

Spatial and temporal patterns of larval dispersal in a coral-reef fish metapopulation: evidence of variable reproductive success

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Abstract

Many marine organisms can be transported hundreds of kilometres during their pelagic larval stage, yet little is known about spatial and temporal patterns of larval dispersal. Although traditional population-genetic tools can be applied to infer movement of larvae on an evolutionary timescale, large effective population sizes and high rates of gene flow present serious challenges to documenting dispersal patterns over shorter, ecologically relevant, timescales. Here, we address these challenges by combining direct parentage analysis and indirect genetic analyses over a 4-year period to document spatial and temporal patterns of larval dispersal in a common coral-reef fish: the bicour damselfish (*Stegastes partitus*). At four island locations surrounding Exuma Sound, Bahamas, including a long-established marine reserve, we collected 3278 individuals and genotyped them at 10 microsatellite loci. Using Bayesian parentage analysis, we identified eight parent-offspring pairs, thereby directly documenting dispersal distances ranging from 0 km (i.e., self-recruitment) to 129 km (i.e., larval connectivity). Despite documenting substantial dispersal and gene flow between islands, we observed more self-recruitment events than expected if the larvae were drawn from a common, well-mixed pool (i.e., a completely open population). Additionally, we detected both spatial and temporal variation in signatures of sweepstakes and Wahlund effects. The high variance in reproductive success (i.e., 'sweepstakes') we observed may be influenced by seasonal mesoscale gyres present in the Exuma Sound, which play a prominent role in shaping local oceanographic patterns. This study documents the complex nature of larval dispersal in a coral-reef fish, and highlights the importance of sampling multiple cohorts and coupling both direct and indirect genetic methods in order to disentangle patterns of dispersal, gene flow and variable reproductive success.

Keywords: larval connectivity, parentage, self-recruitment, sweepstakes effects, Wahlund effect

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Introduction

Many marine animals have a biphasic life cycle that includes a pelagic larval stage followed by comparatively sedentary juvenile and adult stages. One

consequence of this ubiquitous life history strategy is that ocean currents can potentially transport pelagic larvae hundreds of kilometres from where they were spawned (Doherty *et al.* 1995; Weersing & Toonen 2009). Many such species therefore exist within marine metapopulations, where local populations of demersal juveniles and adults are connected by the exchange of planktonic larvae (Kritzer & Sale 2004). However, the

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pattern and magnitude of larval dispersal depends on a complex interplay among ocean currents (Gilg & Hilbish 2003; Cowen & Sponaugle 2009; White *et al.* 2010), larval behaviour (Leis & McCormick 2002; Gerlach *et al.* 2007), pelagic larval duration (Shanks 2009; Weersing & Toonen 2009; Faurby & Barber 2012) and physiological condition (Booth & Hixon 1999; Grorud-Colvert & Sponaugle 2011; Johnson *et al.* 2014). Variation in one or more of these factors can substantially affect both the spatial and temporal patterns of larval dispersal.

Stochastic processes associated with larval dispersal can lead to high larval mortality and may result in variable reproductive success where only a small proportion of adults produce the majority of larvae that survive through their pelagic larval phase (Hedgecock & Pudovkin 2011). Hedgecock (1994a,b) called this phenomenon 'sweepstakes reproductive success', because only a few adults would be 'big winners' in terms of reproductive success. Hallmark characteristics of sweepstakes reproductive success include genetically distinct cohorts and decreased genetic variation and genetic diversity when recruit populations are compared with adult populations (Hedgecock 1994a,b; Hedgecock & Pudovkin 2011). Variable reproductive success can also cause a Wahlund effect, characterized by an excess of homozygotes (Wahlund 1928; Hedrick 2005b). When this phenomenon occurs, genetically distinct cohorts accumulate within a single local population, creating an admixture of genetically differentiated individuals. Evidence of sweepstakes reproductive success and the Wahlund effect indicate spatial as well as temporal variation in larval sources. Documenting both patterns of larval dispersal and variable reproductive success will inform our understanding as to where marine populations lie along the continuum between demographically open (i.e., dependent upon larval connectivity) or closed (i.e., replenished by self-recruitment).

Researchers have directly documented larval dispersal by employing techniques such as otolith microchemistry (Swearer *et al.* 1999; Miller & Shanks 2004), chemical tagging of individuals (Jones *et al.* 1999, 2005), and increasingly, genetic parentage analysis (Jones *et al.* 2005; Planes *et al.* 2009; Christie *et al.* 2010a,b; Harrison *et al.* 2012; Saenz-Agudelo *et al.* 2012). Studies using these methods have documented both self-recruitment (Jones *et al.* 2005; Almany *et al.* 2007; Christie *et al.* 2010a) and larval connectivity (Planes *et al.* 2009; Christie *et al.* 2010b; Harrison *et al.* 2012). However, to date, no study has applied both direct (e.g. parentage analysis) and indirect (e.g. Wright's *F* statistics) genetic methods to monthly cohorts sampled over multiple years. Results from such broad-scale studies are critical for understanding marine metapopulation dynamics (Selkoe *et al.* 2006; Siegel *et al.* 2008; Watson *et al.* 2012),

informing the design of marine reserve networks (Palumbi 2003; McCook *et al.* 2009), and enhancing fisheries management (Gell & Roberts 2003; Fogarty & Botsford 2007).

In this study, we coupled both direct parentage analysis and indirect genetic methods to characterize patterns of larval dispersal in a large marine metapopulation in Exuma Sound, Bahamas, over a 4-year period. Exuma Sound is a semi-enclosed basin characterized by a hydrodynamically complex environment that is punctuated with vigorous, semi-permanent and mesoscale gyres (Colin 1995; Stockhausen & Lipcius 2001). Depending upon their structure and dynamics, these gyres may facilitate connectivity between islands located on opposite sides of the Sound, may cause self-recruitment within an island, or may simply create high mortality for larvae via entrainment and subsequent advection from suitable habitat. For example, simulated oceanographic models of spiny lobster (*Panulirus argus*) dispersal combined with abundance data found spatially variable levels of larval supply at locations throughout the Exuma Sound (Lipcius *et al.* 1997; Stockhausen *et al.* 2000; Stockhausen & Lipcius 2001). These results indicated that different hydrodynamic regimes of the gyres could affect the degree of connectivity between and self-recruitment within local populations in the Exuma Sound.

