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Coral Reef Fish Larvae Settle Close to Home

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Summary

Population connectivity through larval dispersal is an essential parameter in models of marine population dynamics [1–3] and the optimal size and spacing of marine reserves [4–6]. However, there are remarkably few direct estimates of larval dispersal for marine organisms, and the actual birth sites of successful recruits have never been located. Here, we solve the mystery of the natal origin of clownfish (*Amphiprion polymnus*) juveniles by mass-marking via tetracycline immersion all larvae produced in a population. In addition, we established parentage by DNA genotyping all potential adults and all new recruits arriving in the population. Although no individuals settled into the same anemone as their parents, many settled remarkably close to home. Even though this species has a 9–12 day larval duration, one-third of settled juveniles had returned to a 2 hectare natal area, with many settling <100 m from their birth site. This represents the smallest scale of dispersal known for any marine fish species with a pelagic larval phase. The degree of local retention indicates that marine reserves can provide recruitment benefits not only beyond but also within their boundaries.

Results and Discussion

Measuring larval dispersal is regarded as the greatest challenge facing marine ecologists and managers [4–9]. The precise natal origins of juveniles recruiting into adult populations are invariably unknown. Given that the pelagic larval duration of many marine species ranges from weeks to months, the potential for long-distance dispersal by prevailing currents is extremely high [9–11]. However, among those juveniles that survive to reach suitable habitat, this potential is clearly not always reached [11–16]. Although there are numerous indirect measures of the effective scale of dispersal [11–15], the only unequivocal approach is to mark larvae at their birth site and locate them when they recruit to the adult population [16–18]. Most marine organisms produce large numbers of extremely small pelagic larvae that are subject to advection, diffusion, and extremely high rates of mortality, so this approach was once thought impossible. Here, we overcome these impediments by combining mass-marking of larvae in the field with the first application of DNA paternity analyses to estimate self-recruitment in a coral reef fish population. By locating the parents of juveniles recruiting to their natal population, the DNA analyses provide the first measures of fine-scale dispersal distance and direction.

We studied a population of panda clownfish (*Amphiprion polymnus*) associated with a discrete aggregation of anemones (*Stichodactyla haddoni* and *Heteractis crispa*) located in shallow sandy areas adjacent to Schumann Island, in Kimbe Bay, Papua New Guinea (Figure 1A). The population was divided spatially into five subareas, with no individuals found in adjacent sand or coral habitats or within 1 km beyond any of the subareas (Figure 1B). Each anemone was colonized by a maximum of one breeding pair and up to eight juveniles and subadults (Figure 1C). A total of 40 anemones were found in the five subareas, with 33 anemones supporting breeding pairs. Females laid demersal eggs on the upper surface of shells or dead coral next to the anemone (Figure 1C). The embryos developed over a 6–7 day period before hatching [19], providing an opportunity for in situ marking of embryonic otoliths (ear bones) via tetracycline immersion [16]. Late-stage larvae then settled into anemones after a pelagic larval phase lasting 9–12 days [20].

We monitored egg production and labeled the otoliths of all embryos produced by females in the study area with tetracycline over two 3 month periods (April–June, 2002, and August–October, 2003). In the first year, marking was restricted to subareas a–c and then the whole study site in 2003 (Figure 1B). Because larvae always settle into host anemones, it was possible to collect all individuals that recruited to our focal population during the study period by sampling on a daily basis. In 2002, otoliths of ten fish from a total of 63 recruits (16%) collected in subareas a, b, and c tested positive for the tetracycline mark (Table 1). We marked embryos from more adult pairs over the whole population in the following year. This time, 23 fish from a total of 73 newly settled recruits (32%) were marked and had recruited to their natal population (Table 1).

