

1 Experimental demonstration of an Allee effect in microbial populations

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## Abstract

Microbial populations can be dispersal limited. However, microorganisms that successfully disperse into physiologically ideal environments are not guaranteed to establish. This observation contradicts the Bass-Becking tenet: ‘*Everything is everywhere, but the environment selects*’. Allee effects, which manifest in the relationship between initial population density and probability of establishment, could explain this observation. Here, we experimentally demonstrate that small populations of *Vibrio fischeri* are subject to an intrinsic demographic Allee effect. Populations subjected to predation by the bacterivore *Cafeteria roenbergensis* display both intrinsic and extrinsic demographic Allee effects. The estimated critical threshold required to escape positive density dependence is around 5, 20, or 90 *cells mL<sup>-1</sup>* under conditions of high/low carbon resources, or low with predation, respectively. This work builds on the foundations of modern microbial ecology, demonstrating that mechanisms controlling macroorganisms apply to microorganisms, and provides a statistical method to detect Allee effects in data.

## Introduction

The molecular renaissance and technological advances in single cell manipulations have transformed microbiology. Particularly, molecular techniques have allowed investigation of previously intractable questions leading to a conceptual departure from the first clause of Bass-Becking’s (BB) ‘*Everything is everywhere...*’ [1]; multiple studies have shown that microorganisms may be dispersal limited [2]. In contrast, few studies have examined the second clause ‘*...but the environment selects*’ [3]. Updated, BB implies that a population will establish as long as a cell can disperse to a physiologically favorable environment. A corollary is the laboratory dogma that microbial contamination may result from the introduction of a single cell. Microbial systems have long been used to describe the consequences of competition and predator-prey dynamics observed in metazoan populations [4]. Since density-dependent mechanisms limit colonization in metazoans (as reviewed by [5]), establishment for microorganisms may be less straightforward than assumed.

One such density-dependent phenomenon is the Allee effect. An Allee effect (positive density dependence) is characterized by reduced per capita growth rate at small populations [6] compared with large ones, and has been observed in Mollusca, Arthropoda and Chordata (as reviewed in [7]). A strong Allee effect occurs when the per capita growth rate is negative for some small population size, which gives rise to a critical density [5]. This critical density can be detected in the relationship between a population’s probability of establishment and its initial size (see [8]). A strong Allee effect induces a sigmoidal relationship with an inflection point at the threshold [8]. In contrast, for populations without a strong Allee effect, the probability of establishment will be a concave function of initial density due to demographic stochasticity. Previously, Allee effects have been observed in experimental and natural populations of metazoans, with important implications for the management of vulnerable or invasive populations [9, 10]. While microbes have been engineered to display an Allee effect [11, 12], this is the first study to explore their existence in an environmentally isolated microorganism.

Here we report on a combined theoretical/empirical study to detect intrinsic and extrinsic demographic Allee effects in experimental populations of a marine bacterium (*Vibrio fischeri*, strain *ES114 pVSV102*; [13]).

Populations with an intrinsic demographic Allee effect have a lower per capita growth rate at low densities when compared to higher population densities. In contrast, an extrinsic demographic Allee effect results from higher per capita predation risk at low prey densities. In our experiments, *V. fischeri* populations were propagated from a range of inoculum sizes, a subset of which were also exposed to predation by *Cafeteria roenbergensis*. The success or failure of establishment was used to estimate the strength of the demographic Allee effects. This study shows that one cell may not be adequate to initiate a population, especially in nature where growth conditions, including predation, are suboptimal.

## Methods

### Experiment

The presence of a demographic Allee effect was examined using a partial  $2 \times 2$  factorial design for a total of three treatments. Populations of *V. fischeri* were inoculated with geometrically increasing number of viable cells in high carbon (*HC*; 1 to 64 cells  $n = 36$  per density) and low carbon (*LC*; 1 to 2048 cells  $n = 24$  per density) resource environments; a portion of the populations with low resources were exposed to predation (*LCP*;  $n = 12$  per density) by *C. roenbergensis*. High precision inoculum sizes were achieved by flow cytometry; cells were individually sorted into media filled well plates of a final volume of  $200\mu L$  and  $75\mu L$  into *HC* and *LC*, respectively. Well mixed populations were incubated at  $28^\circ C$  for 96 hours before assessing population establishment ( $\Delta OD_{620} \geq 0.25$ ; see supplement).

