Extreme habitat specialisation and population structure of two gorgonian-associated pygmy seahorses

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ABSTRACT: Pygmy seahorses are a group of little-known miniature hippocampid fish that differ considerably in biology and ecology from their larger congeners. We estimated the population density, sex ratio and habitat of 2 sympatric, obligate gorgonian-associated pygmy seahorses, *Hippocampus bargibanti* and *H. denise,* in a 20 km long coastal marine protected area in southeast Sulawesi, Indonesia. Belt transects covering 200 m^2 each were surveyed at 7 sites and 5 depth contours to record the density of seahorses and their host gorgonians. The population density (±SE) was 1.17 (±0.27) per 200 m^2 for *H. denise* and 0.34 (±0.20) per 200 m^2 for *H. bargibanti*, some of the lowest densities for unexploited seahorse populations studied thus far. Male–female pairs (43.9%) were the most common group composition for *H. denise*, with single, 3 or 4 individuals found on 19.5, 7.3 and 29.3% of inhabited gorgonians, respectively. *H. denise* inhabited 7.8% of *Annella reticulata* gorgonians within the survey area but were recorded from a total of 8 gorgonian genera during extensive opportunistic ad hoc searches. *Annella* spp. density was 10.7 times higher than that of *Muricella* spp. (the sole host of *H. bargibanti*), of which 20.0% were inhabited. The small population size, occurrence of pygmy seahorses in groups on their hosts with the resulting skew in sex ratios, and habitat specialisation likely all impact the species' population dynamics, and hence these need to be considered in conservation management strategies.

KEY WORDS: Habitat specialist · Miniature species · Abundance · Rare species · *Hippocampus* · Syngnathidae · Pygmy seahorse · Gorgonian

INTRODUCTION

Ecological specialisation is common on coral reefs and is among the facets that enhance the biological diversity in this ecosystem (Bellwood et al. 2006, Wilson et al. 2008, Hastings & Galland 2010). Habitat specialisation involving a patchy resource can determine population dynamics and social group size; however, the importance of such associations and the role they play in animal abundances and distributions are as yet unclear (Munday et al. 1997, Munday 2002, Schiemer et al. 2009). A spectrum of habitat specialisation is found in coral reef species ranging from generalists, loosely associated with the physical structure of the reef, to extreme specialists found in an obligate association with a single species (Munday et al. 2002, Jones et al. 2004). The populations of habitat specialists tend to be smaller than those of generalists and take longer to recover following perturbation, as a result of invasion by generalists and an inability of specialists to respond plastically to change (Vázquez & Simberloff 2002, Hastings & Galland 2010). The populations of some habitat specialists are closely linked with those of their preferred hosts, whilst others appear more loosely regulated by host abundance (Mitchell & Dill 2005). Reliance on another organism or biologically-derived habitat places specialists at further risk, particularly when this is a habitatforming structure, such as coral or a gorgonian, which is itself affected by disturbance on the reef.

Many organisms that live on coral reefs rely directly on architectural species for their existence (Jones et al. 2004). An association with gorgonians can be beneficial to other organisms due to the gorgonians' temporal stability as hosts, their unpalatability, thus acting as a chemical refuge, and their provision of access to rich food resources via their position in high water flow (Goh et al. 1999, Puglisi et al. 2000, Kumagai 2008). Two of the 7 described species of pygmy seahorses are obligate associates of gorgonian corals and form small groups of individuals that appear to spend their entire post-pelagic life on a single host (Smith 2011). The associations of these miniature seahorses with gorgonian hosts have been documented for *Hippocampus bargibanti*, which lives exclusively with *Muricella plectana* and *M. paraplectana* (Gomon 1997), and *H. denise*, which has a wider range of hosts including *Annella reticulata*, *Muricella* sp. and unconfirmed associations with *Echinogorgia* sp. and *Acanthogorgia* sp. (Gomon 1997, Lourie & Randall 2003, Lourie & Kuiter 2008). Despite a paucity of knowledge regarding the biology and ecology of gorgonian-associated pygmy seahorses, their host specialisation is a factor potentially placing them at an increased risk due to habitat disturbance (Foster & Vincent 2004, Munday 2004, Feary 2007).

Habitat specialists can suffer as a result of their specialisation when those habitats become reduced in availability or disappear altogether (Caley & Munday 2003). The implications of habitat specialisation on populations of coral reef fishes have been difficult to determine (Sale 1991). However, the population size of highly habitat-specific coral-dwelling gobies was linked to host abundance, and thus gobies inhabiting rarer hosts were themselves less common (Munday et al. 1997). The habitat specialisation of gorgonian-associated pygmy seahorses is unique among their congeners and is an aspect of their ecology that would benefit from further research to assess their conservation requirements and the dynamics of their host association.

Small fish species are an important element in the biology of coral reefs both in terms of biodiversity and abundance but they have received limited research focus due to the difficulty of identifying them and estimating their abundance (Munday & Jones 1998, Depczynski & Bellwood 2006). The gorgonian-associated pygmy seahorses *Hippocampus bargibanti* and *H. denise*, which become sexually mature at under 20 mm in length (Lourie & Randall 2003), offer an opportunity to explore the role of habitat specialisation in the ecology and population structure of miniature coral reef fishes. Despite ongoing concern, the con-

servation status of many seahorse species, including pygmy seahorses, is poorly understood (Foster & Vincent 2004, 2005, Lourie et al. 2005, Martin-Smith & Vincent 2006). Given that the majority of seahorses have relatively similar life history strategies and ecological requirements, the biology of lesser-known species has been inferred from data on species that have been the subject of more detailed research (Foster & Vincent 2004). The pygmy seahorses are the smallest, most site-attached, most specialised (Foster & Vincent 2004) and least well-known members of the genus; however, despite the likely differences in biology and ecology from larger, congeneric seahorses, there has been scant research on these fishes, other than limited unpublished observations (D. Tackett pers. comm.). The primary aims of the present study were to identify host associations of these seahorses and quantify their population abundance and distribution, group composition by sex and size, and habitat use to enable an informed assessment of their conservation status and to identify habitats of importance for their management.

MATERIALS AND METHODS

Study species

Hippocampus bargibanti Whitely, 1970, redescribed by Gomon (1997), and *H. denise* Lourie & Randall, 2003 are sympatric throughout much of their range, occurring in Indonesia, Papua New Guinea, Philippines, Micronesia, Solomon Islands and Vanuatu, with *H. bargibanti* also found as far south as New Caledonia and as far north as tropical Japan (Whitley 1970, Lourie & Randall 2003, Foster & Vincent 2004). *H. bargibanti* has been recorded at depths between 13 and 40 m (Lourie et al. 2004, Baine et al. 2008), while *H. denise* has been found between 13 and 90 m (Lourie & Randall 2003). Differing host associations, distinct body shapes and sizes (maximum standard length, SL: *H. bargibanti* 26.9 mm, *H. denise* 24 mm) and the longer snout length of *H. denise* allow ready *in situ* discrimination of the 2 species (Lourie & Randall 2003).

