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Estimating dispersal from genetic isolation by distance in a coral reef fish (*Hypoplectrus puella*)

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Abstract. The spatial scale of dispersal in coral reef fishes eludes ecologists despite the importance of this parameter for understanding the dynamics of ecological and evolutionary processes. Genetic isolation by distance (IBD) has been used to estimate dispersal in coral reef fishes, but its application in marine systems has been limited by insufficient sampling at different spatial scales and a lack of information regarding population density. Here, we present an analysis of IBD in the barred hamlet (*Hypoplectrus puella*, Serranidae) at spatial scales ranging from 10 to 3200 km complemented with SCUBA surveys of population densities covering 94 000 m^2 of reef. We used 10 hypervariable DNA markers to genotype 854 fish from 15 locations, and our results establish that IBD in *H. puella* emerges at a spatial scale of 175 km and is preserved up to the regional scale (3200 km). Assuming a normal or a Laplace dispersal function, our data are consistent with mean dispersal distances in *H. puella* that range between 2 and 14 km. Such small mean dispersal distances is a surprising result given the three-week pelagic larval duration of *H. puella* and the low level of genetic structure at the Caribbean scale (Wright's fixation index, F_{ST}, estimate = 0.005). Our data reinforce the importance of considering population density when estimating dispersal from IBD and underscore the relevance of sampling at local scales, even when genetic structure is weak at the regional scale.

Key words: *barred hamlet; connectivity; coral reef fishes; demography; dispersal;* Hypoplectrus puella; *isolation by distance; marine; pelagic larvae; population genetics; spatial scale.*

Introduction

The vast majority of coral reef fishes go through a pelagic larval stage that develops in the water column before settlement onto the reef (Leis 1991). The duration of this pelagic larval stage varies from a few hours to several months, but typically ranges between two and five weeks. Pelagic larvae provide the potential for long-distance dispersal by marine currents, and most coral reef fish dispersal is thus expected to be mediated by the larvae rather than the relatively sedentary adult stages (Leis 1991). Yet pelagic larvae are notoriously difficult to track directly owing to their small size, and as a consequence the spatial scale of dispersal in coral reef fishes is largely unknown (Mora and Sale 2002, Sale et al. 2005, Cowen et al. 2007).

Improved estimates of marine dispersal are needed owing to the central role played by this parameter in such diverse ecological theories as marine reserve networks (Botsford et al. 2001), metacommunities (Mouquet and Loreau 2003), and adaptive radiation (Gavrilets and Vose 2005). In the few cases in which larvae were marked individually (e.g., Almany et al. 2007), recapture of marked individuals revealed that up to 60% of the larvae can be retained within their natal reef, indicating that local retention of larvae may be significant. However, levels of local retention characterize only part of the larval dispersal distribution. What is the fate of non-retained larvae in terms of spatial scale of dispersal, survival, and fitness?

Population genetics provide an appropriate framework to address questions of larval movement as genetic parameters integrate effective dispersal over several generations and can be estimated at various spatial scales. Spatial genetic structure tends to be generally low among marine populations (Ward et al. 1994, Bohonak 1999), including coral reef fish populations (Shulman and Bermingham 1995; but see, e.g., Taylor and Hellberg [2003] for a counter-example). The apparent paradox of low levels of genetic structure across large geographic distances indicated by genetic studies and high levels of larval retention indicated by mark–recapture studies is sometimes resolved by positing that a handful of effective migrants per generation is sufficient to prevent the establishment of genetic structure. Yet this prediction is specific to the island model of population structure (Wright 1931), which corresponds to a case of extensive dispersal in which all populations exchange migrants with an equal probability.

If dispersal is localized, geographically nearer populations are anticipated to exchange more migrants and

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therefore to be more similar at neutral genetic markers. A pattern of increasing genetic differentiation with distance, referred to as genetic "isolation by distance" (IBD; Wright 1943), is expected in this case. Thus, IBD estimated from neutral genetic markers provides a "genetic signature" of local dispersal. In addition, population genetics theory indicates that when considered at intermediate spatial scales and complemented with population density estimates, IBD can be used to estimate dispersal rate (Rousset 1997, 2004). In the few cases in which estimates obtained with this approach could be compared with direct estimates, both were consistent within a factor of 0.5–1.5 (Rousset 1997). This analytical method appears to outperform simulation-based methods (e.g., Kinlan and Gaines 2003, based on Palumbi 2003), which assume a population density fixed a priori and can yield errors of up to two orders of magnitude (Bradbury and Bentzen 2007).

Here we present an analysis of IBD in the coral reef fish *Hypoplectrus puella* (barred hamlet, Serranidae; inset in Fig. 1; see also Plate 1) and address key assumptions of population genetics theory as it applies to IBD (Rousset 1997). We complement IBD analyses with population density estimates from both SCUBA surveys and genetic data to estimate the spatial scale of dispersal in *H. puella* following Rousset (1997). *Hypoplectrus puella* constitutes a good model species for the assessment of coral reef fish dispersal from genetic data for several reasons. Adults are generally abundant throughout their wider Caribbean range (Domeier 1994), relatively small (<12 cm in standard length), and are conspicuous and easily approached, thus allowing extensive population sampling and monitoring. Spawning can be observed throughout the year above the reef. Typical of many coral reef fishes, fertilization in *H. puella* is external, there is no parental care, and both eggs and larvae remain in the water column for a total of approximately three weeks before settlement onto the reef (Domeier 1994). To the best of our knowledge, this is the first study to integrate analysis of IBD, at spatial scales ranging from tens to thousands of kilometers, with demographic data to estimate dispersal in a coral reef fish. Estimating the spatial scale of dispersal in *H. puella* provides general insights into the ecological and evolutionary significance of larval dispersal and population structure in coral reef fishes. Dispersal in *Hypoplectrus* is also of particular interest because species diversity in the group has been hypothesized to represent a case of recent ecological speciation with gene flow (Puebla et al. 2007).

Methods

Population genetics theory

Following notably Malécot (1950) and Sawyer (1977), Rousset (1997, 2004) provides a formal analysis of isolation by distance at the population level, showing that

$$\frac{F_{ST}}{1-F_{ST}} \approx \frac{A_1}{4D\sigma} + \frac{r}{4D\sigma^2} \quad (1)$$

in a one-dimensional lattice, where F_{ST} is Wright's fixation index between population pairs (Wright 1950), r is the distance between population pairs, D is the density of diploids, A_1 is a constant depending on the dispersal function, and σ^2 is the dispersal rate, corresponding to the mean squared parent–offspring dispersal distance. It follows from Eq. 1 that the slope of the linear regression of $F_{ST}/(1 - F_{ST})$ vs. geographic distance may be used as an estimator of $(4D\sigma^2)^{-1}$. If A_1 is known, the intercept of the linear regression can further be used to estimate both σ^2 and D from the IBD regression. However, this approach is problematic in the absence of information on A_1 since there are no theoretical justifications for neglecting this term except for specific dispersal functions (Sawyer 1977, Rousset 1997). Thus, while $(4D\sigma^2)^{-1}$ may be estimated from the slope of the IBD regression, the assessment of σ^2 from $(4D\sigma^2)^{-1}$ requires an additional estimate of population density D. In addition, the linearity of the IBD pattern implied by Eq. 1 is expected to hold only at intermediate spatial scales of observation, since the dispersal function and mutation become important at small and large spatial scales, respectively. Specifically, Eq. 1 is expected to be reasonably accurate at spatial scales of observation ranging between σ and $0.2\sigma/(2\mu)^{1/2}$ where μ is the mutation rate (Rousset 1997, 2004).

