Single Cell Heterogeneity of Microbial Metabolic Populations in Thermodynamically Constraining Environments

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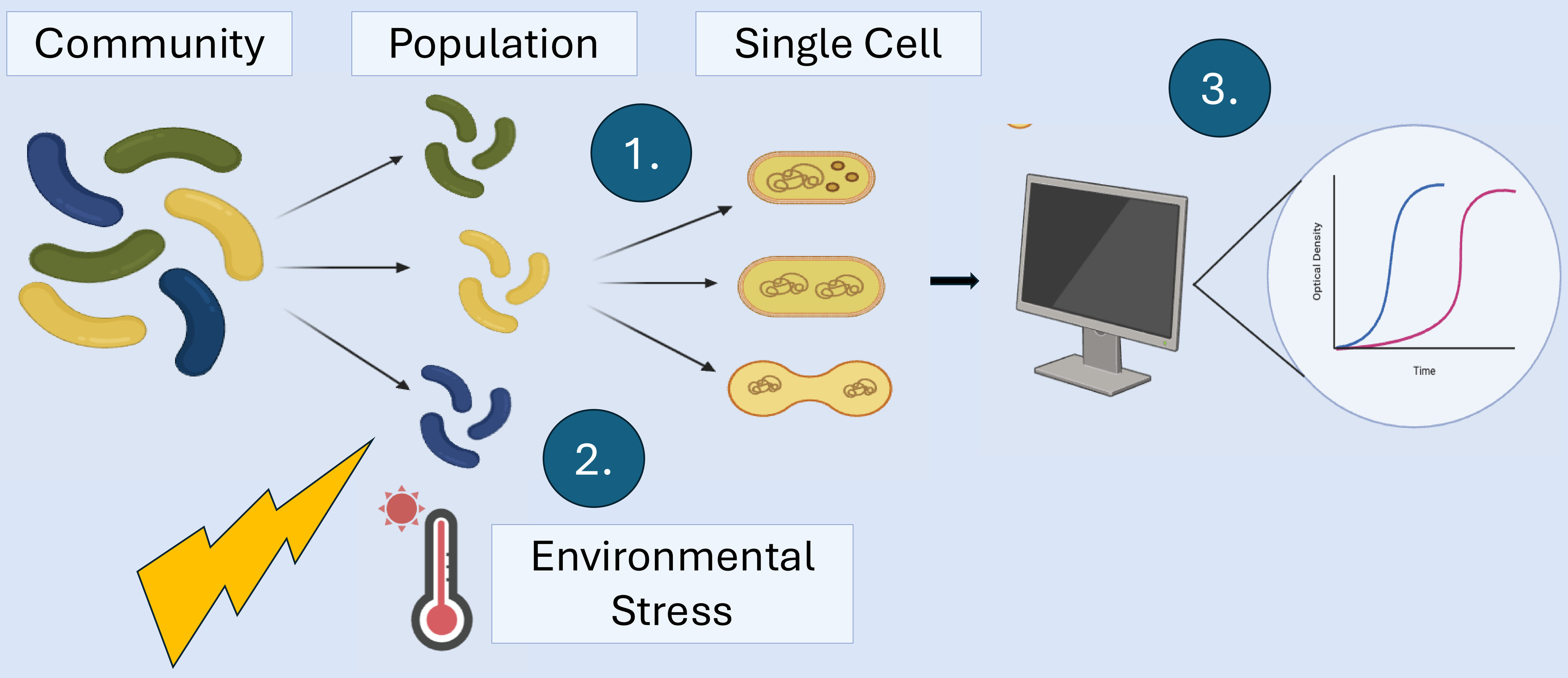
Background

Microbial populations with a lower energy yield are hypothesized to exhibit extremely slow generation times (hundreds to thousands of years)[1-2], but it is unclear if this reflects at a cellular scale, or instead, is an artifact of population averaging.



This study considers heterogeneity in single cell level growth relative to population averages, and whether this heterogeneity must be considered in energetic estimates of cellular growth vs. maintenance within extreme environments.

