# Survival tactics of a microscopic enemy

## Optimisation of bacterial persistence strategies in nutrient-restricted growth conditions

Master's Thesis

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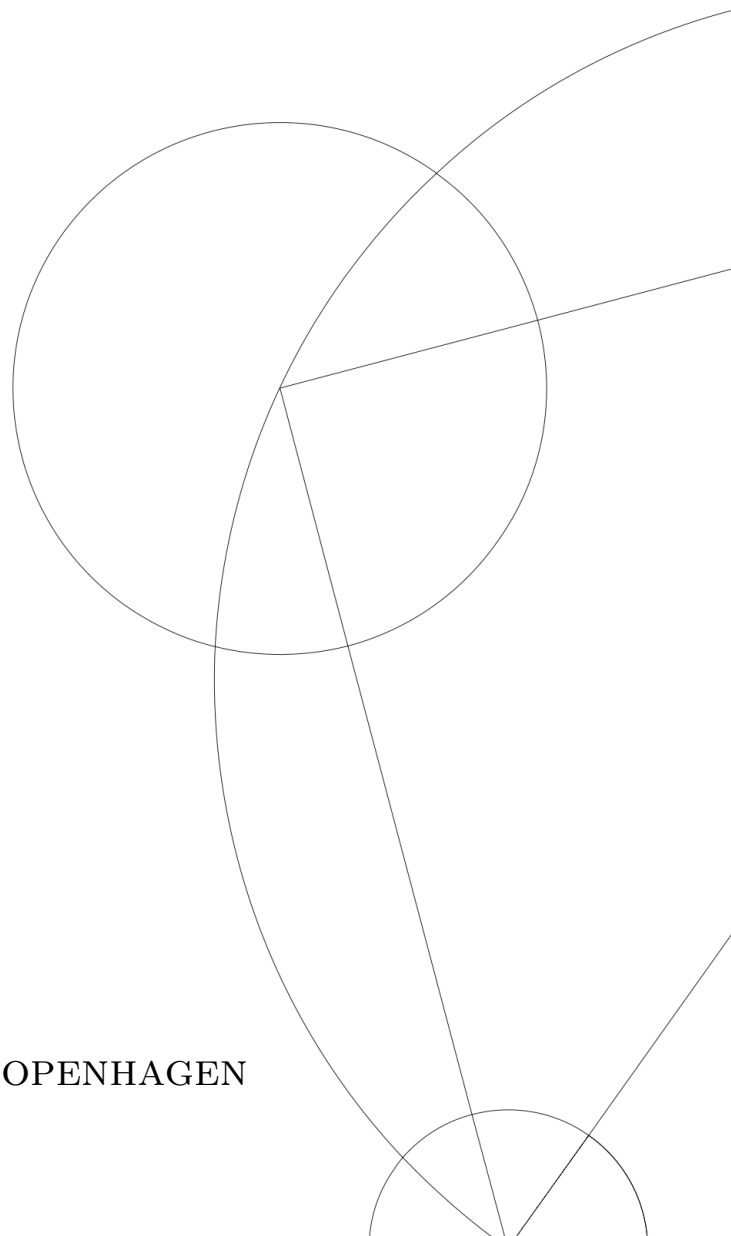

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# Abstract

Antibiotics are considered to be an indispensable part of modern medicine. Recent years have been marked by an increase in therapeutic failure of antibiotic treatments, primarily driven by a rise in antibiotic resistant bacteria. However, this is only one of several phenomena enabling bacterial survival of antibiotics. Antibiotic persistence, i.e. the ability of a subpopulation to survive a lethal dose of antibiotics, have gained more attention recently. While the molecular mechanisms behind persistence are not fully understood, it is known that dormant, non-growing bacteria are more tolerant to antibiotics. The dormancy can be induced by stress such as nutrient starvation, and the variation in the length of lag time from dormancy to growth is believed to be a part the origin of persistent subpopulations. It has also been observed that growing bacterium can spontaneously go into dormancy, which is commonly interpreted as a bet-hedging strategy.

Here, we are motivated by previous work that focused on the starvation-induced persistence under repeated feast-famine cycles, with stochastic applications antibiotics. We extend the work to treat limited amounts of nutrients explicitly, including also competing phenotypes. We first show that this change of setup does not affect the optimal lag time. Then we extend the model of starvation-induced persistence to include also spontaneous persistence, finding that spontaneous persistence can only be optimal when the application of antibiotics is delayed compared to the nutrients. Importantly, we do not consider extinctions explicitly, therefore bet-hedging is not a meaningful strategy here. We study region of antibiotic parameters where the optimal persistence strategy corresponds to a finite rate of spontaneous persistence, the transition to this region being discontinuous. These findings are supported by evolutionary simulations.

# Acknowledgements

First of all I would like to thank my supervisor Namiko for her patience and encouragement, and for providing some structure to my otherwise chaotic workflow. Being a part of the Biocomplexity group at the Niels Bohr Institute has been a pleasure, even though Corona made it a somewhat unusual experience. Thanks to Elias for helping me parallelise my code with "just three lines of code" - especially when I didn't want the help.

I would also like to thank all my family and friends back in Oslo for so staying close, despite living in separate cities for many years now. A special thanks to Guro and Rebekka for being my closest family in Copenhagen these last years. And finally, I would like to thank the Danish tax payers for financing my education. If the world was fair, the privilege of free education would be universal.

# Contents

# List of Figures

# Chapter 1

# Introduction

## 1.1 Motivation

For most people it is impossible to imagine a world without antibiotics. Since modern antibiotics was discovered in the early 20th century [1] it has become an indispensable part of modern society, with applications in medicine as well as in the agriculture sector [2]. The use of antibiotics has lowered the mortality rate from bacterial infections, such as tuberculosis and syphilis, but also from complications following surgeries and even childbirths [1, 3, 4]. However, our medical advantage is threatened by the rise of antibiotic resistant bacteria, and infections and medical procedures that are today relatively harmless are now increasing in mortality. The World Health Organisation states that antibiotic resistance is a "serious threat [that] is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country" [5]. It is estimated that as many as 700,000 people die every year from antibiotic resistance, and this number is expected to increase to 10 million by 2050 [6].

Nature is constantly evolving and mutation of antibiotic resistant bacteria is unavoidable. Antibiotic resistance has even been detected among bacteria in a cave that had been isolated for 4 million years [7]. However, the evolution of resistance is accelerated by overuse and misuse of antibiotics, a behaviour that is reminiscent of a "Tragedy of the commons" [8]. Though public focus is often on antibiotic resistance, this is only one of several phenomena linked to therapeutic failure of antibiotics. Among these are antibiotic persistence, which is the ability of a subset of a bacterial population to survive a lethal dose of antibiotics. In other words phenotypic tolerance of a subpopulation [9, 10]. The persistence phenomenon was originally discovered from its characteristic biphasic killing curve, in experiments that showed that populations of bacteria that were sensitive to antibiotics, were not completely eliminated by antibiotics [11, 12]. Though the mechanics underlying persistence are poorly understood, research indicates that the persistence is evolvable, and furthermore that this phenomenon might trigger the evolution of resistant bacteria [13, 14]. It is known that dormant bacteria

are more tolerant to stresses, such as antibiotics, than growing bacteria. Furthermore, experiments have revealed that distributions of single cell lag times have heavy tails [15, 16]. This phenotypic variation in lag time is believed to be related to the persistence on the population level. Persistence is known to occur as a reaction triggered by some stress, but has also been observed to happen spontaneously in growth friendly conditions [17]. Spontaneous persistence has been interpreted as a bet-hedging strategy in fluctuating environments, with the rate of spontaneous persistence believed to reflect the frequency of fluctuations [18, 19, 20].

Previously, an experiment by Fridman et al. [21] showed that when a bacterial population is subjected to repeated feast-famine cycles with antibiotics applied along with the nutrients, the lag time can evovle to reflect the duration of the antibiotic application Motivated by this experiment, a theoretical study was conducted by Y. Himeoka and N. Mitarai, optimising the wake-up strategy from starvation-induced triggered persistence, for repeated feast-famine cycles with stochastic application of antibiotics [22]. One of their finding is that even simple models of antibiotic persistence display a discontinuity in the optimal lag time, suggesting that evolution of more tolerant bacteria can be avoided with strategic use of antibiotics. Importantly, their work relies on the assumption of the bacteria having infinite access to nutrients, an assumption that is highly unphysical. In the wild, the nutrients will not only be restricted by physical limitations, but there will also be competition from other bacteria for the nutrients. It is therefore not obvious how the optimal lag time will change in such a setup, as there will be a trade-off between waking up early to gain a competitive advantage, and waking up late in order to avoid the antibiotics.

## 1.2 Research Questions

With this in mind we raise the following questions

Q1. How does competition and limitations on nutrients affect the previously computed optimal strategies of triggered persistence?

Q2. For which antibiotic parameters can spontaneous persistence be beneficial, and what is the relation between optimal lag time and optimal rate of spontaneous persistence?

## 1.3 Thesis outline

In **chapter 2** we begin this project with a brief introduction to the biological background necessary to understand the project. This includes a simplistic explanation

[1]Reproduced from https://apps.who.int/mediacentre/events/2015/world-antibiotic-awareness-week/posters/en/index.html

[2]Reproduced from https://www.antibiotikaellerej.dk/hent-materialer

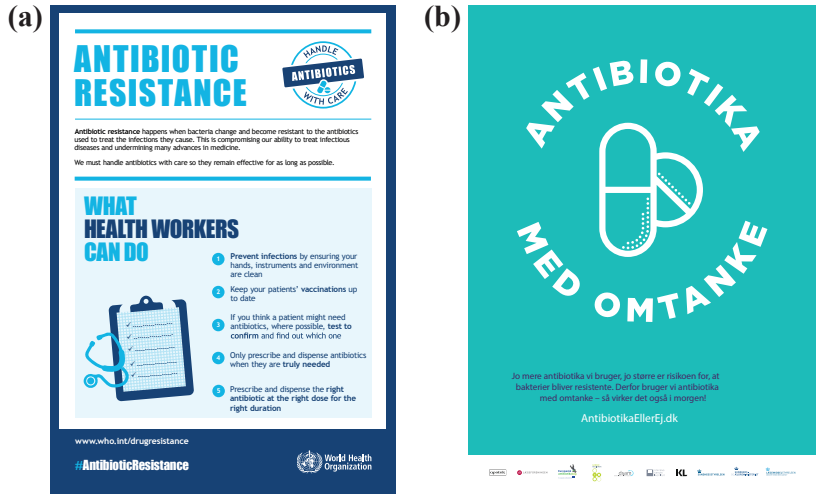


Figure 1.1: ***Example of posters spreading awareness on antibiotic resistance.*** ***a)*** *Material from World Antibiotic Awareness Week 2015* [1]. ***b)*** *Material from "Antibiotika eller ej"* [2].

of the phenomena of persistence, though this phenomena is rather complex and not well-defined.

In **chapter 3** we provide a brief summary of previous the experimental and theoretical works that serve as motivation and inspiration for this project.

In **chapter 4** we then move on to explain the setup that we will use in the rest of this project. This setup being strongly inspired by the before mentioned works. We also provide a summary of assumptions and limitations.

In **chapter 5** we aim to answer the first research question, and we extend the previous work by Y. Himeoka and N. Mitarai on starvation-induced triggered persistence to a model with phenotypic competition for a fixed amount of nutrients. This part is mostly analytical, though the results are supported by numerical results.

In **chapter 6** we extend a model with triggered persistence to include also spontaneous persistence, as we aim to answer the second research question. In this part we rely of simulations to obtain the results.

Lastly, in **chapter 7**, we sum up the thesis and provide a further outlook.

# Chapter 2

# Biological background

## 2.1 Introduction to bacteria

For the most part, this work will be dealing with population dynamics of bacteria, hence extensive knowledge of the cellular components or the mechanics behind these dynamics are not necessary. Even so, it can be useful with a basic introduction to the biological protagonists of this work, for readers unfamiliar with the field.

Bacteria are believed to be the first living organism on Earth. Today they are found in virtually every environment on Earth [23]. The total population of bacteria is estimated to around $10^{30}$ cells, distributed among $10^4 - 10^9$ different species [24, 25]. Only a fraction of these are pathogenic [26]. With so large numbers, bacteria are believed to make up the largest biological domain. Bacteria are primarily single cell prokaryotic microorganisms[1]. Despite their apparent simple structure, the world of bacteria exhibits vast complexity, such as communication [27], or memory [28].

The length of one cell is typically $1 - 5$ µm, though they primarily live in larger communities. In growth friendly conditions, a population of bacteria will usually reach a density of $\sim 10^9$ cells/ml [29]. Bacterial populations are heterogeneous, in that they can contain several phenotypes[2], but also genetic variation [30, 31]. This diversity manifests itself e.g. in distributed growth rates, but also as a part of a n effective survival strategy. We will return to this last point in the section about persistence.

### 2.1.1 Population dynamics

Bacteria reproduce by binary fission, where a mother cell divides into two identical daughter cells [32]. This amounts to exponential population growth, with a growth rate dependent on the doubling time and survival rate of the daughter cells. The growth rate ranges from $\sim 2$ h$^{-1}$ and below, depending on how growth friendly the

[1] Organisms without membrane-enclosed nucleus. Prokaryotes are the smallest life form that can exist individually.

[2] Set of observable traits. Formed by both genes and environment.

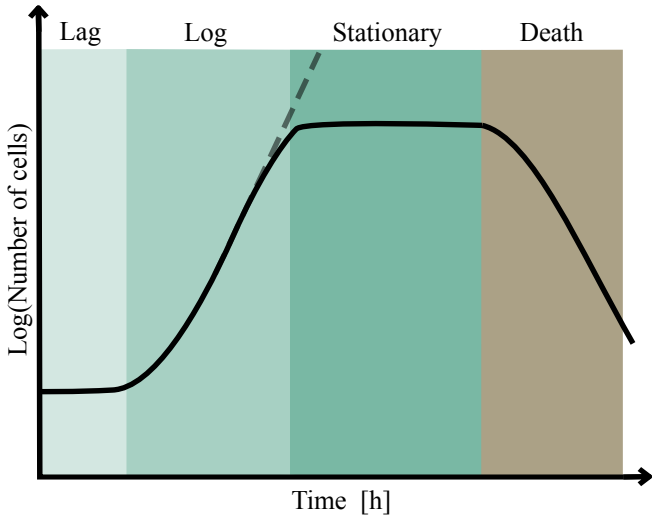


Figure 2.1: ***The four phases of bacterial growth.*** *This work focuses exclusively on the three first phases. The dashed grey line demonstrates theoretical growth under ideal conditions.*

conditions are. Under ideal conditions a bacterial population can therefore reach the mass of Earth within a few days [33]. In a realistic environment bacterial growth is constrained by physical limitations, such as restricted nutrients and space. Bacteria cannot grow in absence of nutrients, and they will often enter a more energy-efficient state of dormancy while waiting for the nutrients to be replenished. Fig. 2.1 illustrates the life cycle of a bacterial population, constituting of the four phases described below:

1. **Lag phase.** When a bacterial population is exposed to new conditions, the bacteria will initially enter some "adaption phase" where the population prepares to grow. Though the population is not growing significantly, this is not a dormant phase but rather the "wake-up" phase from dormancy. The length of the lag phase is highly varying, from $\sim 1$ hour up to several days [34]. Generally, the lag time depends on how much the new conditions differ from the original, in addition to the state of the bacteria just before the change of environment. Research have shown the lag time to be both adaptive and evolvable [35]. In Fig. 2.1 this corresponds to the leftmost constant plateau.

2. **Exponential phase.** When the majority of cells have woken up, the population enters the growth phase (also called log phase). The population grows exponentially, i.e. linearly on the logarithmic axis in fig. 2.1. This is where the binary fission takes place, but also the release of toxins that are the origin of the pathogenic nature of certain species. This phase lasts as long as the amount of nutrients allow, or until some stress prevents the bacteria from growing.

3. **Stationary phase.** As the nutrients are depleted, or a significant amount of inhibitors are formed, the population enters the stationary phase. This phase is characterised by equal growth and death rates, resulting in constant population.

In Fig. 2.1 this corresponds to the second plateau, just before the population starts decreasing.

4. **Death phase.** At some point the death rate becomes larger than the growth rate, and the population starts declining. This will happen when the nutrients run out, or it can be caused by other stressors. In Fig. 2.1 this corresponds to the rightmost part of decreasing slope.

### 2.1.2 Bacteria under stress

In addition to depletion of nutrients, there are several other stresses and potential treats that make life tough for bacteria. Among these are naturally occurring environmental variations such as temperature change or drought, but also deliberately applied stresses such as antibiotics. If the stress is mild, bacteria have several response mechanisms while staying in the growth state [36]. More severe stresses might however be lethal for a growing bacterium, because they target growth processes. In contrast, the dormant state is more robust. It can therefore be beneficial for a population to have some fraction of species remain dormant, even in conditions are seemingly growth friendly.

### 2.1.3 Antibiotic stress

Since its discovery, antibiotics have been our most important weapon against bacterial infections. Generally, antibiotics are divided into two groups depending on how they act on the bacterium: *bacteriostatic* and *bactericidal* antibiotics. Bacteriostatic antibiotics inhibit the bacterial growth, relying on the immune system to ultimately kill the bacteria. Bactericidal antibiotics are antibiotics that also kill the bacteria, yet this distinction is not exact [37]. In the following we will focus exclusively on bactericidal antibiotics, since persistence is only defined for the latter [10]. The rate at which antibiotics kill bacteria is found to be in the same range as the bacterial growth rate [38, 39].

When antibiotics fail to eliminate an infection, it is often related to one of three phenomena: antibiotic *resistance*, *tolerance*, or *persistence*. Antibiotic resistant bacteria are bacteria that do not only survive applications of antibiotics, but can even grow in their presence. Antibiotic tolerant bacteria are susceptible to antibiotics, but are killed at a lower rate. It will therefore take a higher or longer dose of antibiotics to kill a tolerant population, compared to a normally sensitive population. Lastly, antibiotic persistent bacteria are bacteria where a subpopulation has higher tolerance than the rest. We will take a closer look at persistence in the next section.

## 2.2 Persistence

As just mentioned, persistence is phenotypic tolerance of a subpopulation. Importantly, persistence is not a genetic trait, but an emergent behaviour on population-level. Per-

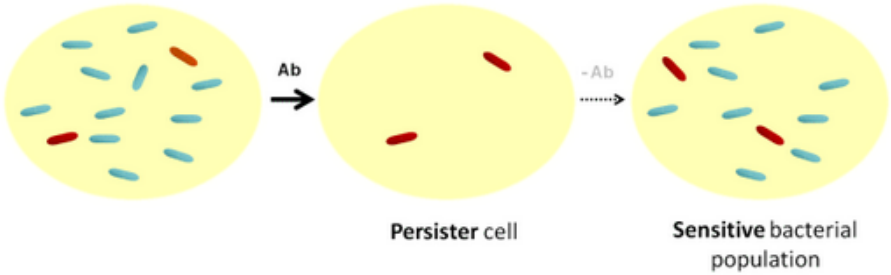


Figure 2.2: ***Bacterial population with persister cells, before, during and after antibiotics.*** *The blue cells represent the normally sensitive cells, and the red denote the persistent subpopulation. Reproduced from [10].*

sistence is not a well-defined phenomena. The mechanics behind are poorly understood, and some of the characteristics are vague or might even appear contradictory [40]. N. Balaban et al. define a persister cell as being a tolerant cell from a population displaying antibiotic persistence [10]. Notably, a population that is regrown from presister cells will be identical to the original population. This is exemplified in fig. 2.2, where a lethal dose of antibiotics is added to a population of bacteria containing some persisters. The persister cells are the only ones to survive, and thus resume growth after the antibiotics are removed. Instead of growing a population purely of persister cells, the new population will contain a fraction of presisters corresponding to that of the original population [10].

Though it is unknown whether the persister phenotype is encoded in the genes of the individual cell or not, it is at least somewhat (epi)genetic on the population level. It has for instants been shown that populations can evolve to change their fraction of persisters [13]. The tolerance of the persister cells might have different origins, such as prolonged lag time, or dormancy.

### Role in therapeutic failure of antibiotics

Persistence was originally discovered from the biphasic killing curve characteristic of populations with persister cells. This is illustrated in fig. 2.3. A population of sensitive (blue) and persister (red) cells are exposed to antibiotics. The gradient line highlights the killing curve of the total population. The sensitive cells are the first to be killed at a high rate. As time goes by there will be fewer and fewer sensitive cells left, therefore the killing rate slows down. Eventually, there are only persisters left in the population, and the killing rate corresponds to that of the persisters. Note that fig. 2.3 shows a simplified curve, and that a population might contain several persister phenotypes resulting in a more complex killing curve.

Fig. 2.3 also illustrates why even antibiotic treatment of sensitive populations might fail. Since the killing rate of persisters is much slower than the population average, there will often be a few persisters left after the antibiotic treatment. In other words,

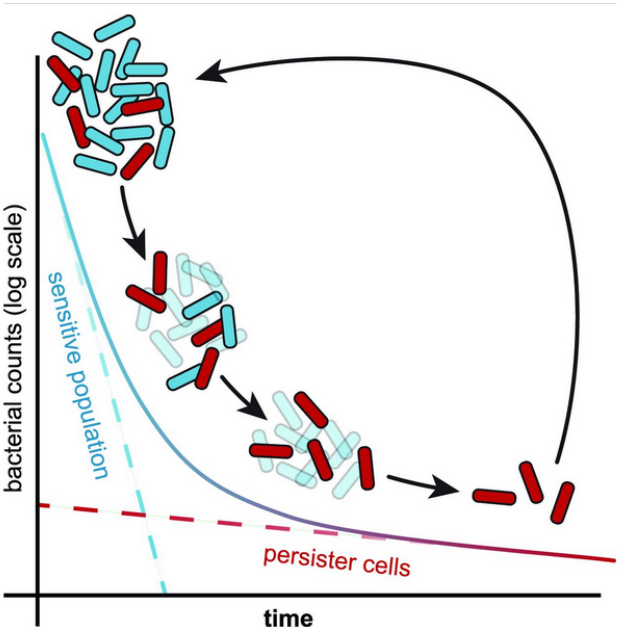


Figure 2.3: ***The biphasic killing curve that is characteristic for a population with persister cells.*** *Antibiotics are applied to a population containing a fraction of persister cells. The blue cells represent the normally sensitive cells, and the red cells denote the persistent subpopulation. Initially the killing curve is dominated by the killing of sensitive cells, but as the population is depleted of sensitive cells the killing rate slows down until there are only persisters left and the killing curve becomes identical to that of the persisters. The dashed lines represent the killing curves of the two phenotypes. Reproduced from [41].*

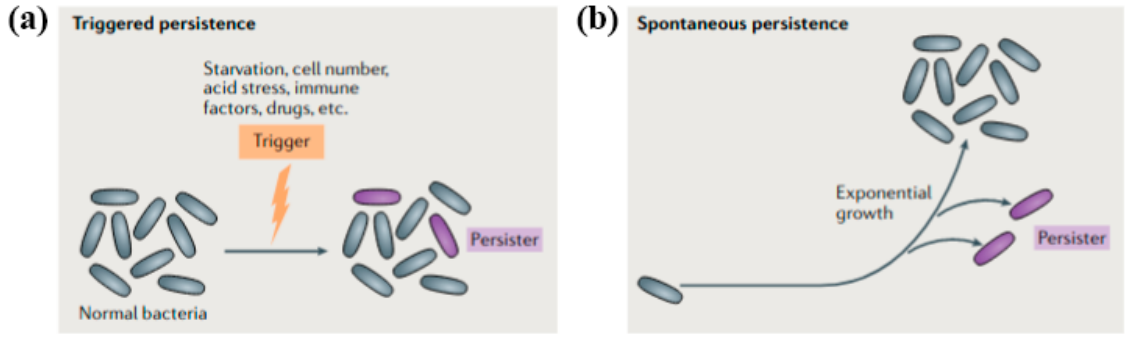


Figure 2.4: ***The two types of persistence.*** *Reproduced from [10]* ***a)*** *Triggered persistence.* ***b)*** *Spontaneous persistence.*

antibiotic persistence contributes to overuse of antibiotics, because persistent infections will often not be cleared at first attempt. Furthermore, research also indicates that antibiotic persistence increases the mutation rate of antibiotic resistant bacteria [42].

### 2.2.1 Types of persistence

We finish of this chapter with an explanation of the two different types of persistence: *triggered* and *spontaneous* persistence. The two types are illustrated in fig. 2.4. Triggered persistence is caused by stress that triggers a fraction of the population to enter the more tolerant state of persistence. Nevertheless, triggered persistors might stay in this phenotype even long after the initial stress is gone. This type makes up most of the experimentally observed persisters, and a typical trigger is starvation.

Spontaneous persistence happens spontaneously during bacterial growth, in absence of stress. It has been interpreted as a survival strategy in fluctuating environments, analogue to that of bet-hedging in gambling [17, 20, 19]. Sensing the surrounding environment is energy consuming and inefficient, however failure to adapt to potential stresses might be lethal. In order to maximise longtime growth in an environment that can contain lethal stresses, it can therefore be advantageous to only have a fraction of the total population grow (gamble). In cases where the growing population dies from the stress, the population as a whole will have another shot at the game of "proliferation or extinction". The rate of spontaneously switching to the persistent state and back is believed to be very low, and is believed to be constant throughout the exponential phase [10]. For *E.coli* it is in the range $10^{-6} - 10^{-3}$ [17, 43, 18].