Our objective was to quantify temporal and spatial patterns of larval dispersal and variable reproductive success in bicolor damselfish (*Stegastes partitus*). We addressed four questions: (i) How consistent are the spatial and temporal patterns of connectivity and self-recruitment in Exuma Sound? (ii) Given the low levels of genetic differentiation previously documented in bicolor damselfish (Purcell *et al.* 2009; Christie *et al.* 2010a), is there greater evidence for connectivity between sites than self-recruitment within sites? (iii) Are directly documented patterns of larval dispersal congruent with those inferred with indirect genetic methods? and (iv) Is there evidence for high variance in reproductive success? To answer these questions, we sampled adults and recruits (i.e., individuals that had recently settled from the plankton) of bicolor damselfish from multiple locations within a regional metapopulation and coupled parentage analyses with calculations of heterozygosity, genetic diversity and genetic differentiation to document patterns of larval dispersal and reproductive success.

Materials and methods

Study site and species

Approximately 500 km of continuous and shallow patch reefs encircle Exuma Sound, a semi-enclosed,

deep ocean basin (>1500 m deep) bordered by the Grand Bahamas Bank (<5 m deep) to the west and north, and the Atlantic Ocean to the east and south (Fig. 1). Circulation is partially self-contained, and consists of predominantly northwesterly along-shore currents (~20 cm/s; Colin 1995) that are punctuated by mesoscale gyres that last from 10 to 30 days and can reach a depth of 200 m (Lipcius *et al.* 1997; Stockhausen & Lipcius 2001).

The bicour damselfish is a common inhabitant of coral reefs throughout the tropical Western Atlantic and greater Caribbean region (Humann & Deloach 2002). Juveniles and adults are highly site-attached and tend to stay within small (1–3 m²) home ranges (Myrberg 1972; Schmale 1981; Hixon *et al.* 2012). Bicolour damselfish do not form long-term mating pairs; instead, territorial males guard demersal nests and may receive eggs from multiple females. Females also mate with multiple males and will travel to male territories up to 10 m away when spawning (Knapp & Warner 1991; Cole & Sadovy 1995; Johnson *et al.* 2010). Spawning activity follows the lunar cycle and overall reproductive effort peaks during the summer months (Hixon *et al.* 2012). Eggs hatch after ~3.5 days of benthic development (Schmale 1981; Johnson *et al.* 2010). The pelagic larval duration (PLD) of bicour damselfish ranges from 24 to 40 days (mean ≈

30 days, Wellington & Victor 1989) before late-stage larvae settle to suitable coral-reef habitat and recruit to the local population (Sponaugle & Cowen 1996). This PLD is typical of many tropical marine fishes (Victor and Wellington 2000, Lester and Ruttenberg 2005). In Exuma Sound, bicour damselfish mature at 6–7 cm total length (TL) and live an average of 0.88 years, with a maximum recorded lifespan of 3.6 years (Hixon *et al.* 2012). Previous work on bicour damselfish dispersal in Exuma Sound directly documented self-recruitment through parentage analysis at two islands (Christie *et al.* 2010a). Christie *et al.* (2010a) also provided evidence for sweepstakes reproductive success, whereby the highly variable reproductive success of adults results in smaller genetic variation and diversity in each cohort (Hedgecock 1994a,b; Hedgecock & Pudovkin 2011).

Sample collection

From 2005 to 2008, we collected tissue samples from 3278 bicour damselfish from three sites adjacent to each of four islands (12 sites total) encompassing Exuma Sound: Cat Island, Eleuthera, Exuma Cays Land and Sea Park and Lee Stocking Island (Fig. 1, the general locations are shown for the sample sites, which were all within 8 km of each other for each island). In

