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Table S1

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Local Replenishment of Coral Reef Fish Populations in a Marine Reserve

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The scale of larval dispersal of marine organisms is important for the design of networks of marine protected areas. We examined the fate of coral reef fish larvae produced at a small island reserve, using a mass-marking method based on maternal transmission of stable isotopes to offspring. Approximately 60% of settled juveniles were spawned at the island, for species with both short (<2 weeks) and long (>1 month) pelagic larval durations. If natal homing of larvae is a common life-history strategy, the appropriate spatial scales for the management and conservation of coral reefs are likely to be much smaller than previously assumed.

Many of the desired outcomes of marine protected areas (MPAs) in fisheries management and biodiversity conservation rely on untested assumptions about the degree to which fish populations are connected by larval dispersal (1–3). Connectivity is a critical parameter in models for optimizing the size and

spacing of MPAs (4–6), but the scarcity of direct information on larval dispersal limits the models' utility (7). Because larvae typically spend times ranging from days to months in the pelagic environment before seeking suitable habitat to begin adult life, direct measurements of connectivity are challenging (7–9). Thus, although larvae have the potential to travel far from their birthplace, realized dispersal distances are seldom known.

We studied populations of two species of coral reef fishes with different reproductive strategies, occupying a 0.3-km² coral reef surrounding a small island that was recently designated an MPA (Kimbe Island in Kimbe Bay, Papua New Guinea). Orange clownfish (*Amphiprion percula*; Pomacentridae) spawn demersal eggs that hatch after several days of parental care, and larvae then spend ~11 days in the pelagic environment.

In contrast, vagabond butterflyfish (*Chaetodon vagabundus*; Chaetodontidae) release gametes directly into the water column (that is, there is no parental care), and larvae spend an average of 38 days in the pelagic environment (Fig. 1). The reproductive characteristics of the vagabond butterflyfish are found in most marine fish species and in nearly all species targeted by fisheries throughout the world's oceans.

In December 2004, we tagged larvae using a method whereby mothers transmit stable barium (Ba) isotopes to their offspring before hatching and dispersal (10). A total of 176 clownfish females and 123 butterflyfish from the reef surrounding Kimbe Island (Fig. 2) were captured and injected with a BaCl₂ solution that was highly enriched in ¹³⁷Ba and depleted in ¹³⁵Ba as compared to natural Ba isotope values. In February 2005, we returned to Kimbe Island and collected 15 clownfish and 77 butterflyfish that had recently settled into benthic reef habitats after completing their pelagic larval phase. The analysis of daily growth increments of sagittal otoliths (ear bones) confirmed that each of the recent settlers was born after the injection of the adults with BaCl₂. We then quantified Ba isotope ratios in the otolith cores of settlers, using laser ablation inductively coupled plasma mass spectrometry (ICP-MS). Ba isotope ratios in the otoliths of all individuals fell on the theoretical mixing curve between the enriched isotope spike and natural Ba, with values that were similar to those from otoliths of larvae from three reef fish species injected with enriched ¹³⁷Ba

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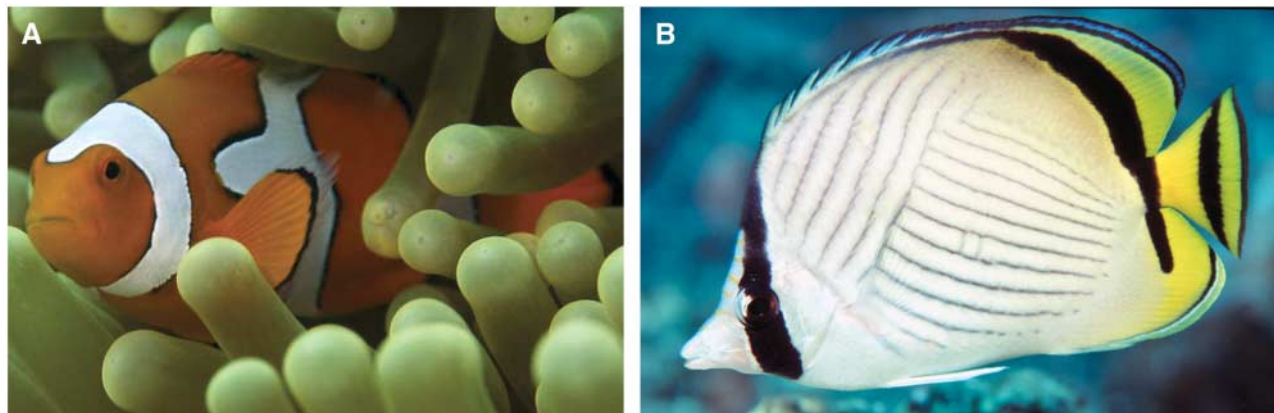


