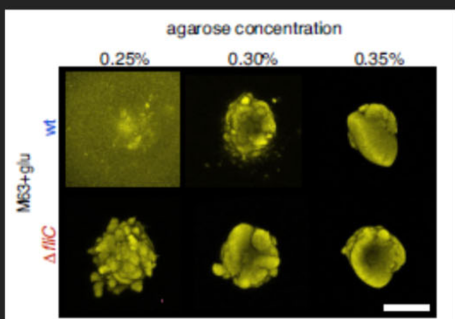
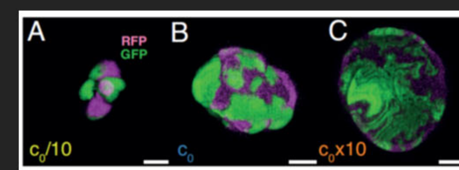
To grow or not to grow? Bacterial growth, dormancy, and death

Namiko Mitarai, Niels Bohr Institute, University of Copenhagen

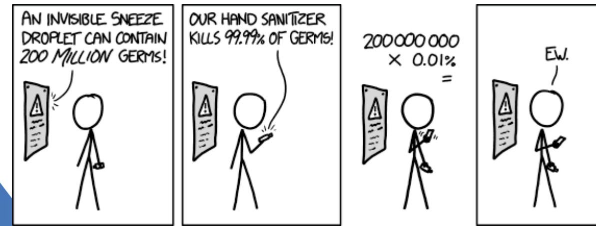


Cordero, NM, Jauffred, BioRxiv



Vazquez, NM, Jauffred, BioRxiv

I'm also involved recent works on 3D colony morphologies. Talk to me if you are interested!



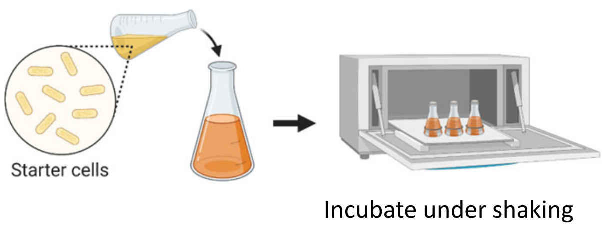
Xkcd: hand sanitizer

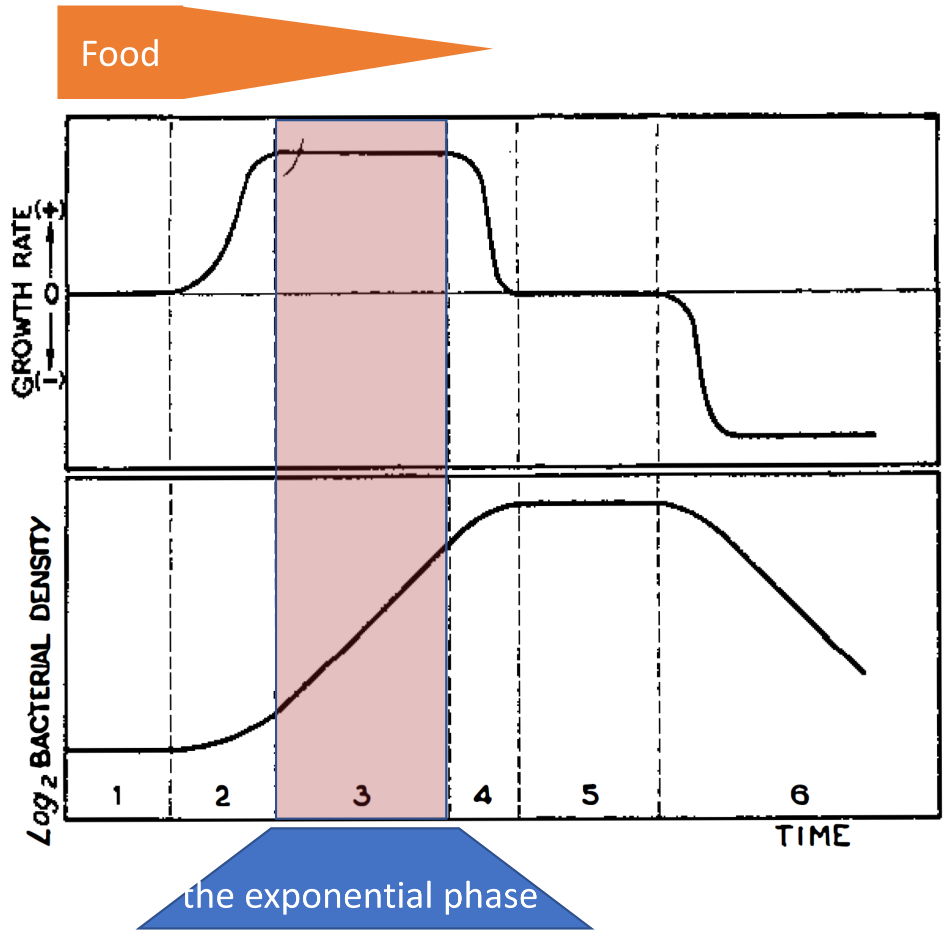
Bacterial growth: Power of exponential

$$\frac{dB}{dt} = kB$$

Stewart EJ et al (2005). PLoS Biol. 3 (2): e45

Bacterial growth phases, Monod(1949)





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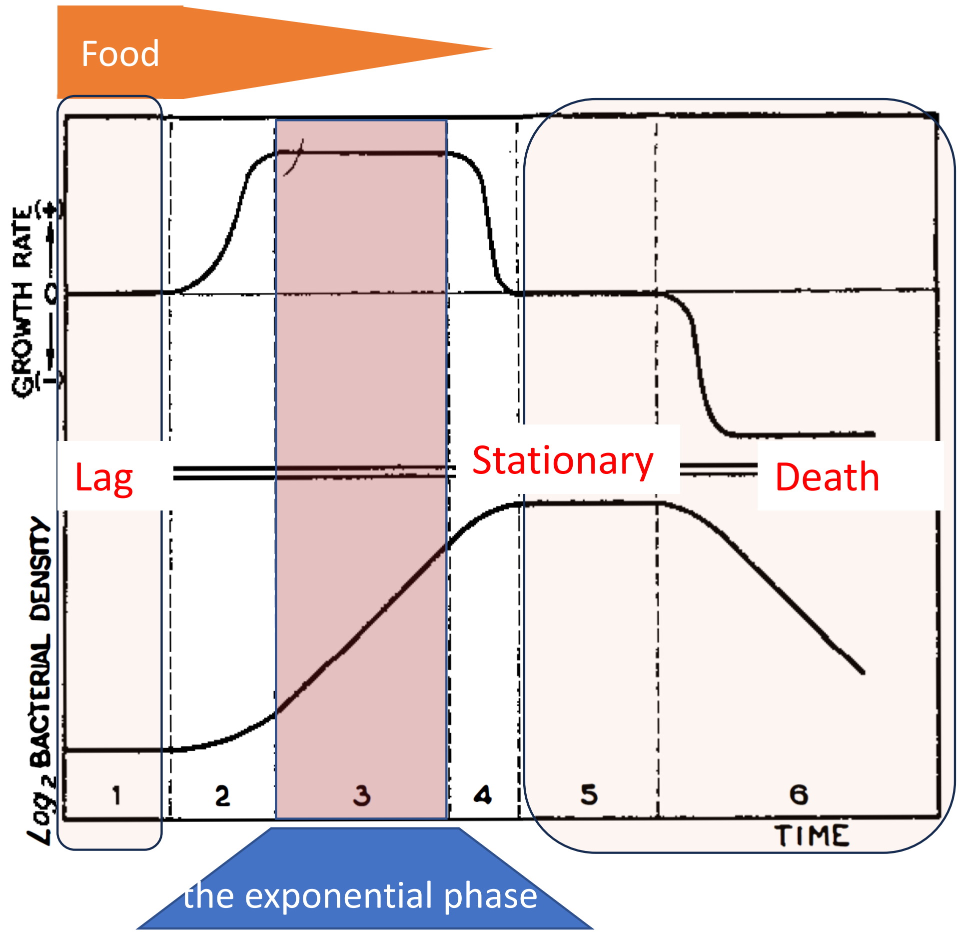
Bacterial growth phases, Monod(1949)

Dormant states are as (more) important for survival of bacteria



Essay

Bacteria grow swiftly and live thriftily

Roberto Kolter[1,*], Nathalie Balaban[2], and Thomas Julou[3]

Current Biology 32, R589–R683, June 20, 2022

Image created with BioRender.com

Dormancy provides stress tolerance

Video from: MS Svenningsen et al. "Birth and resuscitation of (p) ppGpp induced antibiotic tolerant persister cells." *Scientific reports* 9.1 (2019): 1-13.

Antibiotic persistence: Genetically drug sensitive bacteria can still survive (different from resistance)

Bacterial growth phases, Monod(1949)

Dormant states are as (more) important for survival of bacteria

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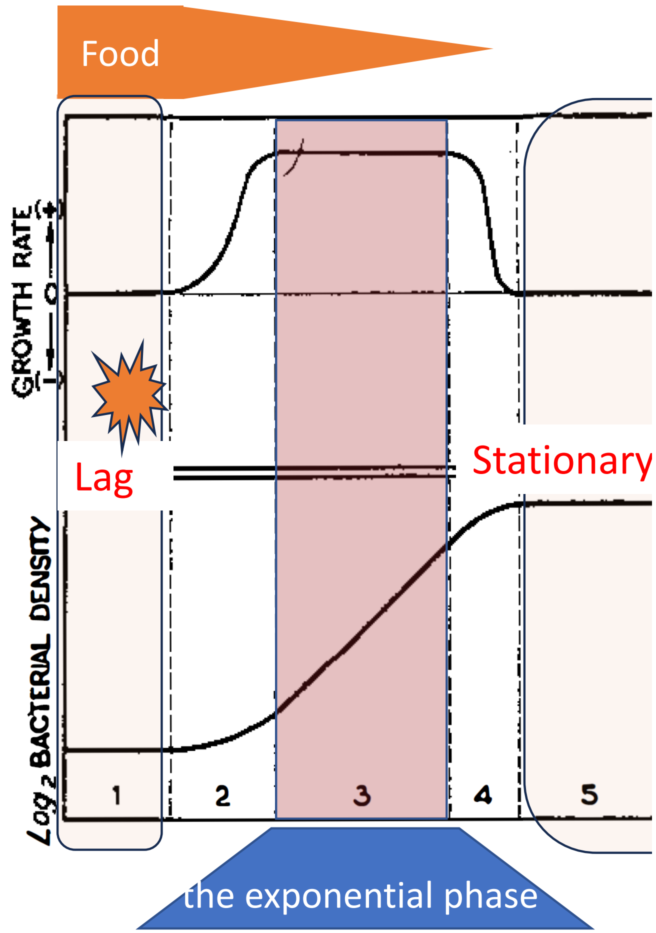