Spatial and temporal patterns in the arrival of marked recruits were similar to those for unmarked recruits. In 2003, most of the marked recruits arrived at subarea c, which was also the location of highest recruitment as a whole (Table 2). Egg production and larval settlement appeared to follow recognizable patterns, with broad cycles of egg production followed by more discrete recruitment pulses at or shortly after the new moon (Figure 2). Larvae returning to the natal population only arrived during the peaks in recruitment in both years. The

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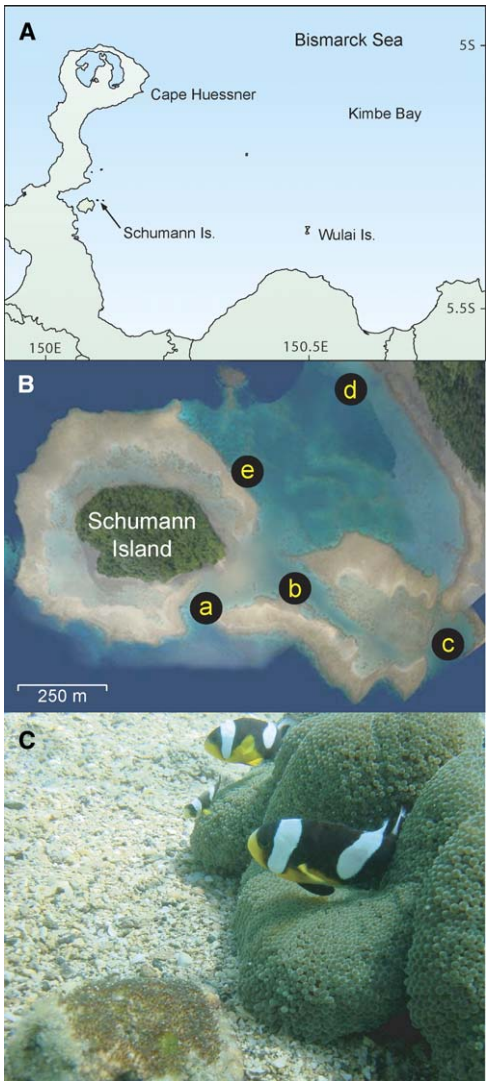

Figure 1. Study Location and Species

(A) Map showing location of Schumann Island in Kimbe Bay, on the northern coast of New Britain, Papua New Guinea.

(B) Aerial photo showing the five subareas (subareas a–e) of shallow sand flat supporting small populations of panda clownfish, *Amphiprion polymnus*.

(C) shows an adult pair of panda clownfish on the anemone *Stichodactyla haddoni* and a clutch of eggs on coral rubble.

coincident spatial and temporal patterns in self-recruitment and recruitment suggest that the arrival of self-recruiters and immigrant juveniles is being driven by the same processes [12].

All resident adult pairs and juveniles arriving in 2003 were genotyped with 11 microsatellite DNA markers to provide accurate identification of the paternity of newly settled recruits. Paternity analysis established the location of the parents that produced offspring returning to the Schumann Island area and a measure of the distance between the settlement site and their natal anemone. We found, on the basis of genetic markers, that 23 individuals from a total of 73 newly settled recruits were spawned by local adult pairs in 2003 (Table 1). The percentage of fish returning to their natal location on the basis of paternity analysis was, therefore, identical to the estimate provided by the mass-marking experiment.

Table 1. Estimates of Self-Recruitment from Tetracycline Marking and Paternity Analysis

	2002	2003	
Total number of pairs marked	22	33	
Total area marking (km^2)	0.2	0.5	
Number of embryos marked	69,250	125,900	
Total number of recruits collected	63	73	
Number of recruits marked	10 tet	23 tet	23 pat
% Self recruitment	15.9%	31.5%	31.5%

Shown are the summary statistics for tetracycline marking of *Amphiprion polymnus* embryos at Schumann island in 2002 and 2003, and paternity analysis in 2003, including the number of pairs for which embryos were marked, the area over which marking took place, the total number of embryos marked, the total number of recruits collected, and the total number of marked recruits collected. tet denotes the number of tetracycline-marked juveniles collected, and pat denotes the number of juveniles collected that were classified to resident parents by paternity analysis.

