

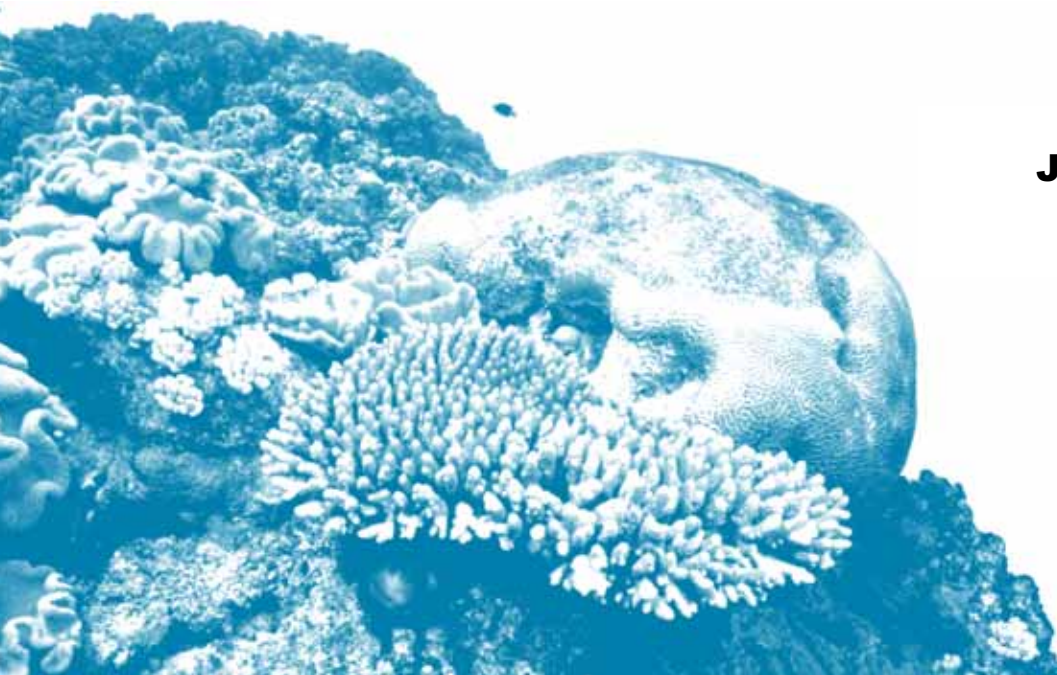


Australian Government
Great Barrier Reef
Marine Park Authority

RESEARCH PUBLICATION NO. 94

**Rapid assessment of anemone and
anemonefish populations at the
Keppel Islands** [electronic resource] :
a report to the Great Barrier Reef Marine Park Authority

**Ashley J. Frisch,
Jean-Paul A. Hobbs**



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Ashley J. Frisch, Jean-Paul A. Hobbs*

*School of Marine and Tropical Biology, James Cook University, Townsville
E-mail: jean-paul.hobbs@jcu.edu.au

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Published by the Great Barrier Reef Marine Park Authority

ISBN 978 1 876945 85 5 (pdf)

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The National Library of Australia Cataloguing-in-Publication entry :

Frisch, Ashley J.

Rapid assessment of anemone and anemonefish populations at the Keppel Islands [electronic resource] : a report to the Great Barrier Reef Marine Park Authority / Ashley J. Frisch, Jean-Paul A. Hobbs.

ISBN 978 1 876945 85 5 (pdf)

Research publication (Great Barrier Reef Marine Park Authority. Online); 94.

Bibliography.

Sea anemones--Monitoring--Queensland--Keppel Islands.

Anemonefishes--Monitoring--Queensland--Keppel Islands.

Sea anemones--Ecology--Queensland--Keppel Islands.

Anemonefishes--Ecology--Queensland--Keppel Islands.

Sea anemones--Conservation--Queensland--Keppel Islands.

Anemonefishes--Conservation--Queensland--Keppel Islands.

Sea anemones--Habitat conservation--Queensland--Keppel Islands.

Anemonefishes--Habitat conservation--Queensland--Keppel Islands.

Hobbs, Jean-Paul A.

Great Barrier Reef Marine Park Authority.

593.6099435

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**Great Barrier Reef
Marine Park Authority**

Director, Communication and Education Group

2-68 Flinders Street

PO Box 1379

TOWNSVILLE QLD 4810 Australia

Phone: (07) 4750 0700

Fax: (07) 4772 6093

info@gbbrmpa.gov.au

Comments and inquiries on this document are welcome and should be addressed to:

Director, Ecosystem, Conservation and Sustainable Use Group

info@gbbrmpa.gov.au

www.gbbrmpa.gov.au

CONTENTS

SUMMARY	1
INTRODUCTION	2
MATERIALS AND METHODS	4
Visual surveys.....	4
Survey sites.....	4
Statistical analyses.....	5
RESULTS	7
Species richness.....	7
Abundance and size structure.....	7
Patterns of distribution.....	8
DISCUSSION	28
REFERENCES	30
APPENDIX 1	32

ACKNOWLEDGEMENTS

The authors are grateful for the information provided by commercial fishers, recreational divers and 'Friends of Keppel'. Thanks are also due to Mark McCormick (JCU) and Ronald Baker (JCU) for providing statistical advice. This project was funded by the Great Barrier Reef Marine Park Authority and fieldwork was undertaken with permission from James Cook University (Authorisation number MBA16).

SUMMARY

This project was commissioned by the Great Barrier Reef Marine Park Authority to address concerns that the abundance of anemonefishes and their host anemones at the Keppel Islands has declined significantly in recent years. Potential causes of this decline include collection by fishers and (or) bleaching events, the effects of which may be depth-dependent. The objectives of this study were therefore to (1) assess the species richness, abundance and size structure of anemones and anemonefishes on reefs in the Keppel Islands, and (2) compare the size and abundance of anemones and anemonefishes among sites that differ with respect to fishing status ('open' or 'closed'), prior bleaching status ('high' or 'low') and depth (3, 7 or 15 m).

Underwater visual surveys (timed-swims) were conducted at 46 sites across the Keppel Islands, resulting in a total search area of 139225 m² (i.e. 1.5% of the known reef area at the Keppel Islands). These surveys found two species of host anemones (*Entacmaea quadricolor* and *Heteractis crispa*) and three species of anemonefishes (*Amphiprion melanopus*, *A. akindynos* and *A. clarkii*). Total counts of anemones and anemonefishes (all species combined) were 1100 and 112, respectively. Of these, approximately 40% were found at a single site (Egg Rock) and 100% were found at just 12 sites. It was concluded that both groups of organisms are currently rare at the Keppel Islands, especially when compared with other locations.

Because of the paucity of anemones and anemonefishes at the Keppel Islands, many of the attempted spatial comparisons [see (2), above] were compromised by low statistical power. Despite this problem, the mean size of host anemones was found to be significantly different between open and closed sites, between sites with high and low bleaching status, and among depths. In particular, closed sites generally had larger anemones than did open sites; low-bleached sites generally had larger anemones than did high-bleached sites; and sites that were surveyed at either 3 or 15 m generally had larger anemones than did sites that were surveyed at 7 m. Whilst it is possible that these differences were caused by collecting and (or) bleaching, it was not possible to reach an unequivocal conclusion with respect to causality.

No statistically significant spatial differences were observed in anemone and anemonefish density, or anemonefish size. Similarly, habitat type and percentage coral cover were observed to have little or no influence on the abundance of either organism. Thus, habitat type and percentage coral cover are probably unsuitable surrogates on which to base any future spatial management scheme. Given the rarity of anemones and anemonefishes at the Keppel Islands, the few sites where significant numbers of these organisms were found may warrant close attention by management agencies.

Because of the unusual biological characteristics of anemones and anemonefishes (e.g. mutual dependence, low reproductive rates, limited dispersal capability, susceptibility to bleaching), they are vulnerable to environmental disturbance and over-exploitation. These factors should be considered in any management plan aimed at ensuring the long-term survival of these organisms at the Keppel Islands.

INTRODUCTION

Anemonefishes and their host anemones are iconic marine organisms, a status that has arisen largely from their exotic association with one another. At least six species of anemonefishes (Pomacentridae: *Amphiprion* spp. and *Premnas biaculeatus*) and six species of host anemones (Actiniaria: *Heteractis* spp., *Stichodactyla* spp. and *Entacmaea quadricolor*) occur on the Great Barrier Reef (GBR) (Randall et al. 1990; Fautin & Allen 1992). Most anemones and anemonefishes appear to be obligate symbionts, since they are seldom found apart and they demonstrably protect each other from predators (Godwin & Fautin 1992; Porat & Chadwick-Furman 2004; Holbrook & Schmitt 2005). Anemones also have a symbiotic relationship with zooxanthellae – small, photosynthetic algae that live in the anemone's tissues. Like hard corals, anemones expel their zooxanthellae during periods of environmental stress, such as that caused by increased water temperature or low salinity (Engebretson & Martin 1994; Hoegh-Guldberg 1999). This expulsion results in whitening, or bleaching, of the anemone – a condition which is not necessarily fatal, but one which probably increases the anemone's risk of mortality.