The one-dimensional model described by Eq. 1 appears appropriate for the description of genetic differentiation between populations separated by a distance larger than the width of the habitat, as opposed to an analogous two-dimensional model, which would be more suitable for populations separated by less than half of the width of the habitat (Rousset 1997). The distances between populations considered in this study (10–3200 km; Fig. 1) are larger than the width of the coral reef habitat (a few kilometers or less), justifying the choice of the one-dimensional model.

Field methods

Fifteen sampling locations were targeted (Fig. 1), including three locations analyzed in Puebla et al. (2007, 2008). Necessary collecting, export, and import permits were obtained prior to fieldwork. Two expeditions were conducted on the Smithsonian Tropical Research Institute research vessel *R/V Urraca* in Kuna Yala (Panama, May 2005) and Honduras (June 2006). Fieldwork was also conducted in Carrie Bow Cay (Belize, July 2004 and August 2005), along the west coast of Barbados (June 2005), and in Bocas del Toro (Panama, March 2004). In order to evaluate temporal genetic variation, sampling was repeated in Bocas del Toro in March 2005 and February 2006. At each site, an average of 15.7 SCUBA transects covering 400 m^2 of reef each were performed to assess *H. puella* density and an average of 50.3 *H. puella* samples were collected (see

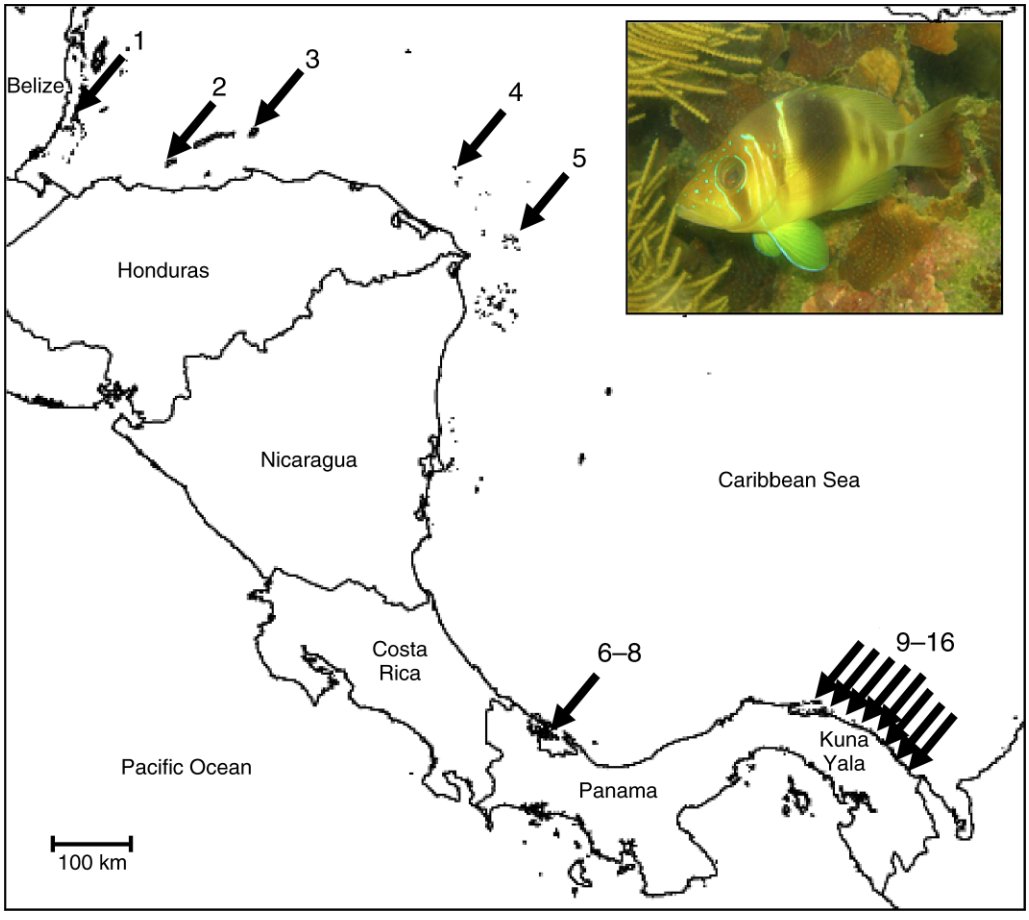

FIG. 1. Sampling scheme of *Hypoplectrus puella* collected for the present study (n = number of individuals): 1, Carrie Bow Cay ($n = 50$); 2, Utila ($n = 55$); 3, Guanaja ($n = 55$); 4, Cayos Becerros ($n = 55$); 5, Cayos de Media Luna ($n = 54$); 6, Bocas del Toro 2004 ($n = 50$); 7, Bocas del Toro 2005 ($n = 35$); 8, Bocas del Toro 2006 ($n = 35$); 9, El Porvenir ($n = 47$); 10, Cayos Holandeses ($n = 54$); 11, Islas Puyadas ($n = 53$); 12, Cayos Ratones ($n = 55$); 13, Cayos Ingleses ($n = 55$); 14, Achutupu ($n = 50$); 15, Isla Dupak ($n = 46$); 16, Goedup ($n = 55$); 17, Barbados ($n = 50$, not shown on the map). Inset: *H. puella* from Bocas del Toro (photograph by O. Puebla, 2004).

Appendix A) following the procedure described in Puebla et al. (2007).

Genetic analyses

Total DNA was extracted using column DNeasy Tissue Kits (QIAGEN, Valencia, California, USA) and genotyped at 10 microsatellite loci (McCartney et al. 2003, Puebla et al. 2007). A detailed description of the 10 microsatellite markers and associated PCR cycling conditions is provided in Puebla et al. (2007). Amplified fragments were separated by capillary electrophoresis on an ABI PRISM 3130*xl* automated genetic analyzer (Applied Biosystems, Foster City, California, USA) with 500 LIZ size standard and analyzed with the software GeneMapper (Applied Biosystems).