# Chapter 3

# Previous work on optimal lag time

## 3.1 Experimental evolution of lag time under antibiotics application

This project is heavily inspired by the work of Y. Himeoka and N. Mitarai [22], which was in turn motivated by experimental work by Fridman et al. [21]. In the latter, bacterial populations that were subjected to repeated applications of antibiotics were shown to evolve longer lag times, matching the application length of the antibiotics. The procedure of the experiment is illustrated in fig. 3.1. A lethal dose of antibiotics were added along with nutrients to the bacteria. After a fixed time $T$, the antibiotics were washed out and the cells that had survived were left to grow overnight. The population, now in the stationary phase, was then diluted before the cycle was repeated. After several repetitions of this procedure, the average lag time $\lambda$ was found to be approximately equal to the duration of the antibiotics $T$. In other words, the bacteria had evolved to become tolerant to the antibiotics.

In the experimental setup just described we consider $\langle\lambda\rangle = T$ [1] to be the optimal lag time. In contrast, the optimal lag time in an environment without any stresses

[1] Here, $\langle\cdot\rangle$ denotes the population average

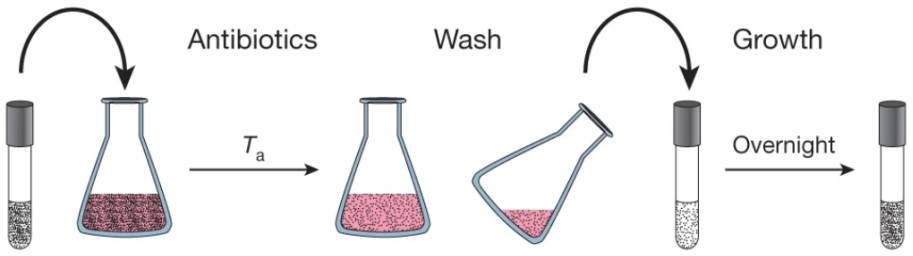


Figure 3.1: ***Illustration of experiment design.*** *Reproduced from [21]*

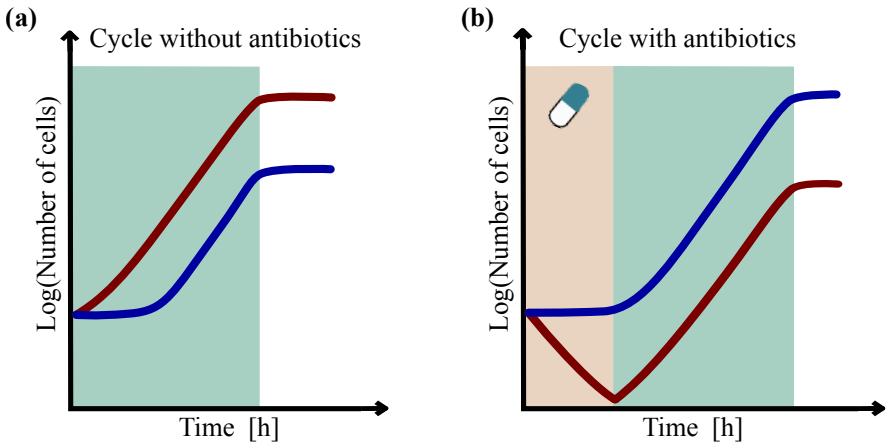


Figure 3.2: ***Illustration of advantage and disadvantage of a long lag time.*** *The light red lines represent growth of a species with short lag time, and the blue lines represent a species with lag time similar to the duration of the antibiotics. The green background highlights the period of nutrients, and the red background highlights the period of antibiotics.* ***a)*** *In absence of antibiotics the red species has an advantage, and is able to consume tha majority of the nutrients.* ***b)*** *In presence of antibiotics the blue species has an advantage, as it is shielded from antibiotic killings.*

would be $\langle\lambda\rangle = 0$. The two cases corresponding to growth in an environment with and without antibiotics are illustrated in fig. 3.2. In wild stresses are not as predictable as in the experiment above. Furthermore, persistence, specifically spontaneous persistence, is considered to be a survival strategy in *fluctuating* environments. It is therefore meaningful to study the optimal lag time strategy in a setup with stochastic application of antibiotics. That is a setup analogue to that of Fridman et al. [21], but where we add antibiotics are during a cycle only with a given probability $p$. In this case, the optimal lag time will reflect the trade-off between advantage and disadvantage of a waking up before the antibiotics are removed, as is illustrated in fig. 3.2.

## 3.2 Theoretical work by Y. Himeoka and N. Mitarai

The effect of stochastic antibiotics on the optimal lag time has first been investigated theoretically by Y. Himeoka and N. Mitarai [22]. Considering the dormant state to be an example of starvation-induced persistence, they studied the optimal lag time in order to maximise growth under stochastic antibiotics. In order to simplify the calculations, they assumed that bacterial growth in each round happens with "unlimited nutrients", and optimised the lag time of a single species to obtain the largest net growth. This corresponds to comparing the net growth at a fixed time very long after addition of the nutrient, in the system of unlimited nutrients. Here, we will briefly go through the results that are relevant for our work.

### Model with delta distributed lag time

In the supplement the authors study the optimal lag time for a population with a delta distributed lag time [2], that is a population where every cell has the same lag time. They find that the optimal lag time, $\lambda^*$ displays a discontinuous transition when the probability of the antibiotics increases above a threshold determined by the killing rate

$$\lambda_I^* = \begin{cases} 0 & \text{if } \ p < (\gamma+1)^{-1}, \\ T & \text{if } \ p \geq (\gamma+1)^{-1}. \end{cases} \tag{3.1}$$

$p$ and $T$ denote the probability and duration of the antibiotics, as described in the previous section. $\gamma$ denotes the killing rate of the antibiotics, that is the death rate of a population subjected to antibiotics.

### Model with exponentially distributed lag time

The starting point of the paper is a model where lag time is exponentially distributed within the population, meaning the dormant cells wake up with a constant rate. They define a fitness measure corresponding to the long time average effective growth of the population, which reads

$$\begin{aligned} F_I(\lambda;\gamma,p,T) = (1-p)\ln\left[\frac{1}{1+\lambda_i}\right] \\ + p\left(-T + \ln\left[\frac{e^{-T/\lambda}-e^{-\gamma T}}{\gamma\lambda - 1} + \frac{e^{-T/\lambda}}{1+\lambda}\right]\right) \end{aligned} \tag{3.2}$$

in the case of exponentially distributed lag times. From maximising $F_I$ with respect to $\lambda$, they find that the optimal lag time undergoes a discontinuous transition also for this model. The discontinuity occurs at some critical probability $p_c$, dependent on the other antibiotic parameters $\gamma$ and $T$

$$\lambda_I^* \approx \begin{cases} 0 & \text{if } \ p < p_c(\gamma,T), \\ pT & \text{if } \ p \geq p_c(\gamma,T). \end{cases} \tag{3.3}$$

### General model

The authors also propose a generalised set up for studying the optimal lag time of a model with any distribution of lag time $r(\lambda)$, and any distribution of antibiotic duration

[2] This could also be considered as antibiotic tolerance, since the entire population is in starvation-induced dormancy with the same lag time.

$q(T)$. The fitness function in this generalised setup reads [3]

$$F[r,q](\gamma,p) = (1-p)\ln\left[\int_0^\infty e^{-\lambda} r(\lambda)\ d\lambda\right] + p\int_0^\infty q(T)\ln\left[\left(e^{-(1+\gamma)T}\int_0^T e^{\gamma\lambda} r(\lambda)\ d\lambda + \int_T^\infty e^{-\lambda} r(\lambda)\ d\lambda\right)\right] dT. \tag{3.4}$$

**Motivation for the current project**

As mentioned before, these results assume that the bacteria have access to unlimited nutrients. A more realistic scenario would be a model with limited nutrients. More specifically, in the experiment in [21], the same media (nutrients) was added in each round and the length of each round was long enough for the population to reach the stationary phase. This means that the amount of available nutrient was in fact limited. In such a case, if there is only one single kind of bacteria, there is not much penalty to have a long lag time. Since if no one is growing, the nutrient will be left unused. It is therefore the competition between phenotypes with different lag times, that drives the selection. In this case, a long lag time would not only amount to a loss of growth in cycles without antibiotics, but also a competitive disadvantage against a species of shorter lag time.

The authors themselves also mention spontaneous persistence as an obvious extension of their work. Specifically, in the case where antibiotics are added later to the bacteria, such that the bacteria can potentially benefit from entering the exponential phase before the antibiotics are applied. Even though triggered persistence is believed to be much more common than spontaneous persistence, it might also be more predictable and easier preventable. If we want to work towards complete elimination of bacterial infections by antibiotics, it is therefore crucial to gain better understanding of also spontaneous persistence. With the endeavour to shred some light on these additional features, we are now ready to propose the setup for our work.

[3] $r(\lambda)$ is normalised, such that $r(\lambda)\ d\lambda$ gives the probability that a cell wakes up between $t = \lambda$ and $t = \lambda + d\lambda$. $q(T)\ dT$ is the concentration of the antibiotics with duration between $T$ and $T + dT$.

# Chapter 4

# Setup of the project

In this chapter we present the setup we will use throughout this project. In general the setup is the same as in [22], with the extension of limited nutrients and one competitor population. Our theoretical setup imitates the following experimental procedure:

- $\boldsymbol{t = 0}$: Two species are inoculated in a fresh medium containing a fixed amount of nutrients, $S_0$. We set $S_0 = 10^9$ cells/ml in all simulations, corresponding to the upper limit on bacterial population density [29].
- $\boldsymbol{t = T_0}$***, with probability*** $\boldsymbol{p}$: Antibiotics are added to the medium, with a fixed probability $p$. We allow antibiotics to be applied after the bacteria are inoculated. Initially we set $T_0 = 0$, such that addition of nutrients and antibiotics are synchronised.
- $\boldsymbol{t = T}$***, with probability*** $\boldsymbol{p}$: Antibiotics are removed from the bacteria. The two species are left to consume the rest of the nutrients, and harvested once they reach the stationary state. Since the twp species share nutrients, they will stop growing simultaneously.
- $\boldsymbol{t \geq T_S}$: All of the nutrients have been consumed, and the populations enter the stationary phase. Both populations are harvested and diluted by a fixed dilution fraction, $f$. We set $f = 10^{-6}$ in all simulations.

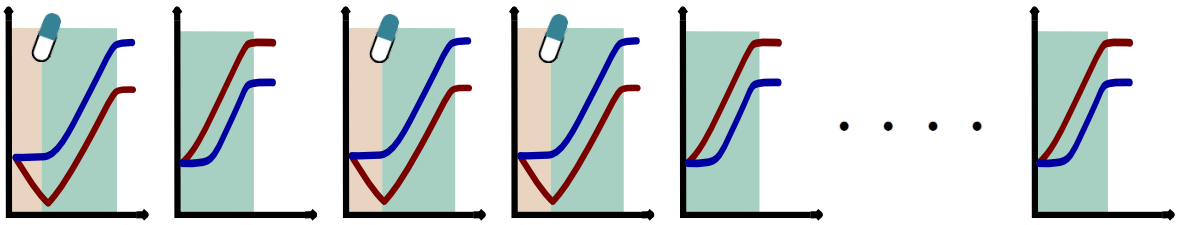

Figure 4.1: ***Illustration of a series feast-famine cycles with stochastic application of antibiotics.*** *Note that this is just an illustration, as the initial densities of each cycle depend on the final densities of the previous cycle.*

We repeat this process infinitely many times, such that we perform an infinite series of feast-famine[1] cycles with stochastic application of antibiotics. This is illustrated in fig. 4.1 for one species with lag time $\lambda \approx 0$ competing against a species with $\lambda \approx T$. Note that fig. 4.1 is only illustrative, as the initial density of each cycle is proportional to the final density of the previous cycle. We also use this setup when we consider spontaneous persistence, to which we will return in chapter 6.

## 4.1 Assumptions and limitations

- **Spatially homogeneous.** We consider an idealised setup that is spatially uniform, meaning we do not consider spacial variations or limitations other than that the one imposed by a fixed $S_0$.

- **Phenotypic competition.** We simplify our model by let competing species vary only in parameters related to persistence, keeping all other bacterial parameters equal for all species. This corresponds to letting different persister phenotypes competing, picking the phenotype with the best persistence strategy. We consider this to be a meaningful simplification, as this project does not concern an overall growth strategy, but is a study of the optimal persistence strategy independently of other bacterial dynamics.

- **Simplified growth dynamics.** In order to simplify analytical calculations we consider a simplified dynamics with sharp transitions between the phases of the growth cycle (see fig. 2.1). This means that the cells start exponentially growing immediately after waking up. Likewise, the transition from exponential growth to the stationary phase happens abruptly. In a more realistic model, the growth rate of the bacteria would depend on the nutrients according to the Monod equation [44]

$$\beta(t) = \beta_{max} \cdot \frac{S(t)}{K + S(t)}. \tag{4.1}$$

  Here, $\beta$ is the growth rate, and $K$ is some constant that regulates its dependency on $S$. In our model we use a special case of eq. 4.1 with $K = 0$, which results in a constant growth rate. We consider this assumption to be reasonable, since a decreasing growth rate according to the Monod equation with our setup will affect both species equivalently.

- **Persistence as dormancy.** We model the lag phase to be equivalent to dormant phase, that is a phase characterised by the absence of events other than the transition to the exponential phase. This reflects the fact that we only consider dynamics on the population level, and do not take the molecular processes in the

[1] Nutrition rich period of growth followed by depletion of nutrients and starvation.

lag phase into account. Persisters are also modelled as dormant cells, meaning they are fully tolerant to antibiotics, though they in experiments suggest that they are rather killed at a slower rate. This further implies that persisters do not grow at all, though this aspect of persisters is not well-defined, in fact they might actually be growing with a very slow growth rate [17]. This also means that we do not distinguish triggered and spontaneous persisters. We will return to this point in the end of chapter 6.

- **Constant antibiotics.** Unless otherwise stated, we consider the effect of antibiotics to be constant during the entire presence of antibiotics. Specifically, the effect of the antibiotics does not decrease with time and we assume the antibiotics to take effect immediately. Physically, this corresponds to applying a high dose of time-dependent antibiotics, and wash out the antibiotics before the concentration drops below the minimal inhibitory concentration. Furthermore, we assume that the antibiotics arrive at exactly the same time $T_0$ in every round. Letting the application time follow a normal distribution seems more realistic, specifically for medical purposes.

### Notes on notation

To avoid any confusion, we use uppercase $*$ to symbolise the optimal persistence strategy, such that $\lambda^*$ denotes the optimal lag time in a given model. Furthermore, we distinguish quantities in a setup with limited nutrients and competition from the original setup of a single species with lowercase $I$ and $I\!I$. This means $F_I$ and $\lambda_I^*$ denote the fitness and optimal lag time in a single species model, respectively. Likewise, $F_{I\!I}$ and $\lambda_{I\!I}^*$ denote the fitness and optimal lag time of a model with competition for nutrients. In cases where the distinction is not relevant, we use $\lambda^*$.

# Chapter 5

# Models of triggered persistence

In this chapter we will revisit the results from section 3.1, extending them to the case of competition for limited nutrients.

## 5.1 Model with delta distributed lag time

We begin with the simplest case, namely the case with delta distributed lag time. In this case the entire population wake up simultaneously[1]. In absence of antibiotics the growth of a population $n_i$ during one feast-famine cycle is given

$$\frac{d}{dt}n_i(t) = \begin{cases} 0 & \text{if } t < \lambda_i, \\ \beta_i n_i(t) & \text{if } t > \lambda_i \text{ and } S(t) > 0. \end{cases} \tag{5.1}$$

Here, $t$ is the time since the nutrients was added to the system. $S(t)$ is the nutrients at time $t$, with $S(0) = S_0$. $\lambda_i$ denotes the lag time of species $i$, such that the first line in eq. 5.1 describes the lag phase of species $i$. The second line describes the log phase where $\beta_i$ is the growth rate of species $i$. For simplicity, we set $\beta_i$ to 1 for all species. With probability $p$ antibiotics is applied along with the nutrients. In this case the differential equations read

$$\frac{d}{dt}n_i(t) = \begin{cases} 0 & \text{if } t < \lambda_i, \\ -\gamma_i n_i(t) & \text{if } \lambda_i < t < T, \\ \beta_i n_i(t) & \text{if } t > \max\{\lambda_i, T\} \text{ and } S(t) > 0. \end{cases} \tag{5.2}$$

The first and last lines are the same as before. The second line describes the killing (death) phase induced by the presence of antibiotics. $\gamma_i$ denotes the rate at which the antibiotics kill population $i$. For simplicity we set this to be the same for all species, i.e. $\gamma_i = \gamma$. $T$ is the time that the antibiotics are removed, such that we reobtain eq. 5.1 by setting $T$ to 0. Depending on whether antibiotics are present or not, the

[1] Again, this is actually antibiotic tolerance.

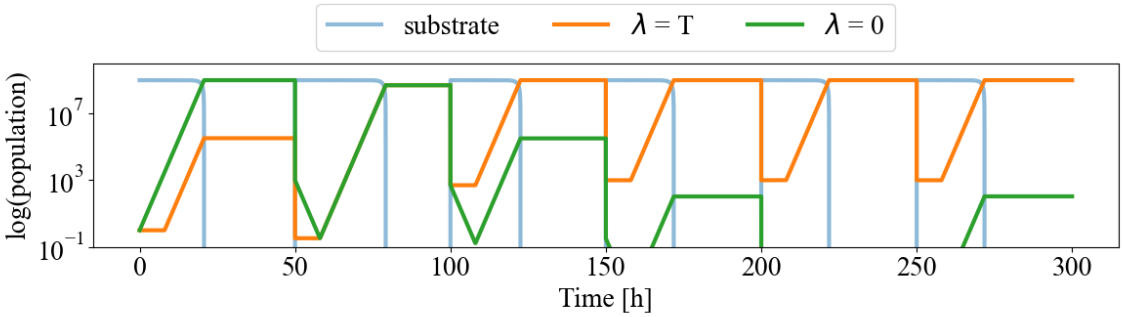


Figure 5.1: ***Example of 6 feast-famine cycles with delta distributed lag time.*** *The probability and duration of antibiotics are $p = 0.6$ and $T = 8$, respectively. The orange line represent a population waking up at $t = T$, and the green line represents a population waking up at $t = 0$. The light blue line represent the nutrient that are left in the medium at a time $t$.*

growth phase of species $i$ lasts from $\max\{T, \lambda_i\}$ until $S(t) = 0$. That is until the entire nutrient has been consumed, which is governed by the following equation

$$\frac{d}{dt}S(t) = \begin{cases} 0 & \text{if } t < \max\{\lambda_i, T\}, \\ -\sum_k \dot{n}_k(t) & \text{if } t > \max\{\lambda_i, T\}. \end{cases} \tag{5.3}$$

Again, we obtain the case without antibiotics by setting $T = 0$. An example of a system with two species governed by eqs. 5.1–5.3 can be seen in fig. 5.1.

With this setup, competition only indirectly affects the growth of species $i$. This can be seen in eqs. 5.1-5.2, where there is no explicit dependence on other species. The competing species interact through eq. 5.3, which in turn determines the length of the growth phase of all species. In this sense, competition is equivalent to setting a dynamic upper limit on $t$. In the following we denote the time that all of the nutrient is consumed as $T_S$. Note that $T_S$ is actually a function of all variables and parameters in the system, which we omit for readability.

We solve eqs. 5.1-5.2 and obtain the bacterial populations as functions of $t$

$$n_i(t) = \begin{cases} n_i(0) & \text{if } t < \lambda_i, \\ n_i(0) \cdot e^{-\gamma(t-\lambda_i)} & \text{if } \lambda_i < t < T, \\ n_i(0) \cdot e^{-\gamma(T-\lambda_i)\cdot\theta(T-\lambda_i)} e^{t-\max\{\lambda_i,T\}} & \text{if } t > \max\{\lambda_i, T\}. \end{cases} \tag{5.4}$$

$\theta(T - \lambda_i)$ is here the step function of $(T - \lambda_i)$, such that $e^{-\gamma(T-\lambda_i)\cdot\theta(T-\lambda_i)} = 1$ if $\lambda_i \geq T$ and the population wakes up after the antibiotics are removed. As before $T = 0$ corresponds to the case without antibiotics, for which $e^{-\gamma(T-\lambda_i)\cdot\theta(T-\lambda_i)} = 1$ for all $\lambda_i$.

We want to find the optimal survival strategy for species $i$. In presence of other species this corresponds to identifying the winning species, that is the species with the highest effective growth given the upper limit on $t$. The effective growth of one feast-famine cycle is $\beta_{eff} = \log\left[\frac{n_i(T_S)}{n_i(0)}\right]$, where $n_i(T_S)$ is the population of species $i$ as

it enters the stationary phase. We define the "fitness" $F_{I\!I}$ of species $i$ to be the average of the effective growth over the probability of antibiotics application $p$

$$F_{I\!I}(\lambda_i;\gamma,p,T) = (1-p)\cdot\log\left[\frac{n_i(T_{S,T=0})}{n_i(0)}\right]_{T=0} + p\cdot\log\left[\frac{n_i(T_{S,T>0})}{n_i(0)}\right]_{T>0}. \tag{5.5}$$

The lowercase $I\!I$ refers to the competition, indicating that this is quantity is conceptually different from the single species fitness in [22]. $n_i(t)$ is of course also a function of $\lambda_i$, $\gamma$ or $T$, though this dependence is not written out explicitly in eq. 5.5, The species with the highest effective growth rate will not always be the largest population after just a few feast-famine cycles. Only in the ideal case of infinite cycles will the species with the highest effective growth rate be certain to win. $F_{I\!I}$ is therefore the long term fitness of a species limited nutrients. We insert $n_i(T_S)$ from eq. 5.4 for the case with and without antibiotics, and rearrange

$$F_{I\!I}(\lambda_i;\gamma,p,T) = (1-p)\ T_{S,T=0} - \lambda_i - p(\gamma+1)(T-\lambda_i)\theta(T-\lambda_i) + pT_{S,T>0}.$$

As previously stated, $T_S$ is a functions of all parameters in the system, including the initial conditions of the cycle. This indicated that there is no cycle independent fitness when fixing the nutrients are fixed. However, to find the fittest species we only need to consider the difference in fitness: $F^i_{I\!I} - F^j_{I\!I}$, where $F^i_{I\!I}$ is short for $F_{I\!I}(\lambda_i;\gamma,p,T)$. This difference is independent of the initial conditions since the time dependent terms cancel out. Species $i$ is fittest when the difference is positive, which is the case when the following inequality is satisfied

$$\begin{aligned} & F^i_{I\!I} > F^j_{I\!I} \\ \Leftrightarrow\ & \lambda_i + p(\gamma+1)(T-\lambda_i)\theta(T-\lambda_i) < \lambda_j + p(\gamma+1)(T-\lambda_j)\theta(T-\lambda_j). \end{aligned} \tag{5.6}$$

In order to determine $\lambda_i$ such that the inequality in eq. 5.6 holds, we need to investigate four cases depending on whether the populations wake up before or after the antibiotics are removed. The four cases are summarised in tab. 5.1. We will now go through the constraints on $\lambda_i$ from tab. 5.1.

| | Species $i$ | Species $j$ | Constraint from $F^i_{I\!I} > F^j_{I\!I}$ |
|---|---|---|---|
| **Case 1** | $\lambda_i \geq T$ | $\lambda_j \geq T$ | $\lambda_i < \lambda_j$ |
| **Case 2** | $\lambda_i \geq T$ | $\lambda_j < T$ | $\lambda_i < \lambda_j + p(\gamma+1)(T-\lambda_j)$ |
| **Case 3** | $\lambda_i < T$ | $\lambda_j \geq T$ | $\lambda_i(1-p(\gamma+1)) < \lambda_j - pT(\gamma+1)$ |
| **Case 4** | $\lambda_i < T$ | $\lambda_j < T$ | $\lambda_i(1-p(\gamma+1)) < \lambda_j(1-p(\gamma+1))$ |

Table 5.1: The four possible versions of eq. 5.6

**In case 1** is the case where both species wake up at $t = T$ or later, hence none of the species are affected by the antibiotics. The fittest species is trivially the species that wakes up first, and thus is able to consume the largest amount of the nutrient.

**In case 2** species $i$ wakes up after the antibiotics, whereas species $j$ wakes up during the antibiotics. Eq. 5.6 now reduces to the following constraint on $\lambda_i$

$$\lambda_i < \lambda_j(1 - p(\gamma + 1)) + pT(\gamma + 1).$$

This inequality only has a solution when $p > (\gamma + 1)^{-1}$, that is when the probability of antibiotics is high relative to how mild the antibiotics are. That is, it can only be beneficial to wake up after the antibiotics if the application is frequent or the antibiotic killing rate is high.

**Case 3** is opposite of case 2, namely the case where species $i$ wakes up early enough to be affected by the antibiotics, whereas its competitor $j$ wakes up after the antibiotics are washed out. Eq. 5.6 now becomes

$$\lambda_i(1 - p(\gamma + 1)) < \lambda_j - pT(\gamma + 1).$$

For $p < (\gamma + 1)^{-1}$ we just reobtain the definition of case 3, namely $\lambda_i < T$. If we combine this with the result from case 1 we get the general constraint $\lambda_i < \lambda_j$ for all $\lambda_j \geq T$.

For $p > (\gamma + 1)^{-1}$ the inequality only has a solution when $\lambda_j > pT(\gamma + 1)$. In this case the inequality reduces to $\lambda_i \geq 0$. A species with lag time shorter than the duration of the antibiotics can therefore only win for severe antibiotics if the competitor wakes up sometime after the antibiotics are removed. The wake-up delay of the competitor depends on the severity of the antibiotics.

