

Assemblage and interaction structure of the anemonefish-anemone mutualism across the Manado region of Sulawesi, Indonesia

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Abstract The Manado area (Indonesia–North Sulawesi), a marine high diversity hot-spot, hosts 7 species of anemonefish (family Pomacentridae, subfamily Amphiprioninae) living in symbiosis with 9 species of sea anemones (family Stichodactylidae and Actiniidae). This high biological diversity –27% and 80%, respectively, of the total known diversity of anemonefish and sea anemones—allows us to test different hypotheses focused on the obligate mutualism between anemonefish and sea anemones. In the Manado area, species richness of anemones and anemonefish across several sites was not correlated, but all anemones contained at

least one fish individual, and there was a strong positive correlation between the numbers of individual anemonefish and anemones. As expected, each fish species had a preferred anemone host; also a partial niche overlap (Pianka's Index) was often detected. The analysis of unique species composition suggests that competition is not an important factor determining the presence or absence of particular combinations of either anemonefish or host anemones (no evidence of competitive exclusion). The NODF algorithm showed that, at both a regional and local scale, the interaction between anemonefish and host anemones is not significantly nested, as a result of a combination of local conditions with competition, forcing species that regionally are more generalist to become more specialist.

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Introduction

The pattern of interactions between partners engaged in mutualistic relationships is determined by a complex interplay of evolution (including co-evolution), historical contingency and ecological opportunity. Identifying these patterns, and their possible causes, is important for understanding why particular species interact with some species but not others. The implications of this interaction structure for determining local ecological

success and stability of the assemblage are many, ultimately the promotion and preservation of local high levels of biodiversity, particularly within hotspots such as tropical coral reefs. For example, local extinction of key species can result in a cascade of further extinctions, which may be predictable from examining the topology and strength of the relationships within an assemblage (Memmott et al. 2004; Srinivasan et al. 2007).

Knowledge of the interaction structure of terrestrial mutualisms continues to grow, particularly plant-focussed relationships such as biotic pollination, seed dispersal and protection (Bascompte et al. 2003; Vázquez et al. 2009). In contrast our understanding of the assemblage topology of marine mutualisms is much less advanced. Several examples of different degrees of association between organisms are known, especially in coral reef areas where the high biological diversity frequently implies high number of interactions (though see Lester and Sewell 1989; Parmentier and Vandewalle 2005; Randall et al. 2005; Floeter et al. 2007; Ollerton et al. 2007). Mutualistic relationships in marine ecosystems are important for determining primary productivity within habitats, for example the zooxanthellae algae which exist within coral polyps (Muscatine 1990). They can be also a factor in maintaining local species diversity (Bshary 2003).

This study focuses on the obligate mutualism between anemonefish and sea anemones. Anemonefish comprise some 26 currently recognized species in the genera *Amphiprion* and *Premnas* belonging to the subfamily Amphiprioninae (Perciformes: Pomacentridae) (Allen 1991; Jang-Liaw et al. 2002). However, a recent study based on DNA analysis suggested a monophyletic origin, thus the 2 genus partition could be incorrect (Santini and Polacco 2006). Natural hybrids also exist which have previously been named as distinct species (Fautin and Allen 1997; Ollerton et al. 2007). Anemonefish are obligate associates of 10 species of sea anemones (Anthozoa: Actiniaria) from three unrelated families (Dunn 1981; Fautin and Allen 1997; Elliott et al. 1999), a mutualistic relationship that extends from the Red Sea, through the Indian Ocean to the western Pacific, and from south-eastern Australia to the latitude of Tokyo. The interaction between anemonefish and hosts anemone provides protection from predators for the fish and their eggs because the tentacles of the anemone are stinging to most other fish. Anemones are in turn protected by the anemonefish from predators such as butterflyfishes (Fautin 1991;

Godwin and Fautin 1992; Holbrook and Schmitt 2005) and waste ammonia excreted by the anemonefish is used by the anemone's symbiotic dinoflagellates (zooxanthellae—see Porat and Chadwick-Furman 2004, 2005; Roopin et al. 2008). This is therefore an extremely rare example of a mutualism in which both groups of organisms provide the same service, in this case physical protection (Ollerton 2006), though mediated in part by a third mutualistic partner, the dinoflagellate (Fautin 1991).

Anemonefish and their host anemones have been used as model systems to test ideas regarding the evolution of specificity and host selection (Arvedlund et al. 1999; Elliott et al. 1999), meta-population theory and species coexistence (Hattori 1995, 2002; Schmitt and Holbrook 2003), the topology of the mutualism (Ollerton et al. 2007), larval recruitment (Jones et al. 2005), social interactions (Buston 2003; Mitchell and Dill 2005; Buston and Cant 2006), and breeding behaviour (Munday et al. 2006), in part because we have a relatively complete knowledge of the diversity of anemonefish and anemones and the extent of their interaction (Fautin and Allen 1997). In common with most other mutualistic interactions, the anemone-anemonefish mutualism is nested in structure (Ollerton et al. 2007). At a regional scale, the most generalist fish interact with both generalist and specialist anemones, and the most generalist anemones interact with generalist and specialist fish; specialist-specialist interactions do not occur (Ollerton et al. 2007). Data on the anemonefish-anemone interaction were collected in the hot-spot of coral reef biodiversity areas around Manado, North Sulawesi (Indonesia), and used to address the following questions: 1—How species-rich are local assemblages of anemonefish and their host anemones within the region and is this richness correlated between the two groups (i.e. does high local anemone diversity support high fish diversity)? 2—Is local species richness determined by the presence of suitable partners or by abiotic factors such as substrate or water depth, and is competition determining the presence or absence of particular combinations of anemonefish and/or host anemone species? 3—Is anemonefish specialisation structured by anemone abundance, with more locally abundant anemones interacting with a greater diversity of fish and vice versa, as suggested by Ollerton et al. (2007)? 4—Is there a predictable interaction structure between the fish and the anemones or is it random, and at what scale (local

or regional)? Specifically, is the interaction structure nested? 5—Does the occurrence of the mutualists co-vary in a predictable way across the sites, or are the local assemblages random with respect to regional species pool? More specifically, is there evidence that local fish assemblages are structured by competitive exclusion?