Number of alleles, allelic richness, gene diversity, and the inbreeding coefficient F_{IS} (Weir and Cockerham 1984) were estimated per locus and sample. Hardy-Weinberg equilibrium was tested for each site and locus with 1000 allele randomizations per test using F_{IS} as a test statistic, with sequential Bonferroni corrections for multiple tests (Rice 1989). Global and pairwise F_{ST} were estimated among samples over all loci following Weir and Cockerham (1984). Global F_{ST} was tested for significant departure from zero using a G test (Goudet et al. 1996) with 10 000 among-sample genotype permutations, using FSTAT version 2.9.3 (Goudet 1995).

Isolation by distance was tested over all samples and loci with a Mantel test (Mantel 1967) between pairwise geographic distances and pairwise $F_{ST}/(1 - F_{ST})$ estimates with 10 000 permutations, using GENEPOP'007 (Rousset 2008). Geographic distances were measured following the shortest path between sampling sites avoiding mainland, yet our results are robust to measuring distances following general near-surface circulation as summarized in Shulman and Bermingham (1995) and Roberts (1997) (Appendix B). The linear regression of pairwise $F_{ST}/(1 - F_{ST})$ to distance was considered at increasing spatial scales of observation, from 10–100 km (samples 12–16, Kuna Yala) to 10–

3200 km (all samples). Approximate bootstrap confidence intervals (ABC; DiCiccio and Efron 1996) on IBD slopes were generated using GENEPOP'007 (Rousset 2008).

Density estimates

Hypoplectrus puella census population densities were estimated from the SCUBA surveys. Moreover, effective population densities were estimated from the genetic data since parameter D in Eq. 1 corresponds to effective density, which is usually lower than census density. Note that D refers to the density over the entire area for which IBD is considered, not density within demes (Rousset 2004). Thus, the estimation of D requires an estimate of coral reef area over the area considered. Population densities were estimated at the scale of Kuna Yala (10–175 km) and Caribbean Panama (10–550 km), the two regions for which we could obtain reliable coral reef estimates (Guzmán 2003, Andréfouët and Guzmán 2005). The linear population density estimate from Panama was considered for dispersal estimates at larger spatial scales of observation.

Census population size in Kuna Yala was estimated by multiplying the density estimate from our Kuna Yala transects by the Kuna Yala coral-dominated area estimate provided by Andréfouët and Guzmán (2005). This estimate is based on maps of the Kuna Yala archipelago in 12 geomorphologic classes developed from Landsat images complemented with ~600 snorkelling observations and SCUBA surveys of more than 50 reefs (see Andréfouët and Guzmán 2005 for details). Knowing that Kuna Yala represents 81% of the Caribbean coral reefs of Panama (Guzmán 2003), the Kuna Yala coral-dominated area estimate was extrapolated to Caribbean Panama and *H. puella* population size in Panama was estimated by multiplying this area estimate by the *H. puella* density estimate from our Panama transects.

Effective population sizes were estimated from the microsatellite data using the estimator based on coalescence theory (Beerli and Felsenstein 1999, Beerli 2006) and implemented in the software MIGRATE version 1.2.5. Bocas del Toro and Kuna Yala were considered two distinct populations for these analyses since there is a gap in the distribution of Caribbean coral reefs between the two locations and genetic differentiation between Bocas del Toro and Kuna Yala (F_{ST} estimate = 0.008) was four times larger than between populations within Kuna Yala (F_{ST} estimate = 0.002; Table 1). Several runs of various lengths were performed to explore the data under different options. Bayesian inference (Beerli 2006) was preferred over maximum likelihood estimation (Beerli and Felsenstein 1999), as the former method appears to be more efficient at exploring the parameter space with highly polymorphic data. Uniform prior functions of 0–100 000 were set for both the effective population size and migration parameters, and the Brownian motion approximation to the stepwise mutation model was used with variable mutation rates following a Gamma function. Final runs were performed with three Markov chain Monte Carlo (MCMC) chains of 10^6 steps preceded by 3×10^6 burning steps. In order to verify the consistency of the results the analysis was repeated several times with different start parameters, including parameter estimates from previous runs.

It is to be noted that our estimation of effective density assumes an equilibrium model and depends on the estimation of μ, which is unknown in practice. Strictly speaking, the effective density D in Eq. 1 corresponds to the inverse of the average probability of coalescence in the previous generation for pairs of genes divided by the linear extent of the population (Rousset 1997, 2004), which cannot be easily estimated. The ratio between effective population size as estimated with MIGRATE and the linear span of the population used as a proxy for the estimation of D is likely to underestimate effective density. Thus, the range of density estimates considered in this study is expected to be fairly conservative.

Linear population density estimates D were obtained by dividing population size estimates by the length of the area considered (Kuna Yala or Panama), measured following the same procedure used to measure the geographic distances between samples.

Dispersal estimates

The slope of the linear regression of pairwise $F_{ST}/(1 - F_{ST})$ estimate to distance was used as an estimator of $(4D\sigma^2)^{-1}$ following Rousset (1997) at increasing spatial scales of observation. Density estimates were then used to estimate σ^2 from $(4D\sigma^2)^{-1}$. We adopted a precautionary approach by considering all values of D ranging from the lower density estimate (i.e., the effective density estimate) to the higher density estimate (i.e., the census density estimate). The most appropriate spatial scale of observation for the estimation of dispersal was evaluated following an iterative procedure (Vekemans and Hardy 2004). A first estimation of σ was calculated on the basis of the IBD regression and associated density estimates over all samples (10–3200 km), and the recommended spatial scale of observation ($\sigma - 0.2\sigma/[2\mu]^{1/2}$; Rousset 1997) was estimated assuming a mutation rate μ of 5×10^{-4} typical of microsatellite loci (Ellegren 2000). A new estimation of σ was calculated over the recommended spatial scale of observation and the procedure was repeated until successive σ estimates stabilized.

Simulations

Extensive simulations of IBD models have been reported by different authors (e.g., Slatkin 1993, Palumbi 2003, Bradbury and Bentzen 2007). Here we use individual-based simulations to evaluate the performance of the method used to estimate dispersal (Rousset 1997) at different spatial scales of observation and with

TABLE 1. Genetic structure and patterns of isolation by distance (IBD) at increasing spatial scales of observation among 15 samples of the barred hamlet (*Hypoplectrus puella*), a coral reef fish genotyped at 10 microsatellite loci.

Spatial scale (km)	Samples	F_{ST} estimate	95% CI	IBD slope ($\times 10^5$)	ABC ($\times 10^5$)	IBD R^2	n
10–100	Kuna Yala (samples 12–16)	0.002	0.000–0.004	−3.346	−12.72–1.440	0.241	10
10–175	Kuna Yala (samples 9–16)	0.002	0.000–0.003	1.061	−1.264–3.276	0.049	28
10–550	Panama	0.004	0.001–0.006	2.653	0.519–6.007	0.814	36
100–650	Honduras and Belize	0.002	0.000–0.004	0.888	0.546–1.135	0.399	10
10–1600	Panama, Honduras, and Belize	0.005	0.003–0.007	0.417	0.167–0.550	0.277	91
10–3200	Barbados, Panama, Honduras, and Belize	0.005	0.003–0.007	0.165	0.054–0.232	0.106	105

Notes: The IBD slope and R^2 are the slope and R^2 of the linear regression between pairwise geographic distances and genetic differentiation estimates ($F_{ST}/[1 - F_{ST}]$), respectively; ABC is the approximate bootstrap confidence interval (DiCiccio and Efron 1996); n is the number of pairwise comparisons. The values for IBD slope and ABC have been multiplied by a factor of 10^5. The R^2 over all samples is highly significant ($P < 0.001$).

different dispersal functions compared to other approaches such as the one implemented in Kinlan and Gaines (2003).

