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Contributed Paper

An experimental test of alternative population augmentation scenarios

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Abstract: *Human land use is fragmenting habitats worldwide and inhibiting dispersal among previously connected populations of organisms, often leading to inbreeding depression and reduced evolutionary potential in the face of rapid environmental change. To combat this augmentation of isolated populations with immigrants is sometimes used to facilitate demographic and genetic rescue. Augmentation with immigrants that are genetically and adaptively similar to the target population effectively increases population fitness, but if immigrants are very genetically or adaptively divergent, augmentation can lead to outbreeding depression. Despite well-cited guidelines for the best practice selection of immigrant sources, often only highly divergent populations remain, and experimental tests of these riskier augmentation scenarios are essentially nonexistent. We conducted a mesocosm experiment with Trinidadian guppies* (Poecilia reticulata) *to test the multigenerational demographic and genetic effects of augmenting 2 target populations with 3 types of divergent immigrants. We found no evidence of demographic rescue, but we did observe genetic rescue in one population. Divergent immigrant treatments tended to maintain greater genetic diversity, abundance, and hybrid fitness than controls that received immigrants from the source used to seed the mesocosms. In the second population, divergent immigrants had a slightly negative effect in one treatment, and the benefits of augmentation were less apparent overall, likely because this population started with higher genetic diversity and a lower reproductive rate that limited genetic admixture. Our results add to a growing consensus that gene flow can increase population fitness even when immigrants are more highly divergent and may help reduce uncertainty about the use of augmentation in conservation.*

Keywords: adaptive divergence, assisted migration, demographic rescue, genetic divergence, genetic rescue, *Poecilia reticulata*, supplementation, translocation

Una Prueba Experimental de Escenarios Alternativos de Aumento Poblacional

Resumen: *El uso humano del suelo está fragmentando los hábitats en todo el mundo y está inhibiendo la dispersión entre poblaciones de organismos previamente conectadas, lo que generalmente resulta en depresión endogámica y un potencial evolutivo reducido de cara al cambio ambiental repentino. Para combatir esto, a veces se utiliza el aumento de poblaciones aisladas con inmigrantes para facilitar el rescate demográfico y genético. El aumento con inmigrantes que son similares genética y adaptativamente a las poblaciones receptoras incrementa efectivamente la aptitud poblacional, pero si los inmigrantes son muy divergentes genética y adaptativamente, el aumento poblacional puede resultar en depresión exogámica. A pesar de las pautas bien citadas para la mejor selección práctica de fuentes de inmigrantes, comúnmente permanecen*

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sólo poblaciones con alta divergencia, y prácticamente no existen las pruebas experimentales de estos escenarios de aumento con mayor riesgo. Realizamos un experimento de mesocosmos con olominas trinitarias (Poecilia reticulata) *para probar los efectos genéticos y demográficos multigeneracionales del aumento a dos poblaciones receptoras con tres tipos de inmigrantes divergentes. No encontramos evidencia de un rescate demográfico, pero sí observamos el rescate genético en una población. Los tratamientos de inmigrantes divergentes tendieron a mantener una mayor diversidad genética, abundancia y aptitud híbrida que los controles que recibieron inmigrantes de la fuente utilizada para sembrar el mesocosmos. En la segunda población, los inmigrantes divergentes tuvieron un efecto ligeramente negativo en uno de los tratamientos, y los beneficios del aumento fueron menos aparentes en general, probablemente porque esta población inició con una diversidad genética mayor y una tasa reproductiva más baja que limitó la mezcla genética. Nuestros resultados se suman a un consenso creciente de que el flujo génico puede incrementar la aptitud poblacional incluso cuando los inmigrantes son más altamente divergentes y puede ayudar a reducir la incertidumbre sobre el uso del aumento en la conservación.*

Palabras Clave: divergencia adaptativa, divergencia genética, migración asistida, rescate demográfico, rescate genético, reubicación, suplemento, *Poecilia Reticulata*

摘要: 人类对土地的利用正引起全球栖息地的破碎化, 并妨碍了原本相连种群间的生物扩散, 这通常导致近交衰退, 并削弱了生物在面临环境快速变化时的演化潜力。为了应对隔离种群的增加, 有时会通过个体迁入来协助种群数量和遗传拯救。迁入在遗传上和适应性上与目标种群相近的个体可以有效提高种群的适合度, 但若迁入与目标种群在遗传上和适应性上差异较大的个体, 则会导致远交衰退。虽然已有常被援引的指导方针来指导选择最佳迁入来源的个体, 但仅存的种群通常是高度趋异的, 且基本没有针对这类更高风险下的种群扩增方案的实验检测。我们利用孔雀鱼 (**Poecilia reticulata**) 开展了一个生态系统围栏实验, 来检测用三种趋异类型的迁入个体扩增两个目标种群, 在多世代后对其种群数量和遗传的影响。我们没有发现种群数量获得拯救的证据, 但在一个种群中观察到了遗传拯救。相比于迁入与围隔生态系统中来源相同的个体, 迁入趋异个体倾向于保持更高的遗传多样性、丰度和杂种优势。在第二个种群中, 迁入的趋异个体在一个处理中有轻微的负面影响, 对种群扩增的益处总体上较为不明显, 这可能是因为这个种群具有较高的起始遗传多样性和较低的繁殖率, 从而限制了遗传混合。我们的结果增强了一个正在逐渐形成的共识, 即使迁入高度趋异的个体, 基因流也可以提高种群的适合度, 这或许有助于减少种群扩增在保护应用中的不确定性。**翻译: 胡怡思; 审校: 魏辅文**

关键词: 适应趋异, 协助迁移, 种群数量拯救, 遗传趋异, 遗传拯救, 孔雀鱼 (*Poecilia reticulate*), 扩充, 迁地

Introduction

Anthropogenic habitat fragmentation is a primary driver of Earth's sixth mass extinction, in part because it hinders natural levels of dispersal and gene flow between previously connected populations (Haddad et al. 2015). Over time this can reduce genetic diversity via genetic drift and inbreeding, contribute to inbreeding depression, and undermine the capacity of populations to adapt to disturbances such as invasive species, pollutants, and climate change (Allendorf et al. 2013; Frankham et al. 2017). Reestablishing connectivity among populations is therefore a top priority in conservation science (Seddon et al. 2014; Haddad et al. 2015).

