Connectivity dominates larval replenishment in a coastal reef fish metapopulation

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Direct estimates of larval retention and connectivity are essential to understand the structure and dynamics of marine metapopulations, and optimize the size and spacing of reserves within networks of marine-protected areas (MPAs). For coral reef fishes, while there are some empirical estimates of self-recruitment at isolated populations, exchange among sub-populations has been rarely quantified. Here, we used microsatellite DNA markers and a likelihood-based parentage analysis to assess the relative magnitude of self-recruitment and exchange among eight geographically distinct sub-populations of the panda clownfish Amphiprion polymnus along 30 km of coastline near Port Moresby, Papua New Guinea. In addition, we used an assignment/exclusion test to identify immigrants arriving from genetically distinct sources. Overall, 82 per cent of the juveniles were immigrants while 18 per cent were progeny of parents genotyped in our focal metapopulation. Of the immigrants, only 6 per cent were likely to be genetically distinct from the focal metapopulation, suggesting most of the connectivity is among sub-populations from a rather homogeneous genetic pool. Of the 18 per cent that were progeny of known adults, two-thirds dispersed among the eight sub-populations and only one-third settled back into natal sub-populations. Comparison of our data with previous studies suggested that variation in dispersal distances is likely to be influenced by the geographical setting and spacing of sub-populations.

Keywords: parentage analysis; microsatellites; dispersal; fish larvae; self-recruitment; marine protected area

1. INTRODUCTION

In marine ecosystems, the extent to which discrete populations are linked by dispersal (either larvae, juveniles or adults) is termed connectivity [1]. Connectivity can have different meanings and implications depending on the scale considered and how it is measured. From an evolutionary perspective, connectivity can be defined as the degree to which gene flow affects evolutionary processes within populations (genetic connectivity) [2]. From an ecological perspective, demographically connected populations are those in which population growth rates are affected by dispersal [3]. Demographic connectivity has been acknowledged as a vital parameter for understanding the dynamics of populations and how they respond to natural and/or human disturbances [4–8]. Most populations of marine organisms are likely to function as metapopulations where numerous sub-populations are connected to varying degrees by larval dispersal [9–11]. Estimates of the magnitude of retention within and connectivity among sub-populations at ecological timescales are essential to understand natural metapopulation dynamics (e.g. [12–14]) and model human impacts on marine ecosystems [15]. In addition, the efficacy of management strategies, such as no-take marine reserve networks, depends on how individual reserve populations function and how they are connected to the metapopulation at larger scale [16,17]. How individual reserves function depends on the degree to which they are self-sustaining, are connected to zones open to fishing and are connected to other reserves in the network [11,17,18]. These functions cannot be confirmed without quantifying patterns of retention within and connectivity among populations. While the nature of demographic connectivity among marine populations is beginning to be described [16,19], the factors that shape its variation remain poorly understood.

The metapopulation concept is particularly applicable to coral reef organisms with pelagic larvae, as adult populations are usually restricted to discrete patches of reef habitat [10,17]. Recent empirical studies have revealed that local replenishment of coral reef fishes is significantly higher than previously envisaged [20–24]. However, in all these studies, a significant proportion of the newly settled juveniles originated from locations beyond the spatial extent of focal populations. Coupled biophysical models have suggested that ecologically relevant larval dispersal in reef fishes occurs over scales of 10–100 km in the
The panda clownfish (Amphiprion polymnus) is a southeast Asian endemic that lives in close association with discrete aggregations of two species of anemone (Stichodactyla hadomi and Heteractis crispa) occurring in sandy habitats associated with coral reefs [29]. Each anemone is usually occupied by one breeding pair and up to eight smaller non-breeders and juveniles. The female (the largest individual) lays demetal eggs on the upper surface of shells or dead coral next to the anemone. Embryos develop over a period of 6–7 days before hatching [29] and post-larvae settle into anemones after a pelagic larval phase lasting 9–12 days [30].

