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# Uncovering the Role of Propagule Pressure in Determining Establishment Success Using a Synthetic Biology Approach

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# Thesis of
# Michael D. Dressler

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HALMOS COLLEGE OF NATURAL SCIENCES AND OCEANOGRAPHY

# Uncovering the Role of Propagule Pressure in Determining Establishment Success Using a Synthetic Biology Approach

By

Michael Dressler

Submitted to the Faculty of
Halmos College of Natural Sciences and Oceanography
in partial fulfillment of the requirements for
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# Abstract

The spread of invasive species poses a major ecological and economical threat. Consequently there are ongoing efforts to develop a generalizable mechanism to predict establishment success of non-native species. One proposed mechanism to predict establishment success is propagule pressure, which is defined as the number of individuals introduced at a given time. Although some studies have demonstrated a positive correlation between propagule pressure and establishment success, others have not, and the effect of propagule pressure on establishment success remains unclear. To address this challenge, a strain of bacteria engineered with an Allee effect,  a growth dynamic that is often associated with establishing species, was used. The timing between successive introduction events that resulted in establishment success was measured. It was observed that if the time between two introduction events was sufficiently long, growth did not occur. By manipulating the growth rate of the bacteria, it was shown that that the minimal time between the two introduction events that resulted in growth was constrained as growth rate decreased. Moreover, it was concluded that asymmetry in the density of bacteria introduced in the introduction events increased the maximum time between introduction events that resulted in growth. These results help to remedy conflicting data in the literature by identifying conditions where propagule pressure has, and does not have, a positive impact on establishment success. These findings can have major implications in understanding and predicting the unique population dynamics of invasive species.

Key words: Allee effect, propagule number, propagule size, engineered bacteria, invasion

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# Chapter I: Establishment, Invasion and Propagule Pressure

Invasive species are widely recognized as a global threat to the preservation of terrestrial and marine ecosystems. By definition in Executive Order No. 13112, an "invasive species" is a non-native organism whose introduction does or is likely to cause economic, environmental harm, and/or harm to human health (Exec. Order No. 13112, 1999). Invasive species account for more than $1.4 trillion in annual economic damages on the global scale (Pimentel et al. 2001). In the United States alone, invasive species result in $78.5 billion in economic losses and $58.3 billion in environmental damages (Pimentel et al. 2001). Second to habitat destruction, invasive species have been described as one of the largest global threats to biodiversity (e.g., Glowka et al. 1994, Williamson 1999).

The number and abundance of invasive species have reached increased levels in temperate regions of Europe, North America, and Australia, primarily due to shipping and aquaculture introductions. According to a global assessment of 329 marine species, only 16% of marine ecoregions had no reported non-native species (Molnar et al. 2008). Marine invasive species are identified across many taxa of plants (e.g., *Caulerpa taxifolia* (seaweed), *Codium fragile* ("green sea fingers"), *Sargassum muticum* (Japanese wireweed) (Smith et al. 2002)), vertebrates (e.g., *Pterois volitans* (red lionfish), *Petromyzon marinus* (sea lamprey), *Gambusia affinis* (mosquitofish) (Smith and Tibbles 1980, Albins and Hixon 2008, Cote et al. 2010)), and invertebrates (e.g., *Mnemiopsis leidyi* (comb jelly), *Carcinus maenas* (shore crab), *Dreissena polymorpha* (zebra mussel) (Grosholz and Ruiz 1996, Johnson and Carlton 1996, Shiganova 1998)). For example, *Crassostrea gigas* (Pacific oyster) has been introduced into more than 45 ecoregions (Molnar et al. 2008). Although oysters yielded an initial economic gain, they caused major ecological damage due to settling in dense aggregations, which limited food and habitat for native species (Eno 1997). Similarly, *Mnemiopsis ledyi* (comb jelly) has had negative impacts in the Black and Caspian Seas. Specifically, *M. ledyi* is a predator of zooplankton, pelagic fish eggs, and larvae of anchovy. As a result, biodiversity, abundance, and biomass of the main components of the pelagic zooplankton-ecosystem have sharply decreased since *M. ledyi* invaded these marine environments (Oguz et al. 2001, Shiganova et al. 2004). Lastly, *Eichhornia crassipes*, commonly referred to as water hyacinth, has become one of the world’s most prevalent invasive aquatic plants. Dense populations can obstruct phytoplankton production and reduce dissolved oxygen concentrations, which in turn can negatively affect organisms at higher trophic levels (Villamagna and Murphy 2010).

Despite the prevalence of invasive species in both marine and terrestrial environments, it is difficult for introduced species to become established, and only a fraction of these become invasive (see below). Invasion can be described as a process of sequential steps or filters that drive the fate of an introduced species. These steps include: (1) vector entrainment, (2) transportation and survival, (3) introduction, (4) establishment, and (5) spread (Carlton 1979, Kolar and Lodge 2001, Miller and Ruiz 2009). According to Kolar and Lodge (2001), vector entrainment is defined as a transport pathway that draws a species from its region of origin and deposits it outside of its native range (Kolar and Lodge 2001). During transportation, a species can either die or survive. Survival determines whether the species will be introduced into a non-native region. At the time of release, the survived species is said to be considered a non-native species (Kolar and Lodge 2001). The second barrier that a species must overcome is establishment. An established population can be defined as one that is unlikely to go extinct in the near future (Drake and Jerde 2009). Within a given time frame, the non-native species becomes established in the non-native region. Interactions with the invaded community then determine how widespread the non-native population will become. Therefore, invasion can be defined as a non-native species that spreads from the point of introduction and becomes abundant, overcoming biotic resistance (Kolar and Lodge 2001). If a non-native species remains localized within a non-native region, it is considered to be a non-invasive species (Kolar and Lodge 2001). According to the “tens rule”, 1-in-10 species imported into non-native areas subsequently appear in the wild, 1-in-10 of those introduced species become established, and 1-in-10 of those established species spread and become invasive (Holdgate 1986, Williamson and Brown 1986, Williamson 1993, Williamson and Fitter 1996, Williamson 1999). Consequently, 99.9% of imported species do not become invasive species. Although this percentage is small, the costs and damages resulting from invasions cannot go unnoticed.

Although the filters of the invasion process have been widely accepted, it is important to understand the underlying mechanisms which result in the development of an invasive species. As a result, a thorough understanding of how these filters operate the invasion process can contribute to current management strategies.

### *Vectors of Invasion*

Multiple vectors have been linked to transporting species that have successfully established and become invasive. Shipping vessels, perhaps one of the most contributing vectors for large introduction sizes (i.e. large numbers of individuals) (MacIsaac et al. 2002, Roman and Darling 2007), can transport non-native organisms both on a surface of the hull and via ballast water (Minchin et al. 2009). Thousands of species (Cariton and Geller 1993), ranging from viruses to fish, have been introduced by transportation of ballast water (Minchin et al. 2009). Organisms such as seaweeds, sponges (*Porifera*), mussels (*Bivalvia*), and barnacles (*Cirripedia*) can attach directly to ship hulls. Shipworms (*Teredinidae*), isopod (*Isopoda*), and amphipod (*Amphipoda*) species can attach to wooden hulls. Lastly, infaunal species can take refuge under sediment accumulation in the bottom of ballast tanks (Minchin et al. 2009).

Other vectors that have been associated with the introduction of organisms between different biogeographical regions are canals, aquaculture, commercial fisheries, trade in ornamental species, the use of live organisms as bait, leisure activities and tourism, research and education, as well as habitat restoration/management (Roman and Darling 2007, Minchin et al. 2009). Canals provide a gateway between different regions either by shipping or by organisms themselves passing through the canal (Gollasch et al. 2006, Minchin et al. 2009). Aquaculture alone can introduce species in multiple ways: growing species directly in the environment, intentional releasement (ocean ranching), escaping pens or cages, larvae released through discharges, and transportation accidents (Minchin 2007, Minchin et al. 2009). As a result, introduced organisms can impact native species through competition, and can themselves serve as vectors for parasites and other pathogens (Minchin 2007, Minchin et al. 2009). Commercial fishery projects have deliberately introduced fishes, crustaceans, and mollusks in various non-native regions, which has had negative consequences for native species and habitats. Even non-living fisheries products (fresh or frozen), often used as bait, have the ability to carry pathogens, including viruses (Minchin et al. 2009). Although release of aquarium species is associated with the introduction of few individuals per event, multiple introduction events can result in the establishment of non-native populations (Roman and Darling 2007). Similar to shipping, recreational boating and activities can be a vector source through accumulation of fouling organisms on hulls and equipment. Activities stimulating transportation of non-native organisms include fishing, diving, sailing, wind-surfing, and jet skiing (Minchin et al. 2009). Lastly, research and education institutions can serve as a source of experimental organism release as well as plantings of non-native marine and estuarine angiosperms for habitat restoration purposes. This action can lead to negative impacts on native ecosystems if not properly controlled (Minchin et al. 2009). Importantly, each of these vectors will introduce different numbers of individuals, at different rates, and over different geographical areas. Consequently, the quantitative experimental study of these vectors, and how they influence establishment success, are likely to be challenging, if not impossible, in a natural setting. Nevertheless, broad scale management strategies have been implemented towards reducing the introduction on non-natives into novel environments.

### *Current Management Strategies*

Various vector management strategies have been employed in efforts to prevent establishment of invasive species. The main objectives of these strategies are to prevent the arrival of non-native species as well as to control their transportation with trade, since it is often more difficult to control spread after arrival and establishment (Minchin et al. 2009). Raising public awareness and implementing codes of practice can reduce the risk of introducing non-native species (Minchin et al. 2009). For example, the ICES Code of Practice aims to reduce the spread of non-native species by encouraging countries to implement policies and procedures. For example, member countries are required to provide a detailed proposal for introductions, using internationally recognized protocols for introduction, meeting current commercial practice codes (e.g., the OIE International Aquatic Animal Health Code), and mandatory licensing for releasing genetically modified organisms (including triploid and polyploid organisms) (ICES 2005). Additional governing documents include treaties within the framework of the International Maritime Organization. These treaties outline specific upper limits for total organismal concentrations in ballast water discharge according to size and taxonomic group

(International Maritime Organization 2004, Minton et al. 2005). Regulations requiring exchanges of port-loaded water for oceanic water when ships pass over deep water also reduce transportation of non-native species (Minchin et al. 2009). Several countries have required ship arrivals from outside of their Exclusive Economic Zones to perform ballast water exchange in the open ocean (Minton et al. 2005).

One of the best options for inhibiting future invasions is preventing the transport of potential invaders through pre-boarder vector management (Johnston et al. 2009). As previously stated, ballast water and fouling organism transport are two critical vectors that result in marine invasions (Johnston et al. 2009). Therefore, current ballast management strategies have focused targeting the vector rather than individual species. However, characterizing species assemblages in each ballast tank and interpreting the risk associated with each assemblage is impractical (Ruiz and Carlton 2003, Minton et al. 2005). In one study, a model was developed that linked ship transportation to existing hotspots of marine invaders. It was found that reducing individual ship visits was more effective in controlling introduction events compared to eliminating key ports (Drake and Lodge 2004, Johnston et al. 2009). In another study, ballast water exchange was estimated to reduce coastal zooplankton numbers from $10^7$ to $10^6$ organisms (Minton et al. 2005). If management strategies focus on altering ship arrival frequency and discharge volume, the probability of invasive establishment can be significantly reduced.

Although an international effort to reduce transportation of non-native species into non-native regions has contributed significantly to the prevention of invasions, a thorough understanding of the dynamics which drive non-native species establishment can provide management with additional insight on implemented policies. In turn, a large scientific effort has been placed on determining which species, or characteristics of organisms, help to promote or detract from establishment and invasion success. This targeted approach has been postulated to be more efficacious at reducing the annual number of introduction events. However, as we will demonstrate below, identifying characteristics associated with establishment success and invasiveness is not complete, and is fraught with challenges.

### *Mechanisms that affect establishment success.*

Several studies have explored the characteristics that promote establishment success (Veltman et al. 1996, Kolar and Lodge 2001, Forsyth et al. 2004, Davis 2009). Previous studies have suggested that establishment requires tolerance of the physical and chemical conditions of new habitat, food acquisition, predator avoidance, reproduction, and environmental suitability (Havel et al. 2015). In one study, the invasive predatory gastropod *Rapana venosa* was investigated for its relationship between establishment success and environmental stress. It was found that *R. venosa* larvae exhibited a broad tolerance to different salinities, ranging from 7 to 32 ppt. As a result from the data gathered on salinity tolerance, future *R. venosa* establishment was predicted to follow the counterclockwise, gyre-like circulation direction within the Chesapeake Bay and distribution of larvae northward and lower sections of all major subestuaries of the western shore of the Bay (Mann and Harding 2003). Another study found interspecific aggression and predation between the invading *Gammarus pulex* and the native *Gammarus duebeni celticus* amphipod species to be the driving force behind species replacement in the environment. Specifically, male *G. pulex* displayed more aggressive behavior towards both male and female *G. d. celticus* engaged in precopula guarding compared to male *G. d. celticus*. Therefore, studies of the behavior of individuals was suggested to be useful in the understanding of species replacement (Jaimie et al. 1995). Lastly, climate change was mentioned to have a positive effect on establishment success. It was found that initiation of recruitment for the three most abundantly introduced ascidian (sea squirt) species, *Ascidiella aspersa*, *Botrylloides violaceous*, and *Diplosoma listerianum*, was strongly negatively correlated with winter water temperature; in other words, recruitment and subsequent establishment occured earlier in years with warmer winters. On the other hand, the native species recruitment was negatively correlated with winter temperature, meaning more recruitment occurred in colder years. Moreover, it was concluded that global warming can facilitate and accelerate the establishment of non-native species (Stachowicz et al. 2002). Given the above examples, it is clear that some parameters have been linked and have served as good predictors for establishment of specific species. However, a global model that predicts invasion success across multiple species remains elusive.