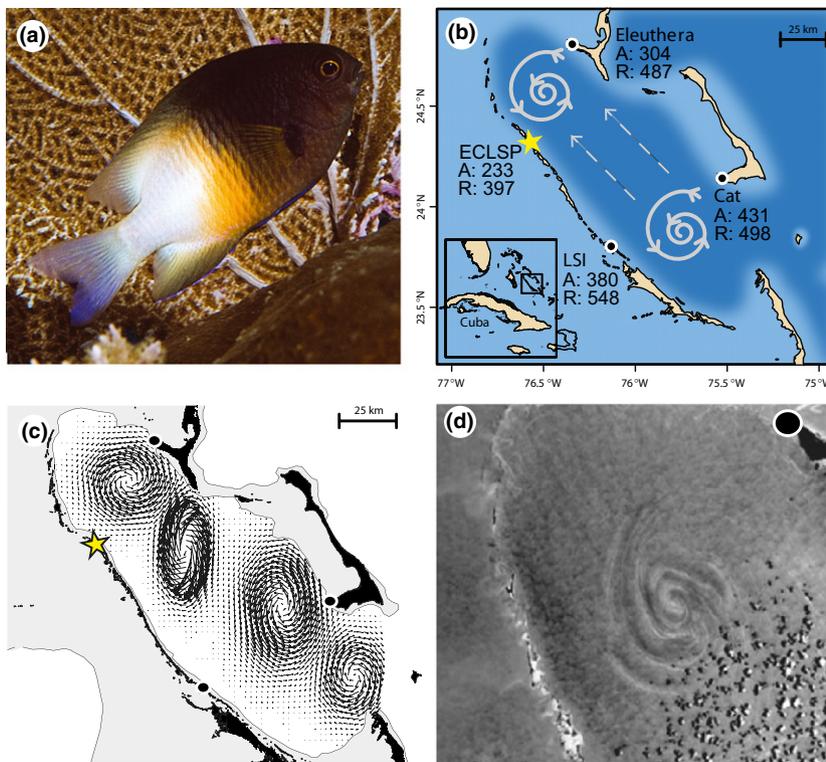


Fig. 1 (a) Adult bicour damselfish, *Stegastes partitus* (courtesy of David Nasser). (b) Map of Exuma Sound, with sampled islands and corresponding adult (A) and recruit (R) sample sizes over all years. The arrows indicate the predominant currents in Exuma Sound, including seasonal mesoscale gyres (thick, grey swirls) and the prevailing northwesterly Antilles Current (thin, grey, dashed arrows). The yellow star represents a marine reserve study area and black circles are unprotected study areas. ECLSP, Exuma Cays Land and Sea Park; LSI, Lee Stocking Island. (c) Near-surface geostrophic currents in Exuma Sound computed from conductivity, temperature and depth (CTD) observations (Stockhausen & Lipcius 2001). Notice that the gyres are closer to ECLSP, Eleuthera and Cat Island than to LSI. (d) Satellite image of one of the northern seasonal gyres adjacent to Eleuthera (courtesy of Larry Ammann, University of Texas).

2005, samples were collected during a single summer visit at each of the four islands (Christie *et al.* 2010a). From 2006 to 2008, we collected adult and recruit samples at the same sites in June, July and August to correspond with the peak summer spawning and recruitment events. Because of the short interval between visits, and by collecting only fish ≤ 30 mm (TL), it was reasonable to assume that all of the sampled recruits in each month belonged to the same cohort (Sponaugle & Cowen 1996).

To facilitate collection with hand nets, SCUBA divers anaesthetized individual damselfish with a solution of 10% quinaldine and 90% methanol mixed with equal amounts of seawater. The anaesthetic solution was targeted at specific fish using squeeze bottles

to minimize exposure to other marine life. We clipped a small portion of the pelvic fins from adults (>5 cm TL, $n = 1348$) before releasing them at the collection site. Recently settled recruits (<3 cm TL, $n = 1930$) were euthanized and their caudal fins were preserved for subsequent analysis. All tissue samples were stored in a urea-based solution (10 mM Tris, 125 mM NaCl, 10 mM EDTA, 1% SDS, 8 M urea, pH adjusted to 7.5 with HCL). The numbers of adults sampled varied by island and year based on the number of new adults that became established in each of the sample sites. We haphazardly collected tissue samples from about 50 recruits at each monthly visit to each island. Thus, we sampled approximately 150 recruits from each island per summer (Table 1).

Table 1 Summary statistics of local populations of bicolor damselfish

Island	Year	N	N_a	k	F_{IS}	H_o	H_e	HWE	% LD
Adults									
Cat	2005	46	23	19	0.102	0.834	0.928	3/7	0
Cat	2006	115	26	18	0.103	0.803	0.895	3/10	0
Cat	2007	123	24	18	0.088	0.814	0.893	3/10	0
Cat	2008	147	26	18	0.076	0.823	0.890	3/10	0
ECLSP	2005	47	22	19	0.060	0.874	0.928	2/7	0
ECLSP	2006	63	20	17	0.104	0.798	0.891	2/10	0
ECLSP	2007	59	21	18	0.097	0.811	0.897	2/10	0
ECLSP	2008	64	22	18	0.011	0.789	0.889	2/10	0
Eleuthera	2005	49	22	19	0.052	0.887	0.936	0/7	0
Eleuthera	2006	43	20	18	0.930	0.809	0.891	2/10	0
Eleuthera	2007	107	24	18	0.091	0.810	0.890	2/10	0
Eleuthera	2008	105	25	18	0.115	0.790	0.892	2/10	0
LSI	2005	43	20	18	0.036	0.899	0.932	0/7	0
LSI	2006	104	28	19	0.120	0.856	0.932	3/7	0
LSI	2007	108	19	18	0.091	0.817	0.893	2/10	0
LSI	2008	125	26	18	0.104	0.799	0.892	3/10	0
Recruits									
Cat	2005	44	22	18	0.090	0.843	0.925	2/7	0
Cat	2006	152	29	18	0.104	0.801	0.894	5/10	0
Cat	2007	155	26	18	0.104	0.799	0.892	6/10	0
Cat	2008	147	26	18	0.110	0.794	0.893	3/10	0
ECLSP	2005	45	22	18	0.014	0.916	0.929	1/7	0
ECLSP	2006	108	25	18	0.091	0.812	0.894	3/10	0
ECLSP	2007	115	25	18	0.094	0.810	0.894	2/10	0
ECLSP	2008	129	24	18	0.107	0.796	0.890	2/10	0
Eleuthera	2005	37	21	19	0.049	0.883	0.927	1/7	4.4
Eleuthera	2006	163	26	18	0.077	0.823	0.892	2/10	0
Eleuthera	2007	145	26	18	0.110	0.791	0.888	4/10	0
Eleuthera	2008	142	26	18	0.121	0.784	0.891	5/10	0
LSI	2005	74	25	19	0.081	0.858	0.933	3/7	0
LSI	2006	162	27	18	0.104	0.804	0.897	3/10	0
LSI	2007	162	28	18	0.124	0.781	0.891	5/10	0
LSI	2008	150	25	18	0.147	0.758	0.888	5/10	0

ECLSP, Exuma Cays Land and Sea Park; LSI, Lee Stocking Island. Column headings are as follows: N , sample size, N_a , mean number of alleles per locus, k , mean allelic richness (corrected for smallest sample size), F_{IS} , Wright's inbreeding coefficient, H_o , observed heterozygosity, H_e , expected heterozygosity, HWE, the number of loci that deviate from Hardy-Weinberg Equilibrium/total number of loci, % LD, percentage of loci pairs in linkage disequilibrium.

