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The role of marine reserves in the replenishment of a locally-impacted population of anemonefish on the Great Barrier Reef

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Abstract

The development of parentage analysis to track the dispersal of juvenile offspring has given us unprecedented insight into the population dynamics of coral reef fishes. These tools now have the potential to inform fisheries management and species conservation, particularly for small fragmented populations under threat from exploitation and disturbance. In this study we resolve patterns of larval dispersal for a population of the anemonefish *Amphiprion melanopus* in the Keppel Islands (southern Great Barrier Reef). Habitat loss and fishing appear to have impacted this population and a network of no-take marine reserves currently protects 75% of the potential breeders. Using parentage analysis, we estimate that 21% of recruitment in the island group was generated locally, and that breeding adults living in reserves were responsible for 79% (31 out of 39) of these of locally-produced juveniles. Overall, the network of reserves was fully connected via larval dispersal; however one reserve was identified as a critical source of larvae for the island group. The population in the Keppel Islands also appears to be well-connected to other source populations at least 60 km away, given that 79% (145 out of 184) of the juveniles sampled remained unassigned in the parentage analysis. We estimated the effective size of the *A. melanopus* metapopulation to be 745 (582-993 95% CI) and recommend continued monitoring of its genetic status. Maintaining connectivity with populations beyond the Keppel Islands and

recovery of local recruitment habitat, potentially through active restoration of host anemone populations, will be important for its long-term persistence.

Introduction

Uncovering patterns of larval dispersal and connectivity is critical for understanding the dynamics of natural populations and how such populations can be managed to minimize impacts from natural or anthropogenic disturbance (Sale *et al*. 2005; Botsford *et al*. 2003; Jones *et al*. 2009). Although determining the fate of larvae in the ocean was once considered practically impossible, significant advances in larval tracking techniques over the past decade have produced empirical observations of larval dispersal patterns in marine environments (reviewed by Cowen & Sponaugle 2009; Leis *et al*. 2011; Jones 2014). Estimates of dispersal distances for coral reef fish larvae based on genetic parentage analysis range from a few metres (Jones *et al*. 2005) to hundreds of kilometres (Christie *et al*. 2010, Simpson *et al.* 2014). However, the magnitude of dispersal appears to decline strongly with distance from the source population (Buston *et al*. 2012; Almany *et al*. 2013; D'Aloia *et al*. 2013) and geographically isolated populations appear to be more dependent on self-recruitment for their replenishment (Pinksy *et al*. 2012; D'Aloia *et al*. 2013; Cuif *et al.* 2015). Although application of these techniques to species conservation has been limited thus far, knowledge of dispersal patterns can inform local management strategies, such as the role of marine reserves in species conservation and the appropriate spatial scales for management (Almany *et al*. 2009). To adequately protect marine species, reserves should be large enough so that protected populations can be self-replenishing and distributed so that they benefit from larval connectivity with other protected populations. Recent studies have now established that populations in reserves can be self-replenishing (Almany *et al*. 2007; Berumen et al. 2012; Harrison *et al*. 2012) and connected to form networks

(Planes *et al*. 2009; Berumen et al. 2012; Harrison *et al*. 2012). Although data on the lifetime reproductive output of protected populations would further improve capacity to manage for their persistence (Botsford *et al.* 2009; Burgess *et al.* 2014), parentage-based measurements of larval dispersal and self-recruitment across reserve networks are the most empirically feasible metrics currently available to assess population persistence (Hogan *et al.* 2012; Lett *et al.* 2015). These same techniques could also be used to identify important sources of larvae to assist in the strategic placement of marine reserves and population restoration of locally impacted coral reef fish species.

In addition to understanding the extent of larval connectivity within and between populations, estimating their effective size is also valuable for evaluating the potential threat to species that are susceptible to natural or anthropogenic disturbance. According to IUCN criteria, species with populations of <1,000 mature individuals are considered vulnerable by virtue of their small size and susceptibility to demographic stochasticity (Mace *et al*. 2008). Population fragmentation can further exacerbate the problem, as geographic isolation may decrease connectivity from distant populations, thereby reducing the input of new individuals that help maintain diversity in the local gene pool (Frankham *et al.* 2002, Segelbacher *et al*. 2003, Coulon *et al.* 2004). Estimates of effective population size (N_e) can provide an indication of the degree of genetic variation and adaptive potential of a population (Luikart *et al*. 2010; Hare *et al.* 2011; Ovenden *et al*. 2013; Frankham *et al*. 2014). However, since reproductive success in natural populations is often highly variable, relationships between N_e and adult census population size are not straightforward (Palstra & Fraser 2012). Estimates of effective population size are therefore currently best used to assess the genetic condition of populations, rather than as a direct proxy for breeder numbers (Hare *et al.* 2011; Ovenden *et al.* 2013). Although conservation

