et al.*, 2003).

The construction of Thesen Islands Marina (Fig. 2.2), an artificial water body (European Commission, 2003), was completed in 2005 and added 25 ha of new estuarine habitat to the system. The last survey work on *H. capensis* within the Knysna estuary was completed in 2001 – long before the completion of Thesen Islands Marina (Lockyear *et* *al.*, 2006). The additional 25 ha of estuarine habitat provided by this development has the potential to offer important habitat to *H. capensis*.

Before a population assessment of *H. capensis* within the marina canals could commence, the physical and biological features within the marina had to be described. Particularly to ascertain if the new environment which resulted from this development had any potential as seahorse habitat. Therefore, the overall objective of this chapter was to describe the environment within the canals of Thesen Islands Marina at a biological and physico-chemical level. And in doing so investigate the habitat potential of this new environment for *H. capensis*. Particular focus was placed on the habitat requirements of seahorses which include the provision of a holdfast (Teske *et al.*, 2007b), suitable protection from predators, food availability, and good water quality (Whitfield, 1995; Foster and Vincent, 2004; Vincent *et al.*, 2011).

2.2 Materials and Methods

2.2.1 Study location

An estuary is defined by Elliot and Whitfield (2011) as: “a semi-enclosed coastal body of water which is connected to the sea either permanently or periodically, has a salinity that is different from that of the adjacent open ocean due to freshwater inputs, and includes a characteristic biota”. The Knysna estuary, with a subtidal area of 10 km^2 (Bell *et al.*, 2003), is classified as an Estuarine Embayment (Whitfield, 1992). This estuary is permanently open and is characterised by three prominent hydrographical regimes (Fig. 2.1) (Largier *et al.*, 2000): (I) the lower bay regime flushes every tidal cycle via tidal pumping and diffusion (tidal prism of 18.5×10^6 m^3 (Switzer, 2003)) and exhibits physico-chemical characteristics similar to the adjacent Indian Ocean; (II) the middle lagoon regime, described as the transitional zone between the bay and the estuary, is characterised by high water temperatures indicative of longer residence times with little direct freshwater influences; (III) the upper estuary regime is directly linked with the Knysna River and is characterised by varied lower salinities (< 30) and stratification (Largier *et al.*, 2000). Residence times varies between 1 and 2 weeks in the lower bay regime, approximately 4 weeks in the lagoon regime and between 3 and 4 weeks in the upper estuary regime (DWA, 2009).

The boundaries of the three regimes fluctuate with tidal and freshwater inputs (Largier *et al.*, 2000). The Ashmead channel (Fig. 2.1), a shallow tidal system, was identified as a fourth regime by Switzer (2003) and is characterised by long residence times, high temperatures in summer, and a low flushing rate. This section of the estuary is very shallow (< 2 m) and receives nutrient rich waste water and stormwater run-off.

2.2.2 Study site

Thesen Islands Marina (34° 2'47.16"S, 23° 3'18.84"E) is a 90.6 ha residential marina estate located on Thesen Island (Figs. 2.1, 2.2) in the lower bay regime of the Knysna estuary. It is one of three, and the largest, residential marina estate in the Knysna estuary (≈ 25 ha canal system). The marina development commenced in 2000 and was completed in 2005. Six hundred housing units were built on the island with 25 ha of newly created canals. The marina is connected to the estuary by two wide access entrances – one at the western entrance connected to the main channel of the Knysna estuary and one at the eastern entrance located off the Ashmead Channel (Figs. 2.2, 2.3). The marina canals, excavated to 1.75 m below mean sea level, were built from vertical gabion walls which rest upon a 2 m wide horizontal Reno mattress™. Gabions are wire cages filled with rocks (Maccaferri, 1915) used in hydrological and environmental engineering applications such as stormwater control, erosion prevention, and canal linings. Additional geotextile material is used as an extra lining to promote sediment retention and erosion control. A Reno mattress refers to a horizontal gabion structure used in the lining of canal beds. Few estuarine developments use gabion structures as material of choice, and no ecological research studies on the suitability of these structures as novel habitats for estuarine fauna were found.

An earlier hydrographical study of the marina (Schumann, 2004) showed no signs of tidal asymmetry with a free exchange of water in the system. There was no significant difference in tidal amplitude between the western and eastern section of the marina, but a lag time of 10 minutes between the two sections was noted. Water temperatures were found to be similar to that of the adjacent estuary (Schumann, 2004).

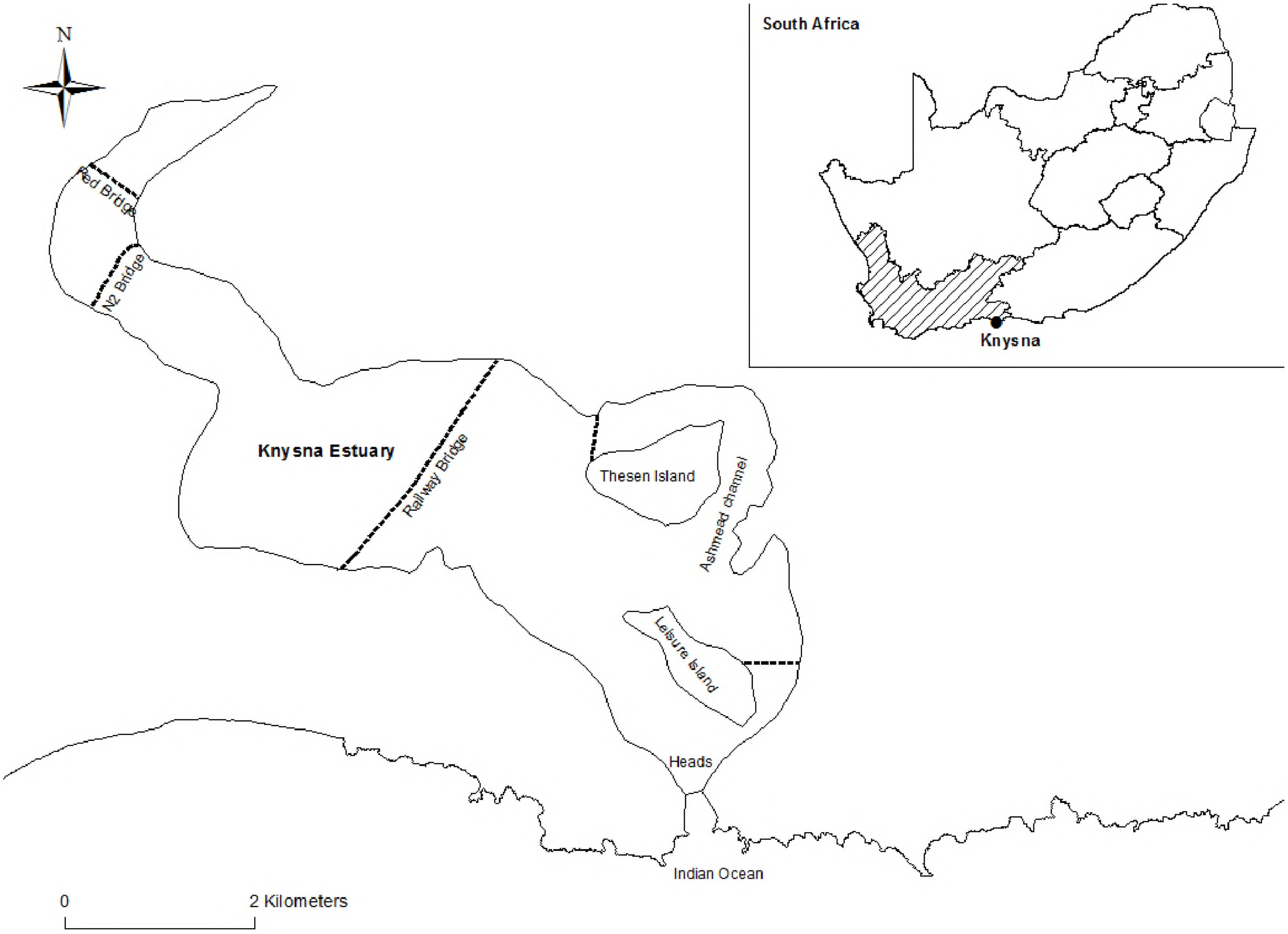


Figure 2.1. Location of Knysna (insert – hatched area shows the Western Cape and the town of Knysna) and the layout of the Knysna estuary. The bay regime stretches from the Heads to the railway bridge; the lagoon regime from the railway bridge to the N2 Bridge and the estuary regime from the N2 Bridge up to the Knysna River (Largier *et al.*, 2000). The Ashmead channel lies on the eastern side of Thesen Island (Switzer, 2003). Both Thesen and Leisure Islands are situated within the bay regime.

Figure 2.2. An aerial photograph of Thesen Islands Marina (photo by Duran de Villiers).

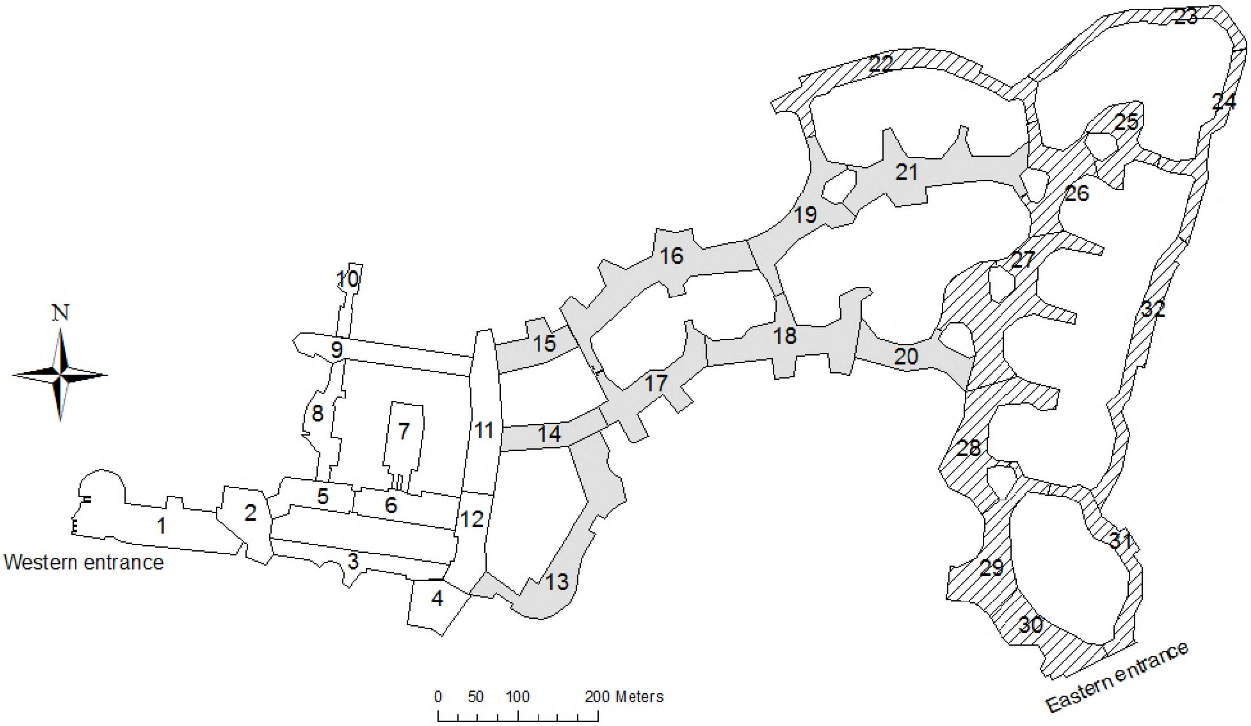


Figure 2.3. The canals of Thesen Islands Marina can be divided into three water current velocity zones along the west-east gradient of the marina: (I) western high velocity zone (white); (II) the middle cross-over zone (grey); and (III) eastern low velocity zone (hatched). For the purpose of convenience, the marina canals were subdivided into 32 smaller sample sites using structural landmarks within the marina.

2.2.3 Physico-chemical features

Thesen Islands Marina can be divided into three distinct water current velocity zones: (I) western high velocity zone; (II) the middle cross-over zone; and (III) eastern low velocity zone (Schumann, 2004) (Fig. 2.3). For the purpose of convenience, the marina canals were subdivided into 32 sample sites using structural landmarks (Fig. 2.3). Ten sampling stations (four in Zone 1, two in Zone 2, four in Zone 3), increasing in distance from the western marina entrance (Fig. 2.4), were selected to assess the physico-chemical features of the marina. Five series of samples were collected from May 2014 to March

2015 on a spring low and high tide. Sampling took place in the middle of the canal during the day. Dissolved oxygen (DO) as percent saturation and temperature were measured along a vertical gradient of 0.5 m with a YSI 550A dissolved oxygen meter (Yellow Springs Incorporated, Yellow Springs, Ohio). Surface turbidity (ntu) samples were taken at each station and measured with a Eutech TN-100 bench-top turbidity meter (Thermo Scientific, Singapore). Vertical conductivity, temperature and depth profiles were taken at each station during high tide using a Sea-Bird Standard Electronic 19-03 CTD (Sea-Bird Electronics, Seattle).

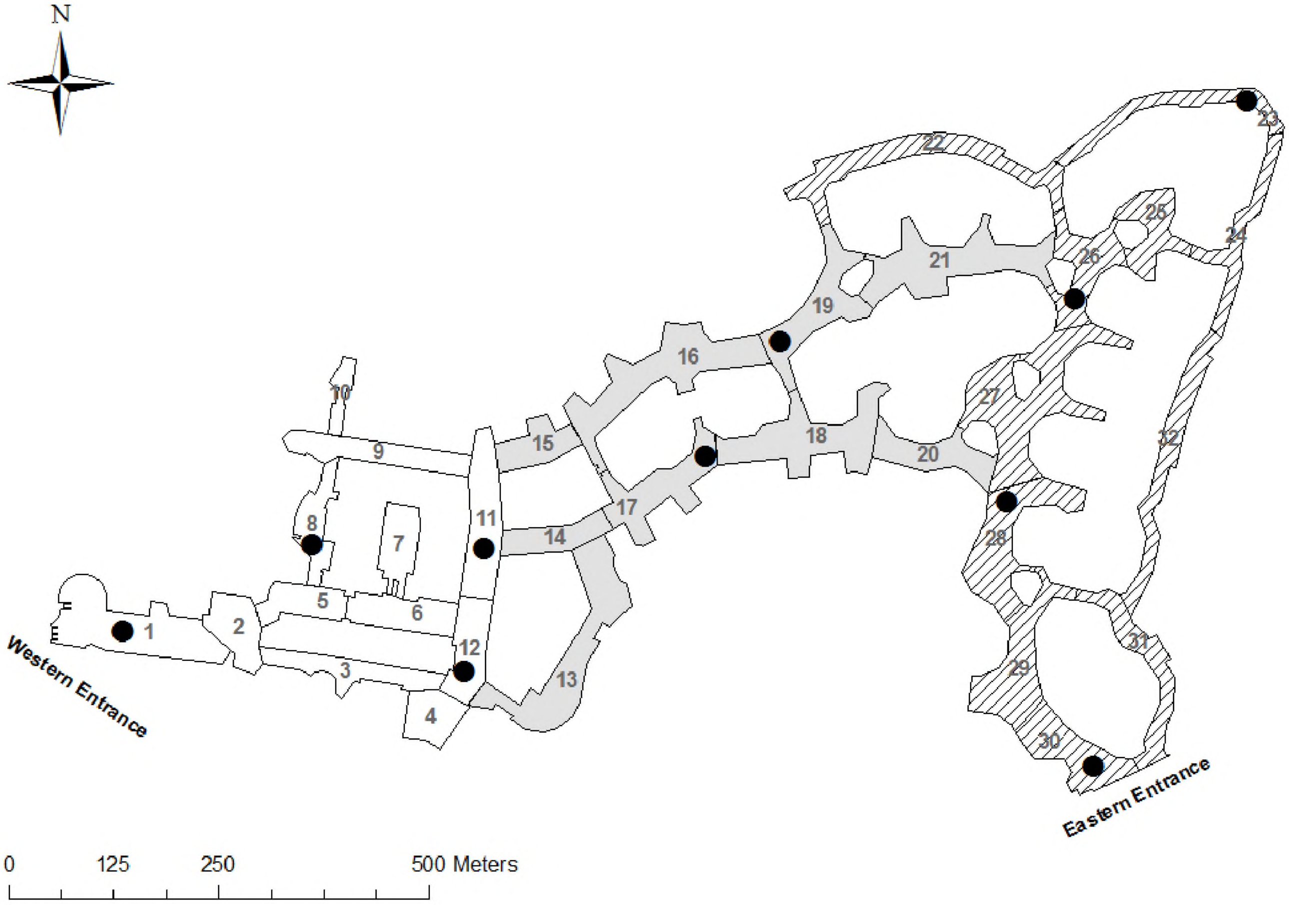


Figure 2.4. Ten water quality sampling stations (•) sampled in 2014 and 2015 within Thesen Islands Marina. Numbers and shading as per Fig. 2.3.

2.2.4 Habitat description

An aquatic vegetation survey took place in the summer and winter of 2014 to obtain an overview of habitats found in the marina canals. Haphazardly located transects (N = 72) with a mean length and search width of 35 m and 1 m respectively were surveyed by snorkelling across the width of the canals. The dominant vegetation type and percentage cover were estimated across each transect.

Major habitat types were assigned to one of the following four categories (Fig. 2.5):

I. Reno mattress habitat. This habitat type was located along the canal edges and only included the horizontal Reno mattress structure and not the vertical gabion wall. The wire and rocks used to fill the mattress were visible. The 2 m wide habitat was exposed to the atmosphere every spring low tide for a period of up to two hours (pers. obs.). The dominant vegetation found on the mattress included *Asparagopsis taxiformis* (Delile) Trevisan de Saint-Léon, 1845 (an invasive red alga species from Indo-Pacific origin (Bolton *et al.*, 2011)) and *Polysiphonia* sp. Greville, 1823.

II. *Codium tenue* (Kützing) Kützing, 1856 beds. These macro-algal stands collected within low water flow areas such as inlets and dead-end canals within the marina canals. The dense vegetation layer lay unattached on the canal bottom and varied in density and thickness. Large diurnal fluctuations in dissolved oxygen took place with super saturation (120 % saturation) during the day and hypoxic (< 20 % saturation) conditions at night.

III. Mixed vegetation (*Zostera capensis* Setchell, 1933, *A. taxiformis*, *Polysiphonia* sp, *Caulerpa filiformis* (Suhr) Hering, 1841, and *Halophila ovalis* (R. Brown) J.D. Hooker, 1858) on sediment. This habitat type was generally found in the middle of the marina canals and shared similar characteristics to the natural seagrass beds found in the larger estuary (Bell *et al.*, 2003; Lockyear *et al.*, 2006; Teske *et al.*, 2007b).

IV. Barren canal floor with sparse vegetation or broken shell beds. The western section of the marina was characterised by marine sediment and large areas of broken shell beds. The character of the sediment changed to mud and fine silt along the west-east gradient.

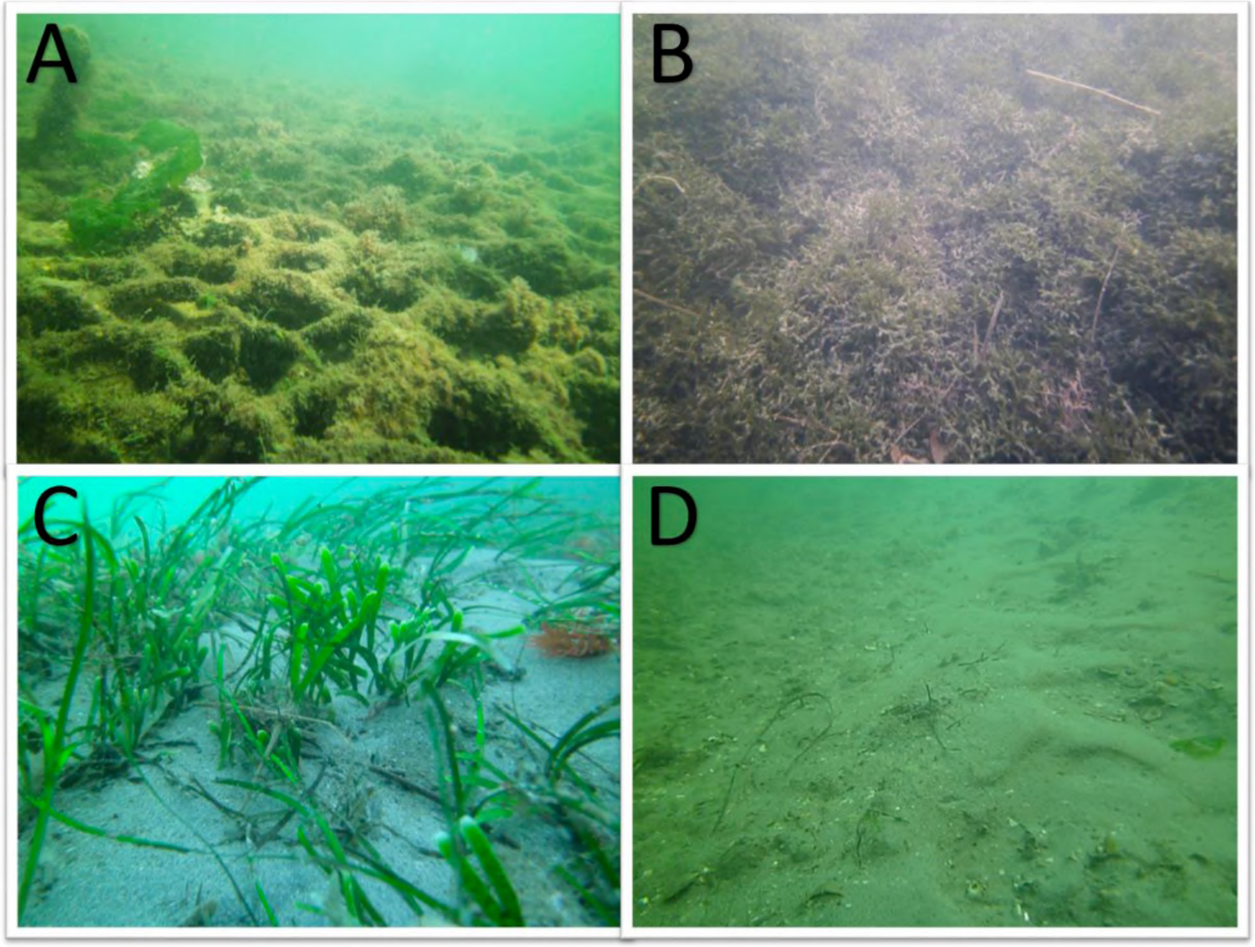


Figure 2.5. Four major habitat types identified within Thesen Islands Marina: A: Reno mattress habitat (wire baskets filled with rocks); B: Dense *C. tenue* beds; C: Mixed vegetation on sediment; and D: Barren canal floor.

2.2.5 Suitability of identified habitat types as seahorse habitat

2.2.5.1 Dissolved oxygen within identified habitat types

To determine the range of oxygen levels in the different habitat types in which seahorses would potentially be found, percent saturation of dissolved oxygen was investigated using a YSI 550A dissolved oxygen meter (Yellow Springs Incorporated, Yellow Springs, Ohio). Samples were collected during two 24-hour sample periods in October 2014 and March 2015. Readings were taken at the surface of the water column and ‘within’ the various habitat types. The probe was positioned within a dense *C. tenue* bed, within a mixed vegetation stand, and in close proximity to the Reno mattress in the respective habitat types. During the 24-hour period readings were taken at noon (12:00 – 14:00), dusk (18:00 – 20:00), midnight (23:00 – 1:00), and dawn (5:00 – 7:00).

2.2.5.2 Potential seahorse prey animals within identified habitat types

Five samples (0.004 m^3) were collected from the three habitat types in each of the three marina zones respectively (N = 45) between March and May 2015. Each sample was collected by vertically placing a 4.2 l plastic container (12.5 cm (w) x 12.5 cm (b) x 27.5 cm (l)) over the specific habitat type. Vegetation underneath the sample was manually loosened and included in the sample. The container lid was slid into position prior to retrieval of the sample. Samples were collected by snorkelling. The contents of the container were deposited into a plastic bag and shaken to dislodge benthic invertebrates from the vegetation. Each sample was washed three times through a 250 µm sieve using unfiltered estuarine water. Ten sub-samples (2 ml) from each collected sample were examined microscopically and invertebrates were identified to major taxonomic groups and quantified. Each 2 ml subsample was replaced into the original sample after each assessment. Focus was placed on invertebrates known to be consumed by seahorses such as small crustaceans (Foster and Vincent, 2004; Kendrick and Hyndes, 2005; Felicio *et al.,* 2006) and nematodes (Castro *et al.,* 2008). All names were verified using the World Register of Marine Species (http://www.marinespecies.org/).

2.2.6 Statistical analysis

The statistical programme R (R Development Core Team, 2014) was used in all analyses. An ANOVA was used to test for any significant differences in physico-chemical features and prey animal abundance spatially and temporally, and statistically significant results 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 in all instances ($p > 0.05$). The ggplot package was used to create all graphs (Wickham, 2009). The ddply function of the Plyr-package was used for all basic descriptive statistics (Wickham, 2011). Table 2.1. provides a summary of all hypotheses tested, the applicable sections within the chapter and the R codes used in each instance.

Table 2.1. A summary of all hypotheses tested in Chapter 2, the applicable sections in the chapter and the R code used in each instance.

Hypothesis	**Section**	**Formula**
H_0: Physico-chemical features do not vary across marina zones or on a seasonal basis	2.2.3 and 2.3.1	Assessment Name=aov(Feature~Season + Zone,) summary.aov(Assessment Name) TukeyHSD(Assessment Name)
H_0: Dissolved oxygen (% sat) does not vary in different habitat types or across marina zones	2.2.5.1 and 2.3.3.1	Assessment Name-aov(Dissolved oxygen %~Habitat type + Zone) Summary.aov(Assessment Name) TukeyHSD(Assessment Name)
H_0: The densities of potential seahorse prey animals do not vary between habitat types or across marina zones	2.2.5.2 and 2.3.3.2	Assessment Name-aov(Prey animal density~Habitat type + Zone) Summary.aov(Assessment Name) TukeyHSD(Assessment Name)

2.3 Results

2.3.1 Physico-chemical features

Physico-chemical features were assessed across seasons and marina zones (Fig. 2.6). Canal water depth ranged from 0.6 m to 3.1 m with a mean (± sd) depth of 2.0 ± 0.7 m. Water temperature varied significantly across seasons (bottom water temperature: $F = 16.0$, $df = 3$, $p < 0.01$; surface water temperature: $F = 38.5$, $df = 3$, $p < 0.01$). Surface water temperatures were significantly colder ($F = 7.9$, $df = 2$, $p < 0.01$) in Zone 1 compared to Zone 3 (Fig. 2.6). Bottom water temperatures were found to be significantly colder than surface water temperature ($F = 91.6$, $df = 1$, $p < 0.01$). Significantly higher bottom ($F = 8.4$, $df = 3$, $p < 0.01$) and surface ($F = 12.0$, $df = 3$, $p < 0.01$) dissolved oxygen saturation was found in autumn and winter. Dissolved oxygen saturation was found to be similar across all marina zones (Fig. 2.6). Bottom dissolved oxygen saturation was significantly higher than surface saturation, particularly during autumn and winter ($F = 182.3$, $df = 1$, $p < 0.01$). Significantly higher turbidity (ntu) was found within Zone 3 (Fig. 2.7) ($F = 5.5$, $df = 2$, $p < 0.01$) compared to Zone 1. Salinity ranged from 28 to 37 with a mean (± sd) of 33.2 ± 2.4.

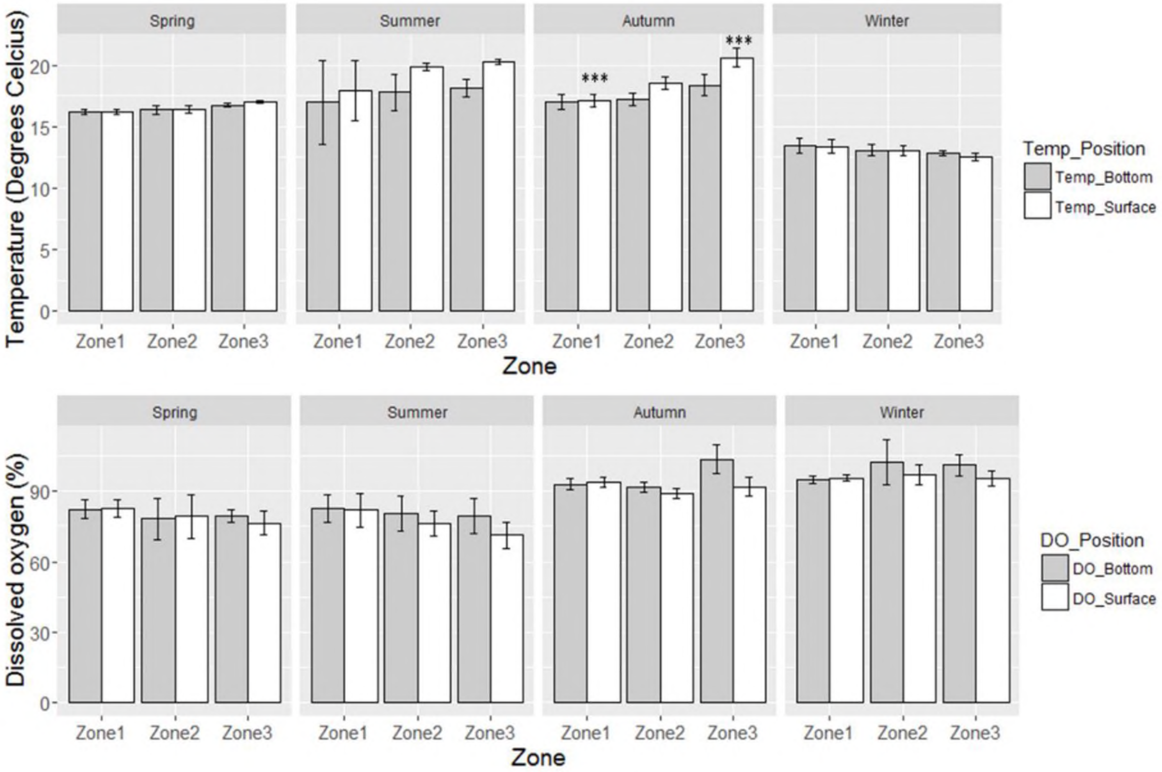


Figure 2.6. Seasonal changes in water temperature and dissolved oxygen saturation (mean ± se) across the three marina zones within Thesen Islands Marina recorded in 2014 and 2015. Pooled data showed that water temperature was significantly ($p < 0.01$) different across seasons and significantly warmer ($p < 0.01$) in Zone 3 compared to Zone 1. Pooled data showed that dissolved oxygen was significantly higher ($p < 0.01$) during winter and autumn. Asterisks indicate significant differences between zones within a season (*** - $p < 0.01$).

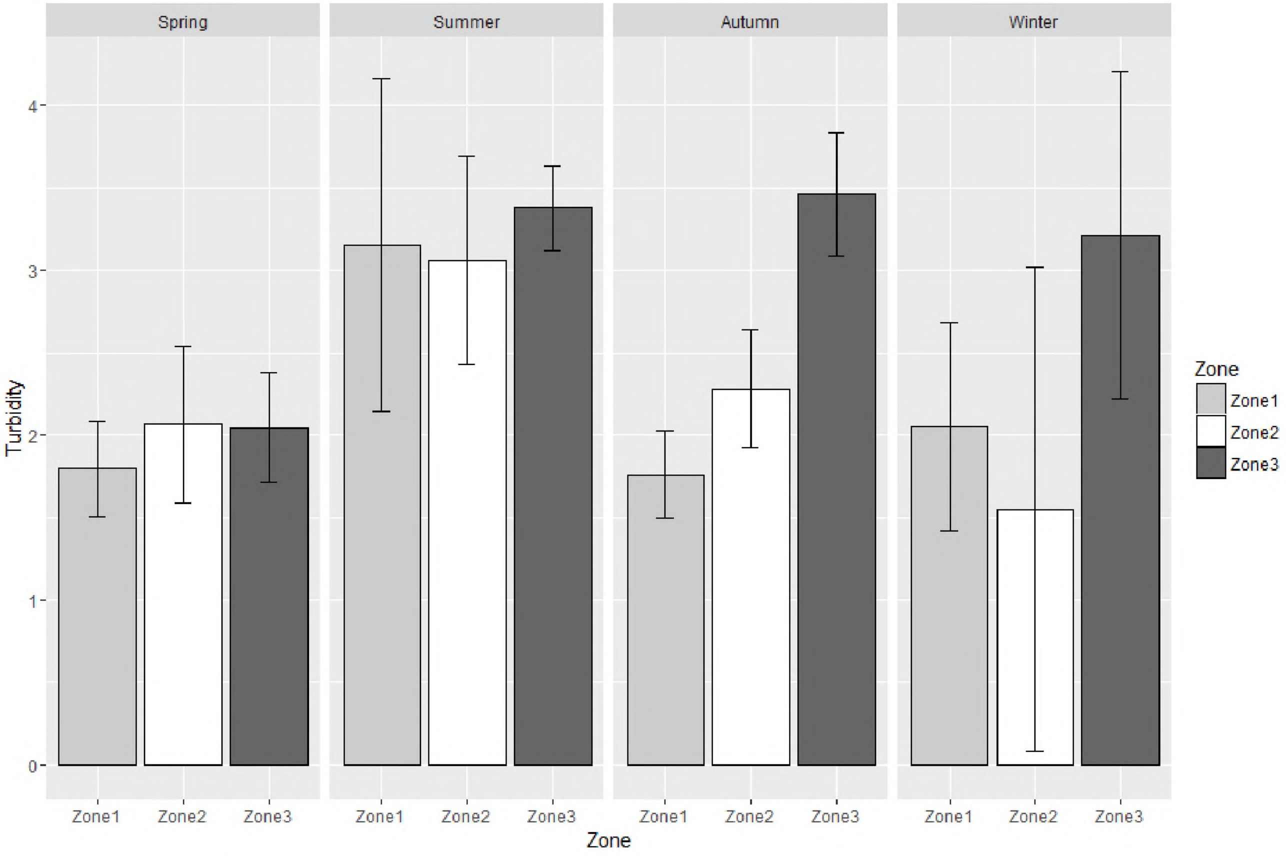


Figure 2.7. Seasonal changes in turbidity (ntu) (mean ± se) across the three marina zones within Thesen Islands Marina recorded in 2014 and 2015. Pooled data showed that turbidity was significantly higher ($p < 0.01$) in Zone 3 compared to Zone 1 across all seasons.

2.3.2 Habitat description

Codium tenue was dominant during the summer 2014 vegetation survey with 32.9 % coverage. Barren sandy areas and *A. taxiformis* had an overall coverage of 26.7 % and 21.9 % respectively. Other prevalent vegetation types included *Z. capensis* (1.8 %), *H. ovalis* (0.3 %), and *C. filiformis* (13.3 %). Only 3.1 % of Reno mattress habitat was present. In winter *C. tenue* cover decreased to 23.0 % with a corresponding increase in barren sandy areas (32.9 %), *H. ovalis* (1.8 %), and *A. taxiformis* (31.2 %). *Caulerpa* *filiformis* and *Z. capensis* coverage decreased to 6.4 % and 1.6 % respectively. The area of Reno mattress habitat remained constant (3.1 %) throughout the year (Fig. 2.8).