When restoring natural levels of dispersal is not feasible, as is frequently the case, the only option for reestablishing connectivity is to actively introduce immigrants. This management practice—variously referred to as augmentation, supplementation, a type of translocation, and assisted migration—can increase the viability of populations through 2 synergistic mechanisms. The first is demographic rescue, which offers a benefit solely by bolstering abundance. Demographic rescue can effectively buffer against demographic stochasticity and Allee effects (Brown & Kodric-Brown 1977; Hufbauer et al. 2015), but it also comes with risks, such as exposure to novel disease pressures and the disruption of social structures (Mills 2013). The second, and potentially greater, benefit of augmentation is genetic rescue, defined by Whiteley et al. (2015) as "an increase in population fitness (growth) owing to immigration of new alleles." Genetic rescue can have a highly positive and lasting impact on populations by increasing genetic diversity and effectively reversing the negative genetic consequences of isolation (Allendorf et al. 2013; Frankham 2016; Frankham et al. 2017).

Genetic rescue is also controversial, in part because it disrupts the unique evolutionary history of isolated populations by adding foreign alleles to local gene pools, an intervention some consider objectionable (discussed in Love Stowell et al. 2017). Also controversial is that although gene flow can benefit populations by increasing genetic diversity, it may in turn break up coadapted gene complexes or introduce maladaptive alleles, both of which contribute to outbreeding depression (Allendorf et al. 2013; Frankham et al. 2017). Empirical studies reflect this duality; genetic rescue attempts are typically highly successful (Frankham 2015; Whiteley et al. 2015), but cases of outbreeding depression do exist, particularly in later-generation hybrids (Fenster & Galloway 2000; Edmands 2007; Waller 2015).

Several authors have synthesized theory and empirical studies to provide guidelines for the selection of immigrant donor populations (Edmands 2007; Hedrick & Fredrickson 2010; Frankham et al. 2011; Weeks et al. 2011), and they suggest that although some degree of neutral genetic and adaptive divergence from the recipient population is necessary to increase genetic diversity, outbreeding depression is a risk at higher levels of divergence that occur when populations are isolated for many years or adapted to different environments. These recommendations are valuable, but the reality is that often the only donor populations that remain are highly divergent at neutral or adaptive loci or both (e.g., Channel Island foxes [*Urocyon littoralis*] [Funk et al. 2016]). This problem is exacerbated by the fact that locally rare and endangered species—those most likely to benefit from augmentation—also tend to have the fewest appropriate donor populations. The applicability of donor selection guidelines would be enhanced by experimental tests of these more extreme augmentation scenarios.

To this end, guppies (*Poecilia reticulata*) from the Northern Range mountains of Trinidad are ideal. Trinidadian guppies inhabit streams punctuated by waterfalls that partially restrict upstream gene flow, resulting in high genetic drift and low genetic diversity in some populations and a broad range of neutral genetic divergence (Suk & Neff 2009; Willing et al. 2010; Baillie 2012) that reflects both the distance between populations and the magnitude of waterfall barriers (Crispo et al. 2006). Waterfalls completely exclude predatory fishes from upstream areas that contain guppy populations, leading to the mostly repeated evolution of two adaptively divergent guppy ecotypes: those adapted to low-predation environments and high-predation environments (Magurran 2005). Low- and high-predation ecotypes differ in a suite of genetically based, fitness-related traits—including life history (Reznick & Endler 1982), behavior (Houde 1997; Huizinga et al. 2009), and male coloration (Endler 1980; Houde 1997). Low-predation ecotypes are at a disadvantage in high-predation environments because they exhibit limited antipredator behaviors (Huizinga et al. 2009) and males are more colorful and therefore conspicuous to predators (Endler 1980). Conversely, high-predation ecotypes are at a disadvantage in low-predation environments because they are less competitive at higher densities (Bassar et al. 2013) and males are less colorful and therefore less sexually attractive to females (Houde 1997).

These features of the Trinidadian guppy system have been used to test potentially risky augmentation scenarios. In a mesocosm experiment, Kronenberger et al. (2017) found that augmentation with highly genetically and adaptively divergent immigrants increased population fitness relative to no augmentation but were unable to distinguish between demographic and genetic rescue. Similarly, Fitzpatrick et al. (2016) found evidence for both demographic and genetic rescue from adaptively divergent immigrants in a field experiment but lacked controls to fully separate the 2 types. We expanded on these studies in a long-term mesocosm experiment that simulated a broad range of augmentation scenarios. We used different populations and included the controls necessary to parse demographic from genetic rescue. In doing so, we examined whether augmentation with divergent immigrants causes demographic or genetic rescue or both; the outcome of augmentation was influenced by attributes of the recipient population; and the outcome of augmentation was influenced by attributes of the donor population. Our aim was to provide generalizable insights into these questions to help reduce uncertainty among wildlife managers considering augmentation.