A one-dimensional lattice model of population structure was adopted, consisting of N_d demes regularly spaced along a circle with N diploid simultaneous hermaphrodites per deme and distance d between adjacent demes (see Puebla et al. [2008] for details). This spatial arrangement is convenient as it eliminates edge effects, and any subsection of the circle approximates a linear one-dimensional model. Here, N_d was set to 1000 with $N = 500$ individuals per deme and $d = 1$. A set of simulations was performed with zygote dispersal implemented as a normal dispersal function with variance $\pi/2$ (mean dispersal distance $\mu_x = 1$, $\sigma^2 = \pi/2$). Another set of simulations was performed with zygote dispersal implemented as a Laplace function where the proportion of zygotes produced at position x dispersing to position y is given by

$$p(x, y) = \frac{a}{2} e^{-a|x-y|}$$

with $\alpha = 1$ ($\mu_x = 1$, $\sigma^2 = 2$). This dispersal function is sometimes used in one-dimensional models of marine dispersal (Botsford et al. 2001, Palumbi 2003) as it is more leptokurtic than the normal function, allowing more long-distance dispersal. A k alleles model of mutation was adopted, with alleles mutating into any of the other $k - 1$ alleles with probability $\mu/(k - 1)$ where μ is the mutation rate and k is the number of alleles. The mutation rate was set to 5×10^{-4} per meiosis, which is representative of microsatellite loci (Ellegren 2000). Simulations were run for 1000 generations with equal initial allele frequencies of 0.05. Ten unlinked loci were simulated by pooling the results from 10 replicate simulations for each set of parameters.

The effect of spatial scale of observation was explored by sampling 10 regularly spaced demes at increasing spatial scales, from 1–10 to 30–300 μ_x. Except for the smallest spatial scale (1–10 μ_x), this scheme leaves unsampled demes between samples, reflecting our sampling of *H. puella* in the Caribbean. Global F_{ST} and IBD slopes, with corresponding confidence intervals, were estimated following the same procedure used for the analysis of the microsatellite data.

RESULTS

Basic genetic statistics

A total of 845 samples were genotyped at the 10 microsatellite loci with <3.5% missing data overall (failed or poor amplification of a locus for a given individual). Sample numbers, number of alleles, allelic richness, gene diversity, F_{IS}, and proportion of randomizations that generated F_{IS} estimates greater and less than observed are presented in Appendix A. A total of 283 alleles were identified (mean = 167 alleles per site sampled), and mean gene diversity per sample ranged between 0.790 and 0.830. For all sites, all loci were at Hardy-Weinberg equilibrium at the 0.05 level after sequential Bonferroni correction for multiple tests. Without corrections for multiple tests, 12 of the 340 tests (10 loci × 17 samples × 2 for heterozygote deficit and excess tests) were significant at the 0.05 level (seven heterozygote deficits and five excesses), quite close to the 5% expected by chance alone.

Temporal genetic variation

The pairwise F_{ST} estimate between samples collected in different years in Bocas del Toro (Panama) was 0.001 for 2004–2005 (95% CI = −0.002 to 0.004), 0.002 for 2005–2006 (95% CI = −0.002 to 0.007), and 0.004 for 2004–2006 (95% CI = −0.001 to 0.010). These differences were not significant and quite low compared to the pairwise spatial F_{ST} estimates, which reached values up to 0.017 (Fig. 2), suggesting that temporal genetic variation is not confounding the spatial patterns reported here. Nonetheless, future sampling is warranted to test whether temporal genetic variation in Bocas del Toro is representative of other Caribbean populations. The larger F_{ST} estimate between 2004 and 2006 than between consecutive years (2004–2005 and 2005–2006) suggests that temporal genetic variation increases with longer delays between sampling events and reinforces the importance of limiting the time between samples for the estimation of dispersal among weakly differentiated populations. In order to keep temporal

