Experimental demonstration of Allee effects in microbial populations

RajReni Kaul, Andrew M. Kramer, Fred Dobbs and John M. Drake

March 2, 2015

Abstract

Italics (non-scientific) are notes to self. Bold sentences are paragraph purpose.

1 Introduction

hook without the straw man, We know that microbes are dispersal limited, but there is still the assumption that dispersal into an ideal environment is the major limiting factor for establishing a new population

Microbiology has been transformed by the molecular renaissance. Molecular techniques allowed exploration of previously intractable questions leading to a conceptual shift of the Becking-Bass(BB) tenet *'Everything is everywhere...'* (Baas Becking [1934], de Wit and Bouvier [2006]). Multiple studies have shown that microbes are dispersal limited, but few studies have examined the second component *'...but the environment selects'* of the BB tenet (Sul et al. [2013], Reno et al. [2009], Hellweger et al. [2014]). The updated tenet implies that a population will establish as long as the cell can disperse to a favorable physiological environment and is akin to the dogmatic view of microbial contamination resulting from the introduction of a single cell. The molecular renasance has shown that populations of micro- and macro-organisms can be subject to similar dynamical processes, so the single cell assumption may not be reasonable when put into the density dependence framework of macro-organisms.

Why is it unreasonable to assume that a single asexual organism can propagate a colony? Need a stronger statement to connect to AE?

Allee effects or positive density dependence can have unexpected impact on population survival

An Allee effect (AE) or positive density dependence is characterized by reduced or negative growth rates at small populations (Allee and Bowen [1932]) and has been observed in Mollusca, Arthropoda and Chordata (as reviewed in Kramer et al. [2009]). Strong Allee effects result in small populations having negative per capita growth rates which result in an extinction vortex. This effect and the minimum population required for a positive growth rate can be detected in the population's probability of establishment when plotted as a function of initial size. Populations not impacted by an Allee effect will have a monotonic increase in the probability of establishment with increasing initial density, demographic stochasticity is driving the relationship. A strong Allee effect would create a sigmoidal relationship centered around the critical threshold, below which the probability of establish would increase slower than above due to negative per capita growth rates (Dennis [2002]). The presence and strength of an Allee effect is important for management of exploited and vulnerable populations as well as nuisance or invasive species. Allee effects have been observed in natural studies (Angulo et al. [2007] predator-induced review Gascoigne and Lipcius [2004]), or experimental manipulations of macro-organisms (Kramer and Drake [2010], Ward et al. [2008]). However, the only study to explore the possibility of an AE in microbes used an engineered yeast, designed to have an Allee effect (Dai et al. [2012]).

We were our study picks up

In the current study, we used a combined theoretical and empirical approach to detect the presence of Allee effects in microbial populations of *Vibrio fischeri*, strain *ES114* containing a plasmid with constitutively expressed green florescence protein (GFP) and kanamycin-resistance cassettes (pVSV102, Dunn et al. [2006]). *V. fischeri* populations were propagated from a range of inoculum sizes, a subset of which were also exposed to predation by the eukaryotic generalist bacterivore *Cafeteria roenbergensis*. The success or failure of establishment was then used to determine the presence and strength an intrinsic or predator-induced Allee effect based on the relationship between probability of establishment and inoculum size.

2 Methods

Experiment

$$y = 1 - e^{(\frac{-x}{\lambda})^k}$$

Figure 1: **Expression used to detect AE** The probability of persistence, y,is a function of the population density, x. An estimate of $k > 1$ indicates the presence of an AE. *Need to determine a way to embed equation without calling it a figure.*

The presence of demographic or predator induced Allee effects were examined using a partial factorial design. Populations of *V. fisheri* were inoculated in high and low resource environments, a portion of the populations with low resources were also exposed to predation by *C. roenbergensis*. Populations of *V. fisheri* with high resources were not exposed to predation due to co-culturing limitations. All populations were grown in mineral salts medium (0.4mM $NaPO_4$ (pH 7.5), 50mM Tris (pH 8.0), 11mM NH_4Cl, 10uM $FeSO_4 \cdot 7H_2O$, 55mM $MgSO_4 \cdot 7H_2O$, 11mM KCl, 0.3M NaCl, 11mM $CaCl_2 \cdot 2H_2O$) containing 20 mM glycerol under kanamycin selection (100mg/mL). The high resource environment had an additional 20mM glycerol.

