Parsing propagule pressure: Simulated and experimental disentanglement of introduction size and number of introductions for colonizing individuals

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ABSTRACT

Colonization of novel habitats is more likely with increasing propagule pressure— the total number of individuals introduced to a site. Two key components of propagule pressure are the number of introduction events and the size of those introduction events. It is unclear which of these components is more important for colonization success, or under what environmental conditions their relative importance might shift. Using demographic simulations paired with a *Tribolium* flour beetle microcosm experiment, we introduced 20 total individuals into replicated novel habitats of stable or fluctuating quality and manipulated the number and size of introduction events used to distribute them through time. After seven generations, we evaluated how different combinations of the number and size of introductions affected establishment probability, size of established populations, and whether effects depended on the variability of the recipient environment. We found no effect of biologically realistic environmental stochasticity on establishment probability or size of established populations in the demographic simulations. However, there was strong evidence that establishment probability was enhanced with more, smaller introductions. In the microcosm, we similarly found no effect of environmental stochasticity on establishment probability, but unlike the simulations found that populations that established were larger in the stable environment, especially with more introduction events. The microcosm experiment yielded greater overall establishment probability and larger populations compared to the demographic simulations. Genetic mechanisms likely underlie these differences in results and thus deserve more attention in efforts to parse propagule pressure. Our results highlight the importance of preventing further introductions of undesirable species to invaded sites, and suggest conservation efforts should focus on increasing the number of introductions or re-introductions of desirable species rather than increasing the size of those introduction events.

KEYWORDS: invasion, reintroduction, propagule pressure, biocontrol, conservation, microcosm, population dynamics, stochasticity, simulation

INTRODUCTION

Colonization is the ecologically fundamental process of population establishment in an unoccupied site, and it underlies the past, present, and future distributions of species. Colonization occurs naturally, but is increasingly prevalent due to anthropogenic influences (Sakai et al. 2001, Cassey et al. 2005, Ricciardi 2007). Incipient populations often face environments that are entirely novel, which is especially likely in the case of anthropogenic colonization (Cassey et al. 2005, Ricciardi 2007). Regardless of whether colonization events to novel habitats are natural (e.g., range expansion) or human-mediated (e.g., biological invasions, reintroductions of rare species, release of biological control agents), their successes or failures have significant implications for natural resource managers and society (Mack et al. 2000).

Most introductions to novel habitats fail, and colonization success can be difficult to predict (Lockwood et al. 2005, Zenni and Nuñez 2013). Incipient populations are commonly small, and face threats from environmental, demographic, and genetic stochasticity (Lande 1988, 1993, Fauvergue et al. 2012). Furthermore, the success of any given population can be idiosyncratic with respect to taxonomy and geography (Lodge 1993, Lockwood et al. 2005). Thus, it is crucial to understand more general features of the colonization process beyond the particular invading organism or the particular invaded environment (Lockwood et al. 2005). Propagule pressure is one such general feature that is the only known consistent predictor of colonization success in novel habitats (Lockwood et al. 2005, Colautti et al. 2006, Simberloff 2009).

Propagule pressure is the total number of potentially reproductive individuals (e.g., adults, eggs, seeds, vegetative material) introduced to an area (Novak 2007). It is often described in this broad sense, which belies its complexity. Two important components of propagule pressure are the number of introduction events— sometimes termed propagule number, and the number of individuals per introduction event— sometimes termed propagule size (*sensu* Fauvergue et al. 2012). Here, we use "introduction regime" to refer to different combinations of the number of introduction events and the number of individuals introduced per event. The same total propagule pressure, N, can be achieved by different introduction regimes depending on how those N individuals are distributed in time or space (Haccou and Iwasa 1996). An introduction regime of N individuals lies on a continuum bounded by maximizing the number of individuals introduced per event (all N individuals introduced in one event to the same location) and maximizing the number of introduction events (one individual introduced in each of N sequential events through time or to each of N unique locations).

Colonization success increases with total propagule pressure, but it is unclear whether the correlation is driven by the number of individuals introduced per event or the number of introduction events (Lockwood et al. 2005, Colautti et al. 2006, Simberloff 2009). Historical data suggest that multiple introductions can facilitate population establishment, but conclusions from these studies are limited by the inability to control for the total number of individuals introduced (Hopper and Roush 1993, Blackburn and Duncan 2001, Grevstad et al. 2011, Fauvergue et al. 2012). Models that hold the total propagule pressure constant agree that multiple, small introductions distributed across space will lead to greater establishment probability compared to a single, large introduction when Allee effects are weak (Haccou and Iwasa 1996, Grevstad 1999, Schreiber and Lloyd-Smith 2009). However, both modeling and empirical approaches have

generated conflicting views on how an introduction regime affects colonization success when introductions are distributed through time and total propagule pressure is constant.