DNA extraction and microsatellite typing

DNA was extracted using a protocol optimized for the urea-based storage solution (Sambrook & Russell 2001; see Christie *et al.* 2010a for details). Samples from 2005 ($n = 385$) were previously extracted and genotyped at seven microsatellite loci (Christie *et al.* 2010a), and because adults sampled in 2006 from Lee Stocking Island ($n = 104$) were needed for quantitative genetic studies (Johnson *et al.* 2010, 2011), they were also genotyped at the same seven microsatellite loci: SpAAC33, SpAAC 41, SpAAC42, SpAAC47, SpAAT40, SpGATA16 and SpGATA40 (Williams *et al.* 2003). The remaining 2839 samples were genotyped at the above seven loci and three additional microsatellite loci: SpTG10, SpTG16 and SpGGA7 (Thiessen & Heath 2007). PCRs were optimized and multiplexed with the Qiagen Multiplex PCR Kit and contained 5.0 μL of Qiagen Multiplex mix, 1.0 μL of each primer and 2.0 μL of approximately 100 ng/ μL DNA template for a total volume of 10 μL . The thermocycle profile consisted of an initial denaturing step at 95 °C for 15 min, followed by 30 cycles of 30 s at 94 °C, 90 s at 58 °C and 60 s at 72 °C. PCR products were screened on an ABI 3100 automated sequencer (Applied Biosystems) at the Center for Genome Research and Biocomputing at Oregon State University. We used GENOTYPER 3.7 to determine allele sizes. We reprocessed 19.5% ($n = 640$) of the individuals (from extraction through scoring) to check for errors in genotyping and to check individuals that were difficult to score at the most polymorphic loci (the error rate was 3.7%).

Parentage analyses

To directly document dispersal on an ecological time-scale, we used a Bayesian parentage analysis (Christie 2010; Christie *et al.* 2013) to identify parent-offspring pairs. Briefly, this method calculates the posterior probability of any given pairwise comparison sharing alleles by chance, given the frequencies of the shared alleles. We compared the genotypes of all biologically plausible adults and all recruits to detect putative parent-offspring pairs. Using the extensive demographic data, we excluded adults that could not be possible parents (due to mortality in previous years) for each cohort of recruits. Pairs unlikely to share alleles by chance (i.e., posterior probability <0.05) after fully accounting for all possible pairwise comparisons were considered to be parent-offspring pairs. All putative pairs were re-analysed from DNA extraction through scoring at all loci to eliminate the possibility of laboratory errors. All pairs shared at least one allele at all loci. None of the identified parent-offspring pairs had missing data.

The possibility of pairs actually being some other first-order relative, that is full-siblings, was very

unlikely due to the size differences between adults and the offspring in each pair. For example, based on average growth rates in our study area (Hixon *et al.* 2012), it would take 430–750 days for a fish to grow to the sizes of the parents. Assuming that the instantaneous mortality rate of adults is 0.0045 per day (Hixon *et al.* 2012), the probability of an adult living long enough to have been the parents of both members of our putative parent-offspring pairs was low (0.034–0.144). We also used a simulation to evaluate the probability that full-siblings could have shared at least one allele at all loci, and thus exhibited the same allele matching pattern as parent-offspring pairs. We used the allele frequencies from the entire population and created genotypes in accordance with Hardy–Weinberg Equilibrium (HWE). Next, we randomly paired individuals and simulated full-sibling offspring based on expected patterns of Mendelian inheritance. We then counted how many full-siblings shared at least one allele at all loci (as did our putative pairs). Because we had eight parent-offspring pairs, we created eight full-sibling pairs (each pair with different parents) and then counted the number of pairs that shared an allele at each locus. This process was repeated 1000 times, and we calculated the probability of observing one full-sibling pair, two full-sibling pairs, three full-sibling pairs, etc. that shared at least one allele across all loci in our data set. We used the R statistical environment for these simulations (R Core Team 2012). Based on the number of shared alleles, the probability of one identified ‘parent-offspring pair’ being full-siblings was not trivial ($P = 0.76$). The probability of two or more pairs being full-siblings was low ($P = 0.04$), and the probability of three or more pairs being full-siblings was very unlikely ($P = 0.001$). These analyses suggest that, at most, one of the identified ‘parent-offspring pairs’ could have been full-siblings. However, this event was unlikely when considered within the context of the above life history information. Given the low probability that one fish lived long enough to produce a 6-cm adult and a 2.5 cm recruit, and the low probability of full-siblings sharing one allele at each loci, it is unlikely that any of the documented parent-offspring pairs were full-siblings or higher-order relatives.

Dispersal distances between identified parent and offspring were determined using both Euclidean distance and along-shore distance (the nearest along-shore path between the adult sample site and the recruit sample site following the fore-reef contour at a resolution of 1 km). Because bicolor damselfish do not move more than a few metres after settling to reef habitat (Hixon *et al.* 2012), the Euclidian distances between parent and offspring represent the minimum dispersal distances of planktonic larvae (i.e., the actual distances travelled by dispersing larvae may have been much greater if they did not follow a direct path).

Given the low genetic differentiation previously documented in this system (Christie *et al.* 2010a), it is possible that larvae from multiple source populations are well mixed while in the plankton. Because parent-offspring pairs record the starting and ending points for larval dispersal, we could test whether or not our observed pattern of larval dispersal (five self-recruitment events and three connectivity events) was consistent with homogenous mixing of larvae from each of the sampled populations. As a null hypothesis, we expected islands to contribute to a randomly mixed pool of larvae that supplies all islands with recruits. However, the probability of observing self-recruitment or connectivity among the sites depends on the number of parents and offspring sampled at each site, and these values were unequal in our study. Using the R statistical environment (R Core Team 2012), we simulated a randomly mating population by creating simulated adults, represented by the numbers of adults we sampled at each island, and placed them together in a combined population. We next sampled this mixed population by the total number of parents we identified, eight individuals, and noted from which population they were sampled. Then, using the same procedure, we sampled simulated recruits and generated locations of parents and offspring expected in an open population, where larvae from each local population joined a well-mixed pool of larvae that contributed offspring to all local populations. We repeated this procedure 10 000 times and calculated the *P*-value as the proportion of simulations that identified equal or greater numbers of self-recruitment events to the one we observed for our empirical data. Using this framework, we tested whether the number of parent-offspring pairs sampled at the same sites (i.e., self-recruitment) was less than or greater than expected.

