EVOLUTION
INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

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Journal:	*Evolution*
Manuscript ID:	10-0099.R1
Manuscript Type:	Original Article
Date Submitted by the Author:	
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Keywords:	Gene Flow, Genetic Drift, Population Biology, Population Genetics, Population Structure

Using isolation by distance and effective density to estimate dispersal scales in anemonefish

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Running Head: Isolation by distance and marine dispersal

Key Words: *Amphiprion clarkii*, continuous population, marine larvae, habitat patchiness

Abstract

Robust estimates of dispersal are critical for understanding population dynamics and local adaptation, as well as for successful spatial management. Genetic isolation by distance patterns hold clues to dispersal, but understanding these patterns quantitatively has been complicated by uncertainty in effective density. In this study, we genotyped populations of a coral reef fish (*Amphiprion clarkii*) at 13 microsatellite loci to uncover fine-scale isolation by distance patterns in two replicate transects. Temporal changes in allele frequencies between generations suggested that effective densities in these populations are 4-21 adults/km. A separate estimate from census densities suggested that effective densities may be as high as 82-178 adults/km. Applying these effective densities with isolation by distance theory suggested that larval dispersal kernels in *A. clarkii* had a spread near 11 km (4-27 km). These kernels predicted low fractions of self-recruitment in continuous habitats, but the same kernels were consistent with previously reported, high self-recruitment fractions (30-60%) when realistic levels of habitat patchiness were considered. Our results suggested that ecologically relevant larval dispersal can be estimated with widely available genetic methods when effective density is measured carefully through cohort sampling and ecological censuses, and that self-recruitment studies should be interpreted in light of habitat patchiness.

Introduction

Dispersal drives population dynamics, range limits, and local adaptation, and can thereby enhance ecosystem resilience (Roughgarden et al. 1988; Gaston 1996; Nyström & Folke 2001; Lenormand 2002). Dispersal also sets the spatial scale of ecological and evolutionary processes, and therefore determines the relative importance of local and regional forces within ecosystems. In the ocean, marine currents and long pelagic larval stages for most organisms create a high potential for long-distance dispersal, despite relatively sedentary adults (Scheltema 1986; Roughgarden et al. 1988; Mora & Sale 2002). High levels of genetic similarity across wide ocean distances support this view of open populations (Palumbi 1992; Mora & Sale 2002), where individual populations primarily receive recruits from other populations rather than from themselves (Jones et al. 1999). However, recent tagging evidence for short-distance larval dispersal (Swearer et al. 1999; Jones et al. 2005; Almany et al. 2007; Planes et al. 2009; Saenz-Agudelo et al. 2009) and sharp genetic breaks in species thought to have high dispersal (Barber et al. 2000; Taylor & Hellberg 2003) suggest that marine dispersal may instead be surprisingly local. As fisheries decline and coastal habitats degrade, identifying typical scales of marine larval dispersal is critical for ecosystem-based management (Sale et al. 2005).

One difficulty in research to date is that most genetic and self-recruitment analyses only measure a small portion of the dispersing individuals. Many genetic methods are strongly influenced by rare, long-distance dispersal events (Slatkin 1987; Waples & Gaggiotti 2006), and these methods are therefore most useful where migration rates are low. However, rare events over evolutionary timescales may be irrelevant to current ecological processes and management decisions. On the other hand, tagging or

parentage studies require recapture of individuals or their offspring, and are therefore conducted over areas of limited extent. One danger of these studies is that typical dispersal distances may be underestimated (Koenig et al. 1996).

Genetic approaches based on isolation by distance theory may present a middle ground that estimates ecologically relevant dispersal parameters (Slatkin 1993; Rousset 1997; Palumbi 2003). When sampled over small spatial scales, these genetic patterns are driven by effective population density and typical dispersal over the past few generations, and are less affected by evolutionary or rare events (Rousset 1997; Hardy & Vekemans 1999; Leblois et al. 2004). Highly polymorphic genetic markers such as microsatellites makes sampling over small spatial scales both possible and informative (Selkoe & Toonen 2006).

Isolation by distance patterns represent a balance between genetic drift and dispersal, and strong isolation patterns can therefore result from either strongly limited dispersal or low effective density. To date, isolation by distance patterns are often interpreted as evidence that dispersal is limited by distance, but that distance remains unknown. To understand dispersal distances quantitatively, we need information on effective population density. While some studies have taken guesses at what effective densities may be (Kinlan & Gaines 2003; Palumbi 2003; Buonaccorsi et al. 2004), estimation of effective density in marine species is difficult. Census sizes for many marine organisms are in the millions, but estimates of effective size in some species are up to six orders of magnitude smaller (Hedgecock 1994; Hauser et al. 2002; Árnason 2004; Hoarau et al. 2005). Practical approaches to empirically estimate effective density are needed for a more accurate understanding of dispersal.

Anemonefish (genera *Amphiprion* and *Premnas*) provide a productive system in which to develop these methods because previous research provides initial expectations for their dispersal scales. They are also intensely exploited for the aquarium trade (Shuman et al. 2005) and therefore strong candidates for conservation within marine protected areas if dispersal scales are known. Larvae hatch after approximately 7 days from benthic eggs laid adjacent to the parents' anemone, then spend 7-11 days in the pelagic ocean (Thresher et al. 1989) before settling onto a host anemone for the rest of their lives (Fautin & Allen 1992). Genetic studies have revealed low genetic distance between populations 1000 km apart on the Great Barrier Reef, and this has been interpreted as 5 migrants per generation dispersing this distance (Doherty et al. 1995). On the other hand, 25-60% self-recruitment fractions have also been measured in anemonefish with artificial otolith tags and genetic parentage analysis (Jones et al. 2005; Almany et al. 2007; Planes et al. 2009; Saenz-Agudelo et al. 2009). These high fractions suggest highly localized dispersal, though the studies were conducted on relatively isolated islands. These disparate pieces of evidence for dispersal scales in anemonefish have appeared difficult to reconcile.

In this study, we searched for isolation by distance patterns in a common coral reef fish (*A. clarkii*) and take multiple approaches to estimate effective density. We then used these estimates to derive more robust estimates of larval dispersal scales than have been available previously. Finally, we determined whether our dispersal estimates were consistent with long-distance or local dispersal.

Methods

Study system

Clark's anemonefish (*A. clarkii*) is a species distributed throughout the Indo-Pacific. Along the islands of Cebu and Leyte in the central Philippines, populations are relatively continuous at the scale of kilometers, except where coral reefs are disrupted by sandy sediment near major river outflows. Currents in this region reverse with the seasonal monsoons, flowing primarily northwards along each coast during the northeast monsoon in January and primarily southwards in August with the southwest monsoon (USAID 2007). Based on an oceanographic model of the region, currents are likely weakest in Ormoc Bay (< 15 cm/s) on the west coast of Leyte and strongest in the shallow water between Bohol and Leyte (up to 100 cm/s) (USAID 2007).

Our study sites were 25 km apart along the east coast of Cebu (n = 10) and the west coast of Leyte (n = 8) (Figure 1). The two coastlines were chosen as replicates to examine common processes affecting dispersal. We intentionally designed our sampling over narrower spatial scales (223-252 km of coastline) than most marine dispersal studies. Genetic differentiation of samples that are close together spatially are more likely to represent recent rather than past migration rates because time to equilibrium is shorter (Slatkin 1993; Hardy & Vekemans 1999).