The number of self-recruiters arriving in the different subareas was unrelated to larval production or adult numbers at the source subareas. A total of 15 parental pairs produced the 23 newly settled recruits that returned the study area as a whole, but the different subareas did not contribute equally (Figure 3). Nine of the self-recruiters came from seven adult pairs in subarea a, where there were only nine adult pairs in total. In contrast, subarea c accounted for only five self-recruiting individuals despite having the highest number (13) of adult pairs (Figure 3) and the greatest overall egg production. The net direction of dispersal over the 3 months potentially indicates a local source-sink dynamic within the broader Schumann Island population. Over half (16 of 23) of the newly settled recruits collected in subareas b and c were produced by adults in subareas a and d (Figure 3). Five individuals returned to their natal subareas, settling <50 m from their parents. None returned to the same anemone as their parents, indicating that direct kin relationships between adults and juveniles are likely to be rare.

Although tetracycline marking and paternity analysis provided the same classification in most cases, there

Table 2. Spatial Distribution of Self-Recruitment

	2002		2003		
Subarea	Total Recruits	Marked Recruits (tet)	Total Recruits	Marked Recruits (tet)	Marked Recruits (pat)
a	28	4	15	4	5
b	17	3	13	4	5
c	18	3	34	12	11
d	X	X	8	3	2
e	X	X	3	0	0

Recruitment and self-recruitment estimates to subareas a–e in 2002 and 2003.

Table 3. Concordance between Tetracycline Marking and Paternity Analysis

	Classification of Recruits on the Basis of Parentage Analysis	
	Marked	Not Marked
Classification of Recruits on the Basis of Tetracycline		
Marked	16	7
Not marked	7	43

Contingency table showing classification of recruits into those that were identified as "marked" (self-recruiters) and "not marked" via tetracycline and parentage analysis.

was not complete agreement between the two techniques (Table 3). Sixteen of the juveniles classified as self-recruiters had tetracycline marks and were also assigned to local parents through genetic analysis (~70% of marked individuals). In addition, 43 juveniles were classified as immigrants by both methods. The probability that the two techniques would select the same 16 juveniles from a sample of 73 individuals if one or both techniques was spurious is extremely low (via hypergeometric distribution, $p = 2.4 \times 10^{-16}$). The discrepancies included seven individuals that were classified as self-recruiters by paternity analysis but did not have tetracycline marks. This may indicate that tetracycline marking in the field chambers was not 100% successful and that this method underestimated self-recruitment. There were also seven individuals with clear tetracycline marks that were not classified as genetically related to any adult pairs. This suggests that there may have been some turnover in the adult population over the 3 months of the study, most likely through the loss of the original adults and subsequent maturation of large juveniles. Again, this would lead to an underestimate of self-recruitment by paternity analysis. If the discrepancies were solely due to false negatives by both methods, actual self-recruitment could have been as high as 42%. However, the less-likely alternative that one or both techniques produce false positives also requires further evaluation. In the future, estimates of self-recruitment may be fine-tuned with improved methods of marking larvae and sampling the DNA of replacement adults.

This is the first time that settled juveniles of any marine fish with pelagic larvae have been traced back to their parents. Although it has been shown that coral reef fish larvae may return to large natal populations [12, 16], our study has detected the smallest scale of dispersal known (i.e., at the scale of tens of meters rather than kilometers). Approximately 32% of the larvae settling into anemones were spawned by adults within a 2 hectare area. Because the 9–12 day larval duration of *A. polymnus* is shorter than is typical for most other coral reef fishes [21], they might be expected to have relatively short dispersal distances. Also, because clownfish have a highly specialized association with a few anemone species [19], there may be a particular advantage to not dispersing away from suitable habitat. Nevertheless, although self-recruitment may be more likely for such species, this was not previously known, and our first direct estimates set a new lower bound to known dispersal distances in coral reef fishes.