Study site

Hippocampus bargibanti and *H. denise* were studied off the small island of Onemobaa adjacent to Tomia Island in the Tukang Besi region of southeast Sulawesi, Indonesia (5° 46′ S, 123° 53′ E; Fig. 1). The

area is typified by rich coral reefs and coral rock islands and is protected by a private marine protected area (MPA) created by Wakatobi Dive Resort (WDR), which has been established for 10 yr and covers 20 km of reef, including all sites surveyed.

Dive surveys were carried out at 7 locations between October and December 2007 and between July and September 2008. Water temperature varied between 26 and 28°C and horizontal visibility ranged from 15 to 30 m. Three of the sites (House Reef, Galaxy and Cornucopia) had steep walls from 2 to 5 m below the surface to approximately 200 m depth. Two sites (Kollo Soha and Pockets) had steep walls from 2 to 20 m depth followed by a gentle slope to over 50 m, and 2 sites (Teluk Waitii and Blade) were coral ridges separated from the main reef. The 'Blade' site also had pinnacles that came off a ridge to around 8 m depth. All ridges began at a depth of approximately 10 m and dropped steeply to over 200 m depth. The proportion of each reef type used in the study represented the diversity and abundance of habitats within the MPA. These reefs were adjacent to shallow lagoons containing corals and seagrasses.
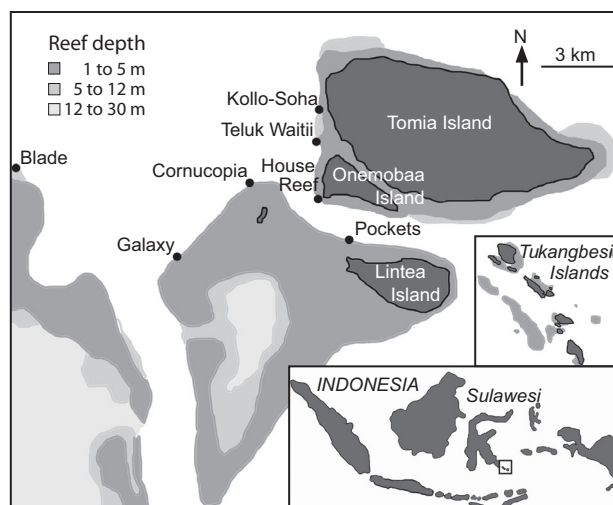

Fig. 1. Study area around Onemobaa Island, southeast Sulawesi, Indonesia, with survey sites highlighted. Sites were grouped into 3 reef types: Wall (a steep wall from 2 to 5 m below the surface to ~200 m; House Reef [also the location of Wakatobi Dive Resort], Galaxy, Cornucopia); Shallow wall (steep wall from 2 to 20 m depth followed by a gentle slope to over 50 m; Pockets, Kollo Soha); Ridge (coral ridge separated from the main reef; Blade, Teluk Waitii)

Assessment of population parameters and habitat occupancy

Out of 40 possible dive sites, 7 were chosen due to anecdotal sightings of pygmy seahorses. At each of these, single 50 m by 4 m belt transect surveys were carried out at 5 depth contours (12, 16, 20, 24 and 28 m, ±1 m), giving a total area surveyed per site of 1000 m^2. Before entering the water, global positioning system location, time, transect bearing and surface conditions were recorded. During each dive, data were collected on the gorgonians *Muricella* spp. Verrill, 1868, *Annella reticulata* (Ellis & Solander, 1786) and a third species known by dive guides to accommodate pygmy seahorses in this area, later identified as *Villogorgia* sp. Duchassaing & Michelotti, 1862. The confusion in the literature concerning the host gorgonians of *Hippocampus denise* led to the sampling of gorgonians known to be possible hosts at the main study location prior to the commencement of data collection. Further gorgonian species were not included in the surveys as searching for such small cryptic animals on a wider range of gorgonian species was precluded by the time frame of the study. Moreover, any other host associations were deemed likely to be rare given the large number of dives that have been spent searching for pygmy seahorses by guides and the first author.

Annella reticulata, *Muricella* spp. and *Villogorgia* sp. gorgonians greater than 100 cm^2 were recorded within 2 m on either side of the transect line, with gorgonian area being estimated by multiplying gorgonian height (measured from the base to farthest point using a tape measure, ±1 cm) by diameter (measured at the widest part of the gorgonian, ±1 cm). In addition to the presence of these gorgonians, we recorded the depth at which the gorgonian was attached to the substratum, estimated exposure of the gorgonian to current (i.e. very protected, protected, semi-protected, semi-exposed, exposed and very exposed, based on surrounding structures), presence of additional organisms living on the gorgonian (recorded as number of different species), gorgonian area, areas of die-off and fouling estimated as a percentage of the total area, and the identity of the fouling species.

Only 1 observer (R.E.S.), who had >5 yr experience observing and locating pygmy seahorses in the wild, searched for these animals on their gorgonian hosts to avoid surveyor bias. Several practice surveys were carried out by this observer prior to data collection to develop and rehearse survey techniques. Once a gorgonian was found to accommodate pygmy seahorses, the number and sex of individual seahorses were recorded. Sex determination was based on a magnified digital image of the urogenital pore, which is slit-
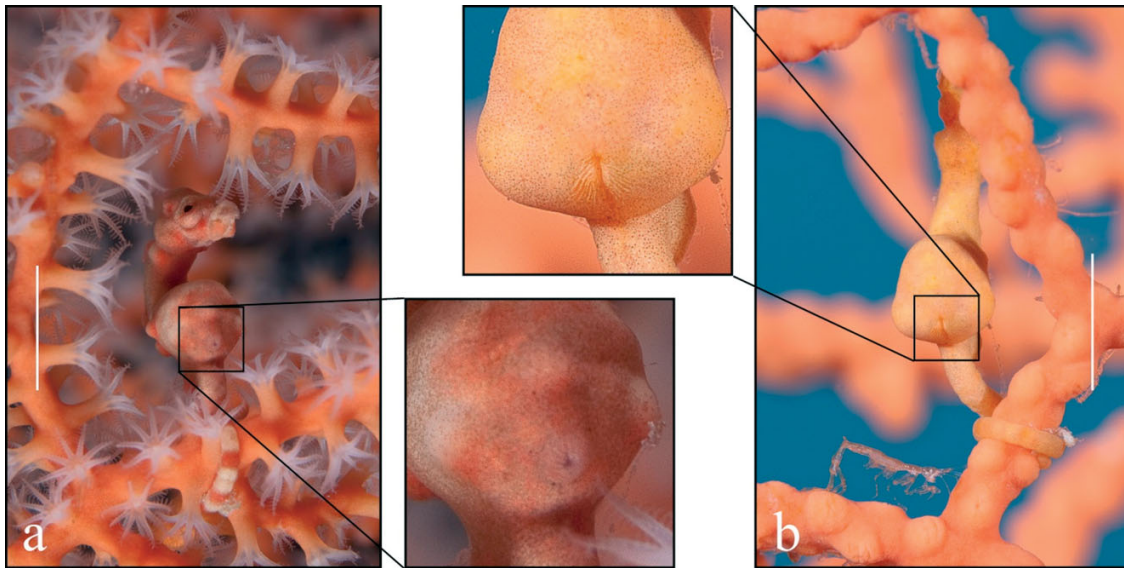
Fig. 2. *Hippocampus denise.* Genital region of (a) female identified by a raised, circular urogenital pore, (b) male identified by a slit-like opening to the pouch at the base of the abdomen. Scale bars: 5 mm

like in males and circular with a raised rim in females (Fig. 2; Lourie & Randall 2003). On occasion, sex discrimination was not possible due to the shape of the gorgonian, reef shape or the location of the pygmy seahorse on the host. If the first attempt at securing an image was unsuccessful, a second attempt was made to determine the sex of individuals at a later date, although this was not always possible. Juveniles were not sexed, as their small size made the process logistically untenable. Due to the difficulty in assessing seahorse length, only a small, recently settled *Hippocampus denise* (recent settlement assertion based on the animal's size and dark non-host specific colouration), was measured to estimate length at settlement. This was done by holding a ruler next to the animal *in situ* and taking a magnified digital image, which was later reviewed to establish SL.