Ecological surveys

Census density of *A. clarkii* along coral reef coastlines was measured with underwater visual transects while on SCUBA during August-October 2008. Visual transects were swum parallel to the fringing reef. Two divers recorded the number and

size of each anemonefish on each anemone in two 5 m swaths that were randomly located from 3 to 12 m deep. The two largest fish per anemone were considered the breeding adults if they were at least 8 cm long (Ochi 1989). One diver towed a GPS unit that recorded position every 15 seconds in order to precisely measure the length of each transect path.

Transects were located exactly 25 km apart on each island, with locations chosen by GPS prior to visiting the site (n = 10 on Cebu and n = 6 on Leyte). Transects were not relocated if habitat was poor to ensure that we estimated an unbiased, mean coastal density (D). Each transect was on average 71 ± 8 minutes and 655 ± 51 m long, for a total area surveyed of 111,000 m^2. We chose to conduct fewer but longer transects so as to average over small-scale spatial variability. The surveys covered 1/40th of the length of the 475 km study area.

Area of each transect was calculated in ArcGIS 9.2 (ESRI, Redlands, CA) from the GPS tracks. We multiplied census densities (fish per m^2) by reef width to calculate linear fish density (fish per km). We measured reef width at our sampling sites from satellite photos in Google Earth.

In addition, two to six additional "reef surveys" were conducted during sample collection dives in the vicinity of each study site specifically on high quality coral reef habitat in Cebu (n = 27) and Leyte (n = 12). The underwater protocol for these reef surveys was the same as for the census transects described above. These reef surveys covered an additional 160,000 m^2 of reef, spanning in total about 1/15th of the length of our study area. When analyzing these reef surveys, however, we had to account for the fact that they represented density on coral reef habitats rather than coast-wide density.

Therefore, we used ArcGIS and the Reefs at Risk coral reef map (Burke et al. 2002) to calculate the area of Cebu and Leyte's coastline covered by reef habitat. We then multiplied reef area (m^2) by the density of fish on reef habitats (fish per m^2) and divided by coastline length to get linear fish density (fish per km). This is the same approach as applied by Puebla et al. (2009).

Finally, we compared our density estimates to those in the literature to ensure that we were not greatly under- or over-estimating typical *A. clarkii* densities.

Genetic samples

We collected non-lethal finclips underwater from *A. clarkii* in August-October 2008 after capturing fish with dip and drive nets at our sampling sites. Sampling was conducted at nearby locations if few or no fish were present at the precise sampling site. The first twenty fish of any size were sampled, and samples were stored in 70% ethanol. Size of each specimen was recorded to the nearest cm and location was marked by GPS.

We extracted DNA from all samples with Nucleospin (Machery-Nagel, Bethlehem, PA) or DNEasy 96 (Qiagen, Valencia, CA) column extraction kits. We amplified and genotyped 13 microsatellite loci (Table 1). Two loci were found through cross-species amplification of loci screened by Beldade et al. (2009), though not published by them. These loci were B6 (F: 5'-3' TGTCTTCTCCCCAAGTCAG, R: 5'-3' ACGAGGCTCAACATACCTG) and C1 (F: 5'-3' GCGACCTTGTTATCACTGTC, R: 5'-3' TTGGTTGGACTTTCTTTGTC).

Final concentrations in 10 µl PCR reactions were 1 µl genomic DNA, 1x Fermentas PCR buffer, 3mM $MgCl_2$, 500 nM fluorescently labeled primer, 500 nM

unlabeled primer, 40 μM each dNTP, and 0.1 μl (0.5 U) Fermentas Taq. Thermal cycling consisted of a 94°C denaturing step for 2 minutes, followed by 30 cycles of 94°C for 45 seconds, annealing temperature for 45 seconds (Table 1), and 72°C for 45 seconds, followed by a final extension at 72°C for 1 minute. Some loci were multiplexed using the Type-it Microsatellite PCR kit (Qiagen) and the manufacturer's PCR protocol with a 60°C or 57°C annealing temperature. PCR products were genotyped on an Applied Biosystems 3730 (MRDDRC Molecular Genetics Core Facility at Children's Hospital Boston) and analyzed in GeneMapper 4.0 (Applied Biosystems, Foster City, CA). All genotypes were checked by eye.

Genotyping error rate was assessed with duplicate, independent PCRs and genotypes for 11 to 66 samples per locus.

Genetic analysis

We assessed genetic linkage and departure from Hardy-Weinberg Equilibrium (HWE) in Genepop with 5000 iterations (Rousset 2008). Linkage and HWE were assessed independently for each locus within each population, then *p*-values were combined across populations with Fisher's method (Sokal & Rohlf 1995). We report Weir & Cockerham's F_{IS} estimate (Weir & Cockerham 1984). We calculated F_{ST} and expected heterozygosity (H_e) in Arlequin 3.11 using the number of different alleles between multilocus genotypes (Excoffier et al. 2005). We use $\alpha = 0.05$ as our Type I error rate throughout and apply Bonferroni corrections where appropriate (Rice 1989).

We assessed the presence of an isolation by distance pattern with a Mantel test for each island and then calculated a combined *p*-value across both islands using Fisher's

method (Sokal & Rohlf 1995). This combination is appropriate because data from each island independently test the hypothesis of isolation by distance. We used the smatr package in R 2.8.1 (Warton & Ormerod 2007) to calculate the slope of the relationship with reduced major axis regression. This method is appropriate when distance between populations is measured with error (Hellberg 1994; Sokal & Rohlf 1995). We also jackknifed over populations to ensure that one outlier population was not having a large influence on our slope estimate.

Isolation by distance

Population genetics theory predicts that the balance between drift and migration in a continuous population will result in a positive correlation of genetic and geographic distance between samples (Rousset 1997). This relationship is called isolation by distance. If the organism is distributed in a linear habitat and samples are taken in discrete locations,

$$\sigma = \sqrt{\frac{1}{4D_e m}} \qquad (1)$$

where σ is the spread of the dispersal kernel, D_e is effective density, and m is the slope of the relationship between $F_{ST}/(1-F_{ST})$ and geographic distance (Rousset 1997). Technically, spread is the standard deviation of parental position relative to offspring position (Rousset 1997), otherwise known as the standard deviation of the dispersal kernel. Dispersal spread (σ) can be estimated from Eq. 1 if the slope (*m)* and effective

density (D_e) are known. Effective density can be thought of as effective population size (N_e) divided by the area occupied by this population. The same set of factors that reduce N_e below census population size (N) (Frankham 1995) also reduce D_e below census density (D) (Watts et al. 2007).

Isolation by distance theory is built on a Wright-Fisher model of reproduction, assumes no selection, and assumes that the population is at drift-migration equilibrium. The one-dimensional formula used here is appropriate when the length of the habitat is greater than the width (Rousset 1997). This assumption seems appropriate on our study reefs, which are hundreds of kilometers long and only hundreds of meters wide.

Effective density from temporal method

Methods to estimate effective density from genetic data are not readily available for continuous populations, though continuous populations are common in the natural world. We take two, independent approaches to estimating effective density in this paper. Our first approach uses temporal genetic change, while our second approach is derived from census density (D).