Individual cells of *Vf* and *C. roenbergensis* were selected to inoculate experimental populations by fluorescence flow cytometry. Exponential phase cultures of *Vf* and *C. roenbergensis* were stained with 3uM propidium iodide (PI),a membrane impermeable nucleic acid dye, for 15 minutes prior to sorting into a well pre-filled with media of a microtiter plate. This method allowed populations to be accurately initiated with geometrically increasing number of viable cells (1 to 64 cells in high resource and 1 to 2048 cells in low resource). The high resource experiment was conducted in 3 replicate 96 well microplates with a final volume of 200uL. The low resource experiment was done in a 384 well microplate with a final volume of 75uL. The volume difference created non-identical but overlapping density treatments of 5, 10, 20, 40, 80, 160 and 320 cells/mL for the high resource treatment (n=36) and 13.3, 26.7, 53.3, 106.7, 213.3, 426.7, 853.3, 1706.7, 3413.3, 6826.7, 13653.3, and 27306.7 cells/mL for the low resource treatment (n=24). A predator-induced AE was tested by adding 10 *C. roenbergensis* per population to half of the low resource treatment replicates.

Immediately following well inoculation the microtiter plates were sealed with optical film to avoid any contamination. Plates were simultaneously incubated at 28C and monitored for growth based on optical density (OD_{620}) and GFP fluorescence (485/528, sensitivity=XX) in a Synergy H1 plate reader (Biotek). Populations were allowed to grow for 72 hours before assessing population establishment. Established populations, scored as 1, were defined as having an increase of at least 0.25 OD_{620} or 100 relative fluorescence units (RFUs) from the initial reading. The threshold used ensured that noise introduced by the plate reader would not be considered growth. *V. fisheri* strain ES114 and *C. roenbergensis* were kindly supplied by Eric Stabb (UGA) and Alexander Bochdansky(), respectively.

Model Fitting

The presence of an AE can be separated from stochastic noise by examining the probability of establishment as a function of initial population density. The probability of small populations establishing when regulated by an Allee effect will be due to a negative per capita growth rate and demographic stochasticity. When a population with an Allee effect is inoculated with more cells than the critical density, probability of establishment will only be reduced due to demographic stochasticity. The different ranges that a population experiences negative per capita growth rate and demographic stochasticity creates a sigmoid when probability of establishment is a function of initial population size. The 2-parameter Weibull function is sigmoidal when the shape parameter is greater than one ($k > 1$). Using this as a statistical test for the presence of an AE, we simultaneously fit the shape (k) and scale (λ) parameters using MLE based on a binomial distribution of establishment against natural log transformed density. Confidence intervals around the point estimates assume a normal distribution.

3 Results

Roughly 90% of the *V. fischeri* culture met the viable cell criteria set by the flow cytometer. The yield or carrying capacity of a population was not effected by initial density within the treatments. The shape parameter,k, for all three treatments was estimated to be greater than 1. The scale parameter,λ, for the three environments was also greater than 0 indicating the density needed for a positive growth rate is larger than 1 $cellmL^{-1}$ (Fig.1) The *V. fischeri* populations in this experimental system are subject to strong Allee effects.