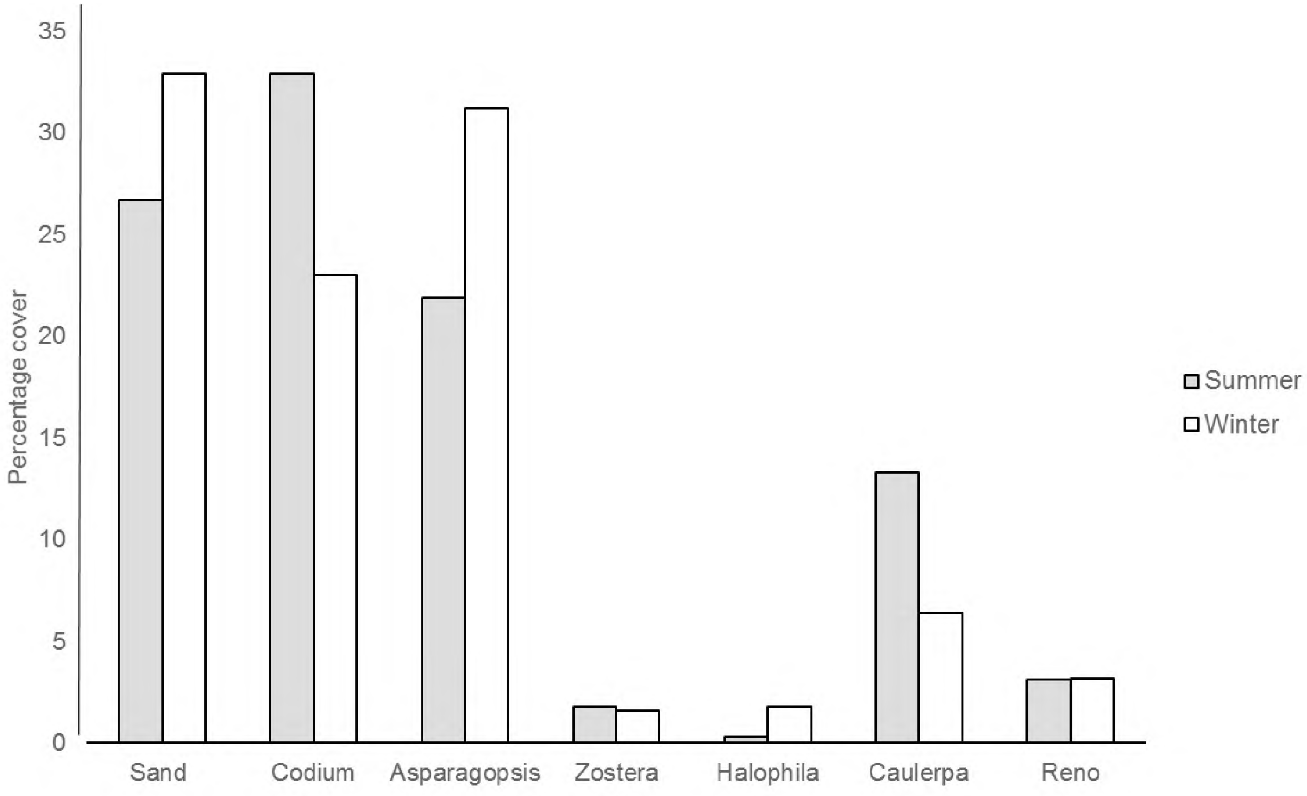


Figure 2.8. Seasonal variation in the percentage cover of the major vegetation types found within the canals of Thesen Islands Marina during the 2014 vegetation surveys. Bare sand and Reno mattress are included in this assessment.

Habitat types also showed a seasonal pattern with a decrease in *C. tenue* habitat from summer to winter (32.9 % to 23.0 %) and corresponding increases in mixed vegetation (37.3 % to 41.1 %) and barren sediment habitats (26.7 % to 32.9 %). Reno mattress habitat remained constant with 3.1 % coverage (Fig. 2.9).

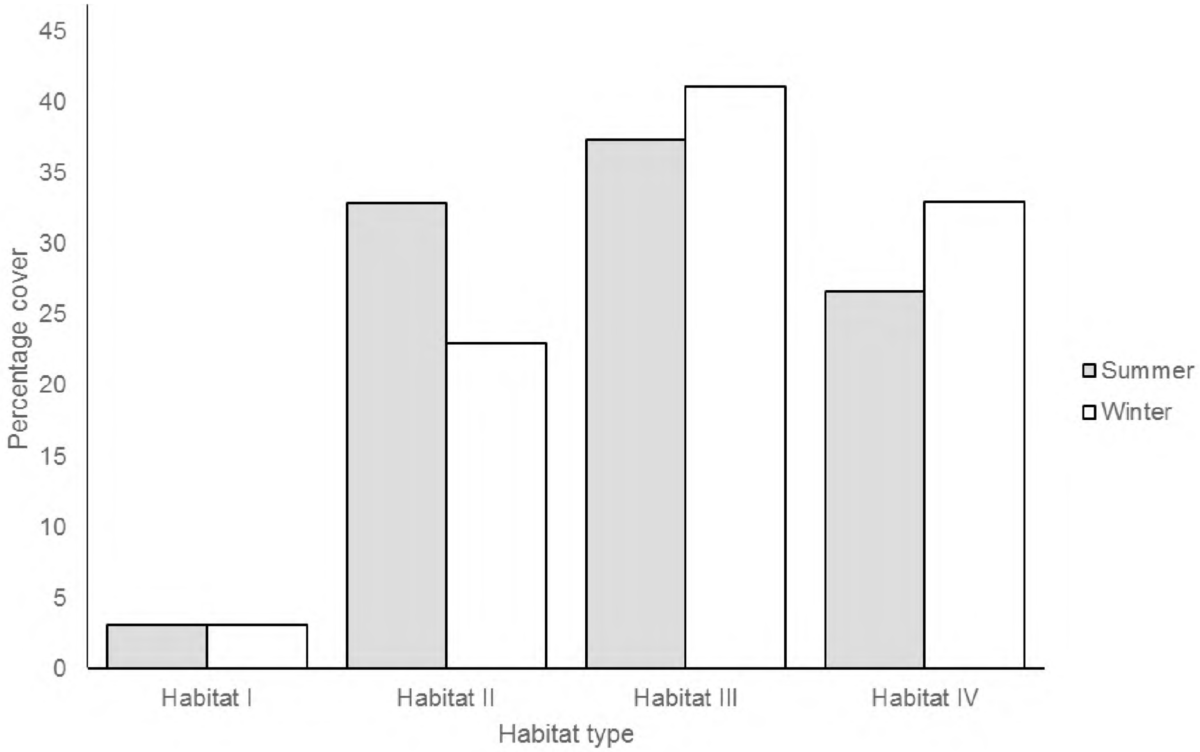


Figure 2.9. Seasonal variation in the percentage cover of the four major habitat types identified during the 2014 vegetation surveys.

The largest extent of Reno mattress habitat was found within the western section of the marina characterised by higher water current velocities (Schumann, 2004). Habitat type changed across marina zones and Reno mattress and mixed vegetation habitats were mostly displaced by *C. tenue* habitat in Zone 3 (Fig. 2.10 A, B).

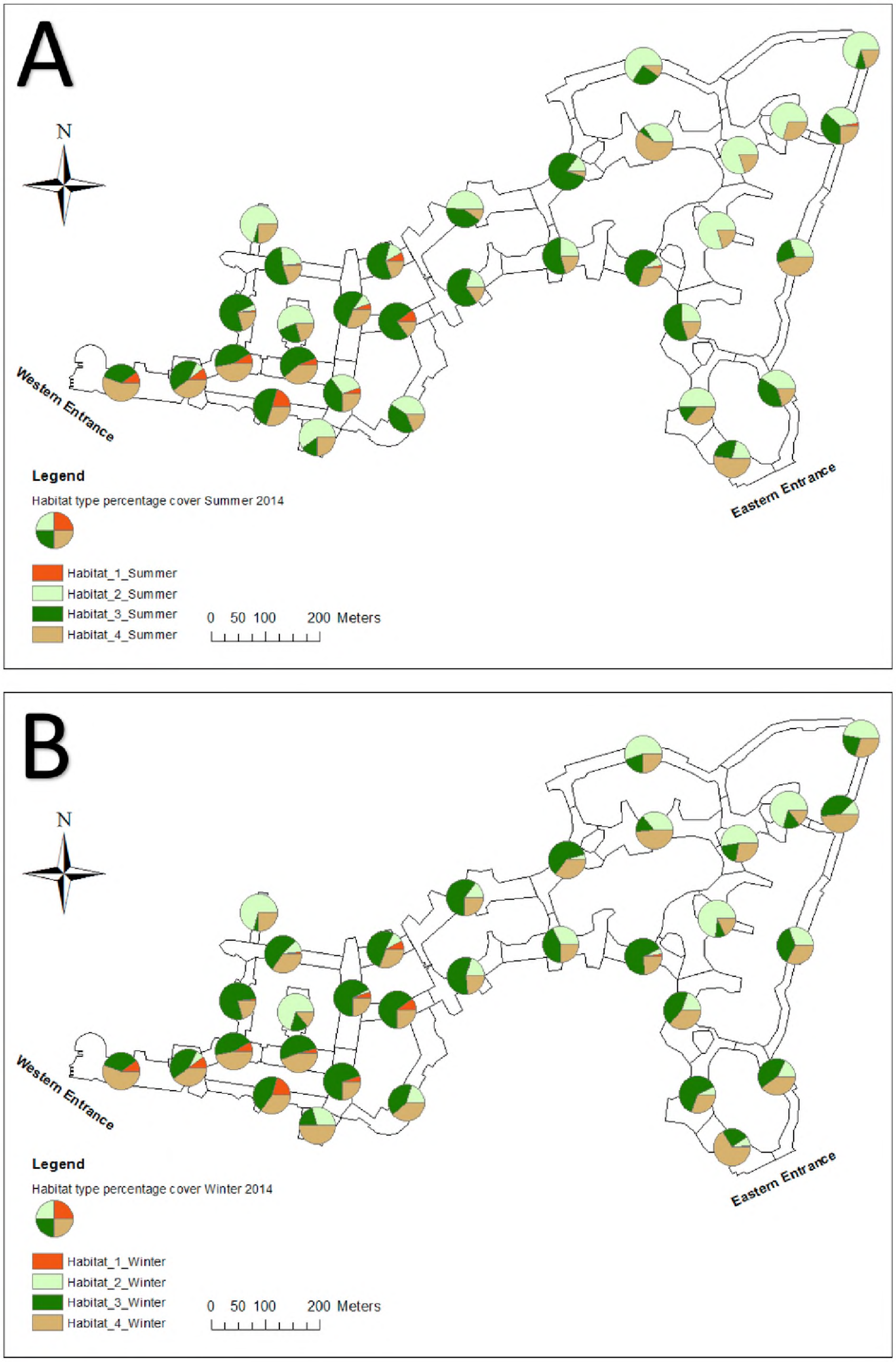


Figure 2.10. Distribution of habitat types within Thesen Islands Marina in the summer (A) and winter (B) of 2014 recorded during the vegetation surveys.

2.3.3 Suitability of identified habitat types as seahorse habitat

2.3.3.1 Dissolved oxygen within identified habitat types

Large diurnal fluctuations in dissolved oxygen saturation were found within *C. tenue* habitat (Table 2.2) with a maximum of 152.0 % and a minimum of 12.1 % recorded during noon and midnight respectively, although no significant differences in mean dissolved oxygen saturation was found between habitat types (F = 0.02, df = 1, p = 0.9). Reno mattress and mixed vegetation habitats showed little diurnal variation. *Codium tenue* habitat also showed marked differences between surface and bottom dissolved oxygen saturation, while the Reno mattress and mixed vegetation habitats were well mixed with little change in dissolved oxygen saturation across depth.

2.3.3.2 Potential seahorse prey animals within identified habitat types

Typical seahorse prey animals identified within the different habitat types are summarized in Table 2.3. Prey densities did not vary significantly across marina zones, but significant differences were noted between habitat types (F = 7.1, df = 2, p = < 0.01) with Reno mattress habitat containing the highest prey densities (Fig. 2.11), particularly high densities of Copepoda. Habitat specific prey densities only varied within Zones 1 and 2 (highest densities within the Reno mattress habitat), whereas densities were similar across habitat types within Zone 3.

Table 2.2. Dissolved oxygen saturation as percent saturation (mean ± sd) within different habitat types measured across 24 hours. Max refers to the maximum percent saturation measured and Min to the minimum percent saturation measured. N refers to the total number of readings taken during each sample.

Time	Habitat	Location	Mean ± sd	N	Max	Min
Dawn	I	Surface	77.0 ± 0.5	10	78.1	76.5
		Bottom	76.8 ± 0.6	10	77.9	75.6
	II	Surface	68.9 ± 0.4	10	69.3	68.2
		Bottom	65.2 ± 2.8	10	67.8	58.7
Noon	I	Surface	94.7 ± 9.3	10	104.5	85.1
		Bottom	96.7 ± 10.2	10	107.0	86.1
	II	Surface	76.9 ± 4.3	30	97.2	71.9
		Bottom	114.2 ± 16.1	27	152.0	91.0
	III	Surface	85.3 ± 0.9	5	86.1	84.0
		Bottom	85.2 ± 0.3	5	85.5	84.8
Dusk	II	Surface	90.9 ± 3.6	35	100.6	86.5
		Bottom	67.3 ± 24.0	25	94.3	20.0
	III	Surface	95.4 ± 0.1	5	95.6	95.3
		Bottom	94.6 ± 0.2	5	94.8	94.5
Midnight	I	Surface	78.6 ± 0.5	10	79.2	77.5
		Bottom	76.1 ± 1.4	10	78.5	74.5
	II	Surface	83.2 ± 2.7	35	87.5	77.7
		Bottom	26.3 ± 13.7	35	68.6	12.1
	III	Surface	89.4 ± 0.4	5	89.7	88.9
		Bottom	88.6 ± 2.7	5	90.0	83.7

Table 2.3. Summary of potential seahorse prey animals identified within Thesen Islands Marina (N = 45).

	Phylum	Class	Subclass	Order	Suborder	Genus
1	Nematoda					
2	Annelida	Polychaeta				
3	Arthropoda		Copepoda			
4	Arthropoda		Copepoda	Harpacticoida		*Porcellidium*
5	Arthropoda	Malacostraca	Eumalacostraca	Tanaidacea		
6	Arthropoda	Ostracoda				
7	Arthropoda	Malacostraca	Eumalacostraca	Isopoda		
8	Arthropoda	Malacostraca	Eumalacostraca	Isopoda	Asellota	

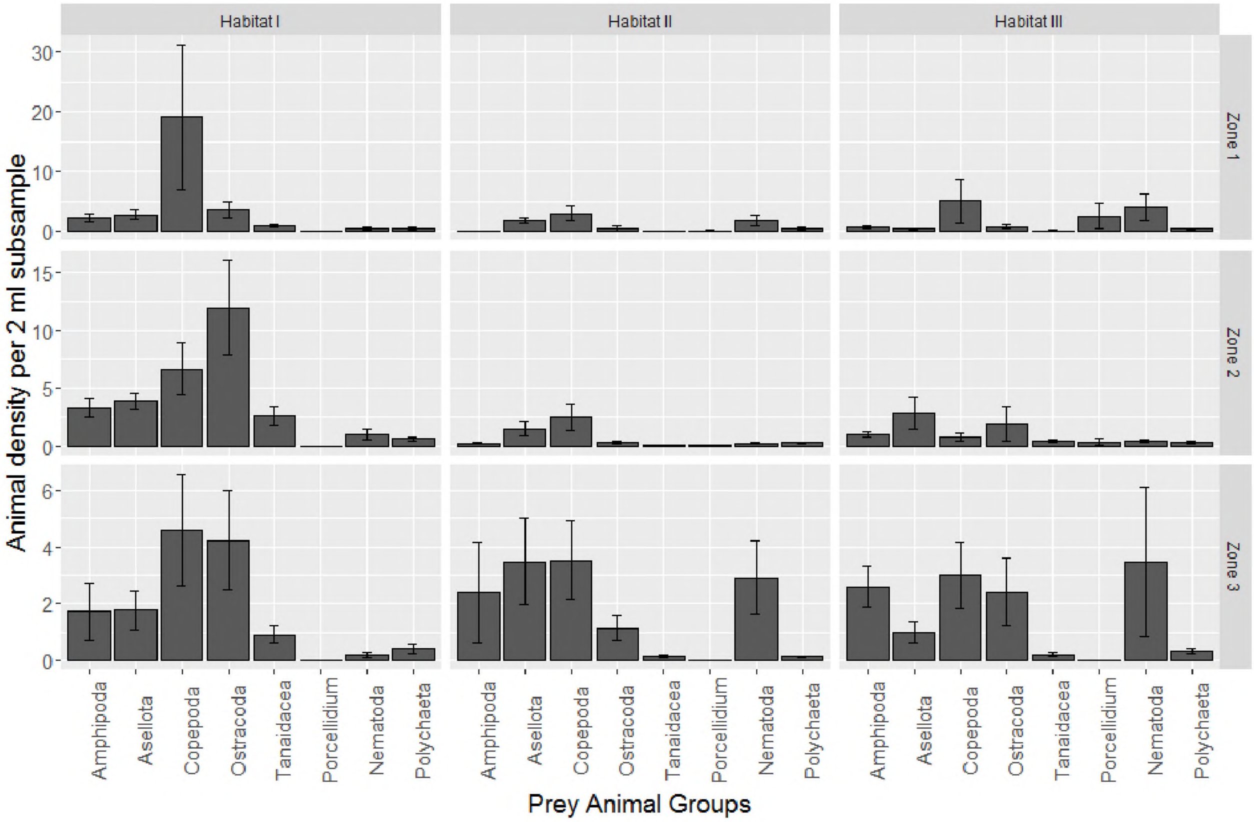


Figure 2.11. Potential seahorse prey animal densities (mean ± se) in different habitat types and marina zones found within Thesen Islands Marina. Density is per 2 ml subsample and 10 subsamples were assessed from each habitat sample collected (N = 45). Sampling was conducted from March to May 2015.

2.4 Discussion

The new aquatic habitat created by the development of the Thesen Islands Marina was found to have potential value for *H. capensis* with regards to habitat availability and water quality. Three habitat types with potential to provide habitat to *H. capensis* were identified (mixed vegetation, *C. tenue* and Reno mattresses) and the water quality within the marina canals was comparative to the main estuary and of high quality.

Historic water quality data for the bay regime of the estuary found a well-mixed system with high dissolved oxygen (82 to 97 % - Allanson *et al.*, 2000) and salinity similar to that of the adjacent ocean (≈ 35) (Allanson *et al.*, 2000), although flooding can result in a marked decrease in salinity throughout the entire estuary (DWA, 2009). Water temperature in the estuary range from 20 °C to 27 °C in summer (but can drop to 10 °C during upwelling events) and from 12 °C to 15 °C in winter (DWA, 2009). Water quality within Thesen Islands Marina was comparable to the estuary bay regime, although areas with a decrease in flow and circulation (Zone 3), similar to conditions within the Ashmead channel as described by Switzer (2003), were found on the eastern side of the marina.

Similarly, Baird *et al.* (1981) found that surface and bottom water temperature, salinity and dissolved oxygen concentrations within a residential marina estate in the Kromme Estuary, St Francis Bay, were similar to that of the adjacent estuary. This marina development showed no signs of stratification and it was concluded that the system was well mixed with high dissolved oxygen content. Marked salinity and dissolved oxygen stratification was, however, found in the Da Gama residential marina estate, another South African marina located in the Zandvlei Estuary, False Bay, owing to specific design features (or rather flaws) of this marina (dead-end canals, canals that are deeper than the adjacent estuary, limited connection to the main estuary) (Davies and Stewart, 1984).

Suitable water flow is a key aim for successful canal developments (Lemckert, 2006) and Thesen Islands Marina's design, with its two connections to the main estuary, mostly achieves this. Reduced water flow within a marina environment can lead to increased water retention (Rivero *et al.*, 2013), and dead-end canal systems are known to present adverse water quality problems that range from severe oxygen and salinity stratification (Davies and Stewart, 1984) to higher sediment contaminant concentrations (Maxted *et al.*, 1997). Temperature stratification (Fig. 2.6) was found to be pronounced within Zone 3 of the marina and was particularly acute during autumn and summer, likely owing to upwelling events that occur regularly during these seasons (Schumann, 2000). The prominent stratification and higher turbidity in Zone 3 could potentially affect seahorses, as high turbidity has been shown to affect prey capture rates (James and Heck, 1994) and mating behaviour (Sundin *et al.*, 2010). Hypoxic conditions that were found to occur within dense *C. tenue* beds owing to large diurnal dissolved oxygen fluctuations also have the potential to affect seahorse feeding behaviour (Ripley and Foran, 2007).

The potential seahorse prey assessments provided evidence that the habitats found in Thesen Islands Marina provide suitable food for *H. capensis*. The specific diet of *H. capensis* is not known, but it is assumed that it would feed on similar types of prey as other seahorse species e.g. small crustaceans (Tipton and Bell, 1988; Foster and Vincent, 2004; Kendrick and Hyndes, 2005; Castro *et al.*, 2008). Harpacticoid copepods were found to be particularly important prey for *Hippocampus zosterae* (Tipton and Bell, 1988) and *H. subelongatus* Castelnau, 1873 (Kendrick and Hyndes, 2005), while nematodes were found to be important prey for *H. reidi* (Castro *et al.*, 2008).

The most abundant habitat type found within the canals of Thesen Islands Marina was mixed vegetation. Past research found that this habitat type, particularly *Z. capensis* and *C. filliformis*, is used by *H. capensis* (Bell *et al.*, 2003; Lockyear *et al.*, 2006; Teske *et al.*, 2007b). The suitability of Thesen Islands Marina's canals as seahorse habitat, therefore, is particularly promising because 67 % of the subtidal area within the marina was covered by some type of vegetation – a necessity for the occurrence of *H. capensis* (Teske *et al.*, 2007b). By contrast, the Knysna estuary has subtidal vegetation cover of a mere 23 % (238 ha) (Adams, 2016). Of particular concern is the threat to mixed vegetation beds, particularly *Z. capensis* and *C. filiformis*, within the estuary owing to an extensive and lengthy *Ulva lactuca* Linnaeus, 1753 bloom (Allanson *et al.*, 2016) – known to displace *Z. capensis* (Human *et al.*, 2016) and saltmarsh vegetation (Watson *et al.*, 2015). The additional habitat found within Thesen Islands Marina have the potential to provide a shelter against habitat loss and adverse environmental conditions for *H. capensis* within a larger, very dynamic system.

Impacts from artificial structures on coastal and estuarine environments vary from being positive (increased biodiversity and biomass (Clynick, 2006; Connell and Glasby, 1999)) to negative (supports invasive species, results in habitat loss and/or fragmentation, alteration of biodiversity (see Bulleri and Chapman, 2010)). A recent review by Bulleri and Chapman (2010) did, however, conclude that artificial structures do not function as surrogates for natural habitats and species compositions on these structures differ from that found in adjacent natural habitats. In many instances, artificial structures are built within a soft substrate system (an estuary), and the species that colonize these structures are non-native to the system (Bulleri 2005). These changes can have adverse effects on the underlying ecological processes of a system.

Most research on the ecological impact of artificial structures found in marinas and harbours have focused on heavily modified water bodies, as opposed to artificial water bodies (see Waltham and Connolly, 2011; Waltham and Connolly, 2013). The latter provides new aquatic habitat to a system, whereas the former changes one habitat type into something else (in many instances natural aquatic habitat is lost). Only a limited number of studies were found that focused on the ecological impact of artificial water bodies e.g. according to Waltham and Connolly (2013), the Burleigh Lake system, Australia, excavated from land (artificial water body) created completely novel fish habitat, and Chovanec (1994) concluded that artificial wetlands can create refuges for endangered species, even in highly frequented urban areas.

Thesen Islands Marina, an artificial water body, added 25 ha of new aquatic habitat to the Knysna estuary, 67 % of which have potential to provide habitat to *H. capensis.* Whether this development is in fact used by *H. capensis* and what role it plays in this species density and distribution will be investigated in Chapter 3.

Chapter 3

Population density, distribution, and structure of *Hippocampus capensis* within a residential marina estate

3.1 Introduction

Successful conservation depends on empirical knowledge of species specific population parameters such as abundance, geographical range, distribution, and habitat requirements (McLean *et al.*, 2016). Changes in these parameters and the reasons behind such changes are central to ecology, specifically, the nature of the relationship between abundance and distribution (Brown, 1984; Guisan and Thuiller, 2005). In a marine environment, variability of populations can be caused by various natural and unnatural factors (Martin-Smith and Vincent, 2005; Correia *et al.*, 2015a), and it is crucial that these are understood for sound management and conservation.

In a review of the fish database, FishBase (http://www.fishbase.org/), a gap in life-history knowledge of small to medium sized fish, which include seahorses, was noted (see Foster and Vincent, 2004). Twenty of the 33 seahorse species listed on the IUCN Red List of Threatened Species are classified as Data Deficient, which highlights the need for population information for this group (www.iucnredlist.org accessed 15 April 2017). The recent review of seahorse taxonomy (Lourie *et al.*, 2016) will not greatly alter the conservation status of those species on the IUCN list. *Hippocampus borboniensis* and *H. fuscus,* both listed as Data Deficient, will now be subsumed under *H. kuda,* listed as Vulnerable, while *H. hendriki* Kuiter, 2001 *(*Data Deficient*)* will be subsumed under *H. angustus* Günther, 1870, also listed as Data Deficient. According to Lourie *et al.* (2016): "population surveys and on-going monitoring are urgently required to accurately ascertain and effectively improve the conservation status of all seahorse species".

Population density information does exist for some seahorse species: *Hippocampus guttulatus* – Curtis and Vincent (2005), Correia *et al.* (2015a), Gristina *et al.* (2015), Woodall *et al.* (2015); *H. hippocampus* – Curtis and Vincent (2005), Correia *et al.* (2015a), Gristina *et al.* (2015), Otero-Ferrer *et al.* (2015); *H. reidi* – Freret-Meurer and Andreata (2008); *H. whitei* – Harasti (2016); *H. capensis* – Bell *et al.* (2003), Lockyear *et al.* (2006); *H. comes* - Marcus *et al.* 2007 and *H. zosterae* – Mason-Jones *et al.* (2010). Many of these studies only presented a snapshot of the status of a population (Bell *et al.*, 2003; Curtis and 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 ≈ 90 % between 2001 and 2009 (see Curtis and Vincent, 2005; Caldwell and 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 and Vincent, 2005). Short term population density estimates, however, are necessary to provide population base-lines, but it is only through long term monitoring that underlying processes responsible for population variability can be understood.

Habitat use is an important aspect in seahorse population assessments (Curtis *et al.*, 2004), because seahorse abundance, density and distribution change across different habitats. Habitat use data are also helpful to understand possible reasons for population variability, and many studies have focused on seahorse density and distribution within different habitat types (e.g. Curtis and Vincent, 2005; Gristina *et al.*, 2015; Otero-Ferrer *et al.*, 2015). Seahorse population declines in the Ria Formosa (Correia *et al.*, 2015a) was attributed to a decline in the number of holdfasts, and in Port Stephens, Australia, (Harasti, 2016) to a decline in preferred habitat.

Seahorses that live in coastal habitats are particularly vulnerable to anthropogenic impacts because human population densities along the coast are three times higher than inland areas (Whitfield and Becker, 2014). The relatively sedentary lifestyle of seahorses and specialised mating behaviour increases the threat from impacts to their environment (see Foster and Vincent, 2004), and habitat alteration, degradation and loss have been identified as a major threat to the Knysna seahorse (*Hippocampus capensis*) specifically (Whitfield, 1995). How seahorses adapt to habitat alterations and additions, if at all, is an important aspect that must be understood to ensure successful future conservation (Vincent *et al.*, 2011).

It was confirmed that the geographical distribution of *H. capensis* is restricted to the Knysna, Swartvlei and Keurbooms estuaries (Bell *et al.*, 2003; Lockyear *et al.*, 2006). Only two published population assessments have been done for *H. capensis* in the Knysna estuary (Bell *et al.*, 2003; Lockyear *et al.*, 2006), the last completed in 2001 (Lockyear *et al.*, 2006). These assessments provided a baseline of the abundance and distribution of this species within the Knysna estuary. Habitat specific density was, however, not taken into consideration, although Teske *et al.* (2007b) concluded that *H. capensis* is likely to occur throughout the system where vegetation is available. With the high development pressures on the Knysna estuary and the construction of three residential marina estates in the past ten years, there was a need to reassess the Knysna seahorse population within this estuary and to focus on the adaptability of *H. capensis* to anthropogenic impacts.

The previous chapter concluded that 67 % of the 25 ha canal system within Thesen Islands Marina was covered by some type of vegetation and/or holdfast and thus provides potential seahorse habitat. The physico-chemical environment within the marina was deemed suitable for seahorse habitation and all habitat types identified as potential seahorse habitat contained typical seahorse prey. The next step was to assess whether seahorses utilize the habitats created by the development of the marina and if so, which habitats.

This chapter aims to:

- Identify which habitat types, if any, are utilized by *H. capensis* within Thesen Islands Marina.
- Assess spatial and temporal variability of seahorse density and population structure within utilized habitat types.
- Assess and compare seahorse populations within the marina development and within the bay regime of the Knysna estuary.

3.2. Materials and methods

3.2.1. At first glance: Seahorse abundance, distribution, and population structure within Thesen Islands Marina

During the habitat survey of the canals of Thesen Islands Marina, seahorses were observed within three of the four habitat types identified (see Chapter 2), thus confirming anecdotal reports. As it was not possible to survey *C. tenue* habitat using standard seahorse survey methods as per Curtis *et al.* (2004), focus was first placed on Reno mattress and mixed vegetation habitats. As stated in chapter 2, Reno mattress habitat is made up of a 2 m wide Reno mattress found along the sides of the marina canals and mixed vegetation habitat from various vegetation types (*Zostera capensis, Caulerpa filiformis, Asparagopsis taxiformis*) located in the middle of the canals.

A preliminary seahorse survey took place from August (late winter) to October (early spring) 2014. Underwater visual survey transects as per Curtis *et al.* (2004) using SCUBA were used. Twelve sites (Fig. 3.1) were surveyed (2230 m^2) during high tide in depths that ranged from 1.5 – 3.0 m. Sites were chosen haphazardly along the west-east gradient of the marina increasing in distance from the western marina entrance. The total number of samples taken in each habitat type depended on the availability of the specific habitat types at each site. Where possible, ten transects (100 m^2) were surveyed per habitat type at each sample site using a stratified sample design. All surveys were undertaken by myself. Each transect sample consisted of a 10 m long transect line with a 0.5 m search width on either side (10 m^2). The coordinates of each transect, taken with a handheld Garmin GPSmap 62, and direction, chosen arbitrarily, were logged. Surface water temperature using a thermometer and turbidity (ntu) using a Eutech TN-100 bench-top turbidity meter (Thermo Scientific, Singapore) were measured at each site.

The height of each observed seahorse, 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 (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. Seahorse sex, brood pouch status for males only and holdfast used by each specimen found were recorded. A distinction was made between a full brood pouch and an empty brood pouch (see Curtis and Vincent, 2005). Males were identified by the presence of a brood pouch. To ensure consistency with previous *H. capensis* surveys, animals larger than 4 cm with no brood pouch were considered to be females and individuals that showed no sexual differentiation and was smaller than 4 cm was considered to be juvenile as established by Lockyear *et al.* (2006).

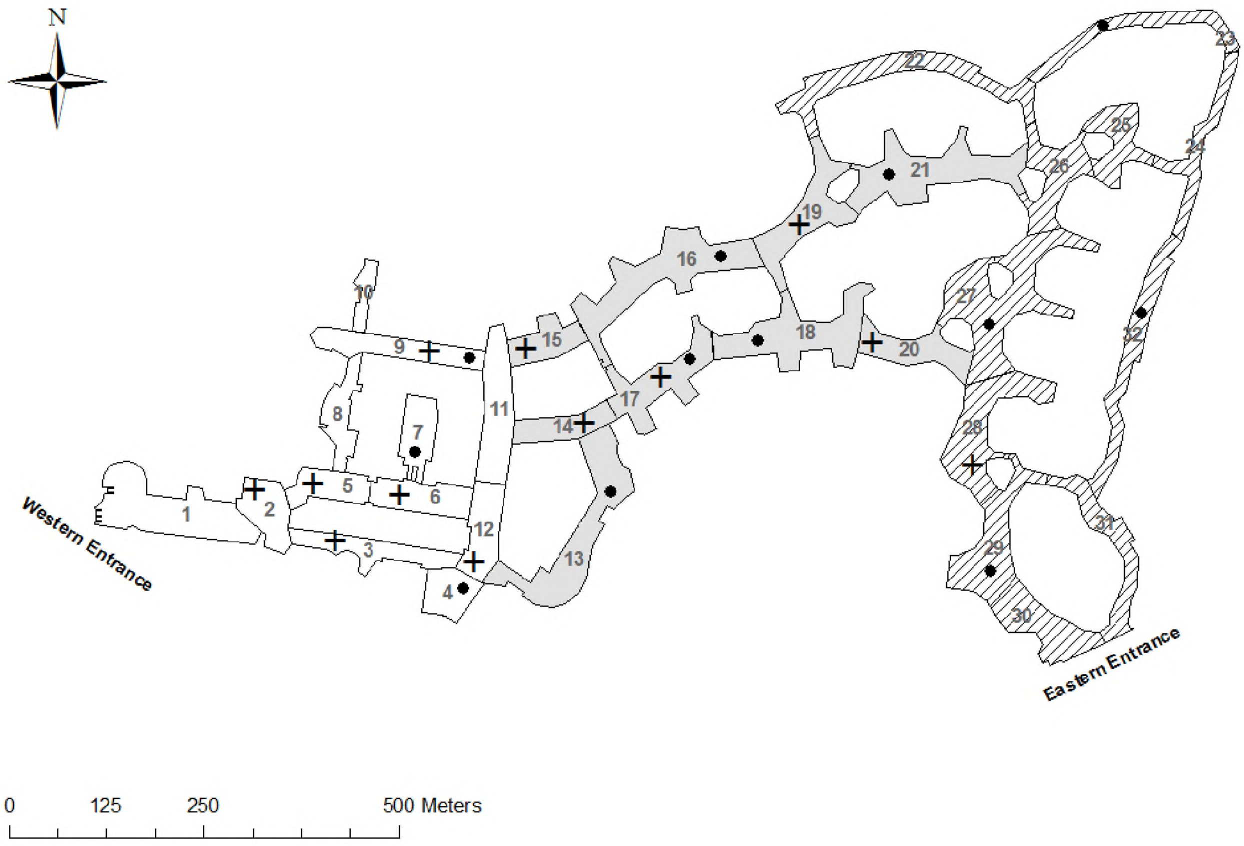


Figure 3.1. The location of sample sites of Reno mattress and mixed vegetation (+) and *Codium tenue* (●) habitats in Thesen Islands Marina. Sample site numbers and marina zone shading as per Figure 2.3. (Zone 1: western high water current velocity zone (white); Zone 2: the middle cross-over zone (grey); and Zone 3: eastern low water current velocity zone (hatched)).

3.2.2. Diving a bit deeper: Seasonal seahorse population trends within Thesen Islands Marina

With the confirmation of seahorses within Thesen Islands Marina, specifically within Reno mattress and *C. tenue* habitats (see Results 3.3.1 and pers. obs), the need arose to investigate population trends spatially and temporally. It was decided to omit the mixed vegetation habitat from ongoing assessments owing to the significantly lower seahorse densities found within this habitat type during the preliminary assessment.

Focus was thus rather placed on habitat types known to be used more extensively by *H. capensis.* Seahorse populations vary temporally and spatially, in some instances significantly (Correia *et al.*, 2015a; Harasti, 2016), and longer term, as opposed to snapshot assessments, are needed to really understand the status of a population. To find out if the population density, distribution and structure of *H. capensis* within this artificial water body remains stable over time, seasonal seahorse surveys took place from January to February (summer) and June to July (winter) in 2015 and 2016 within Reno mattress and *C. tenue* habitats. 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.

The same survey method and seahorse parameters (height, sex, brood pouch status, holdfast used) as described in section 3.2.1 were used for the population assessment within Reno mattress habitat, and in addition the dominant vegetation type and percentage cover were estimated for each 10 m^2 transect. Underwater visual survey transects (Curtis *et al.*, 2004) were not suitable for use in *C. tenue* habitat because animals hid within the algal mass. To overcome this constraint an alternative sampling method was established. A net (38 cm x 19 cm x 30 cm) was used to collect *C. tenue* (two scoops per sample with a mean (± sd) weight of 3.4 ± 1.0 kg (N = 470)). The unattached algae was carefully scooped to make sure the underlying anoxic mud was not disturbed. The collected sample was placed on a floating platform and sorted. The height of all seahorses found was measured, animals sexed and the brood pouch status assessed. The *C. tenue* sample was weighed with a spring balance to the nearest 100 g to establish the drained wet weight. After a sample was processed the vegetation and seahorses were returned to the sample location. The overall percentage cover of *C. tenue* per sample site was recorded. Sampling was done from a boat to prevent disturbance of the *C. tenue* beds and underlying mud. An average seahorse density per drained wet weight of *C. tenue* was established.

Ten transect samples from the Reno mattress habitat and ten samples from the *C. tenue* habitat were collected from the 12 sample sites chosen for each habitat type (Fig. 3.1). Surface water temperature and turbidity (ntu) were measured at each site.

3.2.3. Casting the net wider: Monthly seahorse population trends within the Knysna estuary and Thesen Islands Marina

Different seasonal variations in both seahorse density and structure were noted in both Reno mattress and *C. tenue* habitats (see Results 3.3.2). Does this variability persist on a shorter time scale (monthly) and across all seasons? To answer this question, monthly seahorse surveys were conducted from October 2015 to August 2016 at two sample sites within Reno mattress and *C. tenue* habitat respectively. Sites were chosen based on the highest seahorse densities found during the seasonal population assessments (Sites 2 and 5 within Reno mattress habitat and Sites 4 and 17 within *C. tenue* habitat) (Fig. 3.2). Survey methods as described in section 3.2.1. and 3.2.2. were used within Reno mattress and *C. tenue* habitats respectively. Surface water temperature and turbidity (ntu) were measured at each site.