Materials and methods

Study area and sampling

A total of 233 anemones and 772 anemonefish individuals were observed during 45–60 min SCUBA dives at depth between 1 and 40 m in 16 sampling sites located all over Bunaken Islands and Manado Coast ($\approx 1^{\circ}40' \text{ N}$ – $124^{\circ}39' \text{ E}$ and $1^{\circ}33' \text{ N}$ – $124^{\circ}48' \text{ E}$; Fig. 1), during the month of July 2006. Sampling sites were chosen trying to include the widest range of habitats present in the Bunaken area (vertical slopes, patch reefs, sandy bottom). This area is characterised by fringing reefs on Bunaken islands and mainly by wide bays of volcanic sand and patch reefs along the Manado coast (Tomascik et al. 1997). Seven anemonefishes (*Amphiprion clarkii*, *A. melanopus*, *A. ocellaris*, *A. perideraion*, *A. polymnus*, *A. sandaracinos*, *Premnas biaculeatus*) (Fig. 2) live in the Bunaken area, inhabiting at least nine different sea anemones (*Entacmaea quadricolor*; *Macrodactyla doreensis* (Fam. Actiniidae); *Heteractis aurora*; *H. crispa*; *H. magnifica*; *H. malu*; *Stichodactyla gigantea*; *S. haddoni*; *S. mertensii* (Fam Stichodactylidae)). For each observation, the species (both for anemones and anemonefishes) and the number of anemonefish individuals were recorded. The area covered by the tentacles of each host was regarded as an oval and estimated as (long axial length) $\times$ (short axial length) $\times \pi/4$ (Hattori 2002). In the dimensional analysis, clonal forms of *E. quadricolor* were not considered, because they live in the substrate of branching hard coral of *Acropora spp.* where it is impossible to measure each animal without damaging the reef.

Statistical methods

EcoSim v. 7.0 (Gotelli and Entsminger 2001) was used for niche overlap and co-occurrence calculation. SPSS 13.0 package was used for the one-way

ANOVA statistical calculation and Bonferroni post-hoc tests. Correlations have been calculated using the Pearson or Spearman (for ranked data) coefficients. The “preferred host” of anemonefish was assessed calculating the “Standardized Selection Ratio (SSR)” (values between 0 and 1) (Manly et al. 1993). Manly’s standardized selection ratio represents the probability that an individual will use a particular habitat type, taking into account the different resource availability. For each anemonefish species (i) inhabiting an anemone species (j), SSR was calculated as:

$$\text{SSR} = w_i / \sum w_j$$

(where $w_i = o_i/p_j$)

O_i is the relative frequency of the anemonefish species i and p_j the relative frequency of the anemone species j . Higher values of SSR indicate a strong preference for the selected resource. The Log-Likelihood statistic (χ^2_L) (Manly et al. 1993) was used to check the significance of the observed distribution under a null hypothesis of a random host choice.

Pianka’s Index (O) (Pianka 1973) was used as to estimate the niche overlap among species.

To determine whether the occurrence of the mutualists co-varies in a predictable way across the sites, or whether the local assemblages are random with respect to the regional species pool, we used the Co-occurrence module of EcoSim 7.0 to compare the patterns of fish and anemone presence and absence against two co-occurrence metrics:

- (1) the C-score, which is the average number of “checkerboard units” between all pairs of fish or anemone species (Stone and Roberts 1990). Checkerboard units are sub-matrices with the larger matrix in which pairs of species never co-occur. In a competitively structured community the mean number of checkerboard units (and hence the C-score) is expected to be significantly larger than would occur by, when compared to the distribution of random null models
- (2) The number of unique species combinations represented in different localities. There are 2^n possible species combinations for an assemblage of n species (including sites where no species are present). In an assemblage that is structured by

Fig. 1 Map of the sites in the Manado region



competition, we would expect fewer species combinations than occur by chance, compared to the distribution of random null models. For both of these patterns, EcoSim generated 5000 iterations of the null models with the default equiprobable row and column constraints, as recommended (Gotelli and Entsminger 2001).

Nestedness analysis

To determine whether the host anemone and anemonefish interactions were predictably nested in structure, the local and regional interaction matrices were analysed using the ANINHADO software (Guimarães and Guimarães 2006). ANINHADO is based on the Nestedness Calculator from AICS Research Inc. (Atmar and Patterson 1993), but extends the utility of the previous software by incorporating more realistic null models of species interactions into the analysis. The NODF (nestedness metric based on overlap and decreasing fill) approach was used as this has been shown to be

more rigorous and conservative than other algorithms (Almeida-Neto et al. 2008) and compared against the Ce null model (in which the level of generalisation of each species in the random iterations is approximately equal to that observed in the data matrix), likewise a more conservative (and biologically realistic) model (Bascompte et al. 2003). Only those local sites with ≥ 4 anemonefish and host anemones were included in the analysis (i.e. a 4×4 matrix—see sites in Table 1).

