THE BIOLOGY, ECOLOGY AND CONSERVATION

OF WHITE'S SEAHORSE *HIPPOCAMPUS WHITEI*

by

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CERTIFICATE OF ORIGINAL AUTHORSHIP

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

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ABSTRACT

Seahorses are iconic charismatic species that are threatened in many countries around the world with several species listed on the IUCN Red List as vulnerable or endangered. Populations of seahorses have declined through over-exploitation for traditional medicines, the aquarium trade and for curios and through loss of essential habitats. To conserve seahorse populations in the wild, they are listed on Appendix II of CITES, which controls trade by ensuring exporting countries must be able to certify that export of seahorses is not causing a decline or damage to wild populations. Within Australia, seahorses are protected in several states and also in Commonwealth waters.

The focus of this study was White's seahorse *Hippocampus whitei*, a medium-sized seahorse that is found occurring along the New South Wales (NSW) coast in Australia. The species is listed as 'data deficient' on the IUCN Red List and there is little research information available to assist in the conservation of the species. Research on *H. whitei* was undertaken from 2006-2009 and primarily focused on determining the species' life history parameters, its distribution and relative abundance, habitat preferences and site fidelity, and response to marine protected area (MPA) protection and habitat modification. Research primarily occurred within Sydney Harbour and Port Stephens.

Field surveys found that *H. whitei* is endemic to coastal estuaries along 300 km of NSW coastline. It is a medium-sized seahorse (max L_T 162 mm) that displays rapid

growth (Port Stephens: females L_∞ = 149.2 mm and K = 2.03 per year and males L_∞ = 147.9 mm and K = 2.52; Sydney Harbour: females L_∞ = 139.8 mm and K= 1.28 and males L_∞ = 141.6 mm and K=1.22), becomes sexually mature at approximately 6 months, and can live for up to 5 years in the wild. The species displays life-long monogamy with several pairs observed breeding over three consecutive breeding seasons, and strong site fidelity with seahorses remaining at the same site for up to 56 months for males and 49 months for females. Adult male and female *H. whitei* exhibited a significant preference for sponge and soft coral *Dendronephthya australis* habitats whilst juveniles had a strong preference for gorgonian *Euplexaura* sp. habitat. *Hippocampus whitei* in Port Stephens were significantly less abundant within the no-take MPA and there was a negative correlation with predator abundance. Long-term monitoring of *H. whitei* in Port Stephens found that populations declined over a period of six months for no apparent reason; however, they recovered within three years. A manipulative experiment undertaken on protective swimming nets in Sydney Harbour found *H. whitei* had a positive association with epibiotic growth and proximity to the sea floor. An experiment on the effects of flash photography found it had no significant effect on movements, behaviour, or site persistence of *H. whitei* and concluded that flash photography by divers is a safe and viable survey technique for this species. The information obtained from this study should contribute towards a reassessment of the species under the IUCN Red List and also provides the necessary data to ensure adequate management of the species within NSW waters.

PREFACE

The contents within this thesis constitute my own work with several of the chapters having previously been published or are currently under review. As this thesis is based on chapters made up of published papers, there will be some repetitiveness across each of the chapters. References and formatting are based on the *Journal of Fish Biology* format. The development and implementation of each of the research projects within this thesis were of my own doing; however, I have had assistance from co-authors for various components of this study which I have outlined below.

Chapter 3 has been published as "Harasti, D., Martin-Smith, K., and Gladstone, W. (2013) Population dynamics and life history of a geographically restricted seahorse, *Hippocampus whitei. Journal of Fish Biology* **81**, 1297-1314." All the diving work for this study was undertaken by myself and my two co-authors, my PhD supervisors, provided advice on the study design and guidance in the preparation of the manuscript.

Chapter 4 has been published as "Harasti, D., Martin-Smith, K. & Gladstone, W. (2014). Ontogenetic and sex-based differences in habitat preferences and site fidelity of the White's seahorse *Hippocampus whitei. Journal of Fish Biology*. doi:10.1111/jfb.12492. Both co-authors provided advice and guidance in writing up this research. Professor Gladstone provided advice on the study design to determine

habitat preferences whilst Dr Martin-Smith provided advice on monitoring seahorse movements using a towed GPS system.

Chapter 5 has been published as Harasti, D., Martin-Smith, K. & Gladstone, W. (2014). Does a no-take Marine Protected Area benefit seahorses? *PLOS ONE*. doi: 10.1371/journal.pone.0105462. All of the monthly monitoring for this four year study was undertaken by myself with both my co-authors *Martin-Smith and Gladstone) contributing to the study with assistance with the study design and guidance on the writing of the manuscript.

Chapter 6 has been published as "Harasti, D., Glasby, T., and Martin-Smith, K. (2010) Striking a balance between retaining populations of protected seahorses and maintenance of swimming nets. *Journal of Aquatic Conservation: Marine and Freshwater Ecosystems* **20**, 159-166. Dr Glasby provided advice in the development of the study design and statistical analysis for the manipulative experiment. Dr Martin-Smith provided the data that he had collected previously on seahorses at Clifton Gardens that was incorporated into this manuscript whilst I was responsible for the establishment of the experimental study and subsequent diving surveys.

Chapter 7 was a collaborative study with Beth Moore and Dr Hamilton from the California Academy of Sciences and was supported with funds from the Sea Life Conservation Fund (Sydney Aquarium). The project development, tissue sample collection and final write up were undertaken by myself. Beth Moore was responsible for the genetic analysis and provided advice on the written description

of the methods and results. Dr Hamilton provided advice on the study design and provided comments on the chapter.

Chapter 7 has been published as "Harasti, D and Gladstone, W. (2013). Does underwater flash photography affect the behaviour, movement and site persistence of seahorses?" *Journal of Fish Biology* **83**, 1344-1353. Professor Gladstone assisted with this project by providing guidance on statistical analysis and preparation of manuscript. Dr Keith Martin-Smith also provided feedback on a draft manuscript that helped improve the publication.

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I dedicate this thesis to my father, Dennis Harasti, who passed away unexpectedly in 2005,

aged 52, and my grandfather, Taffy Williams, who passed away in 2013, aged 80.

This PhD project arose from a meeting with Dr Keith Martin-Smith whilst he was working on seahorses at Clifton Gardens in Sydney Harbour. I'm very grateful that Keith took me on board as a student and he has provided excellent guidance, friendship and encouragement since this research commenced.

Professor William (Bill) Gladstone has provided excellent support as my university supervisor, first through the University of Newcastle and now at University of Technology Sydney. Bill has always provided great feedback on the drafts that I have sent and I'm very grateful of his supervision which has helped improve my research and made me a better scientist.

Thanks to Dr Tim Glasby who provided assistance with statistical analysis and filled a great role of 'unofficial supervisor'. Thanks for always responding to my stats questions at such short notice and helping me work through some of the more complicated analyses. You have made me think a lot more about the importance of planning the statistical analysis prior to rushing off and commencing any new study in the future.

I'm grateful to Pam and Chris Norman, former owners of Pro Dive Nelson Bay, who provided great support throughout the duration of the diving surveys and hundreds

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CHAPTER 1

Justification and outline of research

In 2003, a proposal was submitted to the NSW Government to have all syngnathids listed in NSW as protected species. During the assessment and consultation process, it became evident that very little information existed on seahorses in NSW and that their proposed protection was being implemented on the 'precautionary principle'. There had been no research conducted on seahorses in NSW since the work by Amanda Vincent in the early 1990s (Vincent, 1995; Vincent and Sadler, 1995; Vincent and Giles, 2003; Vincent *et al.*, 2004; Vincent *et al.*, 2005) and there was no data available to make an assessment on whether seahorse populations in NSW were under any threat or if populations were in decline. Seahorses were being afforded a total protection status, even though very little was known about their biology, ecology and conservation status in NSW waters at the time.

In light of the apparent lack of recent data on seahorses in NSW, this study was implemented to acquire data that could be used to assist with the conservation and management of seahorses. The focus of this study is *Hippocampus whitei*, the most commonly encountered seahorse in NSW, and although NSW is considered to be the main location for its occurrence, there was no information available on the areas it occurred, the habitats used, its population status or its life history parameters. Its listing on the IUCN Red List as 'Data Deficient' also highlights the lack of information available on this species (IUCN, 2013).

To obtain the data required to assist in the conservation and management of *Hippocampus whitei*, several studies were implemented to help address the knowledge gaps. The details of each study are provided in each chapter with the aims of the individual components of the thesis provided in detail below:

Chapter 2 – provides background information on seahorses, their conservation status and provides a summary of available information on *Hippocampus whitei*, the primary focus of this thesis.

Chapter 3 – details the life history and population parameters for *H. whitei* and provides a detailed assessment on the species' geographical range. The aim was to compare growth, mortality and population abundance of *H. whitei* from two regions at the edges of its range and provide an assessment on its conservation status in the wild based on the data collected.

Chapter 4 – determines the habitat usage and movements of *H. whitei*. The aim was to identify the habitat preferences of all age classes and sexes in Port Stephens and determine their long-term movement patterns and site fidelity.

Chapter 5 – addresses long term changes in population abundances of *H. whitei* within and outside a no-take marine protected area (MPA). The aim was to assess long term changes in population abundance at several sites within Port Stephens and assess the effects that a no-take MPA has on *H. whitei* populations.

Chapter 6 – determines the importance of epibiotic growth on artificial structures as a habitat for *H. whitei*. The aim of this study was to assess the effects of altering epibiotic habitat on *H. whitei* abundance and movements and provide best practise management guidelines to minimise disturbance to seahorse populations in routine cleaning of artificial habitats.

Chapter 7 – provides a preliminary assessment on genetic variation across *H. whitei* populations in NSW. The aim was to collect tissue samples from various *H. whitei* populations throughout its range and determine overall population structure and connectivity using mitochondrial gene methodologies.

Chapter 8 – determines if underwater photography can be used as a non-intrusive method for assisting with research on *H. whitei*. The aim was to assess whether underwater flash photography affected the site persistence, movements and behaviour of *H. whitei*.

Chapter 9 – provides a synthesis of the findings in chapters 3-8 and discusses the key findings from the thesis and makes recommendations for further research and future conservation of *H. whitei*.

CHAPTER 2

Background

2.1   Outline

The aim of this chapter is to provide background information on seahorses and summarise what is known about their biology, distribution, life history parameters and habitat use. As many seahorse species are considered to be under threat, this chapter examines the conservation status of seahorses and provides details on the current threats to seahorses. Additionally, this chapter summarises the known information on *Hippocampus whitei*, the focus of this thesis and provides information on its biology, its distribution, habitat use and home range behaviour, and identifies the knowledge gaps.

2.2   Seahorses

Seahorses are an unusual looking group of bony teleost fish that are members of the family Syngnathidae and of one genus, *Hippocampus*. The genus name is derived from the Greek words 'hippos' (meaning horse) and 'campus' (meaning sea monster) (Lourie *et al* 1999). The Syngnathidae family includes seahorses, pipefishes, pipehorses and seadragons and the word 'Syngnathidae' is derived from the Greek words 'syn' (meaning together or fused) and 'gnathus' (meaning jaws) (Kuiter, 2009). The relatives of syngnathids in the same order (Syngnathiformes or Gasterosteiformes) include the families Aulostomidae (trumpetfishes), Centriscidae

(razorfishes), Fistulariidae (flutemouths or cornetfishes), Macrorhamphosidae (bellows fishes), Pegasidae (sea moths) and Solenostomidae (ghost pipefishes) (Lourie *et al.*, 1999b; Kuiter, 2009).

There are over 300 syngnathids species across approximately 56 genera known in the world with the majority of them pipefish species (approximately 250 species) followed by seahorses (Kuiter, 2009). Estimations of the number of seahorses vary due to taxonomic discrepancies with Vincent *et al.* (2011) indicating there are approximately 48 species worldwide compared to approximately 80 species recognised by Kuiter (2009). Australia is one of the highest diversity regions in the world for seahorses, with approximately 30 species of seahorses known to occur in the region (Kuiter, 2009). Seahorses in Australia are distributed around the entire coastline and occur in a large variety of habitats such as sponge gardens, seagrasses, kelp forests and artificial structures (Kuiter, 2001; 2009). *Hippocampus* spp. are generally found in shallow coastal habitats (less than 20 m depth) but some species are known to occur at depths of 150 m (Lourie *et al*. 1999). Australian species can be found over a large depth range, with species such as *H. abdominalis* and *H. breviceps* occurring in protected shallow water habitats (<15 m) (Moreau and Vincent, 2004; Martin-Smith and Vincent, 2005), whilst species such as *H. minotaur* and *H. paradoxus* are found in much greater depths (> 60 m) (Kuiter, 2009; Foster and Gomon, 2010).

Seahorses can be recognised by their upright posture, body made of ringed bony segments, small mouth at the end of a tubed snout, the head bent at an angle to the

body and a prehensile tail that is used to grip the substratum. *Hippocampus* spp. are unique in that the female transfers her eggs to the male's abdominal pouch where they are fertilised with the male becoming pregnant. After a pregnancy period of approximately 2 to 6 weeks (Foster and Vincent, 2004), the male gives birth to highly developed juveniles that resemble seahorses with no parental care following birth (Kuiter, 2009).

Some seahorse species are known to be monogamous with males and females mating continuously over a breeding season (Vincent, 1995; Kvarnemo *et al.*, 2000; Kvarnemo *et al.*, 2007) however it is unknown if the monogamy continues over successive breeding seasons. Some juvenile seahorses are thought to be planktonic, dispersing on ocean currents and tides and sometimes with large aggregations being found on drifting weed and debris (Kuiter, 2009; Luzzatto *et al.*, 2013). Once the juveniles settle into an area that has suitable habitat it is thought that they will then reside in their settled location maintaining a very small home range (Moreau and Vincent, 2004; Vincent *et al.*, 2005; Rosa *et al.*, 2007).

Seahorses have a broad distribution throughout the world; however, they are not known to occur in the Arctic or Antarctic regions (Kuiter, 2009). Most seahorse species have a small geographic range, being confined to a small number of countries or regions (Kuiter, 2009). Seahorses are predominantly found in marine environments; however, some species can tolerate lower salinities and occur in areas

with freshwater influences such as the Knysna seahorse *Hippocampus capensis* from South Africa that experiences fluctuating salinity in estuaries (Bell *et al.*, 2003).

Seahorses occur in a wide variety of habitats. In temperate waters of Australia and Europe they occur in seagrasses, sponge gardens and on artificial structures (Curtis and Vincent, 2005; Martin-Smith and Vincent, 2005; Vincent *et al.*, 2005; Clynick, 2008) whilst tropical species are known to occur in mangroves, coral reefs and amongst seagrasses (Perante *et al.*, 2002; Job *et al.*, 2006; Morgan and Vincent, 2007; Rosa *et al.*, 2007; Kuiter, 2009; Choi *et al.*, 2012). Some species have very specialised habitats such as the pygmy seahorses *Hippocampus bargibanti* and *Hippocampus denise* that inhabit gorgonian fans (Baine *et al.*, 2008; Smith *et al.*, 2012) whilst other species, such as *Hippocampus abdominalis* are known to use a variety of habitats (Martin-Smith and Vincent, 2005). As they are slow-moving or sedentary, seahorses rely on crypsis to avoid detection by potential predators and hence they are often found hiding amongst their habitats (Kuiter, 2009). Their reliance on, and preference for, complex habitat structure is unknown however it is thought that seahorses prefer more complex habitats as they provide better camouflage which allows for successful ambush predation (Flynn and Ritz, 1999).

In their review of seahorse conservation, Forster and Vincent (2004) determined that life history parameters for most *Hippocampus* species were unknown and that there were few field studies that had assessed the life history attributes of syngnathids. Most life history traits, particularly growth rates in seahorses, have been recorded

from aquaria studies: pot-belly seahorse *Hippocampus abdominalis* (Woods, 2003), tiger-tail seahorse *Hippocampus comes* (Job *et al.*, 2006), Pacific seahorse *Hippocampus ingens* (Ortega-Salas and Reyes-Bustamante, 2006), spotted seahorse *Hippocampus kuda* (Dzyuba *et al.*, 2006; Garcia and Hilomen-Garcia, 2009), slender seahorse *Hippocampus reidi* (Hora and Joyeux, 2009) and the three-spot seahorse *Hippocampus trimaculatus* (Sheng *et al.*, 2007).

In situ studies of life history traits for a few syngnathid species include studies of the Northern pipefish *Syngnathus fuscus* (Campbell and Able, 1998), alligator pipefish, *Syngnathoides biaculeatus* (Takahashi *et al.*, 2003; Barrows *et al.*, 2009), weedy seadragon *Phyllopteryx taeniolatus* (Sanchez Camara *et al.*, 2005; Sanchez-Camara *et al.*, 2011) and for the seahorses: long-snouted seahorse *Hippocampus guttulatus* (Curtis and Vincent, 2006), Japanese seahorse *Hippocampus mohnikei* (Kanou and Kohno, 2001) and dwarf seahorse *Hippocampus zosterae* (Strawn, 1958). Foster and Vincent (2004) indicated that specific studies on *Hippocampus* spp. would be beneficial in establishing these parameters that would help provide the necessary information to assist with their management and conservation.

Natural mortality and longevity of seahorse species are important life history parameters that should be determined to assist in the design of any management actions for species conservation (Foster and Vincent, 2004). Natural mortality is the removal of individuals from a population from natural causes such as disease, old age, predation and competition (King, 1995). Very little is known about natural

mortality of seahorses in the wild with natural rates of mortality estimated for a few seahorse species, such as *Hippocampus comes*, M (year $^{-1}$) = 1.79 (Morgan and Vincent, 2013) and *Hippocampus guttulatus*, M (year $^{-1}$) = 1.13 to 1.22 (Curtis and Vincent, 2006). There is very little information known about the maximum lifespan of seahorses in the wild with only a few species assessed. *Hippocampus comes* has been estimated to live for approximately 2.7 years in the wild (Morgan and Vincent, 2013) whilst *Hippocampus guttulatus* lives longer at approximately 4.3-5.5 years (Curtis and Vincent, 2006).

Estimates of mortality are generally obtained through mark-recapture methods (Pauly, 1980; Seber, 1986; Pollock and Pine, 2007), which can also be used to provide estimates of population abundance (Seber, 1982; Schwarz and Arnason, 1996; White and Burnham, 1999). In seahorses, two methods of tagging have been used to obtain life history parameters and population abundance through mark-recapture/resight analysis. The traditional tagging method for *Hippocampus* spp. involved the use of neck collars (Perante *et al.*, 2002; Martin-Smith and Vincent, 2005) that had a tendency to foul with algal growth. Visible implant fluorescent elastomer tags have been used in recent *Hippocampus* spp. studies (Curtis and Vincent, 2006; Morgan and Vincent, 2013).

More recently, with the popularity of digital underwater photography, studies have utilised natural markings to individually identify animals with the data being used to estimate population abundance. The use of natural markings to obtain population

abundance estimates has been applied in the marine environment for various species, including whale sharks *Rhincodon typus* (Bradshaw *et al.*, 2007; Holmberg *et al.*, 2009; Rowat *et al.*, 2009), nurse sharks *Ginglymostoma cirratum* (Castro and Rosa, 2005), white sharks *Carcharodon carcharias* (Chapple *et al.*, 2011) and has been successfully employed on a syngnathid species, the weedy seadragon *Phyllopteryx taeniolatus* (Martin-Smith, 2011). The use of natural markings of seahorses for identification was recently conducted by photographing the coronet of *Hippocampus reidi* to successfully identify individual animals (Freret-Meurer *et al.*, 2013). However, concerns over the possible impacts of flash photography on seahorses led to it being banned in the United Kingdom (MMO, 2011). This ban was presumably based on the precautionary principle as there was no evidence to suggest that flash photography could have a detrimental impact on seahorses.

Given that many *Hippocampus* spp. have ranges that extended over various countries, it is possible that genetic variation in population structure could lead to differences in life history parameters associated with demographically independent populations (Moritz, 1999; Palsbøll *et al.*, 2007). Genetic divergence in population structure could result from barriers to larval dispersal such as ocean currents and habitat discontinuity (Doherty *et al.*, 1995; Dawson *et al.*, 2002) and given numerous seahorse species show strong site fidelity and lack a pelagic stage (Foster and Vincent, 2004; Vincent *et al.*, 2011), there is potential for limited larval dispersal across the species range. Consideration of the population structure of individual

seahorse species could inform species' conservation practices (Moritz, 1999; Palsbøll *et al.*, 2007). In a study on the population structure of the eastern Pacific seahorse *Hippocampus ingens*, Saarman *et al.* (2010) found a level of genetic divergence between all Pacific coast populations and populations in the Gulf of California and suggested that the Gulf population could be managed as a separate management unit.

Variation in life history parameters between individual species could leave some species more susceptible to over-exploitation and habitat loss while other species with life history characteristics of rapid growth and early maturity could be more resilient (Foster and Vincent, 2004). Conservation planning for seahorses in their natural environments will be assisted by determining life history parameters and assessing genetic variation in population structures.

2.3 Conservation of seahorses

Across the world, seahorses are considered to be under threat from various stressors (Foster and Vincent, 2004). There are concerns they have been over-harvested for traditional medicines and for the aquarium and curio trade, and that several species face population declines as a result of loss of essential habitats (Lourie *et al.*, 1999b; Vincent *et al.*, 2011). Traditional Chinese Medicines (TCM) are one of the largest consumers of seahorses with approximately 95% of all seahorse trade being sold for use in TCM (Vincent *et al.*, 2011). Seahorses are used in TCM as they are thought to be of use in the treatment of a variety of ailments including respiratory problems,

liver and kidney disease, arthritis, infertility and impotence (Vincent, 1996; Kumaravel *et al.*, 2012). Seahorses that are large, pale and smooth are considered the most valuable for TCM (Vincent, 1996) however the emergence of pre-packaged TCM pharmaceuticals means that seahorses that were considered to be less desirable (spiny, small and dark coloured) are now also likely to be utilised (Vincent *et al.*, 2011).

In some countries seahorses are directly targeted by fishers. In the Philippines fishers free dive at night to collect seahorses by hand (Martin-Smith *et al.*, 2004), particularly *Hippocampus comes* which is more active at night. In Brazil and India, divers also collect seahorses during the day by hand from shallow waters (Salin *et al.*, 2005; Rosa et al., 2006). However, the vast majority (estimated to be approximately 95%) of seahorse trade is derived from by-catch from trawl fisheries (particular prawn trawl) (Vincent *et al.*, 2011). Trawl fishing is very indiscriminate and as seahorses utilise similar habitats to prawns, and are very slow-moving, they are swept up in the trawl nets as they cross the ocean floor. Estimates of the weight of seahorses captured each year as by-catch include 6.5 tonnes in Vietnam (Giles *et al.*, 2006), India (9.4 tonnes) (Salin *et al.*, 2005), Malayasia (2.9 tonnes) and Thailand (6.6 tonnes) (Perry *et al.*, 2010).

Trawling also has a substantial impact on seahorse habitats such as sponge gardens and seagrass meadows (Vincent *et al.*, 2011). Trawling in Florida not only harmed or killed *Hippocampus erectus* but also damaged their habitats by physically removing

seagrass (Baum et al., 2003). Some species occurring in shallow coastal estuaries are found adjacent to highly developed coastal city areas (Allen *et al.*, 2002; Bell *et al.*, 2003; Vincent *et al.*, 2005), where anthropogenic disturbances tend to be most frequent and severe. Their existence in these areas can be threatened by habitat degradation and declining water quality.

The biology and ecology of seahorses, such as small home ranges, low population densities, monogamous mating and limited distribution, make them susceptible to localised anthropogenic impacts (Lourie *et al.*, 1999b; Foster and Vincent, 2004). One example is the Knysa seahorse *Hippocampus capensis*, which is restricted to three estuaries in South Africa. The Kynsa estuary is one of the most heavily used water-bodies in South Africa and the large amount of urban and industrial development surrounding the estuary has negatively impacted the estuarine ecosystem (Bell *et al.*, 2003). The effects of these anthropogenic impacts on the estuarine habitats of *H. capensis* are unclear; however, given its restricted geographic range, any loss of habitat would be of major concern for this species.

In Florida, the population size of *Hippocampus zosterae* decreased as a result of damage to seagrass caused by the demolition and construction phases of two adjacent marinas (Masonjones *et al.*, 2010). In Malaysia, *H. kuda* abundance was reduced after extensive port development decimated large areas of seagrass (Vincent *et al.*, 2011), which is considered to be a very important habitat for this species. Damage to coral reefs in the Philippines from dynamite fishing contributed to a

decline in *H. comes* populations (Marcus *et al.*, 2007). Habitat degradation has negatively impacted seahorse species that utilise near shore habitats and, combined with targeted fishing pressures and the large amount of incidental capture from trawl fishing, there are concerns for the long-term conservation of several *Hippocampus* species.

Marine protected areas (MPAs) have been promoted as a useful management tool to protect declining seahorse populations (Martin-Smith *et al.*, 2004; Vincent *et al.*, 2005; Curtis and Vincent, 2006; Morgan and Vincent, 2013). The benefits of MPAs for conserving marine biodiversity have been well documented (Halpern, 2003; Lester *et al.*, 2009), with MPAs shown to have positive effects on marine biodiversity with abundance and size of species within MPAs being greater than non-protected sites (Roberts *et al.*, 2001; Edgar and Stuart-Smith, 2009). Species' abundance and size are more likely to increase with increasing age of an MPA (Barrett *et al.*, 2007; Edgar *et al.*, 2009; Babcock *et al.*, 2010). In the only study that examined the response of seahorses to implementation of MPAs, it was found that MPAs had no significant effect on seahorse densities and that they had little effect on seahorse size (Yasué *et al.*, 2012). The absence of a response to the removal of fishing from the MPA may have been a result of the biology of seahorses, poor habitat quality within the MPA and the small population sizes of seahorses outside the MPA to supply the MPA (Yasué *et al.*, 2012). The response of seahorse populations to MPAs will vary depending on the species, the location and size of the MPA and external impacts

such as land based pollution (Yasué *et al.*, 2012). Further investigation is warranted to determine if implementation of MPAs could benefit declining seahorse populations and assist with their recovery.

The entire genus of *Hippocampus* is listed on Appendix II of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora), which warrants that signatory nations (178 nations as of 2013) must ensure that use of seahorses is undertaken in a sustainable manner. The Appendix II listing still allows for the trade in seahorses; however, exporting countries must certify that their exports are not causing a decline in or damage to wild populations. Some countries provide high levels of protection for seahorses (e.g. the United Kingdom and Australia) whilst other countries, such as Vietnam (Giles *et al.*, 2006), India (Salin *et al.*, 2005), Malaysia and Thailand (Perry *et al.*, 2010) provide little or no protection.

Trade in syngnathids to and from Australia is relatively minor, particularly in comparison with trade involving other countries such as China, India and Hong Kong (Martin-Smith and Vincent, 2006). The collection of seahorses from the wild in Australia for export purposes is considered uneconomical (Kuiter, 2001); however, there is a small amount of collection permitted for aquarium and aquaculture purposes. To ensure their long term sustainability in Australia, all seahorses are protected in Commonwealth waters under the *Environment Protection and Biodiversity Conservation Act* 1999. Seahorses are also protected at the state level with the states of South Australia, Victoria, Tasmania and New South Wales (NSW) legislating

protection measures to conserve wild seahorse populations. For example, in NSW all syngnathid species are protected under the *Fisheries Management Act 1994* (DPI, 2005) making it illegal for them to be taken from the wild, with the justification for protection being to ensure control over trade and to mitigate the potential impact of over-exploitation from aquaculture, aquarists and commercial fishing. When they became protected in 2004, it was acknowledged at the time that there were no current concerns that seahorses in NSW were threatened. However this was a result of lack of data available about population sizes, distribution or knowledge of their physiology and that an assessment on their conservation status could not be made without new research being implemented (Pogonoski *et al.*, 2002).

The IUCN Red List of Threatened Species has ten species of seahorses listed as 'vulnerable', one species as 'least concern' and *Hippocampus capensis* listed as 'endangered' (IUCN, 2013). The majority (approximately 68%) of the seahorse species that are listed are classified as 'data deficient'. One of the species listed as data deficient is *Hippocampus whitei*. Previously, *H. whitei* was listed in 1996 as vulnerable under the IUCN 1994 criteria and this assessment was based on suspected past declines in occupancy, occurrence and habitat, as well as on potential levels of exploitation. The species was reassessed under revised IUCN criteria in 2003 which found that with an increased taxonomic understanding that no appropriate data on biology and ecology, habitat, abundance or distribution were available for this species and hence it was considered to be data deficient. The basis

for the data deficient listing is that there are no published data about population trends or total numbers of mature animals, there is very little available information about its extent of occurrence or its area of occupancy and there have been no quantitative analyses examining the probability of extinction of this species. As a result, the assessors have insufficient data to properly assess the species against any of the IUCN criteria. This thesis aims to undertake research that addresses the knowledge gaps for *H. whitei* to assist with its management and long term conservation.

2.4   *Hippocampus whitei* – current knowledge

2.4.1   Description

The White's Seahorse (*Hippocampus whitei*) was first discovered in 1789 in Port Jackson (Sydney Harbour) and named after John White, surgeon general to the first fleet and author of *Journal of a Voyage to New South Wales 1789*, in which a portrait of *H. whitei* is published and was described by Bleeker in 1855. *Hippocampus novaehollandiae* (Steindachner 1866) is a synonym. Some of the morphological characteristics of *H. whitei* are: dorsal-fin rays 16-17, pectoral-fin rays 15-17; tail-rings 33-34; coronet is high inclined backwards, arranged in five pointed star at apex; spines are variable ranging from low to moderately developed and from round to quite sharp and it has a long snout with sharp eye spines (Lourie *et al.*, 1999b; Kuiter, 2001).

Hippocampus whitei is considered to be a medium-sized seahorse with total length estimates ranging from 100 to 210 mm. Kuiter (2000b; 2001) reported that *H. whitei* grows to a total length of approximately 100 mm, Lourie *et al.* (1999b) reported its maximum length as 130 mm, Edgar (2000) reported a maximum length of 150 mm, whilst Hutchins and Swainston (1999) provided the largest maximum size estimate at 210 mm. Measurements of seahorses can be very variable as a result of the curvature in their body (Lourie *et al.*, 1999b) however the standard method for measuring seahorses is straight line measurements with two measurements: (1) tail length: tail to base of abdomen (bottom of pouch in males) and (2) trunk length: bottom of abdomen or pouch to top of coronet (Figure 2.1). Total length is estimated by combining the two measurements (Lourie *et al.*, 1999b) and this measurement method was employed throughout this study.

2.4.2 Distribution

The distribution of *H. whitei* is limited to the waters of the western Pacific with Lourie *et al.* (1999b) reporting that its distribution includes central NSW, the waters of southern Queensland and the Solomon Islands. In comparison, Kuiter (2000a) indicated the species occurs from southern Queensland to southern Victoria and is also found in South Australia which is similar to the distribution provided by Edgar (2000) of South Australia to Noosa in Queensland. In his revision of Australian seahorses, Kuiter (2001) updated the distribution of *H. whitei* limiting it to the

estuaries of the Sydney and Newcastle region; however, he also notes it may be found in Spencer Gulf - South Australia.
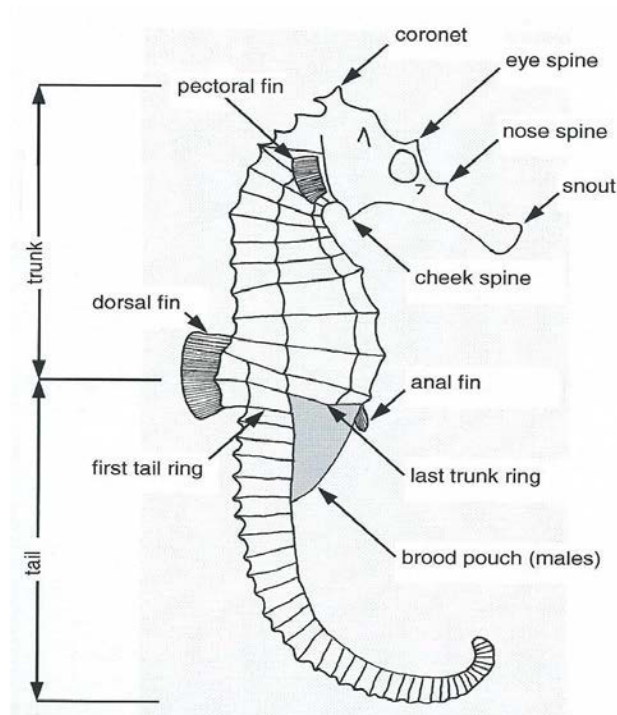
Figure 2.1. Morphology and measuring of seahorses (modified from Lourie *et al*. 1999b).

2.4.3   Reproductive biology

The breeding season of *H. whitei* in Sydney Harbour is considered to be from October to April (Vincent *et al.*, 2004), with males and females forming pairs and displaying monogamy for the duration of the breeding season (Vincent and Sadler, 1995; Vincent and Giles, 2003). It is suggested that they pair bond because it increases reproductive efficiency and increases the potential for higher reproductive success (Vincent and Sadler, 1995). Males and females come together early in the morning during the breeding season and perform a greeting ritual. If the male isn't pregnant the female transfers her eggs into the male's pouch (Vincent, 1995). The pregnancy period in males is 20-22 days (Vincent and Sadler, 1995) with a brood size

of approximately 100-250 with length at birth averaging 8.5 mm (Lourie *et al.*, 1999b). Brood size and the size of the young decrease over the breeding season (Vincent and Giles, 2003). Additionally, Vincent and Giles (2003) found that female size was the key determinant of the number of young released by the male, which was in turn influenced by the number of eggs the female had transferred to the male. Maturity is reached after approximately three months, where a developed pouch can be seen in the males, with reproductive maturity occurring at approximately six months (Kuiter, 2009).

2.4.4   Habitat use

Hippocampus whitei occur in a range of habitat types; however, there have been few studies that have assessed the species' habitat utilisation. The most detailed studies of *H. whitei* (Vincent and Sadler, 1995; Vincent and Giles, 2003; Vincent *et al.*, 2004; Vincent *et al.*, 2005) focused on populations found in *Posidonia australis* and *Zostera muelleri subsp. capricorni* seagrasses within Sydney Harbour whilst Clynick (2008) found that seahorses utilised the artificial habitats of protective swimming nets at various locations in Sydney Harbor. Kuiter (2009) reported that they can also be found in sponge habitats, particularly in coastal estuaries that are subject to tidal currents, and in depths less than 20 m. Prior to the studies in this thesis, there had been no research undertaken that assessed the habitat preferences of *H. whitei* which would provide useful information on the habitats that need to be conserved to ensure long term viability in the wild.

In a study on the protective swimming nets in Sydney Harbour, Clynick (2008) reported that seahorses (*H. abdominalis* and *H. whitei*) were in greater abundance on the permanent swimming net habitats compared to the nets that were only set for the summer months. It was considered that the removal or disturbance of the swimming nets could impact on seahorse populations as the alteration of their habitat could cause them to relocate. As these nets acquire substantive epibiotic growth, they often require cleaning or replacing by maintenance contractors as frequently as every 12 months, which leads to alteration of the seahorse habitat on the nets. It is unknown how this net cleaning affects seahorse abundance and whether this alteration of artificial habitat could cause a decline in local seahorse populations.

2.4.5   Home range

Home range is considered the area in which an animal lives and travels (Burt, 1943). Many species of seahorses have been found to have small home ranges and display strong site fidelity (Perante *et al.*, 2002; Bell *et al.*, 2003; Moreau and Vincent, 2004). In the only study on movements of *Hippocampus whitei*, Vincent *et al.* (2005) found in Sydney Harbour that both females and males exhibited small home ranges during the breeding season, of areas 14.4 m^2 and 9 m^2 respectively. It is hypothesised that they maintained small ranges because the costs of movement were greater than the benefits in terms of survival, growth and reproduction (Vincent *et al.*, 2005). Whilst assessment has been undertaken on the home range of *H. whitei* during the breeding

season, there has been no research on movements outside the breeding season, over a longer time scale or at sites outside of Sydney Harbour.