(b) Sampling and genotyping
A total of 942 individuals were sampled among the eight sites between January and April 2008. Each fish was captured by SCUBA using hand nets, measured (total length TL), fin clipped underwater in situ, and then released back into the same anemone. Fish that were too small to be fin clipped (less than 30 mm) were collected. In addition, all juveniles settling on each anemone over the sampling period were captured using hand nets. Finally, at the end of the experiment 15–30 fertilized eggs were collected (randomly within the clutch) from five egg clutches, each from a different anemone. All samples were preserved in 95 per cent ethanol and returned to the laboratory for subsequent genotyping. For all analyses, fish were divided into three categories according to their size. The first category ‘breeders’ consisted of the female and male (the two biggest individuals) of each anemone. The remaining fish were then divided into two arbitrary categories: ‘non-breeders’ (greater than 50 mm) and ‘juveniles’ (less than 50 mm).

(c) Population structure
We estimated genetic variability within and among sites and between resident breeders, non-breeders and juveniles using F and R statistics via analysis of molecular variance (AMOVA) in Arlequin v 3.11 [35]. Tests for statistical significance for all estimates were based on 10^4 random permutations, and significance levels were adjusted with a sequential Bonferroni correction for multiple tests with p < 0.05. All 18 loci satisfied Hardy–Weinberg and linkage disequilibrium assumptions.

(d) Parentage analysis
Parentage analysis was performed using FAMOZ [36]. The algorithm in this package calculates the log of the odds ratio (LOD) scores for parent–offspring relationships and constructs statistical tests for parentage assignment. Tests are based on simulations that generate offspring from genotyped parents (H0: the most probable parent is the true parent) or from allele frequencies in the population (H1: the most...
probable parent is not the true parent). For each analysis, allelic frequencies were estimated from the 942 genotyped individuals and these estimations were assumed to match the true allele frequencies in the population. Then, simulations of sets of $10^4$ juveniles were carried out under the two possible hypotheses ($H_0$ and $H_1$ above) and subsequent statistical tests were constructed to decide whether a given parent would be selected as the true parent or true parent pair. The distribution of the simulated LOD scores under the two hypotheses was plotted and the intersection between these distributions was designated as the threshold decision value (individuals with LOD scores above the threshold value were accepted as true parents). FAMOZ also allows for the introduction of an error rate in the LOD score calculation that takes into account the hypotheses (individuals with an error, even if it underestimates the real error rate, can reduce type I and II errors related to the parentage tests [37,38]. We evaluated four different error rates and chose an error rate of 0.4% for all further parentage analyses. This approach does not assume that the true candidate population has been sampled and can be advantageous in situations where it is not possible to sample all potential parents [42]. Genotypes of all breeders and non-breeders ($n = 451$) were used as the reference population. The likelihood that a new recruit came from the Bootless Bay population was computed with the partially Bayesian criterion of Rannala and Mountain [43]. Then, this likelihood ratio was compared with a distribution of $10^4$ genotypes simulated ratios from the reference population with a Monte Carlo algorithm [44]. A new recruit was determined to have originated from a different population when the probability of exclusion from Bootless Bay was greater than 95 per cent ($p < 0.05$).

3. RESULTS

(a) Population genetic structure

There was no significant genetic differentiation among the eight sub-populations. Both global $F_{ST}$ and $R_{ST}$ were low ($F_{ST} = 0.0011$, $R_{ST} = 0.0021$) and not
significantly different from zero (p-values 0.11 and 0.08, respectively). Pairwise $F_{ST}$ values among all samples were low (<0.0106) and only one out of 120 pairwise comparisons was significantly greater than 0 after Bonferroni corrections (electronic supplementary material, table S1a). Similarly, pairwise $R_{ST}$ values among all samples were low (less than 0.0219) and none were significantly greater than 0 after Bonferroni corrections (electronic supplementary material, table S1b). We concluded that the eight sites were one single genetic pool for all following analyses.

