

Anemonefish depletion reduces survival, growth, reproduction and fishery productivity of mutualistic anemone–anemonefish colonies

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Abstract Intimate knowledge of both partners in a mutualism is necessary to understand the ecology and evolution of each partner, and to manage human impacts that asymmetrically affect one of the partners. Although anemonefishes and their host anemones are iconic mutualists and widely sought by ornamental fisheries, the degree to which anemones depend on anemonefishes, and thus the colony-level effects of collecting anemonefishes, is not well understood. We tracked the size and abundance of anemone *Entacmaea quadricolor* and anemonefish *Amphiprion melanopus* colonies for 3 yr after none, some, or all of the resident anemonefish were experimentally removed. Total and partial removal of anemonefish had rapid and sustained negative effects on growth, reproduction and survival of anemones, as well as cascading effects on recruitment and productivity of anemonefish in the remaining colony. As predicted, total removal of anemonefish caused acute declines in size and abundance of anemones, although most anemone colonies (76 %) slowly resumed growth and reproduction after the arrival of anemonefish recruits, which subsequently grew and defended the hosts. Partial removal of anemonefish had similar but typically less severe effects on anemones.

Remarkably, the colony-level effects on anemones and anemonefish were proportional to the size and number of anemonefish that were experimentally removed. In particular, anemone survival and anemonefish productivity were highest when one or more adult anemonefish remained in the colony, suggesting that adult fish not only enhanced the protection of anemones, but also increased the recruitment and/or survival of conspecifics. We conclude that the relationship between *E. quadricolor* and *A. melanopus* is not only obligate, but also demographically rigid and easily perturbed by anemonefish fisheries. Clearly, these two species must be managed together as a unit and with utmost precaution. To this end, we propose several tangible management actions that will help to minimize fishing effects.

Keywords Symbiosis · Mutualism · *Amphiprion* · Ornamental fisheries · Fisheries management · Great Barrier Reef

Introduction

Mutualistic relationships, where both partners derive benefit, are common on coral reefs, particularly among fishes, crustaceans, sponges and cnidarians (Fautin and Allen 1997; Wulff 1997; Knowlton and Rohwer 2003). Among mutualistic partners, there is great diversity in the goods or services that are exchanged and in the degree of dependence between partners. For example, trapeziid crabs (*Tetralia*, *Tetraloides* and *Trapezia*) receive protective shelter within and repel predatory starfish from reef-building corals (Family Pocilloporidae and Acroporidae). The distribution and abundance of the crabs are strongly linked to those of the corals, but not vice versa, because the crabs

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(obligate mutualists) depend on the corals for survival, whereas the corals (facultative mutualists) can survive without the crabs, albeit without the benefits (Sin and Lee 2000; Pratchett 2001). Therefore, detailed knowledge of both partners in a mutualism is necessary to understand the ecology and evolution of each partner, and to manage human impacts that disproportionately affect one of the partners.

Twenty-eight species of anemonefishes (*Amphiprion* and *Premnas*) form mutualistic relationships with 10 species of sea anemones (Order Actiniaria) on coral reefs in the Indo-Pacific region (Fautin and Allen 1997; Allen et al. 2008, 2010). Wild anemonefishes are never found without anemones and are rapidly eaten by predators when deprived of their host, indicating that anemonefishes are obligate mutualists (Fautin and Allen 1997). Anemonefishes have a special mucous coating that prevents them from being stung by anemone tentacles, which thereby provide a refuge from potential predators. Anemonefish also depend on anemones for reproduction, since their eggs must be laid on hard substrate beneath an anemone's oral disc. In return for hosting anemonefish, the anemones are supplied with elevated levels of oxygen and nutrients (Cleveland et al. 2011; Szczebak et al. 2013), such that occupied anemones grow faster and reproduce more often than unoccupied anemones (Holbrook and Schmitt 2005; Porat and Chadwick-Furman 2004, 2005). Despite these physiological benefits, the degree to which anemones are dependent on resident anemonefish for survival is poorly understood.

Some species of host anemone, such as the highly conspicuous magnificent anemone *Heteractis magnifica*, survive with or without anemonefish, suggesting they are facultative mutualists (Fautin 1992; Holbrook and Schmitt 2005). Other species, such as the relatively cryptic but widespread bubble-tip anemone *Entacmaea quadricolor*, are often unoccupied on subtropical reefs, but are rarely (if ever) unoccupied on tropical reefs (Scott et al. 2011; Scott and Baird 2014). The difference in occupancy is probably related to the geographic distribution of anemone predators such as corallivorous butterflyfish (Godwin and Fautin 1992; Porat and Chadwick-Furman 2004). On tropical reefs, adult anemonefish aggressively defend their hosts against butterflyfish, such that experimental removal of resident anemonefish can cause *E. quadricolor* to rapidly 'disappear': the anemones either contract and retreat into the protective reef matrix or are eaten by butterflyfishes (Godwin and Fautin 1992; Porat and Chadwick-Furman 2004). Contracted anemones are limited in their ability to feed on zooplankton, receive light for photosynthesis, or efficiently absorb nutrients excreted by resident anemonefish, which together may cause anemones to shrink and eventually die (Porat and Chadwick-Furman 2004, 2005).

If *E. quadricolor* is dependent on resident anemonefish for survival on tropical reefs, as these observations indicate, then recruitment of new anemone–anemonefish colonies presents a puzzling enigma: how can *E. quadricolor* reproduce, disperse and colonize new reefs if anemones cannot survive without resident anemonefish? An extension of this question is whether depletion of anemonefish, either by natural or fishing mortality, spells death for all anemones in a colony or whether some (temporarily hidden) anemones recover by recruiting new anemonefish to defend them from predators. Resolving this enigma has important implications for understanding the ecology and evolution of anemone–anemonefish mutualisms and for effective management of exploited populations.

Due to their bright colors and close association with each other, anemones and anemonefishes are iconic and highly desired by marine aquarists. As a result, anemones and anemonefishes are exported from many Indo-Pacific countries, and anemonefishes are the most traded species in the global marine ornamentals trade, which has an estimated annual value of US \$200–300 million (Wabnitz et al. 2003). Although reliable catch records are not available for any exporting country, anemones and anemonefishes are known to comprise a substantial portion of the marine ornamental trade (up to 60 % in some areas), so fishing pressure on anemones and anemonefishes is thought to be high wherever these organisms are targeted (Wabnitz et al. 2003; Shuman et al. 2005; Jones et al. 2008; Madduppa et al. 2014). Despite the economic value of the trade and the iconic profile of the resource, only five studies have ever investigated the population-level consequences of collecting anemones or anemonefishes anywhere in the world. Unanimously, all five studies reported larger sizes and/or higher densities of anemones and anemonefishes in protected areas relative to fished areas (or higher densities with increasing time since protection), suggesting that many populations are suppressed by collecting (Shuman et al. 2005; Jones et al. 2008; Frisch and Hobbs 2009; Scott et al. 2011; Madduppa et al. 2014). In a fisheries context, the major concerns are that (1) anemones and anemonefishes may be long-lived with low natural mortality (Fautin and Allen 1997; Buston and Garcia 2006); (2) anemone colonies seldom move (Fautin and Allen 1997; Holbrook and Schmitt 2005), thereby enabling repeated visitation by collectors and repeated depletion of anemonefishes; (3) anemonefishes have limited dispersal capabilities and are often self-recruiting (Jones et al. 2005; Almany et al. 2007); and (4) both groups of organisms may be mutually dependent on each other for growth, reproduction and survival (see above). These unique life history characteristics, combined with high market demand and collecting pressure, render anemones and anemonefishes exceptionally vulnerable to over-exploitation (Shuman