### Model Fitting

The presence of a strong Allee effect alters the probability of establishment as a function of initial population density from an inverse exponential decay relationship as expected with demographic stochasticity to sigmoidal. The 2-parameter Weibull function can take on either of these shapes depending on the value of a single parameter,  $k$ . Here, a 2-parameter Weibull is defined as,  $p = 1 - e^{(\frac{-x}{\lambda})^k}$ , where  $p$  is the probability of establishment,  $x$  is the  $\ln(\text{initial population density (cells mL}^{-1}\text{)})$ , and  $\lambda$  is a scale parameter. Interpreting this model as the probability of invasion gives rise to a binomial distribution with likelihood,  $L(p) = L(y|p) = p^y(1 - p)^{(n-y)}$ , where  $y$  is the total number of successes in  $n$  replicates. The shape ( $k$ ) and scale ( $\lambda$ ) parameters were simultaneously estimated by fitting this equation to individual trial data (see supplement).

## Results

The proportion of populations establishing increased and time to detection decreased non-linearly with initial density (Fig. 1), indicating reduced growth rate at low density populations (Table 1). The estimated shape parameter,  $k$ , was greater than 1 for all three treatments, indicating an Allee effect in all cases (Fig. 2). The scale parameter,  $\lambda$ , was also greater than 0, implying that the density needed for a positive growth rate was larger than 1  $\text{cell mL}^{-1}$  (Fig. 2).

## Discussion

This study shows a class of phenomena important in macroscopic systems may be relevant to single celled organisms, questioning the Bass-Becking tenet. Specifically, *V. fischeri* populations were subject to both intrinsic and extrinsic demographic Allee effects. The strength of the effect, represented by the critical density, increased with predation and decreasing carbon (Fig. 1). Possibly, even more pronounced Allee effects would be observed in natural marine populations of heterotrophic bacteria where natural concentrations of dissolved organic carbon are up to three orders of magnitude lower than in our experiments [14]. *V. fischeri* populations subject to *C. roenbergensis* predation at natural concentrations [15] had a significantly higher critical threshold than populations without predation. The difference in critical threshold between the *LC* and *LCP* treatments is due to the additional number of individuals needed to compensate for mortality due to predation. The prey, *V. fischeri* most likely overcame predation by satiation associated with a Type II/III functional response, since *C. roenbergensis* stops filtering when prey fall below 2 cells  $mL^{-1}$  [16].

This study detected the presence of an Allee effect, but the mechanism(s) leading to the critical density in the absence of predation are not yet understood. A candidate mechanism is quorum sensing, which detects density, and is important in *V. fischeri*'s symbiosis with bobtail squid *Euprymna scolopes*. Many other species have similar interactions based on population density [17, 9]. Populations that did not reach densities detectable by our methods were scored as failure to establish. We could not, therefore, differentiate between cell death and extremely slow growth (a doubling time at least an order of magnitude longer than usually observed). Cell dormancy is another possibility for bacteria and might suggest another way of reacting to reduced fitness at low density resulting in an overestimated Allee effect.

In conclusion, this work provides a mechanistic demonstration that our conceptual understanding of processes controlling microbial populations must be more complicated than the historic BB tenant [1], with important implications for health and biotechnology application (see supplement). Microbial ecology has shown that many mechanisms controlling metazoans apply similarly to microorganisms [18]. This study contributes to this literature with an example of positive density dependence.

## Data accessibility

The dataset and analysis has been deposited in Dryad Digital Repository (<http://dx.doi.org/10.5061/dryad.q7qv2>).

## Ethics

No approval for animal research was required. All organisms used in this study are invertebrates, and not regulated by the Institutional Animal Care and Use Committee (IACUC).

## Competing interests

We declare we have no competing interests.

## Authors' contributions

The project was conceived and designed by AMK, FD and JMD. FD conducted preliminary experiments. RBK participated in experimental design, conducted the experiment, analyzed data and drafted manuscript. All authors participated in analyzing data and contributed to the paper. All gave final approval for publication. We agree to be accountable for all aspects of the work.

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## Tables

Table 1: Experimental Summary. Each environmental treatment had populations inoculated with different densities. The average time to establishment and the yield are reported for the established populations.