Methods

Experimental Design

Our mesocosm experiment was set up as a 2 × 5 factorial: 2 recipient populations and 5 augmentation treatments. Three randomized complete blocks of this design were established for a total of 30 experimental mesocosms. The first recipient population was from a low-predation tributary of the Quare River on the southern slope of the Northern Range Mountains in Trinidad, and the second was from a low-predation tributary of the Marianne River on the northern slope (Fig. 1; Supporting Information). Guppy populations from the southern and northern slopes coexist with different suites of predators (Reznick & Endler 1982; Magurran 2005) that originated from mainland Venezuela at different times and via different routes (Suk & Neff 2009; Willing et al. 2010; Baillie 2012) and are sometimes assigned different species designations (Schories et al. 2009). Our test of the effects of divergent immigrants was therefore replicated across two separate evolutionary lineages.

Augmentation treatments were as follows: no immigrants (none); immigrants from the same source as recipients (same); immigrants from a different low-predation tributary of the same river as recipients that were adaptively similar but predicted to be moderately genetically divergent at neutral loci (low-predation close [LPC]); immigrants from a low-predation tributary of a different river that were adaptively similar but predicted to be highly genetically divergent (low-predation far [LPF]); and immigrants from a high-predation reach of the mainstem river, just downstream from recipients, that were adaptively divergent but predicted to be genetically similar (high-predation close [HPC]). Donor populations were unique to each recipient population (Fig. 1). Because immigrants from the same source contribute few novel alleles, any benefit they provide should be due almost exclusively to demographic rescue. In contrast,

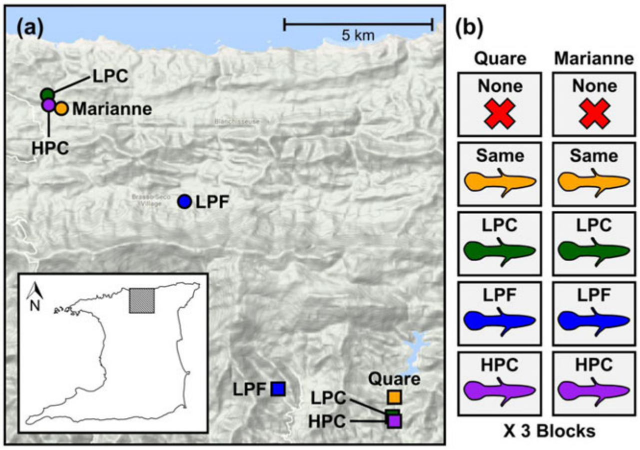

Figure 1. (a) Source locations of our experimental populations of Trinidadian guppies (circles, populations on the northern slope of Northern Range Mountains; squares, populations on the southern slope of the range) and (b) a diagram of our experimental design (none, no immigrants; same, immigrants from the recipient population source). Low-predation close (LPC) and high-predation close (HPC) donor populations are from the same drainages as Quare and Marianne recipient populations and low-predation far (LPF) donors are from different drainages. Both recipient populations are from low-predation environments. The LPC donors are adaptively similar but predicted to be moderately genetically divergent at neutral loci, LPF donors are adaptively similar and predicted to be highly genetically divergent, and HPC donors are adaptively divergent but predicted to be genetically similar.

divergent immigrants do contribute novel alleles, and any benefit they provide over immigrants from the same source should be due almost exclusively to genetic rescue.

Mesocosm Setup and Data Collection

Our 3 experimental blocks were established sequentially, 1–2 months apart, between February and July of 2014. Prior to the establishment of each block, guppies were caught in the wild as subadults, transported to Colorado State University with approval from Trinidad's Fisheries Division of the Ministry of Food Production, and held in sex-specific tanks that were checked for sexing errors daily and corrected to ensure females remained virgins as they reached maturity. Mesocosms consisted of 10-gallon tanks with gravel and vegetation to provide habitat enrichment and refuge for newborn fish. Each was stocked with 16 subadult or newly mature individuals from the Quare or Marianne recipient population at a 1:1 sex ratio, although in 8 of 30 mesocosms the sex ratios were male biased (1:3–1:1.29; average 1:1.72) due to errors in sexing younger subadult fish. Biases were evenly distributed across treatments and sex ratio was unimportant when included as a covariate in the abundance model described below ($F_{1,256} = 0.02, p = 0.897$) and was therefore not modeled. Food quantity, light cycle, and temperature were held constant throughout the experiment. After a 6-month equilibration period, one male and one female immigrant were introduced bimonthly up to month 18 (total of 14 immigrants per mesocosm). Estimates of survival and recruitment (population fitness) and genetic monitoring (individual fitness) continued up to month 24, as resources allowed, and abundance monitoring continued up to month 32.

Mesocosms were sampled bimonthly, during which all adults (≥14 mm) were anesthetized with tricaine methanesulfonate and new immigrants and recruits were given unique marks with visible implant elastomer (Northwest Marine Technology, Shaw Island, Washington). These methods are used widely on guppies with minimal mortality (e.g., Weese et al. 2010). The number of recaptures, recruits, and subadults were recorded, and 2–3 scales were collected from each adult for genotyping at 8 hypervariable microsatellite loci. The microsatellite library (Supporting Information), DNA extraction and polymerase chain reaction (PCR) protocols, and quality controls were identical to those used by Fitzpatrick et al. (2016). The PCR products were sent to Cornell Biotechnology Resource Center for fragment analysis, and fragments were scored with Geneious version 7.1.8 (Kearse et al. 2012). All work was approved by the Colorado State University Institutional Animal Care and Use Committee (number 15-6104A).