managers have long used the 50/500 rule, which recommends a minimum effective population size of at least 500 (Franklin 1980), recent re-evaluation of this rule suggests that $N_e > 1000$ is necessary to ensure the population has sufficient genetic variation to cope with increasing environmental change and avoid extinction in the long-term (Frankham *et al*. 2014). Though these criteria were largely developed in the context of terrestrial species conservation, marine populations are not immune to global extinction (Roberts & Hawkins 1999; Dulvy *et al*. 2003), and habitat loss or overexploitation can also lead to localised extinctions of marine species (Dulvy *et al*. 2003; Reynolds *et al*. 2005). Evidence of reduced genetic diversity and low effective population sizes in exploited marine fishes suggests that these species can be vulnerable to genetic decline despite their high fecundity and relatively large census population sizes (Hauser *et al.* 2002; Hutchinson *et al.* 2003; Hoarau *et al.* 2005). This emphasises a growing need to apply a range of conservation genetic tools to assess the demographic and genetic status of potentially threatened marine species. The relatively recent recognition that populations of marine organisms are not as demographically 'open' as once thought (Cowen *et al.* 2000; Mora and Sale 2002; Steneck *et al.* 2009) suggests that small, geographically isolated populations of marine species may be susceptible to the same precursors to local extinction (e.g. reduced connectivity, genetic diversity loss, inbreeding) as terrestrial species.

Anemonefish are iconic symbols of coral reefs; they inspire feature films, attract millions of tourists to visit reefs each year, and provide a source of income based on the aquarium fish industry (Shuman *et al*. 2005). However, populations of many anemonefish species are vulnerable to decline due to their small local population sizes and highly specialised habitat associations. Most anemonefish live in only a few species of sea anemone (Fautin 1986) and are easy targets for collectors because they rarely venture out of their host. Although the extent of

the collection and global trade in anemonefish and their host sea anemones is unknown, they comprise 60% of the trade in the Philippines (one of the world's major aquarium fish exporters) and several species of anemonefish top the list of aquarium fish imported into the United States (the world's major importer) (Shuman *et al*. 2005; Rhyne *et al*. 2012). Populations of host anemones may also be impacted by bleaching and mortality induced by ocean warming and freshwater inundation (Saenz-Agudelo *et al*. 2011; Hobbs *et al*. 2013). Because host anemones reproduce infrequently and have high juvenile mortality rates (Scott & Harrison 2007), the capacity for recovery of anemonefish habitat following its loss is naturally quite low.

Recent information suggests that a population of the Cinnamon anemonefish, *Amphiprion melanopus*, in the Keppel Islands may be threatened, given its current rarity in the region and potential impacts from habitat loss and aquarium fish collections (Jones *et al*. 2008; Frisch & Hobbs 2009). The objective of this study was to determine the degree to which this population is demographically isolated, whether it is replenished locally or relies on connectivity from beyond the island group. We used genetic parentage analysis to estimate self-recruitment and describe patterns of larval dispersal throughout the island group, and assess whether an existing network of marine reserves contributed to the replenishment of the local population. We also estimate the effective size of the population, in order to provide an indication of its genetic capacity to withstand further pressure from harvest and environmental variability in the long-term.

Materials and methods

Study species and location

Amphiprion melanopus occurs throughout the Western Pacific, primarily in shallow coral reef habitats, where it lives in association with clonal forms of the sea anemone *Entacmaea*

quadricolor (Fautin 1986, Figure 1a). As with most species of potential conservation concern, little is known about reproduction, generation times, and longevity of *A. melanopus* in the wild. These fish typically live in large social groups in which the two largest individuals form a breeding pair and spawn approximately twice per month (Ross 1978a). Strong social control over growth and reproduction in this species makes it impossible to determine its reproductive maturity based on body size alone (i.e. non-lethally). One early observational field study suggested that breeders in this species tend to be at least 60 mm in length (Ross 1978b). A more recent analysis of otoliths and gonads from individuals collected from the southern Great Barrier Reef suggests that *A. melanopus* can live for up to 38 years in the wild, reach a maximum body size of 104 mm, and that > 50% of individuals reach reproductive maturity at 6-7 years of age (Buechler 2005). Newly hatched *A. melanopus* larvae are known to disperse for 8-14 days before returning to the reef to settle (Bay *et al*. 2006), though their dispersal potential remains unknown.