4 Discussion

Findings and applications

This work adds to the growing knowledge of AE impact on natural populations. *V. fischeri* are subject to both intrinsic and predator included Allee effects. The strength of the effect, represented by the critical density, increases with decreasing environmental quality (Fig. 2). We would expect even more pronounced effects in natural marine populations since

dissolved organic carbon (DOC) are three orders of magnitude lower (Pedler et al. [2014]). Understanding this relationship under climate change mediated DOC shifts in marine systems will increase our understanding of heterotrophic microbes in the global carbon cycle *Will this increase or decrease sink strength of aquatic systems?*. Understanding the impacts of Allee effects extends far beyond marine symbionts to the spread of all microbes, determining infectious dose and even the growth of tumors() Regoes et al. [2002], Litchman [2010], Korolev et al. [2014], respectively).

Objections

Our methods did not allow us to differentiate between extremely slow growth and extinction, but both result in a 'failure to establish'. This study detected the presence of an AE, but not the mechanisms leading to the critical density. A candidate mechanism is quorum sensing, which detects density, and is known to be important in *V. fischeri* forming symbiosis with bobtail squid *Euprymna scolopes*. Many other species have similar interactions based on population density (Waters and Bassler [2005], de Kievit and Iglewski [2000], Gascoigne and Lipcius [2004]cites quorum sensing; Pathogens quorum sensing reviewed; cite examples of macro organisms with AE behavior mechanism).

Call to integrate microbial and ecological knowledge to understand microbial populations

Our conceptual understanding of mechanisms controlling microbial populations is much more complex than the original *'Everything is everywhere'* tenet (Baas Becking [1934]). Microbial ecology has shown that many mechanisms that control macro-organisms, also influence microorganisms (Reno et al. [2009], Hellweger et al. [2014], as reviewed in Prosser et al. [2007]). This study continues the trend by presenting an example of density dependence of a microbial population in a rigorous theoretical framework. Work of this nature could be used to further develop long standing microbial concepts such as quorum protection (Macreadie [2015]) and minimum inhibitory concentration (Steels et al. [2000], Galgiani and Stevens [1978]). Furthermore, this work integrates microbial and ecological knowledge to create a highly manipulatable experimental system allowing gains in both fields.

5 References

References

W. C. Allee and Edith S. Bowen. Studies in animal aggregations: Mass protection against colloidal silver among goldfishes. *Journal of Experimental Zoology*, 61(2):185–207, February 1932. ISSN 0022-104X, 1097-010X. doi: 10.1002/jez.1400610202. URL http://doi.wiley.com/10.1002/jez.1400610202.

Elena Angulo, Gary W. Roemer, Luděk Berec, Joanna Gascoigne, and Franck Courchamp. Double Allee Effects and Extinction in the Island Fox. *Conservation Biology*, 21(4):1082–1091, August 2007. ISSN 0888-8892, 1523-1739. doi: 10.1111/j.1523-1739.2007.00721.x. URL http://doi.wiley.com/10.1111/j.1523-1739.2007.00721.x.

L.G.M. Baas Becking. *Geobiologie of inleiding tot de milieukunde*. W.P. Van Stockum & Zoon (in Dutch), The Hague, the Netherlands, 1934.

L. Dai, D. Vorselen, K. S. Korolev, and J. Gore. Generic Indicators for Loss of Resilience Before a Tipping Point Leading to Population Collapse. *Science*, 336(6085):1175–1177, June 2012. ISSN 0036-8075, 1095-9203. doi: 10.1126/science.1219805. URL http://www.sciencemag.org/cgi/doi/10.1126/science.1219805.

T. R. de Kievit and B. H. Iglewski. Bacterial Quorum Sensing in Pathogenic Relationships. *Infection and Immunity*, 68(9):4839–4849, September 2000. ISSN 0019-9567. doi: 10.1128/IAI.68.9.4839-4849.2000. URL http://iai.asm.org/cgi/doi/10.1128/IAI.68.9.4839-4849.2000.

Rutger de Wit and Thierry Bouvier. 'Everything is everywhere, but, the environment selects'; what did Baas Becking and Beijerinck really say? *Environmental Microbiology*, 8(4):755–758, April 2006. ISSN 1462-2912, 1462-2920. doi: 10.1111/j.1462-2920.2006.01017.x. URL http://doi.wiley.com/10.1111/j.1462-2920.2006.01017.x.

