Instituto Superior Técnico

Projecto MEFT

Bacterial Growth in the Presence of Several Resources

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1 Introduction

It is currently estimated that there are more than 10 million different species on our planet, [13]. This incredible biodiversity has been captivating scientists since the 20th century.

Living beings interact with beings of the same species and of different species in order to feed themselves (predation), cooperate (mutualism) or fight for the same resources (competition). These interactions give rise to a highly complex network that governs all the population dynamics and determine whether they lead to the species' coexistence or extinction. It is therefore a great challenge to understand how it is possible for such a vast number of species to coexist in equilibrium and how stable this equilibrium is.

We know that species have the ability to adapt to changes in the environment, [1]. But the connection between this phenomenon and the existing populational models is still missing. Biologically and mathematically we are still far from fully understanding on how adaptative evolution works.

In order to find the answers to all these questions, biologists, physicists and mathematicians have been studying smaller ecosystems and creating mathematical models to describe the dynamics of species, [4] [16] [17]. Although they are deterministic, these models can't be implemented with basic analytical tools given their large number of variables and dependencies, which requires employing methods of dynamical systems theory.

The bacteria *Escherichia coli* are often chosen as subjects of experiments for their simplicity, fast growth, easy access and familiarity. These organisms were first discovered by Theodor Escherich, [2], in 1885 and nowadays hundreds of strains are known, [15]. They have a size of the order of 1 μm, can divide every 20 minutes, are found in the intestines of endotherms (commonly known as warm-blooded animals) and are able to grow in the presence of oxygen, which makes them easy and inexpensive to cultivate in a laboratory, [11].

2 State of the art

In 1934, the ecologist Georgy Gause enounced that in processes of competition for limited resources, one species would drive the others to extinction, [3] — the Gause's Law — based on observations of a culture with two species of single-celled eukaryotes (*Paramecium aurelia* and *Paramecium caudatum*) competing for the consumption of bacteria in an Osterhout's medium[1].

The Gause's Law was later reformulated by Garrett Hardin in 1960 and became known by the name Competitive Exclusion Principle (CEP), [5], stating that the number of species coexisting cannot exceed the number of resources. However, one year later, George Hutchinson noticed that *Plankton* could grow in situations of limited resources, [6], known as the Paradox of the Plankton, as we know today that happens in many other ecosystems, in violation of the CEP. So a question arised: in what circumstances do competitive exclusion and sustainable coexistence take place?

In 1941, Monod studied a culture of *E. coli* in a medium containing glucose and proposed a sigmoidal relation between the culture's growth rate R, and its nutrient concentration c, [4]:

$$R = R_{max}\frac{c}{K + c},\tag{1}$$

where K is the concentration for which $R = R_{max}/2$.

Although revolutionary, this empirical model described a single species in the presence of a single nutrient which is too simplistic, since species depend on different nutrients for growth and are never isolated. Moreover it could only produce accurate results for short-term evolution.

Monod also discovered the diauxic growth. When presented with different nutrients, bacteria evaluate the energy cost of metabolizing each one *versus* the growth rate they will provide (how valuable the resource is), and choose what gene to express in order to ingest the preferred nutrient.

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[1]An Osterhout's medium is a salt solution with 24 mOsm/kg, pH 7.0 and composition 104 mg of NaCl, 8.5 mg of MgCl$_2$, 4 mg of MgSO$_4$, 2.3 mg of KCl, and 1 mg of CaCl$_2$ per liter of water.

In this way, bacteria consume the nutrients sequentially by order of the most to the least favorable, generating different growth rate phases over time, named diauxies. This adaptive mechanism ensures an optimization strategy: it allows the species to have the biggest growth rate when the population is small (and thus, more fragile) and then provides a smaller growth rate when the population becomes bigger (and the risk of extinction is smaller).

> "*Diauxie — This phenomenon is characterized by a double growth cycle consisting of two exponential phases separated by a phase during which the growth rate passes through a minimum even becoming negative in some cases.*"
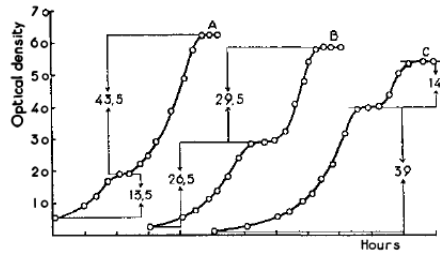
—Monod

Figure 1: Time evolution of *E. coli* density in a medium composed by two different nutrients, glucose and sorbitol, in different proportions: A: Glucose 50 μg/ml; sorbitol 150 μg/ml. B: Glucose 100 μg/ml; sorbitol 100 μg/ml. C: Glucose 150 μg/ml; sorbitol 50 μg/ml. Adapted from [4].