**Lastly, case 4** is characterised by both species having lag time shorter than $T$, hence both are affected by the antibiotics. In this case eq. 5.6 reduces to

$$\lambda_i(1 - p(\gamma + 1)) < \lambda_j(1 - p(\gamma + 1)).$$

When $p < (\gamma + 1)^{-1}$ this corresponds to $\lambda_i < \lambda_j$, which is similar to case 1. When $p > (\gamma + 1)^{-1}$ this corresponds to the opposite inequality, namely $\lambda_i > \lambda_j$.

### Optimal lag time

The results from all four cases are collected in tab. 5.2. We now optimise the fitness $F^i_{II}$ (eq. 5.5) with respect to these, and compare the result with the optimal lag time $\lambda^*_I$ for a single species with unlimited nutrients in eq. 3.1. The optimal wake up strategy when considering competition is the $\lambda$ that maximises the difference in effective growth. When $p < (\gamma + 1)^{-1}$, that is the antibiotic application is mild, this difference

| Domain of $\lambda_j$ \\ Severeness of AB | $p < (\gamma+1)^{-1}$ | $p > (\gamma+1)^{-1}$ |
|---|---|---|
| $\lambda_j \in [0,\ T)$ | $\lambda_i \in [0,\ \lambda_j)$ | $\lambda_i \in (\lambda_j,\ \lambda_j + p(T-\lambda_j)(\gamma+1)]$ |
| $\lambda_j \in (T,\ pT(\gamma+1))$ | - | $\lambda_i \in [T,\ \lambda_j)$ |
| $\lambda_j \in (\max\{pT(\gamma+1),\ T\},\ \infty)$ | $\lambda_i \in [0,\ \lambda_j)$ | $\lambda_i \in [0,\ \lambda_j)$ |

Table 5.2: Constraints from domain of $\lambda_j$ and severeness of the antibiotics (AB).

is minimised when $\lambda_i$ is as short as possible. $\lambda_i$ must be smaller than $\lambda_j$, and the difference in fitness function is monotonically decreasing with $\lambda_i$. The optimal lag time is therefore $\lambda_{II}^* = 0$. When the antibiotic application is severe (last column in tab. 5.2) the difference in fitness increases with $\lambda_i$ until $\lambda_i = T$, after which the difference decreases with $\lambda_i$. Here, the optimal lag time is therefore $\lambda_{II}^* = T$

$$\lambda_{II}^* = \begin{cases} 0 & \text{if } \ p < (\gamma+1)^{-1}, \\ T & \text{if } \ p > (\gamma+1)^{-1}. \end{cases} \tag{5.7}$$

Hence, the optimal wake-up strategy in presence of a competing species is the same as in the case without competition. The case $p = (\gamma+1)^{-1}$ is not well-defined. Only when the competitor has lag time longer than $T$ can we be sure that $\lambda_{II}^* < T$.

### 5.1.1 Discussion

Intuitively, it seems reasonable that introducing competition to the system would favor short lag times. A species with long lag time risks that all the nutrients have been consumed by its competitor before the species itself wakes up. With this in mind it is not surprising that $\lambda_{II}^* = 0$ for low antibiotic stress, also in the case of competition. On the contrary, one could be tempted to assume that for high antibiotic stresses $\lambda_{II}^*$ would be lower than $T$, possibly approaching $T$ as the probability of antibiotic application reaches 1. However, in the same way that waking up early is a competitive advantage in absence of antibiotics, it is a competitive disadvantage in presence of antibiotics. Since, in the latter, the species that wakes up first, will have reduced population density compared to that of its competitor at the time $T$ when the antibiotics are removed, and in turn be able to consume less of the nutrients.

The result in this section is purely analytical, corresponding to an idealised setup without extinction. This means that a population can always recover from extreme antibiotic stress if it is given enough time. In reality, $\lambda = 0$ is not only unfeasible due to physical limitations, but would also make the population very vulnerable to just a single application of antibiotics of duration

$$T > \frac{1}{\gamma} \log \frac{n_i(0)}{n_{ext}},$$

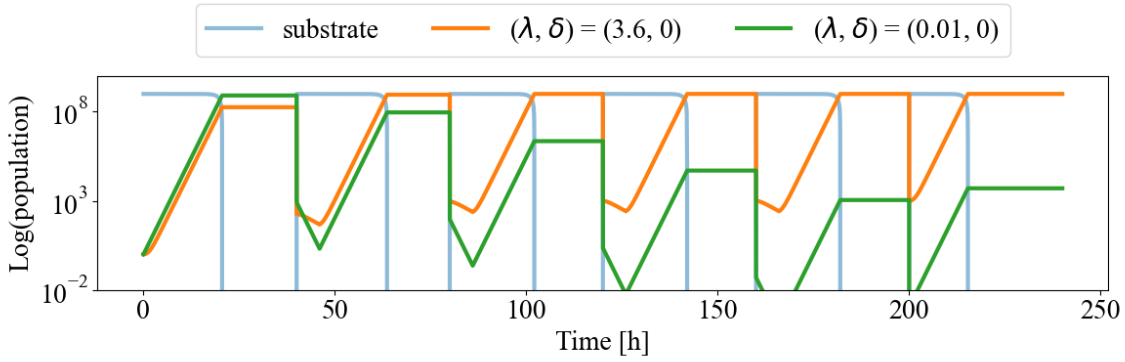


Figure 5.2: ***Example of 6 feast-famine cycles with exponentially distributed lag time.*** *The probability and duration of antibiotics are* $p = 0.6$ *and* $T = 6$*, respectively. The orange line represent a population with wake-up rate* $(pT)^{-1}$*, and the green lines represents a population with wake-up rate* 0.01*. The light blue line represent the nutrient that are left in the medium at a time* $t$*.*

where $n_{ext}$ is some extinction threshold. Whereas we do not expect that including an extinction threshold will affect the optimal lag time for high antibiotic stress, it will likely yield a higher $\lambda^*_{II}$ for mild stresses.

## 5.2 Model with exponentially distributed lag time

We now turn to the slightly more realistic model of exponentially distributed lag times, such that the population wakes up at a constant rate $1/\lambda$. This model is therefore closer to persistence than the previous model, since some cells will have much longer lag times than the majority of the population.

### 5.2.1 Computing the fitness function

As in the previous section we want to find the optimal lag time that maximises the growth of a species $i$ when there is competition for the nutrients. We therefore compute the fitness function as defined in eq. 5.5, also for this model. We split one population into two sub-populations representing two different states: a dormant population $d_i(t)$ (in lag phase) and a growing population $g_i(t)$ (in exponential phase). The total population is therefore $n_i(t) = d_i(t) + g_i(t)$. The sub-populations follow the coupled ordinary differential equations

$$\frac{d}{dt}d_i(t) = -d_i(t)/\lambda_i, \quad \text{if} \quad S(t) > 0, \tag{5.8}$$

$$\frac{d}{dt}g_i(t) = \begin{cases} d_i(t)/\lambda_i - \gamma g_i(t) & \text{if } t < T, \\ d_i(t)/\lambda_i + \beta g_i(t) & \text{if } t > T, \ S(t) > 0. \end{cases} \tag{5.9}$$

As in the previous model $\lambda_i$ is the lag time of species $i$. $\gamma$ and $\beta$ are the killing and growth rates, respectively, and are the same for all species. $t$ is the time after the nutrient is added, $T$ is the time that the antibiotics are removed. As before, the probability that antibiotics are added along with the nutrients is $p$. We obtain the case without antibiotics by setting $T$ to 0, such that the system does never follow eq. 5.9. $S(t)$ is the nutrient and is given by

$$\frac{d}{dt}S(t) = \begin{cases} 0 & \text{if } t < T, \\ -\sum_k \dot{g}_k(t) & \text{if } t > T. \end{cases} \tag{5.10}$$

An example of this model can be seen in fig. 5.2.

Eq. 5.8 is straight forward to solve and we obtain $d_i(t) = d_i(0)e^{-t/\lambda_i}$. At $t = 0$ the entire population is dormant, namely $n_i(0) = d_i(0)$ and $g_i(0) = 0$. We use this along with the solution for $d_i(t)$ to solve eq. (5.9)

$$g_i(t) = \begin{cases} \frac{d_i(0)}{\gamma\lambda_i - 1}\left(e^{-t/\lambda_i} - e^{-\gamma t}\right) & \text{if } t < T, \\ g_i(T)e^{t-T} + \frac{d_i(0)}{1+\lambda_i}\left(e^{-T/\lambda_i}e^{t-T} - e^{-t/\lambda_i}\right) & \text{if } t > T, \end{cases} \tag{5.11}$$

where $\beta = 1$ for simplicity. We again define $T_S$ to be the time that satisfies $S(t) = 0$. The total population at the end of the exponential phase is now

$$n_i(T_S) = d_i(0)\left(\frac{e^{-T/\lambda_i} - e^{-\gamma T}}{\gamma\lambda_i - 1} + \frac{e^{-T/\lambda_i}}{1+\lambda_i}\right) \cdot e^{T_S - T} + d_i(T_S)\frac{\lambda_i}{1+\lambda_i}. \tag{5.12}$$

Before plugging this into eq. 5.5, we make the assumption that at $T_S$ the dormant population is negligible compared to the growing population[2]. We therefore set $\frac{\lambda_i}{1+\lambda_i}d_i(T_S)$ to zero (for further discussion on this approximation see appendix A.1). Finally, we insert $n_i(T_S)$ for $T = 0$ and $T > 0$ in the fitness function eq. 5.5

$$\begin{aligned} F_{II}(\lambda_i; \gamma, p, T) &= (1-p)\ln\left[\frac{1}{1+\lambda_i} \cdot e^{T_{S,T=0}}\right] + p\ln\left[\left(\frac{e^{-T/\lambda_i} - e^{-\gamma T}}{\gamma\lambda_i - 1} + \frac{e^{-T/\lambda_i}}{1+\lambda_i}\right) \cdot e^{T_{S,T>0}-T}\right], \\ &= (1-p)\ln\left[\frac{1}{1+\lambda_i}\right] + p\left(-T + \ln\left[\frac{e^{-T/\lambda_i} - e^{-\gamma T}}{\gamma\lambda_i - 1} + \frac{e^{-T/\lambda_i}}{1+\lambda_i}\right]\right) \\ &\quad + pT_{S,T>0} + (1-p)\cdot T_{S,T=0}. \end{aligned}$$

Once again, the time dependent terms are independent of any species specific parameters. The rest of the the expression corresponds to the single species fitness in eq. 3.2.

[2]In a sense, this corresponds to ignoring "the most persistent persistors". However, at $T_S$ these persisters provide no further benefit and can be considered as just normal cells.

In other words, the difference in fitness of two competing species is just equal to the difference in their single species fitness

$$F_{II}^i - F_{II}^j = F_I^i - F_I^j. \tag{5.13}$$

Here, $F_I^i$ refers to the single species fitness as computed by Y. Himeoka and N. Mitarai. As in the previous section, we therefore expect the intrinsically fittest species to be the fittest species also when competing for nutrients.

### 5.2.2 Numerical results

The result in the previous section relies on the assumption that the dormant populations are negligible at the time that the system reaches the stationary state. An equivalent approximation was done by Y. Himeoka and N. Mitarai for $d(t)$ in the $t \gg \lambda$ region. In our setup, where the nutrient is explicitly considered, $t$ is constrained by an upper limit. We therefore verify our results with a numerical simulation.

We let two species compete for nutrients for $N_c$ cycles in $N_\lambda$ simulations. One species is given the analytically calculated optimal lag time from eq. 5.13 for the given set of antibiotic parameters $p$ and $T$. The other species has lag time $\lambda_i = 10^{-4} + i\Delta_\lambda$, where $i$ is the $i$th simulation of $N_\lambda$. At every cycle antibiotics are applied along with the nutrient with a probability $p$. After every cycle we compute the fraction of the nutrients that is consumed by each species and use the cycle average of this as a numerical measure of fitness[3]. From eq. 5.10 we get that each species consumes a fraction of the nutrients corresponding to $g_i(T_S) - g_i(T)$ divided by the initial amount of nutrients $S_0$. Again, $T = 0$ in absence of antibiotics. The average consumption fraction therefore reads

$$\frac{1}{N_c} \sum_c^{N_c} \frac{1}{S_0} \left( g_{i,c}(T_S) - g_{i,c}(T) \right),$$

where $g_{i,c}(t)$ denotes the growing population of species $i$ after $c$ cycles. We set $N_c$ to 20 000 and $\Delta_\lambda = 0.1$. Both species start with the same concentration of cells in the first cycle of each simulation, and after each cycle both populations are diluted with the dilution factor $f = 10^{-6}$. We set the initial number of cells to $n_0 = f \cdot S_0$, where $S_0 = 10^{-9}$.

In fig. 5.3 we compare the analytical and numerical results for $T = 6$ and $p = 0.2,\ 0.3,\ 0.6,\ 0.9$. In fig. 5.3a the difference in analytically calculated single species fitness is plotted on a logarithmic axis as function of the lag time of the competitor. That is $F_I(\lambda^*; \gamma, p, T) - F_I(\lambda; \gamma, p, T)$. This expression reaches its minimum when $\lambda = \lambda^*$. In 5.3b the consumption fraction of the competitor (with lag time $\lambda$) is

[3]We do not use effective growth as numerical fitness because $\frac{1}{N_c} \sum_c^{N_c} \log \left[ \frac{n_{i,c}(T_S)}{n_{i,c}(0)} \right]$ will be dominated by the cycles when a species with initial density smaller than $fS_0$ consumes most of the nutrients.

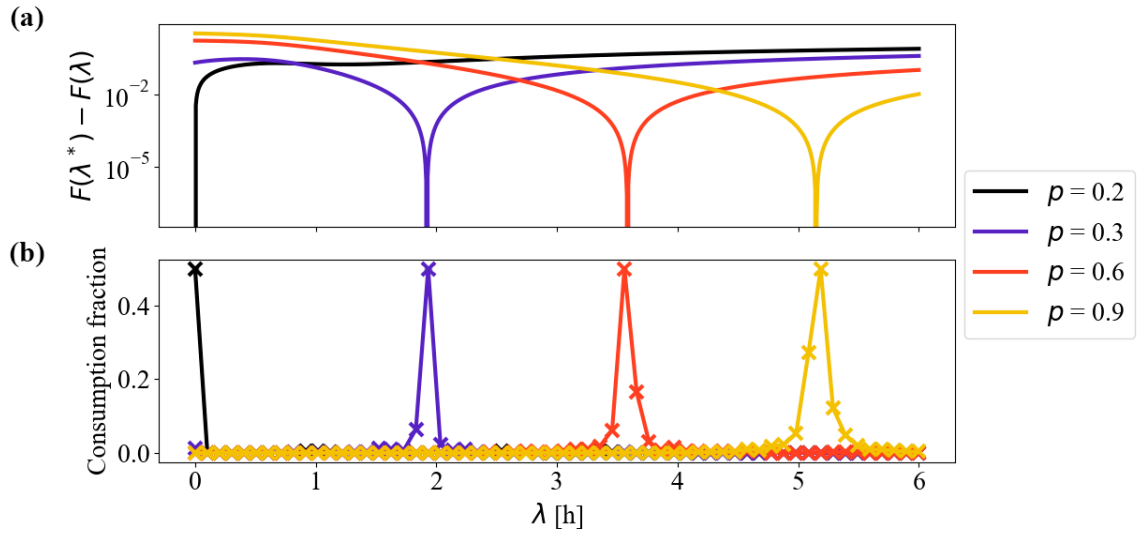


Figure 5.3: ***Comparison of analytically predicted and numerically computed optimal lag times.*** ***a)*** *Difference in analytical fitness of single species optimal and a species with lag time $\lambda$. The minima correspond to $\lambda = \lambda^*$.* ***b)*** *Consumption fraction, i.e. numerical fitness, of species with lag time $\lambda$ when competing against the single species optimal species. Note that the peaks do not exactly correspond to the minima in a), because of the discretisation of $\lambda$.*

plotted against its lag time. We expect the consumption fraction to have a peak at the minimum of $F_I(\lambda^*; \gamma, p, T) - F_I(\lambda; \gamma, p, T)$, with a peak value of 0.5 because the two species are identical here and thus act as one population. We confirm that this is also what we observe. As expected from [22] there is a discrete jump in optimal lag time between $p = 0.2$ and $p = 0.3$, where the optimal lag time jumps from $\lambda^* = 0$ to $\lambda^* \approx pT$. The region around the critical probability is explored in more detail in appendix A.2.

## 5.3 General model

In the previous sections we have showed for two simple models that the optimal lag time is the same when the bacteria compete for a fixed amount of nutrients, as in the case of unlimited nutrients. We will now show that this holds for any model where the dormant subpopulation can be neglected at the time that the population enters the

stationary phase. Adapting the general setup from [22] to a nutrient limited setup

$$F_{II}[r_i, q] = (1-p)\ln\left[\frac{\int_0^{T_S} e^{T_S-\lambda} r_i(\lambda)\ d\lambda + \int_{T_S}^{\infty} r_i(\lambda)\ d\lambda}{\int_0^{\infty} r_i(\lambda)\ d\lambda}\right] + p\int_0^{\infty} q(T)\ln\left[\frac{e^{T_S}\left(e^{-(1+\gamma)T}\int_0^{T} e^{\gamma\lambda} r_i(\lambda)\ d\lambda + \int_T^{T_S} e^{-\lambda} r_i(\lambda)\ d\lambda\right) + \int_{T_S}^{\infty} r_i(\lambda)\ d\lambda}{\int_0^{\infty} r_i(\lambda)\ d\lambda}\right] dT. \tag{5.14}$$

Here, $r_i(\lambda)$ is the distribution of lag times of population $i$, and $q(T)$ is the distribution of antibiotics with duration $T$. Assuming that the entire population is awake at $T_S$ is equivalent to assuming that there are no cells with lag times in $[T_S, \infty]$, hence we have

$$\int_{T_S}^{\infty} r_i(\lambda)\ d\lambda \approx 0 \quad \Rightarrow \int_0^{T_S} e^{T_S-\lambda} r_i(\lambda)\ d\lambda \approx \int_0^{\infty} e^{T_S-\lambda} r_i(\lambda)\ d\lambda.$$

We can now replace the $T_S$ integration limits with $\infty$. $T_S = T_S(r_i, r_j, T)$ is here a function of the distributions of lag time, but not of $\lambda$ itself[4]. We can therefore pull $e^{T_S}$ outside both integrals and logarithms

$$F_{II}[r_i, q] = (1-p)\ln\left[\frac{\int_0^{\infty} e^{-\lambda} r_i(\lambda)\ d\lambda}{\int_0^{\infty} r_i(\lambda) d\lambda}\right] + (1-p)T_S + p\int_0^{\infty} q(T)\ln\left[\frac{\left(e^{-(1+\gamma)T}\int_0^{T} e^{\gamma\lambda} r_i(\lambda)\ d\lambda + \int_T^{\infty} e^{-\lambda} r_i(\lambda)\ d\lambda\right)}{\int_0^{\infty} r_i(\lambda)\ d\lambda}\right] dT + p\int_0^{\infty} T_S \cdot q(T)\ dT.$$

Which corresponds to

$$F_{II}[r_i, q] = F[r_i, q] + (1-p)T_S + p\int_0^{\infty} T_S \cdot q(T)\ dT,$$

where $F[r_i, q]$ is the single species fitness from eq. 3.4 for a normalised $r_i(\lambda)$. And once again we have that

$$F_{II}[r_i, q] - F_{II}[r_j, q] = F[r_i, q] - F[r_j, q],$$

namely that the difference between the fitness of two competing species is equal to the single species fitness of each species. This result is independent of both lag time distributions and the distribution of antibiotic duration, suggesting that the simplification of constant antibiotic killing effect is justified.

[4] $T_S(r_i, r_j, T) = \log[S_0] - \log\left[\sum_{k\in i,j}\left(e^{-(1+\gamma)T}\int_0^{T} e^{\gamma\lambda} r_k(\lambda)\ d\lambda + \int_T^{\infty} e^{-\lambda} r_k(\lambda)\ d\lambda\right)\right]$

### 5.3.1 Discussion

Until now we have seen that the single species optimal lag time is also the optimal lag time when we limit the nutrients for three different models. This result is dependent on the fact that the two species have the same growth rate, as $T_S$ would otherwise not cancel out in the expressions for $F_{II}^i - F_{II}^j$. Furthermore, we have assumed in all three models that all cells are awake at the time that the population enter the stationary phase (in section 5.1 this approximation is exact). With these two assumptions, the final population density will always be on the form $n(T_S; \lambda, \gamma, T) = C(\lambda, \gamma, T) \cdot e^{T_S}$, and the difference on fitness of two populations will always be independent of $T_S$. Since $T_S$ is the term that reflects both the limitation on nutrients and competition, the difference in competition fitness $F_{II}^i$ will therefore always be the same as the difference in single species fitness $F_I^i$.

We have already argued that it is meaningful to fix the growth rate for all species, hence we now turn our attention on the approximation that all species are awake at $T_S$. As mentioned earlier, research indicates that real bacterial populations have long tailed distributions of lag time. It is therefore likely that in a reality there are still cells in a dormant or persistent state at a time $T_S$. However, the fraction of persisters is usually very low [16, 45]. We therefore consider this approximation to be reasonable, even in a general case where we do not know the lag time distribution. At the same time this indicates that the competition optimal might not be identical to the single species optimal, once we include spontaneous persistence. Since we here consider persisters to be dormant cells, spontaneous presistence corresponds to spontaneous reentering to the dormant state. Therefore, the dormant population might no longer be negligible once we include both persistence types.

Lastly, we have still not taken extinction into account, meaning there is no lower threshold on the populations. For combinations of antibiotic parameters that can be lethal with just one cycle of application, we expect the true optimal lag time to be reflecting this. By this we mean that lag times that are too short to protect the population from extinction, are unlikely to be the true optimals.

## 5.4 Evolution of average lag time in model with mutation

So far we have found the analytically optimal lag time and confirmed it with numerical simulations. Since this work is partly motivated by an experimental demonstration evolution of antibiotic tolerance, it is relevant to investigate whether our model can actually allow a population to reach $\lambda^*$ through mutation. We therefore extend our model from two species to $N_\lambda$ species with $\lambda_i = 10^{-2} + i\Delta_\lambda$ and $\Delta_\lambda = 0.1$. Using the model with exponentially distributed lag time (from section 5.2) as a starting point,

we introduce a small mutation rate $\varepsilon$ between the nearest neighbours in terms of $\lambda$

$$\begin{aligned}
\frac{d}{dt}g_0(t) &= d_0(t)/\lambda_0 + (1-\varepsilon)\, g_0(t) + \varepsilon g_1(t), \\
\frac{d}{dt}g_i(t) &= d_i(t)/\lambda_i + (1-2\varepsilon)\, g_i(t) + \varepsilon \left(g_{i-1}(t) + g_{i+1}(t)\right), \\
\frac{d}{dt}g_{N-1}(t) &= d_{N-1}(t)/\lambda_{N-1} + (1-\varepsilon)\, g_{N-1}(t) + \varepsilon g_{N-2}(t),
\end{aligned} \tag{5.15}$$

when $t > T$ and $S(t) > 0$. Mutations therefore only occur in the exponential phase, since it is related to reproduction, thus growth, of bacteria. We set all species except one to 0 at the beginning of every simulation, and then let the system evolve from the initial population for $10^4$ cycles. After every cycle we compute the average lag time weighted by the population densities in the system

$$\langle \lambda \rangle = \frac{\sum_i \lambda_i \cdot n_i(T_S)}{\sum_k n_k(T_S)}$$

.

### 5.4.1 Simulation without extinction

First, we let the system evolve without any lower threshold. In this case, as in previous sections, a population can always recover from the most extreme antibiotic stress. In a model with mutation this means that once a mutation occurs, it will not leave the system. Since all species mutate linearly through lag times, eventually all mutations allowed by the simulation will be present, though most of them with unphysically low densities.

In fig. 5.4 we have plotted the evolution of average lag time as function of cycle number. The antibiotics are still synchronised with the nutrients, and we set the duration of antibiotics to $T = 6$. This corresponds to the antibiotic parameters used in fig. 5.3, which is why we expect a discontinuity in $\langle \lambda \rangle$ in between $p = 0.2$ and $p = 0.3$. The simulation starts from the 0th population, that is the population with $\lambda_0 = 10^{-2}$. We observe that for $p$ below $p_c$ the average lag time stabilises somewhat around $\langle \lambda \rangle = 3 \cdot 10^{-2}$. The dominant lag time is here $\lambda_0$. The average is a little higher because the species with $\lambda_0$ only mutate to species with larger lag time, hence the population densities are not distributed symmetrically around the dominant population. For $p$ above $p_c$ the average stabilises around $\langle \lambda \rangle \approx pT$, which corresponds to what we expect from section 5.2. For $p = 0.2$ and $p = 0.3$ we observe a few spikes between $\langle \lambda \rangle = 0$ and $\langle \lambda \rangle = pT$.

In fig. 5.4b we investigate the region around the critical probability $p_c$ closer. This region is very chaotic with several spikes and jumps between the two optima, indicating that the system is in fact bistable in this region. Here, both the population with $\lambda \approx 0$ and $\lambda \approx pT$ are relatively large, and can therefore quikcly grow large when the conditions are beneficial.