Brian Dennis. Allee effects in stochastic populations. *Oikos*, 96(3):389–401, March 2002. ISSN 00301299. doi: 10.1034/j.1600-0706.2002.960301.x. URL http://doi.wiley.com/10.1034/j.1600-0706.2002.960301.x.

A. K. Dunn, D. S. Millikan, D. M. Adin, J. L. Bose, and E. V. Stabb. New rfp- and pES213-Derived Tools for Analyzing Symbiotic Vibrio fischeri Reveal Patterns of Infection and lux Expression In Situ. *Applied and Environmental Microbiology*, 72(1):802–810, January 2006. ISSN 0099-2240. doi: 10.1128/AEM.72.1.802-810.2006. URL http://aem.asm.org/cgi/doi/10.1128/AEM.72.1.802-810.2006.

John N. Galgiani and David A. Stevens. Turbidimetric Studies of Growth Inhibition of Yeasts with Three Drugs: Inquiry into Inoculum-Dependent Susceptibility Testing, Time of Onset of Drug Effect, and Implications for Current and Newer Methods. 13(2):249–254, 1978.

Joanna C. Gascoigne and Romuald N. Lipcius. Allee effects driven by predation: Predation-driven Allee effects. *Journal of Applied Ecology*, 41(5):801–810, September 2004. ISSN 00218901, 13652664. doi: 10.1111/j.0021-8901.2004.00944.x. URL http://doi.wiley.com/10.1111/j.0021-8901.2004.00944.x.

F. L. Hellweger, E. van Sebille, and N. D. Fredrick. Biogeographic patterns in ocean microbes emerge in a neutral agent-based model. *Science*, 345(6202):1346–1349, September 2014. ISSN 0036-8075, 1095-9203. doi: 10.1126/science.1254421. URL `http://www.sciencemag.org/cgi/doi/10.1126/science.1254421`.

Kirill S. Korolev, Joao B. Xavier, and Jeff Gore. Turning ecology and evolution against cancer. *Nature Reviews Cancer*, 14(5):371–380, 2014.

Andrew M. Kramer and John M. Drake. Experimental demonstration of population extinction due to a predator-driven Allee effect. *Journal of Animal Ecology*, 79(3):633–639, May 2010. ISSN 00218790, 13652656. doi: 10.1111/j.1365-2656.2009.01657.x. URL `http://doi.wiley.com/10.1111/j.1365-2656.2009.01657.x`.

Andrew M. Kramer, Brian Dennis, Andrew M. Liebhold, and John M. Drake. The evidence for Allee effects. *Population Ecology*, 51(3):341–354, July 2009. ISSN 1438-3896, 1438-390X. doi: 10.1007/s10144-009-0152-6. URL `http://link.springer.com/10.1007/s10144-009-0152-6`.

Elena Litchman. Invisible invaders: non-pathogenic invasive microbes in aquatic and terrestrial ecosystems: Invasive microbes. *Ecology Letters*, 13(12):1560–1572, December 2010. ISSN 1461023X. doi: 10.1111/j.1461-0248.2010.01544.x. URL `http://doi.wiley.com/10.1111/j.1461-0248.2010.01544.x`.

Ian Macreadie. Quorum protection, growth and survival. *Microbial Cell*, 2(2):38–42, February 2015. ISSN 23112638. doi: 10.15698/mic2015.02.188. URL `http://microbialcell.com/researcharticles/quorum-protection-growth-and-survival/`.

B. E. Pedler, L. I. Aluwihare, and F. Azam. Single bacterial strain capable of significant contribution to carbon cycling in the surface ocean. *Proceedings of the National Academy of Sciences*, 111(20):7202–7207, May 2014. ISSN 0027-8424, 1091-6490. doi: 10.1073/pnas.1401887111. URL `http://www.pnas.org/cgi/doi/10.1073/pnas.1401887111`.