Figure 1 shows the result of an experiment done by Monod in which he studied the growth of a culture of *E. coli* in a medium with two nutrients. When varying the concentrations of each nutrient, the growth phases are proportional to the nutrient concentrations, showing that bacteria consumed the nutrients separately by order.

In 1969, Robert MacArthur proposed the first mathematical consumer-resource model describing a group of m species competing for p common resources and introduced different timescales for the rates of supply and consumption, [9].

He assumed the rate of growth of a population σ ($\dot{X}_\sigma$) to be proportional to the already existing population (X_σ), the number of resources of each type (R_i), their relative importance measured by the weight parameter (w_i), and the probability of an individual of that population to consume the different nutrients ($a_{\sigma i}$). The number of resources of type i varies according to their current amount, the carrying capacity of the medium (K_i), and to the probability of being eaten by any of the species and their population size. This model is described by the equations:

$$\begin{cases} \dfrac{\dot{X}_\sigma}{X_\sigma} = C_\sigma \left(\sum_{i=1}^{p} a_{\sigma i} w_i R_i - T_\sigma \right) \\ \dfrac{\dot{R}_i}{R_i} = r_i \left(1 - \dfrac{R_i}{K_i} \right) - \sum_{\sigma=1}^{m} a_{\sigma i} X_\sigma \end{cases} \tag{2}$$

where the dot represents time derivative. The constant T_i is the threshold mass of resource necessary to maintain the population, C_i is the proportion between the mass of resource and of the population it originates, and r_i is the maximum rate of resource variation, that takes an exponential form in the absence of consumption.

Inspired by previous works of MacArthur and Edward Wilson, [7] [8], in 2001, the ecologist Stephen Hubbell examined the biodiversity in ecological communities looking at the abundance distribution of species in different locations (fig. 2).
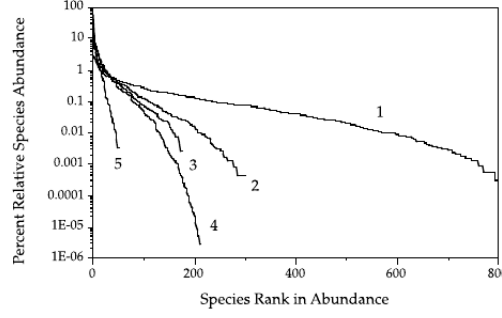
Figure 2: Relative population sizes of 5 ecological communities ranked from the largest to the smallest. 1: Tropical wet forest in Amazonia. 2: Tropical dry deciduous forest in Costa Rica. 3: Marine planktonic copepod community from the North Pacific gyre. 4: Terrestrical breeding birds of Britian. 5: Tropical bat community from Panama. Adapted from [10].

Hubbell noticed that the curves had similar shapes, which lead him to wonder if there would exist a theory behind it and, if so, whether the curves were possible to predict. To explain this pattern, he created the Unified Neutral Theory of Biodiversity [10] in which species were grouped by ecological communities. The theory stated that species that occupied the same geographic area and level in a food chain were seen as equally strong and their differences were irrelevant to their success. In a neutral setup, the individuals were considered identical in terms of average probabilities of birth, death, migration and speciation. Only small random deviations in this quantities were responsible for changes in population which meant that biodiversity arised from stochastic processes.

Based on the MacArthur's model (2), Posfai, Taillefumier and Wingreen presented a resource-competition model in 2016, [16], that accounted for the fact that organisms work with a limited amount of energy and therefore, need to choose how to allocate different fractions in order to favor the traits that maximize the species' probability of survival. This sometimes means reducing certain performances in order to enhance others — trade-offs. This model predicted that coexistence of species could occur in cases where, according to the CEP, could not, reproducing what happens in many ecosystems, [6].