The change in allele frequencies between cohorts contains information about the effective size of the population, but this information can be confounded by migration from surrounding populations. In general, allele frequencies will become more similar to the source population if migration is strong, while frequencies will change independently of the source if drift is strong. The pseudo-maximum likelihood method of Wang and Whitlock (2003) uses this information to estimate effective size independently from immigration rates. Their method considers both temporal changes in gene frequencies in

a focal population and the gene frequencies in a source population from which migrants arrive. The method assumes that there is no selection and negligible mutation, that the source population can be identified, that gene frequencies in the source population are stable, and that sampling does not impact the availability of reproductive individuals (Wang & Whitlock 2003).

For the estimate of effective size as implemented in MNe 2.0 (Wang & Whitlock 2003), we defined two cohorts of *A. clarkii*: the breeding adults (largest pair on each anemone if ≥8cm) and the juveniles (≤6cm). *A. clarkii* grows to 6 cm in 2-3 years, reaches reproductive size at 5-6 years, and is known to live as long as 11 years (Moyer 1986; Ochi 1986). We therefore assume these cohorts are parental and offspring samples about one generation apart, though individual pairs of fish may be a bit closer or further apart in age.

To define the source population, we first combined all non-focal samples because differentiation between populations was low ("MNe-All"). As an alternative definition of the source, we used the two populations flanking the focal population ("MNe-Flanking"). In each case, we repeated the calculation separately with each sampling site as the focal site. We report the median effective size across sites and bootstrap percentile confidence intervals from 10,000 resamples with replacement (Davison & Hinkley 1997).

These two approaches with MNe gave us estimates of local effective population size. These estimates of effective size excluded the fish in other populations centered 25 km and more in each direction. Therefore, we assumed that the spatial extent of each local population extended halfway to each flanking population (12.5 km in each

direction). We converted local effective size to effective density by dividing the effective size by the spatial extent of each local population (25 km).

Effective density from census density

A number of factors reduce effective size below census size, including fluctuations in population size through time, unequal sex ratios, variance in family size, and variance in reproductive success (Frankham 1995). Marine species may be most affected by variance in reproductive success because of family-correlated survival through larval dispersal (sweepstakes recruitment) and age-related increases in fecundity and offspring survival (Hauser et al. 2002; Hedrick 2005). Variance in reproductive success is strongly affected by the mating system, number of mates, fecundity, and longevity of a species, as well as the environmental variability a population experiences (Clutton-Brock 1988). Because anemonefish have similar mating systems and number of mates (permanent pair bonds, (Fautin & Allen 1992)), similarly high fecundity (thousands to tens of thousands of eggs per year, (Richardson et al. 1997)), similar lifespans (around a decade, (Fautin & Allen 1992)), and experience relatively low environmental variability in tropical climates, we expect that variance in reproductive success will be similar across anemonefish species.

To estimate variance in reproductive success for *Amphiprion*, we analyzed genetic parentage studies by Jones et al. (2005) in *A. polymnus* and Planes et al. (2009) in *A. percula*. These studies found that 15 of 33 or 77 of 270 potential breeding pairs (respectively) produced locally recruiting larvae in 23 or 108 larvae sampled (Jones et al. 2005; Planes, pers. comm.). Since observations of non-locally recruiting offspring were

not made, we needed to consider the limited sampling of these studies. We did this by simulating breeding pairs (33 or 270) whose reproductive success was modeled by a negative binomial distribution with a mean of two offspring per breeding pair (a stable population) and a variance that we fit to the data. While a Poisson distribution is often used when all parents have the same probability of reproducing, the negative binomial can allow probability of reproducing to vary among parents (Bolker 2008). From each simulation, we selected a subsample of the offspring (23 or 108) and compared the number of parents represented in this sample to the number of parents observed by Jones et al. or Planes et al. (15 or 77). We conducted 10,000 simulations for each of two hundred reproductive variance values between 2 and 100 on a log10 scale and selected the variance most likely to reproduce the observed values.

Equation 2 in Hedrick (2005) allows us to calculate the ratio of effective (N_e) to census (N) population size as

$$\frac{N_e}{N} = \frac{4}{V_k + 2} \quad (2)$$

where V_k is the variance in reproductive success. We then calculate D_e as

$$D_e = D\frac{N_e}{N} \quad (3)$$

Uncertainty

Each step of our calculations contained a certain degree of uncertainty. Some uncertainty resulted from uncertainty in parameter estimation, while other resulted from

uncertainty in which method to use to estimate effective density. We used both temporal genetic and census methods to explore methodological uncertainty, and we propagated parameter uncertainty through all of our calculations with bootstrap resampling. To do the latter, we sampled the parameter values in Eqs. 1 and 3 from probability distributions that reflected the uncertainty for each parameter (m, D_e, D, or N_e/N) and repeated this 10,000 times. We used normal distributions for D and N_e/N, but used a lognormal distribution for m because confidence intervals from reduced major axis regression are asymmetric. For the MN_e estimates of D_e, we sampled from the bootstrapped distribution of medians (see above). In addition, we replicated our calculations independently on two islands to examine the reliability of our results.

Results

Adult density

Our surveys revealed coast-wide *A. clarkii* densities on Cebu (0.53 ± 0.16 fish/100 m^2, n = 10) that were similar to densities on Leyte (0.55 ± 0.45 fish/100 m^2, n = 6), though Leyte densities were more variable and included numerous zeros (Figure 2). Leyte's reefs are often found as small patches, and some surveys landed on sandy habitat. The densities that we observed do not appear unusual for *A. clarkii* (Table 2). A literature search revealed mean (1.1 ± 0.47 fish/100 m^2, n = 7) and median densities (0.36 fish/100 m^2, n = 7) that were similar to our observations.

Adults made up 13-66% of the fish in each survey, and adult densities were higher on Cebu (0.21 ± 0.071 adults/100 m^2, n = 10) than on Leyte (0.096 ± 0.064 adults/100 m^2, n = 6). From satellite photos, we estimated that reefs in our study region

were approximately 150 m wide. Therefore, mean density (*D*) of *A. clarkii* in Cebu was 317 ± 107 adults/km and 144 ± 96 adults/km in Leyte.

As an estimate of census density with a larger sample size, we also analyzed all of our reef surveys on Cebu (0.25 ± 0.061 adults/100 m^2, $n = 27$) and Leyte (0.16 ± 0.056 adults/100 m^2, $n = 12$). We also estimated reef area to be 47 km^2 along the 252 km east coast of Cebu and 32 km^2 along the 223 km west coast of Leyte. Our calculations therefore suggested a mean linear density from our reef surveys of 457 ± 114 adults/km (Cebu) and 231 ± 80 adults/km (Leyte). The coast-wide densities calculated from reef surveys were higher than those from our census transects, but not significantly so ($p > 0.38$). We would expect these densities to be higher because the reef surveys were biased towards high quality coral reef habitats. In addition, the error bounds on the reef surveys were of similar width to those from the census transects despite twice the sample size, suggesting that additional survey effort would not greatly reduce uncertainty in census density. Because the reef surveys are likely biased high, and because the extra computational steps required to analyze the reef surveys likely introduces additional error and bias that is difficult to quantify (particularly in the calculation of reef area), we use our census transects for all further calculations of effective density.