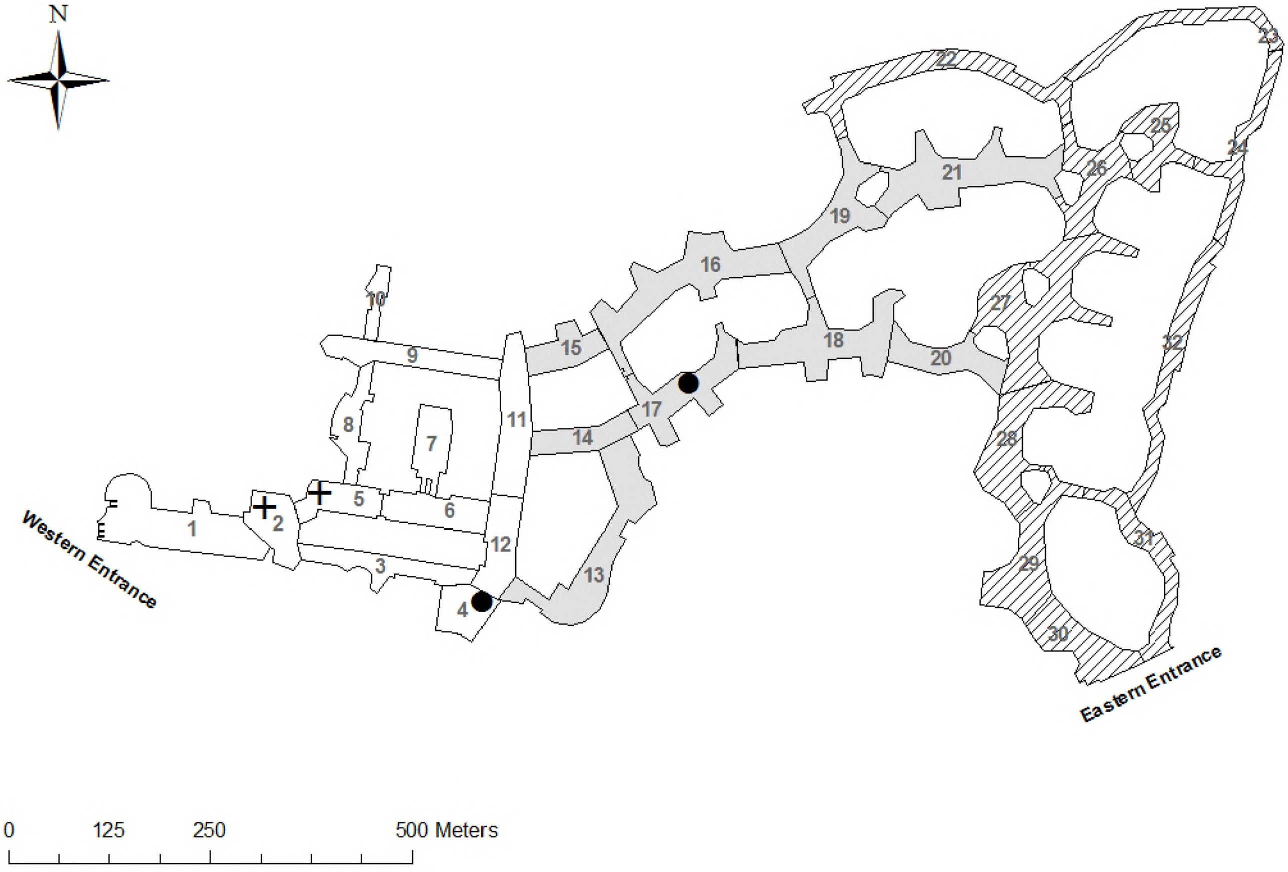


Figure 3.2. Sample sites within Reno mattress (+) and *Codium tenue* (●) habitats within Thesen Islands Marina surveyed on a monthly basis from October 2015 to August 2016. Sample site numbers and marina zone shading as per Figures 2.2 and 3.1.

To assess if there were differences in *H. capensis* population density and structure between the marina and the estuary, two additional sites were chosen in the bay regime of the Knysna estuary and surveyed on a monthly basis from October 2015 to August 2016. These sites were chosen based on high seahorse densities found by Lockyear *et al.* (2006) in 2001: Laguna Grove (0.22 individuals /m^2) and an Inter Island site (0.19 individuals/m^2) (Fig. 3.3).

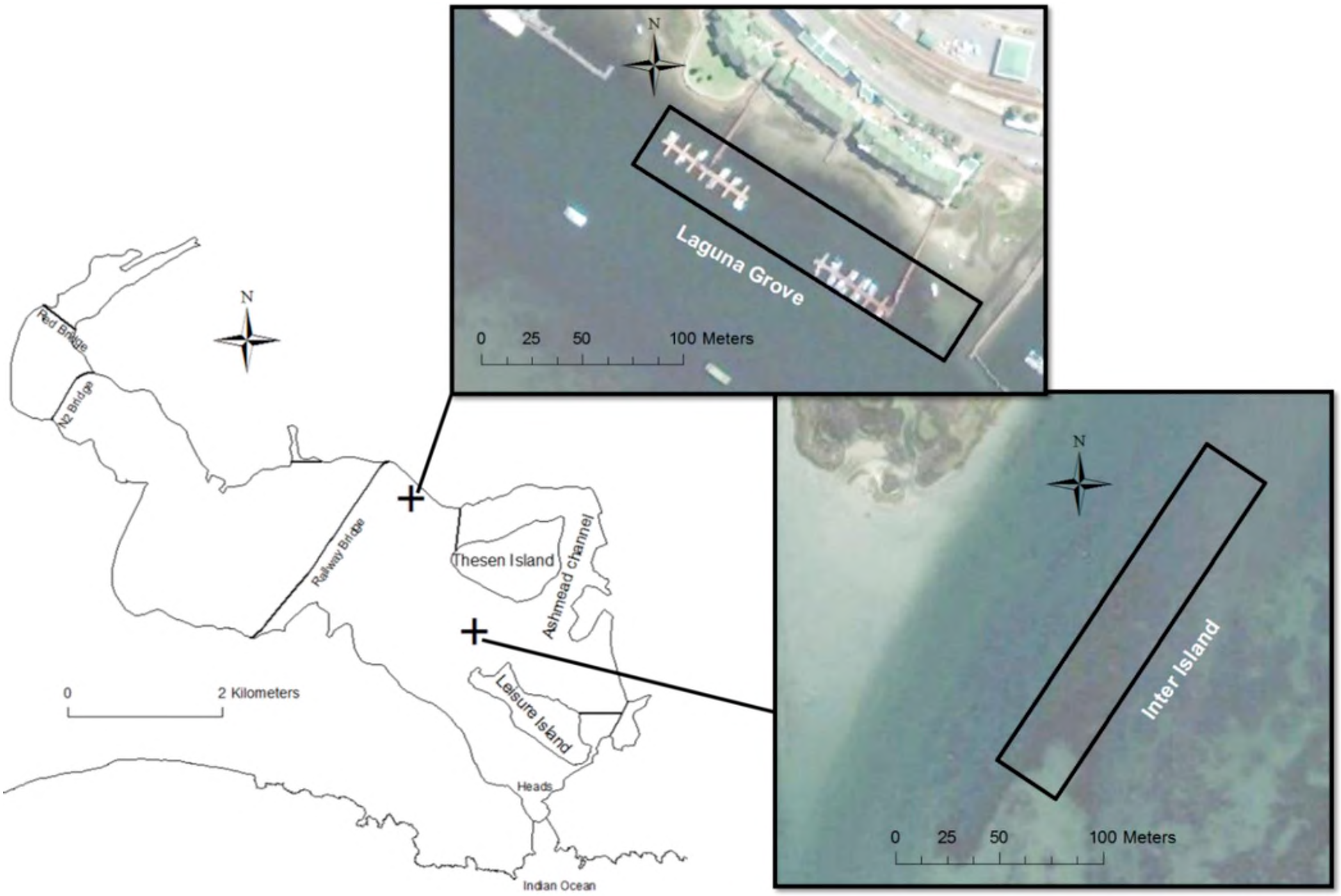


Figure 3.3. Two sample sites (Inter Island and Laguna Grove) within the bay regime of the Knysna estuary, surveyed on a monthly basis from October 2015 to August 2016. (Images sourced from Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community).

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 depth ranges from 1.0 to 2.7 m across the site during high tide. This area is impacted 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. Transects were located parallel to the estuary bank, adjacent to two large jetties. A pier located on the eastern side of the site is a popular fishing spot and marine 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 seahorse habitat at these two sites as well as the presence of seahorses. A similar method as described in section 3.2.2. was used at the two sites with the following adjustments: a 30 m transect line, marked in 10 m intervals, with a 1 m search width was used. The dominant vegetation type and percentage cover per 10 m^2 section were recorded. Sampling was done during high tide. Seven points were marked at each site using a handheld Garmin GPSmap 62 and the survey direction, chosen arbitrarily, recorded and all subsequent surveys used the same starting points and directions. These points were chosen haphazardly within the sample site area. Surface water temperature and turbidity (ntu) were measured at each site.

To compare seahorse densities between Reno mattress habitat within Thesen Islands Marina and the two estuary sites a standard search effort unit had to be established, seeing that 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 seahorses along the transect {*active search time = total search time per transect - (average handling time per seahorse x total number of seahorses found in transect)*}. The average handling time (which included taking measurements, photographing the specimen etc.) was estimated to be 1 min per seahorse. This approach allowed a seahorse density per unit time per unit area (search effort unit) to be established which was used to compare seahorse densities spatially and temporally.

Seven transects (210 m^2) at each estuary site (Laguna Grove and Inter Island) (Fig. 3.3) and ten transects (200 m^2) within Reno mattress habitat in the marina (Sites 2 and 5) (Fig. 3.2) were surveyed on a monthly basis from October 2015 to August 2016 (11-month period). Ten samples were collected on a monthly basis from *C. tenue* habitat at Sites 17 and 4 for the same time period (Fig. 3.2). 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.

3.2.4. Statistical analysis

The statistical programme R (R Development Core Team, 2014) was used in all analyses. The ddply function of the Plyr-package was used for all basic descriptive statistics (Wickham, 2011). 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 Generalised Linear Model (GLM) was used to assess seahorse population differences spatially and temporally. Overall significance was tested using ANOVA. The Car (Fox and Weisberg, 2010) and Mass (Venables and Ripley, 2002) packages were used for Generalised Linear Model analyses. Sex ratios were assessed with a Chi-square test. ANOVA was used to compare temperature and turbidity spatially and temporally. Height differences between males and females and seahorse 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 ($p > 0.05$). Table 3.1.

provides a summary of all hypotheses tested, the applicable sections within the chapter and the R codes used in each instance.

3.3. Results

3.3.1. At first glance: Seahorse abundance, distribution, and population structure within Thesen Islands Marina

From August to October 2014, surface water temperature was significantly warmer ($F = 15.7$, $df = 1$, $p < 0.01$) within mixed vegetation habitat and ranged from 20.5 to 16.0 °C in both Reno mattress and mixed vegetation habitats, with a mean (± sd) temperature in Reno mattress habitat of 17.0 ± 1.2 °C and in mixed vegetation habitat of 17.7 ± 1.4 °C. Turbidity (ntu) in mixed vegetation habitat (mean ± sd = 2.2 ± 1.2 ntu) was significantly ($F = 12.3$, $df = 1$, $p < 0.01$) higher compared to Reno mattress habitat (1.7 ± 0.8 ntu).

The mean (± se) seahorse density within Reno mattress habitat was 0.15 ± 0.03 individuals/m^2 and within mixed vegetation habitat it was 0.02 ± 0.01 individuals/m^2. Significantly lower ($F = 33.2$, $df = 1$, $p < 0.01$) seahorse densities were found in mixed vegetation habitat compared to Reno mattress habitat.

Table 3.1. A summary of all hypotheses tested in Chapter 3, the applicable sections in the chapter and the R code used in each instance.

Hypothesis	Section	Formula
H_0: Water temperature nor turbidity will vary on a seasonal or monthly basis or within different habitat types	3.2.1, 3.2.2, 3.2.3, 3.3.1, 3.3.2, 3.3.3	Assessment Name=aov(Feature~Habitat+ Season+Month,) summary.aov(Assessment Name) TukeyHSD(Assessment Name)
H_0: Seahorse densities will not vary between Habitat types I and III	3.2.1 and 3.3.1	glm.nb(Seahorses/transect~ Habitat type (fixed effect)
H_0: Seahorse densities will not vary between sample sites or on an annual or seasonal basis within Habitat type I	3.2.2 and 3.3.2	glm.nb(Seahorses/transect ~ Sample Site (fixed effect) + Season (random factor) + Year (random factor)
H_0: Seahorse densities will not vary between sample sites or on an annual or seasonal basis within Habitat type II	3.2.2 and 3.3.2	glm.nb(Seahorses/sample ~ Sample Site (fixed effect) + Season (random factor) + Year (random factor)
H_0: Seahorse densities will not differ between sites sampled within the estuary and the marina (Habitat type I) on a monthly basis.	3.2.3 and 3.3.3	glm.nb(Seahorses/transect ~ Sample Site (fixed effect) + Month (random factor) + offset(log(search effort)
H_0: Seahorse densities within the marina (Habitat type I) and the estuary will not vary seasonally	3.2.3 and 3.3.3	glm.nb(Seahorses/transect ~ Sample Site (fixed effect) + Season (random factor) + offset(log(search effort))
H_0: Seahorse densities will not vary on a monthly basis within Habitat type II	3.2.3 and 3.3.3	glm.nb(Seahorses/sample ~ Sample Site (fixed effect) + Month (random factor)
H_0: Seahorse densities will not vary on a seasonal basis within Habitat type II	3.2.3 and 3.3.3	glm.nb(Seahorses/sample ~ Sample Site (fixed effect) + Season (random factor)
H_0: Seahorse heights will not vary temporally or spatially and no height differences will be found between males and females	3.2.1, 3.2.2, 3.2.3, 3.3.1, 3.3.2, 3.3.3	Assessment Name=aov(Seahorse height~Habitat+ Season+Month,) summary.aov(Assessment Name) TukeyHSD(Assessment Name)

An assessment of the pooled data showed an equal female:male sex ratio of 1.2:1 (χ^2 = 2.1, df = 1, p = 0.2). Mean (± se) seahorse height within Reno mattress and mixed vegetation habitats was 6.7 ± 0.1 cm and 6.9 ± 0.1 respectively. Only two juveniles were recorded. Twenty-seven percent of males had a fully inflated brood pouch, indicative of breeding. Reno mattress habitat was exposed to the atmosphere during spring low tide for up to two hours. During these events seahorses were noted in very shallow (< 30 cm) water and in some cases, became stranded (Fig. 3.4). In such instances, they placed their heads against the moist sediment, which probably kept their gills moist, until the tide returned. No seahorse mortalities were noted during these periods, although this may increase their vulnerability to predation e.g. the Grey heron (*Ardea cinerea*) that has been observed to feed on *H. capensis* (Fig. 3.5).

Figure 3.4. Seahorses exposed during spring low tide within Reno mattress habitat in Thesen Islands Marina.

Figure 3.5. A Grey heron (*Ardea cinerea*) feeding on *H. capensis* within Thesen Islands Marina (note the seahorse in the beak).

Eighty-one percent of seahorses were found within Zone 1 of the marina, with 16 % and 3 % in Zones 2 and 3 respectively. Sixty-five percent of seahorses used *A. taxiformis*, 24 % used *C. filiformis*, and 4 % used *Z. capensis, Ulva lactuca and C. tenue* as a holdfast within mixed vegetation habitat. Fifty-eight percent of seahorses used *A. taxiformis* as a holdfast within Reno mattress habitat, 36 % used the wire of the Reno mattress (Fig. 3.6) and 6 % used holdfasts which ranged from twigs to *U. lactuca.*

Figure 3.6. A Knysna seahorse using the wire of the Reno mattress as a holdfast.

3.3.2. Diving a bit deeper: Seasonal seahorse population trends within Thesen Islands Marina

The summer surface water temperatures within Reno mattress habitat ranged from 26.0 °C to 16.0 °C with a mean (± se) of 21.1 ± 0.3 °C, while winter surface water temperatures ranged from 17.0 °C to 13.0 °C with a mean (± se) of 14.4 ± 0.1 °C. Turbidity (ntu) in summer ranged from 13.0 – 0.5 ntu with a mean (± se) of 1.9 ± 0.2 ntu and decreased in winter with a range of 4.1 to 0.8 ntu and a mean (± se) of 1.5 ± 0.1 ntu. Mean (± se) summer surface water temperatures within *C. tenue* habitat were slightly warmer 22.1 ± 0.1 °C with a range of 25.0 °C to 18.0 °C. Winter temperatures within *C. tenue* habitat were similar to that recorded in Reno mattress habitat with a slightly warmer mean (± se) of 15.0 ± 0.1 °C. Turbidity (ntu) within *C. tenue* habitat ranged from 8.9 to 0.4 ntu in the summer to 11.2 to 0.7 in the winter with a mean (± se) of 2.7 ± 0.2 ntu for both summer and winter. Surface water temperatures were significantly warmer in *C. tenue* habitat (F = 29.2, df = 1, p < 0.01), but no significant difference was found among marina zones within a habitat type (Fig.3.7). Turbidity (ntu) did not vary significantly between habitat types, but significantly higher turbidity (ntu) was found in Zone 3 (F = 132.1, df = 2, p < 0.01) compared to Zones 1 and 2, in both habitat types (Fig. 3.8).

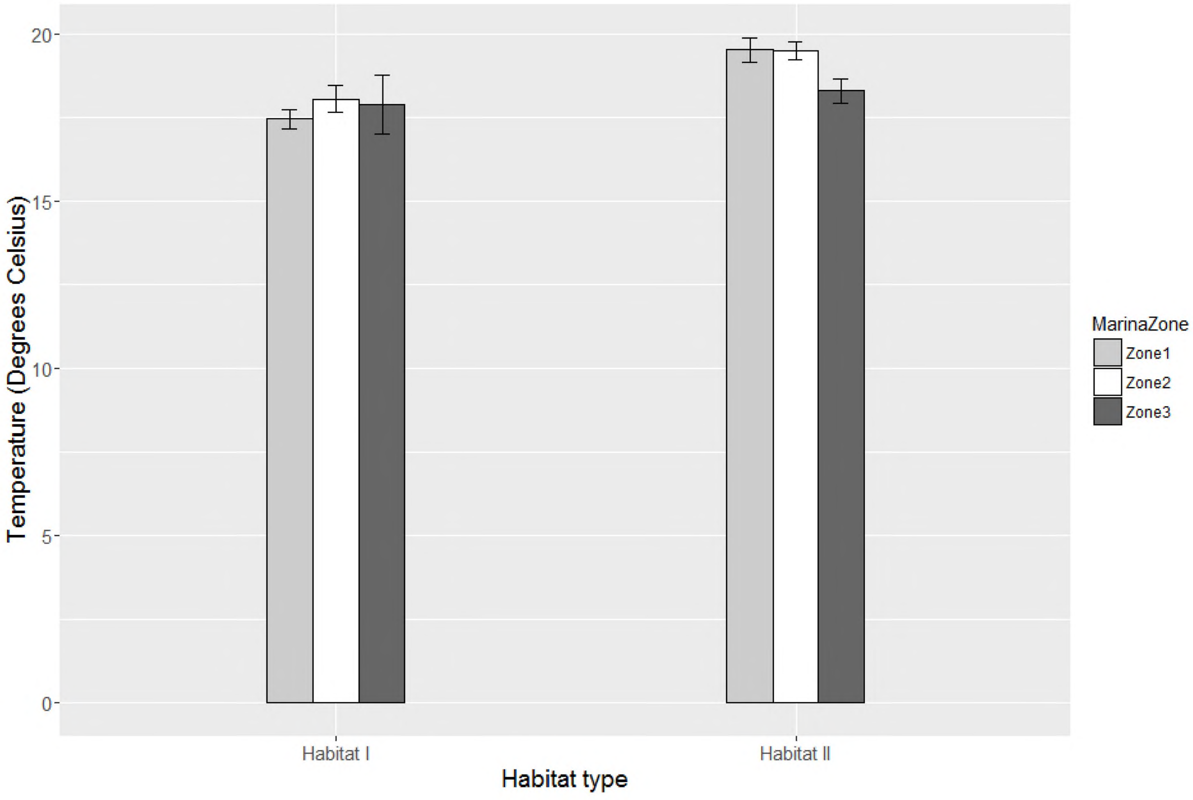


Figure 3.7. Pooled mean (± se) surface water temperatures in Reno mattress and *Codium tenue* habitats within Thesen Islands Marina, across the three marina zones recorded during seasonal seahorse surveys in 2015 and 2016.

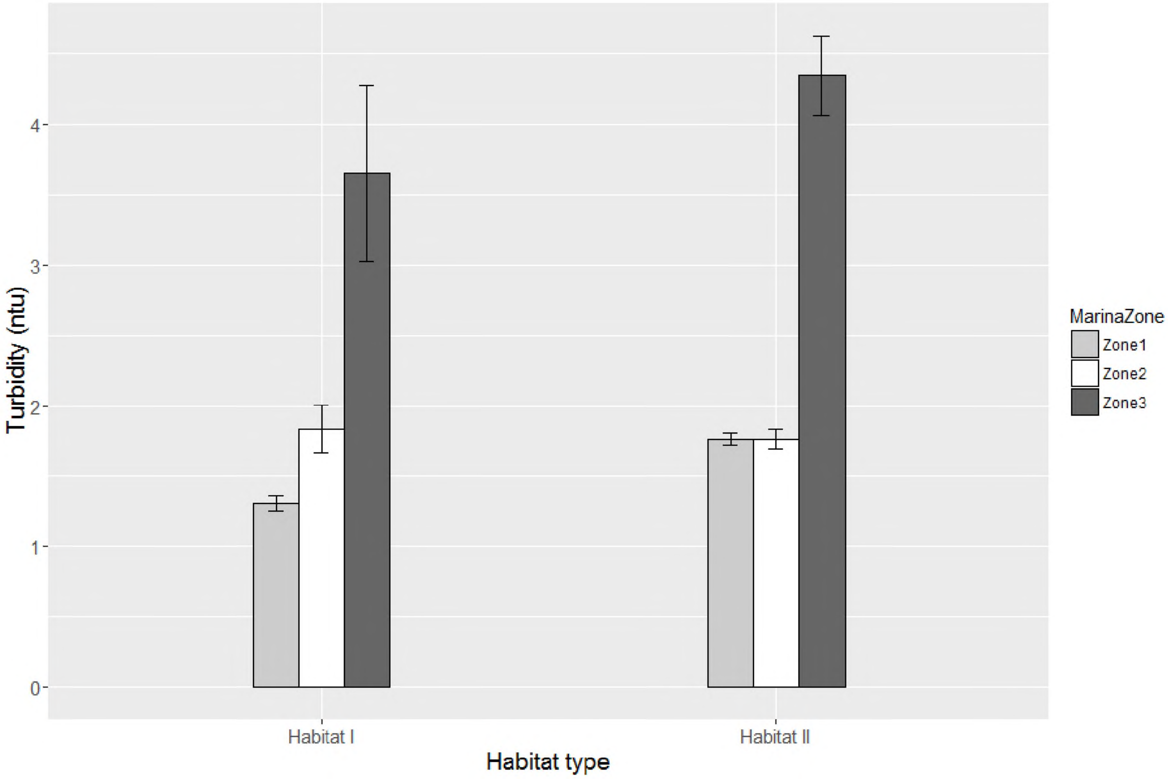


Figure 3.8. Pooled mean (± se) surface water turbidity (ntu) for Reno mattress and *Codium tenue* habitats across three marina zones recorded during seasonal seahorse surveys in 2015 and 2016.

Seahorse densities were significantly higher ($F = 11.5$, $df = 1$, $p = < 0.01$) within Reno mattress habitat in the winter of 2015 and 2016 (Table 3.2). Seahorse densities varied significantly across sample sites within this habitat ($F = 6.9$, $df = 11$, $p < 0.01$) with the highest densities found at Sites 2 and 5 near the western entrance (Zone 1) of the marina (Fig. 3.9) in the summer of 2015 and winter of 2015 and 2016.

Table 3.2. Mean (± se) seasonal *H. capensis* density for both Reno mattress and *Codium tenue* habitats for 2015 and 2016. The density unit used in Reno mattress habitat is individuals per m^2 and in *C. tenue* habitat individuals per kg of *Codium tenue* was used. Effort refers to the area surveyed within the Reno mattress habitat and dive time to the time spent diving during each survey.

Habitat type	Year	Season	Mean ± se	Max*	Effort (m^2)	Dive time
Habitat type I	2015	Summer	0.12 ± 0.02	1.4	820	14.5 h
		Winter	0.17 ± 0.03	1.9	1060	17.4 h
	2016	Summer	0.06 ± 0.01	0.9	990	14.0 h
		Winter	0.13 ± 0.02	1.2	1030	13.8 h
Habitat type II	2015	Summer	0.18 ± 0.03	1.9		
		Winter	0.20 ± 0.03	1.9		
	2016	Summer	0.45 ± 0.06	4.4		
		Winter	0.30 ± 0.04	1.7		

* Max refers to the maximum density of seahorses recorded in Reno mattress habitat as individuals per m^2 and in C. tenue habitat as individuals per kg of *C. tenue* respectively.

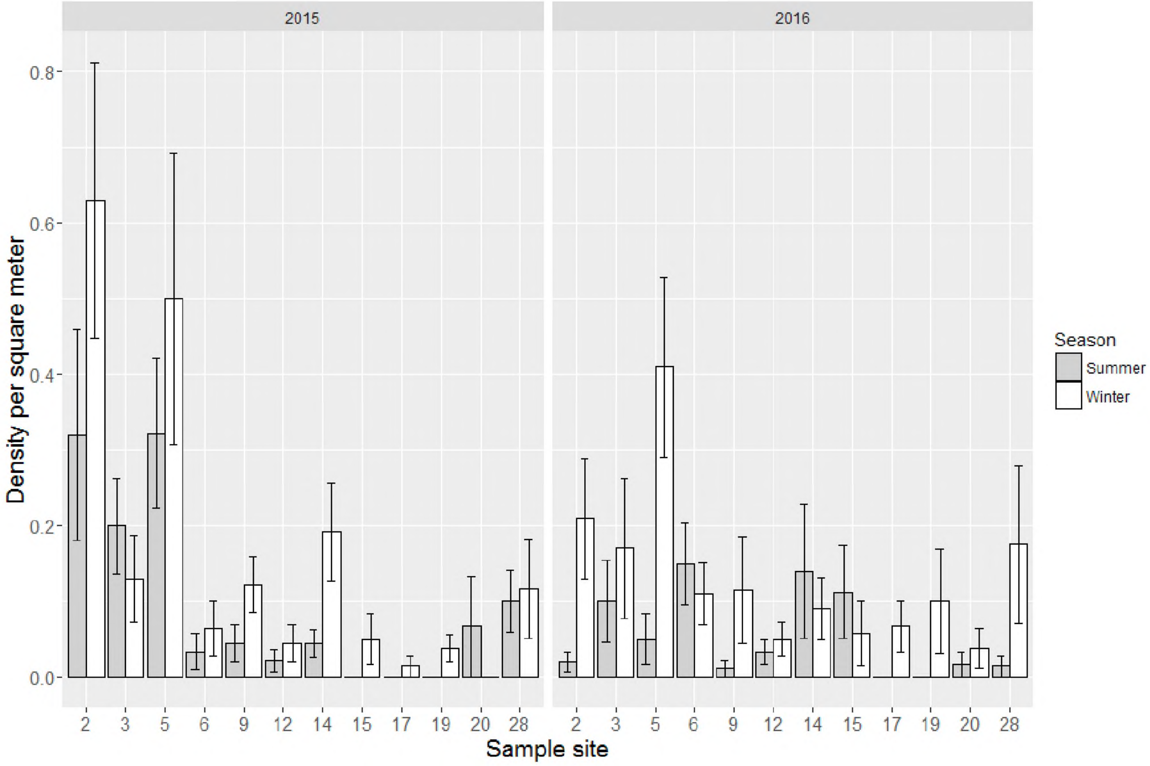


Figure 3.9. Seasonal mean (± se) *H. capensis* densities in Reno mattress habitat within Thesen Islands Marina across all sample sites for 2015 and 2016.

Seahorse densities within *C. tenue* habitat increased significantly (F = 23.0, df = 1, $p < 0.01$) from 2015 to 2016 (Table 3.2) with limited variation between summer and winter. Seahorse densities varied significantly (F = 2.9, df = 11, p = 0.04) across sample sites within the marina and the highest density (mean ± se) of 1.1 ± 0.4 seahorses per kg of *C. tenue* was recorded within sample site 32 in summer 2016 (Fig. 3.10).

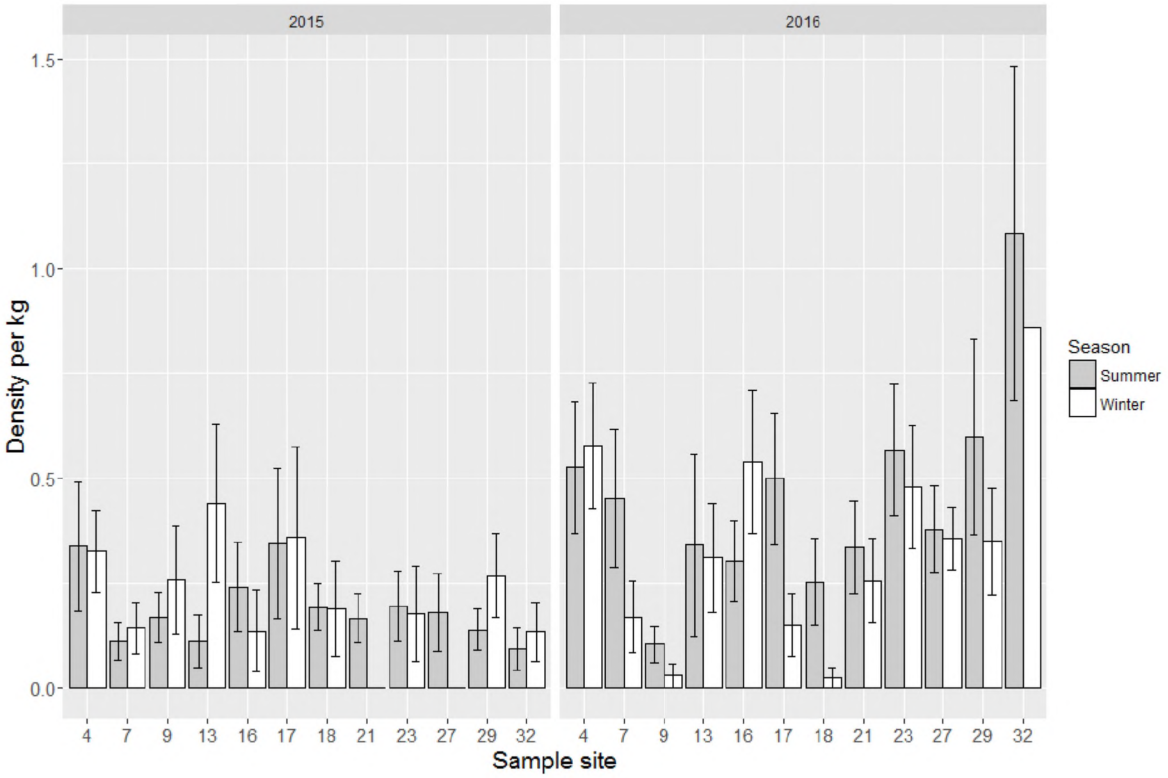


Figure 3.10. Seasonal *H. capensis* densities (mean ± se), across all sample sites in *Codium tenue* habitat within Thesen Islands Marina, for 2015 and 2016.

Seahorse sex ratios within Reno mattress habitat changed from female biased in summer to equal in winter across both years. A female biased sex ratio was found in *C. tenue* habitat during the 2016 winter survey only (Table 3.3). Pooled data showed an overall female biased sex ratio for Reno mattress habitat and an equal sex ratio for *C. tenue* habitat.

Table 3.3. Sex ratio information for *H. capensis* within Reno mattress and *Codium tenue* habitats across seasons for 2015 and 2016.

Habitat type	Season	Male/Female	Chi square	Degrees of freedom	P value	Sex ratio
Habitat type I	Summer 2015	34/62	8.2	1	< 0.01	Female
	Winter 2015	88/77	0.7	1	0.4	Equal
	Summer 2016	16/36	7.7	1	< 0.01	Female
	Winter 2016	59/77	2.4	1	0.1	Equal
Pooled data Habitat type I	2015 and 2016 (all seasons)	197/252	6.7	1	< 0.01	Female
Habitat type II	Summer 2015	44/38	0.4	1	0.5	Equal
	Winter 2015	36/33	0.1	1	0.7	Equal
	Summer 2016	59/49	0.9	1	0.3	Equal
	Winter 2016	39/74	10.8	1	< 0.01	Female
Pooled data Habitat type II	2015 and 2016 (all seasons)	178/194	0.7	1	0.4	Equal

Inflated brood pouches (indicative of breeding) were only noted during the summer surveys. The percentage of breeding males in the Reno mattress habitat decreased from 70 % in the summer of 2015 to 50 % in 2016. The percentage of breeding males in *C. tenue* habitat remained relatively constant at 71 % and 72 % for 2015 and 2016 respectively.

Codium tenue was exclusively used as a holdfast by seahorses in *C. tenue* habitat. A number of holdfasts (different types of vegetation, tunicates, twigs, rocks, wire) was used as holdfasts by seahorses within the Reno mattress habitat and the percentage of use varied across seasons and years. The dominant holdfasts used by *H. capensis* are summarized in Table 3.4.

Table 3.4. Seasonal variation in holdfasts used by *H. capensis* in Reno mattress habitat within Thesen Islands Marina for 2015 and 2016, recorded as percentage of seahorses using a specific holdfast.

Season	Wire	*Zostera*	*Halophila*	*Asparagopsis*	Other
Summer 2015	19	2	0	70	9
Winter 2015	70	2	0	9	19
Summer 2016	48	18	13	0	21
Winter 2016	61	1	0	17	21

Seahorse height ranged from 1.0 to 11.0 cm with a pooled mean (± se) height of 6.8 ± 0.1 and 6.9 ± 0.1 cm in Reno mattress and *C. tenue* habitats respectively. Seahorses were significantly (F = 26.2, df = 1, $p < 0.01$) smaller in the winter of both years compared to the summer, and males were significantly (F = 66.3, df = 1, $p < 0.01$) larger than females in both habitats (Fig. 3.11). Pooled data for both years and seasons showed no significant difference in seahorse height between Reno mattress and *C. tenue* habitats.

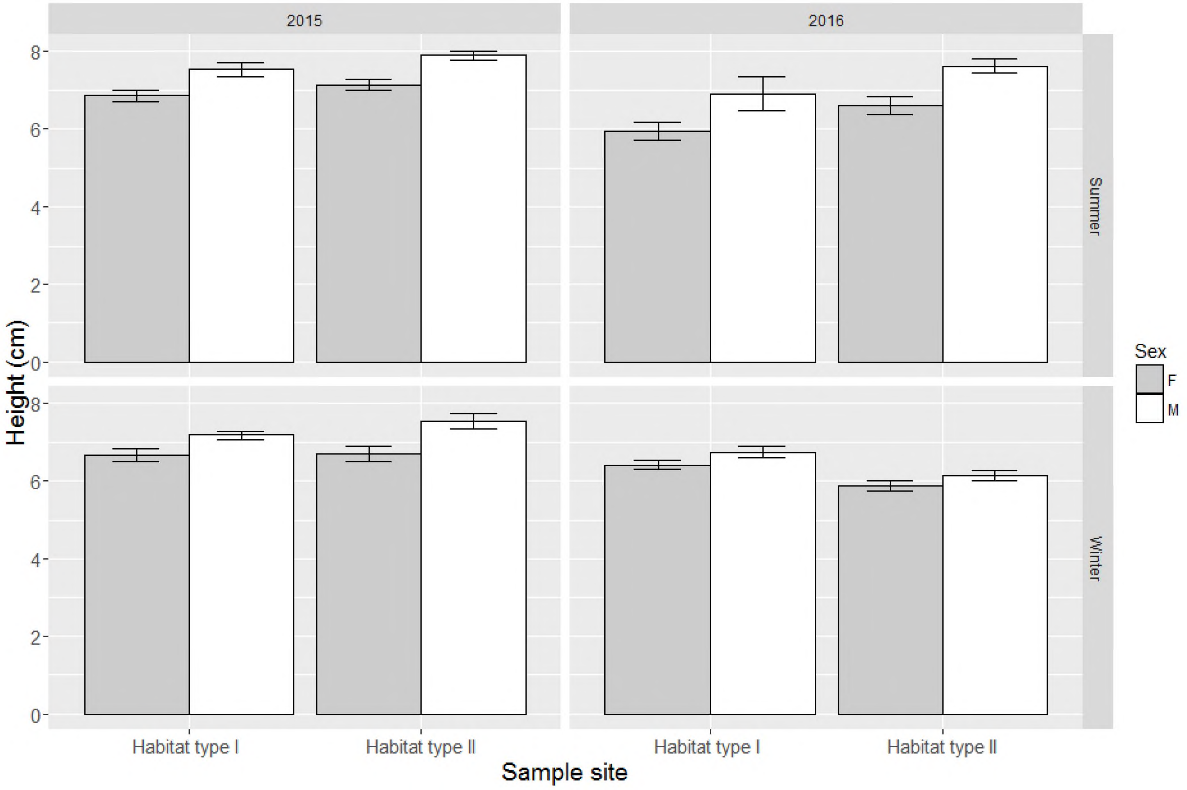


Figure 3.11. Seasonal height differences (mean ± se) between male (M) and female (F) *H. capensis* within Reno mattress and *Codium tenue* habitats in 2015 and 2016.