2.5   Conclusion

Even though seahorses are considered an iconic species that have had considerable research attention, there are still major gaps in our knowledge of *Hippocampus* spp., particularly of the life history, population abundance estimates and habitat preferences of different species. As many species are threatened from over-fishing and potentially habitat loss, it is essential to determine the habitats that are critical for the survival of seahorses and quantify their life history parameters to assess their resilience to threatening processes.

Hippocampus whitei became a protected species in NSW in 2004; however this protection was based on the precautionary principle in the absence of available data on population abundance and threats to the species (DPI, 2005). Whilst there has been extensive research undertaken on pair bonding, reproductive success and home range for *H. whitei*, there is little or no understanding of its population abundance, life history parameters (such as age at first reproduction, growth rate, longevity, mortality rates), habitat usage and response to habitat modification which are considered to be essential knowledge needed for the management of at-risk species (Vincent *et al.*, 2011). Addressing these knowledge gaps for *H. whitei* would assist with management decision-making for the conservation of this species.

CHAPTER 3

Population dynamics and life history of a geographically restricted seahorse,

Hippocampus whitei[1]**.**

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[1] Harasti, D., Martin-Smith, K., and Gladstone, W. (2013). Population dynamics and life history of a geographically restricted seahorse, *Hippocampus whitei*. *Journal of Fish Biology* (2012) **81**, 1297-1314.

3.1   Abstract

Seahorses are considered to be under threat from over-exploitation and loss of essential habitat worldwide. Development of conservation measures is constrained by limited information on population dynamics and life histories. The aim of this study was to collect these data for White's seahorse *Hippocampus whitei*; a geographically-restricted species that is listed as 'Data Deficient' under the IUCN Red List. Data from *H. whitei* populations were collected from two regions, (Port Stephens (north) and Sydney Harbour (south) in New South Wales - Australia, covering most of the known range of *H. whitei*) from 2005 to 2010. Over 1000 individuals were tagged using fluorescent elastomer and on subsequent recaptures were remeasured for growth data that were used in a forced Gulland-Holt plot to develop growth parameters for use in a specialised Von-Bertalanffy growth function model. Growth parameters for Port Stephens were: females L_∞ = 149.2 mm and K= 2.034 per year and males L_∞ = 147.9 mm and K=2.520 per year compared to estimates from Sydney Harbour: females L_∞ = 139.8 mm and K= 1.285 per year and males L_∞ = 141.6 mm and K=1.223 per year. Whilst there was no significant difference in growth between sexes for each region, seahorses in Port Stephens grew significantly quicker and larger and matured and reproduced at a younger age than those from Sydney Harbour. The life span of *H. whitei* is at least five years in the wild with six individuals recorded reaching this age. Data collected on breeding pairs found that *H. whitei* displays life term monogamy with three pairs observed remaining pair

bonded over three consecutive breeding years. Baseline population densities were derived for two Port Stephens' sites (0.035 m^{-2} and 0.110 m^{-2}) and for Manly in Sydney Harbour (1.050 m^{-2}). Even though the life history parameters of *H. whitei* suggest it may be reasonably resilient, precaution should be taken in its future management as a result of its limited geographical distribution and increasing pressures from anthropogenic sources on its habitats.

3.2 Introduction

Worldwide, many seahorse species are considered to be under threat through over-exploitation for traditional medicines, the aquarium trade and for curios and through loss of essential habitat (Lourie *et al.*, 1999b; Foster and Vincent, 2004; Vincent *et al.*, 2011). The entire genus *Hippocampus* is listed on Appendix II of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) and the majority of seahorse species are listed as data deficient (29) with several others listed as vulnerable (10) or endangered (1) (IUCN, 2013).

Protection of seahorses (*Hippocampus* spp.) varies amongst regions, with high levels of protection in some countries, such as Australia, and little or no protection provided in other countries such as Vietnam (Giles *et al.*, 2006), India (Salin *et al.*, 2005), Malaysia and Thailand (Perry *et al.*, 2010). In Australian waters, the States of South Australia, Victoria, Tasmania and New South Wales (NSW) provide some

form of protection to seahorses under their local fisheries or threatened species legislation whilst they are also protected in Commonwealth waters under the *Environment Protection and Biodiversity Conservation Act* 1999.

There are at least seven recognised species of seahorses in NSW (Kuiter, 2009); however only two species are regularly sighted, the pot-belly seahorse *Hippocampus abdominalis* Lesson 1927 and the White's seahorse *Hippocampus whitei* Bleeker 1855. From 2004, all Syngnathidae species were protected in NSW under the *Fisheries Management Act 1994* (DPI, 2005). It was acknowledged at the time that there were no current concerns that syngnathids in NSW were threatened; however this was attributed to little or no available data on population sizes, distribution or knowledge of their physiology.

Most studies assessing life history traits of seahorses have been conducted in aquaria: *Hippocampus abdominalis* (Woods, 2003), spotted seahorse *Hippocampus kuda* Bleeker 1852 (Garcia and Hilomen-Garcia, 2009), slender seahorse *Hippocampus reidi* Ginsburg 1933 (Hora and Joyeux, 2009) and three-spot seahorse *Hippocampus trimaculatus* Leach 1814 (Sheng *et al.*, 2007) whilst studies in situ have reported life history traits for a few seahorse species including the long-snouted seahorse *Hippocampus guttulatus* Cuvier 1829 (Curtis and Vincent, 2006), Japanese seahorse *Hippocampus mohnikei* Bleeker 1854 (Kanou and Kohno, 2001) and dwarf seahorse *Hippocampus zosterae* Jordan & Gilbert 1882 (Strawn, 1958). However, Foster and Vincent (2004) determined that life history parameters for most *Hippocampus* species

are unknown and that species-specific studies would be beneficial in establishing these parameters.

The focus of this study was *H. whitei*, a seahorse that is listed as 'Data Deficient' under the IUCN Red List (IUCN, 2013). The basis for IUCN listing is that there are no published data about populations trends or total number of mature animals for this species, there is little information about distribution or area of occupancy, and no quantitative analyses examining the probability of extinction for this species has been undertaken and that further research is required (IUCN, 2013). Previous research undertaken on *H. whitei* has focused on reproduction, monogamy and pair bonding (Vincent and Sadler, 1995; Vincent and Giles, 2003; Vincent *et al.*, 2004), home range behaviour (Vincent *et al.*, 2005), and the extent to which they use artificial structures (Clynick, 2008; Harasti *et al.*, 2010).

This paper seeks to improve the knowledge on the life history parameters of *H. whitei* to assist with its long term conservation and specifically focuses on (1) the geographical range of the species, (2) development of a growth model, (3) estimates of natural mortality and life span, (4) population abundance from two regions, and (5) assessment of the long term monogamy of breeding partners. As syngnathid species may show a variation in their life history parameters across their geographical range (Sanchez-Camara *et al.*, 2011), this study compares the life history parameters of two populations of *H. whitei* from regions at either end of its

geographical range (Sydney Harbour and Port Stephens), the first such study to do so.

3.3 Materials and Methods

Species Description

Hippocampus whitei occurs in shallow water estuaries and inhabits seagrasses, sponge gardens and artificial structures such as jetties and protective swimming nets (Harasti *et al.*, 2010). *H. whitei* is considered to be monogamous and pair-bonded during the breeding season (Vincent and Sadler, 1995) with a small home range (Vincent *et al.*, 2005). The male brood size of *H. whitei* is 100-250 (Vincent and Giles, 2003) and newborns are approximately L_T 8.5 mm (Lourie *et al.*, 1999b).

Lourie *et al.* (1999b) reports that the distribution of *H. whitei* includes central NSW, the waters of southern Queensland and the Solomon Islands whilst in his revision of Australian seahorses, Kuiter (2001) updated the distribution of *H. whitei* limiting it to the estuaries of the Sydney and Newcastle region; however, he also notes it may be found in Spencer Gulf - South Australia.

Geographical range

To provide an assessment of the species' geographical range, numerous exploratory dives (*n*=100+) from 2005-2009 were undertaken in various coastal estuaries and embayments (*n*=24) along the entire NSW coast to check for occurrence of *H. whitei*.
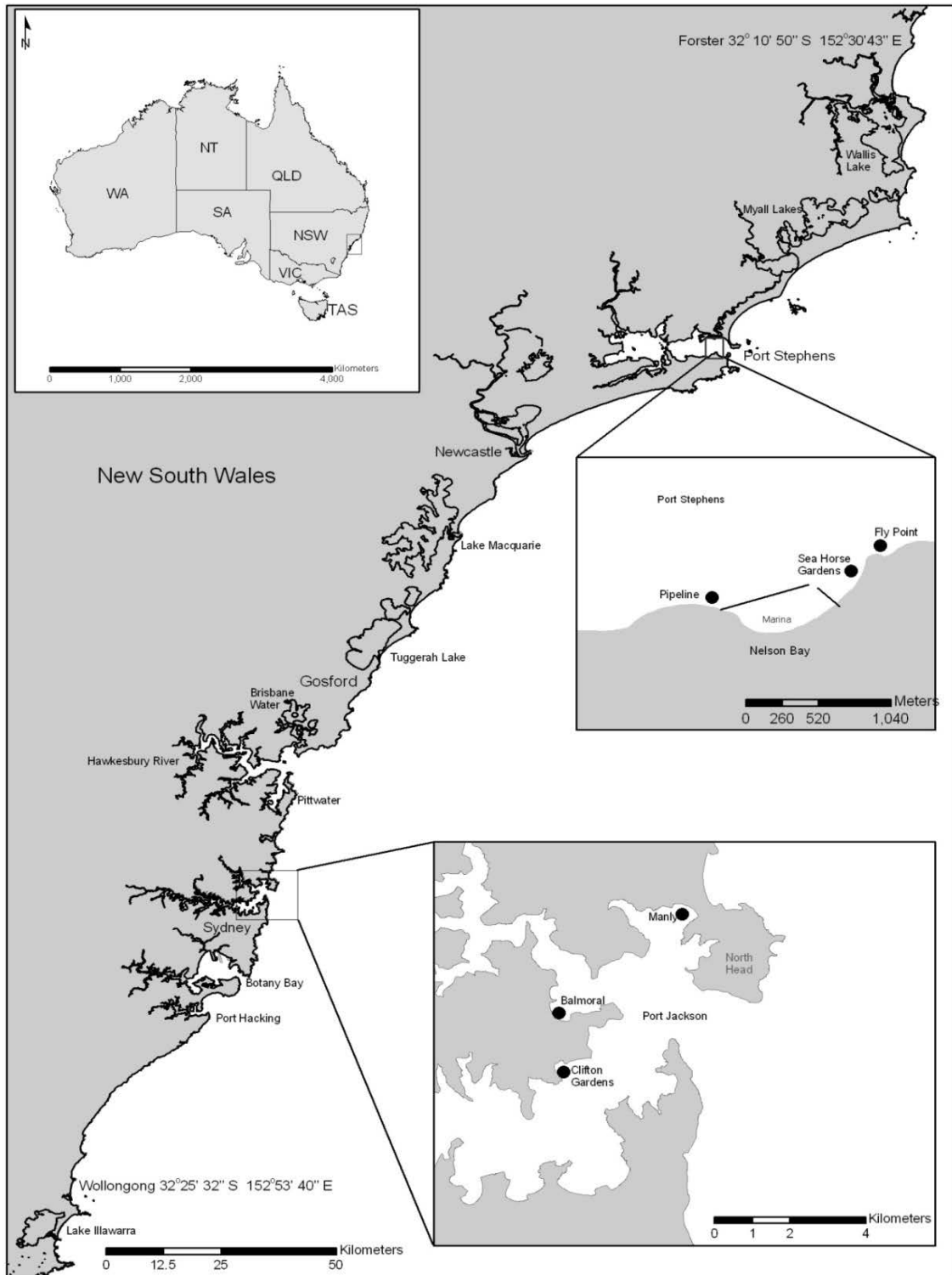
Figure 3.1. Known distribution of *Hippocampus whitei* in New South Wales – Australia with insert boxes of Port Stephens and Sydney Harbour study sites.

Study Sites

Research on life history and population parameters was carried out in two regions; Sydney Harbour (Port Jackson) and Port Stephens in central NSW, Australia (Figure 3.1, Table 3.1). Life history data from Sydney Harbour were collected from three sites: Clifton Gardens, Balmoral and Manly. Data in the Port Stephens region were collected from three sites: the Pipeline, Fly Point and Seahorse Gardens. The sites within Port Stephens are subject to very strong tidal currents and could only be dived on the slack high tide each day for best visibility and safety so the time for each survey varied on each occasion. These sites contain a mixture of sponge garden, soft coral and seagrass habitat and are approximately 6000 m² in size and all located within the Port Stephens-Great Lakes Marine Park which was established in 2005.

Location	Coordinates (Latitude & Longitude)
Clifton Gardens – Sydney Harbour	33⁰50′20.74″S – 151⁰15′11.82″E
Balmoral – Sydney Harbour	33⁰49′37.34″S – 151⁰15′12.84″E
Manly – Sydney Harbour	33⁰47′57.63″S – 151⁰ 16′57.67″E
Pipeline – Port Stephens	32⁰43′04.63″S – 152⁰08′29.27″E
Fly Point – Port Stephens	32⁰42′48.20″S – 152⁰09′11.40″E
Seahorse Gardens – Port Stephens	32⁰42′55.70″S – 152⁰08′58.87″E

Table 3.1. GPS coordinates of survey locations in Sydney Harbour and Port Stephens.

Population abundance

From 2005 to 2010, seahorses were individually tagged using small visible implant fluorescent elastomer tags (VIFE – Northwest Marine Technologies, USA; www.nmt.us) using the techniques described in Woods and Martin-Smith (2004). Every seahorse was given a unique code of three 1-2 mm coloured elastomer tags (green, orange, red or yellow). On several occasions, an additional single tag was added to the seahorse if one of the other tags was too small to detect. This allowed individual growth rates to be monitored over time and provides an estimate of population abundance through mark-resight surveys. Animals were tagged in situ underwater with the capturing, tag and release taking approximately 1-2 minutes per seahorse with the seahorse being placed back on to its holdfast at the completion of tagging. A total of 973 *H. whitei* (L_T range: 33 mm – 150 mm) were tagged in Port Stephens and 400 (L_T range: 53 mm – 143 mm) were tagged in Sydney Harbour. Tag loss was considered to be minimal with only 13 animals in Port Stephens (0.01%) observed to be missing a single tag, which was subsequently replaced on recapture.

To obtain initial population estimates at two sites in Port Stephens, 95 seahorses were individually marked at the Seahorse Gardens and 68 seahorses were marked at the Pipeline in June 2006. Following marking, population size for both locations was estimated using closed population analysis where five dive surveys (average dive time 70 minutes) were conducted on consecutive days where all marked and unmarked seahorses were recorded. Estimates of abundance for both sites were

derived from the joint hypergeometric maximum likelihood estimator (JHE) using Lincoln-Petersen estimates in the program NOREMARK (White, 1996). This estimator assumes that all the marked animals are on the area surveyed for each survey, i.e., that the population is geographically and demographically closed.

Population size was estimated at one site in Sydney, the Manly protective swimming enclosure. A total of 304 individuals were tagged on the Manly net with 15 surveys undertaken between April 2007 and Feb 2008 (with a minimum of 1 survey conducted each month). Each survey consisted of 2 divers counting and recording every marked and unmarked seahorse found living on the net.

As the surveys were conducted over a long time period (10 months), the population was considered to be 'open' as immigration, emigration, births and mortality would have occurred during the survey period. Therefore, an open population Jolly-Seber model was applied to the mark-resight data using the POPAN model in the program MARK (White and Burnham, 1999). The model allows for movement in and out of the population, and provides survivability estimates (ϕ), probability of resighting individuals (P), and an estimate for the total population size (N) (Schwarz and Arnason, 1996).

Natural mortality

Estimates of natural mortality were derived from three models: one direct method and two indirect methods. As *H. whitei* is protected in NSW with no fishing pressure,

all mortality is assumed to be from natural factors such as biological ageing and predation. The direct estimate of natural mortality was obtained using mark-resighting data where recapture probability (P) and apparent survival rate (ϕ) were estimated using a Cormack-Jolly-Seber (CJS) model analysed by Program MARK. Differences between sex for P and ϕ were tested using Akaike's information criteria (AIC) using models that assessed time dependence and sex. The direct estimate of mortality was applied to the seahorse population at Manly in Sydney Harbour for data collected from April 2007 to February 2008 (n=13 surveys) and was also applied to Port Stephens seahorse population for data collected from January 2008 to October 2008 (n=14 surveys) as there were sufficient mark recapture observations to apply the CJS model.

Two indirect estimates of natural mortality were derived from two methods and were applied to populations from Port Stephens and Sydney Harbour. The first method employed the empirical relationship of Pauly (1980): $\log M = -0.0066 - 0.279(\log L_\infty) + 0.6543 (\log K) + 0.4634(\log T)$ where K and L are the von Bertalanffy growth parameters, T is the mean annual water temperature and M is the mortality rate. The mean annual water temperature for Sydney Harbour is 20.6^0C (Bureau of Meteorology data, 2005) and for Port Stephens was calculated at 19^0C using mean monthly temperature data collected from 2005 to 2009 by the primary author (DH). The second method estimated mortality based on the method devised by Jensen

(1996) which is expressed as $M = 1.60K$, where M is the instantaneous mortality rate and K is the derived von Bertalanaffy rate of growth.

Length

Measurements in this study were taken as straight lines (rather than following the curvature of the body) as it was found to be difficult to take curvature measurements whilst handling individuals, particularly underwater. Each seahorse was individually measured to the nearest 1 mm with three measurements recorded, (1) Head length: Tip of snout to mid cleithral ring; (2) Tail length: Tail to base of abdomen (bottom of pouch in males); and (3) Trunk length: Bottom of abdomen/pouch to top of coronet. An estimate for total length (L_T) was obtained by combining trunk and tail lengths.

Mean L_T of males and females from Sydney Harbour and Port Stephens were compared using a two factor analysis of variance (ANOVA). Region and Sex were analysed as fixed orthogonal factors. Data were checked for homogeneity of variances using Cochran's C test and ANOVA analysis was undertaken in SPSS 19.

Length-at-age

Length-at-age was estimated using length increments derived from resighting data from 2006 to 2010 (King, 1995). It was assumed that the growth of *H. whitei* corresponded to the von Bertalanffy growth function (VBGF) (von Bertalanffy, 1938). The VBGF equation is $L_t = L_\infty (1-e^{-K(t-t_0)})$, where L_t is the length at time t, K is the

growth rate constant (measurement of how rapidly a fish approaches its asymptotic length), L_∞ is the maximum asymptotic length and t_0 is the theoretical age at length zero. K and L_∞ were estimated using a 'forced' Gulland-Holt plot (King, 1995). In the forced Gulland-Holt plot, the mean growth rate ($\bar{y}$) and the mean length ($\bar{\upsilon}$) are used to estimate K in the equation: $K = \bar{y}/(L_\infty - \bar{\upsilon})$ with the parameter L_∞ estimated using the mean of the largest 10 male and female lengths (King, 1995). Both K and L_∞ parameters were used in the VBGF to develop a growth model for male and female *H. whitei* from Port Stephens and Sydney Harbour. Comparison of VBGF models was undertaken using an Analysis of Residual Sum of Squares (ARSS) (Chen *et al.*, 1998) that compared VBGF models between sexes within each location and between locations.

Length and age at maturity and first reproduction

At a small size (<70 mm) it was difficult to determine if a juvenile was actually a male or female (because of the absence of a pouch) however the sex could be confirmed through repeated observations of the tagged individual over time to see if a pouch developed. L_T at first reproduction was determined at the Pipeline site by tagging 68 'juvenile' seahorses of L_T 33-80 mm (mean ± S.E.=56.4 ±1.43 mm) between December 2008 and March 2009. These tagged juveniles were monitored until December 2009 with time to maturity and first reproduction recorded and associated lengths.

Life Span

Estimates of life span were derived from mark-resight data of tagged individuals recorded from 2005 to 2010 in Port Stephens. The initial tagging date and L_T were recorded and on each subsequent re-sighting the animal was remeasured with L_T and date recorded to indicate life span. Estimates of actual age were derived from the VBGF model using age at initial tagging compared to the last sighting to provide an indication of life span for males and females from Port Stephens.

Long-term Monogamy

To assess the existence of long-term monogamy in *H. whitei*, 15 pairs of adult seahorses (mean L_T = 122.5 ±2.45 S.E.) were tagged when first observed together in January-February 2008 at the Pipeline in Port Stephens and were monitored monthly until December 2009. Each month, each individual's status was recorded (present or absent) and whether they were paired with the same or a different partner during the breeding season (September–February, Harasti *et al.* (2010)). Seahorses were considered to be in a pair when they were seen greeting their partner in the early morning or were found to inhabit the same holdfast during the day or night (Vincent and Sadler, 1995; Vincent *et al.*, 2004).

Seasonal reproduction

The reproductive frequency of males over a breeding season was assessed by tagging 34 adult males (mean L_T recorded at first reproduction: 121.1 mm ± 2.52 S.E.)

at the Pipeline at the start of September 2008 and monitoring their pregnancy state weekly until the end of March 2009 as this is considered the end of the breeding season (Harasti *et al.*, 2010). Maturity was determined through the presence or absence of a brood pouch in the males, located below the abdomen. Males were considered to be mature if they had a pouch that was observed to be full or was large enough to carry a brood (Curtis and Vincent, 2006). Pregnancy states were recorded as (1) pouch empty – no recent pregnancy, (2) pouch full – evidence of pregnancy (3) pouch very rotund – birthing imminent, and (4) pouch flaccid – birth recently performed. This allowed determination to be made on the number of occasions a male seahorse was able to reproduce during a single breeding season.

3.4   Results

Geographic range

Scuba diving surveys across NSW recorded *H. whitei* in several locations (from north to south): 1. Wallis Lake, 2. Port Stephens, 3. Lake Macquarie, 4. Tuggerah Lakes, 5. Brisbane Water, 6. Pittwater, 7. Port Jackson (Sydney Harbour), 8. Botany Bay, 9. Port Hacking, and 10. Lake Illawarra (see Figure 3.1). The only locations where large abundance of *H. whitei* occurred were Sydney Harbour and Port Stephens with the most found at any of the other locations being eight animals in Port Hacking.

Population abundance

Initial closed population estimates (± 95% CL) from Program NOREMARK for Port Stephens in June 2006 were 213 (169-283) for the Pipeline and 673 (449-1107) for the Seahorse Gardens. Seahorse densities were 0.035 m^{-2} at the Pipeline site and 0.110 m^{-2} at the Seahorse Gardens (each site was approximately 6,000 m^2). The population size on the protective swimming enclosure at Manly (approximate area 300 m^2) was estimated at 315 (304-326), giving a seahorse density of 1.050 m^{-2}. Population abundance fluctuated during the survey period with the highest abundance recorded in November-December 2007 (Figure 3.2); the peak time in the breeding season for *H. whitei*.
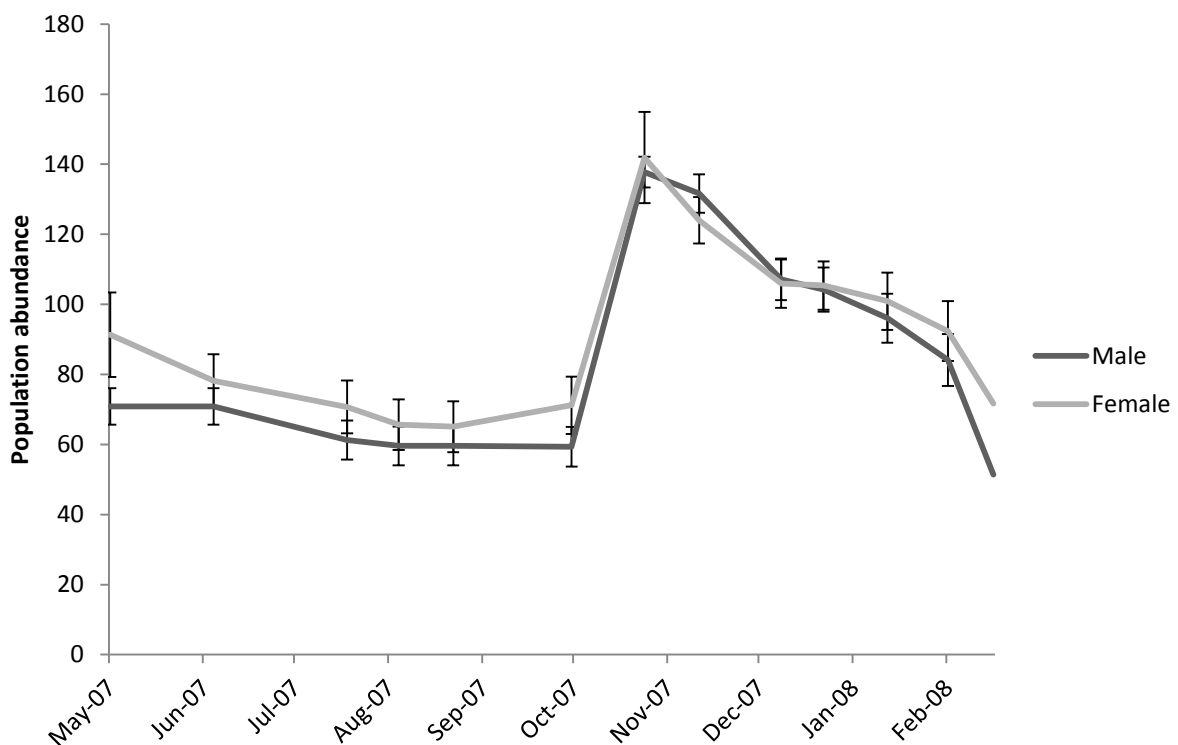
Figure 3.2. Mean ± S.E. population estimates for male and female *Hippocampus whitei* on Manly net swimming enclosure (May 2007 – February 2008).

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Natural mortality

For the two indirect estimates of natural mortality, Jensen mortality estimates were substantially higher for males and females from Port Stephens and Sydney Harbour when compared to the Pauly estimates (Table 3.2). Mortality estimates based on L_∞ and K from the forced Gulland and Holt plot were higher in Port Stephens for both sexes compared to estimates from Sydney Harbour for both sexes. Monthly apparent survival rate (ϕ) and resighting probability (P) varied across surveys for both the Manly and Port Stephens population of seahorses. The most suitable CJS model for Manly (AICC=3039.88, likelihood=1.00, deviance=319.74, parameters=54) and Port Stephens (AICC=770.18, likelihood=1.00, deviance=77.77, parameters=29) was fully time dependent for P and ϕ. The mean probability of resighting tagged individuals (P) across all surveys was 0.62 (±0.04 S.E.) for Manly compared to 0.20 (±0.05 S.E.) for Port Stephens and the average apparent survival rate for the year for Manly was 35.6% whilst Port Stephens was 27.4%.

Length

The largest male specimen recorded from Port Stephens was 162 mm (mean ± S.E. = 115.4 ± 0.86 mm, *n*=541) and the largest female was 155 mm (114.7 ± 0.78 mm, *n*=587). The largest male specimen from Sydney Harbour was 152 mm (107.3 ± 1.41 mm, *n*=180) and the largest female was 145 mm (109.5 ± 1.33 mm, *n*=198). There was a

significant difference in L_T between the two regions ($F_{1,1506} = 0.56$, $P < 0.001$), no significant difference in L_T between sexes for each region ($F_{1, 1506} = 0.43$, $P > 0.51$) and there was no significant region x sex interaction ($F_{1, 1506} = 1.68$, $P > 0.19$).
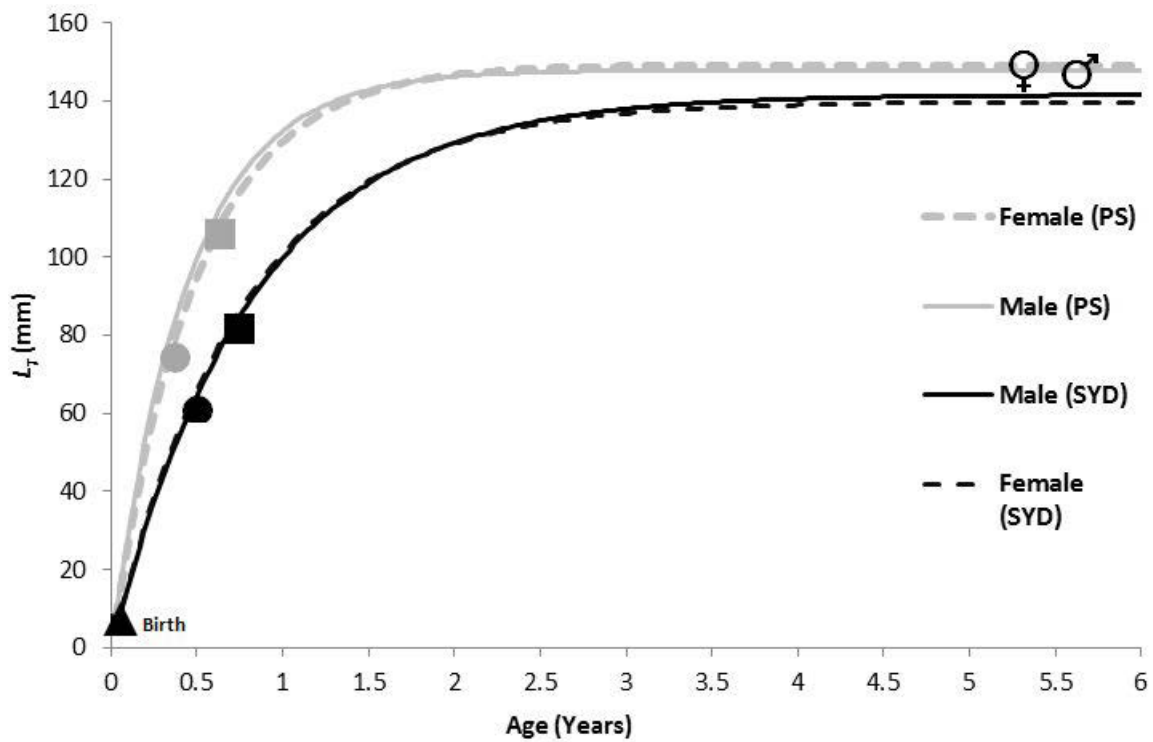
Figure 3.3. Estimated von Bertalanffy growth curve for males and female *Hippocampus whitei* for Port Stephens and Sydney Harbour. ● = mean size at maturity for male and ■ = mean size at first reproduction in Port Stephens. ● = smallest observed mature male (with pouch) and ■ = small pregnant male in Sydney Harbour. ♂ = oldest male and ♀ = oldest female observed in Port Stephens. ▲ = Size at birth of *H. whitei* from Lourie *et al.* (1999).

Table 3.2. Growth parameters (L_∞ and K from the von Bertalanffy growth model) and natural mortality estimates for *Hippocampus whitei* from two different regions. *n* = the total number of growth increments used in model with some individuals having been re-measured on more than one occasion.

	L_∞ (mm)	K (1/Year)	Goodness of fit (R^2)	Mortality (Pauly) (1/Year)	Mortality (Jensen) (1/Year)
PORT STEPHENS					
MALE (*n*=177 from 108 individuals)					
Gulland and Holt (forced)	147.9	2.252	0.89	1.689	3.604
FEMALE (*n*=224 from 120 individuals)					
Gulland and Holt (forced)	149.2	2.034	0.87	1.612	3.254
SYDNEY HARBOUR					
MALE (*n*=76 from 45 individuals)					
Gulland and Holt (forced)	141.6	1.223	0.35	1.145	1.956
FEMALE (*n*=84 from 58 individuals)					
Gulland and Holt (forced)	139.8	1.285	0.61	1.187	2.056

Length at age

Growth parameters varied between the two regions (Figure 3.3 and Table 3.2), with males and females from Port Stephens found to have higher L_∞ and K growth constant than those recorded from Sydney Harbour. ARSS comparisons found there was no significant difference in VBGF growth between sexes for each region (Sydney Harbour: $F_{3,113} = 0.03$, $P > 0.99$ and Port Stephens $F_{3,420} = 0.54$, $P > 0.65$) however there was a significant difference in VBGF growth between locations ($F_{3,514} = 5.38$, $P < 0.001$).

Length and age at maturity and first reproduction

Of the 68 juveniles tagged between December 2008-March 2009, 18 were regularly re-sighted and identified as males from subsequent surveys through the presence of a pouch. The mean ± S.E. L_T for male maturity was 76.8 ± 1.6 mm and using the forced Gulland-Holt plot estimates of L_∞ and K in the VBGF model, the mean age in Port Stephens to reach maturity was 120 days (4 months). The smallest male recorded indicating pouch development was a L_T of 65 mm which is 95 days old from the VBGF model. For Sydney Harbour, the smallest male observed from all surveys with a developing pouch was 60 mm, 165 days on the VBGF model using forced Gulland-Holt plot estimates of L_∞ and K.

From the 68 tagged juveniles, nine were regularly sighted until first observed reproduction. The mean ± S.E. L_T and age at first reproduction of males at Port

Stephens was 106.7 ± 2.4 mm and 210 days. The smallest pregnant male observed across all sampling periods (2005-2010) in Port Stephens was 78 mm (125 days). The smallest pregnant male observed in Sydney Harbour was 80 mm (250 days). At a small size it was difficult to determine if a juvenile was actually a female (because of the absence of a pouch), however the smallest confirmed female observed (determined through observation of partnering with a male and repeated observations over several months) was found to be 72 mm and was seen at the Pipeline site in Port Stephens.

Life Span

The largest time difference from initial tagging to last sighting of a tagged male in Port Stephens was 1713 days (1 August 2005 – 10 April 2010) of an animal that was originally tagged at 113 mm and measured 152 mm on final resighting. For females, the longest resighting period was 1463 days (01 August 2005 – 1 September 2009), that measured 133 mm on initial tagging and 155 mm on final resighting. Five males were re-sighted 4 years after initial tagging whilst only one female was re-sighted 4 year after initial tagging. These animals all occurred at the Seahorse Gardens site with the exception of one male that was found at the Pipeline. Using the forced Gulland-Holt plot estimates of L_{∞} and K in the VBGF model, the oldest recorded male would have been 235 days old at time of initial tagging, making it 1948 days old (5.3 years) on its last resighting whilst the oldest recorded female would have

been 400 days old at time of initial tagging making it 1892 days old (5.2 years) on final resighting.

Long-term monogamy

Of the 15 original breeding pairs observed from the 2007-2008 summer breeding season, nine pairs were observed breeding together during the 2008-2009 season whilst three pairs remained breeding together in the 2009-2010 season (Figure 3.4). These three pairs were all observed breeding together over the three consecutive breeding seasons. On two occasions, a female paired up with another male however in each case their previous male partner had disappeared from the site and was not recorded again. At no stage during the study, did a male or female leave their current partner whilst their partner was still present at the study site even when there were other single partners available.

Seasonal reproduction

In Port Stephens and Sydney Harbour, the first sign of breeding in both regions occurred in late September with males partnering up with a female and displaying pregnant pouches. The latest pregnancy in a male was observed in April however very few pregnant males were encountered after February.

Of the 34 tagged adult males at the Pipeline site, 29 males were recorded reproducing on two occasions or more over the 2008-09 breeding season, the other five animals were observed with a single pregnancy and then not recorded again on the site. The greatest numbers of pregnancies observed over the breeding season was eight which was recorded by two large males (L_T = 139 and 134 cm), with both first observed pregnant in late September and final pregnancy observed in mid-March (approximately 180 days). Both males stayed with their female partners during the entire breeding period. For those males reproducing on six occasions or more (n=10), their mean ± S.E. L_T was 129.4 ± 3.8 mm.

