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The University of Georgia®
Odum School of Ecology

January 11, 2017

Re: *Experimental demonstration of an Allee effect in microbial populations* RSBL-2016-0070

Dear Editor,

Please find enclosed with this letter our revised manuscript "Experimental demonstration of an Allee effect in microbial populations".

We thank the reviewers for pointing out additional areas for improvement. We have made the suggested changes, which has strengthened the paper. Specific responses and changes are detailed below:

Specific replies

Referee: 3

Comments to the Author(s)
In my opinion the authors have addressed the concerns expressed by me and referee 1, and the manuscript is now ready for publication. It is a nice study that will interest a broad community.

Thank you for this endorsement.

Referee: 4

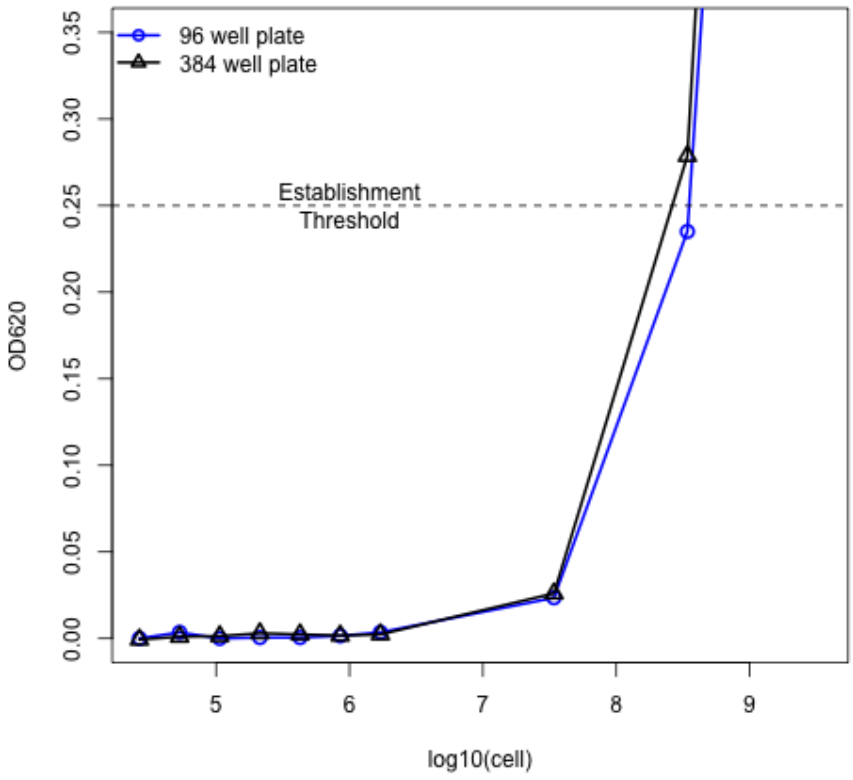
Comments to the Author(s)
I enjoyed this manuscript very much and found the approach and results interesting. As a microbial experimentalist I have always tended to assume, at least with non-model microbes in culture, that this effect was real and it's nice to see experimental evidence that shows this to be the case.

Thank you. We reply point wise to your comments below.

[1] I noticed that between the high and low resource treatments the media volume was changed from 200 to 75 μL. As I see it, this doesn't cause a problem in terms of detecting allee effects within those treatments, but could do so when comparing between treatments. The reason for this is that the detection of establishment is based on $\Delta OD > 0.25$, now for a smaller volume to increase in OD by the same amount as a large volume it would need to increase in cell density by much more. Again, I don't see this as a major problem for the conclusions of this paper, but I can't for the life of me figure out why you would change the volumes in this way. I feel many readers may pick up on this, and there must be a (hopefully sensible) reason for it and would like to see this reported somewhere.

The change of volume is due to the different recommended working volume of 96 and 384 well plates. The low resource treatments were done in a 384 well plate to allow for a reasonably high number of replicates of both treatments (with and without predation) without using a blocked experimental design. We were concerned with spreading predator treatments across blocks/plates/time since we did not have a method to ensure uniform quality of the cultured predator. We have included this point in the supplementary material (line 17).