Questions

1. Is there single cell level heterogeneity in growth relative to the population?
2. Does this heterogeneity change with increased exposure to environmental stress (e.g. temperature)?
3. How might this influence computational predictions of extremophile growth?

Results

Measurements of Single Cell Heterogeneity in Growth

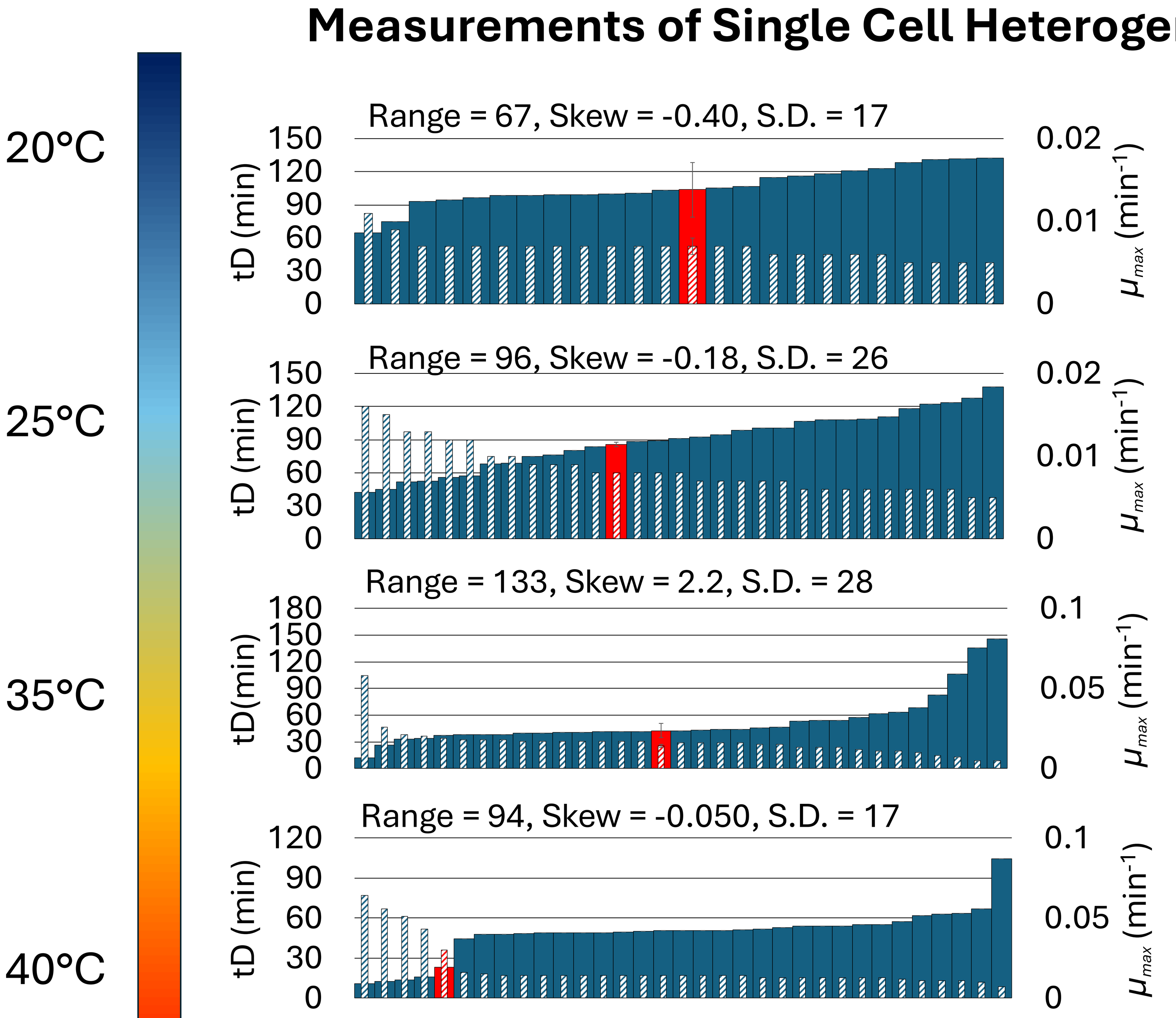


Fig. 1 Doubling time [tD] (solid) and maximum growth rate [μ_{max}] (striped) starting from single cells (blue) vs. a population (red; n=8 error in ±1S.D.).