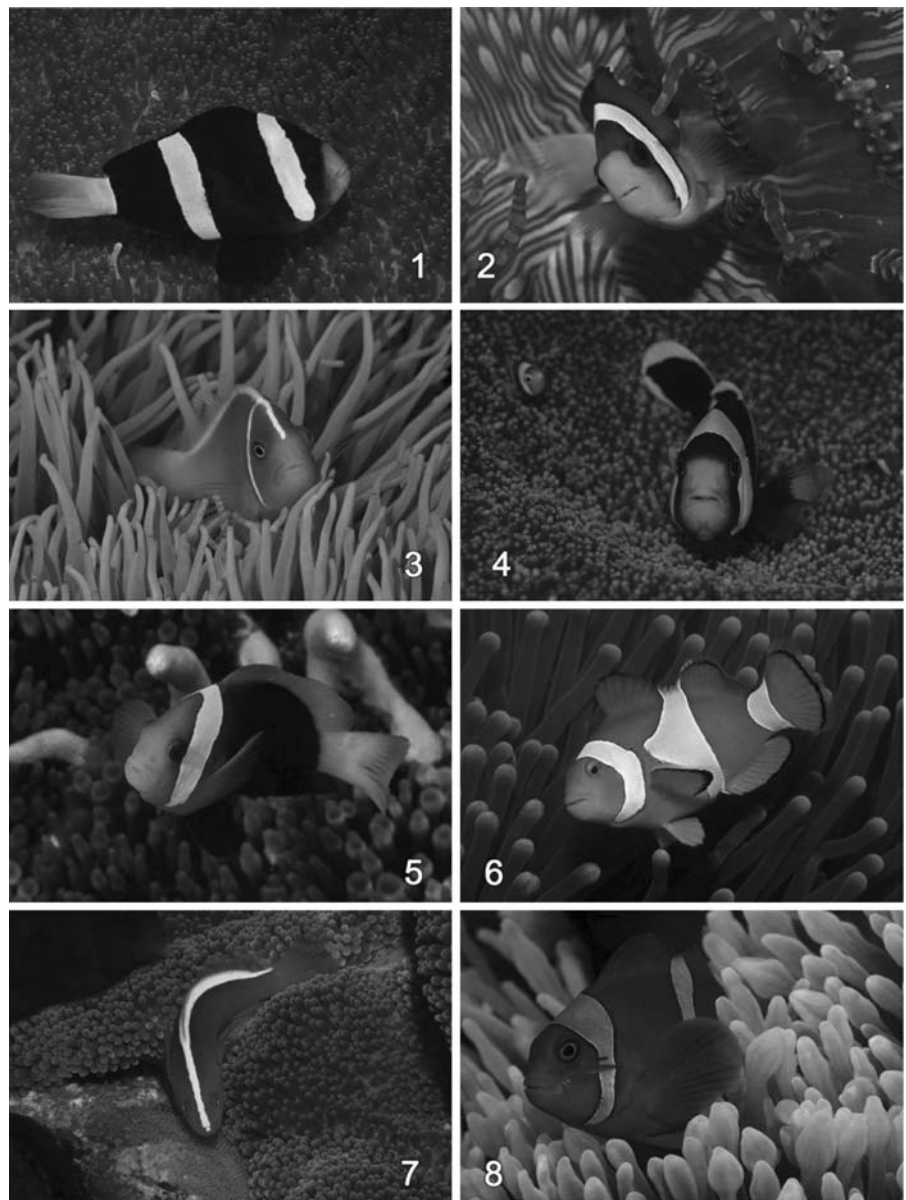
Results

Species richness and abundance of anemones and anemonefish

The survey of 16 sites in the Manado region recorded a total of 7 anemonefish species from the genera *Amphiprion* (6 spp.) and *Premnas* (1 spp.) interacting with 8 anemone species from the genera *Heteractis* (4 spp.), *Stichodactyla* (2 spp.), *Macrodictyla* (1 sp.) and *Entacmaea* (1 sp.) (Table 1). No *S. gigantea*

Fig. 2 Anemonefish and sea anemone species from the Manado area, North Sulawesi, Indonesia

1–*Amphiprion clarkii* (black colour form) on *Stichodactyla mertensii*. 2–*Amphiprion clarkii* on *Heteractis aurora*. 3–*Amphiprion perideraion* on *Heteractis crispa*. 4–*Amphiprion polymnus* on *Stichodactyla haddoni*. 5–*Amphiprion melanopus* on *Entacmaea quadricolor* (clonal form). 6–*Amphiprion ocellaris* on *Heteractis magnifica*. 7–*Amphiprion sandaracinos* (caring for eggs) on *Stichodactyla mertensii*. 8–*Premnas biaculeatus* on *Entacmaea quadricolor* (solitary form)



individual was observed during this survey. The mean number ($\pm$ SD) of anemone species per site was 3.9 ($\pm$ 1.0) and ranged from 2 at Johnson and Bajo to 6 at Bahowo. The mean species richness of anemonefish was 3.9 ($\pm$ 0.9) and ranged from 2 at Batu Kapal to 5 at Tanjung Pisok, Bualo and Bahowo. There was no correlation between the species richness of anemones and anemonefish across the sites (Pearson's $r=0.41$, $n=16$, $p=0.11$). The mean number of individual anemones per site was 14.6 ($\pm$ 8.1) and ranged from 3 at Johnson to 33 at Tanjung Pisok. Every anemone hosted at least one anemonefish, such that there was a

strong positive correlation between the number of individual fish and the number of partner anemones across sites (Pearson's $r=0.99$, $n=16$, $p<0.001$). Sea anemones were detected in a range of depths varying from 1 up to 40 m (Fig. 3). There was no significant difference in species depth except for *H. crispa* and *H. aurora* and *H. magnifica* (ANOVA, Bonferroni post-hoc test, $F=5.186$, $p<0.001$) (as there were only 2 observations of *M. doreensis*, this species was not included in the analysis). Among serean anemones, *S. mertensii* and *H. magnifica* presented larger areas, although a notable variability was recorded (Fig. 3).