There are many reasons why prediction of establishment is difficult to achieve (Williamson 1999). For example, if the prediction is too general, the outcome may not be precise enough. Also, statistical analysis can be unreliable when prediction is based on small but significant $R^2$ values, extrapolation in multidimensional analyses, reliance on a regression predictor (which is always worse than its fit to the original data), production of disproportionate false positives when expected frequency is low, and new sets of variables associated with each new invader. Biological complications include lack of phylogenetic correction, a time delay in reporting invasions (delayed reports of early stages of invasion fail to predict current, later stages of invasions), non-linearity in population dynamics, complexity of ecosystems, and situation-specific detail (Williamson 1999). Consequently, the traditional approach has been to develop separate models for each new invasive species. This approach is impractical, and poses significant time and effort costs. Therefore, conservation biologists have sought to develop a more universal predictor explaining why certain introduced species successfully establish when others fail. Although successful invaders tend to share some key characteristics in their age at maturity (timing of reproduction) and fecundity (number of individuals produced) (Johnston et al. 2009), a single, generic mechanism is needed to identify the establishment of species and has still yet to be explored.

### *Propagule Pressure*

Currently, the most promising determinant of establishment success is propagule pressure. Propagule pressure is the number of individuals released into a specific region over a given time period (Lockwood et al. 2005, Simberloff 2009). Propagule pressure is comprised of two components: propagule size and propagule number. Propagule size is the number of individuals within a single introduction event, whereas propagule number is the rate, or frequency, at which individuals arrive per unit time (Lockwood et al. 2005, Simberloff 2009).

The majority of evidence demonstrating the importance of propagule pressure in determining establishment success stems from theoretical studies or correlative studies. There have been multiple approaches to measuring and modeling propagule pressure. For unintentional introductions, absence of detailed introduction records often leads to the reliance on less direct proxy variables, such as shipping activity (Wonham et al. 2013). Seebens et al. (2016) developed a modelling approach using global ship movements and environmental conditions to simulate the global spread of 40 marine alien species. The model correctly predicted the presence/absence of an alien species in an ecoregion with 77% accuracy (Seebens et al. 2016). Similarly, in response to the International Maritime Organization's mandatory ballast-water exchange policy in 2004, a mathematical framework for optimizing ballast-water exchange was developed, including exchange level, timing, and species salinity tolerance as variables. It was found that ballast water exchange was always effective; species survival was worse in post-exchanged water compared to pre-exchanged water. However, in some cases when survival was equal or better following an exchange, early, intermediate, or late exchange needed to be considered, depending on the species (Wonham et al. 2005). In another example, theoretical propagule pressure models included consideration of river dynamics to predict invasion: Van Oorschot et al (2017) developed a morphological model for the invasive plant species *Fallopia japonica*, in which distribution, native vegetation, and river hydro-morphodynamic processes were influenced by high and low propagule pressure. It was found that high propagule pressure led to a decline in native species cover due to competition and creation of unfavorable native colonization sites. Conversely, low propagule pressure allowed native seedling survival (van Oorschot et al. 2017). Although proxies such as shipping activity offer a method of establishment prediction, the specific components of propagule pressure are difficult to assess individually. For example, time-lags in historical records provide inadequate information in order to make accurate predictions of establishment.

In addition to theoretical studies, there have been empirical studies that report a positive correlation between propagule pressure and establishment success. For example, in a factorial field experiment using larvae of the invasive oyster *Crassostrea gigas*, propagule size and number were quantitatively altered while propagule pressure was held constant. As predicted, the number of oyster colonizers increased with larger propagule sizes. Interestingly, it was found that small, frequent introductions dramatically increased the population size relative to large, infrequent introductions (Hedge et al. 2012). In another study, the effect of propagule pressure on freshwater fish invasions was investigated using nine fish species transferring from an inter-basin water supply to multiple, small irrigation ponds in South Africa. Out of the nine species, five species (*Glossogobius callidus, Gambusia affinis, Gilchristella aestuaria, Oreochromis mossambicus,* and *Cyprinus caprio*) were found to establish in the downstream ponds. In this study, species with more propagules were more likely to become established. Thus, relative interspecific rates of establishment were concluded to be driven by propagule pressure (Woodford et al. 2013). The brook trout *Salvelinus fontinalis* is another example of an invading fish species. Through a combination of data from fish stocking register and field surveys, Korsu and Huusko (2009) studied the roles of propagule pressure and the invader's initial dispersal in driving establishment success. It was determined that one-fourth of the fish introduced to suboptimal habitats were found to be established in the core habitats of such species (Korsu and Huusko 2009). Finally, in an experiment using *Daphnia magna,* different levels of introduction size and introduction number were studied for an effect on population growth and population persistence. On the final day of the experiment, it was found that population size increased with both introduction size and introduction number. The authors also found that the interaction between introduction size and introduction number was positively correlated with persistence (Drake et al. 2005). It can be concluded that empirical studies have reported a direct correlation between propagule pressure and establishment success. Not only do empirical studies provide support to the theory of propagule pressure, but they are consilient with evidence from natural systems.

### *Propagule Size*

Although both components of propagule pressure work in concert, they can each have unique influences on establishment success. Propagule size is predicted to have a straightforward impact on establishment: an increase in introduction size results in an increase in establishment probability (Blackburn et al. 2015). A release of a large number of individuals also increases the likelihood that an introduced population will retain representative samplings of source genetic diversity, thus facilitating establishment (Roman and Darling 2007). Moreover, propagule size has been a strong predictor of establishment success (Memmott et al. 2005).

In one study, Btitton and Gozlan (2003) used mesocosm ponds to identify how propagule size and number predicted the establishment of the invasive fish, *Pseudorasbora parva*. Results concluded that population size was a function of propagule size (Britton and Gozlan 2013). In another analysis of propagule pressure, Casey et al. (2004) studied historical introduction success of 10 different bird families (149 species), concluding that propagule size was non-randomly distributed with respect to a range of other characteristics (e.g., body mass, geographical range, annual fecundity) of non-native species. Therefore, the correlation that was seen between such characteristics and establishment success was confounded in propagule size (Cassey et al. 2004). In a mathematical modelling approach intended to replicate historical bird introductions, it was found that models predicted lower probability of establishment when propagule size was divided between multiple introduction events: one introduction event resulted in greater probability of establishment when compared to multiple introduction events that summed to the same size (Cassey et al. 2014). Liu et al. (2014) performed a greenhouse experiment, testing the effects of habitat conditions and propagule size on the establishment success of the invasive plant, *Hydrocotyle vulgaris*. It was found that both propagule size and habitat conditions significantly affected total biomass, ramet number (population size), leaf area, and stolon length (horizontal connections), especially in grassland communities rather than bare soil (Liu et al. 2014). It was therefore predicted that high propagule pressure, in the form of increased propagule size, enhanced growth of the invasive population. Lastly, in a study using the marine ascidian invader, *Botrylloides violaceus*, varying sizes of larval propagules were released into various *in situ* field mesocosms to test whether invasion success was directly related to the number of arriving propagules (Simkanin et al. 2017). It was found that large propagule sizes confer greater invasion success in marine environments, but the relationship is not consistent across habitat types (Simkanin et al. 2017).

Although the majority of studies report a positive correlation between propagule size and establishment success, there has been evidence that propagule size is not the only determinant of establishment success. In one study, establishment of the invasive psyllid, *Arytainilla spartiophila*, was investigated over six years to determine the effect of propagule size on probability of establishment, probability of persistence, and mediumterm population growth. It was found that even though large release sizes survived the experiment, release size did not guarantee establishment. It was observed that one of the largest release sizes struggled over the duration of the experiment, resulting in a smaller population size than some of the smaller initial releases. Therefore, it was concluded that large populations do not always have larger population growth rates; rather it is annual rate of change that provides information for a species population to become established (Memmott et al. 2005). Overall, there has been substantial evidence that propagule size plays an important role in establishment success. However, experimental evidence is further needed in order to resolve conflicting literature.

### *Propagule Number*

In theory, the repeated release events of individuals into one location functions as a source pool, which then sustains an introduced population even if the initial introduction was of insufficient size to facilitate long-term establishment. Given that the source pool functions as a means to overcome limitations associated with small populations (i.e., demographic effects), propagule number (introduction frequency) is considered to be relevant and important in determining establishment success (Lockwood et al. 2005). Accordingly, it has been suggested that propagule number is crucial in determining persistence in populations with low average individual fitness levels resulting from inter-specific interactions (Drake et al. 2005). As a result, larger and/or more frequent introductions are more likely to establish non-native populations (Cassey et al. 2014).

In a study using bark (*Scolytinae*) and long horned beetle (*Cerambycidae*) species, the relationship between the probability of establishment and arrival rate across an entire pool of potential invading species was analyzed using border interception frequency (port of entry frequency). In this study, boarder interception frequency was used as a proxy for true arrival rate. It was found that there was a positive association between arrival rate and probability of establishment for both bark beetles and long horned beetles (Brockerhoff et al. 2014): the interval between release events affects establishment success. Additionally, Drolet and Locke (2016) demonstrated the importance of propagule number using stochastic population simulations. According to simulation results, when the population was subject to demographic effects, the probability of establishment decreased with increasing propagule number. It was suggested that higher probability of establishment was found in populations introduced by few, but large independent introductions (Drolet and Locke 2016).

Propagule number can have a different influence on genetic diversity compared to propagule size. Propagule number can be dependent on introductions deriving from different source populations. If genetic diversity is different between different source populations, multiple introduction events can result in establishing populations exhibiting greater genetic diversity (Roman and Darling 2007). As a result, more genetically diverse populations can have the ability to overcome demographic stochasticity more easily and increase the probability of establishment. The two factors (propagule number and size) also differ in their response to environmental stochasticity. A high propagule number will allow increased stability in the face of environmental stochasticity, when random environmental disasters can reduce the population size and affect establishment. The inherent temporal conditions of these disasters often do not affect subsequent propagules from establishment. Conversely, an increase in propagule size is more likely to lower the risk of demographic stochasticity (i.e., the variability in population growth) since small populations are often subject to such effects (Lockwood et al. 2005). Given the supporting evidence that propagule size and number have on establishment success, experimental manipulation of each component individually is fundamental in uncovering the mechanism behind establishment success.

### *Genetic Variation*

Small founding populations of introduced species are expected to have lower genetic variation as a result of bottlenecks, compared to variation seen in native populations. As a result, conservation genetics predicts that introduced species would suffer from high risk of inbreeding, which may result in extinction (Roman and Darling 2007). However, introduced species can succeed despite having reduced genetic diversity. For instance, through demographic bottlenecks, deleterious alleles can be eradicated and highly adapted lineages can be preserved through sexual recombination (Roman and Darling 2007). Invasive populations can increase genetic diversity through admixture from multiple native populations (Roman and Darling 2007). This indicates that genetic bottleneck effects can be mitigated by adoption of asexual reproduction and plastic responses to environmental conditions (Roman and Darling 2007). Finally, as in the case of shellfish (e.g., *Crassostrea gigas* and *Crassostrea virginica*), translocations between different geographical locations can enhance genetic diversity, thus increasing establishment probability (Roman and Darling 2007). Moreover, multiple pathways provide a means for introduced species to overcome negative genetic effects and establish.

The genetics of an introduced species may be influenced by propagule pressure in multiple ways. For example, a larger propagule size would be expected to have greater genetic variation to overcome demographic bottlenecks. Multiple releases that derive from different source populations can result in hybridization between individuals from genetically divergent native populations, thus resulting in a more genetically-diverse population (Allendorf and Lundquist 2003). Invasive to temperate estuaries worldwide, a phylogeographic analysis of the oriental shrimp *Palaemon macrodactylus*, was completed by Lejeusne et al. (2014) to determine their genetic inheritance. After comparison of the cytochrome c oxidase subunit I (COI) of *P. macrodactylus*, it was determined that the majority of introduced populations had higher haplotypic diversity than most native populations (Lejeusne et al. 2014). It was hypothesized that global shipping and ballast water provided repeated introduction events from different sources, therefore allowing the population to compensate for deleterious effects of bottlenecks (Lejeusne et al. 2014). In another study, a genetic analysis was performed on seven *Gammaroidea* species, including both invaders (*Gammarus tigrinus*, *Pontogammarus maeoticus,* and *Obesogammarus crassus*) and native species (*Gammarus locusta*, *Gammarus salinus*, *Gammarus zaddachi,* and *Gammarus oceanicus*) in order to evaluate genetic diversity in relation to native range. Although genetic diversity did not differ between invasive and native species, genetic differentiation existed between invasive species. Such evidence supported the claim that geographic and evolutionary distances may explain invasive genetic differentiation. The lack of population genetic structure in invasive populations may, therefore, facilitate distribution of mutations that are beneficial to invasive ranges (Baltazar-Soares et al. 2017). Furthermore, these studies provide evidence that genetic diversity may be a strong driver behind establishment success for introduced species, and that genetic diversity may be used as a nonspecific predictor of invasion potential.