The Keppel Islands are a group of 18 tropical islands located approximately 15 km off the coast of Queensland, Australia, in the southern Great Barrier Reef Marine Park (GBRMP) (Figure 1b). Approximately 700 ha of fringing coral reefs surround the islands and have supported a commercial aquarium harvest of both *A. melanopus* and its host anemone. The fishery is jointly regulated by Queensland's Department of Agriculture and Fisheries (DAF) and the Great Barrier Reef Marine Park Authority (GBRMPA) through traditional fishery management (e.g. gear restrictions and limited entry licensing) and a network of no-take marine reserves (Figure 1c). Eight operators currently hold permits to collect anemones and anemonefish in the areas open to fishing, and their activities are guided by ecological risk assessments (Roelofs 2008a, b) and a Stewardship Action Plan (Donnelly 2013). In fact, the commercial industry enacted a self-imposed moratorium on collection of *A. melanopus* and its

host anemone in the Keppel Islands in 2008 due to concerns about the persistence of these populations following repeated environmental disturbances (Great Barrier Reef Marine Park Authority 2008). In 1991, 2008, 2011 and 2013 major flood plumes from the Fitzroy River, the largest catchment that discharges into the GBRMP, inundated reefs in the Keppel Islands with freshwater, sediment and agricultural pollutants (Devlin & Brodie 2005; Devlin *et al*. 2012). These major floods, combined with a severe coral bleaching event in 2006, caused extensive coral mortality and reductions in fish diversity and abundance (Williamson *et al*. 2014; Jones & Berkelmans 2014). Although population trends during this disturbance period are not available for *A. melanopus* and its host anemone, a 2007 study commissioned by the GBRMPA found that *A. melanopus* are relatively rare in the Keppel Islands, with an average density of 0.62 individuals per 1,000 m^2 of reef habitat (Frisch & Hobbs 2009). This is well below the average population density for this species in other GBRMP locations, with 11.9 to 150 individuals per 1,000 m^2 reported across reefs in the northern GBR (Frisch & Hobbs 2009; Jones *et al*. 2008). Beyond the Keppel Islands, the nearest significant populations of this species are likely to be at least 60 km further offshore at the Capricorn-Bunker reefs at the southernmost end of the Great Barrier Reef (Figure 1b).

Population sampling and genotyping

Between December 2011 and August 2013, teams of 2-4 divers conducted depth-stratified swims along hard-bottomed reef habitat across the Keppel Islands searching for colonies of *A. melanopus*. Initially, search teams used local knowledge from island residents, dive operators and records of anemonefish locations from previous studies (e.g. Frisch & Hobbs 2009) to target search efforts. Once these locations were searched, teams then visited sites previously unexplored by local divers and scientists in order to locate new colonies. Dive teams

visited sites across the Keppel Islands (both inside and outside of marine reserves) and new locations were explored on each of four sampling trips across the 1.8-year sampling period. In total, 472 hectares of the hard-bottomed reef habitat in the Keppel Islands (i.e. 67% of the total reef area) were searched for the presence of this species. When a colony was first located all fish present were captured with hand nets and clove oil (mild anaesthetic). Anaesthetised fish were measured *in situ* to the nearest millimetre total length (TL), sampled by clipping a small piece of tissue from the caudal fin, and immediately returned to their host anemone to recover. The location of the colony was marked with GPS and revisited on subsequent trips to sample recently settled recruits. Three colonies had egg clutches and 4-6 fertilised eggs were collected from each clutch in order to validate parentage assignments. All tissue samples were preserved in 99% ethanol for genotyping.

Genomic DNA was extracted from the tissue samples using NucleoSpin® 96 Tissue kits (Macherey-Nagel) according to the manufacter's protocol. For each individual, a series of five multiplex polymerase chain reactions (PCR) were performed to amplify 22 polymorphic microsatellite loci, with concentrations of primers adjusted for even amplification of loci in multiplex (see Table S1, online Supplementary Information). Further details of these loci and the genotyping procedure are described in Bonin *et al*. 2015. Individual genotypes were scored in GENEMAPPER v4.0 and unique alleles were distinguished using marker specific binsets in MSATALLELE (Alberto 2009). Calculation of the number of alleles per locus, observed and expected heterozygocity and tests of Hardy-Weinberg and linkage disequilibrium were conducted using GENEPOP on the web (Raymond & Rousset 1995; Rousset 2008). Significance levels were adjusted for multiple testing using a false discovery rate (FDR) correction (Benjamini & Hochberg 1995). All 22 loci satisfied Hardy-Weinberg equilibrium

assumptions. Only 5 out of the 231 pairs of loci showed any evidence of linkage disequilibrium and these loci were retained in the parentage data set as this low level of potential linkage is unlikely to affect parentage conclusions given the size of our sample population (Amos *et al*. 1992).