(b) Evaluation of parentage assignment
Parentage analysis assigned 100 juveniles, from a total of 491 that were genotyped to a sampled parent or parent pair from one of the eight sites. Almost half (45%) of these recruits were assigned independently to both the male and female in the same anemone, while the remaining recruits (55%) were assigned to a single parent. We excluded from further analysis all juveniles assigned to only one parent that presented two or more confirmed mismatches between their genotypes and that of the assigned parent (11 juveniles). The remaining 89 recruits were accepted as being true offspring of the parents to which they were assigned. No juveniles were assigned to two parents from different anemones. Overall, missing values accounted for 1.5 per cent of the genetic data and were distributed among all loci (there were no particular loci with consistent missing data).

(c) Self-recruitment and connectivity
Local recruitment ($n = 89$) accounted for 18.2 per cent of total recruitment ($n = 491$) to the focal population (table 1 and figure 2). Of these local recruits, 35 (7.1%) individuals settled into anemones at the same site as

<table>
<thead>
<tr>
<th>source site</th>
<th>BA (57)</th>
<th>LO (37)</th>
<th>MO (29)</th>
<th>TA (48)</th>
<th>LI (31)</th>
<th>MN (13)</th>
<th>BE (57)</th>
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average 7.5 12.3
their parents (self-recruits) while 54 (11.1%) settled in a site other than their natal anemone site (local connectivity). At the site level, self-recruitment averaged 7.5 per cent across all sites, but with variability among sites, ranging from 0 per cent at Lion Island (LI) site to 27 per cent (16 of 59 individuals) at Taurama (TA). The number of juveniles that settled in a given site but came from a different site than that of their natal anemone (local connectivity) averaged 12.3 per cent and varied among sites from 5.7 per cent (4 of 70 individuals) in site Loloata South Bank (BA) to 20 per cent (2 of 10 individuals) in site Motupore North Patch reef (MN; table 1 and figure 2).

We examined larval dispersal as a function of linear distance among sites for those individuals identified by DNA parentage analysis as being offspring of breeders from the focal metapopulation (figure 3). Linear distances among sites were grouped in classes (classes' sizes of 2 km each), with self-recruitment considered a separate class. Approximately 68 per cent of locally spawned recruits (approx. 12.4% of all juveniles) settled within 3 km of their natal site and 75 per cent of these recruits (approx. 13.5% of all juveniles) settled within 7 km of their natal site. The last 25 per cent of the juveniles identified by the parentage analysis (4.7% of all juveniles) dispersed between 7 and 28 km away from their site of origin. The multi-modal dispersal distribution of juveniles differed significantly from the frequency of linear distances among the eight sites (figure 3; $\chi^2 = 20.04$, d.f. $= 9$, $p < 0.05$). We found that higher numbers of larvae recruited back to their natal sites with concomitantly lower numbers of larvae dispersing longer distances than predicted based on the distributions of distances among sites.

Assignment tests revealed that 31 of 491 juveniles had a probability less than 0.05 of being from the same genetic pool as the focal metapopulation. These individuals probably came from one or more genetically distinct populations and accounted for 6.3 per cent of total recruitment. Altogether, parentage analysis and assignment tests accounted for 24.5 per cent of sampled juveniles. The remaining recruits approximately 75 per cent were sourced from a similar gene pool to that of the focal metapopulation but we can infer little more about the origin and dispersal distances of these individuals.

4. DISCUSSION

This study provides the first direct estimates of self-recruitment and demographic connectivity among multiple sub-populations in a coastal coral reef metapopulation. Our results indicated that larval retention within the metapopulation was dominated by local exchange among sites, rather than self-recruitment at the site level. At the other extreme, a small number of individuals came from one or more genetically distinct populations, presumably well beyond the geographical boundaries of our study. The majority of the recruits were genetically indistinguishable from the focal metapopulation, but did not match any of the breeders that we genotyped. Because the sampling within the focal metapopulation was fairly complete, we hypothesize that most of these juveniles represent dispersal from other non-sampled sites along the adjacent coastline.