There is evidence from both models and experiments that colonization success can increase with more, smaller introduction events through time. Branching process models show that, in the long run, several, small introductions will always be more likely to successfully establish a population than a single, large introduction (Haccou and Iwasa 1996, Haccou and Vatutin 2003). This finding was corroborated by simulations with no Allee effects (Drolet and Locke 2016). In a *Daphnia* microcosm experiment, increasing introduction frequency (proportional to the number of introductions) positively affected population growth, but the number of individuals per introduction had no detectable effect. This pattern did not hold for establishment probability, which was only affected by total propagule pressure (Drake et al. 2005). However, Drake et al. (2005) did not continue scheduled introductions if a population went extinct, denying those populations one of the main benefits of repeated introductions and creating variability in the total propagule pressure. In a more recent experiment, several, small introductions led to a 65% increase in abundance of successfully colonizing invasive Pacific Oyster (*Crassostrea gigas*) compared to a single, large introduction (Hedge et al. 2012).

There is also evidence from both models and experiments that colonization success increases with fewer, larger introduction events through time. In simulations of bird invasions, a single, large introduction event always led to the greatest establishment probability (Cassey et al. 2014). When Drolet and Locke (2016) included Allee effects in their simulations, their previously observed pattern reversed and colonization success was instead more likely with fewer, larger introductions than with more, smaller introductions. In a field experiment with the psyllid biocontrol agent, *Arytainilla spartiophila*, the number of individuals per introduction event was a

111 better predictor of establishment success than the number of introduction events (Memmott et al.
112 2005). In this case however, the introduction regimes with the most individuals per event also
113 had the highest total propagule pressure (Memmott et al. 2005). In an experiment that controlled
114 total propagule pressure, a single, large introduction of the non-native mysid, *Hemimysis*
115 *anomala*, led to larger populations and greater survival probabilities compared to several, small
116 introductions through time (Sinclair and Arnott 2015).

117 Environmental stochasticity in the recipient environment may also affect which introduction
118 regime is optimal for colonization. Branching process models show that a more variable
119 environment can reduce the probability of population establishment for all introduction regimes
120 (Haccou and Iwasa 1996, Haccou and Vatutin 2003), and simulations of introductions distributed
121 in space suggest that greater environmental variability will magnify the benefit of multiple
122 introductions (Grevstad 1999). However, simulations of introductions through time did not
123 support either of these outcomes— a single, large introduction was most likely to establish a
124 population even with extreme levels of environmental stochasticity (Cassey et al. 2014).

125 Thus, modeling and empirical approaches have not resolved how different introduction regimes
126 with a fixed total propagule pressure will affect colonization success when introductions are
127 distributed through time. Further, there has been no experimental test of whether variability in
128 the recipient environment interacts with the introduction regime to affect colonization. We paired
129 demographic simulations with a laboratory microcosm experiment using the red flour beetle,
130 *Tribolium castaneum,* to reconcile conflicts in the literature and test how different introduction
131 regimes implemented through time affected colonization success in novel habitats. We explicitly
132 manipulated whether the novel habitat was stable or randomly fluctuating in quality to assess
133 how the success of different introduction regimes may depend upon variability in the recipient

environment. With the total number of individuals introduced held constant at 20, we varied the size and number of introduction events used to distribute those individuals in four different introduction regimes. We evaluated establishment probability and population size over 7 discrete generations to ask: 1) does colonization success increase with more introduction events or with more individuals per introduction event?, and 2) does the effect of the introduction regime on colonization success depend on whether the recipient novel environment is stable or fluctuating through time?

METHODS

General Framework

In simulations and a microcosm experiment, we evaluated the outcome of introducing 20 total individuals to one of two environmental contexts (a stable or fluctuating novel environment), varying the number of introduction events used to distribute those individuals through time. This total was low enough to allow some population extinction within a reasonable timeframe, but high enough to be representative of documented introductions in the literature (Simberloff 1989, Simberloff 2009; Grevstad 1999; Berggren 2001; Taylor et al. 2005; Drake et al. 2005). The introduction regimes were: 20 individuals introduced in the first generation, 10 individuals introduced in each of the first 2 generations, 5 individuals introduced in each of the first 4 generations, and 4 individuals introduced in each of the first 5 generations. To create the fluctuating environment, we imposed a magnitude of variability corresponding to environmental stochasticity in nature, which leads to frequent, mild to moderate perturbations in population growth rate due to external forces (Lande 1993). Populations were tracked for seven generations following the initial introduction.