3.3.3. Casting the net wider: Monthly seahorse surveys within the Knysna estuary and Thesen Islands Marina

Codium tenue habitat within Thesen Islands Marina had significantly higher surface water temperatures whereas the Inter Island site was significantly colder (F = 9.4, df = 3, $p < 0.01$). Reno mattress habitat within the marina and Laguna Grove were not different in terms of surface water temperatures (Table 3.5). Turbidity (ntu) was significantly different across sites (F = 21.8, df = 3, $p < 0.01$). The highest turbidity was found in *C. tenue* habitat and at Laguna Grove and the lowest turbidity at the Inter Island site (Table 3.5).

Table 3.5. Surface water temperatures and turbidity (ntu) (mean ± sd) for all sites sampled from October 2015 to August 2016.

	Site	Mean ± sd	Min	Max
Temperature (°C)	Marina (Habitat type I)	18.0 ± 3.5	13.0	26.0
	Marina (Habitat type II)	19.0 ± 3.5	12.5	23.0
	Laguna Grove	17.9 ± 2.6	13.0	21.0
	Inter Island	16.7 ± 2.4	14.0	20.5
Turbidity (ntu)	Marina (Habitat type I)	1.4 ± 0.8	0.0	3.7
	Marina (Habitat type II)	1.9 ± 1.3	0.9	6.9
	Laguna Grove	1.6 ± 0.6	0.7	2.7
	Inter Island	0.9 ± 0.5	0.3	2.0

Seahorse densities differed significantly (F = 25.1, df = 2, $p < 0.01$) (Fig. 3.12) across all three non-*Codium* sites (Reno mattress habitat, Laguna Grove and the Inter Island site) and the highest densities were consistently found in Reno mattress habitat within the marina. Seahorse densities were lowest at the Inter Island site where no seahorses were found in 87 % of transects surveyed. Seahorse densities varied significantly (F = 3.5, df = 10, $p < 0.01$) across months (Fig. 3.12). The highest seahorse density (mean ± se) in Reno mattress habitat in the marina was recorded in October 2015 (1.80 ± 0.48 individuals per search effort) with a marked decrease in density during January and February. Seahorse density (mean ± se) at Laguna Grove was highest in November 2015 (1.21 ± 0.33 individuals per search effort). Densities at this site showed a continuous, gradual decrease from April 2016 onwards. Monthly seahorse densities remained low at the Inter Island site across the entire sample period (Fig. 3.12). Monthly seahorse densities within *C. tenue* habitat varied significantly (F = 2.1, df = 10, p = 0.02), and the highest mean (± se) density of 0.51 ± 0.11 individuals per kg of *C. tenue* was recorded in January 2016 (Fig. 3.13).

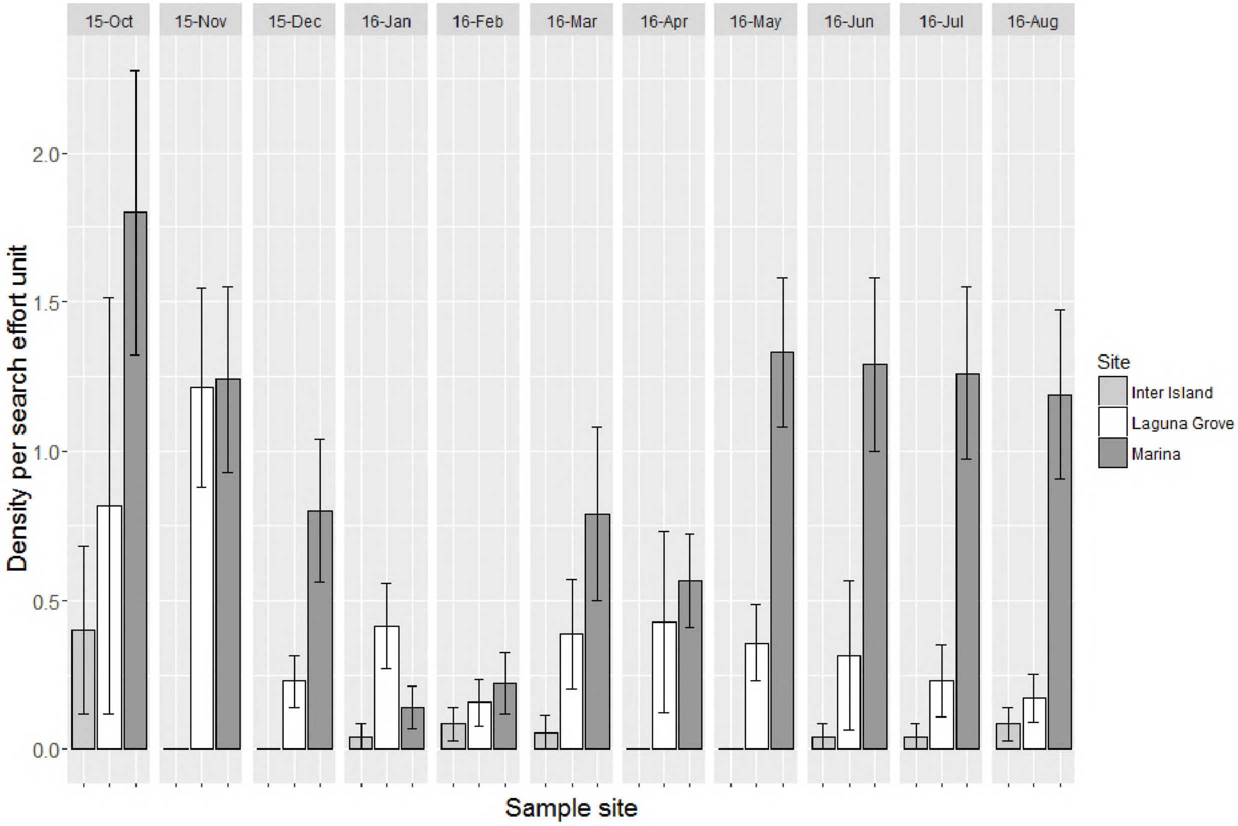


Figure 3.12. Monthly (mean ± se) *H. capensis* densities per search effort at three non-*Codium* sites (Reno mattress habitat within Thesen Islands Marina, Laguna Grove and the Inter Island site within the Knysna estuary) recorded from October 2015 to August 2016.

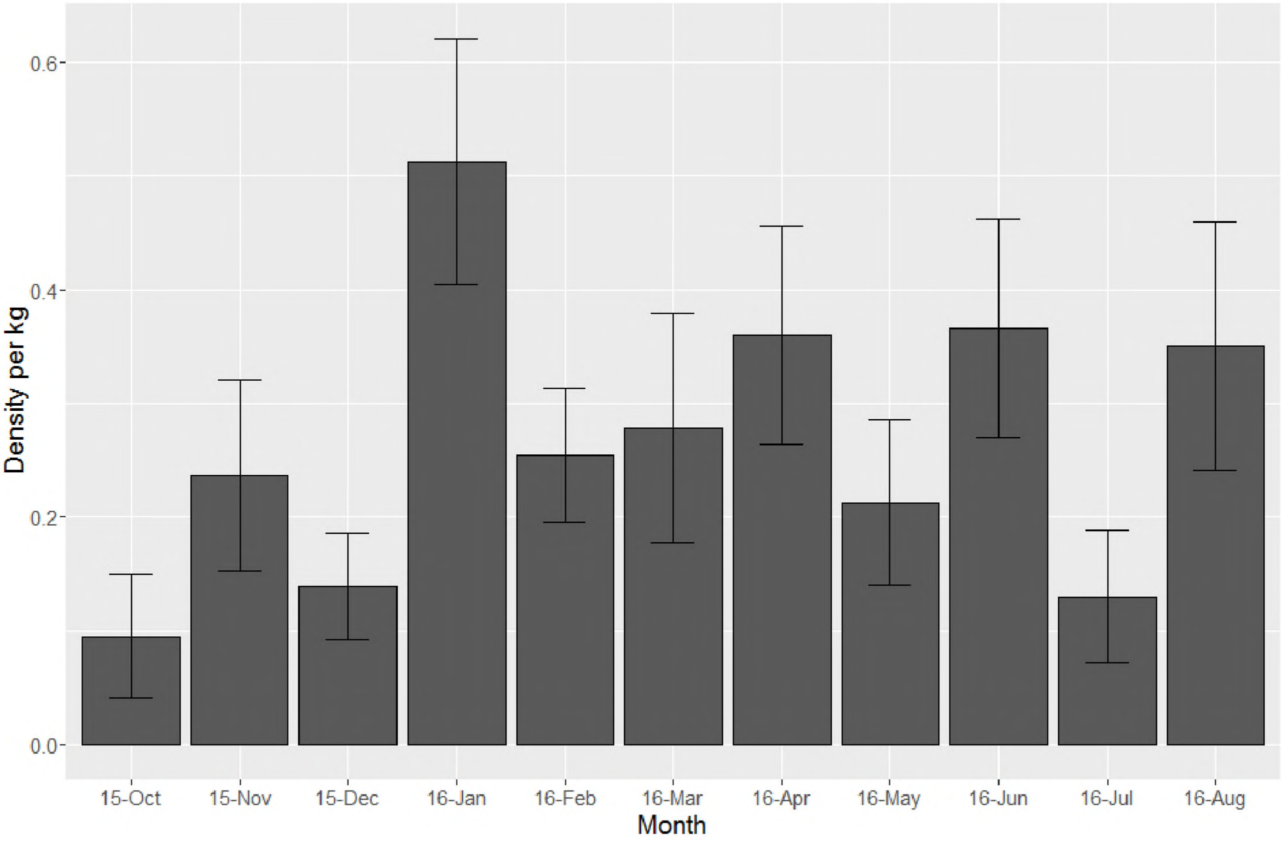


Figure 3.13. Monthly *H. capensis* densities (mean ± se) per kg of *Codium tenue* in *C. tenue* habitat within Thesen Islands Marina recorded from October 2015 to August 2016.

To determine whether monthly variation in seahorse densities persisted on a longer-term basis, data from months were pooled and assessed seasonally (Table 3.6). Seasonal seahorse densities at non-*Codium* sites varied significantly ($F = 9.3$, df = 3, $p < 0.01$) (Table 3.6). Seahorse densities were significantly higher ($F = 25.1$, df = 2, $p < 0.01$) in Reno mattress habitat within the marina across all seasons and highest seahorse densities were recorded in spring for all sites.

Seasonal seahorse densities did not vary significantly in *C. tenue* habitat, although, markedly lower densities were found during spring (Table 3.7).

Table 3.6. Summary of seasonal *H. capensis* densities (mean ± se) for Reno mattress habitat, Laguna Grove and Inter Island (individuals per search effort) recorded from October 2015 to August 2016. Effort refers to the area surveyed and dive time to the time spent diving during each survey.

Season	Site	Mean ± se	Max*	Effort (m^2)	Dive time
Spring Oct + Nov	Marina (Habitat type I)	1.52 ± 0.28	7.6	400	6.4h
	Laguna Grove	1.03 ± 0.36	4.3	420	3.9h
	Inter Island	0.18 ± 0.14	1.7	420	3.5h
Summer Dec + Jan + Feb	Marina (Habitat type I)	0.39 ± 0.10	3.6	600	8.5h
	Laguna Grove	0.27 ± 0.06	1.0	630	4.8h
	Inter Island	0.04 ± 0.02	0.3	630	4h
Autumn Mar + Apr + May	Marina (Habitat type I)	0.90 ± 0.14	5.2	600	9.8h
	Laguna Grove	0.29 ± 0.12	2.2	630	4.8h
	Inter Island	0.02 ± 0.02	0.4	630	4.4h
Winter Jun + Jul + Aug	Marina (Habitat type I)	1.24 ± 0.16	4.8	600	9.9h
	Laguna Grove	0.24 ± 0.09	1.8	630	4.4h
	Inter Island	0.05 ± 0.03	0.3	630	4.1h

* Max refers to the maximum density of seahorses recorded for Reno mattress habitat, Laguna Grove and Inter Island as individuals per search effort.

Table 3.7. Summary of seasonal *H. capensis* densities (mean ± se) for *Codium tenue* habitat (individuals per kg of *C. tenue*) recorded from October 2015 to August 2016.

Season	Mean ± se	Max*
Spring Oct + Nov	0.17 ± 0.05	1.3
Summer Dec + Jan + Feb	0.30 ± 0.05	1.3
Autumn Mar + Apr + May	0.28 ± 0.05	1.5
Winter Jun + Jul + Aug	0.28 ± 0.05	1.8

* Max refers to the maximum density of seahorses recorded for *C. tenue* habitat as individuals per kg of *C. tenue*.

Seahorses were significantly larger (F = 15.7, df = 3, $p < 0.01$) within Thesen Islands Marina in both Reno mattress and *C. tenue* habitats (Fig. 3.14) and seahorse height ranged from 2.0 to 10.0 cm across all sites. Pooled data across all sites showed that male seahorses were significantly larger than females (χ^2 =16.5, df = 1, $p < 0.01$). Height differences between males and females were significant for seahorses found in Reno mattress habitat in Thesen Islands Marina (Fig. 3.15).

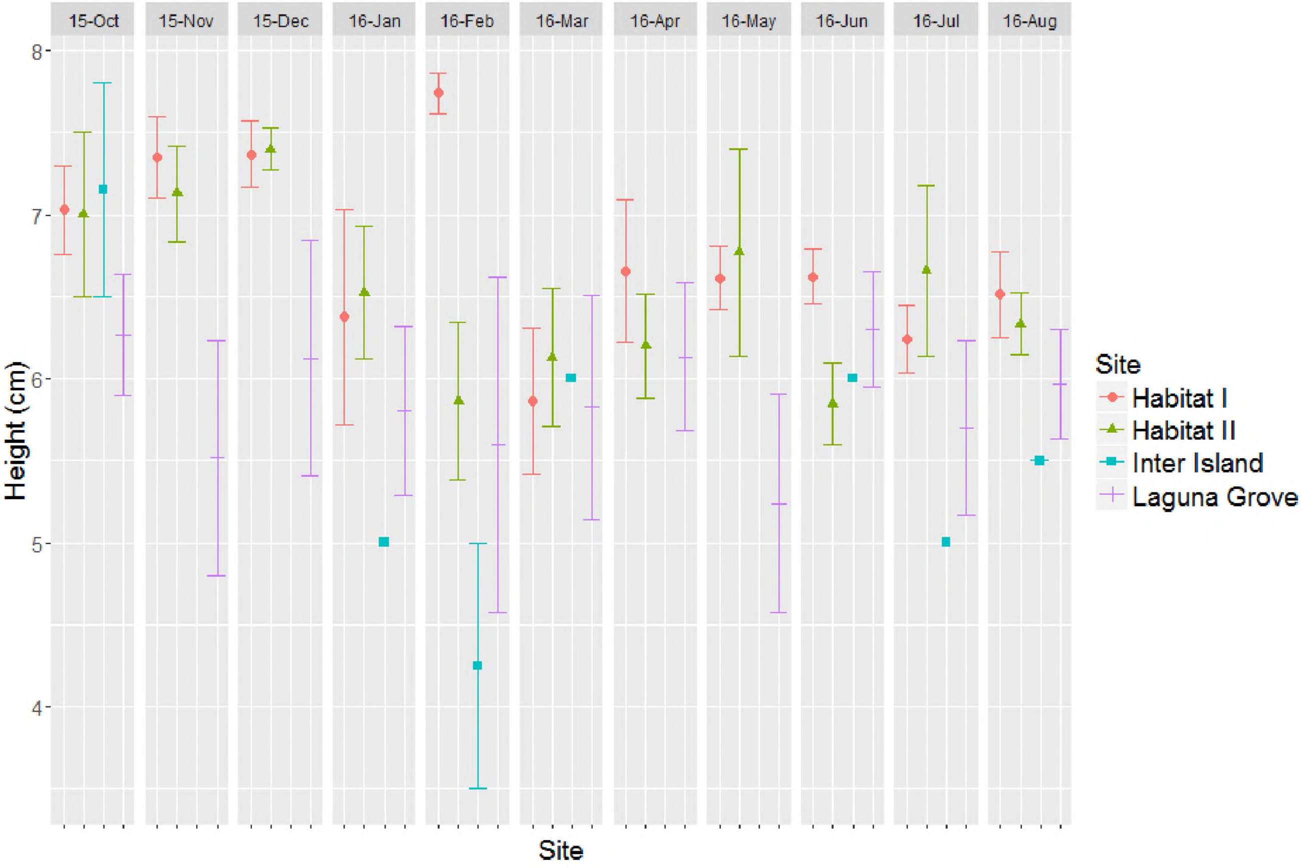


Figure 3.14. Monthly *H. capensis* heights in cm (mean ± se) for all sites recorded from October 2015 to August 2016.

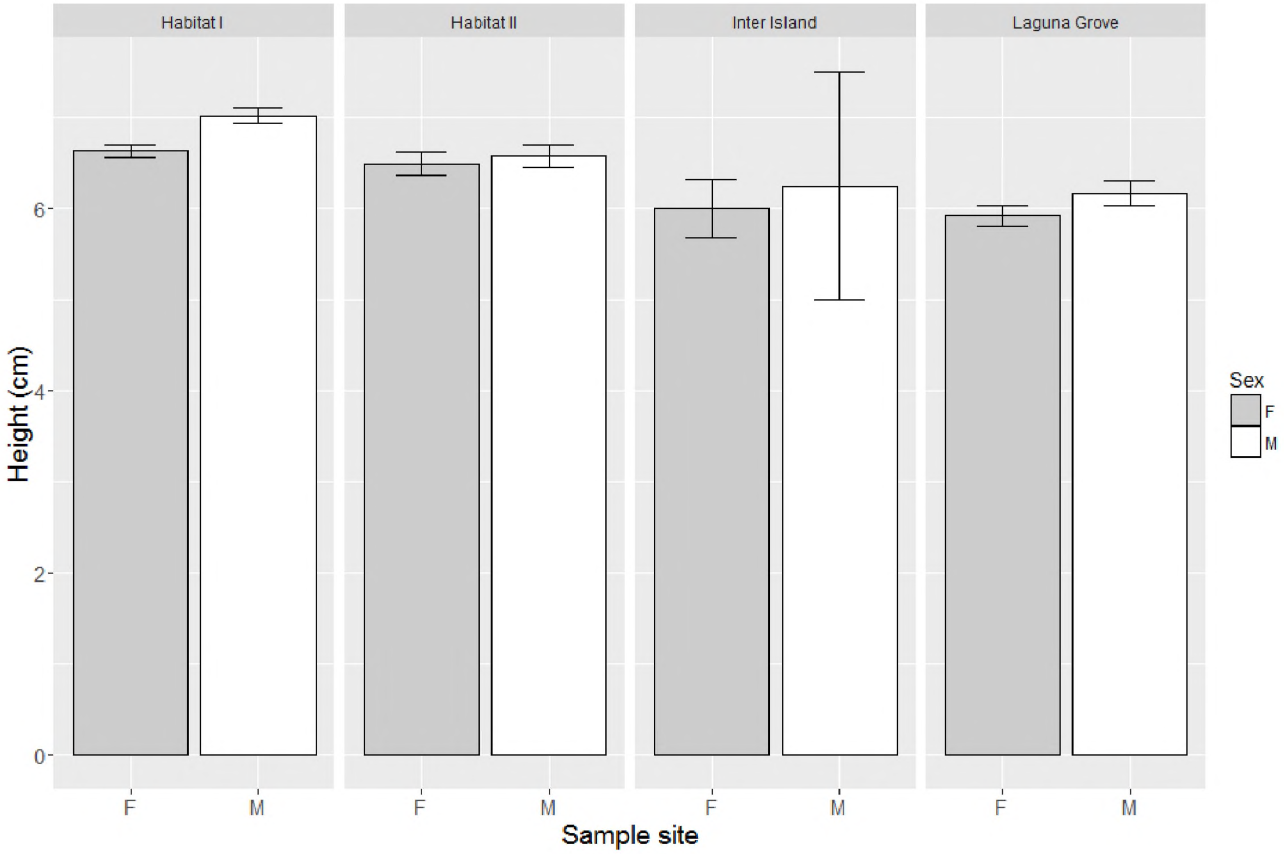


Figure 3.15. Height difference (mean ± se) between male (M) and female (F) *H. capensis* across all sites sampled from October 2015 to August 2016.

Hippocampus capensis in *C. tenue* habitat within Thesen Islands Marina had both an overall and seasonal equal sex ratio. All other sites had a female biased sex ratio (Table 3.8). Pooled data for the entire population (all sites) showed an overall female biased sex ratio. A seasonal change in sex ratio within Reno mattress habitat in Thesen Islands Marina and at Laguna Grove were noted. Because of the limited number of seahorses found at the Inter Island site, seasonal sex ratios for this site could not be established (Table 3.9).

Table 3.8. *Hippocampus capensis* sex ratios for all sites surveyed from October 2015 to August 2016.

Site	Male/Female	Chi square	Degrees of freedom	P value	Sex ratio
Marina (Habitat type I)	224/309	13.6	1	< 0.01	Female
Marina (Habitat type II)	83/90	0.3	1	0.60	Equal
Laguna Grove	40/84	15.6	1	< 0.01	Female
Inter Island	2/10	5.3	1	0.02	Female
Entire population	349/493	24.6	1	< 0.01	Female

Table 3.9. Seasonal changes in the sex ratio of *H. capensis* within Thesen Islands Marina (Reno mattress) and Laguna Grove.

Site	Season	Male/Female	Chi square	Degrees of freedom	P value	Sex ratio
Marina Habitat type I	Spring	60/83	3.7	1	0.05	Female
	Summer	24/30	0.7	1	0.41	Equal
	Autumn	54/74	3.1	1	0.07	Equal
	Winter	86/122	86/122	1	0.01	Female
Laguna Grove	Spring	21/29	1.3	1	0.26	Equal
	Summer	6/11	1.5	1	0.23	Equal
	Autumn	8/25	8.8	1	< 0.01	Female
	Winter	5/19	8.2	1	< 0.01	Female

Holdfast cover, estimated as percentage cover per 10 m^2 transect, within Reno mattress habitat, Laguna Grove, and the Inter Island site 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. filliformis* respectively. 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 3.10). The only available holdfast within *C. tenue* habitat was *C. tenue* and therefore was exclusively used by *H. capensis*.

Table 3.10. Holdfasts used by *H. capensis* within Reno mattress habitat, Laguna Grove and the Inter Island site from October 2015 to August 2016, recorded as percentage of seahorses using a specific holdfast.

Site	Wire	Crevice	*Zostera*	*Asparagopsis*	Other
Marina (Habitat type I)	73%	5%	2%	13%	7%
Laguna Grove	0%	0%	84%	4%	16%
Inter Island	0%	0%	62%	0%	38%

Inflated brood pouches (indicative of breeding) were noted from October 2015 to March 2016 at most sites, although no brooding males were observed at the Inter Island site. Breeding activity was highest during October, November, December and January when > 80 % of males were observed with an inflated brood pouch.

3.4. Discussion

Hippocampus capensis was found to readily inhabit artificial Reno mattress and *C. tenue* habitats in Thesen Islands Marina. The densities of *H. capensis* within Reno mattress habitat, with an overall mean (± se) density of 0.13 ± 0.01 and 0.26 ± 0.02 individuals/m^2 during the seasonal and monthly surveys respectively, exceeded those recorded by Bell *et al.* (2003) (0.0089 individuals/m^2) and Lockyear *et al.* (2006) (0.0114 individuals/m^2) as well as most recorded seahorse densities (Table 3.11). This result is perhaps not surprising as artificial habitats and structures are known to increase seahorse densities (Harasti *et al.*, 2012; Correia *et al.*, 2015b), particularly in areas devoid of natural habitats (Correia *et al.*, 2015b).

Table 3.11. Summary of international seahorse population assessments.

Species	Year	Location	Timeframe	Frequency	Survey method	Area surveyed (m²)	Mean density (SH/m²)	Max density (SH/m²)	Reference
H. capensis	2000	Knysna Estuary (South Africa)	1 Month	Once off	Transects	4920	0.0089	0.25	Bell *et al.* (2003)
H. capensis	2000	Knysna Estuary (South Africa)	1 Month	Once off	Grid	100	0.22	0.46	Bell *et al.* (2003)
H. capensis	2001	Knysna Estuary (South Africa)	12 Months	Once off	Transects	34980	0.0114	0.33	Lockyear *et al.* (2006)
H.guttulatus	2001 and 2002	Ria Formosa (Portugal)	3 Month	Once off	Transects	5760	0.073	0.51	Curtis and Vincent (2005)
H. hippocampus	2001 and 2002	Ria Formosa (Portugal)	3 Month	Once off	Transects	5760	0.007	0.072	Curtis and Vincent (2005)
H. guttulatus	2012	Mar Piccolo (Italy)	4 Months	Once off	Transects	26400	0.018	0.033	Gristina *et al.* (2015)
H. guttulatus	2012	Mar Piccolo (Italy)	4 Months	Once off	Transects	26400	0.0005	0.003	Gristina *et al.* (2015)
H. reidi	2002 - 2004	Ilha Grande (Brazil)	25 Months	Every second month	Grid	175	0.18	0.4	Freret-Meurer and Andreata (2008)
H. whitei	2005 - 2010	Port Stephens (Australia	60 Months		Tagging		0.035 - 0.110		Harasti *et al.* (2012)
H. whitei	2005 - 2010	Sydney harbour (Australia) SWIMMING Nets	60 Months		Tagging		1.05		Harasti *et al.* (2012)
H. guttulatus	2008-2009	Ria Formosa (Portugal)	24 Months	Once off	Transects	12240	0.004	0.035	Caldwell and Vincent (2012)
H. hippocampus	2008-2009	Ria Formosa (Portugal)	24 Months	Once off	Transects	12240	0.001	0.019	Caldwell and Vincent (2012)
H. guttulatus	2010, 2012, 2013	Ria Formosa (Portugal)			Transects		0.053	0.183	Correia *et al.* (2015b)
H. hippocampus	2010, 2012, 2013	Ria Formosa (Portugal)			Transects		0.008	0.024	Correia *et al.* (2015b)
H. comes	2000 - 2002	Bohol Island (Philippines)	24 Months	Rainy/dry seasons	Transects		0.0005		Marcus *et al.* (2007)
H. Zostera	2005 - 2007	Tampa Bay (USA)			Tagging		0.08	0.18	Mason-Jones *et al.* (2010)
H. comes	1996 - 1997	Bohol Island (Philippines)	16 Months	Monthly	Grid	1650	0.019		Perante *et al.* (2002)

The higher densities of seahorses found within the Reno mattress suggests that this habitat is the most important type within the marina development in terms of seahorse use, although the importance of *C. tenue*, confirmed by the sustained presence of seahorses (particularly of juveniles during the 2016 summer survey) within this habitat type, cannot be denied. *Codium* spp. was also identified as an important habitat for *H. guttulatus* in the Ria Formosa (Correia *et al.*, 2013).

The suitability of the marina for *H. capensis* is also confirmed by the higher seahorse densities found in the marina canals throughout the monthly seahorse surveys from October 2015 to August 2016 compared to the estuary sites. Seahorse species found in shallow waters probably live in habitats that are protected against adverse environmental impacts such as storms and which provide suitable habitat in terms of holdfasts (Choo and Liew, 2003). Because *H. capensis* is found in very shallow waters (< 30 cm) and 67 % of the marina canals are covered by some type of holdfast, the marina provides suitable habitat for this species.

The use of artificial holdfasts and habitat (within a highly variable environment) have been recorded for a number of seahorse species (*H. hippocampus* – Otero-Ferrer *et al.* (2015); *H. whitei* – Clynick (2008b), Harasti *et al.* (2012); *H. hippocampus* and *H. guttulatus* – Curtis and Vincent (2005), Gristina *et al.* (2015)). It is, however, the first time the use of an artificial habitat is recorded for *H. capensis*. Not only were significantly higher seahorse densities found within Thesen Islands Marina, an artificial water body, compared to the two estuary sites, but the seahorses were also significantly larger.

The particular distribution pattern of *H. capensis* within Thesen Islands Marina highlights the importance of habitat as well as physico-chemical features in seahorse distributions. Seahorses were found throughout the marina, but densities were linked to the availability of specific habitat types. Two sympatric seahorse 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 and 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.* (2007b) that *H. capensis* will occur wherever suitable vegetation (or holdfasts) are available. The different habitat types utilized by *H. capensis* varied significantly in terms of temperature and turbidity and these physico-chemical features do not seem to control the distribution of *H. capensis*, although optimal physico-chemical conditions for the occurrence of *H. capensis* was not assessed.

A marked decrease in seahorse 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 recorded on a swim net during October and November 2007 increased. This pattern was attributed to the breeding season of this species with movement of seahorses from adjacent sea grass 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 seahorses (see Kleiber *et al.*, 2011). One would thus expect that surveys during a breeding season will result in higher seahorse observations and thus densities. The decrease in density of *H. capensis* during the summer surveys of 2015 and 2016 and the monthly surveys suggest that some external factor or factors might be at play. For example, the occupancy of houses at Thesen Islands Marina increases from 30 % to 100 % during the December and January holiday season (pers. obs.) and this results in a marked increase in boat use within the canals. When exposed to loud noise, *H. erectus* demonstrated physiological, chronic stress responses with reduced mass and body condition (Anderson *et al.*, 2011). Further research on the potential effect of increased noise on seahorses caused by boat activity is required (Vincent *et al.*, 2011).

Some syngnathid species have been noted to undergo seasonal migration to deeper water (e.g. *H. zosterae* and *S. scovelli* populations in Tampa Bay, Florida, 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, was found to migrate offshore after the breeding season (Vincent *et al.*, 2005)), and this might be another explanation for the variability in seahorse densities across months.

There was noticeable variability in juvenile abundance between years. The reason for the low counts of juvenile *H. capensis* during the 2015 summer assessment is not known. This result, however, is similar to that of Bell *et al.* (2003) who only recorded three juveniles during their 2000 transect survey (February to April). Push net sampling used by Lockyear *et al.* (2006) in 2001 during their entire survey period resulted in higher juvenile observations (31 juveniles). No juveniles were however found in 2015 using the method of sampling in dense *C. tenue* (a method similar to that of Lockyear *et al.* (2006) in that all seahorses in a sample are counted). In 2016, 44 juveniles were recorded within *C. tenue*, using the same sample method. This variability between assessments emphasises the need for longer term assessments, as opposed to snapshot investigations. Juvenile sightings in seahorse population surveys are known to vary and range from 21 % to 80 % for *H. guttulatus* in the Mar Piccolo of Taranto, Italy (Gristina *et al.*, 2015); 34 % for *H. breviceps* in Australia (Moreau and Vincent, 2004); 12 % for *H. denise* and *H. bargibanti* (Smith *et al.*, 2012) to 8 % for *H. comes* in the central Philippines (Marcus *et al.*, 2007).

Ontogenetic changes in habitat use might be a reason for the variable observations of juveniles both spatially and temporally. Ontogenetic differences in habitat use by seahorses have been found for *H. comes* in the Philippines (Morgan and Vincent, 2007), *H. whitei* in Australia (Harasti *et al.*, 2014b), and *H. guttulatus* in Portugal (Curtis and Vincent, 2005). According to Whitfield (1989, 1995) *H. capensis* juveniles are planktonic and dispersal via currents is likely. *Ex situ* studies on the reproduction of *H. capensis* (Grange and Cretchley, 1995; Lockyear *et al.*, 1997) lack any data on habitat use by juvenile *H. capensis*, although Teske *et al.* (2007b) found that juveniles preferred *Z. capensis* as a holdfast as opposed to adults who showed a preference for *C. filiformis* where a choice in vegetation types was available. Further research is required to ascertain the suitability of different habitat types within the marina to all life stages of *H. capensis.*

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). The changes in the sex ratio of *H. capensis* across seasons and sites are unusual as most other seahorses have an equal sex ratio (*H. breviceps* – Moreau and Vincent (2004); *H. comes* – Perante *et al.* (2002); *H. reidi* - Rosa *et al.* (2007); *H. bargibanti and H. denise* – Smith *et al.* (2012)). Female biased sex ratios have, however, been noted for *H. hippocampus* in the Macaronesian islands, specifically in artificial habitats (Otero-Ferrer *et al.*, 2015); in *H. erectus* in Chesapeake Bay (Teixeira and Musick, 2001); and Kvarnemo *et al.* (2007) found a wild population of *H. subelongatus* to be female biased with stronger sexual selection on females, a contradiction to the normal male sexual selection found in monogamous species (Vincent, 1994a; Vincent, 1994b; Naud *et al.*, 2009). Sexual selection in *H. capensis* is unknown, but Grange and 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 within the Reno mattress and mixed vegetation habitats compared to females, and that dense *C. tenue* beds ensure male protection against predators. Males are especially more visible during the breeding season owing to courting and competition behaviour (Rosenqvist and 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 these populations.

Males were found to be significantly larger than females across all seasons and sites, although body proportions were not assessed. 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 is 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 seahorses are, however, not commonly found (Vincent and Giles, 2003; Kvarnemo *et al.*, 2007; Otero-Ferrer *et al.*, 2015). Seahorses within the Reno Mattress habitat were significantly larger than seahorses in *Z. capensis*. Larger seahorses, both male and female, have been observed to produce more offspring (Vincent and Giles, 2003; Rosenqvist and Berglund, 2011), although reproductive success is not necessarily linked to size. So, even though seahorses within the Reno mattress habitat can theoretically produce more offspring, the reproductive success of this population is not known which warrants further research.

Conflicting conclusions have been reached with regards to habitat and holdfast use by *H. capensis* within the Knysna estuary. No association between seahorse densities and vegetation cover was found by Bell *et al.* (2003). In fact, higher densities of seahorses were found within low density (< 20 % cover) vegetation stands during this population survey (Bell *et al.*, 2003). Seahorses were also recorded on bare sediment. By contrast, Teske *et al.* (2007b) found a positive correlation between seahorse densities and dense vegetation cover (> 75 %) and suggested that *H. capensis* will only be found in areas with adequate vegetation cover. A recent threat to subtidal vegetation (in particular *Z. capensis*) within the bay regime of the Knysna estuary is an extensive and lengthy *U. 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). The additional habitat found in Thesen Islands Marina provides suitable habitat for *H. capensis* and related biota in a changeable estuarine environment.

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 and 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.

From the assessment, it is clear that the artificial Reno mattress habitat supports the highest densities of seahorses, much higher when compared to present and historical densities within the estuary. The question that now begs to be answered is if this observed distribution is in fact caused by preference for the artificial Reno mattress habitat. Or is it related to some other mechanism? Chapter 4 aims to answer this question.

Chapter 4

An endangered seahorse selectively chooses an artificial structure

4.1 Introduction

Environmental alteration in the form of habitat loss leaves many species vulnerable to extinction (Gibbons *et al.*, 2000; McKinney, 2002; Kerr and Deguise, 2004). The challenge of the conservationist today is to be able to conserve within a heavily modified environment. In particular, scenarios where altered and artificial habitats result in conservation opportunities should be realised (Clynick, 2008b; Garcia-Gomez *et al.*, 2011; Garcia-Gomez *et al.*, 2015).

Seahorses are threatened by habitat loss (Correia *et al.*, 2015b; Harasti, 2016), and many species have shown population declines owing to the destruction and/or alteration of their natural complex habitats (see Vincent *et al.* 2011 and Aylesworth *et al.*, 2015). The importance of finding new and alternative conservation solutions is imperative to ensure sustainable and successful conservation of seahorses and in particular, the usefulness of artificial habitats as a conservation tool should be considered.