Genetic analysis

Among 369 *A. clarkii* samples (Table 3) genotyped at 13 microsatellite loci, the number of alleles per locus ranged from 3 to 18 (mean: 9.5) (Table 1). None of the loci showed significant departure from HWE after combining *p*-values across populations ($p > 0.052$ for all loci), though 13 of the 234 locus-by-population comparisons (5.6%) were

significant. Only one of 78 locus pairs (1.3%) showed significant linkage (APR_Cf8 and NNG_028, $p = 0.047$), but this is likely due to the number of comparisons we made rather than actual linkage. The genotyping error rate was zero for eleven loci, and 3.2% or less for the remaining two loci (Table 1). Expected heterozygosity ranged from 0.102 to 0.890 with a mean of 0.615 ± 0.067.

F_{ST}s between any two sites were low (< 0.028) and 17 of 153 pair-wise comparisons (11%) were significant ($0.05 > p > 0.002$). While more than the 5% of comparisons expected to be significant by chance, none of these comparisons remained significant after Bonferroni corrections. One interpretation of these data would be to conclude that gene flow across the study area is common and that, if any restrictions to gene flow exist, they are weak and not detectable with the current sampling design.

However, as predicted by a drift-migration balance in continuous populations with distance-limited dispersal, a positive relationship between genetic and geographic distance was observed on both Cebu and Leyte with a combined *p*-value of 0.009 (Figure 3). The slope (m) in Leyte (1.89×10^{-4}, 95% CI: 1.60×10^{-4} - 2.23×10^{-4}) was higher than that in Cebu (0.847×10^{-4}, 95% CI: 0.630×10^{-4} - 1.14×10^{-4}). To ensure that one outlier population was not having a large influence on our slope estimates, we jackknifed over populations. Jackknifed mean slopes were slightly steeper that linear model slopes for both Leyte ($2.04 \times 10^{-4} \pm 0.819 \times 10^{-4}$) and Cebu ($0.908 \times 10^{-4} \pm 0.256 \times 10^{-4}$), but were well within the 95% CI for the original estimates. Similarly, removing the two loci with non-zero error rates (Cf29 and 65) led to somewhat steeper slope estimates (2.2×10^{-4} in Leyte and 0.92×10^{-4} in Cebu).

Temporal estimates of effective density

As our first approach to calculating effective density, we estimated population size for single sampling sites with our MNe-All method (the source defined as all non-focal populations). MNe failed while profiling 95% confidence intervals for some sampling sites, potentially due to low sample sizes, but still reported the maximum likelihood estimates of effective size (N_e) in all cases. Median effective size across sampling sites was 92 in Cebu (95% CI: 77 – 196) and 101 in Leyte (95% CI: 54 – 286). Based on 25 km of reef between sampling sites, this would be equivalent to a D_e of 3.7 (Cebu, 95% CI: 3.1 – 7.8) or 4.0 (Leyte, 95% CI: 2.2 – 11) adults/km.

Because MNe is sensitive to misidentification of the source population of immigrants (Wang & Whitlock 2003), we also reran the analysis while defining the source as the two populations flanking each focal sampling site. The N_e estimates for MNe-Flanking were generally larger, with a median size of 526 in Cebu (95% CI: 330 – 2360) and 327 in Leyte (95% CI: 150 – 5020). These higher sizes suggested higher D_e of 21 (Cebu, 95% CI: 13 – 94) or 13 (Leyte, 95% CI: 6 – 200) adults/km.

Effective density from census density

Our second approach to estimating effective density was to consider previously published information on reproductive success in *Amphiprion* and our observed census densities on Cebu and Leyte. Our simulations of reproductive variance revealed that a variance of 4.3 was most likely to produce the Jones et al. (2005) observations in *A. polymnus*, while a variance of 6.0 was most likely to produce the Planes et al. (2009)

observations in *A. percula*. Using equation 2 suggested N_e/N ratios in *Amphiprion* of 0.63 or 0.50, with a mean of 0.57 ± 0.065.

Therefore, considering uncertainty in both D and N_e/N, our demographic estimates of effective density are 178 ± 64 (Cebu) and 82 ± 55 (Leyte) adults/km.

Dispersal distance

By using Eq. 1 and our isolation by distance slopes on Cebu and Leyte, we could define the relationship between effective density and dispersal spread (the diagonal lines in Figure 4), but we could not calculate dispersal spread (σ) without knowing D_e. Our three estimates of D_e on each island provided us with six estimates of dispersal spread (Figure 4). From MNe-All, we estimated a dispersal spread of 27 km (Cebu, 95% CI: 20 - 33) or 18 km (Leyte, 95% CI: 10 - 25). Our estimates from MNe-Flanking were lower because the effective density estimates were higher: 12 km (Cebu, 95% CI: 6 – 16) and 10 km (Leyte, 95% CI: 2.5 - 15). Finally, our demographic estimates of dispersal were the lowest at 4.1 km (Cebu, 95% CI: 2.9 – 7.2) and 3.9 km (Leyte, 95% CI: 2.6 – 12.4). Our estimates of dispersal were generally higher in Cebu because the observed isolation by distance slope was shallower than on Leyte, though this difference was partially compensated by the higher effective density on Cebu.

Comparing the 95% confidence intervals around each estimate to the differences between estimates, it became clear that the greatest source of uncertainty was not in any one parameter's estimate, but rather in which method to use to calculate effective density. Our final range of dispersal spread estimates spanned a factor of seven (4 to 27 km with median 11 km), reflecting remaining uncertainty in the effective density of *A. clarkii*.

Discussion

Understanding dispersal scales in many organisms has been notoriously difficult, and our research demonstrates that increased attention to effective density can aid in the estimation of dispersal spread. Across two replicate coastlines of 220-250 km in the Philippines, we found isolation by distance patterns in populations of *A. clarkii*. These data suggest that dispersal distances are less than 220-250 km, but such patterns cannot easily be compared to high self-recruitment rates in anemonefish. In addition, we used multiple approaches to measure dispersal scale by estimating effective density. We observed temporal shifts in allele frequencies between adult and juvenile cohorts that suggested effective densities of 4-21 adults/km. Census densities of adults in our study area and low variance in reproductive success in *Amphiprion* implied that effective density was perhaps as high as 82-178 adults/km. Using these estimates of effective density with isolation by distance theory suggested that *A. clarkii* dispersal spread is in the range of 4-27 km (median 11 km).

The central role of effective density and census density

Effective density is a central concept in the population genetics of continuous populations because it is needed to convert isolation by distance signals into dispersal estimates. However, little attention has been paid to the estimation of this quantity. By collecting genetic data from multiple cohorts of *A. clarkii* along with ecological census data, we were able to develop two independent estimates of effective density.

The first approach is based on shifts in genetic composition from generation to generation using an explicit model that permits some local retention from a local population as well as input from surrounding populations (Wang & Whitlock 2003). The second is based on census density, because effective population sizes are typically lower than current census sizes (Frankham 1995). For marine species such as cod, snapper, plaice and oysters, evidence suggests that effective density is lower than census density by five to six orders of magnitude (Hedgecock 1994; Hauser et al. 2002; Hoarau et al. 2002; Árnason 2004). If this were true for anemonefish, census density would provide an upper bound that was much too high, and as a result, our dispersal estimate would be much too low.

However, anemonefish occupy individual, easily observed, breeding habitats, and adult density is the density of breeding pairs. This fact brings effective and census densities into closer alignment. Data from parentage studies confirm this assumption for anemonefish (*A. polymnus* and *A. percula*), where 29-45% of parents produced local offspring. This observation suggested that about half of the census density might provide a reasonable effective density estimate. Even using overall census density as an upper bound would provide an informative guideline for these species.