Additional observations

In addition to the assessment of gorgonian–pygmy seahorse associations within transects, gorgonians were searched for the presence of *Hippocampus denise* on an ad hoc basis outside of transects. The uncertainty of *H. denise* host identification in the literature indicated that perhaps other, undescribed, *H. denise*–gorgonian associations exist. These opportunistic ad hoc searches took place during 381 dives at the primary study site and 363 dives within the

species' range over a 3 yr period. Whilst these observations were important for the identification of novel host associations, their necessarily opportunistic nature prevented quantitative estimates of effort used.

Statistical analyses

The effect of depth and site on the number of pygmy seahorse groups was analysed using separate parametric 1-way analyses of variance (ANOVA) for *Hippocampus denise* with data pooled by site and depth, depending on the analysis. Non-parametric Kruskal-Wallis (K-W) tests were used for *H. bargibanti*, as the very low number of groups of this species violated the assumptions of relevant parametric tests. Similarly, low numbers of *H. bargibanti* necessitated the use of Spearman's rank (SR) test to correlate the abundances of this seahorse and *Muricella* spp.; Pearson's correlation was used for *H. denise* and its host *Annella reticulata*. Numbers of occupied hosts, rather than individual seahorses, were used to meet the conditions of the parametric test.

A standard multiple regression was used to predict the influence of habitat variables on the presence of the pygmy seahorses *Hippocampus bargibanti* and *H. denise* as well as their host gorgonians *Muricella* spp. and *Annella reticulata*. Habitat variables tested were depth and reef type in addition to study period for the 4 species, and gorgonian density as a variable

for the seahorses. Abundances of *H. bargibanti* and *H. denise* were tested for correlation with host variables using SR correlation, as the assumptions of multiple regression were not met in this case. Adjustment of p-values from SR correlation was carried out using a Bonferroni correction, when significant differences were found, to account for the use of multiple statistical tests. Host gorgonian variables were host area, exposure, die off, fouling of the host and presence of additional species on the host.

To test whether group sizes of *Hippocampus denise* were generated randomly, a truncated Poisson test, which excluded uninhabited gorgonians, was used. Between 1 and 10 individuals were used as potential group sizes for the Poisson test. A chi-squared test was used to assess the relationship between observed and expected group sizes. Low numbers of individuals prevented this analysis from being carried out for *H. bargibanti*.

Means are reported with ±1 SE. Statistical analyses were carried out using SPSS (PASW Statistics 18.0.2). All statistical probabilities are 2-tailed, and significance was set at $\alpha \le 0.05$.

RESULTS

Pygmy seahorse host associations

Hippocampus denise was found only in association with *Annella reticulata* gorgonians within the area surveyed along transects during this study; however, individuals were found on additional gorgonian genera during ad hoc searches of local reefs. These gorgonians were identified from tissue samples using microscopy as *Acanthogorgia* sp. Gray, 1857, *Verrucella* sp. Milne Edwards & Haime, 1857 and *Villogorgia* sp., as well as the bush-forming whip coral

Ellisella sp. Gray, 1858. The latter 3 species are novel octocoral associations for *H. denise*. These octocorals appeared to be permanent hosts for *H. denise*, based on observations of resident groups lasting between 2 and at least 8 wk. The inhabitation of these other gorgonians was rare; despite 381 dives at the main study location, only single instances of *H. denise* associations with *Verrucella* sp., *Acanthogorgia* sp. and *Ellisella* sp. were observed. The association with *Villogorgia* sp. was slightly more common but was not observed within transect surveys.

During one of the additional dives in the Raja Ampat region of West Papua, New Guinea (0° 11′ S, 130° 18′ E), a single *Hippocampus denise* was observed with a gorgonian thought to be *Echinogorgia* sp. Kölliker, 1865. In addition, around the island of Misool, in southern Raja Ampat (1° 59′ S, 130° 18′ E), *H. denise* was frequently observed, in another novel association for the species, with the gorgonian *Melithaea* sp. Milne Edwards & Haime, 1857.

Hippocampus bargibanti were exclusively found on *Muricella* spp. gorgonians. All results stated here are therefore of *Annella reticulata* gorgonians as hosts for *H. denise* and *Muricella* spp. as hosts for *H. bargibanti*. During impromptu searches of *Muricella* and *Annella* gorgonians, *H. bargibanti* and *H. denise* were recorded as shallow as 4.5 and 7 m, respectively, although these were the only 2 sightings shallower than 10 m. Both were solitary seahorse individuals, and the gorgonians were located in overhangs with low levels of light intensity.

Newly-settled pygmy seahorse biology

One newly settled male *Hippocampus denise* (13 mm SL) was observed in this study on a gorgon-

Fig. 3. *Hippocampus denise.* Colour change *in situ* of a recently settled male (13 mm standard length), from dark orange to pale pink with spots, over a 120 h period (a to c) in southeast Sulawesi, Indonesia

ian with an adult female. The animal was dark orange in colour when photographed on the day of discovery and changed from dark orange to pale pink with dark spots, closely matching the host gorgonian's stems and closed polyps, when photographed 2 more times over the next 120 h (Fig. 3).

Seahorse abundance and distribution

In total, 41 *Hippocampus denise*, in 21 groups, and 12 *H. bargibanti*, in 5 groups, were found at the 7 sites, covering 7000 m^2 (Table 1). *H. denise* were found in 45.7% of transects and *H. bargibanti* in