Altered and artificial habitats are known to provide novel new habitat to aquatic species (Chapman and Clynick, 2006; Clynick, 2008a; Clynick, 2008b; Waltham and Connolly, 2013), but can this fact be used in conservation? Artificial structures have the potential to provide much needed refuge to some endangered species (Clynick, 2008b; Garcia-Gomez, 2011; Perkol-Finkel *et al.*, 2012) e.g. higher abundances of the endangered limpet, *Patella ferruginea,* were found on artificial harbour stones compared to natural rocky shores (Guerra-Garcia *et al.*, 2004). Use of artificial habitat as a conservation tool is particularly applicable in areas devoid of natural habitat (Clynick, 2008b; Correia *et al.*, 2013, Correia *et al.*, 2015b). Artificial Marine Micro-Reserves (AMMRs) is a novel concept proposed by Garcia-Gomez *et al.* (2011, 2015) where artificial habitats, utilized by threatened species, are used as a conservation tool, and protected, within heavily developed areas.

Animals might, however, only use artificial structures because it is the only available habitat e.g. seahorses in the Ria Formosa, Portugal, only used artificial holdfast units in areas devoid of natural habitat (Correia *et al.*, 2015b). Some marine and estuarine biota can adapt to and survive changes to their natural environment, particularly the addition of artificial structures. But is it possible that such structures can become the preferred option?

Preference requires a behavioural outcome by an animal, and non-random association descriptions (regularly dubbed ‘preference’) are insufficient in the description of underlying ecological patterns responsible for said associations (Crowe and Underwood, 1998; Underwood *et al.*, 2004). The ‘Woodhen study’ (see Underwood, 2009) is a perfect example where the limited distribution pattern of a bird species was wrongly assumed to be preference for a particular habitat. The particular distribution of the woodhens was rather owing to the occurrence of predators in habitats not used.

Well thought-out experimentation is required to understand the underlying reasons for resource use (Underwood *et al.*, 2004). Any experimental investigation of habitat choice is, however, fraught with potential pit-falls such as pseudoreplication (Hurlbert, 1984), confounding factors, non-independence, and a lack of appropriate controls (Underwood *et al.*, 2004). When compared to laboratory work, *in situ* experiments have been found to give more convincing results when looking at animal behaviour, as animals tend to behave differently under laboratory conditions (Crowe and Underwood, 1998).

The density of *Hippocampus capensis* was found to be significantly and consistently higher on artificial Reno mattresses (wire baskets filled with rocks) within Thesen Islands Marina (Chapter 3). Could this observed distribution pattern of *H. capensis* be explained by the location of the artificial structures in the marina canals (sides vs middle), some negative feature of other habitats in the marina, or rather by the particular positive features of the Reno mattress structure? To answer these questions, an *in situ* choice experiment was conducted between two microhabitats (definition by Morris, 1987): a Reno mattress structure and *Zostera capensis* (Eel grass). The aim of this experiment was to understand the underlying process responsible for the observed distribution of *H. capensis* (Chapter 3). Findings will help to assess the usefulness of Reno mattress structures in assisting conservation efforts for *H. capensis.*

4.2. Materials and Methods

4.2.1. Choice experiment

Choice experiments took place within the western section of Thesen Islands Marina (Fig. 4.1) during the austral summer from November 2015 to March 2016. Throughout the experimental period temperature, dissolved oxygen, pH and salinity measurements were recorded by a permanently fixed Hydrolab MS5 multiparameter mini sonde (OTT Hydromet, Colorado), as part of the Knysna Estuarine Monitoring Platform (KEMP) project, located about 200 m from the experimental site (Fig. 4.1). This real-time water quality monitoring undergoes calibration every six weeks.

Ten replica Reno mattresses (30 cm x 25 cm x 10 cm) were constructed from double twisted galvanised PVC coated wire mesh and filled with rocks (Fig. 4.2). These materials were similar to those used in the construction and lining of the marina canals. The replica Reno mattresses were placed within the marina on 17 August 2015 for maturation.

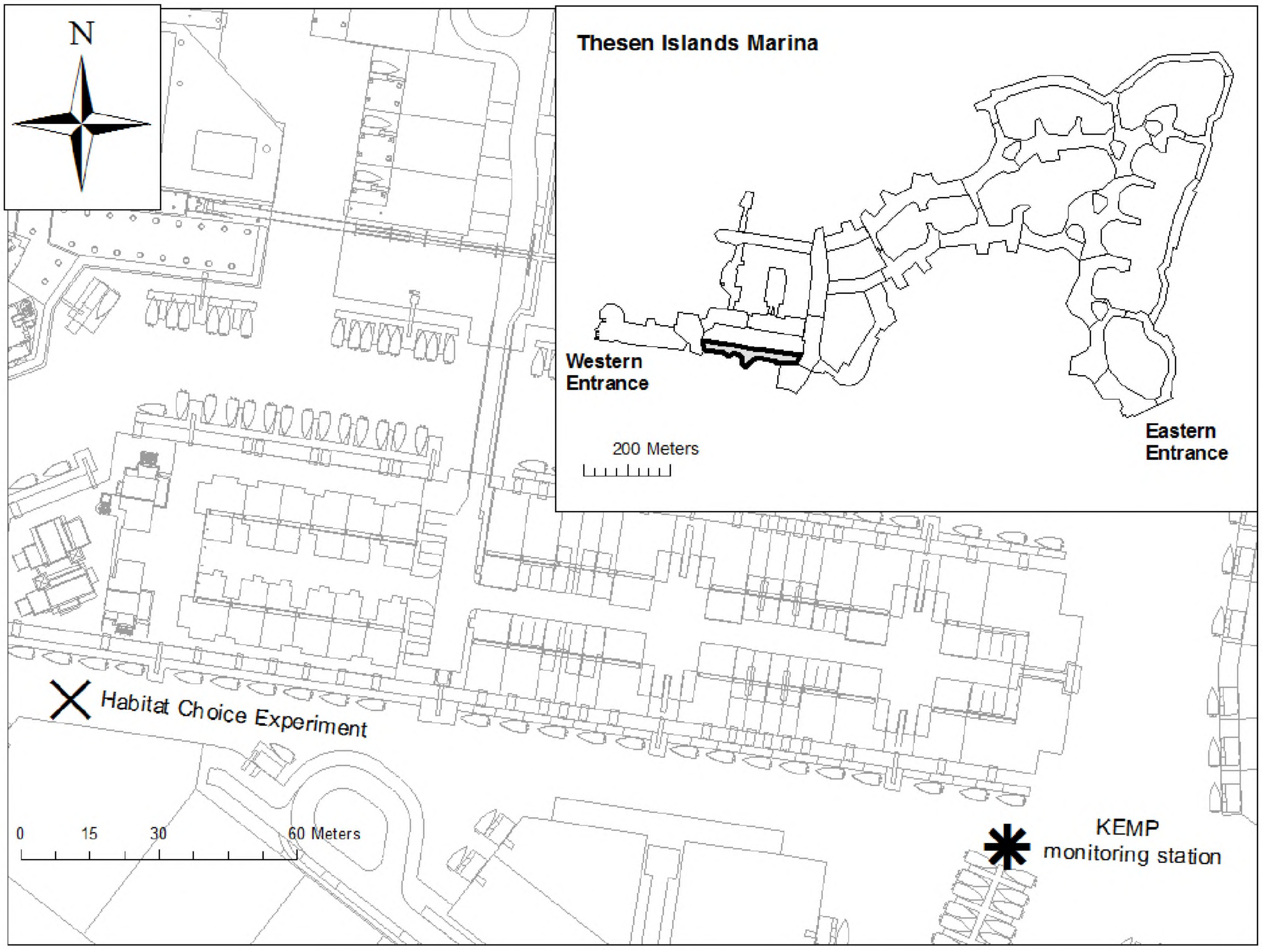


Figure 4.1. The location of the choice experiment, conducted on *H. capensis*, within Thesen Islands Marina and the KEMP water quality monitoring station. Insert shows the location of the experiment within the marina (bold and shaded area).

Figure 4.2. A sample Reno mattress structure used in the choice experiment conducted on *H. capensis* (ruler in picture = 15 cm).

To ascertain the availability of potential seahorse prey animals (small crustaceans (Foster and Vincent, 2004; Kendrick and Hyndes, 2005; Felicio *et al.,* 2006) and nematodes (Castro *et al.,* 2008)) on the replica Reno mattresses, ten epibenthic samples were collected from the structures in December 2015, after a four-month maturation period. A 100 ml syringe, fitted with a 4 mm diameter tube, was used to suck visible biofilm and sediment from the surface of the Reno mattress structure (N = 10). Ten 2 ml subsamples from each syringe were assessed under a microscope and potential prey animals were identified to major taxonomic groups and quantified. Each subsample was replaced. All names were verified using the World Register of Marine Species (http://www.marinespecies.org/).

Ten choice chambers (50 cm x 30 cm x 30 cm) were constructed out of wood and wrapped with green plastic mesh (1 cm diameter). The bottom and top of the chambers were left unlined. Two sandbags were used to submerge and stabilize the chambers. The chambers were placed within a 45 m stretch of marina canal (Fig. 4.3) and positioned 2–3 m from the canal side wall to ensure protection from passing boats. During the 2015 summer population assessment, 70 % of seahorses used *Asparagopsis taxiformis* as a holdfast, but owing to a freshwater flood in September 2015 and the related loss of all *A. taxiformis* from the marina canals, it was decided to use *Z. capensis* in the habitat choice experiment because of previously recorded use by *H. capensis* (Teske *et al.*, 2007b). Each chamber was placed on top of an existing *Z. capensis* stand and the percentage cover of *Z. capensis* in each chamber varied owing to the natural variation in seagrass density. The number of replicates used in the experiment was limited to the availability of *Z. capensis* coverage in the marina canal used. Each chamber was pushed down into the sediment to ensure no gaps or crevices were located along the bottom. At the start of the experimental period, one matured replica Reno mattress was placed within each choice chamber. Half of the chamber area (0.075 m^2) was covered by a Reno mattress structure and the other half by *Z. capensis* (Fig. 4.4). A significant, albeit weak, positive correlation was found between adult seahorse abundance and vegetation height, and Teske *et al.* (2007b) concluded that *H. capensis* will use any holdfast that is available, regardless of vegetation height.

Zostera capensis leaf length was thus not taken into consideration. Most seahorse species require some sort of holdfast within their habitat (see Foster and Vincent, 2004) and *H. capensis* will only occur in the presence of some type of vegetation or holdfast (Teske *et al.*, 2007b). As a consequence, choice chambers with no microhabitat type or only one microhabitat type available to a seahorse that would act as a control, were considered superfluous. To ensure that the position of the Reno mattress within the chamber had no effect on the direction of movement of seahorses when introduced to the experimental chambers, the Reno mattresses in four of the chambers were positioned towards the canal middle, in another four chambers they were positioned towards the canal wall and in two of the chambers they were parallel to the canal wall (Fig. 4.3). Experimental trials were conducted during neap tides to ensure that the choice chambers were submerged throughout the experiment, and consequently consecutive trials were conducted a minimum of 14 days apart.

At the start of the trial, the top of each cage was covered with a Perspex lid attached with cable ties. The surrounding area (≈ 900 m^2) was then searched for ten seahorses using SCUBA. Seahorse sex was established, the brood pouch status assessed, and their heights measured (to the nearest 0.5 cm) using the method of Lourie (2003) before placing one seahorse in each chamber. Only adult seahorses (> 4 cm, Lockyear *et al.*, 2006) were used in the trials. At the onset of an experiment, a seahorse was held parallel between the two microhabitats facing downward within the chamber by the diver. The seahorse was released by the diver. The initial choice (Reno mattress or *Z. capensis*) made by the seahorse was recorded. The seagrass abundance of each chamber was recorded as percentage cover.

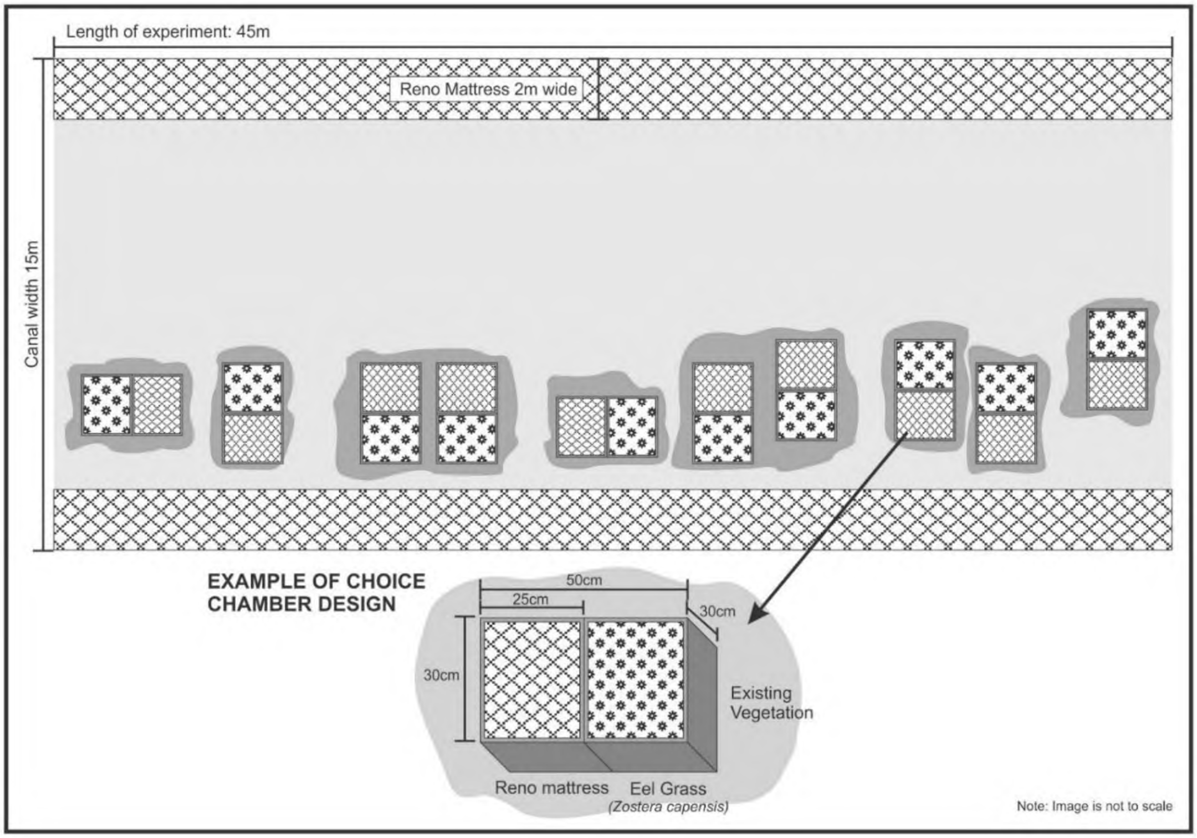


Figure 4.3. Experimental layout of the choice experiment conducted on *H. capensis* from November 2015 to March 2016. The experiment was conducted within the western section of Thesen Islands Marina.

Figure 4.4. The choice chamber used in the choice experiment. Half of the choice chamber was covered in *Z. capensis* and the other half by a Reno mattress. In the photograph the matured Reno mattress structure is located in the forefront, and *Z. capensis* towards the back of the choice chamber.

After a 48-hour acclimation period (Correia *et al.*, 2013), an initial search for the seahorse within each cage was conducted without the removal of the Perspex lid or the disturbance of the choice chamber. If a seahorse was visible, the microhabitat in which it was located and holdfast used were recorded. If it was not possible to locate the seahorse, the lid was removed, and the vegetation section of the cage searched. If the seahorse was not found in the vegetation section, the Reno mattress was searched and if needed moved to search behind and under the structure. Once a seahorse was located, the microhabitat in which it was located and the holdfast used was recorded. After each trial, seahorses were released at the site of collection. Seahorses were collected and placed inside the choice chambers and released after 48 hours during morning periods only between 8:00 and 11:00. One trial consisted of ten choice chambers and only one trial was conducted per neap tide period. Seahorses were not tagged and the possibility exists that seahorses were resampled. The probability of resampling an animal was deemed to be low owing to the relatively low seahorse density recorded for this site in previous seasonal population surveys (mean ± sd = 0.15 ± 0.21 individuals/m^2), the size of the area searched (900 m^2) and, the period between consecutive surveys (> 14 days). Seahorses found as pairs were not used. Seven trials were conducted between November 2015 and March 2016 with 67 data points recorded. During Trial 6 only seven seahorses were found and used in the experiment.

4.2.2. Statistical analysis

The statistical programme R (R Development Core Team, 2014) was used in all analyses. The Plyr-package was used for all basic descriptive statistics (Wickham, 2011). A Chi-square test was used to assess the sex ratio of seahorses used in the experiment and to compare holdfasts used. Microhabitat choice was investigated by the assessment of movement, if any, by seahorses from the microhabitat type initially chosen to that occupied 48 hours later. Movement was coded with a (0) if the seahorse was found on the same microhabitat initially chosen; and a (1) if the seahorse was found in another microhabitat than that originally chosen. A Binomial Generalized Linear Mixed Model was used to assess the likelihood of a seahorse moving from the habitat type initially chosen using the assigned codes (see McCullach and Nelder, 1989; Venables and Ripley, 2002; Dobson and Barnett, 2008). Water quality features (pH, dissolved oxygen, temperature), seahorse sex, location of the replica Reno mattress in the chamber and percentage seagrass cover in each chamber were treated as fixed effects in the model and the variable 'choice chamber' controlled for by treating it as a random effect (each choice chamber was repeatedly sampled). The lme4 package (Bates *et al.*, 2015) was used for the generalised linear mixed effects model. Table 4.1. provides a summary of the hypothesis tested, the applicable sections within the chapter and the R code used.

Table.4.1. A summary of the hypothesis tested in Chapter 4, the applicable sections in the chapter and the R code used.

Hypothesis	**Section**	**Formula**
H_0: *Hippocampus capensis* will show no preference towards a particular microhabitat when given a choice between Reno mattress and *Zostera capensis*	4.2.1 and 4.3	`Choice1=glmer(Move~Initial.Choice+Veg.Percetage.Cover+SH.Gender+Temp+pH+DO+Salinity +(1|Choice.Chamber),data=Choice, family="binomial")` `summary(Choice1)`

4.3. Results

After the three-month maturation period, all replica Reno mattresses were covered by biofilm and colonized by a number of animals which included fish (Gobiidae, Gobiesocidae, Clinidae), feather stars and sea stars (Crinoidea, Asteroidea), urchins (Echinoidea), barnacles (Balanidae), shrimps (Caridae) and snails

(Nassaridae) (Fig. 4.5). Within 7 days of deployment *H. capensis* was noted on the artificial structures (n = 7). At the end of the maturation phase, a total of 14 seahorses were noted on the structures and the maximum number of seahorses found on a single Reno mattress structure during the maturation phase was five. The microscopic assessment of the biofilm on the Reno mattresses found a number of small benthic crustaceans (Copepoda, Tanaidacea, Ostracoda, Amphipoda) as well as nematode and polychaete worms, known prey animals of seahorses (Table 4.2).

Figure 4.5. A matured Reno mattress sample used in the choice experiment.

Table 4.2. Mean (± sd) densities (animals/2 ml) of potential seahorse prey animals found on matured Reno mattress structures.

Prey animal	Mean ± sd	Min	Max
Amphipoda	0.11 ± 0.13	0.00	0.40
Asellota	0.02 ± 0.04	0.00	0.10
Copepoda	7.0 ± 3.72	2.10	13.00
Porcellidium	0.01 ± 0.03	0.00	0.10
Tanaidacea	0.11 ± 0.22	0.00	0.70
Ostracoda	0.53 ± 0.23	0.20	1.10
Nematoda	2.28 ± 1.97	0.20	6.70
Polychaeta	0.15 ± 0.16	0.00	0.50

Temperature ranged from 11.6 °C to 26.4 °C during the experimental period with a mean (± sd) of 21.2 ± 2.7 °C. An upwelling event occurred during the third trial (16 – 18 January 2016) of the experiment with a ≈ 7 °C drop in temperature over one tidal cycle. Dissolved oxygen ranged from 27.5 % to 127.5 % with a mean (± sd) of 86.4 ± 17.2 %. Salinity ranged from 32.2 to 35.4 with a mean (± sd) of 34.8 ± 0.6 and pH ranged from 7.7 to 8.5.

Zostera capensis coverage ranged from 10 % to 90 % among the choice chambers and over the experimental period. For the trials, 60 % of seahorses were collected from adjacent mixed vegetation habitat, 36 % from Reno mattress habitat, and 4 % were found holding onto the outside of the choice chambers. Seahorse height ranged from 4.5 to 9.0 cm with a mean (± sd) of 6.8 ± 1.1 cm. The sex ratio of seahorses used in the experiment was female biased ($\chi 2 = 4.3$; df = 1; $P = 0.04$)

with 42 females and 25 males, of which 13 males had an inflated brood pouch, indicative of breeding (see Foster and Vincent, 2004).

The original microhabitat choice and the microhabitat choice after the 48-hour trial period are summarized in Table 4.3. Instances where seahorses were found swimming around in the cage, no choice was logged. Significantly ($\chi^2 = 29.6$; df = 3; $p = < 0.01$) more seahorses used the wire of the Reno mattress structures (48 %) (Fig 4.6) as a holdfast compared to vegetation (31 %), the green plastic mesh (12 %) or nothing (7 %) after the 48-hour period.

Table 4.3. Summary of the original microhabitat choice and the microhabitat choice after the 48-hour experimental period made by 10 Knysna seahorses per trial (total of 7 trials). In instances where the seahorse was found swimming around in the choice chamber no choice was recorded.

	Initial Habitat Choice		48 Hour Habitat Choice		
Trial	Reno mattress	Vegetation	Reno mattress	Vegetation	None
1	4	6	5	4	1
2	4	6	6	3	1
3	4	6	4	5	1
4	4	6	6	3	1
5	7	3	7	3	0
6	3	4	6	1	0
7	6	4	8	2	0

Figure 4.6. *Hippocampus capensis* on a Reno mattress structure (inside the black circle) within a choice chamber.

Neither the sex of the seahorse, the location of the replica Reno mattress within the choice chambers, physico-chemical water quality features nor percentage vegetation cover had a significant effect on seahorse microhabitat choice and were excluded from the final model. Seahorses were significantly ($Z = 3.0$, $p < 0.01$) more likely to move away from *Z. capensis* towards the Reno mattress structure, or remain on the Reno mattress structure, if given a choice (Table 4.3).

4.4. Discussion

This study found that adult *H. capensis* were significantly more likely to move towards or remain on a Reno mattress structure compared to *Z. capensis*. This behavioural choice made by *H. capensis* suggests that it is the particular features of the Reno mattress structures (e.g. specific materials used to construct a Reno mattress, availability of holdfasts, increased protection provided by crevices, food availability) responsible for the distribution pattern observed in the previous chapter.

Seahorse populations have been found to decline owing to habitat damage caused by anthropogenic activity (see Aylesworth *et al.*, 2015). A 97 % decline in *H. whitei* numbers at one site in Port Stephens, Australia, was recorded owing to a major decline of this species' preferred habitat (Harasti, 2016). In some instances, the alteration and loss of natural habitats goes together with an increase in artificial structures (Gristina *et al.*, 2015; Otero-Ferrer *et al.*, 2015). A number of seahorse species use these artificial structures in their natural habitat (*H. guttulatus* – Curtis and Vincent (2005); Gristina *et al.* (2015); *H. whitei* - Hellyer *et al.* (2011); Harasti *et al.* (2012)) and the potential exists to use artificial structures in areas devoid of natural habitat (Correia *et al.*, 2015b) as a remediation method.

Habitat loss and alteration is one of the three major threats to syngnathid populations worldwide (Vincent *et al.*, 2011). The protection of natural seahorse habitat is integral in successful conservation, but novel new conservation solutions are needed in situations where natural habitats are inevitably lost or altered– particularly within urban environments (Savard *et al.*, 2000). The role of artificial water bodies (European Commission, 2003) in conservation actions show particular promise, as these developments provide novel habitats with no loss of natural habitats (Waltham and Connolly, 2013).

Adult *H. capensis* moved towards a Reno mattress structure in the presence of *Z. capensis*. By contrast, *H. guttulatus* and *H. hippocampus* in the Ria Formosa, Portugal, tended to maintain a preference for natural sea grass habitat over Artificial Holdfast Units (AHU) (Correia *et al.*, 2015b) made from a metal grid covered with a polypropylene base and *Codium*-like, 1.6 cm diameter polyethylene nautical rope holdfasts at a 100 holdfasts/m^2 density (Correia *et al.*, 2013). The usefulness of this specific AHU design was found to be limited in assisting seahorse population recovery in damaged areas only. In Sydney Harbour, Australia, *H. whitei* was found to utilize swim nets, but the temporary nature of seasonal nets and maintenance activities of the permanent nets left seahorses vulnerable within this habitat (Clynick, 2008b). Seahorses on swim nets were exclusively found in areas with epiphytic growth and generally within a meter of the bottom that allowed management and conservation actions to be implemented (Harasti *et al.*, 2010). What is seen here, however, was a serendipitous outcome of the utilization of artificial habitat by a vulnerable species with no thought given prior to the deployment or use of the artificial structures, besides its original purpose of the protection of swimming areas in Sydney harbour. This scenario may also be true in the case of Reno mattresses and *H. capensis* – no thought was specifically given to *H. capensis* habitat provision when it was decided to line the marina canals of Thesen Islands Marina with Reno mattresses. According to

Clynick (2008b), permanent artificial habitat located in close proximity to the seafloor may be beneficial in the provision of suitable, alternative habitat for *H. whitei.* Perhaps the deployment of Reno mattress structures, or similar, in Sydney Harbour would provide this permanent surrogate habitat for *H. whitei*, which has been found to be in decline owing to habitat loss (Harasti, 2016)? Or alternatively, future developments within Sydney Harbour should consider the use of this type of construction material, which should provide suitable habitat for seahorse populations.

An alternative view is that artificial structures might act as fish aggregation devices (Correia *et al.*, 2015b) and may not increase populations *per se*. This seemed to be the case in the Ria Formosa, as highest seahorse densities on the AHUs deployed were found in areas with limited alternative natural habitat (Correia *et al.*, 2015b). Many studies find that artificial reefs and structures concentrate fish populations, rather than increasing biomass (see Grossman *et al.*, 1997 and Pickering and Whitmarsh, 1997). The concept of a successful artificial reef relies on the assumption that it would provide additional habitat and increase the environmental carrying capacity and thus also increase the abundance and biomass of the surrounding biota (see Bohnsack, 1989). The increase in carrying capacity can be owing to increased food availability and feeding efficiency, increased shelter and higher recruitment opportunities (see Bohnsack, 1989). However, fish attraction to an artificial reef can be defined as: “the net movement of individuals from natural to artificial habitats” (Carr and Hixon, 1997). If fish are merely attracted to an artificial reef or structure, no increase in the abundance of the population will occur. Distribution patterns alone are not enough to confirm suitability of a particular habitat and it is crucial to understand the underlying process responsible for the observed distribution using an experimental approach. Only then can insightful conclusions be made with regards to habitat use by a species and its usefulness in conservation actions. The higher densities of seahorses found on the Reno mattress structures do not necessarily equate to an increase in population biomass and ongoing research is needed to establish if these structures increase the environmental carrying capacity or merely attract seahorses from adjacent natural habitats.

The underlying process behind the choice for the artificial structure made by *H. capensis* is still to be determined, but it is hypothesised that the complexity of the Reno mattress structure with its numerous crevices and wire sides could provide more security and a greater number of rigid holdfasts to seahorses. Density of *H. whitei* populations on nets increased when the structural complexity of the net was increased by the addition of frayed net material (Hellyer *et al.*, 2011). *Hippocampus guttulatus* numbers were greater when more holdfast structures were available (Correia *et al.*, 2015b). Holdfast use by *H. capensis* is non-specific and, therefore, it is perhaps not surprising that they would take advantage of the holdfast opportunities presented by the Reno mattress (Teske *et al.*, 2007b). However, holdfast availability may not be the only reason for seahorse habitat choice as Hellyer *et al.* (2011) found more complex artificial habitats supported greater numbers of small mobile epifauna (amphipods and copepods) and *H. whitei* abundance on swim nets were positively correlated to epibenthic growth which provided structural complexity, and the colonization of swim nets by epifauna seems to be an important requirement for seahorse colonization (Harasti *et al.*, 2010). It is possible, therefore, that seahorses are also attracted to Reno mattresses because of increased food availability and structural complexity, particularly when covered by *A. taxiformis* (Chapter 2).

Seahorse densities on artificial structures are usually higher when compared to natural vegetation habitats (Clynick, 2008b; Harasti *et al.*, 2012; Correia *et al.*, 2015b). These higher densities could leave populations vulnerable to human impacts (Correia *et al.*, 2015b), particularly owing to the fact that artificial structures are usually located close to human activities (Clynick, 2008b) and undergo regular maintenance activities which may reduce the diversity of epibiota on these structures (Bulleri and Chapman, 2010). It is thus important to include these structures in management and conservation actions and even in reserves (Harasti *et al.*, 2010; Garcia-Gomez *et al.*, 2015). Artificial Marine Micro-Reserves can be used to protect artificial habitats utilized by vulnerable and endangered species (Garcia-Gomez *et al.*, 2015). In the case of *H. capensis*, if Reno mattress habitats within Thesen Islands Marina are given formal protection, it would assist in the conservation of this endangered species.

Alteration of existing marine infrastructure to enhance structural complexity and thus potential habitat for marine fauna has become increasingly common (Chapman and Underwood, 2011; Hellyer *et al.*, 2011; Dafforn *et al.*, 2015a; Dafforn *et al.*, 2015b). Ecological engineering attempts to: “combine engineering principles with ecological processes to reduce environmental impacts from built infrastructure" (Chapman and Underwood, 2011). The use of Reno mattress structures could contribute to ecological engineering (Chapman and Underwood, 2011) worldwide (as it is a construction material in essence) seeing that this material is already highly complex. Care should, however, be taken to take all potential impacts of the use of these structures into consideration (e.g. facilitating the establishment of alien species, change in original species assemblages) (Bulleri, 2005; Bulleri and Chapman, 2010; Di Franco *et al.*, 2010; Wetzel *et al.*, 2014) and ongoing research on the ecological efficacy of this building material is needed (Chapman and Underwood, 2011; Dyson and Yokom, 2015).

According to Perkol-Finkel *et al.* (2012): "The ability to utilize coastal infrastructures as scaffolds for recovery of threatened species or the enhancement of desirable species has important applications for the conservation of biodiversity in globally expanding coastal urban environments". This approach does not promote a purposeful increase of artificial structures in a natural system (in the name of conservation), but rather to use existing structures (Perkol-Finkel *et al.*, 2012) used by threatened species as a conservation tool e.g. using the Reno mattress structures in Thesen Islands Marina to assist in the conservation of *H. capensis.* And where development is inevitable, to ensure that ecological integrity is maintained by following suitable design philosophies (Bergen *et al.*, 2001).

It is concluded from the current study that *H. capensis* is found in higher densities on artificial Reno mattress habitat owing to the nature of the artificial structure as opposed to the specific location of these structures within the marina canals. The Reno mattress structure was chosen by *H. capensis* and the reasons responsible for this choice (positive or negative (Underwood *et al.*, 2004)) can now be investigated. The potential use of Reno mattresses as an alternative, and even surrogate, habitat for *H. capensis*, and seahorses in general, is an exciting finding, one which warrants further research to really benefit from the potential conservation solution it can provide. The close proximity of *H. capensis* populations in Reno mattress habitats within Thesen Island Marina to human activities could result in adverse effects on these seahorse populations, particularly sub-lethal effects owing to external stressors related to marinas (e.g. boat noise). Chapter 5 investigates this possibility further.

Chapter 5

Insights into the behaviour of Hippocampus capensis within a residential marina estate using GoPro cameras

5.1. Introduction

The behaviour of animals is inherently linked to their survival and reproductive success, and is thus key in successful species conservation (Shumway, 1999). In the past, animal behavioural studies were thought to be less important than more conventional ecological investigations, particularly in terms of conservation (Sutherland, 1998; Shumway, 1999; Anthony and Blumstein, 2000), specifically when linking behaviour with practical conservation management (Caro, 2007). Behavioural studies are important in understanding aspects of conservation such as response to anthropogenic impacts (Caro, 2007).

One of the problems associated with behavioural studies of animals is being able to observe behaviour in the wild without bias. This problem is particularly acute in marine environments, where the additional factor of an underwater study site can confound the observational process. In many instances, researchers have used laboratory studies to overcome this obstacle. Animal behaviour *ex situ* is, however, considered to be different to that in the natural environment (Crowe and Underwood, 1998), which can lead to biased results in the laboratory.

In situ observations of fish using SCUBA tends to yield data that is biased because of the intrusive nature of the diver, the limited ability of divers to identify fish species and, responsive behaviour from fish (Assis *et al.*, 2013). The problem of how to observe underwater species undisturbed has, however, been solved to an extent, by the development of small, affordable, waterproof cameras (Struthers *et al.*, 2015). Underwater research using cameras now includes population studies (e.g. Cappo *et al.*, 2006), habitat use (e.g. Becker *et al.*, 2010; Davis *et al.* 2016), and behavioural assessments (e.g. He, 2003; Ebner *et al.*, 2009; Papadakis *et al.*, 2012; Davis *et al.*, 2016; Filiciotto *et al.*, 2016) of fish and other underwater fauna. According to Wartenberg and Booth (2015) Underwater Visual Transect data recorded on video is more efficient, reliable and objective than that recorded by a diver.

Underwater camera surveys do, however, have limitations such as the inability to detect cryptic species and a dependence on good water clarity, and in a number of studies, underwater camera surveys recorded lower species diversity compared to diver surveys (Assis *et al.*, 2013; Gardner and Struthers, 2013; Davis *et al.*, 2016). The reason for a difference in species diversity recorded for diver and camera surveys could be owing to the positive or negative behavioural response from certain fish species to the presence of a diver (targeted fish species avoid divers, while non-commercial species favoured divers) (Assis *et al.*, 2013). The use of a specific survey method should be project specific which takes the objectives of the study and the targeted species into consideration seeing that there is no “one method fits all” solution (Gardner and Struthers, 2013). According to Ebner *et al.* (2009): “there is scope for using underwater video to study threatened species by non-destructive means and with minimal observer effect”.

Studies of fish using cameras have been, to date, more focused on species richness and abundance than on behaviour, probably owing to the highly mobile nature of most fish species. Sedentary fish species, such as seahorses (*Hippocampus* spp.) should be good candidates for *in situ* behavioural assessments using cameras. Most seahorse behavioural assessments have either been conducted *ex situ* (Mason-Jones and Lewis, 1996; Faleiro *et al.*, 2008; Anderson *et al.*, 2011; Faleiro *et al.*, 2015), or *in situ* by a diver (Vincent and Sadler, 1995; Curtis and Vincent, 2005; Naud *et al.*, 2009). The use of cameras in *in situ* seahorse behavioural studies are limited and include the use of a Remotely Operated Vehicle (ROV) to study *H. denise* at a depth of 102 m at Osprey reef, Australia (Nishikawa *et al.*, 2011), and the use of cameras to assess the impact from flash photography on the movement, behaviour and site persistence of *H. whitei* in Port Stephens, Australia (Harasti and Gladstone, 2013). Detailed behavioural assessments of seahorses using video cameras have, however, not been published.

Thesen Islands Marina presented a perfect environment to test the efficacy of using action cameras to investigate the *in situ* behaviour of *H. capensis* because the site is secure and cameras can be left unattended, and because the Knysna estuary is oligotrophic (Allanson *et al.*, 2000) with low turbidity. Behavioural information for *H. capensis* is limited to *ex situ* mating and courting behaviour (Grange and Cretchley, 1995; Lockyear *et al.*, 1997) and the present study is the first to investigate the *in situ* behaviour of this species. The aims of the study were to investigate the behaviour of *H. capensis* at different times of the day, and see whether seahorse behaviour was affected by human use of the marina, particularly the use of motorized boats.

5.2. Materials and methods

5.2.1 Behavioural analysis

The efficacy of using GoPro cameras (GoPro Inc., USA) to study the behaviour of *H. capensis* within the canals of Thesen Islands Marina was tested during a preliminary assessment from March to April 2015. Five camera deployments were made during this time period and each deployment lasted up to an hour. Seahorses within the Reno mattress habitat in the western section of the marina (where seahorses were most abundant – Chapter 3) were located by snorkelling during high tide, where a single GoPro Hero 3 camera was deployed facing a seahorse. Water depth ranged from 1.5 m to 2.5 m and deployments were made throughout the day. The camera was mounted on a 25 cm long cylindrical pvc pipe with a 2 cm diameter. The pipe was fastened to the Reno mattress wire using a pvc pipe T-junction joint and cable ties. During the preliminary assessments, the camera was placed haphazardly wherever seahorses were found. As the aim of the preliminary assessment was to test the efficacy of using cameras to study seahorse behaviour, the focus was placed on successfully capturing seahorses within the field of view of the camera. The GoPro camera was set on wide angled video with a video resolution of 720p and 60 frames per second.