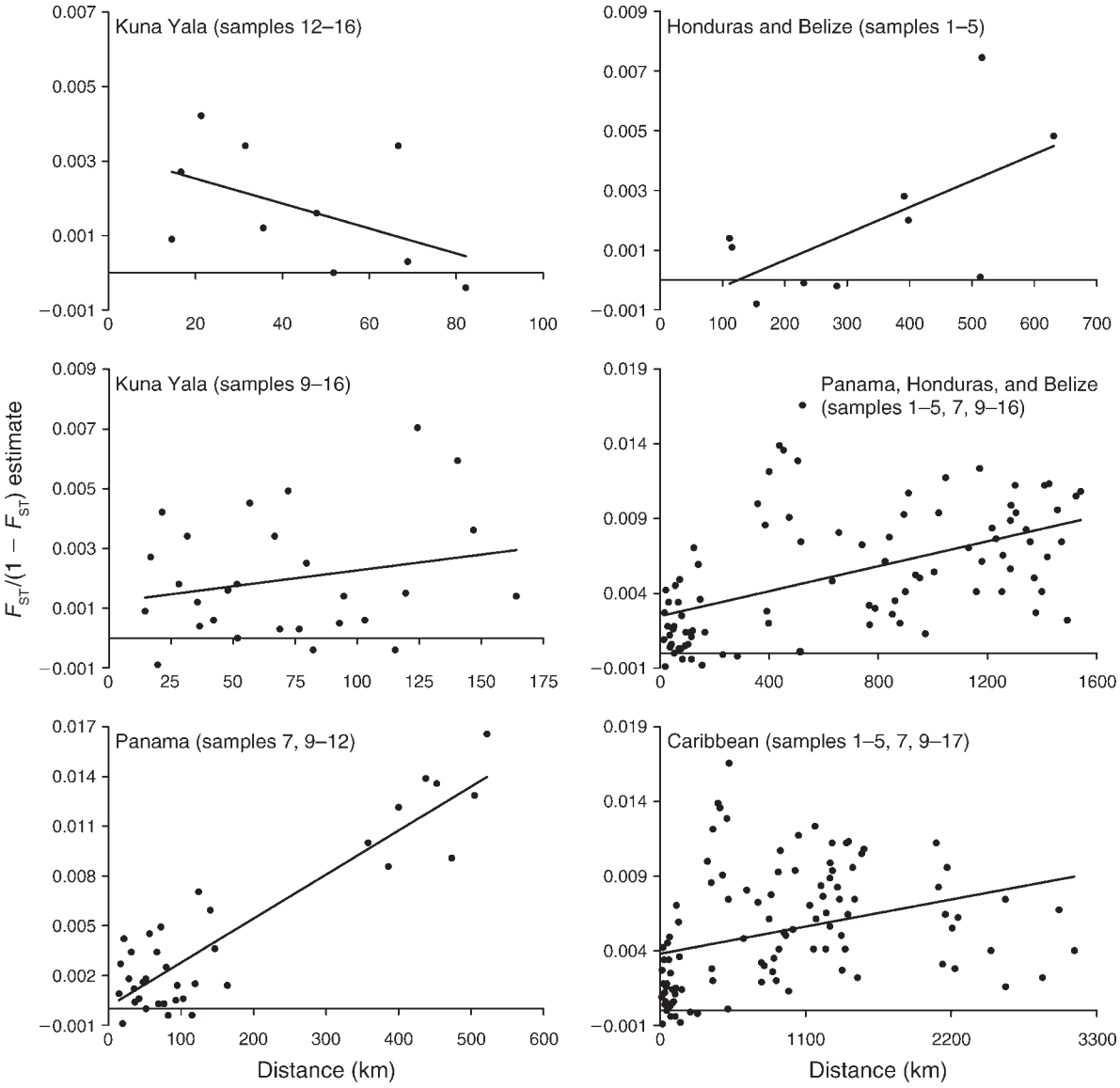

FIG. 2. Relationship between pairwise geographic distances and genetic differentiation estimates ($F_{ST}/[1 - F_{ST}]$) at increasing spatial scales of observation in the coral reef fish *Hypoplectrus puella*. A pattern of isolation by distance (IBD) emerges at a scale of 175 km and is maintained up to the regional scale (3200 km).

genetic variation between samples as low as possible, we used the 2005 sample from Bocas del Toro in all spatial analyses. Thus, the dispersal estimates from Kuna Yala and Panama are based on samples that were all collected in 2005. Note that our results are robust to using the 2004, 2006, or the three samples from Bocas del Toro pooled together (data not shown).

Spatial genetic structure and isolation by distance

Levels of spatial genetic structure were low and tended to increase with increasing spatial scale of observation, with F_{ST} estimates ranging from 0.002 among Kuna Yala samples to 0.005 over all samples (Table 1). Although low, spatial genetic structure was highly significant over all samples of populations (G test $P < 0.001$).

The Mantel test indicated that IBD was highly significant over all samples and loci ($P < 0.001$). The relationship between pairwise $F_{ST}/(1 - F_{ST})$ estimates and geographic distances at increasing spatial scales of observation is illustrated in Fig. 2 and detailed in Table 1. A pattern of IBD emerged at a spatial scale of 175 km and was preserved up to the regional scale (3200 km). Isolation by distance was also observed at all loci individually over all samples (data not shown). The 14 pairwise comparisons at geographic distances >1600 km, which involve the sample from Barbados, did not appear to contribute to IBD (Fig. 2), suggesting that

TABLE 2. Dispersal rate (σ^2) and corresponding mean dispersal distance (μ_x) assuming a normal dispersal function at increasing spatial scales of observation in the coral reef fish *Hypoplectrus puella*.

Spatial scale (km)	σ^2 estimate		μ_x estimate		
	D from transects (km^2)	*D* from genetic data (km^2)	*D* from transects (km)	*D* from genetic data (km)	μ_x estimate (km)
10–100	∞	∞	∞	∞	∞
10–175	**12 (4–∞)**	**287 (93–∞)**	**2.8 (1.6–∞)**	**13.5 (7.7–∞)**	151 (49–∞)
10–550	**8 (4–42)**	**325 (144–1660)**	**2.3 (1.5–5.2)**	**14.4 (9.6–32.5)**	60 (27–309)
100–650	25 (19–40)	971 (760–1579)	4.0 (3.5–5.0)	24.9 (22–31.7)	180 (141–293)
10–1600	52 (40–130)	2069 (1568–5147)	5.8 (5.0–9.1)	36.3 (31.6–57.2)	384 (291–957)
10–3200	132 (94–403)	5226 (3710–15965)	9.2 (7.7–16.0)	57.7 (48.6–100.8)	971 (689–2967)

Notes: Dispersal rate and corresponding mean dispersal distance were estimated from genetic isolation by distance (IBD) analysis (Table 1, Fig. 2) and population density (*D*) estimates, following Rousset (1997). Population density was estimated from both SCUBA transects and genetic data. The estimates considered in this study appear in boldface (see *Methods: Dispersal estimates* and *Results: Dispersal estimates* for a justification of this choice). The μ_x estimates following the simulation-based method implemented by Kinlan and Gaines (2003) are provided for comparison. Negative IBD slopes are interpreted as infinite dispersal distances. Values in parentheses are approximate bootstrap confidence (ABC) intervals (DiCicco and Efron 1996).

processes other than dispersal limitation become important at large spatial scales.

Density estimates

The 235 transects covering 94 000 m^2 of coral reef over all Caribbean sampling locations provided a density estimate of 1.744 ± 0.153 adults per 100 m^2 of reef (mean ± SE). The 130 transects performed along the Caribbean coast of Panama provided a density estimate of 1.629 ± 0.122 adults/100 m^2. Assuming a coral-dominated area estimate of 43 km^2 (see *Methods*), these counts translate into a population size estimate of 700 404 ± 52 503 adults and a linear population density estimate of 1148 ± 86 adults/km for Panama. The 90 transects performed along the Kuna Yala coast gave a density estimate of 1.147 ± 0.119 adults/100 m^2, which, assuming a coral-dominated area estimate of 35 km^2 (see *Methods*), translates into a population size estimate of 401 528 ± 41 650 adults and a linear population density estimate of 1959 ± 203 adults/km.

Bayesian analysis of the microsatellite data provided an estimate of 33.5 (95% CI = 28.5–37.5) for the population size parameter ($\Theta_1 = 4N\mu$, where N is the effective population size and μ the mutation rate) in Kuna Yala. Assuming a mutation rate μ of 5×10^{-4} typical of microsatellite loci (Ellegren 2000), this estimate translates into an effective population size estimate of 16 750 (95% CI = 14 250–18 750) and a linear population density estimate of 81.7 adults/km (95% CI = 69.5–91.5), 24 times lower than the census estimate. The population size paramter Θ_2 was estimated to be 1.5 for Bocas del Toro (95% CI = 0.5–4.5), translating into an effective population size estimate of 750 (95% CI = 250–2250). Thus, the sum of the two effective population size parameters Θ_1 and Θ_2 was 35.0 for Panama (95% CI = 29.0–42.0) or an effective population size estimate of 17 500 (95% CI = 14 500–21 000) and a linear population density estimate of 28.7 adults/km (95% CI = 23.8–34.4), 40 times lower than the census estimate. Repeated and independent MCMC runs with different initial conditions provided generally consistent results, i.e., with overlapping 95% CIs, and the Θ estimates were robust to setting the migration parameters to 0. These estimates suggest that *H. puella* effective population sizes may be 40 times lower than census population sizes. Demographic fluctuations and variance in reproductive success can potentially account for the discrepancy between census and effective population size estimates (Hedrick 2005, Hauser and Carvalho 2008).