James I. Prosser, Brendan J. M. Bohannan, Tom P. Curtis, Richard J. Ellis, Mary K. Firestone, Rob P. Freckleton, Jessica L. Green, Laura E. Green, Ken Killham, Jack J. Lennon, A. Mark Osborn, Martin Solan, Christopher J. van der Gast, and J. Peter W. Young. The role of ecological theory in microbial ecology. *Nature Reviews Microbiology*, 5(5):384–392, May 2007. ISSN 1740-1526, 1740-1534. doi: 10.1038/nrmicro1643. URL `http://www.nature.com/doifinder/10.1038/nrmicro1643`.

R. R. Regoes, D. Ebert, and S. Bonhoeffer. Dose-dependent infection rates of parasites produce the Allee effect in epidemiology. *Proceedings of the Royal Society B: Biological Sciences*, 269 (1488):271–279, February 2002. ISSN 0962-8452, 1471-2954. doi: 10.1098/rspb.2001.1816. URL `http://rspb.royalsocietypublishing.org/cgi/doi/10.1098/rspb.2001.1816`.

M. L. Reno, N. L. Held, C. J. Fields, P. V. Burke, and R. J. Whitaker. Biogeography of the Sulfolobus islandicus pangenome. *Proceedings of the National Academy of Sciences*, 106(21):8605–8610, May 2009. ISSN 0027-8424, 1091-6490. doi: 10.1073/pnas.0808945106. URL `http://www.pnas.org/cgi/doi/10.1073/pnas.0808945106`.

H. Steels, S. A. James, I. N. Roberts, and M. Stratford. Sorbic acid resistance: the inoculum effect. *Yeast*, 16 (13):1173–1183, September 2000. ISSN 0749-503X, 1097-0061. doi: 10.1002/1097-0061(20000930)16:13<1173::AID-YEA617>3.0.CO;2-8.

W. J. Sul, T. A. Oliver, H. W. Ducklow, L. A. Amaral-Zettler, and M. L. Sogin. Marine bacteria exhibit a bipolar distribution. *Proceedings of the National Academy of Sciences*, 110(6):2342–2347, February 2013. ISSN 0027-8424, 1091-6490. doi: 10.1073/pnas.1212424110. URL `http://www.pnas.org/cgi/doi/10.1073/pnas.1212424110`.

Darren M. Ward, Keith H. Nislow, and Carol L. Folt. Predators reverse the direction of density dependence for juvenile salmon mortality. *Oecologia*, 156(3):515–522, June 2008. ISSN 0029-8549, 1432-1939. doi: 10.1007/s00442-008-1011-4. URL `http://link.springer.com/10.1007/s00442-008-1011-4`.