Table 1. Number and percentage of the gorgonians *Annella reticulata* and *Muricella* spp. inhabited by the pygmy seahorses *Hippocampus denise* and *H. bargibanti*, respectively, within 1000 m^2 at each of 7 sites (total 7000 m^2) in southeast Sulawesi, Indonesia. Numbers in parentheses are the numbers of individual seahorses comprising each group

| Site	Site description	No. of inhabited gorgonians (group sizes)		% gorgonians inhabited	
		H. denise	*H. bargibanti*	*A. reticulata*	*Muricella spp.*
House Reef	Steep wall	4 (1, 2, 3, 4)	2 (1,1)	10.3	28.6
Blade	Ridge with pinnacles	3 (1, 2, 4)	0	3.8	0
Pockets	Shallow wall	2 (1,1)	1 (6)	5.9	100
Kollo-Soha	Shallow wall	4 (1,1,2,4)	1 (1)	13.3	25
Teluk Waitii	Ridge	4 (1, 2, 2, 2)	0	23.5	0
Cornucopia	Steep wall	2 (1, 2)	1 (3)	7.4	50
Galaxy	Steep wall	2 (2, 2)	0	4.5	0
Total		21	5	7.8	20
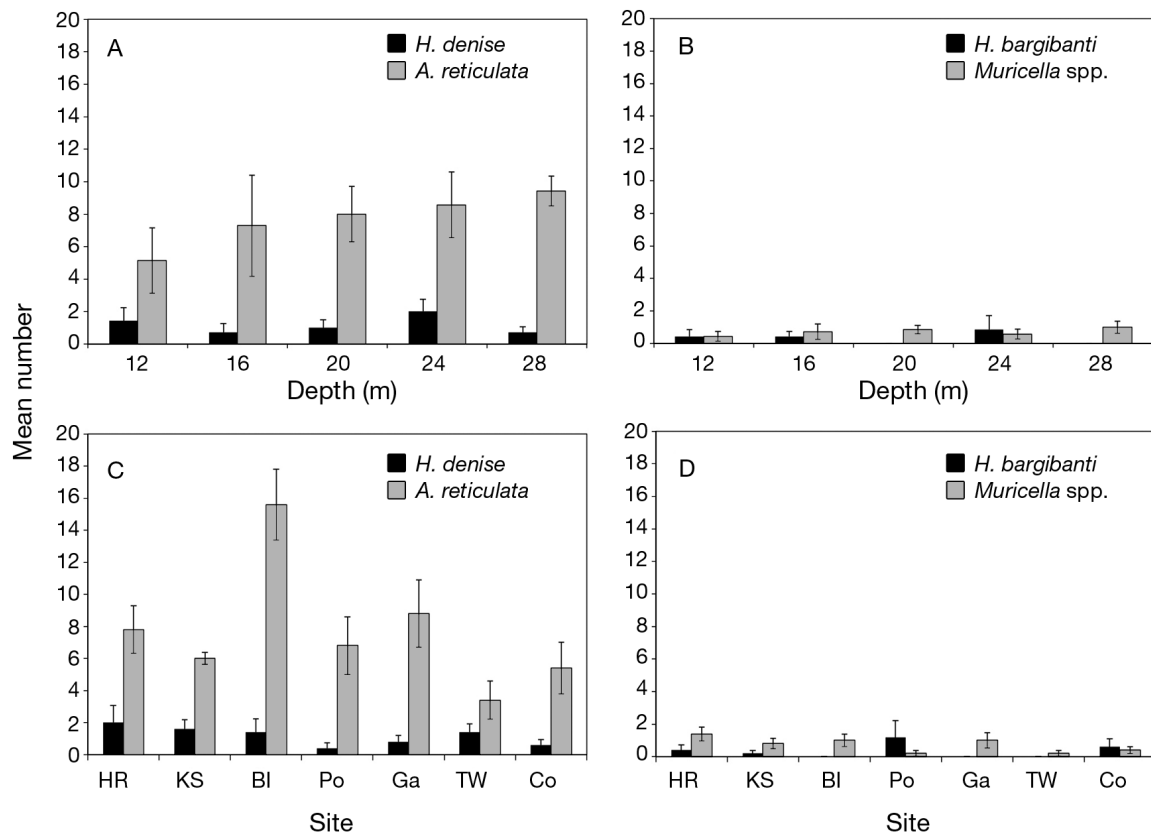
Fig. 4. *Hippocampus* spp., *Annella reticulata* and *Muricella* spp. Mean (±SE) abundance of pygmy seahorses (black) and their host gorgonians (grey): (A,C) *H. denise* and *A. reticulata*, and (B,D) *H. bargibanti* and *Muricella* spp. at (A,B) 5 depth contours and (C,D) 7 sites (House Reef, HR; Kollo-Soha, KS; Blade, Bl; Pockets, Po; Galaxy, Ga; Teluk Waitii, TW; Cornucopia, Co) on a coral reef in southeast Sulawesi, Indonesia

11.4 % of the 35 transects. Densities per 200 m^2 (the area of a single transect) of pygmy seahorses at the different sites ranged from a mean of 0.4 to 2 for *H. denise* and 0 to 1.2 for *H. bargibanti* (Fig. 4). Both species reached their highest density at 6 ind. per 200 m^2 and both were entirely absent from most transects. Mean (±SE) overall density per 200 m^2 was 1.17 ± 0.27 ind. (or 0.0059 ± 0.001 ind. m^{-2}) for *H. denise* and 0.34 ± 0.20 ind. (0.0017 ± 0.0008 ind. m^{-2}) for *H. bargibanti*. Extrapolating these densities to the 20 km of reef within the MPA, which has similar proportions of suitable habitat elsewhere based on observations during additional SCUBA dives within the MPA, yielded an estimated total population of 2343 *H. denise* (95 % confidence interval, CI, calculated using a *t*-distribution: 1266 to 3420) and 686 *H. bargibanti* (95 % CI: 134 to 1505). These estimates do not include the animals below the 28 m transect depth or rare individuals shallower than 12 m (i.e. outside the surveyed depths).
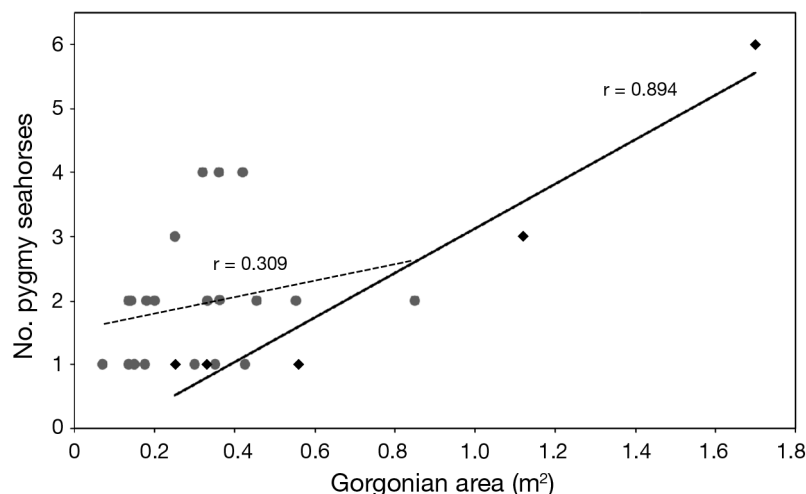

Fig. 5. *Hippocampus bargibanti* and *H. denise.* Relationship between gorgonian (height × diameter) area and number of *H. denise* per host gorgonian (*Annella reticulata*) (●, dashed line) and number of *H. bargibanti* per host (*Muricella* spp.) (◆, solid line) in southeast Sulawesi, Indonesia

The number of *Hippocampus denise* groups was not significantly correlated with *Annella reticulata* abundance (Pearson's correlation, r = 0.245, p = 0.169, n = 33). For this test only, transects without gorgonians present were excluded, since they are a prerequisite for the presence of pygmy seahorses. There was no significant effect of depth (ANOVA, $F_{4,30}$ = 1.279, p = 0.30, n = 35) or site (ANOVA, $F_{6,28}$ = 2.92, p = 0.936, n = 35) on the number of *H. denise* groups. The number of *H. bargibanti* groups was not significantly correlated with *Muricella* spp. abundance, with transects devoid of gorgonians excluded (SR correlation, r = 0.246, p = 0.341, n = 17). The number of *H. bargibanti* groups was not affected by depth (K-W, *H* = 3.538, df = 4, p = 0.472) or site (K-W, *H* = 3.306, df = 6, p = 0.770).