From the preliminary video results and a review of seahorse behaviour in the literature the following ethogram comprising of five behaviours for *H. capensis* was established:

Feeding: the seahorse is actively searching for prey animals (see Foster and Vincent, 2004). During feeding the seahorse holds on to a holdfast and searches the entire surrounding area, in many instances as far wide as the entire length of the outstretched animal, for food. Once the area surrounding a particular holdfast has been searched the seahorse moves to another holdfast and continues to forage (see Video 5.1 in Appendix A).

Irritation: Irritation was identified as increased clicking or tail adjustments (Anderson *et al.*, 2011) made by the seahorse.

Moving from holdfast to holdfast: the seahorse moves from holdfast to holdfast without foraging in-between. Time spent on a holdfast is limited.

Interaction: interaction between two or more seahorses. This can either be between a male and a female as part of courting (Grange and Cretchley, 1995; Vincent and Sadler, 1995) (see Video 5.2 in Appendix A) or between seahorses of the same sex and might entail aggression (Naud *et al.*, 2009) (see Video 5.3 in Appendix A). Displays of aggression can include tail wrestling, using the snout to snap at the opponent, and chasing (Naud *et al.*, 2009). Courting behaviour for *H. capensis* includes the grasping of the female's tail by the male in an attempt to position himself face to face with his partner, followed by a swaying movement (Grange and Cretchley, 1995).

Stationary: the seahorse remains completely still with no foraging activity.

Once the efficacy of using GoPro cameras to study seahorse behaviour within Thesen Islands Marina was established, a behavioural assessment commenced. In the first instance, seahorse behaviour throughout the day was investigated by the deployment of cameras during the morning (6:00–10:00); midmorning to early afternoon (hereinafter referred to as midday) (10:00–14:00); and afternoon (14:00–18:00). Human use of the marina increases from ≈ 30 % to 100 % occupancy during the summer holiday period (pers. obs.). To assess behavioural changes owing to an external stressor, in this case increased boat noise during the summer holiday period, focus was placed on three longer-term periods: Pre-holiday (October to early December 2015); Holiday (mid-December 2015 to Mid-January 2016); and Post-holiday (mid-January to March 2016).

Four GoPro cameras (two Hero 3 cameras and two Hero 3+ cameras) were deployed within the Reno mattress habitat in the western section of Thesen Islands Marina during each deployment (Site 2 as per Fig. 2.3) (Fig. 5.1). The method of deployment and camera settings used were similar to that used during the preliminary assessment. GoPro battery backpacks were used to lengthen the deployment period to a minimum of 2 hours per camera. All recordings were undertaken during an incoming tide. Cameras were collected by snorkelling after each 2-hour deployment.

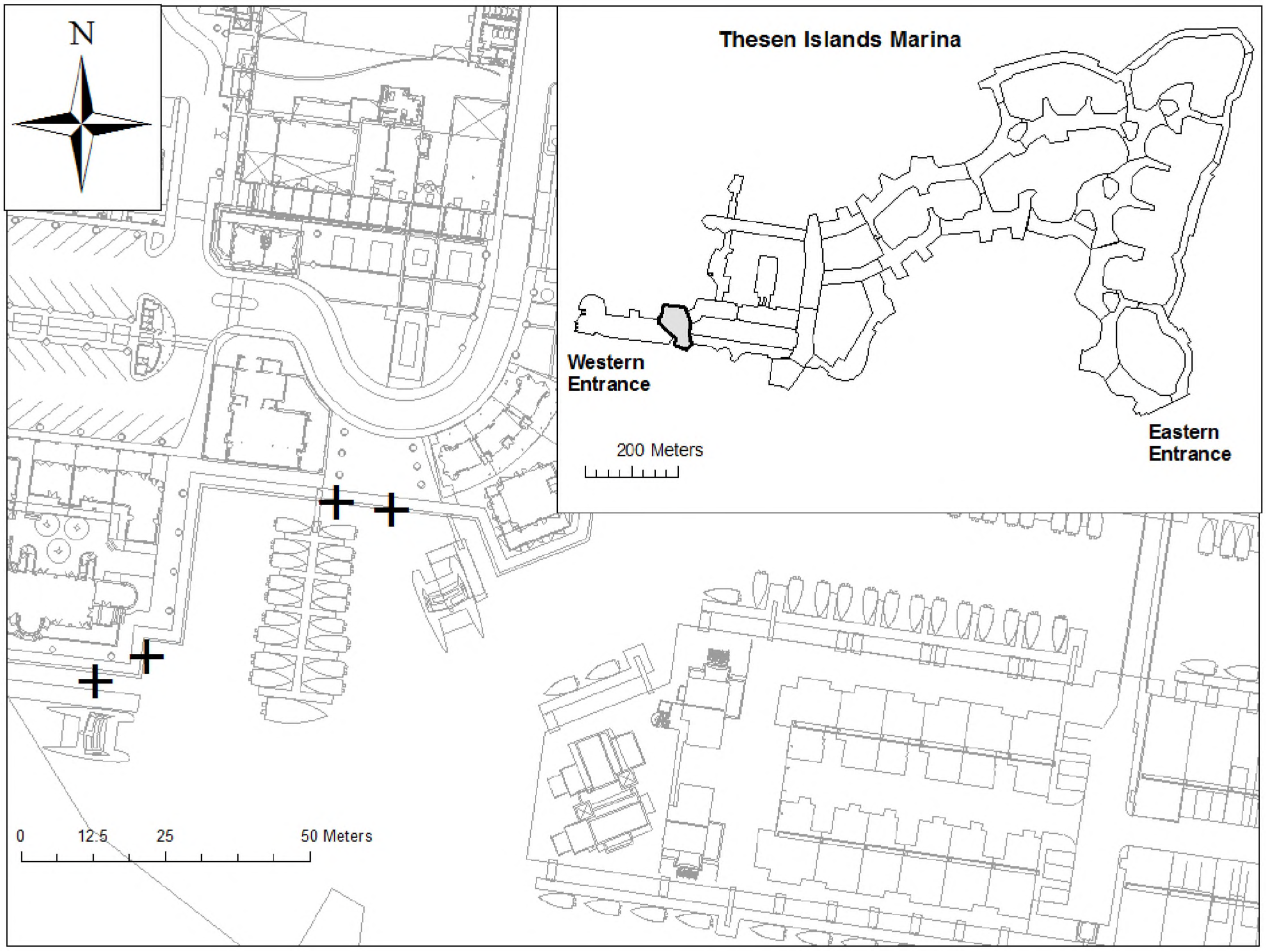


Figure 5.1. Location of camera deployments (+) during the behavioural assessment of *H. capensis* within Thesen Islands Marina. The insert depicts the recording location (bold and shaded area) within Thesen Islands Marina.

Videos were downloaded onto a laptop in ten minute sections, each section considered a sample. The first 10-minute section of each deployment was excluded from the analyses to prevent any behavioural impact that might take place during deployment. This allowed for a 10-minute acclimation period (Becker *et al.*, 2010) after the placement of the camera and the start of the behavioural assessment. Only every third 10-min video sample was included in the assessment to control for any auto-correlation in time. As the individual identity of seahorses could not be ascertained, the possibility exists that animals were resampled.

Each 10-minute video section was assessed separately and one focal animal was assessed in each section. Focal animals were chosen based on the maximum amount of time in the field of view and the ability to assess the behaviour of the animal. Windows Media Player (www.microsoft.com) was used during the video assessments. The ethogram created during the preliminary assessment was used to identify the behaviour of the focal animal in each video section. The video was first watched on fast forward mode to establish the behavioural pattern of the focal animal. Once complete the video was watched from the start and all behaviours noted for the focal animal were timed for the entire 10-minute period. Where possible the sex of the focal seahorse and the number of times it was seen eating (feeding event) were logged. Feeding events were recognized by a sudden jerk of the head accompanied by clicking sounds and/or the expulsion of sediment from the gills (Felicio *et al.*, 2006).

The maximum number of seahorses noted in each video section and the total time the focal animal was visible during the video section (seahorse visibility) were logged. The mean time a seahorse spent on a holdfast was recorded for each video section {*total time spent on a holdfast / total number of holdfasts used*} (Anderson *et al.*, 2011). The occurrence of potential seahorse predators (cormorants and rays – see Kleiber *et al.*, 2011, 2004; cuttlefish, – Harasti *et al.*, 2014a) (all recorded as N_{max}) and the number of boat passes (boat noise used as indicator of a passing boat) were recorded for each video section. Thesen Islands Marina is a 'No Wake' zone, although compliance to this restriction is limited.

5.2.2. Statistical analyses

The statistical programme R (R Development Core Team, 2014) was used in all analyses. The ddply function of the Plyr-package was used for all basic descriptive statistics (Wickham, 2011). The ggplot package was used to create all graphs (Wickham, 2009). Behaviour and all other aspects such as number of boat passes, feeding events etc. were assessed throughout the day (morning, midday, afternoon) and across longer-term periods (Pre-holiday, Holiday, Post-holiday) using ANOVA. Variability in behaviour during longer-term periods were also assessed using ANOVA. Significant differences were further assessed using a Tukey post hoc test. To assess sex differences in behaviour and to prevent pseudoreplication (Hurlbert, 1984), behavioural data from videos that used the same focal animal were pooled. Behavioural differences between males and females were compared using ANOVA and all significant differences 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 in all instances ($p > 0.05$). Table 5.1. provides a summary of all hypotheses tested, the applicable sections within the chapter and the R codes used in each instance.

Table 5.1. A summary of the hypotheses tested in Chapter 5, the applicable sections in the chapter and the R codes used in each instance.

Hypothesis	Section	Formula
H_0: Seahorses will not spend more time doing a specific behaviour	5.2.1 and 5.3	Assessment Name=aov(behaviour time~behaviour) summary.aov(Assessment Name) TukeyHSD(Assessment Name)
H_0: Seahorse behaviour will not vary throughout the day or across long-term periods	5.2.1 and 5.3	Assessment Name-aov(Specific behaviour~time of day + longer time periods + time of day:longer time periods) Summary.aov(Assessment Name) TukeyHSD(Assessment Name)
H_0: Seahorse visibility will remain the same throughout the day and across long-term periods	5.2.1 and 5.3	Assessment Name-aov(Seahorse visibility~time of day + longer time periods + time of day:longer time periods) Summary.aov(Assessment Name) TukeyHSD(Assessment Name)
H_0: The maximum number of seahorses counted within a single video sample will not vary throughout the day or across long-term periods	5.2.1 and 5.3	Assessment Name-aov(Number of seahorses per video sample~time of day + longer time periods + time of day:longer time periods) Summary.aov(Assessment Name) TukeyHSD(Assessment Name)
H_0: The mean time a seahorse spends on a holdfast will not vary throughout the day or across long-term periods	5.2.1 and 5.3	Assessment Name-aov(Mean time spent on a holdfast~time of day + longer time periods + time of day:longer time periods) Summary.aov(Assessment Name) TukeyHSD(Assessment Name)
H_0: The number of feeding events counted during a single video sample will not vary throughout the day or across long-term periods	5.2.1 and 5.3	Assessment Name-aov(Number of feeding events per video sample~time of day + longer time periods + time of day:longer time periods) Summary.aov(Assessment Name) TukeyHSD(Assessment Name)
H_0: The number of potential seahorse predators or boat passages will not vary throughout the day or across long-term periods	5.2.1 and 5.3	Assessment Name-aov(Number of predators/boats per video sample~time of day + longer time periods + time of day:longer time periods) Summary.aov(Assessment Name) TukeyHSD(Assessment Name)

5.3. Results

During the trial period, seahorses were successfully captured on film using the GoPro camera and 49.0 % of footage recorded contained seahorses. Seahorse behaviour was observed, and from the initial trial assessment it was clear that cameras can be used to investigate the behaviour of *H. capensis* in the Reno mattress habitat within Thesen Islands Marina.

During the detailed behavioural assessment, focal seahorse visibility ranged from 46.3 % during the morning of the Pre-holiday period to as little as 1.5 % during midday of the Holiday period (Table 5.2). Seahorses were significantly (F = 8.8, df

= 2, $p < 0.01$) more visible during the morning compared to midday and the afternoon of the Pre-holiday and Post-holiday periods. Overall seahorse visibility varied significantly (F = 22.9, df = 2, $p < 0.01$) across all three longer-term periods with seahorse visibility being the highest during the Pre-holiday period (Table 5.2).

Table 5.2. Summary of video data assessed in different longer-term periods and throughout the day recorded from October 2015 to March 2016. Behavioural assessments were conducted for only one focal seahorse per 10-minute video section.

Time period	Month	No of deployment events	Time of day	Total hours assessed	Total hours with focal seahorse	Percentage of footage with focal seahorse
Pre-holiday	Oct 15	2	Morning	8.2	3.8	46.3
	Nov 15	3	Midday	5.2	1.4	26.9
	Dec 15	2	Afternoon	4.5	1.1	24.4
Holiday	Dec 15	4	Morning	9.3	0.7	7.5
	Jan 16	2	Midday	6.7	0.1	1.5
			Afternoon	3.8	0.5	13.2
Post-holiday	Jan 16	3	Morning	10.3	3.7	35.9
	Feb 16	4	Midday	5.0	0.7	14.0
	Mar 16	1	Afternoon	1.7	0.2	11.8
	Total			54.7 Hours	12.2 Hours	22.3 %

A maximum of seven seahorses was noted during a single video section (Pre-holiday morning) and significantly more seahorses were counted during the morning (F = 9.2; df = 2; p = < 0.01) of the Pre-holiday and Post-holiday periods. The number of seahorses counted was significantly higher (F = 34.7; df = 2; p = < 0.01) during the Pre-holiday period (Table 5.3).

Table 5.3. Mean (± sd) number of seahorses counted during a 10-minute video section throughout the sample period from October 2015 to March 2016. Max refers to the maximum number of seahorses counted during a video section.

Time period	Time of day	Mean ± sd	Max
Pre-holiday	Morning	1.9 ± 1.6	7
	Midday	1.1 ± 1.4	5
	Afternoon	0.6 ± 0.8	3
Holiday	Morning	0.2 ± 0.4	2
	Midday	0.1 ± 0.3	2
	Afternoon	0.5 ± 0.9	3
Post-holiday	Morning	1.1 ± 1.3	6
	Midday	0.4 ± 0.7	3
	Afternoon	0.2 ± 0.4	1

Seahorses spent a significantly shorter ($F = 3.2$, df = 2, $p = 0.05$) time on a holdfast during the afternoon when compared to the morning, particularly during the Pre-holiday and Post-holiday periods (Fig. 5.2). Seahorses spent significantly ($F = 61.7.0$, df = 4, $p < 0.01$) more time feeding compared to all other identified behaviours and pooled data showed that 82.0 % of the video footage examined recorded feeding activity (Fig. 5.3). Feeding was significantly more ($F = 8.6$, df = 2, $p < 0.01$) prevalent during the morning compared to both midday and the afternoon. Time spent feeding decreased significantly ($F = 18.5$, df = 2, $p < 0.01$) from the Pre-holiday period to the Holiday period only (Fig. 5.3).

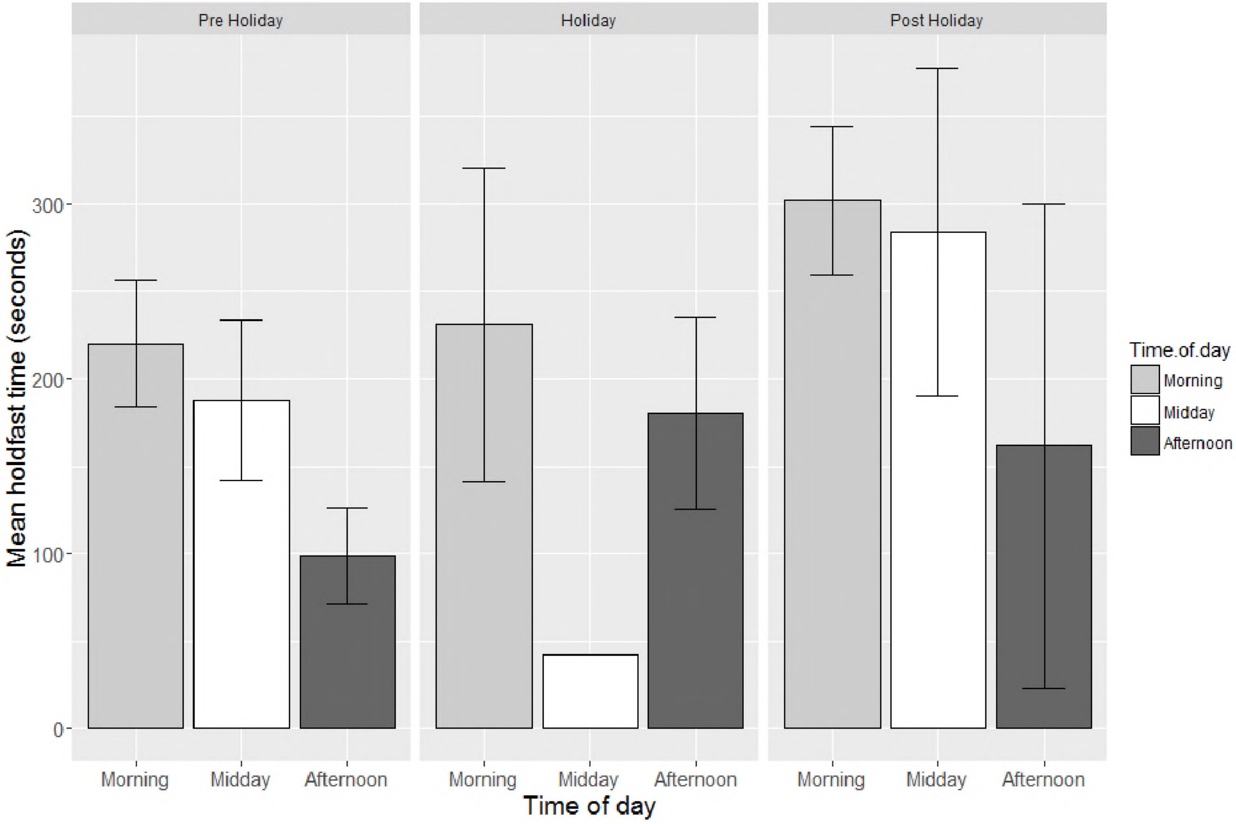


Figure 5.2. Mean (± se) time spent on a holdfast by *H. capensis* per 10-minute video section throughout the day and across longer-term periods recorded from October 2015 to March 2016. Pooled data showed that seahorses spent significantly ($p = 0.05$) longer time on a holdfast during the morning compared to the afternoon across all longer-term periods.

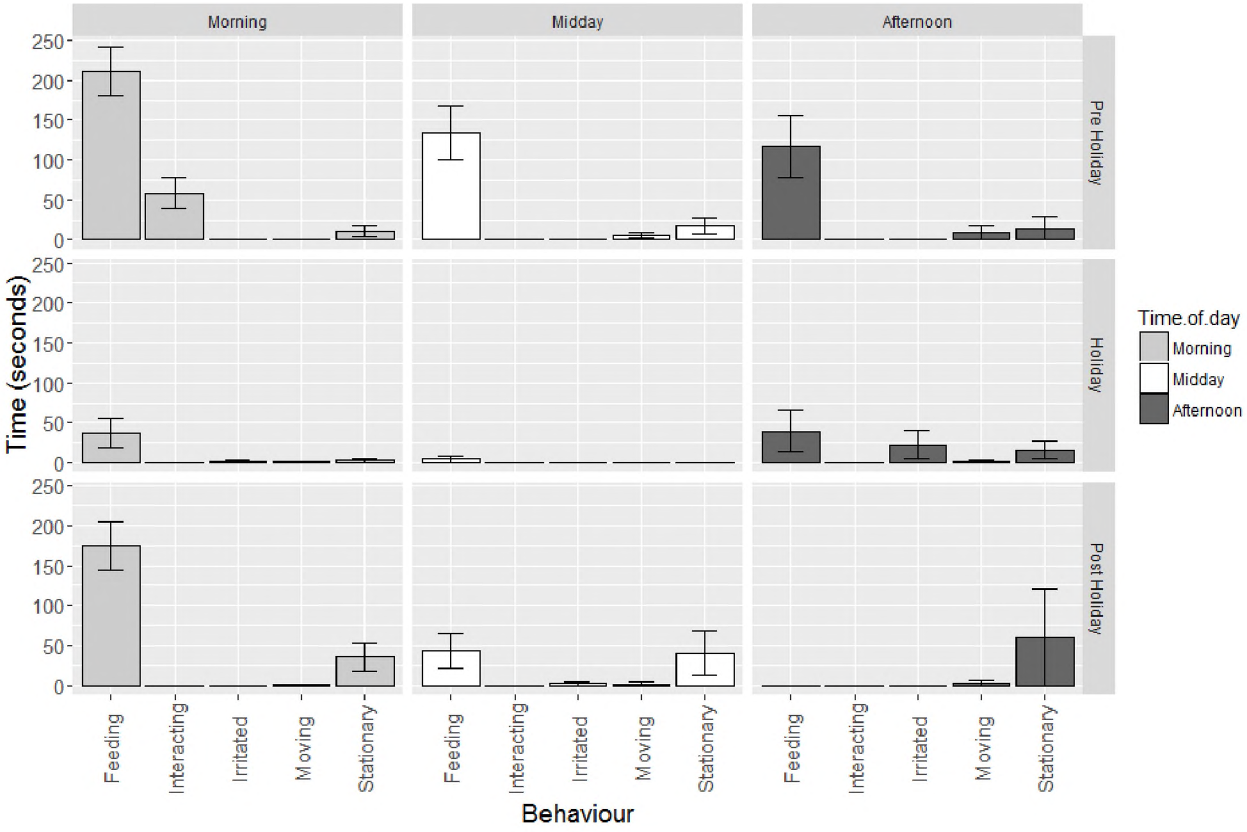


Figure 5.3. Mean (± se) time per 10-minute video section spent doing a specific behaviour throughout the day and across longer-term periods recorded from October 2015 to March 2016.

Pooled data showed a significant decrease (F = 6.9, df = 2, $p < 0.01$) in observed feeding events between the morning and both midday and the afternoon (Fig. 5.4). Observed feeding events were significantly greater (F = 11.9, df = 2, $p < 0.01$) during the Pre-holiday and Post-holiday periods compared to the Holiday period. Observed feeding events decreased significantly (F = 2.6, df = 4, p = 0.04) throughout the day within longer-term periods (Fig. 5.4).

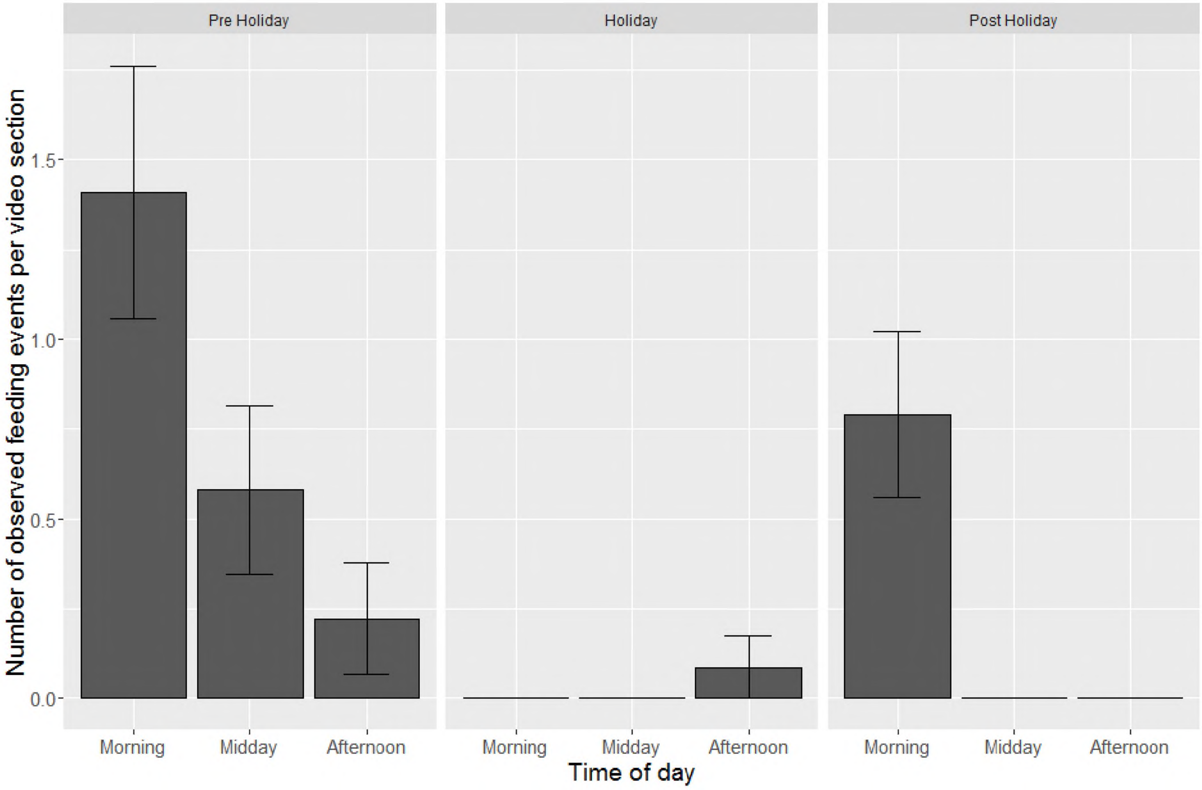


Figure 5.4. Mean (± se) number of feeding events per 10-minute video section observed during the behavioural analysis of *H. capensis* recorded from October 2015 to March 2016.

Seahorse interactions observed during this study were divided into four types: I) reciprocal courting behaviour between a male and a female; II) non-reciprocal courting behaviour between a male and female, instigated by the male; III) aggressive behaviour between two (usually male) or more seahorses; and IV) non-display interaction where seahorses occur in close proximity to each other (< 5 cm apart), but without any aggressive or courting behaviour. Only 4.0 % of the pooled video footage contained seahorse interaction behaviour. Interaction time varied significantly (F = 4.4, df = 2, p = 0.01) throughout the day, and all courting related interactions were noted during the morning. Interaction time between seahorses decreased significantly (F = 10.7, df = 2, P < 0.01) during the Post-holiday period compared to the Pre-holiday period only. Interaction time varied less within the longer-term periods, with significantly more (F = 5.5, df = 4, $p < 0.01$) time interacting during the morning compared to midday or the afternoon of the Pre-holiday period (Fig. 5.3).

Seahorses showed significantly more irritation behaviour in the afternoon compared to both the morning or midday (F = 3.2, df = 2, P =0.04). Irritation behaviour within the longer-term periods varied significantly (F = 2.8, df = 4, p =0.03) and seahorses spent significantly more time displaying irritation behaviour during the afternoon of the Holiday period (Fig. 5.3).

No significant differences were found in the stationary behaviour of *H. capensis* throughout the day, although significantly (F = 4,7, df = 2, p = 0.01) more time was spent stationary during the Post-holiday period compared to the Pre-holiday period (Fig. 5.3). Time spent moving from holdfast to holdfast varied little throughout the day or across longer-term periods (Fig. 5.3).

Sex of the focal seahorse could only be discerned in 41.0 % of the video sections examined and 44 and 42 independent female and male seahorses were identified and used in the sex comparison assessments. Pooled data showed no significant differences in visibility between male and female seahorses (Fig. 5.5), although females tended to be more visible than males in the morning. During interaction events, the male seahorse was used as the focal animal most of the time, which does not mean males interacted more than females *per se* as presented in Fig. 5.5. No significant difference between male and female seahorses were found in the number of observed feeding events nor in the mean time spent on a holdfast.

Sixty percent of male focal seahorses had an inflated brood pouch, indicative of breeding. Juveniles were noted on two occasions, both during the Holiday period.

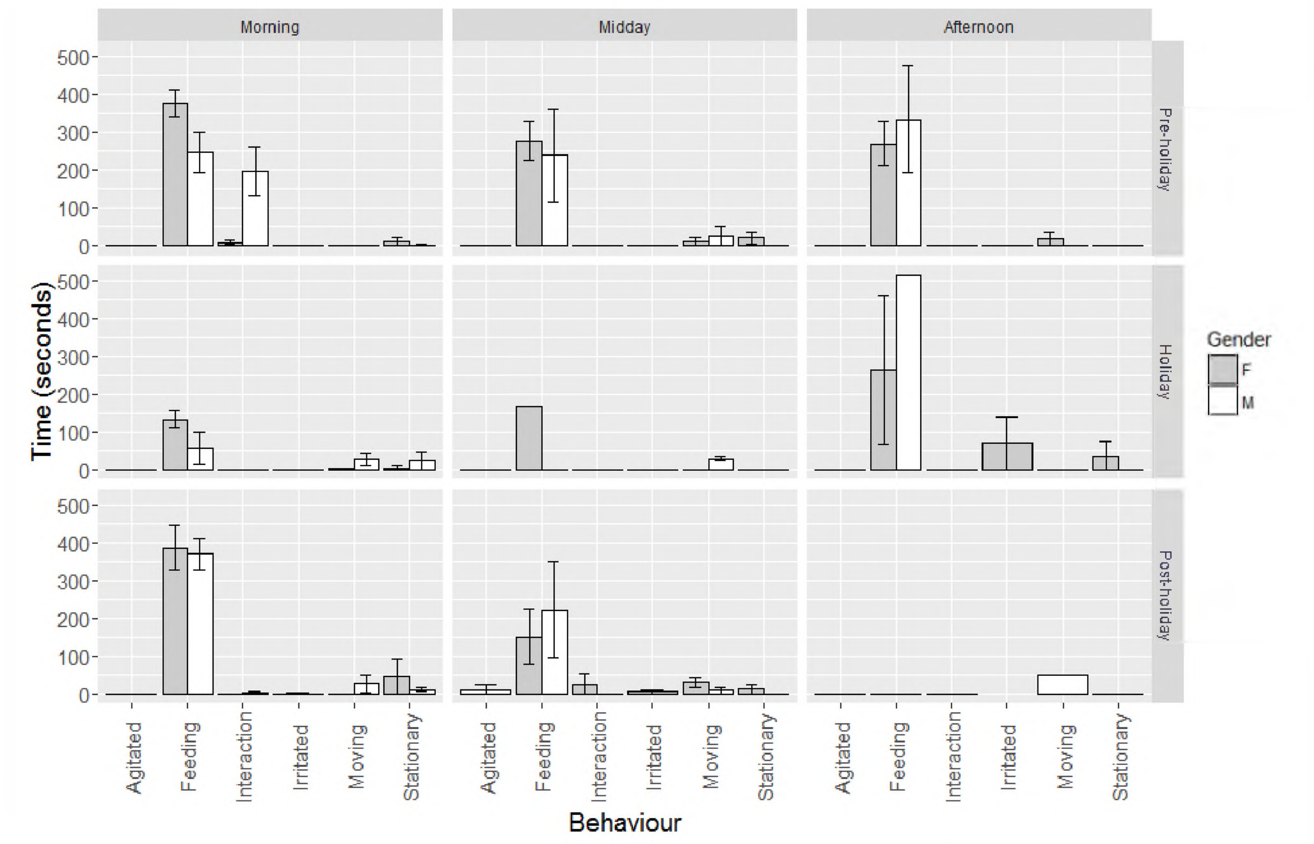


Figure 5.5. Behavioural differences (mean ± se) between male and female *H. capensis* throughout the day and across longer-term periods from October 2015 to August 2016.

The Cape cormorant (*Phalacrocorax capensis*) was the only observed predator feeding on seahorses (Videos 5.4.1 and 5.4.2) and all three feeding events were observed during the morning. Rays and cuttlefish were also observed during the behavioural assessment (Table 5.4), but predation on seahorses by these species were not noted. The number of boat passes varied significantly (F = 14.9, df = 2, $p < 0.01$) throughout the day with significantly more passes recorded during midday. Boat passes were significantly higher (F = 207.4, df = 2, $p < 0.01$) during the Holiday period compared to both Pre-holiday and Post-holiday periods. Boat passes were significantly higher during midday of the Post-holiday period and varied little during the Pre-holiday period (Fig. 5.6).

Table 5.4. Mean (± sd) potential seahorse predator density recorded as N_{max} per 10-minute video sections from October 2015 to August 2016.

Time period	Time of day	Cormorant (mean ± sd)	Ray (mean ± sd)	Cuttlefish (mean ± sd)
Pre-holiday	Morning	0.10 ± 0.47	0.16 ± 0.47	0.03 ± 0.16
	Midday	0	0	0.06 ± 0.25
	Afternoon	0	0.04 ± 0.19	0.07 ± 0.27
Holiday	Morning	0.05 ± 0.30	0.07 ± 0.26	0
	Midday	0	0.05 ± 0.22	0.10 ± 0.40
	Afternoon	0	0	0
Post-holiday	Morning	0.02 ± 0.13	0	0.03 ± 0.18
	Midday	0.07 ± 0.25	0.07 ± 0.25	0
	Afternoon	0	0	0.32 ± 0.54

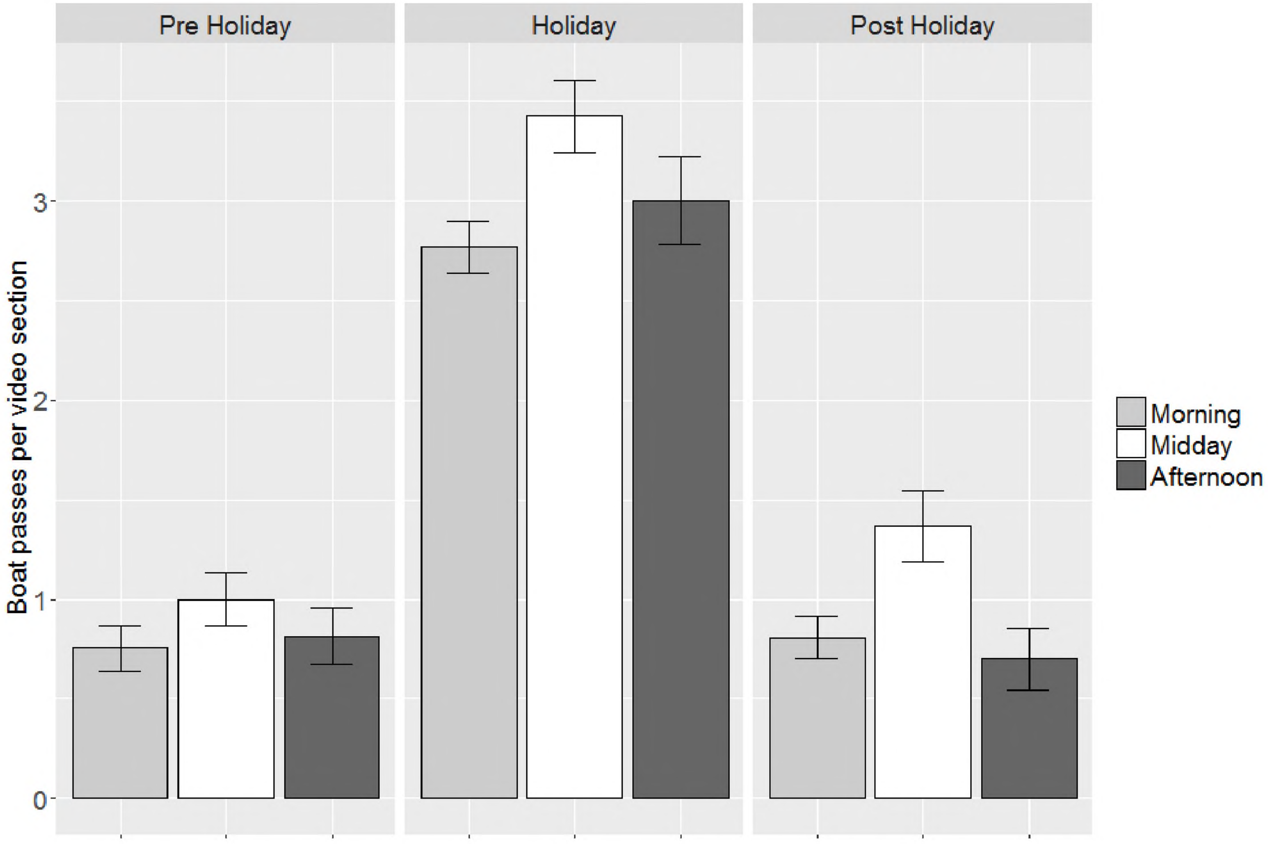


Figure 5.6. Mean (± se) number of boat passes recorded per 10-minute video sections throughout the day and across longer-term periods from October 2015 to March 2016.

