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DEGREE IN MATHEMATICS

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Characterization of *microalgae* growth and the relation with its environment

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Contents

Abstract

This project aims to do an analytical study of the evolution of a population of *Alexandrium minutum* and relate the *microalgae* growth with its environment. The specific goal is to use simple population models to describe the lag and exponential growth phases of this *microalgae* and discuss how variations in the environment conditions (mainly temperature, irradiance and salinity) affect the speed at which the populations increases. We will add into our population model a dilution constant to emulate the effects of dilution in the evolutionary process along time.

To perform simulations and systematically compare the results with the available data in the literature, we have used numerical integration methods for ordinary differential equations combined with algorithms for fitting the parameters of the models. The propagation of errors has been also considered. The results show that considering the *microalgae* environment is important to accurately determine the growth rate.

At the end of this project we have listed a few experiments proposals with the aim to confirm our calculations and provide more insights on the understanding of this *microalgae*.

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Chapter 1

Introduction

An ecosystem is defined as a biological relational class of interacting organisms and their physical environment. This relations are divided into three types: **mutualistic**, when both specie involved take profit of that relation; **competition**, when both specie compete for the same resources; **parasitic**, when one organism, the parasite, lives off of another organism, the host, harming it and possibly causing death.
It is clear that the climatic conditions of recent years are breaking the equilibrium of the different ecosystems around the world with unpredictable consequences. Understanding how this relations work and affect the evolution of each of the specie is key to predict the chain effects of catastrophic events. An specie bloom or the extinction of a specie are events that break the ecosystem's equilibrium and are an example of the root cause of such catastrophes.
Many studies have been made about mutualistic and competitive relations, yet there is a lot to learn about the dynamic formed by a host and their parasite.
This project has been brought in collaboration with **CSIC - Institute of Marine Sciences** and the group **BIOCOMSC1-UPC**, whom provided all the experimental data used in this project and the knowledge and expertise in microbiology, through the fellowship JAEIntroICU-2021-ICM-03 and aims to characterize the evolution of the specie of *Dinoflagellate Alexandrium minutum* and relate the population growth with its environment. This one-celled organism is known to bloom in various coasts around the world and segregates a toxin that can be deathly for humans. Moreover, recent studies like [1] have reported evidences of parasitism in *Alexandrium minutum* cells.
We have divided the manuscript into six chapters. In chapter 2 some preliminary notions are given to explain the scientific interest of this project. In particular, we introduce some mathematical models used in microbiology and some cultivation techniques to carry out biological experiments in a laboratory.

In chapter 3, we describe the methodology to carry out our calculations and show the experimental data that we use along the project to test our results. Before moving on to the results and the conclusions, in chapter 4 we summarize about the mathematical tools that are used along the project.
In the last section we propose a set of experiments to test our calculations and provide new insights on the characterization of this *microalgae*.

Chapter 2

Previous notions

2.1 Scientific challenge

In microbiology, when scientific research is carried out on a particular organism, a sample of this specie is usually taken and isolated within a laboratory culture to study its growth over the days in a closed environment. Apart from being the only viable option for studying the organism in some cases, this technique minimizes noise in observations as the culture is subject to fixed conditions and has no external interaction.
Unfortunately, the image taken of the evolution of the organism in a laboratory is far away from reality. Living beings are in constant interaction with our environment, whether with other living beings or with environmental phenomena. These systems are so complex that predicting and estimating their effects on a specie is impossible. The only solution for understanding, in some way, how an organism interacts with its environment is to isolate it and study the effects of each interaction separately in the laboratory.
In our case, we will study the effect of certain environmental conditions on the growth of a population of *Alexandrium minutum*, as well as the effects of tidal dilution and propose a series of biological experiments to confirm our hypotheses.

2.2 Mathematical models in biology

To study the behaviour of a specie and the interaction with its environment we need to study its evolution over time.