Another potential threat facing anemones and anemonefishes is over-collection, as both groups of organism are highly desired by marine aquarists. Unfortunately, anemonefishes and their host anemones are particularly vulnerable to over-exploitation. This is because (1) anemone colonies seldom move, thereby enabling repeated visitation by collectors, (2) anemones are slow-growing and long-lived, (3) anemonefishes have limited dispersal capabilities, and (4) both groups of organisms are mutually dependent on each other (Fautin & Allen 1992; Wilkerson 1998; Jones et al. 2005; Shuman et al. 2005; Almany et al. 2007).

The Keppel Islands are a group of 18 inshore islands located in the southern section of the GBR. The islands support a number of fringing reefs, although well-developed reefal shoals also exist in the area. Because the Keppel Islands are easily accessed and close to several towns and cities, many of the reefs and shoals are subject to relatively high rates of use. This includes a range of extractive and non-extractive activities such as diving, angling and commercial collecting of aquarium species. Although the latter activity focuses on corals (Scleractinia) and angelfishes (Pomacanthidae), anemones and anemonefishes are also collected, mostly from depths of 7–15 m (commercial fishers, personal communication). Anemonefishes are also collected by recreational fishers, but the extent of this activity is not presently known. Recreational collection of anemones is not permitted by law (see www.gbrmpa.gov.au).

Collection of aquarium species has occurred at the Keppel Islands for about 40 years (Great Barrier Reef Marine Park Authority, personal communication). Commercial collection occurs via two, limited-entry fisheries: the Queensland Coral Fishery (QCF) and the Marine Aquarium Fish Fishery (MAFF). Under Queensland law, a Special Management Area (SMA) for the MAFF exists at the Keppel Islands; at present, eight operators are permitted to access this SMA (Queensland Department of Primary Industries and Fisheries, personal communication). Queensland Government fishery management instruments include the *Fisheries Regulation 2008*, the recently developed *Policy to Manage the Coral Fishery*, and specific fishery license conditions. Commercial collecting in the GBR Marine Park and the adjoining GBR Coastal Marine Park also requires a permit that is jointly issued by the Great Barrier Reef Marine Park Authority and Queensland Parks and Wildlife.

Due to the shallow nature of Keppel Bay and its proximity to the Fitzroy River delta, the reefs of the Keppel Islands are particularly vulnerable to elevated sea temperature and hyposaline flood events, both of which cause coral bleaching (van Woesik et al. 1995; Hoegh-Guldberg 1999). Anecdotal evidence suggests that mass bleaching events (and subsequent recovery of coral communities) have occurred at the Keppel Islands for many decades (van Woesik et al. 1995; GBRMPA 2007). In the summer of 2006, reefs at the Keppel Islands experienced a protracted bleaching event that subsequently caused 40% mortality of hard corals in shallow (≤ 5 m) water (GBRMPA 2007; Schaffelke et al. 2007). Anemones in shallow water may also have bleached to a similar extent, with negative flow-on effects to local anemonefish populations. However, the effects of the 2006 bleaching event on anemones and anemonefishes were not quantified.

Recently, local residents raised concern over the status of anemone and anemonefish populations at the Keppel Islands. The focus of this concern was a perceived decline in the abundance of both organisms to very low levels on shallow reefs, and possibly also deep reefs. Any such decline could be due to the effects of bleaching and (or) collecting by fishers. The objectives of this study were therefore to (1) assess the species richness, abundance and size structure of anemones and anemonefishes at the Keppel Islands, and (2) compare the size and abundance of anemones and anemonefishes between sites that differ with respect to fishing status ('open' or 'closed'), prior bleaching status ('high' or 'low') and depth (3, 7 or 15 m).

MATERIALS AND METHODS

Visual surveys

Because of the expected low abundances of anemones and anemonefishes at the Keppel Islands, 'timed-swims' of 30 minutes duration were chosen as the sampling unit (Shuman et al. 2005). Specifically, a SCUBA diver (i.e. one of the two Authors) swam at a slow, constant speed while following the depth contour of a reef (3, 7 or 15 m, as determined using a standard SCUBA depth gauge). The diver actively searched for anemones and anemonefishes within a 5 m strip (2.5 m either side of the diver) using a stopwatch to record the time. The width of the strip was periodically checked using a flexible metric tape. A Global Positioning System (GPS; Garmin 72, Garmin, Olathe, U.S.A.) was towed at the surface to record the diver's track and the distance swum. This method of survey enabled large areas (~3000 m²) of reef to be surveyed during each dive whilst facilitating an accurate estimate of the area that was searched (Shuman et al. 2005).

When anemones or anemonefish were found, the stopwatch was temporarily paused and all individuals were identified (as per Fautin & Allen 1992), counted, and estimated for size. Anemonefish size (total length; TL) was estimated visually whereas anemone size (oral disc area) was estimated using the formula:

$$L \times W \times \Pi \div 4$$

where *L* and *W* were, respectively, the greatest length and perpendicular width of the oral disc, as measured with a flexible metric tape (Kobayashi & Hattori 2006). If the number of anemones in a colony was large, the size of only ten, haphazardly-selected anemones was recorded. The stopwatch was restarted when the diver continued searching for more colonies. After the completion of each survey, counts of anemones and anemonefishes were standardised by converting to units of density (individuals per 3000 m²). To evaluate the repeatability of results among divers and among surveys, one site was surveyed twice by each diver and another site was surveyed twice by the same diver. All surveys were completed during daylight (0800–1700 hour) between 22 and 31 October, 2007 (inclusive).

Survey sites

Survey sites were spread as widely as possible across the Keppel Islands in order to encompass a range of different habitats, from well-developed coral reefs to rocky shoals. Sandy shoals with low topographic relief were not surveyed because of time constraints. For a complete list of survey sites and associated GPS coordinates, see Appendix 1.

'Open' and 'closed' sites were located in Habitat Protection (dark blue) and Marine National Park (green) Zones, respectively. Commercial collection of aquarium species is permitted in the former but not in the latter, and poaching from green zones is considered to be rare or non-existent (Great Barrier Reef Marine Park Authority, personal communication). Although collection of aquarium species is also permitted in Conservation Park (yellow) Zones, these areas were not surveyed because (1) they contain only a small proportion of the total reef area at the Keppel Islands, and (2)

collecting was prohibited in such areas until recently (2004) which complicates the sampling design.

Survey sites were classified as 'high' or 'low' bleached according to the severity of coral bleaching at each site, as recorded in April 2007 (see GBRMPA 2007). In general, 'high' bleached reefs previously experienced bleaching rates in excess of 40%, whereas 'low' bleached reefs previously experienced bleaching rates below 40%. The survey depth at each site was fixed at 3, 7 or 15 m (refers to depth below mean sea level). This depth range encompasses the majority of reefal area available at the Keppel Islands (authors' personal observation) and is the depth range most commonly occupied by anemone and anemonefish species that inhabit the GBR (Randall et al. 1990; Butler 1991; Fautin & Allen 1992). Each treatment combination was randomly sampled in proportion to its availability. Hence, only one sample could be obtained from 'deep-high bleached-closed' and 'deep-high bleached-open' sites. Two to seven samples were obtained from all other treatment combinations.

To quantitatively describe the habitat at each site, a point sample of the 'substrate type' and 'structural complexity' was recorded every 3 min (~60 m). Substrate type was recorded as L (live, unbleached coral), P (live partially-bleached coral), F (live fully-bleached coral), D (intact dead coral recently colonised by algae), U (unstable substrata such as sand, mud or rubble), R (rock), S (soft coral) or M (macro-algae). Structural complexity was recorded as 1 (flat and sandy), 2 (rubble, small rocks, algae or encrusting coral but highly planar with few refuges), 3 (abundant rocks and coral with limited three-dimensional structure but occasional overhangs), 4 (well developed coral or rock structures with overhangs but few large bommies and caves) or 5 (multi-layered coral matrix with caves, large bommies and abundant overhangs). Each site was also prescribed a 'habitat type', as previously defined by the Great Barrier Reef Marine Park Authority (A = *Acropora*-dominated reef slopes, B = *Acropora*-dominated reefs with a shallow silt base, C = depth-dependent reefs with high turbidity or wave energy, D = rocky reefs or shoals with low coral cover). For more detailed information about habitat types, see GBRMPA (2007).

