THESIS

EXPERIMENTAL TESTS OF RISKY AUGMENTATION SCENARIOS USING TRINIDADIAN GUPPIES

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ABSTRACT

EXPERIMENTAL TESTS OF RISKY AUGMENTATION SCENARIOS USING TRINIDADIAN GUPPIES

Increased isolation of populations, and the subsequent reduction in genetic diversity, can exacerbate global biodiversity loss by contributing to inbreeding depression and reducing the ability of organisms to adapt to rapid environmental change. This has prompted some conservation biologists to consider augmenting isolated populations with immigrants as a means of demographic and genetic rescue. Augmentations are typically highly successful, but they are also controversial due to the risk of outbreeding depression or the introduction of maladapted alleles when immigrants are genetically or adaptively divergent. For my Master's thesis, I tested risky augmentation scenarios using mesocosm populations of Trinidadian guppies (*Poecilia reticulata*) in two separate controlled experiments. In my first experiment (Chapter 1), I augmented mesocosm populations derived from a single recipient source with genetically or adaptively divergent immigrants to assess their short-term demographic effects. Mesocosms that were augmented maintained greater abundance and recruitment than controls that were not. There was also a trend for populations to receive a greater benefit from immigrants that were genetically divergent than those that were adaptively divergent. I expanded upon these results in my second experiment (Chapter 2), in which I augmented mesocosm populations from two different recipient sources with immigrants spanning a greater range of divergence and monitored them over a longer time frame, including an additional control and genetic monitoring to determine the relative impact of demography and genetics. Despite no evidence for demographic rescue, I found genetic rescue in one recipient population. Divergent immigrants did not have a negative effect in almost all cases, and any positive effect they had depended on the genetic diversity, immigrant fitness, and recipient life-history traits.

Together, these experiments provide strong evidence that immigrants can bolster population fitness despite being divergent, thereby supporting the use of augmentation as a management technique in dire situations when no safe immigrant sources are available.

ACKNOWLEDGMENTS

I have received a tremendous amount of support from many people throughout the course of my thesis. Most of all I would like to thank my advisors, Lisa Angeloni and Chris Funk, for helping me weather the trials of graduate school by providing just the right mix of hands-off and hands-on advising, a wealth of experience and scientific knowledge, and a rich academic community. Weekly meetings between the Angeloni lab and that of Cameron Ghalambor, and between the Funk lab and that of Kim Hoke, have vastly improved my writing and presentations and I am indebted to all lab members for their insight. I feel truly fortunate to have found myself in the company of such a talented and ambitious group of mentors and friends.

Much of my success in graduate school was thanks to a National Science Foundation grant (DEB-1146489) awarded to Chris Funk and Lisa Angeloni, and prepared in part by Sarah Fitzpatrick, to study how gene flow affects local adaptation and population dynamics. This grant not only provided me with funding, but also laid out the theoretical basis and informed the design of my two thesis chapters. When I arrived at Colorado State University to begin my degree program, the experiment that would become my first chapter had already been completed and sent out for peer-review by Chris Funk, Lisa Angeloni, Sara Fitzpatrick, Dale Broder, Emily Ruell, and Jedidiah Smith. However, the first attempt at publication was unsuccessful and I was invited by Chris Funk to take over the redrafting process. I overhauled the manuscript and rewrote large sections under a new framework, getting it published and selected as a “featured paper” in Animal Conservation[1], complete with commentaries from three prominent researchers in the field of conservation genetics (Catherine Grueber[2], Scott Mills[3], and David Tallmon[4]) and a response article[5]. This experiment was initially designed as a pilot study for what would become my second chapter, but ended up having scientific merit in its own right due the hard work and perseverance of my coauthors. I am incredibly grateful to them for giving me the opportunity to make it my own.

Throughout the development, implementation, and analysis of my second chapter I was helped immensely by a number of dedicated undergraduates. I would particularly like to thank Jill Gerberich for aiding in collection of guppies in Trinidad on two separate occasions and assisting with guppy processing and the majority of genotyping. Thanks also go to the following: Austin Broberg for his help with collecting in Trinidad, processing, and pre-augmentation behavioral analyses; Emily Mensch for guppy husbandry, help with processing, and post-augmentation behavioral analyses; Sam Coisman for help with processing and color analysis; Heather Schneider and Shelby Walters for husbandry and color analysis; and Morgan Wade and Patrick Katona for husbandry.

Finally, I would like to thank my Trinidadian hosts, Jogi and Mahase Ramlal and Ronni Hernandez for making this all possible. I have spent some of the most amazing moments of my life working with guppies in the field, both before and during my thesis research. The sights, sounds, smells, feels, and tastes of Trinidad's tropical rainforests and mountain streams are forever etched in my mind and serve as a constant reminder of what I am fighting for.

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[1]Kronenberger JA, Funk WC, Smith JW, Fitzpatrick SW, Angeloni LM, Broder ED, and Ruell EW (2017). Testing the demographic effects of divergent immigrants on small populations of Trinidadian guppies. *Animal Conservation* **20**, 3–11.

[2]Grueber CE. Making the best of a bad situation: genetic rescue in the absence of an ideal source population. *Animal Conservation* **20**, 14–15.

[3]Mills LS. Some matchmaking advice when translocated immigrants are a population's last hope. *Animal Conservation* **20**, 12–13.

[4]Tallmon DA (2017). Get a move on: the value of rescue. *Animal Conservation* **20**, 16–17.

[5]Kronenberger JA, Fitzpatrick SW, Angeloni LM, Broder ED, Ruell EW, Funk WC (2017). Playing God with guppies – informing tough conservation decisions using a model experimental system. *Animal Conservation* **20**, 18–19.

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CHAPTER 1: TESTING THE DEMOGRAPHIC EFFECTS OF DIVERGENT IMMIGRANTS ON SMALL POPULATIONS OF TRINIDADIAN GUPPIES[1]

Summary

Augmenting small and isolated populations with immigrants from elsewhere is a potentially powerful, yet controversial management tool. The goal of this approach is to increase population sizes via demographic and/or genetic rescue, but augmentation can also have the unintended consequence of breaking down local adaptation and reducing population fitness through outbreeding depression. In theory, outbreeding depression is more likely the more divergent immigrants are from the recipient population. Managers should therefore choose immigrant populations that are as adaptively and genetically similar as possible. However, for species of conservation concern, divergent source populations are often the only option. A crucial question that remains in applied conservation is whether the positive effects of augmentation with divergent immigrants will outweigh the risks of outbreeding depression. Here, we evaluate the demographic effects of augmenting small, inbred laboratory populations of Trinidadian guppies with two different types of immigrants: (1) adaptively divergent but genetically similar or (2) adaptively similar but genetically divergent, and compare them against the demography of control populations with no immigration. After 1–2 generations, we found that adult abundance remained constant or slightly declined over the duration of the experiment in the control populations. In contrast, adult recruitment and total abundance increased in augmented populations. Furthermore, treatments that received immigrants from the adaptively similar but genetically divergent population attained overall larger population sizes than those that received immigrants from the adaptively divergent but genetically similar population.

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[1]Kronenberger JA, Funk WC, Smith JW, Fitzpatrick SW, Angeloni LM, Broder ED, and Ruell EW (2017). Testing the demographic effects of divergent immigrants on small populations of Trinidadian guppies. *Animal Conservation* **20**, 3–11.

Although our experimental design could not parse out the effects of demographic and genetic rescue, our results do suggest that augmentation can be better than no action, even in situations where only divergent immigrant sources are available.

Introduction

One of the most controversial issues in conservation biology is whether small populations should be supplemented with immigrants from larger populations as a means of increasing population fitness (Tallmon, Luikart & Waples *et al.*, 2004; Hedrick, 1995; Mills & Allendorf, 1996; Ingvarsson, 2001; Frankham *et al.*, 2011; Weeks *et al.*, 2011; Whiteley et al., 2015), a strategy known as augmentation. The controversy stems from uncertainty about whether this management practice will have the desired effect, as well as historical reluctance to actively move individuals from one population to another out of fear of "diluting" local gene pools (Storfer, 1999; Tallmon *et al.*, 2004; Frankham, 2010). According to theory, augmenting populations could have both positive and negative effects on population fitness due to demographic and/or genetic factors. From a demographic perspective, augmentation can increase population fitness by adding more individuals to the population (analogous to births), which decreases demographic stochasticity in a process termed "demographic rescue" (Brown & Kodric-Brown, 1977). However, augmentation can also unintentionally introduce disease, potentially decreasing population fitness (Daszak, Cunningham & Hyatt, 2000). From a genetic perspective, augmentation often results in gene flow that can alleviate inbreeding depression and increase adaptive genetic variation in small populations, thereby increasing population fitness via "genetic rescue" (Thrall *et al.*, 1998; Tallmon *et al.*, 2004). Alternatively, if groups of immigrant and recipient genes are no longer compatible with one another, augmentation can break apart co-adapted gene complexes, reducing population fitness (Mayr, 1963; Edmands, 1999; Storfer, 1999; Lenormand, 2002; Edmands & Timmerman, 2003; Edmands, 2007; Frankham *et al.*, 2011). This uncertainty about the potential outcome of augmenting small

populations is a conservation conundrum and puts wildlife managers in a difficult situation. In many cases, managers choose not to augment out of concerns about the potential negative effects (Frankham, 2010; Frankham *et al.*, 2011).

If managers plan on augmenting a small population, they should ideally use individuals from a similar population to minimize the risk of outbreeding depression. However, for many species of conservation concern, a similar population may not exist because declining populations are often isolated from the rest of the species range and are locally adapted to different environments (e.g., Westemeier *et al.*, 1998; Hogg *et al.*, 2006; Johnson *et al.*, 2010). Theory predicts that outbreeding depression is more likely as adaptive divergence between immigrants and the recipient population increases (Storfer, 1999; Edmands & Timmerman, 2003; Frankham *et al.*, 2011) and as the length of time since last gene flow increases (Bateson, 1909; Dobzhansky, 1937; Muller, 1940; Lynch, 1991; Edmands, 1999, 2002; Edmands & Timmerman, 2003). Augmentation with adaptively and genetically similar immigrants is beneficial in the vast majority of cases (Frankham et al., 2015; Whiteley et al., 2015). However, augmentation with divergent immigrants has received relatively little attention in the literature, and the handful of studies that have explored this issue have yielded mixed results. For example, some studies have found negative effects of augmentation with adaptively divergent (Lacy, 1998; Edmands, 1999, 2002; Fenster & Galloway, 2000; Lee, 2000) or genetically divergent individuals (Hwang *et al.*, 2011; Pekkala *et al.*, 2012). Other studies, however, have found positive effects of augmentation with phenotypically divergent individuals (Willi *et al.*, 2007), individuals from moderately different environments (Johnson *et al.*, 2010), or individuals from populations that are divergent at neutral loci (Hogg *et al.*, 2006; Bossuyt, 2007; Willi *et al.*, 2007; Tortajada, Carmona & Serra, 2010). Thus, two critically important and unresolved questions are: (1) Does adding divergent immigrants increase or decrease the fitness of small populations? (2) If only divergent immigrant sources are available, is it better to choose

populations that are adaptively divergent but genetically similar or adaptively similar but genetically divergent?