Table 1 Interaction matrices for the anemonefish-anemone mutualism at 16 sites in the Manado region of Indonesia

Region	Anemone species	Anemonefish species (genera <i>Amphiprion</i> and <i>Premnas</i>)				
Bahowo		<i>A. clarkii</i>	<i>P. biaculeatus</i>	<i>A. perideraion</i>	<i>A. ocellaris</i>	<i>A. sandaracinos</i>
	<i>E. quadricolor</i>	1	1	0	0	0
	<i>H. aurora</i>	1	0	0	0	0
	<i>H. crispa</i>	1	0	1	0	0
	<i>H. magnifica</i>	0	0	1	1	0
	<i>H. malu</i>	1	0	0	0	0
Bajo	<i>S. mertensii</i>	1	0	0	0	1
		<i>A. ocellaris</i>	<i>A. perideraion</i>	<i>A. clarkii</i>	<i>A. sandaracinos</i>	
	<i>H. magnifica</i>	1	1	0	0	
Batu Kapal	<i>S. mertensii</i>	0	0	1	1	
		<i>A. clarkii</i>	<i>A. sandaracinos</i>			
	<i>H. aurora</i>	1	0			
	<i>H. crispa</i>	1	0			
Bualo	<i>S. mertensii</i>	1	1			
		<i>A. melanopus</i>	<i>P. biaculeatus</i>	<i>A. clarkii</i>	<i>A. perideraion</i>	<i>A. sandaracinos</i>
	<i>E. quadricolor</i>	1	1	1	0	0
	<i>H. crispa</i>	0	0	1	1	0
	<i>H. magnifica</i>	0	0	0	1	0
Fukui	<i>S. mertensii</i>	0	0	1	0	1
		<i>A. clarkii</i>	<i>A. perideraion</i>	<i>A. ocellaris</i>	<i>A. sandaracinos</i>	
	<i>H. crispa</i>	1	1	0	0	
	<i>H. magnifica</i>	0	1	1	0	
Jalan m.	<i>S. mertensii</i>	1	0	0	1	
		<i>A. clarkii</i>	<i>A. perideraion</i>	<i>A. ocellaris</i>	<i>A. sandaracinos</i>	
	<i>H. aurora</i>	1	0	0	0	
	<i>H. crispa</i>	1	1	0	0	
	<i>H. magnifica</i>	0	1	1	0	
Johnson	<i>S. mertensii</i>	1	0	0	1	
		<i>P. biaculeatus</i>	<i>A. clarkii</i>	<i>A. perideraion</i>		
	<i>E. quadricolor</i>	1	0	0		
Kasegaran	<i>H. crispa</i>	0	1	1		
		<i>A. clarkii</i>	<i>A. perideraion</i>	<i>A. polymnus</i>		
	<i>H. crispa</i>	1	1	0		
	<i>H. magnifica</i>	0	1	0		
	<i>Macrodactyla doreensis</i>	1	0	1		
	<i>S. haddoni</i>	0	0	1		
Likuan 1	<i>S. mertensii</i>	1	0	0		
		<i>A. clarkii</i>	<i>A. melanopus</i>	<i>P. biaculeatus</i>	<i>A. perideraion</i>	
	<i>E. quadricolor</i>	1	1	1	0	
	<i>H. aurora</i>	1	0	0	0	
	<i>H. crispa</i>	1	0	0	1	
	<i>S. mertensii</i>	1	0	0	0	

Table 1 (continued)

Region	Anemone species	Anemonefish species (genera <i>Amphiprion</i> and <i>Premnas</i>)				
Mandolin		<i>P. biaculeatus</i>	<i>A. clarkii</i>	<i>A. perideraion</i>	<i>A. ocellaris</i>	<i>A. sandaracinos</i>
	<i>E. quadricolor</i>	1	0	0	0	0
	<i>H. crispa</i>	0	1	1	0	0
	<i>H. magnifica</i>	0	0	0	1	0
	<i>S. mertensii</i>	0	1	0	0	1
Mapia		<i>P. biaculeatus</i>	<i>A. perideraion</i>	<i>A. ocellaris</i>	<i>A. polymnus</i>	
	<i>E. quadricolor</i>	1	0	0	0	
	<i>H. crispa</i>	0	1	0	0	
	<i>H. magnifica</i>	0	0	1	0	
	<i>S. haddoni</i>	0	0	0	1	
Mike		<i>A. clarkii</i>	<i>P. biaculeatus</i>	<i>A. perideraion</i>	<i>A. sandaracinos</i>	
	<i>E. quadricolor</i>	1	1	0	0	
	<i>H. crispa</i>	1	0	1	0	
	<i>H. magnifica</i>	0	0	1	0	
	<i>S. mertensii</i>	1	0	0	1	
Sachiko		<i>P. biaculeatus</i>	<i>A. clarkii</i>	<i>A. perideraion</i>	<i>A. sandaracinos</i>	
	<i>E. quadricolor</i>	1	0	0	0	
	<i>H. crispa</i>	0	1	1	0	
	<i>H. magnifica</i>	0	0	1	0	
	<i>S. mertensii</i>	0	1	0	1	
T. Kopi		<i>A. melanopus</i>	<i>A. clarkii</i>	<i>A. perideraion</i>	<i>A. ocellaris</i>	<i>A. sandaracinos</i>
	<i>E. quadricolor</i>	1	0	0	0	0
	<i>H. aurora</i>	0	1	0	0	0
	<i>H. crispa</i>	0	1	1	0	0
	<i>H. magnifica</i>	0	0	1	1	0
	<i>S. mertensii</i>	0	1	0	0	1
T. Pisok		<i>A. clarkii</i>	<i>P. biaculeatus</i>	<i>A. perideraion</i>	<i>A. ocellaris</i>	<i>A. sandaracinos</i>
	<i>E. quadricolor</i>	1	1	0	0	0
	<i>H. crispa</i>	1	0	1	0	0
	<i>H. magnifica</i>	0	0	1	1	0
	<i>S. mertensii</i>	0	0	0	0	1
Timur		<i>P. biaculeatus</i>	<i>A. clarkii</i>	<i>A. perideraion</i>		
	<i>E. quadricolor</i>	1	0	0		
	<i>H. crispa</i>	0	1	0		
	<i>H. magnifica</i>	0	0	1		
	<i>S. mertensii</i>	0	1	0		