Statistical analyses

Due to the limited occurrence of some habitats (see above), a balanced sampling design was not possible. Also, anemones and anemonefish were absent from a high proportion of the samples (sites). For these reasons, the data could not be analysed using traditional multi-factorial ANOVA techniques (Zar 1999). Instead, the data were pooled and each factor (i.e. fishing status, bleaching status or depth) was considered separately using a Student's *t* test or 1-way ANOVA, depending on the number of categories. If the data were heteroscedastic, either a transformation ($y = \log_{10}[x + 1]$) was applied or a non-parametric test (Kruskal-Wallis test) was used. Bonferroni's adjustment (Zar 1999) was employed to maintain a constant probability of Type I error across multiple comparisons (adjusted $\alpha = 0.017$).

To determine which factor(s) had the greatest influence on the size and abundance of anemones and anemonefishes, least-squares classification and regression tree (CART) analysis was employed (De'ath & Fabricius 2000). This type of analysis successively 'splits' the data into increasingly homogeneous clusters ('leaves') by minimising the residual sums of squares for each split, analogous to least squares regression. In

separate analyses, the size and density of anemones and anemonefishes were used as dependent variables, while combinations of fishing status, bleaching status and depth were used as explanatory factors. Because of the small sample size, the number of leaves in each tree was limited to two (Brieman et al. 1984). Also, to minimise the disproportionate influence of outliers, density data were transformed ($y = \log_{10}[x + 1]$) prior to analysis (Zar 1999; De'ath & Fabricius 2000).

Densities of anemones and anemonefishes were compared between habitat types using a non-parametric Kruskal-Wallis test, since the data were heteroscedastic and non-normally distributed (Zar 1999). Relationships between pairs of variables were analysed by Spearman's rank correlation, again because the data were non-normally distributed (Zar 1999).

Statistical tests were performed using SPSS computer software (SPSS, Chicago, U.S.A.) and CART analyses were performed using S-PLUS 2000 computer software (Mathsoft, Seattle, U.S.A.). All of the data listed in the text and figures are the (untransformed) arithmetic mean \pm one standard error (SE), unless otherwise stated.

RESULTS

A total of 139225 m² of reef (i.e. 1.5% of the known reef area) was surveyed across 46 sites at the Keppel Islands (Figure 1). The mean distance swum during each dive was 605 ± 17 m and the mean area searched at each site was $3,027 \pm 84$ m². The habitat at each site ranged from well-developed coral reef with $\geq 50\%$ cover of live hard coral (e.g. Middle Island and Wreck Beach) to rocky or silty reef with $\geq 50\%$ cover of macro-algae (e.g. Maisy Bay and Corroboree Passage). The mean proportions of each substrate type across all sites were as follows: 40% live hard coral, 23% macro-algae, 20% rock, 8% soft coral, 8% unstable substrata, and 1% intact dead coral. The median structural complexity at each site ranged from 3 (e.g. Monkey Beach) to 4 (e.g. Egg Rock, 15 m) and the mean structural complexity across all sites was 3.3.

There was no significant difference in the size (t test, $t_{29} = 0.55$, $p = 0.59$), number (χ^2 test, $\chi^2_1 < 0.001$, $p > 0.98$) or species richness ($n_1 = 3$, $n_2 = 3$) of anemonefishes that were recorded on different days by the same diver at the same site. Similarly, there was no significant difference in the size (t test, $t_2 = 0.45$, $p = 0.70$), number ($n_1 = 2$, $n_2 = 2$) or species richness ($n_1 = 1$, $n_2 = 1$) of anemonefishes that were recorded on different days when different divers swam the same track at the same site (as determined by a log of GPS coordinates). The number and size of anemones, however, was variable between surveys at the same site. This variability was associated with sea conditions; during periods of high water turbulence, some anemones retracted deep into the reef matrix and effectively disappeared. Their size and number may thus have been underestimated at some sites by as much as 55% and 50%, respectively. However, it is important to note that the surveys were conducted in random order, such that weather-related phenomena were unlikely to bias the results in relation to experimental treatments (fishing status, bleaching status or depth).

Species richness

The species richness of anemones and anemonefishes at the Keppel Islands was limited to two species of anemones (*Heteractis crispa* and *Entacmaea quadricolor*) and three species of anemonefishes (*Amphiprion melanopus*, *A. akindynos* and *A. clarkii*). *Heteractis crispa* ($n = 5$) were always solitary and hosted *A. akindynos* or *A. clarkii*. *Entacmaea quadricolor* ($n = 1095$) were usually found in colonies of 3–362 individuals (mean colony size = 55 ± 20 individuals) and hosted *A. melanopus* and (or) *A. akindynos*. Most anemones hosted only a single species of anemonefish, although large colonies of *E. quadricolor* occasionally hosted both *A. melanopus* and *A. akindynos*, particularly when individuals of the latter species were small (< 3 cm TL). All anemonefishes were associated with one or more anemones and all anemones were associated with one or more anemonefishes.

Abundance and size structure

Anemones and anemonefishes were encountered during only twelve (26%) of the 46 surveys (Table 1). Total counts of anemones and anemonefishes were 1100 and 112 individuals, respectively, which together comprised 25 separate colonies (n.b. a sub-sample of 163 anemones were measured for size). The maximum number of anemones and anemonefishes encountered during any single survey was 490 and 45 individuals,

respectively. That particular survey was undertaken at Egg Rock (depth = 15 m). The overall density of anemones and anemonefishes at the Keppel Islands was estimated to be 12.2 ± 7.7 and 1.2 ± 0.7 individuals (respectively) per 1000 m² of reef habitat.

Total counts of *A. melanopus*, *A. akindynos* and *A. clarkii* across all sites were 59, 48 and 5, respectively. The mean colony size for each species of anemonefish was 4.2 ± 0.8 , 2.8 ± 0.6 and 2.5 ± 0.5 individuals, respectively. There was a significant correlation between the number of anemones and anemonefishes (all species) in each colony (Spearman's rank correlation, $\rho = 0.77$, $p < 0.001$; Figure 2a) and at each survey site ($\rho = 0.99$, $p < 0.001$; Figure 2b).

The length range of *A. melanopus* and *A. akindynos* at the Keppel Islands was 2–11 cm TL (Figure 3). The modal length class for both species was 8–10 cm TL, although a significant number of small (2–3 cm TL) *A. akindynos* were also observed. Most of these small *A. akindynos* were associated with colonies of larger *A. melanopus* (see above). The absence of anemonefishes less than 2 cm TL was not unexpected because reproduction (and thus recruitment) is restricted to late Spring and Summer when water temperature is higher (Fautin & Allen 1992; Wilkerson 1998).

Patterns of distribution

Anemones and anemonefishes were observed at 12 different sites across the Keppel Islands, from Forty-Acre Patch in the west to Egg Rock in the east (Table 1). With respect to fishing status, closed sites tended to have greater densities of anemones and anemonefishes than open sites (Figure 4a, c). Also, closed sites tended to have larger anemones and anemonefishes than open sites (Figure 4b, d). These differences, however, were not statistically significant, with the exception of the disparity in anemone size (Table 2). Another potential difference between open and closed sites was the catchability of anemones. At open sites, many of the anemones were deeply embedded in rock crevices, making them potentially difficult to collect. In contrast, many of the anemones at closed sites were attached to pieces of (potentially removable) dead coral (Figure 5).

Low-bleached sites tended to have greater densities of anemones and anemonefishes than did high-bleached sites (Figure 6a, c). Also, low-bleached sites tended to have larger anemones and anemonefishes than did high-bleached sites (Figure 6b, d). These differences, however, were not statistically significant, except for the disparity in anemone size (Table 2). Interestingly, bleached anemones were observed at two high-bleached sites (Figure 7). However, anemones in this condition were rare ($n = 4$) and comprised less than 1% of the population.

The density of both anemones and anemonefishes increased with increasing water depth (Figure 8a, c). However, these patterns were not statistically significant (Table 2). Interestingly, the mean size of anemones observed at 7 m was significantly smaller than the mean sizes of anemones observed at 3 and 15 m (Figure 8b). The mean size of anemonefishes at 7 m was also smaller than the mean sizes of anemonefishes at 3 and 15 m (Figure 8d), but the differences were not statistically significant (Table 2).

Classification and regression tree (CART) analyses revealed that depth accounted for most of the variation in anemone and anemonefish density, and anemonefish size

(Table 3). In contrast, fishing status accounted for most of the variation in anemone size. Bleaching status explained the least amount of variation in anemone density and anemone size, while fishing status explained the least amount of variation in anemonefish density and anemonefish size.

