

Evaluation of Schink et al.

Having the Gem Shine through a Fog

Laman Trip, Diederik S.; Maire, Théo; Youk, Hyun

DOI

[10.1016/j.cels.2019.07.004](https://doi.org/10.1016/j.cels.2019.07.004)

Publication date

2019

Document Version

Accepted author manuscript

Published in

Cell Systems

Citation (APA)

Laman Trip, D. S., Maire, T., & Youk, H. (2019). Evaluation of Schink et al. Having the Gem Shine through a Fog. *Cell Systems*, 9(1), 3-7. <https://doi.org/10.1016/j.cels.2019.07.004>

Important note

To cite this publication, please use the final published version (if applicable).
Please check the document version above.

Copyright

Other than for strictly personal use, it is not permitted to download, forward or distribute the text or part of it, without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license such as Creative Commons.

Takedown policy

Please contact us and provide details if you believe this document breaches copyrights.
We will remove access to the work immediately and investigate your claim.

Evaluation of Schink et al.: Having the gem shine through a fog

Diederik S. Laman Trip, Théo Maire, and Hyun Youk*
Delft University of Technology, The Netherlands

*Correspondence to: h.youk@tudelft.nl

A. Summary and novelty of the work

Researchers have known for a long time that the number of surviving *E. coli* cells exponentially decreases over time (Fig. 1B) after they have entered a stationary phase - a result of running out of food (e.g., glycerol) (Fig. 1A). While other researchers have attributed the exponential decay to the simplest possible mechanism - namely, a cell has a certain chance of dying in a given time window independently of whether the other cells are dying or not - the authors found that, surprisingly, a collective mechanism - one in which cells can extend their viability by feeding on the contents liberated by partially/fully lysed, dead cells (indicated by the lag time in Fig. 1C) - can produce the same exponential decay. To me, their even more surprising discovery, is that if there are enough dead cells, then the remaining, intact *E. coli* cells can consume the liberated biomass to grow and divide (Fig. 6), rather than just prolonging their survival without dividing. These cells eventually run out of the carcass to feed on, and thus start to die (Fig. 6C). This feeding-on-the-carcass mechanism has remained hidden in front of our eyes all this time, and this makes the work novel. While several works, mainly in synthetic biology, have previously shown that (usually drug-induced) lysis of engineered *E. coli* cells can help the other *E. coli* cells grow by releasing a specific enzyme (e.g., Egbert et al., bioRxiv - <https://doi.org/10.1101/086900>), the authors' work is the first that I am aware of that shows that wild-type (K-12), un-engineered *E. coli* cells can feed on each other. This is an important finding that I think *Cell Systems'* readers would be interested in. To explain their work, the authors use the simplest possible mathematical model (Fig. 3) with just two parameters, and they measured these values (one in Fig. 5 and the other (I think) in Fig. 4). The authors give, in the introduction and discussion sections, an evolutionary interpretation. This is one aspect of the manuscript that I think contains logical flaws because, for one thing, their work shows that a "fitness" of an individual depends on the others and thus their proposed "selection coefficient" (last equation in the discussion section), which treats the "mutant" and "wild-type" as independent of each other, doesn't make sense (more on this below). To me, a more accurate description of the work's importance, which does not diminish the novelty, is that survival and growth of individuals depend on feeding on the others' dead corpses. From a biophysicist's perspective, this work also has implications of seeing a cell culture as a truly "open system" that is out of equilibrium, in which millions of cells are energetically "connected" to each other (due to the repression of the "supply" shown in Fig. 3A). The authors investigated this to some extent. This may be too technical for non-biophysicists but I think this is important as well.