Environment	Initial Density ( <i>cell mL</i> <sup>-1</sup> )	Established (%)	Time to Establishment (Hour)	Yield ( <i>OD</i> <sub>620</sub> )
High Carbon ( <i>HC</i> )	5	53 (19/36)	31.9 ± 9.5	0.39 ± 0.053
	10	72 (26/36)	32.6 ± 8.9	0.38 ± 0.045
	20	97 (35/36)	31.6 ± 9.1	0.39 ± 0.052
	40	100 (36/36)	31.1 ± 8.8	0.39 ± 0.049
	80	100 (36/36)	30.7 ± 8.3	0.39 ± 0.039
	160	100 (36/36)	29.1 ± 7.5	0.39 ± 0.041
	320	100 (36/36)	27.3 ± 7.3	0.39 ± 0.036
Low Carbon ( <i>LC</i> )	13.3	8.3 (1/12)	95.0 ± NA	0.26 ± NA
	26.7	75 (9/12)	84.3 ± 8.326	0.41 ± 0.092
	53.3	100 (12/12)	63.2 ± 6.537	0.66 ± 0.018
	106.7	100 (12/12)	58.2 ± 6.043	0.65 ± 0.025
	213.3	100 (12/12)	52.5 ± 4.042	0.65 ± 0.019
	426.7	100 (12/12)	48 ± 3.767	0.65 ± 0.024
	853.3	100 (12/12)	35.1 ± 11.574	0.7 ± 0.084
	1706.7	100 (12/12)	37.4 ± 1.56	0.69 ± 0.023
	3413.3	100 (12/12)	35.2 ± 1.513	0.7 ± 0.011
	6826.7	100 (12/12)	31.9 ± 1.165	0.7 ± 0.017
	13653.3	100 (12/12)	29.7 ± 0.829	0.71 ± 0.012
	27306.7	100 (12/12)	26.9 ± 0.454	0.7 ± 0.012
Low Carbon with Predation ( <i>LCP</i> )	13.3	8.3 (1/12)	86.2 ± NA	0.37 ± NA
	26.7	8.3 (1/12)	94.0 ± NA	0.27 ± NA
	53.3	50 (6/12)	89.7 ± 6.196	0.35 ± 0.108
	106.7	33 (4/12)	87.8 ± 3.942	0.38 ± 0.073
	213.3	100 (12/12)	69.4 ± 5.437	0.56 ± 0.032
	426.7	100 (12/12)	69.5 ± 10.154	0.54 ± 0.089
	853.3	100 (12/12)	37.6 ± 1.677	0.69 ± 0.011
	1706.7	100 (12/12)	35.7 ± 1.943	0.7 ± 0.034
	3413.3	100 (12/12)	34.8 ± 1.211	0.7 ± 0.014
	6826.7	100 (12/12)	31.6 ± 1.632	0.73 ± 0.021
	13653.3	100 (12/12)	27.8 ± 1.396	0.72 ± 0.012
	27306.7	100 (12/12)	26 ± 1.46	0.71 ± 0.019

## Figure Captions

Figure 1. **Probability of establishment from fitted Weibull curve.** The outcome of each population per inoculum size (small points, colored by treatment) were used to calculate the average probability of establishment with a binomial confidence interval (open symbols) and fit the Weibull function (solid line). The estimated critical thresholds (closed symbols; 95% confidence interval in shaded region) are: 4.85 (2.76 – 6.67), 23.8 (19 – 29.6), and 89.4 (60.2 – 126) *cells mL<sup>-1</sup>* for the *HC* (40mM glycerol), *LC* (20mM glycerol) and *LCP* (20mM glycerol plus 133 *C. roenbergensis mL<sup>-1</sup>*), respectively.

Figure 2. **Parameter estimates suggest Allee Effect present in all treatments.** The shape parameter,  $k$ , tests for the presence of an Allee effect; values greater than 1 indicate a sigmoidal relationship between density and probability of establishment. The critical threshold, as determined by the inflection point, has a theoretical upper bound of  $\lambda \left( \tilde{x} = \lambda \sqrt[k]{\frac{k-1}{k}} \right)$ . Values of  $\lambda$  less than zero implies a critical threshold less than 1 *cell mL<sup>-1</sup>*, which were considered biologically irrelevant. Point estimates are presented with 95% confidence region.