Christopher M. Waters and Bonnie L. Bassler. QUORUM SENSING: Cell-to-Cell Communication in Bacteria. *Annual Review of Cell and Developmental Biology*, 21(1):319–346, November 2005. ISSN 1081-0706, 1530-8995. doi: 10.1146/annurev.cellbio.21.012704.131001. URL `http://www.annualreviews.org/doi/abs/10.1146/annurev.cellbio.21.012704.131001`.
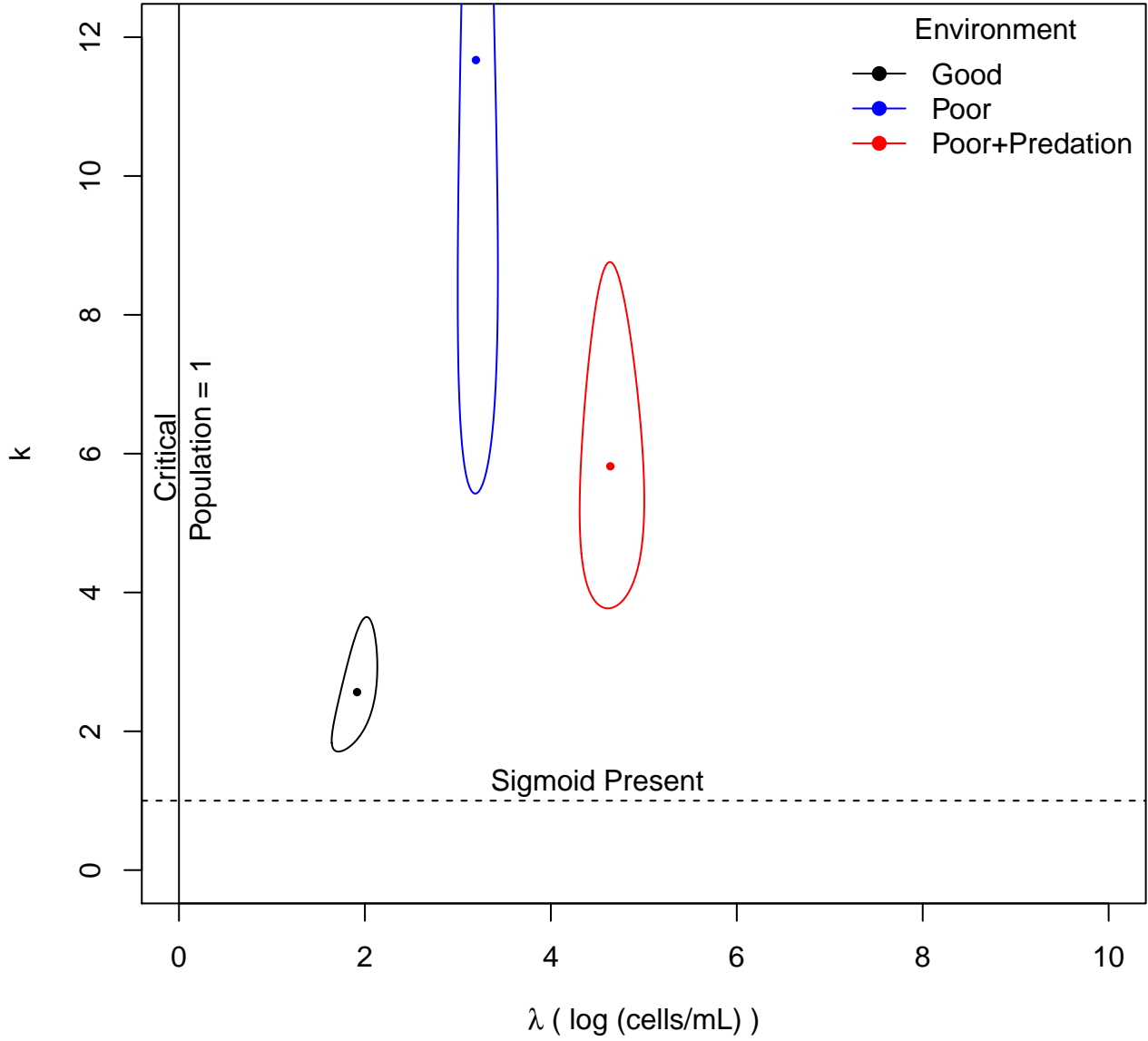
6 Figures