Although the local panda clownfish population is clearly sustained by a significant degree of self-recruit-

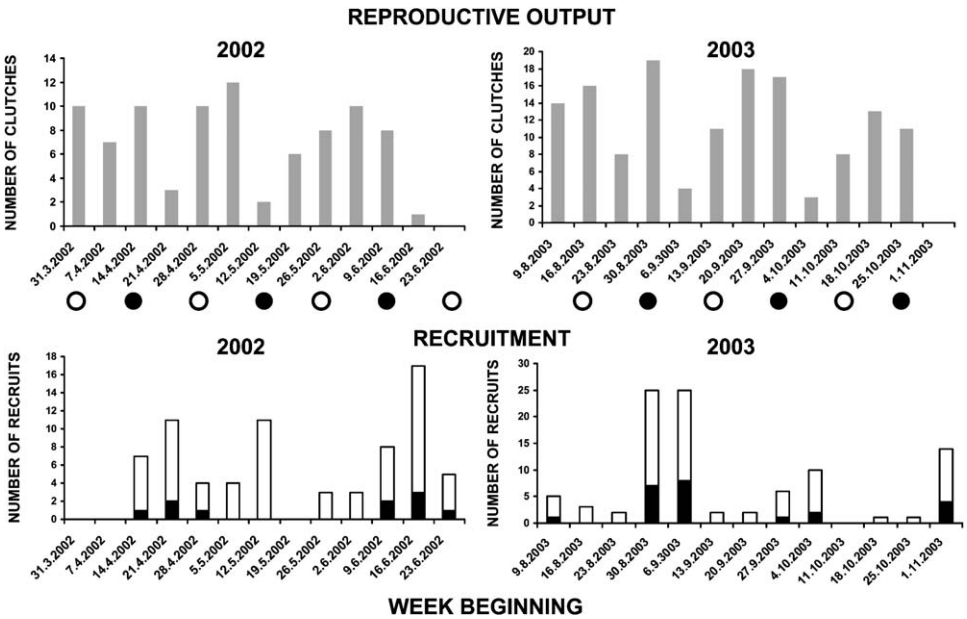

Figure 2. Timing of Egg Production and Recruitment

The timing and magnitude of egg production (number of clutches laid), recruitment, and self-recruitment on a weekly basis over 3 month periods in 2002 and 2003. For recruitment, the bars represent all recruits, and filled portions represent numbers of self-recruiters on the basis of the presence of tetracycline marks. Open circles denote time of full moon, and filled circles denote time of new moon. The date given is the first day of each weekly interval.

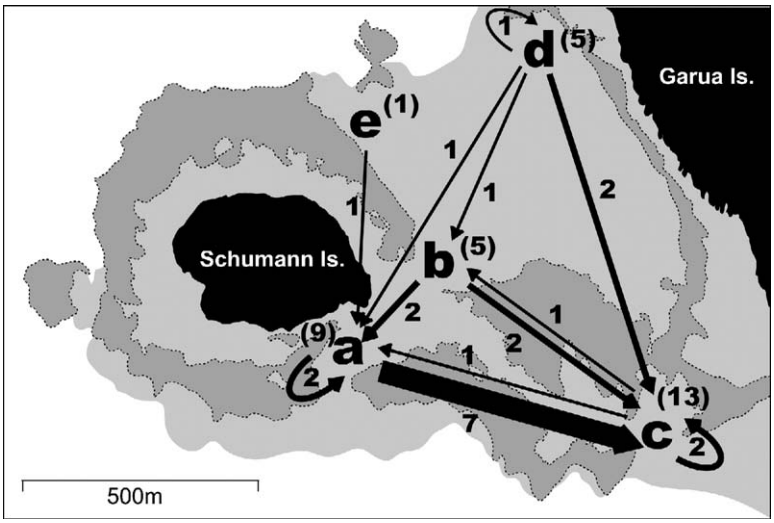

Figure 3. Local-Scale Connectivity Network

Map showing distance and direction of fine-scale dispersal of all juvenile panda clownfish settling within their natal population at Schumann Island, as determined from parentage analysis. The thickness of the arrows reflects numbers of juveniles either moving between subareas a–e or returning to the subarea of their birth. The number of adult pairs at each subarea is indicated in brackets. Total reproductive output in each subarea is proportional to the number of adult pairs.

ment, the 9–12 days in the pelagic environment do not preclude a considerable potential for dispersal. We currently do not know where the other 68% of larvae settling at Schumann Island come from or whether juveniles born at Schumann Island are successfully recruiting to anemones in other locations within or outside of Kimbe Bay. This species appears to have a sparse distribution, and there are no other known aggregations in Kimbe Bay (although a few isolated anemones have been recorded >10 km away). It seems likely that many of the juveniles that recruited to the study site and that were not sourced from the local population had traveled a considerable distance.