Anemones and anemonefishes were found in all four habitat types (Figure 9). In general, the highest densities of both organisms were found in habitat A (*Acropora*-dominated reef slopes) and the lowest densities were found in habitat B (*Acropora*-dominated reefs with a shallow silt base) which are generally more susceptible to bleaching. However, variability within each habitat type was extensive (n.b. anemones and anemonefishes were absent from more than half of all surveys, regardless of habitat type) such that any differences were not statistically significant (anemone density: Kruskal-Wallis test, $\chi^2_3 = 4.5$, $p = 0.21$; anemonefish density: $\chi^2_3 = 4.9$, $p = 0.18$).

The density of anemones and anemonefishes did not appear to be restricted by the amount of live coral; the percentage of live coral cover was not correlated with either anemone density (Spearman's rank correlation, $\rho = 0.04$, $p = 0.79$, Figure 10a) or anemonefish density ($\rho = 0.05$, $p = 0.73$, Figure 10b). In contrast, the density of anemones and anemonefishes tended to increase with increasing structural complexity (Spearman's rank correlation, $\rho = 0.32$, $p = 0.028$, Figure 11a; $\rho = 0.33$, $p = 0.027$, Figure 11b).

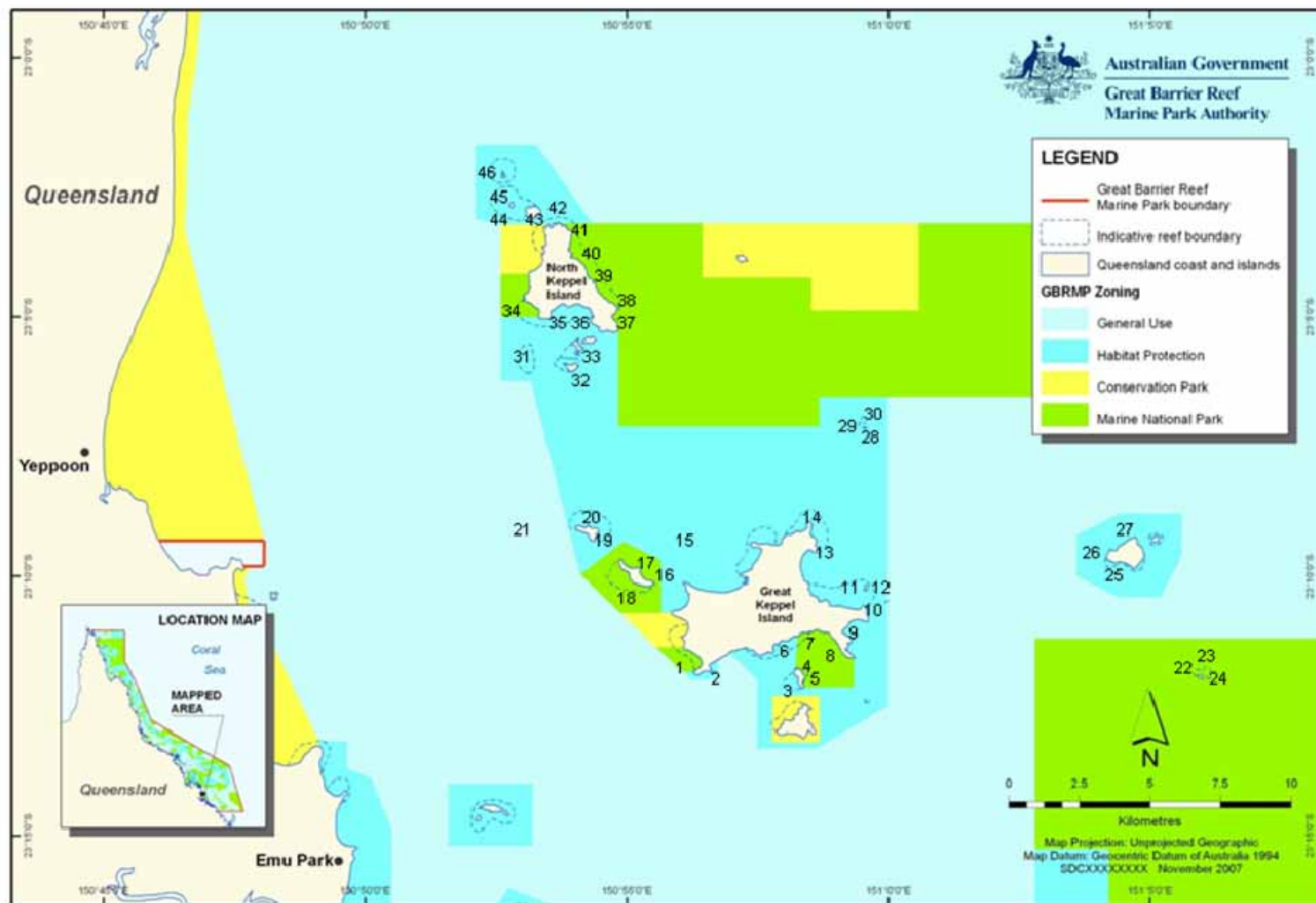


Figure 1. Approximate locations of the 46 survey sites at the Keppel Islands. Refer to Appendix 1 for descriptions of survey sites.

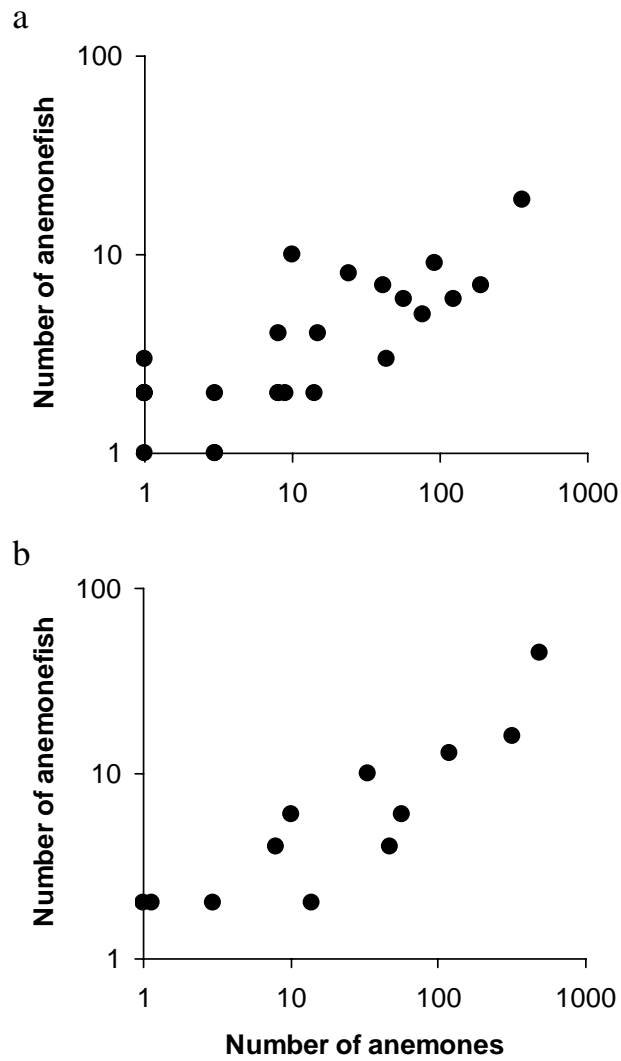


Figure 2. The relationship between the number of anemones and anemonefishes (a) per colony and (b) per survey site (~3000 m²). Both relationships were statistically significant ($p < 0.001$), as determined by Spearman's rank correlation (Zar 1999).

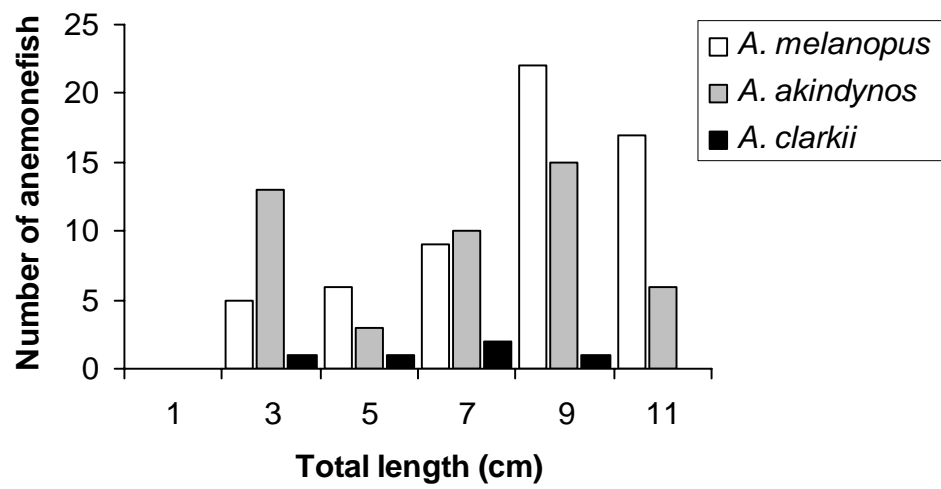


Figure 3. Length-frequency distribution of anemonefishes (*Amphiprion melanopus*, *Amphiprion akindynos*, *Amphiprion clarkii*) at the Keppel Islands. The x-axis labels are size-class midpoints (e.g. 0–1.9, 2–3.9).