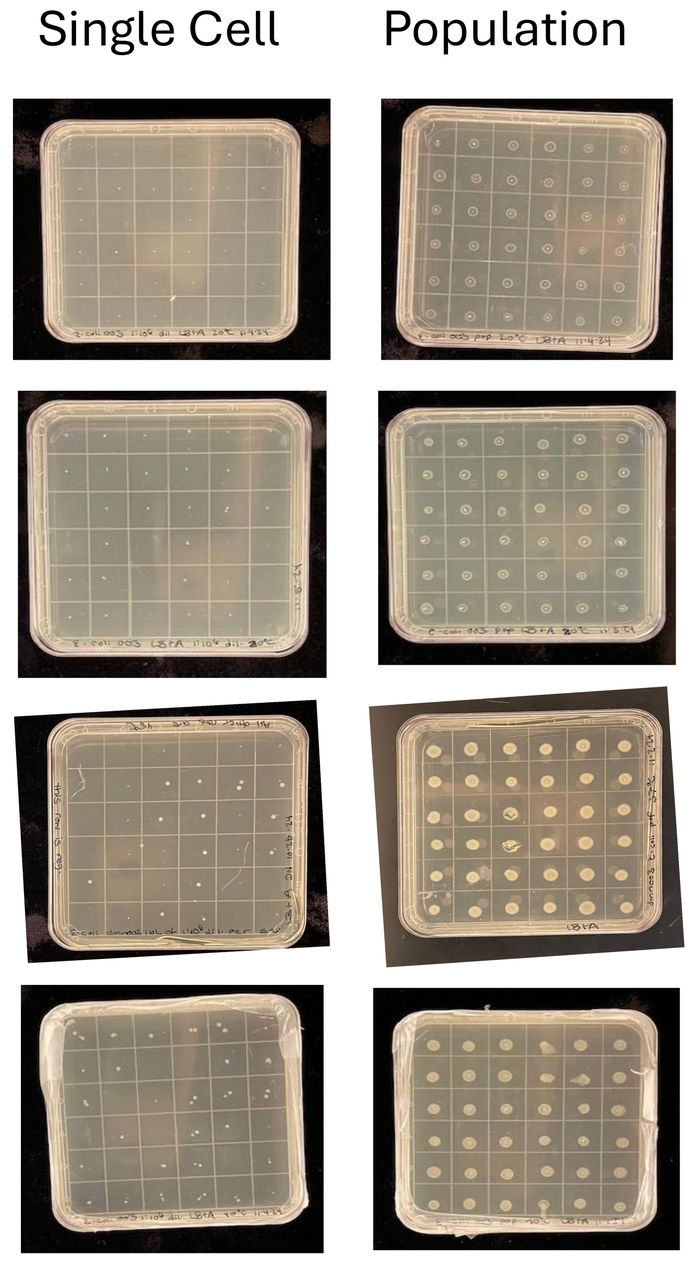


Fig. 2 Colony images from overnight growth of single cell (left) or population (right) starting inoculum.