Gorgonian coral abundance

The density (per 200 m^2) of *Annella reticulata* (7.7 ± 0.91 ind.) was 10.7 times higher than that of *Muricella* spp. (0.71 ± 0.15 ind.; Fig. 4). The density of *Villogorgia* sp. was the lowest of the 3 species surveyed (0.38 ± 0.4 ind. 200m^{-2}), with only 13 individuals found within the 7000 m^2 study area. Extrapolation of

gorgonian densities to the 20 km of reef within the MPA yielded a total *A. reticulata* population of 15 371 (95 % CI: 8163 to 22 580), 743 *Villogorgia* sp. (95 % CI: 293 to 1192) and 1429 *Muricella* spp. (95 % CI: 578 to 2279). Again, these estimates only include abundances for transects between 12 and 28 m depth. There was a significant effect of site on *A. reticulata* abundance (ANOVA, $F_{6,28}$ = 4.170, p = 0.004, n = 35), this largely was due to the site 'Blade' having a significantly higher abundance than other sites. There was no significant effect of site on *Muricella* spp. abundance (ANOVA, $F_{6,28}$ = 1.423, p = 0.241, n = 35).

Habitat occupancy

Percentage occupancy of the 2 hosts was: 7.8 % of *Annella reticulata* occupied by *Hippocampus denise* and 20.0 % of *Muricella* spp. occupied by *H. bargibanti* (Table 1). The smallest gorgonians inhabited by *H. bargibanti* and *H. denise* measured 0.25 and 0.07 m^2, respectively (Fig. 5). Recorded habitat variables (depth, host density, reef type, with study period included along with habitat variables) and host variables (host area, gorgonian exposure, die off, fouling, number of other species on host) did not significantly influence seahorse abundance for either species (Table 2). Habitat variables accounted for 8.2, 16.5, 7.5 and 10.2 % of the total variance in *H. denise*, *A. reticulata*, *H. bargibanti* and *Muricella* spp. abundance, respectively. For *H. denise* and *H. bargibanti*,

Table 2. Results of statistical tests used to assess the influence of various variables on the abundance of pygmy seahorses *Hippocampus denise* and *H. bargibanti* and their host gorgonians *Annella reticulata* and *Muricella* spp. in southeast Sulawesi, Indonesia. For habitat variables, r^2 and *F* are shown for each of the 4 species, while remaining values are standardised β values of multiple regressions; for host variables, r-values from Spearman rank correlations (non-parametric) are shown. (–): not available

|	*H. denise*	*A. reticulata*	*H. bargibanti*	*Muricella* spp.
Habitat variables				
r^2	0.082	0.165	0.075	0.102
F	0.673	2.035	0.608	1.178
Depth	−0.083	0.255	−0.089	0.161
Gorgonian density	0.277	–	0.225	–
Study period	−0.043	0.286	−0.083	0.221
Reef type	0.013	0.088	−0.076	−0.216
Host variables				
Area	0.309	–	0.894	–
Gorgonian exposure	−0.209	–	–	–
Gorgonian die off	0.357	–	0.395	–
Gorgonian fouling	0.115	–	−0.013	–
No. of additional species on gorgonian	0.207	–	0.395	–
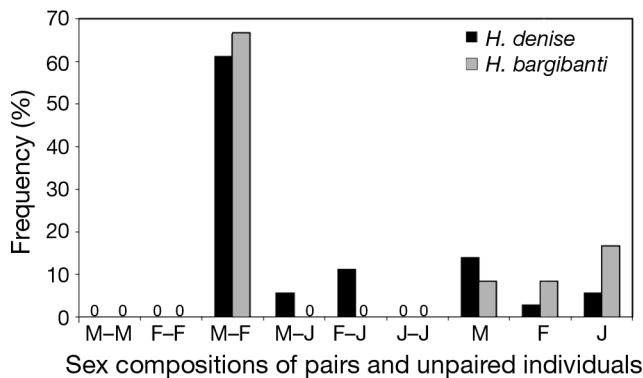
Fig. 6. *Hippocampus denise* and *H. bargibanti*. Pairings of seahorse individuals; the number of sexed individuals and juveniles was 36 for *H. denise* and 12 for *H. bargibanti*. Where more than 2 individuals were present on a gorgonian, males and females were grouped together in a putative pair (male–female) and the remaining unpartnered individuals were recorded as an unpartnered male (M), female (F), juvenile (J) or, if possible, a pairing of M–J and F–J. The sex of juveniles was not determined

the density of host gorgonians made the largest contribution to explaining their abundance (Table 2).

Group size and composition

Of the 41 *Hippocampus denise* observed, 5 were juvenile (12.2%): of the remaining 36 adults (87.8%), it was not possible to sex 5, leaving 31 sexed adults comprising 18 males (58.1%) and 13 (41.9%) females. This was found to be an unbiased sex ratio (1:1.38, chi-squared test: $\chi^2 = 0.806$, p = 0.369, n = 31). Of the 12 *H. bargibanti*, 2 were juveniles (16.7%) and 10 were adults (83.3%: 5 males, 5 females).

Of the 41 *Hippocampus denise* recorded during the study, 8 were alone on their gorgonian host, 18 were in pairs, 3 in a single group of 3, and 12 in 3 groups of 4 individuals. A truncated Poisson test found that by random allocation of the 41 *H. denise* individuals, one would expect 6.8 solitary individuals, 6.6 groups of 2 individuals, 4.3 trios and 2.2 groups of 4 individuals. The observed group sizes fitted a random distribution of individuals ($\chi^2 = 3.64$, df = 2, p = 0.162). It is important to note that this analysis omitted uninhabited gorgonians; had they been included, these group sizes would not be expected by chance. Of the 12 *H. bargibanti* recorded, 3 were solitary on the host, 3 were together in a group, and 6 were together in a group. Due to the low numbers of *H. bargibanti*, it was not possible to fit these data to a Poisson distribution.

Male–female pairs were the most commonly encountered combination of 2 individuals for both species, representing 61% of *Hippocampus denise* and 67% of *H. bargibanti* (Fig. 6), while 22% of *H. denise* and 33% of *H. bargibanti* were unpaired. Adult–juvenile pairings represented 17% of observed *H. denise* individuals, but adult–juvenile pairing was not found in *H. bargibanti*.

There was no correlation of pygmy seahorse group size with gorgonian host area for *Hippocampus denise* (SR, r = 0.309, p = 0.174, n = 21), whereas a positive correlation was found for *H. bargibanti* (SR, r = 0.894, p = 0.041, n = 5; Fig. 5).

Additional observations

The study sites also supported populations of the free-living pygmy seahorses *Hippocampus pontohi* Lourie & Kuiter, 2008 and *H. severnsi* Lourie & Kuiter, 2008, as well as the diminutive pygmy pipehorse *Kyonemichthys rumengani* Gomon, 2007. Rarely, shallow seagrass beds harboured the seahorse *H. kuda* Bleeker, 1852. Pygmy seahorses were the most common hippocampids in the area.