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Bacteria can evolve to have longer lag-time under antibiotic application

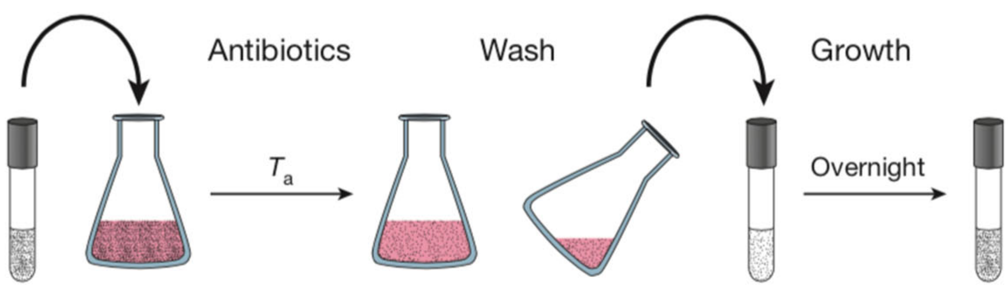
LETTER

doi:10.1038/nature13469

Optimization of lag time underlies antibiotic tolerance in evolved bacterial populations

Ofer Fridman[1], Amir Goldberg[1], Irine Ronin[1], Noam Shoresh[2] & Nathalie Q. Balaban[1]

a



Fridman et al. Nature (2014)

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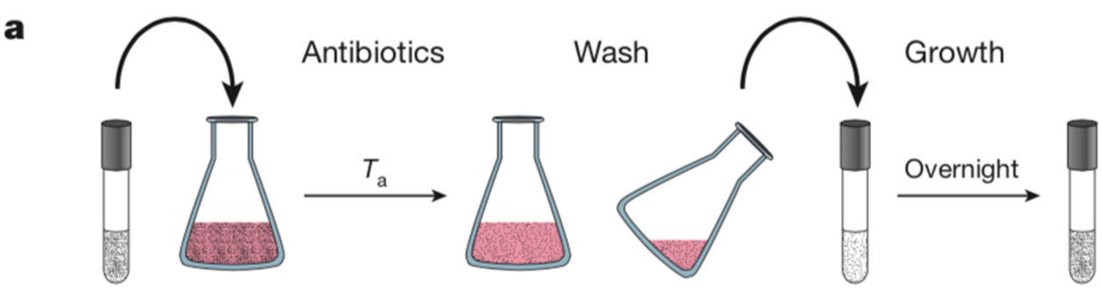
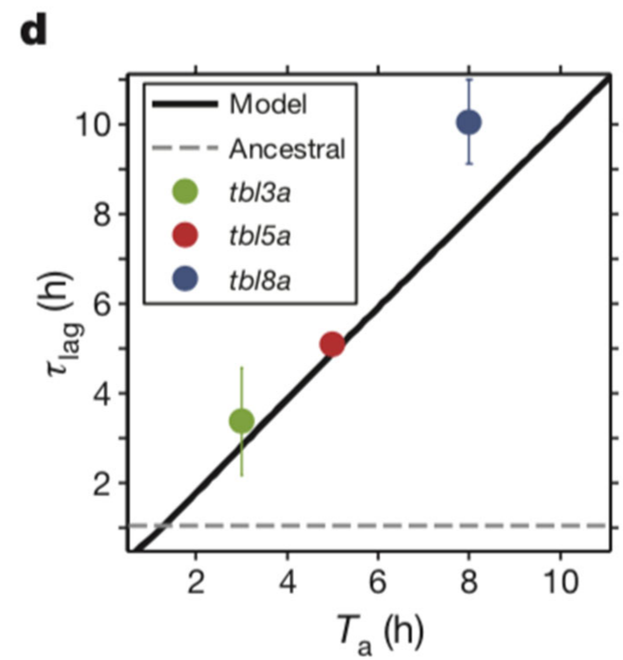


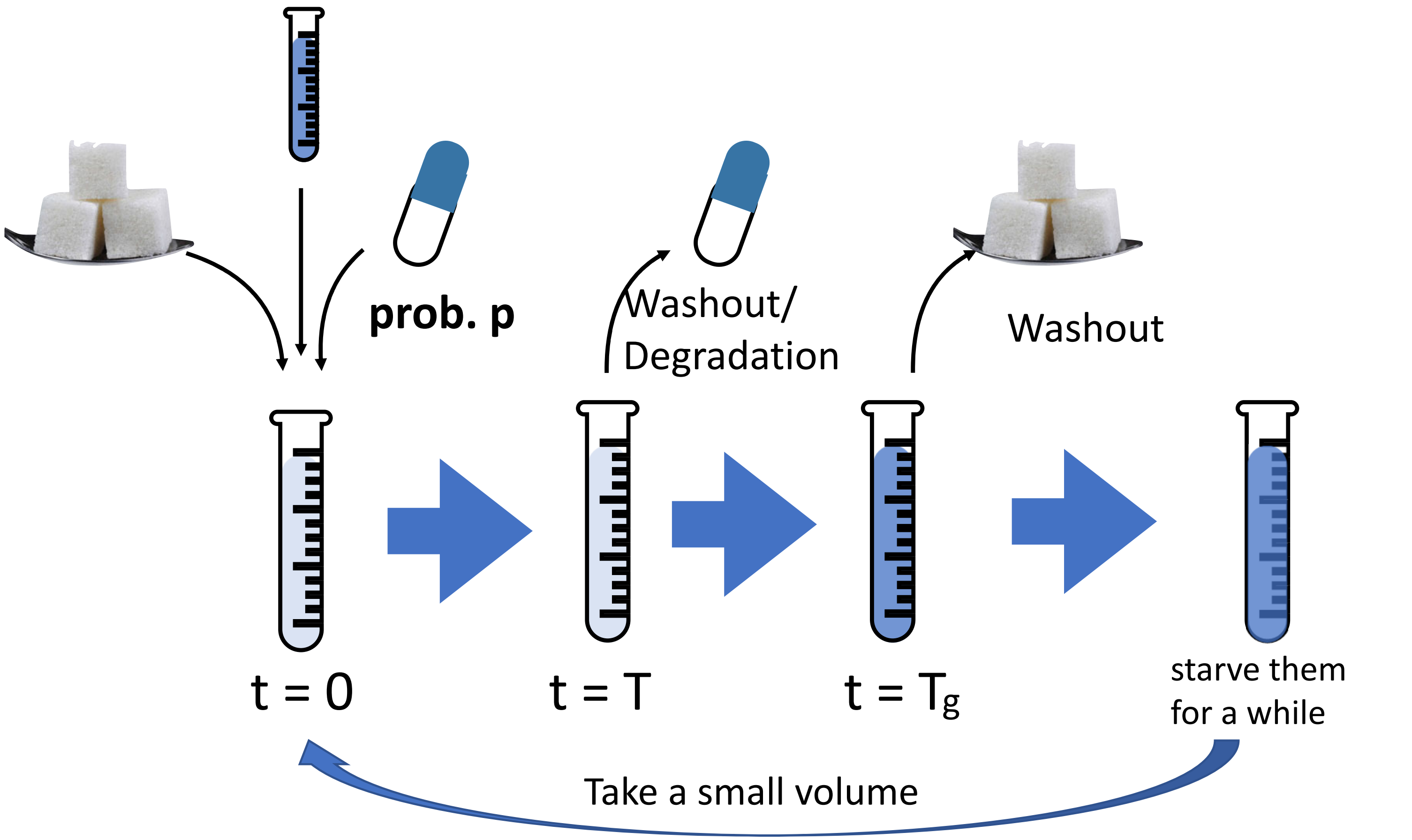
Fridman et al. Nature (2014)