Without empirical estimates of effective density, the range of possible effective densities in marine species is extremely large, and simple assumptions about effective density could be dramatically incorrect. The methods we proposed with *A. clarkii* narrowed this uncertainty considerably and allowed us to estimate dispersal distance within an order of magnitude. Even with the remaining uncertainty, this is a substantial improvement over previous knowledge.

Effective density from genetic diversity?

Beyond the temporal genetic and census data that we used in this paper, it may also be possible to estimate effective density from genetic diversity. For example, Puebla et al. (2009) proposed a method that used the program MIGRATE to estimate effective population size, and then divided population size by reef length to estimate density. However, this method assumes the island model of migration and requires discrete and isolated populations within which isolation by distance processes do not affect genetic diversity. These criteria are often difficult to meet in widely dispersing marine species.

An alternative approach for estimating effective density may come from the population genetic theory for continuous populations. For example, Wright showed that in a continuous population N_e is affected not only by the number of individuals in the population, but also by the size of what he called the genetic neighborhood (Wright 1969). The neighborhood refers to the number of adults from which an individual's parents can be treated as if drawn at random. Wright provides equations for the effective size, length, and neighborhood size of one-dimensional, continuous populations (Wright 1969, pp. 298 and 302). These equations can be combined to show that:

$$N_e = D_e\sqrt{ka\sigma} \qquad (4)$$

where a varies from about 1.5 to 3.5 depending on the shape of the dispersal kernel, and k is the length of habitat occupied by the population. Effective population size (N_e) can be estimated from genetic diversity (e.g., $N_e = \theta/(4\mu)$, where θ is a measure of genetic

diversity and μ is mutation rate). Eq. 4 may be most useful for estimating bounds on effective density (D_e), as it relies on specifying a maximum or minimum dispersal spread (σ). However, the scale of analysis is crucial, and it is difficult to know what value to assume for *k*. If we use the diversity in our data set measured over 475 km of coastline and assume that $\sigma < 475$ km, then $D_e > 1.6$ adults/km. If we instead assume that the diversity we observe applies across the Philippines ($k \approx 24{,}000$ km), then $D_e > 0.23$ adults/km. Estimating local θ in the context of an isolation by distance model based on empirical data would be a valueable topic for future research and theory.

Assumptions of dispersal calculations

A major assumption of our calculations is that the isolation by distance pattern has reached a stationary phase close to drift-migration equilibrium under the current demographic parameters. This stationary phase is reached within a few generations for populations separated by 10σ, but may take tens or hundreds of generations for populations separated by 100-1000σ (Hardy & Vekemans 1999; Vekemans & Hardy 2004). If *A. clarkii* spatial genetic patterns are not yet stationary, they are likely becoming stronger with time because *A. clarkii* is exploited for the aquarium trade in the Philippines and its density has likely declined as a result (Shuman et al. 2005). Given the relatively small spatial scale of our study and the 5-10 yr generation time in *A. clarkii* (see Methods), it appears that our estimate of dispersal should reflect an ensemble average over the last few decades or perhaps century of dispersal. This period is likely an ecological timescale relevant to ecology, conservation, and management.

Isolation by distance estimates of dispersal also assume that effective density is constant across space (Leblois et al. 2004). In reality, densities vary at both large and small spatial scales. Simulations by Leblois revealed that isolation by distance patterns can be biased upwards if small sampling areas are immediately surrounded by a lower density (Leblois et al. 2004), as might occur if coastlines are scouted for high population densities and only sampled in those locations. We did not select our study areas based on high density, and therefore do not expect this to be a problem. We are not aware of analyses that examine the effects of spatial variation in effective density within a study region.

In addition, we assume that demography, not selection, drives patterns of genetic differentiation. Hardy-Weinberg Equilibrium at all of our loci and consistent patterns of isolation by distance across multiple loci support this assumption (data not shown). While the large variation in allelic richness across loci (3 to 18) suggests that mutation rate may vary among the loci we examined, mutation rate does not strongly affect isolation by distance patterns unless rates are much higher or lower than typical microsatellites (Leblois et al. 2003).

Comparison to other measures of larval dispersal

Previous evidence for larval dispersal in *Amphiprion* appeared contradictory because separate studies reported both relatively high effective migrant exchange (5 migrants/generation) between populations 1000 km apart on the Great Barrier Reef (Doherty et al. 1995) and high fractions of self-recruitment (30-60%) to small, local reefs (Jones et al. 2005; Almany et al. 2007). In comparison, the dispersal kernel that we

estimated predicts an effectively zero probability of any larvae traveling 1000 km. However, many larvae moving shorter distances over multiple generations (stepping-stone dispersal) can produce relatively low genetic divergence over large distances. In fact, extrapolating the isolation by distance pattern that we observed to 1000 km predicts that F_{ST} should equal 0.03 at this distance. This low predicted F_{ST} matches well to the F_{ST} of 0.05 observed by Doherty et al. (1995), though our interpretation based on an isolation by distance framework differs from their island model calculation.

When compared to observations of high self-recruitment, our estimated dispersal kernel for *A. clarkii* does not at first appear compatible. Typical dispersal of 4-27 km appears unlikely to provide 30-60% self-recruitment. To investigate this further, we simulated larval dispersal across a continuous habitat as a Gaussian random number with the median dispersal spread calculated in our study (11 km) (Figure 5a). We then measured self-recruitment to a 500 m section of reef, which is similar in size to those studied for *Amphiprion* self-recruitment (Jones et al. 2005; Almany et al. 2007). Under this model, we found that only 2% of arriving larvae on a continuous reef were born by parents on the same reef (2% self-recruitment) (Figure 5a). The self-recruitment rate was similarly low for Laplacian dispersal kernels.

Reef patchiness and comparison to self-recruitment

While our results were not compatible on continuous habitats, another possibility is that reef patchiness may strongly influence self-recruitment. Most self-recruitment studies have been conducted on small habitat patches with the nearest population more than 10 km away (Jones et al. 2005; Almany et al. 2007). We tested the idea that reef

patchiness is important by simulating larval dispersal as above, but used 500 m patch reefs spaced every 10 or 15 km in place of a continuous reef. In this patchy environment, self-recruitment rose to 36% or 56% (10 or 15 km spacing, respectively) (Figure 5b). Levels of self-recruitment similar to this have been measured for a number of reef fish in habitats that are patchy at this spatial scale (Jones et al. 1999; Jones et al. 2005; Almany et al. 2007).

This high self-recruitment fraction in patchy habitats results from a low influx of non-local larvae, not from large numbers of larvae remaining on local reefs. We note that self-recruitment as measured by Jones et al. (2005) and similar studies (percent of recruiting larvae that are from local parents) is different from local retention (percent of dispersing larvae that recruit locally) (Botsford et al. 2009). Our finding suggests that habitat patchiness may play an important role in creating high self-recruitment but low local retention (Figure 5c). This hypothesis should be testable by conducting self-recruitment studies in both continuous and patchy habitats.

A number of other explanations for this discrepancy are possible. Some authors have suggested that marine fish larvae may have a bimodal strategy in which some larvae are actively retained while others passively disperse (Armsworth et al. 2001). This possibility could be represented by a strongly leptokurtic dispersal kernel with a very strong mode at zero distance and long tails away from the parents.