Dispersal estimates

The slope of the linear regression of pairwise $F_{ST}/(1 - F_{ST})$ to distance provided a $D\sigma^2$ estimate of 23 553 individuals × km (ABC = 7632–∞) in Kuna Yala. The lower approximate bootstrap confidence interval of the IBD slope was negative in this case, which is interpreted as an infinite $D\sigma^2$ estimate. The $D\sigma^2$ estimate for Caribbean Panama was 9423 individuals × km (ABC = 4162–48 148). The larger $D\sigma^2$ estimate for Kuna Yala than for Panama is an expected result since Kuna Yala contains 81% of the Caribbean coral reefs of Panama (Guzmán 2003) while representing only approximately one-third of the Caribbean coast of Panama. Thus, *H. puella* density *D* is anticipated to be higher in Kuna Yala than across Panama. The $D\sigma^2$ at spatial scales of observation of 100–650, 10–1600, and 10–3200 km were estimated to be 25 154 (ABC = 22 034–45 785), 59 998 (ABC = 45 465–149 273), and 151 543 (ABC = 107 584–462 976), respectively.

The σ^2 estimates obtained by substituting the *D* estimates in the $D\sigma^2$ estimate are presented in Table 2. The iterative procedure (Vekemans and Hardy 2004) stabilized at 7–285 km (7 km considering the census density estimate and 285 km considering the effective density estimate), indicating that Kuna Yala (10–175 km) and Panama (10–550 km) constitute the most appropriate spatial scales of observation for the estimation of dispersal. The IBD pattern at 10–550 km (Panama) is largely driven by the differentiation between Bocas del Toro and Kuna Yala, perhaps owing to the gap in coral reef distribution between Kuna Yala and Bocas del Toro. Nonetheless, the σ estimate from

TABLE 3. Simulation results: genetic structure and patterns of isolation by distance (IBD) among 11 regularly spaced samples ($n = 55$ pairwise comparisons) at increasing spatial scales of observation from 1–10μ_x to 30–300μ_x, where μ_x is the mean dispersal distance.

Spatial scale	F_{ST} estimate (95% CI)	IBD slope (ABC) ($\times 10^3$)	IBD R^2	$D\sigma^2$ estimate (ABC)	μ_x estimate (ABC)
Normal dispersal function ($D\sigma^2 = 785$, $\mu_x = 1$)					
1–10μ_x	0.002 (0.001–0.003)	0.510 (0.312–0.771)	0.623	490 (324–802)	3.1 (2.1–5.1)
2–20μ_x	0.004 (0.003–0.005)	0.491 (0.352–0.722)	0.818	509 (346–710)	3.3 (2.2–4.5)
3–30μ_x	**0.006 (0.004–0.007)**	**0.411 (0.293–0.553)**	**0.839**	**608 (452–853)**	3.9 (2.9–5.5)
4–40μ_x	**0.007 (0.006–0.009)**	**0.404 (0.304–0.543)**	**0.871**	**618 (460–823)**	4.0 (2.9–5.3)
5–50μ_x	**0.008 (0.006–0.010)**	**0.268 (0.203–0.349)**	**0.767**	**933 (715–1931)**	6.0 (4.6–7.9)
6–60μ_x	**0.009 (0.008–0.010)**	**0.294 (0.206–0.387)**	**0.885**	**851 (645–1213)**	5.5 (4.1–7.8)
7–70μ_x	0.010 (0.009–0.012)	0.219 (0.159–0.271)	0.741	1143 (924–1576)	7.3 (5.9–10.1)
8–80μ_x	0.011 (0.009–0.013)	0.230 (0.178–0.284)	0.756	1085 (880–1406)	6.9 (5.6–9.0)
9–90μ_x	0.012 (0.010–0.013)	0.199 (0.157–0.255)	0.786	1256 (980–1597)	8.0 (6.3–10.2)
10–100μ_x	0.012 (0.011–0.014)	0.178 (0.128–0.233)	0.756	1403 (1073–1954)	9.0 (6.9–12.5)
20–200μ_x	0.019 (0.017–0.020)	0.078 (0.047–0.118)	0.562	3187 (2126–5298)	20.4 (13.6–33.9)
30–300μ_x	0.020 (0.018–0.022)	0.028 (0.015–0.045)	0.263	9025 (5533–17141)	57.8 (35.4–109.8)
Laplace dispersal function ($D\sigma^2 = 1000$, $\mu_x = 1$)					
1–10μ_x	0.002 (0.001–0.003)	0.629 (0.483–0.795)	0.538	397 (314–518)	2.5 (2.0–3.3)
2–20μ_x	0.004 (0.003–0.005)	0.446 (0.309–0.683)	0.648	561 (366–809)	3.6 (2.3–5.2)
3–30μ_x	0.005 (0.004–0.006)	0.483 (0.340–0.667)	0.821	518 (375–735)	3.3 (2.4–4.7)
4–40μ_x	0.008 (0.005–0.010)	0.374 (0.252–0.595)	0.892	668 (420–990)	4.3 (2.7–6.3)
5–50μ_x	0.009 (0.007–0.010)	0.348 (0.244–0.468)	0.886	718 (534–1024)	4.6 (3.4–6.6)
6–60μ_x	**0.009 (0.008–0.010)**	**0.298 (0.238–0.378)**	**0.841**	**839 (662–1049)**	5.4 (4.2–6.7)
7–70μ_x	**0.011 (0.008–0.013)**	**0.284 (0.213–0.376)**	**0.858**	**879 (666–1172)**	5.6 (4.3–7.5)
8–80μ_x	**0.011 (0.009–0.013)**	**0.241 (0.188–0.323)**	**0.869**	**1036 (774–1331)**	6.6 (5.0–8.5)
9–90μ_x	**0.012 (0.010–0.014)**	**0.224 (0.170–0.292)**	**0.746**	**1118 (854–1468)**	7.2 (5.5–9.4)
10–100μ_x	0.013 (0.011–0.014)	0.151 (0.103–0.207)	0.622	1651 (1210–2427)	10.6 (7.7–15.5)
20–200μ_x	0.015 (0.013–0.016)	0.057 (0.037–0.076)	0.514	4832 (3275–6759)	28.1 (21.0–43.3)
30–300μ_x	0.018 (0.017–0.019)	0.040 (0.031–0.059)	0.458	6324 (4266–7997)	40.5 (27.3–51.2)

Notes: Two sets of simulations were performed, with zygote dispersal implemented as a normal and a Laplace function. $D\sigma^2$ estimates following the method implemented in this study (Rousset 1997) were accurate at intermediate spatial scales of observation (estimates consistent with the simulated $D\sigma^2$ value within a factor 0.75–1.25 appear in boldface). The μ_x estimates following the simulation-based procedure implemented by Kinlan and Gaines (2003) tended to overestimate dispersal. The IBD slope and R^2 are the slope and R^2 of the linear regression between pairwise geographic distances and genetic differentiation estimates ($F_{ST}/[1 - F_{ST}]$), respectively; ABC is the approximate bootstrap confidence interval (DiCiccio and Efron 1996). The values for IBD slope have been multiplied by a factor of 10^3.