Similarly to the MacArthur' model, the population growth rates ($\dot{n}_\sigma$) vary according to the current population densities (n_σ) and death rates (δ_σ), the nutritional values (v_i) and available amounts (r_i) of nutrients, and the consumption rates ($\alpha_{\sigma i}$) of every resource i by each species σ, called "metabolic strategies". The variations of resources concentrations ($\dot{c}_i$) are proportional to their supply rates (s_i) and decrease with the rates of consumption and degradation (μ_i):

$$\begin{cases} \dot{n}_\sigma = n_\sigma \left[\sum_{i=1}^{p} v_i \alpha_{\sigma i} r_i(c_i) - \delta_\sigma \right] \\ \dot{c}_i = s_i - \sum_{\sigma=1}^{m} n_\sigma \alpha_{\sigma i} r_i(c_i) - \mu_i c_i \end{cases} \tag{3}$$

where

$$r_i(c_i) = \frac{c_i}{K_i + c_i}, \tag{4}$$

K_i being the concentration for which $r_i = r_i^{max}/2$. The metabolic strategies are constrained by the maximum uptake rate they can have, E_σ:

$$\sum_{i=1}^{p} w_i \alpha_{\sigma i} = E_\sigma. \tag{5}$$

Posfai et al. tested the possibility of coexistence of a system consisting of three species in the presence of three nutrients with different supply rates. In this case, by equation 5, given two metabolic

strategies for a certain species, the third one is automatically determined. Thus, a triangular plot, where the axes go from 0 to 1, i.e. a simplex plot, is the perfect way to visualize the distribution of metabolic strategies.
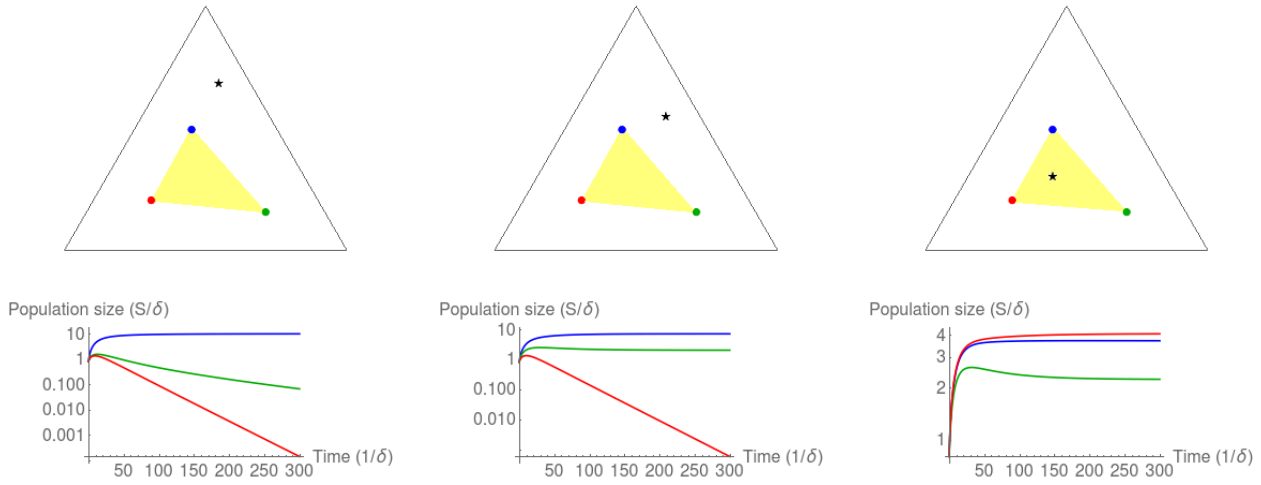