Effective population size estimation

Contemporary genetic effective population size (N_e) for *A. melanopus* in the Keppel Islands was estimated using the Linkage Disequilibrium (LD) method under the assumption of monogamous mating. The LD method estimates effective size based on the observed linkage disequilibrium between unlinked loci, which is expected to occur more often in small breeding populations due to genetic drift. This method is particularly promising for conservation applications because it only requires one sample of the population (Schwartz *et al.* 2007; Luikart *et al*. 2010; Waples & Do 2010; Ovenden *et al.* 2013) and evaluation of the method has shown that it can provide precise estimation of N_e with 10-20 microsatellite loci (Waples & Do 2010). Our data set for N_e estimation consisted of 19 microsatellite loci, with a mean of 6.9 ± 1.2 SE alleles per locus, for the 412 anemonefish sampled in the Keppel Islands. Three of the loci used in parentage analysis (*alat22*, *bicin98* and *am9*) were excluded to estimate N_e, eliminating all potential linkage among markers in the data set. Genetic effective size was estimated using the software program NeESTIMATOR v2 (Do *et al*. 2014) and a number of steps were taken in the analysis to ensure that the resulting estimate was as precise and accurate possible, based on recommendations from a sensitivity analysis conducted by Waples & Do (2010). First, genotypes of all 412 individuals were included in the analysis because precision of the estimate has been shown to increase substantially with sample size. Second, a critical p-value of 0.02 was set to screen out low frequency alleles that could introduce upward bias in the estimate. Finally, 95%

Confidence Intervals (CIs) were estimated using a jackknife method, which overcomes issues with lack of independence in pairwise comparisons among loci and provides a more conservative range estimate than parametric CIs. Because our analysis included samples taken across generations (e.g. adults and juveniles), the effective size estimate should be interpreted as the genetic equivalent of the number of breeders that produced all the cohorts from which samples were taken (Waples & Do 2010).

Resolving patterns of connectivity with parentage analysis

Parent-offspring relationships were assessed in the software program FAMOZ using a maximum likelihood approach (Gerber *et al*. 2003). This program computes log of the odds ratio (LOD) scores for assigning offspring to candidate parents based on the observed allelic frequencies at each locus. Data simulations were used to determine the minimum threshold LOD scores for parent-offspring assignments. Based on the simulation of 10,000 offspring with a calculation error rate of 0.01% these thresholds were set at 5 for single-parent assignments and 16 for parent-pair assignments. As previous work on this species (Ross 1978b) and our own observations suggested that breeders tend to be at least 60 mm TL, we considered all fish > 60 mm TL as potential parents and those < 60 mm TL as potential offspring in the analysis. One allele mismatch between putative parent-offspring genotypes was allowed in order to account for potential genotyping error and *de novo* mutation of microsatellite markers. Allowing for some genotyping error is necessary to minimise false negatives and increase the accuracy of assignments (Harrison et al. 2013a, b), however we adopted a conservative approach in this study by removing any assignments that had an excessive probability of being false positive assignments.

The capacity of FAMOZ to identify true parent-offspring pairs using our set of 22 microsatellite markers was validated using the fertilised eggs sampled from three separate colonies. For one of these clutches only one of the true parents was sampled in the field (we could not catch the other) and FAMOZ correctly assigned 3 out of 4 eggs back to this parent with single-parent LOD scores ranging from 10.76-12.71. The remaining egg sampled from this clutch was missing data across 8 of the 22 loci and was not assigned to any parent in the sample population. For the other two clutches both true parents were sampled and FAMOZ correctly assigned eggs back to their parents with parent-pair LOD scores ranging from 24.81-29.93 when little to no data was missing across parent and offspring genotypes. However the ability of the analysis to assign eggs back to both parents diminished when data was missing across 6 or more loci. The consequence of missing data was that only one of the two true parents was assigned; it never resulted in mis-assignment of eggs to incorrect parents.

In addition to the validation of parentage with eggs of known parenthood, we used simulated data sets with known parent-offspring relationships to assess our ability to correctly assign true parents and exclude false assignments. Following the methods described in Harrison *et al*. (2014), we simulated 5 datasets from the observed allelic frequencies each with 150 female and male parents, and 200 offspring of known and unknown decent. Each dataset was analysed in FAMOZ with the same settings and assignments were compared to true relationships. Averaged across all simulations, we were able to correctly assign or correctly exclude 93.0% ± 0.9% SE of simulated offspring. Approximately 5.6% ± 0.7% SE of simulated offspring were falsely assigned to a single parent that was not its true parent (false positive), and 0.6% ± 0.3% SE were falsely excluded when the true parent was present in the sample (false negative).

Dispersal distances

In order to estimate the minimum distances traveled by *A. melanopus* larvae that were assigned to parents in the Keppel Islands, we measured the shortest in-water distance between their natal and settlement site using spatial coordinates of individual anemonefish colonies. A chi-square test of independence was then used to examine whether these empirical dispersal distances were independent of the distribution of distances between sites. A significant result would indicate that the larval dispersal distances we observed were *not* independent from the distribution of distances between sites (i.e. that dispersal was at least partially driven by the distances between sites where *A. melanopus* colonies were located).