et al. 2005; Jones et al. 2008). Therefore, a thorough understanding of the colony-level effects of collecting anemones and particularly anemonefishes is required to complement knowledge of population-level effects and to inform management initiatives that aim to minimize the effects of collecting one or other (or both) of the partners.

Due to regional differences in market demand and socioeconomic conditions, anemonefish collection practices vary substantially among countries. In the Philippines, anemonefishes of all sizes are collected, leaving anemones without residents, except for the occasional young recruit (AJF, pers. obs.). In contrast, on Australia's Great Barrier Reef (GBR), standard practice for commercial collectors is to remove a breeding pair of adults and/or multiple subadults from each anemone or colony, leaving at least one (sometimes juvenile) anemonefish remaining (Jones et al. 2008; M. Atkinson, pers. comm.). However, these practices may be suboptimal or even detrimental because (1) fecundity increases exponentially with increasing body size (Sadovy 1996), so systematic removal of large adult fish, such as breeding pairs, greatly reduces the reproductive output of the population; (2) the absence of adult fish may expose anemones to predation by butterflyfishes, thereby reducing anemone size and/or survival, and creating a positive feedback loop that reduces the available habitat for future recruitment of anemonefishes (Godwin and Fautin 1992; Porat and Chadwick-Furman 2004); and (3) natural mortality of juvenile anemonefish is high and density-dependent (Buston 2003a), indicating that productivity (harvest potential) of juvenile fish may be much greater than that of adult fish. As these observations suggest, the colony-level effects of collecting anemonefishes are likely to be dependent on the size and/or number of anemonefishes that are removed from each anemone. Therefore, an evaluation of the various collection practices and their effects on the productivity of anemone–anemonefish colonies is warranted.

Entacmaea quadricolor, the dominant species of anemone on the GBR (Hobbs et al. 2013; Scott and Baird 2014), has been commercially collected in the region for at least 45 yr, which may have contributed to observed population declines in high-use areas (Jones et al. 2008; Frisch and Hobbs 2009). Unlike most other anemones, which are typically solitary and host relatively small groups of anemonefish (Fautin and Allen 1997), *E. quadricolor* can form vast colonies (up to 362 polyps) that host large groups of anemonefish: 50 or more individuals in the case of the cinnamon anemonefish *Amphiprion melanopus* (Richardson et al. 1997a; Frisch and Hobbs 2009). *Amphiprion melanopus* is also one of the most aggressive anemonefishes and least willing to share its host, presumably due to its greater host specificity relative to other anemonefishes (Fautin and Allen 1997; Elliot and Mariscal 2001; Ollerton

et al. 2007) and/or the high palatability of its preferred host (*E. quadricolor*) to butterflyfishes (Godwin and Fautin 1992; Porat and Chadwick-Furman 2004). Therefore, *A. melanopus* is expected to influence the colony dynamics of *E. quadricolor* to a greater extent than most other combinations of anemone and anemonefish species. For this reason, fisheries that target *E. quadricolor* and *A. melanopus* may be most in need of revised collection practices.

Here, we report results from a 3-yr manipulative field experiment designed to evaluate the effects of anemonefish depletion on the colony-level dynamics and fishery productivity of anemone–anemonefish colonies (*E. quadricolor* and *A. melanopus*). Our specific objectives were to (1) determine the extent to which *E. quadricolor* is dependent on *A. melanopus* for growth, reproduction and survival; (2) evaluate whether *E. quadricolor* has the capacity to recover from fish depletion via settlement and recruitment of new *A. melanopus*; and (3) develop sustainable harvest strategies that optimize colony productivity. To do this, resident *A. melanopus* of different sizes and numbers were removed from host *E. quadricolor* followed by quantification of colony attributes at various times thereafter.