Fig. 1. Study species. An adult (A) *A. percula* (photo by S. R. Thorrold) and (B) *C. vagabundus* (photo by R. Patzner).

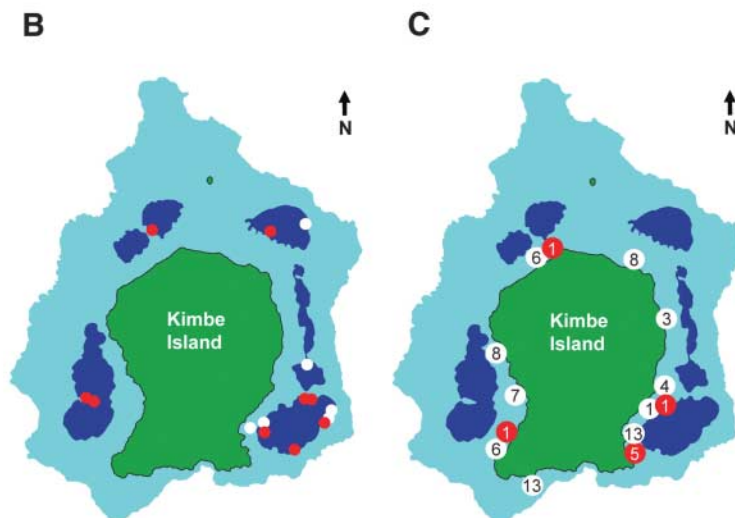
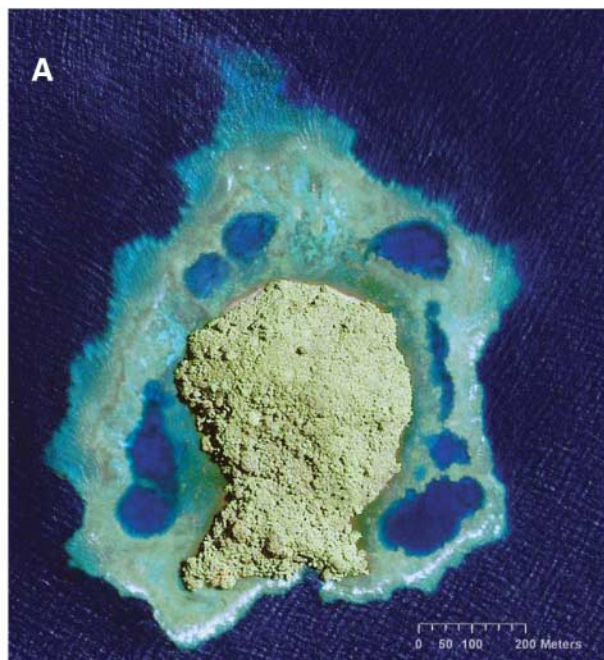


Fig. 2. (A) Satellite image of the Kimbe Island MPA (taken by the IKONOS-2 satellite at a resolution of 1 m). (B and C) Schematic diagrams of Kimbe Island showing the locations of tagged (red circles) and untagged (white circles) juveniles collected in February 2005. The locations of juvenile (B) *A. percula* ($n = 15$) and (C) *C. vagabundus* ($n = 77$) are shown. In (C), the number in each circle corresponds to the number of juveniles collected from that location.

under controlled laboratory conditions (Fig. 3). Definitive identification of tagged fish was based on otolith $^{138}\text{Ba}/^{137}\text{Ba}$ ratios because they converged on the natural ratios much more slowly than did $^{138}\text{Ba}/^{135}\text{Ba}$ values (Fig. 3). A fish was considered tagged if the $^{138}\text{Ba}/^{137}\text{Ba}$ ratio of its otolith core was more than 3σ lower than the mean value from measurements of control otoliths ($n = 86$) assayed throughout the ICP-MS runs.