Another possibility is that dispersal spread (σ) varies dramatically among species, from *A. clarkii* (this study) to *A. polymnus* (Jones et al. 2005) and *A. percula* (Almany et al. 2007). Alternatively, *Amphiprion* dispersal spread might vary between regions, from the Philippines (this study) to Papua New Guinea (Jones et al. 2005; Almany et al. 2007),

perhaps as a result of larval behavior or oceanographic currents. Using our continuous habitat model above, dispersal spread would have to be very low (600 m) for self-recruitment to reach 30%, and even lower (300 m) to reach 60% self-recruitment. These dispersal spreads are one to two orders of magnitude below our estimates of *A. clarkii* dispersal spread in the Philippines, and such strong variation among species or regions appears unlikely.

We suggest that high fractions of self-recruitment in patchy habitats as well as regular dispersal to surrounding reefs are both consistent with a single larval dispersal strategy and do not require dispersal kernels to change shape dramatically either among *Amphiprion* species or among study regions.

Conclusions and future directions

In the future, we predict that greater attention to effective density will provide more robust estimates of dispersal and greater ability to interpret isolation by distance patterns. Our study suggests that two additions to typical genetic sampling can aid in the estimation of effective density. First, samples from two (or more) distinct cohorts can be used to examine temporal changes in allele frequencies with MNe and derive point estimates of effective density. Second, ecological surveys can put an upper bound on effective density. These additions entail more field effort, but the gain is an enhanced ability to understand dispersal.

Going forward, there is a clear need for further development of effective density methods relevant to continuous populations. Our study showed that remaining uncertainty in dispersal distances derives largely from differences among methods for

calculating effective density rather than from uncertainty in parameters. New theory or simulations that indicate the most appropriate methods or suggest new methods would be quite useful at this point. As discussed above, genetic diversity may provide insights into effective density if certain challenges can be resolved. In addition, the intercept of the isolation by distance pattern may contain important but rarely used information on effective density if one can make assumptions about the shape of the dispersal kernel (see Rousset 1997).

Understanding ecological scales of dispersal in a wide range of organisms has been complicated by methods that focus on exceptional rather than typical dispersers, but isolation by distance approaches can address this problem when effective density is estimated. In *A. clarkii*, our median dispersal spread estimates of 11 km appear consistent with high self-recruitment rates if habitat patchiness is considered. Our estimates of dispersal spread suggest that marine reserves for anemonefish would need to be ten or more kilometers wide to be self-sustaining (Lockwood et al. 2002), or integrated in dense marine reserve networks (Kaplan et al. 2006; Gaines et al. 2010). Further efforts to integrate multiple sources of information on dispersal, such as studies that combine both isolation by distance and parentage methods, will continue to improve our understanding of dispersal.

As populations of many species continue to decline, accurate measurement of dispersal distances will aid in effective management and conservation. Isolation by distance genetic studies that account for the effective density of populations can provide this important information.

Acknowledgments

MLP gratefully acknowledges support from a NSF Graduate Research Fellowship, a NDSEG Fellowship, an International Society for Reef Studies/Ocean Conservancy grant, a Stanford Biology SCORE grant, and a Myers Oceanographic Trust grant. The authors also want to thank the Project Seahorse Foundation, the Coastal Conservation and Education Foundation, G. Sucano, and A. Vailoces for critical assistance in the field. G. Bernardi generously shared primer sequences for us to screen. R. Waples, S. Planes, and an anonymous reviewer provided helpful comments on earlier versions of the manuscript.

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Tables

Table 1. Microsatellite loci used in this study.

Locus	Annealing Temp (°C)	# of alleles	H_e	F_{IS}	% error (# tested)	Reference
1359	53	7	0.602	0.040	0 (23)	(Liu et al. 2007)
1578	58	5	0.439	0.117	0 (63)	(Liu et al. 2007)
915	53	4	0.102	-0.012	0 (29)	(Liu et al. 2007)
B6	53	14	0.878	-0.009	0 (32)	*this paper*
C1	53	8	0.756	0.002	0 (30)	*this paper*
D1	53	18	0.890	0.015	0 (27)	(Beldade et al. 2009)
Cf29	53	18	0.823	-0.038	3.2 (31)	(Buston et al. 2007)
Cf8	53	3	0.621	0.034	0 (27)	(Buston et al. 2007)
45	58	12	0.695	0.038	0 (66)	(Quenouille et al. 2004)
65	53	12	0.700	-0.017	1.9 (52)	(Quenouille et al. 2004)
LIST12_004	58	3	0.203	0.026	0 (31)	(Watts et al. 2004)
LIST12_012	53	4	0.558	0.060	0 (11)	(Watts et al. 2004)
LIST12_028	53	15	0.730	0.027	0 (29)	(Watts et al. 2004)

Table 2. Densities of *A. clarkii* compiled from the literature.

Country	Site	Density (fish/100 m^2)	Reference
Japan	Murote Beach, Shikoku Island	3.2	(Ochi 1985)
Japan	Miyake-jima	0.25	(Moyer 1980)
Japan	Sesoko Island, Okinawa	0.36	(Hattori 1994)
Philippines	Olango inside MPA*	2	(Shuman et al. 2005)
Philippines	Olango outside MPA	0.15	(Shuman et al. 2005)
Papua New Guinea	Madang	1.8	(Elliott & Mariscal 2001)
Australia	Keppel Islands	0.0036	(Frisch & Hobbs 2009)

* MPA: Marine Protected Area

Table 3. Sample sizes of *A. clarkii* at each site. Sites are listed south to north on each island. Adults are the two largest fish on each anemone if they are at least 8 cm. Juveniles are defined as fish up to 6 cm.

Site Name	Island	# adults	# juveniles	Total samples
Santander	Cebu	10	5	20
Boljoon	Cebu	8	7	20
Argao	Cebu	9	8	20
Carcar	Cebu	15	4	20
Minglanilla	Cebu	12	6	20
Danao	Cebu	12	7	20
Sogod	Cebu	4	15	21
Tabogon	Cebu	13	3	21
Daanbantayan	Cebu	10	9	20
Malapascua	Cebu	14	4	20
Pintuyan	Leyte	11	4	17
Padre Burgos	Leyte	11	6	19
Maasin	Leyte	11	5	18
Inopacan	Leyte	23	6	34
Baybay	Leyte	12	6	19
Albuera	Leyte	10	7	20
Ormoc City	Leyte	11	9	20
Palompon	Leyte	14	3	20
			Total	369

Figure Legends

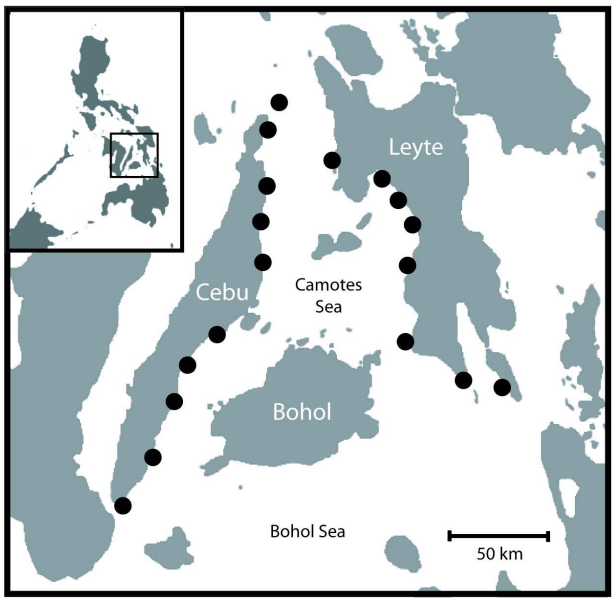
Figure 1. Cebu and Leyte Islands in the central Philippines. Black dots indicate study sites. Rectangle in inset map shows location of primary map within the Philippines.