Trinidadian guppies (*Poecilia reticulata*) are small, live-bearing freshwater fish, and they are ideal for addressing these questions for several reasons. First, adaptive divergence between guppy populations in “low predation” (LP) and “high predation” (HP) environments is well characterized from decades of evolutionary research in this system (Haskins *et al.*, 1961; Seghers, 1974; Reznick & Endler, 1982; Magurran, 2005), and can thus be treated as a predictor variable in factorial experiments. HP guppies are found in large, low-elevation streams with diverse communities of piscivorous fish, including a major guppy predator, the pike cichlid (*Crenicichla* spp.; Reznick & Endler, 1982; Magurran, 2005). In contrast, LP guppies inhabit small, high-elevation headwater streams and coexist only with a killifish (*Rivulus hartii*), a gape-limited predator of juvenile and small-sized guppies. LP and HP guppies differ predictably across a suite of life history (Reznick, 1982, Reznick & Endler, 1982), morphological (Haskins *et al.*, 1961), color (Endler, 1983), and behavioral (Seghers, 1974; Houde & Endler, 1990) traits, which increase individual fitness in the respective environments and strongly suggest a repeated pattern of local adaptation. Second, LP populations are typically highly isolated (with low genetic variation and a high degree of inbreeding) and upstream immigration is rare due to the presence of downstream barrier waterfalls (Barson, Cable & van Oosterhout, 2009; Suk & Neff, 2009; Willing *et al.*, 2010). This makes LP guppies an excellent model for small populations of conservation concern (van Oosterhout *et al.*, 2007). Lastly, guppies have short generation times (3–4 generations/year; Magurran, 2005) and can be reared in the laboratory (Reznick & Endler, 1982; Torres-Dowdall *et al.*, 2012), making it possible to rapidly test the effects of population augmentation across generations.

We used a replicated factorial experiment in the laboratory to test whether augmenting small, inbred guppy populations with immigrants from divergent sources had a positive or negative effect on population fitness relative to no augmentation. Specifically, if augmentation

has a net positive effect, we would expect abundance, recruitment, and survival to increase more in populations with immigrants than in those without immigrants. But, if augmentation has a net negative effect, abundance, recruitment, and survival should decrease in populations that received immigrants relative to those that did not. To gain further insight into the effects of different *types* of divergent immigrants, we compared the demographic impacts of augmentation with immigrants from an adaptively divergent but genetically similar source to those of immigrants from an adaptively similar but genetically divergent source. Although our experiment was not designed to differentiate demographic from genetic rescue, our results shed light more generally on how divergent immigrant sources can differ in their ability to increase population fitness.

Methods

Experimental mesocosms

To address our question about the effects of divergent immigrants on small populations, we used replicate 10-gallon glass aquaria, or mesocosms, in the laboratory to simulate small LP populations in upstream pools. Each mesocosm was seeded with 10 LP fish from the Aripo LP site: 1 wild-caught, 3 F_1, and 1 F_2 lab-bred individuals per sex. Generations were mixed in this way so that we could use all available LP fish and maximize the number of mesocosm replicates. Females were impregnated by randomly-selected males from their population upon introduction. Although we were not able to estimate individual inbreeding coefficients or provide direct evidence for inbreeding depression in the recipient population, it did have low observed heterozygosity relative to HP streams (H_o = 0.58 vs. 0.77, respectively; Baillie, 2012), a trend consistently observed in LP compared to HP guppy populations (Barson et al., 2009; Suk & Neff, 2009; Willing et al., 2010). Aripo LP mesocosm populations received one of 3 treatments: (1) HP immigrants from a population in the same drainage, Aripo HP; (2) LP immigrants from a distant population on the opposite side of the Northern Range of Trinidad, Marianne LP; or (3)

no immigration as a control. All immigrants were the F_1 offspring of wild-caught individuals, captured together at the same site per population. Importantly, the Aripo HP immigrants were adaptively divergent from the recipient Aripo LP populations (coming from a typical high-predation source) but were only moderately genetically divergent at 10 neutral microsatellite loci (F_{ST} = 0.12; Baillie, 2012), indicating relatively recent divergence and/or recent gene flow between LP and HP Aripo populations. Conversely, the Marianne LP immigrants were adaptively similar to the recipient Aripo LP populations, but considerably more genetically divergent at 10 microsatellite loci (F_{ST} = 0.35; Baillie, 2012), indicating longstanding isolation between these populations. We included 3 replicates per treatment according to a randomized controlled block design for a total of 9 experimental mesocosm populations.

Full spectrum fluorescent bulbs were hung at a constant distance (30 cm) from each mesocosm. Three equally sized pieces of floating aquarium grass were kept in each tank to provide shelter for juvenile guppies. Fish in each tank were fed twice daily (AM: 165 µl Tetramin™ tropical fish flake paste; PM: 250 µl hatched *Artemia* cysts). Food quantity, temperature (25 ± 1°C), and light cycle (12 hours light and 12 hours dark) were held const ant throughout the experiment. Sponge filter and partial water changes occurred once every 2 weeks, at which point tank surfaces were scrubbed of algae.

Population censuses

During the course of the study, we conducted monthly censuses for 6 months (7 total; one at the beginning of the study and each month thereafter). At each census, all adult fish (defined as having a standard length of 14 mm or longer) were anesthetized with a dilute solution of MS-222 and marked with visible implant elastomer (VIE; Northwest Marine Technology, Inc.) to label new recruits. Previous capture-mark-recapture studies on guppies demonstrate high mark retention and low marking mortality using these methods (Reznick *et al.*, 1996). Individuals smaller than 14 mm were not processed but were counted in the total number of sub-adult fish.

After processing, individuals were placed in an aerated recovery tank and then returned to their experimental tank once they revived. We were able to conduct complete censuses of all individuals within each mesocosm.

Immediately after the 3rd monthly census (2 months after the beginning of the study), we began adding one adult female and one adult male immigrant per month to the immigration treatment mesocosms from the appropriate source population. Guppies have a gestation of approximately one month and reach sexual maturity at approximately 2 months of age. Thus offspring resulting from matings between resident guppies and the first immigrants were most likely born by the 4th monthly census, sexually mature by the 6th monthly census, and could have produced offspring by the final census. Immigrant females were sexually mature and pregnant upon addition to experimental mesocosms. Therefore, the demographic contribution of immigrant females included offspring in utero in addition to themselves. Despite this, we were still able to resolve treatment effects because our aim was to assess the overall effect of divergent immigrants on population fitness regardless of immigration rate.

Statistical analyses

We calculated total abundance, adult abundance, adult recruitment, and adult survival for each mesocosm each month. Total abundance was measured as the total number of individuals (adults + sub-adults) in the tank at the time of each census, adult abundance was the number of adults at each census, adult recruitment was the number of new adults, and adult survival was the proportion of adults that had survived since the previous census. To remove the direct demographic contribution of immigrants we also calculated total and adult abundance for each mesocosm with immigrants excluded from the analysis.

For each of the above population metrics (total abundance, adult abundance, adult survival, and adult recruitment), we fit Bayesian univariate generalized linear mixed models (GLMM) with the MCMCglmm function in the MCMCglmm R package (Hadfield, 2010) using R

version 3.0.2 (R Development Core Team, 2011). These additive random effects models can be thought of as the Bayesian equivalent of repeated measures models and therefore incorporate data from all months. Immigration treatment was modeled as a fixed effect and month nested within mesocosm and block were treated as random effects. Models were run for 300,000 iterations with a burn-in of 150,000 and a thinning interval of 200 with an inverse-Wishart proper prior (Hadfield, 2010). We estimated the means of controls, the effects of each immigration treatment, and their 95% credible intervals (CI) using Markov chain Monte Carlo (MCMC) sampling of their posterior distributions, conditioned on the random effects. Effects were considered significant if the 95% CI did not overlap zero and the estimated pMCMC value was ≤ 0.05 (Longdon *et al.*, 2011). pMCMC values can be interpreted as the Bayesian equivalent of a p-value, and were calculated as 2 times the smaller MCMC estimates of: 1) the probability that the parameter estimate is greater than zero, or 2) the probability that the parameter estimate is less than zero.

Results

By the end of the study, total and adult abundance was generally higher in mesocosms that received immigrants than in control mesocosms with no immigration (Table 1.1). Mean total abundance was significantly higher in the adaptively similar but genetically divergent immigrant treatment mesocosms than in control mesocosms, regardless of whether or not the immigrants themselves were included in total abundance: 95% CI of effects is (3.82, 19.83) individuals with immigrants and (0.10, 18.52) individuals without (Table 1.1). Moreover, mean total abundance started to decrease slightly in the control mesocosms after the 3rd census, but continued increasing in both immigrant treatments for another 1–2 months before leveling off or starting to decrease (Fig. 1.1, Supporting Information Fig S1.1).

Mean adult abundance was significantly higher in immigrant treatment mesocosms than in control mesocosms when immigrants were included: 95% CI (1.01, 6.01) for adaptively

divergent but genetically similar treatments and (1.92, 6.63) for adaptively similar but genetically divergent treatments (Table 1.1). When immigrants were excluded, mean adult abundance was marginally significantly higher in the adaptively similar but genetically divergent treatment mesocosms than in control mesocosms: 95% CI (-0.09, 3.61) (Table 1.1). Mean adult abundance declined slightly in control mesocosms (Fig. 1.2), but consistently increased throughout the study in the immigrant treatment mesocosms when immigrants were included (Fig. 1.2a) and stayed constant when immigrants were excluded (Fig. 1.2b).

Mean adult survival rate was high (roughly 85%) and did not differ significantly among treatments (Table 1.1). However, mesocosms that received adaptively divergent but genetically similar immigrants had significantly higher mean adult recruitment than controls, and the adaptively similar but genetically divergent treatment had marginally significantly higher mean adult recruitment than controls: 95% CI (0.29, 3.22) and (-0.11, 2.58), respectively (Table 1.1). Mean recruitment leveled off in the controls after the 5th census, but continued increasing in immigrant treatment mesocosms (Fig. 1.3).

Discussion

Our results support the hypothesis that immigrants have a net positive demographic effect on the short-term fitness of small populations, despite originating from divergent sources. Mean abundance and recruitment were higher in the immigrant mesocosms compared to the controls without immigrants. In the control mesocosms, population sizes declined slightly over the duration of the study. In the mesocosms that received immigrants from the adaptively divergent but genetically similar population, total population sizes either reflected the demographic contribution of immigrants and their babies or growth above this baseline (i.e., genetic rescue); our experiment was not designed to separate out the effects of demographic and genetic rescue. Our analyses excluding immigrants removed their direct demographic contribution, but not that of any offspring the female immigrants may have been carrying. It is therefore possible

that the higher total abundance in these mesocosms after immigrant exclusion is the result of immigrant offspring rather than genetic rescue. Surprisingly, the mesocosms that received immigrants from the adaptively similar but genetically divergent (LP) population had a greater increase in total abundance than in adult abundance or recruitment, suggesting that population growth was driven by higher birth rates in these mesocosms. LP guppies typically have lower fecundity than HP guppies, with smaller brood sizes and longer interbrood intervals, and take longer to reach sexual maturity (Reznick, 1982; Reznick & Endler, 1982). Our finding that LP guppies had higher fecundity suggests that the difference in ability of these divergent immigrant sources to increase population fitness was not solely an artifact of their fecundities in their respective native environments; we would have expected HP guppies to have higher reproductive output, but we found just the opposite. One potential explanation for this is that LP guppies, being better adapted than HP guppies to the competitive, predator-free mesocosm environment, were able to allocate resources toward reproduction more appropriately. We found that adaptively similar but genetically divergent immigrants were overall more beneficial to population fitness than adaptively divergent but genetically similar immigrants. This may come as a surprise because (coming from a LP environment) the adaptively similar but genetically divergent immigrants likely had low genetic variation and therefore may contribute relatively little novel genetic information. But because recipients and immigrants came from population sources that have been long-isolated from one another, and genetic drift is random, introgression between two inbred sources can still result in genetic rescue (Heber *et al.*, 2013). However, our results should be interpreted with some caution because we could only test one source population for each divergent immigrant treatment, and findings may differ depending on the genetic and demographic characteristics of divergent immigrants in other systems.