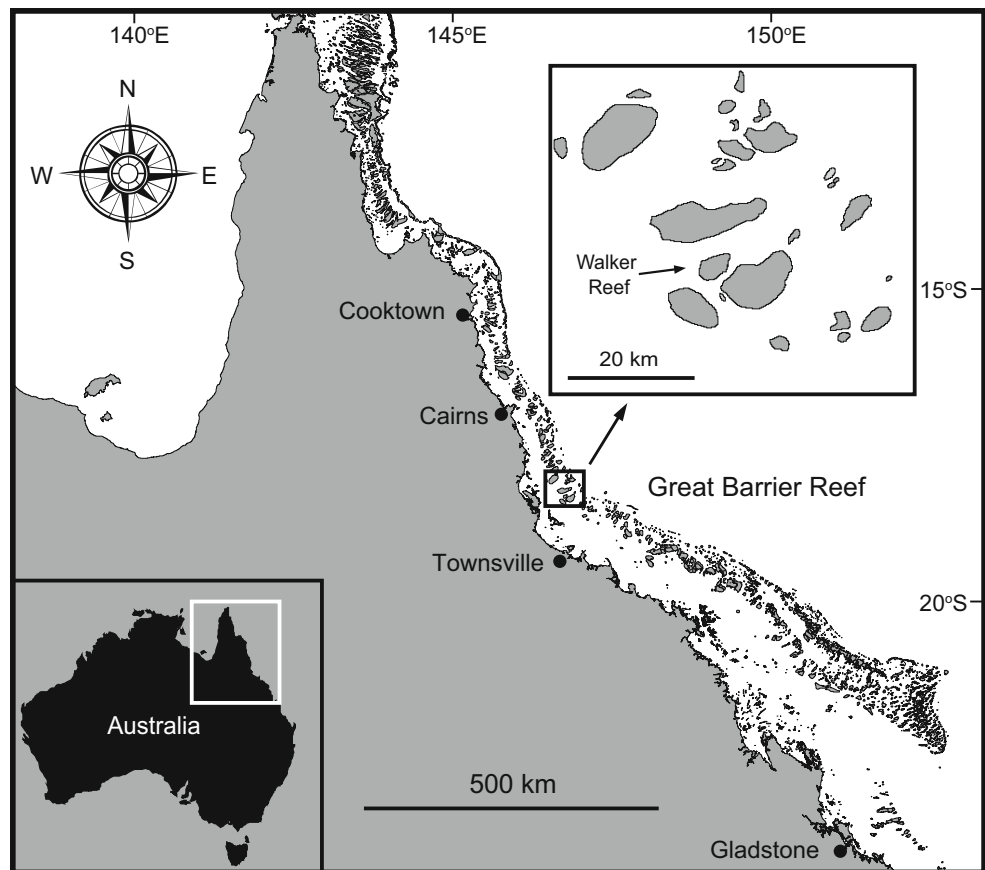
Materials and methods

Location and species

The study was undertaken at Walker Reef (18°19'S, 146°45'E), which is a typical platform reef located ~50 km from the mainland coast in the central section of the GBR, Australia (Fig. 1). Like other reefs in the area, Walker Reef supports a high diversity and abundance of corals and fishes, including anemone predators such as *Chaetodon lineolatus* and *C. ulietensis* (Williams and Hatcher 1983). Moderate densities of *E. quadricolor* (~158 ha⁻¹) and *A. melanopus* (~34 ha⁻¹) can be found on hard substrates throughout the reef. Unlike anemone and anemonefish populations elsewhere (Jones et al. 2008; Hobbs et al. 2013), those at Walker Reef showed no evidence of mass bleaching of anemones and no recorded history of commercial harvesting (verified by fishery logbook data, available at <http://qfish.fisheries.qld.gov.au>). Due to Walker Reef's remote offshore location, it is seldom (if ever) visited by recreational fish collectors, and terrestrial nutrient and sediment inputs are likely to be low or negligible.

Entacmaea quadricolor are typically brown with long, bulb-tipped tentacles. In shallow water (e.g., reef crest), polyps tend to be numerous, small (7–15 cm oral disc diameter) and clustered together in crevices, so that

Fig. 1 Map of the study area, Walker Reef, Great Barrier Reef, Australia



tentacles are confluent and form an extensive colony that resident anemonefish usually regard as a single large anemone. All the polyps in these colonies are thought to be clones produced asexually by longitudinal fission (Fautin and Allen 1997; Scott et al. 2014). In deeper water (e.g., reef slope), polyps tend to be large (20–40 cm diameter) and exist as small groups or solitary individuals (Fautin and Allen 1997; Richardson et al. 1997a). In both colonial and solitary forms, the pedal disc of each polyp is typically anchored in a deep hole or crevice so that only emergent tentacles are visible. Although *E. quadricolor* is host to many anemonefish species, those at Walker Reef are most often occupied by mono-specific groups of *A. melanopus*. Reproduction (and recruitment) of *A. melanopus* occurs from mid-spring to mid-autumn, but is highest in summer (December to February; Fautin and Allen 1997; Richardson et al. 1997b).

Data collection

A systematic search of a 1.5 km² area of shallow coral reef (3–7 m depth) was undertaken in September 2005 to determine the distribution and abundance of anemones and anemonefish. Forty-seven colonies of *E. quadricolor* and *A. melanopus* were located and individually marked with

benthic tags and GPS coordinates to ensure easy relocation. All 47 colonies were well separated in space (>50 m apart), indicating that the probability of adjacent anemone colonies merging (or anemonefish moving between colonies) was extremely low (Elliot and Mariscal 2001; Hattori 2006).

Anemonefish size (total length, *L*) was estimated visually by a single observer (AJF) who periodically practiced underwater estimation of *L* using 12 plastic fish models of different sizes. In all five trials, differences between estimated and actual *L* of models were not significantly different from zero (paired *t* test). Based on estimates of *L*, anemonefish were categorized as recruits (<20 mm), juveniles (20–50 mm) or adults (>50 mm), and the number of recruits in a colony was inferred to represent instantaneous recruitment (Elliot and Mariscal 2001; Buston 2003b). To provide a standardized measure of the quantity of anemonefish associated with each anemone colony, the weight and abundance of resident anemonefish were used to calculate group biomass (*B*). To do this, a subsample of captured anemonefish (see below) were weighed to the nearest 0.01 g on an electronic balance, measured to the nearest 0.5 mm *L* using Vernier calipers, and the bivariate data were used to construct a length–weight curve (Fig. 2). Biomass of each anemonefish colony was then calculated as follows:

$$B = \Sigma(0.015 \times L_i^{3.15}),$$

where B and L are denoted in g and cm, respectively.

Anemone polyp size (oral disc area, ODA) was calculated using the formula:

$$\text{ODA} = L_{\max} \times W \times \Pi \div 4,$$

where L_{\max} and W are the maximum length and perpendicular width of the oral disc, respectively, as measured with a flexible tape (Holbrook and Schmitt 2005). If the number of anemones in a colony was large, the size of 20 haphazardly selected polyps was measured as a representative sample. Anemone colony area was calculated as the sum of the ODAs (ΣODA_i) of constituent anemones or, for large colonies, the product of mean ODA and the number of anemones in the colony. Anemone colony area (or group biomass, in the case of anemonefish) was regarded as the best indicator of group size because it incorporates both polyp (or fish) size and abundance information (Elliot and Mariscal 2001). When counting colonial anemones, each individual was lightly touched by hand (causing slight polyp contraction) to differentiate between confluent anemones. Any temporal increase in the abundance of anemones was used as a retrospective indicator of asexual reproduction. To avoid bias caused by diurnal expansion behavior of anemones, all measurements of ODA were taken on calm, sunny days between 1000 and 1500 hrs. All anemones and anemonefishes appeared healthy at commencement of the experiment.

Experimental design

Each of the 47 anemone–anemonefish colonies was randomly assigned to one of three treatment groups. In the first group (‘total removal’, $n = 17$ colonies), all anemonefish were removed by capture with a scoop-net and clove oil

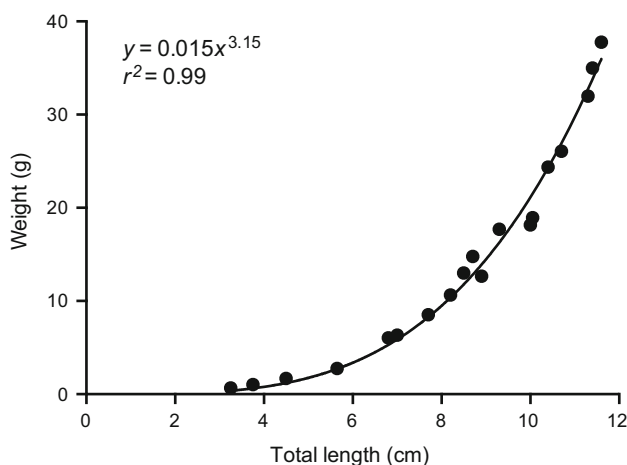


Fig. 2 Length–weight relationship for cinnamon anemonefish *Amphiprion melanopus*