Establishment probability and the size of established populations were used as measures of colonization success. Populations were deemed 'established' for any time step in which they were extant and population size was noted for all extant populations in every time step. For analysis, establishment probability and mean population size were assessed in generation 7.

Study System

Our simulations and microcosm experiment use *Tribolium castaneum* (red flour beetles) to model the life history of organisms with discrete, non-overlapping generations (e.g., annual insects and fishes) following Melbourne and Hastings (2008). Individual simulated colonists were randomly sourced from a randomly mating, infinitely large population. Individual experimental colonists came from a thoroughly mixed source population maintained at 800 individuals in each of the 4 temporal blocks over which the experiment was replicated. To obtain colonists, beetles from the source population were mixed freely on a plate, selected from many sections of the plate, and introduced to subsets of the experiment populations in a random order (about 24 subsets per block). All migrant females were assumed to arrive mated from the source population. The strong maternal effects exhibited by *Tribolium* flour beetles were reduced by rearing individuals from the source population on novel growth medium for 1 generation prior to using them as colonists (Van Allen and Rudolf 2013, Hufbauer et al. 2015).

Simulations

Population dynamics were simulated with a Negative Binomial-Binomial gamma (NBBg) Ricker model (Melbourne and Hastings 2008) with an additional mating function of the form:

$$N_{t+1,i} \sim NegBinom\left(\mu_i, k_D F_{mated_{(t,i)}}\right)$$

$$\mu_i = \frac{F_{mated_{(t,i)}}}{p} R_{E_i} e^{-\alpha N_{t,i}}$$

$$R_{E_i} \sim Gamma\left(k_E, \frac{R_0}{k_E}\right)$$

$$N_{t,i} = N_{migrants_{(t,i)}} + N_{residents_{(t,i)}}$$

$$F_{mated_{(t,i)}} = \begin{cases} F_{migrants_{(t,i)}}, & F_{migrants_{(t,i)}} + F_{residents_{(t,i)}} = N_{t,i} \\ F_{migrants_{(t,i)}} + F_{residents_{(t,i)}}, & F_{migrants_{(t,i)}} + F_{residents_{(t,i)}} < N_{t,i} \end{cases}$$

$$F_{migrants_{(t,i)}} \sim Binom\left(N_{migrants_{(t,i)}}, p\right)$$

$$F_{residents_{(t,i)}} \sim Binom(N_{residents_{(t,i)}}, p)$$

$$p = 0.5$$

$$R_0 \sim Gamma(2.6, 1)$$

$$\alpha \sim Gamma(0.0037, 1)$$

$$k_E \sim Gamma(17.6, 1)$$

$$k_D \sim Gamma(1.07, 1)$$

(Eq. 1)

Where $N_{t+1,i}$ is the size of population i at time $t+1$, k_D represents stochasticity due to demographic heterogeneity, μ_i is the expectation of the size of population i in the next time step, $F_{mated(t,i)}$ is the latent number of mated females in population i at time t, p is the probability of an individual being female, R_{Ei} is the latent density-independent population growth rate for environment E, α is the egg cannibalism rate, $N_{t,i}$ is the total size of population i at time t, k_E represents environmental stochasticity, R_0 is the density-independent population growth rate for the average environment, $N_{migrants(t,i)}$ is the total number of migrants to population i at time t, and $N_{residents(t,i)}$ is the total number of residents in population i at time t.

We imposed a mating function such that a population would deterministically go extinct if it comprised only non-migrant females. In cases with an all female population and a mixture of

187 residents and migrants, we only included the number of migrant females in the density-
188 dependent effect of the demographic heterogeneity term $k_D * F_{mated(t,i)}$ because this effect
189 manifests via the number of eggs laid by females and only migrant females, being pre-mated,
190 would lay eggs. Weakly regularizing gamma priors were taken from Melbourne and Hastings
191 (2008). The expected equilibrium population size for the model is:

192 $$K = \frac{\log(R_0)}{\alpha} \quad \text{(Eq. 2)}$$

193 For each combination of the 4 introduction regimes and 2 recipient environment types (described
194 above), we simulated 500,000 replicate populations for 7 generations. All simulations were
195 performed in R (R Core Team 2016). This model captures the stochastic and deterministic
196 ecological dynamics of the *Tribolium* system well, but does not include evolutionary processes
197 (Melbourne and Hastings 2008, Hufbauer et al. 2015).