Outbreeding depression is expected be the strongest in the F_2 generation, once recombination has shuffled alleles from both populations (Bateson, 1909; Dobzhansky, 1937; Muller, 1940; Templeton, 1986; Lynch, 1991; Edmands, 2007). Our experiment ended shortly

after the start of the F_2 generation and therefore only captured the effects of interpopulation recombination via some of the sub-adults; many negative outbreeding effects may not manifest until later in adulthood, and if our experiment had run for a longer period or our sample size were larger we would have been better able to detect them. However, outbreeding depression is still possible in F_1 hybrids if immigrant genes are maladapted, there is underdominance, or detrimental epistatic interactions exist between heterozygotes or sex chromosomes (Edmands, 2007). Reduced fitness of F_1 hybrids has been demonstrated widely, including in plants (Quilichini *et al.*, 2001; Heiser & Shaw, 2006; Galloway & Etterson, 2005), invertebrates (Lonsdale *et al.*, 1988; Lee, 2000; Hwang *et al.*, 2012), and vertebrates (Sasa et al., 1998; Gilk et al., 2004; Neff, 2004). Therefore, while a thorough test for outbreeding depression requires monitoring over multiple generations, the lack of negative outbreeding effects in our experiment, though short-term, is notable. There is also the question of whether the dynamics observed in our laboratory populations can be extrapolated to wild populations. We believe they generally can, in part because carrying capacity was not reached in these mesocosms (based on populations sizes that reached ~50 individuals before declining in a subsequent experiment; Kronenberger et al., unpubl. data), and therefore density-dependent limits on population growth should not have influenced our results. Furthermore, our results are consistent with a similar increase in population fitness following immigration from a divergent population of guppies in the wild (Fitzpatrick et al., 2016).

Previous studies testing the effects of non-divergent immigrants on population fitness have generally found positive results (reviewed by Frankham et al., 2015; Whiteley et al., 2015). When immigrants are divergent, however, studies have been inconsistent, with some describing a positive effect (Hogg *et al.*, 2006; Bossuyt, 2007; Willi *et al.*, 2007; Johnson *et al.*, 2010; Tortajada *et al.*, 2010) and others a negative effect (Lacy, 1998; Edmands, 1999, 2002; Fenster & Galloway, 2000; Lee, 2000; Hwang *et al.*, 2011; Pekkala *et al.*, 2012). These inconsistencies may be due to a variety of factors, including the type and degree of divergence of immigrants

(Frankham et al., 2011), the severity of inbreeding depression in recipient populations (Ingvarsson, 2001), and the duration of population monitoring (Tallmon et al., 2004).

Future studies are needed to build upon our experiment and previous research. First, longer-term studies will provide a more complete understanding of the ultimate effects of augmentation on population dynamics, as has been argued previously (Whitlock *et al.*, 1995; Fenster, Galloway & Chao, 1997; Edmands 1999, 2007; Tallmon *et al.*, 2004; Willi *et al.*, 2007). Second, studies that manipulate the degree of divergence of immigrants and the level of inbreeding of recipient populations will identify key thresholds when the positive effects of gene flow become negative. Third, the goal of our study was to determine the overall effect of divergent immigrants on demography, rather than tease apart the contributions of demographic versus genetic rescue. Future studies should attempt to parse out the relative importance of these two mechanisms in rescuing populations (*sensu* Hufbauer et al., 2015). To avoid unaccounted-for variation in immigration rates, immigrants should not be pregnant upon introduction. We also suggest including a treatment with immigrants from the same population to control for the numerical input of the immigrants themselves; these immigrants would contribute little novel genetic information, and therefore their impact on population fitness would be almost entirely demographic. Contrasts between treatments with immigrants from the same population and those with immigrants from elsewhere would be able to differentiate demographic from genetic rescue. Finally, when managers decide to augment declining or small populations, they should utilize the opportunity to test the effects of immigrants on population dynamics, for example by using an adaptive management framework in which management actions are adjusted depending on the effectiveness of previous approaches (Walters, 1986; Williams, Nichols & Conroy, 2002).

Conservation implications

If managers decide to augment a population, they should ideally choose an immigrant source that is adaptively and genetically similar to the recipient population. However, our results suggest that if no similar source exists, augmenting with individuals from a divergent population may be better than doing nothing, particularly for very small populations that are highly vulnerable to extinction from demographic stochasticity. Individual mesocosms in our experiment showed widely varying population responses due to small initial population sizes (Fig S1.1), but overall mesocosms that received divergent immigrants maintained higher abundances than controls that did not.

Our study is relevant to many species of conservation concern in which the only potential sources for augmenting small, isolated populations are adaptively and/or genetically divergent. For example, many small populations of Great Basin Columbia spotted frogs (*Rana luteiventris*; a candidate for listing under the U.S. Endangered Species Act) are separated from each other by tens to hundreds of kilometers of inhospitable desert habitat and are also highly genetically differentiated from each other (Funk *et al.*, 2008; Robertson *et al.*, unpubl. data). Similarly, island foxes (*Urocyon littoralis*; listed as Endangered under the U.S. Endangered Species Act) have been subdivided into 6 subspecies, each on a different island (Goldstein *et al.*, 1999; Aguilar *et al.*, 2004; Funk *et al.*, 2016). In both species, the only potential sources for augmentation are highly divergent from recipient populations.

If deemed necessary for increasing population fitness and averting extinction, augmenting with individuals from divergent populations should at least be considered in these and other cases in which a similar source population is not available. We realize, however, that a single empirical study in guppies or any other experimental system does not necessarily inform all species and cases. Ultimately, the effects of immigration and gene flow will depend on many factors, particularly the degree of divergence of immigrants and the degree of inbreeding depression in recipient populations. More studies are needed to determine how these factors

and others influence the outcome of gene flow if we are to come to a consensus about when demographic and/or genetic rescue via augmentation will work.

Table 1.1 – Summary of the estimated means for the control mesocosms and the effects of the immigration treatments (relative to the mean values for the controls) on mesocosm abundances, number of adult recruits, and survival rate, estimated from the posterior distributions of Bayesian univariate GLMMs.

Response variable	Mean of control mesocosms with no immigrants (95% CI)	Adaptively divergent but genetically similar immigrant treatment (95% CI)	Adaptively similar but genetically divergent immigrant treatment (95% CI)
Total abundance including immigrants	12.09 (4.10, 20.24)	6.20 (-3.11, 14.75)	11.43 (3.82, 19.83)*
Total abundance excluding immigrants	12.17 (4.44, 20.05)	4.14 (-3.35, 13.08)	9.01 (0.10, 18.52)*
Adult abundance including immigrants	7.93 (5.77, 10.15)	3.62 (1.01, 6.01)**	4.22 (1.92, 6.63)***
Adult abundance excluding immigrants	8.01 (6.08, 9.86)	1.43 (-0.38, 3.25)	1.77 (-0.09, 3.61)·
Number of adult recruits	1.94 (-0.60, 4.63)	1.75 (0.29, 3.22)*	1.21 (-0.11, 2.58)·
Adult survival rate including immigrants (%)[a]	84.76 (70.17, 100.00)	0.10 (0.00, 3.39)	0.06 (0.00, 3.77)
Adult survival rate excluding immigrants (%)[a]	85.90 (71.58, 100.00)	0.003 (0.00, 5.77)	0.06 (0.00, 3.51)

The estimated level of significance (pMCMC) of effects is denoted by asterisks: ·$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
[a]Survival rates were arcsine square root transformed prior to running the model. The means, effects, and 95% CI presented here are back transformed. We corrected upper and lower bounds of back transformed 95% CIs using B(X) = 100.00 when X > π/2 and B(X) = 0.00 when X < 0.

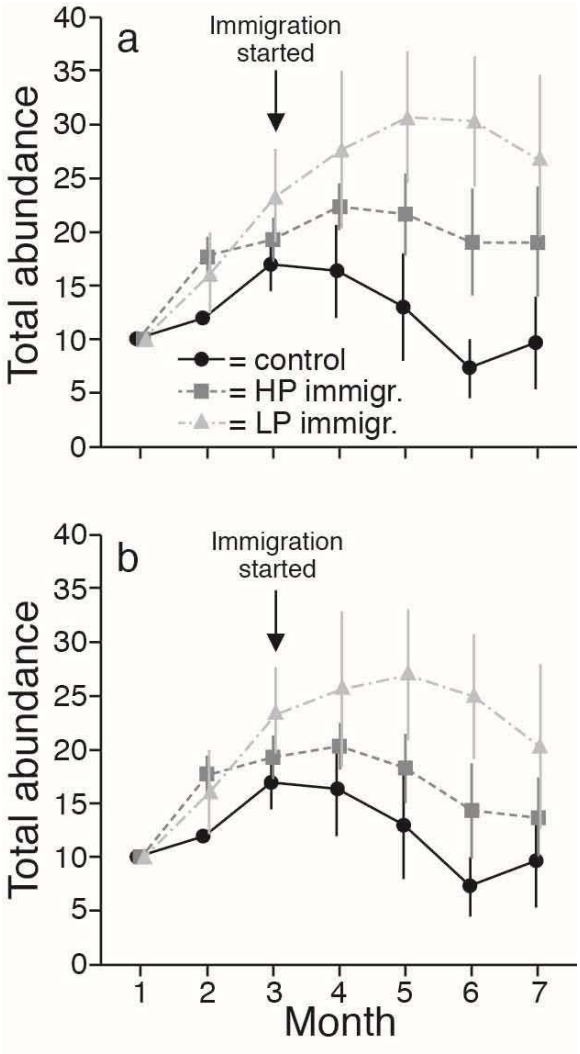

Figure 1.1 – Mean monthly total abundance (adults + sub-adults) in control, adaptively divergent but genetically similar (HP), and adaptively similar but genetically divergent (LP) immigrant treatment mesocosms including (a) or excluding (b) immigrants. Error bars show standard errors. Monthly addition of immigrants (one female and one male per month) in immigrant treatment mesocosms started immediately after the 3rd census.

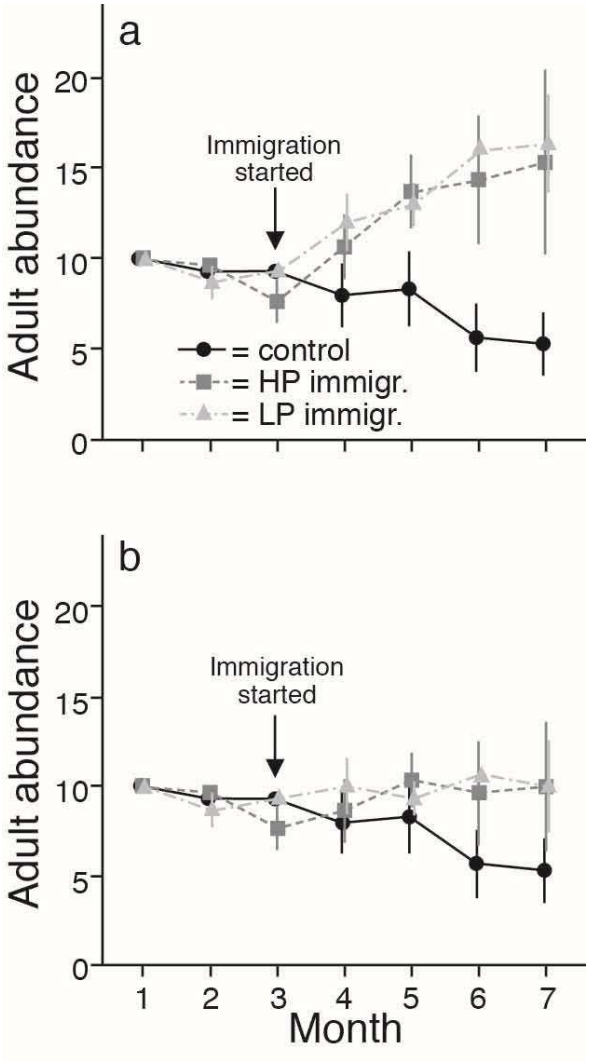

Figure 1.2 – Mean monthly adult abundance in control, adaptively divergent but genetically similar (HP), and adaptively similar but genetically divergent (LP) immigrant treatment mesocosms including (a) or excluding (b) immigrants. Error bars show standard errors. Monthly addition of immigrants (one female and one male per month) in immigrant treatment mesocosms started immediately after the 3rd census.