Figure 3: Top: simplex plots with metabolic strategies of 3 species ($m = 3$) relative to 3 different resources ($p = 3$) represented by colored dots: $\alpha_{1i} = (0.30, 0.20, 0.50)$ in blue, $\alpha_{2i} = (0.20, 0.65, 0.15)$ in green, $\alpha_{3i} = (0.60, 0.20, 0.20)$ in red, the convex-hull of metabolic strategies in yellow triangles, and the supply rates $s = (0.10, 0.20, 0.70)$, $s = (0.10, 0.30, 0.50)$ and $s = (0.40, 0.30, 0.30)$ from left to right respectively in black stars. Bottom: simulations obtained with model equations (3), (4) and (5) for the evolution of the 3 population densities with parameters $\mu = 0.1$, $\delta = 0.1$, $c = 1$, $v = 1$, $K = 1$, $w = 1$, $E_\sigma = 1$ for times between 0 and 300 δ. Note that we have taken the same parameters for all three species.
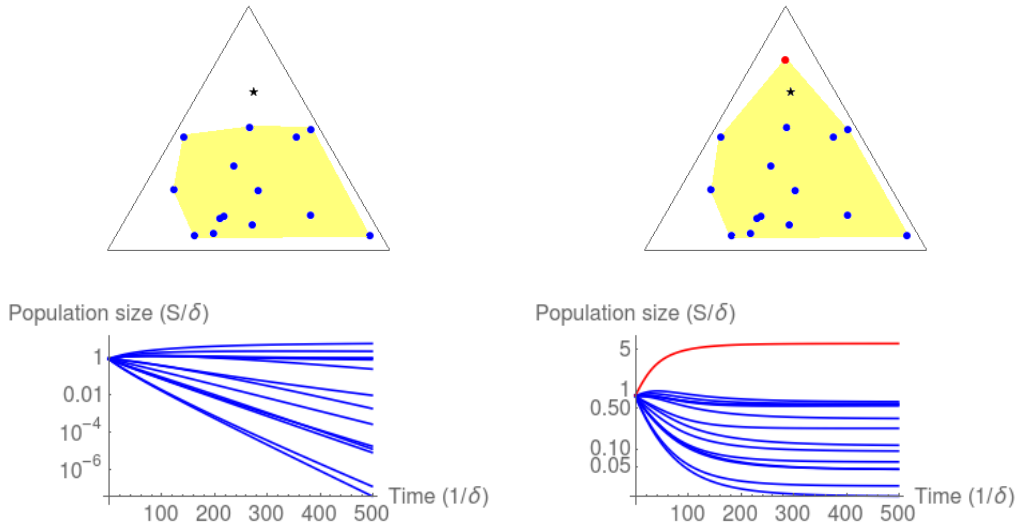

Figure 4: Top: simplex plots with metabolic strategies of 15 species ($m = 15$) relative to 3 different resources ($p = 3$) represented by blue (14) and red (1) dots, convex-hull of metabolic strategies in yellow polygons, and supply rates $s = (0.15, 0.19, 0.66)$ in a black star. The metabolic strategies in blue were chosen randomly between 0 and 1 such that their convex-hull would not include the supply rates. The metabolic strategies in red were chosen such that the new convex-hull formed by the 15 species would include the supply rates. Bottom: simulations obtained with model equations (3) for the evolution of the same 14 and 15 population densities with parameters $\mu = 0.1$, $\delta = 0.1$, $c = 1$, $v = 1$, $K = 1$, $w = 1$, $E_\sigma = 1$ for times between 0 and 500 δ.

Figure 3 shows three simplex plots containing the initial settings of the simulations, with dots representing the metabolic strategies of each species and a star with the nutrients' supply rates. Below, there are respective evolutions of the population densities over time. The results indicate that, from left to right, as the supply rates fall into the area delimited by the metabolic strategies — the convex-hull — the coexistence becomes possible. This, of course, discloses the importance of having certain species in an ecosystem. The addition of a species whose metabolic strategies allow to enclose the supply rates is determinant to the future of all the other species.

In the first system of figure 4, composed by 14 species, it can be seen that most go to extinction. However, if a new determinant species is inserted in the system, this no longer happens and all species can coexist.

Motivated by the Unified Neutral Theory of Biodiversity of Hubbell, [10], Posfai et al. decided to incorporate demographic stochasticity in their model considering the following set of equations:

$$
\begin{cases}
\dot{n}_\sigma = \left[\sum_{i=1}^{p} \left(\alpha_{\sigma i} + \xi_{\sigma i} \right) c_i - \delta_\sigma \right] n_\sigma \\
c_i = \dfrac{s_i}{\sum_{\sigma=1}^{m} n_\sigma \left(\alpha_{\sigma i} + \xi_{\sigma i} \right)} \\
\delta_\sigma = 1 + \xi_\sigma
\end{cases}
\tag{6}
$$

where $\xi_{\sigma i}$ and ξ_σ are random variables with Gaussian distribution $\mathcal{N}(0, \Sigma^2)$, and with metabolic strategies and supply rates normalized such that

$$
\sum_{i=1}^{p} \alpha_{\sigma i} = 1 \quad \text{and} \quad \sum_{i=1}^{p} s_i = \sum_{\sigma=1}^{m} n_\sigma(0). \tag{7}
$$