198 To parameterize the model, we censused 125 *Tribolium* populations one generation after
199 establishing them at various, known densities (between 5 and 200 individuals) on the novel
200 growth medium following the rearing procedure described in "Microcosm Experiment" below.
201 We fit the hierarchical model (Eq. 1) to these data in a Bayesian framework to generate posterior
202 distributions for each of the parameters using the nimble package in R (de Valpine et al. 2016).
203 We used a Metropolis-Hastings random walk sampler with 3 chains having 50,000 samples each
204 (including 10,000 samples that were removed for burn in). The chains converged (multivariate $\hat{R}$
205 for the 4 key parameters =1.01) and produced a sufficient number of effective samples (R_0: 757,
206 α: 548, k_E: 4103, k_D: 1703).

207 A single novel growth medium mixture was used for these 125 populations (mixture 5,
208 Supplementary Table 1), so we treated the estimated environmental stochasticity parameter, k_E,
209 as the expectation for a stable environment. We parameterized a fluctuating environment model
210 by increasing the standard deviation of the density-independent per capita population growth rate
211 by approximately 10% for any given population at any given time step. This was accomplished
212 by multiplying all of the samples from the posterior distribution of the environmental
213 stochasticity parameter, k_E, by 100/121 (derived by moment matching for the gamma
214 distribution). By using random sets of parameters drawn from the samples of the posterior
215 distributions, we were able to propagate the model uncertainty through the simulations and
216 combine it with the deterministic and stochastic dynamics represented by the model itself.

217 *Microcosm Experiment*

218 We founded 842 *Tribolium* populations with different introduction regimes (20 individuals in the
219 first generation, 10 individuals in the first 2 generations, 5 individuals in the first 4 generations,
220 or 4 individuals in the first 5 generations) and environments (stable or fluctuating) with between
221 96 and 120 replicate populations per treatment combination (Supplemental Table 3). Each
222 population was reared in a 4cm x 4cm x 6cm plastic box (AMAC Plastic Products) with 2
223 tablespoons (approximately 15 g) of freshly prepared growth medium that had been humidified
224 for at least 24 hours. The growth medium used for the source population (the natal environment
225 of the colonists) comprised 95% wheat flour (Pillsbury Co. or Gold Medal Products Co.) and 5%
226 brewers' yeast (Sensient Flavors). Colonists were introduced into a novel growth medium
227 comprising a small percentage of natal medium mixed with corn flour (Bob's Red Mill). All
228 populations were reared in one of two dark incubators at 31° and approximately 70% relative
229 humidity (standard conditions) and were haphazardly rotated between incubators weekly.

230 For each population in each generation, a known number of adults laid eggs for 24 hours in fresh
231 medium, and were then removed. Offspring were given 35 days to develop, and adults were then
232 censused. Censused adults laid eggs on freshly prepared growth medium for 24 hours,
233 completing their laboratory life cycle. In censusing trials, we estimated the maximum
234 observation error to be 4.6% (median: 0%, mean: 0.26%) with no detectable difference across
235 observers or populations with different sizes.

236 Each replicate population experienced a novel environment that was either stable or randomly
237 fluctuating through time. The same novel growth medium mixture containing 99.05% novel corn
238 flour and 0.95% natal medium (mixture 5, Supplementary Table 1) was used for the stable
239 environment for the duration of the experiment, which preliminary results indicated would yield
240 a population growth rate of $\lambda = 1.2$ compared to a mean population growth rate of 3.36 on 100%
241 natal medium. To create the fluctuating environment, we randomly selected a novel growth
242 medium mixture from one of 9 possible media mixtures for each population in each generation.
243 Each population in the fluctuating environment therefore experienced a unique series of
244 environmental conditions. The 9 possible media mixtures represented a gradient of corn flour to
245 natal medium ratios, and were designed to yield expected population growth rates between 0.88
246 and 1.33 (Supplementary Table 1). We chose this range to mimic environmental stochasticity
247 measured in nature (Sæther and Engen 2002) while remaining within the bounds of biologically
248 realistic population growth (λ between 0.5 and 1.5) (Morris et al. 2008). The random choice was
249 constrained such that the expected geometric mean population growth rate for each population
250 resembled expected growth of populations in the stable environment ($\lambda_{expected} = 1.2 \pm 0.05$).