(10 %, in ethanol). In the second group (‘partial removal’, $n = 17$ colonies), haphazardly derived proportions of resident anemonefish were removed from each colony using the same method. The proportions of anemonefish that were removed ranged from 20 to 86 % by number (median 50 %) and 5–99 % by biomass (median 68 %), and included a haphazard distribution of recruit, juvenile and adult anemonefish. Captured anemonefish were transported to the mainland and subsequently used to construct a length–weight curve (see above) or used in other experiments (e.g., Roy et al. 2013). In the third group (‘control’, $n = 13$ colonies), all fish were captured (as above) and immediately returned to their host anemone (as control for the potential effects of clove oil: Frisch et al. 2007). All anemonefish removals occurred at commencement of the experiment, after which there were no further removals. The abundance, mean polyp size (ODA) and total colony size (ΣODA_i) of sea anemones, and the abundance, mean size (L) and group size (B) of anemonefish in each treatment group were then assessed at 0, 2, 6, 12 and 36 months, commencing on September 10, 2005. If a colony could not be located, the surrounding reef (within a 50 m radius) was thoroughly searched. Due to the limited movement capacity of anemones, colonies that disappeared were presumed to have died (Holbrook and Schmitt 2005).

Data analysis

Bivariate relationships between the size and number of anemones and anemonefish in each colony were evaluated by Spearman’s rank correlation, and the distribution of dead anemone colonies among treatment groups was analyzed by a χ^2 homogeneity test. To investigate the effect of anemonefish removal on abundance, mean polyp size (ODA) and anemone colony area (ΣODA_i), temporal observations were first standardized to an index of percent change ($y = [x_2 - x_1]/x_1 \times 100$) to reduce variability caused by differences in initial abundance, size and area (x_1) within each treatment. Data were then analyzed by two-way, repeated-measures analysis of variance (RM-ANOVA) with treatment and time as factors. Anemonefish data (abundance, group biomass and recruitment) were analyzed in the same way, except that temporal observations were converted to an index of absolute change ($y = x_2 - x_1$) to avoid zeros in treatments where anemonefish were experimentally removed. In all cases, homoscedasticity and sphericity (assumptions of RM-ANOVA) were checked a priori with Levene’s and Mauchly’s tests, respectively. Where necessary, data were transformed ($y = \log_{10}[x + 101]$ or $[x + 101]^2$) and the Greenhouse–Geisser correction was applied. To resolve differences between groups within each time period, data were analyzed either by one-way analysis of variance (ANOVA) and Tukey’s post hoc test, or the

Kruskal–Wallis test if the data were nonparametric (Bonferroni's correction was applied to ensure a constant probability of Type I error). For some of the analyses, data from the 'partial removal' group were retrospectively divided into two subgroups ('adults present' and 'adults absent') to differentiate between colonies that contained recruits, juveniles and adults from colonies that contained recruits and juveniles only. The effect of anemonefish removal on anemone colonies was further investigated by Spearman's rank correlation, with the magnitude of anemonefish removal expressed as a continuous variable (i.e., as a percentage of initial abundance or biomass, from 0 to 100 %). All data provided in the text and figures are expressed as the arithmetic mean \pm standard error (SE) of untransformed data. Statistically significant differences were considered to exist if $P < 0.05$, except when Bonferroni's correction was used.

Results

The sizes of anemone and anemonefish colonies at Walker Reef were highly variable but correlated. There were 1–110 anemones per colony (median 9) and 1–30 anemonefish per colony (median 4), with significant positive relationships between anemone colony area and anemonefish abundance ($r_s = 0.88$, $P < 0.001$; Fig. 3a) and between anemone colony area and anemonefish group biomass ($r_s = 0.86$, $P < 0.001$; Fig. 3b). Although not directly quantified, the behavior of anemonefish was highly variable and dependent on size (L). When a diver approached the host, many of the large (adult) anemonefish became aggressive and occasionally bit the diver, whereas small (recruit and juvenile) anemonefish always retreated and hid among anemone tentacles. Prior to commencement of the experiment, there were no significant differences in abundance, mean size (ODA or L), size distribution (ODA or L) and colony size (ΣODA_i or B) of anemones and anemonefish among treatment groups (one-way ANOVA and Kolmogorov–Smirnov tests; $P > 0.39$ in all cases), suggesting that experimental results presented hereafter were unlikely to be confounded by initial (pre-treatment) colony characteristics.

Effects of anemonefish removal on anemones

No colonies moved > 2 m, but five of 47 colonies could not be found despite extensive searching and were presumed dead. The proportion of dead colonies was not significantly different among treatment groups, potentially due to small sample sizes (Fig. 4a). However, the proportion of dead colonies among groups with and without adult anemonefish was significantly different ($\chi^2_1 = 5.36$, $P = 0.021$; Fig. 4b), suggesting the absence of adult fish increased the likelihood of anemone colony mortality.

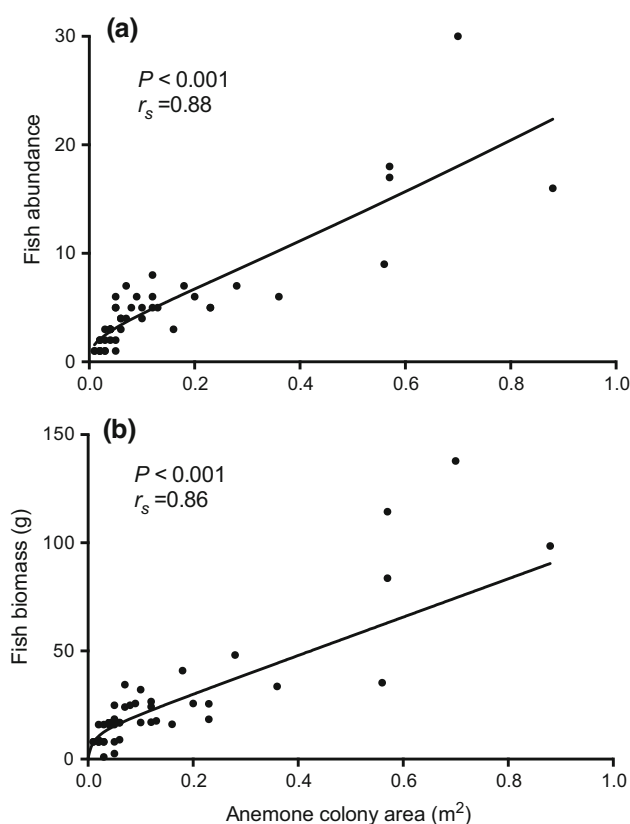


Fig. 3 Relationships between anemone colony size (combined surface area of oral discs) and **a** abundance and **b** group biomass of anemonefish in each colony. Significant relationships were identified by Spearman's rank correlation coefficient (r_s) and are depicted by power curves

Removal of anemonefish had rapid and sustained effects on anemone colonies. Polyp abundance (Fig. 5a), mean polyp size (Fig. 5b) and colony area (Fig. 5c), which are proxies for asexual reproduction, growth and quantity of anemonefish habitat, respectively, increased steadily in 'control' colonies, but typically decreased or remained unchanged in 'partial removal' and 'total removal' colonies, depending on the quantity of anemonefish that were removed. Differences between control and manipulated groups formed rapidly (within 2 months) and typically increased through time (up to 3 yr). However, the responses of individual colonies to manipulation were highly variable, such that treatment effects and interactions were not always statistically significant (Table 1).