Genetic Divergence and Diversity

To confirm our predictions about the degree of neutral genetic divergence between recipient and donor populations and to quantify their genetic diversity, we used the genotypes of the wild-caught individuals to calculate mean pairwise F_{ST} values with Genepop version 4.2 (Rousset 2008), expected heterozygosity with Arlequin version 3.5.2.2 (Excoffier & Lischer 2010), and allelic richness with HP-Rare version 1.1 (Kalinowski 2005). Mean expected heterozygosity and allelic richness were also calculated for each mesocosm population at each bimonthly sampling period to monitor changes in genetic diversity over time. Finally, we calculated mean effective population sizes of the recipient populations with the linkage disequilibrium method provided in NeEstimator version 2.01 (Do et al. 2014) and all alleles.

Population Fitness

A strength of our mesocosm study was that all individuals were censused at each sampling session, eliminating the need to account for imperfect capture probability as in most mark-recapture studies. Our primary indicator of population fitness was abundance, defined as the total number of individuals (adults and subadults) at a given time point. To help interpret abundance patterns, we calculated bimonthly survival (proportion of adults surviving between sampling periods) and bimonthly recruitment (number new adults each sampling period).

Mean abundance, survival, and recruitment were estimated with repeated measures linear mixed models with PROC MIXED in SAS version 9.4 (SAS Institute, Cary, North Carolina) (Supporting Information). We assumed a Toeplitz covariance structure in which the covariance between sampling periods decreases exponentially with time. Fixed effects included the three-way interaction between recipient population (Quare or Marianne), treatment (none, same, LPC, LPF, or HPC), and sampling period, as well as all pairwise 2-way interactions and main effects. Fitting population, treatment, and sampling period together in a common interaction term reflected our underlying experimental design and the possibility that demographic parameters varied over time in different ways and in different augmentation treatments depending on the recipient population. The final fixed effect was baseline abundance, a covariate representing the abundance in each mesocosm just prior to the start of augmentation. Block was included as a random effect.

Individual Fitness

We used genotypes to reconstruct a pedigree for each mesocosm population with (I Colony2) (Jones & Wang 2010). We specified a polygamous mating system with inbreeding, a genotyping error rate of 0.005, and the full-likelihood analysis method with very high likelihood precision and very long runs that were repeated 5 times to maximize correct parentage assignment. Individuals were retained only if parents were correctly assigned at a probability of 0.7 or greater. Following pedigree reconstruction, individual fitness (defined as lifetime reproductive success) was estimated by summing parentage assignments. Individuals were then classified as either recipients, immigrants, or hybrids by visually following the family lines of known recipients and immigrants across generations. We pooled F_1 and later-generation hybrids for analysis because we lacked the power needed to estimate their fitnesses separately. However, in Quare populations—where the majority of hybrids were produced—F_1 and later-generation hybrids from divergent immigrant treatments had the same monthly reproductive success ($n = 114$, $S = 1296$, $p = 0.333$) per a Wilcoxon rank-sum test performed using PROC NPAR1WAY in SAS version 9.4. This, coupled with the fact that abundance in these treatments increased toward the end of the experiment (Fig. 5), suggests that later-generation hybrids did not experience outbreeding depression.

Mean individual fitness was estimated with a generalized linear mixed model using PROC GLIMMIX in SAS version 9.4 (Supporting Information). Individual fitness data were not normally distributed and were overdispersed ($\mu = 0.92$, $\sigma^2 = 4.5$) due to a high incidence of zeros. We therefore assumed a negative binomial distribution because it produced the lowest corrected Akaike information criterion value when compared with all available distributions and because it is appropriate for overdispersed count data (Ver Hoef & Boveng 2007). Fixed effects included the three-way interaction between population (Quare or Marianne), treatment (none, same, LPC, LPF, or HPC), and genetic ancestry (recipient, immigrant, or hybrid), as well as all pairwise two-way interactions and main effects. The three-way interaction reflects our underlying experimental design and the possibility that genetic ancestry classes had different fitness values in different augmentation treatments depending on the recipient population. We also included as fixed effects 2 covariates: abundance upon reaching adulthood (which accounts for density dependent effects on individual fitness) and sampling period upon reaching adulthood. Sampling period at adulthood was modeled as fixed, because sampling periods occurred at regular intervals (i.e., not randomly selected), and categorical to control for factors that changed nonlinearly over time, such as fluctuating disease pressure from *Mycobacterium* spp. that were likely introduced into our mesocosms from the wild (P. Schaffer, personal communication). It also controls for the fact that later recruits were younger when genotyping ended and therefore had less opportunity to produce offspring. Recruits captured at the end of genetic monitoring had zero chance of producing counted offspring and were not modeled. Mesocosm nested within block was included as a random effect.

Results

Genetic Divergence and Diversity

Mean pairwise F_{ST} values between recipient and donor population sources ranged from 0.08 to 0.25 (Table 1), which, for Trinidadian guppies, indicates low to relatively high genetic divergence at neutral loci (for comparison, see Suk & Neff 2009 and Baillie 2012). Genetic divergence was higher between Quare than Marianne populations in all cases. Contrary to our expectations, the Quare LPC donor population was the most divergent and the Marianne LPC and HPC donors were similarly divergent.

Genetic diversity of recipient population sources was lower in Quare than Marianne, with mean expected heterozygosities of 0.71 versus 0.87, allelic richnesses of 9.03 versus 13.24, and effective population sizes of 247 (95% CI, 144–552) versus 949 (95% CI, 504–4437), respectively

Table 1. Mean pairwise F_{ST} values between recipient and donor populations, expected heterozygosity (H_E), and allelic richness (A_R) estimated with 8 microsatellite loci from wild-caught Trinidadian guppies.