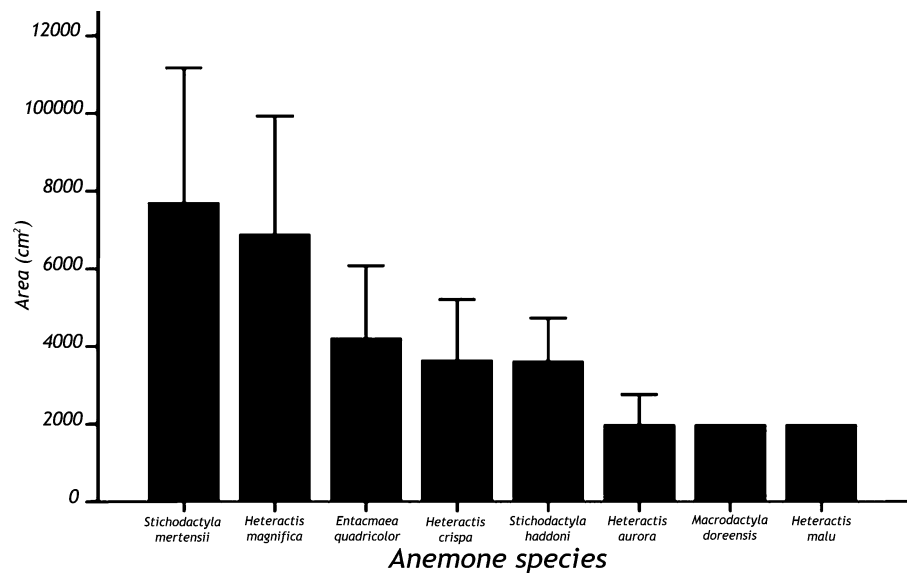
1 indicates an observed interaction; 0 indicates no interaction. Sites are listed alphabetically

Clonal forms of *E. quadricolor* were not included in the analysis due to the impossibility of an accurate estimate of the area. All hosts were relatively abundant in the Manado and Bunaken area, except *S. haddoni*, present only in sandy lagoons of Manado coast. No data of anemone density have been collected.

Host anemone specialisation

Across the region, anemone species hosted 1, 2 or 3 species of anemonefish with *E. quadricolor* being the single most generalist anemone species. Within sites, the majority of anemone species (51.6%) hosted a

Fig. 3 Mean area of sea anemone species ($m \pm SD$)



single anemonefish species, 45.2% hosted two species, whilst 3.2% host three species (Table 1). There was a statistically significant correlation between host anemone specialisation (i.e. the number of anemonefish species hosted) and the abundance of that anemone species at a site (Spearman's $r=0.60$, $n=62$, $p<0.001$) (Fig. 4). A statistically significant correlation also existed between anemonefish specialisation (i.e. the number of anemone species used by fish as hosts) and the abundance of that anemonefish

species at a site (Spearman's $r=0.65$, $n=64$, $p<0.001$) (Fig. 5).

Anemonefish specialisation

Host use

Anemonefish species utilised 1, 2 or 6 host anemone species across all sites, with *A. clarkii* being the most generalist species (Table 2). On the

Fig. 4 Relationship between anemone specialisation (number of anemonefish hosted per species per site) and anemone abundance (number of individuals per species per site) Spearman's $r=0.60$, $n=62$, $p<0.001$. Between parenthesis the number of observations of each pair of values (shown only if >1)

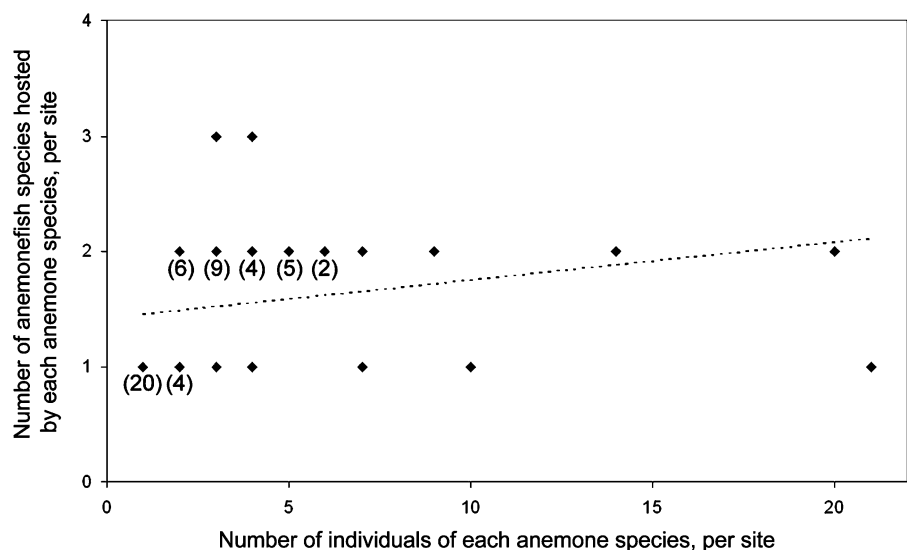
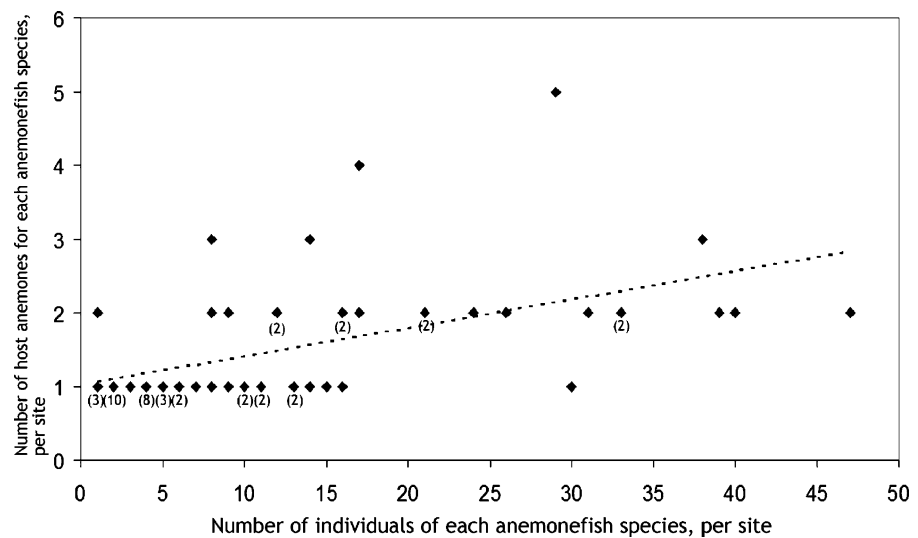