In general, total removal of anemonefish had a greater effect on anemones than did partial removal of anemonefish. There was a significant negative relationship between the proportion of anemonefish removed (measured either as percent frequency or percent group biomass) and the proportional change in anemone colony area, regardless of time after manipulation ($P < 0.001$ in all cases). Spearman's correlation coefficient (r_s), a measure of the strength

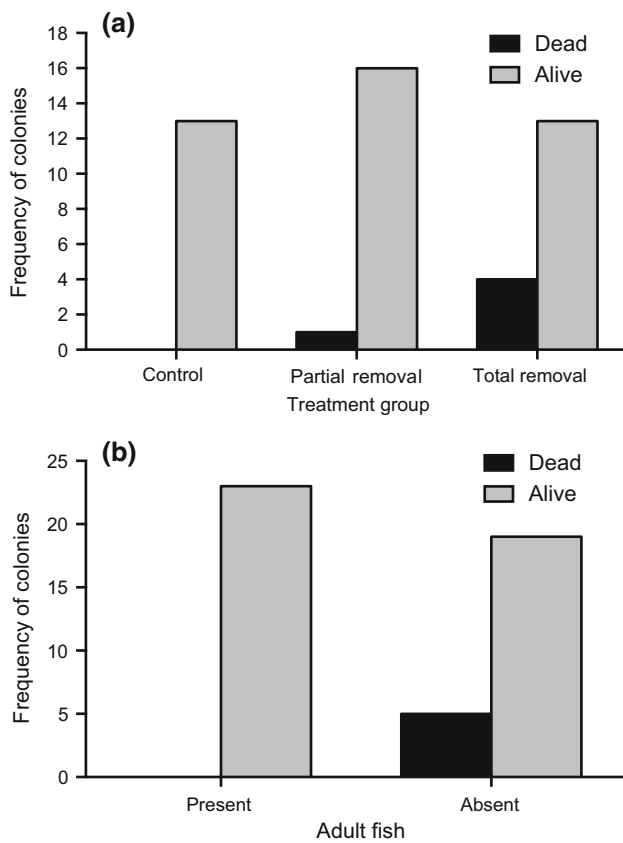


Fig. 4 Frequency distribution of dead anemone colonies after 3 yr in relation to **a** treatment groups and **b** the presence of adult anemonefish. Control, partial removal and total removal refer to the quantity of anemonefish that were removed from each colony at the commencement of the experiment. Statistical significance (*P*) of differences between frequency distributions was 0.085 (**a**) and 0.021 (**b**)

of a relationship, was in the range -0.79 to -0.50 and typically increased (weakened) with increasing time since manipulation (Fig. 6). In the short term (0–2 months), unoccupied anemones typically retreated into the reef matrix and shrank in size. In the long term (1–3 yr), these anemones either died (24 %) or resumed growth (76 %), depending on whether or not they recruited new anemonefish (which subsequently grew and defended them). Anemones occupied by small anemonefish only (i.e., no adults) responded similarly, except that fewer of them died (9 %) and more of them resumed growth (91 %).

Effects of anemonefish removal on anemonefish

In contrast to control anemone colonies, which increased in polyp abundance and total area (Fig. 5a, c), group size of anemonefish colonies in the control treatment was relatively stable through time (Fig. 7a, b), suggesting that anemonefish colonies expand slowly relative to their hosts (cf. Fig. 3).

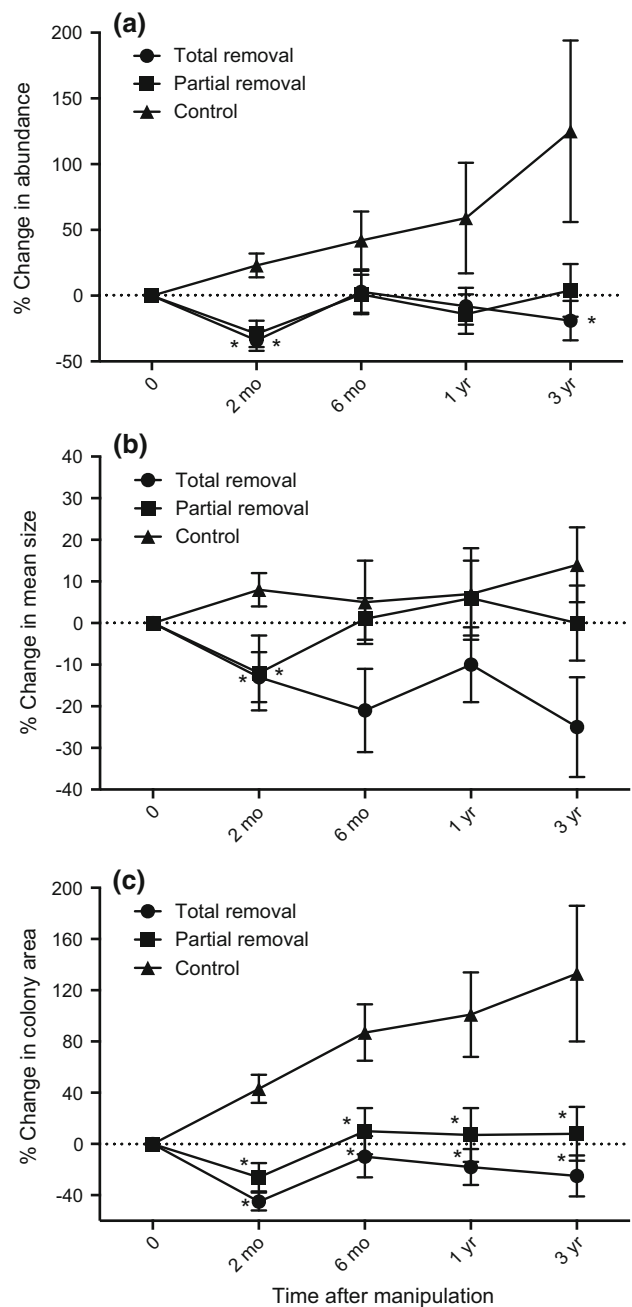


Fig. 5 Effects of anemonefish removal on **a** anemone abundance, **b** anemone mean size (oral disc area), and **c** anemone colony area (combined surface area of oral discs) as a function of time after manipulation (per colony, mean \pm standard error; $n = 13$ –17). Treatment groups (control, partial removal and total removal) refer to the quantity of anemonefish that were removed from each colony at the commencement of the experiment. Dashed horizontal lines depict zero-change trajectories. Results of statistical tests are listed in Table 1. Groups that were significantly different from controls (within time) are denoted by asterisks. Note x-axis is not to scale