Results

Population sampling and effective size

Intensive searches of approximately 472 hectares of reef (67% of the hard-bottomed reef habitat) revealed that the local population size of *Amphiprion melanopus* is relatively small in the Keppel Islands, with only 228 adults and 184 juveniles located across the region. The majority of these individuals—75% of adult anemonefish and 73% of juvenile anemonefish—were located within marine reserves (Table 1). Colonial anemones were never unoccupied whether inside or outside reserves, and 66% of the anemone colonies (i.e. 31 out of the 47 colonies discovered) were located within marine reserves (Table 1). Colonies in reserves also supported larger groups of anemonefish, with an average of 9.9 ± 1.7 SE fish per colony in reserves compared to 6.6 ± 1.1 SE per colony in the sites open to fishing. The marine reserves at Monkey Bay and Egg Rock supported the largest numbers of adult *A. melanopus*, with 64 and 70 adults respectively.

The genetic effective population size (N_e) of *A. melanopus* in the Keppel Islands was estimated to be 745 (582-993 95% CI).

Larval dispersal and network connectivity

Parentage analysis assigned 21% of all sampled juveniles (39 out of 184) back to parents sampled in the Keppel Islands population (Table 1). Of the 39 identified parent-offspring pairs, 23 were to single parents and 16 were to parent pairs in the same colony. Single parent LOD scores ranged from 5.12 to 14.29 (average 9.25 ± 0.53 SE), parent pair LOD scores ranged from 18.12 to 32.51 (average 24.27 ± 0.94 SE), and only two of the assignments included a genotype mismatch (both were at only a single locus).

These assignments revealed that the *Amphiprion melanopus* population is connected across the network of marine reserves in the Keppel Islands and that colonies located within the reserves play a role in supplying recruits across the island group (Figure 2). Colonies in all marine reserves produced anemonefish larvae that dispersed to colonies located within both reserves and open (fished) sites. Overall, adults living in reserves were responsible for generating 79% of the assigned offspring (31 out of 39), suggesting that these protected adults are the primary source of locally-produced recruitment (Figure 3). Colonies in two of the open sites, Big Peninsula and Wreck Bay, also contributed to local network connectivity through the supply of larvae to colonies located within reserves (Figure 2). The *A. melanopus* colonies open to harvest around Corroboree Island at the northernmost tip of the Keppel Island group received larvae from four other sites to the south (3 reserve sites and 1 open site), but no offspring that settled elsewhere in the Keppel Islands were assigned back to parents at this site.

Of the 39 offspring that were assigned to local adults in Keppel Islands, 14 of them not only remained within the island group but also settled back onto their natal reef (Figure 3). This was largely due to self-recruitment at Monkey Bay, where 12 out of the 83 juveniles sampled in the reserve were offspring of adults in the reserve (i.e. 14% self-recruitment for this site, Table 1). The other location where self-recruitment was observed at the site-level was the marine reserve at Egg Rock, with 2 out of 12 juveniles were assigned back to parents at that location (i.e. 17% self-recruitment for this site, Table 1). Egg Rock is relatively isolated in comparison to the other sites and overall levels of recruitment were relatively low (i.e. 12 juveniles in total between December 2011 and August 2013) despite having the largest number of adults and the highest number of *A. melanopus* colonies of any site in the region (Table 1).

Connectivity among colonies within a marine reserve

The Monkey Bay marine reserve was particularly vital to ensuring connectivity between sites and maintaining the replenishment of the local *A. melanopus* population. Colonies in Monkey Bay received the highest levels of recruitment with 45% of the juveniles (83 out of 184) sampled across the Keppel Islands settling in this reserve. Furthermore, 51% of the assigned offspring (20 out of 39) were produced from parents living in Monkey Bay. The large numbers of larvae settling to and assigned offspring originating from this single reserve allowed us to explore fine-scale connectivity dynamics across the seven *A. melanopus* colonies located in Monkey Bay (Table 1). Six out of the seven colonies in this reserve contributed to population replenishment through the export of larvae to other colonies in the Keppel Islands (Figure 4). There was also a substantial influx of anemonefish larvae to Monkey Bay from unknown sources, with 6 out of the 7 colonies each receiving between 4 -16 unassigned recruits (Figure 4).

We also observed a significant degree of larval exchange among the seven colonies in Monkey Bay, with 10 *A. melanopus* larvae dispersing between colonies that were less than 1 km apart and two of these settling into colonies that were less than 25 m from their parents (Figure 3). Remarkably, one anemonefish recruit that settled into a small *E. quadricolor* colony in marginal habitat at the opposite end of the same reef as its parents was re-sampled six months later as a subadult in its natal colony (Figure 4). This shows that a juvenile anemonefish less than 54 mm TL was able to successfully migrate across at least 340 m of reef to find another colony. Perhaps even more remarkable are the two larvae that not only returned to their natal reserve after a pelagic phase lasting more than 1 week, but actually settled back into the same colony as their parents (Figure 4). To our knowledge, these are the first accounts of coral reef fish larvae that have a pelagic stage settling back into the same social group as their parents.