### *Climate Change*

Many species, especially those engaged in long distance dispersal, can adjust geographic ranges in response to climate change (Viana 2017). It has been observed that changes in water temperature are likely to promote establishment of non-native species with the expense of native species (Roman and Darling 2007). Increasing sea temperatures have been shown to correlate with both growth and reproduction of marine fish and invertebrates, which can lead to population increases (Brown et al. 2004, Côte and Green 2012). In addition to increases in water temperature, increases in air temperature due to global warming result in decreased ice cover, increased evaporation, change in hydrology volume due to increased runoff, increased in nutrient inputs, change in habitat stratification, and change in habitat size (Rolls et al. 2017). Such environmental changes could perpetuate the establishment of non-native species into novel regions. For example, the climate boundary between temperate and subarctic temperatures is a natural limit for the distribution of many freshwater fish species, including *Cyprinids*, *Percids*, and *Centrarchids* (Rolls et al. 2017). Therefore, invasions may be associated with poleward range expansions of temperate organisms if subarctic temperatures rise (Rolls et al. 2017). If non-native species are able to interact favorably with a climate-changing environment, competition between native and non-native species may result in the reduced abundance of native species.

In one study, Sheppard et al. (2014) investigated the role of climate and suitability on the establishment of alien plants (*Archontophoenix cunninghamiana*, *Psidium guajava* and *Schefflera actinophylla*), in comparison to native species (*Rhopalostylis sapida*, *Lophomyrtus bullata* and *Schefflera digitate*). Under different climate and suitability conditions, non-native performance was greater than native species. It was concluded that the non-native species were able to persist in a wide range of temperature and precipitation conditions, as long as temperatures do not drop below levels required for survival (Sheppard et al. 2014). Liu et al. (2011) used ecological niche models to predict the impact of climate change on native and non-native species, which included the invasive red swamp crayfish, *Procambarus clarkii*. Their model predicted that the distribution of invasive species was due to complex effects of climate change, rather than simply promoting invasion risk. Interestingly, it was found that some regions would become prone to invasion, while others would reduce invasion risk by decreasing invasive potentials or no longer offer climatically suitable areas for *P. clarkii* establishment (Liu et al. 2011). Finally, a study was conducted in which the effects of climate change were assessed in relation to the establishment of the invasive smallmouth bass, *Micropterus dolomite*, on populations of native lake trout, *Salvelinus namaycush*. Results showed that the number of Ontario lakes with vulnerable lake trout populations are predicted to increase from 118 to 1612 by 2050 following a projected increase in climate temperature. It was suggested that establishment was attributed to climate change, as lake trout is considered a cold water species and smallmouth bass is considered a warm water species. The increase in temperature could result in longer stratification periods, thereby decreasing hypolimnetic (deep water) oxygen concentrations and leading to increased frequency of anoxic waters. These anoxic waters are detrimental to lake trout populations (Sharma et al. 2009).

### *Predation*

Predation, or lack thereof within the context of the enemy release hypothesis, may facililate establishment success. Cuthbert et al. (2018) investigated the role of functional response and prey switching of the native predatory amphipod, *Gammarus duebeni*. Larvae of the native mayfly, *Baetis rhodani*, and larvae of the invasive gammarid *Crangonyx pseudogracilis* were used as prey species to *G. duebeni* (Cuthbert et al. 2018). Functional response is defined as the relationship between resource use and availability (Dick et al. 2014). In the context of invasive species, comparative functional responses reveal the relationship between resource consumption rate (e.g., by a predator) and resource density (e.g., prey) between an invader and a native species (Dick et al. 2013). The premise behind prey switching is that predators that attack a variety of prey feed most heavily on the most abundant species. When a particular prey species declines in number, the predator “switches” to the next most abundant in number (Murdoch 1969). In the study, when both native and invasive prey species were simultaneously presented, it was found that *G. duebeni* disproportionally fed on more native prey than invasive prey. Thus, prey switching was not exhibited in the presence of invasive prey (Cuthbert et al. 2018).

### *Allee Effects*

As stated above, genetic variation, climate change, and predation are three influences that have been shown to promote establishment success for non-native species. However, Allee effects is a component that has been shown to reduce establishment success. Allee effects can be classified into two types: component Allee effects and demographic Allee effects. Component Allee effects describe the level of components of individual fitness, such as juvenile survival, whereas demographic Allee effects are the level of the overall mean of individual fitness, viewed through the demography of the whole population (Courchamp et al. 2008). At low densities, individual fitness is considered to be higher due to lower intraspecific competition. Conversely, at high densities, intraspecific competition increases. At low densities, if one fitness component decreases, another, higher fitness component may compensate in order to keep intraspecific competition low. This gives rise to a negative density dependence. However, if compensation is not sufficient for low densities, lower individual fitness values domineer. Thus, the result is an Allee effect, in which a lower *per capita* population growth rate exists (Courchamp et al. 2008).

The strength of an Allee effect can also vary depending on the *per capita* growth rate. If the Allee effect is weak, the *per capita* growth rate at lower densities is lower compared to higher densities, but still remains positive. If the Allee effect is strong, the *per capita* growth rate at lower densities is negative below a certain value, often termed the Allee threshold (Courchamp et al. 2008). Indeed, empirical studies have used thresholds as indicators of establishment (Drake and Jerde 2009). With a strong Allee effect, *per capita* growth increases from negative values at small population sizes, becomes positive at some critical population size, and then increases with population size until competition becomes a factor (Wittmann et al. 2014).

Allee effects may arise from a number of mechanisms such as mate finding or interbreeding when genetic diversity of the founder population is low (Primack 2006, Clark and Johnston 2009). Allee effects can be correlated with reproductive mechanisms, including fertilization in sessile organisms, mate finding in mobile organisms and cooperative breeding (Courchamp et al. 2008). They can also be correlated with mechanisms related to survival. Such examples include environmental conditioning (allelopapthy), predation, and aggregation by predators. Finally, Allee effects can arise due to cooperative behaviors. Some researchers have argued that highly social species, which demonstrate a need for conspecifics, would be correlated to increased susceptibility to Allee effects (Munoz-Duran 2002, Courchamp et al. 2008).

### *Allee effects in invasive species*

Allee effects provide opportunities to study population dynamics during the process of establishment, whether successful or not. This is due to the notion that invasion speed decreases as the Allee effect increases in strength (Taylor and Hastings 2005). This is of particular interest in the field of invasion biology, as uncovering mechanisms that drive establishment can provide insight towards management strategies.

Due to large effective population sizes, Allee effects associated with marine populations have been considered unimportant due to marine populations often being large (Gascoigne and Lipcius 2004). However, Allee effects can still exert effects on large populations. For example, many marine species have larvae that are retained locally, thereby producing confined populations (Jones et al. 1999), which can result in an Allee effects. Many marine species have subpopulations that collectively contribute to metapopulations through larval transport (Lipcius et al. 1997, Crowder et al. 2000, Lipcius et al. 2001, Gascoigne and Lipcius 2004). If density is low and Allee effects are constructed in subpopulations, the metapopulation, as whole, can suffer demographically, and thus be subject to Allee effects. Thus, large population sizes and metapopulation structure are not necessarily accurate predictors for the absence of Allee effects (Gascoigne and Lipcius 2004).

Some species are able to overcome Allee effects using alternative strategies. It is very common for fish species to exhibit the compensatory process of fast growth and rapid maturation in response to low introduction numbers (Britton and Gozlan 2013). Factors that increase propagule resilience to environmental change (e.g. adaptivity and tolerance to temperature and salinity), thereby making them more prone to overcome Allee effects (Johnston et al. 2009). Environmental modification is one key mechanism that can overcome Allee effects. In fact, this is the type of Allee effect that was first observed by the scientist of whom the dynamic is named after, Warder Allee. He demonstrated the Allee effect using goldfish in synthetic pond water containing dissolved salts of high chemical purity in distilled water. Allee demonstrated that goldfish conditioned the water by giving off organic matter and carbon dioxide. He then showed that goldfish grew better in water that other goldfish had lived in and conditioned, thus suggesting that organisms can modify their environment in order to improve survival (Allee 1938).

Notably, the Allee effect has been used to make successful predictions of marine establishment and subsequent invasion. For example, Leung et al. (2004) used survival analysis and maximum likelihood techniques to theoretically predict the establishment of the invasive zebra mussel, *Dreissena polymorpha,* in Michigan inland lakes. It was determined that the fitted models correctly predicted probabilities of invasion more times using an Allee model versus a non-Allee model (Leung et al. 2004). Additionaly, although not invasive, the gamete morphology of the sea urchin, *Strongylocentrotus droebachiensis,* is density-dependent (Levitan 2002, Berec et al. 2018). At times of low density, larger eggs and slow, long-lived sperm are produced, whereas at higher densities, smaller eggs and fast, short-lived sperm are released (Levitan 2002). That being said, the density-dependent selection associated with mate-finding-related traits can provide insight on invasive population control if invasive species demonstrate similar traits. Allee effects have been observed in parasites in coastal marine ecosystems. In a study by Krkošek et al. (2011), they investigated the colonization of the juvenile Pacific salmon by the ectoparasitic salmon lice, *Lepeophtheirus salmonis*. These parasitic lice demonstrated Allee effects in colonization in that pair formation between pre-adult females and adult male lice was dependent on both local abundance of lice and mate-searching dispersal of males among host fish (Krkošek et al. 2012). Furthermore, it can be suggested that models that consider Allee effects can improve the accuracy of establishment predictions. Overall, Allee effects are important to consider during the establishment phase of invasion, and appear to have some influence on the establishment and spread of a variety of marine species.

### *Challenges with studying invasions in the natural setting*

Researchers have turned to experimental studies to address the validity of the theory of propagule pressure and seek evidence for the driving forces behind establishment success. It is agreed that an ideal experimental design would include quantifying establishment of an invasive species in a natural, biological system. Under such studies, organisms and their interactions can be observed in direct relation to the natural environment, thus taking into account a wide array of environmental variables that would be otherwise difficult to simulate in a mathematical or theoretical framework.

However, studying establishment and invasion dynamics in marine environments is challenging. For example, small initial population sizes are challenging to locate and track, multiple confounding environmental factors can obscure invasion dynamics, and invasive species are often not identified until long after establishment has occurred. In addition, invasion is a multi-step and trans-disciplinary process, involving population, community, evolutionary, and physiological biology and their distinct roles in the different stages of invasion (Moyle and Light 1996). Therefore, it is challenging for empirical studies to report on all aspects of the invasion process (Blanchet 2012).

### *Synthetic Biology*

To overcome the issues relating to the study of invasion dynamics in natural systems, researchers have used synthetic biology to explain complex, ecological and evolutionary processes. Synthetic biology can be defined as the engineering of biology and synthesis of biological systems (Serrano 2007). The premise behind synthetic biology is that rather than looking at a list of parts that comprise organismal complexity, research focus should be directed towards how such parts combine together (Mukherji and van Oudenaarden 2009). Synthetic biology aims to engineer novel, model-guided behaviors into cells using gene circuits (Zomorrodi and Segre 2016). Gene circuits are assembled using genes from diverse sources, which are used to implement a variety of functions including growth and division, signaling, and differentiation (Nandagopal and Elowitz

2011). Elementary gene circuits are circuits in which gene expression is regulated by a single protein in response to a signal. A signal is defined as a molecule that causes a physiological response. Once the signal acts on the protein, the protein then binds to the DNA region that codes for a specific gene, resulting in the expression of that gene. However, depending on how the circuit is designed, proteins can either bind to DNA sequences to activate or repress gene expression (Wall et al. 2004). By building together series of activators and repressors that interconnect, one can build a gene circuit that produces a novel behavior in the cell. In summary, each component of the gene circuit works in concert along with the cellular components to simulate a core behavior observed in nature.

Synthetic systems offer an advantage over other measurements of natural systems by incorporating both engineering and biological aspects. Specifically, synthetic systems are able to test the function of core dynamics with minimal interference from background processes. Synthetic systems offer a method to culture, control, manipulate, and achieve rapid reproduction rates in correlation with a natural occurrence that would be otherwise difficult to measure in natural systems (Yu et al. 2012, Dressler et al. 2017). To date, synthetic systems have contributed to the understanding of complex natural systems, particularly in the field of ecology and evolutionary biology. For example, engineered microbes have been used to study cooperative dynamics within and between populations (Dressler et al. 2017), predator-prey relationships (You et al. 2004), and mechanisms that maintain biodiversity (Kerr et al. 2002). As a result, synthetic biology can be used as an alternative approach to studying establishment dynamics, and could thus be used to study propagule pressure.