Panama is consistent with the one from Kuna Yala (10–175 km) where no gap is apparent.

While σ^2 is estimated from IBD without making restrictive assumptions about dispersal distribution (Rousset 1997, 2004), the estimation of other parameters such as mean dispersal distance depends on the dispersal function. For symmetric dispersal in one dimension, σ^2 is simply the variance (second moment) of the dispersal function:

$$\sigma^2 = \int_{-\infty}^{\infty} g(x)x^2\,dx \qquad (2)$$

where x is the signed parent–offspring distance and $g(x)$ the density of the dispersal function. In this case $g(x)$ is centered on 0 and the mean of the signed parent–offspring distance is 0. Yet from an ecological perspective we are interested in the mean of the unsigned (i.e., absolute value) parent–offspring distance μ_x, defined as the mean (first moment) of the dispersal function:

$$\mu_x = \int_{-\infty}^{\infty} g(x)|x|\,dx. \qquad (3)$$

Mean dispersal distance μ_x can be predicted from σ^2 for specific dispersal functions $g(x)$. The normal function is considered here since simulations of Lagrangian larval transport suggest that larval dispersal might follow such a function (Siegel et al. 2003). For a normal function in one dimension, $\mu_x = \sigma \times (2/\pi)^{1/2}$. In this case our σ^2 estimates of 8–325 km^2 would translate into mean parent–offspring distances ranging between 2.3 and 14.4 km (Table 2).

Simulations

Global F_{ST} estimates increased from 0.002 to 0.020 with increasing spatial scale of observation (Table 3). A pattern of IBD emerged in all simulations regardless of the dispersal function and spatial scale considered, as indicated by the positive slope of the IBD regression. The occurrence of IBD despite low levels of genetic structure is consistent with the microsatellite analyses (Table 1). The $D\sigma^2$ estimates obtained with the method implemented in this study (Rousset 1997) were consistent with the simulated values within a factor 0.75–1.25 at spatial scales of observation ranging between 3 and 60 μ_x for the normal dispersal function and between 6 and 90 μ_x for the Laplace dispersal function (note that this is considering $D = 500$, the census linear population density). The μ_x estimate as implemented in Kinlan and Gaines (2003) overestimated dispersal in all

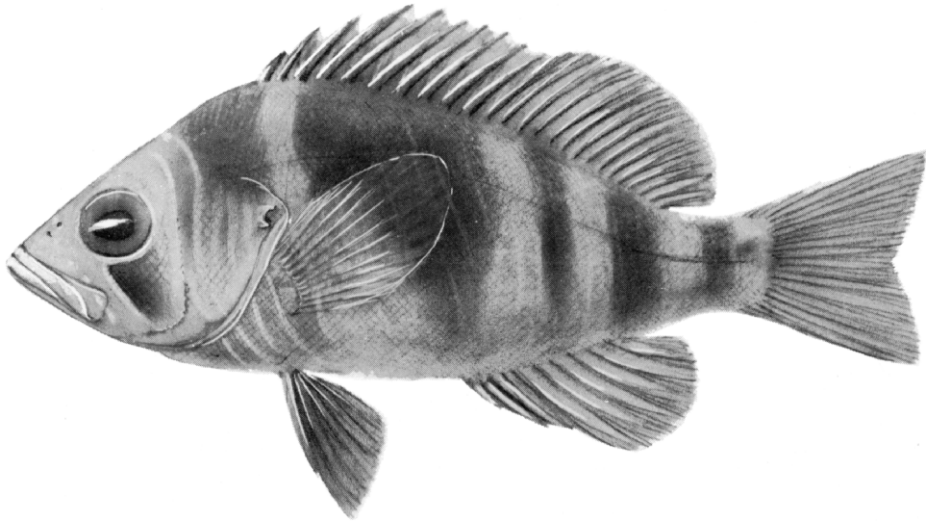

PLATE 1. *Hypoplectrus puella* (Cuvier). Reproduced with permission from Böhlke and Chaplin (1968 [illustrated by S. P. Gigliotti and F. Janschka]). Credit: The Academy of Natural Sciences, Ewell Sale Stewart Library.

simulations, by more than one order of magnitude at large spatial scales of observation.

DISCUSSION

The multi-scale approach implemented here reveals that genetic IBD in *H. puella* emerges over a spatial scale as small as 175 km and is preserved up to 3200 km, notwithstanding very low levels of genetic structure at the Caribbean scale (global F_{ST} estimate = 0.005). Furthermore, the inclusion of population density estimates provides an opportunity to estimate dispersal rate (σ^2) from IBD. Assuming a normal dispersal function, our data predict mean dispersal distances <15 km, which contrasts with the potential for long-distance dispersal provided by the three-week pelagic larval stage of *H. puella* and typical of many coral reef fishes. Our dispersal estimates also contrast with simulation-based estimates that ignore the effect of population density and spatial scale of observation on dispersal estimates (Table 2), indicating that these parameters are critical for the assessment of marine dispersal from genetic data.

Isolation by distance

Genetic isolation by distance (IBD) appears to be a robust pattern in *H. puella*. It is statistically highly significant and is observed independently in different areas (Panama and Honduras–Belize), over spatial scales ranging from 175 to 3200 km, and at all microsatellite loci whether analyzed together or individually.

The spatial scale at which IBD emerges in *H. puella*, 175 km, is of interest in comparison to genetic analyses of coral reef fish populations conducted over large spatial scales, e.g., ~14 000 km in *Acanthurus triostegus* (convict surgeonfish; Planes and Fauvelot 2002). In this respect our results underscore the relevance of considering local scales for the estimation of marine dispersal, even when genetic structure is weak at the regional scale.

The decrease of the slope of IBD at large spatial scales (observed in both the microsatellite data and the simulations) may be due to several factors: (1) the greater importance of mutation at large spatial scales (Rousset 1997); (2) nonequilibrium conditions between migration and genetic drift (Slatkin 1993); or (3) F_{ST} might have reached its upper limit at the loci considered (Bradbury and Bentzen 2007). The latter hypothesis can be excluded since we have reported F_{ST} values three times larger than the largest pairwise F_{ST} observed here (Puebla et al. 2008).