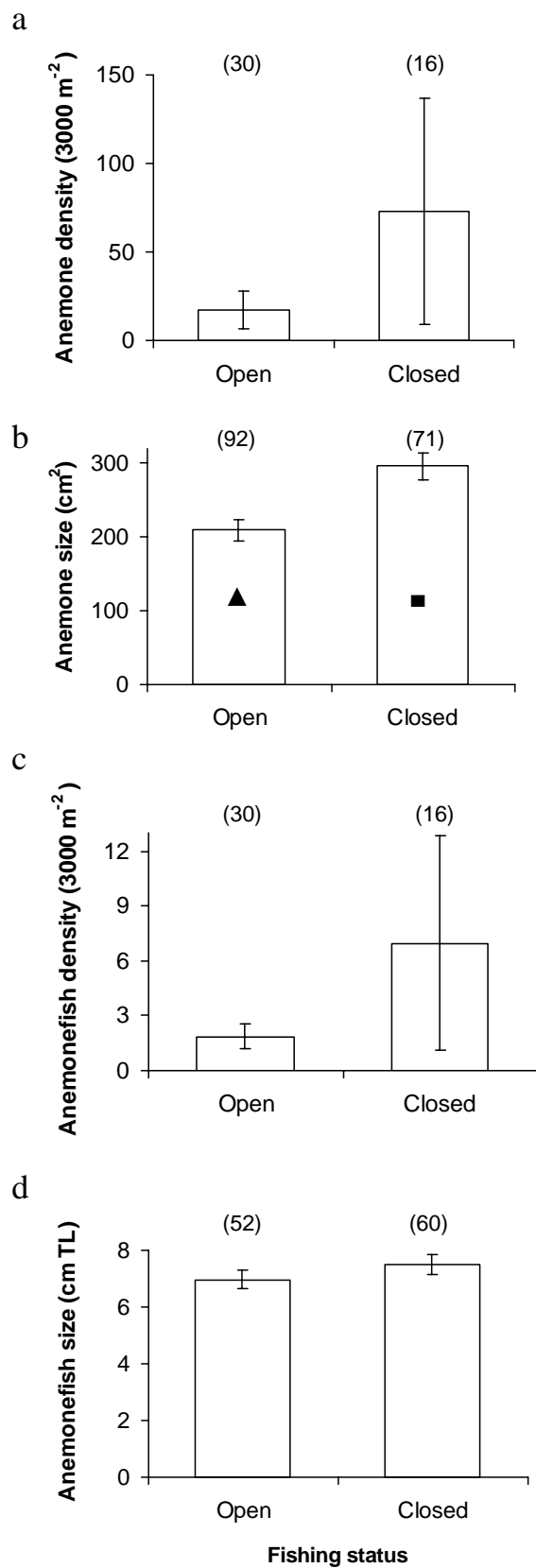


Figure 4. Mean density and mean size of anemones (a, b) and anemonefishes (c, d) in relation to fishing status. The size of anemones and anemonefishes was estimated in terms of oral disc area and total length, respectively. Sample sizes (in parentheses) reflect the number of surveys (a, c) or the number of individuals (b, d). Note that estimates of anemone size were calculated from a sub-sample of 163 anemones (see Materials and Methods section for further information). Symbols (▲ or ■) denote groups that are significantly different ($p < 0.017$).

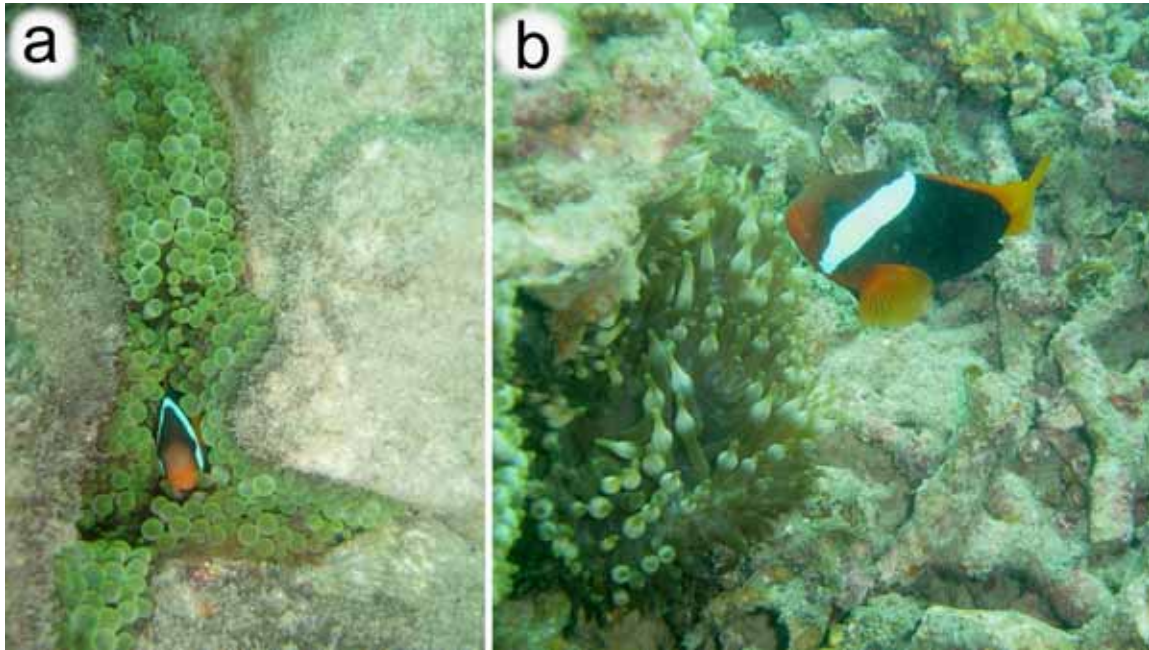


Figure 5. (a) Anemones at 'open' sites were often deeply embedded in rock crevices. (b) Anemones at 'closed' sites were often attached to dead coral. The anemones in both photographs are *Entacmaea quadricolor* and the anemonefish are *Amphiprion melanopus*. Photographs: © A. Frisch.

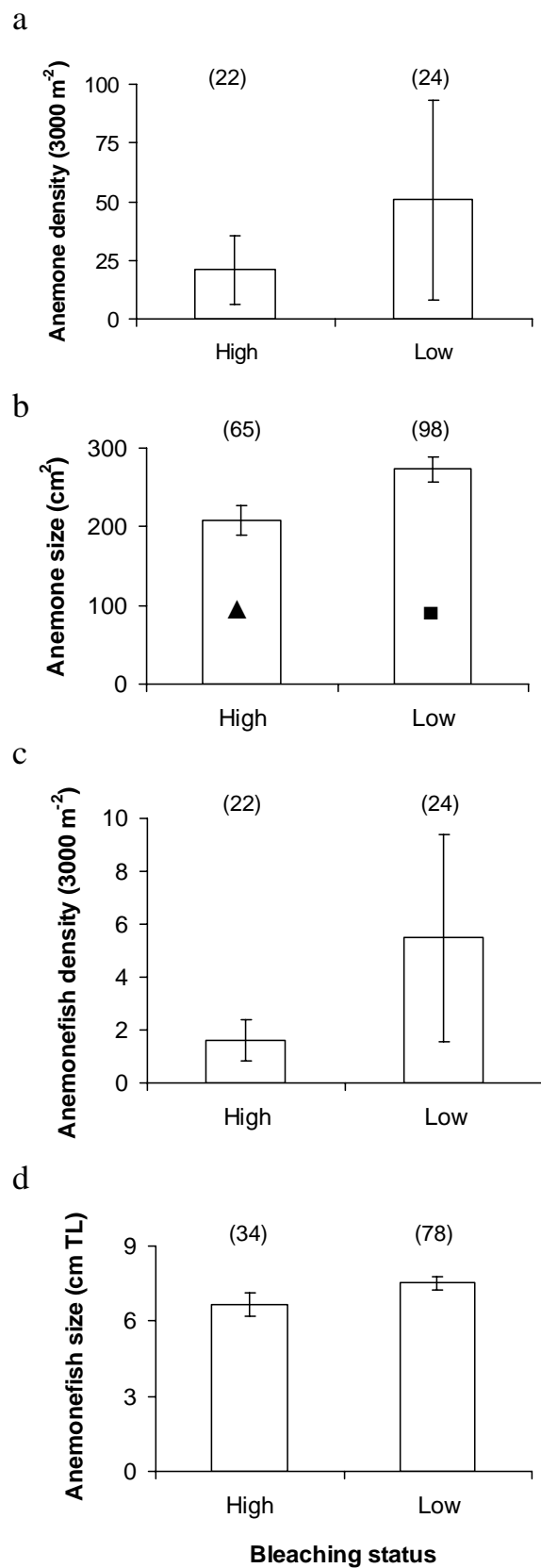


Figure 6. Mean density and mean size of anemones (a, b) and anemonefishes (c, d) in relation to bleaching status. The size of anemones and anemonefishes was estimated in terms of oral disc area and total length, respectively. Sample sizes (in parentheses) reflect the number of surveys (a, c) or the number of individuals (b, d). Note that estimates of anemone size were calculated from a sub-sample of 163 anemones (see Materials and Methods section for further information). Symbols (▲ or ■) denote groups that are significantly different ($p < 0.017$).