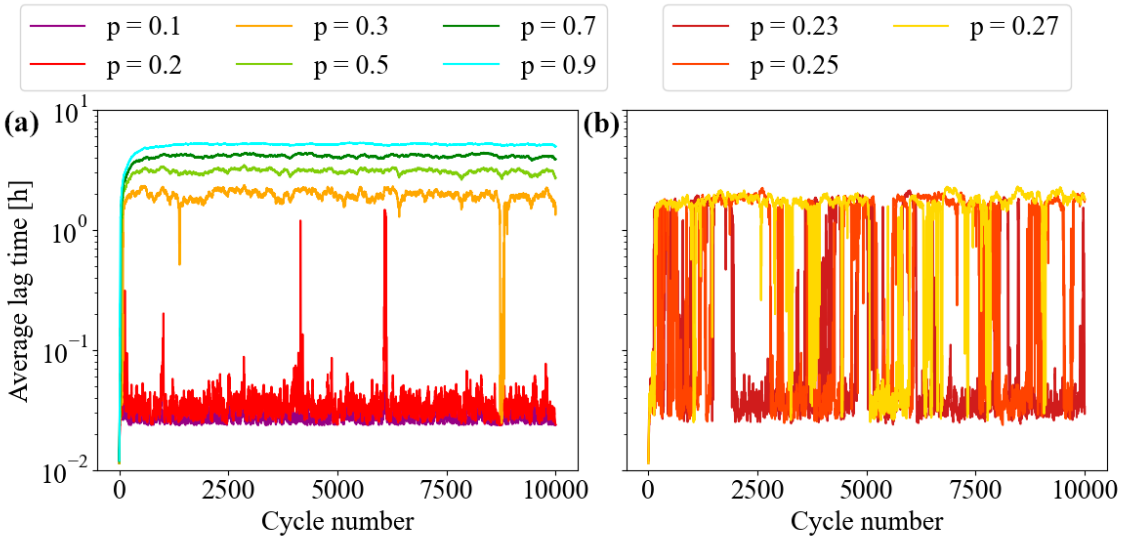


Figure 5.4: ***Evolution of average lag time in simulation with mutation, without extinction.*** *At every time step each population mutates to nearest neighbours with the mutation rate* $\varepsilon = 10^{-3}$. ***a)*** *Evolution of average lag time for different* $p$. ***b)*** *Evolution of average lag time around the critical probability* $p_c \approx 0.25$.

### 5.4.2 Simulation with extinction

We now implement a lower threshold of one cell $n_{min} = 1$ such that we truncate $d_i(t) + g_i(t)$ to zero if $d_i(t) + g_i(t) < n_{min}$. We check the system for extinctions after every antibiotic application and every dilution. In reality, cells are discrete therefore it would be more correct to use the threshold on $d_i(t)$ and $g_i(t)$ individually. Since we are working with at continuous model, with transition rates instead of discrete events, we chose not to apply this individual threshold.

In fig. 5.5a we let the system evolve from $\lambda_0 = 10^{-2}$ as in the case without extinction, and find that the system gets stuck in a local optimum close to the lower limit on $\lambda$. Contrary to what one would expect, we observe that the average lag time is lower the more frequent the antibiotic application is. This is because the simulation starts from $\lambda_0 = 10^{-2}$, which is the optimal lag time in cycles where the antibiotics are absent. Mutations only occur during the growth phase, and are introduced to the system with a very small initial population. Mutations with higher lag times are therefore prone to go extinct by dilution the same cycle as they appear, because they are much smaller than the population they originated from. Since new mutations usually go extinct the same cycle as they occur, the fittest species during a cycle with antibiotics will at best have a lag time only a few generations away from the original population with $\lambda_0 = 10^{-2}$. Furthermore, this species will also have the smallest density at the beginning of the cycle. When we take the population density into account, the highest lag time is therefore not large enough to provide a real evolutionary advantage.

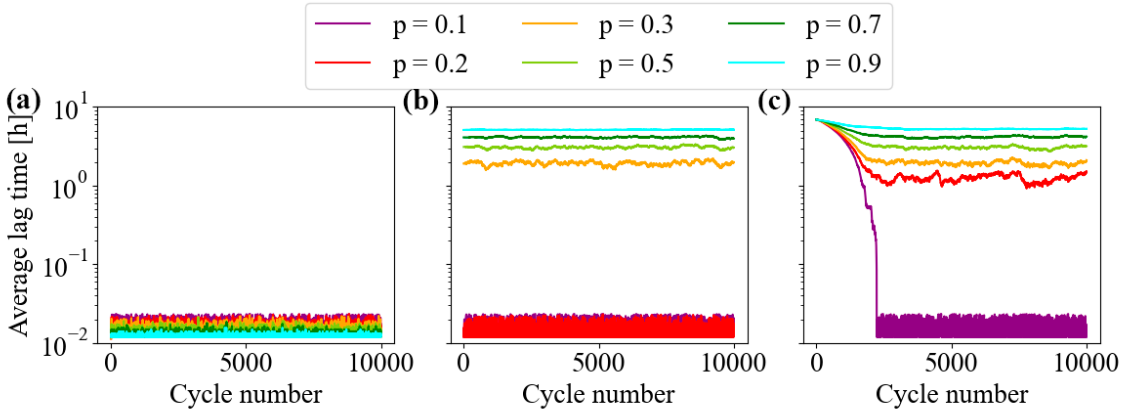


Figure 5.5: ***Evolution of average lag time in simulation with mutation and extinction.*** *At every time step each population mutates to nearest neighbours with mutation rate* $\varepsilon = 10^{-3}$ *and extinction threshold* $n_{min} = 1$. ***a)*** *Evolution starting at* $\lambda_0 = 10^{-2}$. ***b)*** *Evolution starting at* $\lambda_i = \lambda^*$. ***c)*** *Evolution starting at* $\lambda_{N-1} = T + 10^{-2}$.

Put differently, the intrinsically fittest species might also be the most vulnerable to extinction. For the system to be able to evolve from the initial lag time, a necessary condition is that the mutations are able to grow large enough to survive at least one round of antibiotics. Only then is a longer lag time a feasible advantage. For example a species with $\lambda = pT$ would need an initial population of

$$d_0 \geq \frac{pT\gamma - 1}{e^{-1/p} - e^{-\gamma T}} \cdot n_{min} = \frac{pT - 1}{e^{-1/p} - e^{-T}} \approx (pT - 1)e^{1/p},$$

in order to survive one round of antibiotics. For $p = 0.3$ and $T = 6$ this corresponds to an final population of $73/f$ cells/ml or more. New mutation typically reach a population of $\sim 10^2$ cells/ml before dilution, hence they they do no even survive the dilution event. Decreasing $1/f$ does not help noticeable, since this will increase the initial populations of all species, hence shorten the exponential phase where the mutations take place, because there of the restrictions imposed by $S_0$. Another option would be to lower the extinction threshold, $n_{ext}$. If we want populations of $\sim 100$ cells to survive dilution by a factor $10^{-6}$, this would require an extinction threshold of $n_{min} \leq 10^{-4}$. Increasing the mutation rate can improve the situation, however it requires an unphysically large mutation rate for the new mutation to survive at least one dilution. Another option is to consider only extinctions from antibiotics, since most extinctions occur during the dilution event.

Motivated by this we therefore change the initial population that we let the system evolve from. In the middle plot of fig. 5.5 we start the simulation from the analytically found optimal lag time $\lambda^*$. The average lag times does not change notably, confirming that these correspond to a local optimum. The behavior is different from the case without extinction (fig. 5.4), in that there are no fluctuations around the critical probability. The reason for this is the same as why $\langle\lambda\rangle \approx \lambda_0$ for all $p$ in fig.5.5a,

namely that most new mutations are diluted to extinction. In fig. 5.5b we start the simulation from the highest lag time, that is $\lambda_{N-1} = T$. We observe that for most $p$ the average lag time converges to the analytic optimal lag time. For $p = 0.2$ the system seems to be stuck in the local maximum around $\lambda \approx pT$, though it is likely that it requires more cycles to reach the true optimal.

When the system gets stuck in $(\lambda_0, \delta_0)$ here, but not in reality, it is probably related to the simplifications of our model. Specifically, since we do not consider space, a population is considered to be extinct when the number of cells per ml drops below 1. In a real system in absence of antibiotics, *one* species is enough to keep the population alive, independently of the volume of the space. This indicates that the extinction threshold imposed here, might in fact be unphysically strict.

# Chapter 6

# Models with spontaneous persistence

We now extend the model with triggered persistence to allow also spontaneous persistence. First we consider the simple model where all persisters (both triggered and spontaneous) are modelled by the same dormant state. This means that spontaneous persistence amounts to a spontaneous re-entering to the dormant state. A consequence of this simplification is that the lag time or "wake up" rate from triggered persistence and spontaneous persistence are the same, despite being two different phenomena. We use the model with exponentially distributed lag time from section 5.2 as a starting point, adding a term of spontaneous persistence to all equations. Since spontaneous persistence is expected to play a bigger role when antibiotics are applied during the exponential phase, we now allow the nutrients and antibiotic to be desynchronised, meaning we set $T_0 \geq 0$. With this setup, $T$ thus denotes the removal time of the antibiotics, whereas we denote the duration by $T_{AB} = T - T_0$.

In absence of antibiotics, the differential equations read

$$\frac{d}{dt}d_i(t) = -d_i(t)/\lambda + \delta g_i(t) \qquad \text{if } \; S(t) > 0, \tag{6.1}$$

$$\frac{d}{dt}g_i(t) = d_i(t)/\lambda + (1-\delta_i)\cdot g_i(t), \qquad \text{if } \; S(t) > 0. \tag{6.2}$$

$\lambda_i$ is the species specific lag time, and $\delta_i$ is the species specific rate of spontaneous persistence. As in the model of triggered persistence, $d_i(t)$ and $g_i(t)$ denote the dormant (persister) and growing populations, respectively. $S(t)$ denotes the nutrients at a time $t$. With probability $p$ antibiotics are applied at $t = T_0$. In this case $\dot{g_i}(t)$ reads

$$\frac{d}{dt}g_i(t) = \begin{cases} d_i(t)/\lambda - (\gamma+\delta_i)\cdot g_i(t) & \text{if } T_0 < t < T, \\ d_i(t)/\lambda + (1-\delta_i)\cdot g_i(t) & \text{if } t < T_0 \text{ or } T < t, \; S(t) > 0. \end{cases} \tag{6.3}$$

Again, $\gamma$ is the antibiotic killing rate. $S(t)$ follows almost the same differential equation

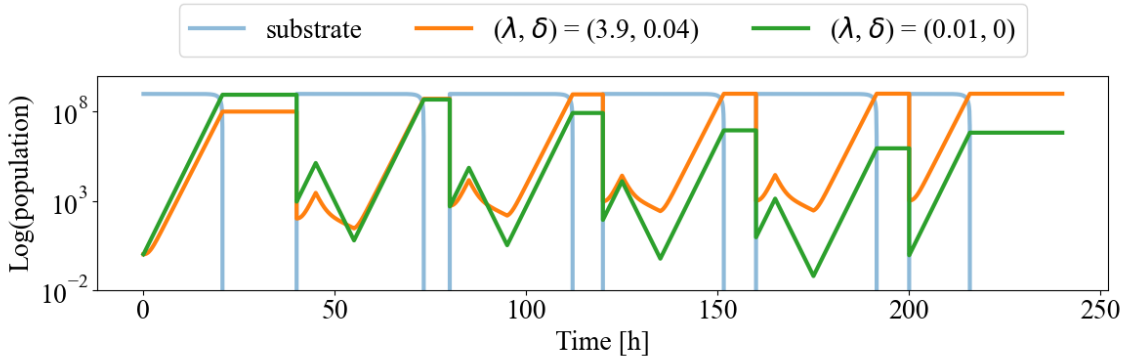


Figure 6.1: ***Example of 6 feast-famine cycles with spontaneous persistence.*** *Antibiotics are added at* $T_0 = 5$*, and the probability and duration of antibiotics are* $p = 0.6$ *and* $T_{ab} = 10$*, respectively. The orange line represents a population with wake-up rate* 3.9 *and a rate of spontaneous persistence* 0.04*. The green line represents a population with wake-up rate* 0.01 *and no spontaneous persistence. The light blue line represents the nutrient that is consumed by both populations.*

as before (eq. 5.10), namely

$$\frac{d}{dt}S(t) = \begin{cases} 0 & \text{if } T_0 < t < T, \\ -\sum_i \dot{g}_i(t) & \text{if } t < T_0 \text{ or } T < t, \end{cases} \tag{6.4}$$

where we obtain the case without antibiotics by setting $T_0 = T = 0$. Even though spontaneous persistence is specifically defined as spontaneously occuring persisters during exponential growth, we assume for simplicity that cells enter the dormancy at the constant rate $\delta_i$ independent of the presence of antibiotics. Letting spontaneous persistence occur only in absence of antibiotics yields qualitatively the same results as we obtain with this model. An example of two populations evolving according to eq. 6.1–6.4 can be seen in fig. 6.1.

## 6.1 Single species optimal strategy

In the previous chapter we optimised the wake-up strategy of a species that has to compete for fixed nutrients, that is we extended some of the results from [22] to the case of competition. When we now also consider spontaneous persistence, the expression for competition fitness becomes too complicated to solve analytically. We therefore start by identifing the optimal single species persistence strategy, which we will later use in simulations to determine the optimal persistence strategy of a competing species. Conceptually, optimising the single species strategy corresponds to evolving several species in parallel, picking the fittest population after infinitely many cycles. We will return to what is meant by "fittest" in the end of the next subsection.

### 6.1.1 Deriving a measure of fitness

We solve eqs. (6.1) and (6.2) by turning the set of coupled first order differential equations into a set of second order differential equations. This is achieved by adding $\dot{d}(t)$ and $\dot{g}(t)$, and then integrating the sum. We then obtain $d$ as function of $g$: $d(g) = \int g\ dt - g$, which we can plug into eq. (6.2) in order to eliminate the $d$-dependence. Lastly, we differentiate to get the equation on the form of a second order differential equation. The corresponding equation for the killing phase is obtained by replacing $\int g\ dt$ with $-\gamma \int g\ dt$ in $d(g)$.

$$\lambda\ddot{g} + [\lambda(\gamma+\delta)+1]\dot{g} + \gamma g = 0, \tag{6.5}$$

$$\lambda\ddot{g} - [\lambda(1-\delta)-1]\dot{g} - g = 0. \tag{6.6}$$

We solve eq. 6.5-6.6 in appendix B.1. The total size of a population at $t > T$ reads

$$\begin{aligned} n(t) = &\frac{d_0}{(a_p-b_p)(a+b)^2}\left[(b+b_p)e^{-aT_0} + (a-b_p)e^{bT_0}\right](b+a_p)e^{-b_pT_{AB}}\cdot ae^{b(t-T)} \\ &- \frac{d_0}{(a_p-b_p)(a+b)^2}\left[(b+a_p)e^{-aT_0} + (a-a_p)e^{bT_0}\right](b+b_p)e^{-a_pT_{AB}}\cdot ae^{b(t-T)} \\ &- \frac{d_0}{(a_p-b_p)(a+b)^2}\left[(b+b_p)e^{-aT_0} + (a-b_p)e^{bT_0}\right](a-a_p)e^{-b_pT_{AB}}\cdot be^{-a(t-T)} \\ &+ \frac{d_0}{(a_p-b_p)(a+b)^2}\left[(b+a_p)e^{-aT_0} + (a-a_p)e^{bT_0}\right](a-b_p)e^{-a_pT_{AB}}\cdot be^{-a(t-T)}. \end{aligned} \tag{6.7}$$

Here, $d_0$ is the population at $t = 0$, and as mentioned $T_{AB}$ is the duration of the antibiotics. Like in the previous chapter the entire population is initially dormant. $a, b, a_p$, and $b_p$ are non-linear combinations of $\lambda, \delta, \gamma$, and the growth rate $\beta = 1$, defined as

$$\begin{aligned} a &= -\frac{\lambda(1-\delta)-1}{2\lambda} + \frac{\sqrt{(\lambda(1-\delta)-1)^2+4\lambda}}{2\lambda}, \\ b &= \phantom{-}\frac{\lambda(1-\delta)-1}{2\lambda} + \frac{\sqrt{(\lambda(1-\delta)-1)^2+4\lambda}}{2\lambda}, \\ a_p &= \phantom{-}\frac{\lambda(\gamma+\delta)+1}{2\lambda} + \frac{\sqrt{(\lambda(\gamma+\delta)+1)^2-4\lambda\gamma}}{2\lambda}, \\ b_p &= \phantom{-}\frac{\lambda(\gamma+\delta)+1}{2\lambda} - \frac{\sqrt{(\lambda(\gamma+\delta)+1)^2-4\lambda\gamma}}{2\lambda}. \end{aligned}$$

The signs are chosen such that $a, b, a_p$ and $b_p$ are positive for all $\lambda, \delta$ and $\gamma$. When $\delta = 0$, they reduce to

$$a = \frac{1}{\lambda}, \qquad b = 1, \qquad a_p = \gamma, \qquad b_p = \frac{1}{\lambda}.$$

These parameters express how the parameters from the model of triggered persistence are transformed when we include spontaneous persistence. $b$ can be interpreted as the

effective growth rate of a population with persisters, and it decreases with $\delta$ because the fraction of growing cells in this model is lower compared to the model of triggered persistence. $a$ and $b_p$ can be interpreted as the effective wake up rates in absence and presence of antibiotics, respectively. They have opposite dependency on $\delta$, with $a$ increasing and $b_p$ decreasing. In this sense, a system with spontaneous persistence is able to have a "dynamic" effective lag time that is shorter in absence of antibiotics and longer, thus more tolerant, when antibiotics are present. However, this is at the expense of a lower growth rate. This interpretation is not faultless, as it implies that eqs. 6.1-6.2 can be written on the form of eqs. 5.8-5.9. Though this is not the case, the interpretation still provides some insight on how the model with spontaneous persistence is different from that of only triggered persistence.

In the case without antibiotics eq. 6.7 reduces to

$$n(t) = \frac{d_0}{a+b}\left(ae^{bt} + be^{-at}\right).$$

At long times $t$ this expression is dominated by the first term, and maximising the population density corresponds to maximising $b$, which is the largest when $\delta = 0$. Since $b$ plays the role of a growth rate, this is also what we would expect in an environment without antibiotic stress. The case with antibiotics is more complicated. If we let $t$ become large enough it will always be optimal to maximise the effective growth rate, that is $\delta = 0$, independently of the antibiotic parameters. In other words spontaneous persistence is only meaningful when there is a restriction of the growth phase, i.e. if there is an upper bound on $t$. This can be achieved by either fixing the amount of nutrients or by fixing the cycle length. Whereas there are physical meaningful constraints on $S_0$, these are less obvious for $T_S$. We therefore constrain the growth phase by a fixed amount of nutrients $S_0$, and define a new measure of single species fitness: $\langle T_S \rangle_p$, that is the average time it takes a single population to consume $S_0$.

To find an expression for $\langle T_S \rangle_p$ we first need to isolate $T_S$, both for a cycle with and without antibiotics. From eq. 6.4 we have

$$0 = S_0 - g(T_S) + g(T) - g(T_0). \tag{6.8}$$

We first compute $T_{S,T=T_0}$ for cycles without antibiotics. In absence of antibiotics both $g(T)$ and $g(T_0)$ are zero, and $g(T_S) = d_0 \frac{ab}{a+b}\left(\exp[bT_S] - \exp[-aT_S]\right)$ (see appendix B.1 for details). Now we make the approximation that the second term can be neglected at $t = T_S$. Since $a \geq 1/\lambda$ for any $\delta$ and we further expect $\lambda \leq T_S$, we consider this approximation to be reasonable [1]. We now have

$$\begin{aligned} S_0 &= d_0 \frac{ab}{a+b} e^{bT_S}, \\ \Rightarrow T_{S,T=T_0} &= \frac{1}{b} \log\left[\frac{a+b}{ab} \cdot \frac{S_0}{d_0}\right]. \end{aligned}$$

[1] This corresponds to an upper limit of $\exp[-aT_S] \approx 0.37$

We can replace the dependence on $S_0$ and $d_0$ with $f = S_0/d_0$, choosing the initial population to be $d_0 = f \cdot S_0$, where $f$ is the dilution factor. The case with antibiotics is computed in appendix B.2. We make the same approximation as in the case without antibiotics, namely that all terms with the factor $\exp(-aT_S)$ can be neglected.

$$T_{S,T>T_0} = \frac{1}{b}\log\left[\frac{(a+b)^2(a_p-b_p)}{ab}\frac{1+fg'(T)-fg'(T_0)}{fD}\right] + T,$$

where $D$ is the sum of the factors that the two first terms in eq. 6.7 do not share. $g'(t) = g(t)/d_0$ such that also this expression is independent of the initial conditions (see appendix B.2 for the exact form of $D$). Finally, taking the average of $T_S$ weighted by the probability of antibiotics, we obtain

$$\langle T_S\rangle_p = \ (1-p)\frac{1}{b}\log\left[\frac{a+b}{ab}\cdot\frac{1}{f}\right] + \ p\left(T + \frac{1}{b}\log\left[\frac{(a_p-b_p)(a+b)^2}{ab}\frac{1+fg'(T)-fg'(T_0)}{fD}\right]\right).$$

We define the fitness to be the inverse of the average consumption, since short $T_S$ is analogue to a high effective growth in a model with unlimited nutrients. Finally, we therefore have

$$F_I(\lambda,\delta;\gamma,p,T_0,T,f) = \frac{1}{\langle T_S\rangle_p}. \tag{6.9}$$

Note that in cycles with antibiotics the shortest consumption time does not necessarily yield the largest final density. Minimising consumption time is therefore a different measure of fitness from effective growth, as used in the previous chapter. With fixed nutrients and no competition, the effective growth becomes

$$\beta_{eff} = -\log[bf] + p\log\left[1-\frac{g(T_0)-g(T)}{S_0}\right],$$

which is dominated by the first term, except for when $T_0$ and $T$ are both very long, such that $g(T_0)-g(T) \approx S_0-0$. Maximising this expression corresponds to minimising $b$ and the impact of antibiotics (i.e. $g(T_0)-g(T)$). Using the effective growth with fixed nutrients will therefore trivially always yield the same answer, namely that it is optimal with long lag time to avoid antibiotics. Counter-intuitively, it is also optimal with a high rate of spontaneous persistence independently of all other parameters. This trivial result reflects that for a single species with fixed nutrients the nutrient will be left unused, if the species is not growing. Maximising effective growth is therefore equivalent to minimising the antibiotic impact. Consequently, the word "fitness" will in the following refer to eq. 6.9.

Next, we maximise eq. 6.9 numerically. We let $\lambda$ range from $10^{-2}$ to $T$, and $\delta$ range from 0 to 1, and pick the set $(\lambda,\delta)$ that yields the highest $F_I(\lambda,\delta;\gamma,p,T_0,T,f)$ for a given set of antibiotic parameters. As earlier, we set $\gamma = 1$ and $f = 10^{-6}$.

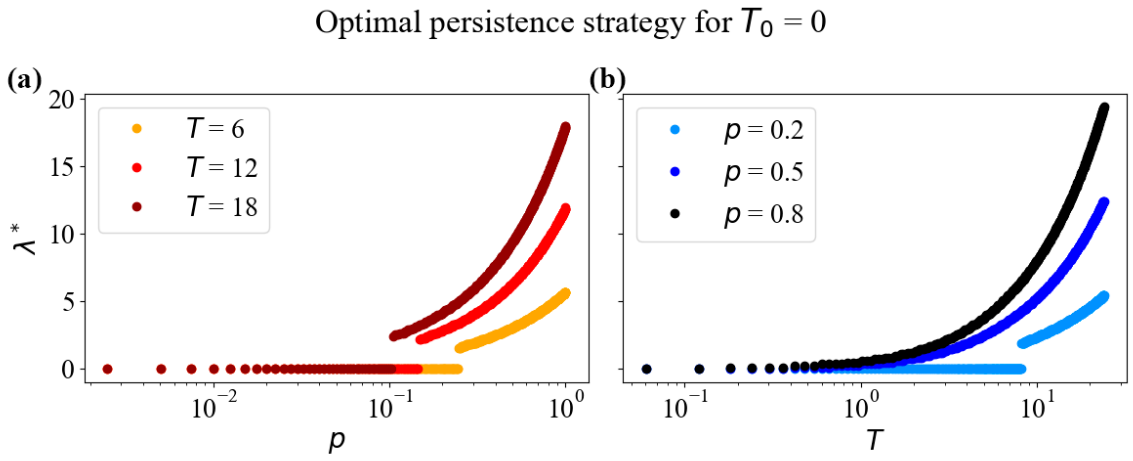


Figure 6.2: ***Optimal persistence strategy when antibiotics and nutrients are synchronised.*** ***a)*** *The optimal lag time as function of probability.* ***b)*** *The optimal lag time as function of the duration of antibiotics.*

### 6.1.2 Optimal strategies for synchronised nutrients and antibiotics

First, we consider $T_0 = 0$. Since antibiotics and nutrients are always applied simultaneously, it is less beneficial to wake up before antibiotics are removed and we expect spontaneous persistence to be less relevant here. Independently of $p$ and $T_{AB}$, we find that the rate of spontaneous persistence is always 0. This means that when antibiotics and nutrients are synchronised, the model with optimal spontaneous persistence strategy reduces to the model with only triggered persistence. We confirm that we obtain the same optimal strategy as in sections 3.1 and 5.2. In fig. 6.2 the optimal lag time $\lambda^*$ is plotted against $p$ and $T = T_{AB}$. As expected we find that $\lambda^* \to 0$ for low antibiotic severeness, and $\lambda^* \approx pT$ for high antibiotic severeness. The jump between $\lambda^* \to 0$ and $\lambda^* \approx pT$ occurs later the less severe the antibiotic application is. When $p \gtrsim 0.5$ there is no jump and $\lambda^* \approx pT$ for all $T$. Heat maps of $\lambda^*$ and $\delta^*$ for $p \in [0, 1]$ and $T \in [0, 24]$, can be found in appendix B.3.