Recipient and donor populations[a]	F_{ST} *(SE)*	H_E *(SE)*	A_R *(SE)*
Quare	–	0.71(0.092)	9.03(1.41)
LPC	0.25(0.08)	0.67(0.054)	6.44(0.77)
LPF	0.19(0.05)	0.78(0.079)	14.01(1.82)
HPC	0.17(0.08)	0.89(0.027)	18.20(1.56)
Marianne	–	0.87(0.047)	13.24(2.89)
LPC	0.08(0.04)	0.79(0.082)	12.41(2.56)
LPF	0.13(0.05)	0.76(0.088)	9.94(1.89)
HPC	0.08(0.04)	0.80(0.095)	16.92(3.02)

[a] *Recipient populations, n = 282; Marianne LPF, n = 41; donor populations, n = 42. Abbreviations: LPC, low-predation genetically close; LPF, low-predation genetically dissimilar ; HPC, high-predation genetically close.*

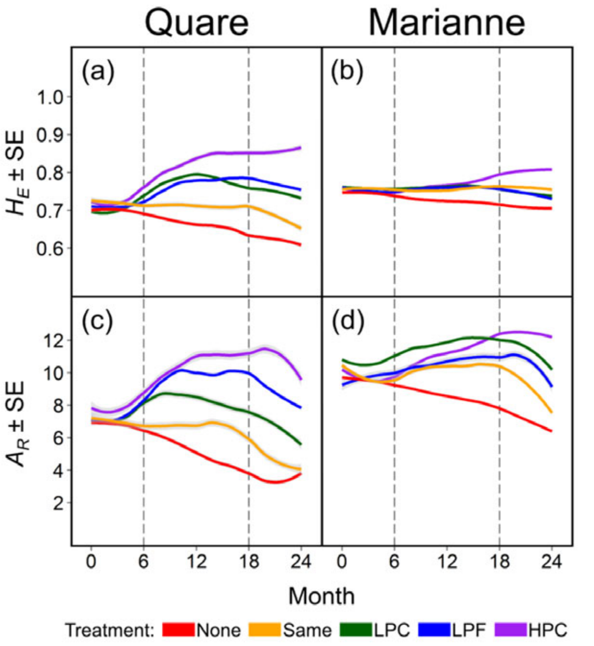

Figure 2. Means of (a, b) expected heterozygosity (H_E) and (c, d) allelic richness (A_R) over time for all augmentation treatments in Quare and Marianne populations of Trinidadian guppies fit with a loess smoothing function (dashed lines, start and end of augmentation. Each solid line represents 3 mesocosms, 1 from each experimental block, except for the Quare none treatment at month 24, which represents 1 mesocosm due to extinctions.

(Table 1). Following augmentation, genetic diversity metrics in the Quare divergent immigrant treatments increased in proportion to those of the donor populations and were highest in the HPC treatment, followed by LPF and LPC (Fig. 2). In the same and none treatments, genetic diversity metrics generally declined over time. Marianne treatments showed little differences in heterozygosity, but allelic richness increased slightly in treatments that were augmented and was highest in the HPC treatment, followed by LPC, LPF, and same. In the none treatment, allelic richness declined over time.

Population Fitness

Abundance patterns revealed clear effects of immigrants from each donor population on recipient population fitness. In Quare populations, divergent immigrants increased abundance more than immigrants from the same source or no immigrants at all (Fig. 3). Specifically, LPC, LPF, and HPC treatments followed a cycle of growth and decline, reaching maximum abundance several months after augmentation began and again toward the end of the experiment. In contrast, the none and same treatments did not exhibit the same initial growth period and abundance slowly declined over time. Our repeated-measures model, which controls for baseline abundance, showed no significant difference ($p > 0.05$) in estimated mean abundance between the none and same treatments (Fig. 4). However, for the none treatment, estimated mean abundance was greater in LPC ($t_{282} = 2.23$, $p = 0.027$) and HPC ($t_{282} = 2.78$, $p = 0.006$) and nearly so in LPF ($t_{282} = 1.85$, $p = 0.066$). Likewise, for the same treatment, estimated mean abundance was greater in LPC ($t_{282} = 2.03, p = 0.043$) and HPC ($t_{282} = 2.59, p = 0.010$) but was less pronounced in LPF ($t_{282} = 1.66, p = 0.097$).

Marianne populations exhibited more variable patterns of abundance, the most obvious being rapid growth in LPF and HPC treatments that began prior to augmentation (Fig. 3). This growth was unexpected and primarily driven by unusually rapid reproduction in only one replicate mesocosm from each treatment (Supporting Information). These populations peaked shortly after augmentation began and ultimately declined in the LPF treatment or declined before once again increasing in the HPC treatment. None, same, and LPC treatments followed a more gradual cycle of growth and decline. Estimated mean abundance did not differ significantly, although it was nearly greater in the LPC treatment than in LPF ($t_{282} = 1.74$, $p = 0.083$; Fig. 4). Finally, despite similar overall abundance in Quare and Marianne populations, survival was lower ($t_{197} = 4.99$, $p < 0.001$) and recruitment higher ($t_{197} = -4.33$, $p < 0.001$) in Quare than in Marianne (Fig. 4). Survival and recruitment was similar among treatments within populations.