251 We estimated the amount of environmental stochasticity that we achieved in the fluctuating
252 environment as the difference in mean total stochasticity between populations in the fluctuating

253 and stable environments (Sæther and Engen 2002). We assumed that total stochasticity was a
254 combination of demographic and environmental stochasticity for populations in the fluctuating
255 environment, and that demographic stochasticity was the sole contributor to total stochasticity
256 for populations in the stable environment. Total stochasticity (demographic plus environmental)
257 of each population that did not experience extinction (n=667) was calculated as the variance of
258 the natural logarithms of its population growth rates through 7 generations:

$$s_{total} = s_{demographic} + s_{environmental} = var(\log(\lambda_t)) \quad \text{(Eq. 3)}$$

259 where, for a particular population, s_{total} is its total stochasticity, $s_{demographic}$ is its demographic
260 stochasticity, $s_{environmental}$ is its environmental stochasticity (assumed to be 0 for populations
261 in the stable environment), and λ_t is its per capita population growth rate between generation *t-1*
262 and generation *t* (*t=1, 2, … , 7*). We only calculated total stochasticity for populations that did
263 not experience any extinction in order to capture the full temporal extent of environmental
264 fluctuations and because extinctions would have an infinite effect on this measure of
265 stochasticity.

266 *Statistical analyses*

267 We evaluated how our environment treatment affected variability in population growth rate (total
268 stochasticity from Eq. 3) using a linear mixed effects model with environment (stable or
269 fluctuating) as a fixed effect and block as a random intercept effect.

270 We used a mixed effects logistic regression with a logit link to predict the binary response of
271 establishment, and a mixed effects Poisson regression with a log link to analyze population size.
272 In both models, introduction regime, environment treatment, and their interaction were treated as
273 fixed effects, and block was treated as a random intercept effect.

274 We assessed the effect of temporary extinctions on the establishment probability and mean
275 population size by fitting the generalized linear mixed effects models described above to data
276 from the multiple introduction regimes (i.e. not the 20x1 regime) and with additional predictor
277 variables. To assess the effect of the presence of a temporary extinction, we included an
278 additional Boolean predictor for whether a population went temporarily extinct or not. To assess
279 the role of the total propagule pressure, we included a numeric predictor representing the number
280 of beetles introduced after the latest temporary extinction.

281 Group-level significance of fixed effects was tested using likelihood ratio tests on nested models,
282 and least-squares contrasts were used to compare levels of the fixed effects. All statistical
283 analyses were performed in R, version 3.3.2 (R Core Team 2016). Generalized linear mixed
284 models were fit using the lme4 package, version 1.1-12 (Bates et al. 2015) and pairwise
285 comparisons were made using the lsmeans package, version 2.25 (Lenth 2016).

286 *Data availability*

287 The raw experiment data, simulated population trajectories, R code for the simulations, R code
288 fitting the NBBg model, samples from the posterior distributions of the NBBg parameters, and R
289 code fitting the mixed effects models are available as supplemental material, on Figshare
290 (https://doi.org/10.6084/m9.figshare.4648865.v1), and on GitHub
291 (https://github.com/mikoontz/ppp-establishment).

292 *Note on egg contamination*

293 Laboratory procedures after generation 3 resulted in occasional egg contamination between
294 replicate populations of the same treatment. Some populations of 1 individual persisted when

they should have deterministically gone extinct, so we manually edited those population trajectories such that they went extinct in the generation after having only 1 individual.

RESULTS

Simulations

Summary statistics for the posterior distributions of the four NBBg model parameters are given in Supplementary Table 2.

Our simulations showed introduction regimes with more introduction events were more likely establish a population by the seventh generation (Figure 1). Introductions into a stochastically fluctuating environment resulted in slightly lower population establishment for all introduction regimes (difference of ~1%), but did not favor any particular regime (not shown). The mean population sizes for each introduction regime/environment combination were approximately equal in simulations. Mean population sizes only incorporated extant populations, so they were slightly larger than the expectation for the equilibrium population size (Figure 2).

Microcosm experiment

Mean environmental stochasticity of populations in the fluctuating environment was 0.052 (95% CI = 0.0073 to 0.0966; p = 0.023). This value is near the median value of 0.055 measured in nature by Sæther and Engen (2002) in a metaanalysis of 35 avian populations, indicating that we achieved biologically realistic fluctuations in population growth rate.