When some or all anemonefish were experimentally removed from hosts, mean abundances of anemonefish per colony increased rapidly (within 2 months) and resulted in

Table 1 Results of statistical tests for the effects of anemonefish removal on various colony parameters

Variable	Factor	<i>F</i> statistic	Degrees of freedom*	Probability	Figure no.
Anemone abundance	Treatment	3.24	2, 44	0.049	5a
	Time	1.34	3, 135	0.263	5a
	Interaction	1.45	6, 135	0.197	5a
Anemone mean size	Treatment	3.57	2, 44	0.037	5b
	Time	1.74	2, 108	0.173	5b
	Interaction	1.26	4, 108	0.288	5b
Anemone colony area	Treatment	7.87	2, 44	0.001	5c
	Time	1.61	3, 135	0.188	5c
	Interaction	2.29	6, 135	0.037	5c
Fish abundance	Treatment	10.7	2, 44	<0.001	7a
	Time	16.4	3, 132	<0.001	7a
	Interaction	3.97	6, 132	0.001	7a
Fish biomass	Treatment	11.5	2, 44	<0.001	7b
	Time	14.9	3, 145	<0.001	7b
	Interaction	9.24	6, 145	<0.001	7b
Fish recruitment	Treatment	12.5	2, 44	0.016	7c
	Time	21.6	3, 146	<0.001	7c
	Interaction	1.8	6, 146	<0.001	7c

Results in bold indicate statistical significance

* In some cases, the degrees of freedom were rounded down to the nearest integer, after Greenhouse-Geisser correction for sphericity

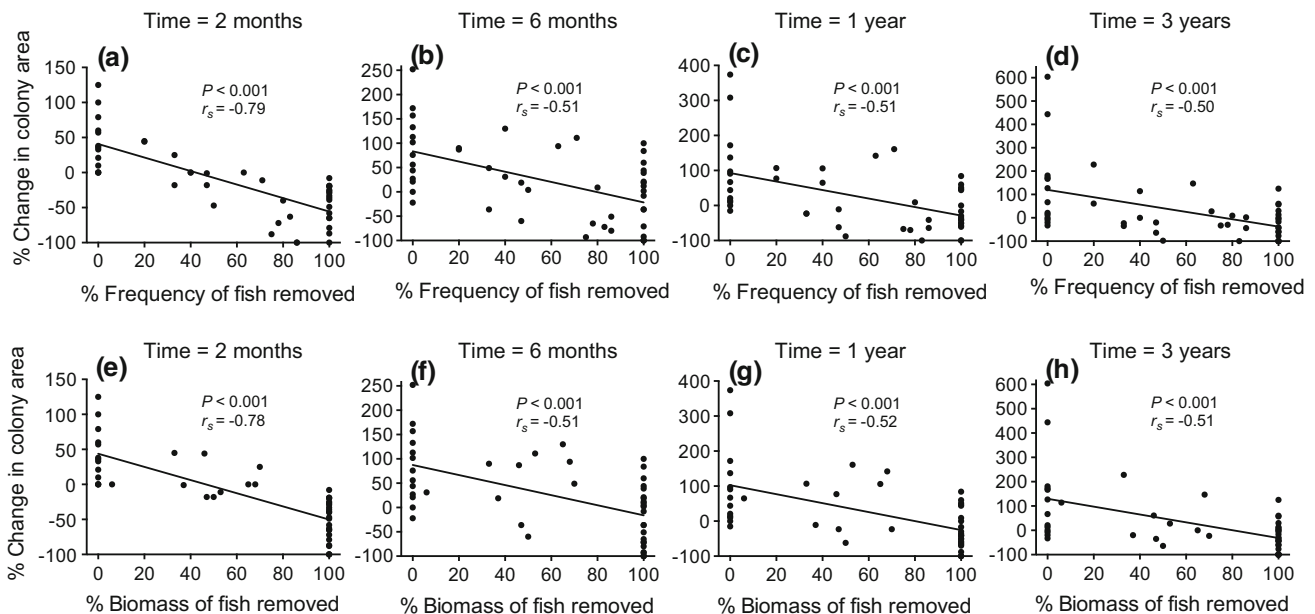


Fig. 6 Effects of anemonefish removal on anemone colony area (combined surface area of oral discs) after 2 months (a, e), 6 months (b, f), 1 yr (c, g) and 3 yr (d, h). The magnitude of anemonefish removal was measured in percent frequency (a–d) or percent group

biomass (e–h). Significant relationships were identified by Spearman's rank correlation coefficient (r_s) and are depicted by lines of best fit. A change in colony size of -1 to -99 % indicates colony shrinkage and a change of -100 % indicates colony death

significant inter-group differences that remained for the duration of the study (Fig. 7a). Group biomass of 'partial removal' colonies increased rapidly and was significantly

different to control colonies from 6 to 36 months (Fig. 7b). Group biomass of 'total removal' colonies also tended to increase through time, but less than in 'partial removal'