SEX	TAG	Jan-08	Feb-08	Mar-08	Apr-08	May-08	Jun-08	Jul-08	Aug-08	Sep-08	Oct-08	Nov-08	Dec-08	Jan-09	Feb-09	Mar-09	Apr-09	May-09	Jun-09	Jul-09	Aug-09	Sep-09	Oct-09	Nov-09	Dec-09
M	G2G4G4		1		1	1	1	1	1	1	1	1	1	1											
F	G2G9G10G10		1	1	1					1	1	1													
M	G3G4G9		1								1	1	1	1											
F	G3G3G10		1								1	1	1	1											
M	P6P7P9	1			1		1	1	1		1	1	1	1	1	1	1	1	1	1	1	1	1	1	
F	G7G9G10	1			1					1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M	G1G2G9		1			1				1	1	1		1	1	1				1	1	1	1	1	1
F	G1G2G10		1			1				1	1	1	1	1	1	1	1	1	1	1					
M	G6G9G9		1	1					1		1	1	1	1	1	1		1	1					1	1
F	G9G9G10		1	1						1	1	1	1	1	1	1		1	1		1			1	1
M	G2G5G7G10		1							1	1	1	1	1	1	1									
F	G2G9G9G10		1							1	1	1	1	1	1	1									
F	G7G8G9		1		1		1			1	1	1	1	1	1	1		1	1	1	1	1	1		1
M	G7G9G9		1		1	1	1			1	1	1	1	1	1	1	1	1	1	1	1	1	1		1
F	G6G6G6		1	1			1	1	1	░	░	░	░	░	░	░	1	1	1	1	1	1	1	1	
M	G6G6G7		1																						
░	G1G5G░									░	░	░	░	░	░	░									
F	G1G5G5		1				1	1		1	1	1	1	░	░	1		1		░	1	░	░	░	
M	G3G3G3		1	1						1	1	1													
░	G1G1G░													░	░	░				░		░	░	░	

Figure 3.4. Long term monogamy in *Hippocampus whitei* over three breeding seasons. Data is shown for those pairs (*n*=9) that were resighted paired over consecutive breeding seasons. 1= Sighting of individual. Shading shows when individuals were found together during a survey while hatched shading indicate one of the original paired seahorses from the 2008-2009 breeding season was seen to pair up in the following breeding season with another individual.

3.5   Discussion

Geographic Range

This study has confirmed that *H. whitei* has a limited geographical distribution in Australia and appears to be endemic to just nine estuaries, coastal lakes and embayments from Wallis Lake-Forster in the north to Lake Illawarra in the south, along approximately 300 km of the NSW coast. Records from the Australian Museum ichthyology collection and NSW Department of Primary Industries estuarine fish catch data, and discussions with scuba divers along the NSW coast, also confirm that *H. whitei* has only been recorded within the same 300 km region. Records of *H. whitei* from Queensland and the Solomon Islands (Lourie *et al.*, 1999b) are most probably the high-crown seahorse *Hippocampus procerus* (Kuiter, 2001), which is similar in appearance and taxonomy but has a tropical distribution, a taller and spinier coronet, higher fin ray count and generally a spinier body. Similar to the endangered seahorse *H. capensis* in South Africa, *H. whitei* does not venture into oceanic waters and its range is limited to estuarine environments. As the distribution of *H. whitei* covers the most developed and populated areas of NSW (DECCW, 2009), the estuaries where it is known to occur are subject to various anthropogenic impacts (such as dredging, runoff, land-based development) and habitats for *H. whitei* (i.e. sponge gardens, softcorals and seagrasses) are considered under threat and in decline in several of these locations (West *et al.*, 2004; DECCW, 2009; Smith *et al.*, 2010).

Growth, length and life span

This study was successful in following several individual *H. whitei* over several years and it was surprising that *H. whitei* were found to live for more than five years in the wild as data from individuals kept in aquaria is that they only live for approximately three years (Sydney Aquarium and Manly Oceanworld *pers. comm*). The longevity of *H. whitei* is similar to that of *H. guttulatus*, a similar sized seahorse species that was estimated to live for 4.3 - 5.5 years in the wild (Curtis and Vincent, 2006). Having six individuals resighted four years after initial tagging highlights the benefits of using VIFE as a tagging method for long term studies on seahorses, as tagged animals were still easily identified after four years with no indications of any harm caused by the tags.

This study assessed life history parameters from two different regions of the range of *H. whitei*, which approximated the northern and southern limits. *H. whitei* from Port Stephens (near the northern range limit) were found to grow quicker, mature and reproduce earlier and grow larger (L_T) than individuals from Sydney Harbour. L_∞ estimates indicate *H. whitei* grows to a medium size for seahorses and displays similar rates of rapid initial growth as recorded in *H. abdominalis* (Martin-Smith *unpublished data*) and *H. kuda* (Dzyuba *et al.*, 2006). The reason for the difference in growth parameters between Sydney and Port Stephens may be attributed to habitat variation. Populations in Port Stephens lived in what is considered to be their natural habitats (sponges, softcorals and seagrasses; Kuiter (2009)) compared to the

animals monitored in Sydney Harbour that lived on artificial structures (swimming net enclosures and jetty pylons). The structural complexity of the natural habitats, i.e the long-bladed seagrass *Posidonia australis* Hooker 1858 and dense *Dendronephthya australis* Kükenthal 1905 soft corals, may provide good cryptic protection for seahorses when compared to artificial structures. *Hippocampus whitei* prefer habitats with dense epibiotic growth and avoid areas devoid of growth, possibly in relation to the greater availability of shelter and prey in these areas (Harasti *et al.*, 2010). Flynn and Ritz (1999) demonstrated that *H. abdominalis* also showed a preference for complex vegetated habitats as this increases their prey capture efficiency whilst assisting them in remaining hidden from potential predators. The availability of seahorse prey is considered to be greater in more complex habitats as abundances of mobile epifauna (amphipods and copepods) being significantly greater in habitats that provided more structural complexity (Hellyer *et al.*, 2011). Another possibility for variation in growth between the two regions could be attributed to genetic variation in *H. whitei* across its range and a future genetic study would be required to assess this theory.

The von Bertalanffy growth model does not allow for the influence of water temperature on growth to be considered. Wong and Benzie (2003) found that daily growth rate in *H. whitei* was almost doubled when contained in 26°C water compared to 17°C. However, the effect of water temperature is unlikely to be the cause of growth variation between Port Stephens and Sydney Harbour as the annual

mean water temperatures were similar (19°C and 20.6°C respectively). Indirect mortality estimates were found to be lower in Sydney Harbour than Port Stephens which can be attributed to values of L_∞ and K being significantly lower in Sydney Harbour. Direct estimates of mortality were also found to be higher in Port Stephens than Sydney Harbour and the differences in mortality between the two regions could be attributed to levels of predation varying between locations. Known predators of *H. whitei* have not been recorded however the authors observed during the various surveys that there were fewer species of fish, particularly recreational targeted species such as snapper *Pagrus auratus* (Bloch & Schneider 1801), at Sydney Harbour sites compared to Port Stephens. An assessment of predation on *H. whitei* would provide insight into effects on mortality across regions and the average apparent annual survival rate in Sydney Harbour of 35.6% and Port Stephens of 27.4% is similar to the survival rate of *H. guttulatus* in Portugal (29.4%-32.2%; Curtis and Vincent (2006)).

Maturity, seasonal reproduction and long term monogamy

Kuiter (2009) reports that the sex of *H. whitei* could be determined after three months due to presence of a pouch and that they were ready to breed in six months in aquaria. This study produced similar results with males in Port Stephens maturing at four months and reproducing at seven months. The maximum recorded number of pregnancies in the current study by a single male was eight (*n*=2), comparable with the value of seven found by Vincent and Sadler (1995). Using a mean brood size

of 157 (Vincent and Giles, 2003), there is potential for approximately 1200 offspring to be born per male per season in Port Stephens; however, Vincent and Giles (2003) also found that brood size was lower in later broods so the subsequent number of offspring produced would decrease over the breeding season.

Pair bonding and monogamy in seahorses has been well studied (Vincent and Sadler, 1995; Kvarnemo *et al.*, 2000; Mattle and Wilson, 2009; Bahr and Wilson, 2011); however, these studies were limited to investigating pair monogamy over single breeding seasons. This study documented pair bonding in *H. whitei* over three consecutive breeding seasons and shows that *H. whitei* can form long term bonds for reproduction if both partners remain alive. Kvarnemo *et al.* (2000) assessed long-term genetic monogamy in the West-Australian seahorse *Hippocampus subelongatus* Castelnau 1873 and found that some individuals were faithful throughout breeding whilst others switched mates during the season. Whilst this study did not assess genetic parentage of offspring, there was no evidence to suggest that any of the observed breeding pairs that mated over consecutive seasons bred with any other animals as pairs were commonly seen in close proximity and observed performing morning greetings as described by Vincent and Sadler (1995).

The reason for long term monogamy in *H. whitei* is unknown. Vincent and Sadler (1995) suggested that monogamy in seahorses may be beneficial as it could increase reproductive success; the 'mate familiarity effect'. The mate familiarity effect allows partners to synchronise their reproductive breeding cycles permitting them to

rebreed soon after a birth, thus reducing the interbrood interval, and increasing their potential reproductive rates. In a study on the messmate pipefish *Corythoichthys haematopterus* (Bleeker 1851), Sogabe *et al.* (2007) found that mate changes disadvantaged reproductive success because of the time constraints associated with finding a new partner and the time taken for a female to produce mature eggs, whilst Sogabe and Yanagisawa (2008) found partners also remained pair bonded during the non-reproductive season for the same species. It is most likely that *H. whitei* will remain pair bonded whilst both partners remain alive as it improves reproductive efficiency and increases the potential for higher reproductive success.

Population abundance

This study found several locations where large populations of *H. whitei* occurred and baseline population estimates were obtained for these locations. The seahorse population abundance on the Manly net was found to vary over time with a large influx of animals observed during October-November; the commencement of the breeding season for *H. whitei*. This increase in population size on the net could be attributed to seahorses moving from the adjacent seagrass (as reported in Harasti *et al.* (2010)) onto the net in search of a breeding partner as this artificial habitat is a suitable aggregation area for seahorses, possibly because of the dense epibiotic growth that provides protection and potential prey (Hellyer *et al.*, 2011).

The large abundance of *H. whitei* at the Seahorse Gardens site (Port Stephens) was surprising as it was not known as an area where seahorses occurred (the name

'Seahorse Gardens' was given to the site at the completion of this study). In the first tagging session at this site in 2005, 91 animals were encountered by two divers in only 45 min. The large abundance was confirmed with the mark-resight density estimate of 0.110 m^{-2}. Densities of *H. whitei* in Port Stephens were similar to densities recorded previously in seagrass in Sydney Harbour, 0.22 m^{-2} and 0.09 m^{-2} (Vincent *et al.*, 2005). Densities of *H. whitei* were also similar to other seahorse species elsewhere, including *H. capensis* 0.22 m^{-2} (Bell *et al.*, 2003) and the short-headed seahorse *Hippocampus breviceps* Peters 1869 0.21 m^{-2} (Moreau and Vincent, 2004). The high densities of *H. whitei* within Port Stephens could be linked to the expansive *Dendronephthya australis* soft coral habitat and sponge gardens that *H. whitei* inhabited. An assessment of habitat usage by *H. whitei* is required to help predict other areas where the species may occur and determine what habitats are important for the long term conservation of this species.

Conservation of the species

This study has determined that *H. whitei* has a small distribution in NSW, displays rapid growth and early maturity and can be found in abundance in several locations. As this species is considered to have no pelagic stage (Kuiter, 2009) and found only in coastal estuaries, it is most likely that recruitment is limited to areas where adults are breeding as offspring are less likely to be dispersed by oceanic currents. With the potential for limited mixing amongst the various estuarine populations, there is the possibility for a degree of genetic differentiation amongst these populations,

particularly at the northern and southern ends of its range. A study on *H. whitei* genetics may resolve whether populations along the NSW coast represent a single genetically connected population, or if geographically separate populations are genetically distinct and should be managed as separate evolutionarily significant units.

Even though this species has a very limited geographic range, there is no indication at this stage that it is threatened with extinction in the immediate future. As it is listed as a protected species in NSW and not permitted to be taken from the wild, it is unlikely that populations will decline as a result of fishing pressures. However, precaution should be taken in its future management as a result of its limited distribution and the increasing pressures from coastal developments and anthropogenic inputs on its estuarine habitats.

3.6 Acknowledgements

CHAPTER 4

Ontogenetic and sex-based differences in habitat preferences and site fidelity of the White's seahorse *Hippocampus whitei*[2].

4.1 Abstract

The aim of this study was to determine and compare habitat preferences for male and female adult and juvenile White's seahorse *Hippocampus whitei* and assess their movements and site fidelity over 4 years. Data were collected from 3 sites along 1.5 km of estuarine shoreline in Port Stephens, New South Wales, Australia, from 2006 to 2009 using seahorses which had been tagged with visible implant fluorescent elastomer. Relative availability of 12 habitats and habitat preferences of *H. whitei* was determined, based on the habitat that *H. whitei* used as a holdfast. *Hippocampus whitei* occurred in nine different habitats; adults preferred sponge and soft coral *Dendronephthya australis* habitats with no difference between male and female habitat preferences whilst juveniles preferred gorgonian *Euplexaura* sp. habitat. There was a significant preference by adults for *D. australis* colonies with height >40 cm and avoidance of colonies <20 cm. Neither adults nor juveniles used sand or the seagrasses *Zostera muelleri* subsp. *capricorni* and *Halophila ovalis*. Seahorses showed cryptic behaviour with ~ 50% of adult sightings cryptic and ~ 75% for juveniles with crypsis occurring predominantly in *Sargassum* sp. for adults and *Euplexaura* sp. habitat for juveniles. Within sites, females moved significantly larger distances (maximum 70 m) than males (maximum 38 m) over 20 months. Strong site fidelity was displayed by *H. whitei* with males persisting at the same site for up to 56 months and females for 49 months and no seahorses moved between sites. The longest period that a *H. whitei* was recorded on the same holdfast was 17 months for a male

and 10 months for a female. As this species displays strong site fidelity, specific habitat preferences and has a limited distribution, future management needs to minimise the risk of habitat disturbance as loss of key habitats could have a negative impact on species abundance and distribution.

4.2   Introduction

Conservation of species threatened with extinction requires understanding of the key processes that influence their distribution and abundance (Brown, 1984). Habitat loss has detrimental effects on biodiversity, leading to many species being threatened with extinction (Mace & Lande, 1991; Stuart *et al.*, 2004). Habitat loss and alteration can be natural or anthropogenic and the conservation of habitats and the ecological processes maintaining habitats are important for maintaining biodiversity, ecological processes and ecosystem function (Groombridge, 1992; Mora *et al.*, 2011). The conservation of threatened species is intrinsically linked to the protection of habitats that are essential for feeding, reproduction and shelter. To assist with species' conservation, it is important to distinguish between the habitat use and habitat preference. Habitat 'use' refers to those habitats where a species occurs whilst habitat 'preference' is a species disproportionate use of a habitat in relation to its relative availability and is a consequence of habitat selection (Manly *et al.*, 1992). Loss and alteration of important marine habitats can lead to population declines (Paddack *et al.*, 2009; Pratchett *et al.*, 2009; Bonin *et al.*, 2011). Numerous studies have

detailed habitat relations of marine fish, such as habitat use by a threatened serranid species (Harasti & Malcolm, 2013), habitat specificity of juvenile tropical fish (Wilson *et al.*, 2010), and effects of habitat degradation on coral reef fish settlement (Feary *et al.*, 2007). Seahorses (*Hippocampus* spp.) of the family Syngnathidae include 48 recognised species worldwide (Vincent *et al.*, 2011). They are deemed to be under threat globally with several species listed as threatened on the IUCN Red List; ten species are listed as 'vulnerable', one species is listed as 'endangered', one species as 'least concern' and the remaining species are listed as 'data deficient' (IUCN, 2013). Seahorses are considered to be threatened from incidental capture in trawl fisheries, over-exploitation from commercial fishing, and through the loss of essential habitats (Vincent *et al.*, 2011).

Seahorses are known to utilise a wide range of habitats including seagrasses (Bell *et al.*, 2003; Vincent *et al.*, 2004), mangroves (Rosa *et al.*, 2007), coral reefs (Perante *et al.*, 2002), and artificial structures (Harasti *et al.*, 2010), as well as faunal substrates such as sponges and soft corals (Kuiter, 2009) and macroalgae (Moreau & Vincent, 2004). Some *Hippocampus* species are very habitat specific, such as the pygmy seahorses *H. bargibanti* Whitley 1970 and *H. denise* Lourie & Randall 2003, that are associated only with gorgonian fans (Baine *et al.*, 2008; Smith *et al.*, 2012) whilst other species such as the pot-belly seahorse *H. abdominalis* Lesson 1927 use a variety of habitats (Martin-Smith & Vincent, 2005). At a small spatial scale, the habitat of a seahorse is its 'holdfast' i.e. the substrate to which it is attached via its prehensile tail. The alteration or destruction of habitats has negative effects on populations of seahorses.

Populations of the long-snouted seahorse *H. guttulatus* Cuvier 1829 were reduced in seagrass meadows that were damaged by seine netting (Curtis *et al.*, 2007) and populations of the tiger tail seahorse *H. comes* Cantor 1850 were reduced on coral reefs that had been subjected to blast and poison fishing (Marcus *et al.*, 2007). Populations of the dwarf seahorse *H. zosterae* Jordan & Gilbert 1882 declined following degradation of seagrasses caused by the construction of two adjacent marinas (Masonjones *et al.*, 2010) and abundance of *H. whitei* declined when artificial habitats had been deliberately modified (Harasti *et al.*, 2010).

To protect seahorses in the wild from loss of essential habitats, the habitats that are used and preferred must be first determined. There have been very few studies that have assessed the habitat usage or preferences of seahorses: Kynsna seahorse *Hippocampus capensis* Boulenger 1900 preferentially used aquatic vegetation in estuaries (Teske *et al.*, 2007), *H. comes* used a variety of habitat types on a coral reef with no holdfast preference and habitat usage differed with ontogeny (Morgan & Vincent, 2007), whilst two different studies have compared habitat utilisation of *H. guttulatus* and short-snouted *H. hippocampus* (*L.* 1758) (Curtis & Vincent, 2005; Gristina *et al.*, 2014). The focus of this study was *Hippocampus whitei*, a medium-sized seahorse that is considered endemic to eight coastal estuaries of central New South Wales (NSW), Australia (Harasti *et al.*, 2012). *Hippocampus whitei* grows to a maximum length (L_T) of 162 mm, exhibits rapid initial growth and maturity, lives for up to 5 years in the wild (Harasti *et al.*, 2012), and has a small home range (~9 – 14 m^2) over short time scales of days-weeks (Vincent *et al.*, 2005). This species occurs in

seagrasses, artificial structures, and sponge garden habitats (Vincent *et al.*, 2005; Harasti & Gladstone, 2013) to depths of 20 m (Kuiter, 2009). However, whilst these studies have reported habitats that *H. whitei* occupy, it is not known if they actually prefer these habitat types or whether they are using these habitats because their preferred habitats are not available. It is also not known if *H. whitei* show any preferences to more complex habitats as seahorses are considered to rely on crypsis to hide from predators (Kleiber *et al.*, 2011)

All seahorses are protected in NSW and are not at risk from fishing pressures, with the greatest potential threat being loss of essential habitats. Given the limited distribution of *H. whitei* and its distribution being located around the most highly developed region in Australia (NSW EPA, 2012), there is a need to determine the habitats that are of importance to this species to assist with its long term conservation. In addition, as Morgan & Vincent (2007) found differences in habitat use between juvenile and adult *H. comes*, it is important to assess if *H. whitei* also displays ontogenetic differences in habitat preference. It is unknown if *H. whitei* prefers specific habitat types or whether it is a habitat generalist showing no significant preference or avoidance of habitats. If *H. whitei* prefers specific habitats, any loss of essential habitats could have a negative impact on population abundance and its distribution. Therefore, the aims of this study were to 1) determine and compare the habitat preferences of adult male and female and juvenile *H. whitei*, 2) assess if they displayed cryptic behaviour within particular habitats and 3) assess the long-term movements and site fidelity of *H. whitei*.

4.3   Materials and Methods

Study Sites

This study was undertaken at Nelson Bay in the Port Stephens-Great Lakes Marine Park in Port Stephens on the New South Wales (NSW) mid-north coast, Australia ($32^0 43'04.63''$S – $152^0 08'29.27''$E) (Figure 1). The area was selected because *Hippocampus whitei* is known to be abundant (Harasti *et al.*, 2012) and its distribution within Port Stephens includes a large number of distinct habitats that are defined in Table I. The study was undertaken at three sites extending over 1.5 km of coastline, each site was approximately 6000 m^2 in extent, and contained a variety of habitat types (Table 1). The sites were similar in structure and configuration in that they were dominated by sponge garden reef habitat interspersed with sand and the soft coral *Dendronephthya australis* with seagrass meadows present in the shallow (2-5 m) sections. The depth range for each site was limited from 2-13 m as no *H. whitei* have been observed > 12 m depth, even though numerous dives (100+) were conducted in the 13-25 m depth range at each of the sites from 2006-2009. It was observed at depths of 13-25 m that there was still continuous habitat available, particularly with extensive sponge garden and *Dendronephthya australis* habitats, but no seahorses were ever recorded deeper than 12 m.

Habitat preference

The null hypothesis that *H. whitei* would not display a significant preference for a specific habitat was tested by comparing habitat availability with habitat use and determining habitat preference scores (Manly *et al.*, 1992; Light & Jones, 1997; Gladstone, 2007; DeMartini *et al.*, 2010). A point-transect method was used to provide an estimate of the relative availability of each habitat type (Choat & Bellwood, 1985) where 20 transects measuring 30 m in length were haphazardly placed at each site. To avoid transects overlapping, the initial transect was placed at one end of the site with all subsequent transects laid one after the other towards the other end of the study site. The habitat occurring directly below the transect at 1 m intervals was recorded (Table 1), providing a total of 600 habitat points for each site. These surveys were undertaken in January 2009. In addition, the height of *Dendronephthya australis* was also recorded in three size classes (<20 cm, 20-40 cm and >40 cm) with the size class estimated visually by the diver. The area to 50 cm on both sides of the transect was thoroughly searched for seahorses. When a seahorse was found the following information was recorded: habitat (being the habitat used as a holdfast by the seahorse using its prehensile tail to anchor itself), sex, and depth. However, the number of seahorses encountered using the transect point-count method was very low (n=21 seahorses across all three sites from 60 transects) and insufficient for any meaningful statistical analysis on habitat association.

In addition to seahorses being recorded on the habitat transects, data on seahorse habitat use were also collected monthly at each site from January 2008 to December 2009 using a random 60 min roaming diver survey technique (Kingsford & Battershill, 1998) which included the diver actively searching through habitats with hands to find any hiding seahorses. When a seahorse was observed, its depth, holdfast habitat, sex, and state of maturity were recorded. Adult males were determined by presence of a brood pouch whilst females were determined as those without a brood pouch and were greater than L_T 75 mm. Juveniles were those animals considered to be not mature and less than L_T 75mm as ~75mm is considered to be the average size for sexual maturity for *H. whitei* in Port Stephens (Harasti *et al.*, 2012). When seahorses were observed on *D. australis*, the height of the colony was estimated using the height classes described above. For sightings in all habitat types, it was also recorded if the seahorse sighting was cryptic. For the purpose of this study, crypsis refers to 'was the seahorse hidden within its habitat and could not be visually seen by the diver?' It was considered to be cryptic when the seahorse was obscured from initial view until the habitat was actively searched thoroughly by the diver using hands and the seahorse was detected within or underneath the habitat. All encountered habitats that were considered complex were actively searched for hidden seahorses. Whilst the classification of crypsis was subject to interpretation by the diver, the cryptic recording was kept consistent throughout the surveys as data was only collected by the lead author (DH). The results from each monthly survey (*n*=24) were combined for each site for habitat preference analysis. Water

temperature was recorded monthly at each site from two Mares dive computers (www.mares.com).

Long-term movement

As part of a larger research project on the biology and ecology of *H. whitei* by the authors (Harasti *et al.*, 2012), seahorses at each of the three study sites had been previously tagged using small visible implant fluorescent elastomer tags (VIFE – Northwest Marine Technologies, USA; www.nmt.us) using the techniques described in Woods & Martin-Smith (2004). Seahorses were individually tagged with three 1-2 mm elastomer tags, inserted into unique locations on the body to allow for individual identification on subsequent resighting. The use of elastomer tags in seahorses has not been shown to alter their behaviour or growth (Woods & Martin-Smith, 2004) and even though these tags are considered permanent, they are unlikely to affect seahorse cryspis as animals have been observed alive 4 years after initial tagging (Harasti *et al.*, 2012). Specific elastomer colours were used for each site (Pipeline = red, Seahorse Gardens = yellow and Little Beach = orange). A total of 948 seahorses were tagged across the three sites between 2006 and 2009 (Pipeline: *n* = 548, Seahorse Gardens: *n* = 387 and Little Beach *n* = 13).

An initial survey was undertaken in January-February 2008 to mark the location of each seahorse in each site. The location of tagged seahorses was recorded by a diver towing a tethered Garmin Map 60 GPS (www.garmin.com) on a surface float that

was time synchronised to a MARES (www.mares.com) dive computer. The time the seahorse was recorded was matched to the correct GPS coordinates when the GPS track was downloaded into Google Earth software (www.google.com/earth). The location of each seahorse that was recorded during the initial survey was monitored bi-monthly using the roaming diver survey technique until August 2009. Distance moved between surveys was measured by plotting points in Google Earth and measuring the distance between each point. For each individual, a small washer with numbered flagging tape was pushed into the substrate marking the seahorse location to assist estimating movements on subsequent resighting and if a seahorse was found within close proximity to its marker (<5 m), the distance moved was estimated by the diver underwater. If the seahorse was found on the same holdfast as the previous survey the distance moved was recorded as 0 m.
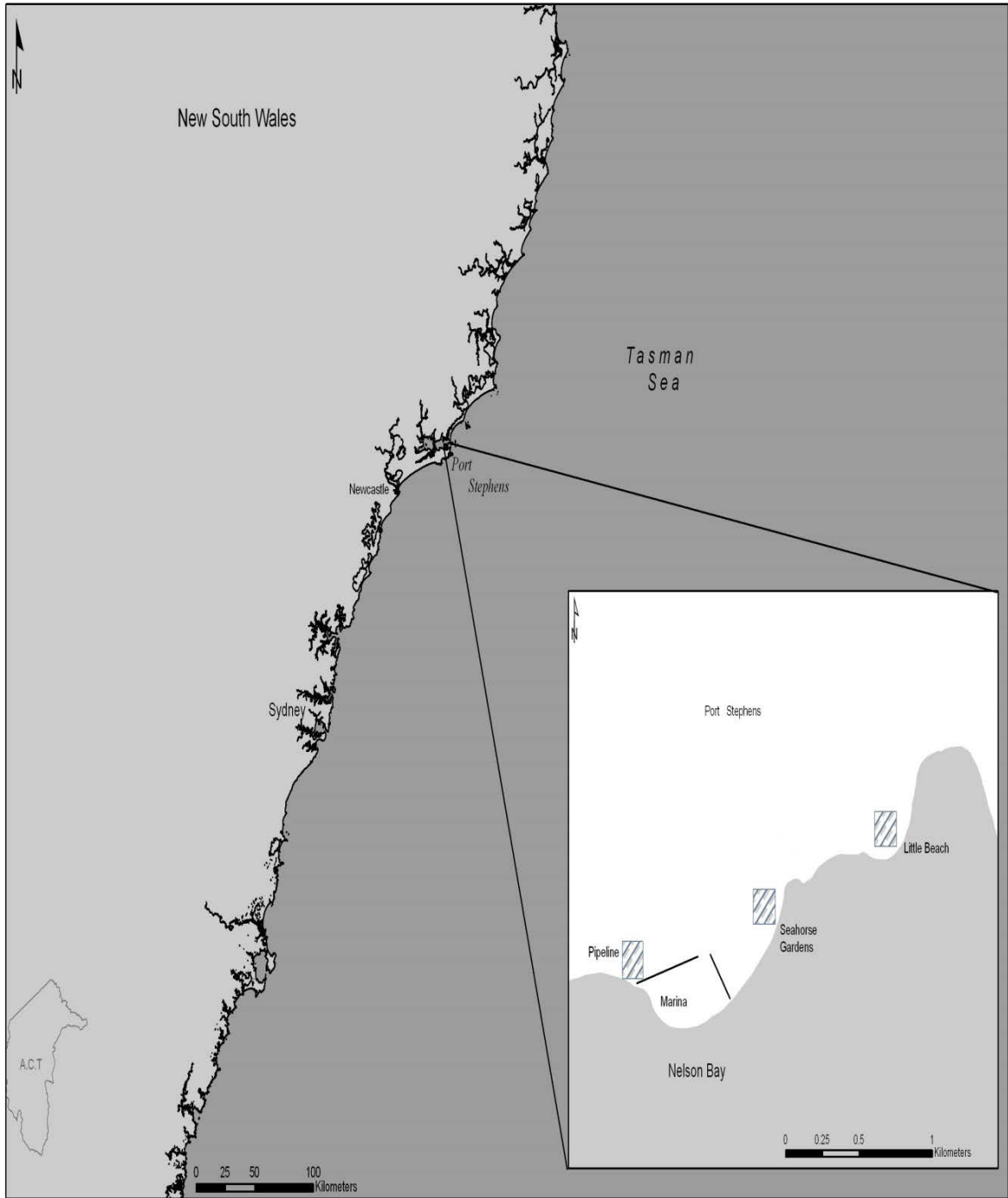
Figure 4.1. Location of study sites, Port Stephens, New South Wales – Australia.

Table 4.1. Descriptions of habitat types from habitat preference study and observed habitat depth range.

Habitat type	Description	Depth range (metres)
Algae	Algal habitats included small turfing and clumping brown and red algae's such as *Hormophysa* sp., *Euptilota* sp. *Zonaria* sp. and *Codium* sp.	1 – 25
Ascidian	Constituted several different species in class Ascidiacea such as *Clavelina meridionalis* and *Sycozoa* sp.	3 – 25
Carijoa sp.	Family: Clavulariidae. Undescribed species of *Carijoa* sp. The colony is coated by an orange sponge and has large white polyps. Colony length to 25 cm.	5 – 25
Dendronephthya australis	Family: Nephtheidae. Highly branched and bushy soft coral colony that has pink stalk and branches with white sclerites. Colony grows to a max height of ~ 70 cm and was found to vary greatly in size.	4 – 23
Euplexaura sp.	Family: Plexauridae. Yellow gorgonian fan found to grow to a max height 40 cm and width 70 cm.	5 – 25
Halophila ovalis	Family: Hydrocharitaceae. Has ovate leaves with stalk-like petioles. Commonly referred to as paddle weed with a max leaf length of 4 cm.	2 – 10
Ecklonia radiata	Family: Alariaceae. Large brown-green coloured seaweed that has several fronds arising from a central supporting stipe stem. Grows to a max height to 2 m. Commonly referred to as kelp.	1 – 25
Posidonia australis	Family: Posidoniaceae. Commonly referred to as strapweed with tough leaves with a leaf length of 45 cm and width of 2 cm.	1 – 5
Sand	Patches of bare sand that contained no other habitat type.	0 – 25

Sargassum sp.	Family: Sargassaceae. Constitutes brown alga in genus *Sargassum*, such as *Sargassum vestitum and is brown-yellow in colour. Maximum observed length is 1.5 m.*	4 – 18
Sponge	Sponge habitats constituted a variety of species from phylum Porifera. Some of the more common and identifiable species included *Echinoclathria* sp., *Holopsamma laminaefavosa*, *Plumohalichondria australis*, and *Mycale australis*.	5 – 25
Zostera muelleri subsp. *capricorni*	Family: Zosteraceae. Commonly referred to as ribbon weed with a narrow blade that grows to max length of 50 cm.	1 – 6

Site fidelity

To gain an understanding of site fidelity, monthly surveys were undertaken from January 2006 to December 2009 at the Seahorse Gardens and Pipeline, as these were the two sites where large populations of tagged *H. whitei* occurred (Harasti *et al.*, 2012). On each occasion a tagged seahorse was resighted, its locality details were recorded through the use of the diver-towed GPS or by its proximity to a marked position.

Data analysis

Habitat Preferences

One-factor permutational multivariate analysis of variance (PERMANOVA) was used to determine if the habitat composition of the three sites differed, using PERMANOVA+ Version 1.0.5 within PRIMER-E 6 (Plymouth Routines in Multivariate Ecological Research http://www.primer-e.com/) (Anderson, 2001). The number of occurrences of each habitat on each transect was summed, and data were square-root transformed. The analysis was done on the Bray-Curtis similarity matrix, with site analyzed as a fixed factor, and significance determined from n=9999 permutations. Habitat composition of the 3 sites did not differ ($F_{2, 59} = 1.84$, $P > 0.05$) and hence the habitat preferences for *H. whitei* are reported with all three sites combined.

To determine the habitat availability and preferences of *H. whitei* in Port Stephens, the resource selection probability function (RSPF) was used (Manly *et al.*, 1992). RSPF is a function that computes the probability that a particular resource, as characterized by a combination of environmental variables, will be used by an individual animal (Manly *et al.*, 1992). The use of the RSPF has been widely applied in terrestrial and marine studies for assessing habitat preferences (Aarts et al., 2013; Gladstone, 2007; Hooten *et al*. 2013). The RSPF is generally estimated from observations of (1) presence/absence (used vs. unused), or (2) presence/available (used vs. available) resource units (Boyce *et al.*, 2002). The RSPF was used to determine the preferred habitats of *H. whitei* using the formula $\hat{W}_i = O_i/\pi_i$ where O_i is the proportional use of habitat type i, π_i is proportional availability of habitat type *I*, and $\hat{W}_i$ is the preference score for habitat type. To determine the statistical significance of preference scores, 95% confidence intervals were used for $\hat{W}_i$, where the upper confidence interval was <1, the habitat was significantly avoided. If the confidence interval was between < 1 and > 1, the habitat was used in proportion to its availability (i.e. seahorse showed no preference or avoidance to habitat) and if the lower confidence interval was > 1, the habitat was significantly preferred. Those habitat types where no seahorses were recorded were excluded from the habitat preference analysis (Manly *et al.,* 1992).

Long term movements and depth

To test the null hypothesis that the depth seahorses occurred did not differ among sites or between sexes and juveniles, a two-factor Analysis of Variance (ANOVA) was conducted. The factors site and sex were treated as fixed orthogonal factors. The null hypothesis that the distance moved by seahorses over 17 months did not differ between sexes and among sites was tested by a two-factor ANOVA. The factors sex and site were treated as fixed orthogonal factors. The data analysed were the total distances moved by 36 seahorses across all nine bi-monthly surveys from the Seahorse Gardens (7 males: avg L_T 124.4 ± 2.6 cm S.E., 8 females avg L_T 125.9 ± 3.2 cm) and Pipeline (11 males: avg L_T 126.2 ± 2.1 cm, 10 females avg L_T 125.1 ± 3.6 cm) sites. To meet the assumptions for repeated measures ANOVA, only seahorses that were recorded on each of the 9 surveys were included in the analysis. Data did not assume normality (Sharpiro-Wilk; df = 36, $P < 0.05$) and were square root transformed. All statistical analysis for movements was undertaken in the software package SPSS 20 (www.ibm.com/spss).

4.4 Results

Habitat availability and preferences

Sand was the most dominant habitat present across all sites combined (33% of habitat present) followed by sponge habitat (16%), algal habitat (15%) and soft coral

Dendronephthya australis habitat (13%) (Figure 4.2). The monthly 60 minute surveys recorded a total of 1146 *H. whitei* observations. *Hippocampus whitei* occurred in 9 of the available habitats; no seahorses were recorded in sand, or the seagrass habitats *Zostera muelleri* subsp. *capricorni* and *Halophila ovalis*. Juveniles were also not observed in ascidian and *Carijoa* sp. habitats. There was no significant difference in habitat preference scores between male and female *H. whitei* as the confidence intervals for male and females were similar. Adult males and females exhibited a significant preference for sponge and *D. australis* habitats, showed no preference for ascidian, *Carijoa* sp., and gorgonian *Euplexaura* sp. habitats, and showed a significant avoidance of algal, *Posidonia australis*, *Ecklonia radiata* and *Sargassum* sp. habitats (Figure 4.3a). Juveniles displayed different habitat preference to adults with a significant preference for *Euplexaura* sp. gorgonian habitats, no preference for *Ecklonia radiata*, *Sargassum* sp., sponge and *D. australis* habitats, and a significant avoidance of algal and *P. australis* seagrass habitat (Figure 4.3b).