We found no evidence that the probability of establishment was affected by a main effect of environment (χ^2=0.72, df=1, p=0.40), nor by an interaction between environment and

315 introduction regime (χ^2=3.49, df=3, p=0.32). However, there was strong support for an effect of
316 introduction regime on establishment probability (χ2=59.76, df=3, p<0.0001). Pairwise
317 comparisons of the different introduction regimes averaged across the environment treatments
318 revealed that the 4x5 regime was the most likely to establish populations, with a probability of
319 about 0.98, whereas the 20x1 and 10x2 regimes were the least likely to establish populations,
320 with a probability reduced to about 0.8 (Figure 1).

321 The mean size of populations that persisted until generation 7 was shaped by significant effects
322 of introduction regime (χ^2=91.65, df=3, p<0.0001), environment treatment (χ^2=117.83, df=3,
323 p<0.0001), and their interaction (χ^2=44.62, df=3, p<0.0001). Populations established via more
324 introduction events were larger when averaged across the environment treatments. Extant
325 populations in the stable environment were larger than those in the fluctuating environment when
326 averaged across the introduction regimes. The interaction manifests as the benefit of a stable
327 environment increases with more, smaller introduction events (Figure 2).

328 Extinctions accumulated regularly throughout the experiment period, with 101 out of 842
329 populations (12.0%) going extinct by generation 7 (Supplementary Figure 2). The additional
330 introductions that some populations received often restored a population that had temporarily
331 gone extinct. Out of 602 populations that received more than 1 introduction (i.e. not the 20x1
332 introduction regime), 104 of them (17.3%) temporarily went extinct at least once before being
333 replenished by additional colonizing individuals. Twelve populations were rescued in this way at
334 least twice, and one population was rescued in this way three times.

335 Temporary extinctions significantly affected colonization success. The presence of a temporary
336 extinction significantly reduced average establishment probability from 92.4% to 82.1%

(difference = -1.13 on logit scale, 95% CI = -0.22 to -2.04, χ^2 = 5.44, df = 1, p = 0.020) and mean population size from 47.8 to 45.4 (difference = -0.052 on log scale, 95% CI = -0.02 to -0.09, χ^2 = 9.42, df = 1, p=0.0021). Each additional colonist contributing to a population after the latest temporary extinction significantly increased the mean population size (estimate = 0.005 on the log scale, 95% CI = 6.4e-05 to 0.01, χ^2 = 3.94, df = 1, p=0.047).

Results from the simulations are expected to represent the dynamics in the microcosm experiment if the assumption of the model on which the simulations were built holds—that only demographic processes are responsible for the dynamics observed in the experiment. However, establishment probability at generation 7 was equal to or greater than that expected from the simulations (Figure 1) and mean population sizes were much larger in the experiment than in the simulations (Figure 2), together suggesting an influence of non-demographic mechanisms.

DISCUSSION

We assessed how the number and size of introduction events through time drive colonization success in a novel environment when the total number of individuals introduced to a site is fixed. We considered novel environments that were either stable or randomly fluctuating in quality through time, and evaluated populations through 7 discrete generations. We approached this question in two ways: 1) stochastic simulations of a demographic population dynamics model parameterized with empirical data, and 2) a highly replicated laboratory microcosm experiment. By coupling these approaches, we were able to expand and test the theoretical understanding of how the introduction regime affects colonization in stable and fluctuating environments as well as to develop new avenues for research when observations did not align with predictions. We

found that several, small introductions increase colonization success and that demographic processes alone are insufficient to explain the dynamics observed in the experiment.

We found minimal to no effect of a biologically realistic level of environmental stochasticity on establishment probability in demographic simulations. This finding is inconsistent with the prediction made by Grevstad (1999) who found with simulations that several, small introductions would produce an especially high establishment probability compared to a single, large introduction in a variable environment. The difference in findings is perhaps due to a difference in the kinds of introduction regimes modeled: Grevstad (1999) simulated multiple introductions distributed in space, while we focused on introductions separated in time. Additionally, we may not have detected the same effect of environmental stochasticity that Grevstad (1999) noted because of differences in the magnitude of environmental variation imposed. At the highest levels of simulated environmental variation imposed in Grevstad's study, over 65% of environments would result in population decline and over 40% of environments would result in population loss of greater than 90%. This kind of environmental variability might be characterized as random catastrophes—major events causing immediately disastrous effects to the population, rather than a level of environmental stochasticity leading to more moderate fluctuations in growth rates (Lande 1993). Our simulations and microcosm results do corroborate the results from simulations by Cassey et al. (2014) who also used introductions through time and found a minimal effect of environmental stochasticity on establishment probability. Cassey et al. (2014) further found a minimal effect of random, infrequent catastrophes and bonanzas, suggesting that increasing environmental variability through time in the broadest sense (i.e., encompassing environmental stochasticity, random catastrophes, and bonanzas) does not

380 magnify the benefit of several, small introductions through time in the same way that Grevstad
381 (1999) found with several, small introductions across space.