Predictions of Single Cell Heterogeneity in Growth

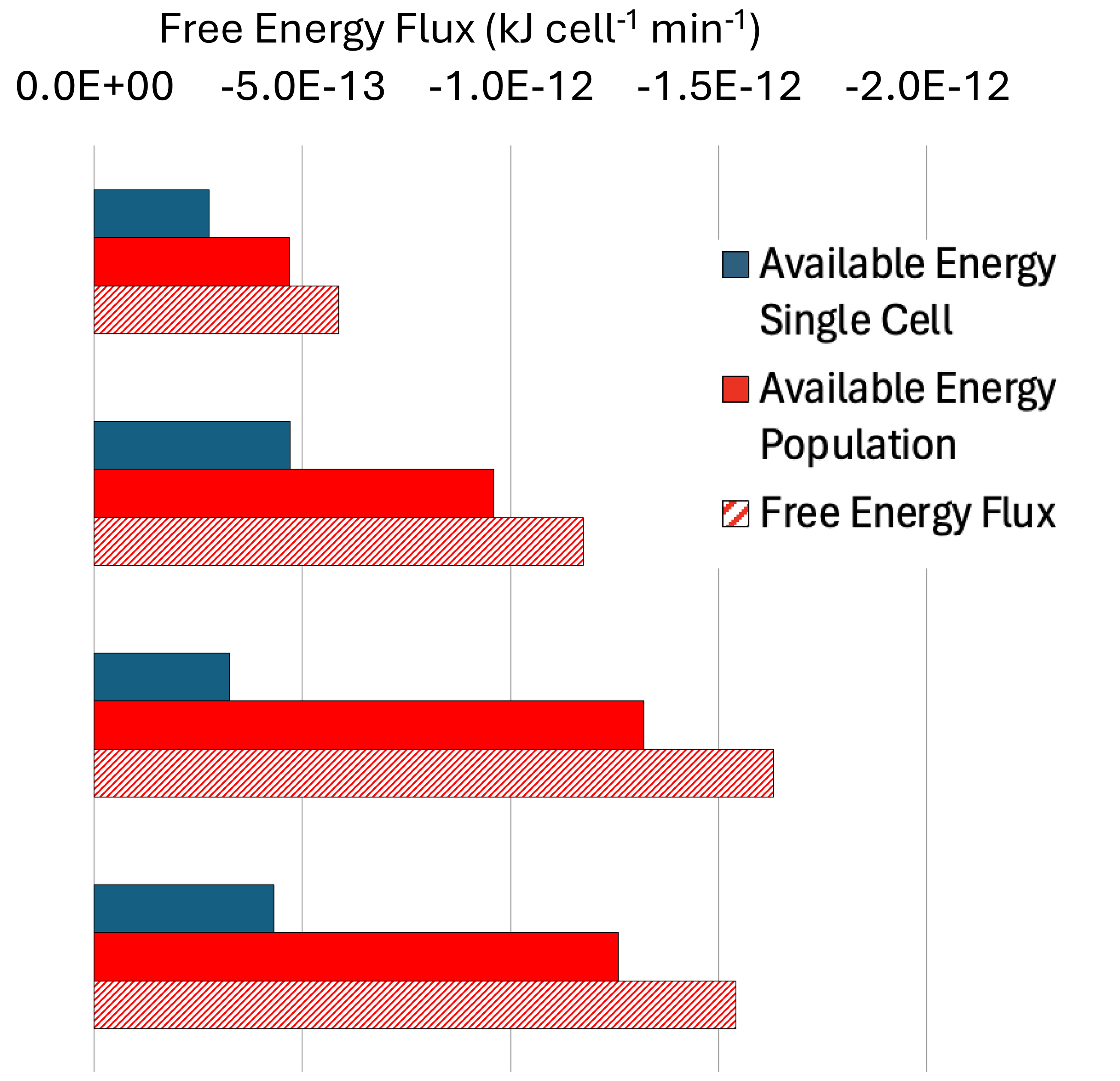
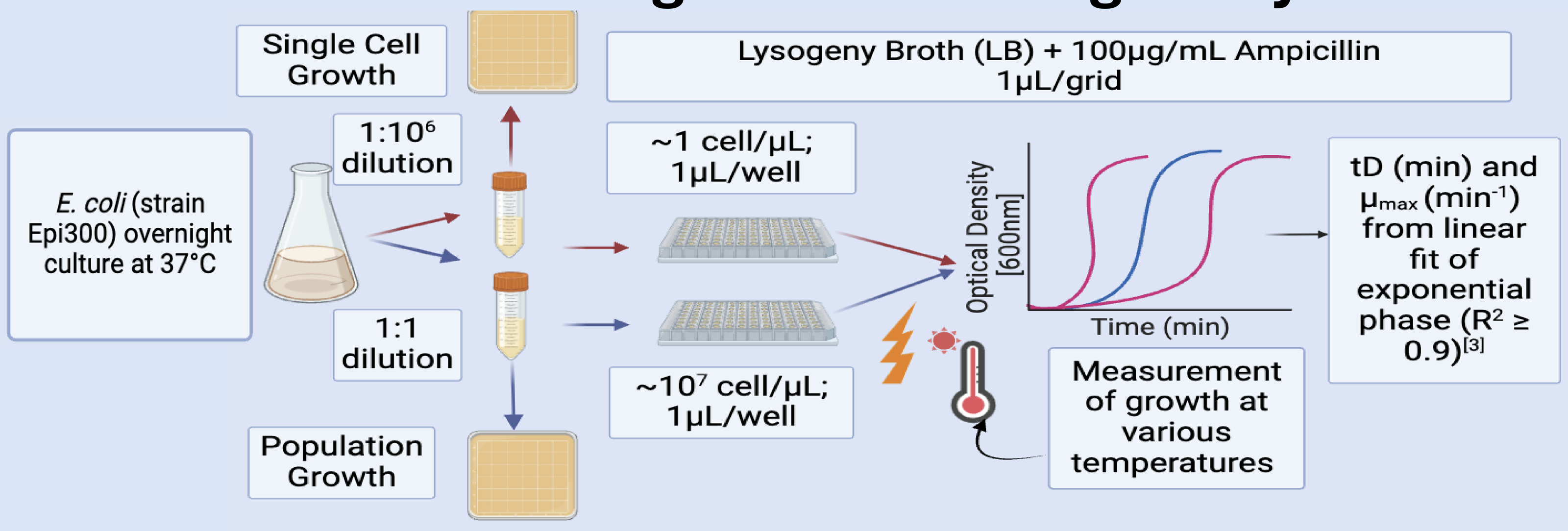