A total of 262 occurrences of *D. australis* were recorded across all three sites, and the occurrence of the three size classes was similar (Figure 4.4). The standardised habitat preference scores combined for all sites found that both adult and juvenile *H. whitei* had a strong preference for the larger *D. australis* soft corals (>40 cm height), no preference for soft corals of 20-40 cm, and a significant avoidance of soft corals <20 cm (Figure 4.5).
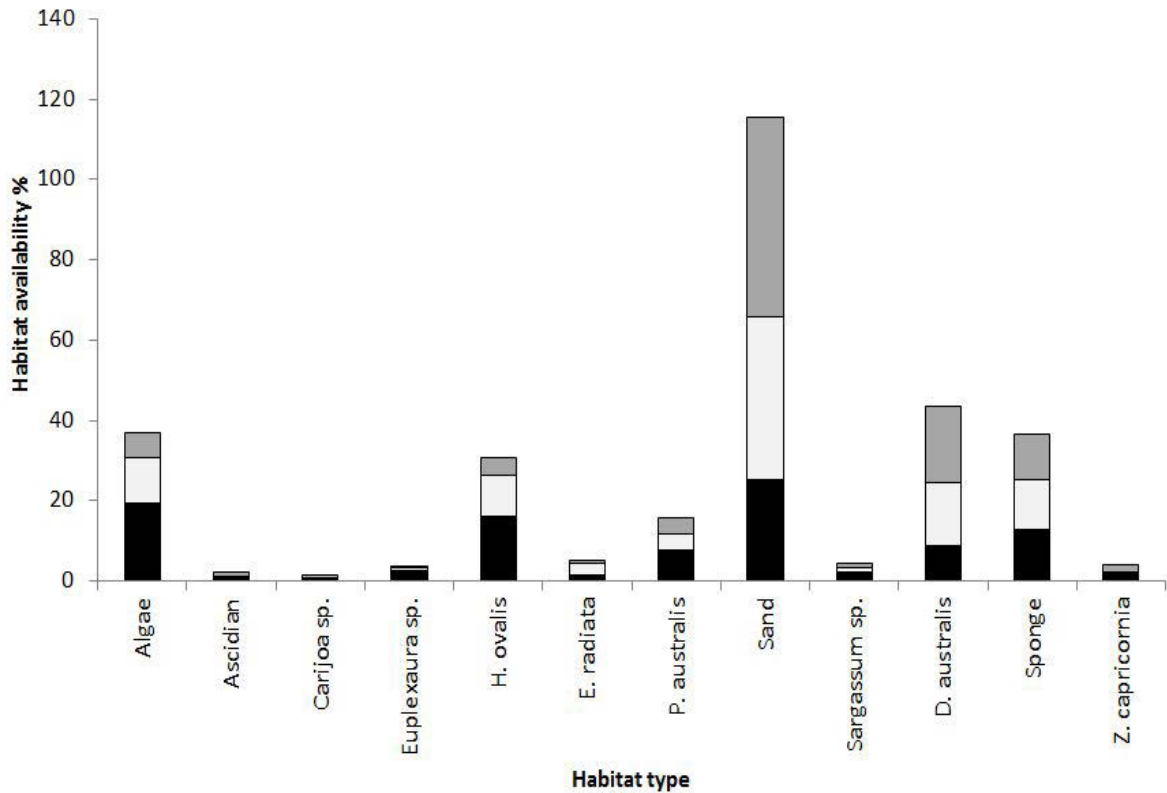
Figure 4.2. Availability (expressed as % occurrence in all transects in each site) of habitats at the three study sites in January 2009. Seahorse Gardens = grey bars, Pipeline = white bars and Little Beach = black bars.

Cryptic sightings

Across the three sites, 50.3% of adult seahorse sightings were of cryptic individuals. Adults were found to be most cryptic in *Sargassum* sp. (91.7% of sightings cryptic) and *D. australis* (73.9%) whilst they were most easily detected in sponge habitats (26.1%). Juveniles were more cryptic (75.3% of all sightings cryptic) and were most cryptic in gorgonian *Euplexaura* sp. (85.4%) and *D. australis* (75.0%) habitats compared to sponge habitats (42.3%).
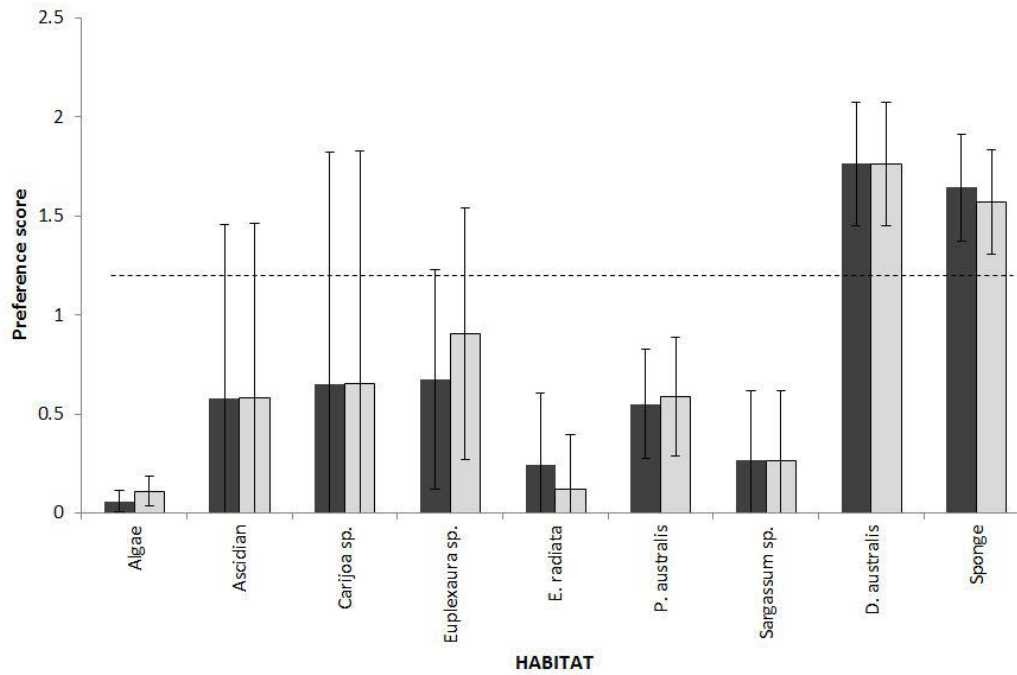
Figure 4.3a. Habitat preferences scores (±95% CIs) of adult *Hippocampus whitei* (males = dark bars, females = light bars) across 3 sites in Port Stephens, New South Wales, Australia based on 1146 individual *H. whitei* observations from Jan 2008 – Dec 2009. Preference scores with a lower confidence interval >1 (indicated by dashed line) indicate a significant habitat preference.
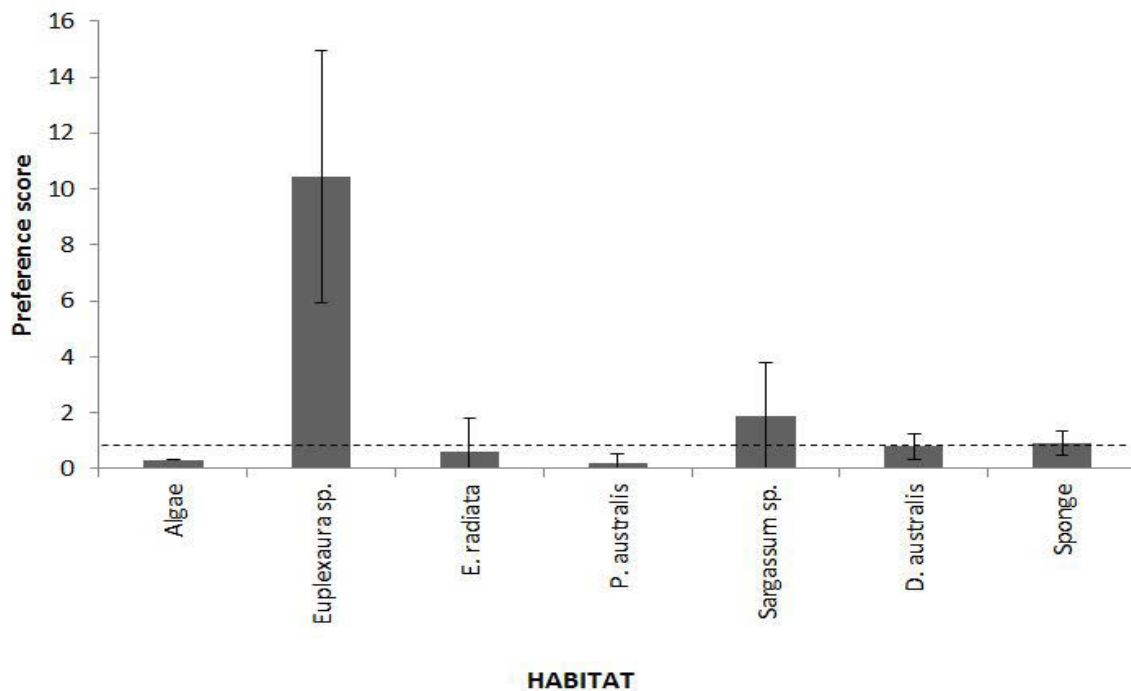
Figure 4.3b. Habitat preferences of juvenile *Hippocampus whitei* across 3 sites in Port Stephens, New South Wales, Australia based on 100 observations from Jan 2008 – Dec 2009.

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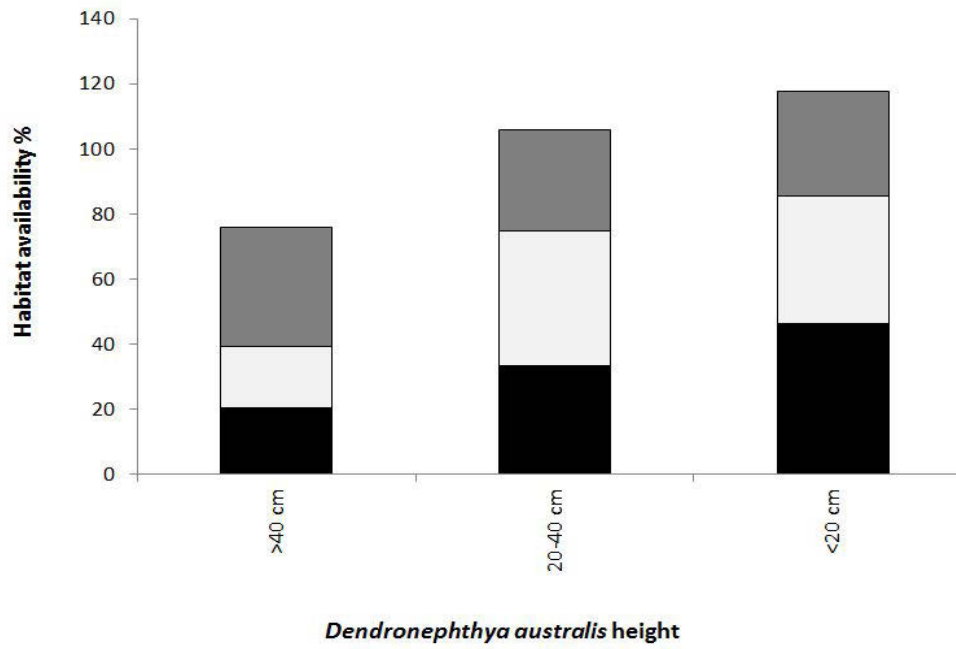
Figure 4.4. Habitat availability (%) for the various size classes of the soft coral *Dendronephthya australis* across all three sites in Port Stephens. Seahorse Gardens = grey bars, Pipeline = white bars and Little Beach = black bars.
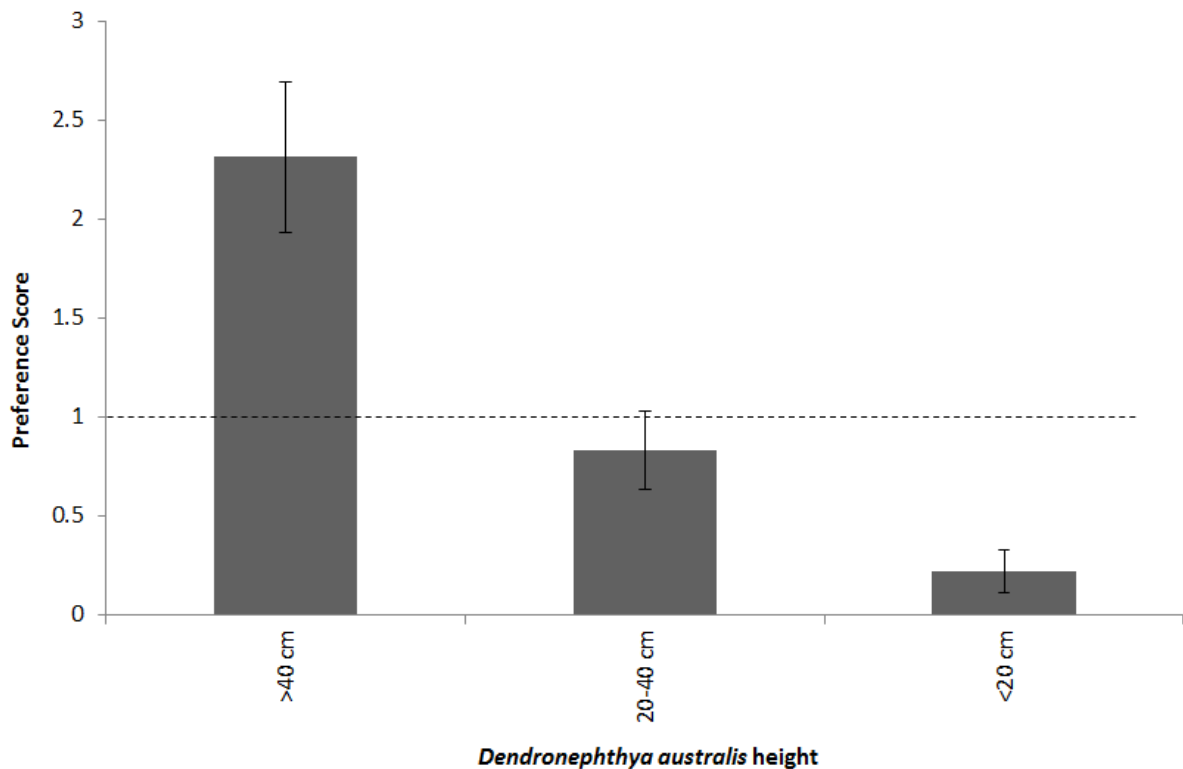
Figure 4.5. Standardised habitat preference scores for adult and juvenile *Hippocampus whitei* combined across all three sites in relation to height classes for *Dendronephthya australis*.

Depth

There was no significant difference in the depths recorded between adult males and females and juveniles (two factor ANOVA, $F_{2,1213}$ = 1.5, $P > 0.05$), there was no significant difference in the depth seahorses occurred across sites (two factor ANOVA, $F_{2,1213}$ = 0.02, $P > 0.05$) however there was a significant site x sex interaction (two factor ANOVA, $F_{4,1146}$ = 12.3, $P = 0.000$). This interaction occurred at the Seahorse Gardens site where the mean depth for juveniles was 6.5 m ± 0.4 compared to a mean depth of 8.5 m ± 0.1 S.E. for males and 8.4 m ± 0.1 S.E. for females. The mean depth of *H. whitei* observed at the Pipeline was 7.8 m ± 0.1 S.E. which was similar to the mean depth at Little Beach of 7.8 m ± 0.2 S.E. The minimum observed depth for *H. whitei* was 1.7 m and a maximum of 11.8 m.

Long-term movement

The mean total distance moved over 17 months for seahorses at the Seahorse Gardens was 21.8 m ± 3.9 m for males and 38.4 m ± 5.5 m for females compared to 11.6 m ± 3.2 m for males and 26.3 m ± 2.5 m for females at the Pipeline. The total distance moved significantly differed between the two sites ($F_{1, 28}$ = 7.87, $P < 0.05$) and between sexes ($F_{1, 28}$ = 16.51, $P < 0.001$), and the sex x site interaction was not significant ($F_{1, 28}$ = 0.26, $P > 0.05$). The greatest distance an individual was found to move across all surveys combined (*n*=9) was for a female at the Seahorse Gardens that moved a combined distance of 70 m compared to only 38 m for a male at the

Seahorse Gardens. The largest distanced moved by a seahorse between two survey periods (~50 days) was 43 m for a female seahorse compared to only 15 m for a male, both from Seahorse Gardens.

Site fidelity

No seahorses moved between any of the three sites, with tagged seahorses being resighted only at the site at which they were initially tagged. From 2006 to 2009, the longest time individuals were recorded at the Seahorse Gardens site was 56 months for a male and 49 months for a female, and the longest times that tagged individuals were recorded at the Pipeline site was 52 months for a male and 42 months for a female. The longest period that a seahorse was recorded on the same holdfast, and not observed on any other holdfasts, was 17 months for a male and 10 months for a female, both observed at the Pipeline. A juvenile that was originally tagged at 33 cm resided on the same gorgonian holdfast for 5 months at the Pipeline site.

4.5   Discussion

Various studies have found that individual fish species are associated with particular habitat types and many species show ontogenetic changes in habitat use between adult and juvenile stage (Light & Jones, 1997; DeMartini *et al.*, 2010; Boaden & Kingsford, 2013). It is important to understand how habitat use can vary between

different life stages of species as ontogenetic differences may require protection or individual management of various habitat types if the species is considered threatened. Determining the habitat preferences for *Hippocampus whitei* is a useful measure that can assist with the species long-term conservation through the protection of habitats that it uses. It is evident from this study that *H. whitei* utilises a variety of habitat types, and that habitat preference by juveniles was found to differ from adults.

As seahorses are slow moving, sedentary species (Kuiter, 2009), they rely on crypsis for survival as they have little ability to swim quickly away from predators (Kleiber *et al.*, 2011). The *Dendronephthya australis* habitat offers a complex structure that provides excellent camouflage for seahorses to hide from predators, hence the high level of crypsis (74%) displayed by seahorses using this habitat. The larger the soft coral colony, the greater the structural complexity because of the increased number of 'branches', and hence this is probably why the seahorses had a significant preference for large *D. australis* (>40 cm height) whilst showing significant avoidance to smaller ones that lacked complexity. Similar to *D. australis*, the gorgonian *Euplexaura* sp. habitat that was significantly preferred by juvenile seahorses is also a very complex habitat with juveniles displaying strong crypsis (85%) by hiding amongst the gorgonian branches. The reliance and preference for habitat complexity by seahorses is unknown; however, it is thought that seahorses prefer more complex habitats as they provide better camouflage to avoid predators but also allows for successful ambush predation (Flynn & Ritz, 1999). Complex habitats potentially

provide a good source of prey for seahorses as amphipods and copepods, considered a main prey of seahorses (Kendrick & Hyndes, 2005; Kuiter, 2009), were significantly more abundant in habitats that provided more structural complexity (Hellyer *et al*., 2011). With both the sponge and *D. australis* habitats being structurally complex, there is greater potential for Hippocampid prey to occur within these habitats providing a food resource for *H. whitei*.

 This ontogenetic change in habitat preference between juveniles and adults is similar to *H. comes*, which preferred macroalgal habitats as juveniles and corals as adults (Morgan & Vincent, 2007). The reason for ontogenic variation in habitat use is unknown; however, the small size of the *Euplexaura* sp. gorgonians made it difficult for larger adults (L_T >100 mm) to hide so they possibly move to larger more complex habitats as they mature for greater crypsis. As juveniles and adults were found in close proximity to each another on their preferred habitats, there was no evidence of any size segregation as reported in other studies (Morgan & Vincent, 2007). It has been suggested that ontogenic habitat changes may be attributed to reproductive opportunity (Whiteman & Côté 2004; Morgan & Vincent, 2007); however; this is unlikely for *H. whitei* as pregnant males were found occurring across 9 different habitat types and were found using the same habitat types as juveniles.

Adult seahorse were found to display a strong preference for the soft coral habitat *D. australis*, which is considered a rare habitat in NSW and has a very limited distribution within Port Stephens with it being most prevalent in the same area as this study (NSW DPI *unpublished data*). The *D. australis* habitat has been found to

contain high biodiversity when compared with other adjacent habitats and is considered an important nursery habitat for many fish species (Poulos *et al.*, 2013). In addition to its limited distribution in Port Stephens, *D. australis* has only been confirmed to occur from Port Hacking (Sydney) to Port Stephens (NSW DPI *unpublished data*) which coincides with most of the known range of *H. whitei* (Harasti *et al.*, 2012).

Female *H. whitei* moved significantly greater distances than males over the 17 month observation period and males were more likely to remain on the same holdfast for longer duration than females. In a previous study, *H. whitei* displayed small home ranges in seagrass habitat over 2-3 months, with females having a larger (~14.4 m^2) home range than males (~9 m^2) (Vincent *et al.*, 2005). The authors speculate that males potentially move less to conserve energy for brooding embryos in their pouch with the large pouch also hindering their movements through increased weight and drag (Vincent *et al.*, 2005). It has been hypothesised that *H. whitei* maintain small ranges because the costs of movement are greater than the benefits in terms of survival, growth and reproduction (Vincent *et al.*, 2005) and it is probably a combination of these factors that have led to *H. whitei* displaying site fidelity within Port Stephens.

There was no evidence to suggest any of the *H. whitei* moved between sites in Port Stephens and they displayed site persistence which is similar to a study of *H. whitei* in seagrass (Vincent *et al.*, 2005) and for other seahorse species (Perante *et al.*, 2002; Curtis & Vincent, 2006; Rosa *et al.*, 2007). Whilst this study indicates *H. whitei* is very

site-associated, individual seahorses can still move large distances as showed by a female seahorse that was found to move 43 m within 50 days. In a displacement experiment using *H. guttulatus*, it was found that tagged individuals were able to travel large distances in a short time period (150 m over 8 days) with a single animal moving 60 m in one day (Caldwell & Vincent, 2013). However, unlike the present study, Caldwell & Vincent (2013) found no significant difference in movements between males and females. Additionally in a pilot study, 70% of displaced seahorses were able to move 20 m or less to return to their original home location indicating the ability of seahorses to navigate back to their preferred habitat when displaced (Caldwell & Vincent, 2013). Even though some individual *H. whitei* were observed moving around their site, they displayed strong site fidelity as evident through repeated observations of the same individuals existing at the same sites and even on the same holdfasts for large durations of this study. Seahorses from the Seahorse Gardens moved significantly greater distances than individuals from the Pipeline and this could be attributed to the available habitat being sparser at the Seahorse Gardens which was the site with the most sand habitat (38% sand versus 30% at Pipeline), a habitat seahorses did not use. Seahorses may therefore have had less available habitat to choose from and were therefore forced to move larger distances over sand when changing holdfasts.

 It was observed during the surveys that the Seahorse Gardens site was subjected to frequent anchoring as it provides a safe anchorage for vessels in the majority of weather conditions and is closer to Nelson Bay town than the other sites. This

frequent anchoring caused damage to the habitats, particularly *D. australis* soft corals which were easily dislodged from the benthos by moving anchor chains. This anchor damage may have forced individual seahorses to involuntarily move to alternate holdfasts through changes and/or loss of their existing habitat. Since 2010, both *D. australis* and sponge habitats within Port Stephens have been adversely affected as a result of sand inundation (NSW DPI *unpublished data*) however no apparent decline in available habitats was observed during this study. The degradation of habitats important for *H. whitei* could have a detrimental impact on this species given its very limited distribution (Harasti *et al.*, 2012) and its range occurring along some of the most heavily populated estuaries in Australia (NSW EPA, 2012). Even though this species is protected within NSW from fishing pressures, the loss of essential habitats through anthropogenic impacts could have ramifications for the future conservation of this species.

4.6   Acknowledgements

CHAPTER 5

Does a no-take Marine Protected Area benefit seahorses?[3]

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[3] Harasti, D., Martin-Smith, K. & Gladstone, W. (2014). Does a no-take Marine Protected Area benefit seahorses? *PLOSONE*. doi: 10.1371/journal.pone.0105462

5.1 Abstract

Seahorses are iconic charismatic species that are often used to 'champion' marine conservation causes around the world. As they are threatened in many countries by over-exploitation and habitat loss, marine protected areas (MPAs) could help with their protection and recovery. MPAs may conserve seahorses through protecting essential habitats and removing fishing pressures. Populations of White's seahorse, *Hippocampus whitei*, a species endemic to New South Wales, Australia, were monitored monthly from 2006 to 2009 using diver surveys at two sites within a no-take marine protected areas established in 1983, and at two control sites outside the no-take MPA sites. Predators of *H. whitei* were also identified and monitored. *Hippocampus whitei* were more abundant at the control sites. Seahorse predators (3 species of fish and 2 species of octopus) were more abundant within the no-take MPA sites. Seahorse and predator abundances were negatively correlated. Substantial variability in the seahorse population at one of the control sites reinforced the importance of long-term monitoring and use of multiple control sites to assess the outcomes of MPAs for seahorses. MPAs should be used cautiously to conserve seahorse populations as there is the risk of a negative impact through increased predator abundance.

5.2 Introduction

Human uses of the marine environment have caused declines in species worldwide (Butchart *et al.*, 2010). Over-fishing, pollution, introduction of invasive species, climate change and habitat loss continue to threaten marine species (McClenachan *et al.*, 2010). It has been estimated that the global abundance of marine fishes has declined ~38% between 1970 and 2007 (Hutchings *et al.*, 2010) and the IUCN Red List has approximately 800 marine species listed as threatened (IUCN, 2013). One group of fishes, the seahorses (*Hippocampus* spp.) of the family Syngnathidae, have 11 species assessed as threatened on the IUCN Red List. In several countries they have been over-harvested for traditional medicines, curios and the aquarium trade and several species face population declines as a result of loss of essential habitats and over-fishing (Foster and Vincent, 2004; Vincent *et al.*, 2011). Concerns over the unsustainable trade in seahorses led to them being listed on Appendix II of the Convention on International Trade in Endangered Species (CITES) (Vincent *et al.*, 2011). Appendix II still allows trade in *Hippocampus* spp. however exporting countries must be able to certify that export of seahorses is not causing a decline or damage to wild populations.

Various management options have been proposed or implemented to protect *Hippocampus* spp. in the wild including the application of minimum size limits (Foster and Vincent, 2005), implementation of temporary fishing closures during recruitment periods (Vincent *et al.*, 2007), the protection of essential habitats (Vincent

et al., 2011), providing seahorses with a conservation status prohibiting collection (DPI, 2005), and the implementation of no-take marine protected areas (MPAs) (Martin-Smith *et al.*, 2004; Marcus *et al.*, 2007; Vincent *et al.*, 2007; Morgan and Vincent, 2013).

The benefits of MPAs for conserving marine biodiversity are well documented (Roberts *et al.*, 2001; Halpern, 2003; Lester *et al.*, 2009; Babcock *et al.*, 2010); however, the potential benefit of MPAs for conserving seahorse populations is relatively unknown. It has been suggested that *Hippocampus* spp. with small over-lapping home ranges would benefit from the creation of small scale no-take MPAs (Vincent *et al.*, 2005) by protecting critical spawning biomasses (Curtis and Vincent, 2006). The creation of no-take MPAs would also contribute towards conserving seahorse habitats by removing damaging processes, such as destructive fishing practises including dynamite fishing (Marcus *et al.*, 2007) and demersal seine netting (Curtis *et al.*, 2007).

As seahorses are charismatic species that garner considerable public support, it has been suggested they could be used as flagship species to assist with the protection of marine biodiversity around the world (Vincent *et al.*, 2011). It has been shown that selecting MPAs for estuarine seagrass habitats, based on the density and assemblage variations of syngnathids, would benefit other fish species (Shokri *et al.*, 2009). Seahorses have been used as a flagship marine species to help establish MPAs in the Philippines; however, the MPAs had no significant effect on seahorse densities and little effect on seahorse size (Yasué *et al.*, 2012). In this example, the removal of

fishing from the MPA did not increase densities of seahorses. This may have been because of poor habitat quality within the MPA, the biology of seahorses, and the small population sizes of seahorses outside the MPA to supply the MPA (Yasué *et al.*, 2012). Calls for MPAs to be used generally for syngnathid conservation should be treated cautiously. The biological attributes of syngnathids, such as limited movement and strong site fidelity (Harasti and Gladstone, 2013), small home range (Vincent *et al.*, 2005), early reproduction (Harasti *et al.*, 2012), and (for some species) lack of a dispersive pelagic larval phase (Kuiter, 2009), suggest that local populations are likely to respond positively to an MPA. However, there are other reasons why MPAs may not be effective for syngnathids, including specific habitat preferences of all life stages of syngnathids not being met within an MPA (Yasué *et al.*, 2012), habitat changes that follow MPA establishment leading to a decline in the availability of preferred habitat (Babcock et al., 1999; Shears et al., 2008), larval dispersal by some species limiting opportunities for local recruitment and population replenishment (Morgan and Vincent 2007), and the build-up of predators within an MPA causing a decline in prey species (Graham *et al.*, 2003), potentially including syngnathids. In addition, the effectiveness of an MPA for syngnathids might be compromised by activities occurring outside the boundaries that affect habitats within the MPA, such as pollution (Yasué *et al.*, 2012). To date, apart from Yasué *et al.*, 2012, there have been no studies that have specifically tested the effects of an MPA for syngnathids.

The aim of this study was to assess the benefits of no-take MPAs on seahorses. This was done by quantifying the relative abundance of the White's seahorse *Hippocampus whitei* within multiple no-take MPAs and multiple control sites, by identifying and quantifying predators of *H. whitei*, and testing for correlations between the abundance of predators and *H. whitei*. *Hippocampus whitei* is a medium-sized seahorse (maximum length (L_T) of 162 mm) that is considered endemic to several estuaries along the New South Wales (NSW) coast (Harasti *et al.*, 2012) and is protected under NSW fisheries legislation ensuring it cannot be taken from the wild (DPI, 2005). The species exhibits initial rapid growth, reaches sexually maturity at approximately 6 months and has a lifespan in the wild of 5-6 years (Harasti *et al.*, 2012). It occurs in a range of habitats including artificial structures (Harasti *et al.*, 2010); however, it has been found to prefer soft coral, sponge and gorgonian habitats and displays strong site fidelity (see chapter 4).

5.3   Materials and Methods

Study Sites

This study was undertaken at four sites near Nelson Bay in the Port Stephens-Great Lakes Marine Park in Port Stephens, NSW, Australia ($32^0 43' 04.63''$S, $152^0 08' 29.27''$E) (Figure 5.1). Each site was approximately 6000 m² and ranged in depth from 2-13 m with a variety of habitat types, such as *Dendronephthya australis* soft coral, *Posidonia australis* seagrass and sponge gardens, located at each of the sites. Two of the sites (Fly Point and Little Beach) are located within the Fly Point Sanctuary Zone, a no-

take zone that has been protected since 1983 with all forms of fishing excluded. The other two sites (Pipeline and Seahorse Gardens) are located in a Habitat Protection Zone, which has restrictions on commercial fishing activities such as no trawling whilst fishing and anchoring are permitted, and both are popular fishing locations (personal observations). Habitats across the four sites consisted of sponge, soft coral and seagrass habitats and it was found that there was no significant difference in habitat availability amongst three of the sites (Pipeline, Seahorse Gardens and Little Beach) (see Chapter 4). Fly Point was found to contain significantly more available habitat for seahorses, as this site had the most extensive sponge garden habitat and the least amount of sand (*unpublished data*).

Relative abundance of *H. whitei*

The hypothesis that seahorse abundance would not differ between the sanctuary and non-sanctuary sites was tested with data gathered during monthly surveys of each site between January 2006 and December 2009 (*n*=48 monthly surveys). Seahorse abundance in each site was assessed with a 60 min random roving diver search (Kingsford and Battershill, 1998), which involved the observer (DH) haphazardly swimming over the site searching for seahorses amongst the various habitats while swimming at a constant speed. To minimise problems associated with non-independence, the start and end point for each survey varied from survey to survey. When a seahorse was encountered, it was classified as male, female or juvenile.

Adult males were determined by the presence of a brood pouch whilst females lacked a brood pouch and were greater than L_T 75 mm. Juveniles were considered less than L_T 75 mm as ~75 mm was found to be the mean size for sexual maturity for *H. whitei* in Port Stephens (Harasti *et al.*, 2012).
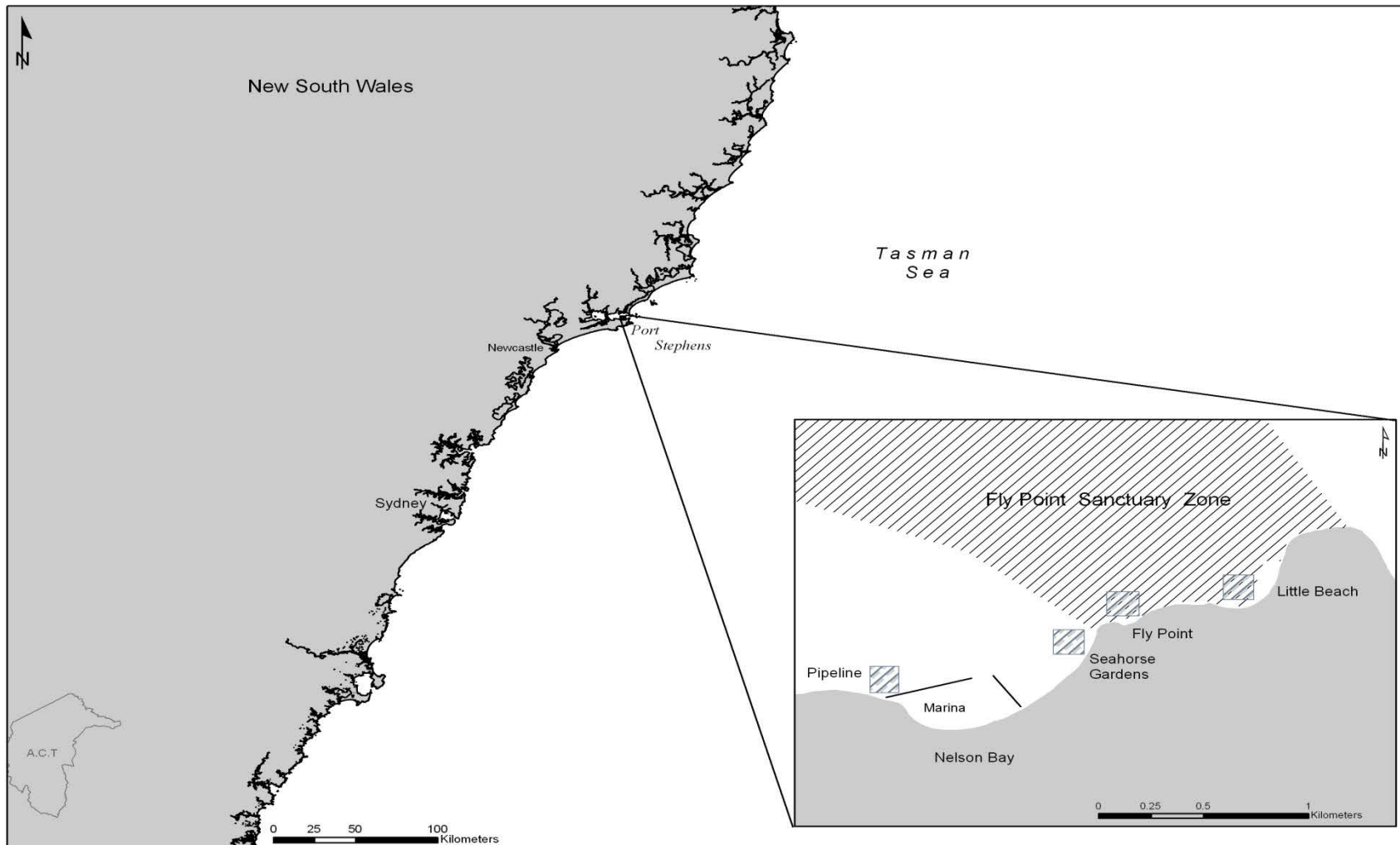
Figure 5.1. Location of study sites, Port Stephens, New South Wales – Australia.