DISCUSSION

The present study provides the first estimates of population size in a pygmy seahorse. *Hippocampus bargibanti* and *H. denise* had the lowest mean densities of an unexploited seahorse population recorded to date. Mean densities of 0.0017 ± 0.0008 ind. m^{-2} and 0.0059 ± 0.001 ind. m^{-2} were observed for *H. bargibanti* and *H. denise*, respectively. Comparable but higher mean densities of 0.007 to 0.0089 ind. m^{-2} were found in transect surveys of *H. abdominalis, H. hippocampus* and *H. capensis* (Bell et al. 2003, Foster & Vincent 2004, Curtis & Vincent 2005, Martin-Smith & Vincent 2005). Additionally, the mean density of *H. zosterae*, a dwarf species found in shallow Caribbean seagrass habitats and the most similar seahorse in size to be the focus of population estimates, was 0.08 ind. m^{-2} (Masonjones et al. 2010). The range of densities for both *H. bargibanti* and *H. denise* within individual 200 m^2 transects was between 0 and 0.03 ind. m^{-2}. The higher end of the range is comparable to a focal grid study (i.e. in a defined area of suitable habitat usually selected for a high density of animals) of *H. comes* in the Philippines, where a mean density of 0.02 ind. m^{-2} was recorded in a previously exploited population (Perante et al. 2002). However, such comparisons between focal study grids and transect surveys are difficult due to higher densities of seahorses associated with focal grids compared to transects (Foster & Vincent 2004).

Transect surveys carried out in the present study revealed the patchiness of inhabited gorgonians, with 54 and 89% of transects yielding no animals for *H. denise* and *H. bargibanti*, despite their hosts being present in 94 and 49% of transects, respectively. In common with other seahorse populations, the patchy distribution and site attachment of gorgonian-associated pygmy seahorses are likely to place them at greater risk of habitat disturbance (Foster & Vincent 2004). The very low densities of both *H. bargibanti* and *H. denise* highlight the need for careful monitoring of these species.

Population estimates of 2343 (95% CI: 1266 to 3420) for *Hippocampus denise* and 686 (95% CI: 134 to 1505) *H. bargibanti* within surveyed depths of the 20 km of this privately managed MPA are likely to be relatively high in comparison to other Southeast Asian reefs, particularly given the study area's extreme remoteness, low human population density and protection for a decade. Many areas within the Coral Triangle are suffering rapid loss of reefs (due to overexploitation, destructive fishing practices and pollution) that is likely to affect pygmy seahorse popula-

tions as a result of their obligate association with gorgonians (Cesar et al. 1997, Hoegh-Guldberg 1999, Bruno et al. 2003, Fabricius & McCorry 2006). Gorgonians are especially susceptible to detachment from the substratum caused by physical disturbance such as dynamite fishing and contact by divers (Coma et al. 2004). In this study, we found small populations of pygmy seahorses in an area with low levels of human disturbance, requiring cautious application of these data to other sites where human impacts are greater.

Low numbers of juveniles were found for both *Hippocampus bargibanti* and *H. denise*, which is similar to findings for other seahorse species (Bell et al. 2003, Curtis & Vincent 2005, Martin-Smith & Vincent 2005, Rosa et al. 2007). The record of a recently settled juvenile male *H. denise* in the present study, at 13 mm SL, indicates that sexual maturity is attained soon after settlement. Sexual maturity was recorded in an animal of 13.3 mm SL in the description of *H. denise* (Lourie & Randall 2003), which indicates that maturity may be attained sooner than in congeneric seahorses, which mature between 3 and 12 mo post settlement (Foster & Vincent 2004). In general, small size of pygmy seahorses likely limits their reproductive output (Munday & Jones 1998, Depczynski & Bellwood 2006), but early maturation in H. denise may counteract the size-based limitation and increase reproductive output.

In the present study, *Hippocampus denise* inhabited 7.8% of *Annella reticulata* whereas *H. bargibanti* occupied 20.0% of *Muricella* spp. The absence of statistical correlation between pygmy seahorse density and host density indicates that the association with gorgonians does not determine seahorse population dynamics. These results contrast with expectations for many of the reef's habitat specialists, such as the fishes *Amphiprion* and *Gobiodon*, whose population sizes tend to be linked with those of their hosts (Kuwamura et al. 1994, Munday et al. 1997, Mitchell & Dill 2005). However, in the case of pygmy seahorses, a large proportion of potential hosts is vacant. Factors that may influence inhabitation rates of hosts by pygmy seahorses, based on data from other reef fishes, are patterns of settlement and differential mortality as well as the presence and distribution of larvae in the water column (Booth 1992). Occupancy rates of hosts varied considerably, with 23.5% of *A. reticulata* inhabited by *H. denise* at one site and as few as 3.8% at another. Interestingly, the site with the highest density of *A. reticulata* had the lowest percentage of occupied gorgonians, whereas the site with the lowest density of *A. reticulata* had

the highest percentage occupancy. This suggests that larval abundance of *H. denise* may be an explanation for inhabitation rates in its host species, as a result of constant settlement rates by planktonic juveniles regardless of gorgonian density.

Hippocampus denise was exclusively found in association with *Annella reticulata* gorgonians during transect surveys, but outside of the surveyed area, they were observed with a further 6 gorgonian genera, 4 of which are novel associations. Although not observed during the present study, the addition of *Muricella* brings the total number of reported host genera for *H. denise* to 8. *H. bargibanti*, however, was only found living in association with *Muricella* spp. gorgonians. In these terms, *H. denise* is a relative generalist in comparison to *H. bargibanti*, which is an extreme habitat specialist found exclusively in association with a single genus of gorgonian coral.

In this study, neither species exhibited a sex ratio bias, which is consistent with *in situ* population estimates of the seahorses *Hippocampus whitei*, *H. comes* and *H. breviceps* (Vincent & Sadler 1995, Perante et al. 2002, Moreau & Vincent 2004). Whilst the overall sex ratio for the population was not skewed, perhaps more important in gorgonian-associated pygmy seahorse ecology is the sex ratio of distinct groups, particularly since animals appear isolated on their gorgonian host following settlement (Smith 2011). Interestingly, no same-sex pairs were observed in the survey area. A possible explanation for this is the preferential settlement of animals to hosts that already accommodate a member of the opposite sex, given that seahorses are gonochoristic. Since post-settlement movement appears not to occur, and solitary individuals remained on their host in isolation for extended periods (Smith 2011), relocation to find a partner appears unlikely.

Pygmy seahorses are assumed to be monogamous, at least between broods, as has been found in most other seahorse species and reported for *Hippocampus bargibanti* (Jones & Avise 2001, Foster & Vincent 2004, Vincent et al. 2004, Naud et al. 2008). In our study, some seahorse groups were found to have a skewed sex ratio, suggesting that pygmy seahorses may benefit from plasticity in their mating system. The naturally low density of seahorses has been proposed to explain the adaptive benefit of monogamy in the genus at large (Foster & Vincent 2004). Polygamy is predicted where one sex is able to monopolise partners, which is possible in pygmy seahorses given that groups are isolated together on a host gorgonian and at a comparatively high density (Emlen & Oring 1977, Ihara 2002, Kokko & Rankin 2006, Hernaman &

Munday 2007). Other recent studies of seahorses have shown that the strict social and genetic monogamy observed in early research of seahorse reproductive biology is subject to variation (Kvarnemo et al. 2000, Moreau & Vincent 2004), thus the mating systems of pygmy seahorses would be an interesting direction for future research.