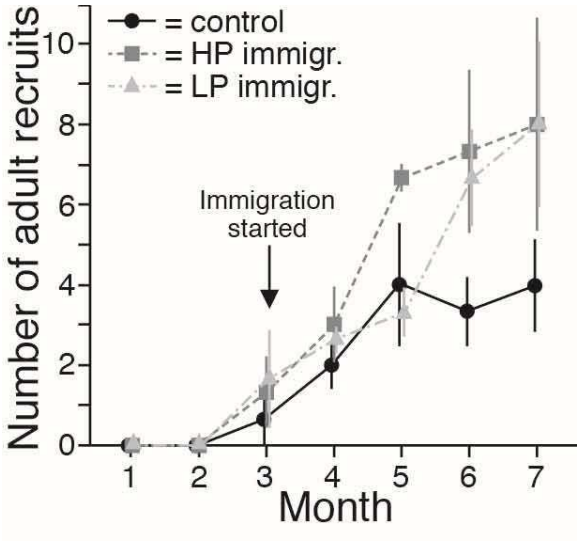

Figure 1.3 – Mean monthly number of adult recruits in control, adaptively divergent but genetically similar (HP), and adaptively similar but genetically divergent (LP) immigrant treatment mesocosms. Error bars show standard errors. Monthly addition of immigrants (one female and one male per month) in immigrant treatment mesocosms started immediately after the 3rd census.

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CHAPTER 2: GENETIC RESCUE IN SMALL POPULATIONS OF TRINIDADIAN GUPPIES FOLLOWING AUGMENTATION WITH DIVERGENT IMMIGRANTS

Summary

Human land use is fragmenting habitats worldwide and impeding 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, some conservation biologists are considering the augmentation of vulnerable populations with immigrants as a means of demographic and genetic rescue. Augmentation with immigrants that are genetically and adaptively similar to the target population is a highly effective strategy for increasing population fitness, but when only divergent immigrant sources remain these efforts can lead to outbreeding depression. Despite well-cited guidelines for the selection of immigrant sources, experimental tests of riskier augmentation scenarios are essentially nonexistent. Here, we present the results of a mesocosm experiment in which we used Trinidadian guppies to test the multigenerational demographic and genetic effects of immigration from a range of divergent sources into two populations. We found no evidence for demographic rescue, but we did observe genetic rescue in one population. Specifically, treatments that received divergent immigrants maintained 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 gene flow were less apparent overall, possibly because this population had higher genetic diversity at the start and a lower reproductive rate. Our results support a growing body of research suggesting that immigrants can increase population fitness even when they are genetically or adaptively divergent, helping to relieve uncertainty about the use of augmentation in lieu of clearly suitable immigrant sources.

Introduction

Anthropogenic habitat fragmentation is a primary driver of the population declines that constitute Earth's sixth mass extinction (Ceballos et al. 2015; Haddad et al. 2015), in part because it hinders natural levels of dispersal and gene flow among previously connected metapopulations. Over time, the resulting isolation can reduce genetic diversity via genetic drift and thereby 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. 2014; Hedrick & Garcia-Dorado 2016). Because of these negative impacts, reestablishing connectivity among populations is a top priority in conservation biology.

When restoring natural dispersal between populations 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 variety of translocation, and, when reducing maladaptation is the primary goal, assisted gene flow—can increase the viability of populations through two synergistic mechanisms. The first is demographic rescue, or a benefit received solely from bolstering the size of the population (Brown & Kodric-Brown 1977; Hufbauer et al. 2015). Demographic rescue works primarily by buffering against demographic stochasticity and is thus especially likely in populations with small effective population sizes where Allee effects may be of concern (Carlson et al. 2014). However, attempts at demographic rescue also come with risks, such as exposure to novel disease pressures and disruption of social structures, suggesting that immigrants should be carefully screened prior to introduction (Frankham 2015; Mills 2017). 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 an enormously positive and lasting impact on populations by increasing genetic diversity and thereby relieving inbreeding depression and providing the novel alleles

required for adaptation to rapid environmental change (Allendorf et al. 2013; Hedrick & Garcia-Dorado 2016), effectively reversing the genetic consequences of isolation.

Genetic rescue is also controversial. This is in part because it disrupts the unique evolutionary history of isolated populations by altering local gene pools with foreign alleles, a human intervention that some consider distasteful (Frankham 2015; Tallmon 2017). Another reason for the controversy is that predicting the ultimate outcome of gene flow is difficult (Tallmon et al. 2004; Garant et al. 2007; Gompert & Buerkle 2016). While gene flow is predicted to benefit isolated populations by increasing genetic diversity, it may also cause harm by breaking up co-adapted gene complexes or introducing maladaptive alleles, both of which contribute to outbreeding depression (Edmands 2007; Allendorf et al. 2013). Empirical studies reflect this duality; while genetic rescue attempts are typically highly successful (Frankham 2015; Whiteley et al. 2015)—as famously demonstrated in greater prairie chickens (Westemeier et al. 1998), adders (Madsen et al. 1999), Florida panthers (Johnson et al. 2010), and Scandinavian wolves (Åkesson et al. 2016)—cases of outbreeding depression are not unusual, particularly in later generation hybrids (Edmands 2007; Waller 2015).

In an effort to reduce uncertainty, several authors have synthesized theory and empirical studies to provide guidelines for the selection of immigrants, suggesting that outbreeding depression is most likely when the “recipient” and “donor” populations are long isolated from one another (and therefore divergent at neutral genetic loci) or adapted to different environments (and therefore divergent at adaptive loci; Edmands 2007; Hedrick and Fredrickson 2010; Frankham 2011; Weeks et al. 2011). Recommendations such as these are highly valuable, but the unfortunate reality is that (with continued habitat fragmentation worldwide; Haddad et al. 2015) often the only donor populations that remain *are* divergent and therefore risky if used for augmentation (e.g., Funk et al. 2016). This problem is exacerbated by the fact that locally rare and endangered species—those that would receive the greatest benefit from augmentation—also tend to have the most fragmented ranges and the fewest appropriate

donor populations. The utility of donor selection guidelines in these riskier augmentation scenarios is limited and would be greatly enhanced by supplementing theory and empirical studies with manipulative experiments.

To this end, guppies (*Poecilia reticulata*) from the Northern Range mountains of Trinidad are ideal. Trinidadian guppies inhabit streams that are punctuated by waterfalls that partially restrict their upstream dispersal and reduce gene flow into some populations, resulting in a broad range of genetic divergence and within-population genetic diversity (Crispo et al. 2006; Suk & Nef 2009; Willing et al. 2010). Furthermore, while guppies can occasionally circumvent waterfall barriers, most predatory fishes are excluded from upstream reaches. This has led to the repeated evolution of two distinct ecotypes of guppies: those adapted to low-predation environments and those adapted to high-predation environments. Low- and high-predation guppies differ in a suite of genetically-based traits, including life-history, behavior, morphology, body size, and male coloration (Magurran 2005) and are therefore considered adaptively divergent. Occasional gene flow occurs from low-predation populations into downstream high-predation populations, making these ecotypes relatively similar at neutral loci despite being adapted to different environments (Crispo et al. 2006; Suk & Nef 2009; Willing et al. 2010). This natural range of genetic and adaptive divergence provides a unique opportunity to test the effects of divergent immigrants within a single system.

We took advantage of the Trinidadian guppy system to simulate risky augmentation scenarios in a controlled mesocosm experiment. In doing so, we addressed three interrelated questions with implications for applied management: 1) How does augmentation with divergent immigrants affect population fitness, and through what mechanism? 2) How is the outcome of augmentation influenced by attributes of the recipient population? 3) How is the outcome of augmentation influenced by attributes of the donor population? Our aim was to provide generalizable insights into these questions as a means of reducing uncertainty about the use of augmentation when divergent immigrants are the only option.

Methods

Experimental design

Our mesocosm experiment was set up as a two-by-five factorial, with two recipient populations and five immigration treatments. Three randomized controlled blocks of this design were established for 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 from a low-predation tributary of the Marianne River on the northern slope (Fig. 2.1; see Table S2.1 for UTM coordinates). Guppy populations from the southern and northern slopes coexist with different suites of predators (Reznick & Endler 1982; Magurran 2005), colonized Trinidad from mainland Venezuela at different times and via different routes (Suk & Neff 2009; Willing et al. 2010), and are distinct to the degree that some researchers assign them separate species designations (Schories et al. 2009). Our test of the effects of divergent immigrants was therefore replicated across recipient populations representing two separate evolutionary lineages.

Augmentation treatments were as follows: no immigrants (None); immigrants from the same population as recipients (Same); low-predation immigrants from a source close to the recipient population, which were adaptively similar but predicted to moderately genetically divergent (LPC); low-predation immigrants from a source far from recipients, which were adaptively similar but predicted to be highly genetically divergent (LPF); and high-predation immigrants from a source close to recipients, which were adaptively divergent but predicted to be genetically similar (HPC). Donor populations were unique to each recipient population (Fig. 2.1). Because immigrants from the same population contribute few novel alleles, any benefit that Same immigrants provide should be due almost exclusively to demographic rescue. In contrast, divergent immigrants do contribute novel alleles, and any benefit that LPC, LPF, and HPC immigrants provide over that of Same immigrants should be due almost exclusively to genetic rescue.

Mesocosm setup and data collection

Our three experimental blocks were established sequentially, 1–2 months apart, between February and July of 2014. Prior to the establishment of each block, guppies were wild-caught in Trinidad 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 to ensure they remained virgins. Mesocosms consisted of 10-gallon tanks, each with a gravel substrate, sponge filter, floating plastic grass to provide refuge for newborn fish, and two rocks, a clay pot, and a live *Philodendron* plant for habitat enrichment. Each was stocked with 16 individuals at a 1:1 sex ratio, although in 8 of 30 mesocosms the sex ratios were male-biased (1:3–1:1.29, or 1:1.72 average) due to errors in sexing 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. Guppies were fed once daily (alternating between 400 µl ground flake paste and 800 µl newly hatched *Artemia* cysts), overhead full-spectrum lights were on a 12-hour light cycle, and water temperature was kept at 25 ± 1℃. Food quantity, light, and temperature were held constant for the dur ation of the experiment. After a 6-month equilibration period, immigrants were introduced at a rate of one male and one female bimonthly up to month 18, for a total of 14 immigrants per mesocosm. Full demographic and genetic population monitoring continued up to month 24, and reduced monitoring of abundance only continued up to month 30.

Mesocosms were sampled bimonthly, during which all adults (≥14 mm in standard length) were anesthetized with tricaine methanesulfonate and new immigrants and recruits were given unique marks with visible implant elastomer (Northwest Marine Technology, Inc.), methods widely used on guppies with minimal mortality (e.g., Weese et al. 2010). The numbers 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 (Table S2.2), DNA extraction and PCR protocols, and quality controls were identical to those used by

Fitzpatrick et al. (2015, 2016). PCR products were sent to Cornell Biotechnology Resource Center for fragment analysis and fragments were scored with Geneious 7.1.8 (Kearse et al. 2012). All work was approved by the Colorado State University Institutional Animal Care and Use Committee (#12-3818A).