That any set with finite $\delta$ is always sub-optimal to $(\lambda^*, 0)$, confirms that in this model there is no benefit from waking up earlier. $\lambda^*$ from 5.2 is the optimal lag time independently whether we consider effective growth or consumption time. This result thus supports that eq. 6.9 is a meaningful fitness measure. In this setup where extinction is not considered, spontaneous persistence does not act as an insurance against extinction like it would in a more realistic scenario. Using the analogy of bet-hedging, in the setup without extinction it is less relevant to bet-hedge because one is in fact always able to gamble again. Spontaneous persistence provides insurance against extinction, but does not shield the population density significantly from the antibiotics (unless the rate of spontaneous persistence is unphysically high). A finite rate of

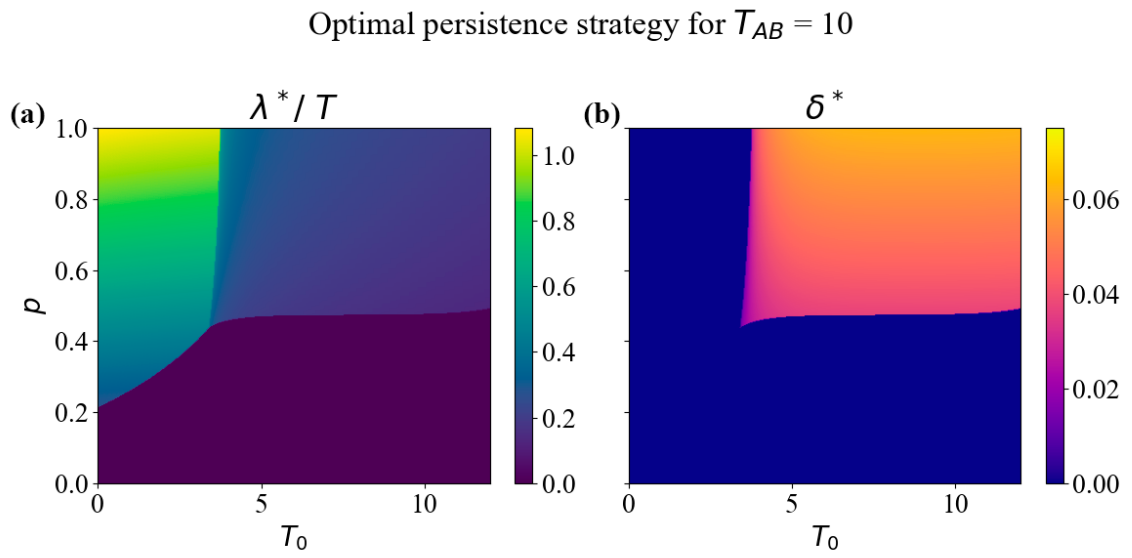


Figure 6.3: ***Optimal persistence strategy when antibiotics and nutrients are desynchronised.*** *The duration of antibiotics is set to $T_{AB} = 10$.* ***a)*** *The optimal lag time divided by the time that the antibiotics are removed.* ***b)*** *The optimal rate of spontaneous persistence.*

spontaneous persistence will therefore provide relatively little protection against the antibiotics compared to a long lag time. Furthermore, spontaneous persistence affects the effective growth rate after the antibiotics are removed, which is not the case for a long lag time.

### 6.1.3 Optimal strategies for desynchronised nutrients and antibiotics

Next we desynchronise the application of nutrients and antibiotics, meaning we let $T_0$ range from 0 to 12. This range approximately corresponds to times below the lower bound on the consumption time in absence of antibiotics, for $f = 10^{-6}$. Later application of antibiotics would therefore reduce to the case without antibiotics.

In fig. 6.3a and 6.3b we have plotted two heat maps corresponding to the optimal lag time and optimal rate of spontaneous persistence, respectively, as functions of $p$ and $T_0$. The duration of the antibiotics is fixed to 10. In the heat map of optimal lag time, we observe three different regions. When the probability of antibiotics is low, $\lambda^* \to 0$. For higher probability and early application of antibiotics, $\lambda^* \approx pT$. These are the two cases we know from triggered persistence. We also observe a third region where $\lambda^*$ is finite, but considerably shorter than $pT$. The three transitions between the regions all appear to be discontinuous, a point we will return to later. The heat map of optimal rate of spontaneous persistence only contains two regions, though the borders coincide with the optimal lag time. For low probability or early application of

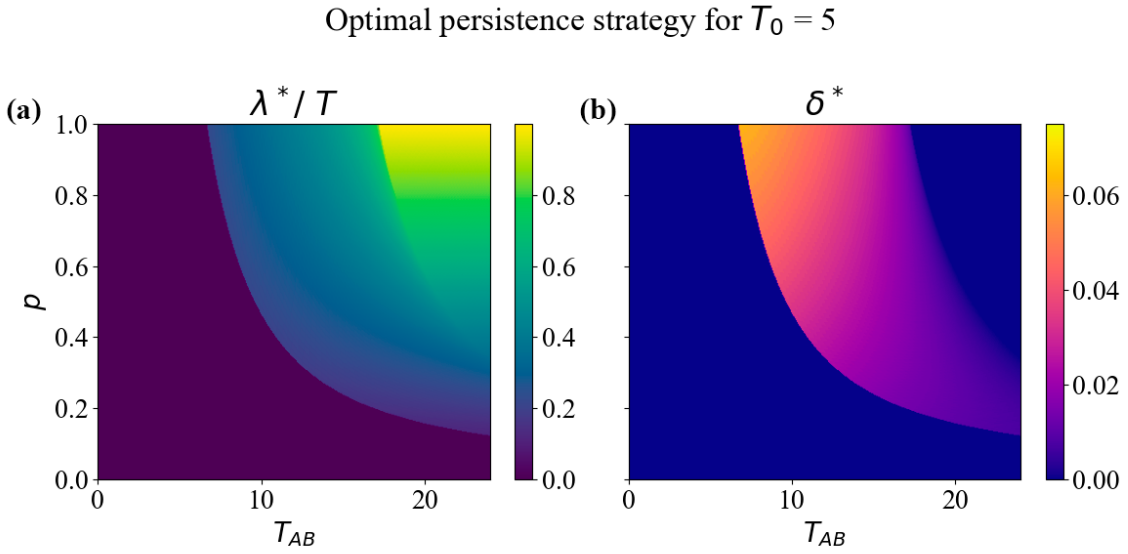


Figure 6.4: ***Optimal persistence strategy for desynchronised nutrients and antibiotics.*** *The duration of antibiotics is set to $T_0 = 5$.* ***a)*** *The optimal lag time divided by the time that the antibiotics are removed.* ***b)*** *The optimal rate of spontaneous persistence.*

antibiotics, we always have $\delta^* = 0$. This is in agreement with what we observed in the synchronised case. When antibiotics are frequent and delayed, $\delta^*$ takes values within $0.03 - 0.06$.

In order to study the optimal persistence strategies as function of antibiotics stress, we vary the duration of antibiotics and fix the application time to $T_0 = 5$. The result is plotted as two new heat maps in fig. 6.4. Again we observe three regions of $(\lambda^*, \delta^*)$. For low probability or short duration of antibiotics, $\lambda^* \to 0$ and $\delta^* = 0$. For high probability and very long duration of antibiotics we have $\lambda^* \approx pT$ and $\delta^* = 0$. As in the previous figure, these are the regions we are familiar with from chapter 5 and [22]. In between these two regions, we again observe a region of $0 < \lambda^* < pT$ and $\delta^*$ approximately ranging from 0.01 to 0.06. Both transitions between the regions appear to be discontinuous. We will study these transitions more thoroughly, but first we will look at how the antibiotic characteristics differ for the three regions.

### Regions in parameter space

The three regions are highlighted in fig. 6.5 and roughly summarised in tab. 6.1.

**In region I** the antibiotic stress is low, either because it is rare, or because it is of short duration and applied significantly after the nutrients (see fig. 6.5). In the former, the application is rare enough that it is more beneficial to behave as if antibiotics are never applied to the system. In the latter, the antibiotics might be very frequent, yet the duration is short compared to the application time. The antibiotic impact is not

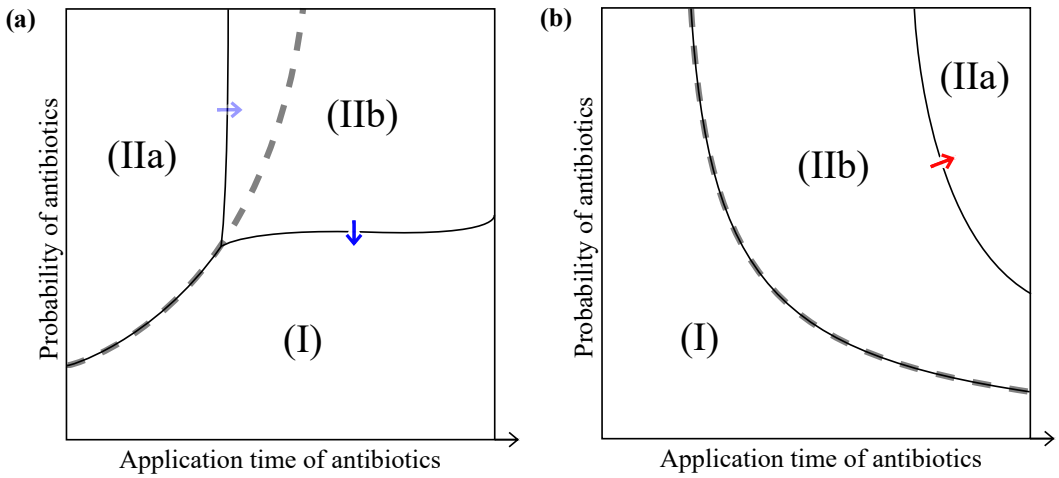


Figure 6.5: ***Three regions of optimal persistence strategies.*** *The dashed line highlights the boundaries between region I and IIa when $\delta$ is fixed to 0.* ***a)*** *The three regions in the space of probability and application time. $T_{AB} \approx 10$. The blue arrows show how the boundaries change when we increase $T_{AB}$. The stronger the color, the higher is the impact. This figure approximately corresponds to fig. 6.3.* ***b)*** *The three regions in the space of probability and duration, for $T_0 \approx 5$. The red arrow show how the boundary between region I and IIa change when we increase $T_0$. For very small $T_0$ region IIa does cover region IIb entirely, and also the upper part of region I. This figure approximately corresponds to fig. 6.4.*

| Region | Characterisation | Antibiotic parameters | $(\lambda^*, \delta^*)$ |
|---|---|---|---|
| **I** | Low stress | Low $p$ **or** (low $T_{AB}$ **and** high $T_0$) | $(0, 0)$ |
| **IIa** | High, immediate stress | High $p$ **and** low $T_0$ | $(pT, 0)$ |
| **IIb** | High, delayed stress | High $p$ **and** high $T_{AB}$ **and** high $T_0$ | $(\lambda(p, T_0, T), \delta(p, T_0, T))$ |

Table 6.1: Rough characteristics of the three regions in fig. 6.5

large enough to delay the consumption time significantly, and the priority is to wake up as fast as possible, which translates to $\lambda^* \to 0$ and $\delta^* = 0$.

**In region II** the antibiotics stress is high, as it is frequently applied and of relatively long duration compared to the delay in application time (which translates to severe antibiotic stress compared to the population density at $t = T_0$). When the antibiotics come shortly after the nutrients (See fig. 6.5a), a medium to high frequency is sufficient to trigger this region, independently of the duration of the antibiotics. For longer $T_0$, the duration of antibiotics also need to be long in order to trigger region II. Overall, $p$ or $T$ are so large that the term of $T_{S,T>T_0}$ dominates $\langle T_S \rangle_p$ (eq. 6.9).

In fig. 6.5 region II is split in two. In terms of antibiotic parameters, the main difference between the two is the delay $T_0$. Region IIa corresponds to a short delay, whereas region IIb corresponds to a long delay. For a given $T_0$, region IIa corresponds

to more extreme combinations of $p$ and $T_{AB}$ than region IIb. In region IIa the loss from waking up early is larger than the gain, and and the priority is to limit the population loss during antibiotics. With a lag time of $\lambda^* \approx pT$, spontaneous persistence provides no further benefit, which is why we also have $\delta^* = 0$. In region IIb the loss and gain from waking up early is more balanced because the antibiotics are delayed considerably.

Then we turn our attention to the transitions between the different regions, and their dependency on the antibiotic parameters. We begin with the application time $T_0$. As highlighted in fig. 6.5b, $T_0$ controls the boundary between region IIa and IIb. For $T_0$ below $\sim 4$, only region IIa exist. As $T_0$ increases there is a range where both regions exist, but above $T_0 \approx 6$ region IIa disappears[2] and is replaced by region IIb. The exact values of $T_0$ depends on $f$, that is the dilution factor, but also the maximal fold increase of one cycle. The other boundary in fig. 6.5, that is the boundary between region I and IIb, is practically independent of $T_0$.

Next, we consider how the boundaries depend on $T_{AB}$ and $p$. The two boundaries in fig. 6.5 have the same functional form, just translated. The shape is on the form $p \propto 1/T_{AB}$, but is also dependent on $T_0$.

### Optimal persistence strategy in region IIb

Finally, we study the optimal persistence strategies in region IIb in more detail, where the spontaneous persistence can be beneficial. In fig. 6.6 we have plotted the optimal lag time and rate of spontaneous persistence as function of $p$. We set $T_0 = 5$, such that this corresponds to lines in fig. 6.4. The small inset in fig. 6.6 highlights the connection between the two figures. We observe a discrete jump in $\lambda^*$ from 0 to $\approx 3$ for all $T_{AB}$. From there the lag time increases almost linearly with $p$. Just before reaching region IIa the slope increases exponentially until $\lambda^* \approx pT$. There is also a jump in $\delta^*$ from 0 to some finite value that is dependent on $T_{AB}$. Far away from region IIa $\delta^*$ increases somewhat linearly, but closer to IIa $\delta^*$ starts exponentially decreasing until $\delta^* = 0$.

When we vary $p$ the optimal persistence strategy in a cycle with or without antibiotics is constant, respectively. However, the weight given to each term in the fitness function (eq. 6.9) varies with $p$. Decreasing $p$, increases the weight of $T_{S,T=T_0}$ in $\langle T_S \rangle_p$, that is it increases the weight of the cycles without antibiotics. It is therefore not surprising that decreasing $p$ also decreases both $\lambda^*$ and $\delta^*$, and conversely the optimal parameters must increase with $p$. We note that the transition from region I to region II (i.e. the discontinuity in fig. 6.6) must happen simultaneously for $\lambda^*$ and $\delta^*$. When $p$ is small it is unlikely that $\delta > 0$ can be a good strategy, because this will decrease the effective growth rate in all cycles, and provide poor protection in the rare events of antibiotics. It therefore makes sense that $\delta^* = 0$ for all antibiotic parameters that yield $\lambda^* \to 0$. We also note that the transition from 0 to a finite value can not happen

[2]Though not completely, for $T_0 = 6$ region IIa appears around $T_{AB} \approx 48$.

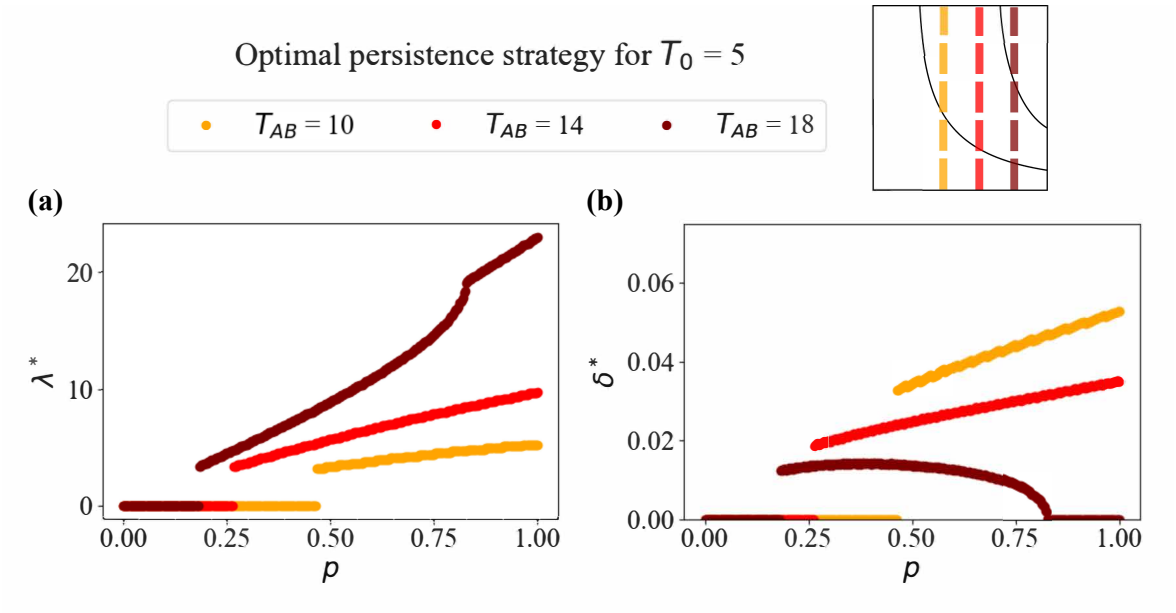


Figure 6.6: ***Optimal persistence strategy as function of*** $p$ ***for*** $T_0 = 5$**.** The small inset highlights the relation to fig. 6.4. **a)** Optimal lag time a function of probability of antibiotics. **b)** Optimal rate of spontnaeous persistence as function of probability of antibiotics.

later for $\delta^*$ than for $\lambda^*$, because in this case $\lambda^*$ would behave as in the case without spontaneous persistence, and must in this case jump directly from $\lambda^* \to 0$ to $\lambda^* \approx pT$. As we have seen, spontaneous persistence provides no further benefit when $\lambda^* \approx pT$ (see fig. 6.2). Y. Himeoka and N. Mitarai showed that for the single species model corresponding to section 5.2 there is always a critical $p$ for which the jump in optimal $\lambda^*$ occurs between region I and II. Though we do not prove this mathematically for our model, it still seems reasonable that eq. 6.9 can yield a discontinuity in $p$. For $p = 0$ the fitness is dominated by the term representing cycles without antibiotic, and the optimal persistence strategies correspond to the optimal in absence of antibiotics: $(\lambda^*, \delta^*) = (0, 0)$. Even though this might not be the optimal strategy in cycles with antibiotics, the disadvantage of $(\lambda^*, \delta^*) = (0, 0)$ during antibiotics is not noticeable for small $p$, hence this is still the overall optimal strategy. For the $p$ where $T_{S,T>T_0}$ can no longer be neglected compared to $T_{S,T=T_0}$, a continuous change in $(\lambda^*, \delta^*)$ is not enough to even out the antibiotic impact.

The second transition in fig. 6.2, from region IIb to region IIa, in continuous in $p$. Once $\lambda^*$ reaches a certain size, spontaneous persistence becomes less advantageous and $\delta^*$ starts decreasing. As $\delta^*$ decreases, it in turn becomes more important with a high lag time, hence we interpret the exponential behavior close to region IIa as resulting from a positive feedback loop between $\lambda^*$ and $\delta^*$.

Before introducing competition to the model, we consider the optimal persistence as function of $T_{AB}$. In fig. 6.7 we have plotted lines corresponding to three different $p$. As before we set $T_0 = 5$, such that these are also lines in fig. 6.4, but orthogonal

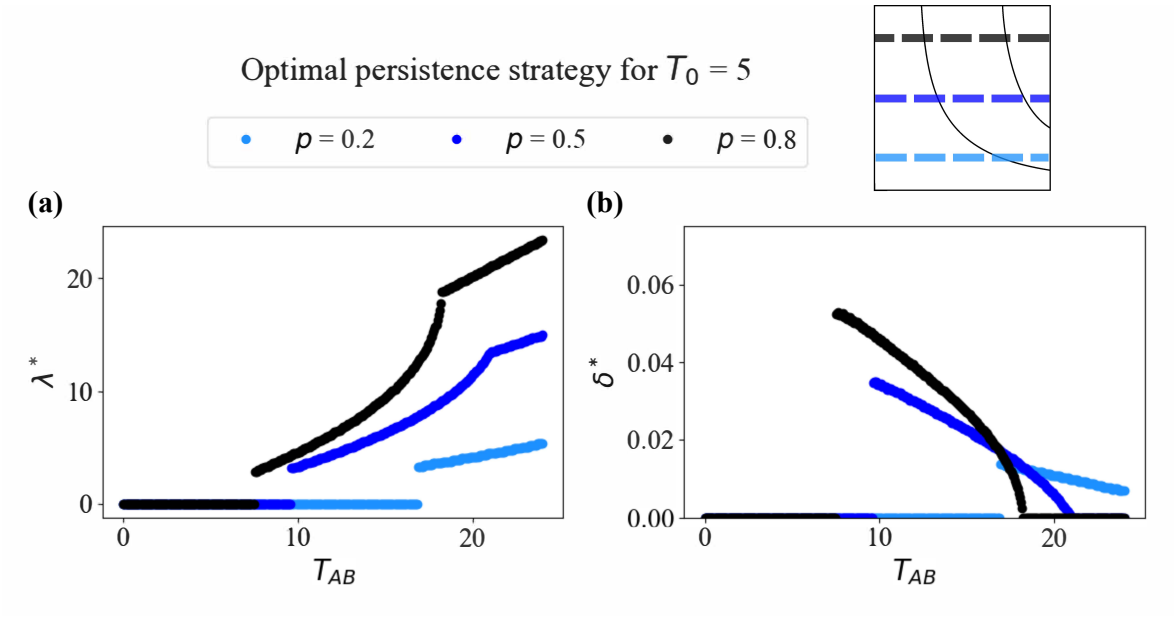


Figure 6.7: ***Optimal persistence strategy as function of*** $T_{AB}$ ***for*** $T_0 = 5$***.*** *The small inset highlights the relation to fig. 6.4.* ***a)*** *Optimal lag time a function of the duration of antibiotics.* ***b)*** *Optimal rate of spontaneous persistence as function of the duration of antibiotics.*

to those from fig. 6.6 $\lambda^*$ behaves fairly similar as in fig. 6.6. It jumps from $\lambda^* \to 0$ to $\approx 3$, then it increases somewhat linearly before it $\lambda$ starts increasing exponentially close to region IIa. $\delta^*$ also does a discontinuous jump from region I to IIb, after which it decreases for all $T_{AB}$, that is opposite of what we observed as function of $p$ in fig. 6.6. Far away from region IIa the decrease appears to be linear, before it decreases exponentially until $\delta^* = 0$.

Increasing $T_{AB}$ does not affect the weights of $T_{S,T=T_0}$ and $T_{S,T>T_0}$ in $\langle T_S \rangle_p$. However, it increases the antibiotic impact of one cycle. This affects both the optimal persistence strategy of cycles with antibiotics, and the length of $T_{S,T>T_0}$. For a given $p$ it is therefore not surprising that $\lambda^*$ increases with antibiotic duration. On the contrary $\delta^*$ decreases, to counteract the slow consumption resulting from a longer lag time on the cycles without antibiotics. In other words, as $\lambda^*$ increases it becomes more important for the population to have a high effective growth rate since the frequency of antibiotics does not increase. The optimal parameters display the same discontinuous jump and kink in fig. 6.7 as they do in fig. 6.6. The discontinuity makes sense from the same arguments as in fig. 6.6, since for $T_{AB} = 0$ we trivially have $(\lambda^*, \delta^*) = (0, 0)$. The arguments for why $(\lambda^*, \delta^*)$ in region II are $C^0$ functions of $p$ also applies to $T_{AB}$.

Importantly, we emphasise once more that in this model triggered persisters and spontaneous persisters wake up at the same rate. The advantage of spontaneous persistence in a model of stochastic antibiotics is that the population can wake up earlier, and thus grow more in cycles without antibiotics, and still maintain some protection

in cycles with antibiotics. We would therefore expect that spontaneous persistence is optimal when coupled with a relatively short lag time. This is also what we have observed, with $\lambda^*$ in region IIb being lower than $\lambda^*$ in region IIa. If spontaneous persistence is not allowed in the model, the entire region IIb becomes region IIa. However, a short lag time also constrains the spontaneous persisters to wake up shortly after they went dormant, necessitating a relatively large rate of spontaneous persistence in order to maintain the benefit of persistence. This might therefore partially explain why the rate of spontaneous persistence that our model yields, is higher than what is observed experimentally. In a model with separated wake-up rates, spontaneous persistence might therefore be beneficial, even in the case without an antibiotic delay.

## 6.2 Competition optimal strategy

We now extend the results from previous section to include competition between two phenotypes. As we have seen, with the addition of spontaneous persistence effective growth rate becomes species specific. This is why we can no longer isolate $T_S$, and accordingly not write down a cycle independent expression for the competition fitness $F_{II}^i$. As mentioned in the beginning of section 6.1, as the model is now too complicated to be solved analytically, we will now study the optimal persistence strategy for competition numerically.