The link between group size and host size has been studied in various species of reef fishes where a correlation between these factors has been attributed to resource limitation or as an indirect consequence of patch size effects, such as through dominance of 1 group member (Elliott & Mariscal 2001, Mitchell & Dill 2005). We found no significant effect of gorgonian host area on the number of resident *Hippocampus bargibanti* or *H. denise*. Interestingly, there was an apparent minimum size of gorgonian able to support a particular group size of *H. denise*. However, since the majority of gorgonians accommodate fewer than the apparent maximum number of seahorses, this indicates that often the carrying capacity is not reached on a given gorgonian. Presently, data on other aspects of pygmy seahorse ecology are insufficient to speculate on explanations of these patterns.

Our finding that *Hippocampus denise* is a relative generalist compared to the extreme habitat specialist *H. bargibanti* has implications for their conservation and management. Habitat specialists are expected to have smaller populations, a prediction confirmed for this population of *H. bargibanti* and *H. denise*, which is postulated to put them at a higher risk of extinction compared to generalist species (Hawkins et al. 2000, Caley & Munday 2003, Munday 2004, Feary et al. 2007). Both pygmy seahorses rely on gorgonian corals as hosts, but the absolute requirement of *Muricella* spp. by *H. bargibanti* means that reductions in populations of this gorgonian will have corresponding effects on seahorse populations. *H. denise*, however, demonstrates a preference for *Annella reticulata* gorgonians at our study location but also lives in association with up to 7 other gorgonians. These additional associations provide a buffer for this species if *A. reticulata* populations were to suffer increased mortality rates as a result of pathogen infection or anthropogenic pressures. Our results suggest that conservation efforts for the rarer habitat specialist *H. bargibanti* should be paramount. However, as both species are obligate associates of a biologically derived habitat, it is vital to protect the hosts of both from disturbance.

In common with many other members of the genus, gorgonian-associated pygmy seahorses have small brood sizes, lengthy parental care and low mobility, all of which reduce their ability to re-colonise areas

where populations have been diminished (Foster & Vincent 2004, Smith & Tibbetts 2008). This places both species at a higher risk of extinction and indicates the need for conservation strategies that are different from those applied to their congeners (Feary 2007, Wilson et al. 2008). Their capacity to recolonise an area is impeded by their reliance on the presence of gorgonian corals, with *Hippocampus bargibanti* having a particular preference for well-established gorgonians. We found the smallest *Annella* accommodating *H. denise* to be 0.07 m^2 and a *Muricella* sp. of 0.25 m^2 supporting *H. bargibanti*. Gorgonians themselves have life history parameters favouring slow growth and low levels of recruitment, leading to a slow recovery following perturbation (Garrabou 1999, Coma et al. 2004). The combination of 2 organisms with such traits makes the persistence of pygmy seahorse populations precarious where anthropogenic impacts reduce gorgonian abundance, and is likely to make habitat loss the primary threat to pygmy seahorses (Sanchez-Camara et al. 2006).

The results of this study indicate that *Hippocampus bargibanti* and *H. denise* have a strong dependence on specific habitat conditions, which places them at high risk from habitat degradation. Their popularity within the SCUBA diving community also indicates that diver damage to gorgonian hosts needs investigation. As habitat specialists, the 2 gorgonian-associated pygmy seahorses are at an increased risk of extinction, which is particularly true for the extreme specialist *H. bargibanti*, and this should be taken into account when planning management approaches for these species.

LITERATURE CITED

Baine MSP, Barrows APW, Ganiga G, Martin-Smith KM (2008) Residence and movement of pygmy seahorses, *Hippocampus bargibanti*, on sea fans (*Muricella* spp.). Coral Reefs 27:421

Bell EM, Lockyear JF, McPherson JM, Marsden AD, Vincent ACJ (2003) First field studies of an endangered South African seahorse, *Hippocampus capensis.* Environ Biol Fishes 67:35–46

Bellwood DR, Wainwright PC, Fulton CJ, Hoey AS (2006) Functional versatility supports coral reef biodiversity. Proc Biol Sci 273:101–107

Booth DJ (1992) Larval settlement-patterns and preferences by domino damselfish *Dascyllus albisella* Gill. J Exp Mar Biol Ecol 155:85–104

Bruno JF, Petes LE, Harvell CD, Hettinger A (2003) Nutrient enrichment can increase the severity of coral diseases. Ecol Lett 6:1056–1061

Caley MJ, Munday PL (2003) Growth trades off with habitat specialization. Proc R Soc Lond B Biol Sci 270:S175–S177

Cesar H, Lundin CG, Bettencourt S, Dixon J (1997) Indonesian coral reefs — an economic analysis of a precious but threatened resource. Ambio 26:345–350

Coma R, Pola E, Ribes M, Zabala M (2004) Long-term assessment of temperate octocoral mortality patterns, protected vs. unprotected areas. Ecol Appl 14:1466–1478

Curtis JMR, Vincent ACJ (2005) Distribution of sympatric seahorse species along a gradient of habitat complexity in a seagrass-dominated community. Mar Ecol Prog Ser 291:81–91

Depczynski M, Bellwood DR (2006) Extremes, plasticity, and invariance in vertebrate life history traits: insights from coral reef fishes. Ecology 87:3119–3127

Elliott JK, Mariscal RN (2001) Coexistence of nine anemonefish species: differential host and habitat utilization, size and recruitment. Mar Biol 138:23–36

Emlen ST, Oring LW (1977) Ecology, sexual selection, and evolution of mating systems. Science 197:215–223

Fabricius KE, McCorry D (2006) Changes in octocoral communities and benthic cover along a water quality gradient in the reefs of Hong Kong. Mar Pollut Bull 52:22–33

Feary DA (2007) The influence of resource specialization on the response of reef fish to coral disturbance. Mar Biol 153:153–161

Feary DA, Almany GR, McCormick MI, Jones GP (2007) Habitat choice, recruitment and the response of coral reef fishes to coral degradation. Oecologia 153:727–737

Foster SJ, Vincent ACJ (2004) Life history and ecology of seahorses: implications for conservation and management. J Fish Biol 65:1–61

Foster SJ, Vincent ACJ (2005) Enhancing sustainability of the international trade in seahorses with a single minimum size limit. Conserv Biol 19:1044–1050

Garrabou J (1999) Life-history traits of *Alcyonium acaule* and *Parazoanthus axinellae* (Cnidaria, Anthozoa), with emphasis on growth. Mar Ecol Prog Ser 178:193–204

Goh NKC, Ng PKL, Chou LM (1999) Notes on the shallow water gorgonian-associated fauna on coral reefs in Singapore. Bull Mar Sci 65:259–282

Gomon MF (1997) A remarkable new pygmy seahorse (Syngnathidae: *Hippocampus*) from south-eastern Australia, with re-description of *H. bargibanti* Whitley from New Caledonia. Mem Mus Vic 56:245–253