Genetic divergence and diversity

To confirm our predictions about the degree of genetic divergence between recipient and donor populations, and to estimate within-population genetic diversity, we used genotypes of the wild-caught individuals used in our experiment to estimate pairwise F_{ST} values and expected heterozygosity with Arlequin 3.5.2.2 (Excoffier & Lischer 2010), and allelic richness with HP-Rare 1.1 (Kalinowski 2005). Expected heterozygosity and allelic richness were also estimated for each mesocosm population at each bimonthly sampling period to monitor changes in genetic diversity over the course of the experiment. Finally, we estimated effective population sizes of the recipient populations with the linkage disequilibrium method provided in NeEstimator 2.01 (Do et al. 2014), including all alleles. Confidence intervals of effective population sizes were calculated with the jackknife method of Waples and Do (2008).

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 of wild populations. We focused on abundance as the primary indicator of population fitness. Abundance was defined as the total number of individuals (adults and subadults) at a given time point, but abundance patterns were qualitatively the same when subadults were excluded. We did not use population growth rate as an indicator of population fitness because mesocosms occasionally reached carrying capacity and density-dependent factors made growth rate less informative. To interpret abundance patterns, we also calculated

bimonthly survival (defined as the proportion of adults surviving between sampling periods) and bimonthly recruitment (defined as the number new adults each sampling period).

Abundance, survival, and recruitment were estimated with repeated measures linear mixed models using PROC MIXED in SAS 9.4 (SAS Institute, Cary, NC; see Table S2.3 for tests of fixed effects). 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 two-way interactions and main effects. The final fixed effect was baseline abundance, a covariate representing the abundance in each mesocosm just prior to the start of augmentation at month 6. Block was included as a random effect. Recruitment and survival data spanned from month 6 to 24 (18 months) and abundance data from month 6 to 30 (24 months).

Individual fitness

To quantify individual fitness, we used genotypes to infer a pedigree for each mesocosm population using 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 only retained for subsequent analyses if correctly assigned parents 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 hybrids, F_2+ hybrids, and backcrosses for analysis because we lacked the power needed to estimate the fitness of later-generation hybrids separately. Later-generation hybrids are expected to be more susceptible outbreeding depression because many genetic

incompatibilities are only revealed after recombination (Edmands 2007). In Quare populations—where nearly all of the later-generation 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) according to a Wilcoxon rank-sum test performed using PROC NPAR1WAY in SAS 9.4 (SAS Institute, Cary, NC). This, coupled with the fact that abundance in divergent immigrant treatments increased after genotyping ended at month 24 (Fig. 2.5), suggests that outbreeding depression did not occur in later-generation hybrids.

Differences in individual fitness were estimated with a generalized linear mixed model using PROC GLIMMIX in SAS 9.4 (SAS Institute, Cary, NC; see Table S2.4 for tests of fixed effects). Individual fitness was not normally distributed due to a high incidence of zeros, so we assumed a negative binomial distribution as it produced the lowest corrected Akaike Information Criterion value when compared against all other available distributions (Burnham & Anderson 2002). 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. We also included as fixed effects two covariates: abundance upon reaching adulthood and sampling period upon reaching adulthood. Sampling period was included to account for factors that changed over time in the study, such as the degree of disease pressure from *Mycobacterium* spp., which were inadvertently introduced into the mesocosms from the wild (as confirmed by P. Schaffer at Colorado State University's Veterinary Diagnostic Laboratories). This covariate also controls for the fact that later recruits were younger when genotyping ended and therefore had less opportunity to produce offspring that would be counted. Recruits captured the final month had zero chance of producing counted offspring and were excluded from the model. Mesocosm nested within block was included as a random effect.

Results

Genetic divergence and diversity

The F_{ST} analysis revealed moderate to high genetic divergence between all donor and recipient population pairs, although the Quare LPC donor population was more divergent than LPF, contrary to our expectations (Table 2.1). Genetic divergence was greater among Quare than Marianne source populations in all cases. Quare recipients also had lower genetic diversity than Marianne, with expected heterozygosities of 0.71 and 0.87, allelic richness levels of 9.03 and 13.24, and effective population sizes of 247 (95% CI: 144, 552) and 949 (95% CI: 504, 4437), respectively. By the end of the experiment, Quare treatments followed a rank-order for expected heterozygosity and allelic richness, with HPC having the highest genetic diversity, followed by LPF, LPC, Same, and then None (Fig. 2.2). In the LPC, LPF, and HPC treatments, genetic diversity increased following augmentation before leveling off or beginning to decline. In contrast, genetic diversity in the None and Same treatments remained flat or declined throughout the experiment. Marianne treatments were less variable and did not follow a clear pattern, although the None treatment consistently had the lowest genetic diversity.

Population fitness

In Quare populations, divergent immigrants had a more positive impact than immigrants from the same population or no immigrants at all (Figs. 2.3 & 2.4). Specifically, LPC, LPF, and HPC treatments followed a cycle of growth and decline, increasing to a peak several months after immigration began before declining and then once again increasing. This contrasted with the None and Same treatments, which did not experience the same initial growth and slowly declined throughout the course of the experiment. The repeated measures model, which controls for baseline abundance just prior to immigration, found no difference in estimated mean abundance between the None and Same treatments. However, relative to None treatments, estimated mean abundances were greater in LPC (t_{257}=2.31, p=0.022), LPF (t_{257}=1.76,

p=0.079), and HPC (t_{257}=3.13, p=0.002). Likewise, relative to Same treatments, estimated mean abundances were greater in LPC (t_{257}=2.03, p=0.044) and HPC (t_{257}=2.85, p=0.005). This trend was nonsignificant in the LPF treatment (t_{257}=1.50, p=0.136). Estimated mean abundance was similar in LPC, LPF, and HPC treatments (all p≥0.189).

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. 2.5). This initial pre-immigration growth was unexpected and primarily due to unusually rapid reproduction in only one replicate mesocosm from each of the LPF and HPC treatments (Fig. S2.1). Populations peaked shortly after immigration began and ultimately declined in the LPF treatment or declined and increased slightly again in the HPC treatment. None, Same, and LPC treatments increased more gradually to peak and then declined before leveling off or increasing slightly again by the end of population monitoring. The repeated measures model found the LPC treatment had greater estimated mean abundance than LPF (t_{257}=2.02, p=0.044), but all other treatments were similar (all p≥0.127; Fig. 2.4).

We found no population-level difference in abundance (t_{257}=0.78, p=0.438), but did find lower survival (t_{197}=4.99, p<0.001) and higher recruitment (t_{197}=-4.33, p<0.001) in Quare than in Marianne populations (Fig. 2.4). All treatment-level differences in survival or recruitment were nonsignificant for both Quare (all p≥0.106) and Marianne (all p≥0.174).

Individual fitness

Quare populations had a higher reproductive rate than Marianne, with more offspring (436 versus 325) and generations (2–6, or 3.7 average, versus 1–4, or 2.5 average) over the full 24 months of genetic monitoring. This resulted in many more hybrids being produced in Quare than in Marianne populations (137 versus 13, respectively). In Quare populations, individual fitness of the three genetic ancestry classes was similar in the Same treatment (all p≥0.101), but otherwise immigrants had the highest fitness, followed by hybrids, and then recipients (Fig. 2.5).

Specifically, immigrants had higher fitness than recipients in 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), and hybrids had higher fitness than recipients in the LPC (t_{1435}=1.76, p=0.079) and LPF (t_{1435}=2.32, p=0.020) treatments, but not in HPC (t_{1435}=1.32, p=0.186). Immigrants had greater fitness than hybrids in the LPF (t_{1435}=-1.72, p=0.086) and HPC (t_{1435}=-2.27, p=0.024) treatments, but not in LPC (t_{1435}=-0.57, p=0.566). In Marianne populations, we had little power to resolve pairwise comparisons of hybrid fitness (all p≥0.653) and there were no significant differences between recipients and immigrants in any treatments (all p≥0.110).

Discussion

How does augmentation with divergent immigrants affect population fitness, and through what mechanism?

We found that immigrants can rescue small populations despite being genetically or adaptively divergent. All divergent immigrant treatments positively affected the fitness of Quare mesocosm populations, and while results were mixed in Marianne populations, divergent immigrants did not have a negative effect overall (but see the discussion of LPF immigrants below). Populations that received immigrants from the same source did not fare better than those without augmentation, but particularly in Quare populations, those that received divergent immigrants had consistently higher genetic diversity (Fig. 2.2), abundance (Figs. 2.3 & 2.4), and hybrid fitness (Fig. 2.5). Together, these results indicate that demographic inputs did not strongly affect abundance and that increases in population fitness were primarily due to genetic rescue.

These findings corroborate those of other augmentation studies in the Trinidadian guppy system. In a shorter duration mesocosm experiment with different source populations, Kronenberger et al. (2017a) found that augmentation with genetically and adaptively divergent immigrants had a positive effect relative to no augmentation, but were unable to determine the

mechanism of rescue without the appropriate controls. Fitzpatrick et al. (2016), again using different source populations, found evidence for both demographic and genetic rescue from adaptively divergent immigrants in the wild, but lacked controls to fully separate the two. Few studies have determined the relative contribution or demography versus genetics in rescue. In the most thorough to date, Hufbauer et al. (2015) found genetic rescue in both small and large experimental populations of flour beetles (*Tribolium castaneum*), but long-term demographic rescue only in large populations. The authors argued that their small populations were too stochastic for immigrants to buffer against declines in abundance. We believe a similar situation was at play in our study, given considerable within-treatment variation in abundance among individual mesocosms (Fig. S2.1).

In Quare populations, divergent immigrants caused genetic rescue by producing hybrids with greater fitness than recipients, but hybrid fitness was still lower than that of immigrants. This could arise through a variety of mechanisms—for example, if fixed deleterious recessive alleles were masked by immigrant alleles with incomplete dominance—but it does not explain why immigrants had higher fitness than recipients despite having comparable genetic diversity (Table 1). One possibility is a sort of "behavioral rescue" that reflects unique attributes of our study system. Trinidadian guppy males have highly polymorphic coloration both within- and between-populations, and females are known to prefer males with novel coloration (Houde 1997; Hughes 2013), so novelty could have played a role in elevating the fitness of divergent immigrant males. We also assayed a subset of males for sexual displays and forced copulations (well-documented metrics of reproductive effort in guppies; Houde 1997) and found that divergent immigrants typically performed more of these behaviors than recipients in both populations (unpublished data). However, fitness was elevated regardless of sex (unpublished data) and the reason for high female fitness is unclear. Whatever the mechanism, high fitness of divergent immigrants in Quare populations clearly increased genetic admixture and thereby quickened the pace of genetic rescue.

How is the outcome of augmentation influenced by attributes of the recipient population?

Quare recipient populations started out with lower genetic diversity (Table 2.1, Fig. 2.2) and were the only ones to experience genetic rescue. This is exactly what we would expect from theory suggesting that lower genetic diversity increases the probability of genetic rescue (Tallmon et al. 2004; Garant et al. 2007; Allendorf 2013). We did not observe genetic rescue in Marianne populations possibly due to their high genetic diversity, but also because they had very low rates of genetic admixture. It is feasible that with greater hybridization they would have experienced genetic rescue (or outbreeding depression), but the fact that reproduction was low suggests that these populations had relatively high fitness to begin with.