Dispersal distances

Observed larval dispersal distances for *A. melanopus* ranged from 0 to 28 km and were independent of the distances between sites ($\chi^2 = 13.595$, df=7, p=0.06) suggesting that the larval dispersal patterns we observed are not driven simply by the geographic distances between colonies in the Keppel Islands. Approximately 49% of assigned offspring settled within 4 km of their natal colony; substantially more than would be expected based on the distribution of distances between the sampling sites (Figure 5).

Discussion

Small, fragmented populations are threatened by both demographic and genetic stochasticity that can increase their susceptibility to population decline, inbreeding and localised extinction (Mace *et al*. 2008; Frankham *et al*. 2014). While it is often quite challenging to determine the

status of potentially threatened species due to their small local population sizes, tools from conservation genetics can provide valuable information to assess demographic processes and guide management practices (Almany *et al.* 2009; Schwartz *et al.* 2007; Hare *et al.* 2011). Marine reserves are the primary tool used in the conservation of species in coral reefs ecosystems and will be valuable if they can make a contribution to the persistence of small, geographically isolated populations. A growing number of empirical studies have shown that a substantial proportion of coral reef fish larvae settle close to home (Jones *et al.* 2009) and that marine reserve networks have the potential to contribute to the local persistence of marine species (Almany *et al.* 2009; Harrison *et al.* 2012). Here we show that marine reserves have become the primary refuge of a coral reef fish population impacted by a combination of fishing pressure and habitat loss. Extensive searches uncovered 228 potentially breeding adult *Amphiprion melanopus* across 472 hectares of reef and 59% of these adults were concentrated at only two sites—the marine reserves at Monkey Bay and Egg Rock. Genetic parentage analysis assigned 39 out of the 184 juvenile anemonefish sampled in the Keppel Islands back to local adults, suggesting 21% self-recruitment at the scale of the island group. Breeding adults protected by marine reserves produced 31 of these self-recruiters and were therefore the primary source of local population replenishment.

Given that the 472 hectares of reef that we searched accounts for 67% of the total reef area in the Keppel Islands, we estimate the total breeding population size of *A. melanopus* in the Keppel Islands to be approximately 340 individuals. However, this may represent an over-estimate because the 33% of reef area that we did not search is largely composed of marginal habitat that is unlikely to host anemonefish, particularly at the densities we observed at the two sites with large colonies of breeding adults. Regardless, if we assume that a large proportion of the adult

population in the Keppel Islands was sampled, the fact that 79% of juveniles (145 out of 184) remained unassigned in the parentage analysis suggests that other source populations must have contributed to the replenishment of this small local population. Despite its geographic isolation, the *A. melanopus* population in the Keppel Islands appears to be well-connected to other source populations that are likely to be at least 60 km away. This degree of connectivity should promote the resilience of the Keppel Islands population from localised anthropogenic and environmental impacts because, if local breeders are lost, distant source populations can still replenish the local population.

We estimated the genetic effective size of the *A. melanopus* population in the Keppel Islands to be 745 (582-993 95% CI). However the LD method that we used to estimate N_e assumes a closed population, and yet the results of the parentage analysis suggest that this population receives migrants. Waples & England (2011) explored the effects of violating this assumption on estimates of effective size using the LD method and found that when immigration rates are high (e.g. more than 10%), N_e estimates based on samples of a local population converge on a value that more closely reflects the genetic effective size of the metapopulation. Thus, the estimated effective size of 745 (582-993) not only includes the local breeders that contributed to the replenishment of the Keppel Islands population, but also those adults that contributed larvae from other populations further away. When interpreting this value, it is important to note that it is not synonymous with census population size, and indeed the actual number of adults in the *A. melanopus* metapopulation is likely to be several orders of magnitude greater than the effective population size, as is typically observed for marine fish species with high fecundity and high juvenile mortality (e.g. Hauser *et al.* 2002; Hoarau *et al.* 2005). Instead this effective size estimate provides an indication of the evolutionary potential of the population, and from a

conservation perspective maintaining a minimum N_e of 1000 has been advised to maintain adaptive capacity in a changing environment (Frakham *et al.* 2014). At face value, the fact that the N_e of the *A. melanopus* metapopulation is below this threshold suggests a degree of vulnerability over evolutionary timescales, however this interpretation requires caution because the metapopulation as a whole was under-sampled in this study. Further sampling of this species in the Keppel Islands to compare N_e estimates in this local population over time, or alternatively sampling across larger spatial scales, would be useful to monitor the genetic status of this species.