5.4. Discussion

GoPro cameras were successfully used to study the *in situ* behaviour of *H. capensis* within a residential marina estate. Previously published studies of *in situ* seahorse behaviour (Vincent and Sadler, 1995; Moreau and Vincent, 2004; Curtis and Vincent, 2005; Felicio *et al.*, 2006; Naud *et al.*, 2009) have been from diver observations which could have resulted in observation bias. Cameras can be used to control for observation bias and should be considered in seahorse behavioural assessments – particularly in artificial habitats where the deployment of cameras is generally easier. An extensive literature review found no other instances of cameras being used during *in situ* seahorse behavioural assessments and it would appear that this is the first time that video cameras have been used to study *in situ* seahorse behaviour.

Hippocampus capensis was found to be more visible during the morning which suggests greater activity, particularly feeding, during this time. Activity then decreases throughout the day, a similar pattern found for *H. breviceps* in Australia (Moreau and Vincent, 2004). James and Heck (1994) found that a reduction in light levels significantly decreased prey capture rates in *H. erectus* and Felicio *et al.* (2006) found that the feeding frequency of *H. reidi* was directly proportional to luminous intensity. Nocturnal behaviour for this species is unlikely, owing to the decrease in light availability, although this aspect was not assessed during the present study as it was not possible to use GoPro cameras at night. Male/female interactions were limited to the morning only and courting behaviour included the clasping of tails, swaying and moving vertically through the water column as described by Grange and Cretchley (1995). Morning greetings to confirm pair bonds is a specific behaviour noted in monogamous seahorse species (Vincent, 1995) and is suggested to synchronize mating between male and female. Seahorses were not tagged in this study and it was not possible to individually track a specific seahorse. The morning courting behaviour could not be confirmed as “daily greetings” as described by Vincent (1995) and monogamous behaviour could not be confirmed.

No significant behavioural differences were noted between male and female *H. capensis* although, in general, female seahorses have been found to be more active than males (Freret-Maurer *et al.*, 2012). In particular, male *H. reidi* were found to be less active during their gestation period and females were more active in foraging behaviour (Freret-Maurer *et al.*, 2012). The decrease in productivity and movement in pregnant male seahorses, suggests a higher cost of production compared to females (Freret-Maurer *et al.*, 2012). Female seahorses have also been found to have larger home-ranges compared to males (Vincent and Sadler, 1995; Moreau and Vincent, 2004).

Aggressive behaviour has been noted in other seahorse species (*H. fuscus*, Vincent, 1994a; *H. guttulatus* – Naud *et al.*, 2009), specifically between males. Because aggression was only noted between male seahorses in this study, and only the male was seen to instigate courting, it suggests that *H. capensis* exhibits conventional sex roles, with male sexual selection, commonly associated with monogamous species (Kvarnemo *et al.*, 2007). Conventional sex roles have been found in most seahorse species (Mason-Jones and Lewis, 1996; Naud *et al.*, 2009), but Kvarnemo *et al.* (2007) did find female sexual selection in *H. subelongatus* in Australia owing to size assortative pairing and a female-biased adult sex ratio.

Seahorses tend to spend most of their time feeding and foraging (Felicio *et al.*, 2006), which was confirmed in the present study. Differences in foraging behaviour have been noted in some seahorse species (*H. erectus* – James and Heck, 1994), particularly between sympatric species (*H. hippocampus* and *H. guttulatus* – Curtis and Vincent, 2005). The foraging behaviour noted in *H. capensis* (Video 5.1) is consistent with other seahorse species (see Foster and Vincent, 2004), and from this assessment it is clear that the Knysna seahorse actively hunts for prey animals and is not a “sit and wait” predator as described for other seahorses (see Foster and Vincent, 2004).

Cormorants have previously been noted as a seahorse predator (see Foster and Vincent, 2004). In a review by Kleiber *et al.* (2011), rays are noted as potential seahorse predators (one of 82 predators identified) and although rays were prevalent during the morning, there were no recordings of rays feeding on *H. capensis.* Octopus and cuttlefish were found to prey on seahorses in Australia (Harasti *et al*., 2014a), but in this study, *H. capensis* was found to live alongside octopuses with no obvious signs of stress or avoidance, which suggests that octopus (*Octopus vulgaris*) do not prey on this species. Seahorses did, however, display avoidance behaviour in the form of hiding and suspending feeding to remain completely stationary in the presence of cuttlefish (*Sepia vermiculata*), but an actual feeding event was not noted.

Motor boats have been shown to have an impact on the biology and ecology of fishes, although the effects are species and size specific (Whitfield and Becker, 2014). According to Becker *et al.* (2013) underwater inhabitants of estuaries are particularly vulnerable to boat related impacts owing to the shallow and narrow nature of many estuaries and the high boating pressures, especially during holiday periods. Noise pollution is a concern for syngnathid populations, particularly in relatively sedentary seahorses, as avoidance of noise via movement is unlikely (Vincent *et al.*, 2011). Noise related stress, both acute and chronic, can not only lead to mortality but also sub-lethal effects which can result in suboptimal production (Anderson *et al.*, 2011). Noise has been found to increase stress levels in seahorses (*H. erectus* – Anderson *et al.*, 2011), and cause an increase in the occurrence of disease, longer gestation periods and fewer, smaller and slower growing offspring (*H. zosterae* – as referred to in Anderson *et al.*, 2011).

The marked decrease in the visibility of seahorses and time spent feeding during the December holiday season, might be explained by the increase in boat use in the marina and the related increase in noise. Thesen Islands Marina is a 'No Wake' zone, which probably lessens the impact from boats in the marina canals, as opposed to the impact of boats in the estuary with no speed limit. It is unlikely that the seahorses moved away from the Reno mattress habitat and it is thought the animals rather moved into the crevices of the mattresses, which decreased visibility. Boat noise has been found to have an effect on the breeding behaviour of certain fish species (Whitfield and Becker, 2014) and the dearth of courting behaviour observed during the holiday period might be ascribed to the increase in boat related noise. Some seahorse species have been found to be able to acclimate to external noise stressors (*H. erectus* – Anderson *et al.*, 2011), although the acclimation ability of *H. capensis* to increased boat noise still needs to be determined.

Interactions related to courting decreased during the holiday period. This is of concern because this period is part of the breeding season of *H. capensis*. There are, however, other factors that may have influenced seahorse behaviour at this time. For example, the decrease in time spent feeding and increase in time spent stationary, particularly during the Post-holiday period, could be ascribed to a lengthy upwelling event in which water temperature declined by ≈ 9 °C across a tidal cycle in December 2015 and January 2016, which could have led to a decrease in fish activity (Buxton and Smale, 1989; Hanekom *et al.*, 1989; Tilney and Buxton, 1999).

GoPro cameras were successfully used to investigate the behaviour of *H. capensis* within an artificial habitat. The results of this study suggest a possible relationship between increased boat noise and a change in behaviour of *H. capensis*, a relationship that needs further research to confirm and quantify. Increased activity and visibility of *H. capensis* during the morning should be considered in future surveys, to ensure more accurate population assessments. Behaviour was found to be a useful tool in the assessment of an external stressor e.g. boat noise, and the behavioural baseline for *H. capensis*, created during this study, can be used in future to ascertain the behavioural status of this population.

The use of video cameras to study seahorse behaviour presents a number of limitations: i) resampling of animals, ii) limitation in estimation of size, observation of sex and reproductive status and, iii) requirement of good water clarity. The efficacy of using this method in natural seagrass habitats also needs to be tested, as the ability to see a focal animal might be limited by dense vegetation. This method permits the revision of recorded data and viewing the sample in fast forward mode allows the establishment of behavioural patterns, which can be difficult to do during *in situ* diver observations owing to the slow movement of seahorses. The non-invasive nature of cameras is particularly useful in studying threatened species, as adverse impacts are minimal. The use of cameras in future seahorse behavioural assessments should be considered, particularly in the investigation of seahorse populations within artificial environments.

Chapter 6

General discussion, conservation recommendations and future research

6.1 *Hippocampus capensis*: here and now

Thesen Islands Marina, an artificial water body, provided 25 ha of additional suitable habitat for *H. capensis*. In particular, artificial Reno mattresses were found to be readily inhabited by *H. capensis*, and this habitat was chosen over *Z. capensis* by seahorses during an *in situ* choice experiment. This suggest that this artificial habitat provides suitable surrogate habitat to *H. capensis*. Whether these structures increase population biomass must, however, still be determined. This study provides the first density and distribution estimates of *H. capensis* within the Knysna estuary's bay regime, since 2001 (see Lockyear *et al.*, 2006), and significantly higher seahorse densities were consistently found within Reno mattress structures within Thesen Islands Marina. Habitat specific seahorse population densities did vary significantly over an eleven-month period with seasonal fluctuations, and distributions were patchy, common features of seahorse populations globally (see Correia *et al.*, 2015a).

Seahorse population trends and the interpretation thereof can depend on the time scale of the assessment, and in some instances what appears to be an overall decline in population abundance may be a factor of the assessment period. For example, Correia *et al.* (2015a) found a decrease in *H. guttulatus* and *H. hippocampus* in Portugal over a six-year period (see Curtis and Vincent, 2005; Caldwell and Vincent, 2012), but after eight years from the original assessment, the population was in a state of recovery.

Seahorse density can be markedly higher in artificial habitats (Harasti *et al.*, 2012; Gristina *et al.*, 2015; Otero-Ferrer *et al.*, 2015). These higher densities are ascribed to the increased complexity of some artificial structures (Hellyer *et al.*, 2011; Gristina *et al.*, 2015), the availability of safe and permanent holdfasts (Gristina *et al.*, 2015), increased prey availability (Hellyer *et al.*, 2011; Otero-Ferrer *et al.*, 2015), and increased protection and shelter against predators or fishing impacts (Gristina *et al.*, 2015; Otero-Ferrer *et al.*, 2015). Several reasons for the higher density of *H. capensis* on the Reno mattress habitat may include the availability of rigid and permanent holdfasts, shelter (crevices) against predators, and increased prey availability. The higher seahorse densities recorded during this study compared to those reported by Bell *et al.* (2003) and Lockyear *et al.* (2006) does, however, not necessarily equate to an overall population increase of *H. capensis.* Suitable seahorse habitat (mixed vegetation beds which consists of *Z. capensis* and *C. filiformis* (Teske *et al.*, 2007b)) within the estuary has decreased in the past few years owing to an extensive bloom of *Ulva lactuca* (Adams, 2016; Allanson *et al.*, 2016; Human *et al.*, 2016) and damage to seagrass meadows by bait diggers (pers. obs.). The decrease in the area of coverage of natural habitats within the larger estuary, and the confirmed suitability of the Reno mattress structures as habitat for *H. capensis,* can perhaps explain the high seahorse densities found on these structures as more seahorses are inhabiting a smaller area of available habitat. The variation in the population structure between habitat types (Reno mattress and mixed vegetation habitats had a female biased sex ratio and *C. tenue* an equal sex ratio) emphasises the importance of understanding the underlying processes which govern the ecology of species. Ongoing monitoring and experimental assessments are needed to establish which underlying processes are responsible for the higher seahorse densities in the Reno mattress habitat, long term population trends, and the preference shown by *H. capensis* for this artificial habitat.

The use of a mean seahorse density for the entire Knysna system, I believe, has led to a flawed estimation of the total population size of *H. capensis*. Both Bell *et al.* (2003) and Lockyear *et al.* (2006) extrapolated their mean seahorse density results to estimates for the entire Knysna estuary. I suggest that this method is highly inaccurate because vegetation, a prerequisite for the occurrence of *H. capensis* (Teske *et al.*, 2007b), is not distributed evenly within the estuary. According to Bell *et al.* (2003), their total population estimate of 89 000 seahorses within the Knysna estuary was preliminary and should be used with caution as it did not take the variability of vegetation cover in the system into consideration. Only between 238 ha (Adams, 2016) and 390 ha (Barnes and Ellwood, 2011) of the 10 km^2 Knysna estuary is covered by subtidal vegetation, most of which consists of *Z. capensis*. Using the conservative subtidal vegetation coverage estimate of 238 ha and the mean seahorse densities recorded by Bell *et al.* (2003) and Lockyear *et al.* (2006), the total *H. capensis* population estimate would be 21

182 and 33 320 seahorses respectively, far less than what was originally estimated by these authors (89 000 and 62 120 seahorses). The problem of using mean seahorse densities for an entire system and extrapolation should be evident, especially when one considers that seahorse densities vary within different, and even the same, habitat types (Harasti *et al.*, 2014b; Otero-Ferrer *et al.*, 2015; this study). It is for this reason that conservation and management of this endangered species would make more sense at a habitat level, as opposed to a general, one solution fits all basis.

The last IUCN assessment of *H. capensis* (Czembor and Bell, 2012) stated that this species occupies an area of only 27.0 km^2. The authors of the assessment concede that this estimate is probably too liberal, particularly when one considers that only 2.4 km^2 of the Knysna estuary's subtidal area (the largest system occupied by *H. capensis*) is covered by vegetation (Adams, 2016). The observed adaptability of *H. capensis* to habitat alteration and addition by no means frees them from their endangered status, although, the reasons behind the need for this classification have shifted. Seagrass habitats are still under threat within the Knysna, Keurbooms and Swartvlei estuaries (Adams, 2016), and *H. capensis* is therefore threatened by the loss of these habitats. The ability of *H. capensis* to utilise different habitat types enhances the survival potential of this species. It might, however, be argued that the high seahorse densities within Thesen Islands Marina, found in a relatively small area (particularly Sites 2 and 5 within the marina as per Figs. 2.3 and 3.1), leave these populations particularly vulnerable to exploitation, pollution events, or other human impacts (see Correia *et al.*, 2015b).

The behavioural assessment found that increased boat noise within the marina during holiday periods may have an adverse impact on the breeding and feeding behaviour of these seahorse populations. Further research on the communal living of *H. capensis* and humans, and whether this arrangement is viable in the long run, is required. Such results could be of relevance on a global seahorse conservation scale, particularly in areas where humans and seahorses have encroached into each other's space (Curtis and Vincent, 2005; Harasti *et al.*, 2012, Aylesworth *et al.*, 2015; Correia *et al.*, 2015a; Gristina *et al.*, 2015; Otero-Ferrer *et al.*, 2015).

Habitat provision is usually not a consideration when planning marina developments. In fact, these developments are rather known for adverse impacts on the surrounding environment which include habitat loss and/or fragmentation (Bulleri and Chapman, 2010), spread of alien invasive species (Arenas *et al.*, 2006), and alteration of natural species composition (Connell and Glasby, 1999; Connell, 2001; Bulleri and Chapman, 2010). Poor water quality is another major concern related to marina developments (Cosser, 1989; Davies and Stewart, 1984; Maxted *et al.*, 1997). In the case of Thesen Islands Marina, roughly 25 ha of estuarine habitat was added to the Knysna estuary and the development can be classified as an artificial water body (terminology European Commission, 2003). This additional estuarine area was found to contain viable seahorse habitat with adequate water quality.

Studies to identify the ecological processes responsible for particular patterns of distribution of fish in artificial habitats are few (Clynick, 2008a). In the present study, the underlying process of habitat choice by *H. capensis* was investigated and Reno mattresses were confirmed to be suitable surrogate habitat for this species. It was found that *H. capensis* prefers an artificial Reno mattress structure to *Z. capensis* (a known habitat for this species (Teske *et al.*, 2007b)) when given a choice. A number of different habitat types within Thesen Islands Marina, however, were utilized by *H. capensis*, which ranged from artificial Reno mattress to dense *C. tenue* beds, each with very different physical and biological features. Dense *C. tenue* within Thesen Islands Marina is characterised by limited seahorse prey availability, large diurnal dissolved oxygen fluctuations (with hypoxic conditions commonly found during the night), and non-permanent holdfasts because the algae is unattached. Artificial Reno mattress habitats within the marina, by contrast, contains a myriad of potential seahorse prey animals, well oxygenated water, permanent holdfast structures and a large number of crevices. Not only are these two habitats different, but they also occur in different environments. *Codium tenue* was found within low flow, dead-end canals where the alga smothered everything, whereas visible Reno mattresses were found within canals characterised by higher water flow. Both were, however, readily inhabited by *H. capensis*, despite physico-chemical and biological differences. *Hippocampus capensis* is a habitat generalist, a fact confirmed by the ability of this species to utilize new and various habitat types. For example, *Asparagopsis taxiformis,* first recorded in the Knysna estuary in 2009 (Bolton *et al.*, 2011), is readily used by *H. capensis* as a holdfast, and artificial Reno mattress, also a new addition to the estuary, is confirmed as being favoured by this species. Similar adaptability was observed in *H. guttulatus* in both the Ria Formosa, Portugal, and Mar Piccollo, Italy, (Correia *et al.*, 2015a; Gristina *et al.*, 2015), and *H. whitei* (Clynick, 2008b; Harasti *et al.*, 2012) in Sydney Harbour, Australia. This adaptability could be key to future conservation endeavours, and alternative, artificial habitats could be central to the conservation of those seahorse species negatively impacted by habitat loss. It is clear from this study that Thesen Islands Marina provides valuable habitat to *H. capensis* and acts as a kind of refuge against habitat loss within a larger, variable estuary, a stark contrast to the anticipated impact of the development prior to completion (Lockyear *et al.*, 2006). Artificial structures were favoured by seahorses in the Mar Piccolo, Italy, a heavily polluted and impacted system (Gristina *et al.*, 2015). *Hippocampus whitei* colonized swim nets in Sydney Harbour, Australia, (Clynick, 2008b) and *H. gutulatus* populations recovered in the Ria Formosa, Portugal, owing to the utilization of different, and in many instances artificial, habitats (Correia *et al.*, 2015a). Many seahorse species are thus able to adapt to changes to their natural habitats. In most, if not all cases, the usefulness of artificial structures as seahorse habitat was serendipitous. The results from the present study indicates that building material in the form of gabions and Reno mattresses, known to provide habitat to at least one seahorse species, should be considered in future coastal developments, such as the development of increasingly popular residential marina estates. Habitat creation can then be a proactive goal of coastal developments, as opposed to a reactive consequence.

The *modus operandi* of *in situ* seahorse behavioural research entails observations made by a diver. This study presents an alternative method using GoPro cameras as a means to investigate seahorse behaviour. The sedentary behaviour and small home ranges of most seahorse species should allow successful observations using cameras. The cryptic nature of seahorses could, however, present a possible problem with this technique as well as the inability to use standard GoPro cameras during the night. Nevertheless, the non-invasive nature of cameras makes them particularly useful in researching threatened species. Behaviour was successfully used to assess the potential impact of an external stressor (boat noise) on the Knysna seahorse. This assessment was conducted *in situ*, which ensured the authenticity of the observed seahorse behaviour. The baseline behavioural information generated by this study can be used in future to assess the status of this seahorse population.

Several known biological features of *H. capensis* have been confirmed by this study, while new information was also generated. Table 6.1 presents a summary of historic biological information of *H. capensis* compared to findings from this study.

Table 6.1. Summary of historic knowledge of *H. capensis* and knowledge generated during this study.

Hippocampus capensis **Historic information**	***Hippocampus capensis*** **Findings of current study**
Maximum length 12 cm. (Whitfield, 1995; Lourie *et al.*. 2004)	Mean (± sd) height 6.7 ± 1.3 cm and maximum recorded height of 11 cm.
Depth range of 0.5 to 20 m. (Whitfield. 1995)	Seahorses found at a depth of 14 m in the Knysna Estuary (pers. obs.). Minimum depth recorded was < 30 cm (with intermittent exposure during spring low tides).
Breeds from September to April. (Lourie *et al.*. 2004)	Males with inflated brood pouches (indicative of breeding) noted from October to March.
Conventional sex roles with male sexual selection. (Fourie, 1997)	Aggressive behaviour noted between males, males noted to always instigate courting – suggests male sexual selection.
No behavioural information available.	Ethogram for *H. capensis* established. More active/visible during the morning, spends majority of time (≈ 80 %) feeding. Increased boat noise found to potentially affect feeding and courting behaviour.
Male biased sex ratio found during a transect survey and an even sex ratio during a focal grid study (2000); even sex ratio found during a transect survey (2001). (Bell *et al.*, 2003; Lockyear *et al.*, 2006)	Female biased sex ratios found within artificial Reno mattress and mixed vegetation habitats; even sex ratio found within *Codium tenue* beds.
Vegetation used by this species include: *Caulerpa filiformis, Codium extricatum, Halophila ovalis, Ruppia cirrhosa and Zostera capensis.* (Teske *et al.*, 2007b)	Vegetation used by this species included: *Z. capensis*, *H. ovalis*, *C.filiformis*, *Codium tenue*, *Ulva lactuca*, *Asparagopsis taxiformis and Polysiphonia* sp.
No report of artificial habitat use or any preference for a specific habitat type. (Teske *et al.*, 2007b)	Used artificial habitats and showed a preference for Reno mattress habitats.

6.2 Seeing into the future: conservation and management implications

A recurring theme in coastal and estuarine research is the effects of habitat loss and alteration on species diversity, abundance, and composition (Connell and Glasby, 1999; Bulleri and Chapman, 2010; Dugan *et al.*, 2011; Aylesworth *et al.*, 2015; Harasti, 2016). In many instances, such research goes hand in hand with a plea to conserve natural habitats, and marine conservation actions usually focus on the protection of natural habitats e.g. Marine Protected Areas. Potential does, however, exist in the use of artificial habitats for species conservation (Garcia-Gomez *et al.*, 2011), and in some instances artificial structures can be viewed as a tool to ensure the conservation of endangered or vulnerable species (Guerra-Garcia *et al.*, 2004; Clynick, 2008b). More effective enforcement of species protection on artificial structures is possible because these structures generally do not attract public interest, and structures can be monitored closely owing to their locality (Garcia-Gomez *et al.*, 2011). Reno mattresses are introduced here as a potential tool that can be used in the conservation of *H. capensis*.

Formal protection of the seahorse population within Thesen Islands Marina, particularly at Sites 2 and 5 (Figs. 2.3 and 3.1), should be considered. Specifically, the Reno mattresses in these two sites should be classified as an Artificial Marine Micro-Reserve (terminology Garcia-Gomez *et al.*, 2011; Garcia-Gomez *et al.*, 2015). An important aspect of a micro-reserve is its small size (usually < 20 ha) and its conservation success depends on a small home range of the species being conserved. Although the home range of *H. capensis* is not known, in general, seahorses maintain a relatively small home range (usually < 20 m^2) (Rosa *et al.*, 2007; Vincent *et al.*, 2005), and are thus promising candidates to benefit from Artificial Marine Micro-Reserves. Micro-reserves need to be exposed to clean marine water and should be permanent in nature (Garcia-Gomez *et al.*, 2011) – all features of the Reno mattress habitat within the western section of Thesen Islands Marina. Maintenance activities common to a marina, such as jetty maintenance, should then be managed and monitored accordingly using a reserve management plan. Co-existence between humans and seahorses is possible (Harasti *et al.*, 2010), and Thesen Islands Marina and the Reno mattresses found within this system provide a perfect opportunity to conserve this endangered seahorse species via the implementation of artificial reserves. The establishment of Artificial Marine Micro-Reserves should however not be done in isolation, as protection and rehabilitation of natural environments remain important. The proposed approached of using artificial structures does not suggest that these structures should be deployed "in the name of conservation", and rehabilitation and protection of natural habitats should always remain the first priority (Harasti, 2016).

Presented below is a number of conservation actions that should be considered:

1. Proclaim the Reno mattress habitats within the western section of Thesen Islands Marina as an Artificial Marine Micro-Reserve.
2. Conservation measures of these reserves should include:

- Prevention of human impacts such as walking on or damaging the structures.
- Limitation of boat activity near the reserves, in particular, on top of the Reno mattress structure.
- Continued monitoring of the seahorse populations found in these reserves, which should include water quality and habitat monitoring to ensure a healthy overall environment for the population. Ongoing monitoring should particularly investigate the reproductive success of seahorse populations on artificial structures.

- Involvement of owners of shops, commercial boat operators, restaurants as well as marina residents located next to the reserves to promote a joint conservation approach.

3. Monitor the extent of natural seahorse habitat within the Knysna estuary and the related seahorse populations found in these habitats and implement measures to prevent adverse human impacts, such as bait digging and damage from boats, on these habitats.

4. Investigate the usefulness of Reno mattress structures in the rehabilitation of damaged natural seahorse habitat within the Knysna estuary (and the Keurbooms and Swartvlei estuaries) and the potential to use these structures to promote the recovery of seahorse populations.

These conservation actions will be presented to management agencies responsible for the Knysna estuary (SANParks and Knysna Municipality) as well as to the Thesen Islands Marina Home Owners Association and the Thesen Islands Harbour Town Association in the hope that implementation will take place.

6.3 The way forward

Future research on *H. capensis* should be focused on three aspects: I) Population dynamics (which should include an estimation of site fidelity and home range, population size, age and growth, fecundity and survival rates, and population structure); II) habitat (with a specific focus on ontogenetic habitat use); and III) behaviour.

To ensure sound and successful conservation, the entire area and habitat types required by a species (for all life stages) should be conserved. To do this, the home range of a species is a key requirement. The home range of *H. capensis* could be assessed by using Visible Implant Fluorescent Elastomer (VIFE) tags (Woods and Martin-Smith, 2004; Harasti *et al.*, 2012). Tagging would allow various aspects of *H. capensis* to be studied e.g. population range, population abundance, natural mortality, length at age, length and age at maturity, life span, monogamy, and seasonal reproduction (Harasti *et al.*, 2012). All these aspects should be compared between seahorse populations within Thesen Islands Marina and the larger Knysna estuary. Many seahorse species have a small home-range (e.g. 1 m^2 range recorded for *H. comes* – Perante *et al.*, 2002; home range of *H. breviceps* range from 1 to 12 m^2 – Moreau & Vincent, 2004) and show strong site fidelity (e.g. *H. whitei* - Harasti *et al.*, 2014b). Home-range, movement and habitat use can, however, vary between different life stages (Curtis and Vincent, 2005; Morgan and Vincent, 2007; Harasti *et al.*, 2014b) and locations (Harasti *et al.*, 2014b). The establishment of the home-range and site fidelity of *H. capensis* would allow immediate management recommendations and specific conservation areas could be identified which would allow effective conservation of entire seahorse populations. Focus could be placed on ontogenetic and seasonal differences in home-range extent and habitat use. Without this basic understanding of seahorse movement and range within the Knysna estuary, effective conservation of the species is impossible – as specific conservation areas cannot be identified and the threat of habitat fragmentation remains.

This study found that Reno mattresses is a preferred habitat type of *H. capensis* and that this habitat type is extensively used by this species within an artificial water body. The underlying processes responsible for this preference need to be assessed (e.g. what features of the Reno mattress make it preferential?). Another question that warrants an answer is whether these structures can be used to enhance habitat in natural areas where seagrass beds have been damaged? And, if so, are there any requirements in the successful use of these structures (e.g. adequate water flow, minimum size requirement of artificial structure to be successful, adverse side effects in using these structures). Perhaps any marina or harbour development, regardless of the construction material used, is beneficial to seahorses. An investigation of seahorse populations within other marina and harbour developments located within the Knysna estuary, particularly those that were not constructed from gabions and Reno mattresses, will help to prove (or disprove) the conclusions made in this study. The efficacy of using Reno mattress structures in international seahorse conservation endeavours should also be considered, particularly as a tool to re-establish seahorse populations (Correia *et al.*, 2015b; Harasti, 2016).

The initial behavioural assessment of *H. capensis* concluded that increased boat noise has an adverse effect on the behaviour of *H. capensis*, in particular on its feeding and courting behaviour. A lengthy upwelling event experienced during the behavioural assessment could, however, have confounded these results. An *in situ* experiment, with suitable controls, would be able to confirm (or refute) the observations made in this study.

6.4 Final thought

The Knysna seahorse has been able to survive within the Knysna estuary since its discovery in 1900, despite the highly dynamic nature of this system and increasing anthropogenic pressure, in particular, the expansion of the built environment and human population. If the historic rate of anthropogenic development within the Knysna estuary continues, a tipping point might be reached, which could have dire consequences for the Knysna seahorses within this system. Practical conservation action is needed to ensure that the extant Knysna seahorse population evident in 2016 prevails.

Appendix A

Videos of seahorse behaviour

Appendix B presents video examples of the seahorse behaviour recorded during the behavioural assessment of *Hippocampus capensis* discussed in Chapter 5. Five videos are available on the attached USB drive.

Video 5.1 (1:55 min): Feeding behaviour

This video is presented in fast forward mode. In this video a pregnant male is seen feeding. Feeding events can be observed by a sudden jerk of the head (0:10 min; 0:11 min), and the expulsion of sediment from the gills (0:34 min; 0:38 min). The seahorse is clearly seen foraging all the while holding onto a holdfast. Feeding takes place from the adjacent structures (epibenthic prey). At 1:38 min he moves to another holdfast and continues to forage for prey animals.

Video 5.2 (1:21 min): Courting behaviour

The courting behaviour between a female and male (pregnant) seahorse is presented in this video. The seahorses grasp each other’s tails, move alongside each other and sway from side to side. A third seahorse (pregnant male) tries to disrupt the courting behaviour (0:15 min), but the pair prevail. The wire of the Reno mattress, used as a holdfast, can clearly be seen and a lot of the courting behaviour takes place within the crevices of the mattress.

Video 5.3 (1:06 min) Aggressive behaviour

This video is presented in fast forward mode. The video begins showing a pregnant male seahorse within the Reno mattress habitat, feeding. At 0:28 min a second seahorse (also a pregnant male) enters the field of view in the bottom right-hand corner. At 0:40 min the first seahorse notices the second and begins to 'chase' the intruder away. An example of head butting can be seen at 0:55 min. The original seahorse manages to chase the intruder away at the end of the video.

Videos 5.4: Cormorant predation

Video 5.4.1 (0:18 min):

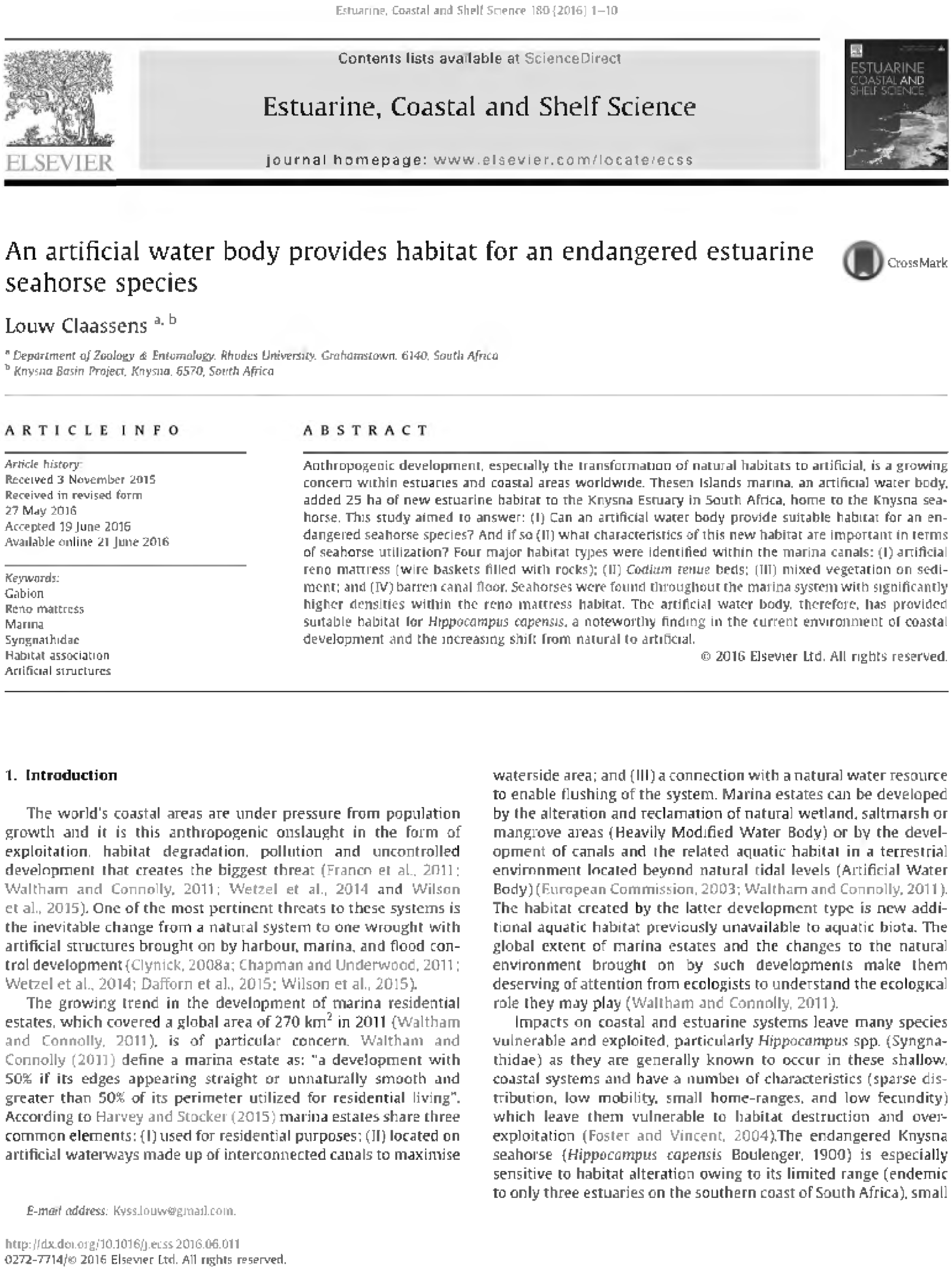
This video is presented in slow motion. The seahorse is located in the top left-hand corner of the field of view and the cormorant enters from the left (0:09 min). The seahorse is grabbed by the cormorant at 0:10 min and pulled from its wire holdfast, clearly seen at the end of the video.

Video 5.4.2 (0:08 min):

This video is presented in slow motion. Two seahorses are visible in the field of view (centre). The cormorant enters from the bottom left at 0.03 min and grabs the second seahorse located further back and the seahorse is pulled from its wire holdfast.

Appendix B

Publication



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An artificial water body provides habitat for an endangered estuarine seahorse species

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ABSTRACT

Anthropogenic development, especially the transformation of natural habitats to artificial, is a growing concern within estuaries and coastal areas worldwide. Thesen Islands marina, an artificial water body, added 25 ha of new estuarine habitat to the Knysna Estuary in South Africa, home to the Knysna seahorse. This study aimed to answer: (I) Can an artificial water body provide suitable habitat for an endangered seahorse species? And if so (II) what characteristics of this new habitat are important in terms of seahorse utilization? Four major habitat types were identified within the marina canals: (I) artificial reno mattress (wire baskets filled with rocks); (II) *Codium tenue* beds; (III) mixed vegetation on sediment; and (IV) barren canal floor. Seahorses were found throughout the marina system with significantly higher densities within the reno mattress habitat. The artificial water body, therefore, has provided suitable habitat for *Hippocampus capensis*, a noteworthy finding in the current environment of coastal development and the increasing shift from natural to artificial.

1. Introduction

The world's coastal areas are under pressure from population growth and it is this anthropogenic onslaught in the form of exploitation, habitat degradation, pollution and uncontrolled development that creates the biggest threat (Franco et al., 2011; Waltham and Connolly, 2011; Wetzel et al., 2014 and Wilson et al., 2015). One of the most pertinent threats to these systems is the inevitable change from a natural system to one wrought with artificial structures brought on by harbour, marina, and flood control development (Clynick, 2008a; Chapman and Underwood, 2011; Wetzel et al., 2014; Dafforn et al., 2015; Wilson et al., 2015).

The growing trend in the development of marina residential estates, which covered a global area of 270 km^2 in 2011 (Waltham and Connolly, 2011), is of particular concern. Waltham and Connolly (2011) define a marina estate as: "a development with 50% if its edges appearing straight or unnaturally smooth and greater than 50% of its perimeter utilized for residential living". According to Harvey and Stocker (2015) marina estates share three common elements: (I) used for residential purposes; (II) located on artificial waterways made up of interconnected canals to maximise waterside area; and (III) a connection with a natural water resource to enable flushing of the system. Marina estates can be developed by the alteration and reclamation of natural wetland, saltmarsh or mangrove areas (Heavily Modified Water Body) or by the development of canals and the related aquatic habitat in a terrestrial environment located beyond natural tidal levels (Artificial Water Body) (European Commission, 2003; Waltham and Connolly, 2011). The habitat created by the latter development type is new additional aquatic habitat previously unavailable to aquatic biota. The global extent of marina estates and the changes to the natural environment brought on by such developments make them deserving of attention from ecologists to understand the ecological role they may play (Waltham and Connolly, 2011).