Lag time after starvation

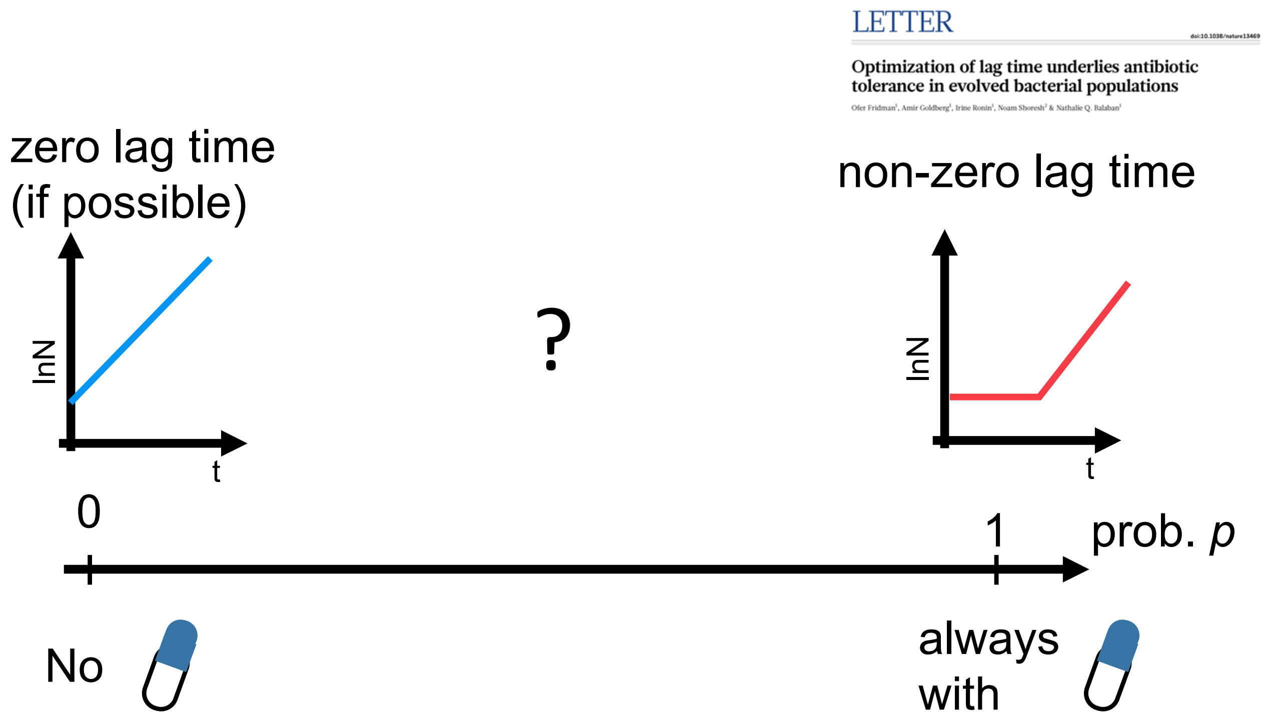


Antibiotic application time

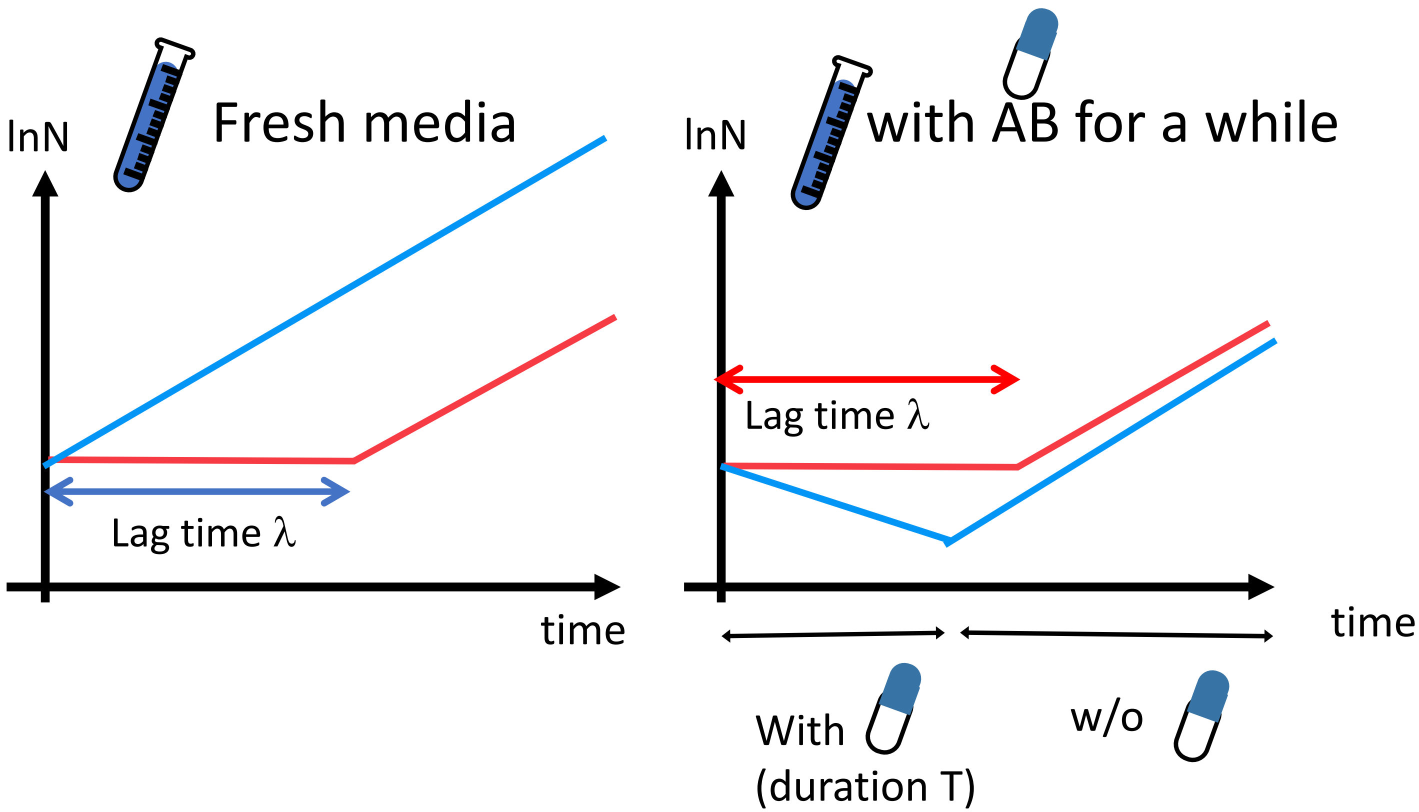
Repeated growth-starvation cycle with stochastic AB application
-> Will the longer-lag time phenotype selected?



How does the lag time evolve in an intermediate region?

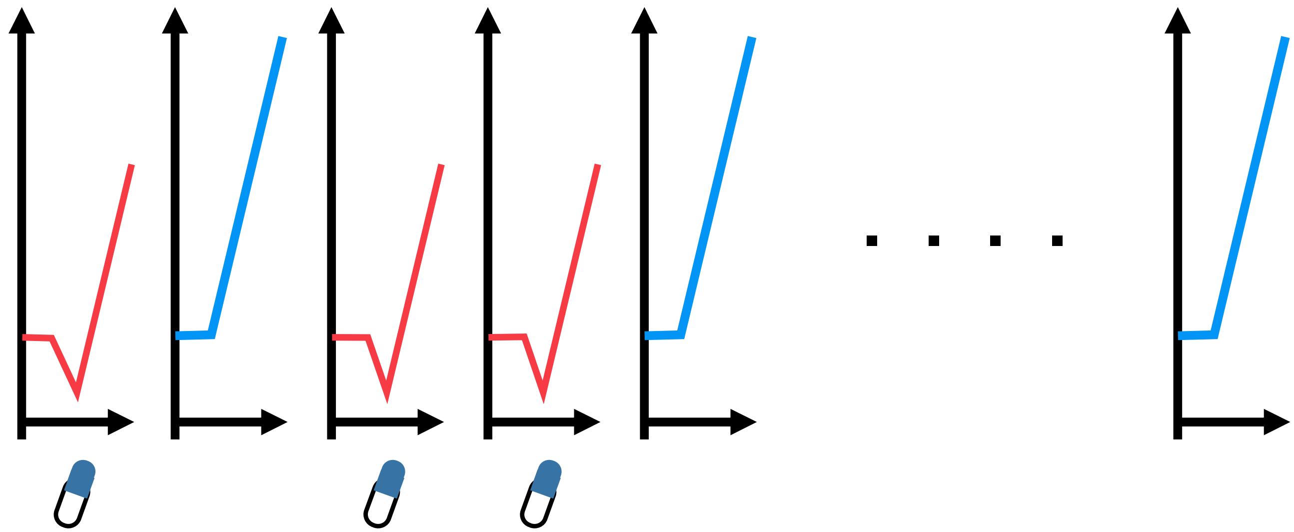


Trade-off between growth and tolerance



General Setup

Repeat feast-famine cycle, with stochastic AB application

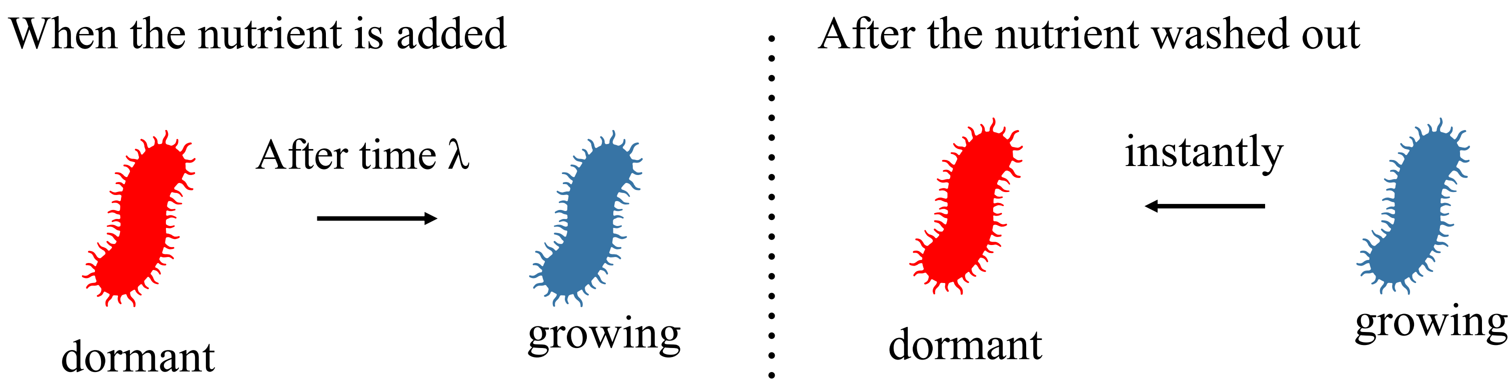


Q. What is the best waking-up strategy?

(optimize the cumulative population gain)

- Yusuke Himeoka and NM, Plos Comp17(2): e1008655 (2021)

The simplest case: Deterministic Lag Time (delta-distributed)



<u>Dormant</u>

- No growth
- No death

<u>Growing</u>

- Grows at rate μ=1 if there's no antibiotics
- Dies at rate γ if there're antibiotics

If the wakeup is completely deterministic

The population of the cells at time $t > \max\{\lambda, T\}$ for each condition.

	+ AB (prob. p)	- AB (prob. $1-p$)
$\lambda < T$	$\exp[-\gamma(T-\lambda)]\exp[t-T]$	$\exp[t-\lambda]$
$\lambda > T$	$\exp[t-\lambda]$	$\exp[t-\lambda]$

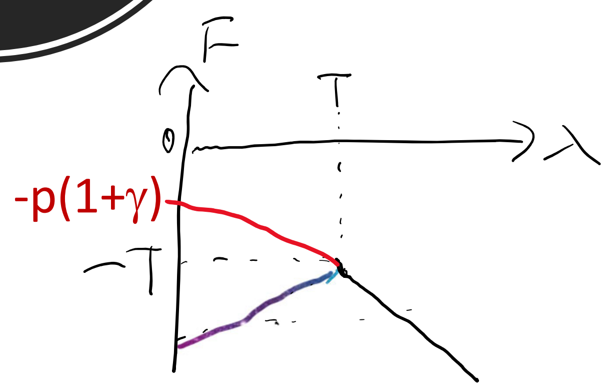
AB application duration T, lagtime λ, Growth rate fixed to 1

Average growth after N repeats:

$$(+AB\ growth)^{pN}(-AB\ growth)^{(1-p)N}$$

Fitness to maximize:

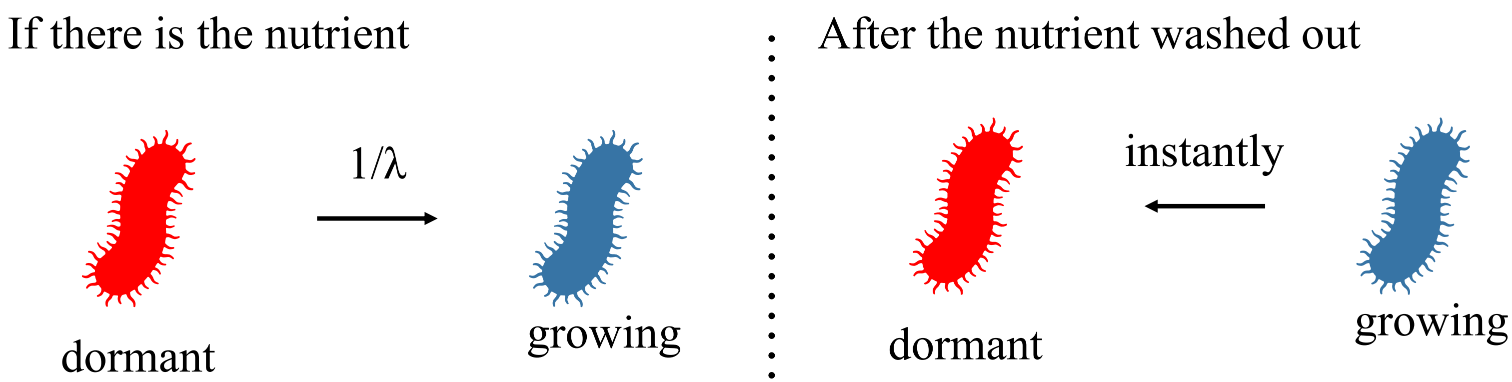
$$F_I^\delta(\lambda,\gamma,p,T) = \begin{cases} -p(T-\lambda)(1+\gamma)-\lambda & (\lambda < T) \\ -\lambda & (\lambda > T). \end{cases}$$