Fig. 5 Relationship between anemonefish specialisation (number of anemone species used as hosts per fish species per site) and anemonefish abundance (number of fish individuals per species per site). Spearman's $r=0.65$, $n=64$, $p<0.001$. Between parentheses the number of observations of each pair of values (shown only if >1)



contrary, *A. ocellaris*, *A. sandaracinos*, *A. melanopus* and *P. biaculeatus* inhabited only one anemone species (both with a preference for *E. quadricolor*). All anemonefish species monitored revealed a preferred sea anemone host, identified by the highest standardized selection ratio (SSR) between all the possible sea anemone species (Table 3). Usually, only one species of anemonefish inhabits a specific anemone, but in the Manado area there was a relatively high frequency of cohabitations among different anemonefish species. Particularly, a very

high cohabitation frequency (0.31) was observed in anemones inhabited by *A. clarkii*, which shared its microhabitat with *A. perideraion* (in *H. crispa*) and *A. sandaracinos* (in *S. mertensii*) (Table 4). *A. clarkii* resulted as the dominant species [bigger size, aggressive behaviour, actively in charge of sea anemone defence—the first to react to the observer's approaching—(Godwin and Fautin 1992)] in 89% (16/18) of cohabitations with *A. perideraion* and in 91% (10/11) with *A. sandaracinos*. The small *A. sandaracinos* was found in cohabitation in the 50% of

Table 2 Quantitative matrix of the interactions between anemone hosts and anemonefish across the Manado region. Data show the number of times an anemone species is recorded as hosting a

fish species across all 16 sites. Also included is the number of host anemone species used by each anemonefish species and the number of anemonefish species hosted per anemone

	<i>A. clarkii</i>	<i>A. perideraion</i>	<i>A. polymnus</i>	<i>A. melanopus</i>	<i>P. biaculeatus</i>	<i>A. ocellaris</i>	<i>A. sandaracinos</i>	Total number of anemonefish hosted per anemone species
<i>E. quadricolor</i>	6	0	0	5	13	0	0	3
<i>H. crispa</i>	52	64	0	0	0	0	0	2
<i>S. mertensii</i>	26	0	0	0	0	0	22	2
<i>M. doreensis</i>	1	0	1	0	0	0	0	2
<i>H. magnifica</i>	0	28	0	0	0	25	0	2
<i>H. aurora</i>	8	0	0	0	0	0	0	1
<i>H. malu</i>	1	0	0	0	0	0	0	1
<i>S. haddoni</i>	0	0	8	0	0	0	0	1
Total number of host anemone species per anemonefish species	6	2	2	1	1	1	1	

Table 3 Host preference in anemonefish of Bunaken area, expressed as SSR (Standardized Selection Ratio, Manly et al. 1993). In bold the preferred anemone host, identified by the higher SSR value for each species

	Freq.	<i>A. clarkii</i>	<i>A. perideraion</i>	<i>A. polymnus</i>	<i>A. melanopus</i>	<i>A. ocellaris</i>	<i>A. sandaracinos</i>	<i>P. biaculeatus</i>
<i>H. crista</i>	0.43	0.13	0.48					
<i>H. magnifica</i>	0.23		0.52			1.00		
<i>S. mertensii</i>	0.17	0.18					1.00	
<i>E. quadricolor</i>	0.10	0.04			1.00			1.00
<i>H. aurora</i>	0.03	0.26						
<i>S. haddoni</i>	0.03			0.67				
<i>M. doreensis</i>	0.01	0.13		0.33				
<i>H. malu</i>	0.01	0.26						
χ^2_L		67.84	65.15	57.24	23.13	74.14	42.95	60.13
Sig.		***	***	***	**	***	***	***

Freq relative frequency of anemone host. *A.*: *Amphiprion*; *P.*: *Premnas*; *H.*: *Heteractis*; *S.*: *Stichodactyla*; *E.*: *Entacmaea*; *M.*: *Macroactyla*

** $p < 0.01$; *** $p < 0.001$

observations, and it was the subaltern species in the 91% of cohabitations. In *H. crista* there was a significant difference between the area of the anemones inhabited only by *A. clarkii* against those occupied only by *A. perideraion*. Indeed, the first one was present in anemones with areas significantly lower than those inhabited by *A. perideraion* (ANOVA, Bonferroni post-hoc test, $p < 0.01$), while anemones with an intermediate area were often shared by both species. An opposite situation was detected in cohabitations between *A. clarkii* and *A. sandaracinos* inside *S. mertensii*. In this case, *A. clarkii* inhabited larger anemones, while *A. sandaracinos* preferred the smaller ones (Table 5).

Anemonefish niche overlap

The Pianka's niche overlap Index (O) (Table 6) showed that an almost complete niche overlap occurs between *A. melanopus* and *P. biaculeatus* ($O = 1.00$), as already highlighted by the same preferred host choice (*E. quadricolor*). However, *A. melanopus* seems to prefer shallow water anemones than *P. biaculeatus*, but this difference in depth was not significant (ANOVA, Bonferroni post-hoc test, $F = 5.186$, n.s.). All specimens of *E. quadricolor* at depth greater than 9 m are occupied by *A. clarkii* (Fig. 6). A high value of O has been detected also for *A. perideraion* and *A. ocellaris* ($O = 0.74$), while the

Table 4 Number of anemones inhabited simultaneously by two anemonefish species (*A.* = *Amphiprion*; *P.* = *Premnas*)

	<i>A. clarkii</i>	<i>A. perideraion</i>	<i>A. polymnus</i>	<i>A. melanopus</i>	<i>A. ocellaris</i>	<i>A. sandaracinos</i>	<i>P. biaculeatus</i>
<i>A. clarkii</i>	–	18		1		11	
<i>A. perideraion</i>	18	–					
<i>A. polymnus</i>			–				
<i>A. melanopus</i>	1			–			
<i>A. ocellaris</i>					–		
<i>A. sandaracinos</i>	11					–	
<i>P. biaculeatus</i>							–
Total cohabitations	30	18	0	1	0	11	0
Total hosts occupied	96	92	0	4	0	22	0
Ratio	0.31	0.20	0.00	0.25	0.00	0.50	0.00