Compared with our previous study in this location [45], by doubling the number of microsatellite markers used, we reduced the statistical errors linked to likelihood-based parentage assignments to less than 5 per cent (both type I and II errors based on simulated data). In addition, we were able to increase substantially the spatial scale and provide for the first time direct estimates of larval exchange among sub-populations spaced up to approximately 28 km from each other. At this geographical scale, levels of self-recruitment were highly variable among sites, but sites with higher numbers of breeders tended to have more self-recruits than sites with fewer breeders (table 1). The exception was site TA, which had by far the highest level of self-recruitment despite not representing the largest breeding population. Site TA was located in a relatively protected location close to the head of the bay, while all the other sites with larger breeding populations were outside the bay (Manubada Island (BE) and FI) or in more exposed locations (BA). Interestingly, in terms of proportions, the site with the second highest self-recruitment rate was MN, a site with a small breeding population also sheltered within the head of the bay. Larvae spawned at these sheltered sites (TA and MN) would therefore likely be less susceptible to advection by alongshore current flows than larvae from more exposed locations outside Bootless Bay. In addition, the proportion of larvae locally spawned that recruited to their natal sites was over-represented compared with the proportion expected based on the geographical scale, levels of self-recruitment were highly variable among sites, but sites with higher numbers of breeders tended to have more self-recruits than sites with fewer breeders (table 1). The exception was site TA, which had by far the highest level of self-recruitment despite not representing the largest breeding population. Site TA was located in a relatively protected location close to the head of the bay, while all the other sites with larger breeding populations were outside the bay (Manubada Island (BE) and FI) or in more exposed locations (BA). Interestingly, in terms of proportions, the site with the second highest self-recruitment rate was MN, a site with a small breeding population also sheltered within the head of the bay. Larvae spawned at these sheltered sites (TA and MN) would therefore likely be less susceptible to advection by alongshore current flows than larvae from more exposed locations outside Bootless Bay. In addition, the proportion of larvae locally spawned that recruited to their natal sites was over-represented compared with the proportion expected based on the distribution of distances among sites. However, almost half of these self-recruits were from site TA, indicating that shorter dispersal distances may be a feature of the most protected sites in coastal embayments. Overall, the frequency distribution of known dispersal trajectories appears to be largely explained by the geographical spacing, location and size of the sub-populations. Certainly, the different modes in this distribution coincide with the frequency of spacing between sites.

The high variation in levels of self-recruitment among sites, and the relationship between self-recruitment and population size is consistent with the model of James et al. [27] for the Great Barrier Reef whereby large reefs
contributed more than smaller ones to the local larval pool. Our mean estimate of self-recruitment per site (7.5%) is similar to mean simulated values among 321 relatively continuous reefs along the Great Barrier Reef. In their simulations, James et al. estimated that virtual larvae returning to their natal reef comprised less than 10 per cent of the settling cohort for most of the reefs. While local retention of larvae may be an advantage in environments where habitats are limited or separated by great distances [17], this advantage may not be extended to situations where habitats are more continuously distributed as in Bootless Bay. Particular sites, with high replenishment rates, such as TA site in this study, could play a crucial role in sustaining the stock in the entire metapopulation [12,46].

The coastal geographical setting may be critical in explaining the low self-recruitment pattern of our focal clownfish metapopulation. In the present study, levels of self-recruitment at both ‘site’ (ranged from 0 to 27%, average 7.5%) and ‘metapopulation’ level (18%) were relatively low compared with published values for *A. polymnus* and other clownfish species (*A. percula*) at more isolated locations in Kimbe Bay (Papua New Guinea) [20,22,23]. These values also correspond to the lowest empirical estimate of self-recruitment measured so far among coral reef fishes (reviewed in [17]). However, our estimate of self-recruitment at the metapopulation level for 2008 (18%) is close to that of our previous estimate of 25 per cent obtained at a smaller spatial scale in Bootless Bay (excluding MN, BE and FI) sampled in 2005–2006 [45], suggesting that these results are not atypical of this region and that the geographical settings do have an important role in determining the observed dispersal pattern.