### *Question and Objective*

In this thesis, the overarching goal was to use bacteria engineered using the principles of synthetic biology to contain an Allee effect to examine how propagule pressure dictates establishment success. Within this overarching goal, we studied three aims: (1) to determine how the timing between introduction events affects establishment success, (2) to determine how varying initial introduction density affects establishment success, and (3) to determine how reducing generation time in a population expressing an Allee effect affects establishment success.

### *Aim 1: Determine how the timing between introduction events affects establishment success*

Propagule number is one component of propagule pressure that has yet to be explicitly studied. Conflicting literature in combination with the lack of empirical studies that specifically investigate propagule number provide the need for a mechanistic understanding of its role in establishment success for populations suffering from Allee effects. We examined how the timing between two successive introductions influences establishment success, with a specific focus on propagule sizes that are below an Allee threshold. By doing so, the conditions by which propagule pressure dictates establishment success can be defined in a clear, concise way.

*Hypotheses*

$H_0$: A second introduction of bacteria will not increase the survival of a population that is below the Allee threshold.

$H_a$: A second introduction of bacteria will increase the survival of a population that is below the Allee threshold.

$H_0$: Total cell density is not dependent on the timing of a second introduction of bacteria.

$H_a$: Total cell density is dependent on the timing of a second introduction of bacteria.

### *Aim 2: Determine how varying initial introduction density affects establishment success*

Intuitively, a sufficiently large propagule size that is above the Allee threshold will allow establishment and growth. However, when propagule size is below the Allee threshold, growth may or may not occur. It is known that propagule size is not a fixed value. Rather, propagule size can fluctuate between introduction events. Consequently, we examined how varying propagule size between two introduction events influenced population growth. To accomplish this, we kept the total number of cells (i.e. volume) constant, but varied the proportion of total cells introduced initially and at the second introduction event. Through observation of population growth with different propagule sizes (as a function of time to second introduction), we provide evidence for the importance of each component (propagule size and propagule number) in establishment success.

*Hypotheses*

$H_0$: Varying the volume of bacteria introduced during the first and second introduction will not affect the period over which equal volume introductions result in growth of total cell density.

$H_a$: Varying the volume of bacteria introduced during the first and second introduction will affect the period over which equal volume introductions result in growth of total cell density.

### *Aim 3: Determine how reducing generation time in a population expressing an Allee effect affects establishment success*

A major benefit of using engineered bacteria is the ability to fine tune and perturb the behavior of the gene circuit. This can be accomplished using factors that directly (e.g., the amount of inducer) or indirectly (e.g., the composition of the medium) influence the function of the gene circuit. Previous studies have noted that the environmental conditions will not be consistent during an invasion, which may affect the ability of a species to establish. One proposed factor that affects establishment success is growth rate (McDermott and Finnoff 2016). In our study, the concentration of casamino acids in the medium controls the growth rate of the population. By altering the concentration of casamino acids in the medium and measuring cell density, we were able to determine how generation time (i.e. growth rate) had an effect on propagule size and number. An understanding of how generation time affects establishment success will allow us to develop a potential mechanism for patterns observed in establishing species demonstrating Allee effects. Furthermore, findings may be used to establish stronger control/limitation of establishing non-native species.

*Hypotheses*

$H_0$: Decreasing the concentration of casamino acids in the medium will not result in a change in period over which equal volume introductions result in growth of total cell density.

$H_a$: Decreasing the concentration of casamino acids in the medium will result in a change in period over which equal volume introductions result in growth of total cell density.

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# Chapter II: Symmetry in propagule size reduces establishment success in bacteria engineered with an Allee effect

## Introduction

Invasive species threaten biodiversity and can have widespread economic impacts (Mack et al. 2000, Pimentel et al. 2001, Molnar et al. 2008). In an effort to inform strategies that prevent establishment and spread of invasive species, several studies have tried to identify broadly defined characteristics that correlate with establishment success and invasiveness (Veltman et al. 1996, Kolar and Lodge 2001, Forsyth et al. 2004, Davis 2009). Previously identified characteristics include body mass, genetic diversity, reproductive strategy, diet, thermal tolerance, and environmental suitability (Foreman et al. 2012, Duncan 2016, Estrada et al. 2016, Peoples et al. 2017). While these characteritsics have proven somewhat effective in their ability to predict establishment and invasiveness success at a species-specific level (Wang and Jackson 2011), a strong, generalized predictor to identify potential invaders is desired.

One theoretically proposed determinate of establishment success is propagule pressure, which is defined as the number of individuals released into a specific area over a given time period (Lockwood et al. 2005, Simberloff 2009). Propagule pressure is composed of two components: propagule size and propagule number. Propagule size is the number of individuals released in a single introduction event, whereas propagule number is the frequency at which individuals are introduced per unit time (Lockwood et al. 2005, Simberloff 2009). One unique property of propagule pressure is its predicted ability to overcome a population dynamic that can inhibit establishment, the Allee effect, which is defined as a positive relationship between the fitness of an individual and population density (Taylor and Hastings 2005, Smith et al. 2014). For species with an Allee effect, the population will only become established and grow if a minimal density of individuals is present. If this minimal density is not met, the population will grow slowly (weak Allee effect), or not at all (strong Allee effect), both of which can limit establishment success (Taylor and Hastings 2005). Allee effects have been observed to regulate the establishment and spread of invasive species (Johnson et al. 2006), and are often due to the inability to initiate cooperation between individuals at low initial density (Allee 1951).

While propagule pressure remains a promising metric to predict potential invasiveness, its true contribution to predicting establishment success remains somewhat unresolved. On the one hand, several studies have shown that propagule pressure is positively correlated with establishment success (Supplementary Table S1). For example, it has been found that by increasing propagule pressure of water fleas, the probability of establishment increases, even in the presence of an Allee effect (Drake 2004, Gertzen et al. 2011). On the other hand, several studies show a weak, or apparently non-existent, correlation between propagule pressure and establishment success (Supplementary Table S1). For example, in an analysis of passeriform birds, differences in propagule sizes did not predict establishment success (Moulton et al. 2013).

These conflicting data may be due to a lack of understanding as to how the relative roles of propagule size and propagule number affect establishment success. It has been acknowledged that both components of propagule pressure have unique relationships with establishment probability, and that, for a given scenario, one element may become more important than another (Lockwood et al. 2005). For example, propagule size has a greater influence on propagule pressure when a population is subject to a strong Allee effect and demographic stochasticity (Lockwood et al. 2005, Wittmann et al. 2014), whereas propagule number has a more influence during times of environmental stochasticity (Lockwood et al. 2005). Moreover, this conflicting data may also be due to an inability to sufficiently detect, quantify, and track populations in nature during their early stages of invasion (Silveira et al. 2017). It is also confounded by the limited number of experimental studies that have evaluated the ability of propagule pressure to determined establishment success (Drake et al. 2005). Overall, it remains unclear how propagule pressure affects establishment success for a population with an Allee effect.

To understand how propagule pressure affects establishment success for a population with an Allee effect, we used a strain of *Escherichia coli* that contains a gene circuit that confers an Allee effect to the population. Gene circuits are used in the field of synthetic biology to engineer novel behaviors into cells. To date, synthetic systems have contributed to our understanding of complex ecological and evolutionary dynamics (Tanouchi et al. 2012). Accordingly, synthetic systems have been used to study cooperation (Dressler et al. 2017), predator-prey relationships (You et al. 2004), and mechanisms that allow maintenance of biodiversity (Kerr et al. 2002). Studying these processes using synthetic systems can remove or reduce confounding factors that can obscure dynamics observed in nature, and allow biological behaviors to unfold in controllable and easily quantifiable environments (Yu et al. 2012, Dressler et al. 2017). They can also confirm previous theoretical predictions, thus providing empirical evidence to support theoretical findings (Smith et al. 2014).

## Results

### *An experimental approach to examine propagule pressure and establishment success*

A previously engineered strain of bacteria that had an Allee effect was used (Smith et al. 2014, Wilson et al. 2017) (Fig. 1a, see Supplementary Fig. S1 for gene circuit). The gene circuit in these bacteria consisted of the LuxR/LuxI quorum-sensing system from *Vibrio fischeri* (Visick et al. 2000) and the CcdA/CcdB toxin-antitoxin module from the F conjugative plasmid (Bernard et al. 1993). Expression of both is induced by isopropyl-D-1-thiogalactopyranoside (IPTG). CcdB causes cell death by inhibiting DNA replication (Dao-Thi et al. 2005). However, *N*-Acyl homoserine lactone (AHL) synthesized by LuxI/LuxR system can rescue the population, as it is diffusible and can be shared amongst all of the members. If the concentration of AHL synthesized by the entire population is sufficiently high, CcdB is inhibited by CcdA, and the bacteria grow. If an insufficient concentration of AHL is synthesized, the population grows slowly, and can go extinct. As each bacterium synthesizes on average the same amount of AHL, whether or not a sufficient concentration of AHL is achieved is based on the initial density of the population. Consequently, the population of these bacteria have an Allee effect (Allee et al. 1949, Courchamp et al. 2008). Similar to some characteristics of non-native species, such as aggregation for mate finding (Wells et al. 1998), *Myxococcus xanthus* formation of a fruit body structure with stress-resistant spores (Kadam and Velicer 2006), litter production by the hybrid cattail *Typha latifolia* and *Typha angustifolia* (Farrer and Goldberg 2009), flucturations in cell growth relating to dispersal and spread of *Escherichia coli* (Smith et al. 2014), these bacteria cooperate to modify their environment via the production of AHL to overcome the Allee effect.

To investigate how propagule pressure influences establishment and growth of our engineered bacteria, we designed an experimental protocol that we termed a ‘multiple introduction experiment’. At time zero, a group of bacteria at a given density were added into the center of a well in a 24-well plate (Fig. 1b). A 24-well plate was chosen due to the size of the well allowing for uniform mixing while shaking as well as an adequate time period for observation of growth. These bacteria were allowed to synthesize AHL for a given period of time, whereupon a second introduction of a group of bacteria of a given initial density occured. Thirty-six hours after the initial introduction of bacteria, cell density was quantified using $OD_{600}$. Changing the density of bacteria inoculated into the well could be used as a surrogate of changing propagule size. Changing the timing between introduction events served to manipulate a facet of propagule number, the time period between releases. This experimental setup therefore mimics group dispersal, where multiple individuals will attempt to disperse to and colonize a new area, which has been observed in invasive species (Jongejans et al. 2008, Murray and Phillips 2010).

To establish that the engineered bacteria could function properly in our multiple introduction experimental setup, we inoculated medium containing various initial densities of bacteria into the 24-well plate and measured colony forming units (CFU, Fig. 1c) and $OD_{600}$ (Fig. 1d) after 24 hours. When the medium was not supplemented with IPTG growth was observed at all initial densities using both CFU and $OD_{600}$. However, when the medium was supplemented with IPTG, we observed that if the initial density of the bacteria was above ~$10^5$ CFU/mL, growth was observed as measured by an increase in CFUs and $OD_{600}$. If the initial density of bacteria was below ~5 x $10^3$ CFU/mL, CFUs decreased and an increase in $OD_{600}$ was not observed. Between these two initial densities, growth was variable: for some biological replicates CFUs and $OD_{600}$ increased slightly, whereas in others, CFUs and $OD_{600}$ decreased. This region, which we designate the region of Allee threshold, approached the bifurcation point of the system. In line with previous publications that have examined the Allee effect, growth was variable within this region (Méndez et al. 2011, Poole et al. 2012, Cai et al. 2013). Studying populations near the Allee threshold is important as previous studies have indicated that noise in this region can be critical in determining establishment and invasion success (Potapov and Rajakaruna 2013).