382 There also did not appear to be a strong effect of environmental stochasticity on population size
383 in the demographic simulations. All treatments in the simulations reached similar mean
384 population size by generation 7, which was slightly higher than the equilibrium size due to our
385 measure only including extant populations. This suggests that strong density dependence in the
386 demographic simulations was the primary influence on population size.

387 We observed striking differences in the measures of colonization success between the
388 demographic simulations and the microcosm experiment. We found that establishment
389 probability increased with the number of introduction events (as in the simulations), but that all
390 microcosm establishment probabilities equaled or exceeded expectations from simulations. In the
391 microcosm, mean population size in stable environments was greater than in fluctuating
392 environments. There was also an interaction between environment and introduction regime,
393 whereby the mean population size was increasingly greater in stable compared to fluctuating
394 environments as the number of introductions increased. Furthermore, populations grew larger by
395 generation 7 in the microcosm experiment than in the simulations.

396 Departures from the simulation expectations in the microcosm experiment suggest that the
397 demographic processes captured by the model do not account for all of the biological processes
398 that occurred in the microcosm. Recent work shows that adaptation to the novel, harsh
399 environment from standing variation is possible in this species (Hufbauer et al. 2015), and likely
400 explains the greater establishment probability and population sizes in the microcosm compared
401 to expectations derived from demographic simulations which do not include adaptation.

402 The rescue effect of immigration can act demographically by increasing the size of populations
403 (Brown and Kodric-Brown 1977). Certainly, demographic rescue played a critical role for the
404 104 populations that went extinct temporarily until another introduction event revived them.
405 Those temporary extinctions had lasting effects on colonization success. Colonization success
406 declined for populations that experienced a temporary extinction, and the mean population size
407 significantly increased if more colonists contributed to the population after a temporary
408 extinction. These results reflect the overarching importance of total propagule pressure
409 regardless of introduction regime.

410 The rescue effect can also act genetically by increasing the fitness of populations (Brown and
411 Kodric-Brown 1977, Frankham 2015, Whiteley et al. 2015). The immigrants all came from the
412 same large external source population, so it is unlikely that gene flow from migrants produced
413 novel genetic combinations on which selection could act (Novak 2007). Thus, a more likely
414 mechanism by which immigration increased mean population size was by relieving inbreeding
415 depression or counteracting drift-induced allele loss. Small populations are more prone to
416 experiencing increased homozygosity and inbreeding depression, which can reduce population
417 growth rates and increase extinction risk (McCauley and Wade 1981, Saccheri et al. 1998,
418 O'Grady et al. 2006, Szűcs et al. 2014, 2017). Even small amounts of gene flow can alleviate
419 these effects, so the additional small introductions of mated individuals from the external source
420 population were well-suited to bring about longer-term relief (Slatkin 1985, Mills and Allendorf
421 1996). However, Cassey et al. (2014) found that simulated inbreeding depression was especially
422 detrimental for several, small introductions through time, so other mechanisms might be at play.

423 Sustained immigration may affect adaptation of the incipient population. Introductions to a harsh,
424 novel habitat can result in adaptive evolution with the right amount of gene flow if additional

immigrants to a declining population prevent extinction long enough to allow for adaptation to occur (Holt and Gomulkiewicz 1997). However, too much gene flow can lead to genetic swamping whereby the homogenizing effects of gene flow overpowers ongoing local adaptation (Mills and Allendorf 1996, Lenormand 2002). This may have been the case for populations in the 10x2 introduction regime, which had the highest average migration rate, lowest establishment probability, and lowest mean population size. Alternatively, negative density dependence may have reduced population fitness when migration rates were high, reducing population growth rates and hampering the spread of adaptive alleles (Holt and Gomulkiewicz 1997). Though not significant, the lower establishment probability and mean population size in the 10x2 introduction regime warrants further work to assess whether they exemplify the yet-unseen scenario described by Blackburn et al. (2015) in which maladaptive gene flow from multiple introductions hampers population establishment in a novel range. More broadly, further study is necessary to evaluate how immigration affected adaptation in this system, if at all (Gomulkiewicz and Holt 1995, Boulding and Hay 2001).