We examined the possibility of introducing bias by changing the volume, but not threshold value. The difference in the manufacture's specification for well depth (optical path) is 11.43mm and 10.67mm for the 384 and 96 well plate, respectively. Based on the path length, we'd expect the 384 well plate to be more sensitive despite the smaller volume. We tested this by creating dilutions of a culture which were read in both plates. The density of the cultures were determined by direct counts (instead of CFUs). When the two standard curves are compared, the 384 well plate is marginally more sensitive (Fig 1.). For this reason, we believe the results between the 2 plates are comparable.



Comparing standard curves *The lower detection limit in the 96 and 384 well plates are comparable.*

[2] The second question regarding experimental approach also concerns the detection of establishment using ΔOD. I notice that in table 1, the time to establishments for LC treatments at low initial densities approaches 96 hours (the maximum time allowed). Therefore, is it not possible that in the low (e.g. 13 cells mL-1) density LC treatments some populations could have eventually established? I feel the time allowed should have been longer, allowing for much inhibited growth at low densities (still an allee effect), or at least some back of the envelope calculations to show that in fact 96 hours is adequate to detect as sufficient allee effect.

Yes, it is possible that if run longer some additional populations could have eventually established. But when should we end the experiment?

We set the time limit before running any experiments due to the unique properties of bacteria. Unlike, the higher order organisms that have previously been used to study Allee effects, bacteria can enter dormancy in unfavorable environments. For this reason we decided that 'failure to establish' would be anything with an order of magnitude slower growth. The Vibrio strain used in these experiments have a doubling time of approx. 20min in rich media. Starting at $5\ cells\ mL^{-1}$*, the population would reach the plate's lower detection limit of* $\sim 10^7 cells\ mL^{-1}$ *in 70 hours with a doubling time of 200 minutes, which is an order of magnitude larger than the normal doubling time. We rounded up to 96 hours which would allow detection of a populations with a doubling time of 275 minutes. You'll notice from the figure above the establishment threshold is greater than the plate's lower detection limit. The establishment threshold was increased to avoid the observed noise in turbidity in the LCP treatment (most likely from predation). However, we did not observe any wells with a consistent upward trend in OD that fell short of the establishment threshold at 96 hours. Therefore, failure to score as established indicates growth of more than an order of magnitude slower than normal, and there were no borderline cases.*

[3] Table 1 is missing data. In the methods (SI) you state that LC treatment has a density of 13 cells mL-1, which is missing from the table, and that LCP treatment has densities of 13 and 27 cells mL-1 which are both missing from the table. The data is visible in figure 1, so it must exist and it needs to be reported in the table.

Yes, this was an overlooked compiling error. We have added the additional data to the table.

[4] My final suggested revision regards the use of "The critical threshold" (Abstract)", "The estimated critical thresholds" (Figure 1 legend), and "The scale parameter, lambda, is the upper bound of the critical density required to escape the Allee effect" (Figure 2 legend). First of all, I'm unsure if these things are the same or different. If it is essentially the same (which I think it should be, in essence), then the numbers being different is slightly odd and/or inconsistent (I'm imagining that exp(lambda)=critical threshold in cells mL-1). Could the authors either clarify why these numbers are different, if they are supposed to be, or revise them to make consistent and less confusing.

This was poor expression on our part. The abstract should also read "estimated critical threshold". This has been added. The different reported critical threshold values in the abstract and Figure 1 legend is due to rounding. We think the rounded values in the abstract increase readability, and have not altered the values.

The λ *values reported in Figure 2 are not the log transformed estimated critical thresholds. The scale parameter,* λ*, is the theoretical upper bound of the inflection point as* $k \to \infty$ *(supplemental material ; see equation 2). We now realize that the legend is misleading when read alone. The revised legend now reads:*

"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 λ less than zero implies a critical threshold less than 1 cell mL^{-1}, which were considered biologically irrelevant. Point estimates are presented with 95% confidence region."

I assert that these results are original and not under consideration for publication elsewhere. All figures have been produced by the authors.

I can be reached by email (reni@uga.edu), phone (706-583-5538) or post (Odum School of Ecology, University of Georgia, Athens GA 30602). Thank you for your consideration.

Sincerely,
RajReni Kaul