### 6.2.1 Numerical setup

Like in section 5.2.2 we use average consumption fraction as a numerical measure of fitness. In the simulations we let the single species optimal $(\lambda^*, \delta^*)_I$, with the optimal parameters corresponding to a given set of antibiotic parameters, compete against a competitor with $(\lambda, \delta) = (\lambda_i, \delta_j)$. Each set of $(\lambda_i, \delta_j)$ corresponds to an entry in a $N_\lambda \times N_\delta$ matrix of bacterial parameters, where $\lambda_i = 10^{-2} + i\Delta_\lambda$ and $\delta_j = j\Delta_\delta$. We set $N_\lambda = 100$ and $\Delta_\lambda = T/100$, such that $\lambda_i$ ranges from 0.01 to $T$. Likewise, we set $N_\delta = 100$ and $\Delta_\delta = 10^{-3}$, meaning $\delta$ ranges from 0 to 1. We let the matrix of competitors compete against the single species optimal in parallel for $N_c$ cycles. After every cycle we compute the consumption fraction of the single species winner and its competitor. Finally, we take the cycle average of the consumption fraction, such that the numerical fitness measure becomes[3]

$$F_{II}(\lambda_i, \delta_i; \lambda_j, \delta_j; \gamma, p, T_0, T, f) = \frac{1}{N_c} \sum_c^{N_c} \frac{1}{S_0} \left( g_{i,c}(T_S) - g_{i,c}(T) + g_{i,c}(T_0) \right). \tag{6.10}$$

[3]Here, we have not writen out the explicit dependencies. $g_{i,c}(T_S) = g(d_0, T_S; \lambda_i, \delta_i; \gamma, T_0, T)$, where $d_0$ and $T_S$ are functions of all parameters in the system, in addition to the sequence of cycles with and without antibiotics before the current cycle $c$, that is the "history" of antibiotic applications. When we let the number of cycles become very large ($N_c \to \infty$), this sequence dependency reduces to a dependency on $p$.

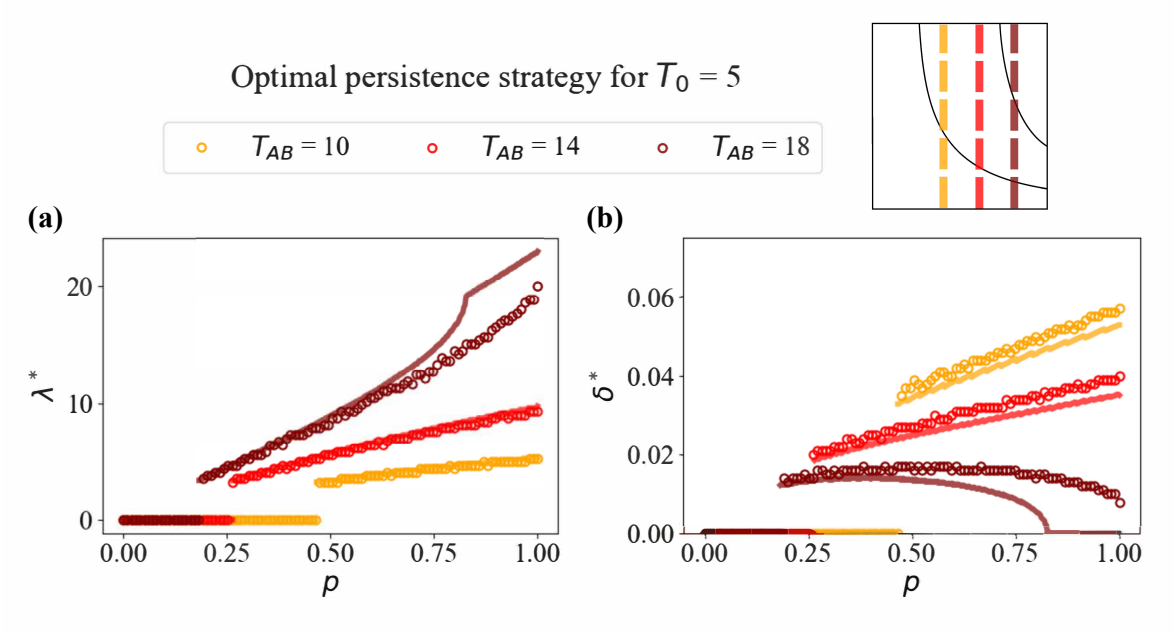


Figure 6.8: ***Optimal persistence strategy during competition as function of*** $p$ ***for*** $T_0 = 5$***.*** *The circles denote the competition optimal from* $F^i_{II}$*, and the solid lines denote the single species optimal from* $F^i_I$*. The small inset highlights the relation to fig. 6.4.* ***a)*** *Optimal lag time a function of the probability of antibiotics.* ***b)*** *Optimal rate of spontaneous persistence as function of the probability of antibiotics.*

After $N_c$ cycles we pick the set of $(\lambda_i, \delta_j)$ that yields the largest value for eq. 6.10 as the set of optimal competitor parameters. If the consumption fraction of the optimal competitor is $\approx 1$, this is not only the strongest competitor, but the optimal persistence strategy when taking competition into account. We run each competition for $N_c = 10^4$ cycles. Since we solve this model numerically, we do not make use of the approximation in section 6.1.

### 6.2.2 Results

We find that the single species optimal $(\lambda^*, \delta^*)$ from the previous section to a large extent is also the optimal persistence strategy during competition. Since the single species fitness $F_I(\lambda, \delta; \gamma, p, T_0, T, f)$ is defined as the inverse average consumption time for a fixed nutrient, and consuming the nutrients fast is an obvious advantage during competition, this is not very surprising. In fig. 6.8 we have plotted the optimal persistence strategy according to eq. 6.10 against that from eq. 6.9. The circles represent the competition optimal $(\lambda^*, \delta^*)_{II}$, whereas the solid lines represent the single species optimal $(\lambda^*, \delta^*)_I$ corresponding to those in fig. 6.6. The circles do not form a completely smooth curve, stemming from both a low resolution on $\lambda$ and $\delta$, and a finite number of cycles. The complete heat map of competition optimal persistence

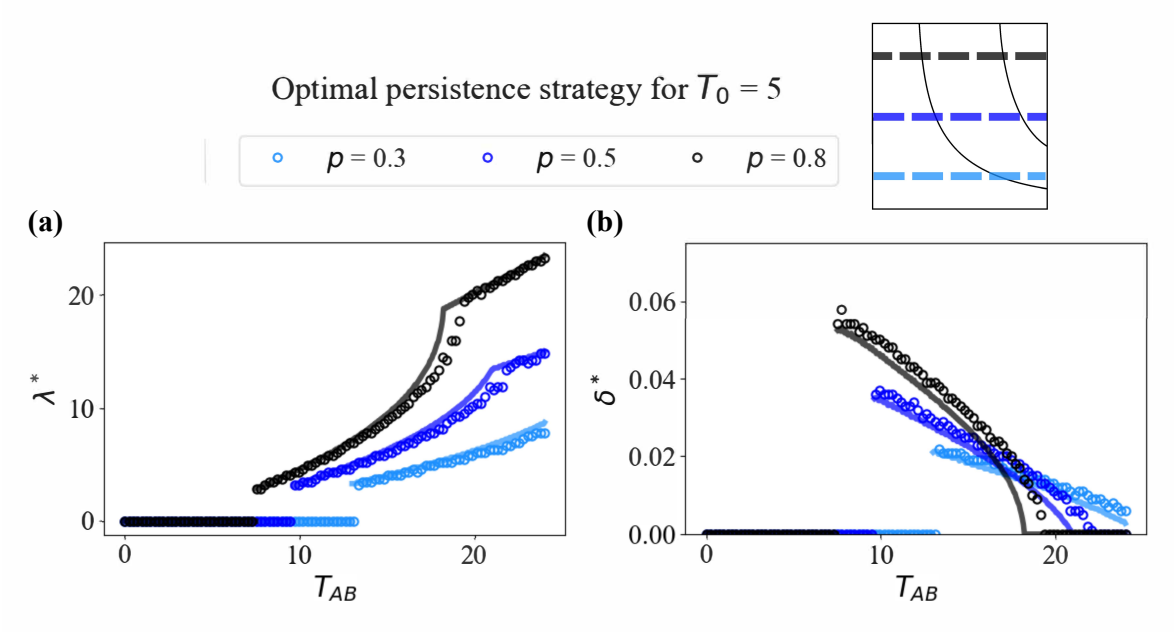


Figure 6.9: ***Optimal persistence strategy during competition as function of*** $T_{AB}$ ***for*** $T_0 = 5$***.*** *The circles denote the competition optimal from* $F^i_{II}$*, and the solid lines denote the single species optimal from* $F^i_I$*. The small inset highlights the relation to fig. 6.4.* ***a)*** *Optimal lag time a function of the duration of antibiotics.* ***b)*** *Optimal rate of spontaneous persistence as function of the duration of antibiotics.*

strategies can be found in appendix B.3.

The competition optimal display the same three regions as described in the previous subsection. In region I $(\lambda^*, \delta^*)_{II} = (\lambda^*, \delta^*)_I = (0, 0)$, and the jump to region IIb happens simultaneously in the two setups. Far away from region IIa, the optimal lag time is approximately the same, that is $\lambda^*_{II} \approx \lambda^*_I$. However, as we approach region IIa we observe that the single species optimal $\lambda^*_I$ increases faster than $\lambda^*_{II}$. The transition to $\lambda^* \approx pT$ thus occurs later for $\lambda^*_{II}$ than for $\lambda^*_I$. From fig. 6.8 we find that competition appears to have a larger impact on the optimal rate of spontaneous persistence than on the optimal lag time. $\delta^*_{II}$ is a little larger than $\delta^*_I$ in all of region IIb, and this difference seems to increase slightly with $p$.

Then we look at $(\lambda^*, \delta^*)_{II}$ as function of $T_{AB}$, and compare this to $(\lambda^*, \delta^*)_I$ from fig. 6.7. In fig. 6.9 we have plotted $(\lambda^*, \delta^*)_{II}$ and $(\lambda^*, \delta^*)_I$ as function of $T_{AB}$ correspond to those in fig. 6.7. We observe the same here as in fig. 6.8, namely that the transition from IIb to IIa occurs later for $(\lambda^*, \delta^*)_{II}$ and that $\delta^*_{II} > \delta^*_I$ in region IIb. The transition is less delayed in $T_{AB}$ than in $p$, which makes sense when we consider the shape of the boundary in fig. 6.4. In the range of $T_{AB}$ that we consider, the boundary is much steeper as function of $T_{AB}$ than of $p$. Delaying the transition will therefore affect $(\lambda^*, \delta^*)$ a lot in a small range of $p$, and affect $(\lambda^*, \delta^*)$ a little for a large range of $T_{AB}$.

In chapter 5 about triggered persistence we argued that the competition optimal and

single species optimal was the same as long as the dormant population is negligible when the population enters the stationary state. An important condition for this was that the competing phenotypes had the same growth rate. When now allowing spontaneous persistence, we have seen that the growth rate becomes species specific. Furthermore, with the rate of growing species that re-enter dormancy (i.e. when $\delta > 0$), the dormant populations might no longer be negligible at $T_S$. Therefore, it is no longer clear whether $(\lambda^*, \delta^*)_{II}$ should be identical to $(\lambda^*, \delta^*)_I$ or not. At the same time triggered persisters are expected to be far more common than spontaneous persisters hence results implying that the dormant (persistent) population is no longer negligible would be unphysical. Based on experimental observation, the rate of spontaneous persistence is expected to be in the range $10^{-6} - 10^{-3}$. This range corresponds to an effective growth rate of $b \approx 1$, though the effective growth rate we observe from our simulations are in actually in the range $b \approx 0.95 - 0.99$.

The differences $(\lambda^*, \delta^*)_{II}$ and $(\lambda^*, \delta^*)_I$ are likely to stem from the effective dilution factor or maximal fold increase in the two setups. We have set $f$ to $10^{-6}$ in both models, but since there are two species in the competition setup the nutrients will be consumed faster, which roughly translates to $f_{eff} = f/2$ in cycles where the species have approximately the same population density. The difference in optimal persistence strategy of a model with $f$ and $f/2$ can be found in appendix B.3. We can consider the exponential phase as a linear function with a negative offset dependent mostly on $\lambda$, and a slope that depends negatively on $\delta$. The optimal parameters are the parameters of the line that first crosses some threshold value, determined by $f$. If we set the threshold infinitely high, the line with the steepest slope will always be the first to cross the threshold, independently of its offset. However, for a finite threshold there can be a line of less steep slope with a smaller offset that reaches the threshold first, depending on whether the two lines intersect before or after the threshold. As the threshold value decreases, the two lines are less likely to intersect before the threshold, hence it becomes more important to reduce the (negative) offset than to have a steep slope. It therefore seems reasonable that for an lower maximal fold increase $f$, the transition from region IIb (with smaller offset and less steep slope) to region IIa (large negative offset, steep slope) occurs later. This interpretation further signalises that the competition optimal strategy depends on the number of competitors, since the maximal fold increase with fixed nutrients is $\approx S_0/\sum_i n_i(0)$. In the same line of arguments, $\delta^*_{II}$ should be higher than $\delta^*_I$, and inversely $\lambda^*_{II}$ should be shorter than $\lambda^*_I$. This corresponds to what we observe in figs. 6.8–6.9 for the rate of spontaneous persistence. It is therefore more surprising that $\lambda^*$ is approximately the same in the two setups, however this simplistic explanation is not capable of capturing the full complexity of the equations in section 6.1. It seems intuitive that waking up earlier can be a competitive advantage, however this is seemingly just the case for severe antibiotics.

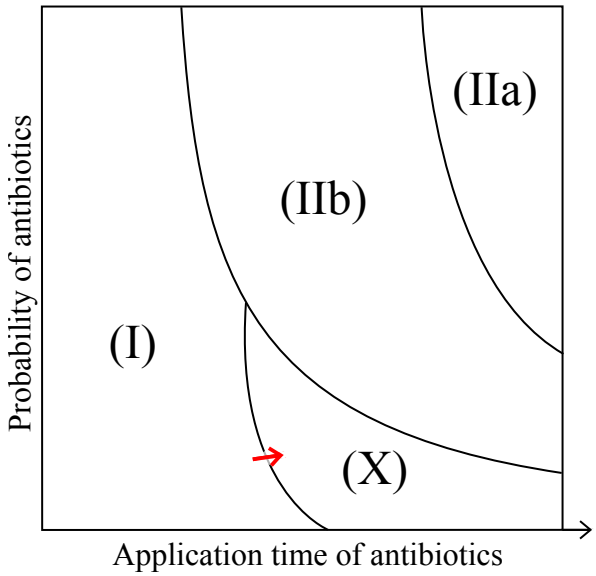


Figure 6.10: ***Suggested 4th region of high risk of extinction.*** *The red arrow highlights how we expect region X the change with $T_0$, that is we expect this region to appear at longer antibiotic duration the longer the delay.*

### 6.2.3 Region of vulnerability to extinction

Like in the chapter about triggered persistence, we are not considered extinction. In region I the optimal strategy is to ignore the antibiotics, i.e. a strategy that is very vulnerable to extinction. In fig. 6.10 we have highlighted the region where we expect the optimal $(\lambda^*, \delta^*)$ to change if we include extinction. Region X is where the frequency of antibiotics is very low, hence the optimisation of $(\lambda^*, \delta^*)$ is dominated by cycles without antibiotics despite the duration being rather long. Populations with $(\lambda, \delta) \approx (0, 0)$ are therefore very vulnerable to extinction during the rare antibiotic events. Specifically, we expect the optimal persistence strategy to be different where $T_{AB} > T_0 + \log\left[fS_0/n_{min}\right]$ because this is where a species with initial density $fS_0$ and parameters $(\lambda, \delta) = (0, 0)$ would go extinct with just one cycle of antibiotics. The red line highlights how we expect this region boundary to change with $T_0$.

## 6.3 Evolution of average lag time and rate of spontaneous persistence

Once more we verify the optimal persistence strategy with simulations evolving $\lambda$ and $\delta$ through mutation. We extend eq. 5.15 by adding spontaneous persistence, also allowing mutation in $\delta$. For $t > T$ and $S(t) > 0$ the equations in the growth phase now

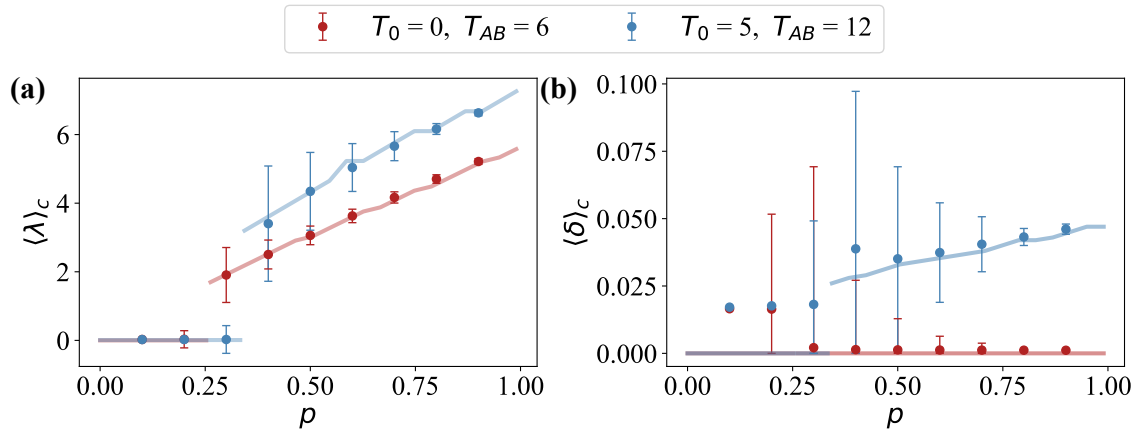


Figure 6.11: ***Average lag time and rate of spontaneous persistence from simulation with mutation and no extinction.*** *The rate of mutation is $\varepsilon = 10^{-3}$ and the simulation is without extinction. Lines represent $\lambda^*_{II}$ and $\delta^*_{II}$, and the dots represent the mutation average, with the standard deviation as error bars. We use cycles $10^3 - 10^4$ in the average, to allow the system to settle. Red denotes a simulation with synchronised nutrients and antibiotics, whereas blue denotes a simulation with $T_0 = 5$.* ***a)*** *The average lag time.* ***b)*** *The average rate of spontaneous persistence.*

become

$$\frac{d}{dt}g_{i,j}(t) = d_{i,j}(t)/\lambda_i + \left[1 - \delta_j - \alpha\varepsilon\right] g_{i,j}(t) + \varepsilon \sum_{k=1,-1} \left[g_{i+k,j}(t) + g_{i,j+k}(t)\right] \tag{6.11}$$

where $\alpha \in 2, 3, 4$ depending on the number of nearest neighbours in the matrix, and $g_{-1,j}(t)$, $g_{N,j}(t)$, $g_{i,-1}(t)$, $g_{i,N}(t)$ are 0 for all $t$. Our system is now a matrix where every entry corresponds to a of combination of lag time and rate of spontaneous persistence, $(\lambda_i, \delta_j)$, that mutate to the nearest neighbour entries. We let $\lambda_i$ range from $10^{-2}$ to $T = T_0 + T_{AB}$ with step size $\Delta_\lambda = 0.2$, such that $\lambda_i = 10^{-2} + i\Delta_\lambda$. Likewise, we let $\delta_j$ range from 0 to 0.2 with step size $\Delta_\delta = 0.01$, such that $\delta_j = j\Delta_\delta$.

As previously, we compute the average $\lambda$ and $\delta$ at each iteration and let the system evolve for $10^4$ cycles. After every cycle we compute the average lag time weighted after the population densities in the system

$$\langle\lambda\rangle = \frac{\sum_{i,j} \lambda_i \cdot n_{i,j}(T_S)}{\sum_{k,l} n_{k,l}(T_S)}, \qquad \langle\delta\rangle = \frac{\sum_{i,j} \delta_j \cdot n_{i,j}(T_S)}{\sum_{k,l} n_{k,l}(T_S)}.$$

### 6.3.1 Simulations without extinction

First we let the system evolve without extinction, for both a synchronised and desynchronised setup. We initialise the simulation from $(\lambda_0, \delta_0) = (10^{-2}, 0)$, since without

extinctions this is not an absorbing state. In fig. 6.11 we have plotted the cycle average of the average $\lambda$ and $\delta$ at each iteration. That is

$$\langle\lambda\rangle_c = \frac{1}{N_c}\sum_c \langle\lambda\rangle, \qquad \langle\delta\rangle_c = \frac{1}{N_c}\sum_c \langle\delta\rangle. \tag{6.12}$$

We take the average from the 1000th cycle, in order to avoid effects from the transient period before system has stabilised.

The synchronised case we have set $T_0 = 0$ and $T_{AB} = 6$, the same antibiotic parameters as used in fig. 5.4. Here the optimal persistence strategy is $(\lambda^*, \delta^*)_{II} = (\lambda_I^*, 0)$, and is represented by the red lines in fig. 6.11. The lines are not smooth because both the resolution on the parameters, and the number of cycles were relatively low. The red dots represent the averages $\langle\lambda\rangle_c$ and $\langle\delta\rangle_c$, with the standard deviation as error bars. With the error bars most of the mutation averages are in accordance with $(\lambda^*, \delta^*)_{II}$. The exception is for the rate of spontaneous persistence at $p \leq 0.2$, which stems from the asymmetric distribution of population densities around the optimal $\delta^* = 0$.

The system displays frequent fluctuations around $p_c$, where the discontinuity in $\lambda^*$ occurs, but decreases rapidly away from the jump in $\langle\lambda\rangle_c$ The large uncertainties indicate that there are several "good" persistence strategies in this region. In cycles with antibiotics populations with $\delta$ increase rapidly, and in cycles without antibiotics they decline again. The relative uncertainty is much larger on $\langle\delta\rangle_c$ than $\langle\lambda\rangle_c$. This reflects that around the critical probability $(\lambda^*, \delta^* \pm \Delta_\delta)$ is a better strategy than $(\lambda^* \pm \Delta_\lambda, \delta^*)$. The evolution of average lag time and rate of spontaneous persistence from which fig. 6.11 is taken can be found in appendix B.3.

The blue lines and dots represent the desynchronised simulation, where we have set $T_0 = 5$ and $T_{AB}$. For this set of antibiotic parameters, we expect spontaneous persistence to be optimal for all $p > p_c$ as demonstrated by the solid blue lines. Again, the mutation averages are in mostly in accordance with $(\lambda^*, \delta^*)_{II}$, expect for when $\delta^* = 0$. $\langle\lambda\rangle_c$ is now fluctuating even more frequently around $p = p_c$, but approaches $\lambda_{II}^*$ for higher probabilities. A similar behaviour is observed for $\langle\delta\rangle_c$. Around $p = p_c$ we observe that $\langle\delta\rangle_c > \delta_{II}^*$, but as $p$ increases, $\langle\delta\rangle_c$ approaches the expected value $\delta_{II}^*$.

Qualitatively, the averages behave similarly in the two regions, that is fluctuations in both $\langle\lambda\rangle_c$ and $\langle\delta\rangle_c$ around the critical $p_c$, and for other $p$ both simulations stabilise around the optimal values from section 6.1-6.2. The reason that the desynchronised setup displays more fluctuations than the synchronised setup might be that with the delay in antibiotics, the loss and gain from waking up early is more balanced, hence the optimal strategy is less well-defined. Note that the arithmetic mean and standard deviation as used here, do not provide a good description of the underlying distributions, since they are not symmetric (see B.3).

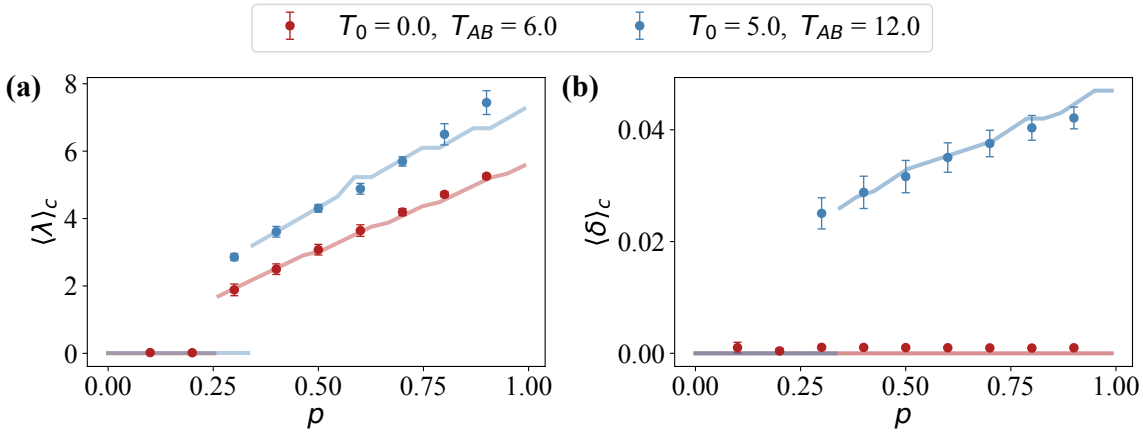


Figure 6.12: ***Average lag time and rate of spontaneous persistence from simulation with mutation and extinction.*** *The rate of mutation is $\varepsilon = 10^{-3}$ and the simulation is without extinction. Lines represent $\lambda^*_{II}$ and $\delta^*_{II}$, and the dots represent the mutation average, with the standard deviation as error bars. We use cycles $10^3 - 10^4$ in the average, to allow the system to settle. Red denotes a simulation with synchronised nutrients and antibiotics, whereas blue denotes a simulation with $T_0 = 5$.* ***a)*** *The average lag time.* ***b)*** *The average rate of spontaneous persistence.*

### 6.3.2 Simulations with extinction

Then we include extinction, expecting that spontaneous persistence is more important here, even though mutation also act as some security against extinction, since an extinct species might reappear through mutation. We observe the same as in section 5.4, namely that the system gets stuck in the initial state when the duration is not long enough to kill the initial species, and otherwise they all go extinct (See appendix B.3 for figures). We therefore initialise the system from $(\lambda_{N-1}, 0)$, using the same extinction threshold as before, namely $n_{min} = 1$. In fig. 6.12 we have plotted the average lag time and rate of spontaneous persistence $\langle\lambda\rangle_c$ and $\langle\lambda\rangle_c$ for the same antibiotic parameters as in fig. 6.11. Again, the dots and error bars represent the average lag time and rate of spontaneous persistence according to eq. 6.12. The solid lines denote the optimal strategies $\lambda^*_{II}$ and $\delta^*_{II}$. The most prominent difference, is the standard deviations that are much smaller when we include extinction. Considering the results from section 5.4, this is not surprising, since when we include a lower threshold, the mutations will often go extinct in the same round as they appear. Hence, the system with extinctions is less volatile than the system without extinctions, which is reflected in the standard deviation.