Figure 7. Photograph showing a bleached anemone. On the left is a normal anemone of the same species (*Entacmaea quadricolor*). Photograph: © A. Frisch.

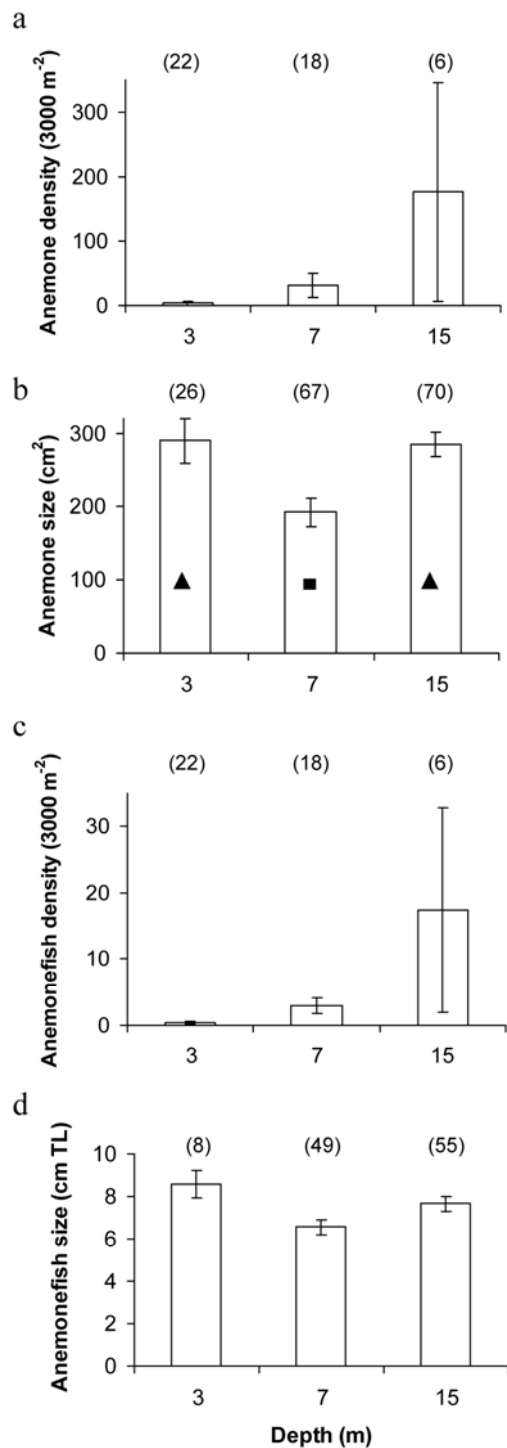


Figure 8. Mean density and mean size of anemones (a, b) and anemonefishes (c, d) in relation to water depth. The size of anemones and anemonefishes was estimated in terms of oral disc area and total length, respectively. Sample sizes (in parentheses) reflect the number of surveys (a, c) or the number of individuals (b, d). Note that estimates of anemone size were calculated from a sub-sample of 163 anemones (see Materials and Methods section for further information). Symbols (▲ or ■) denote groups that are significantly different ($p < 0.017$).

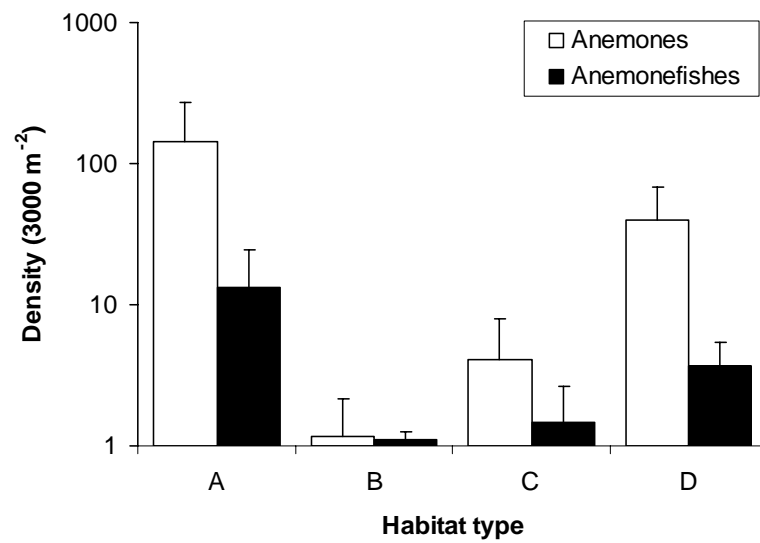


Figure 9. The mean density of anemones and anemonefishes in each of four habitat types at the Keppel Islands. See Materials and Methods section for definitions of each habitat type. Samples sizes for habitats A, B, C and D were 8, 17, 9 and 12, respectively. Differences between groups were not statistically significant ($p > 0.05$). For clarity, 'minus' error bars are not shown because they extend below the x-axis (n.b. plus and minus error bars are asymmetrical because of the logarithmic scale).

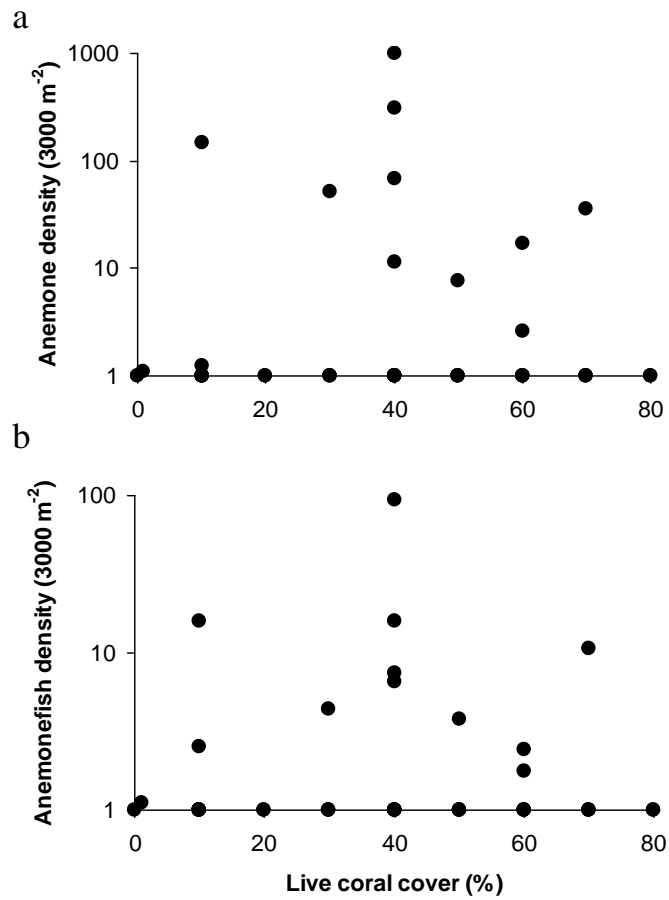


Figure 10. The density of (a) anemones and (b) anemonefishes with respect to percentage live coral cover at 46 sites in the Keppel Islands. Data points lying directly on the x -axis depict sites where anemones and anemonefishes were absent.

Table 1. The number and density of anemonefishes and host anemones (all species combined) in relation to habitat indices at each of 46 survey sites in the Keppel Islands. See Materials and Methods section for definitions of each habitat type and structural complexity.