Otoliths of nine clownfish and eight butterflyfish were identified as tagged based on $^{138}\text{Ba}/^{137}\text{Ba}$ ratios, providing incontrovertible evidence that these fish had returned to their natal reef (Fig. 3). Assuming that we tagged all clownfish larvae produced from Kimbe Island, 60% of juveniles made the return journey—a conservative estimate if we failed to locate any breeding pairs. For butterflyfish, we estimated the proportion of the total adult population captured and injected with Ba as 17.3% [95% confidence interval (CI): 14.4 to 20.0%] via a population survey conducted after 123 adults had been injected with Ba and externally tagged. Scaling the proportion of tagged juveniles (8 of 77) to the proportion of adults injected with Ba indicated that a remarkable 60.1% (95% CI: 52.0 to 72.2%) of juvenile butterflyfish returned to their natal reef. Tagged juveniles were found in a variety of locations scattered around Kimbe Island, although the larvae of both species that returned settled in the greatest numbers at the southeastern corner of the island (Fig. 2).

Our direct estimate of ~60% self-recruitment for these two species demonstrates that larvae are capable of returning to a very small target reef (only 0.3 km²), even after an extended larval duration. Although there is much recent indirect evidence for the limited dispersal of marine lar-

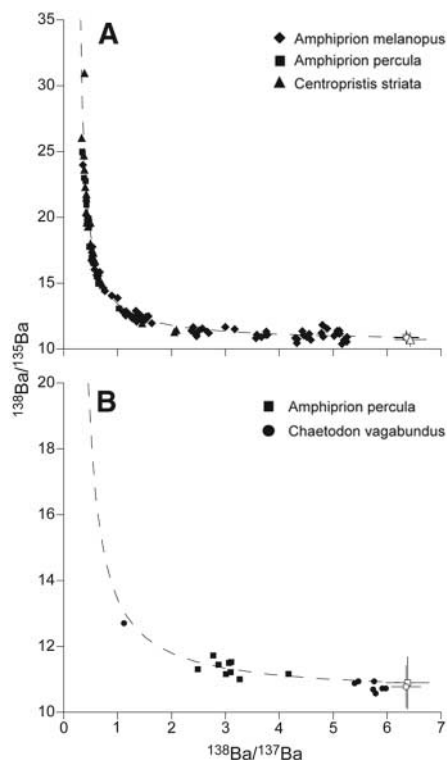


Fig. 3. (A) Ba isotope ratios in otolith cores from juveniles of three reef fish species: *A. melanopus* (diamonds), *A. percula* (squares), and *Centropomus striata* (triangles), spawned from females injected with $^{137}\text{BaCl}_2$ up to 3 months after the injections and reared in the lab through settlement (10); and mean values ($\pm 3\sigma$) from controls for each species (open symbols), along with the theoretical mixing curve (dashed line) between the enriched Ba isotope spike and natural Ba ratios. (B) Ba isotope ratios in otolith cores of tagged *A. percula* (squares) and *C. vagabundus* (circles) juveniles and mean ($\pm 3\sigma$) values from the otoliths of all unmarked juveniles (open symbols), along with the theoretical mixing curve (dashed line) between the enriched Ba isotope spike and natural Ba ratios.

vae (11), our results, in combination with two previous mark/recapture studies of larval dispersal (12, 13), suggest that self-recruitment in marine fish populations may be common and take place on a smaller scale than previously realized. For example, a recent Caribbean-wide biophysical model of population connectivity in reef fishes highlighted larval capabilities as a key factor determining levels of self-recruitment (14). When active larval behavior was introduced in

the model within a few days after hatching, self-recruitment of virtual larvae averaged ~21% to reef areas delineated by 450 km². In our study, the proportion of self-recruitment was three times greater to a reef more than three orders of magnitude smaller.

The observation that parental habitat is demonstrably of sufficient quality for survival and reproduction provides a compelling argument for the presence of some degree of self-recruitment

in fish populations. Selection may therefore favor the retention of many larvae, especially if the probability of encountering better adult habitat by dispersing is low (15) or advantages accrue through local adaptation (16). A number of mechanisms may be used by larvae to avoid being swept away from natal reefs. Field evidence suggests that reef fish larvae migrate vertically in the water column to exploit currents at different depths and thereby avoid dispersal away from spawning locations (17). Larvae are also capable of sustained directional swimming soon after hatching (18), and possess a range of well-developed sensory systems to locate and orient to reefs, including sight, smell, and sound (18–21).