Implementing the previous model they obtained the results shown in figure 5. The model was able to reproduce the curves identified by Hubbell.
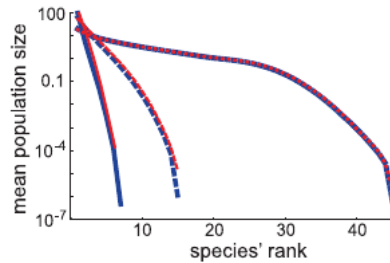

Figure 5: Simulation of rank-abundance curves obtained with model equations (6) and (7) for a total population of 100 individuals competing for 3 resources equally supplied. The solid, dashed and dotted curves correspond to immigration probabilities of 0.001, 0.01 and 0.1 respectively. Adapted from [16].

In December 2018, Pacciani-Mori, Suweis and Maritan constructed a model that assumed the same equations as Posfai et al., (3), (4) and (5), but dynamic metabolic strategies instead of fixed ones, [17]. By doing this, they introduced the fact that species have the ability to adapt to changes in the environment, in this case, nutrients' concentrations and number of competing species. To do this, Pacciani-Mori et al. required species to adapt in a favorable way, i.e., so that they would evolve in order to maximize their growth rate:

$$
\dot{\alpha}_{\sigma i} \propto \frac{\partial}{\partial \alpha_{\sigma i}} \left(\sum_{i=1}^{p} v_i \alpha_{\sigma i} r_i - \delta_\sigma \right). \tag{8}
$$

Since δ_σ is the death rate of species σ, $1/\delta_\sigma$ is a natural choice for the characteristic time scale of the evolution of population σ. Therefore, the characteristic time scale of evolution of the metabolic strategies can be written as a multiple of $1/\delta_\sigma$, given by a parameter d.

Now that the metabolic strategies are dynamic, there is a maximum uptake rate (E_σ^*) for each species: $\sum_{i=1}^{p} w_i \alpha_{\sigma i}(t) = E_\sigma(t) \leq E_\sigma^*$. Similarly, the nutrient uptake rates E_σ^* can be written as $Q\delta_\sigma$. Therefore, the evolution of metabolic strategies is described by the equation

$$\dot{\alpha}_{\sigma i} = \alpha_{\sigma i}\, d\, \delta_\sigma \left[v_i r_i - \Theta\left(\sum_{i=1}^{p} w_i \alpha_{\sigma i} - Q\delta_\sigma \right) \frac{w_i}{\sum_{k=1}^{p} w_k^2 \alpha_{\sigma k}} \sum_{j=1}^{p} v_j r_j w_j \alpha_{\sigma j} \right]. \tag{9}$$

The full calculation of equation (9) is rather lengthy and can be found in the supplemental material of [17], page 4. The Heaviside-theta function prevents the metabolic strategies from taking negative values.

Pacciani-Mori et al. tested the model of equations (3), (4) and (5) with the addition of equation (9), for the growth of one species only in the presence of two different nutrients in order to reproduce the observations of Monod. The results are shown in figure 6.
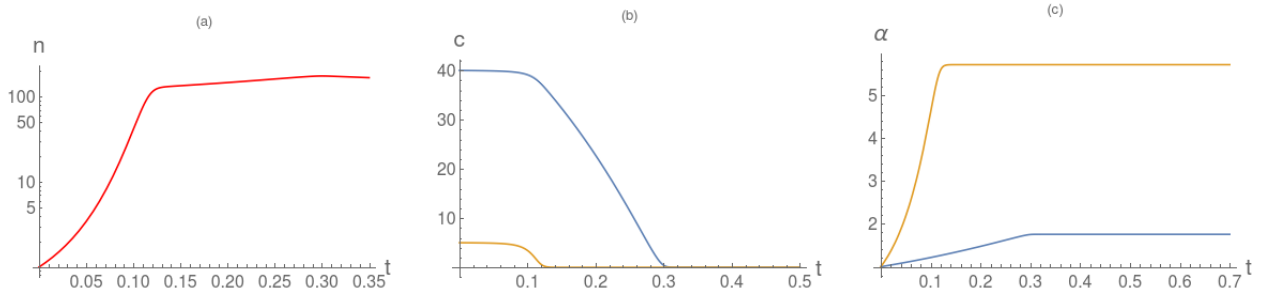

Figure 6: Simulations obtained with model equations (3), (4), (5) and (9) for 1 population of individuals of the same species with access to 2 resources of different properties. From left to right are the results of the simulation for the population density, nutrient concentrations and respective metabolic strategies with parameters $\vec{v} = (2, 25)$, $\vec{w} = (1, 4)$, $\vec{K} = (1, 3)$, $Q = 25$, $\Delta = 1$ and $d = 1$ for times between 0 and 500 δ.