Site no.	Location	Habitat type	Live coral cover (%)	Structural complexity (median)	Anemonefishes		Anemones	
					<i>N</i>	3000 ⁻²	<i>N</i>	3000 ⁻²
1	Monkey Beach	B	50	3	-	-	-	-
2	Monkey Point	B	80	3	-	-	-	-
3	Halfway Island	B	60	3	-	-	-	-
4	Halfway Island	A	40	3.5	-	-	-	-
5	Halfway Island	C	90	3	-	-	-	-
6	Clam Bay	B	40	3	-	-	-	-
7	Clam Bay	B	70	3	-	-	-	-
8	Clam Bay	B	30	3	-	-	-	-
9	Southeast Bay	A	40	3	6	7.1	57	67.6
10	Reef 23-032	B	60	3	2	2.4	14	16.9
11	Bald Rock	A	30	4	4	4.4	47	51.5
12	Bald Rock	C	10	3	-	-	-	-
13	Wreck Beach	B	60	3	2	1.8	3	2.6
14	Big Peninsula	D	50	3	4	3.8	8	7.6
15	Half Tide Rocks	B	60	3	-	-	-	-
16	Passage Rocks	B	80	3	-	-	-	-
17	Middle Island	B	70	3	-	-	-	-
18	Middle Island	B	50	3	-	-	-	-
19	Miall Island	B	60	3	-	-	-	-
20	Miall Island	B	50	3	-	-	-	-

Table 1. (continued)

Site no.	Location	Habitat type	Live coral cover (%)	Structural complexity (median)	Anemonefishes		Anemones	
					<i>N</i>	3000 ⁻²	<i>N</i>	3000 ⁻²
21	Forty-Acre Patch	D	40	3	6	6.9	10	11.5
22	Egg Rock	D	40	3	-	-	-	-
23	Egg Rock	D	70	3	-	-	-	-
24	Egg Rock	A	40	4	45	93.8	490	1020.8
25	Barren Island	A	40	3	-	-	-	-
26	Barren Island	A	30	3	-	-	-	-
27	Barren Island	A	60	3	-	-	-	-
28	Man & Wife Rocks	C	70	3.5	10	10.8	33	35.5
29	Man & Wife Rocks	C	10	3.5	-	-	-	-
30	Man & Wife Rocks	C	10	3	-	-	-	-
31	Square Rocks	C	10	3	2	2.5	1	1.3
32	Sloping Island	C	40	3	-	-	-	-
33	Pumpkin Island	B	20	3	-	-	-	-
34	Environment Centre	B	20	3	-	-	-	-
35	Maisy Bay	B	30	3	-	-	-	-
36	Maisy Bay	A	10	3	-	-	-	-
37	North Keppel Island	D	10	3.5	13	15.9	119	145.7
38	North Keppel Island	D	10	3	-	-	-	-
39	North Keppel Island	D	50	3	-	-	-	-
40	North Keppel Island	D	0	4	-	-	-	-

Table 1. (continued)

Site no.	Location	Habitat type	Live coral cover (%)	Structural complexity (median)	Anemonefishes		Anemones	
					<i>N</i>	3000 ⁻²	<i>N</i>	3000 ⁻²
41	North Keppel Island	D	40	3	6	6.9	10	11.5
42	Corroboree Passage	D	10	3	-	-	-	-
43	Corroboree Island	D	40	3	16	15.9	317	314.4
44	Conical Island	C	10	3	-	-	-	-
45	Conical Island	D	40	3	-	-	-	-
46	Conical Rocks	C	40	3	-	-	-	-

Table 2. Results of statistical tests comparing the density and size of anemones and anemonefishes in relation to fishing status, bleaching status and depth.

Variable	Factor	Statistical test	Test statistic (t , χ^2 or F)	Degrees of freedom	Prob. ^a	Figure no.
Anemone density	Fishing status	Student's t	0.35	44	0.73	4a
Anemone size	Fishing status	Student's t	3.71	161	<0.001	4b
Anemonefish density	Fishing status	Student's t	0.03	44	0.97	4c
Anemonefish size	Fishing status	Student's t	1.11	110	0.27	4d
Anemone density	Bleaching status	Student's t	0.57	44	0.57	6a
Anemone size	Bleaching status	Student's t	2.66	161	0.009	6b
Anemonefish density	Bleaching status	Student's t	0.39	44	0.70	6c
Anemonefish size	Bleaching status	Student's t	1.65	110	0.10	6d
Anemone density	Depth	Kruskal-Wallis	3.81	2	0.15	8a
Anemone size	Depth	ANOVA	8.13	2/160	<0.001	8b
Anemonefish density	Depth	Kruskal-Wallis	4.26	2	0.12	8c
Anemonefish size	Depth	ANOVA	3.67	2/109	0.029	8d

^a Significant differences were considered to exist when $p < 0.017$ (as per Bonferroni's adjustment; see Zar 1999).

Table 3. Summary of classification and regression tree (CART) analyses of anemone and anemonefish density and size.

Dependent variable	Explanatory factor	Split ^b
Anemone density ^a (3000 m ⁻²)	Fishing status, bleaching status, depth	Depth
Anemone density ^a (3000 m ⁻²)	Fishing status, bleaching status	Fishing status
Anemone size (cm ²)	Fishing status, bleaching status, depth	Fishing status
Anemone size (cm ²)	Bleaching status, depth	Depth
Anemonefish density ^a (3000 m ⁻²)	Fishing status, bleaching status, depth	Depth
Anemonefish density ^a (3000 m ⁻²)	Fishing status, bleaching status	Bleaching status
Anemonefish size (cm ²)	Fishing status, bleaching status, depth	Depth
Anemonefish size (cm ²)	Fishing status, bleaching status	Bleaching status

^a Indicates data that were transformed ($y = \log_{10}[x + 1]$) prior to analysis

^b Refers to the explanatory variable that accounted for the greatest amount of variability among tree clusters ('leaves')

Table 4. Density estimates of anemonefishes at various locations in the west Pacific region. Results from the present study are included for comparison.

Location	Species	Density ^a (1000 m ⁻²)	Reference
Lizard Island, Great Barrier Reef	<i>A. melanopus</i>	11.9	Srinivasan et al. 1999
Kimbe Bay, Papua New Guinea	<i>A. melanopus</i>	4	Srinivasan et al. 1999
Guam, Mariana Islands	<i>A. melanopus</i>	1	Ross 1978
Keppel Islands	<i>A. melanopus</i>	0.62	This study
Solitary Islands, New South Wales	<i>A. akindynos</i>	100–520	Richardson 1999
One Tree Island, Great Barrier Reef	<i>A. akindynos</i>	5–25	Sale et al. 1986
Julian Rocks, New South Wales	<i>A. akindynos</i>	6–8	Richardson 1996
Keppel Islands	<i>A. akindynos</i>	0.55	This study
Murote Beach, Japan	<i>A. clarkii</i>	29–39	Ochi 1989
Madang, Papua New Guinea	<i>A. clarkii</i>	0–31	Elliot 1992
Olango, Philippines ^b	<i>A. clarkii</i>	20	Shuman et al. 2005
Olango, Philippines ^c	<i>A. clarkii</i>	1.5	Shuman et al. 2005
Keppel Islands	<i>A. clarkii</i>	0.04	This study

^a Data are means or ranges

^b Protected reefs

^c Exploited reefs

Table 5. Selected quotes from the logbook of a recreational SCUBA diver at the Keppel Islands (1988–1994). Each quote refers to one dive (25–55 minutes in duration).

Location	Date	Max. depth (m)	Quote ^a
Humpy Island	28-8-88	14	‘Huge anemones with abundant clownfish’
Man & Wife Rocks	18-2-90	18	‘Found heaps of anemones. About 10 colonies. Both <i>akindynos</i> and <i>melanopus</i> ’
Monkey Beach	26-8-90	3	‘12 <i>melanopus</i> and 11 anemones’
Egg Rock	5-7-92	17	‘Heaps of tomato clowns’
Man & Wife Rocks	5-1-94	12	‘Saw ... many clownfish’

^a ‘Clownfish’ refers to any species of anemonefish. ‘Tomato clowns’ refers only to *Amphiprion melanopus*.

DISCUSSION

In general, anemones and anemonefishes were found to be rare at the Keppel Islands; only 1100 anemones and 112 anemonefishes (all species combined) were found within 139225 m² of reef habitat. Furthermore, almost half of these organisms were found at a single site (Egg Rock). It is unlikely that this result was an artefact of the sampling methodology because (1) both divers are competent (i.e. over 20 years combined experience) at finding anemones and anemonefishes, (2) timed-swims enabled adequate sampling of each site by facilitating extensive surveys across large tracts of reef, (3) the same anemonefishes were found when the same site was re-surveyed on different days, and (4) survey sites covered the vast majority of reefs at the Keppel Islands. The degree to which anemonefishes at the Keppel Islands are rare can be further demonstrated by considering the relevant published literature. In particular, estimated densities of *A. melanopus*, *A. akindynos* and *A. clarkii* at other west Pacific locations are typically an order of magnitude greater than those of conspecifics at the Keppel Islands (Table 4).