In contrast to low self-recruitment estimates in Bootless Bay for *A. polymnus*, Almany et al. [20] reported consistent high self-recruitment rates in Kimbe Island for two species with contrasting life-history characteristics (*A. percula*: benthic eggs and approx. 11 days of pelagic larval duration (PLD) and *Chaetodon vagabundus*: pelagic eggs and approx. 38 days of PLD). Both *Amphiprion* species have similar life-history characteristics and differences between studies in Bootless Bay and Kimbe Island suggest that, at ecological timescales, dispersal kernels may be more influenced by the relative isolation or geographical setting of the focal populations than species-specific life-history characteristics [47]. Still, this trend clearly needs to be tested in more species and locations before any conclusion can be made. Besides, other studies based on geochemical signatures in otoliths suggest that this is not a general rule. Patterson et al. [48] showed that *Pomacentrus coelestis* on Lizard Island exhibited 75 per cent self-recruitment even though it has many other reefs relatively close by, while Patterson & Swearer [49] showed that *Coris picta* exhibited 26–65% self-recruitment on isolated Lord Howe Island. However, until all existing methods to estimate self-recruitment are cross-validated, comparisons among them should be made cautiously [17].

Parental analysis suggested that most sites received a higher proportion of recruitment from larvae spawned at different sites within the metapopulation than from self-recruitment. This high connectivity among sites was probably underestimated, in particular that between the inside and outside of Bootless Bay, as it was not possible for us to exhaustively search all potential areas outside of the Bay. This lack of sampling presumably explains a significant proportion of the approximately 300 juveniles that settled in our study area and were left unassigned either by parentage analysis or assignment tests. It seems that a much larger sampling effort along the coast line will be necessary to find the origin of those juveniles.

Assignment tests detected that a non-negligible percentage (6.3%) of the juveniles sampled in this location were genetically distinct from the focal metapopulation. We hypothesize that these recruits were long-distance immigrants, but unfortunately, even if this was confirmed, we could not estimate how far these juveniles had travelled. This would require much more extensive sampling of genetic signatures at greater distances to the east and west of Bootless Bay. If indeed these genetically distinct recruits are long-distance immigrants, they may play an important role in buffering extinction risk in this metapopulation [50]. However, the fact that these individuals apparently belonged to a different genetic pool suggests that either we have fortuitously captured a very rare dispersal event, or that the juveniles that we collected would not have successfully reproduced if we had not captured them. This is because a constant exchange of this magnitude with successful reproduction of these individuals should lead to homogenization of these genetic pools [2]. The question that remains is how variable this contribution is over time and whether or not these individuals are capable of successfully integrating into their new population.

In conclusion, given the relatively low-observed self-recruitment rates, a high proportion of connectivity among sites, and the relatively high proportion of long-distance dispersal, it appears that connectivity and not self-recruitment dominates larval replenishment in this focal clownfish metapopulation. We found that 18 per cent of juveniles in Bootless Bay settled between 0 and 28 km from their place of origin while over 80 per cent were likely to have dispersed from populations beyond our studied sites. These results have significant implications for the design of MPAs in this area as they indicate that a single MPA inside Bootless Bay may not be sufficient to maintain the metapopulation if unprotected sources were to collapse. In addition, while there is consistent evidence that life-history characteristics of individual species can play an important role in terms of dispersal at evolutionary (genetic) timescales [51–54], the suggestion that the spatial distribution of suitable habitats may have more impact on levels of demographic connectivity than life-history characteristics of individual species clearly deserves more attention in future studies. If this happens to be true, it will have encouraging implications for the use of MPAs to offer protection to coral reef fish assemblages [55]. Testing this hypothesis at more locations, and on more species, remains a top priority for conservation biologists working in coral reef ecosystems.

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