B. Overall recommendation

Overall, this is an important work that I recommend for publication but after a major revision in which the authors rewrite and re-arrange large parts of the manuscript without doing any additional experiments. Despite the novelty stated above, it took me a very long time to understand the paper and there are several points that I am uncertain about, which I ask the authors to clarify. The difficulty stems from two reasons:

B1. Logical jumps and non-linear flow of the storyline: Many parts of the manuscript left me needing to interpret and "read between the lines" to understand what the authors did, why they did it, and what they learned and how it motivates the next part of the manuscript. More fully explaining the work, including the authors' logical reasoning and conclusion from

each experiment in simpler terms, would do more justice to the work. One way to resolve this problem is by not stating the conclusion before explaining the supporting experiment. This occurs, for example, when explaining Fig. 1C. The authors first state that "dead cells leak biomass that is taken up by the viable cells", before explaining (even a bit) the experiments that led to this conclusion. Such examples occur throughout the manuscript and hinder one's understanding.

B2. Difficult-to-comprehend (cryptic) figures: Many of the figures are not self-explanatory (e.g., to understand Fig. 2A, I had to read its 3/4-page-long caption, which should really be in the main text). By re-arranging, re-plotting, and modifying many of the figures, the figures would flow more logically and be more self-explanatory.

I would think many readers (and reviewers) may not understand the significance of - what I think is - a beautiful and important work. The manuscript as it is presented just doesn't do justice to it. For this reason, I am recommending a major rewrite after which the manuscript would look very different (yet without new experiments). Below I give specific comments on each section and the figures associated with each section. Please understand that the length of my comments below does not mean at all that the work contains scientific errors. On the contrary, I find that the authors have been very careful with controls and performed very difficult, technically challenging experiments that yielded very novel results. It's just that they are not so clearly explained, which I find is a shame given that I think this is an important work. I'm only trying to suggest ways to improve the presentation so that it's more accessible to the readers.

C. Major points

C1. Summary section

C1.1. The summary is too dry, technical, and obfuscates the findings. For example, "... is a physiological steady state..." (what does this mean?) and "...death rate is quantitatively determined by the ratio of maintenance flux per viable cell to nutrient yield per perished cell" (what does this mean?). I would just replace the last sentence with much simpler one, something like: "cells can survive by feeding on contents liberated by lysed, dead cells."

C1.2. Eliminate the "fitness" part (more on this below).

C2. Highlights section

C2.1. First highlight: "collective steady-state" is an unclear term. I would just say "collective feature".

C2.2. Second highlight: This is in regards to the evolutionary angle of the story, which I think actually does not make sense due to the cells feeding on each other. The "fitness" does not make sense and I would thus eliminate this second highlight altogether.

C2.3. Third highlight: Replace "fitness" with "death rate". And even then, this sentence obfuscates what they really mean: why not say something like, "Balancing the supply of biomass liberated from lysed cells and amount of feedstock required to sustain oneself determines the death rate"? You can break this into two sentences - one to replace the second highlight and the remainder as a third highlight.

C2.4. Fourth highlight: Replace "fitness" with "collective survival".

C3. Introduction section

C3.1. The first paragraph (and particularly the first sentence) does not make sense nor is it the strong angle to introduce the story. The authors' focus on "fitness" really does not make sense because the whole point of the story is that cell-survival is a collective phenomenon, not an attribute (such as fitness) that one can assign to a "mutant" or a "wild-type" as they do in the discussion section. By the virtue of the fact that the mutants and wild-type are mixed in the same population, they would feed on each other, and thus one cannot just assign a "fitness" (growth rate) to a mutant and to a wild-type cell. This would contradict their own finding. I would eliminate all mentions of "fitness" and evolutionary angle in this section and throughout the manuscript.

Instead, I would just explain the exponentially decaying number of surviving cells has been known (which they do later), that people conventionally think of this as a cell-autonomous phenomenon (which can indeed explain the mechanism), but that the authors will show that there is a different mechanism that can explain this. Such a simpler, more down-to-earth introduction does not reduce the novelty and the value of the work.

C3.2. The focus on "steady state" is confusing. Indeed, we sometimes say that an exponentially growing population has a steady-state growth rate. But this is only an approximation - on a more careful examination, a logistic growth better explains the population dynamics, not the simple exponential growth. So, I am not sure why this is stressed, and particularly for death. I think one just needs to say that the number of surviving cells goes down exponentially over time (Fig. 1B) and that this has been known.