At the present time, habitat loss appears to be the most prominent threat to the *A. melanopus* population in the Keppel Islands. The levels of connectivity we observed suggest that the numerical supply of larvae is sufficient to sustain the local population in marine reserves, but this will only be possible if there is settlement and breeding habitat available in those reserves. Although marine reserves can be effective at protecting species from overfishing, reef organisms living within reserves are still vulnerable to a multitude of other environmental impacts that affect the local habitat (Jones *et al*. 2004; Williamson *et al*. 2014). The conservation of species in marine reserves will therefore depend on the existence of refuges that are able to maintain local breeding populations through self-recruitment and connectivity, while avoiding the worst impacts of environmental disturbance (Williamson *et al*. 2014). In the Keppel Islands, the two marine reserves with the largest numbers of adult *A. melanopus* vary substantially in their vulnerability to environmental impacts. The Monkey Bay reserve is within 20 km of the mouth of the Fitzroy River and is highly exposed to freshwater, sediment and pollutants from flood plumes, whereas the Egg Rock reserve is more than 30 km offshore and is less exposed to riverine flood plumes (Devlin *et al*. 2012). However, our study revealed that the more impact-

prone Monkey Bay reserve also protects the adults that contributed most to local population replenishment. *Amphiprion melanopus* colonies in Monkey Bay produced >50% of the assigned offspring and also received 83 juveniles, almost 50% of all juveniles sampled across the region. In comparison, the Egg Rock population produced 10% of assigned offspring and received only 12 juveniles during the entire 1.8-year sampling period. This striking contrast in the contribution of these two reserves to local population replenishment is unlikely to be an artifact of sampling, given that both locations were thoroughly checked for juveniles on each of the four sampling trips. Although the Egg Rock reserve appears least susceptible to local environmental impacts and harbours the largest population of adults in the region, our empirical measurement of larvae originating from this reserve suggests it is not a significant source of local recruitment. From a conservation perspective, these findings reinforce the need to identify and protect key settlement and nursery habitats of species in order to promote population replenishment (Steneck *et al*. 2009; Wen *et al*. 2013). Although selection of sites for marine reserves that are more sheltered or resistant to environmental impacts can ensure the preservation of some individuals (McLeod *et al*. 2009), it is important to recognise that these sites must also export larvae (i.e. function as sources rather than sinks) if they are to ensure persistence of the wider network.

Habitat loss may also impact the ability of the marine reserve network in the Keppel Islands to support the local *A. melanopus* fishery. Larval export from marine reserves to reefs open to fishing was substantially lower for *A. melanopus* (4.3%; 8 of 184), compared to estimates for two other fishery-targeted species in this same system of reserves. Harrison *et al.* (2012) reported that reserves generated ~56.8% of coral trout (*Plectropomus maculatus)* and ~44.9% of stripey snapper (*Lutjanus carponotatus*) larvae dispersing from reserves to reefs open to fishing in the Keppel Islands. The comparatively lower export of *A. melanopus* larvae from reserves may be

due to differences in life history traits among these species (e.g. reproductive behaviour, pelagic larval duration) and/or a lack of recruitment habitat for *A. melanopus* in areas open to fishing. Not only were there fewer host anemone colonies outside of reserves (i.e. 16 colonies in total across the Keppel Island group), there were no colonies available to settlers that were not already inhabited. From a fisheries management perspective, this data indicates that the local system of reserves is unlikely to support a re-opened *A. melanopus* fishery until recruitment and breeding habitat recover in areas that are open to fishing. A continued moratorium on collection of both *A. melanopus* and its host anemone *E. quadricolor* across the Keppel Islands is recommended in order to re-build stocks of both these species. Given the naturally slow capacity for recovery of *E. quadricolor* populations (Scott & Harrison 2007), active restoration of anemone populations could benefit both this benthic invertebrate and the populations of fish that they support (Scott *et al.* 2014).

It is clear that the scale of disturbance affecting coral reefs is expanding (e.g. Bruno & Selig 2007) and their condition globally is rapidly deteriorating (e.g. Frieler *et al.* 2013). Despite some indication that coral reef fish larvae have the potential to disperse long distances (e.g. Simpson *et al.* 2014), the scale of demographic connectivity amongst coral reefs appears smaller than previously thought. Levels of self-recruitment at a scale of 30 km or less typically range between 30-60% (Jones *et al.* 2009) and dispersal declines strongly with distance from the source population (Buston *et al*. 2012; D'Aloia *et al*. 2013). The expanding scale of reef degradation, conflicting scales of larval dispersal, and the small number of case studies pose a significant challenge when attempting to incorporate connectivity into the conservation and management of coral reef species (McCook et al. 2009). Our study supports the notion that coral reef fish populations are influenced by both local and distant demographic processes. Although the local

network of marine reserves is demographically well-connected at both local and regional scales and provides an effective refuge from fishing, the reserves alone may not be sufficient to ensure the long-term persistence of this anemonefish population in the face of ongoing, and potentially widespread, habitat loss. Reserves, fisheries restriction, and in some cases active habitat restoration, may all be required for the conservation of species in coral reef ecosystems.