The system evolves much slower when we include extinctions, and it takes long to converge to a steady state. We therefore take the average of only the 3000 last cycles to avoid too much influence from the transient period, though we should also increase the total number of cycles. Overall, $\langle\lambda\rangle_c$ and $\langle\delta\rangle_c$ follow the curve of the previously

computed optimal, but there are a few differences in the simulation of desynchronised nutrients and antibiotics. First, both $\langle\lambda\rangle_c$ and $\langle\delta\rangle_c$ seem to be stuck in some local optimum for $p = 0.3$. This is similar to what we observed in section 5.4.2 for the evolution of average lag time of in the simulation with extinction. For antibiotic parameters corresponding to the synchronised case here (fig. 6.12) we observe $\langle\lambda\rangle_c \approx pT$ for $p = 0.2$, for which we actually expect $\lambda^* = 0$. This suggests that this is a stochastic effect stemming from a finite number of total cycles. Close to the critical $p$ the system takes longer to converge. A second difference from the synchronised case is that there are no averages for $p = 0.1$ and $p = 0.2$. Here, all populations go extinct in what appears to be a "tragedy of the commons". The $\langle\lambda\rangle$ and $\langle\delta\rangle$ decreases during cycles without antibiotics, but eventually becomes too small to survive one cycle with antibiotics, thus goes extinct.

### 6.3.3 Three-state model

Throughout this project, we have mentioned several times that we model spontaneous and triggered persisters as the same dormant state, with the same wake-up rate $1/\lambda$. We believe that a more realistic model would allow three separate states or subpopulations:

- $\boldsymbol{d(t)}$: a dormant population waking up at the rate $1/\lambda$ representing triggered persisters.
- $\boldsymbol{g(t)}$: a growing population of antibiotic sensitive cells.
- $\boldsymbol{r(t)}$: a population of spontaneous persisters waking up at the rate $1/\omega$.

Though temporal constraints prevent us from exploring this model in detail, we still allow ourselves to evolve such a model through mutation, comparing it the the previous subsections. Before adding mutation, the differential equations of the system in absence of antibiotics read

$$\frac{d}{dt}d_i(t) = -d_i(t)/\lambda, \qquad \text{if } S(t) > 0, \tag{6.13}$$

$$\frac{d}{dt}g_i(t) = d_i(t)/\lambda + (1-\delta_i)\cdot g_i(t) + r_i(t)/\omega_i, \qquad \text{if } S(t) > 0, \tag{6.14}$$

$$\frac{d}{dt}r_i(t) = \delta_i g_i(t) - r_i(t)/\omega_i, \qquad \text{if } S(t) > 0. \tag{6.15}$$

Though $r(t)$ is equivalent to the dormant population in that the spontaneous persisters do not grow or die, $r(t)$ is distinguished from $d(t)$ by separate wake-up rates. Here, $1/\omega_i$ is the wake-up rate from spontaneous persistence and otherwise all other parameters are the same as previously. In cycles with antibiotics, we have

$$\frac{d}{dt}g_i(t) = d_i(t)/\lambda - (\gamma+\delta_i)\cdot g_i(t) + r_i(t)/\omega_i \text{ if } T_0 < t < T. \tag{6.16}$$

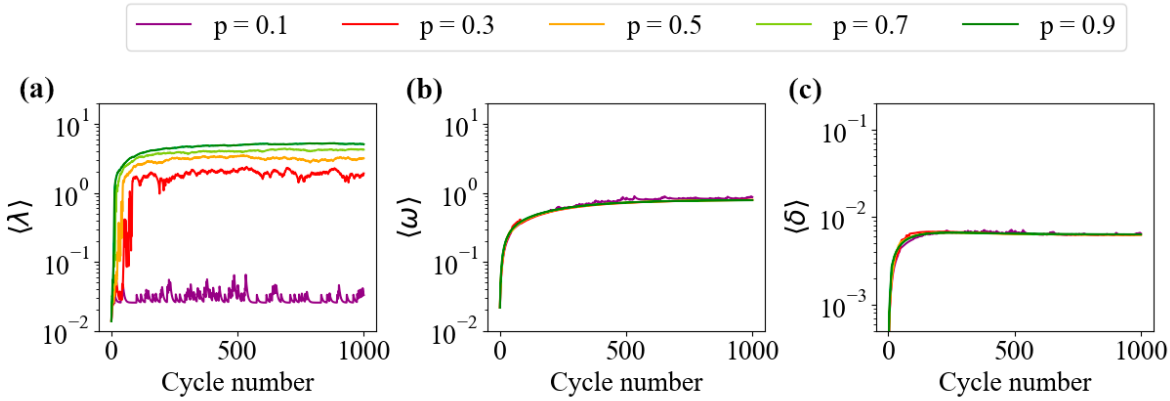


Figure 6.13: ***Evolution of average persistence strategy for*** $T_0 = 0$ ***and*** $T_{AB} = 6$***.*** *The simulation is without extinctions and with the mutation rate* $\varepsilon = 10^{-3}$*.* ***a)*** *The evolution of average lag time from dormancy.* ***b)*** *The evolution of average time spent in the state of spontaneous persistence.* ***c)*** *The evolution of average rate of spontaneous persistence.*

The nutrient follows the same differential equation as in eq. 6.4.

We allow mutations to occur in all persistence parameters, that is $\lambda_i$, $\delta_i$, and $\omega_i$. This also means that the matrix that represented the system of competing phenotypes in previous subsections is now replaced with a 3-dimensional tensor. With mutation, the differential equation of the growing population in absence of antibiotics becomes

$$\begin{aligned}\frac{d}{dt}g_{i,j,l}(t) = \frac{d_{i,j,l}(t)}{\lambda_i} + \left[1 - \delta_j - \alpha\varepsilon\right] \cdot g_{i,j,l}(t) + \frac{r_{i,j,l}(t)}{\omega_l} \\ + \varepsilon \sum_{k=1,-1} \left[g_{i+k,j,l}(t) + g_{i,j+k,l}(t) + g_{i,j,l+k}(t)\right].\end{aligned} \tag{6.17}$$

Here, $\alpha = 3, 4, 5$ or 6, depending on the number of nearest neighbours. As in all previous simulations, we set mutation rate to $\varepsilon = 10^{-3}$. We use the same range of $\lambda$ and $\delta$ as before, and discretise $\omega$ like $\lambda$, assuming that the same range of values are meaningful for the two lag times. That is $\lambda_i = 10^{-2} + i\Delta_\lambda$, $\omega_l = 10^{-2} + l\Delta_\lambda$ and $\delta_j = j\Delta_\delta$ Now, we consider only the evolution without extinction, and let the system evolve from $(\lambda_0, \omega_0, \delta_0) = (0.01, 0.01, 0)$. Because the system now can contain more than $10^4$ species and differential equations, and we are restricted by temporal constraints, we only let the system evolve for 1000 cycles. This is not ideal, especially considering previous results from simulations with extinction indicating that $10^4$ cycles is not enough to allow the system to converge.

We first use the synchronised case corresponding to $T_0 = 0$ and $T_{AB} = 6$. The evolution of average persistence parameters can be observed in fig. 6.13. The average lag time still corresponds to the optimal lag time as from the model of only triggered persistence. This is perhaps not so surprising, as $d(t)$ in this model reduces to that in the model of triggered persistence in section 5.2. In addition, with $T_0 = 0$ and

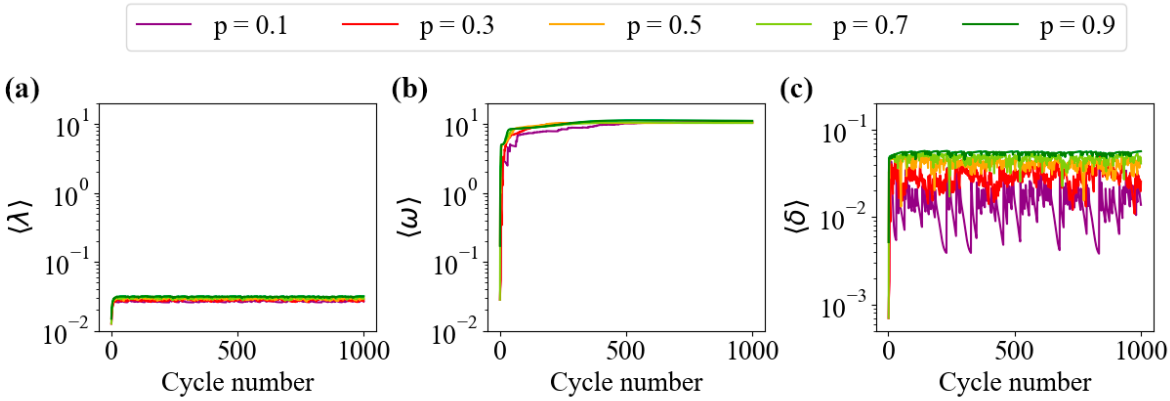


Figure 6.14: ***Evolution of average persistence strategy for*** $T_0 = 5$ ***and*** $T_{AB} = 12$***.*** *The simulation is without extinctions and with the mutation rate* $\varepsilon = 10^{-3}$*.* ***a)*** *The evolution of average lag time from dormancy.* ***b)*** *The evolution of average time spent in the state of spontaneous persistence.* ***c)*** *The evolution of average rate of spontaneous persistence.*

no extinction, spontaneous persistence has limited relevancy. Furhtermore, $T_{AB} = 6$ corresponds to somewhat mild antibiotics as any species with $\lambda \approx 0$ and initial density $n(0) = f \cdot S_0$ can survive the antibiotics. The average wake-up rate from spontaneous persistence is low, though higher than the lowest $\langle\lambda\rangle$, and seems to be independent of $p$. This is also the case for $\langle\delta\rangle$. The main difference from the equivalent simulation of the other models is the lack of fluctuations around $p_c$, though with only 1000 cycles, this might simply be a stochastic result. Note also that all phenotypes with $(\lambda^*, \omega_l, \delta_0 = 0)$ are the same species, independent of the value of $\omega_l$, because $\delta = 0 \Rightarrow r(t) = 0$.

Lastly, we desynchronise antibiotics and nutrients, setting $T_0 = 5$ and $T_{AB} = 12$ as previously. The evolution of average persistence parameters can be observed in fig. 6.14. Now we observe the strategy that was suggested in the end of section 6.1, namely a short $\langle\lambda\rangle$ coupled with finite $\langle\delta\rangle$ and long $\langle\omega\rangle$. However, contrary to what was assumed, $\langle\delta\rangle$ is still much larger than experimental values. The two wake-up rates are approximately independent of $p$, with $\langle\lambda\rangle \approx 3 \cdot 10^{-2}$, that is the same as for $p = 0.1$ in the synchronised case. We observe that $\langle\omega\rangle \approx 10$, which is larger than all $\langle\lambda\rangle$ in the model with only one wake-up rate. Only $\langle\delta\rangle$ seem to vary with $p$, though still within a range that is higher than experimentally observed. Surprisingly, even the when $p = 0.9$ is the average $\langle\lambda\rangle \approx \lambda_0$. It would be interesting to study this three-state model for $0 < T_0 < 5$, in order to study the transition between fig. 6.13–6.14.

Overall, it would be useful to study this last model in more details. Now $d(t)$ is the same as in the model with triggered persistence in section 5.2, and the dynamics between $g(t)$ and $r(t)$ are the same as in the previous model of spontaneous persistence. Eqs. 6.13–6.16 describe a set of inhomogeneous second order differentials equations, which we can evaluate according to the fitness described in section 6.1.

# Chapter 7

# Summary and conclusions

We have shown that the optimal strategy of triggered persistence does not change when we introduce fixed nutrients and competition between phenotypes for nutrients. This reflects the fact that triggered persistence does not affect the effective growth rate of a species, and that even though waking up early can be a competitive advantage in cycles without antibiotics, it can also be a competitive disadvantage in cycles with antibiotics. This seems to be the case, independently of the distribution of lag times and duration of the antibiotic application, though the general model with triggered persistence is only treated superficially. Our results therefore strengthen the significance of the previous work by Y. Himeoka and N. Mitarai, suggesting that their work is more general than what their assumptions suggest. This is encouraging, since the existence of a discontinuity suggests that the application of antibiotics can be streamlined to be as efficient as possible, while avoiding the evolution of more tolerant species.

After extending our model to allow also spontaneous persistence, we have seen that whether spontaneous persistence can be beneficial or not depends on how delayed the antibiotics are compared to the replenishment of nutrients. Only when the delay is significant can a population gain from having spontaneous persisters, though what we find to be the optimal rate of spontaneous persistence is significantly higher than the experimentally observed rate. The optimal finite rate of spontaneous persistence is coupled with a lower optimal lag time relative to the model without spontaneous persistence. We observe a discontinuous jump in both lag time and rate of spontaneous persistence, much like what was found in the model of starvation-induced triggered persistence, suggesting that also evolution of the fraction of spontaneous persistence can be avoided. These findings thus support the assumption that spontaneous persistence can be beneficial, but disagree with experimentally found rates [17, 18]. Furthermore they disagree with research suggesting that spontaneous persistence is beneficial when stress arrives at low frequency. This is possible linked to the fact that we do not treat extinctions, meaning a species can always recover from any stress. In such a setup there is not much benefit from very low rates of spontaneous persistence, as any species can survive antibiotics even without antibiotics.

## 7.1 Outlook

There are two main extensions to this project that are very obvious, as they have been referred to throughout the entire project That is the inclusion of an extinction threshold and distinguishing the two persister types with the introduction of separate wake-up rates. We believe extinction to be especially significant for the optimal persistence strategy in regions of rare, but severe antibiotics. Furthermore we believe that extinction might alter the significance of the delay $T_0$ of the antibiotics, since in our model the main incentive for spontaneous persistence is the possibility of growing before the antibiotics. Hence, in our project spontaneous persistence does not really act as bet-hedging, but in a model with extinction it could.

In the very last section, we did a minimal investigation of the three-state model, that is a model that distinguishes spontaneous persisters from triggered persisters through the introduction of separate lag times. While the results from the synchronised simulation were very similar to those from the earlier models, the results from the desynchronised simulation were not. A more thoroughly investigation of this model would therefore be interesting.

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# Appendices

# Appendix A

# Appendices to chapter 3

## A.1 Approximation in section 5.2.1

We investigate the approximation $\lambda d(T_S)/(1+\lambda) \approx 0$ numerically. $\lambda d(t)/(1+\lambda)$ in monotonically decreasing with $t$. The smallest value $T_S$ can take, and thus the largest value of $d(T_S)$, is obtained in the case without antibiotics[1]. We now compute a lower limit on $T_S$ by integrating eq. (5.10) in $t \in [0, T_S]$, and setting it equal to 0. For a cycle without antibiotics, this yields

$$\begin{aligned} S_0 - \sum_k g_k(T_S) &= 0, \\ \Rightarrow S_0 - \sum_k \frac{d_k(0)}{1+\lambda_k}\left(e^{T_S} - e^{-T_S/\lambda_k}\right) &= 0. \end{aligned} \tag{A.1}$$

From a physical point of view it intuitively clear that the shorter the lag time is, the faster the nutrient is consumed, why the lower limit on $T_S$ must correspond to the shortest $\lambda_k$. Mathematically, we have that $e^{-T_S/\lambda_k}$ is monotonically increasing with $\lambda_k$, hence $e^{T_S} - e^{-T_S/\lambda_k}$ is decreasing with $\lambda_k$. The bigger this difference is, the lower $T_S$ is needed to satisfy eq. (A.1), why $\inf\{T_S\}$ corresponds to $\lambda_k \to 0$. We can therefore compute a lower limit on $T_S$ by setting $\lambda_k = 0\ \forall k$ [2] and use that $e^{T_S} \geq \left(e^{T_S} - e^{-T_S/\lambda_k}\right)$

$$\inf\{T_S\} \geq \log\left[\frac{1}{2f}\right],$$

where we have also used that the ratio $S_0/d_k(0)$ is the smallest when $d_k(0) = fS_0$. For a dilution factor of $10^{-6}$ $\inf\{T_S\}$ is approximately 13.

The ratio $\frac{\lambda}{1+\lambda} d(t)/g(t)$ is plotted in Fig. (A.1) for two fixed $t$. The dashed line corresponds to $t = \lambda$. The blue line corresponds to the lower limit on $T_S$ and goes

[1] Because $T_{S,T>0} - T \geq T_{S,T=0}$ for any combination of parameters.

[2] So the two populations are the same

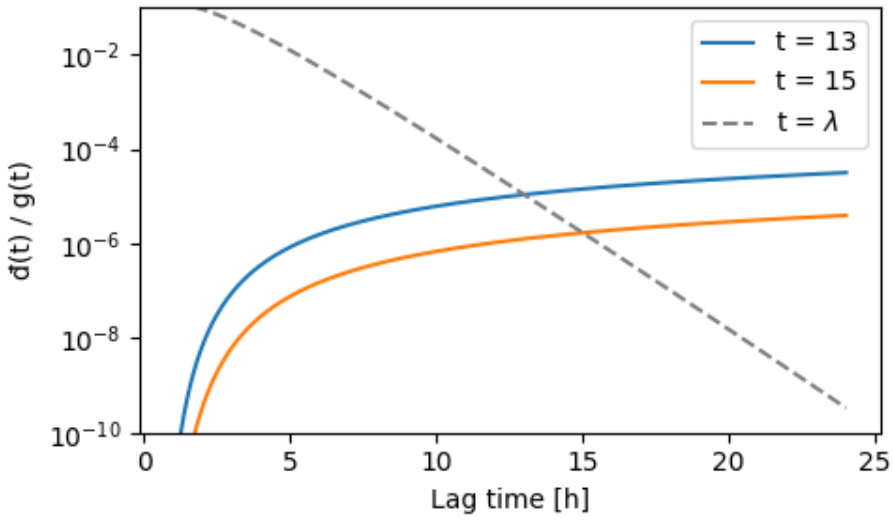


Figure A.1: ***Value of*** $\frac{\lambda}{1+\lambda} d(t)/g(t)$ ***which is approximated to 0.***

toward $10^{-4}$ as the lag time exceeds a day. For small lag times the ratio is always below the region of reasonable dilution factors, hence the approximation is reasonable here.

## A.2 Numerical average in region around $p_c$

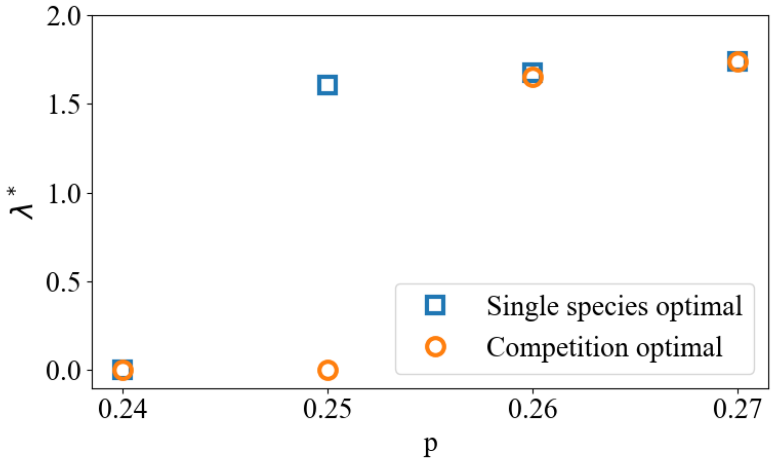


Figure A.2: ***Optimal lag time around critical probability in model with exponentially distributed lag time.*** *Single species optimal is the analytical optimal according to* $F_I(\lambda; \gamma, p, T)$*, and competition optimal is the numerical consumption fraction.*

Here, we investigate the region around the critical p in fig. 5.3 closer . The analytical and numerical optimal lag times $\lambda^*$ are plotted against the probability of antibiotics. The jump appears to occur slightly later for the numerical than the analytical optimal. However, analytically, the jump lies between $p = 0.249$ and $p = 0.25$. For $p$ this close to the critical $p_c$ the system is very vulnerable to statistical fluctuations, hence we would need more than 10 000 cycles to compute the true numerical optimal. It is unlikely that this is related to the approximation we did in the calculations (see appendix A.1). The approximation is more accurate the smaller $\lambda$ is, therefore the approximation "favours" short $\lambda$ and any effect from the approximation would yield a higher analytical $p_c$ than the numerical $p_c$. Here, we see the opposite, namely that the analytical jump in $\lambda^*$ occurs earlier than in the numerical case.

# Appendix B

# Appendices to chapter 4

## B.1 Solving eq. (6.5-6.6)

We want to solve the following set of second order differential equations

$$\lambda\ddot{g} - [\lambda(1-\delta) - 1]\dot{g} - g = 0, \tag{B.1}$$
$$\lambda\ddot{g} + [\lambda(\gamma+\delta) + 1]\dot{g} + \gamma g = 0. \tag{B.2}$$

From this we obtain the characteristic equations

$$\lambda x^2 - [\lambda(1-\delta) - 1]x - 1 = 0, \tag{B.3}$$
$$\lambda y^2 + [\lambda(\gamma+\delta) + 1]y + \gamma = 0. \tag{B.4}$$

**First, solving eq. (B.3) for $\mathbf{t \leq T_0}$**

$$x_\pm = \frac{\lambda(1-\delta)-1}{2\lambda} \pm \frac{1}{2\lambda}\sqrt{[\lambda(1-\delta)-1]^2 + 4\lambda}$$

We now define $b \equiv x_+$ and $a \equiv -x_-$. From studying $b$ and $a$ we realise that $\lambda = 1/ab$ and $\delta = (a+1)(1-b)$. The solution of eq. (6.5) is on the form

$$g(t) = B_0 \cdot e^{bt} + A_0 \cdot e^{-at}, \tag{B.5}$$

with the boundary conditions

$$g(0) = B_0 + A_0 = 0 \qquad \Rightarrow A_0 = -B_0,$$
$$\dot{g}(0) = bB_0 - aA_0 = ab \cdot d_0 \qquad \Rightarrow B_0 = d_0\frac{ab}{a+b}.$$

Plugging this into eq. (B.5) yields

$$g(t) = d_0\frac{ab}{a+b}\left(e^{bt} - e^{-at}\right). \tag{B.6}$$

We find the equivalent expression for the dormant population by considering eq. (6.2) and isolating $d(t) = \lambda\dot{g} - \lambda(1-\delta)g$. Then inserting $\lambda = 1/ab$ and $\delta = (a+1)(1-b)$

$$\begin{aligned} d(t) &= \frac{1}{ab}\left(\dot{g} + (a - ab - b)g\right), \\ \Rightarrow d(t) &= \frac{d_0}{a+b}\left(a(1-b)e^{bt} + b(a+1)e^{-at}\right). \end{aligned}$$

The total population for $t \leq T_0$ is therefore

$$n(t) = \frac{d_0}{a+b}\left(a \cdot e^{bt} + b \cdot e^{-at}\right).$$

**Then solving eq. (B.4) for $\mathbf{t} \in [\mathbf{T_0}, \mathbf{T}]$**

$$y_\pm = -\frac{\lambda(\gamma+\delta)+1}{2\lambda} \pm \frac{1}{2\lambda}\sqrt{(\lambda(\gamma+\delta)+1)^2 - 4\lambda\gamma}.$$

And we again define $b_p = -y_+$ and $a_p = -y_-$, and we realise that $\gamma = a_p b_p/ab$. The solution to eq. (B.4) is on similar form as before, namely

$$g(t) = B_p \cdot e^{-b_p t} + A_p \cdot e^{-a_p t}.$$

The boundary conditions are now

$$\begin{aligned} g(T_0) &= B_p \cdot e^{-b_p T_0} + A_p \cdot e^{-a_p T_0} = d_0 \frac{ab}{a+b}\left(e^{bT_0} - e^{-aT_0}\right), \\ \dot{g}(T_0) &= -b_p B_p \cdot e^{-b_p T_0} - a_p A_p \cdot e^{-a_p T_0} = ab \cdot d(T_0) - (a_p + b_p - ab)g(T_0), \end{aligned}$$

where we have used that $\gamma + \delta = a_p + b_p - ab$. From this we obtain that

$$\begin{aligned} B_p &= \quad d_0 \frac{ab}{a+b}\left[\frac{(a-b_p)e^{bT_0} + (b+b_p)e^{-aT_0}}{a_p - b_p}\right] e^{b_p T_0}, \\ A_p &= -d_0 \frac{ab}{a+b}\left[\frac{(a-a_p)e^{bT_0} + (b+a_p)e^{-aT_0}}{a_p - b_p}\right] e^{a_p T_0}. \end{aligned}$$

Inserting this yields

$$g(t) = d_0 \frac{ab}{a+b}\left(\left[\frac{(a-b_p)e^{bT_0} + (b+b_p)e^{-aT_0}}{a_p - b_p}\right] e^{-b_p(t-T_0)} - \left[\frac{(a-a_p)e^{bT_0} + (b+a_p)e^{-aT_0}}{a_p - b_p}\right] e^{-a_p(t-T_0)}\right).$$

And we find the dormant population by isolating $d(t)$ in eq. (6.2), that is $d(t) = \lambda\dot{g} + \lambda(\gamma+\delta)g$ such that we can find $d(T)$ for $t \in [T_0, T]$

$$d(t) = \frac{1}{ab}\left(\dot{g} + (a_p + b_p - ab)g\right).$$

We thus obtain

$$\begin{aligned} d(t) =& \frac{d_0}{a+b} \cdot \frac{a_p - ab}{a_p - b_p}\left[(a-b_p)e^{bT_0} + (b+b_p)e^{-aT_0}\right]e^{-b_p(t-T_0)} \\ &- \frac{d_0}{a+b} \cdot \frac{b_p - ab}{a_p - b_p}\left[(a-a_p)e^{bT_0} + (b+a_p)e^{-aT_0}\right]e^{-a_p(t-T_0)}. \end{aligned}$$