Discrete transition in the optimal lag time:

$$\lambda^* = \begin{cases} 0 & (\gamma < 1/p - 1) \\ T & (\gamma \geq 1/p - 1). \end{cases}$$

A Simple Model: Lag time as a constant rate process

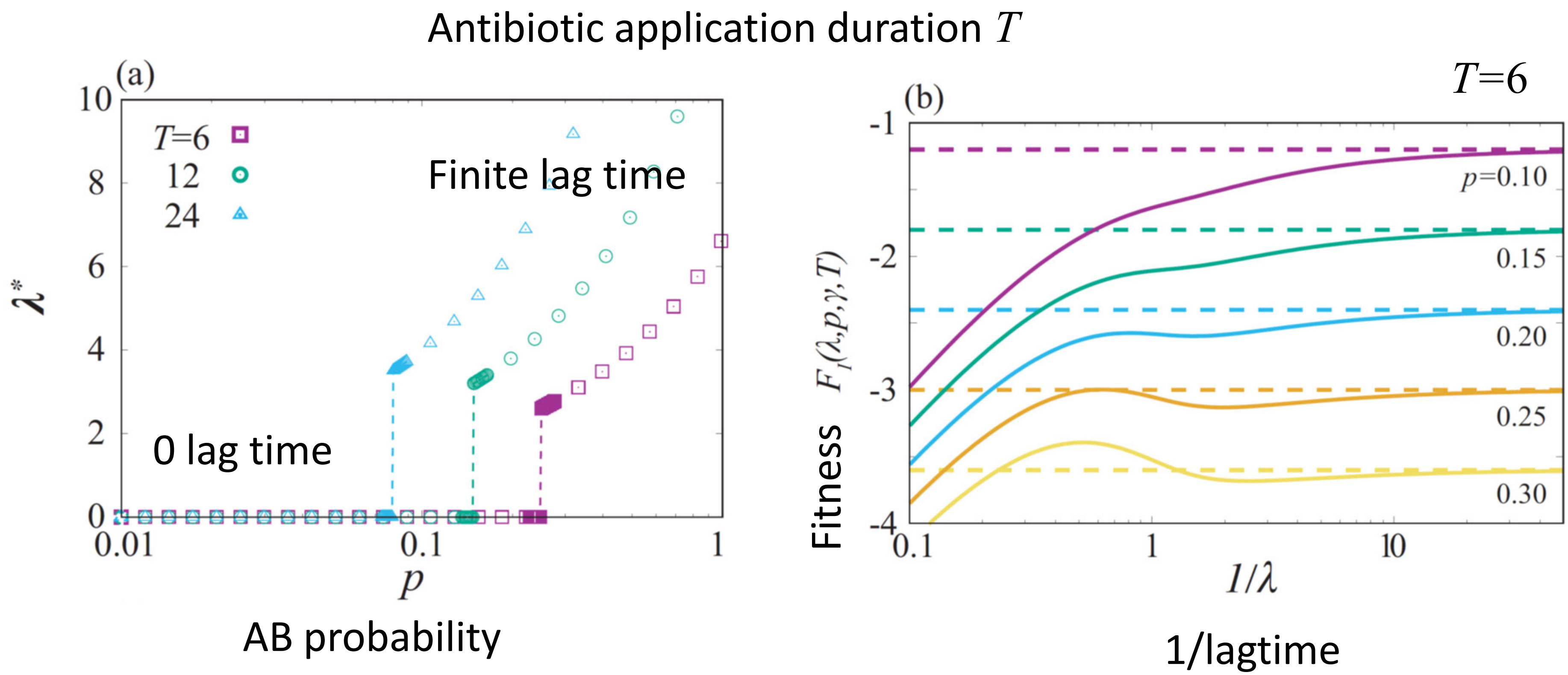


$$\frac{d}{dt}d(t) = -d(t)/\lambda,$$

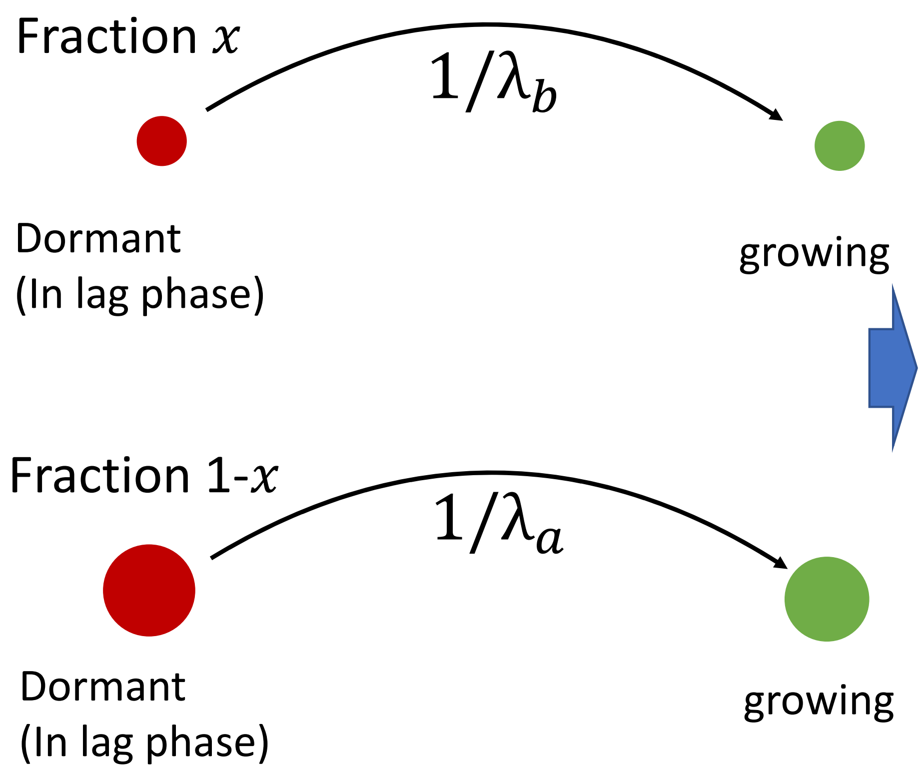
$$\frac{d}{dt}g(t) = \begin{cases} d(t)/\lambda - \gamma g(t) & (t < T) \\ d(t)/\lambda + g(t) & (t > T), \end{cases}$$

Discontinuous transition of the optimal lagtime

(Growth rate and Death rate set to 1)



Bet-hedging? Two populations



Total fitness

$$F_{II}(x, \lambda_a, \lambda_b; \gamma, p, T) = p \ln\Big[(1-x) f_a(T) + x f_b(T)\Big] + (1-p)\ln\Big[(1-x) f_a(0) + x f_b(0)\Big],$$

Find optimal
λ_a, λ_b, x

$(\lambda_a \leq \lambda_b)$

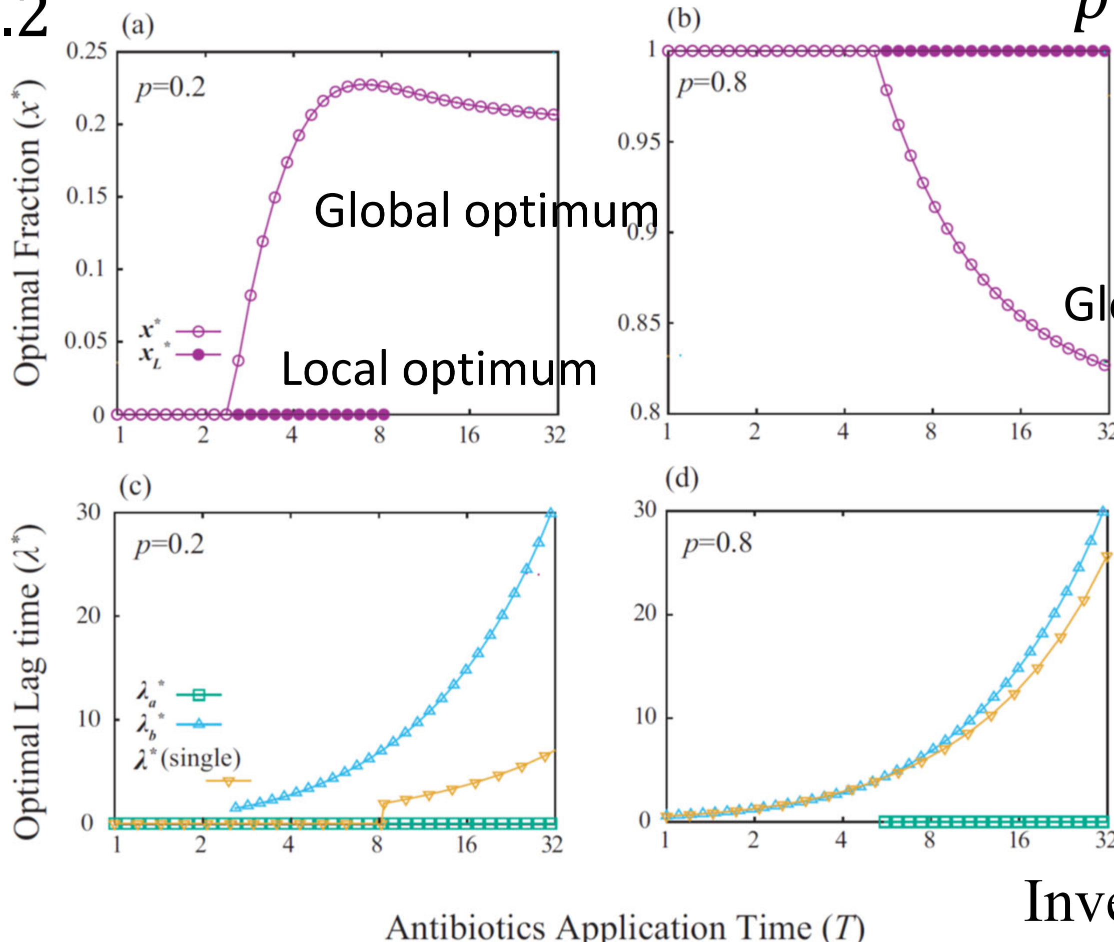
i.e. $\boldsymbol{x}$ is fraction of slow wakeup population