Pilot study

To determine if time of day affected the observability of *H. whitei* at the sites, a pilot study was done to test the hypothesis that *H. whitei* abundance would not differ between day and night, as has been found for another similar sized seahorse, *H. comes*, that was considered to be easier to detect at night (Perante *et al.*, 2002). The pilot study involved conducting a 60 min diver search (as described above) at the site during daylight hours (0700-1700) then followed up by a repeat survey during the night (1800-0600); both dives were done on the high tide approximately 12 hr apart. Surveys were conducted at one of the no-take sanctuary sites (Fly Point) and one of the non-sanctuary sites (Pipeline) with each site being surveyed on six occasions between October and December 2005. Sites were both sampled within 48 hours of each other. The hypothesis that seahorse abundance would not vary between day and night was tested with a 2-factor analysis of variance (ANOVA) with the factor time treated as fixed with two levels (day, night) and the factor site treated as random and orthogonal with two levels. There was no significant difference in the mean abundance of *H. whitei* between night and day surveys ($F_{1,24}$ = 0.45, $P > 0.5$), and the time x site interaction was also non-significant ($F_{1,24}$ = 0.02, $P > 0.5$). Therefore, time of surveying was considered irrelevant and all sampling occurred between 0600 and 2200.

Predator abundance

During 2006, as part of the 60-min monthly surveys and additional dives at the four locations (n=~100 dives across four sites), predation events on *H. whitei* were observed and recorded. Species that were classified as predators of *H. whitei* were observed to attack or feed on *H. whitei*. From 2007-2009, during the 60-min monthly abundance surveys (n=36 monthly surveys), the numbers of predators observed at each site were identified and recorded.

Data analysis

The hypothesis that mean seahorse abundance would differ between the sanctuary zone and non-sanctuary zone sites was tested by 3-factor permutational multivariate analysis of variance (PERMANOVA) using PERMANOVA+ 1.0.5 within PRIMER-E 6 (Plymouth Routines in Multivariate Ecological Research http://www.primer-e.com/) (Anderson, 2001). The factor Status was analysed as fixed with 2 levels (sanctuary, non-sanctuary), the factor Site was analysed as random with 2 levels and nested in Status, and the factor Year was analysed as random with 4 levels (2006, 2007, 2008, 2009). Each monthly survey was treated as a replicate (n=12) for each year. The analysis was done on the Euclidean distance similarity matrix with significance determined from n=9999 permutations. The same 3-factor PERMANOVA design was applied to test the hypotheses that predator abundance would differ between sanctuary and non-sanctuary sites with the factor Year having 3 levels (2007, 2008, 2009). Post-hoc evaluations of significant results were done

using pair-wise *t*-tests. The hypothesis that there would be a relationship between the abundance of seahorses and abundance of predators was tested, using the combined data for all sites from all monthly surveys between 2007 and 2009, by Pearson product-moment correlation coefficient with SPSS 20.

5.4 Results

Relative abundance of *H. whitei*

A grand total of 2,104 *H. whitei* (1953 adult and 151 juvenile) were observed in the monthly surveys from 2006-2009, with 1802 observed in the non-sanctuary zone (control) sites and 302 observed in the sanctuary zone sites. Mean monthly abundance of *H. whitei* in the sanctuary zone (mean 3.1 ± 0.3 S.E.) was significantly less than the non-sanctuary zone (18.8 ± 0.9) (Table 5.1, Figure 5.2) therefore the hypothesis that seahorse abundance would not differ between the sanctuary and non-sanctuary sites was rejected. The significant year x site(MPA) interaction occurred because mean seahorse abundance differed between the two non-sanctuary zone sites in some years but not all years and did not differ between the two sanctuary zone sites in any year (Table 5.1(a)).

Numbers of *H. whitei* varied greatly at the non-sanctuary sites with a large decline in the *H. whitei* population at the Seahorse Gardens in 2007. The decline commenced in October 2006 and continued until March 2007 (Figure 5.3), during which the monthly mean abundance of *H. whitei* was 4.8 ± 1.8 compared to the mean monthly

abundance of 17.2 ± 1.4 for the site across all years. From January to February 2007, 0 adult *H. whitei* and only 1 small juvenile were observed at the Seahorse Gardens. This was the only time across all four sites and all years when no adult seahorses were observed.
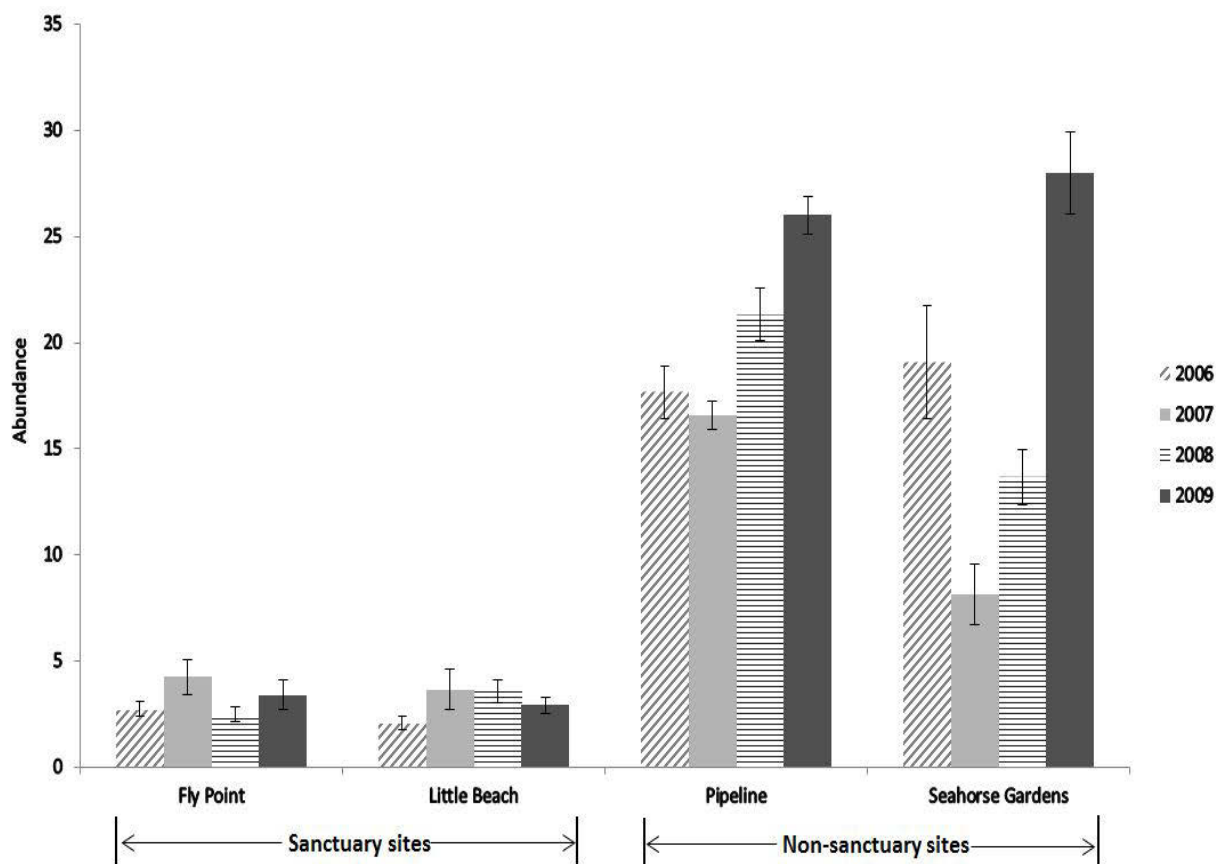
Figure 5.2. Mean monthly abundance of *H. whitei* (± S.E.) at four sites within Port Stephens for 2006-2009.
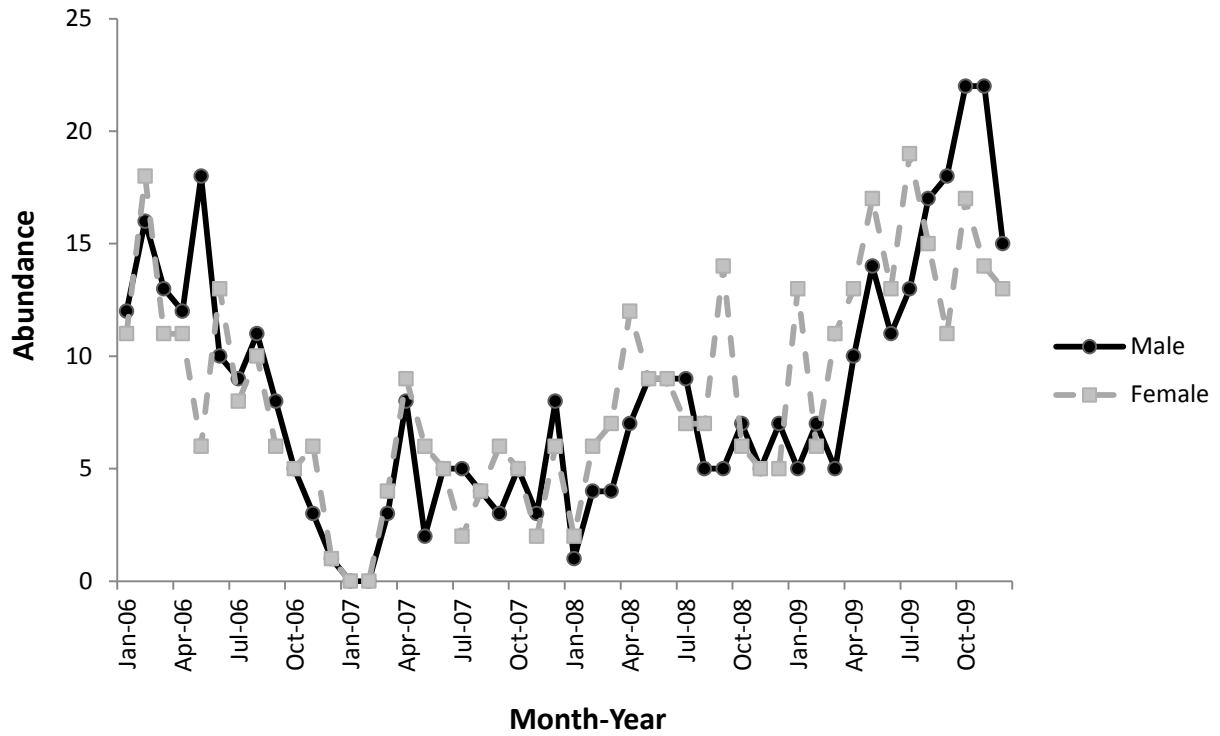
Figure 5.3. Monthly abundance of adult *H. whitei* recorded in 60 min dive surveys from 2006-2009 at the non-sanctuary site Seahorse Gardens, Port Stephens.
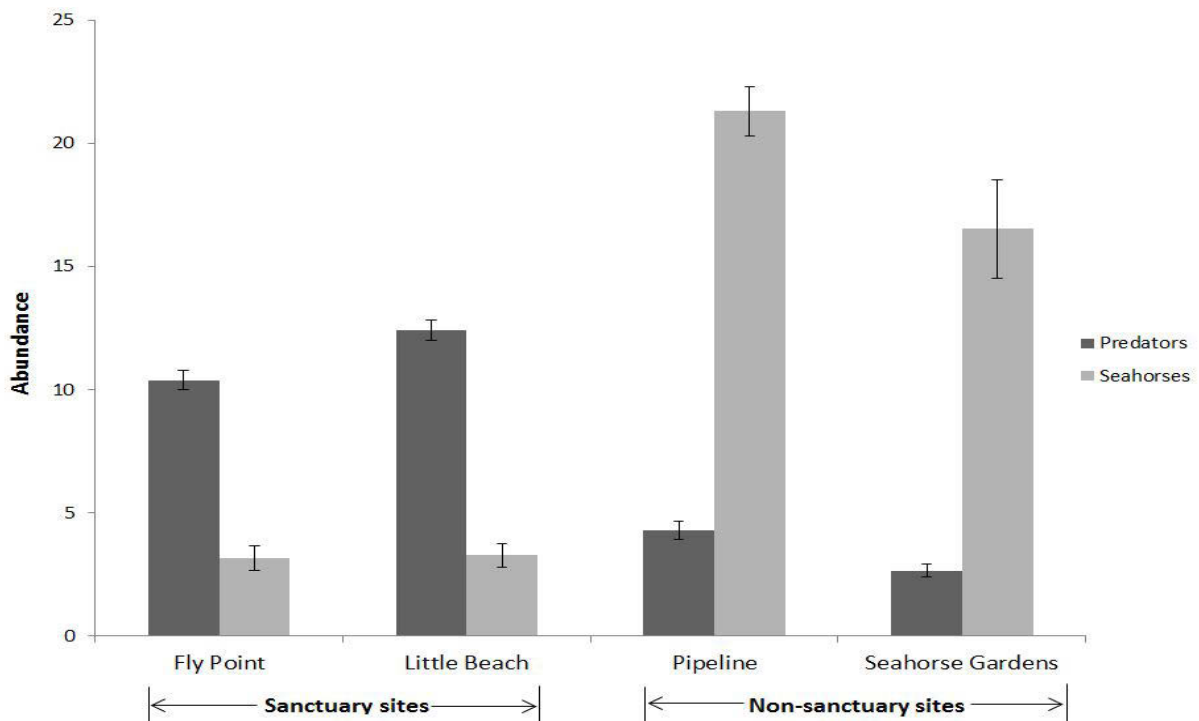
Figure 5.4. Monthly mean abundance (± S.E.) for *H. whitei* and predators (fish and octopus) for each site from 2007-2009.

Table 5.1. Summary of hypotheses tested in the long term monitoring of *H. whitei* and predator abundance within and outside a marine protected area (MPA) with details of statistical analysis performed and PERMANOVA results.

Hypotheses	Source	df	MS	Pseudo-*F*	*P*(perm)
(a) Seahorse abundance would not vary between sanctuary zone sites and non-sanctuary zone sites and years.	MPA site (fixed)	1	11781	19.67	0.012
	Year (random)	3	409.54	4.19	0.067
	Site(MPA) (random)	2	120.67	1.24	0.358
	MPA x Year	3	483.21	4.95	0.052
	Year x Site(MPA)	6	97.61	6.01	0.001
	Residual	191			
(b) Predator abundance would not vary between sanctuary zone sites and non-sanctuary zone sites and years.	MPA site (fixed)	1	2272.11	36.24	0.006
	Year (random)	2	13.03	1.99	0.24
	Site(MPA) (random)	2	61.18	9.33	0.04
	MPA x Year	2	1.69	0.26	0.79
	Year x Site(MPA)	4	6.55	2.17	0.09
	Residual	143			

Predator abundance

Five different species preyed on *H. whitei* across the four sites. Three species of fish (dusky flathead *Platycephalus fuscus*, eastern red scorpionfish *Scorpaena jacksoniensis*, and striped anglerfish *Antennarius striatus*) and two species of octopus (Sydney octopus *Octopus tetricus* and blue-lined octopus *Hapalochlaena fasciata*), were recorded either attacking or feeding on *H. whitei.* A total of 13 predation events were recorded from 2006 to 2009 (9 in the sanctuary sites and 4 in the non-sanctuary), with the most frequently observed predation events involving *S. jacksoniensis* (*n*=5) and *O. tetricus* (*n*=4). These five species were surveyed monthly from 2007 to 2009 and it was found that the mean number of predators in the sanctuary zone sites (11.4 ± 0.4 S.E.) was significantly greater than the mean number of predators in the non-sanctuary zone sites (3.5 ± 0.3) (Table 5.1(b), Figure 5.4). Therefore, the hypothesis that predator abundance would not differ between the sanctuary and non-sanctuary sites was rejected. The significant Site(MPA) effect occurred because mean predator abundance differed between sites in the non-sanctuary zone but not between the two sites in the sanctuary zone. The most abundant predators in the sanctuary zone sites were *S. jacksoniensis*, *O. tetricus* and *P. fuscus* (Figure 5.5). There was a significant, negative correlation between monthly seahorse abundance and predator abundance (*r*= -0.69, *n* = 144, *P* < 0.001; Figure 5.6), with high abundance of predators associated with lower abundance in seahorses.
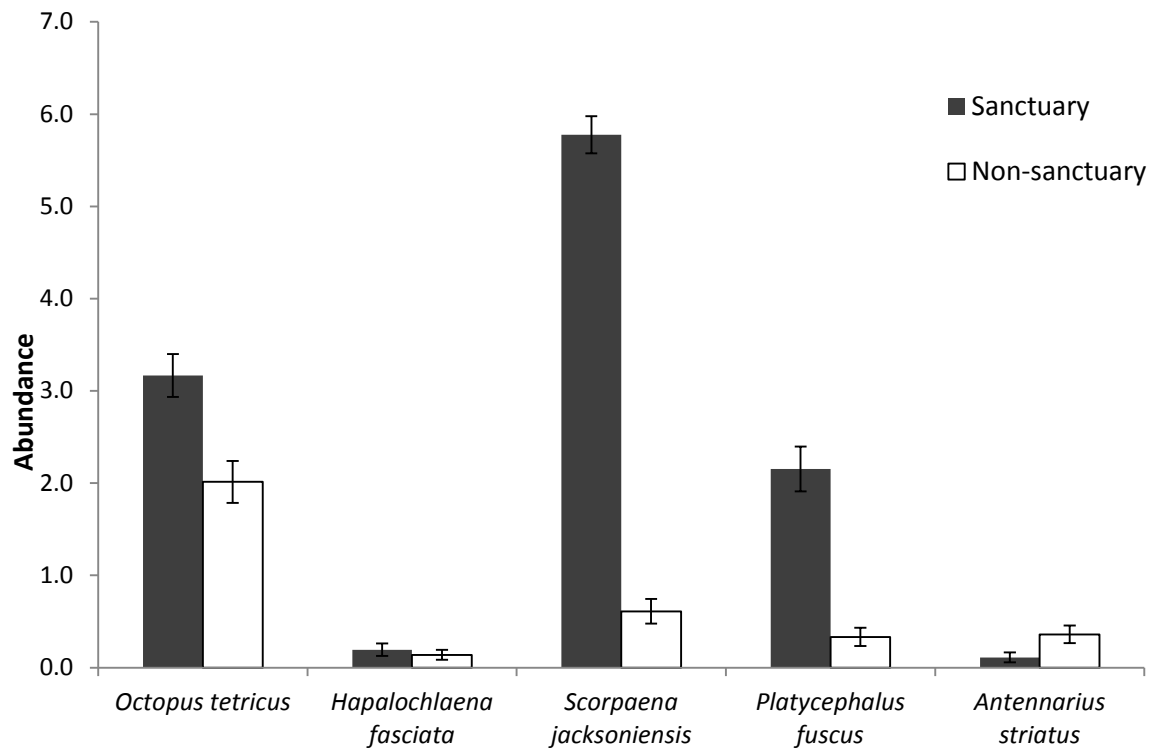
Figure 5.5. Monthly mean abundance (± S.E.) in 2007-2009 of seahorse predators at two sites within the Fly Point Sanctuary Zone and at two sites outside the Sanctuary Zone.
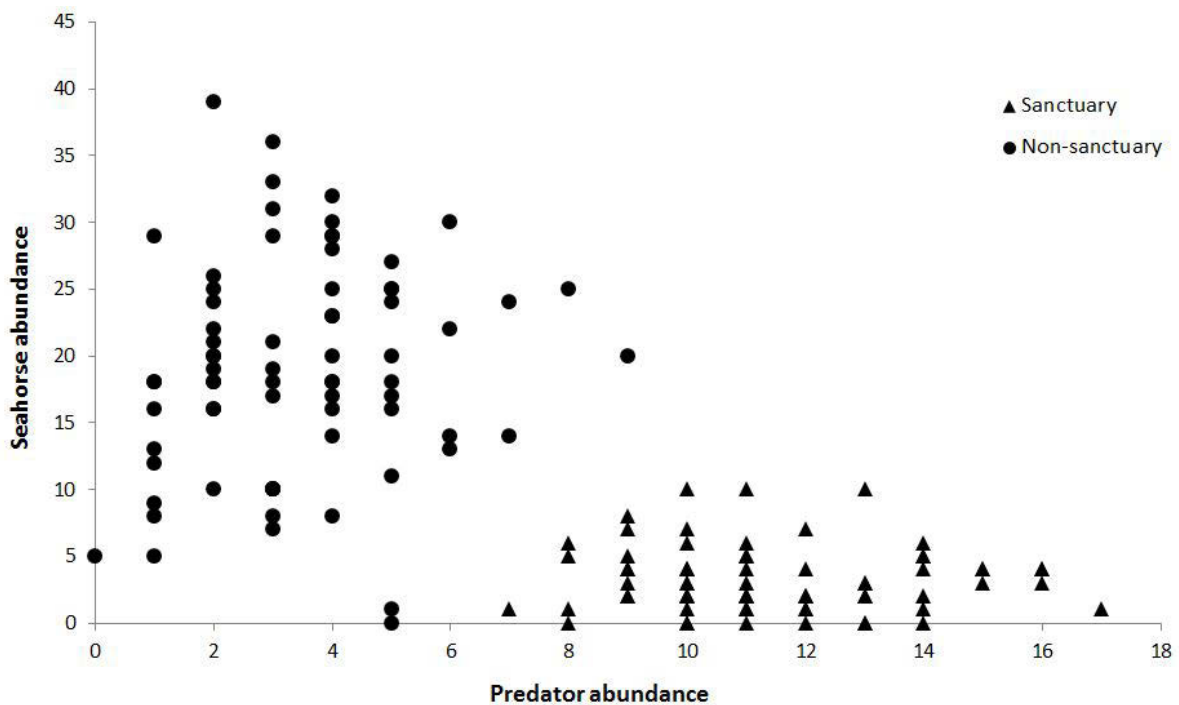
Figure 5.6. Relationship between monthly seahorse abundance and predator abundance from 2007 – 2009 at each site.

5.5   Discussion

Seahorse abundance

The main finding of this study that the abundance of seahorses was significantly lower within a no-take MPA, compared with sites open to fishing, was unexpected as it has been suggested that seahorse abundance would benefit from small-scale no-take MPAs (Martin-Smith *et al.*, 2004; Vincent *et al.*, 2005; Marcus *et al.*, 2007; Morgan and Vincent, 2013). The most likely cause of this was the greater risk of predation in the no-take sanctuary zone sites, as suggested by the greater abundance of seahorse predators in these sites and the negative correlation between predator abundance and seahorse abundance. In a study on marine reserves in the Philippines, it was found that syngnathid abundance was lower within the no-take MPA compared to the fished sites outside; however, the difference was not significant and possibly confounded by differences in habitat (Samoilys *et al.*, 2007). In this example, the authors acknowledged that it was difficult to determine if observed effects were real responses to changed increases within the protected MPA (e.g. increased predator abundance) or other factors such as increased species visibility in the non-protected MPA sites as result of less structurally-complex habitats (Samoilys *et al.*, 2007).

It is unlikely that the significant difference in *H. whitei* abundance between the no-take sites and control sites was related to habitat differences. Habitat types did not differ between sites and Fly Point, one of the sanctuary zone sites, had the greatest coverage of sponge habitat; a preferred habitat for *H. whitei* (see chapter 4). The

observed differences between the no-take and control sites are also unlikely to have been confounded by differences in the detectability of seahorses. Although seahorse detectability differs among habitat types, there were no differences in the relative abundance of habitats among sites and no evidence that the occurrence of cryptic behaviour among seahorses differed among the sites (see chapter 4).

One of the often-stated goals of MPAs is the preservation of areas with species and assemblages occurring in an undisturbed state, at least from the exclusion of fishing pressure, for the benefit of scientific research, education and public awareness (Allison *et al.*, 1998; Agardy *et al.*, 2003). Seahorses and other syngnathids are charismatic species that attract support for marine conservation (Scales, 2009; Vincent *et al.*, 2011). The findings of this study suggest that seahorses might not benefit from the use of MPAs for marine conservation; however, the finding of this study linking decreased seahorse abundance with increased predator abundance is based on correlative evidence. There are no data available on the abundances of seahorse and their predators prior to the establishment of the no-take MPA. To test and validate this finding, field experiments are needed to determine the actual rates of seahorse predation between sites closed and open to fishing, and to determine other predator species (Mislan and Babcock, 2008; Bassett *et al.*, 2009).

Predators of *Hippocampus whitei*

As seahorses are a slow-moving species, they rely on crypsis through colour changes and algal-like filaments that mimic their habitat, to avoid predation (Schmid and

Senn, 2002; Kuiter, 2009). Eighty-two predators of syngnathids are known, including fishes, turtles, sea birds, invertebrates and marine mammals (Kleiber *et al.*, 2011). None of the predators recorded in this study were included in the Kleiber *et al.* (2011) review, with the only recorded predator of *H. whitei* in the literature being the little penguin *Eudyptula minor* whilst Kuiter (2009) observed the striated frogfish *Antennarius striatus* predating on *H. abdominalis*, a seahorse known to occur in the same region as *H. whitei* (Harasti *et al.,* 2010*)*.

The cephalopod *Octopus tetricus* and the scorpionfish *Scorpaena jacksoniensis* are believed to the most frequent predators of *H. whitei* as they were responsible for the majority of observed predation events and were the two most abundant predators. However, given the large diversity and size of fishes found within the Fly Point no-take MPA site (Edgar *et al.*, 2009), there are potentially other predators of *H. whitei* that were not detected. During monthly surveys from 2008-2009, both snapper *Pagrus auratus* and leatherjacket *Nelusetta ayraudi* were observed to attack *H. whitei* following their release after being handled underwater (for measuring or tagging as part of other studies). This occurred if the seahorse swam away from the holdfast it was placed on after handling. However, there were no observations of either species attacking *H. whitei* that had not been 'disturbed'. Another cephalopod species, the mourning cuttlefish *Sepia plangon*, was observed to prey on juvenile *H. whitei* on two separate occasions within the sanctuary zone however it was not included in the monthly predator surveys as the observations occurred in 2008 and 2009, prior to the predator study reported here.

Predator abundance

Seahorse predators were more abundant within the no-take sanctuary zone sites, which is similar to findings of other studies from around the world that have reported greater abundance and/or biomass of predator fishes in areas protected from fishing (Williamson *et al.*, 2004; Ashworth and Ormond, 2005; Samoilys *et al.*, 2007; Currie *et al.*, 2012). The three most abundant predator species (*Platycephalus fuscus*, *Octopus tetricus* and *Scorpaena jacksoniensis*) were more abundant within the sanctuary zone and are considered to be important recreational and commercial species that are targeted by fishers in NSW (DPI, 2013), therefore these species are likely to benefit from the exclusion of fishing. Additionally, data collected from baited underwater remote video systems has found that the Fly Point sanctuary zone has greater diversity and larger fish species than the non-sanctuary zone sites (NSW DPI *unpublished data*). This is also supported by the findings of Edgar *et al.* (2009) that demonstrated that Fly Point was high in fish biomass and in density of larger fish species. The increased numbers of predators within the sanctuary zone sites is not surprising, as the sanctuary zone has been protected for 30 years (since 1983) with no fishing allowed, and numerous studies have shown that fish biomass and density increased over time within MPA's (Halpern, 2003; Williamson *et al.*, 2004; Edgar *et al.*, 2009; Babcock *et al.*, 2010; Currie *et al.*, 2012)

With the implementation of MPAs, there will be 'winners and losers', with some species benefiting from protection by increases in size and abundance (Barrett *et al.*,

2007; Watson *et al.*, 2009). Other species showed no change in abundance or abundance decreased as a result of increased predation and interspecific competition (Graham *et al.*, 2003; Watson *et al.*, 2007; Götz *et al.*, 2009), particularly the smaller cryptic fishes (Willis and Anderson, 2003; Samoilys *et al.*, 2007; Edgar and Stuart-Smith, 2009). Protected areas have been shown to help promote recovery of predatory species (Willis *et al.*, 2003; Williamson *et al.*, 2004), which potentially can have indirect negative effects on prey species in the protected areas (Graham *et al.*, 2003).

Whilst this study suggests that *H. whitei* has been negatively impacted by a no-take protected area most likely through increased predation, other species of seahorse and other syngnathids might be affected in different ways by MPAs. Species' responses to MPAs will depend on a range of factors including the availability of preferred habitats, potential predators in the area and factors occurring outside an MPA.

Decline in *Hippocampus whitei* abundance

Population estimates and monthly relative abundance data show that *Hippocampus whitei* populations across the four sites in Port Stephens were stable with the exception of the Seahorse Gardens, which experienced a large population decline in late 2006. As the species is protected and not exploited by fishing, such an abrupt decline is unusual and the cause of the decline is unknown. Population declines in *Hippocampus sp.* in the absence of fishing pressure have been recorded elsewhere,

with *H. abdominalis* populations declining 79-98% over 3 years (Martin-Smith and Vincent, 2005) and populations of *H. guttulatus* and *H. hippocampus* declining by 94% and 73%, respectively over a seven year period (Caldwell and Vincent, 2012). Given that the decline of *H. whitei* in this study occurred only at the Seahorse Gardens site, it is unlikely that the decline can be attributed to ecosystem-wide stressors such as disease or environmental variables as populations at the nearby three sites should also have been affected. Throughout the study, there was no noticeable change in currents or water temperature at the Seahorse Gardens. These two variables were however considered to influence changes in seahorse abundance in Ria Formosa lagoon (Caldwell and Vincent, 2012). There were also no recorded incidents of illegal collecting, nor was the site affected by trawling, netting or dredging which are prohibited in the area. Seahorse predator abundance at the site did not increase during the study, nor were there observations of increases in other species that may prey on *H. whitei*.

A potential hypothesis for the decline is that the seahorses may have moved off the site into deeper water; however, *H. whitei* displays strong site fidelity and does not move large distances (see chapter 4). Support for the movement hypothesis is that several seahorses tagged at the site in 2006, disappeared during the decline period, but started to be resighted again from late 2007 until 2010 (Harasti *et al.*, 2012). Numerous exploratory dives were undertaken in the deeper water (12-18 m) surrounding the Seahorse Gardens site from 2006-2009 however no *H. whitei* were encountered deeper than 12 m (maximum depth of study site), so the location to

which seahorses might have migrated is unknown. With such a population decline there is concern that reproduction would be reduced as a result of Allee effects (Kramer *et al.*, 2009), especially with the high level of monogamy displayed by *H. whitei* (Harasti *et al.*, 2012), as mature animals could find it difficult to find a mate. Although there was a rapid decrease in population abundance, the actual recovery of the population to almost pre-decline levels occurred within three years, with the highest number of juveniles at the site occurring in 2009. As *H. whitei* is considered an R-selected species with rapid growth, early age at maturity and sexually mature at approximately six months (Harasti *et al.*, 2012), the species has the potential to repopulate a site if sufficient breeding adults return to the site, or recruitment from adjacent sites is successful.

This study illustrates the importance of long-term monitoring of seahorse populations as it was shown that seahorse numbers varied considerably over a 12-month period. Long-term monitoring of multiple sites is necessary for a good understanding of seahorse population changes in the wild and allows for better assessment on the status of seahorse populations. This study indicates that caution should be used when investigating the use of MPAs to conserve seahorse populations as there is potential for negative impacts on seahorse abundance through potentially increased predator abundance. Other management interventions may be more suitable such as entire protection of the seahorse species, removal of destructive fishing practises that damage essential habitats, restoration of natural habitats or creation of artificial habitats. A range of management measures are

needed to conserve threatened populations of seahorses and the declaration of a marine protected area may not be the ideal solution.

5.6   Acknowledgements

CHAPTER 6

Striking a balance between retaining populations of protected seahorses and maintenance of swimming nets. [4]

6.1   Abstract

The fish family Syngnathidae (seahorses, pipefish, pipehorses and seadragons) is fully protected in New South Wales, Australia, and in some countries certain species are threatened by unsustainable collecting, capture as incidental bycatch and habitat degradation. Within Sydney Harbour, two species of seahorses (*Hippocampus abdominalis* and *Hippocampus whitei)* have been found to colonise artificial structures such as jetty pylons and protective netted swimming enclosures. These protective nets are subject to fouling from epibiotic growth (e.g. algae, ascidians, bryozoans, etc.) and rubbish which causes the nets to collapse from the additional weight. Local authorities employ diving contractors on an ad hoc basis to remove the epibiota from nets.

Surveys showed a significant decline in the numbers of both seahorse species at one site following the replacement of a net and recovery of the *H. whitei* population took more than 15 months. A manipulative experiment tested the importance of epibiotic growth for seahorses. *Hippocampus whitei*, tagged with individual marks, were allocated to sections of a net which had undergone different cleaning procedures. Seahorse size, position on the net and total population abundance were recorded every two weeks over a three month period. It was demonstrated that seahorses have a significant positive association with epibiotic growth and proximity to the seafloor. Seahorse populations also showed seasonal variation in abundance with increased numbers on the net during the breeding season (spring – summer). This

project has led to the development of best practice net cleaning procedures for local authorities in Sydney Harbour to manage growth on the nets whilst minimising impacts on seahorse populations.

6.2   Introduction

Estuarine ecosystems around the world are threatened by increasing coastal development and declining water quality (Weinstein, 2008). Within many Australian estuaries and harbours, there are various types of artificial structures (such as wharfs, pylons, sea walls, pontoons) that provide important habitat for various marine species. These structures provide unique marine habitats (Glasby and Connell, 1999; Connell, 2001; Holloway and Connell, 2002) and are known to be utilised by a variety of fishes (Coleman and Connell, 2001; Brickhill *et al.*, 2005; Clynick, 2008) and molluscs (Cole *et al.*, 2005; Blockley, 2007). Seahorses have been found commonly to utilise artificial structures as habitat, with species such as *Hippocampus abdominalis*, *H. reidi* and *H. subelongatus* recorded on pilings and piers (Kvarnemo *et al.*, 2000; Pogonoski *et al.*, 2002; Martin-Smith and Vincent, 2005; Rosa *et al.*, 2007), *H. abdominalis and H. kuda* being recorded on fish cages (Marshall, 2004; Choo and Liew, 2006), and *H. whitei* on protective swimming nets (Pogonoski *et al.*, 2002; Clynick, 2008).

All syngnathids are legislated as protected species in New South Wales (NSW) under the *Fisheries Management Act* (1994). The White's seahorse, *H. whitei*, is one of

the most common seahorse species in shallow estuarine waters of NSW (Kuiter, 2000b; Pogonoski *et al.*, 2002). The pot belly seahorse, *H. abdominalis* also occurs in NSW coastal waters and is generally found in deeper 'sponge gardens' or on algal covered reefs (Kuiter, 2000b). The natural habitats of *H. whitei* in Sydney Harbour include seagrasses (Vincent *et al.*, 2005) and sponge gardens (Kuiter, 2000b). Both species occur in Sydney Harbour; a large highly modified estuary where the foreshores have been subject to significant pressures from high density urban and industrial development since European settlement.

Throughout the world, most species of seahorse are associated with complex structures such as marine vegetation (Moreau and Vincent, 2004; Rosa *et al.*, 2007; Teske *et al.*, 2007a), coral reef habitats (Lourie and Randall, 2003; Morgan and Vincent, 2007) and sponges (Kuiter, 2000b) so their association with artificial structures is not surprising. Whilst artificial structures are often used to enhance fish abundances (Baine, 2001), the netted swimming enclosures in Sydney Harbour were installed to ease public concerns about potential encounters with sharks. The permanent nets within the harbour can support large amounts of epibiotic growth (e.g. ascidians, bryozoans, sponges and algae) that causes the net to sag and break from its surface support (floats or wharfs) due to the increased weight. As a result, Sydney authorities (such as local councils) hire commercial contractors to clean the nets on an ad-hoc basis or to replace heavily fouled nets. The impact of removing epibiota from a net on local seahorse populations is uncertain, but anecdotal

evidence from local divers suggests that seahorse abundance in the vicinity of the net decreases dramatically as a result of net cleaning.