The total population for $t \in [T_0, T]$ is therefore

$$\begin{aligned} n(t) =& d_0 \frac{a_p}{a+b}\left[\frac{(a-b_p)e^{bT_0} + (b+b_p)e^{-aT_0}}{a_p - b_p}\right]e^{-b_p(t-T_0)} \\ &- d_0 \frac{b_p}{a+b}\left[\frac{(a-a_p)e^{bT_0} + (b+a_p)e^{-aT_0}}{a_p - b_p}\right]e^{-a_p(t-T_0)}. \end{aligned}$$

**Lastly, solving eq. (B.4) for $\mathbf{t \geq T}$.** The solution is on the same form as for $t \leq T_0$, but the boundary conditions are different. Introducing the notation $T_{ab} \equiv T - T_0$

$$\begin{aligned} g(T) &= Be^{bT} + Ae^{-aT} = B_p e^{-b_p T} + A_p e^{-a_p T}, \\ \dot{g}(T) &= bBe^{bT} - aAe^{-aT} = ab \cdot d(T) + (ab - a + b)g(T) \end{aligned}$$

which yields

$$\begin{aligned} B &= \frac{1}{a+b}\left[(b+a_p)B_p e^{-b_p T} + (b+b_p)A_p e^{-a_p T}\right]e^{-bT}, \\ A &= \frac{1}{a+b}\left[(a-a_p)B_p e^{-b_p T} + (a-b_p)A_p e^{-a_p T}\right]e^{aT}. \end{aligned}$$

Which yields the growing population for $t \geq T$

$$\begin{aligned} g(t) =& \frac{1}{a+b}\left[(b+a_p)B_p e^{-b_p T} + (b+b_p)A_p e^{-a_p T}\right]e^{b(t-T)} \\ &+ \frac{1}{a+b}\left[(a-a_p)B_p e^{-b_p T} + (a-b_p)A_p e^{-a_p T}\right]e^{-a(t-T)}. \end{aligned}$$

And we find $d(t)$ for $t \geq T$ as before

$$\begin{aligned} d(t) =& \frac{1-b}{b}\frac{1}{a+b}\left[(b+a_p)B_p e^{-b_p T} + (b+b_p)A_p e^{-a_p T}\right]e^{b(t-T)} \\ &- \frac{a+1}{a}\frac{1}{a+b}\left[(a-a_p)B_p e^{-b_p T} + (a-b_p)A_p e^{-a_p T}\right]e^{-a(t-T)}. \end{aligned}$$

Which yields the total population

$$\begin{aligned} n(t) =& \frac{1}{b}\frac{1}{a+b}\left[(b+a_p)B_p e^{-b_p T} + (b+b_p)A_p e^{-a_p T}\right] e^{b(t-T)} \\ & -\frac{1}{a}\frac{1}{a+b}\left[(a-a_p)B_p e^{-b_p T} + (a-b_p)A_p e^{-a_p T}\right] e^{-a(t-T)}. \end{aligned}$$

Finally, replacing $A_p$ and $B_p$ with $a, b, a_p$ and $b_p$

$$\begin{aligned} n(t) =& \frac{a\cdot d_0}{(a+b)^2}\cdot\frac{b+a_p}{a_p-b_p}\left[(a-b_p)e^{bT_0} + (b+b_p)e^{-aT_0}\right] e^{-b_p T_{ab}} e^{b(t-T)} \\ & -\frac{a\cdot d_0}{(a+b)^2}\cdot\frac{b+b_p}{a_p-b_p}\left[(a-a_p)e^{bT_0} + (b+a_p)e^{-aT_0}\right] e^{-a_p T_{ab}} e^{b(t-T)} \\ & -\frac{b\cdot d_0}{(a+b)^2}\cdot\frac{a-a_p}{a_p-b_p}\left[(a-b_p)e^{bT_0} + (b+b_p)e^{-aT_0}\right] e^{-b_p T_{ab}} e^{-a(t-T)} \\ & +\frac{b\cdot d_0}{(a+b)^2}\cdot\frac{a-b_p}{a_p-b_p}\left[(a-a_p)e^{bT_0} + (b+a_p)e^{-aT_0}\right] e^{-a_p T_{ab}} e^{-a(t-T)}. \end{aligned}$$

The full forms of $g(t)$ and $d(t)$ are easily found changing the prefactor of each term in $n(t)$, as given by the equations above.

## B.2 Computing $T_S$ in case with antibiotics

We have that

$$g(T_S) = S_0 + g(T) - g(T_0), \tag{B.7}$$

where

$$\begin{aligned}
g(T_0) &= d_0 \frac{ab}{a+b}\left(e^{bT_0} - e^{-aT_0}\right), \\
g(T) &= d_0 \frac{ab}{a+b}\frac{1}{a_p - b_p}\left[(a-b_p)e^{bT_0} + (b+b_p)e^{-aT_0}\right] e^{-b_p(T-T_0)} \\
&\quad - d_0 \frac{ab}{a+b}\frac{1}{a_p - b_p}\left[(a-a_p)e^{bT_0} + (b+a_p)e^{-aT_0}\right] e^{-a_p(T-T_0)}, \\
g(T_S) &\approx d_0 \frac{ab}{(a+b)^2}\cdot\frac{b+a_p}{a_p - b_p}\left[(b+b_p)e^{-aT_0} + (a-b_p)e^{bT_0}\right] e^{-b_p(T-T_0)}\cdot e^{b(T_S-T)} \\
&\quad - d_0 \frac{ab}{(a+b)^2}\cdot\frac{b+b_p}{a_p - b_p}\left[(b+a_p)e^{-aT_0} + (a-a_p)e^{bT_0}\right] e^{-a_p(T-T_0)}\cdot e^{b(T_S-T)}.
\end{aligned}$$

We have here neglected terms with the factor $\exp(-at)$ in $g(T_S)$, as discussed in section 6.1.1. We now isolate $T_S$ in eq. (B.7)

$$T_{S,T>0} = \frac{1}{b}\log\left[\frac{(a+b)^2(a_p - b_p)}{ab}\frac{S_0 + g(T) - g(T_0)}{d_0 D}\right] + T,$$

where

$$D = (b+a_p)\left[(b+b_p)e^{-aT_0} + (a-b_p)e^{bT_0}\right] e^{-b_p T_{ab}} - (b+b_p)\left[(b+a_p)e^{-aT_0} + (a-a_p)e^{bT_0}\right] e^{-a_p T_{ab}}.$$

Pulling $d_0$ out of the expression for $g(t)$, such that $g(t) = d_0 g'(t)$, and inserting $d_0 = fS_0$

$$T_{S,T>0} = \frac{1}{b}\log\left[\frac{(a+b)^2(a_p - b_p)}{ab}\frac{1 + fg'(T) - f'g(T_0)}{fD}\right] + T,$$

## B.3 Supplementary plots of optimal persistence strategy

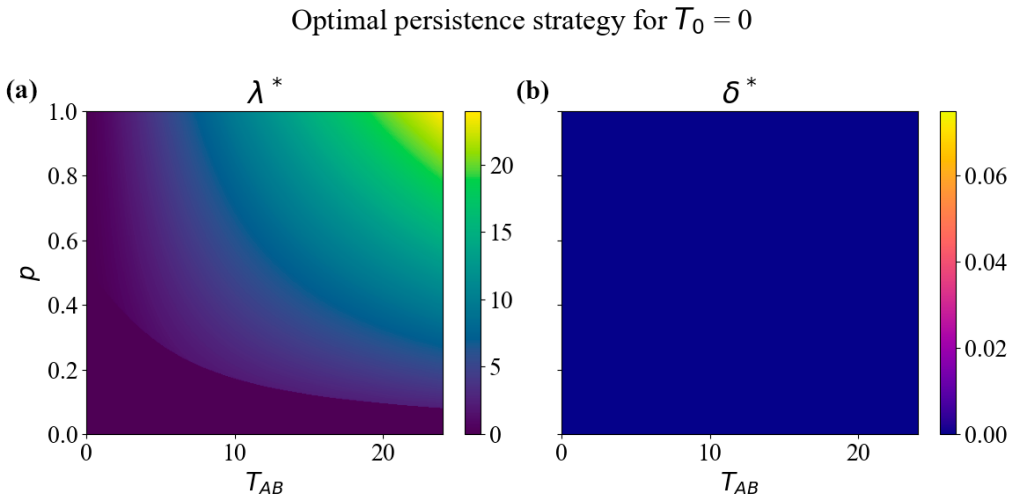


Figure B.1: ***Heat map of optimal persistence strategy when*** $T_0 = 0$***.*** ***a)*** *The optimal lag time.* ***b)*** *The optimal rate of spontaneous persistence.*

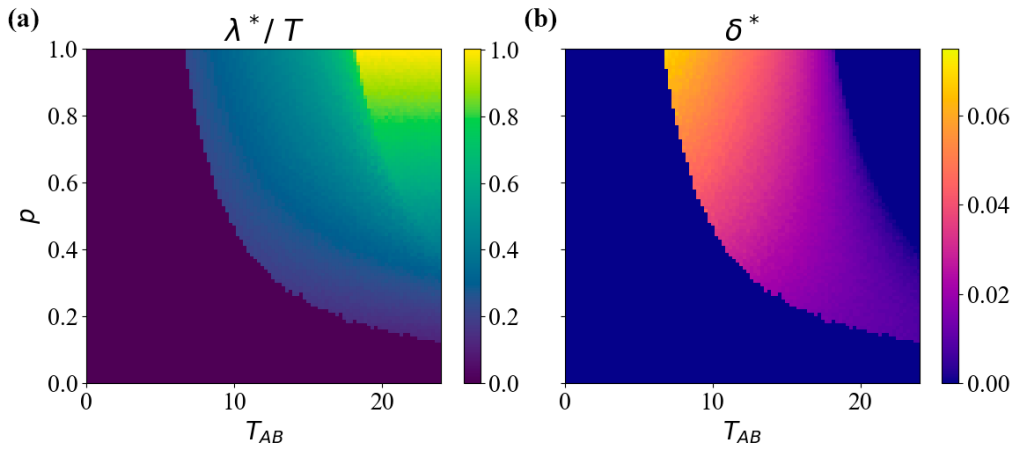


Figure B.2: ***Heat map of optimal persistence strategy for competition and*** $T_0 = 5$***.*** ***a)*** *The optimal lag time.* ***b)*** *The optimal rate of spontaneous persistence.*

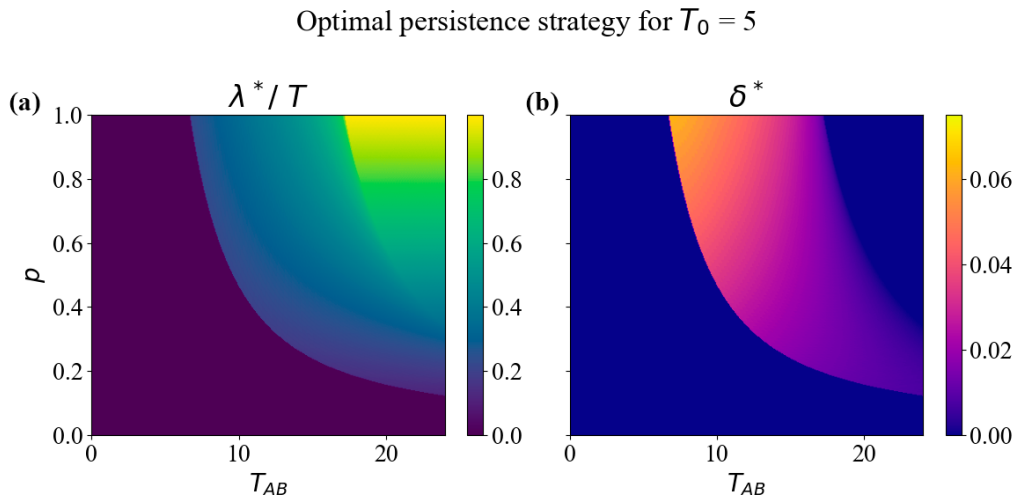


Figure B.3: ***Heat map of optimal single species persistence strategy for*** $T_0 = 5$ ***for comparison.a)*** *The optimal lag time.* ***b)*** *The optimal rate of spontaneous persistence.*

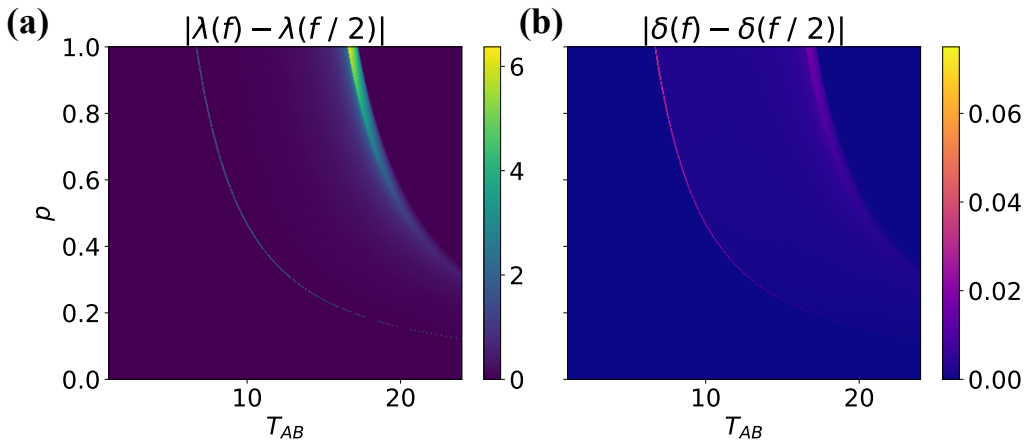


Figure B.4: ***Absolute difference in optimal persistence strategies for*** $f = 10^{-6}$ ***and*** $f = 0.5 \cdot 10^{-6}$***. a)*** *The optimal lag time.* ***b)*** *The optimal rate of spontaneous persistence.*

## B.4 Supplementary plots of evolution of average persistence strategy

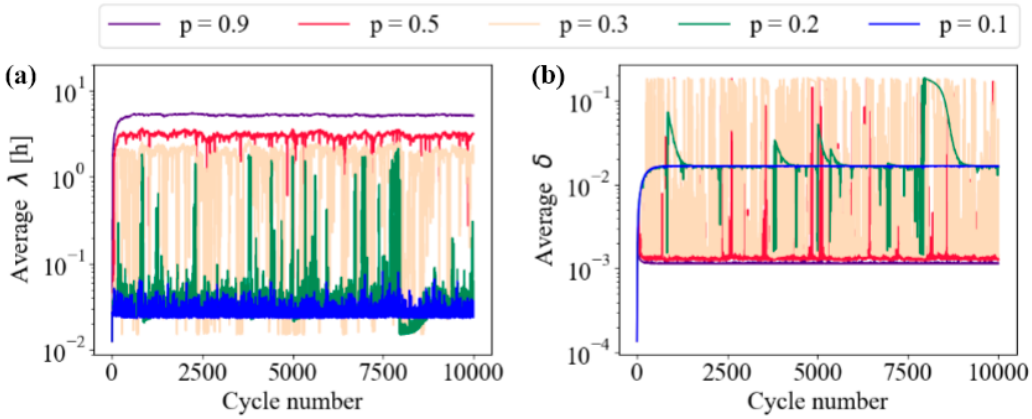


Figure B.5: ***Evolution of average persistence strategy with*** $T_0 = 0$, $T_{AB} = 6$. ***a)*** *Average lag time.* ***b)*** *Average rate of spontaneous persistence*

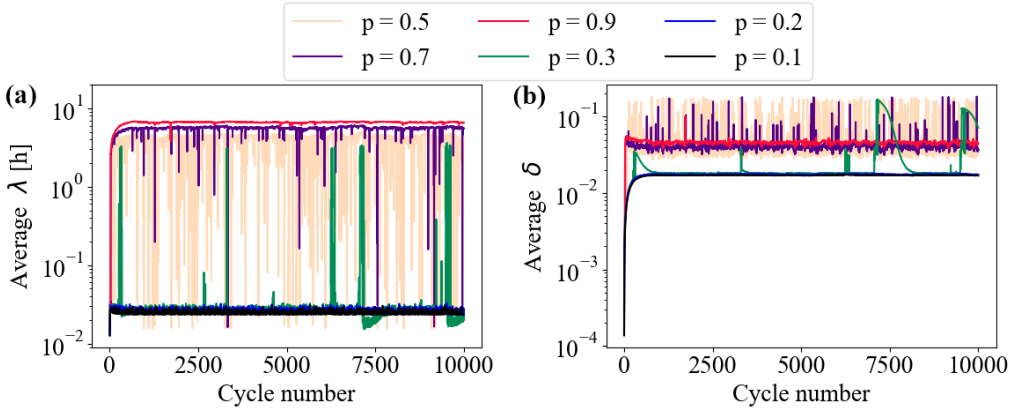


Figure B.6: ***Evolution of average persistence strategy with*** $T_0 = 5$, $T_{AB} = 12$. ***a)*** *Average lag time.* ***b)*** *Average rate of spontaneous persistence*

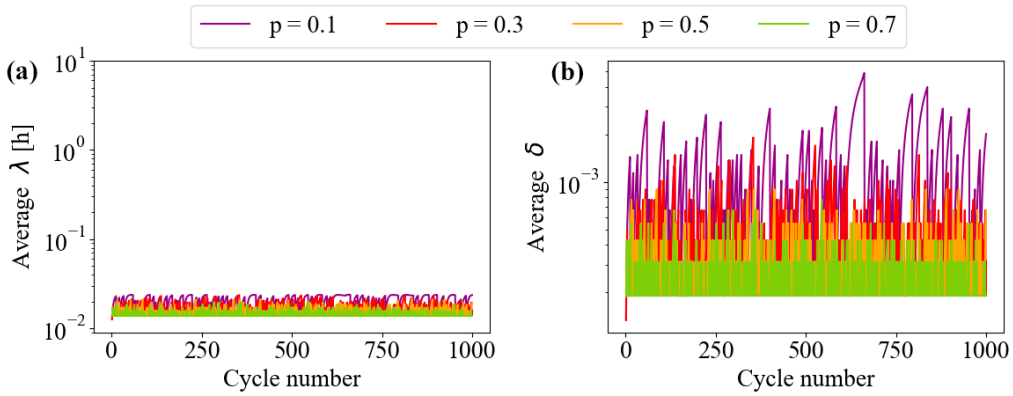


Figure B.7: ***Evolution of average persistence strategy with extinction and*** $T_0 = 0$, $T_{AB} = 6$. *The evolution starts from* $\lambda_0 = 0.01$ *and* $\delta_0 = 0$. ***a)*** *Average lag time.* ***b)*** *Average rate of spontaneous persistence*

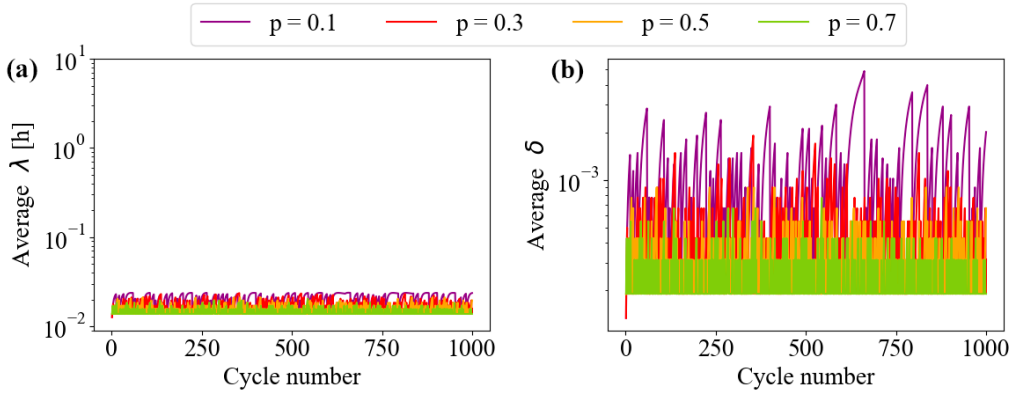


Figure B.8: ***Evolution of average persistence strategy with extinction and*** $T_0 = 0$, $T_{AB} = 6$. *The evolution starts from* $\lambda_0 = 0.01$ *and* $\delta_0 = 0$. ***a)*** *Average lag time.* ***b)*** *Average rate of spontaneous persistence*

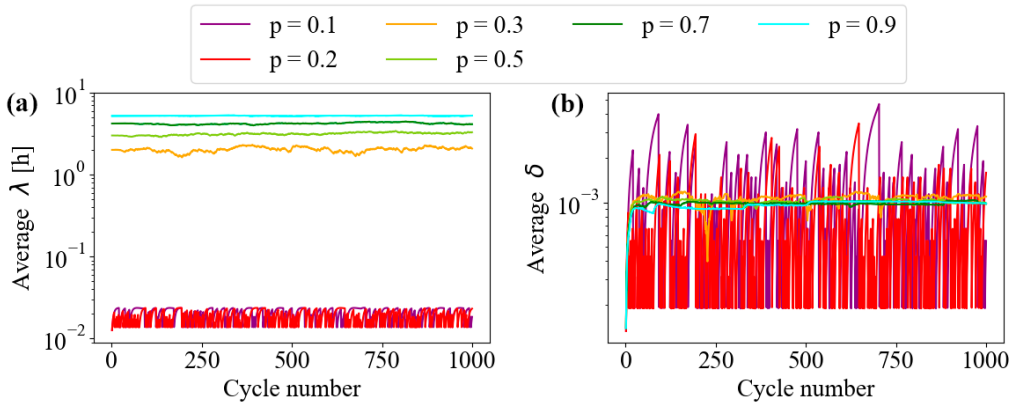


Figure B.9: ***Evolution of average persistence strategy with extinction and*** $T_0 = 0$, $T_{AB} = 6$. *The evolution starts from* $\lambda = \lambda^*$ *and* $\delta = \delta^*$. ***a)*** *Average lag time.* ***b)*** *Average rate of spontaneous persistence*

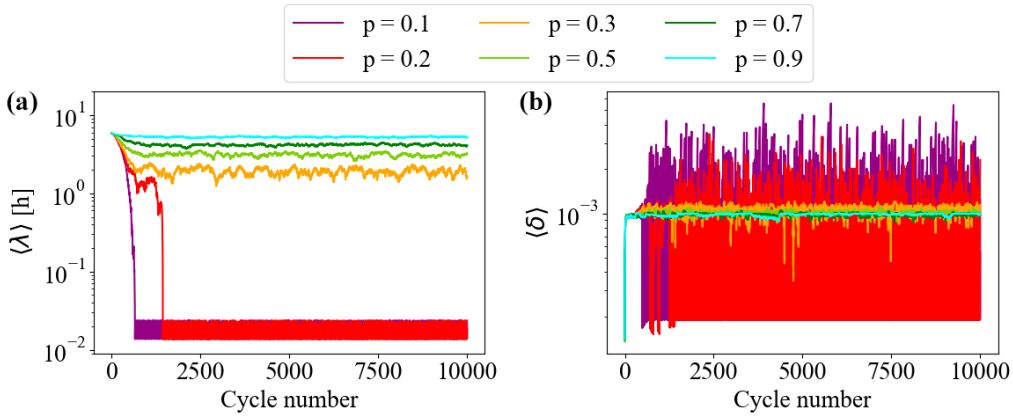


Figure B.10: ***Evolution of average persistence strategy with extinction and*** $T_0 = 0$, $T_{AB} = 6$. *The evolution starts from* $\lambda_{N-1} = T$ *and* $\delta_0 = 0$. ***a)*** *Average lag time.* ***b)*** *Average rate of spontaneous persistence*

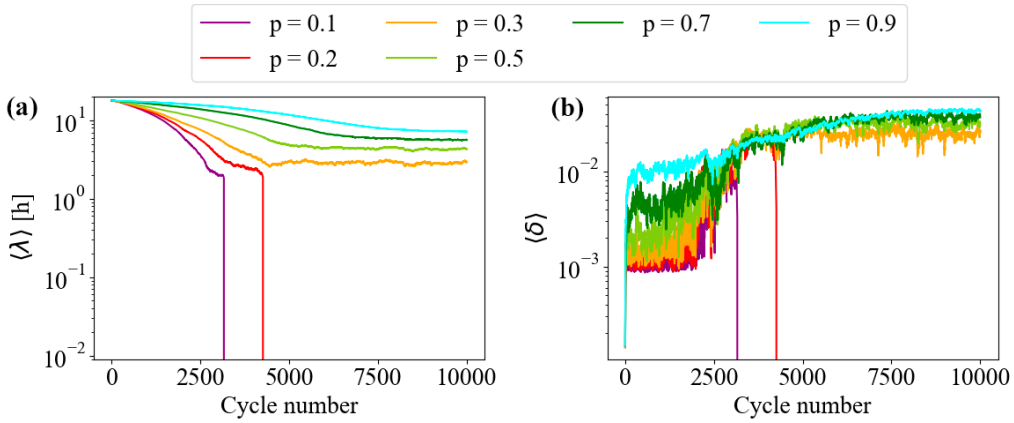


Figure B.11: ***Evolution of average persistence strategy with extinction and*** $T_0 = 5$***,*** $T_{AB} = 12$***.*** *The evolution starts from* $\lambda_{N-1} = T$ *and* $\delta_0 = 0$*.* ***a)*** *Average lag time.* ***b)*** *Average rate of spontaneous persistence*