Population-genetic analyses

GENEPOP v. 4.0.11 (Raymond & Rousset 1995; Rousset 2008) was first employed to determine whether any locus pairs were in linkage disequilibrium, and to test for departures from HWE for adult and recruit samples collected each year at each island. For each measure, a total of 10 000 batches and 5000 iterations per batch were used to reduce the standard errors below 0.01. We tested for large allele dropout, null alleles and stuttering that could explain deviations from HWE using MICRO-CHECKER v2.2.3 (Van Oosterhout *et al.* 2004). We used ARLEQUIN v3.11 (Excoffier *et al.* 2005) to calculate observed and expected heterozygosities and F_{ST} v2.9.3.2 (Goudet 2001) to calculate F_{IS} values, mean number of alleles per locus, mean allelic richness (*k*) and Nei's gene diversity (1987). Allelic richness was corrected for sample size of adult and recruit population at each island among years. To test for population

differentiation, we used a variety of metrics based on allele frequencies in each population. We first used F_{STAT} to calculate pairwise, unbiased F_{ST} , (Weir & Cockerham 1984) and we adjusted the corresponding *P*-values for multiple comparisons with a sequential Bonferroni procedure ($\alpha = 0.05$). We also used randomization tests within F_{STAT} (5000 permutations) to compare F_{ST} values between recruits pooled by year. For comparisons between groups with ten and seven loci, only the matching seven loci were used. Because highly polymorphic loci can artificially reduce F_{ST} values (Hedrick 2005a; Meirmans & Hedrick 2011), we also used SMOGD v1.2.5 (Crawford 2010) to calculate pairwise D_{est} values (Jost 2008) and GENODIVE (Meirmans & Van Tiernderen 2004) to calculate standardized versions similar to F_{ST} including multilocus G'_{ST} (Hedrick 2005a) and pairwise population G''_{ST} (Meirmans & Hedrick 2011).

Variable reproductive success

To test for the Wahlund effect, we compared the number of loci out of HWE due to a homozygous excess (averaged across cohorts) to cohort groups that were pooled together, both temporally (each island across all years) and spatially (each month across all islands). To examine the evidence for a temporal Wahlund effect, we compared the average number of loci that deviated from HWE due to homozygote excess for each recruit cohort at each island (averaged across months) to the samples pooled across all months and years at that island. To examine the evidence for a spatial Wahlund effect, we compared the average number of loci that deviated from HWE due to homozygote excess for each monthly recruit cohort (averaged across islands) to the samples pooled across all islands in a given month. If a Wahlund effect occurred, then the number of loci with a homozygous excess would be less in the cohort average compared with the pooled group. To test for sweepstakes reproduction, we first tested for genetic differences among each cohort using a randomization test (5000 permutations) in F_{STAT} to compare F_{ST} among pooled recruit samples by islands and years. Second, we used randomization tests (5000 permutations) in F_{STAT} to test for differences in genetic variation (observed heterozygosity), genetic diversity (expected heterozygosity and allelic richness), and F_{IS} between recruits and adults sampled in each year.

Results

Parentage analysis

We detected eight parent-offspring pairs, two each year from 2005 to 2008. We documented five pairs that exhibited self-recruitment within an island (two of

which were previously reported by Christie *et al.* 2010a) and three pairs that documented connectivity between islands (Fig. 2, Table 2). Remarkably, in three of the five self-recruitment events, the recruit fish returned to the exact reef from which it originated (Table 2). For the other two self-recruitment events, the recruits returned to a nearby site at the same island (<5 km away). The longest dispersal distance detected was 111.2 km in Euclidean distance, or 129.4 km in along-shore distance (Table 2). Given the sample sizes we collected and the number of parent-offspring pairs we detected, it is unlikely that we would have documented so many self-recruitment events, if the larvae were drawn from a common, well-mixed pool (randomization test, P -value = 0.03).

Population-level analyses

Across all adult and recruit populations grouped by yearly collection at each island, the mean number of alleles per population over all 10 loci ranged from 19 to 29. The mean allelic richness per population over all loci, calculated from a minimum sample size of 43 for adults and 37 for recruits, ranged from 17 to 19 alleles. Observed heterozygosity ranged from 0.758 to 0.916 (Table 1). Based on the MICRO-CHECKER analysis for deviations from HWE, homozygote excess was the most frequent cause of deviation. There was no evidence for

large allele drop-out, and we detected low average numbers of null alleles in our 10 loci: 2.4 [0.22 standard error or the mean (SEM)] in adult populations and 2.8 (0.18 SEM) in recruit populations. We suspect that a large proportion of homozygous genotypes are from population admixture (i.e., a Wahlund effect: see 'variable reproductive success' below).