CONCLUSION

Our experimental results suggest that several, small introductions through time lead to greater colonization success in a novel habitat, and that introductions into a stable recipient environment lead to larger population sizes, but not greater establishment probability. Further, introductions to a stable recipient environment are especially beneficial to populations established with more introduction events. These results defied our expectations derived from parallel simulations of a model that included demographic processes but not evolutionary ones, so we suspect a genetic mechanism might be at work. Genetic mechanisms are rarely incorporated when simulating the effect of introduction regime on colonization (but see Cassey et al. 2014), and our multi-

generation microcosm is unique in bringing evolutionary processes to bear on parsing two key components of propagule pressure in an experimental setting.

For invasions, our results highlight the importance of preventing further introductions to the same location, even for established species. For conservation and biological control, our results suggest that emphasis should be placed on increasing the number of introductions or re-introductions to a location, rather than increasing the size of those events if Allee effects are weak. Sustained introduction efforts should also bring about concomitant benefits in the form of longer-term monitoring, increased data collection, and more opportunities for experimentation and adaptive management (Lockwood et al. 2005, Godefroid et al. 2011).

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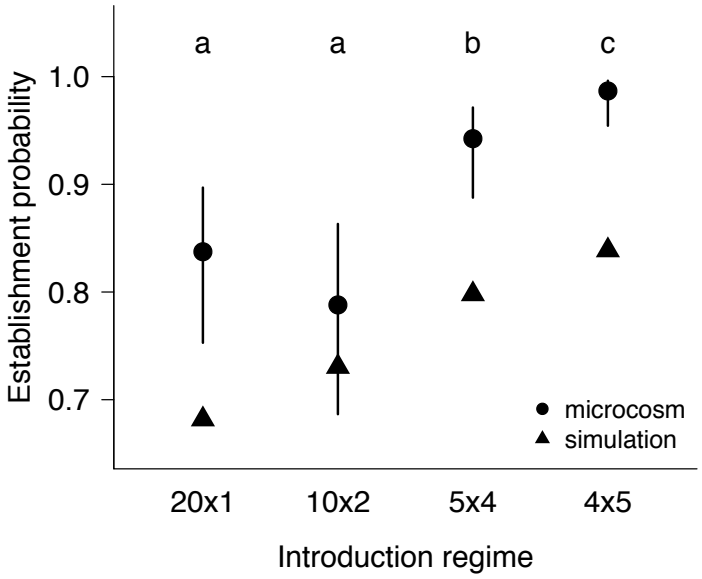

Figure 1. Establishment probabilities for each introduction regime assessed at generation 7. Triangles represent results from simulations. Dot-whiskers represent estimates and 95% confidence intervals from the mixed effects logistic regression model fit to data from the microcosm experiment. Different letters over dot-whiskers represent introduction regimes with significantly different establishment probabilities.

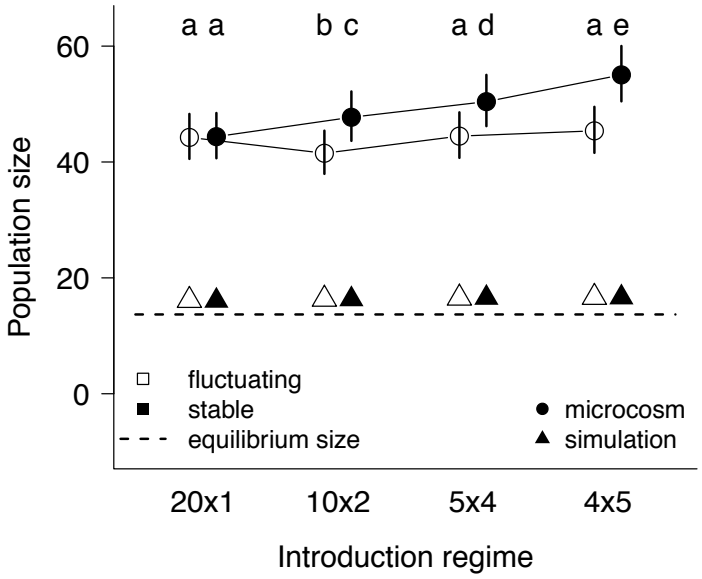

Figure 2. Mean sizes of extant populations for each introduction regime/environment combination in generation 7. Triangles represent results from simulations. Dot-whiskers represent estimates and 95% confidence intervals from the mixed effects Poisson regression model fit to data from the microcosm experiment. Different letters over dot-whiskers represent introduction regimes with significantly different mean population sizes. The dashed line represents the theoretical equilibrium population size derived from the NBBg model, which includes extinction and is therefore lower than the mean population size from the simulations.