Two-Species Case (with exp. wake up)

$p = 0.2$ $p = 0.8$

Slow-wake-up fraction

x^*



Local optimum

Everyone wakes up ASAP ➡ Secure some

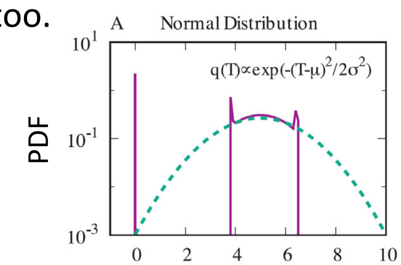
Secure all ➡ Invest some for early wake-up

Take-home message: long-lag subpopulation can be selected

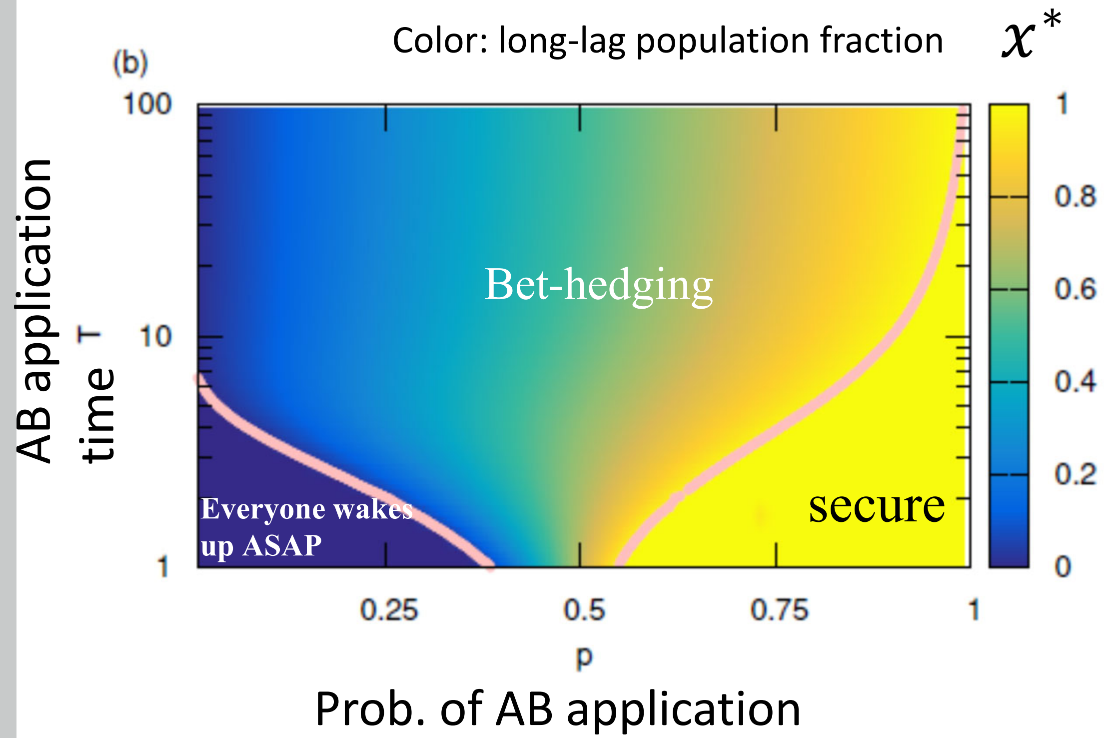
In AB application strategy space, there are regions that select for variable drug tolerant population

Yuske Himeoka and NM, Plos Comp17(2): e1008655 (2021)

Generalization such as variable T has been analyzed, too.

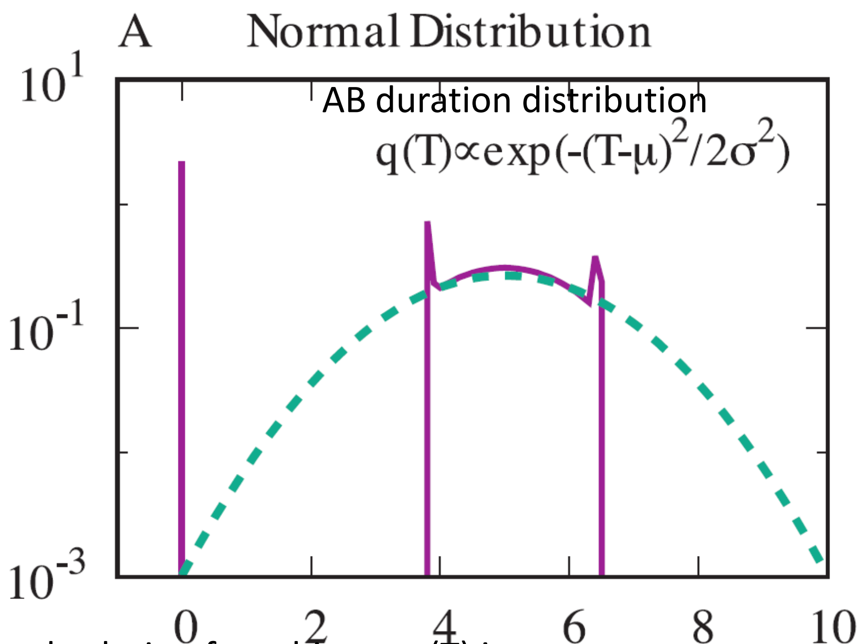


Analysis with spontaneous persistence by Silja B. Låstad (in preparation)



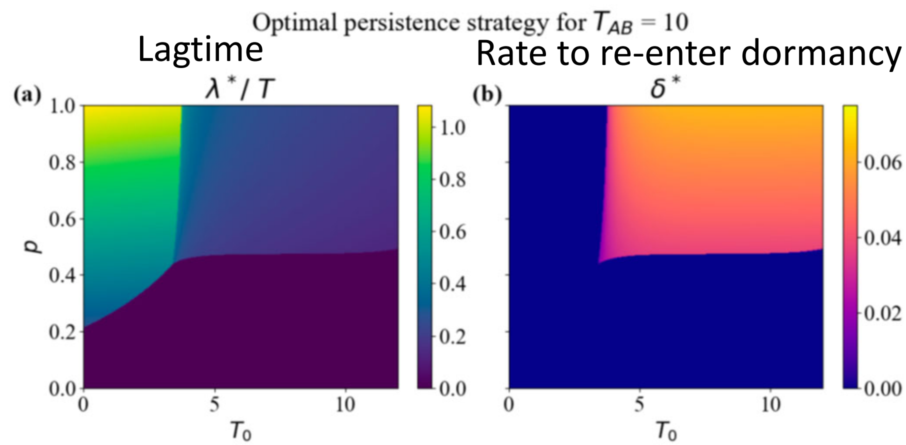
Some more extensions..

General solution: Optimal lagtime distribution $r^*(l) = \alpha\delta(l) + (1-\alpha)s(l),$



General solution for arbitrary q(T) in
Yuske Himeoka and NM, Plos Comp17(2): e1008655 (2021)

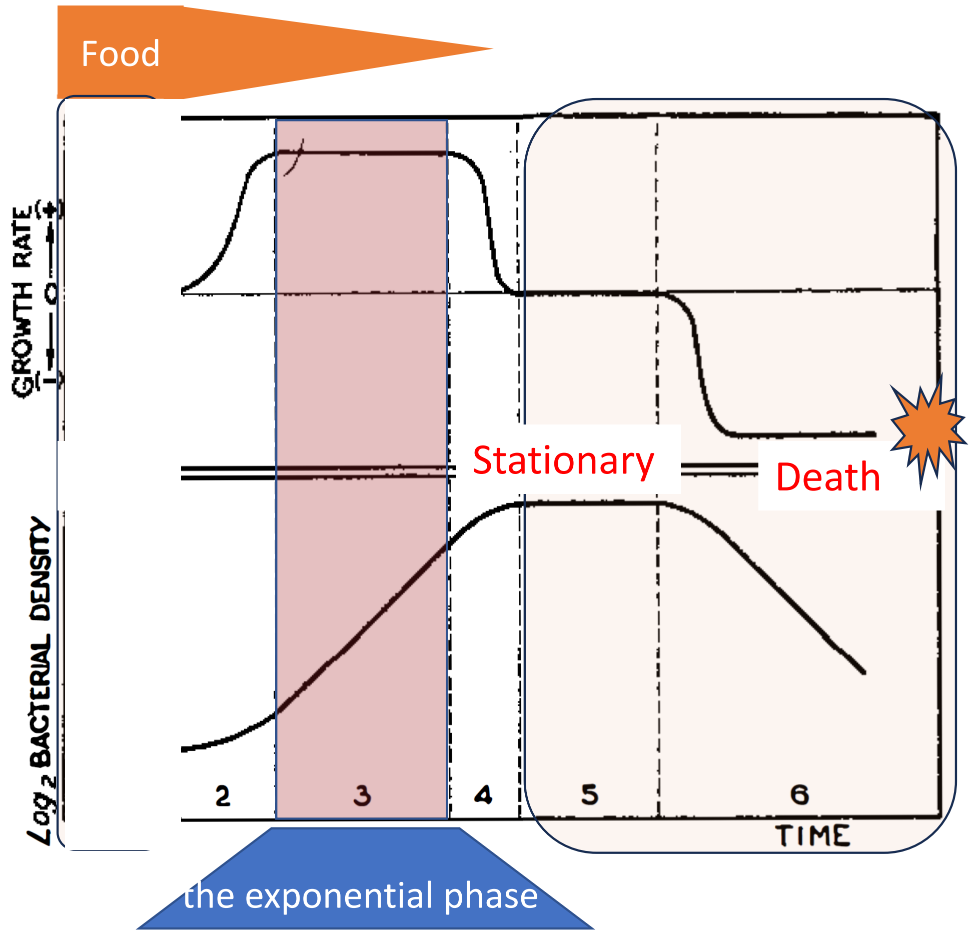
If awake bacteria can go dormant again (Låstad and NM, in prep.)