Figure 2. Census densities of *A. clarkii* in Cebu and Leyte, Philippines. Lines are the adult density (solid with dots) and total density (dashed with dots) at standard survey sites. Also shown are the total densities on sites chosen for having high quality coral reefs (x).

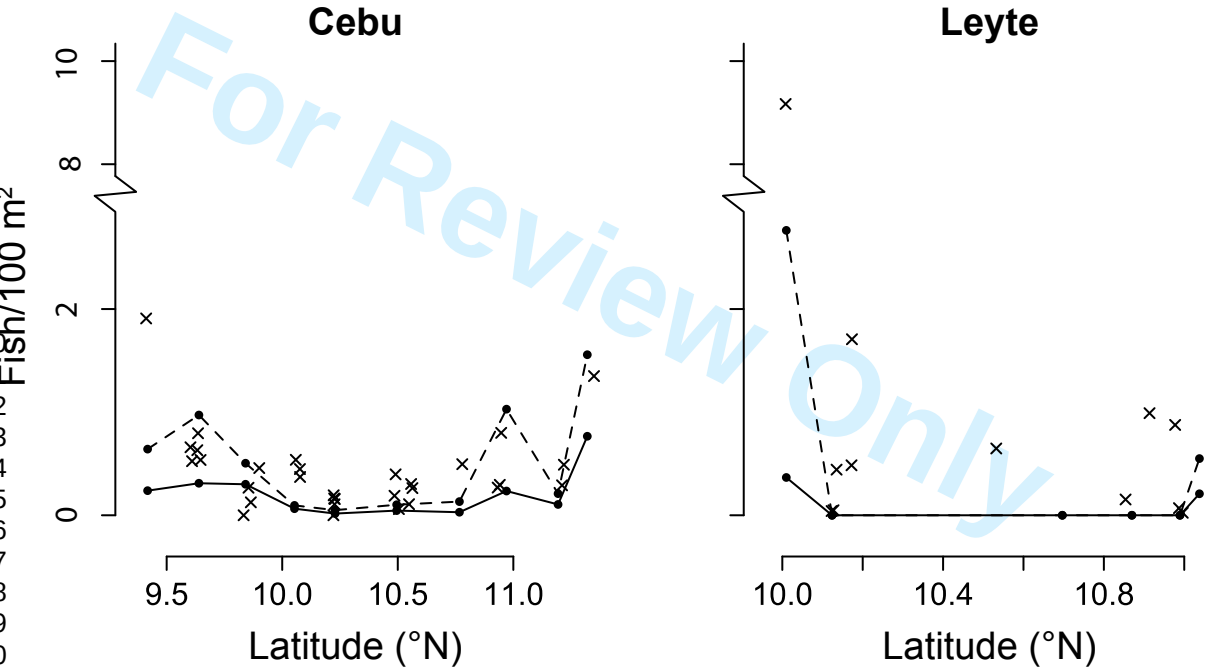
Figure 3. Genetic distance between *A. clarkii* populations in Cebu and Leyte, shown with a reduced major axis regression against geographic distance. Cebu: $p = 0.11$, $r^2 = 0.04$, $m = 0.847 \times 10^{-4}$, 95% CI: 0.630×10^{-4} - 1.14×10^{-4}. Leyte: $p = 0.01$, $r^2 = 0.31$, $m = 1.89 \times 10^{-4}$, 95% CI: 1.60×10^{-4} - 2.23×10^{-4}).

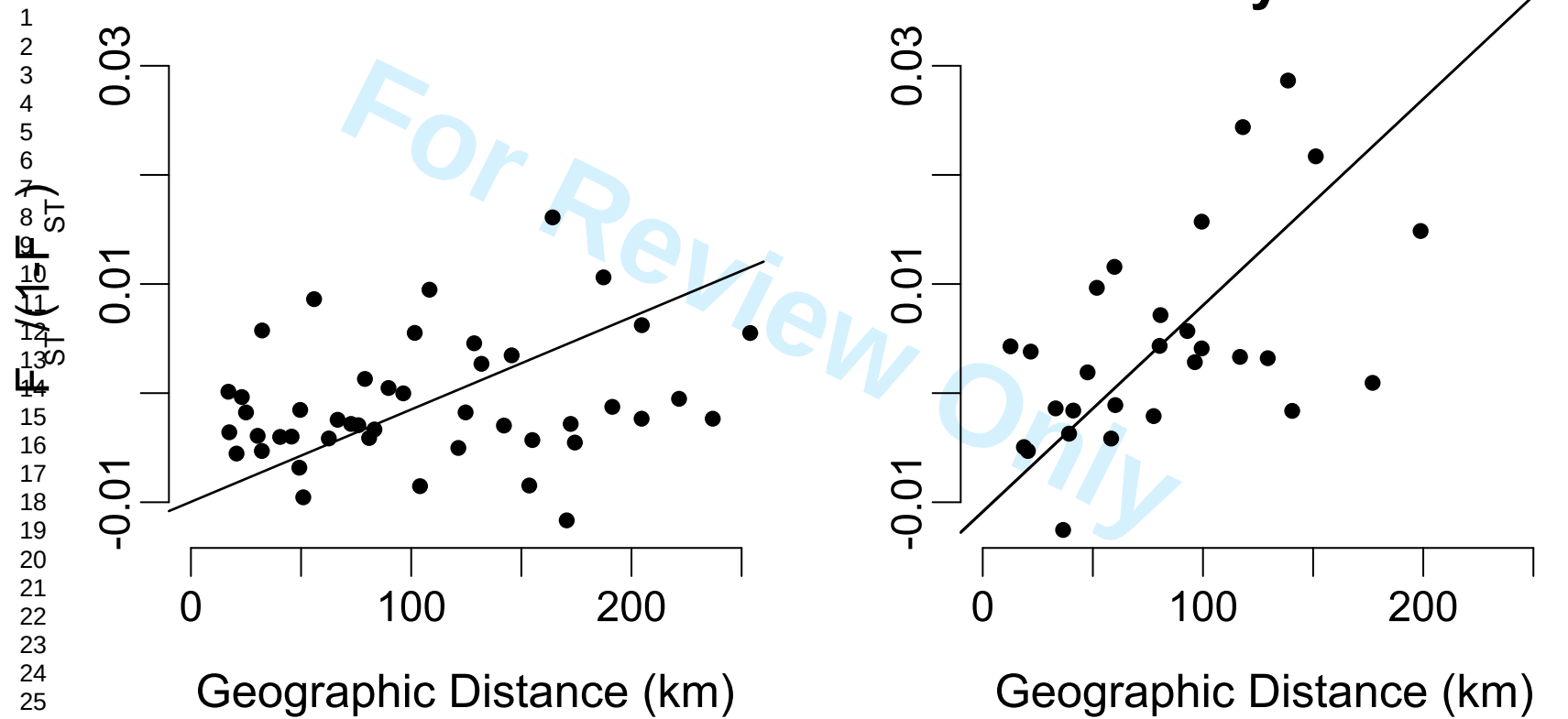
Figure 4. Graph illustrating the calculation of dispersal spread from the slope of the isolation by distance relationship and estimates of effective density. By knowing the slope, we can draw the solid lines (Cebu in black, Leyte in grey). By also knowing the effective density, we can calculate the corresponding dispersal spread (various dashed and dotted lines). Three estimates of effective density are shown for each island: MNe-All (dashed), MNe-Flanking (dotted), and a demographic estimate from census density (dash-dotted).