Table 5 Areas (cm²) of *H. crispa* and *S. mertensii* anemones inhabited by two symbiotic anemonefish, both singly and in cohabitation

	<i>N</i>	Mean	Std. Deviation	Minimum	Maximum
<i>H. crispa</i>					
<i>A. clarkii</i>	39	2845	1290	491	5672
<i>A. clarkii</i> + <i>A. perideraion</i>	18	4307	2206	1256	9499
<i>A. perideraion</i>	74	5082	2787	79	14307
<i>S. mertensii</i>					
<i>A. clarkii</i>	17	8882	4221	4416	22687
<i>A. clarkii</i> + <i>A. sandaracinos</i>	11	7532	2940	3847	14307
<i>A. sandaracinos</i>	11	5939	1936	3847	8655

generalist species *A. clarkii* presented a partial overlap with *A. sandaracinos* ($O=0.40$), *A. perideraion* ($O=0.20$), *A. melanopus* and *P. biaculeatus* (both $O=0.10$).

Group composition and anemone size

A positive correlation between anemone area and anemonefish group numerosness was present in *S. mertensii* (considering the total of dominant and subordinate individuals; $r=0.74$, $p<0.01$, $n=39$), *E. quadricolor* ($r=0.74$, $p<0.01$, $n=22$) and *H. aurora* ($r=0.76$, $p<0.01$, $n=8$), which hosts only a dominant species. A lower correlation was detected in *H. crispa* ($r=0.38$, $p<0.05$, $n=97$). For *H. magnifica* and *H. haddoni* this correlation was not significant.

Predictability of fish-anemone assemblages

For the Manado data sets, the C-scores and the unique species combinations were not statistically significant

for either the anemonefish or the host anemones (Table 7).

Predictability of local and regional interaction structure

Neither the regional interaction matrix nor any of the site matrices were significantly nested in structure when analysed using the NODF algorithm (Table 8). The same result was obtained using the more traditional temperature metric *T* of Atmar and Patterson (1993) (results not shown).

Discussion

The 7 anemonefish and 8 anemone species observed in Bunaken coral reefs represent some 27% and 80%, respectively, of the total known diversity of anemonefish and the anemones which host them (Fautin and Allen 1997; Ollerton et al. 2007). Within sites the

Table 6 Results of the niche-overlap analysis using EcoSim. Values of Pianka's Index (*O*) were generated using 5000 iterations of the null models; see text for details. Pianka's index varies between 0 (total separation) and 1 (total overlap)

	<i>A. clarkii</i>	<i>A. perideraion</i>	<i>A. polymnus</i>	<i>A. melanopus</i>	<i>A. ocellaris</i>	<i>A. sandaracinos</i>	<i>P. biaculeatus</i>
<i>A. clarkii</i>	–	0.20	0	0.10	0	0.40	0.10
<i>A. perideraion</i>		–	0	0	0.74	0	0
<i>A. polymnus</i>			–	0	0	0	0
<i>A. melanopus</i>				–	0	0	1.00
<i>A. ocellaris</i>					–	0	0
<i>A. sandaracinos</i>						–	0
<i>P. biaculeatus</i>							–

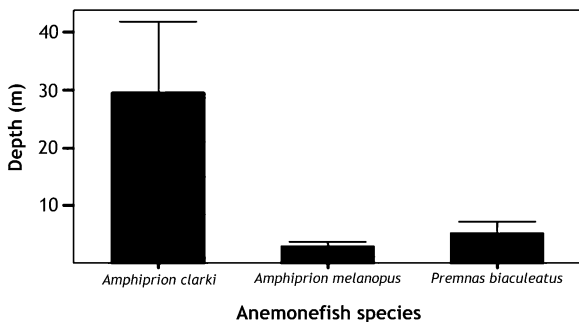


Fig. 6 Mean depth (m $\pm$ SD) of sea anemones *E. quadricolor* inhabited by three anemonefish species

diversity of anemones and anemonefish can be as high as 60% (anemones) and almost 20% of the total diversity of Amphiprioninae. This is clearly a region of high diversity for this mutualism and compares with, for example, the assemblage of 9 anemonefish and 10 host anemones observed by Elliott and Mariscal (2001) at Madang, Papua New Guinea. The species richness of anemones and anemonefish across several sites in the Manado region was not correlated, such that sites with high anemone diversity may have low anemonefish diversity and vice versa. However, because anemonefish are obligate symbionts of the anemones, and anemone survival is enhanced by the presence of the fish (Fautin 1991; Godwin and Fautin 1992; Holbrook and Schmitt 2005), all anemones were observed to contain at least one fish and there was a strong positive correlation between the numbers of individual anemonefish and anemones.

Species richness seems not be correlated either with the typology of reef site, as the 3 sites with highest richness are a fringing reef on an island (Bualo), a slow-descending reef slope on the coast (Tanjung Pisok) and a coastal site with sandy bottom and reef patches (Bahowo).

Anemonefish of the Manado region have a specific preferred host, and it was possible to observe some

niche overlaps as showed by the Pianka's index (Table 6), leading to a multiple habitat utilisation which may cause a variable association frequency with not-preferred host anemones. When the preferred host was the same, as *H. magnifica* for *A. perideraion* and *A. ocellaris*, some other factors may determinate differential use of resources: in our study, *A. melanopus* was exclusively associated with the clonal form of *E. quadricolor*, while *P. biaculeatus* only with the solitary form (Elliott and Mariscal 2001). Differentiation with depth is a consequence: *E. quadricolor* forms colonies only after asexual reproduction, in association with large colonies of hard branched corals (*Acropora* spp. or *Porites* spp.), that in Bunaken area, due to the reef conformation, are present only in very shallow waters. However, depth is not significantly different between groups then probably also other factors may be involved in the (partial) niche differentiation, as predicted by niche theory (MacArthur and Levins 1967). From a phylogenetic point of view, it is interesting to highlight that closely related species (E.g. *A. perideraion* and *A. sandaracinos*, see Santini and Polacco 2006) occur on distinct anemone host, while species living in the same host are phylogenetically distant, as for *A. clarkii*, *P. biaculeatus* and *A. melanopus* hosted by *E. quadricolor*.