The individuals consume the first resource until it ends at $t \approx 0.11$, [see figure 6(b) in orange]. When this happens, the metabolic strategy corresponding to this resource changes, [see figure 6(c) in orange], and the population suffers a diauxic shift, [see figure 6(a)]. Then, the individuals consume the second resource until it ends at $t \approx 0.30$, [see figure 6(b) in blue]. When this happens, the metabolic strategy corresponding to this resource changes, [see figure 6(c) in blue], and the population starts having a negative growth, [see figure 6(a)].

We've already seen that with the model constructed by Posfai et al., when the supply rates fall outside of the convex-hull of metabolic strategies, the coexistence of the initial set of species becomes impossible. However, they did not consider the adaptability of species. Pacciani-Mori et al. repeated the same simulations as Posfai et al. did, adding this detail and compared the results.

Figure 7 shows the results of the simulations obtained with model equations (3), (4), (5) and (9). Observing the simplex plot, it is possible to understand that, over time, the metabolic strategies evolved in a way such that their convex-hull would include the supply rates. By doing this, species become more fit to survive, as the consumption rates of each resource become compatible with their supply rates. Analyzing the plots that display this evolution (fig. 7 bottom), we can identify that adaptation period ($0 < t \lesssim 100$) following by the stabilization of the populations and metabolic strategies ($t \gtrsim 100$), in contrary of what is obtained in the fixed metabolic strategies model of Posfai et al., figure 7 top right. These results exhibit that adaptation is crucial to the survival of species when in a sub-ideal initial setting.
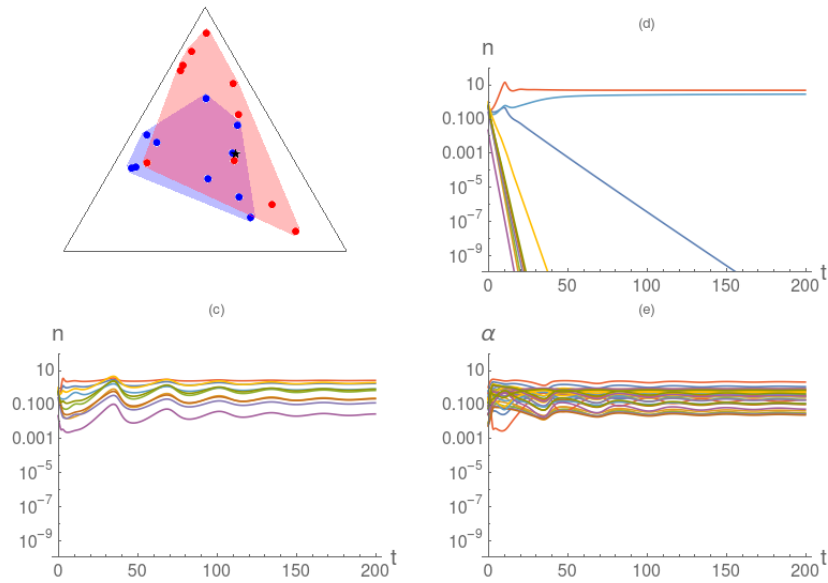