Horizontal axis:
Time between nutrient addition and AB addition

Bacterial growth phases, Monod(1949)

Dormant states are as (more) important for survival of bacteria



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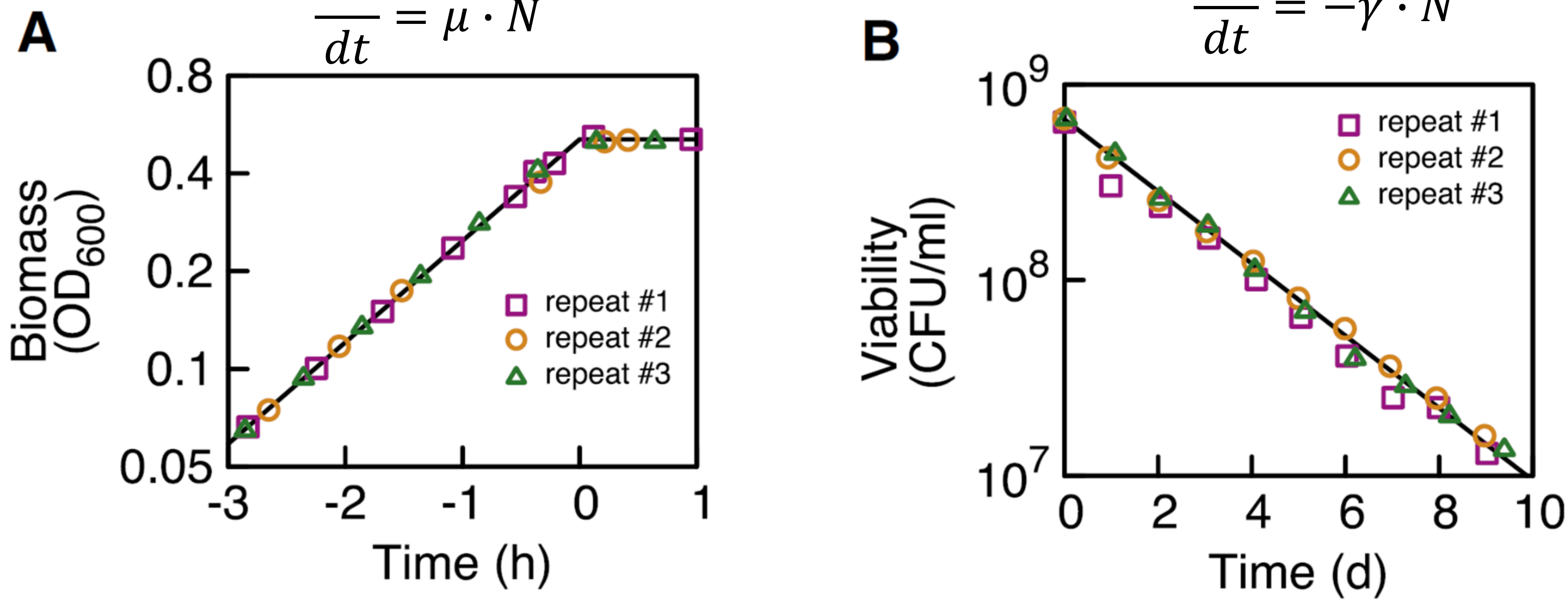
Current Biology 32, R589–R683, June 20, 2022

Image created with BioRender.com

Grown in a well-defined, minimal medium
Starve by running out of a carbon source (provide energy)

$$\frac{dN}{dt} = \mu \cdot N$$

$$\frac{dN}{dt} = -\gamma \cdot N$$



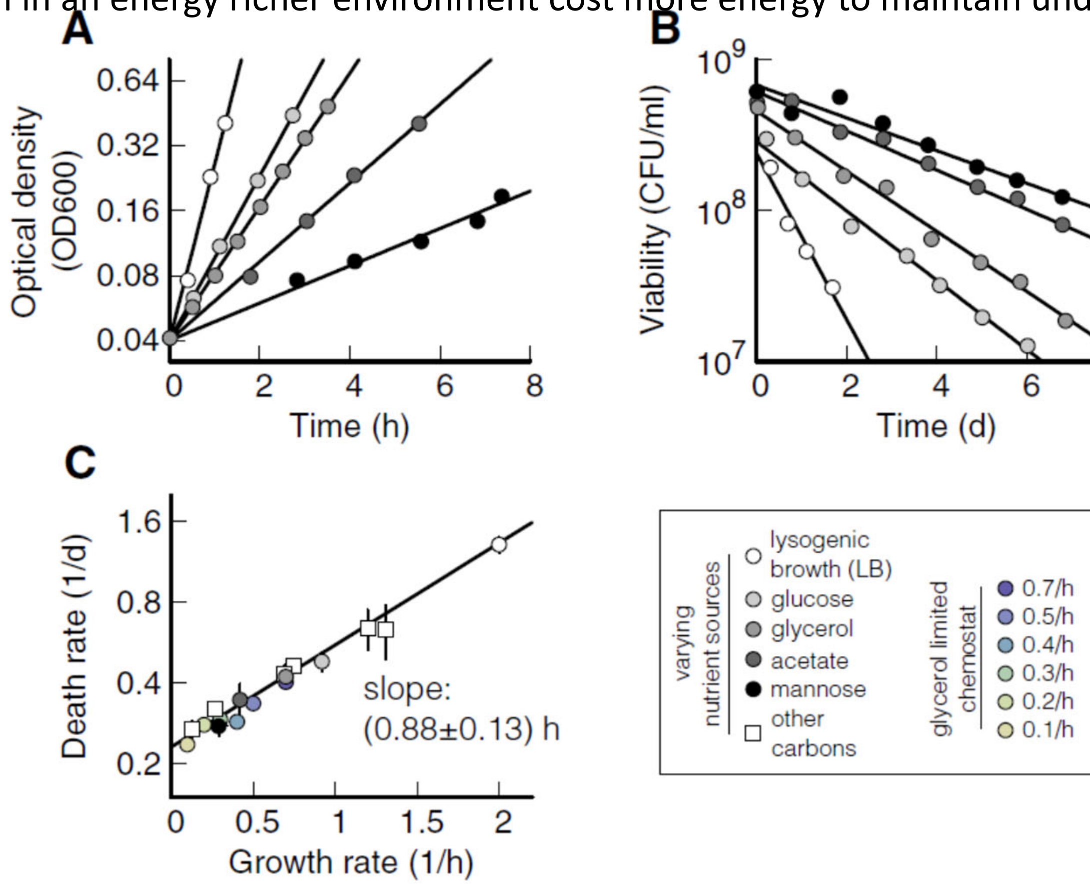
$\mu = 0.7/h$

Schink, S. J., Biselli, E., Ammar, C., & Gerland, U. (2019). Death rate of E. coli during starvation is set by maintenance cost and biomass recycling. Cell systems, 9(1), 64-73.

Linear trade-off in *E. Coli* grown in different media/rate

Biselli, E., Schink, S. J., & Gerland, U. (2020). Slower growth of Escherichia coli leads to longer survival in carbon starvation due to a decrease in the maintenance rate. *Molecular systems biology*, *16*(6), e9478.

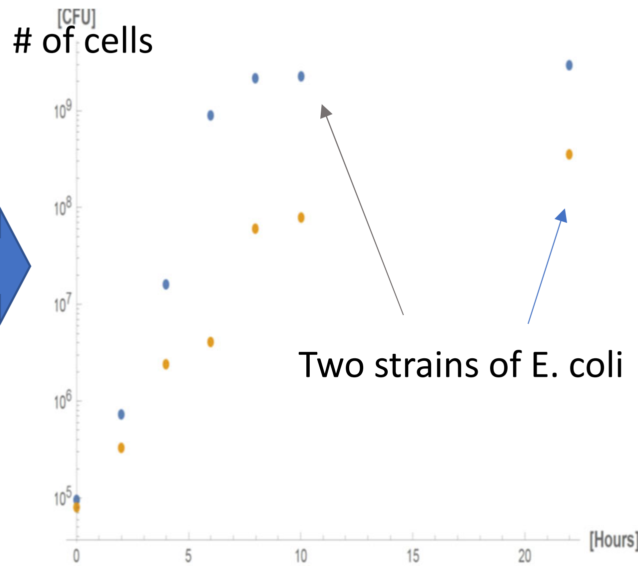
Cells grown in an energy richer environment cost more energy to maintain under starvation



Role of death rate in fitness?

If we focus on exp growth phase, fastest grower=fittest

Two strains grown
in a flask



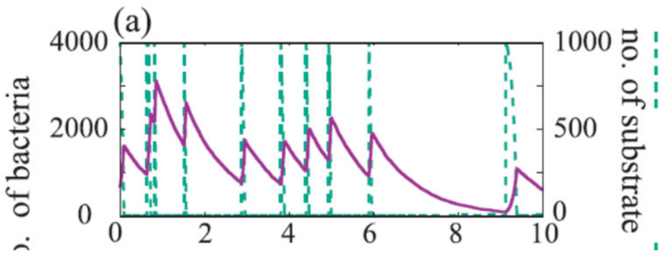
Dilute and repeat

The faster growing strain will take over the whole

What if faster growing cells also die faster under starvation?

• Himeoka, Y., & Mitarai, N. (2020). Dynamics of bacterial populations under the feast-famine cycles. *Physical Review Research*, *2*(1), 013372.