Fig. 1

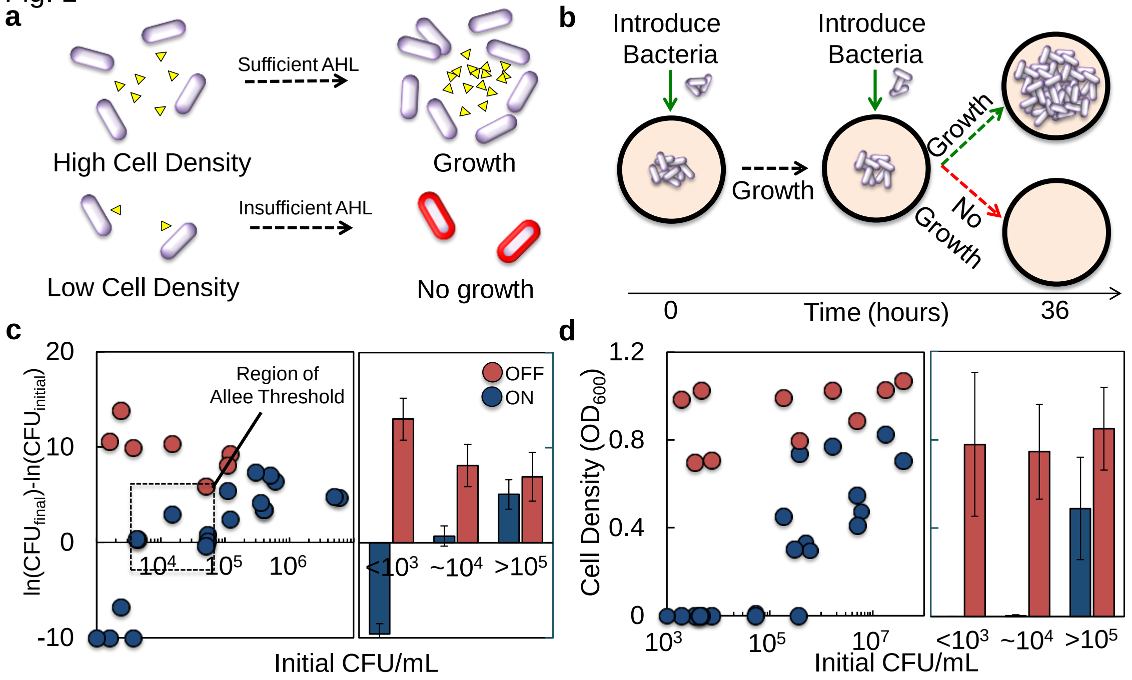

**Fig. 1. An experimental approach to studying propagule pressure. (a)** Bacteria engineered to have an Allee effect. At low initial density, insufficient AHL is produced over time, which does not allow the population to grow. At high initial density, sufficient AHL is produced, and growth occurs. **(b)** Multiple introductions experiment. At time zero, bacteria at a given initial density are introduced. After a set amount of time, a second introduction of bacteria occurs. Thirty-six hours after the initial introduction, cell density is measured using $OD_{600}$ . **(c)** Experimental verification of Allee effect using CFU. Left panel: When the circuit was OFF (red circles, no IPTG in the medium), an increase in CFUs was observed at all initial densities. When the circuit was ON (blue circles, IPTG in the medium), an increase in CFUs was observed if the initial density was greater than $10^5$ CFU/mL. In between these two initial densities, CFUs either increased slightly, or remained unchanged. This region represented the range of initial densities over which the Allee threshold occurs. Right panel: The change in CFUs plotted as a function of binned initial densities (CFU/mL). For panels c and d, data points plotted and standard deviation from a minimum of three individual experiments. **(d)** Experimental verification of Allee effect using $OD_{600}$. Left panel: If the initial density was above $10^5$ CFU/mL an increase in cell density ($OD_{600}$) was observed after 24 hours. However, if the initial density was below $10^5$, including the region of Allee threshold ($P = 0.18$ when $OD_{600}$ is compared to zero, one-tailed t-test), an increase in $OD_{600}$ was not observed. Right panel: Final $OD_{600}$ plotted as a function of binned initial densities (CFU/mL).

### *The timing between two introduction events determines establishment success*

Previous studies that have experimentally evaluated the contribution of propagule pressure to establishment success have yielded somewhat conflicting data. It has been observed that interactions between propagule size and number affect population establishment, but both components alone have little influence (Drake et al. 2005). A previous study found that one introduction of high propagule size led to establishment more often as compared to than smaller, frequent introduction events (Sinclair and Arnott 2016). In contrast, a previous study has found that propagule number was more important than size in determining establishment success (Koontz et al. 2017). Taken together, even in experimental systems, there is debate about the role of propagule pressure in determining establishment success. One potentially overlooked component that could affect establishment success is the time between introduction events. In theory, two introduction events that occur sufficiently close together, could increase establishment success. To test this idea, we used our multiple introductions experimental approach. We hypothesized that for an initial introduction that was above the Allee threshold (initial density of $>10^5$ CFU/mL), establishment and growth would always occur. Conversely, if an initial introduction of bacteria was near the Allee threshold, establishment and growth may not occur, even if the initial introduction was supplemented with a second introduction event. We focused our experiments on the scenario where the first introduction event did not result in successful establishment and growth. This scenario allowed us to examine how a second introduction, which varied in time relative to the first event, would impact establishment and growth.

We initially used our multiple introduction experiment (Fig. 1b) to determine the ability of two introductions (an initial 10 μL introduction, then a second 10 μL introduction) of equal density to result in growth and establishment. We observed that if the initial density of bacteria introduced was greater than $10^5$ CFU/mL, $OD_{600}$ increased regardless of time between the first and second introductions (Fig. 2a). When the density of bacteria introduced was near the Allee threshold, $OD_{600}$ was observed to increase under certain circumstances. If a single introduction resulted in an increased in $OD_{600}$, a similar increase was observed in all wells regardless of time between the first and second introductions (Fig. 2b). However, when the initial density of bacteria introduced at first introduction event did not result in an increase in $OD_{600}$, an increase in $OD_{600}$ was only observed within a range of time between the first and second introductions (Fig. 2c). We observed that if the time between introduction events was greater than 16 hours, $OD_{600}$ was not significantly greater than zero (one-tailed t-test, P values listed in Table S2). Otherwise, if the time between introduction events was 16 hours or less, an increase in $OD_{600}$ observed. These data suggested that growth is maximized when the timing between two successive introduction events is sufficiently short.

### *AHL production is important in determining growth in our multiple introduction experiments*

To explain these findings, we hypothesized that AHL produced by the first introduced was insufficient to rescue itself, but sufficient to rescue the second introduced population. Since death due to CcdB is not instantaneous and occurs over several hours (You et al. 2004), populations inoculated in the first introduction event can produce AHL over an extended period of time before succumbing to CcdB. This AHL would be available to the second population, which in turn would also create its own AHL. The combination of AHL produced by these population could then rescue the second population and allow for growth.

To demonstrate that AHL produced by the first population was critical for rescuing the second population, we performed our multiple introductions experiment. We inoculated 10 µL of bacteria in medium with IPTG and allowed the first population to synthesize AHL for 8 hours (Fig. 2d). We then filtered the medium to remove bacteria, but not AHL. We placed the filtered medium back into the plate, introduced an additional 10 µL of bacteria, and measured cell density after 36 hours. We observed an increase in $OD_{600}$ in populations grown in filtered and non-filtered medium. This indicated that AHL produced from the first population was sufficient to rescue the second.

To demonstrate that removing AHL produced by the first population does not result in growth of the second population, we performed our multiple introduction experiment using 10 µL of bacteria. We allowed the first population to synthesize AHL for 8 hours (Fig. 2e). We then collected the medium, pelleted the bacteria using centrifugation, and resuspended them, along with a second 10 µL introduction of bacteria, in fresh M9 medium containing IPTG. After 36 hours, we did not observe a significant increase in $OD_{600}$ in bacteria resuspended in fresh medium, whereas an increase in $OD_{600}$ was observed for bacteria that were not collected via centrifugation. This indicated that removing AHL produced from the first population did not allow the second population to grow.

Finally, to demonstrate that AHL could rescue populations where the time to reintroduction was greater than 16 hours, we performed our multiple introduction experiment. We introduced 10 µL bacteria in medium with IPTG, and allowed them to synthesize AHL for 24 hours (Fig. 2f). We then introduced a second 10 µL aliquot of bacteria along with 100 nM of purified AHL. After 36 hours of total growth, we observed a significant increase in growth for populations supplemented with purified AHL, but did not observe an increase in $OD_{600}$ for populations not supplemented with purified AHL. This indicated that the lack of growth past 16 hours observed in our multiple introduction experiments was predominantly due to an insufficient concentration of AHL, and not due to nutrient exhaustion or changes in cell physiology outside of the circuit dynamics.

### *A reduction in generation time decreases establishment success*

Previous studies have shown that a fast generation time (e.g., number of broods per season (O'Connor 1986, Duncan et al. 1999), early maturity (Richardson and Rejmanek 2004), and frequent litters (Capellini et al. 2015)) is positively correlated with establishment success. However, it has also been found that there is no significant correlation between generation time and establishment success (Veltman et al. 1996). For species with an Allee effect, a faster generation time helps to overcome the Allee threshold (Shaw and Kokko 2015). Given that generation time may play an important role in determining establishment success, we sought to examine how changing the generation time of our engineered bacteria affected the range of times to second introduction that led to growth.

To manipulate the growth rate of our engineered bacteria, we altered the amount of casamino acids in the growth medium. We first confirmed that increasing the percentage of casamino acids in the growth medium increased growth rate (Fig. 3a). To accomplish this, we grew our engineered bacteria in M9 medium with increasing

Fig. 2

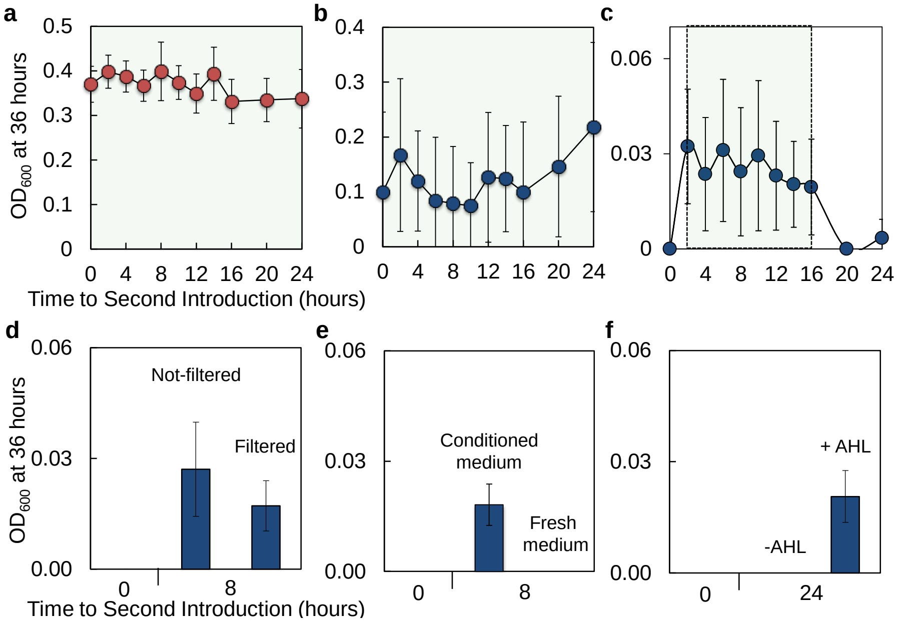

**Fig. 2. Timing between the first and second introduction event determines growth. (a)** Cell density after two introduction events of the same cell density and above the Allee threshold (4.42 x $10^5$ CFU/mL +/- 2.35 x $10^5$ in two, 10 μL introductions). $OD_{600}$ increased regardless of the time to second introduction. For panels a, b and c, standard deviation from a minimum of three replicates and $OD_{600}$ at 36 hours. Green shaded regions indicate times at which growth occurred ($P \leq 0.05$, when $OD_{600}$ is compared to zero (one-tailed t-test, Supplementary Tables S2)). **(b)** Cell density when a single introduction event led to an increase in $OD_{600}$. When the initial bacteria introduced (2.66 x $10^4$ CFU/mL +/- 2.04 x $10^4$) was within the Allee threshold, and a single introduction event was sufficient to result in an increase in $OD_{600}$ (time = 0), $OD_{600}$ increased regardless of the time to second introduction. **(c)** Cell density of bacteria when a single introduction did not lead to an increase in $OD_{600}$. Initial density of bacteria introduced (2.61 x $10^4$ CFU/mL +/- 1.25 x $10^4$) was within the Allee threshold. If a second introduction event occured up to 16 hours after the first introduction event, an increase in $OD_{600}$ was observed. After 16 hours, an increase in $OD_{600}$ is not observed. **(d)** After 8 hours of growth, we filtered, or did not filter as a control, the medium through a 0.22 μm filter to remove cells, but allowed AHL to remain in the medium. We then made a second introduction into the filtered medium. We observed growth in both filtred and non-filtered medium, the difference of which was insignificance ($P = 0.08$, two-tailed t-test). Standard deviation from eight biological replicates. **(e)** After 8 hours of growth, we removed, or did not remove as a control, the medium containing bacteria from the well. We centrifuged the cells, and resuspended them in fresh medium with IPTG. We observed no growth in cells that had been resuspended in fresh medium, but observed growth from cells in conditioned media (not fresh), that of which was significant ($P < 0.001$, two-tailed t-test). Standard deviation from seven biological replicates. **(f)** After 24 hours, we performed a second introduction event. In one of the wells, we added 100 nM of purified AHL resuspended in ethanol and 10μL of bacteria. In a second well (control), we only added 10μL of bacteria. We observed that the addition of purified AHL was sufficient to allow population growth ($P < 0.001$, one-tailed t-test), whereas no growth was observed in the populations without exogenously added AHL ($P = 1.0$, one-tailed t-test).

percentages of casamino acids (without IPTG), and measured $OD_{600}$ over 24 hours. We then determined growth rate by determining the slope of a linear line fit through the region of exponential growth. We observed that as the concentration of casamino acids increased in the growth medium, the growth rate of the bacteria increased.