The observed correlation between anemone area and group numerosness may result from the effects of area on the dominant fish size, especially when microhabitat is occupied by a single social group (Buston 2003; Buston and Cant 2006). Our observations confirmed a positive correlation between anemone area and number of adult fish, as already demonstrated in other studies (Mitchell and Dill 2005). Only in *H. magnifica* and *S. haddoni* the correlation between area and number of fish was not significant: probably in these anemones (the former occupied by *A. perideraion* or *A. ocellaris*, the latter only by *A. polymnus*) other parameters may influence the numerosness of the inhabiting anemone-

Table 7 The results of the C-score and Unique species combinations analyses using EcoSim. Probabilities were generated using 5000 iterations of the null models; see text for details

	C-score observed	C-score from null models	p observed $\geq$ null model	Unique species combinations observed	Unique species combinations from null models	P observed $\leq$ null model
Anemonefish	7.48	7.32	0.29 (ns)	10.00	11.49	0.14 (ns)
Anemones	4.04	4.02	0.45 (ns)	11.00	10.66	0.82 (ns)

Table 8 Results of the nestedness analysis using the ANINHADO software (Guimarães and Guimarães 2006) implementing the NODF algorithm and the null model. Probabilities were calculated from comparison using 1000 CE null model simulations

	NODF value	<i>P</i>
Regional matrix	29.85	0.41
Bahowo	38.49	0.49
Bualo	43.84	0.68
Jalan M	47.03	0.63
Likuan 1	49.59	0.25
Mandolin	32.12	0.79
Mike	46.37	0.77
Sachiko	40.58	0.78
T Kopi	34.83	0.73
T Pisok	35.89	0.86

fish social group (Richardson 1999). There is also some evidence that the largest dominant female anemonefish of some species, which stimulates the anemone's growth, could move in a limited reef area using different anemones as multiple hosts (Kobayashi and Hattori 2006). It is also possible that the collection of anemonefish for the aquarium trade could affect the anemonefish population structure (Shuman et al. 2005); although there is no evidence that this is valid for the Bunaken area.

Our observations of different anemonefish species cohabitation in the same sea were consistent with those of Elliott and Mariscal (2001), asserting that most derived species may share their anemone with other species, founding their strategy in the small size. According to Hattori (2002), also if *A. clarkii* when cohabiting with *A. perideraion* showed a dominant behaviour because of the bigger size, aggressiveness and higher mobility (Hattori 1995), the subaltern is a better competitor in terms of space displacement. When the anemone area is large, *A. clarkii* is not able to control the whole territory permitting *A. perideraion* settlement and, gradually, it may prevail on this larger species (see Table 5): adult specimens of *A. clarkii* often transfer from a cohabiting anemone towards a smaller one probably due to high energetic costs of the interaction with *A. perideraion*. *A. clarkii*, indeed, is also known as a better disperser compared to other anemonefish species (Hattori 1995, 2002). A similar situation may be hypothesized for the cohabitation among *A. clarkii* and *A. sandaracinos*.

The analyses of the C-score [the average number of “checkerboard units” in which pairs of species never co-occur (Stone and Roberts 1990)] and of the number of unique species combinations in the different sites both strongly suggest that competition is not an important factor determining the presence or absence of particular anemonefish/anemone combinations, i.e. fish assemblages in anemone hosts are not structured by competitive exclusion. It is possible that, at least in some cases, a stochastic event on a chronological basis (“who arrives first”) may determine the presence of an anemonefish in an anemone for long periods, and not a clear niche differentiation or a superiority as competitor against other species. Each species of a guild has similar ecological requirements and a random probability of using available resources, unrelated to the numerosness of the population, theory supported by some observations of some small Pomacentridae (Sale et al. 1984), fishes with planktonic larval stage, living in a small territory that may change ownership. However, for both cohabitation and habitat use results, it has to be considered that many anemonefish species are able to inhabit several different hosts present in their territory, moving between anemone hosts to acquire new partners or bigger hosts, depending on different factors as reproductive period, host availability, and development (Hattori and Yamamura 1995; Kobayashi and Hattori 2006) as well as the probably different timing of larval recruitment for different species. As it was not possible to assess the temporal stability of this assemblage, more observations at different temporal scales are necessary to obtain a more complete overview.

Across the whole of its distributional range and taxonomic diversity, the anemonefish-anemone nested interaction is highly statistically significant (Ollerton et al. 2007; Ollerton unpublished, using the NODF algorithm). However at both a regional and a local scale, the interaction between anemonefish and host anemones is not significantly nested, which confirms the reanalysis of the interaction data collected from Madang, Papua New Guinea by Elliott and Mariscal (2001) (Ollerton et al. 2007). Why this should be so is not clear, though it may be related to the pelagic dispersal abilities of fish and anemone larvae, or a combination of local conditions with competition, forcing species that regionally are more generalist (e.g. *A. perideraion*, *A. melanopus*, *A. ocellaris*) to become more specialist.

Host anemone and anemonefish specialisation (i.e. the number of interacting partners at each site) is strongly influenced by the relative abundance of potential hosts. That is to say, those anemone species which are most abundant at each site tend to interact with more species of anemonefish; and conversely, the most abundant anemonefish at a site interact with a wider diversity of anemone species. This supports the suggestion by Ollerton et al. (2007) regarding the importance of local population size “ (...) with abundant species interacting more frequently with one another”, something which has also been noted for plant-pollinator mutualisms (Dupont et al. 2003; Ollerton et al. 2003; Vázquez and Aizen 2004) and for marine cleaner fish relationships (a review in Floeter et al. 2007).

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