Impacts on coastal and estuarine systems leave many species vulnerable and exploited, particularly *Hippocampus* spp. (Syngnathidae) as they are generally known to occur in these shallow, coastal systems and have a number of characteristics (sparse distribution, low mobility, small home-ranges, and low fecundity) which leave them vulnerable to habitat destruction and over-exploitation (Foster and Vincent, 2004).The endangered Knysna seahorse (*Hippocampus capensis* Boulenger, 1900) is especially sensitive to habitat alteration owing to its limited range (endemic to only three estuaries on the southern coast of South Africa), small

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population size, and habitat vulnerability (Whitfield, 1995; Bell et al., 2003; Lockyear et al., 2006; Teske et al., 2007). Changes to natural estuarine systems owing to development and the replacement of seagrass meadows with hard structures have the potential to seriously affect this species (Whitfield, 1995; Teske et al., 2007). How seahorses adapt to habitat alterations and additions, if at all, is an important aspect which must be understood to ensure successful future conservation (Vincent et al., 2011).

The Knysna Estuary (Fig. 1), an estuarine embayment (Whitfield, 1992), is located on the southern coast of South Africa (34° 4′56.19″S, 23° 3′34.85″E) and is considered to be the country's most important estuary in terms of biodiversity (Turpie et al., 2002). Three residential marina estates have been developed in the estuary in the past ten years of which Thesen Islands Marina (Figs. 1 and 2) is the largest (≈25 ha of canals). Thesen Islands Marina can be classified as an Artificial Water Body (European Commission, 2003) as it was developed on an existing island within the Knysna Estuary.

Early anecdotal observations of seahorses within the marina system allowed an opportunity to investigate the adaptability of *Hippocampus capensis* to anthropogenic impacts on and additions to its natural environment. Two questions were considered: (I) Can artificial water bodies provide suitable habitat for an endangered seahorse species? And if so (II) what characteristics of this new habitat are important in terms of seahorse utilization? The physico-chemical and habitat features of the marina are described, together with a population assessment of *H. capensis*. This is the first study of *H. capensis* in the Knysna Estuary since the last population survey of 2001 (Lockyear et al., 2006) which was undertaken prior to the development of Thesen Islands Marina.

2. Materials and methods

2.1. Study species

Hippocampus capensis is an estuarine seahorse species found exclusively in the Knysna, Swartvlei and Keurbooms Estuaries, all in close proximity to each other on the south coast of the Western Cape of South Africa. It was the first seahorse species to be listed as Endangered on the IUCN Red Data List in 2000 (Hilton-Taylor, 2000). The Knysna seahorse has a maximum recorded standard length of 12 cm (Whitfield, 1995) with an adult range of 5.3–11.2 cm (Lourie et al., 2004) and is found in depths of 0.5 m–20 m (Whitfield, 1995). It breeds from late September to early April when water temperatures rise above 20 °C (Lourie et al., 2004). Sexual maturity is reached within a year at a standard length of 6.5 cm and a gestation period of two to four weeks (dependant on water temperature) has been recorded (Grange and Cretchley, 1995; Whitfield, 1995; Lockyear et al., 1997; Lourie et al., 2004).

2.2. Study site

Thesen Islands Marina (34° 2′47.16″S, 23° 3′18.84″E) is a 90.6 ha residential marina estate located on Thesen Island (Figs. 1 and 2) in the lower bay regime of the Knysna Estuary (Largier et al., 2000; Rademeyer, 2008). The marina development commenced in 2000 and was completed in 2005. Six hundred housing units were built on the island with 25 ha of newly created canals. The marina is connected to the estuary by two wide access entrances – one at the

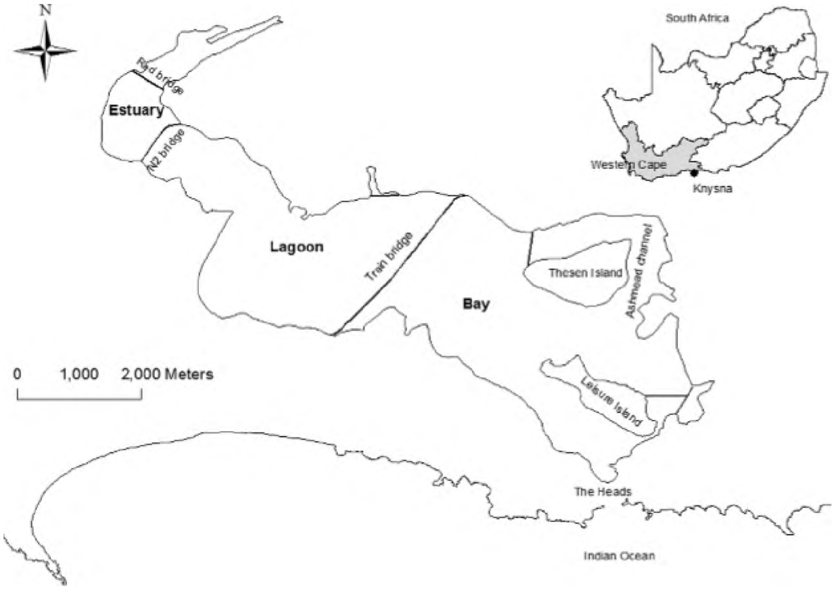

Fig. 1. Locality of Knysna (insert) and the layout of the Knysna Estuary. The bay regime stretches from the Heads to the train bridge; the lagoon regime from the train bridge to the N2 bridge and the estuary regime from the N2 bridge up to the Knysna River (Largier et al., 2000). The Ashmead channel lies on the eastern side of Thesen Island (Switzer, 2003). Both Thesen and Leisure Islands are situated within the bay regime.

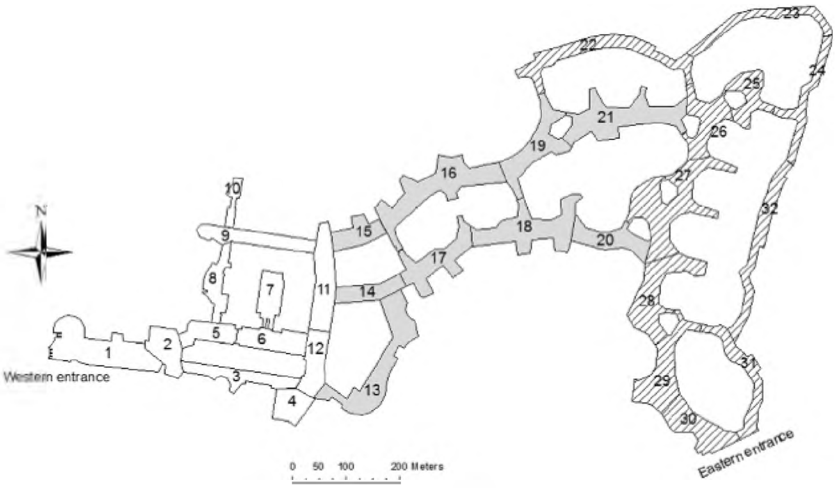

Fig. 2. The canals of Thesen Islands Marina can be divided into three zones along the west–east gradient of the marina: (I) western high velocity zone (white); (II) the middle cross-over zone (grey); and (III) eastern low velocity zone (hatched). The canals were also divided into 32 smaller sample sites. Numbering starts on the western section of the marina.

western end connected to the main channel of the Knysna Estuary and one at the eastern end located off the Ashmead Channel (Fig. 2). The marina canals, excavated to −1.75 mean sea level, were built from vertical gabion walls which rest upon a 2 m wide horizontal reno mattress. Gabions are wire cages filled with rocks (Maccaferri, 1915) used in hydrological and environmental engineering applications such as stormwater control, erosion prevention and canal linings. Additional geotextile material is used as an extra lining to promote sediment retention and erosion control. A reno mattress refers to a horizontal gabion structure used in the lining of canal beds. Few estuarine developments use gabion structures as material of choice and no ecological research studies on the suitability of these structures as novel habitats for estuarine fauna were found.

An earlier hydrographical study of the marina (Schumann, 2004) showed no signs of tidal asymmetry with a free exchange of water in the system. There was no significant difference in tidal amplitude between the western and eastern section of the marina, but a lag time of 10 min between the two sections was noted. Temperatures were found to be similar to that of the adjacent estuary (Schumann, 2004).

2.3. Physico-chemical features

Thesen Islands Marina can be divided into three distinct current velocity zones: (I) western high velocity zone; (II) the middle cross-over zone; and (III) eastern low velocity zone (Schumann, 2004) (Fig. 2). Ten sampling stations, increasing in distance from the western marina entrance (Fig. 2), were selected to assess the physico-chemical features of the marina. Samples were collected throughout the year. Dissolved oxygen concentrations and temperature were measured along a vertical gradient of 0.5 m with a YSI 550A dissolved oxygen meter (Yellow Springs Incorporated, Yellow Springs, Ohio). Surface turbidity samples were taken at each station and measured with a Eutech TN-100 turbidity meter (Thermo Scientific, Singapore). Vertical conductivity, temperature and depth profiles were taken at each station during high tide using a Sea-Bird Standard Electronic 19-03 CTD (Sea-Bird Electronics, Seattle).

2.4. Habitat description

An aquatic vegetation survey took place in the summer and winter of 2014 to obtain an overview of the likely habitats found in the marina canals. Haphazardly located transects (N = 72) with a mean length and search width of 35 m and 1 m respectively were surveyed by snorkelling across the width of the canals. The dominant vegetation type and percentage cover were estimated by the diver across each transect.

Major habitat types were assigned to one of the following four categories (Fig. 3):

I Reno mattress habitat. This habitat type is located along the canal edges. The wire and rocks used to fill the mattress are visible. The 2 m wide habitat is exposed to the atmosphere every spring low tide for a period of up to two hours. The dominant vegetation types found include *Asparagopsis taxiformis* (an invasive red alga species from Indo-Pacific origin (Bolton et al., 2011)) and *Polysiphonia* sp.

II *Codium tenue* beds. These macro-algal stands collect within low current velocity areas such as inlets and dead-end canals within marinas and harbours. The dense vegetation layer lies unattached on the canal bottom and varies in density and thickness. Large diurnal fluctuations in dissolved oxygen concentrations take place with super saturation (120% saturation) during the day and hypoxic (<20% saturation) conditions at night.

III Mixed vegetation (*Zostera capensis*, *A. taxiformis*, *Polysiphonia* sp, *Caulerpa filiformis*, and *Halophila ovalis*) on sediment. This habitat type is generally found in the middle of the marina canals and shares similar characteristics to the natural seagrass beds found in the larger estuary (Bell et al., 2003; Lockyear et al., 2006; Teske et al., 2007).

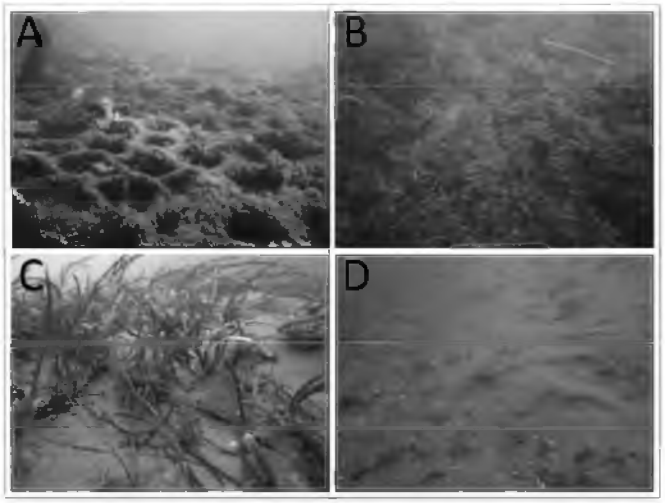

Fig. 3. Four major Habitat types identified within Thesen Islands Marina: A: (I) Reno mattress habitat (wire baskets filled with rocks); B: (II) Dense *C. tenue* beds; C: (III) Mixed vegetation on sediment and D: (V) Barren canal floor.

IV Barren canal floor with sparse vegetation or broken shell beds. The western zone of the marina is characterised by marine sediment and large areas of broken shell beds. The character of the sediment changes to mud and fine silt along the west-east gradient.

2.5. *Hippocampus capensis* population assessment

2.5.1. *Habitat types I (reno mattress) and III (mixed vegetation)*

A preliminary seahorse population assessment took place from August (late austral winter) to October (early austral spring) 2014. The marina was subdivided into 32 smaller sample sites (Fig. 2) to ensure a systematic sample approach. Structural features of the marina were used for this demarcation. Underwater visual survey transects as per Curtis et al. (2004) using SCUBA were used. The preliminary assessment focused on Habitat type I situated along the canal edges, and type III in the middle of the canals, using a stratified sampling design. Twelve sites (Fig. 4) were sampled during high tide in depths which ranged from 1.5 to 3 m. Ten transect samples, 10 m long with a 1 m search width (100 m^2), were surveyed per habitat type at each sample site by a single diver and a total area of 2230 m^2 was surveyed. The coordinates of each transect, taken with a handheld Garmin GPSmap 62, and the direction, chosen arbitrarily, were logged. Surface water temperature and turbidity were measured at each site.

The dominant vegetation type and percentage cover were estimated by the diver for each 10 m^2 transect. Height (Lourie, 2003), sex, brood pouch status and holdfast used by each specimen found were recorded. Males were identified by the presence of a brood pouch. Animals larger than 4 cm with no brood pouch were considered to be females. An individual which showed no sexual differentiation and was smaller than 4 cm was considered to be juvenile (Lockyear et al., 2006). The same methods were used in subsequent seasonal seahorse surveys within Habitat type I (January and June 2015).

2.5.2. *Habitat type II (C. tenue beds)*

Underwater visual survey transects (Curtis et al., 2004) were not suitable for use in Habitat Type II because animals were found to hide within the algal mass. To overcome this constraint an alternative sampling method was established. A net (38 cm × 19 cm × 30 cm) was used to collect *C. tenue* (two scoops per sample with an average weight of 3.5 kg). The collected sample was placed on a floating platform and sorted. All seahorses found were measured (Lourie, 2003), sexed and the brood pouch status assessed. The *C. tenue* sample was weighed to the nearest 100 g to establish the drained wet weight. The vegetation sample and seahorses were returned to the sample location. Sampling was done from a boat to prevent disturbance of the *C. tenue* beds and underlying mud. Habitat type II sampling took place in the summer (February) and winter (June) of 2015. Ten samples were collected from each of the twelve sample sites selected (Fig. 4). An average seahorse density per drained wet weight of *C. tenue* was established.

2.6. *Statistical analysis*

The statistical programme R (R Development Core Team., 2014) was used in all analyses. A Kruskal Wallis test was used to test for any significant differences in physico-chemical features across marina zones and seasons and a Kruskal Nemenyi post-hoc test to assess those features found to be significantly different. The PMCMR package was used for Kruskal Wallis and post-hoc analyses (Pohlert, 2014).

The distribution of *H. capensis* was found to be over-dispersed (variance > mean) and followed a negative binomial distribution. A Negative Binomial Generalised Linear Model (GLM) was used to test the hypothesis that seahorse densities varied across Habitat

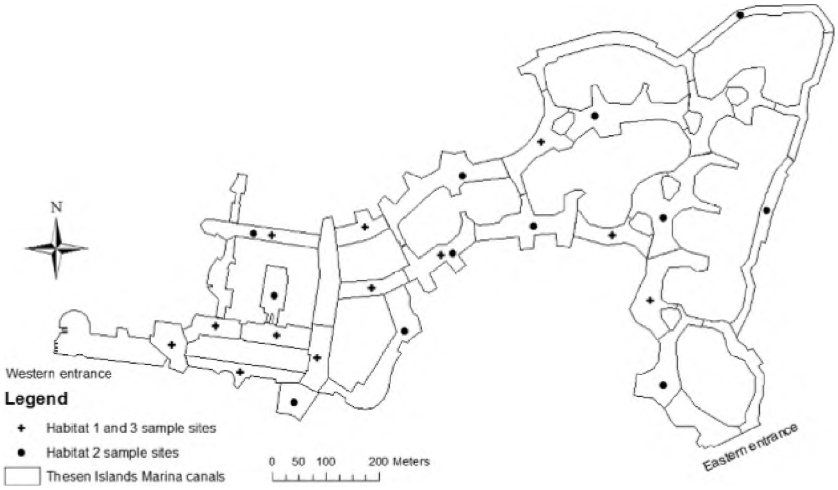

Fig. 4. The twelve sample sites within Thesen Islands Marina surveyed during the preliminary (2014) seahorse survey within Habitat type I and III (+) as well as the comparative summer and winter (2015) survey within Habitat type I (+) and during the summer and winter (2015) seahorse survey within Habitat type II (●). The numbers used refer to the 32 demarcated sample sites (Fig. 2).

types I and III. Sex ratios were assessed with a Chi-square test. The Car (Fox and Weisberg, 2011) and Mass (Venables and Ripley, 2002) packages were used for Generalised Linear Model analyses.

3. Results

3.1. Physico-chemical features

The physico-chemical features of the marina ranged within historic limits recorded for the Knysna Estuary (Table 1). Both surface ($\chi^2 = 42.7$; d.f. = 3; $P < 0.01$) and bottom ($\chi^2 = 29.7$; d.f. = 3; $P < 0.01$) water temperature changed significantly across seasons and surface temperatures within zone 1 were significantly colder ($\chi^2 = 6.8$; d.f. = 2; $P = 0.03$) compared to Zone 2 and 3. Dissolved Oxygen concentrations were similar across seasons and marina zones. Significantly higher turbidity was found within Zone 3 ($\chi^2 = 8.5$; d.f. = 2; $P = 0.01$).

3.2. Habitat description

Codium tenue was dominant during the summer 2014 vegetation survey with 32.9% coverage. Barren sandy areas and *Asparagopsis taxiformis* had an overall coverage of 26.7% and 21.9% respectively. Other major vegetation types found included *Zostera capensis* (1.8%); *Halophila ovalis* (0.3%) and *Caulerpa filiformis* (13.3%). Only 3.1% of reno mattress habitat was present. In winter *C. tenue* cover decreased to 23% with a corresponding increase in barren sandy areas (32.9%) and *A. taxiformis* (31.2%). *C. filiformis* coverage decreased to 6.4% while *Z. capensis* (1.6%) and *H. ovalis* (1.8%) showed a slight increase. The area of reno mattress habitat remained constant (3.2%) throughout the year.

Table 1
Summary of the physico-chemical features recorded in Thesen Islands Marina 2014–2015. The table includes the maximum, minimum, mean and standard deviation (Std. Dev.) recorded for each feature.

	Max	Min	Mean	Std. dev.
Depth (m)	3.1	0.6	2	0.7
Temperature (°C)	25	11.5	17.6	2.9
Dissolved Oxygen (% Sat)	121.8	54.4	91	12.4
Turbidity (ntu)	7.03	0.08	2.61	1.36
Salinity	37	28	33.2	2.4

Habitat types showed a similar seasonal pattern with a decrease in Habitat type II from summer to winter (32.9%–23%) and corresponding increases in Habitat type III (37.3%–41.1%) and IV (26.7%–32.9%). Habitat type I remained constant with 3.1% coverage.

3.3. Hippocampus capensis population assessment

3.3.1. Habitat types I (reno mattress) and III (mixed vegetation on sediment)

Seahorse densities varied across habitat types and significantly ($Z = -3.8$; Std. Error = 0.54; $P < 0.01$) lower seahorse densities were found in Habitat type III compared to Habitat type I. An assessment of the pooled data showed an equal female: male sex ratio of 1.2:1 ($\chi^2 = 1.6$; d.f. = 1; $P = 0.2$). Average height was (mean ± s.d.) 6.4 ± 2.0 cm. Two juveniles were recorded. Only 27% of males had a fully inflated brood pouch. Habitat type I was exposed to the atmosphere during spring low tide for up to two hours. During these events seahorses were noted in very shallow (<30 cm) water and in some cases became stranded. In such instances, they kept their gills moist by placing their heads against the moist sediment until the tide returned. No seahorse mortalities were noted during these periods.

Eighty-one percent of seahorses were found within the western marina zone, with 16% and 3% in the transitional zone and eastern zone respectively.

Seahorse density within Habitat type I decreased in January 2015 (Table 2) and the sex ratio (1.8:1) was female biased ($\chi^2 = 8.1$; d.f. = 1; $P < 0.01$). Mean (±s.d.) height was 5.5 ± 3.2 cm. No juveniles

Table 2
Summary of the preliminary seahorse survey within Habitat III (2014); preliminary seahorse survey within Habitat I (2014); summer seahorse survey within Habitat I (January 2015) and winter seahorse survey within Habitat I (June 2015). The area surveyed and dive hours are included to indicate sampling effort for the respective surveys.

Date	Habitat type	Habitat description	Seahorse abundance	Females	Males	Juveniles	Average seahorse density (Indv/m^2)	Max seahorse density (Indv/m^2)	Area surveyed (m^2)	Dive hours
Sep-Oct 14	III	Mixed veg	23	13	9	0	0.02	0.11	1200	13.6
Aug -Oct 14	I	Reno mattress	182	95	81	2	0.18	0.64	1030	14.6
Jan 15	I	Reno mattress	100	62	34	0	0.12	0.32	820	14.5
June 15	I	Reno mattress	179	80	88	0	0.17	0.63	1060	17.4

were found. Nearly three quarters (73.5%) of males had a fully inflated brood pouch indicative of breeding. Sexually mature males (inflated brood pouch) were noted at a height of 5 cm. An increase in seahorse densities were found during the winter survey (Table 2) with an equal sex ratio ($\chi^2 = 0.4$; d.f. = 1; P = 0.5). Mean (±s.d.) height was 6.2 ± 2.4 cm. No juveniles or any indication of breeding were noted. The highest densities of seahorses were continually found within the western section (Zone 1) of the marina (Fig. 5).

3.3.2. *Habitat type II (C. tenue beds)*

Seasonal seahorse densities within Habitat type II were stable (Table 3). Inflated brood pouches were noted in 70% of males during the summer survey and none of the males during the winter survey. Mean (±s.d.) heights of 7.5 ± 0.9 cm and 7.1 ± 1.2 cm were recorded for summer and winter respectively. Seahorse height was significantly different ($\chi^2 = 13.8$; d.f. = 1; P < 0.01) between Habitat types I and II. An equal female: male sex ratio of 1: 1.2 ($\chi^2 = 0.4$; d.f. = 1; P = 0.5) and 1:1.1 ($\chi^2 = 0.2$; d.f. = 1; P = 0.6) was found during the summer and winter survey respectively. The distribution of seahorses within Habitat type II was similar across all sample sites and showed limited variation across seasons (Fig. 6).

3.4. *Habitat use*

During the preliminary survey (2014) 50% of seahorses used *Asparagopsis taxiformis* as a holdfast and 42% used the reno mattress wire mesh (Fig. 7).The use of *A. taxiformis* as a holdfast increased to 70% during the summer (2015) while the use of wire mesh decreased to 17%. During winter (2015) 69% of seahorses used the wire (Fig. 7) mesh as a holdfast and 9% *A. taxiformis*. *Codium tenue* was exclusively used as holdfast within habitat type II.

4. Discussion

Four habitat types were identified within the canals of a residential marina estate within the Knysna Estuary, South Africa, and

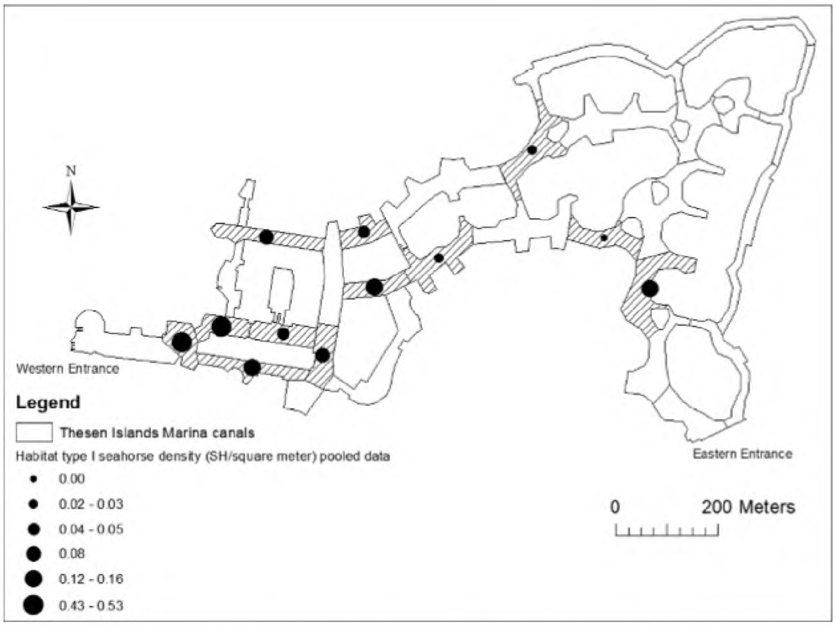

Fig. 5. Seahorse density and distribution within Habitat type I (Reno mattress habitat) for all survey data.

Table 3
Summary of the summer (February 2015) and winter (June 2015) seahorse survey within Habitat II. Densities were calculated as seahorses per kilogram of *Codium tenue*.

Date	Habitattype	Habitat description	Seahorse abundance	Females	Males	Juveniles	Average seahorse density (Indv/kg)	Max seahorse density (Indv/kg)	Kg *Codium* surveyed
Feb 15	II	Dense *C. tenue* beds	82	38	44	0	0.17	0.31	470.7
June 15	II	Dense *C. tenue* beds	68	32	36	0	0.19	0.44	365.5

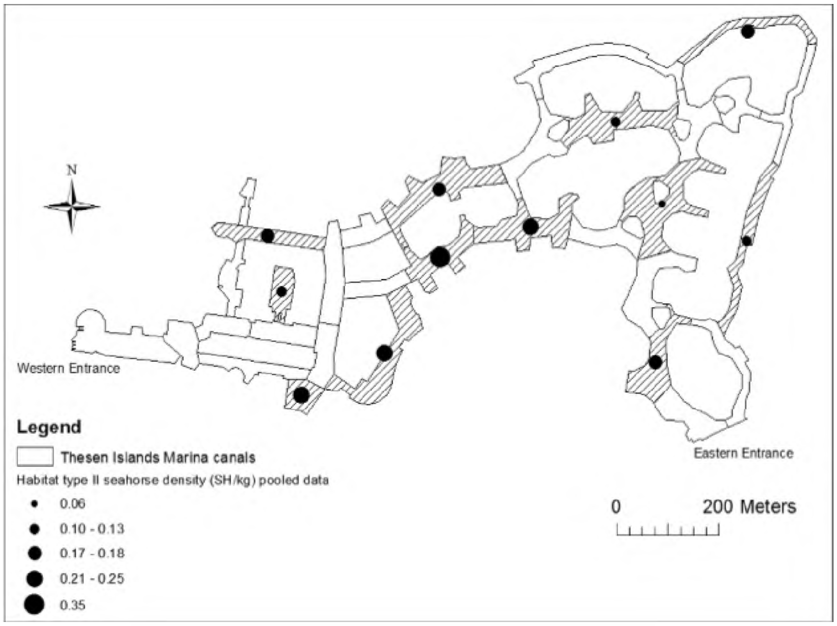

Fig. 6. Seahorse density and distribution within Habitat type II (*Codium tenue* beds) for all survey data.

Hippocampus capensis was found to occur throughout this system. The most important habitat type in terms of seahorse use was the artificial reno mattress habitat (Habitat type I). The functionality of this habitat type is related to current velocity and in areas with reduced current velocity (dead-end canals), an increase in sedimentation or the presence of *Codium tenue* which cover the reno mattress make the habitat unsuitable for use by seahorses. The largest extent of Habitat type I was found within the western zone of the marina characterised by higher current velocities. An overall change in habitat type dominance from Habitat type I (reno mattress) and III (mixed vegetation on sediment) within the western zone to Habitat type II (*C. tenue* beds) within the eastern zone of the marina was found. This change could be ascribed to the decrease in current velocity along the west-east gradient of the marina which favours the growth of *C. tenue*. This pattern persisted across seasons although the respective abundance of the different habitat types changed. The impact of this habitat dominance change on *Hippocampus capensis* is noted by the change in the habitat type used (reno mattress vs *C. tenue* beds) by the seahorses in the different marina zones. The eastern side of the marina showed similarities to typical dead-end canal systems such as high turbidity, which have been shown to effect syngnathid prey capture rates (James and Heck, 1994) and mating behaviour (Sundin et al., 2010). By contrast the western marina zone showed similar physico-chemical characteristics to historical data for the bay regime of the Knysna Estuary (Allanson et al., 2000).

The maximum seahorse density within Habitat type 1 (0.64 individuals/m^2) exceeded historic seahorse densities within the Knysna Estuary (0.25 individuals/m^2 in 2000 and 0.33 individuals/m^2 in 2001) (Bell et al., 2003; Lockyear et al., 2006). Conventional transect surveys (Curtis et al., 2004) were not suitable to use within Habitat type II. Lockyear et al. (2006) used push net sampling in very dense (>80% cover) vegetation stands, but this method was also deemed unsuitable. An alternative method was established which allowed a seahorse density estimate per kilogram of *C. tenue*. Seahorse densities were found to be similar across all sites within Habitat type II. It is proposed that this method be considered during seahorse surveys in dense, unattached macro-algal beds.

The low observations of juvenile seahorses in this study is a concern. Bell et al. (2003) recorded three juvenile observations during her 2000 transect survey which correlates with the juvenile sightings of this study. Push net sampling used by Lockyear et al.

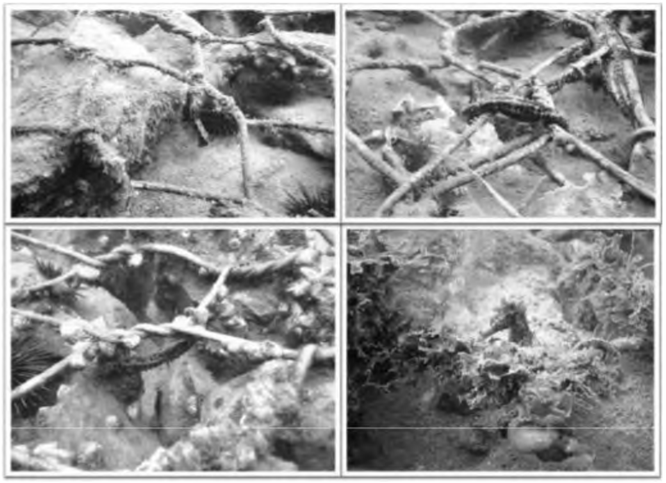

Fig. 7. Artificial habitat (reno mattress), found within the canals of Thesen Islands Marina, utilized by *H. capensis*. In each instance the wire of the mattress is used as a holdfast by the seahorse.

(2006) in 2001 resulted in higher juvenile observations (31 juveniles). No juveniles were however found in the Habitat type II sampling method used in dense *Codium tenue* beds (a method similar to that of Lockyear et al., 2006). Juvenile sightings in seahorse population surveys are known to vary and range from 21% to 80% for *Hippocampus guttulatus* in the Mar Piccolo of Taranto (Gristina et al., 2015); 34% for *Hippocampus breviceps* in Australia (Moreau and Vincent, 2004); 12% for *H. denise* and *H. bargibanti* (Smith et al., 2012) to 8% for *Hippocampus comes* in the central Philippines (Marcus et al., 2007). The size of juvenile seahorses plays a role in their visibility during surveys. Larger seahorse species such as *H. guttulatus* (maximum height of 18 cm) are considered juvenile at < 50% size at maturity (Gristina et al., 2015) and would consequently be easier to see during a transect survey. The dearth of juvenile observations during the present study could be ascribed to ontogenetic differences in habitat use by *H. capensis*. Ontogenetic differences in habitat use by seahorses have been found for *H. comes* in the Philippines (Foster and Vincent, 2004; Morgan and Vincent, 2007) and *H. guttulatus* (Foster and Vincent, 2004). According to Whitfield (1989, 1995) *H. capensis* juveniles are planktonic and dispersal via currents is likely. *Ex situ* studies on the reproduction of *H. capensis* (Grange and Cretchley, 1995; Lockyear et al., 1997) lack any data on habitat use of juvenile *H. capensis*. Teske et al. (2007) found that juveniles preferred *Zostera capensis* as a holdfast as opposed to adults who showed a preference for *Caulerpa filiformis* where a choice in vegetation types was available. Further research is required to ascertain the suitability of different habitat types within the marina to all life stages of *H. capensis*.

The decrease in seahorse densities between the preliminary (late winter to early spring 2014) and the summer survey (2015) might be ascribed to the increase in recreational activities during the December holiday season. The occupancy of houses on the island increases from 50% to 100% during this time with a related increase in boat use. Anderson et al. (2011) found that seahorses exposed to loud noise demonstrated physiological, chronic stress responses with reduced mass and body condition. Further research on the potential effect of increased noise on seahorses caused by boat activity is required (Vincent et al., 2011). Another explanation for the decrease in seahorse density may be the fact that seahorses hide within the crevices of the reno mattress during this time and therefore surveys were unable to account for all individuals present.

Conflicting conclusions have been reached with regards to habitat and holdfast use by *H. capensis* within the Knysna embayment. Bell et al. (2003) found no association between seahorse densities and vegetation cover, and in fact found higher densities of seahorses within low density (<20%) vegetation stands. Seahorses were also recorded on bare sediment. By contrast Teske et al. (2007) found a positive correlation between seahorse densities and dense vegetation cover (>75%) and suggested that *H. capensis* will only be found in areas with adequate vegetation cover. This fact limits the distribution of *H. capensis* in the Knysna Estuary as it was found that only 11% of the sub-tidal area in the estuary is covered by vegetation (Teske et al., 2007). In the winter of 2014, 67% of the subtidal marina area was covered by some type of vegetation (mixed vegetation on sediment, *C. tenue* beds, *A. taxiformis* on reno mattress). A recent threat to the subtidal vegetation of the embayment 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). The additional habitat found in Thesen Islands Marina could thus be a refuge for *H. capensis* and related biota in a changeable estuarine environment.

The highest densities of seahorses were found in the reno mattress habitat although it is the habitat type with the least percentage cover (only 3.1% cover) within the marina. A number of seahorse species have been noted to utilise artificial holdfasts. *Hippocampus reidi* will use fish coral screens and nylon ropes (Rosa et al., 2007), both *H. guttulatus* and *Hippocampus hippocampus* use ropes, bricks and nets as holdfasts (Curtis and Vincent, 2005; Correia et al., 2013; Gristina et al., 2015) while *H. whitei* use swimming nets within Sydney Harbour (Clynick, 2008b). Gristina et al. (2015) reports that *H. guttulatus* and *H. hippocampus* within the Mar Picco di Taranto, Italy, prefer to shelter and feed in artificial habitats such as mussel farms and rocky artificial substrata. This preference might, however, be owing to the large decline in natural seagrass beds within the area which leave artificial habitat as the only choice for seahorses. Another reason could be the exemption of such areas from fishing (Gristina et al., 2015). Hellyer et al. (2011) and Gristina et al. (2015) found that more complex artificial structures have a higher abundance of epifauna which include potential seahorse prey animals among the Amphipoda and Copepoda. This may help to explain the suggested preference of these habitats by seahorses.

Detailed experimental research on artificial habitat and holdfast use by seahorses is limited to two studies. Correia et al. (2015) found that *H. guttulatus* densities increased with the deployment of Artificial Holdfast Units (AHU) in the Ria Formosa, Portugal, while Hellyer et al. (2011) showed that more complex frayed net material supported higher densities of *H. whitei* in Sydney Harbour, Australia. *Hippocampus capensis* can be regarded as a holdfast generalist (Teske et al., 2007) as opposed to seahorse species associated with a specific type of holdfast {*H. bargibanti* and *H. denise*} (Smith et al., 2012). Their adaptability in the use of a new holdfast (reno mattress wire mesh) can thus be expected. In some transects the availability of wire mesh was patchy due to sedimentation. Seahorses found in these transects were located on the wire patches as opposed to adjacent vegetation on sediment. This observation helps to strengthen the association between *H. capensis* and the reno mattress habitat.

In conclusion, the development of the canals in Thesen Islands Marina, which have relatively stable environmental conditions, has created important new habitat for the endangered Knysna seahorse. The highest density of seahorses (0.64 individuals/m^2) were found in the western zone of Thesen Islands Marina within the reno mattress habitat, characterised by high current velocity. Seasonal variation in seahorse abundances was not significant and the decrease in numbers found during the summer 2015 survey recovered during the winter 2015 survey. Seahorses were also found within dense *C. tenue* beds and this habitat type should be regarded as important – especially in areas of low current velocities where the availability of alternative habitat is limited. This study provides a first glimpse of the current population dynamics of *H. capensis* within an artificial water body and provides insights into the usefulness of this type of development in the creation of suitable estuarine habitat. Future research will focus on long term population trends of the species as well as the process of habitat selection and use.

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Chapter 7

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