This emphasizes the importance of recipient life-history in determining the outcome of augmentation. Not only did Quare populations have lower genetic diversity, they also had lower survival and higher recruitment (Fig. 2.4). It is likely that increased recruitment in Quare populations was, at least in part, a compensatory response to decreased survival and the resulting declines in abundance. Fast life-history traits have been well documented in Trinidadian guppies, particularly in response to predation (Reznick & Endler 1982; Magurran 2005), but also in response to disease pressure (Fitzpatrick et al. 2014) like that from *Mycobacterium* spp. we observed in our mesocosms. It is unclear whether the different life-history traits we observed Quare and Marianne populations were immediate responses to levels of mortality or evolved strategies of their source populations, but they functioned to either magnify (Quare) or dampen (Marianne) the genetic effects of divergent immigrants and ultimately influence the outcome of augmentation.

How is the outcome of augmentation influenced by attributes of the donor population?

We found few differences in population fitness among divergent immigrant treatments. Genetically and adaptively divergent immigrant treatments followed very similar trajectories, although in Marianne the genetically divergent LPF treatment declined rapidly towards the end

of the experiment (Fig. 2.3) and our repeated measures model found it to have the lowest estimated mean abundance (Fig. 2.4). This result suggests that genetic divergence had a greater impact than adaptive divergence by, if not decreasing population fitness, putting a limit on its increase. We did not observe any hybrids in this treatment by the end of genetic monitoring, which could be due to either none being produced or none surviving into adulthood. We believe the former is more likely given the slow life-history traits of Marianne recipients. Interestingly, the Quare LPF treatment also had the lowest estimated mean abundance of the three divergent immigrant treatments, but, in this case, LPF immigrants were not the most genetically divergent (Table 2.1) and LPF hybrids had higher fitness than recipients (Fig. 2.5), suggesting that outbreeding depression did not occur.

In addition to divergence, we found that genetic diversity of the donor population can strongly influence genetic diversity of the recipient population after augmentation. For example, both HPC donor populations had the highest genetic diversity (Table 2.1) and HPC treatments maintained the highest genetic diversity over time (Fig. 2.2). Conversely, Quare LPC and Marianne LPF donors had the lowest genetic diversity, and so too did their respective treatments. These results highlight the importance of considering genetic diversity in addition to divergence when evaluating the risks and benefits of potential donors.

Conclusion and recommendations

Augmenting isolated populations can be a daunting task, particularly when the only donor populations that exist would be considered risky according to established guidelines (Edmands 2007; Hedrick and Fredrickson 2010; Frankham 2011; Weeks et al. 2011). Our study corroborates others (Fitzpatrick et al. 2016; Kronenberger et al. 2017a) finding that rescue can occur even when immigrants are genetically or adaptively divergent, and adds to current understanding by simultaneously testing several risky augmentation scenarios across two separate evolutionary lineages in a controlled experiment. Our results suggest that

augmentation is unlikely cause demographic rescue in highly stochastic populations. However, if immigrant fitness and recipient life-history traits encourage admixture, genetic rescue is likely and can have a highly positive impact on population fitness, even when immigrants are divergent. We echo other authors (Frankham et al. 2014; Whiteley et al. 2015) encouraging complete documentation of augmentations to help inform future projects, as well as adaptive management so augmentations can be adjusted in response to new information. Finally, we would like to emphasize the ability of manipulative experiments to reduce uncertainty about the outcome of risky augmentations (Whiteley et al. 2015; Grueber 2017; Kronenberger et al. 2017b; Tallmon 2017). Augmentations are typically considered for populations at the brink of extinction that cannot be ethically manipulated to test hypothesis-driven research questions, but through experimentation in model systems, we can inform theory and refine management recommendations without harming the populations we seek to rescue.

Table 2.1 – Pairwise F_{ST} values between recipient and donor populations, expected heterozygosity (H_E), and allelic richness (A_R).

	F_{ST}	H_E	A_R
Quare	—	0.71	9.0
LPC	0.25	0.67	6.4
LPF	0.19	0.78	14.0
HPC	0.17	0.89	18.2
Marianne	—	0.87	13.2
LPC	0.08	0.79	12.4
LPF	0.13	0.76	9.9
HPC	0.08	0.80	16.9

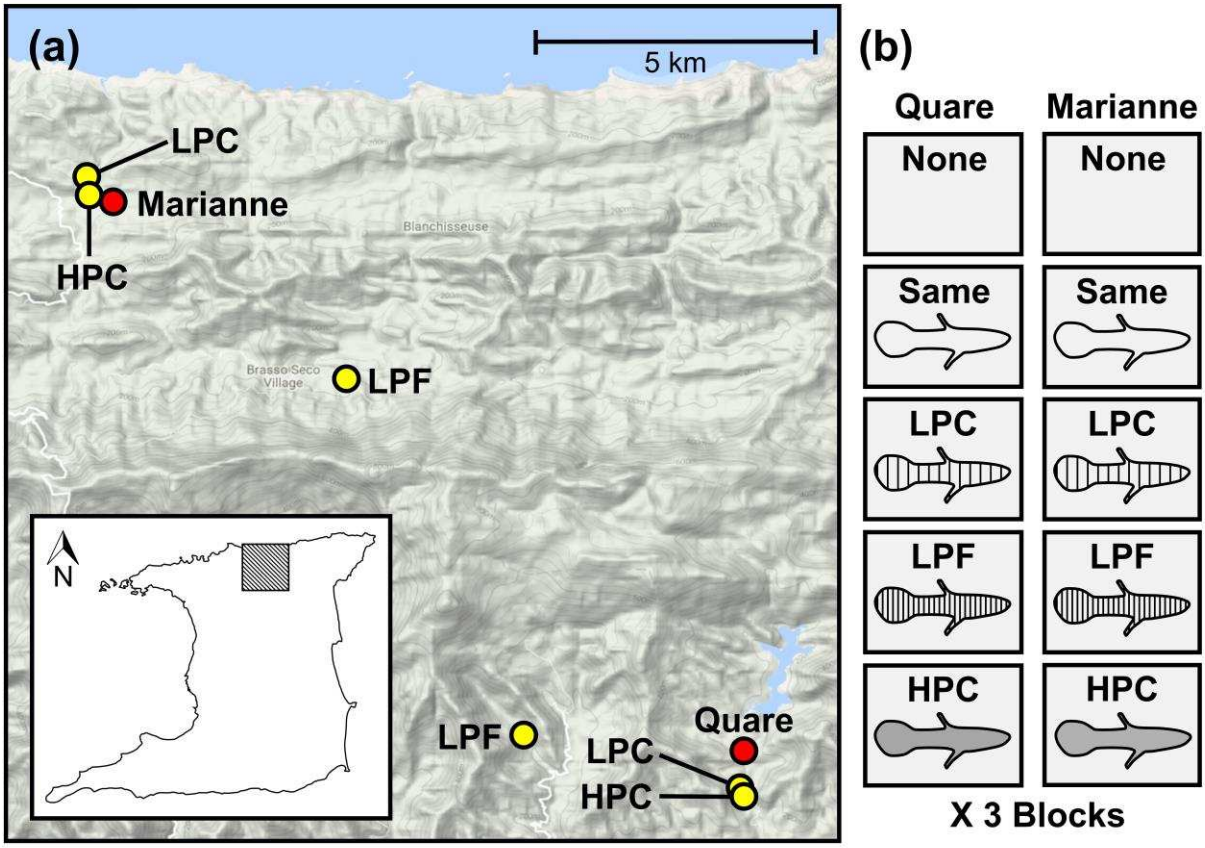

Figure 2.1 – Source locations of our experimental populations (a) and our experimental design (b). Low-predation close (LPC) and high-predation close (HPC) donor populations are from the same drainage as recipient populations and low-predation far (LPF) donors are from different drainages. Both recipient populations, Quare and Marianne, are in low-predation environments. In the diagram of our experimental design, vertical lines on guppies signify predicted genetic divergence and the darker shade signifies adaptive divergence. Specifically, LPC donors were adaptively similar (background shade) but predicted to be moderately genetically divergent (few vertical lines), LPF donors were adaptively similar (background shade) and predicted to be highly genetically divergent (many vertical lines). And HPC donors were adaptively divergent (darker shade) but predicted to be genetically similar (no vertical lines).

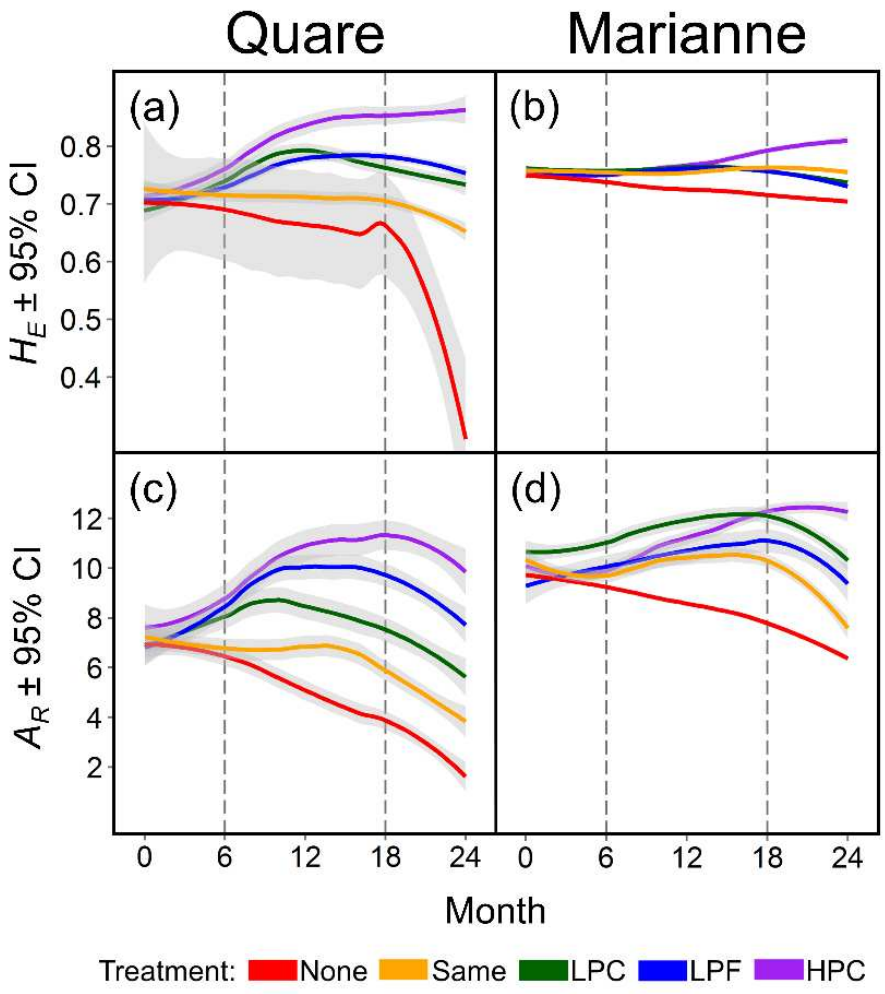

Figure 2.2 – Mean expected heterozygosity (H_E), allelic richness (A_R), and 95% confidence intervals over time for all treatments in Quare (a/c) and Marianne (b/d) populations, fit with a loess smoothing function. Vertical dashed lines indicate the start and end of immigration.