Fig. 3 Free energy flux and available energy after temperature maintenance costs based on population or single cell growth of aerobic glucose oxidation.

Methods

Measurements of Single Cell Heterogeneity in Growth



Predictions of Single Cell Heterogeneity in Growth

Cellular Free Energy Flux[4]

$$Free\ Energy\ Flux\ (kJ\ cell^{-1} min^{-1}) = 4\pi r D C \Delta G$$

- r = average cell radius (5 × 10^{-7} cm)
- D = diffusivity constant for the limiting substrate at T in (cm^2/min)
- C = Concentration of limiting substrate (mol/cm^3)
- ΔG is the *in situ* free energy change for aerobic oxidation of glucose calculated in Geochemist's Workbench.

Cellular Maintenance Energy*[5]

$$m_E (kJ\ cell^{-1} min^{-1}) = 4.5 \cdot e^{\left[-\frac{69{,}400}{R\left(\frac{1}{T}-\frac{1}{298}\right)}\right]}$$

- R is the universal gas constant (8.314 $\frac{J}{K \cdot mol}$)
- T is the temperature in Kelvin

*maintenance requirements dependent only upon the ambient temperature.

Available energy represents the FEF after accounting for maintenance costs. For single cell adjustments, the maintenance cost was adjusted by the fractional difference of the population growth and minimum cellular growth rate for each temperature.

Key Takeaways

1. There is heterogeneity in growth at the single cell level for *E. coli*.
2. When exposed to a temperature gradient, a greater proportion of *E. coli* cells show significantly different growth rates relative to the population.
3. Predictions of metabolic energy yield do not currently capture the energy flux as defined by cellular growth rates, with minimum estimates resulting in a much lower amount of available energy for growth.

References: [1]Jϕrgensen et al., 2016. *Ann. Rev. Marine Sci*. 8:311-332. [2] D'Hondt et al., 2004. *Science*. 306:2216-2221. [3] Wirth et al., 2023 *Nat. Protocol*. 8:2401-2403. [4] Lau et al., 2016 *PNAS* 113:E7927-E7936 . [5] Tijhuis et al., *1993 Biotechnol. Bioeng*. 42:509-519

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