The population evolution after an infinitesimal time increment is mainly proportional to the population. This leads to a linear equation and shows that the population's experiences an exponential evolution described as follows:

$$\begin{cases} \dot{N}(t) = \mu\, N(t) \\ N(t_0) = N_0 \end{cases} \implies \int_{N_0}^{N(t)} \frac{1}{N}\, dN = \mu \int_{t_0}^{t} dt \implies \ln N(t) - \ln N_0 = \mu\,(t - t_0)\,.$$

Therefore, the so-called specific growth rate, μ, is calculated as follows:

$$\mu = \frac{\ln N - \ln N_0}{t - t_0} \tag{2.1}$$

Mathematical models let us predict, not only the current status of a specie, but the end-to-end evolution during the course of time. In the upcomming subsections we will define a set of mathematical models used in microbiology.

2.2.1 Primary Models

Logistic model

The logistic model is the most basic and commonly used model to describe a population's evolution over time. Considering the Specific growth rate during the exponential phase μ and the carrying capacity N_{max} (the maximum number of individuals a population can reach), the logistic model is given by the evolution law:

$$\frac{dN}{dt}(t) = \mu\left(1 - \frac{N(t)}{N_{max}}\right)N(t), \quad \mu \geq 0, \quad N_{max} > 0\,. \tag{2.2}$$

On the one hand, population growth is assumed to be linear with a growth rate μ, hence, the term $\mu N(t)$. On the other hand, the more a population grows the more pressure is applied to the population itself. In essence, when the number of individuals in a population increases, so does the difficulty on maintaining the population itself. Hence, the term $\mu N(t)\frac{N(t)}{N_{max}}$ is substracted.

Baranyi-Roberts model

Note that in the logistic model, growth starts immediately but for real cultures such thing does not happen many often. Usually, cells need some time to adapt to the medium (this adaptation time is called *lag phase*, which might depend on the environment and the specie itself).

The Baranyi-Roberts (**BR**) [2] model is an adaptation of the logistic model (2.2) which considers this lag phase and is defined by the evolution law:

$$\frac{dN}{dt}(t) = \mu\, \frac{e^{bt}}{1 + ae^{bt}} \left(1 - \frac{N(t)}{K}\right) N(t), \tag{2.3}$$

where $K > 0$ is the carrying capacity, μ is the Specific growth rate and a, $b > 0$ are parameters representing intrinsic properties of the cell (in this project the physical meaning of these parameters is unknown).
This equation induces a Cauchy Problem when considering t_0 as the time (in days) of the first observation and $N(t_0; t_0, N_0) = N_0$ in the following way:

$$\begin{cases} \frac{dN}{dt}\,(t; t_0, N_0) = \mu\ \frac{e^{bt}}{1+ae^{bt}}\left(1 - \frac{N(t;t_0,N_0)}{K}\right)\ N(t), \\ N(t_0; t_0, N_0) = N_0 \end{cases} \tag{2.4}$$

The solution of (2.4) is

$$N(t; t_0, N_0) = \frac{N_0\ K\ \left(\frac{1+ae^{bt}}{1+ae^{bt_0}}\right)^{\frac{\mu}{ab}}}{N_0\left(\frac{1+ae^{bt}}{1+ae^{bt_0}}\right)^{\frac{\mu}{ab}} - N_0 + K} \tag{2.5}$$

Note that (2.3) is a non-autonomous differential equation yet it approaches an autonomous differential equation when t goes to infinity:

$$\lim_{t\to\infty} \frac{e^{bt}}{1+ae^{bt}} = \frac{1}{a} \implies \frac{e^{bt}}{1+ae^{bt}} = \frac{1}{a}\left(1 - \frac{e^{-bt}}{a} + O(e^{-2bt})\right) \iff$$

$$\mu\ \frac{e^{bt}}{1+ae^{bt}}\left(1 - \frac{N(t)}{K}\right)\ N(t) = \frac{\mu}{a}\left(1 - \frac{N(t)}{K}\right)\ N(t)\ \left(1 + O(e^{-2bt})\right)\ ,$$

from which one recovers the logistic-like law ignoring the exponential correction term.