In this study, data from a long-term population monitoring programme of seahorses at one netted site in Sydney Harbour were used to assess the effect of net replacement on local seahorse abundances. The relationship between epibiota and seahorse distribution and abundance on a protective swimming net was also investigated. It has been documented that the majority of seahorses on swimming nets tend to occur close to the seafloor (Clynick, 2008). Thus, it was predicted that seahorse abundances would not be affected significantly by cleaning epibiota from the top of the net, whereas abundances would decrease if epibiota was removed from the bottom of the net. A manipulative experiment was used to test this prediction and to help determine which of various cleaning options could minimise localised impacts on a seahorse population. Results from seahorse population surveys were used to determine the most appropriate time of year for cleaning nets.

6.3 Materials and Methods

Study Sites

This study was conducted at two sites in Sydney Harbour, New South Wales, Australia. Long-term monitoring of the seahorse populations on a swimming net enclosure was undertaken at Clifton Gardens (33^0 50' 20.74"S – 151^0 15' 9.51"E) while

a manipulative experiment was undertaken on the swimming net enclosure at
Manly (33^0 47′ 57.63″S – 151^0 16′ 57.67″E) (Figure 6.1).
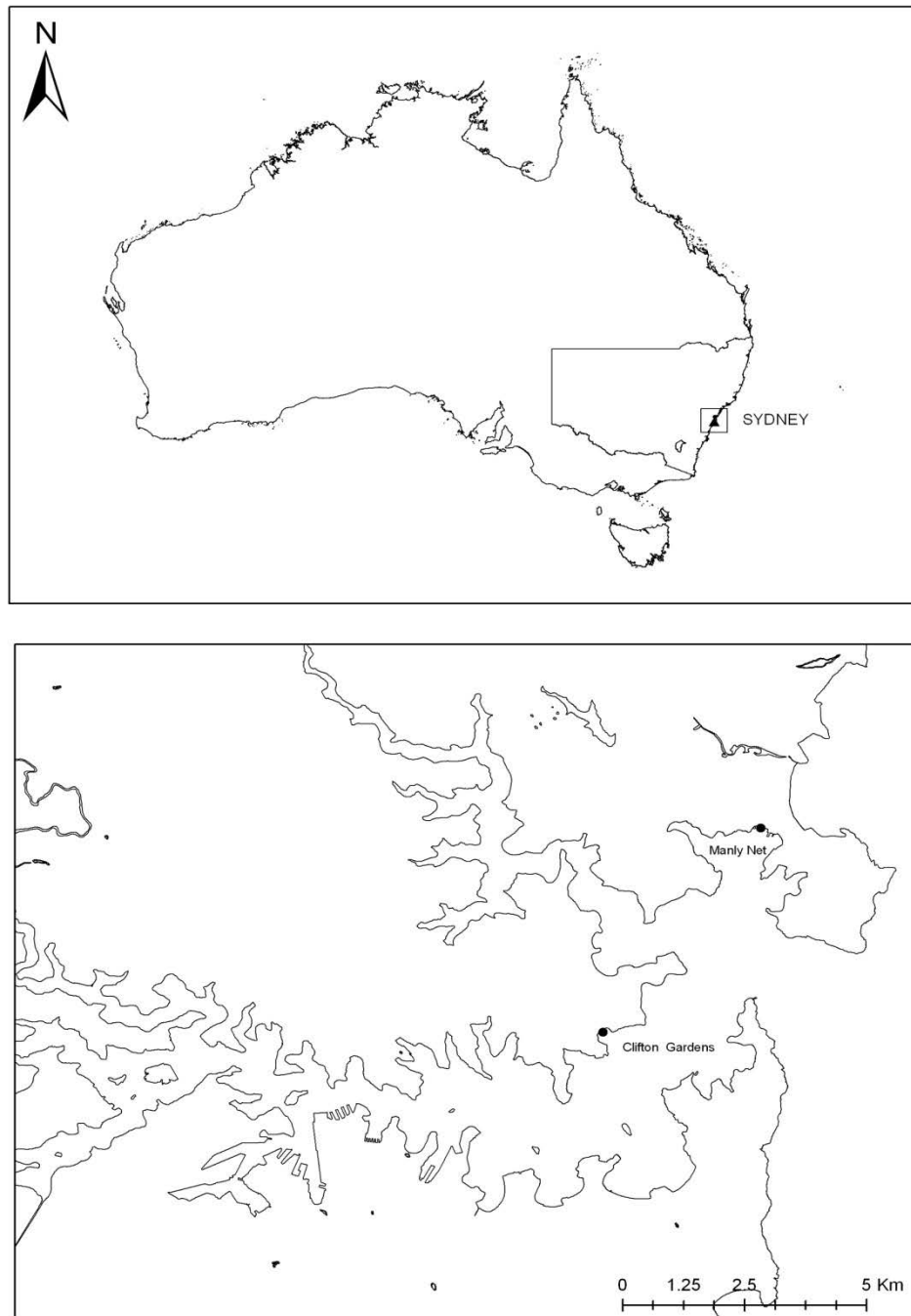
Figure 6.1. Location of study sites in Sydney Harbour.

Clifton Gardens

The swimming net at Clifton Gardens has a perimeter of ~200 m, running under a pier for ~140 m and between posts for the remaining 60 m. The deepest part of the net is submerged to a depth of 3.2 m at mean low tide. The original net consisted of metal rings of 15 cm diameter but this was replaced in 2000 by a polypropylene net of 10 cm square mesh (J. Clark-Jones, *pers. comm*). The epibiota observed on the net included bryozoans (*Schizoporella errata*, *Watersipora subtorquata*), mussels (*Mytilus galloprovincialis planulatus*), green algae (particularly *Codium cuneatum*), brown algae (*Zonaria* sp., *Sargassum* sp., *Ecklonia radiata*), colonial and solitary ascidians (*Diplosoma listerianum*, *Botrylloides leachi*, *Pyura stolonifera*, *Styela plicata*, *Herdmania grandis*), and several sponges. Seagrass beds of *Halophila ovalis* and *Zostera capricorni* are found inside the swimming enclosure while beds of *Posidonia australis* and *Zostera capricorni* are found outside the net.

Manly

The swimming net at Manly consists of a 10 cm square mesh and is 150 m long. The average maximum depth of the net is 2.8 m with the deepest section of the net at 3.5 m at mean low tide. The net is strung between nine wooden pilings which are spaced 25 m apart. The net is held up by surface floats and is in direct contact with the sandy bottom. The epibiota observed on the net is similar to the species recorded on the Clifton Gardens net.

The protective swimming net is surrounded by seagrass meadows consisting of *Halophila ovalis*, *Posidonia australis* and *Zostera capricorni*. The closest rocky reef is approximately 100 m away. Cleaning of the net is generally undertaken on an annual basis, with the last cleaning occurring in October 2006 (T. McDonald *pers. comm*).

Long-term seahorse population monitoring

Seahorse populations on the net at Clifton Gardens were enumerated on 12 occasions between January 2003 and August 2005 at 39–253 day intervals. In late August 2005 the old net was replaced with a new net by a contractor employed by the local council. Subsequent to this net replacement a further five population surveys were undertaken from November 2005 to July 2008 at 78–533 day intervals (Figure 6.2). All seahorses of both species on the net were counted by a pair of divers, one swimming on each side with a survey taking approximately 90 minutes. There were 12 surveys prior to net replacement and five surveys subsequently.

Net cleaning experiment

From May 2007 to April 2008, monthly surveys were undertaken on the Manly net using two divers on opposite sides of the net to count and record every seahorse living on the structure. Data on sex, size and location were recorded. The same diver (DH) was present for every survey to ensure consistent data collection. A complete survey of the net took 2 to 3 hours. Seahorses found on the net were individually tagged with small visible implant fluorescent elastomer tags (VIFE – Northwest

Marine Technologies, USA) using the techniques described in Woods and Martin-Smith (2004). Each animal was given three permanent tags, pink or yellow in colour, in unique locations to enable identification of individuals. In total, 304 animals were tagged on the net prior to the experiment commencing but not all tagged animals were used in the experiment. By marking individuals many weeks before the experiment, it was hoped to minimise potential effects on their normal behaviour (i.e. seahorses were expected to have recovered from any stress due to handling and tagging).

Sections of the net were allocated to be cleaned or left untouched as controls. Each experimental section was 4 m wide and 2.5–3.5 m high (i.e. the total height of the net). Replicate sections were separated by buffer zones of 1m where no epibiota was removed; hereafter referred to as buffer zones. This distance was deemed acceptable as seahorses tend to be territorial and typically remain within areas of $< 1m^2$ over three months (Harasti *unpubl. data*, and see results below).

The usual method for net cleaning by commercial contractors is to remove all the growth on the net using metal scrapers and a similar technique was employed in this study. Three cleaning options were compared to a control treatment (A). The cleaning treatments were: (B) total clean (all growth removed), (C) top section clean (all growth removed from the surface to a depth of 1.2 m) and (D) bottom section clean (all growth removed from the seafloor to a height of 1.2m on the net). Each

treatment was randomly assigned to various locations on the net and replicated four times.

The cleaning experiment was set up over two days (November 12 and 13, 2007). Prior to growth being removed from each treatment, the tagged seahorses were carefully removed from each replicate section (including controls) and placed in catch bags and hung off the net away from the cleaning works. This ensured that seahorses were minimally impacted during the cleaning process. After cleaning, each seahorse was returned to the same section of the net from which it had been removed. The location of seahorses was then recorded each month for four months. To determine if animals from the net were utilising the surrounding seagrass as habitat, four dives of 30 minutes duration were conducted in the seagrasses over different months.

Data analysis

Long-term seahorse monitoring

As estimates of seahorse abundance were not considered to be independent between successive time periods, an auto-regressive integrated moving-average (ARIMA) impact analysis was used to examine the effect of net replacement (Box and Jenkins, 1976; McDowall *et al.*, 1980). ARIMA models are the most general class of models for analysing time series and are particularly suitable for comparing trends before and after an intervention, in this case the replacement of the net (McDowall *et al.*, 1980).

When data points, such as the population of seahorses, are serially dependent an autoregressive coefficient (*p*) can be estimated which describes consecutive data from previous, time-lagged data. Autoregression will only be stable if the data are stationary and the number of differencing passes required to achieve stationarity is given by the coefficient *d*. Independent from the autoregressive process, each data point in the series can be affected by past error, which is estimated using the moving average coefficient (*q*). While, primarily developed and used in economics and social sciences, ARIMA is increasingly being recognised as a tool to investigate changes in ecological systems (e.g. Bergamino *et al.* (2007)).

In order to determine the values of the parameters *p*, *d* and *q*, correlograms of autocorrelation and partial autocorrelation were generated as recommended by Pankratz (1983). Alternative ARIMA (p,d,q) models were run on log-transformed data and those with the lowest Akaike Information Criteria accepted.

Net cleaning experiment

In order to balance numbers of replicates across all treatments for seahorse movements, only 11 marked individuals were considered per treatment (defined by the lowest number of tagged animals found in a single treatment at the commencement of the experiment). Therefore, only 176 of the 304 tagged seahorses were used in the study (4 treatments x 4 replicates, each with 11 seahorses).

Surveys were undertaken on a fortnightly basis following the cleaning experiment, with seven surveys conducted from November 2007 to February 2008. During each

survey, data were collected on the location of seahorses on the net (including treatment found in), their abundance, size, sex and position on the net relative to the seafloor. The distance each seahorse had moved from one survey to the next was recorded in metres. It is acknowledged that this is not an absolute measure of total movement; rather it was used as a relative comparison among treatments.

Data were checked for homogeneity of variances using Cochran's C test and abundances or distances moved were compared among treatments or times using one (fixed) factor ANOVAs. Post-hoc comparisons of means were done using Student-Neuman-Keuls tests. Details of hypotheses tested and details of each analysis are indicated in Table 6.1. A single factor repeated measures ANOVA, using data from four surveys prior to net cleaning and four surveys after cleaning, was used to determine if net cleaning affected seahorse abundances.

When comparing abundances of seahorses over time for the same treatment in an analysis, we randomly selected a subset of $n = 7$ different individuals per treatment for each time (from a total of 44 available; 4 treatments x 11 seahorses) to ensure that data were independent. Seahorses that were not resighted (22 in total) were not included in any of the analyses as it was not known if they were alive or had migrated off the net.

6.4   Results

Seahorse population monitoring

Two species of seahorses were found on the Clifton Gardens net; *H. abdominalis* and *H. whitei*. Prior to replacement of the net, numbers of both species varied with little apparent pattern although *H. abdominalis* populations were more variable than *H. whitei* (coefficient of variation = 0.56 vs 0.37 respectively) (Figure 6.2). For both species the best models were described by ARIMA (0,0,1) indicating that populations were stationary (there was no trend in abundance over time) but had first order serial dependency (abundance was auto-correlated between successive time points). Both species showed significant declines following replacement of the net (b = -1.377, T-ratio = -3.378, P = 0.005 for *H. abdominalis*, b = -0.985, T-ratio = -7.405, P < 0.001 for *H. whitei*). Numbers of *H. whitei* had recovered at the last population census (February 2008) while numbers of *H. abdominalis* remained low.

Seahorse abundance and distribution

Both *H. abdominalis* and *H. whitei* were found on the Manly net but numbers of *H. abdominalis* were too small for any meaningful analysis (only 6 males and 4 females recorded over the 12 months). Individuals of *H. abdominalis* tended to be much more mobile than those of *H. whitei* with individuals of the former moving from one end of the net to the other over a period two weeks. Furthermore, the majority of *H. abdominalis* were seen within 1.5 metres of the surface. In contrast, virtually all *H. whitei* were found close to the seafloor (details below).
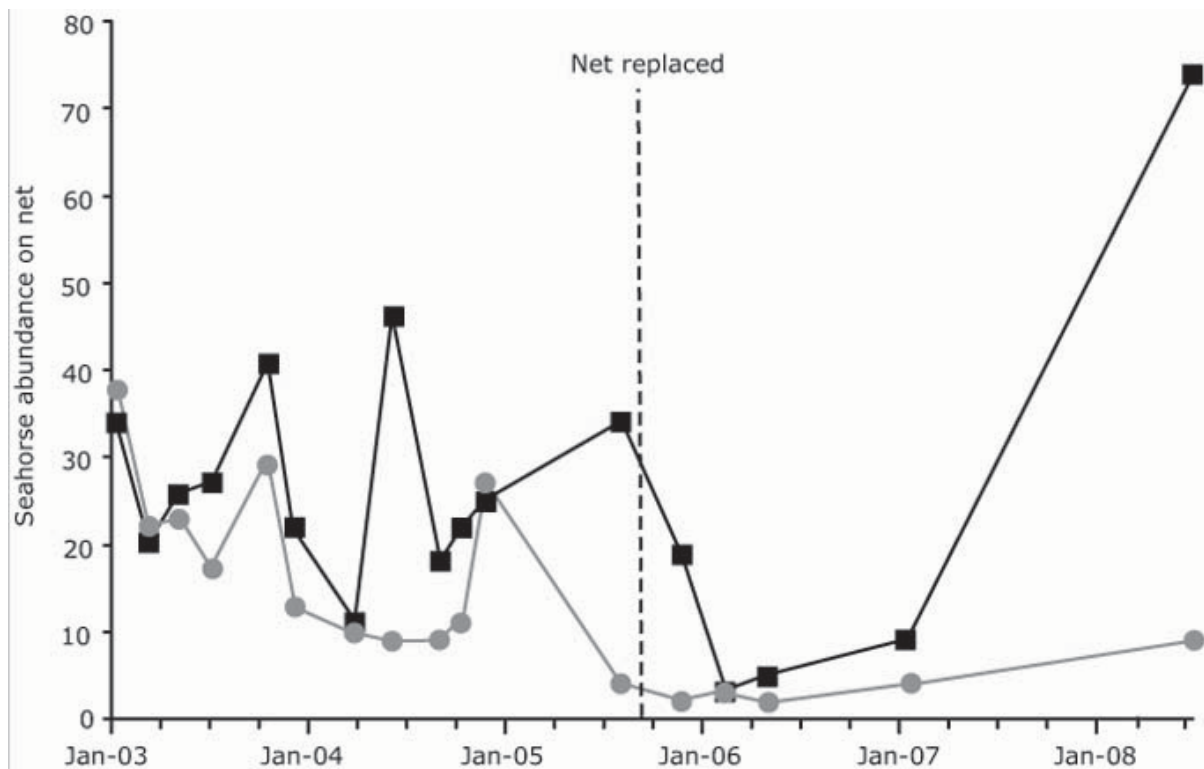
Figure 6.2. Total abundance of seahorses on swimming enclosure net at Clifton Gardens, Sydney Harbour, over the period 2003-2008. ■ = *Hippocampus whitei*, ● = *Hippocampus abdominalis*.

Of the 304 *H. whitei* tagged on the net, 86% were resighted during the 8 month period after initial tagging. A mean of 175 (±18.2 S.E.) *H. whitei* were seen on the entire net each month (between May 2007 and April 2008). There were more *H. whitei* seen on the net between September-February (austral spring-summer) (mean 219 ± 12.4 S.E. animals per month) compared to 131 ± 23.33 S.E. animals per month between March and August (Figure 6.3). The appearance of pregnant males correlated with this increased abundance; pregnant males were first observed on the net in late September and were present through to the end of February. During this period animals were often observed paired up with a partner, however, there was no

apparent difference in sex ratio between the breeding and non-breeding period (Figure 6.3).

Depth

The majority of *H. whitei* on the net were observed within 1.2 m of the seafloor. Prior to the cleaning experiment (July 07–November 07), 95.9% of seahorses observed over six surveys were found within the bottom 1.2 m of the net. Following cleaning of certain sections of the net (November 07– February 08), significantly more (99%) of all observed seahorses were found within 1.2 m of the seafloor (Table 6.1a). In addition to using the actual net as a holdfast, seahorses were observed with their tails holding onto epibiota, particularly sponges and algae. Seahorses were also observed hiding under the loose folds of the net on the seafloor, particularly on the net sections that were devoid of epibiota near the seafloor (treatments B and D).

Table 6.1. Summary of hypotheses tested in the manipulative cleaning experiment on the Manly net with details of statistical analysis performed and Analysis of Variance (ANOVA) results.

Hypothesis	Source	df	MS	F
(a) There will be more seahorses on lower section of net (within 1.2 m of seafloor) than on upper section (>1.2 m from seafloor) irrespective of cleaning treatment.	Height	1	23.14	89.9 ***
	Residual	10	0.26	
(b) Seahorse abundance on net will not vary among treatments prior to net cleaning experiment.	Among treatments	3	1.75	0.17 ns
	Residual	12	10.38	
(c) Total seahorse abundance on the net will not decrease following the implementation of the various cleaning treatments.	Before vs After cleaning	1	1711.12	1.69 ns
	Residual	3	1011.8	
(d) Seahorse distribution on the net will change following cleaning, with abundance increasing in the top clean treatment and controls and decreasing in bottom clean and complete clean treatments.	Among treatments	4	0.21	4.08 **
	Residual	135	0.05	
(e) Seahorse abundance will not change from before to after cleaning in controls (A) or top cleaning treatment (C), whereas abundance will decrease in treatments with epibiota removed from bottom of net (treatments B and D).	Initial Treatment (A)	4	0.31	10.52 ***
	Residual	30	0.03	
	Initial Treatment (B)	4	0.41	1.08 ns
	Residual	30	0.04	
	Initial Treatment (C)	4	0.36	8.21 ***
	Residual	30	0.04	
	Initial Treatment (D)	4	0.9	1.89 ns
	Residual	30	0.5	
(f) Seahorses in treatments with bottom growth removed will move greater distances than animals from top clean treatments	Greatest Distance moved	3	1.45	3.01 *
	Residual	132	0.48	

* $P<0.05$, ** $P<0.01$. *** $P<0.001$, ns $P> 0.05$. Refer to text and Figures 6.4-6.6 for results of post-hoc comparisons.
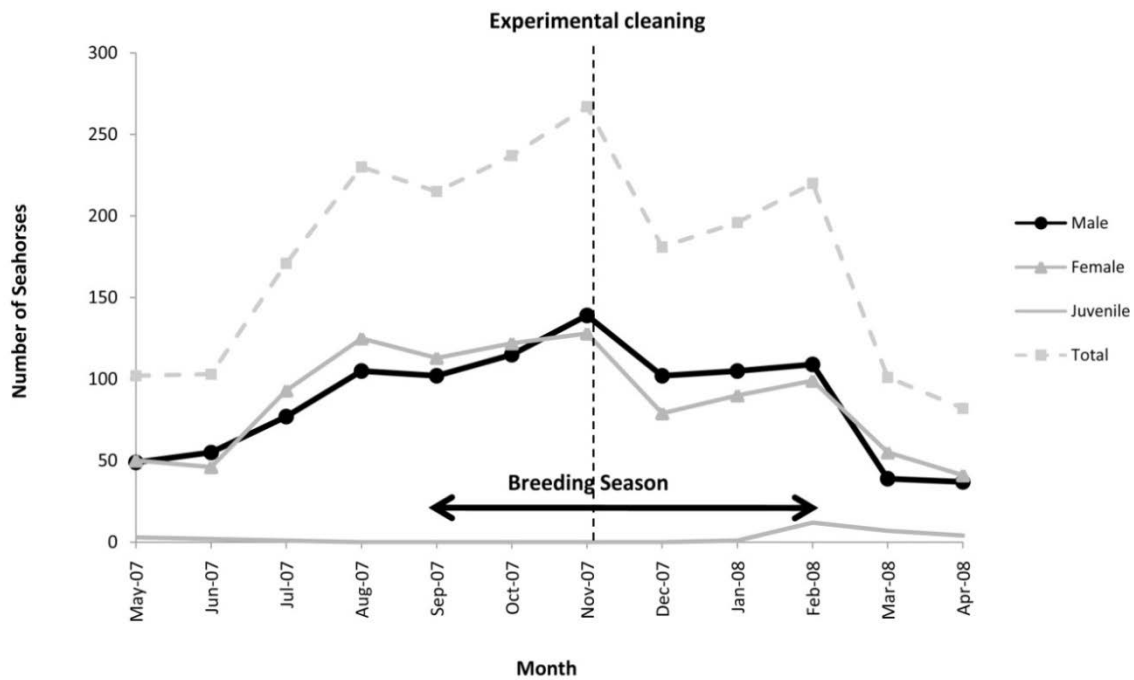
Figure 6.3. Total abundance of *Hippocampus whitei* on Manly swimming enclosure net over 12 month period (May 2007 – April 2008). Dashed line indicates when manipulative cleaning was undertaken. Breeding season is shown with double arrow from September to February.

Effects of cleaning treatments on seahorse abundance

Abundances of animals were not significantly different among the four treatments on the day prior to cleaning (A=13.7 ± 0.47 S.E. seahorses, B=15.3 ± 2.5 S.E., C=14.5 ± 1.3 S.E., D=14 ± 1.4 S.E.: Table 6.1b). Following cleaning, the bottom clean treatment (D) had the lowest return rate for tagged individuals with 10 animals not being resighted. The top clean treatment (C) had the highest resighting rate, with only one animal not being resighted over the four month period. There was no significant difference in total seahorse abundance on the net after the cleaning occurred (repeated measures ANOVA: Table 6.1c).

Following the cleaning process in mid-November, the total abundance of seahorses observed in each of the four treatments differed significantly (Control (A) = Top clean (C) > Total clean (B) = Bottom clean (D) with treatment other being intermittent between A–C and B–D (Table 6.1d; Figure 6.4). Treatment 'Other' includes the 1 m buffer zones between each replicate and other sections of the net not included in the experiment, and are considered to be similar to the control site (Treatment A) as the habitat was not modified during the experiment.

Seahorse movements

Of the seahorses that were initially located in treatment A (control), significantly more were still found in this treatment at the end of the experiment than were found in the other treatments, (Table 6.1e; Figure 6.5a), and similar results were found for the top clean treatment C (Table 6.1e; Figure 6.5c). That is, the majority of animals that began in treatments A or C, stayed in these treatments. Conversely, animals that were initially located in treatments B (total clean) or D (bottom clean dispersed among treatments such that there was no significant difference in their densities among treatments at the end of the experiment (Table 6.1e; Figures 6.5b and 6.5d).

Seahorses that were observed in Treatment C (top clean) were all observed within the bottom 1.2 m of the net where the habitat had not been modified; there were no animals observed in the clean section above 1.2 m. Similar observations were made for treatments B and D, with the majority of seahorses observed hiding amongst the

loose folds at the base of the net. The only treatment where animals were found above 1.2 m from the seafloor was treatment A (control).
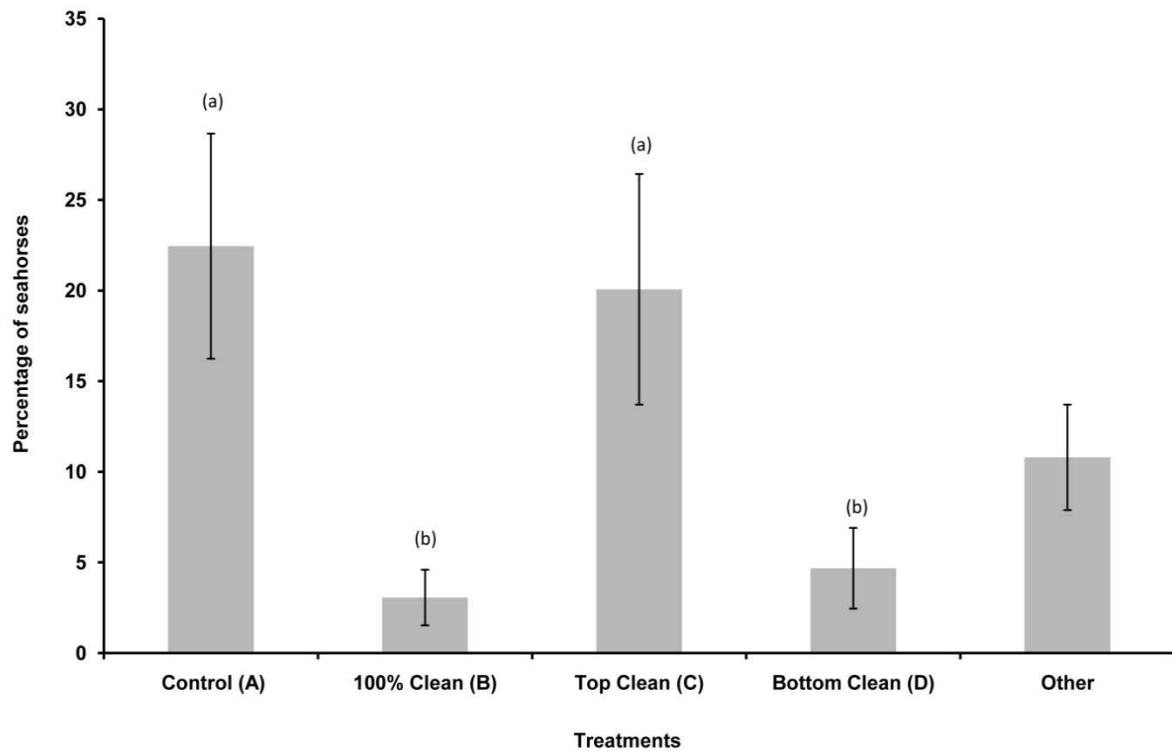
Figure 6.4. Mean percentage (±S.E.) of total abundance of seahorses (average of randomly selected 28 animals per treatment each time) across treatments following net cleaning (average of seven surveys November 07– February 08). Treatment descriptions are Control (A) = 100% epibiotic growth; 100% Clean (B) = all epibiotic growth removed; Top Clean (C) = epibiotic growth removed from surface to 1.2m from seafloor; Bottom Clean (D) = epibiotic growth removed from seafloor to a height of 1.2m from seafloor; Other = Buffer zones and other sections of net not included in experiment (100% epibiotic growth). Treatments not significantly different in post-hoc tests marked with same letter (a or b) above bars.
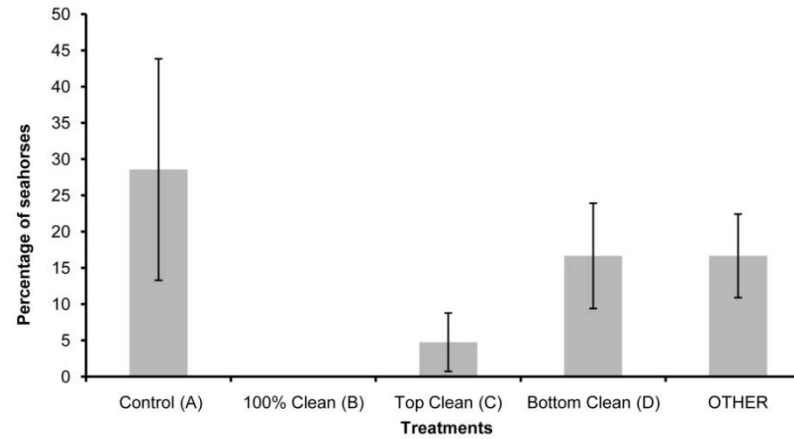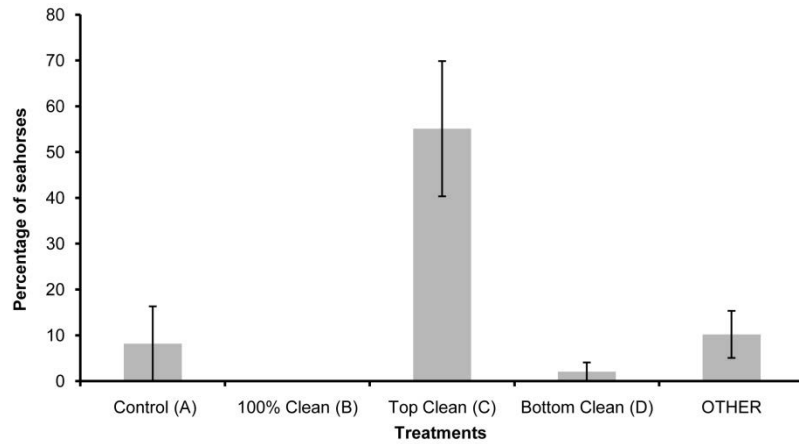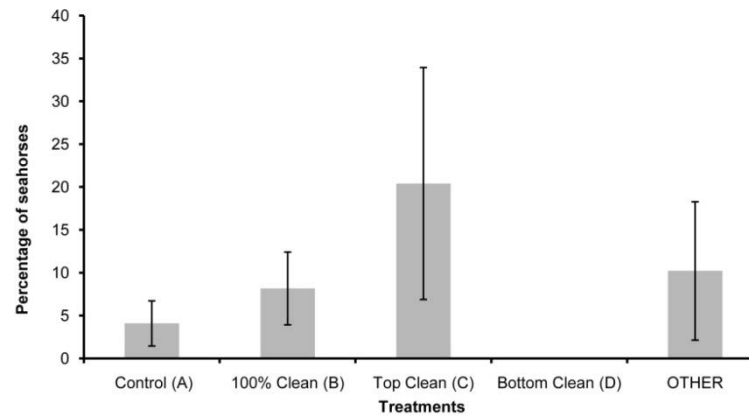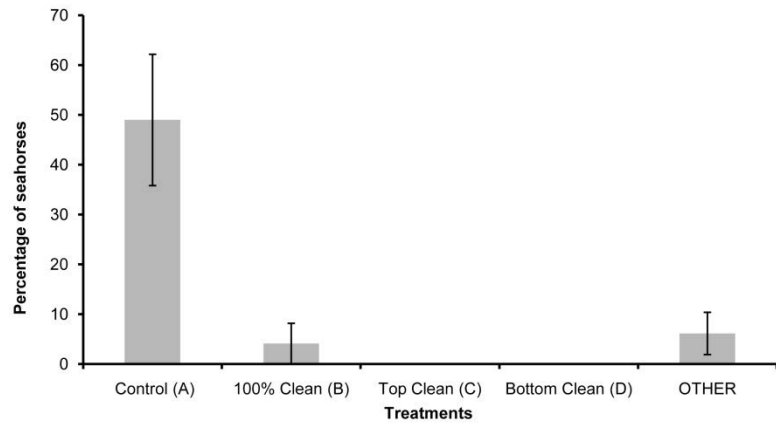
Figures 6.5a–d. Distribution of seahorses among treatments four months after allocation to different net cleaning treatments (initially *n*=28 seahorses per treatment). (a) seahorses initially in Treatment A, (b) seahorses initially in Treatment B, (c) seahorses initially in Treatment C, (d) seahorses initially in Treatment D. Treatments same as those described in Figure 6.4.

The mean distance moved by all seahorses following the manipulative cleaning experiment was 8.3 m ± 0.8 m S.E. The greatest distance moved by an individual seahorse on the net was 60 m while the distance moved by several individuals was less than 1 m over the seven surveys following cleaning. There was a significant difference in the distance moved by seahorses from each treatment (Table 6.1f), which was due to animals initially in Treatment D (bottom clean) moving more than animals from all other treatments (Figure 6.6).

Seahorses in seagrass

Small numbers of seahorses were found within the adjacent seagrass beds (6, 5, 3, 7 respectively over different surveys) including 5 of the tagged animals from the net.
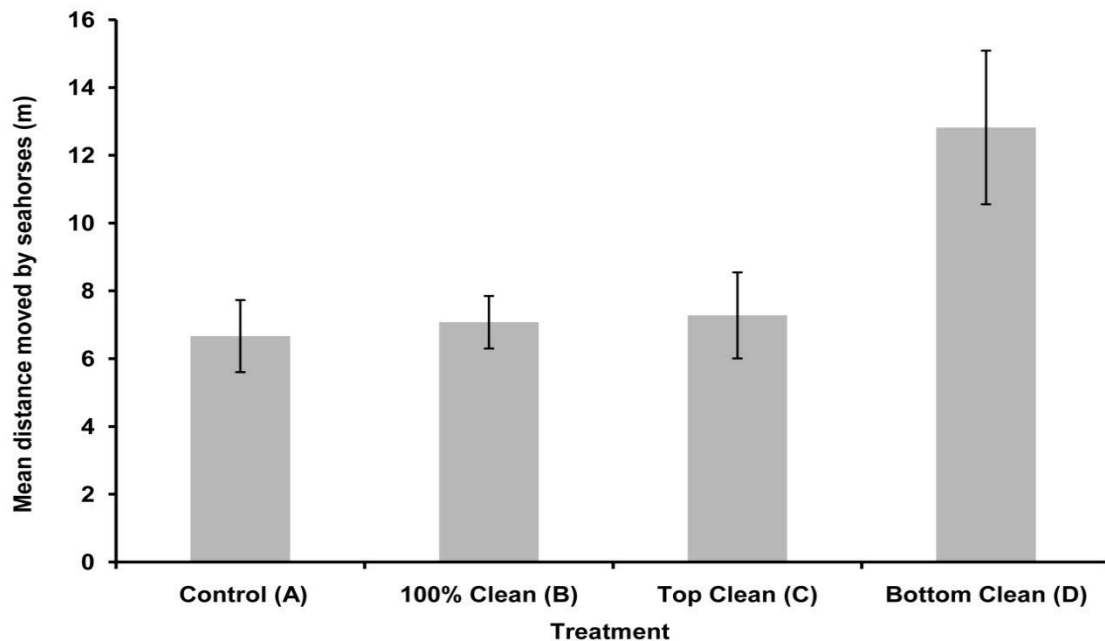
Figure 6.6. Mean (±S.E.) of maximum distance moved by animals from each treatment. Treatments same as those described in Figure 6.4.