Figure 7: Simulation obtained with model equations (3), (4), (5) and (9) for the evolution of 10 population densities ($m = 10$) competing for 3 different resources ($p = 3$) during 200 time steps. Top left: simplex plot with initial and final metabolic strategies (and respective convex-hulls) represented in blue and red respectively and supply rates in a black star; Top right: evolution of the population densities in the case where the metabolic strategies are fixed; Bottom left: evolution of the population densities in the case where the metabolic strategies are adaptative; Bottom right: evolution of the metabolic strategies in the adaptative case; The parameters used were $Q = 2$, $\mu = 0$, $\delta_\sigma \in \mathcal{U}[1, 1.5]$ ($\mathcal{U}$ being the uniform distribution), $E_\sigma \in \mathcal{U}[0, Q\delta_\sigma]$, $v_i \in \mathcal{U}[1, 2]$, $w_i \in \mathcal{U}[0, v_iQ]$, $n_\sigma(0) \in \mathcal{U}[0, 1]$, $c_i(0) \in \mathcal{U}[0, 1]$, $K_i \in \mathcal{U}[1, 5]$, $s_i \in \mathcal{U}[0, 5]$, $\alpha_{\sigma i}(0) : \sum_{i=1}^{p} w_i \alpha_{\sigma i}(0) = E_\sigma(0)$.
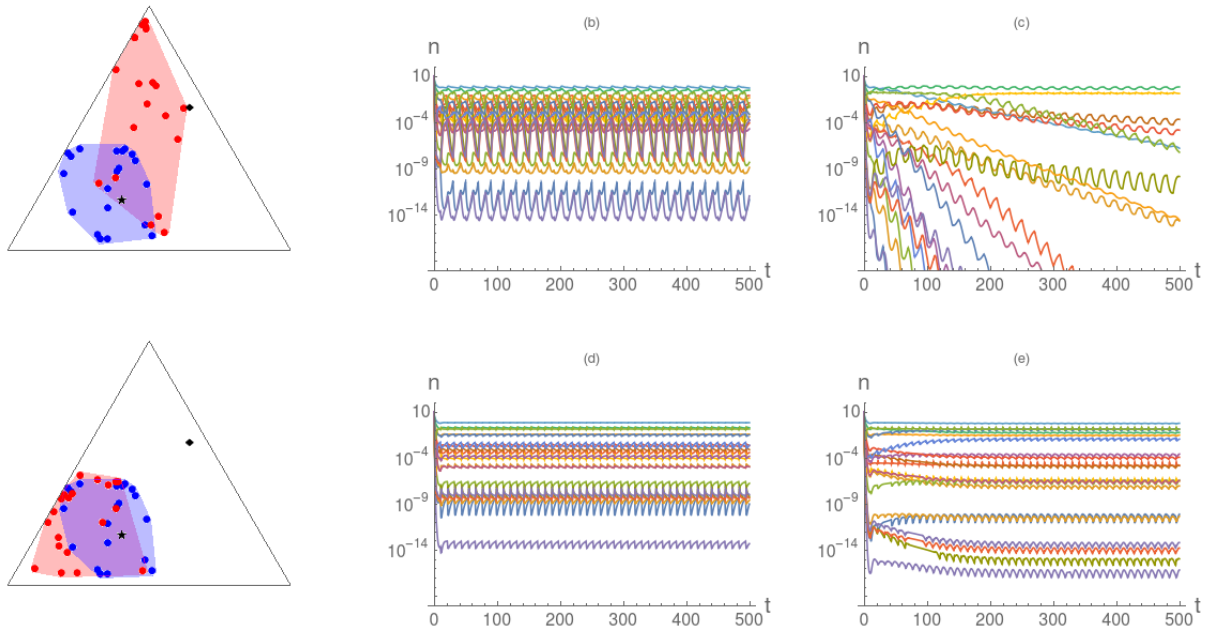

Figure 8: Simulation obtained with model equations (3), (4), (5) and (9) for the evolution of 20 population densities ($m = 20$) competing for 3 different resources ($p = 3$) during 500 time steps. $Q = 2$, $\mu = 0$; Top Left: Simplex plot with initial and final metabolic strategies (and respective convex-hulls) represented in blue and red respectively for $\tau_{in} = \tau_{out} = 10$; In a black star and diamond are represented respectively the supply rates that lie inside and outside the convex-hull of metabolic strategies; Bottom left: same as previous but with $\tau_{in} = 10$ and $\tau_{out} = 1$; (b): adaptative metabolic strategies, $\tau_{in} = \tau_{out} = 10$; (c): fixed metabolic strategies, $\tau_{in} = \tau_{out} = 10$; (d): adaptative metabolic strategies, $\tau_{in} = 10$ and $\tau_{out} = 1$; (e): fixed metabolic strategies, $\tau_{in} = 10$ and $\tau_{out} = 1$.