C3.3. Last paragraph of introduction: "...not even the exponential form of the survival kinetics has been understood so far." - this is untrue. It's more that the others have attributed the exponential form to the simple mechanism of each cell having a certain (fixed) chance of dying in a given time window, regardless of whether the other cells die or not during that time. And then the authors can say that "here we will show that a different, collective mechanism can produce the same exponential form, which suggests that the commonly accepted mechanism is, in fact, incorrect". Again, this seems more honest and does not diminish at all the work's novelty.

C4. "Survival kinetics..." section and Fig. S1

C4.1. Eq.1: Get rid of the time-dependence of γ . It's unnecessary since the authors will focus on it being constant right away ($\gamma = 0.43/d$) and it is just distracting.

C4.2. Fig. S1B: Why show the mutants? This raises more questions and is unnecessary for the rest of the story since the focus will be on just the early (0-48h) phase of death where the number of survivors exponentially decreases. I would eliminate Fig. S1B and just keep Fig. S1A. And thus, also eliminate the mention of "alternating phases of decay and regrowth" in the manuscript right after Eq. 1.

C5. "Exponential decay..." section and Fig. 1

C5.1. "Alternatively, the exponential decay may be a collective behavior": Here's an example of a logical gap. The only reason to say this is because the authors already know the answer that the readers, including myself, would not have suspected before reading the

manuscript. That's what makes the work novel and I think the authors are doing themselves disservice with this sentence. It also breaks the logical flow - I had to stop and wonder what I have been missing thus far. Nothing in the work (up to Fig. 1B) suggests a collective behavior. Eliminate this sentence.

C5.2. Revise Fig. 1C and more fully explain the logic behind it: It took me a very long time to understand the UV-killing experiment, its results, and the "viability" plotted in Fig. 1C. My understanding is that: the authors took some volume V out of a culture, then exposed that volume of cells to UV which either fully or partially lyses a cell (which one and what percentage of the cells in the volume V is intact afterwards?), then puts the entire volume V back into the original culture, then sets the time to "zero" in Fig. 1C for the purple data points, and then observed the lag time T , which the authors interpret as the untreated cells taking up the leaked molecules from the UV-treated cells to remain viable until the leaked biomass runs out. Is this correct? If so, just fully state this. To understand this, I had to look at the methods (to see how the authors normalized the purple data to 100%, how they actually performed the UV-treatment).

C5.3. Fig 1D: The authors can plot several curves in Fig. 1C, each with a different lag time, and then in Fig. 1D, show this plot. This way, we will see that the different mixing ratios (N_{UV}/N) yield different lag times, but the same slope as the black line in Fig. 1C (thus the fully/partially lysed cells only affect the lag-time, not the death rate after the leaked biomass runs out). Explain why this would be the case.

C5.4. Lines in Figs. 1C & 1D: These lines are not linear regressions on the data points - I think they are actually from their yet-to-be-introduced model. If so, I would not plot these lines here and leave them for Fig. 3, where one introduces the model. One can plot interpolation lines between the data points as a guide to the eyes.

C5.5. Fig. S3: Move this to Fig. 1 (e.g. Fig. 1E) because it is an important control for Fig. 1C and an important experiment on its own. My issue with Fig. 1C is that it involves UV-treated cells whereas it would be nicer to show the same effect (i.e., lag time arising from cells feeding on leaked biomass) without an artificial treatment such as UV. So I was skeptical throughout the manuscript until, much later, the authors referred me to Fig. S3. Fig. S3 shows that old cultures, containing dead cells due to "natural death", can be mixed with a recently starved cells, and that this yields the same effect. This is crucial and should be part of Fig. 1.

C5.6. Another control: As another control, the authors should show what happens when they just take out some volume V of media without cells, and then add this back to the starved culture. Then there should be no lag time. This can be a supplementary figure.