6.5   Discussion

This study demonstrated a clear relationship between the abundance of *Hippocampus whitei* and epibiotic growth on a swimming net. The seahorses were found to inhabit complex structure, be it growth on the net or the folds of the net gathered on the seafloor. The seahorses moved away from sections of the net which had growth removed (treatments B and D). Surveys of the seahorse populations on the two nets were conducted on a regular basis and on each occasion it was likely that most animals on the nets were counted, ensuring an accurate estimate of the total population. Tagged animals that were not seen on the nets were possibly utilising the surrounding seagrasses. Some individuals were seen on the net only occasionally during the surveys, indicating that they were possibly moving to and from adjacent natural habitats.

The manipulative net cleaning experiment clearly showed that the abundance of seahorses is directly related to epibiotic growth on the net (most likely because it provides complex physical structure); the removal of epibiota will reduce seahorse abundance on the net. This was indeed observed at Clifton Gardens where the seahorse population on the net declined after the old net was replaced. The long-term monitoring study at Clifton Gardens demonstrated a clear decline in numbers of *H. whitei*, following the replacement of the heavily fouled net. Similar to the findings of the manipulative experiment, seahorse decline was likely caused by the lack of epibiotic growth on the newly deployed net. Recolonisation of the net by

seahorses took a considerable amount of time (15 months) and this is probably linked to the rate of epibiotic growth occurring on the net as suggested by Clynick (2008).

The ARIMA models suggest that both species of seahorse on the Clifton Gardens net declined in response to net replacement but statistical support was not as strong for declines in populations of *H. abdominalis*. There was a large decline in abundance from November 2004 to August 2005 before the net was replaced. Other studies have shown large declines in *H. abdominalis* populations unrelated to direct anthropogenic disturbances (Martin-Smith and Vincent, 2005) It is possible that the changes in populations of *H. abdominalis* were caused by factors other than net replacement, particularly since this species shows little site fidelity and much greater capacity for long distance movements than other seahorses including *H. whitei* (Martin-Smith and Vincent, 2005).

The reason for attraction of seahorses to epibiota on the protective swimming nets is not clear. It has previously been shown that the abundance and diversity of fishes on pilings within Sydney Harbour was strongly associated with epibiotic growth and that when epibiota was removed there was a significant decline in diversity and abundances of some fish species (Coleman and Connell, 2001; Clynick *et al.*, 2007). Clynick (2008) indicated that limited cover of epibiota on nets might result in only small numbers of seahorses being seen; this was confirmed in this study as it was evident that seahorses avoided sections of the net that were devoid of growth. Their

relationship with epibiota may be related to camouflage and/or food as suggested for other small fishes (Coleman and Connell, 2001). Seahorses are slow moving species (Foster and Vincent, 2004) that are susceptible to predation and rely on camouflage to avoid detection (Kuiter, 2000b; Schmid and Senn, 2002). The association between epibiota and food for seahorses is also unknown but it is likely that the growth on the net encourages an increase in small crustaceans, which are the predominant prey for seahorses (Foster and Vincent, 2004).

It is important to recognise that protective net swimming enclosures in Sydney Harbour may either be providing new habitat and effectively increasing the total seahorse population in the harbour, or they may be acting as 'sinks' and effectively taking seahorses away from their natural habitats. The seagrass beds (*Posidonia australis*) adjacent to the Manly net were found to contain *H. whitei*, including tagged animals from the net, similar to earlier studies (Vincent and Giles, 2003; Vincent *et al.*, 2005). Clearly if the natural habitats of seahorses are being removed, then there is potential for artificial structures such as nets to be used to help the recovery of declining seahorse populations. Seahorse habitats are under threat worldwide (Foster and Vincent, 2004); the installation of artificial habitats in areas where traditional habitats have been lost could be used as a potential conservation tool. For example, within Sydney Harbour large areas of *P. australis* have declined in the past 100 years (West *et al.*, 2004). The use of artificial structures by seahorses in Sydney Harbour may be attributed to a decline in their natural habitats and if there

was no possibility of recovery in the natural habitats such as seagrasses, then artificial structures may become a viable management option. This study has indicated that any installed structures should be located close to the seafloor and relatively complex to make them more attractive to seahorses. There may, however, be other ramifications of installing artificial structures in estuaries, such as providing surfaces that are used primarily by introduced rather than native epibiota (Glasby, 2007).

The breeding season for *H. whitei* has been reported as October to April for Watsons Bay in Sydney Harbour (Vincent, 1995). It was found that the population on the Manly net started breeding in late September and continued until the end of February during this one year study, but clearly the exact duration of breeding may vary among years. The abundance of seahorses decreased at the conclusion of the breeding season and it is believed they moved off the net into the surrounding seagrass meadows and deeper waters (Vincent, 1995; Vincent *et al.*, 2004). This differs from the findings of Clynick (2008) who found no seasonal variation in seahorse populations on nets at Balmoral and Clifton Gardens. The latter result may be related to the fact that sampling was only conducted every 2-3 months and that only five sub-sections of each net were sampled instead of surveying the entire population as in the present study. Sampling of sub-sections on a net could produce less precise population estimates if amounts of epibiotic growth vary along the net and sampling is not stratified accordingly. Further investigations of populations of

H. whitei from other regions will help determine the extent to which populations may fluctuate in size, particularly during the breeding season.

The results from the studies at Clifton Gardens and Manly provide useful information for local authorities on how to clean their swimming nets whilst minimising localised impacts on seahorse populations. Given that seahorse abundance and distribution on the net is strongly related to the presence of epibiota, not cleaning nets of epibiota would minimise impacts on seahorses. This may not, however, be viable as the excess weight can cause the net to break from its support. It is therefore recommended that future net cleaning regimes involve removing growth only from the top section of the net, leaving epibiota on the bottom (within 1.2 m of the seafloor). This would ensure that seahorses still have suitable habitat and food whilst reducing the weight of the net. If the bottom sections of a net must be cleaned, this should be done in small sections at a time to ensure that sufficient suitable seashore habitat is always present on the net. It is also recommended that the cleaning of the net be undertaken during the winter period (non-breeding season) when seahorse abundance on the net is reduced and ensures that mating animals are not affected by the cleaning. Finally, to minimise large scale disturbance to the seahorses in one event, it is recommend that only a maximum of half the net be cleaned or replaced each year to ensure there is sufficient epibiotic growth to maintain the seahorse population.

6.6   Acknowledgements

CHAPTER 7

Are populations of the White's seahorse *Hippocampus whitei* genetically distinct across localities in New South Wales?

7.1 Abstract

This aim of this study was to assess if there was any genetic variation in *Hippocampus whitei* across its known range in New South Wales (NSW), Australia. Tissue samples were collected from 85 adult *H. whitei* at seven sites along the species' known geographic range in NSW. Samples were collected whilst scuba diving using stainless steel clippers with approximately 1 mm of dorsal fin collected and stored in NaCl-saturated DMSO solution. A pilot study determined that a 650 bp fragment of cytochrome oxidase I (CO1) was most suitable to determine the degree of sequence variability across locations.

Genetic variability within the COI locus among the sampled *H. whitei* sequences showed no evidence of significant population substructure. The pair-wise distance matrix and visual inspection showed that no samples collected south of Port Stephens showed any variability, with the exception of two specimens from Sydney, which had one variable locus in common. Samples from Port Stephens and north, however, showed more variation with several specimens from Port Stephens showing at least one variable nucleotide, and one individual having two. Specimens from Forster showed more variability with two specimens having 4 and 5 variable nucleotide. These variable sites were unique to the Forster area, and were not seen in any other variable samples. The largest distance observed for samples in the study was 1.07% and was recorded between a sample from Port Stephens and a sample

from Forster. The lowest distance between closely related species within the clade that contains *H. whitei* is 1.99%, observed between *H. subelongatus* and *H. angustus*.

Based on this study using mitochondrial gene methodology, it appears that CO1 is not sufficiently variable to form any conclusions regarding the overall population structure and connectivity of *H. whitei*. However, it does show that populations in the northern most range of the species (Forster and to a lesser degree Port Stephens) appear to have a notably higher genetic diversity from sampled populations at the southern extent of the range and further investigation using microsatellite loci is required to confirm if there is any variation in population structure occurring.

7.2 Introduction

The advent of DNA sequencing technologies has allowed biologists to assess the underlying genetic structure of populations, assess patterns of genetic variation between and within populations and providing taxonomic clarity in phylogenetic relationships amongst taxa (Avise, 1998; Haig, 1998; Avise, 2000). Molecular analysis has shown that species previously known to have wide geographic distribution are often complexes with two or more genetically different species (Shaffer and McKnight, 1996; Mah *et al.*, 2010). Genetic variation in population structure could lead to differences in life history parameters associated with demographically independent populations and consideration of the population structure of individual taxa could inform species' conservation practices (Moritz, 1999; Palsbøll *et al.*, 2007).

In seahorses, direct access to measures of genetic differentiation has led to the identification of cryptic species, the recognition of synonymy for distinctly named species (Teske *et al.*, 2005), and the identification of genetically distinct populations (Saarman *et al.*, 2010). The degree of genetic differentiation among sampled populations of seahorse species can suggest the degree of dispersal, or level of population connectivity, and can reveal unique populations worthy of management consideration. Population connectivity has been previously studied in seahorses using comparative mitochondrial sequence data for several species found throughout the tropical Indo-Pacific (Lourie and Vincent, 2004; Lourie *et al.*, 2005;

Lourie, 2006). These studies revealed a high degree of population differentiation, most likely associated with sea level fluctuations in the Sunda Shelf area leading to repeated cycles of isolation and recolonisation of Sunda shelf shallow water habitats. Mitochondrial and nuclear DNA sequences have also been used to understand spatial and temporal patterns of evolutionary divergence among closely related seahorse species (Teske *et al.*, 2005). These studies revealed that a great deal of information about genetic relatedness among populations can be extracted from comparative sequence analysis sampled from specimens across a species geographic range.

The focus of this study is the White's seahorse *Hippocampus whitei*, a seahorse that is considered to be endemic to the waters of New South Wales (NSW), Australia (Harasti *et al.*, 2012). The geographic range of *H. whitei* is limited to a 300 km stretch of NSW coastline from Forster in the north to Lake Illawarra (Wollongong) in the south (Figure 7.1). There have been several previous studies on *H. whitei* that have primarily focused on their reproductive behaviour and pair bonding (Vincent, 1995; Vincent and Giles, 2003; Vincent *et al.*, 2004), home range behaviour (Vincent *et al.*, 2005), and their use of artificial structures (Clynick, 2008; Harasti *et al.*, 2010). More recently, studies have investigated life history parameters for *H. whitei* and found that is displays rapid growth, matures at approximately 3-4 months and is able to commencing reproduction at approximately 7 months (Harasti *et al.*, 2012). It grows to a maximum length of approximately 162 mm (Harasti *et al.*, 2012) and it has been

found to display strong site fidelity that includes inhabiting the same holdfast for over 12 months (see chapter 4).

Hippocampus whitei is a protected species in NSW under the *Fisheries Management Act 1999* meaning that it cannot be taken from the wild and it is also listed on the IUCN Red List as 'data deficient' (IUCN, 2013). The basis for the IUCN listing is that there is no published data about populations trends or total number of mature animals for this species, there is little information about its distribution or area of occupancy and no quantitative analyses examining the probability of extinction for this species has been undertaken (IUCN, 2013). In their review of threatened marine species in Australia, Pogonoski *et al.* (2002) determined that the conservation status of *H. whitei* is 'data deficient' and indicated that further research on the distribution and abundance of this species is necessary.

This species is known to occur in shallow water estuaries where it can be found inhabiting seagrasses, sponge gardens and artificial structures such as jetties and protective swimming nets (Harasti *et al.*, 2010). As these estuaries are located adjacent to heavily populated areas (DECCW, 2009), each of these estuaries is subject to various anthropogenic impacts (such as dredging, runoff) and seahorse habitats (i.e. sponge, softcorals and seagrass) are under threat in several of these locations. Large populations of *H. whitei* have been found occurring within Port Stephens and Sydney Harbour (Harasti *et al.*, 2012), however no research has been conducted within the other estuaries across its range and its abundance in these other locations

is unknown. There is some concern that some of these populations may be locally threatened, from pollution impacts and habitat loss, and as the species is considered to have no pelagic stage (Kuiter, 2009), there is the possibility that they may be genetically distinct from other *H. whitei* populations. Some populations of *H. whitei* may need to be treated as evolutionarily significant units in that they are considered distinct for purposes of conservation (Moritz, 1994).

This project aims to use mitochondrial gene methodology to determine if populations of *H. whitei* along the NSW coast represent a single genetically connected population, or if geographically separate populations are genetically distinct and should be managed as separate evolutionarily significant units.

7.3 Materials and Methods

Tissue samples from 85 *Hippocampus whitei* were collected from various sites along the species' known geographic range in NSW (Figure 7.1 and Table 7.1). The average distance between sampling locations was 40.5 km ± 12.1 S.E. Tissues were obtained while SCUBA diving, using stainless steel clippers or scissors to remove a small amount (approximately 1 mm) of dorsal fin. Tissue samples were preserved in a NaCl-saturated DMSO solution and shipped to the California Academy of Sciences, in San Francisco, USA for molecular analysis. As a pilot for the study, whole genomic DNA was extracted from 32 of the preserved tissues, comprising a

preliminary representation of individuals from each of the seven sampled geographic locations (Table 7.1). Extractions were completed using Qiagen DNeasy Blood & Tissue Kits and the included extraction protocol.

Table 7.1. Locations of tissue sample collection for *Hippocampus whitei* in New South Wales.

Location	GPS Coordinates
Wallis Lake – Forster	$32^0 11'10.11''$S – $152^0 30'37.70''$E
Nelson Bay – Port Stephens	$32^0 43'04.63''$S – $152^0 08'29.27''$E
Tuggerah Lake - Wyong	$33^0 20'34.86''$S – $151^0 28'03.57''$E
Empire Bay – Brisbane Water	$33^0 29'32.56''$S – $151^0 21'55.39''$E
Manly – Port Jackson	$33^0 47'57.63''$S – $151^0 16'57.67''$E
Clifton Gardens – Port Jackson	$33^0 50'20.74''$S – $151^0 15'11.82''$E
Gunamatta Bay – Port Hacking	$34^0 03'30.22''$S – $151^0 08'53.10''$E
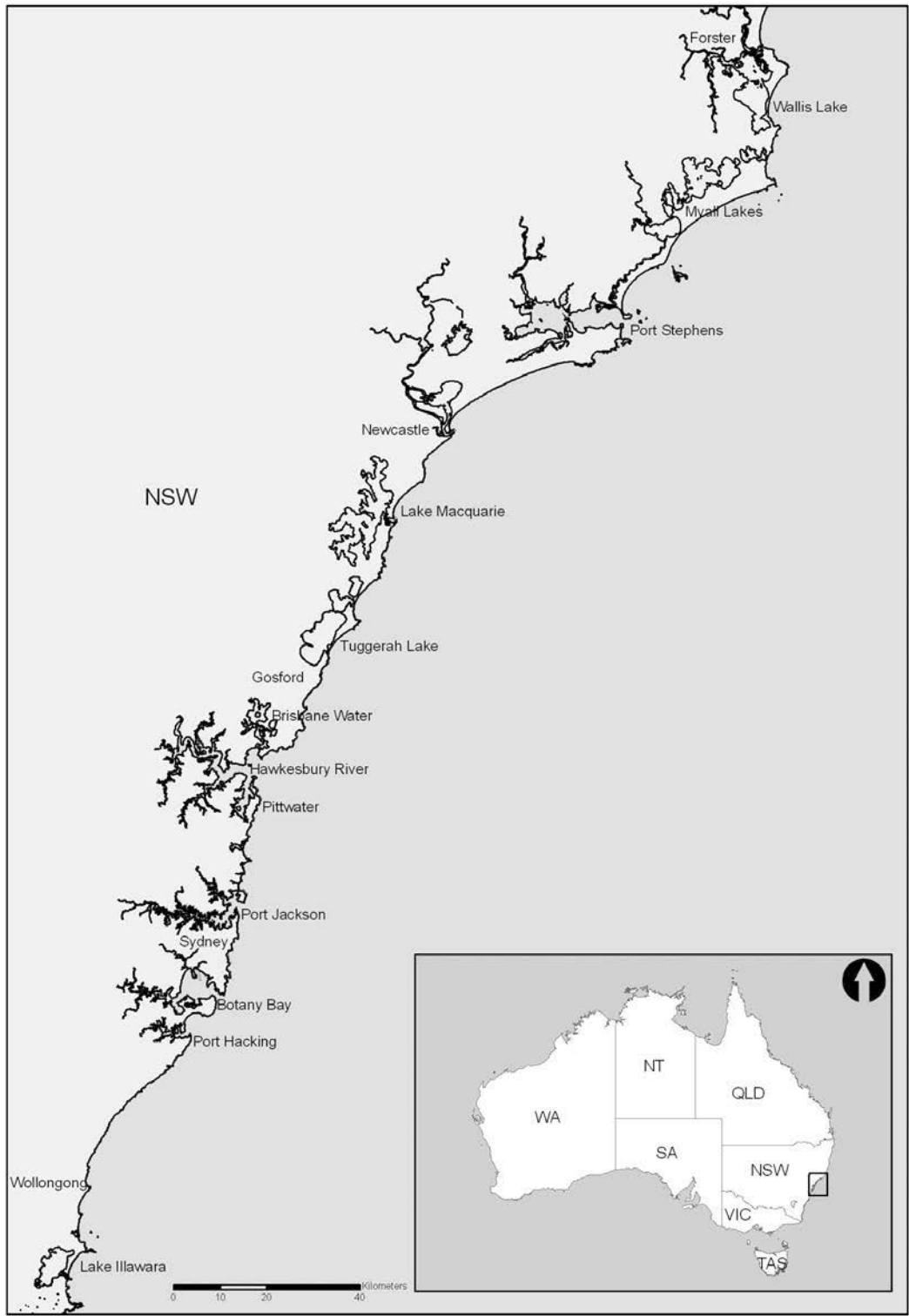
Figure 7.1. Geographic distribution of *Hippocampus whitei* in New South Wales and sampling locations.

To determine which genetic marker would provide the greatest amount of information about *H. whitei* population connectivity, a preliminary survey of genetic variability was conducted across three previously studied loci. An extensive amount of mitochondrial cytochrome b data has been generated and used for phylogenetic and phylogeographic studies of seahorses (Lourie *et al.*, 1999a; Lourie and Vincent, 2004; Lourie *et al.*, 2005; Teske *et al.*, 2007b; Saarman *et al.*, 2010). Based on these methods, a 700 bp fragment of cytochrome b (cyt b), as well as two other mitochondrial loci that have been useful for seahorse research, a 650 bp fragment of cytochrome oxidase I (CO1) (Barcode of Life effort, and experience with unpublished data) and a 610 bp fragment of the hypervariable region, or D-loop (Saarman *et al.*, 2010), were tested on 5 samples to determine the degree of sequence variability at each of these three loci.

Polymerase chain reactions (PCR) were used to amplify fragments of all loci. All amplifications were performed in 25 µL reaction volumes containing 2 µl of whole genomic template DNA, 15.75 µl of ultra-pure water, 2.5 µl of 10X *Taq* reaction buffer (Invitrogen, included with Invitrogen *taq*), 0.5µl of each dNTP (10mM) (Promega), 2.5 µl of 50 mM MgCL (Invitrogen, included with Invitrogen *taq*), 0.75 µl of each primer (10 µM stock), and 0.25 µl of Invitrogen *taq* polymerase (5 units/µl) (Invitrogen). All reactions were run on BioRad MyCycler thermal cyclers. For cyt b, the following primers were used: forward ShF2 5'- TTGCAACCGCATTTTCTTCAG -3' and reverse ShR2 5'- CGGAAGGTGAGTCCTCGTTG -3' (Lourie and Vincent,

2004), under the following conditions: 94 °C for 3 min; 35 cycles of 94°C for 30 s, 52°C for 30 s, and 72°C for 1 min; and then a final extension step of 72°C for 7 min. For CO1, the following primers were used: forward FishF2 5′-TCGACTAATCATAAAGATATCGGCAC -3′ and reverse FishR1 5′-TAGACTTCTGGGTGCCCAAAAGAATCA -3′ (Ward *et al.*, 2005), under the following conditions: 94°C for 3 min; 35 cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 1 min; and then a final extension step of 72°C for 7 min. For D-loop, the following primers were used: forward HCBF1009 5′-TGAATTGGGGGAATACCTGT -3′ and reverse HCRLrv 5′-AAGCGTCGATGAAAGTGTG -3′ (Saarman *et al.*, 2010) cycled under the same conditions that were used for CO1.

PCR products were visualized on a standard 1% agarose gel with ethidium bromide, and following successful amplification, were cleaned using 2 µl of ExoSap-IT enzyme (USB) in 5 µl of PCR product under the following conditions: 37°C for 15 min followed by 80°C for 15 min. ExoSap-IT-cleaned products were then used directly in cycle sequencing. Cycle sequencing was performed in both directions with BigDye Terminator™ (vers. 3.0, Applied Biosystems Inc.) in 10 µl reaction volumes using the following reaction mix: 5.13 µl of H2O, 1.63 µl of BigDye Terminator™ buffer (5X), 0.75 µl of BigDye Terminator™, 0.5 µl primer (10 uM), and 1.0 µl PCR product. Reactions were cycled using the fast cycle sequencing conditions outlined in (Platt *et al.*, 2007), using a 55°C annealing temperature. Cycle sequencing products were precipitated using a standard ethanol precipitation method

(Sambrook and Russell, 2001). Precipitated cycle-sequencing products were re-suspended in 10 μl of ABI Hi-Di formamide, denatured at 95°C for two minutes followed by five minutes on ice, and then sequenced on an Applied Biosystems ABI Prism 3130xl automated genetic analyser.

Complementary sequences for the first 5 samples were edited and assembled into contigs (the sequence that results from aligning overlapping fragments that run in opposite directions) using Sequencher 4.7 software (GeneCodes Corp). Consensus sequences from confirmed contigs were used in nucleotide alignments for each locus. Multiple sequence alignments were performed using Clustal X 2.02 (Larkin *et al.*, 2007) software and manually edited in MacClade 4.08 (Maddison and Maddison, 1989). At this point the three different gene sequences can be compared for their degree of variability. Visual inspection of the alignments showed that D-loop sequences were completely invariable across the 5 samples, cyt b sequences had only one sample with a single nucleotide difference, and CO1 had two samples with a single nucleotide difference. Based on this result, and also previous experience using other seahorse data (B. Moore *pers obs* and *unpublished data*), it was determined that cytochrome oxidase 1 (COI) was more variable than the other two loci, and was chosen for use in the rest of the study. The remaining 27 extracted samples were sequenced for COI and aligned using the protocol above.

Once all sequences were aligned, a pair-wise distance matrix using uncorrected p-distances was produced using PAUP 4.0 (Swofford, 1989). The alignment was also

visually inspected for sites that differed from the rest of the aligned sequences. In addition, published seahorse CO1 sequences from *H. comes, H. subelongatus, H. barbouri, H. angustus,* and *H. histrix*, species which were found to be in the same seahorse evolutionary lineage as *H. whitei* (Teske *et al.*, 2004), were downloaded from GenBank (unpublished barcodes from the Redpath Museum) and aligned using the alignment method described above. This was done to compare distances seen between other closely related seahorse species at this locus to the distances seen within the *H. whitei* sampled for this pilot study.

7.4 Results

Genetic variability within the COI locus among the sampled *H. whitei* sequences showed no evidence of significant population substructure. In spite of the general homogeneity of the sampled sequences, the pair-wise distance matrix and visual inspection of alignments showed some interesting results (Table 7.2). With the exception of two specimens from Sydney, which had one variable locus in common, no samples collected south of Port Stephens (Nelson Bay) showed any variability. Samples from Port Stephens and north, however, showed more variation. Four seahorses from Port Stephens showed at least one variable nucleotide, and one in particular (1295) had two. Two of these Port Stephens' samples (1305 and 1353) had a variable site in common, but the other two samples had unique nucleotide substitutions in their sequences. Samples 1365 and 1364 from Forster, had 4 and 5

variable sites respectively. All variable sites for these samples were shared between these two specimens, except for one additional variable site found in sample 1364. These variable sites were unique to the Forster area, and were not seen in any other variable samples. All other sequences that have not been directly discussed were identical.

The largest distance observed for samples in the pilot study was 1.07%, and was measured between sample 1295 from Port Stephens, and sample 1364, from Forster (Table 7.3). The lowest distance between closely related species within the clade that contains *H. whitei* was 1.99%, observed between *H. subelongatus* and *H. angustus* (Table 7.3).

Table 7.2. Specimen information and collection localities for *H. whitei* specimens used in the pilot study. "X" is used to mark sequences that were used for the pilot study, while "X*" marks sequences that were only used initially to determine the variability at each locus. Variable sites are listed in numbers of nucleotide substitutions for CO1 sequences. Alignment locations are listed as the base numbers where substitutions were found in the CO1 alignment.

CAS #	Collection Location	cyt B	D-loop	CO1	Variable Sites	alignment location
1363	Forster			X	0	0
1364	Forster			X	5	174, 342, 378, 489, 513
1365	Forster			X	4	174, 342, 378, 489
1366	Forster			X	0	0
1367	Forster			X	0	0
1368	Forster			X	0	0
1287	Nelson Bay			X	0	0
1295	Nelson Bay			X	2	259, 495
1299a	Nelson Bay			X	0	0
1300a	Nelson Bay			X	1	81
1305	Nelson Bay			X	1	412
1352	Nelson Bay			X	0	0
1353	Nelson Bay			X	1	412
1354	Nelson Bay			X	0	0
1357	Nelson Bay			X	0	0
1359	Nelson Bay			X	0	0
1290	Tuggerah Lakes			X	0	0
1291	Tuggerah Lakes			X	0	0
1292	Tuggerah Lakes			X	0	0

1276	Empire Bay			X	0	0
1277	Empire Bay			X	0	0
418	Sydney	X*	X*	X	1	429
419	Sydney	X*	X*	X	0	0
469	Sydney	X*	X*	X	0	0
470	Sydney	X*	X*	X	1	429
667	Sydney	X*	X*	X	0	0
1321	Port Hacking			X	0	0
1322	Port Hacking			X	0	0
1329	Port Hacking			X	0	0
1330	Port Hacking			X	0	0
1340	Port Hacking			X	0	0
1341	Port Hacking			X	0	0

Table 7.3. CO1 pair-wise distance matrices, with distances (uncorrected *p*) listed as percentages. A) Distances between only the variable samples of *H. whitei* found in the pilot study. The highest observed distance is highlighted in yellow. B) Distances of closely related seahorse species, using CO1 sequences obtained from GenBank. The lowest distance between two species is shown in bold with *.

A							
	1364	**1365**	**1295**	**1300a**	**1305**	**1353**	**418**
1364; Forster							
1365; Forster	0.15%						
1295; Nelson Bay	**1.07% ***	0.92%					
1300a; Nelson Bay	0.92%	0.76%	0.46%				
1305; Nelson Bay	0.92%	0.76%	0.46%	0.31%			
1353; Nelson Bay	0.92%	0.76%	0.46%	0.31%	0.00%		
418; Sydney	0.92%	0.76%	0.46%	0.31%	0.31%	0.31%	
470; Sydney	0.92%	0.76%	0.46%	0.31%	0.31%	0.31%	0.00%

B							
	H. angustus	*H. barbouri*	*H. comes*	*H. histrix*			
H. angustus; GQ502122							
H. barbouri; GQ502124	7.36%						
H. comes; GQ502135	3.37%	7.06%					
H. histrix; GQ502147	8.28%	9.36%	8.28%				
H. subelongatus; GQ502170	**1.99% ***	6.90%	2.91%	8.28%			

7.5   Discussion

Based on the results of this study, it would appear that CO1 is not sufficiently variable to form any conclusions regarding the overall population structure and connectivity of *H. whitei*. Instead, this preliminary analysis shows low variation across all three mitochondrial loci examined, suggesting either: 1) *H. whitei* is a relatively young evolutionary lineage that has not yet had sufficient divergence time to accumulate sequence differentiation at these relatively rapidly evolving loci, or 2) dispersal is generally sufficient among *H. whitei* populations to prevent the accumulation of sequence differences within the sampled populations. The only way to distinguish between these two hypotheses is 1) a genetic comparison between *H. procerus* and *H. whitei*, as well as other members of the *H. whitei* clade, to assess divergence times among lineages, and 2) assessing genetic diversity among *H. whitei* populations with a more rapidly evolving genetic marker, such as microsatellites, which are more time consuming, costly and somewhat more complex to sequence and analyse than the mitochondrial markers chosen for this study.

Specimens living within the northern most range of the species (Forster and to a lesser degree in Port Stephens) appear to have a notably higher genetic diversity from sampled populations at the southern extent of the range. Although only two specimens from Forster showed evidence of this variability, the degree to which they differ from other individuals is quite striking. The highest distance (1.07%) observed in the study distance matrix is between a specimen from Forster and a specimen

from Port Stephens (Table 7.3). This particular Forster sample (1364) is the most variable sequence in the pilot study, and the Port Stephens sample (1295) is the only other sample with more than one unique variable site (Table 7.2). Although this distance is still too low to clearly suggest that the two samples might be different species (the lowest distance between species of other closely related seahorses is 1.99%), it is an indication of a distinct genetic history. Also from Port Stephens, are three additional variable samples, two of which share the same nucleotide substitution. This, in conjunction with the two variable Forster samples, represents a great deal of variability in a very limited range, especially considering the conserved nature of this gene across the rest of the species range.

The single substitution seen at the same site in two of the Sydney samples (418 and 470) is quite intriguing and may possibly have resulted from *H. whitei* being transported from their northern range into the southern waters of Sydney Harbour and Port Hacking under the influence of the East Australian Current (EAC) (Roughan *et al.*, 2011). The shared substitution between these two samples is unique among all the other variable sites found in sequences from other locations. However, with sample sizes this low, it is also possible to obtain these results by chance. Dispersal on the EAC into southern waters is unlikely given that that *H. whitei* is considered to have no pelagic stage (Kuiter, 2009) and its unlikely that adults migrate large distance along the NSW coast as they display strong site fidelity and limited movements (Harasti *et al.*, 2012). It is possible that over time these estuarine

populations may become more genetically separated as there is no evidence to suggest that any individuals may be moving between estuaries and reproducing with other populations. Further sampling at the northern (Forster and Port Stephens) and southern end (Lake Illawarra and Port Hacking) of the *H. whitei* range is required to support or refute this theory.

In addition, a critical next step is to clarify the genetic relatedness of *H. whitei* and its putative sister taxon, *H. procerus* which is known from the waters of south east Queensland and Solomon Islands (Lourie *et al.*, 1999b). Analysing specimens of *H. procerus*, the sister to *H. whitei* (Teske *et al.*, 2004), would support a better understanding of evolutionary boundaries between these two species. It would also support the investigation of possible hybrid zones between the two species and could provide perspective on the observed genetic variation in the northern most sampled populations of *H. whitei*.

The large amount of variation seen in CO1 sequences from *H. whitei* in the northern part of the species range suggests that there is some population structure occurring between northern localities, and possibly around southern areas like Sydney. Unfortunately, this locus is not variable enough to allow for the in-depth study that would be required to elucidate this kind of information. A study using microsatellite loci would yield the best results, and also would be feasible considering the amount of work that has already been done with microsatellites for other seahorse species (Jones *et al.*, 1998; Galbusera *et al.*, 2007; Pardo *et al.*, 2007; Wilson and Martin-Smith,

2007). However, microsatellite analysis is more costly than using the mitochondrial gene methodology employed in this study but would help address the question on whether there is any population structure occurring in *H. whitei* across its range or if they represent a single genetically connected population.

7.6   Acknowledgements

CHAPTER 8

Does underwater flash photography affect the behaviour, movement and site

persistence of seahorses?[5]

[5] Harasti, D and Gladstone, W (2013). Does underwater flash photography affect the behaviour, movement and site persistence of seahorses? *Journal of Fish Biology* **83**, 1344-1353.

8.1 Abstract

This study assessed the impact of flash photography on the White's seahorse *Hippocampus whitei*, a medium-sized seahorse species endemic to Australia, as the effect of flash photography on a seahorse species has never been tested. An experiment was established to test the effect of flash photography and/or the handling of *H. whitei* on their behavioural responses, movements and site persistence. A total of 24 *H. whitei* were utilised in the experiment with eight in each of three treatments (flash photography, handling and control). The effect of underwater flash photography on *H. whitei* movements was not significant; however the effect of handling *H. whitei* to take a photo had a significant effect on their short-term behavioural responses to the photographer. Kaplan-Meier log rank test found there was no significant difference in site persistence of *H. whitei* from each of the three treatments and that flash photography had no long term effects on their site persistence. The findings from this study conclude that the use of flash photography by divers is a safe and viable technique with *H. whitei*, particularly if photos can be used for individual identification purposes.

8.2   Introduction

Long-lasting individual variation in the body markings of fishes has been used as the basis for many non-invasive methods for identification of individuals (Barker and Williamson, 2010; Anderson *et al.*, 2011). With the increasing sophistication of underwater digital photographic techniques, their use to record the natural markings of animals for individual identification has become a popular alternative to traditional tagging methods. Photographs recording inter-individual variation in body markings and features have been employed for studies of movements and population abundance of whale sharks *Rhincodon typus* Smith 1828 (Holmberg *et al.*, 2009), grey nurse sharks *Carcharias taurus* Rafinesque 1810 (Bansemer and Bennett, 2008), manta ray *Manta alfredi* (Krefft 1868) (Marshall and Bennett, 2010) and great white sharks *Carcharodon carcharias* (Linnaeus 1758) (Domeier and Nasby-Lucas, 2007). Recently, Martin-Smith (2011) used photography to focus on the unique patterns of spots and blotches in the Weedy Seadragon *Phyllopteryx taeniolatus* (Lacepède 1804) to develop estimates of abundance through capture-mark-recapture methods.

Seahorses (*Hippocampus* spp.) are considered an iconic group of fishes and are highly sought after by underwater photographers (Uyarra and Cote, 2007), and there is potential for photographers to assist ichthyologists through 'citizen science' type programs (Goffredo *et al.*, 2004). For example, Kuiter (2009) provides a guide to syngnathids that incorporates images taken by photographers from around the

world, which assist in understanding species' distributions, behaviours and habitat utilisation. In the only known study that used photography to identify individual seahorses, Baine *et al.* (2008) monitored the movements of the pygmy seahorse *Hippocampus bargibanti* Whitley 1970 for up to 40 weeks.

Many seahorse research projects utilise a method of tagging, such as neck collar tags (Curtis, 2006) or visible implant fluorescent elastomer (Harasti *et al.*, 2010), with no apparent impacts on behaviour, growth (Woods and Martin-Smith, 2004) or longevity (Harasti *et al.*, 2012). However, the tagging of seahorses can be time-consuming and labour-intensive. There is potential for photography of individual seahorses to be used as an alternate method to physical tagging if the species shows individual variation in a distinguishable pattern of marks (such as spots or blotches) that is recognisable for at least the duration of the study. Whilst photos of seahorses can be taken without the use of an underwater flash, the attenuation of colour with increasing depth in the marine environment, particularly red (Michiels *et al.*, 2008), can make it difficult to determine individual marks on seahorses. As seahorses are cryptic, a flash is required to ensure that the seahorse in the image is correctly exposed with all natural markings visible, however, there has been no assessment of the effects of scuba divers using flash photography on seahorses and it is not known if this sampling method could disrupt their behaviour, physiology or longevity and therefore compromise a study's objectives.

Research that has assessed the environmental impacts of scuba diving has examined habitat damage (Rouphael and Inglis, 2001; Barker and Roberts, 2004), changes in fish behaviour (Dearden *et al.*, 2010; Dickens *et al.*, 2011), but, as far as known, the potential effects of flash photography on a marine fish species have not been investigated. Seahorses have been found to have advanced eye structure (Mosk *et al.*, 2007; Lee and Bumsted O'Brien, 2011); however, the effect of bright flashes on seahorse eyesight is unknown. Seahorses require good eyesight to feed on their tiny fast moving prey of amphipods and copepods (Kuiter, 2009) and any harm caused to the eyes could result in the inability to catch prey and/or reduce their longevity.