Despite the high levels of self-recruitment we detected, ~40% of juveniles of both species came from outside the MPA. The reef nearest to Kimbe Island is 10 km away, and reefs in this region are typically separated by 5 to 20 km. Ecologically important larval exchange must occur between populations at these scales. Thus, the Kimbe Island MPA is likely to be self-sustaining as well as providing recruitment subsidies to populations beyond its boundaries. Although levels of retention and connectivity may differ where reefs are closer and populations are less isolated, the Kimbe Island example sets a new boundary condition for the scale at which self-recruitment can occur.

Ideally, the size and spacing of marine reserves should be predicated on an understanding of larval dispersal distances (3–6, 22). The optimal design should be one in which individual

MPAs are large enough so that populations within reserves can sustain themselves, yet small enough and spaced so that a proportion of larvae produced inside the MPA is exported to unprotected areas (3, 5, 12). Our study suggests that the spatial scale at which coral reef MPAs can achieve these dual goals may be relatively small. However, if natal homing and larval retention are common, some MPAs may fail to deliver substantial recruitment subsidies to locations beyond their boundaries. We therefore support recent suggestions (23, 24) that MPA networks should be combined with conventional management strategies to both protect threatened species and ensure the sustainability of fisheries on coral reefs.

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Materials and Methods

References

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Developmentally Regulated piRNA Clusters Implicate MILI in Transposon Control

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Nearly half of the mammalian genome is composed of repeated sequences. In *Drosophila*, Piwi proteins exert control over transposons. However, mammalian Piwi proteins, MIWI and MILI, partner with Piwi-interacting RNAs (piRNAs) that are depleted of repeat sequences, which raises questions about a role for mammalian Piwi's in transposon control. A search for murine small RNAs that might program Piwi proteins for transposon suppression revealed developmentally regulated piRNA loci, some of which resemble transposon master control loci of *Drosophila*. We also find evidence of an adaptive amplification loop in which MILI catalyzes the formation of piRNA 5' ends. *Mili* mutants derepress LINE-1 (L1) and intracisternal A particle and lose DNA methylation of L1 elements, demonstrating an evolutionarily conserved role for PIWI proteins in transposon suppression.

Known piRNAs are not expressed until spermatocytes first enter mid-prophase (pachytene stage) at ~14 days after birth (P14) (1–4). However, *Mili* expression begins in primordial germ cells at embryonic day 12.5 (5, 6), and transposons, such as L1, can be expressed in both premeiotic and meiotic germ cells (7, 8). We therefore probed a connection between *Mili* and transposon control by

examining MILI-bound small RNAs in early-stage spermatocytes. Notably, MILI-associated RNAs could be detected at all developmental time points tested (Fig. 1 and fig. S1). Northern blotting revealed that pre-pachytene piRNAs join MILI before pachytene piRNAs become expressed at P14 (Fig. 1B). The appearance of pre-pachytene piRNAs was MILI-dependent, suggesting a requirement for this protein in either

their biogenesis or stability (Fig. 1C). These results raised the possibility that MILI might be programmed by distinct piRNA populations at different stages of germ cell development.

To characterize pre-pachytene piRNAs, we isolated MILI complexes from P10 testes and deeply sequenced their constituent small RNAs. Like pachytene populations, pre-pachytene piRNAs were quite diverse, with 84% being cloned only once. The majority of both pre-pachytene (66.8%) and pachytene (82.9%) piRNAs map to single genomic locations. However, a substantial fraction (20.1%) of pre-pachytene piRNAs had more than 10 genomic matches, as compared to 1.6% for pachytene piRNAs.

Annotation of pre-pachytene piRNAs revealed three major classes (Fig. 2A). The largest (35%) corresponded to repeats, with most matching short interspersed elements (SINEs) (49%), long interspersed elements (LINEs) (15.8%), and long terminal repeat (LTR) retrotransposons (33.8%). Although pachytene piRNAs also match repeats (17%), the majority (>80%) map

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