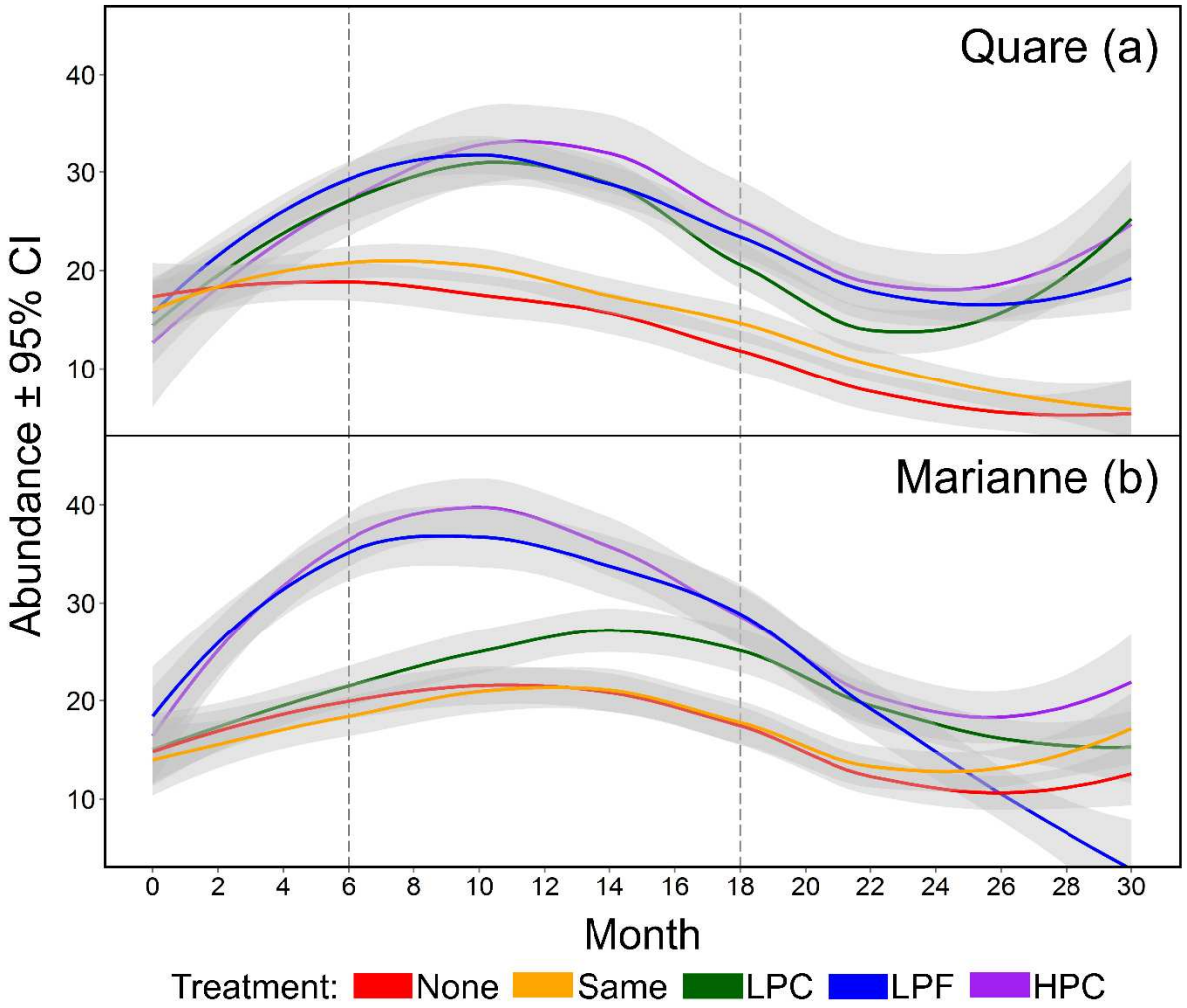

Figure 2.3 – Mean abundance and 95% confidence intervals over time, fit with a loess smoothing function, for Quare (a) and Marianne (b) populations. Vertical dashed lines indicate the start and end of immigration.

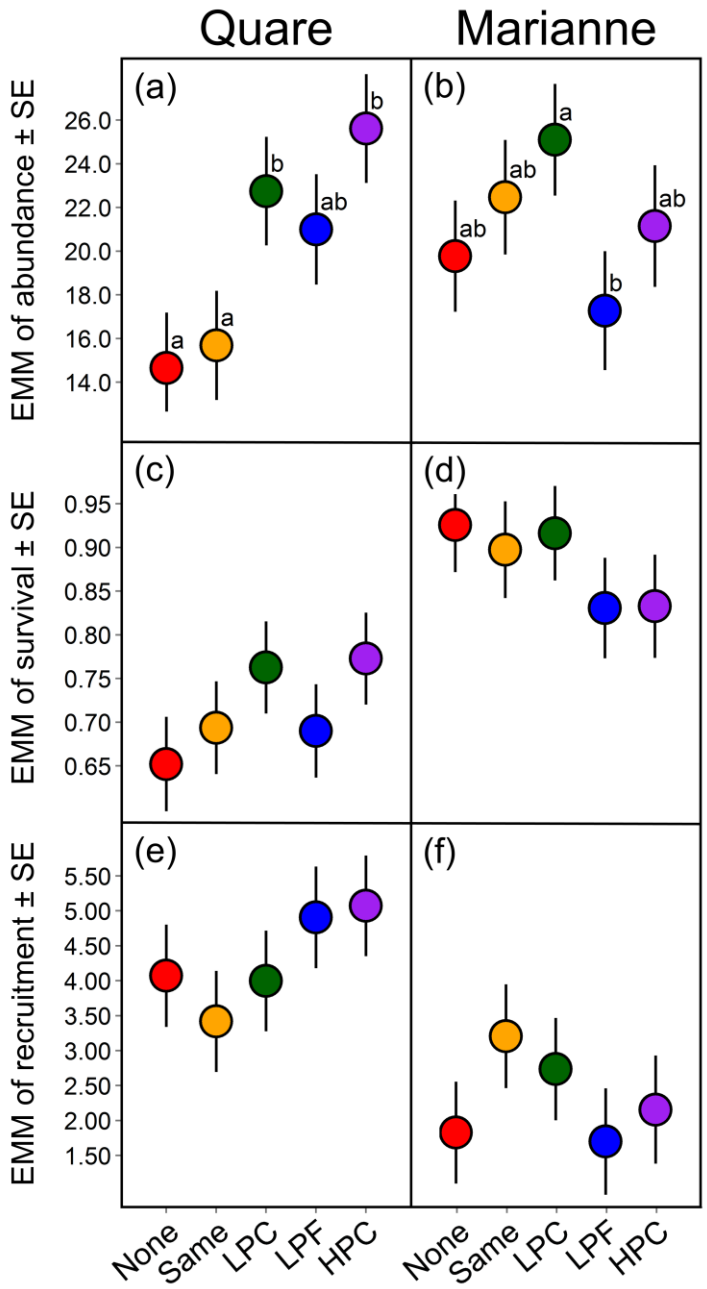

Figure 2.4 – Estimated marginal means and standard errors of abundance, survival, and recruitment for Quare (a/c/e) and Marianne (b/d/f) populations. Treatments are significantly different at the *p*=0.05 level for abundance only, as denoted with different letters.

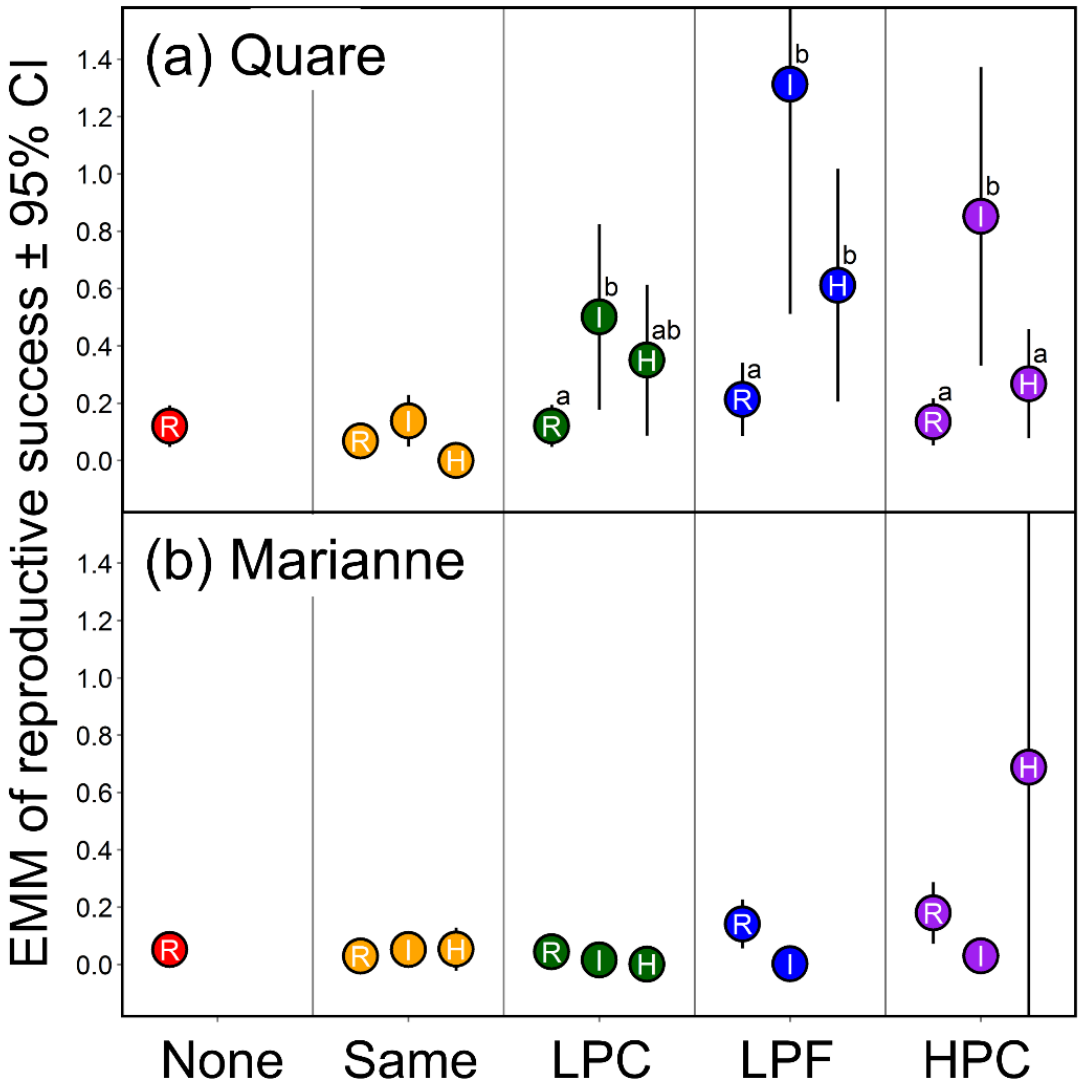

Figure 2.5 – Estimated marginal means and standard errors of lifetime reproductive success of all genetic ancestry classes within each treatment for Quare (a) and Marianne (b) populations. "R" signifies recipient genotypes, "I" immigrants, and "H" hybrids. Because Same immigrants are from the same source as recipients, "hybrids" in this case is not literal. No hybrids were produced in the Marianne LPF treatment. Groups are significantly different at the p=0.05 level for Quare LPC, LPF, and HPC treatments only, as denoted with different letters.