Individual Fitness

Guppies from Quare populations had higher reproductive rates than those from Marianne, producing more offspring (436 vs. 325) and generations (range 2–6 average 3.7 vs. range 1–4, average 2.5 average) over the 24 months of genetic monitoring. This resulted in the production of many more hybrid individuals in Quare than in

Marianne populations (137 vs. 13). In Quare populations, individual fitness of the three genetic ancestry groups was similar in the same treatment, but otherwise immigrants had the highest fitness, followed by hybrids, and then recipients (Fig. 5). Immigrants had higher fitness than recipients in the LPC ($t_{1435} = 3.49, p = 0.001$), LPF ($t_{1435} = 5.21, p < 0.001$), and HPC ($t_{1435} = 5.30, p < 0.001$) treatments. Hybrids had higher fitness than recipients in the LPF treatment ($t_{1435} = 2.32, p = 0.020$) and the trend was nearly significant in LPC ($t_{1435} = 1.76, p = 0.079$), but not in HPC. Immigrants had higher fitness than hybrids in the HPC treatment ($t_{1435} = -2.27, p = 0.024$), and the trend was nearly significant in LPF ($t_{1435} = -1.72, p = 0.086$), but not in LPC. In Marianne populations, there was little power to resolve pairwise comparisons of hybrid fitness, and recipients and immigrants had similar fitness in all treatments.

Discussion

Augmentation with Divergent Immigrants and Demographic Versus Genetic Rescue

We found that immigrants can rescue small populations even when moderately or highly genetically divergent or adaptively divergent. All divergent immigrant treatments increased the fitness of Quare mesocosm populations, and although results were mixed for Marianne populations, divergent immigrants did not have an overall negative effect (but see the discussion of LPF immigrants below). Populations that received immigrants from the same source did not fare better than those without augmentation. In contrast, recipient populations, especially those from Quare, that received divergent immigrants tended to have higher genetic diversity (Fig. 2), abundance (Figs. 3 & 4), and hybrid fitness (Fig. 5) than controls with immigrants from the same source. Together, these results indicate demographic inputs did not strongly affect abundance and that increases in population fitness were primarily due to genetic rescue.

Few researchers have been able to isolate the relative contribution of demography versus genetics in rescue. In the most thorough study to date, Hufbauer et al. (2015) found genetic, but not demographic, rescue in small populations of flour beetles, arguing that high demographic stochasticity hindered the ability of immigrants to buffer against declines in abundance. We believe a similar situation occurred in our study, given considerable within-treatment variation in abundance among individual mesocosms (Supporting Information). This variation was probably caused by small differences in birth and death rates that compounded over time, eventually resulting in the extinction of 6 out of 30 populations. Three of these extinctions occurred in the none treatment and one in the same treatment. However, 2 more from the same treatment were nearly extinct, suggesting that demographic inputs did not prevent high extinction risk. It is difficult to pinpoint the specific factors leading some populations to extinction and others to growth, and more research is needed to better understand demographic stochasticity and to mitigate its impact on small populations.

In Quare populations, divergent immigrants caused genetic rescue by producing hybrids with increased

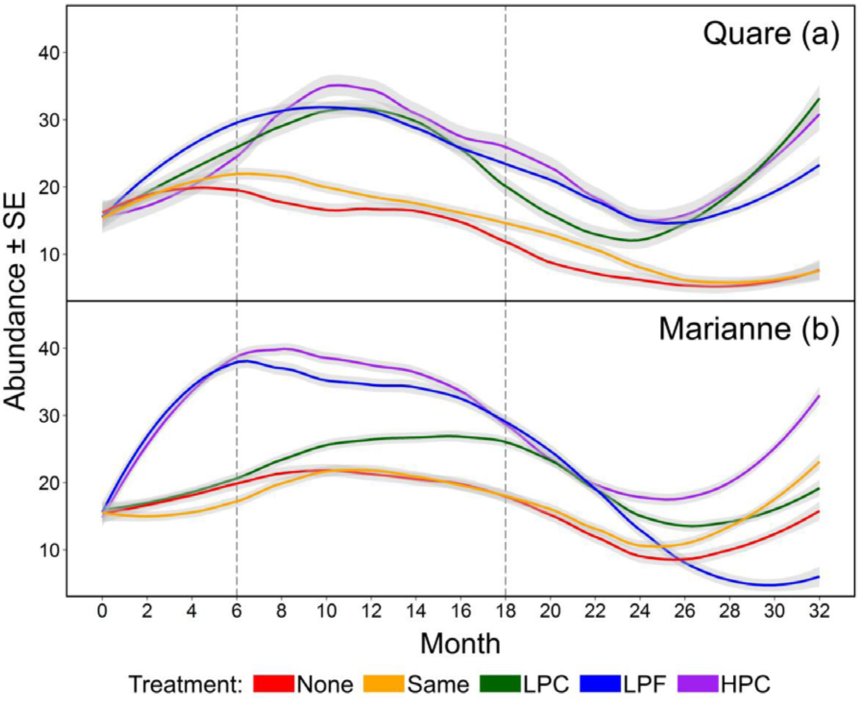

Figure 3. Mean abundance (SE) over time for all augmentation treatments in (a) Quare and (b) Marianne populations of Trinidadian guppies fit with a loess smoothing function (vertical dashed lines, start and end of augmentation). Each solid line represents 3 mesocosms, 1 from each experimental block.