Global F_{ST} was low and not significantly different from zero (95% confidence interval: 0.000–0.001). Pairwise comparisons of F_{ST} revealed low genetic differentiation across all years and islands for both adult and recruit populations, ranging from -0.0032 to 0.0046 (Table S1, Supporting information). The low F_{ST} values were not simply an artefact of the highly polymorphic loci. Similar metrics that account for polymorphic loci were also relatively low: G'_{ST} ranged from -0.003 to 0.005 , G''_{ST} ranged from -0.045 to 0.065 , and D_{est} ranged from -0.016 to 0.057 with a mean for each estimator of 0.0004 , 0.006 and 0.004 , respectively. Nevertheless, after correcting for multiple comparisons, there were 21 significant F_{ST} pairwise comparisons among adult populations out of the 120 total possible comparisons among islands across years. Lee Stocking Island was one of the populations involved in the majority of those significant comparisons (86%; Table S1, Supporting information). Comparisons of adult to recruit populations across all years and islands revealed 20.3% of the pairwise comparisons of F_{ST} values to be

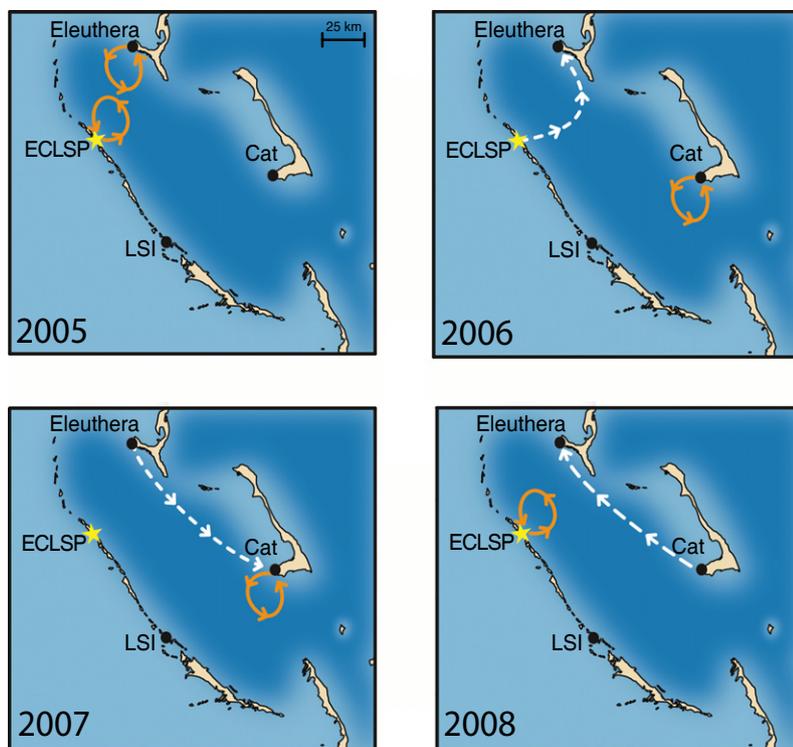


Fig. 2 Parent-offspring pairs of bicolor damselfish documented in each of 4 years. Orange solid arrows indicate larval connectivity and white dashed arrows show self-recruitment. The yellow star is the marine reserve study area, and black circles are unprotected study areas. ECLSP, Exuma Cays Land and Sea Park; LSI, Lee Stocking Island.

Table 2 Euclidean and along-shore distance between members of each parent-offspring pair of bicour damselfish

Adult island (spawning island)	Offspring island (recruitment island)	Year	Month	Distance (km)		P-value
				Euclidean	Along shore	
Eleuthera	Eleuthera	2005	July	0	0	0.0360
ECLSP	ECLSP	2005	July	0	0	0.0110
Cat	Cat	2006	August	0	0	0.0116
ECLSP	Eleuthera	2006	August	61.1	129.4	0.0433
Cat	Cat	2007	June	3.1	3.2	0.0259
Eleuthera	Cat	2007	August	111.2	129.0	0.0377
Cat	Eleuthera	2008	July	111.2	129.0	0.0332
ECLSP	ECLSP	2008	July	4.4	4.7	0.0136

ECLSP, Exuma Cays Land and Sea Park.

Along-shore distance was measured by tracing the contour of the 20-m isobaths with a resolution of 1 km. The 'P-value' is the posterior probability of the pair being false given the shared allele frequencies (Christie 2010). Notice that three offspring returned to the exact same reef inhabited by their parents (distance = 0).

significant (Table S2, Supporting information). Pairwise comparisons among all recruits among islands across years revealed that 22 of 120 pairwise comparisons were significant (18.3%) at an alpha equal to 0.05 after a sequential Bonferroni correction, and populations sampled at Lee Stocking Island were one of the pairs involved in 68% of the significant comparisons (Table S3, Supporting information). Finally, genetic differentiation among cohorts of recruits was significant when the following years were compared (F_{STAT} randomization test): 2005 vs. 2006 (P -value = 0.0056); 2005 vs. 2008 (P -value = 0.0146); 2006 vs. 2007 (P -value = 0.0034); and 2007 vs. 2008 (P -value = 0.0252; 2005 vs. 2007 was marginally significant; P -value = 0.105).

Variable reproductive success

We found evidence for both temporal and spatial Wahlund effects. We compared the average number of loci that deviated from HWE for each recruit cohort at each island (averaged across months) to recruit samples pooled across all months and years at that island. We found that the pooled estimate had considerably more loci out of HWE due to homozygote excess (Fig. 3a), indicating a temporal Wahlund effect (i.e., samples across time were more different from one another than samples within the same month). We also compared the average number of loci that deviated from HWE for each monthly recruit cohort (averaged across islands) to the samples pooled across all islands in a given month and found that the pooled estimates had considerably more loci out of HWE due to homozygote excess (Fig. 3b), indicating a spatial Wahlund effect (i.e., samples across islands in a given month were more different from one another than samples within a site).

As predicted by the sweepstakes hypothesis, we detected evidence for lower genetic variance and diversity in recruit populations compared with adult populations. The randomization tests in F_{STAT} revealed lower observed heterozygosity ($P = 0.016$) and expected heterozygosity ($P = 0.019$) in recruits compared with adults (Fig. 4a, b). Also the numbers of alleles (standardized by sample size) present in recruit populations were lower than adult populations ($P = 0.032$; Fig. 4c). Finally, we also documented a positive and significantly higher F_{IS} in recruit populations compared with adult populations ($P = 0.034$; Fig. 4d; again using F_{STAT}).