The majority of photos taken of seahorses are for pleasure and seldom used for research/conservation purposes; however, the impact of divers taking photos of seahorses has not been investigated. In May 2011, the use of flash photography was banned on seahorses within the United Kingdom by the Marine Management Organisation that issues licenses that allow diving with seahorses (MMO, 2011). Seahorses are protected in the United Kingdom under Schedule 5 of the *Wildlife and Countryside Act 1981* and divers require a licence if they dive with the intention of carrying out an activity that is likely to disturb seahorses (such as photography, filming or surveys). The MMO website states '*We are no longer issuing licences that permit flash photography on seahorses due to the potential impact of flash photography. This follows advice from our statutory advisors and is on a precautionary basis while we develop our evidence base on potential impacts.*' The basis for banning flash photography on

potential impacts is intriguing as there is no published research that demonstrates that flash photography has an impact on any marine fish.

The aim of this study was to assess the impact of using underwater flash photography on the behaviour, movements and site persistence of seahorses and determine if photography could be utilised in the future as a non-harmful and minimally invasive method for data collection. The species selected for use in this study was the White's seahorse *Hippocampus whitei* Bleeker 1855, that is common in various estuaries along the central coast of New South Wales (NSW), Australia, and endemic to this region (Harasti *et al.*, 2012).

8.3   Materials and Methods

Species description

Hippocampus whitei occurs in estuaries, coastal lakes and coastal embayments along the NSW central-mid north coast from Forster to Wollongong, a linear distance of approximately 300 km (Harasti *et al.*, 2012). This species is a medium-sized seahorse [max total length (L_T) 162 mm: L_T is taken as straight line measurement from tip of coronet to bottom of tail] that grows rapidly and lives for at least 5 years in the wild (Harasti *et al.*, 2012). Over the breeding season (September-February) they are pair-bonded and monogamous (Vincent *et al.*, 2005; Harasti *et al.*, 2012) and have small

home range (~9-14 m^2) with females having larger home ranges than males (Vincent *et al.*, 2005).

Study Site

This study was conducted in the Port Stephens estuary within the Port Stephens-Great Lakes Marine Park at a dive site known as the 'Pipeline' (32^{0}43'04.63"S – 152^{0}08'29.27"E). The study site contained a mixture of seagrass, sponge, kelp and soft coral habitats and *H. whitei* was regularly observed prior to the study. This study site and species were chosen because *H. whitei* is abundant at this site (Harasti *et al.*, 2012), it has easy access for diver entry and is a shallow depth (less than 10 m) that allowed for a sufficient bottom time (approximately 90 min) to complete each survey.

Hippocampus whitei in the study site still retained individual markings created in a previous study (Harasti *et al.*, 2012) using small visible implant fluorescent elastomer tags (VIFE – Northwest Marine Technologies, USA; www.nmt.us) (Woods and Martin-Smith, 2004). Each *H. whitei* was tagged with three 1-2 mm elastomer tags inserted in unique locations on the body to allow for individual identification on subsequent resighting. These elastomer tags are considered to be permanent as they still occur in *H. whitei* more than 4 years after initial tagging (Harasti *et al.*, 2012).

Experimental design

Twenty-four adult *H. whitei* (12 male mean L_T 114.8 cm ± 2.3 cm S.E, min 100 cm, max 125 cm and 12 female mean L_T 115.8 cm ± 2.5 cm S.E, min 105 cm, max 140 cm) were randomly selected from the pool of tagged seahorses that had been observed regularly on the site over the previous 3 months (and represented approximately 12% of the *H. whitei* present at the site). The chosen *H. whitei* were found spread out in an area of approximately 900 m² that consisted predominantly of sponge habitat and as the study commenced towards the end of the breeding season for *H. whitei*, several of the males (*n*=7) were observed to be pregnant. The position of each *H. whitei* was marked with a small piece of flagging tape tied to a large stainless steel washer that was pushed into the sand adjacent to the holdfast (defined as the habitat feature to which the *H. whitei* was attached by its prehensile tail).

Hippocampus whitei were randomly allocated to three experimental treatments, with eight *H. whitei* (four males and four females per treatment). The treatments were: (1) flash photography, (2) handling and flash photography, and (3) control (photography without flash, no handling). These treatments were established to test three separate hypotheses: (1) the reaction of *H. whitei* would not differ amongst the three treatments; (2) the distance moved by *H. whitei* would not differ amongst the three treatments; and (3) the site persistence of *H. whitei* would not differ amongst the three treatments.

The no flash photography treatment was considered to be equivalent to no photography, because it involved the same behaviour by a diver getting close enough to the *H. whitei* to see individual markings. It was not necessary to have a treatment that involved the *H. whitei* being handled with no flash photography because previous studies on *H. whitei* had found no effect of handling (Harasti *et al.*, 2010; 2012).

For Treatment 1, the camera used to photograph *H. whitei* was a Nikon D300 using a Nikon 60 mm lens in an Ikelite underwater housing (www.ikelite.com) with a single Ikelite DS125 strobe to provide the flash. The strobe was set to half-power as this is considered to be the general setting an underwater photographer would use when photographing *H. whitei* at close range. The strobe at this setting fired with an average flash intensity of 55 watt-sec for a duration of 1.5 ms (J Brigham - Ikelite *pers. comm*). The distance from the camera to the individual was approximately 30-40 cm. Each *H. whitei* in treatment 1 was subject to six photos being taken within a period of 1 min. Whenever possible, three photos of each side of the individual were taken; however, it was not always possible if the *H. whitei* did not turn around.

In Treatment 2, the handling of *H. whitei* involved the photographer using a gloved hand to gently coax the *H. whitei* into a better position to photograph. The *H. whitei* were never grabbed or held in any way. Once the *H. whitei* was in a good position for photography, it was then photographed using the same method as described for Treatment 1. If the *H. whitei* swam away after being handled, it was not pursued and

recorded as 'swam away'. In Treatment 3, the *H. whitei* were not touched and they were photographed in their observed position using the same methods as the other treatments with the exception that the flash was turned off. The photography in Treatments 2 and 3 took approximately the same time as Treatment 1.

Weekly experiments were carried out in January-March 2009 (total experiments=10) with each seahorse subject to its allocated treatment over 10 weeks. The senior author was the photographer for each of the surveys carried out and to ensure there was no impact or effect from the presence of other divers, surveys were only conducted when there were no other divers present in the study area. There is the possibility that during the study other divers may have photographed the *H. whitei* in each treatment, however this was minimised by putting requests through local dive shops for divers to try to avoid photographing *H. whitei* in the study site and near flagging tape and there were no observations made of other divers photographing or interfering with the *H. whitei* from each treatment.

Response

The reaction of a *H. whitei* to a treatment was recorded in four categories: 1. No reaction – there was no apparent change in the behaviour or position of the *H. whitei*, 2. Actively hid – the *H. whitei* moved to a different position on the holdfast that obscured it from the photographer, 3. Changed holdfast – the *H. whitei* detached itself from the holdfast and swam to another holdfast < 50 cm away, and 4. Swam away – the *H. whitei* detached itself from the holdfast and swam at > 50 cm away. A

response was recorded as soon as the *H. whitei* reacted to the treatment and if no reaction was observed with 1 min after the six photos, then 'no reaction' was recorded. When a *H. whitei* changed its holdfast, the flagging tape was moved to the new position.

Movement

When a *H. whitei* was first found during the weekly experiments, its location was noted and the distance between that location and its previous survey location (identified by holdfast being marked with the *H. whitei* tag ID on flagging tape) was estimated (to the nearest 10 cm). Distance moved was estimated prior to any photography or handling. If the *H. whitei* was using the same holdfast as the previous week, the distance moved was recorded as 0 cm. At the completion of photography, the seahorse's new holdfast was marked with flagging tape so that at the following survey the distance moved by the *H. whitei* would be from its last photographed location. Nine surveys assessing movement were carried out; survey one was not included as there were no data to estimate movement.

Site Persistence

The site persistence of *H. whitei* in the three treatments in the 9 months following the experiment was assessed by undertaking 10 additional dive surveys (each 60 minutes duration) monthly from March until December 2009. Individuals were recorded as either absent or present.

Data analyses

The hypothesis that the behavioural response of *H. whitei* would not differ amongst the three treatments was tested using chi-square 4 x 3 contingency table analysis that compared the frequency distribution of each response across all treatments. Cramer's V was calculated to measure strength of association among the levels of the row and column variables.

The hypothesis that distance moved by *H. whitei* would not differ amongst treatments was tested using a repeated-measures, mixed design, two-way ANOVA using the software package SPSS 19 (www.ibm.com/spss). Treatment and time were defined as fixed orthogonal factors, and Mauchly's Test of Sphericity was used to assess if the sphericity assumption had been violated. The hypothesis that the site persistence of *H. whitei* would not differ amongst the three treatments was tested by using a Kaplan-Meier Log Rank test (Kaplan and Meier, 1958; Pollock *et al.*, 1989) in SPSS 19.

8.4   Results

Response

The frequency of responses by *H. whitei* to the three treatments differed significantly (χ^2 = 10.47, df = 6, P < 0.001; Figure 8.1) and there was little association between the categorical variables in the contingency table (Cramer's V = 0.47). *Hippocampus whitei*

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that were handled were more likely to swim away from the photographer, attempted to hide or change their holdfast (Figure 8.2).
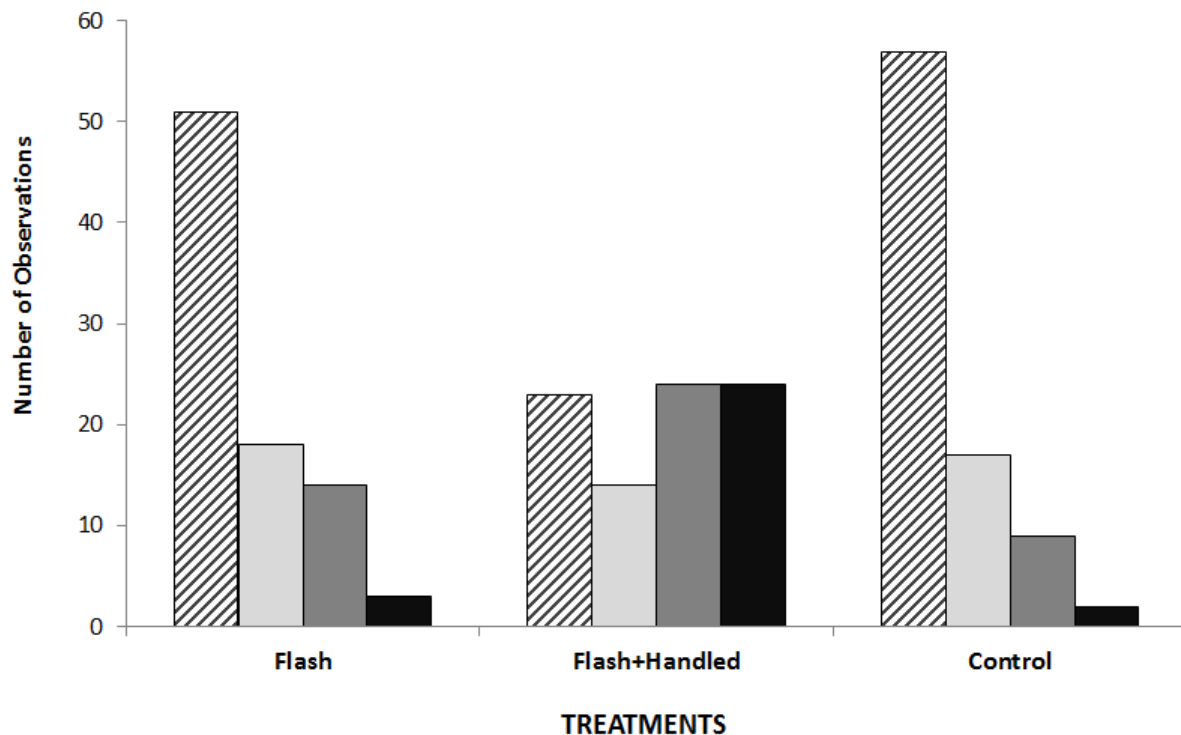
Figure 8.1.   Frequency of occurrence of responses of *Hippocampus whitei* (*n*=10 surveys combined) to three experimental treatments (*n*=8 individuals per treatment): flash photography, flash photography + handling, and photography without flash and no handling (control). No response = ▱ (white bars with black diagonal stripe), actively hid = ▪ (light grey bars), changed holdfast = ▪ (grey bars), swam away = ■ (black bars).

Movement

The mean weekly distance moved by *H. whitei* did not differ among treatments (Figure 8.2) and the mean ± S.E. weekly distance moved by each *H. whitei* was 32.2 ± 2.9 cm. Analysis is based on six *H. whitei* per treatment as two animals from each treatment disappeared during the course of the nine surveys. Mauchly's test of

sphericity was not significant (χ^2 = 48.9, df=35, $P > 0.05$); therefore, sphericity was assumed and within-subject effects for movement over time was not significant ($F_{8,128}$ = 0.38, $P > 0.05$). The interaction between movements over time and treatments was not significant ($F_{16,128}$ = 0.48, $P > 0.05$).
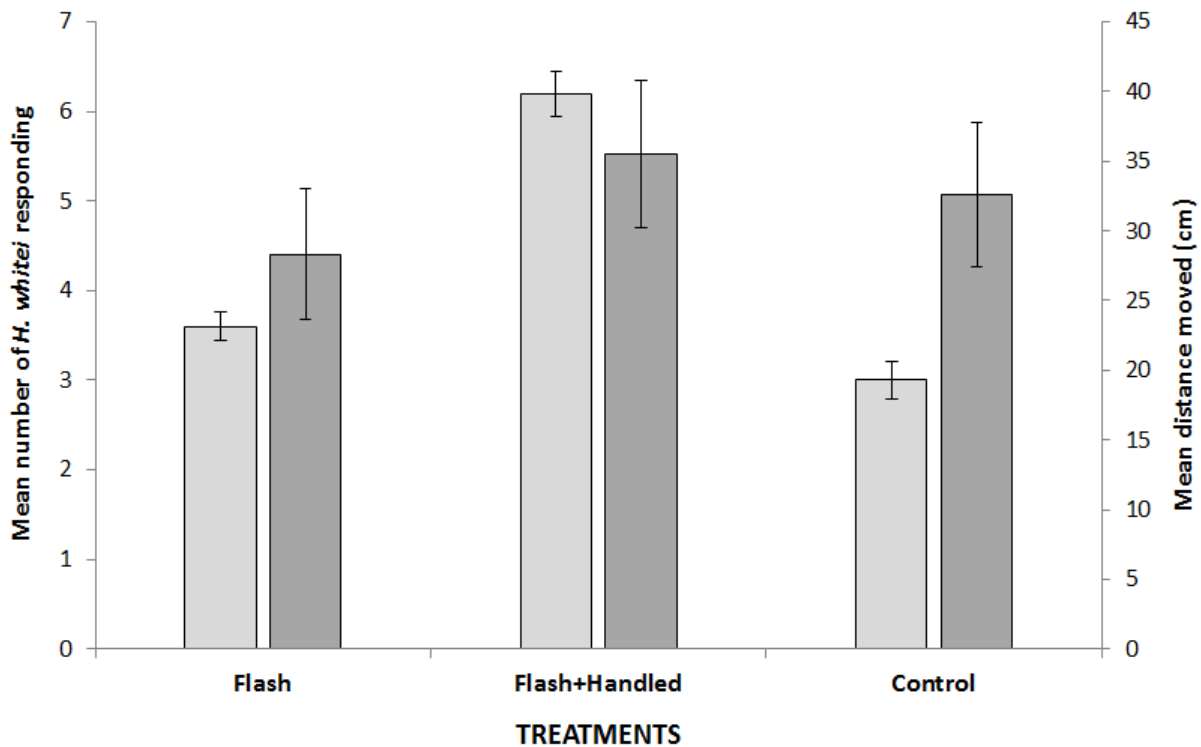
Figure 8.2. Mean (±S.E) number of *H. whitei* across three treatments (*n*=8 per treatment), responding (with all responses combined) for each weekly survey (*n*=10) [light coloured bars (▢)]. Mean (±S.E) distance moved by *H. whitei* across each treatment (*n*=6) for each weekly survey (*n*=9) [dark coloured bars (▮)].

Site persistence

Kaplan-Meier comparisons of site persistence found there was no significant difference in the number of *H. whitei* observed on the site over time across the three treatments (χ^2 = 1.43, df=2, $P > 0.05$; Figure 8.3). For the last survey in December 2009,

there were four *H. whitei* remaining from the flash treatment and three remaining from each of the control and handled treatments. Additionally, random dives in April-May 2010 at the site found that seven of the experimental *H. whitei* were still present on the site (three from flash treatment and two each from control and handled treatments).
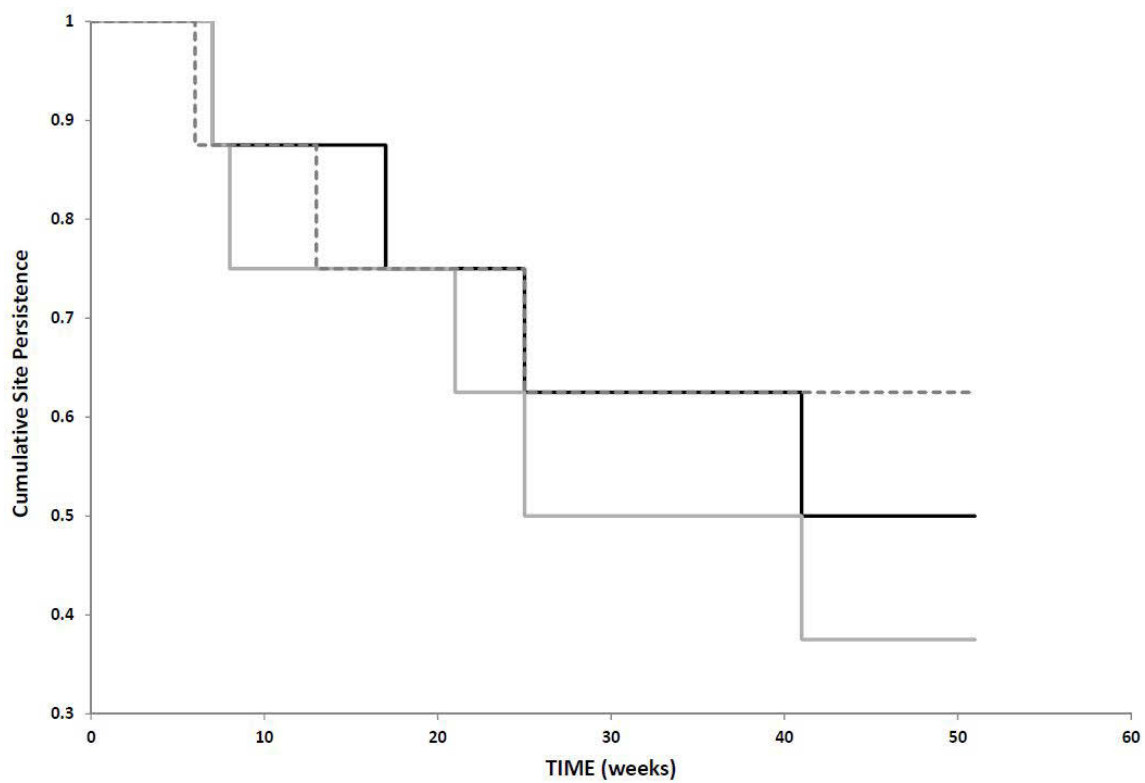
Figure 8.3. Cumulative site persistence of *Hippocampus whitei* across all three treatments from January 2009 – December 2009. Flash = ——— (black line), Flash + handled = ——— (grey line) and Control (no flash) = — — (dashed grey line).

8.5 Discussion

This study set out to assess the effects of underwater flash photography on the seahorse *H. whitei* and found negligible effects on behaviour, movements and site persistence. Whilst this study did not assess the effectiveness of using spot pattern markings as an identification method for *H. whitei*, the use of flash photography as a method to assist with individual identification could be implemented with minimal effect on this species if conducted with similar frequencies to those employed in this study. Martin-Smith (2011) found in a population dynamics study of *P. taeniolatus*, that individuals were identifiable 18 months after being initially photographed using spot and blotch patterns and that some animals were photographed over 20 separate occasions at one of the sites. As individual *P. taeniolatus* were repeatedly encountered throughout the study, it is unlikely that the flash photography had an effect on their movements or site persistence similar to the findings of this study.

Handling of *H. whitei* altered their short-term behaviour, leading to avoidance behaviours such as seeking shelter or changing holdfast. However, this handling effect was only considered short-term as there was no long-term effect on individual's movements or site persistence. The gradual disappearance from the site of *H. whitei* from all treatments could be attributed to death or movement off the site. Harasti *et al.* (2012) estimated the average annual survival rate of *H. whitei* in Port Stephens to be 27.4%, so it is not unusual that several of the *H. whitei* in the study disappeared over the duration of the experiment.

If photographers are to take images of seahorses for scientific purposes, the least invasive method would be to not handle the seahorse. Though this study only focused on one species of seahorses, with a set number of photos taken, the effects of repetitive flash photography and handling of other seahorse species is unknown. Species such as the pygmy seahorses, *H. bargibanti*, and *H. denise* Lourie & Randall 2003, are heavily sought after by underwater photographers, and one of the authors (DH) has witnessed numerous occasions where these pygmy seahorses have been handled by photographers and been subject to large amounts of flash photography on a daily basis. The effect of this handling and constant flash photography on these pygmy seahorse species is unknown and merits study.

The decision to ban underwater flash photography of seahorses in the United Kingdom was not based on scientific evidence, although it is recognised that it was a precautionary approach. The results from this study demonstrated that there is no significant effect of flash photography on the behaviour, movements or site persistence of *H. whitei* which is of similar size to the two species of seahorse found in the United Kingdom: long-snouted seahorse *H. guttulatus* Cuvier 1829, and short-snouted seahorse *H. hippocampus* (Linnaeus 1758) (Lourie *et al.*, 1999b). As divers are able to provide valuable information to scientists through the provision of photographs, there is significant potential for this form of citizen science (Goffredo *et al.*, 2004). Photos taken by underwater photographers are often provided to scientists and help present information that may fill in gaps on species distribution, habitat

use, food sources, behaviour and even in the discovery of new species. An example of this is the website Nudipixel (www.nudipixel.net) where photographers from around the world provide images of opisthobranchs, and several new identification guides (Debelius and Kuiter, 2007; Coleman, 2008) incorporate information and images provided to the Nudipixel website by underwater photographers. The recent establishment of the iSeahorse website (www.iseahorse.org) provides an opportunity for photographers to assist researchers learn more about the biology and ecology of seahorses and help contribute towards their long term conservation.

For any projects that plan on implementing photography to individually identify seahorses, as an alternative to traditional tagging methods, there would need to be some form of validation process implemented to ensure that individuals are clearly identifiable in photos over time. As seahorses are known to change colour and skin filaments regularly (Kuiter, 2009), the validation would need to be repeated frequently and should involve an independent party to the research to test that the seahorses can be individually recognised on a long term basis without any tags.

8.6   Acknowledgements

CHAPTER 9

General Discussion and Conclusion

9.1   Introduction

Whilst seahorses are considered to be an iconic species, there is still much to learn about the biology and ecology for the majority of *Hippocampus* spp. When all syngnathids were listed as protected species in New South Wales in 2004, they were protected based on the precautionary principle as there were no data available to provide a detailed assessment on their current conservation status (DPI, 2005). The only detailed research undertaken on any syngnathid species in NSW was the extensive work undertaken by Amanda Vincent on *Hippocampus whitei* in the 1990s in Sydney Harbour (Vincent, 1995; Vincent and Sadler, 1995; Vincent and Giles, 2003; Vincent *et al.*, 2004; Vincent *et al.*, 2005); however, these studies focused predominantly on pair bonding, reproduction and home range behaviour with none of these studies assessing population abundance, life history parameters, distribution or habitat use.

To date, there have been very few other *Hippocampus* species that have been studied extensively in the wild, with the exception of *H. guttulatus* and *H. hippocampus* (Curtis and Vincent, 2005; 2006; Curtis, 2007; Curtis *et al.*, 2007; Caldwell and Vincent, 2012; Caldwell and Vincent, 2013) and *H. comes* (Perante *et al.*, 2002; Job *et al.*, 2006; Morgan and Vincent, 2007; Morgan and Vincent, 2013). The conservation

and management of seahorses requires decisions to be based on adequate biological and ecological data. The overall aim of this study was to provide the necessary data to assist with the conservation and protection of *H. whitei* across its range. This project set out to collect important information on *H. whitei* life history parameters, population abundance, habitat preferences and effects of a marine protected area on *H. whitei* through diving and mark-recapture surveys. Experiments were implemented to assess the relationship of *H. whitei* with epibiotic growth on artificial structures, the effects of underwater photography on the movements and behaviour of *H. whitei* and genetic analysis of collected tissue samples to determine if there was any variation in genetic structure of *H. whitei* populations across its range. With the knowledge gained from this study on *H. whitei*, combined with data collected on other *Hippocampus* spp., there is now considerable information that can be used to help inform decision-making regarding seahorse conservation world-wide.

9.2   Study Results

9.2.1   Life History Parameters

One of the key findings from this study was the quantification of the life history parameters for *H. whitei*. It was determined that *H. whitei* displays rapid growth, early maturity and reproduction indicating that it has the ability to develop large populations if conditions are appropriate, such as the availability of suitable habitat

and few predators. *Hippocampus whitei* were observed to be living for > 5 years in Port Stephens and it was found that *H. whitei* displayed monogamy over a period of three consecutive breeding seasons.

9.2.2   Habitat usage and preferences

Previous studies on *H. whitei* had recorded the species occurring in seagrasses and on artificial structures in Sydney Harbour (Vincent *et al.*, 2005; Clynick, 2008; Hellyer *et al*. 2011). However, an assessment of the full range of habitats occupied by *H. whitei* and their preference for particular habitats had not previously been undertaken. In areas with up to 12 different habitat types, it was found that adult *H. whitei* had a significant preference for sponge and *Dendronephthya australis* soft coral habitats whilst the juveniles showed significant preference to *Euplexaura* sp. gorgonian habitats. *Hippocampus whitei* is considered to be a very cryptic species with both adults and juveniles displaying high levels of crypsis during surveys. *Hippocampus whitei* is a slow moving fish susceptible to predation and it is believed that these more complex habitat types provide better camouflage to avoid predators and also allow better ambush predation (Flynn and Ritz, 1999). The study on the Manly protective swimming net supports the hypothesis that *H. whitei* prefer habitats with more complexity as the manipulative experiment demonstrated that *H. whitei* had a significant positive association with epibiotic growth. It was found that removal of epibiota caused a decrease in *H. whitei* abundance and that *H. whitei* showed significant avoidance to areas devoid of epibiotic growth.

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9.2.3   Population abundance

Surveys found that the range of *H. whitei* in NSW appears to be confined to approximately 300 km of NSW coast with the only locations found to have significant abundance of *H. whitei* being Port Stephens and Sydney Harbour. Population abundance densities for *H. whitei* in Port Stephens were comparable with densities recorded previously in Sydney Harbour (Vincent *et al.*, 2005) and similar to that recorded for other *Hippocampus* species (Bell *et al.*, 2003; Moreau and Vincent, 2004). Monitoring of *H. whitei* populations in Port Stephens over four years showed stability in the Fly Point, Little Beach and Pipeline populations whilst the Seahorses Gardens was found to rapidly decline and then subsequently recover after three years. This rapid decline and recovery suggests that the life history parameters of *H. whitei* make the species rather adaptable to recovery if the population declines; however, any recovery would depend on suitable habitat still being available for *H. whitei* to use. It was found that *H. whitei* populations within a no-take marine protected area (MPA) were significantly lower than non-protected sites adjacent to the protected area and that there was a significant correlation between increasing predator abundance and declining *H. whitei* abundance. Whilst no-take MPAs have been suggested as a management option to protect seahorses in the wild (Martin-Smith *et al.*, 2004; Morgan and Vincent, 2013), consideration must been given on a species-by-species basis to the possible ramifications of a no-take protected area on

local predator populations which could have a confounding effect on seahorse abundance.

9.2.4   Genetics

The genetic research undertaken as part of this study found that there were some levels of genetic variation in CO1 sequences across *H. whitei* populations, particularly with the populations in the northern part of its range however results are not definitive. This suggests that there could be some variation in genetic population structure across its range. However, to conclusively assess population structure, a study using microsatellite loci would be most applicable, similar to the techniques that have been employed on other seahorse species (Jones *et al.*, 1998; Galbusera *et al.*, 2007). If microsatellite analysis found that geographically separate populations of *H. whitei* are genetically distinct, then consideration would need to be given to managing them as separate evolutionary significant units.

9.2.5   Impacts of flash photography

The recent ban on flash photography of seahorses in the United Kingdom (UK) is the only known occurrence where the use of flash photography has been banned on a marine species. Whilst the ban was not based on scientific evidence as none exists, the restriction was implemented based on the precautionary principle to minimise any potential impact. This study found that the use of flash photography had no significant effect on *H. whitei* behaviour, site persistence or movements. Therefore,

the use of flash photography is considered a safe method to capture images of *H. whitei*, particularly if they could be used for education and research purposes. The methods used in this study could potentially be employed in the UK to test if flash photography of *H. guttulatus* and *H. hippocampus* actually has any effect on their behaviour and site persistence.

9.3   Conservation implications

During the course of this study, several large populations of *H. whitei* were regularly monitored. All populations were stable with the exception of the decline and subsequent recovery at the Seahorse Gardens (Port Stephens) between 2007 and 2009. Since the completion of data collection for this thesis in 2009, it has been anecdotally noticed that the *H. whitei* populations within Sydney Harbour and Port Stephens appear to have dramatically declined. In 2008, the Manly net population was estimated to be approximately 300 animals however from 2011-2013 only a handful of animals have been found on each dive with a similar reduction in abundance on the protective swimming net at Clifton Gardens when compared to previous levels. However, a potential cause for the apparent *H. whitei* decline in Sydney Harbour is unknown. A similar situation has been observed in Port Stephens with seahorse abundance across all four sites anecdotally declining since 2011. The cause of the apparent decline is unknown; however, there have been problems of sand inundating all habitats across all sites, particularly the soft coral *D. australis*

habitat which has completely disappeared from Fly Point and Little Beach where it previously occurred.

Given the limited distribution of *H. whitei* and its range occurring along some of the most populated estuaries in Australia, the degradation of essential habitats could have a detrimental impact on this species. Seagrass habitats within Sydney Harbour have been shown to decline (West *et al.*, 2004) and anecdotal information suggests that the soft coral habitat *Dendronephthya australis* has also declined substantially within Sydney Harbour (R. Kuiter *pers. comm*) to the point it is no longer found occurring in any abundance. *Hippocampus whitei* has been found to display strong site fidelity, specific habitat preferences with a small home range, and the loss of any essential habitats could have serious ramifications for the long term conservation of this species. Its use of artificial structures as a habitat is beneficial as it has been demonstrated that there is potential for artificial habitats to be used as a habitat restoration tool for seahorses (Correia *et al.*, 2013).

Consideration also needs to be given to the potential impacts of climate change as the south-east region of Australia is expected to experience the greatest increase in water temperature as a result of climate change (Hobday and Lough, 2011) and this covers the entire known range of *H. whitei*. With its very limited distribution, strong site fidelity and apparent lack of pelagic dispersal, it could potentially struggle to adapt to changing environments if water temperature increases across its range. In a study on *H. guttulatus* it was found that newborn seahorses were impacted by future

warming via metabolic depression (i.e. heat-induced hypometabolism) and that they were more likely to suffer from rising water temperatures potentially affecting their growth and survival (Aurélio *et al.*, 2012). The potential impact of increasing water temperature on *H. whitei* warrants further investigation, particularly given its limited distribution along the NSW coast.

Hippocampus whitei is currently listed on the IUCN Red List for threatened species as 'data deficient' and the data collected from this study should be used to assist in a reassessment of the species classification. Based on the results from this study and previous work by Amanda Vincent (Vincent and Sadler, 1995; Vincent and Giles, 2003; Vincent *et al.*, 2004; Vincent *et al.*, 2005), the species should no longer be considered as 'data deficient'; however, whether it warrants listing as a threatened species would depend on the IUCN listing criteria and deliberation from other hippocampid experts.

9.4   Future research

Following the completion of this study, it has been identified that there are four research priorities for *H. whitei*. They are in relation to: (1) use of photos for identification through natural markings, (2) resurvey of established study sites to assess population abundance, (3) assessment of genetic variation in populations across the range using microsatellite analysis, and (4) the potential impact of climate change and associated increasing water temperature on the species.

It was considered during this study that there could be potential use for *H. whitei* to be individually identified through natural markings, similar to the study on the Weedy Seadragon *Phyllopteryx taeniolatus* (Martin-Smith, 2011). Photographs were collected on various individuals throughout this study and it was noticed that colouration changes in *H. whitei*, particularly when they were dark in colour, made it difficult to see natural markings which could hinder identification from markings on the body. As flash photography was found to have no significant impact on *H. whitei* behaviour, movements and site persistence, it would be worthwhile to implement a study to assess the use of natural markings as an alternative to traditional elastomer tagging, as the injecting of tags into seahorses is considered intrusive. As part of the study, consideration would need to be given to the time taken to match individuals through photos over time as the use of elastomer is relatively cheap and time efficient as individual identification can be determined *in situ* through tag positions rather than analysed later on a computer through photo identification software that may be more time consuming.

A resurvey of the populations in Port Stephens and Sydney Harbour in the near future is necessary to determine if the populations have indeed declined from the population abundance estimates recorded in this study. If the population abundance has declined, consideration would need to be given to having the species considered for increased threatened species listing under state and Commonwealth legislation

(NSW *Fisheries Management Act 1994* and Commonwealth *Environment Protection and Biodiversity Conservation Act 1999*) and the IUCN Red List.

Following on from the study using mitochondrial gene methodology, a study should be implemented using microsatellite genetics as this will help better determine if there is any population structure occurring across the range of *H. whitei*. Samples would need to again be collected across the entire range of *H. whitei*, particularly the northern and southern limits, to determine if any populations are genetically separate from each other.

With the impacts of climate change becoming more evident in south-east Australia and the predictions that water temperatures will increase in NSW, there is a need to assess the potential ramifications of increasing ocean water temperature on *H. whitei*. Research could be implemented, similar to the recent study on *H. guttulatus* (Aurélio *et al.*, 2012), that would assess the physiological and behavioural responses of *H. whitei* to increased ocean warming.

9.5   Conclusion

This study implemented several aims to help increase the knowledge on the biology, ecology and conservation of *Hippocampus whitei*. The research undertaken addressed the project aims through the use of diving surveys, the establishment of manipulative experiments, collection of tissue samples to allow for genetic analysis and tagging that allowed animals to be individually identified for the duration of the study. These methods helped determine life history parameters, mortality and

population abundance estimates for *H. whitei* from two different regions (Port Stephens and Sydney) and determined that the species shows ontogenic differences in habitat preferences. The species was shown to display strong site fidelity and it was shown that abundance of *H. whitei* decreased within a no-take marine protected area with seahorse abundance negatively correlated with predator abundance. Manipulative experiments found that *H. whitei* had a strong association with epibiotic growth on artificial structures that led to development of best practise management guidelines for net cleaning and it was demonstrated that underwater flash photography had a negligible impact on the species site persistence, movements and behaviour.

With the completion of the various studies undertaken in this thesis, and with the previous work done by Amanda Vincent in the 1990's, our understanding of *Hippocampus whitei* is now considered comprehensive when compared to numerous other seahorse species. The data obtained from this study provides the information necessary to ensure suitable decisions can be made regarding the conservation of this protected species. Whilst the data collected from this study will contribute towards the future management of this species in Australia, the lessons learnt from this study could also be used to assist with management of other *Hippocampus* spp. around the world.

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