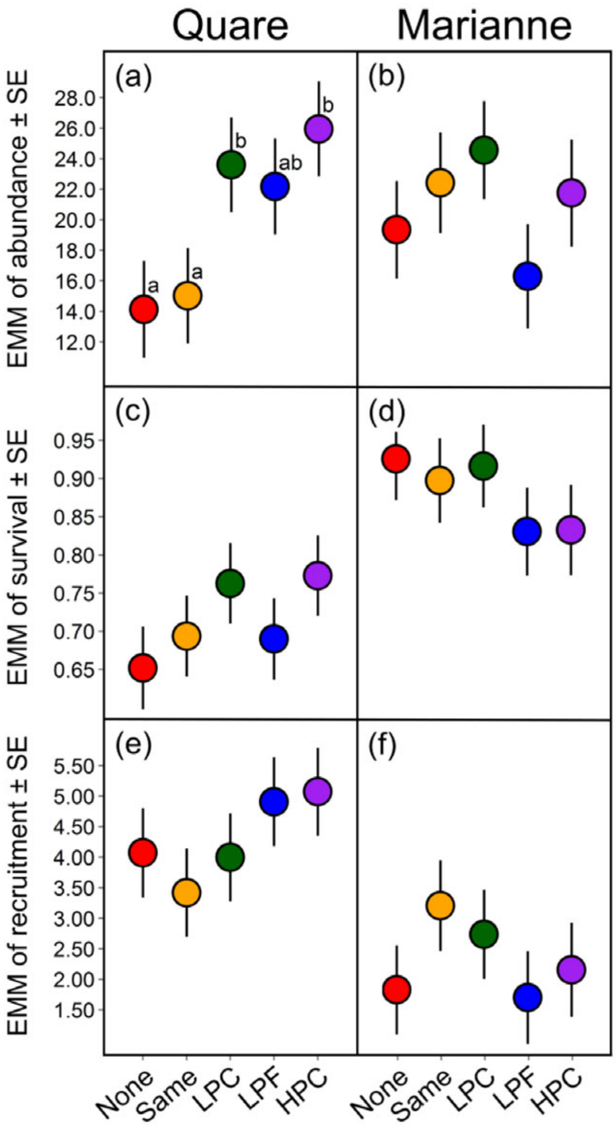

Figure 4. Estimated marginal mean (adjusted for other effects in the model) of (a, b) abundance, (c, d) survival, and (e, f) recruitment for all augmentation treatments (x-axis) in Quare and Marianne populations of Trinidadian guppies. Each point represents 3 mesocosms, 1 from each experimental block. Treatments are different at the p = *0.05 level for Quare abundance only, as denoted by different letters.*

fitness relative to recipients (a trend significant in the LPF treatment [Fig. 5]), but hybrid fitness was still lower than that of divergent immigrants (significant in the HPC treatment). Intermediate hybrid fitness could have arisen through a variety of mechanisms, for example, if fixed deleterious recessive alleles were masked by immigrant alleles with incomplete dominance. However, this does not explain why immigrants were more fit than recipients despite having comparable genetic diversity (Table 1). One plausible mechanism relates to certain attributes of guppy mating behavior. Male Trinidadian guppies have highly polymorphic coloration and females are known to prefer males with novel coloration (Houde 1997), potentially increasing the fitness of divergent immigrant males. Furthermore, subset of males in our mesocosm populations was assayed for sexual displays and forced copulations (well-documented metrics of reproductive effort in guppies [Houde 1997]) and divergent immigrants performed more of these behaviors than recipients in Quare, but not Marianne, populations (E. Mensch, personal communication). However, divergent immigrant fitness was elevated for both sexes, and the reason for high immigrant female fitness is unclear. Whatever the mechanism, high divergent immigrant fitness in Quare populations clearly increased genetic admixture and thereby accelerated the pace of genetic rescue.

Influence of Recipient Population on Augmentation Outcome

Quare populations started out with lower genetic diversity (Table 1 & Fig. 2) and, in line with theoretical expectations (Allendorf et al. 2013; Frankham et al. 2017), were the populations that experienced genetic rescue. We did not observe genetic rescue in Marianne populations, possibly due to relatively high levels of starting genetic diversity and low reproductive rates that resulted in few hybrid individuals. Had there been greater admixture, augmentation may have resulted in genetic rescue in these populations, but the fact that survival was high (Fig. 4) suggests they were well-adapted to the mesocosm environment to begin with and likely had little to gain from the addition of new alleles.

Differences in the effects of augmentation between Quare and Marianne populations emphasize the importance of considering recipient life history when carrying out and evaluating augmentations. Quare populations not only had lower survival than Marianne, but also had higher recruitment (Fig. 4). The evolution of fast life history (i.e., early maturity and high fecundity) has been well documented in Trinidadian guppies in response to high mortality levels caused by predation (Reznick & Endler 1982; Magurran 2005), disease, and floods (Fitzpatrick et al. 2014). It is unclear whether the differences in reproductive rates we observed were immediate responses to different levels of mortality or evolved strategies in their source populations, but they had consequences for the outcome of augmentation, either magnifying (in Quare populations) or dampening (in Marianne populations) the genetic contribution of immigrants.

Influence of Donor Population Attributes on Augmentation Outcome

We found few differences in population fitness among divergent immigrant treatments. Genetically and adaptively