Figure 2: **Parameter estimates suggest Allee Effect present in all treatments.** The k parameter is used to test the presence of an AE. Values greater than 1 indicate a sigmoidal relationship between density and probability of establishment. The shape parameter, λ, is the upper bound on the inflection point or critical density required to escape the AE. The point parameter estimates are presented with 95% confidence intervals. *Include explanation of treatments?*
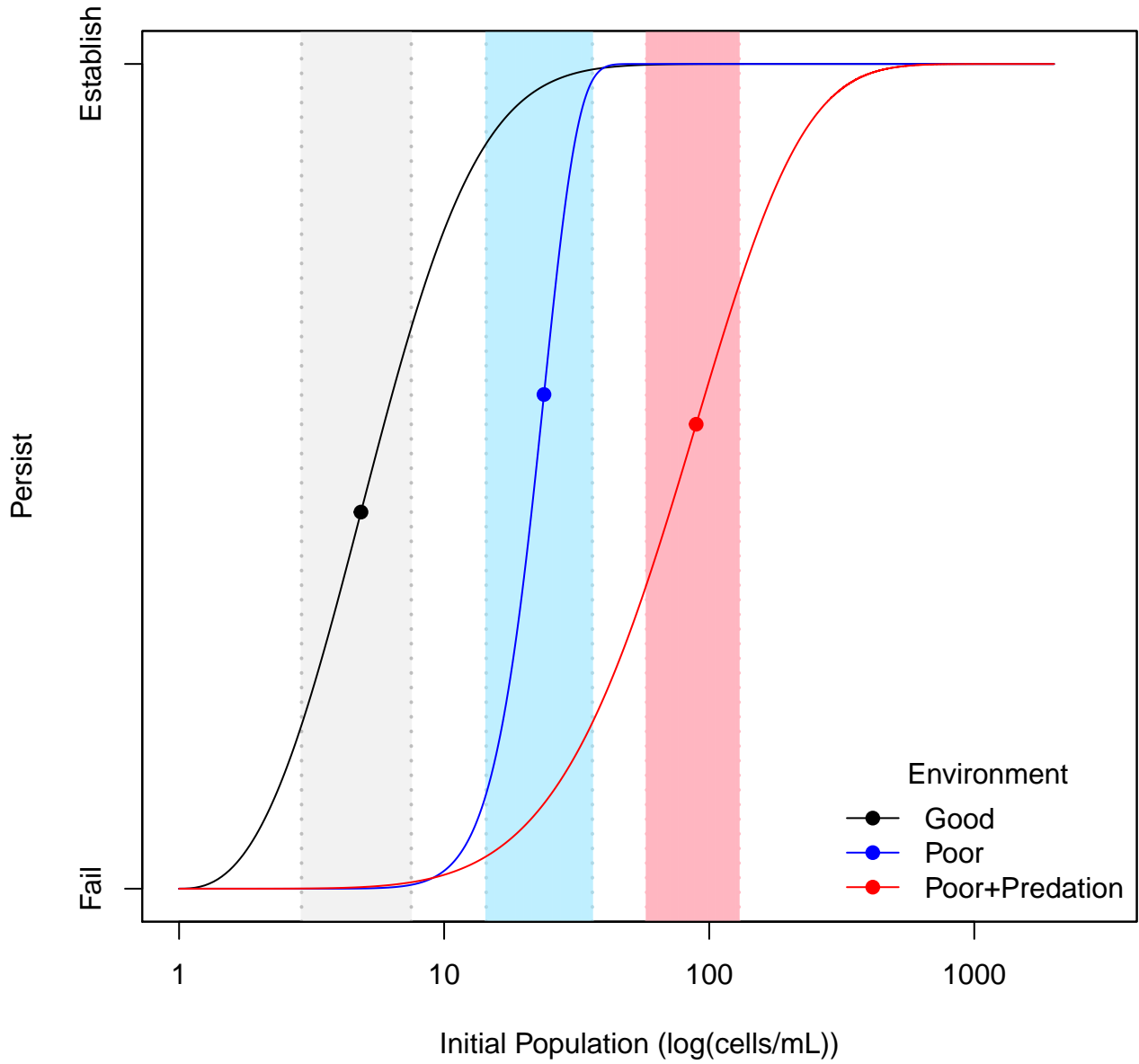
Figure 3: **Weibull curve based on estimated parameters.** Point is based on parameter point estimate, the shaded area is based on the 95% confidence interval. The values are: X-X for the high resource (40mM glycerol), Y-Y low resource (20mM glycerol) and Z-Z low resource with predation (20mM glycerol plus 134 Cafeteria/mL)