Spatial scale of dispersal

Dispersal rate (σ^2) is the parameter estimated from IBD without making restrictive assumptions about dispersal distribution. The estimation of σ^2 is expected to be valid for any dispersal function, including asymmetric ones, as long as the first, second, and third moments are finite (Rousset 1997). The smallest spatial scales over which we observed IBD (10–175 km and 10–550 km) were used for the estimation of σ^2 *in H. puella* following the iterative procedure of Vekemans and Hardy (2004). This choice is consistent with the fact that the analytical approximation underlying our estimate is expected to be more accurate at local scales (Rousset 1997). Moreover, IBD first develops at local scales under nonequilibrium conditions between genetic drift and migration (Slatkin 1993).

The estimation of other parameters of dispersal, such as mean dispersal distance, depends on the dispersal function, which provides an opportunity to predict mean dispersal distances for specific dispersal functions. Considering our σ^2 estimate interval of 8–325 km^2 and

assuming a normal dispersal function, our results predict mean parent–offspring dispersal distances in *H. puella* ranging from 2.3 to 14.4 km. The swimming abilities of coral reef fish larvae, the early development of their sensory systems, and sophisticated behaviour (Leis and McCormick 2002) can potentially contribute to local settlement.

It is to be noted that our estimates reflect effective dispersal, i.e., individuals that survive and reproduce. Also, the samples considered for the estimation of σ^2 (Bocas del Toro and Kuna Yala) were collected within one region of connectivity identified by Cowen et al. (2006) on the basis of their biophysical model of the Caribbean (i.e., the Panama–Colombian Gyre subregion). Thus, the dispersal estimates reported here probably reflect typical dispersal distances not subject to the influence of exceptionally strong barriers to larval movement (e.g., Taylor and Hellberg 2003).

Mean dispersal distances in the tens of kilometers or less do not exclude the possibility of long-distance dispersal. Coral reef fish larvae are frequently collected hundreds of kilometers away from coral reefs (e.g., Victor 1987), and Rosenblatt and Waples (1986) as well as Lessios and Robertson (2006) reported genetic evidence of dispersal for several species of coral reef fish across the Eastern Pacific Barrier, a stretch of 5000 km of deep water separating the eastern and central Pacific. These studies provide strong evidence that coral reef fish larvae are capable of dispersal across large geographic distances, yet long-distance dispersal might be rare at ecological timescales. Dispersal in coral reef fishes might therefore follow "fat tail" leptokurtic functions, with the majority of larvae settling locally and only a small proportion of larvae dispersing long distances. The estimation of σ^2 implemented here is robust to high kurtosis since the fourth moment of the dispersal function can be infinite (Rousset 1997, 2004). Assuming a leptokurtic dispersal function such as the Laplace function and following Buonaccorsi et al. (2004), our data predict mean parent–offspring dispersal distances in *H. puella* ranging between 2.0 and 12.7 km.

The simulation-based procedure implemented by Kinlan and Gaines (2003) to estimate dispersal in several species of coral reef fish also assumes a Laplace function, but ignores the effect of population density and spatial scale of observation by simply estimating dispersal distance as $0.0016 \times (\text{IBD slope})^{-1.0001}$. When applied to our data, this simulation procedure yields dispersal values ranging from 60 to 971 km (Table 2), more than one order of magnitude larger than our estimates. In addition, Table 2 indicates that dispersal estimates increase at large spatial scales such as the ones considered in Kinlan and Gaines (2003) owing to the lower IBD slope at these spatial scales. To the extent that our dispersal estimate from Kuna Yala and Panama (2.3–14.4 km) are reasonably accurate, the comparisons between methods show how the absence of empirical data on demographic and scale effects can bias dispersal estimates. Our results suggest that the spatial scale of marine dispersal was overestimated by Kinlan and Gaines (2003). This is further suggested by our simulations (Table 3) in which the method considered in Kinlan and Gaines (2003) systematically overestimated dispersal by more than one order of magnitude at large spatial scales of observation.

The method implemented here (Rousset 1997) does not rely on the a priori identification of sampled populations and does not require the sampling of all populations to estimate dispersal. It assumes a homogenous population density D and requires that the distance between samples is known. Clearly, the method cannot be implemented in the absence of IBD, which could result from extensive dispersal but also from nonequilibrium conditions between migration and genetic drift or a lack of statistical power. This approach also requires an estimation of population density to estimate dispersal, which could be challenging for highly mobile species such as pelagic fishes.

Localized dispersal and genetic structure

The low F_{ST} estimates reported in this study are consistent with decades of genetic studies that have evidenced generally low levels of population genetic structure among marine populations (Ward et al. 1994, Bohonak 1999), including coral reef fish populations (Shulman and Bermingham 1995). Nonetheless, low levels of genetic structure across large geographic distances do not rule out local dispersal and settlement, as shown here by the emergence of IBD at a spatial scale of 175 km in *H. puella* and mean dispersal distance estimates ranging from 2.3 to 14.4 km (see also Purcell et al. 2006). Low levels of genetic structure over large geographic areas such as the ones reported in this study do not even necessarily imply long-distance (i.e., between nonadjacent populations) dispersal; they can result from stepping-stone (i.e., between adjacent populations) migration exclusively (Rousset 2004).

Conclusions

Our combination of genetic and demographic data indicates that *H. puella* recruits are likely to disperse only about 10 km or less, on average, from their parents. These are counterintuitive values of mean dispersal distance for a fish such as *H. puella* capable of spending three weeks in the plankton as larvae. The extent to which these results can be generalized to other coral reef species remains to be assessed, but the dispersal estimates reported here are consistent with similar studies of non-coral-reef fishes such as rockfishes (Buonaccorsi et al. 2004, 2005, Gomez-Uchida and Banks 2005).

Our results reinforce the importance of considering population density when estimating dispersal from IBD and suggest that simulation-based studies (e.g., Kinlan and Gaines 2003) have led to the overestimation of dispersal distances in coral reef fishes. Furthermore, our

study emphasizes the importance of estimating dispersal at local scales, even in cases for which regional assessments of population genetic structure over thousands of kilometers establish very low F_{ST} estimates.

From a conservation and management perspective our results suggest that marine protected areas may not be as dependent upon regional sources of recruits as previously considered (Roberts 1997). Dispersal limitation also informs models of speciation with gene flow, such as the one we put forward to explain the recent radiation of species in *Hypoplectrus* (Puebla et al. 2007, 2008). We suggest that studies of IBD at spatial scales ranging from tens to thousands of kilometers coupled with quantitative assessment of population density will dramatically improve understanding of the role that larval dispersal plays in both the ecology and evolution of coral reef fishes.

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APPENDIX A

Basic statistics per locus of 17 samples of the coral reef fish *Hypoplectrus puella* (Serranidae) genotyped at 10 microsatellite loci (*Ecological Archives* E090-221-A1).

APPENDIX B

Relationship between pairwise geographic distances and genetic differentiation estimates among 15 samples of the coral reef fish *H. puella* genotyped at 10 microsatellite loci (*Ecological Archives* E090-221-A2).