Discussion

We found direct evidence for both connectivity and self-recruitment in a marine metapopulation of bicour damselfish. Expanding the temporal scale of Christie *et al.* (2010a) by 3 years, we documented dispersal events ranging up to ~130 km, which were previously inferred indirectly, but not directly quantified. Importantly, we also found that a long-established marine reserve, the Exuma Cays Land and Sea Park, not only was self-seeding (i.e., self-recruitment was documented twice at this island), but also generated larvae that seeded unprotected areas. While Lipcius *et al.* (2001) had inferred indirectly that this marine reserve is capable of both self-recruitment and exporting larvae to other islands, we provide the first direct evidence. Additionally, we documented a spatial Wahlund effect, which suggests that different sets of adults contributed to the offspring sampled at each island within a given month. We also documented to a lesser extent a temporal Wahlund effect, which suggests that each monthly cohort within a site originated from different sets of

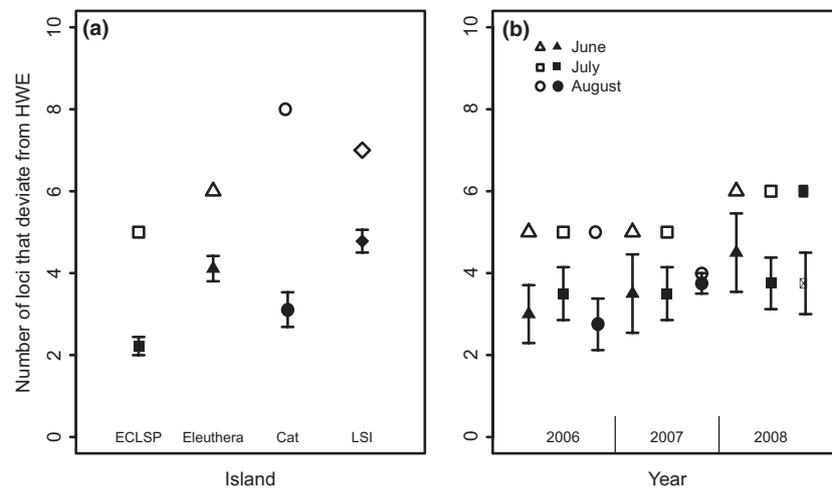


Fig. 3 Temporal and spatial Wahlund effects. Each solid symbol is the number of loci that deviate from Hardy–Weinberg Equilibrium (HWE) averaged across cohorts [± 1 standard error of the mean (SEM)]. Each open symbol is the number of loci that deviate from HWE when all cohorts are pooled. (a) Temporal Wahlund effect where closed symbols represent recruit populations averaged across months at each island and open symbols represent all recruit cohorts pooled at a given island. (b) Spatial Wahlund effect where closed symbols represent the number of loci out of HWE averaged across islands for recruits in a given month and open symbols represent recruits pooled across islands in the same month.

parents. Moreover, because we observed lower heterozygosity (observed and expected) and allelic richness in recruit cohorts compared with adult populations, it appears that sweepstakes reproduction was also prevalent in this system.

While many studies have indirectly inferred connectivity and self-recruitment, few studies have addressed the question of temporal variability of these events using direct methods (but see Saenz-Agudelo *et al.* 2012). To our knowledge, our study is the first to directly document larval dispersal patterns over 4 years, using multiple cohorts within each year. Our findings suggest that studies based on a single year may not sufficiently capture the variation in patterns of larval dispersal that can exist in marine systems. Conducting multi-year studies that sample multiple cohorts within each year to describe the variability in dispersal will facilitate effective planning for fisheries management and enhance the design and placement of marine reserves.

The pattern of gene flow inferred from F_{ST} suggested similar patterns of dispersal compared with the parentage analysis: the greatest number of significantly different pairwise comparisons of population structure was found at Lee Stocking Island, where no parent-offspring pairs were documented. The fact that one island, Lee Stocking Island, had the majority of genetic difference compared with itself as well as to other locations, suggests that dispersal may not be equal throughout the Exuma Sound. Similar levels of genetic differentiation have been documented in bicolor damselfish populations from other regions (e.g., Lacson 1992;

Ospina-Guerrero *et al.* 2008; Hepburn *et al.* 2009; Hogan *et al.* 2010; Salas *et al.* 2010) and across the entire Caribbean region (Purcell *et al.* 2009). The temporal stability of genetic differences involving Lee Stocking Island means that these differences were not simply due to sampling error (Waples 1998), and may indicate that the patterns observed on an ecological timescale correspond to patterns on an evolutionary timescale.

Similar to other studies in marine systems, we detected a Wahlund effect despite low F_{ST} values (Tracey *et al.* 1975; Johnson & Black 1984; Addison & Hart 2004; Levitan 2005). These studies attributed localized genetic differentiation to be the mechanism causing the observed pattern. In our system, it is likely that sweepstakes reproduction was the mechanism resulting in the Wahlund effect. Our findings concur with previous work in this system by Christie *et al.* (2010a) that provided evidence of sweepstakes reproductive success. Because evidence of the sweepstakes reproduction in bicolor damselfish was also found from studies in the Mesoamerican Barrier Reef System (Hepburn *et al.* 2009; Villegas-Sanchez *et al.* 2010), this effect may be prevalent throughout the geographic range of this species. More generally, sweepstakes reproduction, and not necessarily restricted larval dispersal, may be responsible for patterns of genetic differentiation observed in many marine species.