Stochastic feast-Famine cycle with growth-death trade-off



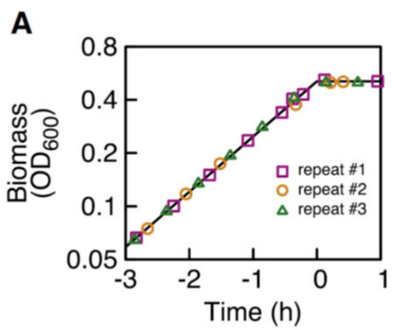
Setup

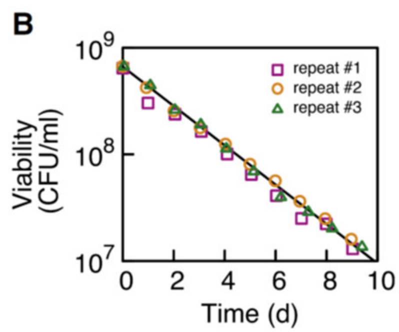
With food

$$\frac{dN}{dt} = \mu \cdot N$$

Without food

$$\frac{dN}{dt} = -\gamma \cdot N$$





γ: *an increasing function of* μ

If nutrients comes sporadically

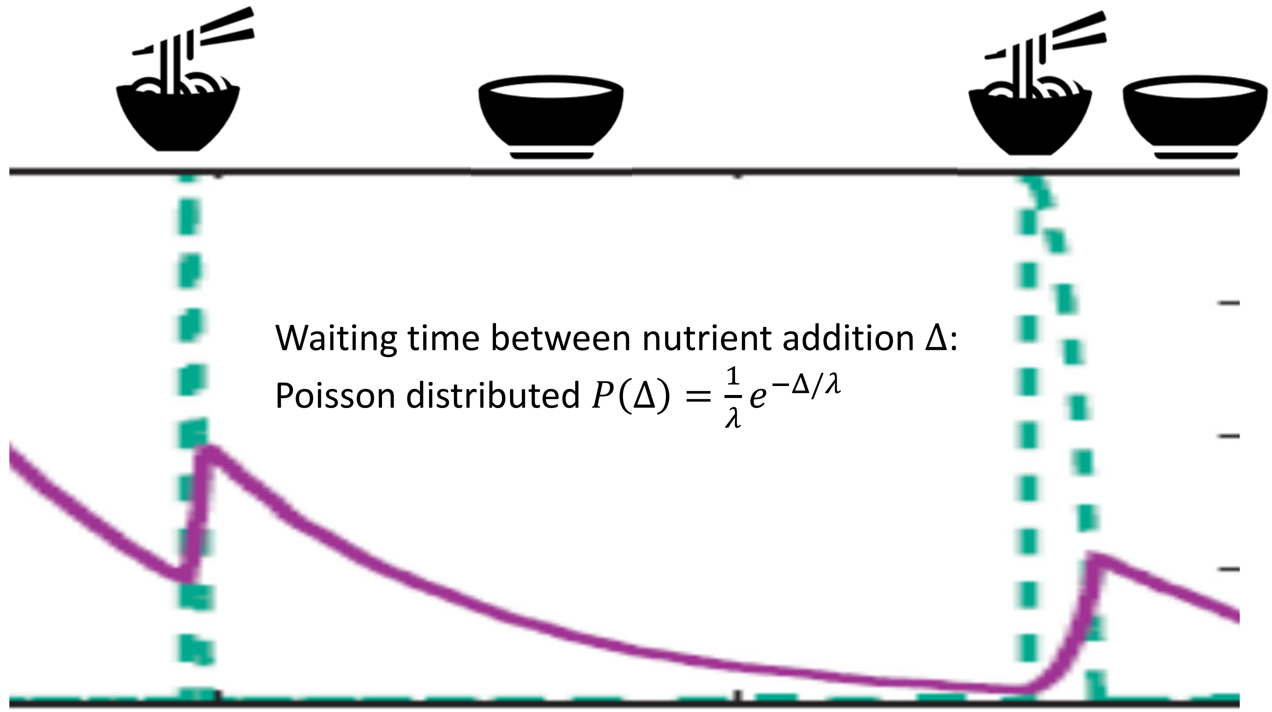
- Better to eat it fast (and grow fast) in the feast period with nutrients
- Need to survive the famine period without nutrient – slower death desirable
- **What is the fitness in this setup?**
- **Competition in growth may result in “TOC”?**

Set up: Multiple species compete for stochastic addition of food

Bacteri a $N_i \xrightarrow{\text{rate } \mu_i N_i} N_i + 1$

Food $S \xrightarrow{\text{rate } \sum_i \mu_i N_i} S - 1$

Bacteria $N_i \xrightarrow{\text{rate } \gamma_i N_i} N_i - 1$



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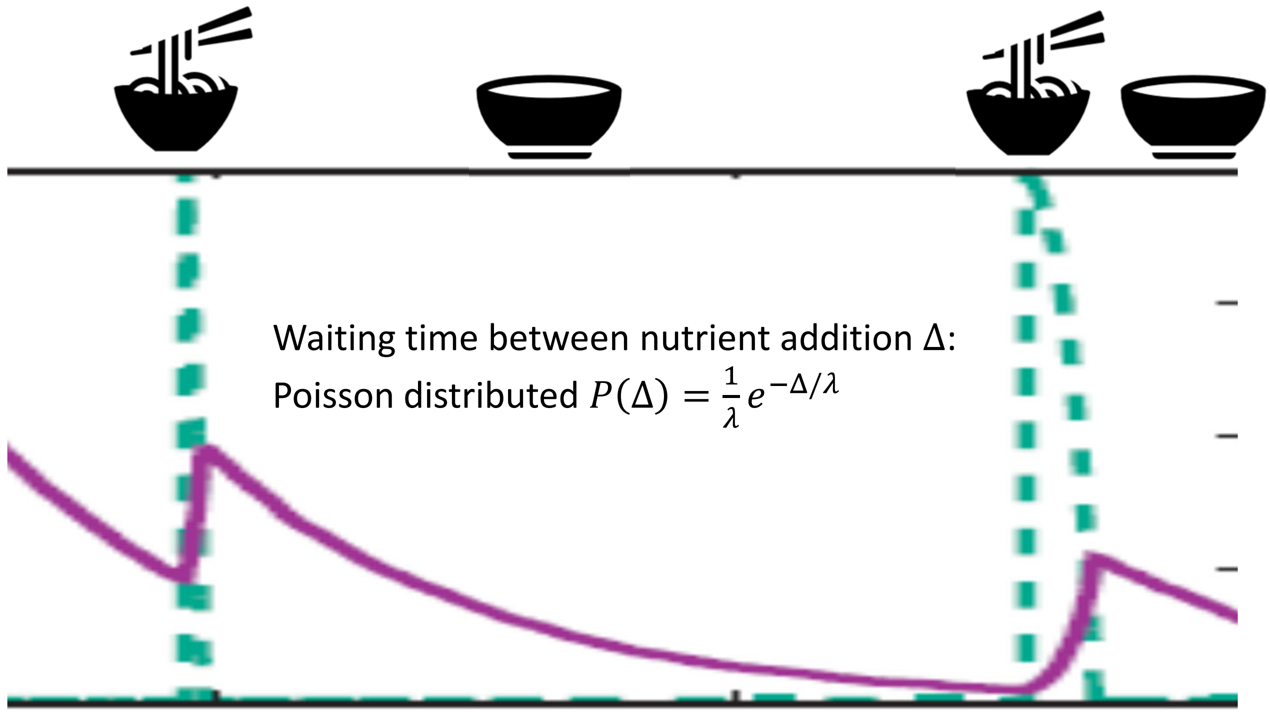

$\text{Trade} - \text{off:}\, \gamma_i = f(\mu_i)$, species can mutate to another growth rate

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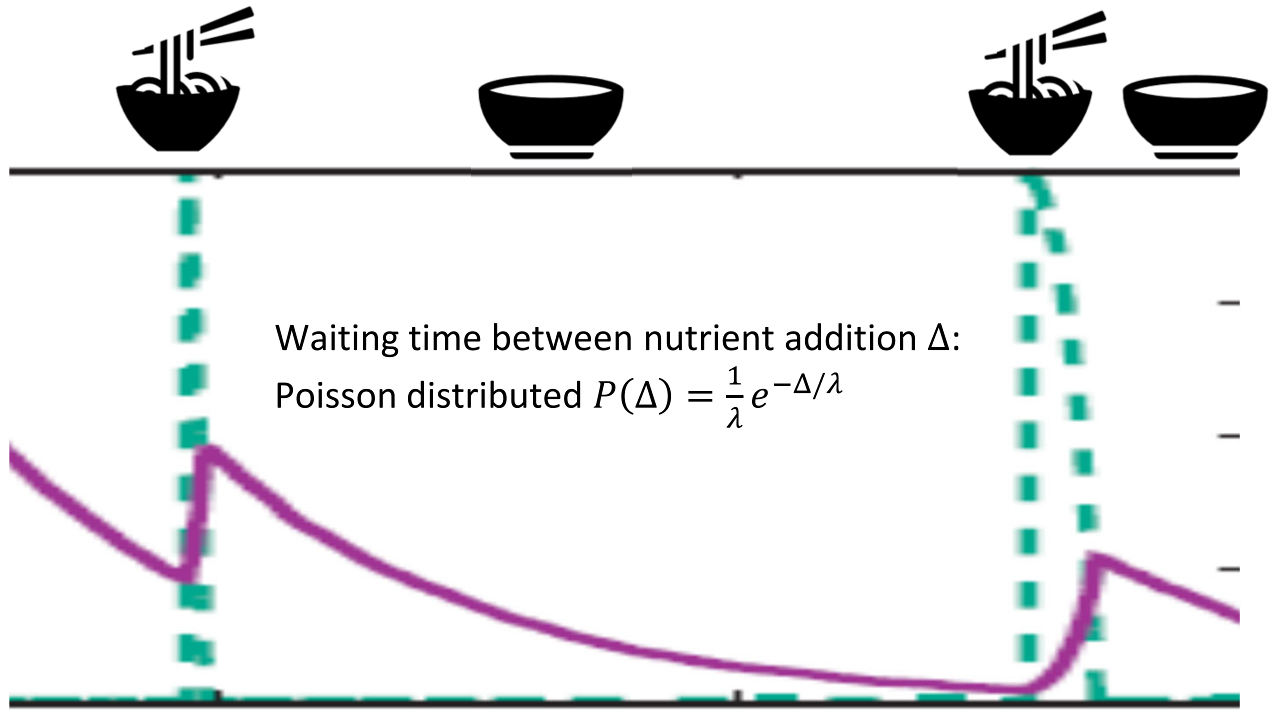
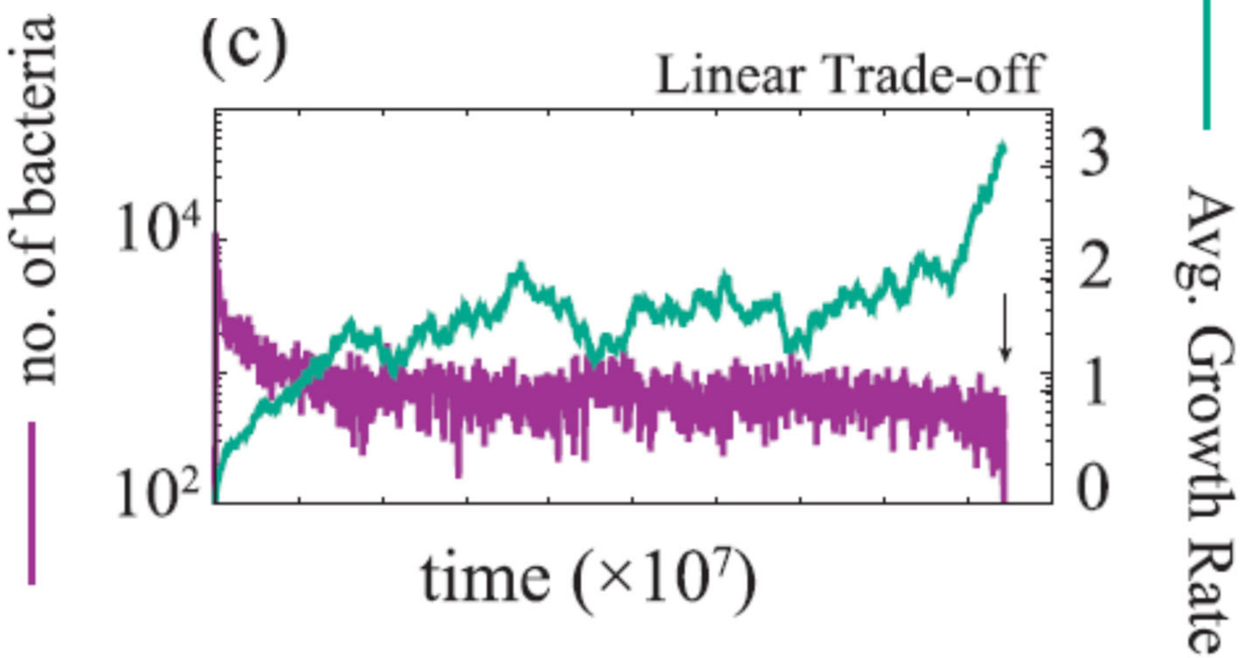