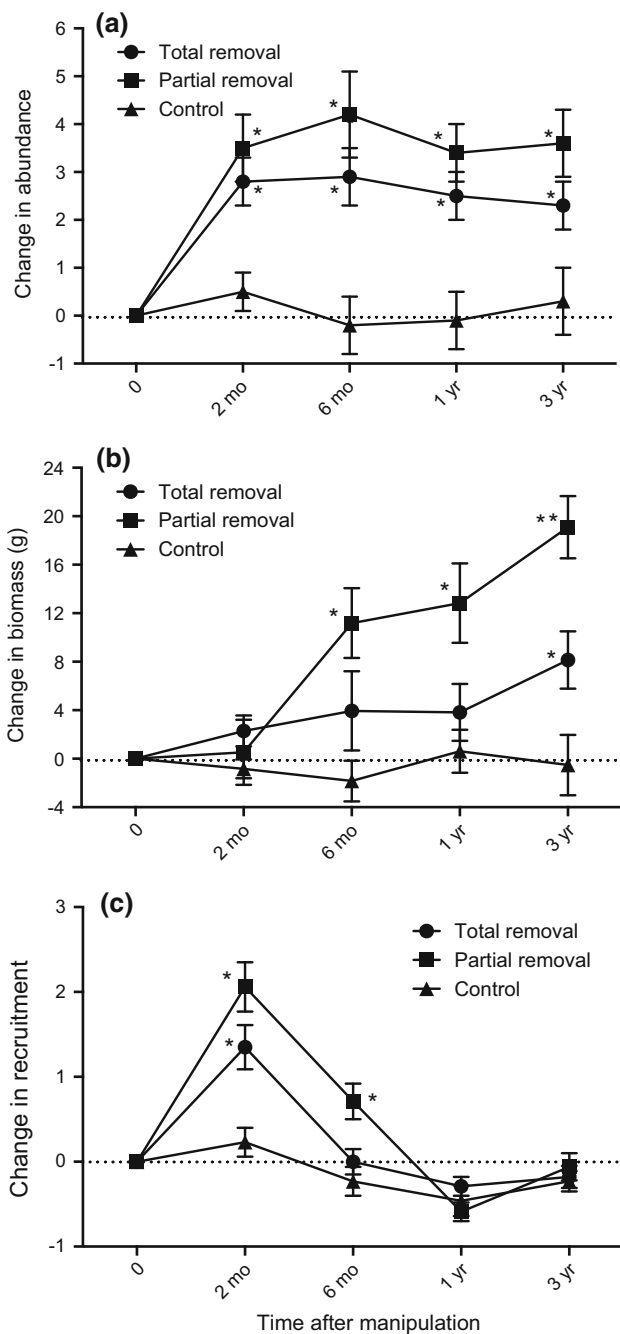


Fig. 7 Effects of anemonefish removal on **a** abundance, **b** group biomass and **c** recruitment of anemonefish (per colony, mean ± standard error) as a function of time after manipulation ($n = 13–17$). Treatment groups (control, partial removal and total removal) refer to the quantity of anemonefish that were removed from each colony at the commencement of the experiment. Horizontal lines depict zero-change trajectories. Results of statistical tests are listed in Table 1. Groups that were significantly different from controls (within time) are denoted by asterisks. Note x-axis is not to scale

colonies, such that all three treatment groups were significantly different from each other at 3 yr (Fig. 7b). Mean recruitment of anemonefish was relatively stable through

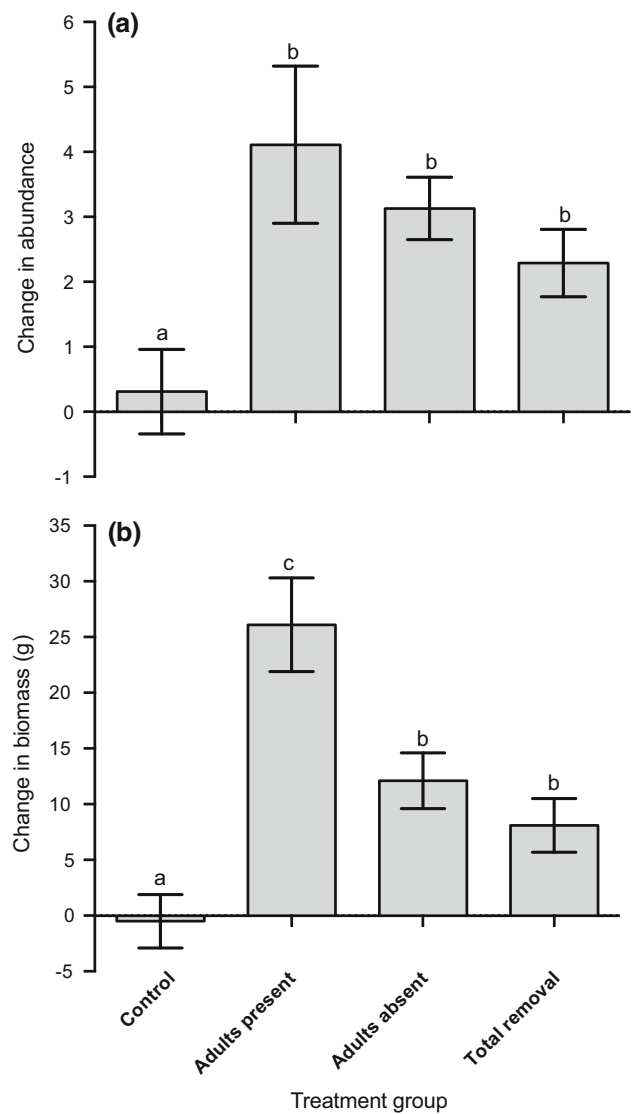


Fig. 8 Effects of anemonefish removal on **a** abundance and **b** group biomass of anemonefish after 3 yr (per colony, mean ± standard error). Treatment groups refer to the status of colonies at the commencement of the experiment. The labels ‘adults present’ and ‘adults absent’ together constitute the ‘partial removal’ treatment shown in Fig. 7. Groups with the same alphabetic letter were not significantly different from each other. Sample sizes for each treatment group were 13 (control), 9 (adults present), 8 (adults absent) and 17 (total removal)

time in control colonies, whereas a pulse of recruitment occurred in ‘partial removal’ colonies at 2 and 6 months, and in ‘total removal’ colonies at 2 months (Fig. 7c). Among ‘partial removal’ colonies, those that contained one or more adult anemonefish attained larger group sizes (in terms of abundance and biomass) than those that did not contain adult anemonefish (relative to initial group sizes), suggesting that adult fish in some way enhanced the recruitment and/or survival of conspecifics (abundance: $F_{3,43} = 4.80$, $P = 0.006$; biomass: $F_{3,43} = 13.85$, $P < 0.001$; Fig. 8).

Discussion

Although several previous studies have demonstrated the importance of anemonefish to anemones (Godwin and Fautin 1992; Porat and Chadwick-Furman 2004, 2005; Holbrook and Schmitt 2005), the question of whether anemones can recover from anemonefish depletion has gone unanswered, and the colony-level effects of ornamental fishery practices are yet to be evaluated. In this study, partial or total removal of anemonefish were found not only to have rapid and sustained effects on growth, (asexual) reproduction and survival of anemones, but also cascading effects on recruitment and productivity of anemonefish in the remaining colony. Remarkably, colony-level effects on anemones and anemonefish were found to be proportional to the size and number of anemonefish that were experimentally removed. Unexpectedly, a high proportion of anemone colonies survived after total removal of anemonefish, albeit with long-lasting effects. These results shed new light on anemone–anemonefish symbioses and have important implications for management of ornamental fisheries.

As in other anemone–anemonefish species combinations (Fautin 1992; Elliot and Mariscal 2001; Mitchell and Dill 2005), group sizes of *E. quadricolor* and *A. melanopus* colonies at Walker Reef were highly variable but positively correlated (Fig. 3), indicating that one or both of the partners strongly influenced the size and abundance of the other partner. When some or all of the resident anemonefish were experimentally removed, there were corresponding decreases in the size and abundance of host anemones (Figs. 5, 6), presumably as a direct or indirect result of predation by corallivorous butterflyfish, or reduced nutrient transfer (Godwin and Fautin 1992; Porat and Chadwick-Furman 2004; Roopin and Chadwick 2009; Roopin et al. 2011). Taken together, these results indicate that the size and abundance of *E. quadricolor* is strongly limited by the size and abundance of resident *A. melanopus*. That is, for a host anemone to grow and multiply into a large colony, it requires protection and/or nutrients from large and numerous anemonefish. Thus, the mutualistic relationship between *E. quadricolor* and *A. melanopus* (and potentially of other anemone–anemonefish species combinations) is not only obligate, but also relatively rigid in terms of mutual demography.