C5.7. conclusion: Even after all the above revisions, at this point in the manuscript, one cannot conclude that "...interpretation of Figure 1 implies that dead cells leak biomass that is taken up by the viable cells." at the beginning of the 2nd paragraph. This would be a logical jump. The authors would need to then present Fig. 2B (the assay for membrane-permeability) first and, if possible, plot the % of cells with compromised membrane (i.e., % of the red cells in Fig. 2B) as a function of the number of days in starvation. I would either

move Fig. 2B to end of Fig 1, or, more suitably, wait until Fig. 2 before making the conclusion that cells feed on the dead cells' leaked biomass. One can move the picture of Fig. 2B to supplementary if one plots the % of cells with compromised membrane vs. days in starvation as a main figure.

C5.8. Fig 2C-H: I think these are irrelevant and distracting from the main storyline. I would eliminate Figs. 2C-H and the elaborate experiments involving the two antibiotics (ampicillin and Chloramphenicol, which is never even mentioned in the main text in the first place). The authors' point with these experiments is to claim that the dead cells' biomass is used for preventing the intact cells from dying (i.e., the "maintenance cost") and that the intact cells do not replicate by feeding on the biomass in Fig. 1. First of all, the last statement is a contradiction of their own (beautiful) result later in Fig. 5. Secondly, the experiment in Fig. 1D (lysing different amounts of cells with UV and then adding them back to the original culture) already shows that the lag time increases as more lysed cells are added, but that the viability never goes above "100%" in Fig. 1C which means that one never ends up with more cells - which would arise from replicating cells - than the total number of cells that one started with. I think this already shows that the leaked biomass is used for delaying population decrease (obvious from Fig. 1C & 1D) than for replication, at least in the regime of N_{UV}/N that the authors test in Fig. 1. So the whole description and Figs. 2C-H are just distracting and I recommend eliminating them.

C5.9. Last paragraph of this section: This section's conclusion, that "Taken together, these observations suggest ... γ is a constant and well-defined measure of bacterial fitness during exponential death" - I'm unsure why this is a big deal, unless I'm missing something. I think what the authors show up to this point is that leaked biomass can feed the population, that this delays the population's extinction, and that since naturally (without UV) dying cells would leak biomass too (shown by their membrane-integrity test on days-long starved cells), the result suggests that the well-known exponential decay in the number of survivors is likely resulting from the interaction between the rate at which biomass is leaked by the dying cells and the rate at which the survivors consume that leaked biomass. I think the authors should say this, and it leads naturally to the next section - the mathematical model. The authors can also emphasize here that it is not trivial that the amount of leaked biomass that they initially added to the culture (Fig. 1) only tunes the lag-time but not the exponential decay-rate (i.e., the slope of the purple and black lines being the same in Fig. 1C). Understanding this would require a model, which again can be used as a natural springboard into their next section.

C6. "Balance between..." section (section on the mathematical model)

C6.1. The "supply" and "demand" analogy: This analogy from microeconomics only partially worked for me. The "supply" part is clear, but the "demand" wasn't since it seemed weird to have a "demand" arrow leading to death (Fig. 3A). I would recommend not using this analogy but it's just my personal taste.

C6.2. Fig. 3A: The drawing is only partially correct. The "demand" arrow leads to cell death, and the null sign, indicating dead cells, should also have an arrow going to the "dead cells" shown on the left side of the figure - this would complete a feedback loop. It's the

completion of this loop that enables the authors to correctly claim that the population survival is a collective phenomenon.

Also, like in the main text, label the "supply" and "demand" arrows with the symbols "J_s" and "J_d" respectively, and include a legend for "N", "alpha", and "beta" in the figure like the "epsilon" that's already there.

C6.3. Plotting the predictions of the model: Missing right now is a simple plot of viability vs. time in starvation that the model predicts (something like Fig. 1C). Having such a simple plot in Fig. 3 would show that the model can sufficiently reproduce the data (flat horizontal line (lag time) following by a single exponential decay). I recommend adding this.