2.2.2 Secondary Models

In this section we will describe a set of models to relate the specific growth rate μ with the environmental variables which we will work with from now on.

Ratkowsky model

Van't Hoff and Arrhenius [4] put forward the concept that the rate constant for chemical reactions might be described by the following expression in exponential form:

$$k = A\ e^{-\frac{E}{R\,T}}, \tag{2.6}$$

where k is the specific reaction rate constant, R is the universal gas constant, T the absolute temperature, E is an empirically determined quantity called the activation energy and A is a parameter. This equation has become generally known as the **Arrhenius Law** and it has had great success in describing the temperature

dependence of chemical reactions [5].
In microbiology, it has been recognized that temperature is also a cardinal factor controlling the rate of development of microbial populations and microbiologists have substituted growth rate constant μ for rate constant k in equation.

D.A Ratkowsky discussed in [11] the fact that when $\ln \mu$ is plotted against reciprocal temperature $\frac{1}{T}$ a curve is obtained instead of a straight line and came up with an adapted expression of (2.6) that fits empirical data. This expression was known as the "square-root" relationship and it takes as parameters b and T_0 (T_0 is presented as a "conceptual temperature of no metabolic significance"):

$$\sqrt{\mu} = b(T - T_0) \tag{2.7}$$

Afterwards, this relationship was adapted in [11] to describe bacterial growth throughout the entire temperature range. Considering T_{min}, T_{max} as the maximum and minimum temperatures, respectively, at which the rate of growth is zero and b, c as two parameters which will be adjusted via linear regression, the final empirical relationship described by Ratkowsky has this form:

$$\sqrt{\mu} = b(T - T_{min})(1 - e^{c(T - T_{max})}) \tag{2.8}$$

This model is used in Section 5.1.3, while in Section 5.1.1 we use a modification of it to describe the effect of salinity in the growth of rate of *microalgae*.

Blackman model

The Blackman model [9] was thought to describe the direct effect of irradiance in bacterial growth and describes the photosynthetic response to irradiance when there are no inefficiencies in photon usage. Assuming irradiance as limiting substrate for photosynthesis, Blackman (1905) observed that there was a clear linear relationship between irradiance saturation and the limiting substrate until saturation. He described this relationship as follows:

$$\begin{cases} \mu = \mu_{max} \frac{I}{I_k}, & \text{if } I \leq I_k \\ \mu = \mu_{max} & \text{otherwise} \end{cases}, \tag{2.9}$$

where I_k indicates the irradiance saturation. This model is used in Section 5.1.2 to describe the effect of irradiance in the growth of *microalgae*.

Remark 2.1. Another model which has been widely used to describe the rate of enzymatic reactions by relating reaction rate with the concentration of substrate is the **Monod model**.

Monod (1942) proposed that the growth kinetics of a culture changes with substrate concentration the same way the enzymatic reaction rate does. **Monod kinetics** is given by the relation

$$\mu = \mu_{max} \frac{S}{S + S_k},$$

where S is the substrate concentration, μ_{max} the maximum Specific growth rate and S_k the substrate saturation.

2.3 Scientific method

In this section we give a quick view of the differences of the two types of cultivation techniques and explain a commonly used method for pythoplankton quantitative analysis.

2.3.1 Continunous and non-continuous cultivation techniques

The two main types of cultivation technique are continuous culture and batch culture (non-continuous). The key difference between both techniques is that batch culture is used to grow microorganisms under limited nutrient availability in a closed system while continuous culture is a technique used to grow microorganisms under optimum and continual supply of nutrients in an open system of cultivation. Below are the main features of each cultivation technique.

Batch culture technique

Batch culture technique is a closed system of cultivation. In this technique at first nutrient solution is prepared with inoculum (culture organism) and added in the fermentation tank along with some aeration. Neither fresh medium is added nor used up media is removed from the cultivation vessel. Thus, the culture's volume remains constant. Since fresh media is not added during the course of incubation, concentration of nutrition decreases continuously. Furthermore, various toxic metabolites also accumulates in the culture vessel. Therefore batch culture technique gives characteristics growth curve with lag phase, log phase, stationary phase and decline phase.