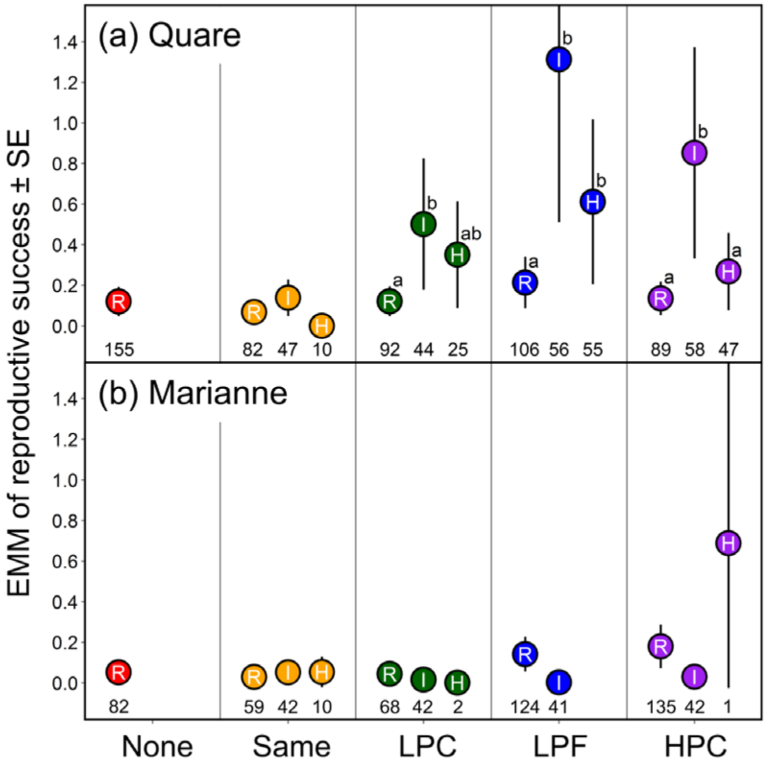

Figure 5. Estimated marginal means adjusted for other effects in the model of lifetime reproductive success of all genetic ancestry groups and augmentation treatments in (a) Quare and (b) Marianne populations of Trinidadian guppies (R, recipient genotypes; I, immigrants; H, hybrids; numbers below points, sample size of potential parents). Because same immigrants are from the same source as recipients, hybrids in this case is not literal. No hybrids were produced in the Marianne LPF treatment. Groups are different at the p = *0.05 level for Quare LPC, LPF, and HPC treatments only, as denoted with different letters.*

divergent immigrants produced similar outcomes, as did immigrants with different levels of genetic divergence. One exception was the moderately genetically divergent Marianne LPF treatment, which declined rapidly toward the end of the experiment (Fig. 3) and had the lowest estimated mean abundance (Fig. 4). This could have been due to a unique feature of this specific donor population (e.g., harboring high levels of deleterious alleles or a harmful pathogen) or it may suggest that genetically divergent immigrants pose a greater threat than adaptively divergent, but neutrally genetically similar, immigrants. However, we do not think the decline provides evidence for outbreeding depression because no hybrids were observed by the end of genetic monitoring (Fig. 5). This could be due to a lack of hybrid survival, but it is more likely that no hybrids were produced given the slow life history traits of Marianne recipients (for comparison, we found only one hybrid in Marianne LPC, 2 in HPC, and 10 in same). The Quare LPF treatment also had the lowest estimated mean abundance of the 3 divergent immigrant treatments, but in this case many hybrids were produced and they had higher fitness than recipients (Fig. 5), indicating that outbreeding depression did not occur.

Relative to divergence, the influence of donor population genetic diversity on recipient populations was clear: as long as donors were somewhat divergent (not from the same source as recipients), those with greater genetic diversity increased recipient genetic diversity more following augmentation. For example, both HPC donor populations had the highest genetic diversity (Table 1), and HPC treatments maintained the highest genetic diversity over time (Fig. 2). Conversely, Quare LPC and Marianne LPF donors had the lowest genetic diversity, and so too did their respective treatments. High diversity treatments also tended to have the highest abundances, emphasizing the importance of considering genetic diversity in addition to divergence when evaluating the risks and benefits of potential donors.

Recommendations

Augmentation of isolated populations can be a daunting task, particularly when the only available donor populations may pose risks of outbreeding depression. Our results are consistent with those of others (Fitzpatrick et al. 2016; Kronenberger et al. 2017) in that rescue, not outbreeding depression, occurred even when immigrants were highly divergent and add to current understanding because we tested several alternative augmentation scenarios across two separate evolutionary lineages in a controlled experiment. Our results also suggest demographic rescue alone is not enough to limit extinction risk in small and highly stochastic populations. However, if recipient genetic diversity is low and augmentation results in admixture, genetic rescue can have a highly positive impact on population fitness.

We uncovered clear and positive effects of divergent immigrants on one population (Quare), but effects on the second population (Marianne) were less clear due to demographic stochasticity and limited genetic admixture. These pitfalls could be avoided by including ample replication, especially when populations are small, and a duration of genetic monitoring sufficient to reliably estimate the fitness of later-generation hybrids. Additional testing of more extreme augmentation scenarios in different contexts and across diverse taxonomic groups is needed to increase the applicability of donor-selection guidelines. However, defining acceptable levels of divergence that are both broadly applicable and precise is unlikely because F_{ST} and similar statistics vary widely among taxa (Leinonen et al. 2008) and are influenced by genetic diversity and the molecular markers chosen (Whitlock 2011). It is therefore important that wildlife managers consider what level of divergence is acceptable on a case-by-case basis. In our study, for comparison, the most genetically divergent donor populations were in the middle to high end of the F_{ST} range for our study species, and adaptively divergent donors had experienced strong, often opposing, selection pressures for many generations. For a given level of divergence, we recommend managers choose donors with the highest genetic diversity possible and traits that facilitate genetic admixture, whether related to life history, behavior, or some other aspect of the target species' biology. Higher levels of divergence may present a greater risk of outbreeding depression, but our results show that forgoing augmentation or relying on demographic inputs alone (i.e., playing it safe) can sometimes be an even riskier option.

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Supporting Information

Geographic coordinates for each of our source populations (Appendix S1), details of our microsatellite library (Appendix S2), tests of fixed effects for population fitness (Appendix S3) and individual fitness (Appendix S4) models, and abundance trajectories of individual mesocosms (Appendix S5) are available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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