C6.4. The model: The authors used the simplest possible model to explain the phenomenon, with just two adjustable parameters (alpha and beta). This is great. The model also shows why we can call this phenomenon collective: cell survival is coupled to the "supply flux" from the dying cells, which in turn is determined by the amount of biomass required to sustain survival ("demand flux"), meaning that a given cell's survival is coupled to the death of the others. I would really explain this, in simple terms, for the lay audience.

C6.5. Fig. 3B-D: I would move these to the supplement or, better yet, eliminate them. I found these very difficult to understand. First of all, the "fluxed per cell" axis in Figs. 3B-D should really say "d(epsilon)/dt" in Equation 5. This would make it less confusing. Secondly, the description of Figs. 3B-D (starting just after Equation 4), was really difficult for me to understand and I think a non-specialist (i.e., those not conversed in coarse grained models / statistical physics) would barely understand this explanation and it's purpose.

To me, the purpose is to show how the rate of death and the rate of consumption of the leaked biomass can balance each other in a self-sustaining manner (otherwise, the number of survivors would not decay as a single exponential with a fixed gamma, as in Fig. 1C). So I would first re-state this in a simple manner. And then better explain what the "internal energy state" (epsilon) is - it's reflective of the survivor's metabolic state. And then say that Fig. 3B-D are bifurcation diagrams from the theory of dynamical systems in which one can find the stable fixed point where the two aforementioned rates are exactly balanced (black point in Fig 3B-D) - for a wide variety of "supply rate" and "demand rate". The authors can then explain its biological implications. First, the authors would have shown that what they experimentally observed is not a finely tuned system, in which the balance only occurred because the "supply" and "demand" were just right (since widely varying supply and demand fluxes can all yield a single, stably fixed point). Secondly, the stableness of the fixed point means that this is self-policing operation - any fluctuations in the demand or the supply fluxes would be corrected by the population. Somehow, all this down-to-earth explanation is missing and instead, the authors have given a very elaborate explanation of "coarse-grained" models and such, which I would relegate to the supplementary section.

C7. "Determination of the average maintenance rate ..." section and Fig. 5.

C7.1. Move Fig 5 to Fig 4.: The authors refer to Fig. 5 before Fig. 4. So swap Fig. 5 with Fig 4.

C7.2. Remove this section / move it to supplement / simplify it: The two main points of this section and of Fig. 5 are that (1) the authors experimentally measured the "beta" - one of

the two free parameters in their model - and that (2) the lag time seen in Fig. 1 is due to the presence of biomass. Of the many panels in Fig. 5, the only ones that are of importance are Fig. 5A, B, and H. Since Fig. 5C-G all show the same thing on the different days, and none of them are actually important for the main story - and so they are very distracting - I would eliminate Fig. 5C-G. Fig. 5H shows that they obtained the "beta" as a constant from this experiment of adding food (glycerol).

C.7.3. Text for this section: I would move this whole section to supplement because it is very technical, distracting from the main storyline, contains an explanation that is too elaborate for the purpose - they measured the beta (Fig. 5H) - and is just not well explained for a general audience. It is indeed nice to see that the authors could relate the beta to the number of ATPs required to sustain viability - but this, to me, is a very technical fine point.

C8. "Fitness cost..." section and Fig. 4

C8.1. Fig. 4: I would eliminate Fig. 4 or move it to the supplement. It is irrelevant for the main storyline. The fact that wasteful enzyme - either by its production or the fact that it performs a task that interferes with growth - decreases viability or growth rate seems irrelevant for the main point of their story - dying cells feed live cells and that this is a self-policing system. Moreover, Fig. 4 is unclear as it's presented because it lacks informative legends in the figure itself.

C8.2. Text for this section: Likewise, I would either eliminate this whole section or move it to the supplement. I guess the authors want to mention "fitness" here because they try to connect the work to evolutionary dynamics. I think their argument regarding fitness contradicts their own finding here (more on this in my comments on the discussion section). Thus, I think they should just eliminate this section altogether.