2.3.2 Continuous culture technique

Continuous culture technique is an open system of cultivation. In this technique fresh sterile medium is added continuously in the vessel while used up media with bacterial culture is continuously removed. The flux at which medium is

added/removed is key to determine the effect of dilution in the culture's growth. In essence, the biomass increases as a consequence of growth, yet it decreases as a consequence of the flux.

Considering Q as the flux and V the volume of the bio-reactor, we have $Q = \frac{d\,V}{d\,t}$. Hence, the dilution factor is:

$$D = \frac{1}{V}\,\frac{d\,V}{d\,t}\ . \tag{2.10}$$

Note that D^{-1} is the time needed to fully renew the bio-reactor's medium.

Chapter 3

Materials and methods

The environmental variables which relationship with specific growth rate will be studied are temperature (T), salinity (S) and irradiance or light intensity (I).

3.1 Methodology

In this project we will use the primary model (2.3) and substitute the constant μ for an expression that describes the growth rate of *Alexandrium minutum* during the exponential phase as a function of temperature, salinity and irradiance (note that the exponential phase corresponds to the fast increase of cell concentration. In Fig. 3.1 one can see the exponential phase of this particular culture corresponds to the time frame starting on day 4 until day 21). We will estimate the sample error produced during the cell count and calculate the propagation of error based on the variability of the population function with respect to the initial condition. This estimation provides a regime where the growth is expected to be well-defined by the primary model we obtain below. The steps that we will take are the following:

- Fit the secondary models described in section 2.2.2 into our experimental data.
- Use these models to define a function on μ with salinity , temperature and irradiance as variables.
- Compare the results with the classic Baranyi-Roberts model (2.3) where μ is given by (2.1).
- Describe the possible variability of S, I and T during the lifetime of a population of *Alexandrium minutum*

- Include this variability in the function found for μ and compare the resulting primary model with the classic Baranyi-Roberts (2.3).
- Consider the hypothetical role of a dilution factor produced by tidal and study its effect on the population's function for different dilution values.
- With the results obtained, propose a set of experiments to confirm our calculations and provide new insights on our study.

Before starting this methodology we must choose carefully the experimental data that will be used to test our calculations.

3.2 Experimental data

We have chosen data from a set of experiments to test the goodness of the calculations we will perform.

The first set of experiments have been collected from [3]. This paper studies the effect of salinity, temperature and irradiance alone with the growth of *Alexandrium minutum*. The control conditions of the experiments are shown in table 3.2.

Strain	Temperature (^{o}C)	Salinity (p.s.u.)	Irradiance (mmol photon m^{-2} s^{-2})	Volume (mL)
A. minutum T1	25	15	120	500

Table 3.1: Control conditions of the experiments brought in reference [3].

To fit the secondary models (Section 2.2.2) that relate temperature, salinity and irradiance with μ into our experimental data, we will use the tables in 3.2 collected from [3].

When it comes to fitting the Ratkowsky model (2.8) into experimental data, only 3 observations are not enough to do an accurate fit. For this reason, we have collected another experiment brought in [6] to confirm the goodness of Ratkowsky (2.8).

We have the same problem for irradiance treatments. Unfortunately, we could not find any other reliable experiments that could be used in this project.

Note that μ values have been calculated considering the exponential phases of each experiment and using equation (2.1).

Finally, we will use the observations from the salinity treatment (collected from [3]) shown in 3.2 for $S = 15$ p.s.u to test the primary models and our hypothesis.