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APPENDIX 1: CHAPTER 1 SUPPLEMENTARY INFORMATION

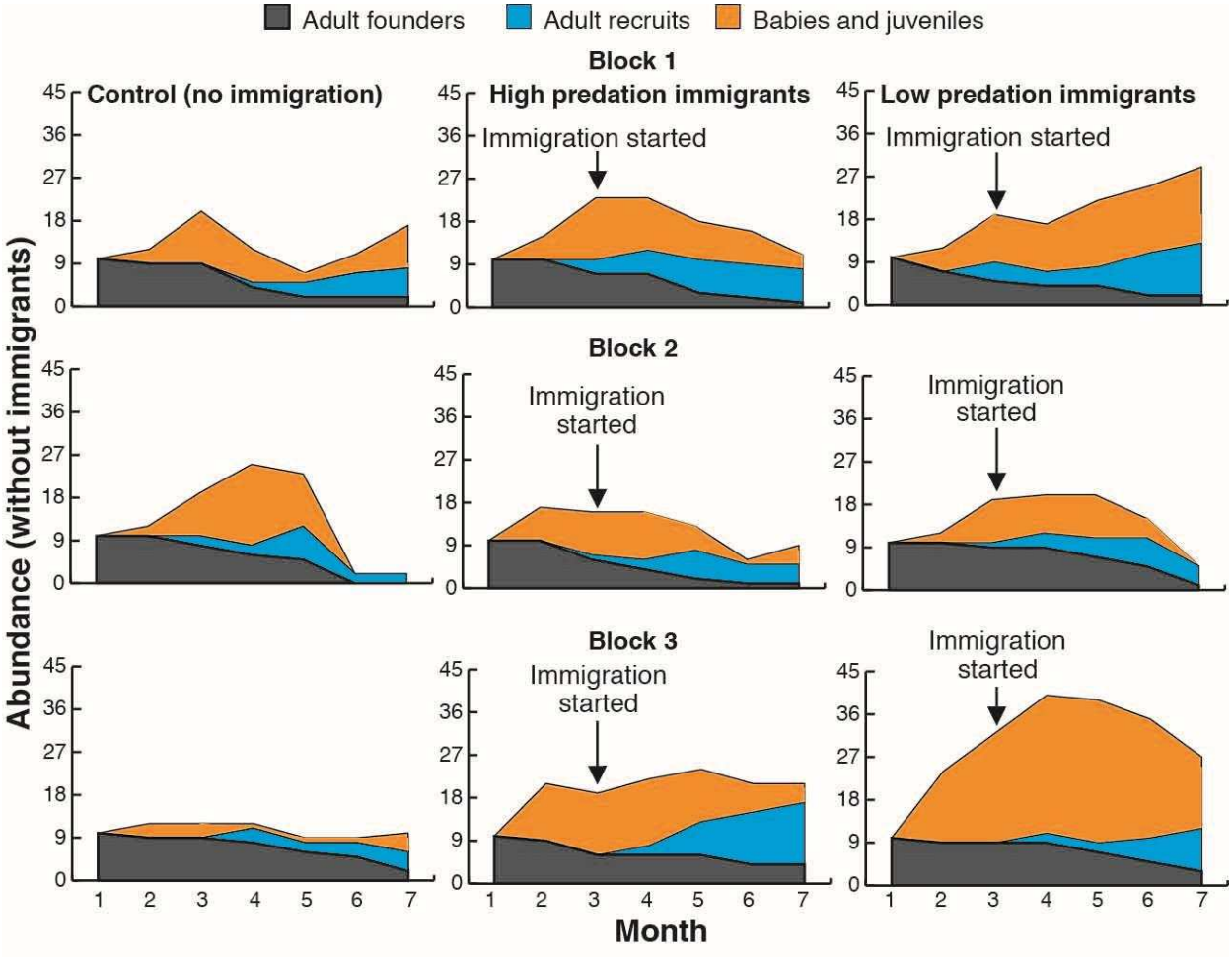

Figure S1.1 – Abundance of adult founders, adults recruited into the population, and sub-adults (< 14 mm) for each treatment and block over time.

APPENDIX 2: CHAPTER 2 SUPPLEMENTARY INFORMATION

Table S2.1 – Universal Transverse Mercator (UTM) coordinates of the sampling location for each source population, displayed as easting followed by northing.

	UTM coordinates
Quare	697227, 1181501
LPC	697161, 1180846
LPF	690134, 1188405
HPC	697242, 1180673
Marianne	685974, 1191669
LPC	685507, 1192151
LPF	693322, 1181783
HPC	685539, 1191789

Table S2.2 – Details on the microsatellite library used in this study, including the number of alleles (N_A), observed heterozygosity (H_O), and expected heterozygosity (H_E) per locus for both recipient populations.

						Quare			Marianne		
Locus	Panel	Dye	Repeat motif	Primer	Size range (base pairs)	N_A	H_O	H_E	N_A	H_O	H_E
Prgf006	2	FAM	AGAT	F: AAGAAACAAAGCCAGTCCAACAC R: TGCCTCTGGTTGGATTTATTGAC	152–324	15	0.85	0.87	32	0.94	0.95
Prgf021	1	VIC	AGAT	F: CAGGTTGCTGTCTTGTTGCTTC R: TGTCGATGTTGTCTACTGCAAAG	160–324	11	0.83	0.79	20	0.93	0.92
Prgf027	1	NED	AGAT	F: GTGGATGCAGTGTCTCTATCATG R: TTGTCACTGTTTAAGCATCTGGG	146–298	15	0.84	0.89	16	0.89	0.90
Prgf034	1	FAM	AAAG	F: CCCATTCACCCTATTTCCCAAAG R: GCCCACTCCCTTTCCGTAATATC	148–360	12	0.83	0.84	28	0.95	0.94
Prgf038	1	PET	AGAT	F: GGTCACGTGGTTTGGAAATGTC R: AAAGCATCCCGACAGTATGATTC	158–318	20	0.90	0.90	18	0.94	0.90
Prgf039	2	NED	AAAC	F: TCCCTTTCCTTGCTGAAGTTTAAG R: ACAAAGGTCTGCATAATTGTGATG	212–296	8	0.66	0.70	0	0.00	0.00
Prgf042	2	VIC	AGAT	F: ACATAACATTCCTTTAGTGCACG R: AGGAGCAATAAGAAGAAGGGTTC	126–242	4	0.12	0.14	5	0.57	0.60
Prgf043	2	PET	ATCC	F: CCTTTCCCTGTGGTGAATATTGG R: AGTCTTTGCCTCCCTACTTAGAC	179–275	7	0.56	0.54	12	0.86	0.87

Table S2.3 – Type 3 tests of fixed effects from the abundance, survival, and recruitment repeated measures linear mixed models.

Response	Fixed effect*	Num. df	Den. df	*F*	*p*
Abundance	Population	1	257	0.60	0.438
	Treatment	4	257	2.79	0.027
	Period	12	257	9.03	<0.001
	Population*Treatment	4	257	1.85	0.120
	Population*Period	12	257	0.82	0.630
	Treatment*Period	48	257	0.69	0.941
	Population*Treatment*Period	48	257	1.24	0.149
	Baseline abundance	1	257	39.78	<0.001
Survival	Population	1	197	24.89	<0.001
	Treatment	4	197	0.55	0.701
	Period	9	197	4.03	<0.001
	Population*Treatment	4	197	1.05	0.382
	Population*Period	9	197	1.42	0.180
	Treatment*Period	36	197	0.64	0.942
	Population*Treatment*Period	36	197	1.48	0.048
	Baseline abundance	1	197	1.52	0.219
Recruitment	Population	1	197	18.79	<0.001
	Treatment	4	197	0.20	0.938
	Period	9	197	6.90	<0.001
	Population*Treatment	4	197	1.35	0.252
	Population*Period	9	197	1.79	0.073
	Treatment*Period	36	197	0.92	0.605
	Population*Treatment*Period	36	197	1.21	0.203
	Baseline abundance	1	197	36.05	<0.001

*Block was included as a random effect in all models.

Table S2.4 – Type 3 tests of fixed effects from the fitness (lifetime reproductive success) generalized linear mixed model.

Response	Fixed effect*	Num. df	Den. df	*F*	*p*
Fitness	Population	1	1435	0.34	0.558
	Treatment	4	1435	0.25	0.912
	Population*Treatment	4	1435	0.39	0.819
	Group	2	1435	0.02	0.979
	Population*Group	2	1435	5.39	0.005
	Treatment*Group	6	1435	0.44	0.854
	Population*Treatment*Group	5	1435	2.15	0.057
	Period at adulthood	11	1435	7.64	<0.001
	Abundance at adulthood	1	1435	96.83	<0.001

*Mesocosm nested within block was included as a random effect.

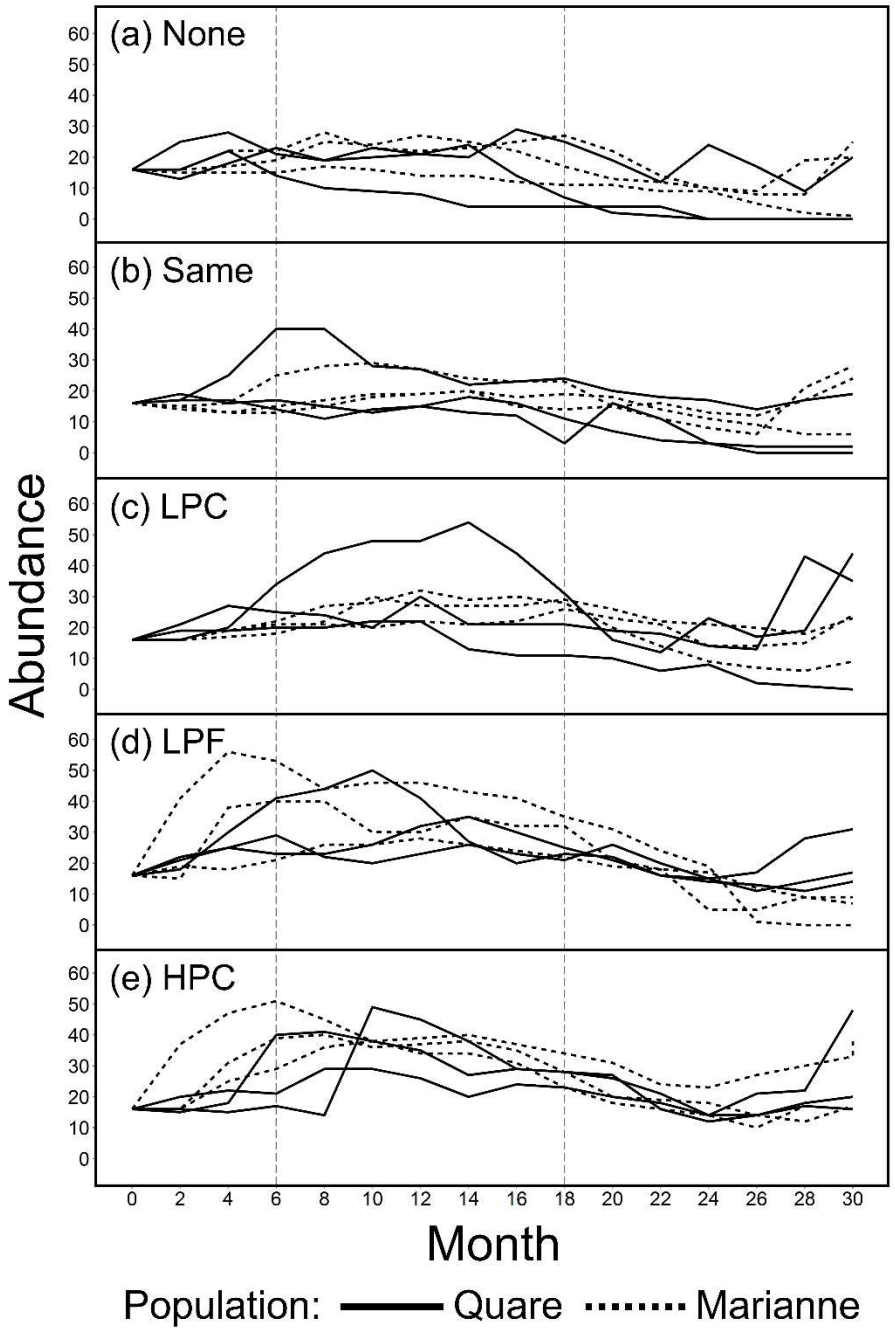

Figure S2.1 – Trends in abundance for individual mesocosms from the None (a), Same (b), LPC (c), LPF (d), and HPC (e) treatments. Vertical dashed lines indicate the start and end of immigration.