Next, we sought to determine if translation rate, which could impact AHL production rate independently of growth rate, was affected by changing the percentage of casamino acids in the medium. We grew a GFP expressing strain of *E. coli* for 24 hours in the M9 medium with increasing percentages of casamino acids (Fig. 3b). At 20-minute intervals, we measured $OD_{600}$ and GFP (a.u.). We then plotted normalized GFP (GFP (a.u.)/$OD_{600}$) as a function of time, and determined the translation rate by determining the slope of a linear line fit through the region of exponential growth. We observed that as the percentage of casamino acids increased in the medium, the rate at which GFP was produced also increased, which indicates that translation rate also increased in the cell.

Since increasing translation rate may affect the circuit functionality and dynamics, we sought to determine if changing the percentage of casamino acids in the growth medium changed the region of Allee threshold. We inoculated medium with increasing percentages of casamino acids and containing various initial densities of bacteria into the 24 well plate and measured colony forming units (with IPTG, as was performed to generate Fig 1c). We observed that the region of the Allee threshold remained unchanged ($5 \times 10^3$ - < $10^5$ CFU/mL) when casamino acids were varied between 0.1% and 2% (Fig. 3c). Furthermore, we observed that $OD_{600}$ remained a consistent indicator of growth. When experiments were initiated with an initial density above the Allee threshold, there was an increase in $OD_{600}$ (Fig. 3d). Conversely, there was no observable increase in $OD_{600}$ when the initial density was in the region or below the Allee threshold.

To determine the influence that growth rate had on the range of times to second introduction that led to growth, we performed our multiple introductions experiment (Fig. 1b), but changed the concentration of casamino acids in the medium. Our previous results (Fig. 2c) had demonstrated that with two inoculations of 10 μL, we did not observe growth if the time to second introduction was greater than 16 hours. We observed that decreasing the percentage of casamino acids to 1% served to constrain the times to second introduction

Fig. 3

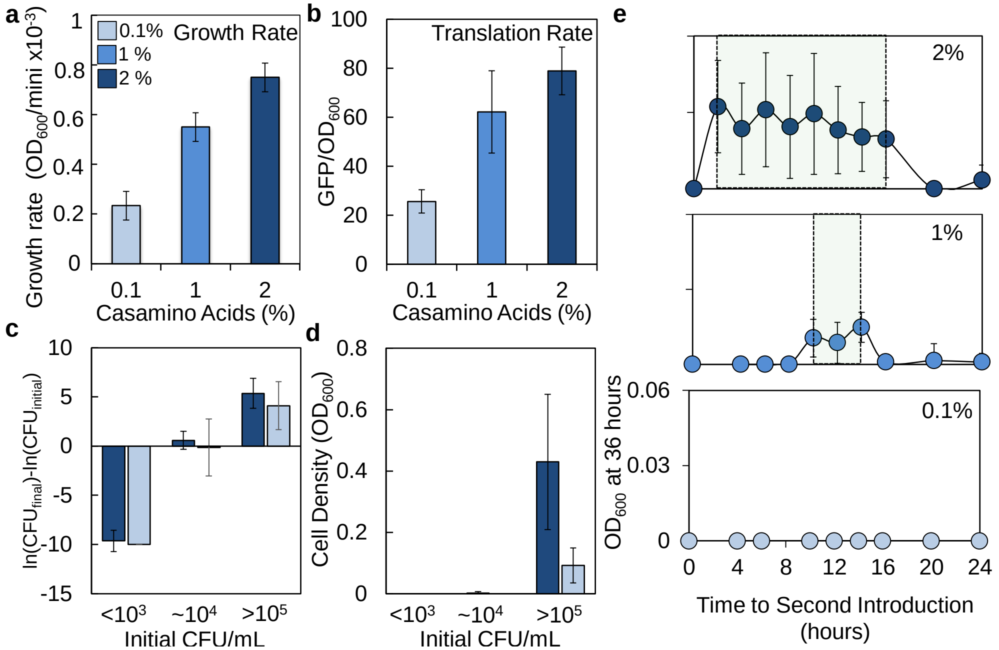

**Fig. 3. Reducing growth rate of bacteria constricts the time to second introduction that leads to growth. (a)** Bacteria were grown (without IPTG) in medium with increasing percentage of casamino acids. Standard deviation from three biological replicates. Comparison between 0.1% and 1.0 %, $P < 0.01$ (two-tailed t-test). Comparison between 0.1% and 2.0%, $P < 0.001$. Comparison between 1.0% and 2.0%, $P < 0.01$. For all panels, 0.1% = light blue, 1% = blue, 2% = dark blue. **(b)** GFP (arbitrary units) normalized by cell density ($OD_{600}$) of bacteria (no IPTG) growing in medium with increasing percentage of casamino acids. Increasing the percentage of casamino acids increased the rate of GFP production. Comparison between 1% and 2 %, $P = 0.01$ (two-tailed t-test). All other comparisons, $P < 0.001$. **(c)** Change in CFUs of bacteria grown in medium for 24 hours with different percentages of casamino acids (with IPTG) binned according to initial cell density. We observed that the region of the Allee threshold did not change when the percentage of casamino acids was increased. P values (two-tailed t-test) > 0.24 for all comparisons. Raw data in Supplementary Fig. S4. **(d)** $OD_{600}$ at 24 hours of bacteria grown as in panel c. $OD_{600}$ did not increase in the region of the Allee threshold ($P > 0.08$ when ~$10^4$ is compared to zero, both growth media, one-tailed t-test). Raw data in Supplementary Fig. S4. **(e)** Cell density ($OD_{600}$) after 36 hours of bacterial populations grown in medium with different percentages of casamino acids when a single introduction ($2.88 \times 10^4$ CFU/mL +/- $1.32 \times 10^4$) within the Allee threshold event does not lead to an increase in $OD_{600}$. As the percentage of casamino acids increased, the times to second introduction that allowed for growth increased (green shaded region). With 0.1% casamino acids in the growth medium, growth was not evident at 36 hours. Standard deviation from a minimum of three replicates. P values (one-tailed t-test) in Supplementary Table S2.

that led to growth (Fig. 3e): an increase in $OD_{600}$ was only observed if the second time to introduction was between 10 and 14 hours. A further reduction in casamino acids to 0.1% resulted in no growth at all times to second introduction examined. Overall, we observed that reducing growth rate constrained the times to second introduction that led to growth.

### *Asymmetry in the density of bacteria introduced increases establishment success*

Our analysis using the same cell density for both introduction events demonstrated that growth of the population is constrained by the timing between the first and second introductions. However, in nature, it is likely that propagule size will vary between introduction events (Lockwood et al. 2005), which may impact the aforementioned dynamics. Accordingly, we explored how varying the density of bacteria between introduction events influenced growth. We performed our multiple introduction experiment (Fig. 1b) but varied the total density of bacteria that was inoculated at the first and second introduction events by changing the volume of cells that were inoculated. The total volume of bacteria introduced in both inoculation events remained the same (20 μL). This allowed us to rule out how changes in the volume of medium in the well might impact the accumulation of AHL, and competition through increased nutrient, thus allowing our experiments to focus on the effect of bacterial density.

We observed that as the cell density between the first and second introduction diverged, longer times to second introduction resulted in $OD_{600}$ values significantly greater than zero (Fig. 4a and b). When the introduction volumes used were combinations of 5 μL and 15 μL, growth was not observed if the time to second introduction exceeded 20 hours. When the introduction volumes used were combinations of 1 μL and 19 μL, growth was observed at all times to second introduction measured. In general, we observed that as the cell density of the two introduction events became more asymmetrical (Fig. 4b), the time to second introduction that resulted in an increase in $OD_{600}$ occurred increased.

Fig. 4

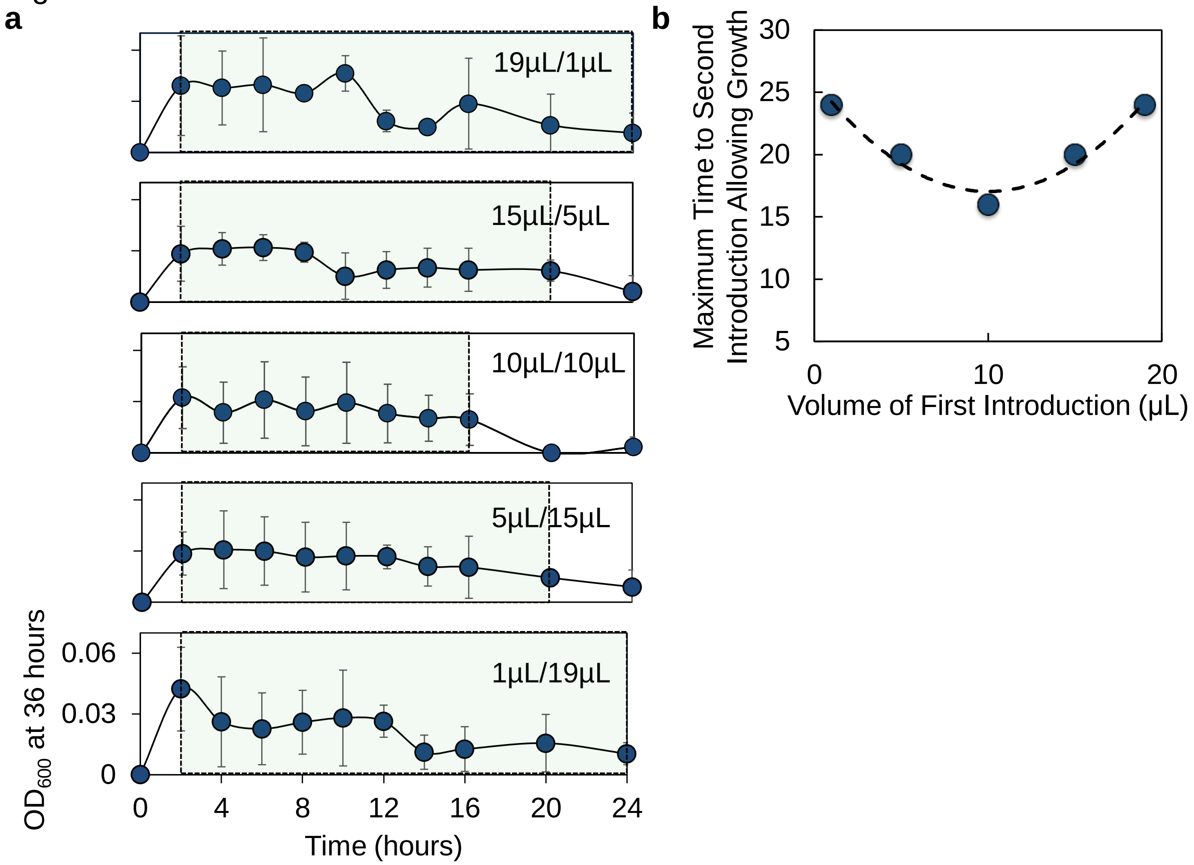

**Fig. 4. Asymmetry in propagule size increases the range of time over which a second introduction leads to growth. (a)** Cell density OD$_{600}$ at 36 hours of bacterial populations after two introduction events, where the cell density of each introduction event varied. As the densities of the two introduction events became more asymmetrical, the range of time over which the second introduction event led to an increase in OD$_{600}$ increased (green shaded region). Middle panel (10µL/10µL) replotted from Fig. 2c. Average cell density for all experiments = 2.55 x $10^4$ +/- 1.2 x $10^4$ CFU/mL. Standard deviation from a minimum of three experiments. P values (one-tailed t-test) in Supplementary Table S2. **(b)** Summary plot showing the longest time between first and second introduction events that led to an increase in OD$_{600}$. Data from panel a.

## Discussion

In efforts to better understand the conditions under which propagule pressure does or does not influence establishment, we used bacteria engineered with an Allee effect. For populations with initial densities within the region of the Allee threshold, we observed that growth rate, propagule size, and time to second introduction can determine establishment success and growth. We observed that when two introduction of equal density are made, growth is not observed if the timing between two introduction events is too long. Reducing the growth rate of the bacteria served to limit the timing between introduction events that resulted in growth. Furthermore, we observed that as the cell density of the two introduction events became more asymmetrical, the times to second introduction over which growth occurred increased. Overall, our results suggest that a critical determinant of the success of propagule pressure in determining establishment success is the timing between multiple introductions. If the timing between these introductions is too long, then establishment and growth may not occur. As the timing between introduction events would likely be unknown during natural invasions, this dynamic may account for the lack of congruence between studies that do, and do not, demonstrate a positive correlation between propagule pressure and establishment success.

One mechanism that can lead to an Allee effect is through environmental modification, the process of which is simulated by our engineered bacteria. Indeed, previous studies have suggested that invasive species can overcome Allee effects through environmental modification (Allee 1938, Cappucinno 2004, Farrer and Goldberg 2009, Laland et al. 2015). We surmise that the reason why growth was not observed if the time between the first and second introduction was too long (Fig. 2c, Fig. 3e, Fig. 4a) is due to interactions between production and decay of AHL, or more broadly, modification of the environment. As each bacterium produces (on average) the same amount of AHL, increasing either the growth rate or the initial population density would serve to increase the total amount of AHL produced over a given period of time. However, the AHL that is produced is not stable and is degraded in the medium according to first order decay (Wang and Leadbetter 2005). Therefore, any AHL available to a given cell would be a function of the production of AHL by the entire population minus its decay in the medium. These dynamics can be used to offer an explanation as to why, in some cases, growth was not observed after a second introduction of bacteria. Experiments where we manipulated the presence or absence of AHL (Fig 2d-f) strongly suggest that AHL is a critical determinant of growth in our experiments.