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Data Accessibility

Full microsatellite genotypes for all anemonefish and egg samples used in parentage analysis and effective population size estimation, as well as all the raw data used to create figures, are available in the Dryad Digital Repository (doi:10.5061/dryad.dj050). GenBank accession numbers for microsatellites are provided in Supplementary Information Table S1.

Author Contributions

GPJ and MCB initiated the study. MCB, DHW, AJF and PSA conducted field work and MCB conducted laboratory work with assistance from HBH. MLB contributed reagents and analytical tools. MCB and HBH analysed the data and MCB wrote the paper, with all authors contributing to revisions.

Table and figure captions

Table 1. Details of *Amphiprion melanopus* population sampling and parentage assignments across both reserve and open (fished) sites in the Keppel Islands. Percentages indicate the proportion of the total juveniles sampled at that site that were offspring of parents at that site (SR = self-recruitment), offspring of parents at other sites in the Keppel Islands (LC = local connectivity), or were not assigned (UA = unassigned).

Figure 1. *Amphiprion melanopus* is highly specialised in its habitat requirements, with both juveniles and adults using the host sea anemone *Entacmaea quadricolor* (a). The population of *A. melanopus* located in the Keppel Islands at the southern end of the Great Barrier Reef (b) is susceptible to both habitat loss and aquarium harvest and is currently managed using traditional fisheries management and a network of no-take marine reserves (c).

Figure 2. Larval connectivity of the anemonefish, *Amphiprion melanopus* across a marine reserve network in the Keppel Islands, Great Barrier Reef. All six marine reserves (green symbols) are connected through larval dispersal to at least one other marine reserve in the network. Four out of the six sites open to harvest (blue symbols) also receive larvae from adults living in reserves.

Figure 3. Percentage of the assigned offspring (39 larvae) that recruited back to a natal marine reserve or dispersed among reserves and open (fished) areas in the Keppel Islands. Offspring produced from adults in marine reserves accounted for 79% of the local replenishment of the *Amphiprion melanopus* population in the Keppel Islands.

Figure 4. Dispersal dynamics among seven *Amphiprion melanopus* colonies in the Monkey Bay marine reserve. This particular reserve made the largest contribution to connectivity and replenishment of the Keppel Islands population, with five of the seven colonies both exporting and receiving larvae from other sites in the region. Within-site exchange among the colonies was also significant, with 12 offspring settling to colonies within a few 100 metres of their parents and an additional two offspring settling back into their natal colony.

Figure 5. Frequency distributions of the observed dispersal distances of assigned offspring (black bars) and the distances between the 12 sites where anemonefish colonies were located (grey bars). Observed dispersal distances were independent of the distances between sampling sites, with 49% of assigned offspring settling within 4 kilometres of their natal colony.

Table 1

reserve status	site name	site code	latitude	longitude	# colonies	# adults	# juveniles	# assigned	% SR	% LC	% UA
reserve	Monkey Bay	MB	23°11'34.60"S	150°56'9.83"E	7	64	83	20	14	10	76
	Egg Rock	EGG	23°12'0.06"S	151° 5'55.24"E	11	70	12	3	17	8	75
	Middle Is.	MI	23°10'9.81"S	150°55'40.07"E	4	17	25	3	0	12	88
	Clam Bay	CB	23°11'18.11"S	150°58'45.14"E	3	6	6	2	0	33	67
	Halfway Is.	HWI	23°11'58.80"S	150°58'24.22"E	2	5	4	1	0	25	75
	North Keppel Is.	NKI	23°4'51.88"S	150°54'50.22"E	4	9	6	1	0	17	83
open	Coroborree Is.	CRI	23°3'7.47"S	150°53'13.65"E	3	17	20	6	0	30	70
	Wreck Bay	WB	23°10'32.86"S	150°59'23.78"E	4	12	11	1	0	9	91
	Big Penninsula	BP	23° 9'9.81"S	150°58'4.19"E	4	13	10	1	0	10	90
	Halftide Rocks	HTR	23° 9'21.26"S	150°56'4.12"E	1	3	1	1	0	100	0
	Humpy Is.	HPI	23°12'29.07"S	150°58'29.17"E	2	5	3	0	0	0	100
	Man & Wife Rock	MWR	23° 7'2.67"S	150°59'32.87"E	2	7	3	0	0	0	100
Keppel Islands TOTAL					**47**	**228**	**184**	**39**	**21**	**--**	**79**