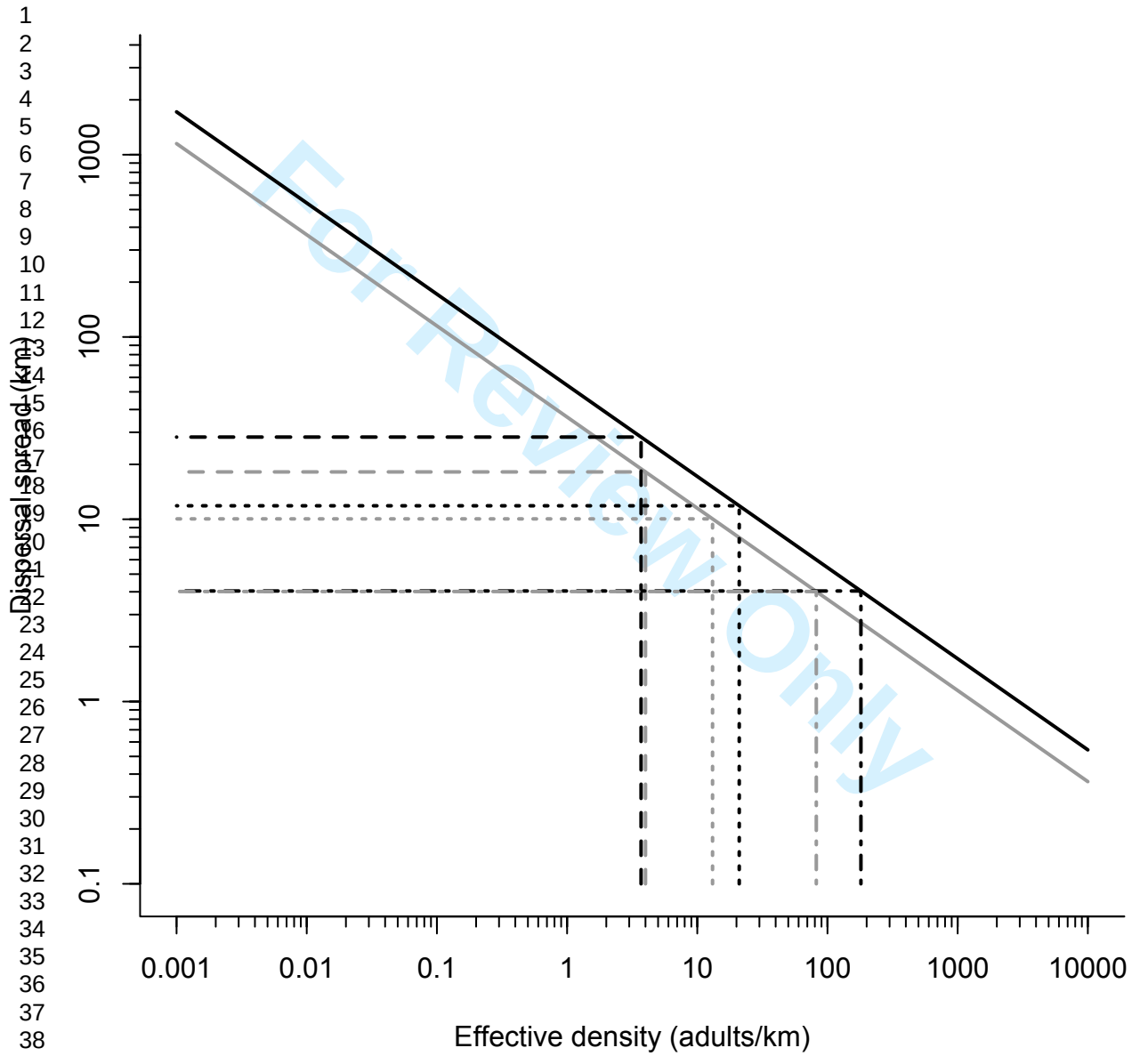
Figure 5. Effects of reef patchiness on the self-recruitment fraction. In continuous habitats (a), many of the recruiting larvae on a small patch of reef (center of diagram) come from surrounding reefs and the self-recruitment fraction (fraction of recruiting larvae that are from local parents) is low. In a patchy reef seascape (b), there are few surrounding reefs from which larvae can arrive and the number of non-local recruiting larvae will be low. Therefore, the self-recruitment fraction is high. However, because self-recruitment is a measure of the larvae arriving at a local reef, the few larvae that self-recruit may in reality be only a small fraction of all larvae that disperse from a reef, leading to both high self-recruitment and low retention (c).



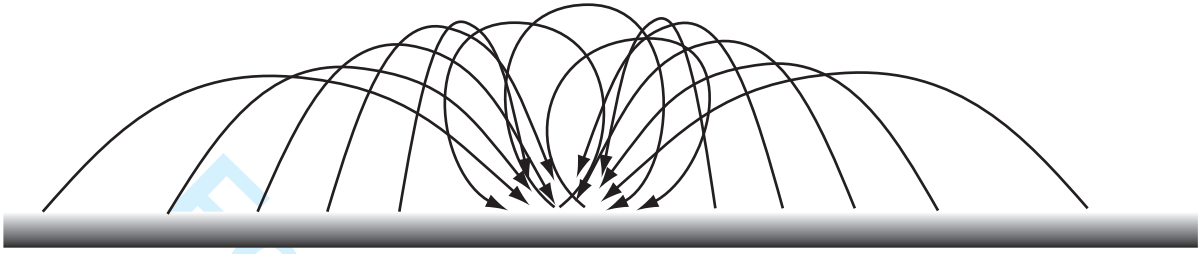
Cebu and Leyte Islands in the central Philippines. Black dots indicate study sites. Rectangle in inset map shows location of primary map within the Philippines.
173x114mm (300 x 300 DPI)



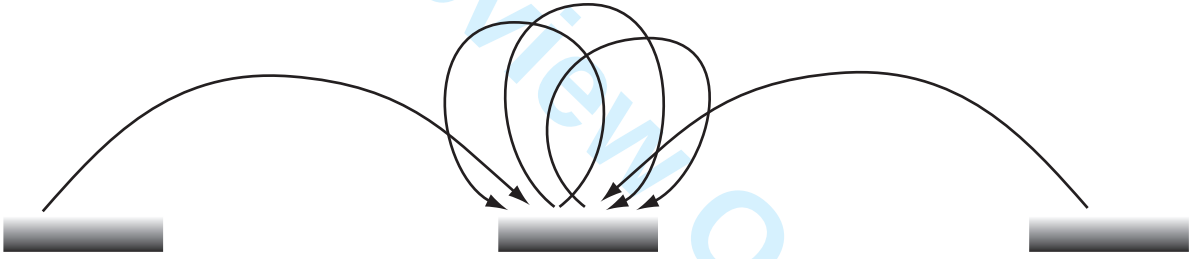




a) Recruitment from the regional population (low self-recruitment)



b) Recruitment primarily from the local reef (high self-recruitment)



c) High self-recruitment with low local retention