The mechanisms by which larvae were able to maintain their position or find their way back to their natal population are as yet unknown. At Schumann Island, panda clownfish embryos often hatch in strong tidal currents, so posthatch larvae are likely to be transported away from the immediate area. Interactions between physical oceanographic processes and larval behavior may lead to significant retention of larvae in near-shore waters adjacent to the natal population [11, 22]. Alternatively, embryos of species that spawn demersal eggs may imprint on local sounds and/or chemical cues [22–24], allowing late-stage larvae to actively home to their natal location. Distinguishing between these two mechanisms will be important if realistic models are to be developed to predict connectivity over larger spatial scales.

The presence of significant self-recruitment on an extremely local scale has important implications for the conservation and management of coral reef fishes [4–6]. Patterns of larval duration and dispersal are poorly understood for small, specialized reef fishes that are closely associated with corals and other sessile invertebrates. Local extinctions as a result of habitat devastation may progress into global extinctions in the absence of sufficient connectivity to allow recovery [25–28].

There is universal acceptance that understanding patterns of larval retention and population connectivity are critical for sizing and spacing closed areas in marine-reserve networks [4–7, 29–31]. Indirect measures of dispersal distances cannot be used to parameterize models of optimal reserve design until they have been validated. Our results show that both extremely localized and longer-distance dispersal must be occurring in the panda clownfish. Although it is widely speculated that marine reserves may provide a recruitment subsidy to fished areas *beyond* their boundaries [4], our results also indicate that there will also be significant recruitment benefits *within* marine reserves. The dispersal pattern supports the contention that marine reserves can be sized for optimal protection of resident populations and spaced to allow a significant recruitment subsidy from reserves to adjacent exploited populations [6, 30]. The first validated measures of larval dispersal in a marine fish provided here indicate that these dual management objectives may be achievable.

Experimental Procedures

Tetracycline Marking

Eggs were laid on the upper surface of small (5 × 10 cm) terra cotta tiles placed next to anemones. All embryos (>4 days old) were sealed for 2 hr in oxygenated marking chambers containing 250 mg/l of tetracycline positioned beside the anemone. After this, all nests were photographed to assess the number of embryos produced and then returned to previous position. All recruits were collected from the same anemones on a daily basis over the 3 months of the study, beginning 9 days after embryo marking began (to allow for the larval duration) and ending 12 days after embryo marking ceased. Otoliths were dissected from all juveniles, ground to a thin section that encompassed the otolith core, and then examined under a fluorescence microscope for evidence of a tetracycline mark.

Parentage Analysis

All reproductive individuals were caught, tail clipped underwater, and released back onto their anemones in June 2003 (an operation taking less than 3 min per fish). The fin clips were then preserved in 95% ethanol and returned to the lab for subsequent genetic analyses. In most anemones, adults were easily recognized as the two larger individuals; however, when size was not a clear indicator, we collected fin clips from additional individuals to make sure all potential adults were sampled (85 individuals in total). We screened 11 microsatellites [32] from the 85 potential parents and all 73 new recruits (offspring) collected from anemones in the five subareas over the 3 month period in 2003. Five loci demonstrated significant

homozygote excess, which we attributed to null alleles. These loci were therefore rescreened and found to conform to Hardy-Weinberg equilibrium. Paternity was assessed with a likelihood approach [33]. We used FAMOZ [34] to compute log-likelihood-based paternity. FAMOZ provides log of the odds ratio (LOD) scores that are calculated for assigning parentage. The program used simulations based on a comparison of offspring assignment with allelic frequencies and genotype frequencies to build a statistical test for parentage assignment. We introduced an error rate (10%) to include mistakes in scoring parental or offspring genotypes, the presence of null alleles, and marker mutation [35]. Results from the simulation indicated that a minimum LOD score of 8.57 results in a 99% probability of accurately identifying paternity, provided all potential adults are sampled [35].

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