The data presented in Fig. 2c shows that, for two introduction of 10 μL, growth does not occur if the time to second introduction was greater than 16 hours. Taking into account the production and decay of AHL, we believe that after 16 hours, AHL has degraded to such a point that it cannot rescue the population that is inoculated in the second introduction event. However, if the time to second introduction occurs at or before 16 hours, the AHL produced from the first population, in combination with AHL produced from the second population, is sufficient to rescue the second population, and thus growth is observed. Decreasing the percentage of casamino acids in the medium served to constrain the times to second introduction that resulted in growth. Here, reducing the percentage of casamino acids reduces growth rate (Fig. 3a) and translation rate (Fig. 3b), both of which would reduce the amount of AHL produced by each bacterium, and thus the amount available for the population as a whole. Insufficient AHL is produced by all populations when grown in medium with 0.1% casamino acids, and thus growth is not observed. When grown in medium with 1% casamino acids, growth was not observed if the time to second introduction was less than 10 hours or greater than 14 hours. We surmise that between 10 and 14 hours represents a ‘sweet spot,’ where production of AHL from the first population is sufficient to rescue the second population. After 14 hours, AHL has degraded sufficiently so as to not rescue the second population. Before 10 hours, due to the decrease in growth and AHL production rate caused by the reduction in casamino acids, the combined population cannot synthesize a sufficient amount of AHL to rescue the entire population. Overall, reducing growth rate serves to limit the positive effect that propagule pressure can have on establishment success.

Previous studies have suggested that large propagule sizes can increase the likelihood of establishment (Simberloff 2009). The results presented in our study appear to confirm these findings (Fig. 4a-b). Moreover, our results suggest that, when considering the same total amount of propagules, asymmetry in propagule size between two introductions may increase the likelihood of establishment success. We surmise that this is due, as described above, to the production and decay kinetics of AHL (Fig. 5). Populations with higher densities would produce more AHL relative to those with smaller densities. Similarly, it would take a longer time for AHL produced by a population with a high density to degrade below a concentration that is insufficient to rescue a second introduction. In experiments with a large initial introduction density (19 µL/1 µL and 15 µL/5 µL), the increased amount of AHL produced by the first population, coupled with the longer time required for its decay, served to increase the times to second introduction that allowed growth. In experiments with a smaller first population, (1 µL/19 µL and 5 µL/15 µL), the amount of AHL produced by the population in the second introduction, coupled with the small amount produced by the first introduction, is sufficiently large to rescue the population. Since the amount of AHL produced by the population in the second introduction is large, it serves to extend the times to second introduction that still allow growth to occur. Overall, asymmetry in propagule size also serves to increase the times to second introduction that allow population growth.

Fig. 5

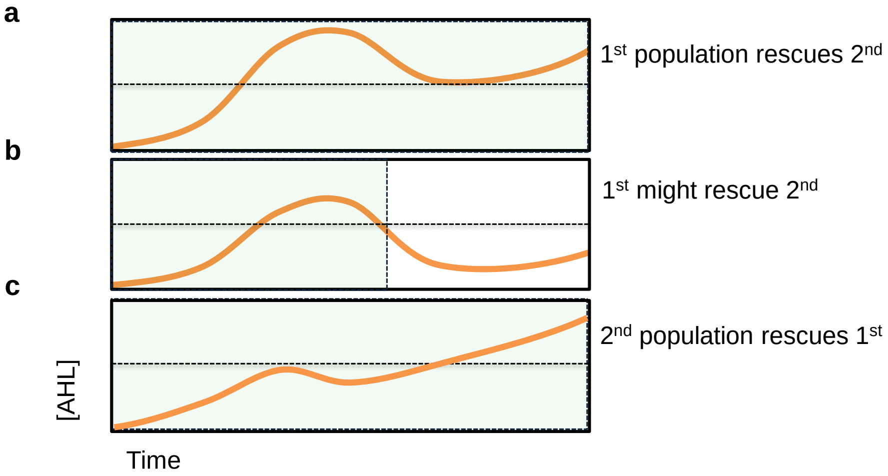

**Fig. 5: We hypothesize that the production and decay dynamics and AHL can explain our results. (a)** The amount of AHL produced by the first population is sufficiently large to rescue the second population (19µL/1µL introduction). The second population continues to produce AHL (orange line), thus allowing growth. **(b)** AHL produced by the first population may be insufficient to rescues the second population. If the second population is introduced at a time where AHL produced by the first population has degraded below a threshold (dotted line), the second population cannot take advantage of the AHL produced by the first population, and growth does not occur. However, if the second population is introduced prior to AHL degraded below the threshold, population growth occurs (15µL/5µL, 10µL /10µL, and 5µL /15µL cases). **(c)** AHL produced from the 1st population does not reach the threshold to rescue the second population. However, because the second population is sufficiently high in density, the AHL produced from the second population serves to rescue the first population, and growth occurs (1µL /19µL case).

Although the role of propagule pressure in biological establishment has been widely attributed, the concept can also be applied to reintroduction biology, or more broadly, establishment, in general. To achieve a high probability of establishment success, management strategies should evaluate factors including environmental conditions and life history parameters (Tabak et al. 2018). In addition to these factors, our results suggest that one initial introduction of high density close to the Allee threshold can rescue smaller populations introduced at later times. Furthermore, repeated introduction events that are within or near the Allee threshold, and at similar density, can result in population extinction if the timing between introduction events is too long.

Our results may have implications in the management of invasive species. Previous management approaches have emphasized reducing the number of individuals in an established by reducing the propagule size per introduction event below the Allee threshold (Johnson et al. 2006). As our results showed that one introduction of sufficiently high density can rescue an introduction of low density, this approach appears to be warranted. However, our results suggest that simply reducing the propagule size of dispersers below the Allee threshold may not be sufficient if multiple introduction events are predicted to occur. Here, it would be important to extend the timing between potential dispersal or introduction events through management strategies that attempt to limit the frequency and number of vectors to particular locations, such as the regulation of shipping pathways (Johnston et al. 2009).

Overall, our results suggest that the timing between two introduction events is a critical determinant of the ability of propagule pressure to facilitate establishment success. While our study considered populations that are well-mixed, marine environments are heterogeneous. Therefore, it would be interesting to examine how these dynamics factor into environments that are spatially heterogeneous. Moreover, while highly quantitative empirical data on the establishment of cooperative, non-native species is sparse, it would be interesting to examine such data to determine if there is evidence to support our findings. As we turn to the future, we are one step closer to understanding the complex process by which invasions operate.

## Methods and Materials

### *General growth conditions*

*Escherichia coli* DH5αPRO (Clontech, Mountain View, CA) was used throughout this study unless otherwise indicated. The majority of our experiments were performed in modified M9 medium [1X M9 salts (48 mM $Na_2HPO_4$, 22 mM $KH_2PO_4$, 862 mM NaCl, 19 mM $NH_4Cl$), 0.4% glucose, 2% casamino acids (Teknova, Hollister, CA), 0.5% thiamine (Alfa Aesar, Ward Hill, MA), 2 mM $MgSO_4$, 0.1 mM $CaCl_2$] buffered to pH 7.4 with 100 mM 3-(N-morpholino)propanesulfonic acid (MOPS, Amresco, Solon, OH). We inoculated single colonies from an agar plate (1% agar) into 3 mL Luria–Bertani (LB) medium (Genessee Scientific, El Cajon, CA). Overnight cultures were allowed to grow for approximately 24 hours. Short-term agar storage plates containing individual colonies were replaced approximately every 2 weeks. Unless otherwise indicated, all media contained 25 μg/mL chloramphenicol (Alfa Aesar, Heysham, England) and 50 μg/mL kanamycin (Amresco). The gene circuit was activated using 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG, Promega, Madison, WI).

### *Determining the region of Allee threshold*

From an overnight culture, 1 mL of bacteria was transferred into a separate microfuge tube. After centrifugation for 1 min at 12,000 rpm, the supernatant was removed and cells were resuspended in 1 mL of fresh modified M9 medium. A 10-fold dilution series was then performed. 10 μL from each tube of the dilution series was inoculated into 0.5 mL of M9 medium (that did, or did not, contain IPTG) that was contained in the well of a 24 well microplate (Costar 3524, Corning, Oneonta, New York). To prevent evaporation, the medium was covered with 500 μL of mineral oil (Fisher Scientific, Fair Lawn, NJ). Culture plates were incubated at 37 ºC while shaking at 160 revolutions per minute (RPM) for 36 hours. Cell density was measured using $OD_{600}$ in a Victor X4 microplate reader (Perkin Elmer, Waltham, MA). Colony forming units (CFU) were determined as previously described (Smith et al. 2014). To determine the region of the Allee threshold (e.g., near the bifurcation point in the system), we plotted ($\ln(CFU_{final}) - \ln(CFU_{initial})$) versus initial density, and located the range of initial densities where growth did not increase significantly, or decreased, over 36 hours. A minimum of three biological replicates were used for our analysis.

To determine how changing the percentage of casamino acids affected the Allee threshold, we performed the experiment described above but changed the percentage of casamino acids in the M9 medium.

*Multiple introduction experiments*

Bacteria were serially diluted in M9 medium without IPTG. 10 μL from the serial dilutions was inoculated into 24 well plates as described in “Determining Region of the Allee Threshold” that contained 500 μL of M9 medium supplemented with 1 mM IPTG. We focused on using initial densities in the region of the Allee threshold, which was verified by measuring CFU as previously described (Smith et al. 2014). This initial inoculation represented the first introduction event in our experiments. The 24 well plate was shaken at 160 RPM at 37 ºC. The serial dilutions from which the bacteria were inoculated from was placed at 4ºC so that their density did not change over the course of the experiment. After a given period of time (ranging from 2 hours to 24 hours), the serial dilution was removed from 4ºC, and 10 μL from the serial dilution was added into the same well in the 24 well plate. This represented the second introduction event. Plates were then shaken at 160 RPM at 37 ºC for a total of 36 hours, regardless of the timing of the second introduction event. Cell density was measured using $OD_{600}$. In experiments where propagule size was varied, we varied the volume of the first and second introduction events, but kept the total introduction volume (20μL) consistent for all experiments. All $OD_{600}$ values reported have been blanked using cell free medium.

To confirm that cell density does not significantly change when bacteria were held at 4 ºC, CFU measurements of serially diluted bacteria (no IPTG in the medium) were conducted for each culture at 0 hours, 12 hours, and 24 hours as previously described (See Supplementary Fig. S2) (Smith et al. 2014).

*Filtration experiment*

Bacteria were serially diluted and introduced into 24 well plates as described in “multiple introductions experiment”. After 8 hours of growth, the medium containing initial introduced bacteria was removed from the well, and filtered through a 0.22 µm syringe filter (Genessee Scientific). The filtered medium was placed into a new well in the 24 well plate and overlaid with 500 µL of mineral oil. A second aliquot of bacteria was then introduced as described in "Experiments with multiple introductions." Plates were shaken at 160 RPM at 37ºC for a total of 36 hours. Cell density was measured using $OD_{600}$. Eight biological replicates were performed.

### *Medium replacement experiment*

Bacteria were serially diluted and introduced as described in "multiple introductions experiment". After allowing a 10 µL introduction to grow for 8 hours, we removed the medium from the well, and centrifuged it at 12,118 x g for 2 minutes to pellet the cells. The pelleted cells were washed twice in fresh M9 medium, and were subsequently resuspended in 500µL of fresh M9 medium (with IPTG). The media was transferred to a new well on the 24 well plate and overlaid with 500µL of mineral oil. A second introduction 10 µL aliquot of bacteria was then added as described in "multiple introductions experiment," and the plates were shaken at 160 rpm at 37 ºC for a total of 36 hours. Cell density was measured using $OD_{600}$. Seven biological replicates were performed.

### *AHL rescue experiment*

Bacteria were serially diluted and introduced as described in "multiple introductions experiment". After allowing a 10 µL introduction to grow for 24 hours, a second 10 µL introduction of the same cell density was made. To the same well we added, or did not add as a control, 100 nM of purified AHL (3OC6HSL, Cayman Chemical Company, Ann Arbor, MI). The plates were shaken at 160 RPM at 37ºC for a total of 36 hours. Cell density was measured using $OD_{600}$. Five biological replicates were performed.

### *Growth rate*

An overnight culture was diluted 1000-fold into 200 µL of fresh M9 medium (without IPTG) that was contained in the well of a 96-well plate (Genesee Scientific). The media in the microplate was overlaid with 70 µL of mineral oil, and placed in a Victor X4 microplate reader that was preheated to 37ºC. Every 10 minutes, the plate was shaken with a radius of 0.1 mm for 10 seconds on the fast setting, and $OD_{600}$ was measured. Growth was determined by fitting a linear line through the region of the growth curve where there was a linear increase in growth. We then compared the slopes of the these linear lines ($R^2 \geq 0.99$). Three biological replicates were performed.