C9. "Dissection of the fitness ..." and Figs. 6 and 7

C9.1. Text for this section: This section distracts one from following the main story line. I would eliminate this section altogether. Again, this section is dealing with "fitness" of a cell and - due to its origins from evolutionary biology and the authors later use of it - implies an evolutionary angle to the story which I find is distracting and incorrect.

C9.2. Fig. 7: Likewise, I would eliminate this figure, which contains typos (square instead of triangle in front of all instances of "rpoS" and is not self-explanatory in the first place. If included, it should come before Fig. 6 since the authors refer to it before Fig. 6.

C9.3. Fig. 6: This is one of the authors' key findings. It shows that by adding biomass to very few cells, you can cause those few cells to not only survive but divide as well. But instead, somehow, the text for this section doesn't say this. Instead, the text focuses on how one obtains the "alpha" - a parameter in the model - from the experiment here. The text should be clearer - it took me an enormous amount of time to understand Fig. 6 - this is a shame since it's such a novel finding. One way to improve this is to label and explain, directly in the figure, what $N_{\{G\}}$ is. The fact that cells do not grow (and die) if they are concentrated but can grow if you keep the same supernatant + dead cells while decreasing the concentration of cells (by diluting) - this is not at all straightforward and should be better explained in the text and in the figure.

C10. "Discussion" section

I would recommend that the authors completely rewrite the discussion section. I would focus on restating their findings and its (non-evolutionary) implications. Right now, they focus on the implications of the work for evolutionary biology - particularly on how one should interpret competitions between "mutants" and "wild-type" cells. As a reviewer, my job is not to define the scope of their work - that is the authors' right. But my problem is that the last equation in the discussion section is just wrong and contradicts the authors' own findings in this work - unless I'm missing a fundamental point here. Here's my logic: The first equation in the discussion section (which should have a number) defines, correctly, the "selection coefficient" s as it is commonly used. This basically stems from the fact that in a competition experiment between a mutant and a wild-type strain, if you know each strain's growth rate, then you can predict which one will overtake the population. But then the authors propose the last equation in this section as a "generalization" of s , in light of their beautiful finding that cell survival depends on the others' death (which in turn depends on the surviving cells - Fig. 3A). But this extension is incorrect because, if you co-culture two strains - "wild-type" and "mutant" - then their survival would depend on the death of each other (i.e., mutant would depend on the lysis of both the mutant and wild-type cells) and so this new selection coefficient evidently would not give any information about the outcome of such competition experiments under starvation conditions. Furthermore, the new selection coefficient is motivated by the paragraph just above it, which contains logical gaps: "Since bacterial cells typically grow much faster in the presence of nutrients than they die in the absence of nutrients" (what does this really mean? I don't think this is actually true) and "...they must spend a proportionally larger fraction of time in death phase than in exponential growth" (this is not self-evident and seems incorrect).

In other words, exponential growth tells one about a strain's fitness - this is a single cell feature - and one cannot do the same with the death rate because this is collective feature, meaning that doing competition experiments in starving conditions seems non-sensical.

C11. General comments on the figures

C11.1. Understanding the figures without the figure captions is very difficult (e.g. Fig. 1C). There are often big logical jumps in going from one sub-panel to another within a figure (e.g, Fig. 6B to 6C). I would recommend adding more self-explanatory legends directly in the figures, and the specific revisions that I recommended above.

C11.2. I recommend moving some of the figure captions to the main text. As an example, the caption for Fig. 2A is 3/4-pages long. And while the main text refers to Fig. 2A, it doesn't mention how that experiment was carried out with sufficient details (e.g., name of an antibiotic "cm" can only be found in the figure caption, but not in the figure and the main text). Expanding the main text by migrating the details in the figure captions to them will likely improve one's comprehension.

Overall, I recommend publication after majorly revising the presentation. I hope you find these comments of some help.