$\text{Trade} - \text{off:}\, \gamma_i = f(\mu_i)$, species can mutate to another growth rate

$\text{Mutation for evolution : a newly born bacterium } \mu_i \xrightarrow{\text{rate } \rho} \mu_i \pm \Delta\mu$

Linear Growth-Death trade-off in Repeated Feast-Famine cycle (with small mutation rate)



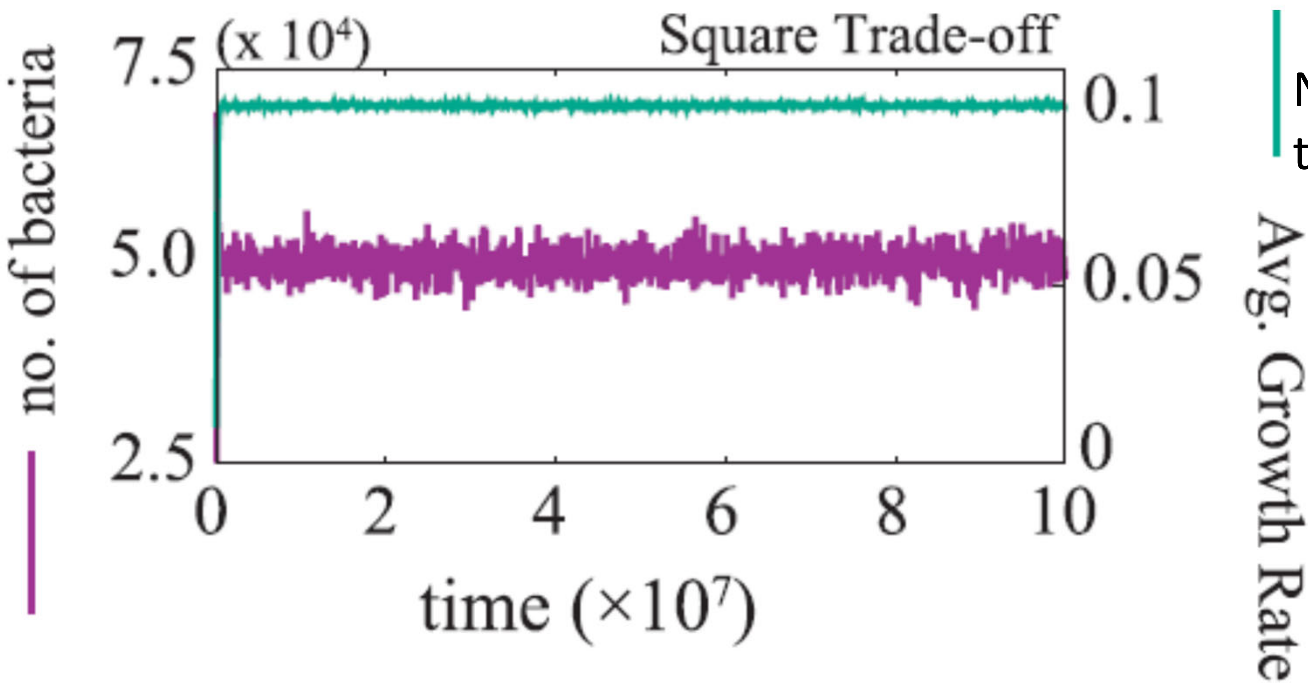
Linear death-growth trade-off

$$\gamma = a + b\mu$$

Tragedy of commons

$$a = 10^{-3},\ b = 0.1,\ \delta\mu = 10^{-2}, \qquad \rho = 10^{-5} \qquad \lambda = 1.28,$$

Square Growth-Death trade-off in Repeated Feast-Famine cycle (with small mutation rate)



Non-linear death-growth trade-off

$$\gamma = a + b\mu^2$$

Optimal growth rate exists

$a = 10^{-3},\ b = 0.1,\ \delta\mu = 10^{-2},$ $\rho = 10^{-5}$ $\lambda = 1.28,$

Intuitive candidates for the fitness?

$$\mu - \gamma$$

- If growth and death happens at the same time

$$\frac{dN}{dt} = (\mu - \gamma) \cdot N$$

the difference gives the effective growth rate

$$\mu / \gamma$$

- If we consider logistic growth

$$\frac{dN}{dt} = \mu \cdot N(1 - N) - \gamma \cdot N$$
$$= \mu \cdot N[(1 - N) - \gamma / \mu]$$

the efficient usage of the carrying capacity is determined by the ratio

(cf. Haerter, NM, Sneppen ISMEJ 2014)

In our set up, $\frac{\mu}{\gamma}$ determines the fitness

Linear trade-off $\gamma = a + b\mu$

$\frac{\mu}{\gamma} = \frac{\mu}{a+b\mu} = \frac{1}{a/\mu+b} =$

The higher growth rate the better

-> Tragedy of commons

Square trade-off $\gamma = a + b\mu^2$

$\frac{\mu}{\gamma} = \frac{\mu}{a+b\mu^2} = \frac{1}{a/\mu+b\mu}$

The optimal growth rate at

$$\mu = \sqrt{a/b}$$

-> Stable system

"Derive" $\frac{\mu}{\gamma}$ determines the fitness against invasion

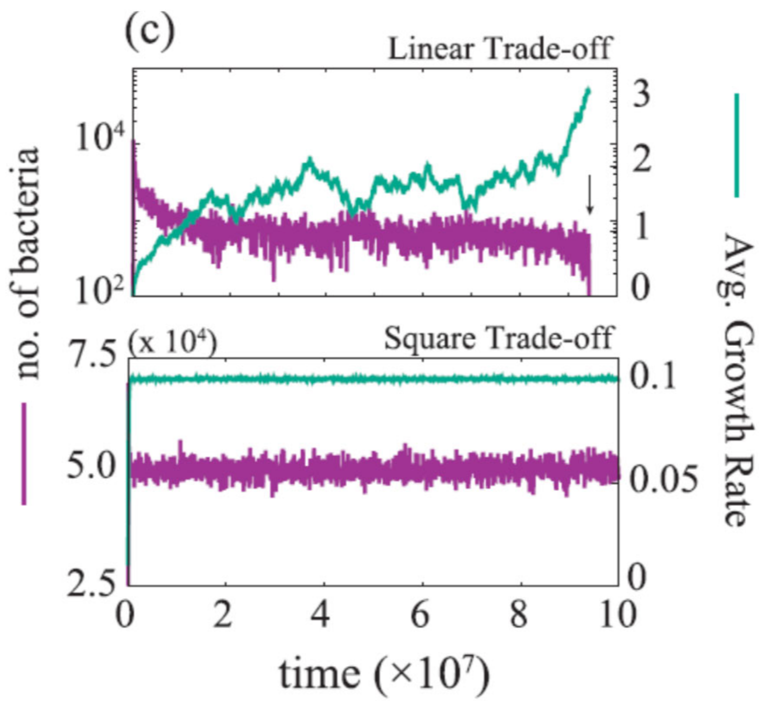
- Waiting time to the next nutrient Δ : Stochastic variable
- Time for the nutrient to run out τ : Determined by bacteria in the system

It is easy to show Effective growth fold $G_{eff} = \begin{cases} \mu\tau - \gamma(\Delta - \tau)\ (\text{for } \Delta > \tau) \\ \mu\Delta(\text{for } \Delta < \tau) \end{cases}$

If the waiting time to the next nutrient Δ is Poisson distributed with average λ , then $\langle G_{eff} \rangle = \left[\mu\left(1 - \mathrm{e}^{-\tau/\lambda}\right) - \gamma \mathrm{e}^{-\tau/\lambda}\right]\lambda$

$\rightarrow$In steady state, τ is determined by $\langle G_{eff} \rangle$=0, i.e. $\mathrm{e}^{-\tau/\lambda} = \frac{\mu}{\mu+\gamma}$

$\rightarrow$Condition for the second species with $\tilde{\mu}$ and $\tilde{\gamma}$ to invade the system: $\frac{\tilde{\mu}}{\tilde{\gamma}} > \frac{\mu}{\gamma}$



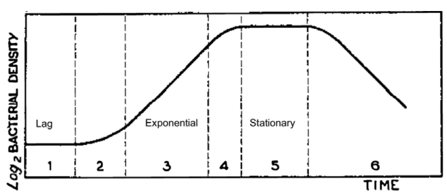
Take-home message

Under repeated Feast Famine Cycles, fitness is determined by (Growth rate)/(Death rate in starvation)

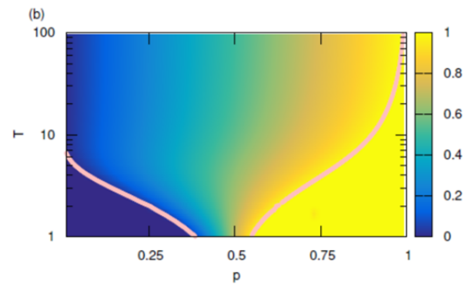
-> Different trade-off results in different evolutionary consequences

- **Derivation of fitness is tedious – is it possible to make a general statement?**

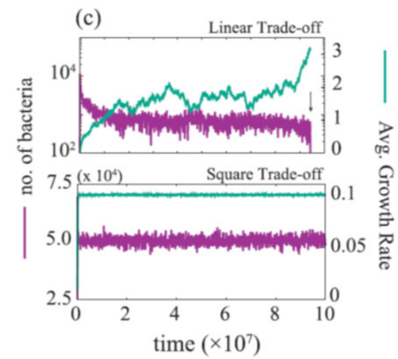
Himeoka, Y., & Mitarai, N. (2020). Dynamics of bacterial populations under the feast-famine cycles. *Physical Review Research*, *2*(1), 013372.



Dormancy happens when environment force it to



Dormancy can be selected since it provide stress tolerance



Growth and death trade-off determines evolutionary outcome

BASP

Danish Research Foundation

VILLUM FONDEN

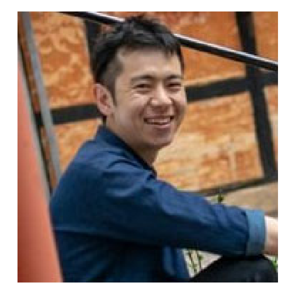
Villum foundation experiment

novo nordisk foundation

NERD
Bacteria growth and virus (phage):
Postdoc and PhD positions available

Growth, Dormancy, and Death

Himeoka, Y. and Mitarai, N., 2021. *PLoS computational biology*, *17*(2), p.e1008655.
Himeoka, Y., & Mitarai, N. 2020 *Physical Review Research*, *2*(1), 013372.
(related works: Himeoka et al. mSpheres (2022), Himeoka and Mitarai Rhys. Rev. Res. (2022))



Yusuke Himeoka NBI-> U Tokyo