Hastings PA, Galland GR (2010) Ontogeny of microhabitat use and two-step recruitment in a specialist reef fish, the browncheek blenny (Chaenopsidae). Coral Reefs 29: 155–164

Hawkins JP, Roberts CM, Clark V (2000) The threatened status of restricted-range coral reef fish species. Anim Conserv 3:81–88

Hernaman V, Munday PL (2007) Evolution of mating systems in coral reef gobies and constraints on mating system plasticity. Coral Reefs 26:585–595

Hoegh-Guldberg O (1999) Climate change, coral bleaching

and the future of the world's coral reefs. Mar Freshw Res 50:839–866

Ihara Y (2002) A model for evolution of male parental care and female multiple mating. Am Nat 160:235–244

Jones AG, Avise JC (2001) Mating systems and sexual selection in male-pregnant pipefishes and seahorses: insights from microsatellite-based studies of maternity. J Hered 92:150–158

Jones GP, McCormick MI, Srinivasan M, Eagle JV (2004) Coral decline threatens fish biodiversity in marine reserves. Proc Natl Acad Sci USA 101:8251–8253

Kokko H, Rankin DJ (2006) Lonely hearts or sex in the city? Density-dependent effects in mating systems. Philos Trans R Soc Lond B Biol Sci 361:319–334

Kumagai NH (2008) Role of food source and predator avoidance in habitat specialization by an octocoral-associated amphipod. Oecologia 155:739–749

Kuwamura T, Yogo Y, Nakashima Y (1994) Population-dynamics of goby *Paragobiodon echinocephalus* and host coral *Stylophora pistillata.* Mar Ecol Prog Ser 103: 17–23

Kvarnemo C, Moore GI, Jones AG, Nelson WS, Avise JC (2000) Monogamous pair bonds and mate switching in the Western Australian seahorse *Hippocampus subelongatus.* J Evol Biol 13:882–888

Lourie SA, Kuiter RH (2008) Three new pygmy seahorse species from Indonesia (Teleostei: Syngnathidae: *Hippocampus*). Zootaxa 1963:54–68

Lourie SA, Randall JE (2003) A new pygmy seahorse, *Hippocampus denise* (Teleostei: Syngnathidae), from the Indo-Pacific. Zool Stud 42:284–291

Lourie SA, Foster SJ, Cooper EWT, Vincent ACJ (2004) A guide to the identification of seahorses. Project Seahorse and TRAFFIC North America. University of British Columbia and World Wildlife Fund, Washington, DC

Lourie SA, Green DM, Vincent ACJ (2005) Dispersal, habitat differences, and comparative phylogeography of Southeast Asian seahorses (Syngnathidae: *Hippocampus*). Mol Ecol 14:1073–1094

Martin-Smith KM, Vincent ACJ (2005) Seahorse declines in the Derwent estuary, Tasmania in the absence of fishing pressure. Biol Conserv 123:533–545

Martin-Smith KM, Vincent ACJ (2006) Exploitation and trade of Australian seahorses, pipehorses, sea dragons and pipefishes (Family Syngnathidae). Oryx 40:141–151

Masonjones HD, Rose E, McRae LB, Dixson DL (2010) An examination of the population dynamics of syngnathid fishes within Tampa Bay, Florida, USA. Curr Zool 56: 118–133

Mitchell JS, Dill LM (2005) Why is group size correlated with the size of the host sea anemone in the false clown anemonefish? Can J Zool 83:372–376

Moreau MA, Vincent ACJ (2004) Social structure and space use in a wild population of the Australian short-headed seahorse *Hippocampus breviceps* Peters, 1869. Mar Freshw Res 55:231–239

Munday PL (2002) Does habitat availability determine geographical-scale abundances of coral-dwelling fishes? Coral Reefs 21:105–116

Munday PL (2004) Habitat loss, resource specialization, and extinction on coral reefs. Glob Change Biol 10:1642–1647

Munday PL, Jones GP (1998) The ecological implications of small body size among coral reef fishes. Oceanogr Mar Biol Annu Rev 36:373–411

Munday PL, Jones GP, Caley MJ (1997) Habitat specialisation and the distribution and abundance of coral-dwelling gobies. Mar Ecol Prog Ser 152:227–239

Munday PL, Pierce SJ, Jones GP, Larson HK (2002) Habitat use, social organization and reproductive biology of the seawhip goby, *Bryaninops yongei.* Mar Freshw Res 53: 769–775

Naud MJ, Curtis JMR, Woodall LC, Gaspar MB (2008) Mate choice, operational sex ratio, and social promiscuity in a wild population of the long-snouted seahorse *Hippocampus guttulatus.* Behav Ecol 20:160–164

Perante NC, Pajaro MG, Meeuwig JJ, Vincent ACJ (2002) Biology of a seahorse species, *Hippocampus comes* in the central Philippines. J Fish Biol 60:821–837

Puglisi MP, Paul VJ, Slattery M (2000) Biogeographic comparisons of chemical and structural defenses of the Pacific gorgonians *Annella mollis* and *A. reticulata.* Mar Ecol Prog Ser 207:263–272

Rosa IL, Oliveira TPR, Castro ALC, Moraes L and others (2007) Population characteristics, space use and habitat associations of the seahorse *Hippocampus reidi* (Teleostei: Syngnathidae). Neotrop Ichthyol 5:405–414

Sale PF (1991) Habitat structure and recruitment in coral reef fishes. In: Bell SS, McCoy ED, Mushinsky HR (eds) Habitat structure, the physical arrangement of objects in space. Chapman & Hall, London, p 197–210

Sanchez-Camara J, Booth DJ, Murdoch J, Watts D, Turon X (2006) Density, habitat use and behaviour of the weedy seadragon *Phyllopteryx taeniolatus* (Teleostei: Syngnathidae) around Sydney, New South Wales, Australia. Mar Freshw Res 57:737–745

Schiemer L, Niedermüller S, Herler J (2009) The influence of colony size and coral health on the occupation of coral-associated gobies (Pisces: Gobiidae). Coral Reefs 28: 137–142

Smith RE (2011) The biology and conservation of gorgonian-associated pygmy seahorses. PhD thesis, The University of Queensland, Brisbane

Smith RE, Tibbetts IR (2008) Mating and birth of Denise's pygmy seahorses (*Hippocampus denise*) observed in the wild. Coral Reefs 27:617

Vázquez DP, Simberloff D (2002) Ecological specialization and susceptibility to disturbance: conjectures and refutations. Am Nat 159:606–623

Vincent ACJ, Sadler LM (1995) Faithful pair bonds in wild seahorses, *Hippocampus whitei.* Anim Behav 50: 1557–1569

Vincent ACJ, Marsden AD, Evans KL, Sadler LM (2004) Temporal and spatial opportunities for polygamy in a monogamous seahorse, *Hippocampus whitei.* Behaviour 141:141–156

Whitley GP (1970) Abstract of proceedings. Ordinary general meeting 26th November 1969. Proc Linn Soc NSW 94:292–295

Wilson SK, Burgess SC, Cheal AJ, Emslie M and others (2008) Habitat utilization by coral reef fish: implications for specialists vs. generalists in a changing environment. J Anim Ecol 77:220–228