### *Translation rate*

A plasmid (colE1 replication origin, chloramphenicol resistance) containing the $P_{tet}$ promoter (Lutz and Bujard 1997) controlling the expression of *gfp*(*mut3b*) was transformed into competent (Transformation Kit & Buffer Set (Zymo Research Corp., Irvine, CA)) DH5αPRO bacteria (Clontech, Palo Alto, CA). Isolation of plasmid DNA was acheieved using a Zippy Plasmid Miniprep Kit (Zymo Research Corp.). An overnight culture of bacteria containing the $P_{tet}$-GFP(mut3b) plasmid were diluted 1000-fold into 200 µL of M9 media containing difference concentrations of casamino acid (0.1%, 1.0%, and 2.0%). GFP expression was activated using 100 ng/mL of anhydrotetracycline (atc). 200 µL of media containing cells was placed into each well of a 96-well plate, was subsequently overlayed with 70 µL of mineral oil, and placed in a Perkin Elmer Victor X4 microplate reader that was preheated to 37°C. Every 20 minutes, the plate was shaken with a radius of 0.1 mm for 10 seconds on the fast setting, and both GFP (arbitraty units, a.u.) and $OD_{600}$ were measured. GFP (a.u.) was normalized to $OD_{600}$ and plotted as a function of time. Average plotted from three technical replicates from five biological replicates.

### *Statistical significance*

As indicated in the figure or table legends, we used a student's one- or two-tailed t-test (un-paired, unequal variance) to determine statistical significance ($P \leq 0.05$) using Microsoft Excel (Redmond, WA). One-tailed t-tests were used to determine if data was significantly greater than zero. We chose this statistical test as $OD_{600}$ values cannot be less than zero, only greater. Two-tailed t-tests were used for all other statistical tests.

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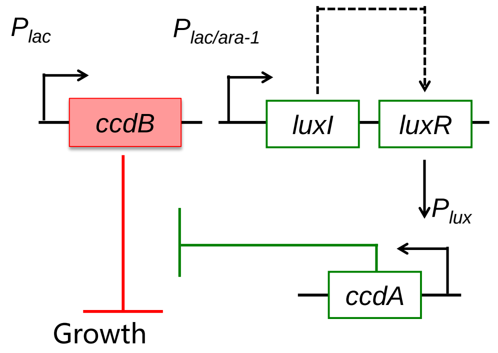
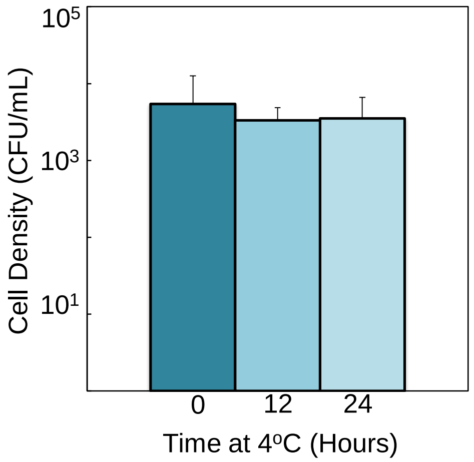
# Supplemental Material

Supplementary Fig. S1



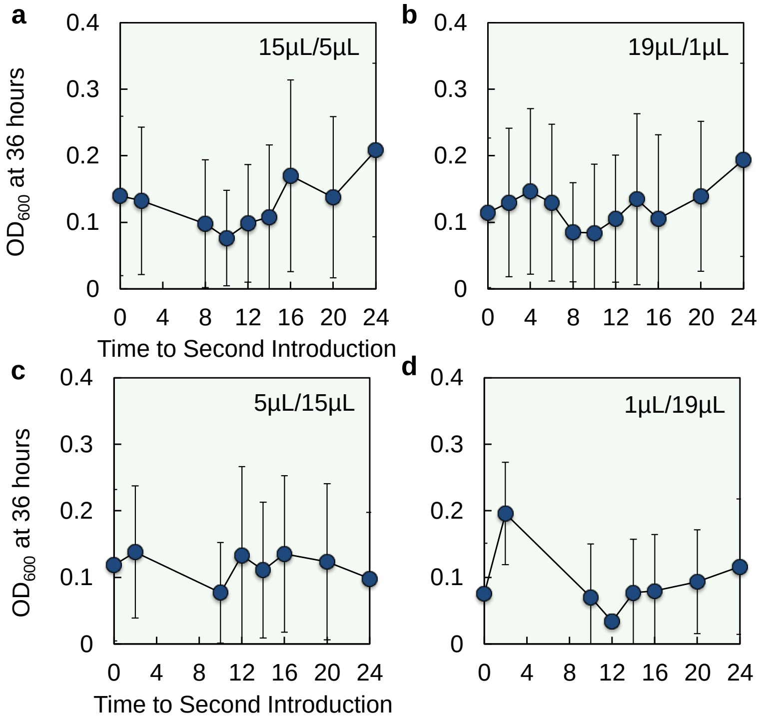
**Supplementary Fig. S1. Circuit diagram conferring the Allee effect to our engineered bacteria.** An IPTG inducible $P_{lac}$ promoter regulates expression of the toxin protein *ccdB*, which kills the cell by inhibiting gyrase (Dao-Thi et al. 2005). The *luxR*/*luxI* quorum sensing system is activated by IPTG via the $P_{lac/ara}$ promoter. LuxI produces an acylhomoserine lactone (3OC6HSL, AHL), which can be shared amongst all members of the bacterial population. Once the AHL has reached a sufficiently high concentration, it binds to the LuxR protein, and drives expression of the *ccdA* antitoxin protein (regulated by the $P_{lux}$ promoter). This prevents cell death by interfering with the CcdB protein (Afif et al. 2001). Construction and additional testing of circuit functionality can be found in (Smith et al. 2014).

Supplementary Fig. S2



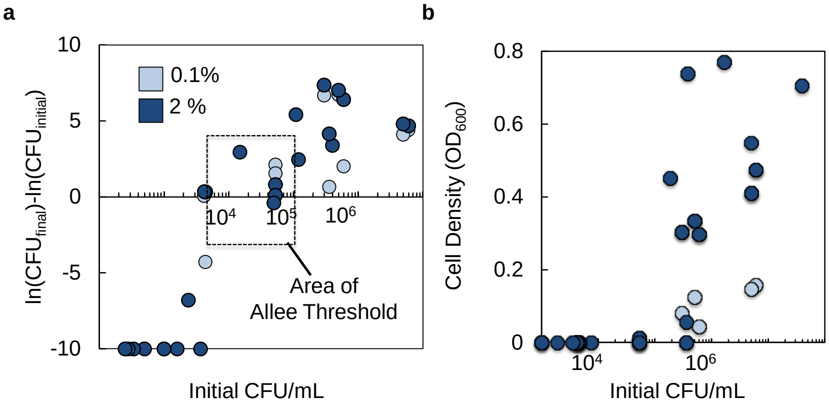
**Supplementary Fig. S2. Temporarily storing the engineered bacteria at 4°C does not decrease their cell density.** We serialy diluted bacteria near the Allee threshold in M9 medium (without IPTG), and subsequently stored the bacteria at 4°C. CFUs were measured upon serial dilution (time = 0 hour), and after 12 and 24 hours. $P > 0.5$ when the 0 hour measurement is compared to 12 and 24 hour measurements (two-tailed t-test). Standard deviation from three replicates. This indicates that in our expeirments with multiple introductions, large changes in CFU in the second introduction event cannot primarily account for growth dynamics observed (Fig. 2, 3 and 4 , and Supplementary Fig. S3).

Supplementary Fig. S3



**Supplementary Fig. S3. Growth of engineered bacteria occurs regardless of the time to second introduction if growth is observed in a single introduction event (t = 0).** Initial CFU near the Allee threshold for each experiment (indicate below) and volumes introduced indicated in the top right of each panel (a, average density = 2.00 x $10^4$ CFU/mL +/- 1.27 x $10^4$; b, average density = 2.41 x $10^4$ CFU/mL +/- 1.45 x $10^4$; c, average density = 2.19 x $10^4$ CFU/mL +/- 1.26 x $10^4$; d, average density = 2.29 x $10^4$ CFU/mL +/- 8.90 x $10^3$). Standard deviation from a minimum of three replicates. P values are indicated in Supplementary Table S2.

Supplementary Fig. S4



**Supplementary Fig. S4. Raw data showing the effect of changing the percentage of casamino acids in medium on the growth dynamics of the engineered bacteria. (a)** Raw data showing the change in CFU over 24 hours for engineered bacteira grown in medium with different percentages of casamino acids. Allee threshold is indicated with the square box. For both panels, 0.1% = light blue, 2% = dark blue. Data corresponds to Fig. 3c in the main text. **(b)**Raw data showing the final cell density (OD$_{600}$) for engineered bacteria grown in medium with different percentages of casamino acids. Data corresponds to Fig. 3d in the main text.

**Table S1. A summary of conflicting results linking propagule pressure to establishment success.**

| | Main Finding | Reference |
|---|---|---|
| **Propagule pressure promotes establishment success** | Canopy disturbance and propagule pressure of exotic plant invasion are most important predictors of *Alliaria petiolata*, *Berveris thunbergii*, and *Microstegium vimineum* establishment and invasion. | (Eschtruth and Battles 2009) |
| | A positive correlation exists between propagule pressure and probability of *Bythotrephes longimanus* establishment. | (Gertzen et al. 2011) |
| | There is a positive coorelation between interception frequency (disruption of propagule pressure) and establishment for *Scolytinae* and *Cerambycidae* species. | (Brockerhoff et al. 2014) |
| | Using an ecological modelling approach, the product of propagule size and frequency correlate more strongly with invasion success than either single component alone. | (Wittmann et al. 2014) |
| | Propagule pressure is positively related to establishment success from re-analysis of New Zealand bird introduction data. | (Blackburn et al. 2011) |
| **Propagule pressure does not promote establishment success** | Initial releases of New Zealand bird species explain establishment success as opposed to introduction frequency and high introduction number | (Moulton et al. 2012) |
| | Three estimates of propagule pressure (number of introduced individuals, number of areas introduced, and number of years during which species was planted) for 130 species of woody plants did not predict successful establishment and invasion. | (Nunez et al. 2011) |
| | Propagule pressure does not determine invasion success for communities with high invasion resistance, particularly for species *Urochloa platyphylla, Digitaria sanguinalis, Abutilon theophrasti,* and *Amaranthus* retroflexus, which experience strong density-dependent inhibition | (Barney et al. 2016) |
| | Using *Pseudomonas chlororaphis*, *Pseudomonas putida*, and *Escherichia coli*, establishment and invasion success does not depend only on propagule pressure. | (Ketola et al. 2017) |
| | Propagule pressure did not influence establishment for *Linepithema humile* in field studies. | (Sagata and Lester 2009) |

**Table S2. A summary of P-values obtained in our multiple introduction experiments. P values were determined using a one-tailed t-test and compared against zero.**

| IPTG | Percentage of Casamino Acids | Introduction Volumes (μL) | | 0 hr | Time to second introduction | | | | | | | | | | Fig |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 2 hr | 4 hr | 6 hr | 8 hr | 10 hr | 12 hr | 14 hr | 16 hr | 20 hr | 24 hr | |
| NO | 2 % | 10 | 10 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | 2a |
| YES | | | | < 0.001 | < 0.001 | 0.013 | 0.013 | 0.002 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | 2c |
| | | | | 1 | 0.008 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | 0.013 | 1 | 0.211 | 2c, 3a, 4b |
| | | 15 | 5 | 1 | 0.019 | < 0.001 | < 0.001 | < 0.001 | 0.008 | < 0.001 | 0.046 | 0.014 | 0.001 | 0.101 | 3a |
| | | 19 | 1 | 1 | 0.020 | 0.002 | 0.004 | 0.003 | 0.008 | 0.001 | 0.004 | 0.023 | 0.042 | 0.048 | 3a |
| | | 5 | 15 | 1 | 0.010 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | 0.002 | < 0.001 | 0.088 | 3a |
| | | 1 | 19 | 1 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | 0.007 | 0.002 | 0.002 | 0.003 | 3a |
| | 1 % | 10 | 10 | 1 | - | 1 | 1 | 1 | 0.003 | 0.004 | 0.008 | 0.172 | 0.165 | 0.081 | 4b |
| | 0.1 % | | | 1 | - | 1 | 1 | - | 1 | 1 | 1 | 1 | 1 | 1 | 4b |
| | 2 % | 19 | 1 | < 0.001 | 0.002 | < 0.001 | 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | 0.006 | 0.040 | S3 |
| | | 15 | 5 | < 0.001 | 0.002 | - | - | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | 0.006 | 0.023 | S3 |
| | | 5 | 15 | < 0.001 | 0.002 | - | - | - | < 0.001 | 0.058 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | S3 |
| | | 1 | 19 | < 0.001 | 0.015 | - | - | - | 0.042 | 0.005 | 0.010 | 0.016 | 0.033 | 0.038 | S3 |

## Supplemental Material References

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