Logbook records from a local recreational SCUBA diver (1988–1994) show that anemonefish sightings were once a common occurrence at the Keppel Islands, including sites that were surveyed during the present study (Table 5). As an example, ‘12 *melanopus* and 11 anemones’ were recorded during a single dive at Monkey Beach in 1990. Not a single specimen of either organism was seen at this site during the present study. If anemonefish populations at the Keppel Islands have declined by as much as this anecdotal evidence suggests, the ‘baselines’ set by the present study will instead reflect ‘low’ levels of abundance. Hence, a caution is issued with respect to future stock assessments that evaluate changes based on the results presented here.

Because of the lack of previous quantitative information about the density of anemones and anemonefishes at the Keppel Islands, it is difficult to determine the cause of the purported declines in abundance. This problem is exacerbated by the current paucity of anemones and anemonefishes at the Keppel Islands, since the high frequency of zero values in the dataset compromises the statistical power of comparisons between experimental treatments (Zar 1999). Hence, small (but potentially important) divergences may have gone unnoticed because the minimum detectable difference for each of the statistical analyses was relatively large.

Despite the problem of low statistical power, the mean size of host anemones was found to be significantly different between sites of dissimilar fishing status, bleaching status and depth. Interestingly, closed sites generally had larger anemones than did open sites and low-bleached sites generally had larger anemones than did high-bleached sites. Whilst it is possible that these differences were caused by collecting and bleaching (respectively), an unambiguous result can only be achieved after temporal replication. With respect to depth, anemones at 7 m were generally smaller than those at 3 and 15 m. This result may reflect the fact that two of the most important (and potentially most impacted) collecting sites at the Keppel Islands were sampled at 7 m (n.b. information on the relative importance of collecting sites was provided by commercial fishers). This hypothesis is supported by CART analyses, which revealed that fishing status (as opposed to depth or bleaching status) accounted for the greatest amount of variability in anemone size between

sites. It should also be noted that anemone size may be influenced directly by collecting, or indirectly by removal of anemonefish (see below).

Field and laboratory observations indicate that individual anemones live for many decades, that successful recruitment is rare, and that asexual 'budding' is the predominant mode of reproduction for species such as *E. quadricolor* (Fautin & Allen 1992). Also, it has been experimentally demonstrated that host anemones require the protection of anemonefish to grow and reproduce (Godwin & Fautin 1992; Porat & Chadwick-Furman 2004; Holbrook & Schmitt 2005). Thus, it can be inferred that whole anemone colonies may be very old and that their natural rate of increase (in terms of both size and number) is extremely slow. For this reason, it may take several decades for anemone (and thus anemonefish) populations to recover from severe disturbance or over-exploitation. Contemporary comparisons of 'open' versus 'closed' sites may therefore be confounded by historical collecting that occurred before closed sites became protected (i.e. before 1988 in the case of Middle Island or 2004 in the cases of Clam Bay, Monkey Beach, Environment Centre and North Keppel Island). This concept will also apply to any future stock assessment undertaken at the Keppel Islands during the next few decades.

Habitat type (A, B, C or D) and amount (%) of live coral cover were found to have little or no influence on the densities of anemones and anemonefishes at the Keppel Islands. Thus, habitat type and percentage live coral cover are probably unsuitable surrogates on which to base any future spatial management scheme. A more appropriate surrogate for this purpose is probably structural complexity. It should also be noted that a high proportion of all anemones and anemonefishes at the Keppel Islands were found at just a few sites. Given the rarity of anemones and anemonefishes at the Keppel Islands, these 'special' sites may warrant close attention by management agencies. In any case, it will be crucial to manage anemone and anemonefish populations simultaneously, because these organisms are mutually dependent on each other.

In summary, anecdotal evidence suggests that the abundance of anemones and anemonefishes at the Keppel Islands has declined significantly in recent years. More importantly, this study has demonstrated that anemones and anemonefishes are currently rare at the Keppel Islands, at least at the locations sampled. Comparisons of abundance across different sites revealed spatial patterns that would be expected if anemone and anemonefish populations were previously impacted by both bleaching and collecting. However, it was not possible to reach an unequivocal conclusion with respect to causality because of the lack of temporal replication and the overall rarity of anemones and anemonefishes at the Keppel Islands. Regardless of what may have caused the apparent decline in abundance, the unique biological characteristics of anemones and anemonefishes dictate that future management will need to be careful and considered.

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Appendix 1. Description and coordinates of the 46 survey sites at the Keppel Islands.

Site no.	Location	Coordinates	Survey area (m ²)	Fishing status	Depth (m)	Bleaching status ^a
1	Monkey Beach	S23 11.796 E150 56.241	2655	Closed	3	High
2	Monkey Point	S23 11.751 E150 56.604	3040	Open	3	High
3	Halfway Island	S23 12.155 E150 58.104	2735	Open	7	High
4	Halfway Island	S23 11.725 E150 58.250	3190	Closed	7	High
5	Halfway Island	S23 11.970 E150 58.406	3120	Closed	15	High
6	Clam Bay	S23 11.294 E150 58.133	2830	Open	7	High
7	Clam Bay	S23 11.304 E150 58.885	3175	Closed	3	High
8	Clam Bay	S23 11.428 E150 59.023	3850	Closed	7	High
9	Southeast Bay	S23 10.896 E150 59.557	2530	Open	7	High
10	Reef 23-032	S23 10.572 E150 59.659	2480	Open	3	High
11	Bald Rock	S23 10.191 E150 59.591	2740	Open	3	High
12	Bald Rock	S23 10.341 E150 59.624	3705	Open	15	High
13	Wreck Beach	S23 09.539 E150 58.554	3415	Open	3	High
14	Big Peninsula	S23 08.988 E150 58.599	3150	Open	7	High
15	Half Tide Rocks	S23 09.261 E150 56.282	2920	Open	3	High
16	Passage Rocks	S23 10.081 E150 55.812	2450	Open	3	High
17	Middle Island	S23 09.877 E150 55.302	3725	Closed	3	High
18	Middle Island	S23 10.238 E150 55.145	3480	Closed	3	High
19	Miall Island	S23 09.211 E150 54.025	4970	Open	3	Low
20	Miall Island	S23 09.016 E150 54.206	3685	Open	7	Low

Appendix 1. (continued)

Site no.	Location	Coordinates	Survey area (m ²)	Fishing status	Depth (m)	Bleaching status ^a
21	Forty-Acre Patch	S23 09.002 E150 53.312	2615	Open	7	Low
22	Egg Rock	S23 11.978 E151 05.916	2770	Closed	7	Low
23	Egg Rock	S23 11.974 E151 06.078	2595	Closed	3	Low
24	Egg Rock	S23 11.965 E151 05.909	1440	Closed	15	Low
25	Barren Island	S23 09.721 E151 04.149	2650	Open	15	Low
26	Barren Island	S23 09.552 E151 04.121	2640	Open	7	Low
27	Barren Island	S23 09.441 E151 04.325	2425	Open	3	Low
28	Man & Wife Rocks	S23 07.141 E150 59.489	2790	Open	15	Low
29	Man & Wife Rocks	S23 07.157 E150 59.526	3680	Open	7	Low
30	Man & Wife Rocks	S23 07.147 E150 59.521	3740	Open	3	Low
31	Square Rocks	S23 06.295 E150 53.073	2385	Open	7	Low
32	Sloping Island	S23 06.027 E150 54.045	2785	Open	7	Low
33	Pumpkin Island	S23 05.525 E150 54.208	2515	Open	7	Low
34	Environment Centre	S23 04.794 E150 53.033	3070	Closed	3	High
35	Maisy Bay	S23 05.171 E150 53.718	3240	Open	3	High
36	Maisy Bay	S23 05.144 E150 54.218	3580	Open	7	High
37	North Keppel Island	S23 04.991 E150 54.763	2450	Closed	7	Low
38	North Keppel Island	S23 04.845 E150 54.813	3275	Closed	15	Low
39	North Keppel Island	S23 04.353 E150 54.429	3290	Closed	3	Low
40	North Keppel Island	S23 03.931 E150 54.172	2885	Closed	3	Low

Appendix 1. (continued)

Site no.	Location	Coordinates	Survey area (m ²)	Fishing status	Depth (m)	Bleaching status ^a
41	North Keppel Island	S23 03.532 E150 53.922	3050	Closed	7	Low
42	Corroboree Passage	S23 03.177 E150 53.424	3895	Open	3	Low
43	Corroboree Island	S23 03.023 E150 53.342	3025	Open	7	High
44	Conical Island	S23 02.958 E150 52.794	2870	Open	3	Low
45	Conical Island	S23 02.842 E150 52.703	2720	Open	3	Low
46	Conical Rocks	S23 02.165 E150 52.565	3000	Open	3	Low

^a Refers to the severity of coral bleaching, as recorded in April 2007 (see GBRMPA 2007).