Of course, as species have a certain dynamic, so do their surroundings. In nature, resources are subject to large fluctuations over time, with many factors, for example the seasons. Pacciani-Mori et al. tested the robustness of an ecosystem subject to these fluctuations and the importance of having adaptative mechanisms to react to them. They considered variable supply rates with some periodicity where their values would lie inside the convex-hull of metabolic strategies (s_{in}) for a time τ_{in} and then change to another outside (s_{out}) for a time τ_{out}.

In the cases where the species had the ability to adapt to the fluctuations of nutrient supplies, using equation 9, we can see that they all survived (fig. 8 middle), only varying the population with the same periodicity as the supply rates did. However, when removing the adaptation system, by fixing the metabolic strategies in time, this behavior changes (fig. 8 right). If the supply rates lie outside the convex-hull of metabolic strategies for too long, the coexistence becomes compromised (fig. 8 top right). Species can only coexist as long as the time outside the convex-hull, τ_{out} is small compared to τ_{in} (fig. 8 bottom right). We can also observe that the metabolic strategies evolve in a way that they include the supply rates s_{out} when they spend enough time outside the convex-hull (fig. 8 top left), and that it does not happen when that time interval is small (fig. 8 bottom left).

This model was able to explain both the violation of the Competitive Exclusion Principle and the diauxic growth.

The populational models developed until now allow us to describe the growth rate of populations in terms of parameters but they lack an explanation for what mechanisms originate variations on the rate of growth. Also, they do not take into account the necessity of more than one nutrient for growth.

3   Thesis planning

To describe the growth, adaptation and survival of m species and p nutrients, Posfai et al. introduced a mathematical model based on a modified MacArthur growth model where the nutrient consumption is described by the classical Tessier-Monod sigmoidal growth function. In his PhD thesis, Monod calibrated the growth of the bacteria E. coil based on this approach. The Tessier-Monod function has been used in different modeling contexts (Monod [4], Dilão [14], Hwa [12]). However, a clear calibration in complex environments with different resources is lacking.

As it is well known, the fit of a model to some experimental data does not validate the model. The validation results from the consistency of the biological and physical assumptions of the model (internal consistency) and its ability to predict new phenomena. The numerical modeling work of Posfai et al., [16], predicted some important properties of multi-species and multi-nutrient communities, namely, the existence of metabolic strategies for species in a community, the possibility of coexistence if a convex envelop property is observed and the importance of key species for the community stability. All these hypothesis need a biological validation.

On the other hand, in the Tessier-Monod modeling approach, without considering death rates, the sigmoidal growth law does not allow for the explosion of the number of individuals of a single species in the presence of continuous supply of resources. This is an inconsistency of the Tessier-Monod model. In this context, the mass action approach is an alternative (Dilão, [14]) that should be compared with the Posfai et al. approach, [16]. Another issue that should be investigate is related with the absence of diauxies in the simulations of Posfai et al. model. To solve this problem, Pacciani-Mori et al. [17] introduced a dynamic metabolic strategy with the assumption that bacteria would maximize their growth rate. This mechanism should be analyzed in detail experimentally.

The goal of this project is to preform growth experiments in a community of two (or three) species, in a stirred reactor tank experiment, with at least two food sources. We want to model and calibrate the laws of population growth, and to analyze the possibility of sustained coexistence and the circumstances leading to competitive exclusion. Our approach will give precise answers to the right approach to simulate bacterial populations growth and precise calibration of model parameters.

From a more theoretical point of views, this will answer the questions of Hwa on the existence of first principles growth models.

Other questions we would like to answer are related with complementarity of food sources. If there are no complementary food sources, we would expect that a species with one nutrient would attain a steady state value, say n_1, and if there are two nutrients in the media, the steady state should be $n_2 > n_1$. Thus, we raise the question: do complementary nutrients for growth exist?

Our thesis calendar will be divided into five tasks:

1. Development of new mathematical models describing multi-species and multi-nutrient growth, in order to eliminate inconsistencies in the growth models — 1 month.

2. Development of new mathematical models to explain diauxies without a maximization principle but with dynamic metabolic strategies — 1 month.

3. Experimental planning to calibrate and validate the mathematical models in colonies of E. Coli — 1 month.

4. Laboratory experiments — 2 months.

5. Thesis writing — 1 month.

Tasks 3 and 4 will be done in the experimental facility of Isabel Gordo at Instituto Gulbenkian de Ciência.

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