As *E. quadricolor* is dependent on resident anemonefish for survival, it is logical to ask: does anemonefish collection spell death for anemones, and how can anemones colonize new reefs via larval dispersal? As in previous studies, undefended anemones contracted into the reef matrix and ‘disappeared’ within hours to days (Godwin and Fautin 1992; Porat and Chadwick-Furman 2004).

Surprisingly, however, we found that most (76 %) of these (temporarily hidden) colonies recruited new anemonefish over the following months (Fig. 7c) and subsequently resumed colony expansion (Fig. 5). We conclude that recovery of fish-depleted *E. quadricolor* colonies (and, by inference, colonization of new reefs via larval dispersal) is possible, but critically dependent on the supply of anemonefish larvae. If larval supply is limited (e.g., due to natural hydrographic events, reduced spawning stock biomass or non-breeding season), anemones will remain unoccupied (undefended) for long periods of time, which is eventually fatal because they are unable to expand their tentacles to collect plankton and sunlight (Porat and Chadwick-Furman 2004). Thus, collection of anemonefish during periods when larval supply is limited is anticipated to be highly detrimental and may be sufficient to facilitate local extirpation of anemones. This combination of events (collection and reduced larval supply) may have contributed to the collapse of anemone populations seen previously in other parts of the GBR (Keppel Islands; see Jones et al. 2008; Frisch and Hobbs 2009).

Although *E. quadricolor* is capable of recovering from anemonefish depletion, it is evident that this process is slow (>3 yr, on average) and strongly influenced by mutual constraints: growth and asexual reproduction of *E. quadricolor* are limited by the size and abundance of *A. melanopus*, which is limited, in turn, by habitat size (anemone size and abundance) as well as strict social regulation of colony membership (Buston 2003b; this study). Furthermore, growth of individual anemonefish is precisely regulated to maintain discrete size differences between potential rivals so as to reduce conflict and avoid eviction from the host (Buston 2003c). In combination, these pervasive mutual constraints suggest that the natural rate of increase in anemone–anemonefish colonies is extremely slow and that large colonies (e.g., Figure 1a of Thomas et al. 2014) are probably very old, perhaps decades or centuries (see also Fautin and Allen 1997). The consequence for anemone and anemonefish populations is that they can only support relatively light fishing pressure and that recovery from natural disturbance (e.g., mass bleaching) or over-collection may be extraordinarily slow, at least in the tropics (cf. Scott et al. 2011). Therefore, suspension of collecting in places where anemones and anemonefishes have already been depleted (e.g., Shuman et al. 2005) or are exposed to frequent natural disturbances (e.g., Jones et al. 2008) is justified and may be essential to prevent local extirpation of these iconic species.

Depending on the location, anemonefish fisheries remove either all the juvenile and adult individuals in each anemone or colony (e.g., Philippines) or only the larger individuals, leaving one or more juvenile fish remaining

(e.g., Australia; Jones et al. 2008; M. Atkinson, pers. comm.). These practices are driven by market demand and, in the latter case, by fishers' belief that the remaining juvenile(s) will grow to defend the host. However, results from the present study demonstrate that these fishery practices are suboptimal or even detrimental. Size and number of anemonefish increased more rapidly (and anemone mortality was lower) in 'partial removal' colonies than in 'total removal' colonies (Figs. 5a, 7), and anemone survival and anemonefish productivity were higher when at least one adult anemonefish remained in the colony (Figs. 5b, 8). Visual observations confirm that anemonefishes' defensive abilities are strongly size-dependent (see also Ross 1978), so preservation of one or more adult individuals is expected to provide the host with greater opportunity for growth and reproduction, which ultimately leads to more habitat for additional anemonefish. Furthermore, the strongly hierarchical social system of anemonefish facilitates rapid replacement of juveniles, but not of adults (Fautin 1992; Elliot and Mariscal 2001), and replacement of juveniles enhances productivity because smaller individuals have a greater weight gain per unit of time than larger individuals, since lifetime growth is asymptotic (Choat and Robertson 2002). It should also be noted that the mean monthly increase in biomass for the 'partial removal' treatment was maximal between 2 and 6 months (Fig. 7b), which provides a useful guide in terms of optimal harvest interval. Together, these results provide strong justification for regulation of fishery harvest practices. In particular, we recommend fish collectors undertake harvests no more than once per 6 months, preferentially target juvenile fish (the smaller, the better), and leave a minimum of one adult anemonefish (>50 mm *L*) in each solitary anemone, or in the case of colonies, a minimum of one adult anemonefish in each ~0.14 m² of oral disc area (based on observed minimum adult occupancy rates, extracted from data in Fig. 3a) or ~1 m² of reef substratum (based on average adult territory size; Ross 1978). In addition, it may be prudent to limit collecting to periods when supply of larval anemonefish is maximal (e.g., mid-spring to mid-autumn on the GBR).

In summary, this study demonstrates that, at Walker Reef, demography of *E. quadricolor* is inexorably linked to that of *A. melanopus*. Removal of anemonefish resulted in fewer and smaller hosts, which in turn had lasting effects on the hosts' ability to support anemonefish, thereby creating a positive feedback cycle that reduced the productivity of the mutualistic partnership as a whole. Importantly, the colony-level effects on anemones and anemonefish were proportional to the size and number of anemonefish that were experimentally removed, such that preferential collection of juvenile fish and preservation of at least one adult fish per anemone or unit of colony area are recommended to

minimize the impact of anemonefish fisheries. Total removal of resident anemonefish reduced growth, (asexual) reproduction and survival of *E. quadricolor*, but was not necessarily fatal if recruitment of new anemonefish occurred soon after, analogous to when anemone larvae colonize new reefs. These results enhance our understanding of anemone–anemonefish symbioses and provide a sound basis for management of anemonefish collection practices, which are yet to be formally prescribed anywhere in the world. Strong, precautionary management of anemones and anemonefish is imperative given the severe declines in some populations (Shuman et al. 2005; Jones et al. 2008; Frisch and Hobbs 2009; Madduppa et al. 2014; Thomas et al. 2014) and the intensifying threats that they will face in the future (e.g., mass bleaching: Saenz-Agudelo et al. 2011; Hobbs et al. 2013; Lonnstedt and Frisch 2014). Lastly, we urge caution with respect to extrapolation of our results to fringe habitats (e.g., subtropical reefs), where the absence of corallivorous butterflyfishes may render *E. quadricolor* a facultative mutualist.

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