The variable pattern of larval dispersal and reproductive success documented in this study has wide-ranging ecological and evolutionary implications. First, while variance in reproductive success is appreciated in many

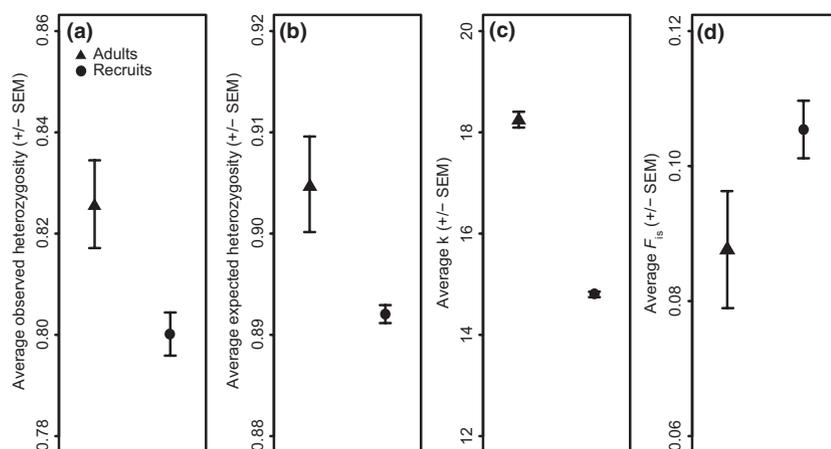


Fig. 4 Comparisons of the average observed heterozygosity, gene diversities (expected heterozygosity and allelic richness— k) and F_{IS} between adult (triangles) and recruit (circles) bicolour damselfish.

terrestrial systems (Clutton-Brock 1988), the contribution of this variance is not well understood in marine systems. Second, many marine populations are characterized by high rates of dispersal (Levin 2006; Gaines *et al.* 2007) and gene flow (Waples 1998; Hedgecock *et al.* 2007). However, if there is high variance in reproductive success, then the resulting changes to allele frequency distributions (Hedgecock & Pudovkin 2011) can cause samples from populations to be genetically differentiated from one another even though there may be little to no correlation between dispersal and the documented genetic differentiation. Because this high variance in reproductive success is simply one form of genetic drift, and is thus random, there is often no relationship between geographic distance and genetic differentiation (sometimes termed ‘chaotic genetic patchiness’; Johnson and Black 1982, Johnson & Black 1984). We suggest that there is nothing ‘chaotic’ about these observed patterns of genetic differentiation, and that by greatly extending the duration of sampling presented in Christie *et al.* (2010a), we have identified that (i) there is substantial dispersal in this system, as characterized by parents and offspring being as far as 129 km apart; and (ii) the signature of high gene flow in this system is continually eroded by high variance in reproductive success (i.e., sweepstakes reproduction). Thus, disentangling dispersal, gene flow and genetic drift can be achieved only by sampling multiple cohorts of recruits over multiple years or spawning cycles.

Although mechanisms for variance in reproductive success have rarely been documented, region-wide genetic homogeneity may be eroded by the predominant mesoscale gyres that commonly occur in Exuma Sound (Colin 1995; Stockhausen & Lipcius 2001). Depending upon their characteristics, each gyre may promote connectivity or self-recruitment and may also entrain larvae and advect them away from suitable habitat, causing high levels of larval mortality. Using

biophysical models (reviewed by Werner *et al.* 2007) in our system could help to explain the mechanisms underlying the observed patterns. For example, at islands where offshore gyres have been previously observed—Exuma Cays Land and Sea Park, Eleuthera, and Cat Island—we detected self-recruitment, whereas no parent-offspring pairs were observed at Lee Stocking Island, where gyres are less prevalent (Stockhausen & Lipcius 2001). However, the hypothesis that gyres facilitate self-recruitment remains to be tested. Moreover, seasonal and annual fluctuations in location, duration and strength of the gyres may further affect patterns of successful larval dispersal. Such variation in the gyres may dictate whether oceanic conditions are favourable for successful settlement of larvae (Hedgecock & Pudovkin 2011), and thus may be the primary mechanism driving sweepstakes reproduction in this system. The other hydrodynamic feature of Exuma Sound, the prevailing northwesterly Antilles Current, could have transported larvae from Cat Island to Eleuthera, and when this current reverses direction, which can happen for short periods of time (Colin 1995), the opposite pattern may have occurred. This possibility was corroborated by the fact that we detected larval connectivity in both directions between these islands.

This study illustrates the temporally and spatially complex nature of larval dispersal in a coral-reef fish. Here, we show that by sampling multiple cohorts and coupling both direct and indirect genetic methods, it is possible to disentangle patterns of dispersal, gene flow and variable reproductive success. The next steps towards a comprehensive understanding of marine metapopulation dynamics include quantifying larval retention and linking genetic analyses with local demographic, oceanographic and environmental data. Synthesis of these different fields will greatly advance our understanding of metapopulation dynamics and enhance fisheries management and conservation

efforts by identifying the spatial boundaries of marine populations.

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C.D.S, D.W.J., M.A.H, and M.R.C planned the study. C.D.S, D.W.J., M.R.C, M.A.H, and T.J.P conducted field work. M.R.C. and T.J.P conducted the laboratory work. T.J.P. analysed the data, and wrote the paper with guidance by M.R.C., with all authors contributing to revisions.

Data accessibility

All microsatellite genotypes and R scripts have been deposited in the Dryad Digital Repository (doi:10.5061/dryad.qr7f5).

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Pairwise F_{ST} values for adult vs. adult bicolor damselfish populations (above diagonal) and their corresponding P -values (below diagonal).

Table S2 Pairwise G'_{ST} values for both adult vs. adult (above diagonal) and recruit vs. recruit (below diagonal) bicolor damselfish populations.

Table S3 Pairwise G''_{ST} values for both adult vs. adult (above diagonal) and recruit vs. recruit (below diagonal) bicolor damselfish populations.

Table S4 Pairwise D_{est} values for both adult vs. adult (above diagonal) and recruit vs. recruit (below diagonal) bicolor damselfish populations.

Table S5 Pairwise F_{ST} values among all adult vs. recruit populations. Black boxes with white text indicate significant F_{ST} values based on a sequential bonferroni correction.

Table S6 Pairwise F_{ST} values recruit vs. recruit bicolor damselfish populations (below diagonal) and their corresponding P -values (above diagonal).