Temperature treatment	
Temperature (^{o}C)	μ (day^{-1})
10	0.0783581
25	0.179788
30	0.1590288

Salinity treatment	
Salinity (p.s.u)	μ (day^{-1})
7.5	0.122547
15	0.194140
25	0.164341
30	0.119644
37.5	0.007224

Irradiance treatment	
Irradiance (mmol photon m^{-2} s^{-2})	μ (day^{-1})
15.0	0.001
120.0	0.169751
240.0	0.179887

Table 3.2: Specific growth rates for different temperatures (top left), salinities (top right) and irradiances (bottom) collected from [3].

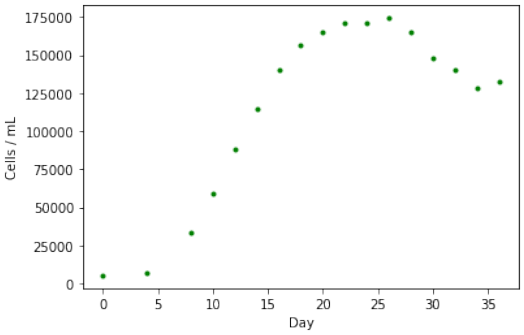


Figure 3.1: Cell concentration with $S = 15$ p.s.u. regarding the experiment brought in [3].

Temperature treatment	
Temperature (^{o}C)	μ (day^{-1})
13	0.16
15	0.23
18	0.3
20	0.37
22	0.31

Table 3.3: Specific growth rates for different temperature values collected from [6].

Day	Cells / mL
0	5338
4	7389
8	33686
10	58964
12	87727
14	115022
16	140300
18	156357
20	165061
22	171132
24	171132
26	174251
28	165061
30	148111
32	140300
34	128186
36	132901

Table 3.4: Cell concentration counting with $S = 15$ p.s.u regarding the experiment brought in [3].

Chapter 4

Mathematical tools

In this section we will expose a series of concepts, methods and tools seen during the degree of Mathematics that will be of great use throughout this project.

4.1 Ordinary differential equations

The theory of ordinary differential equations studies evolutionary processes which are deterministic, finite-dimensional and differentiable with respect the evolution (time) variable.

In the case of our study, the temporary law of evolution of a population of *Alexandrium minutum* defines an evolutionary process which is assumed to be expressed by ordinary differential equations. The BR method, presented in section 2.2.1, is the known model that best describes such processes in microbiology. Note that this approximation ignores space diffusion properties of the studied population.

Next, we recall the definition of evolutionary process. In many common real situations there is no way to compute the evolution process analytically. Hence, we will describe below a numerical method that will allow us to find the solution of the Cauchy problem induced by the differential equation when the initial condition, in this case the initial concentration of *Alexandrium minutum* cells is N_0.

When the evolution law is given by a differentiable function, then the evolutionary process is differentiable with respect to initial condtions (and parameters). We will see below how the solution of an initial value (or Cauchy) problem given by the ordinary differential equations and the initial condition is affected by changes in the initial condition. This will allow us to estimate the propagation of the error given by the observation of the initial values.

Definition 4.1. *An evolutionary process with phase space an open set $\Omega \subset \mathbb{R} \times \mathbb{R}^n$ and domain $D \subset \mathbb{R} \times \Omega$ is a continuous application*

$$\begin{aligned} \Phi\colon D &\longrightarrow \mathbb{R}^n \\ (t; t_0, x_0) &\mapsto \Phi(t; t_0, x_0) \end{aligned}$$

such that:

- *D is open and, for all $(t_0, x_0) \in \Omega$, $I(t_0, x_0) = \{t \in \mathbf{R} |\ (t; t_0, x_0) \in\ D\}$ is an open interval.*
- *For every $(t_0, x_0) \in \Omega$, $t_1 \in I(t_0, x_0)$:*
  - *$t_0 \in I(t_0, x_0)$ and $\Phi(t_0; t_0, x_0) = x_0$,*
  - *$t_2 \in I(t_1, \Phi(t_1; t_0, x_0)) \iff t_2 \in I(t_0, x_0)$ and $\Phi(t_2; t_1, \Phi(t_1; t_0, x_0)) = \Phi(t_2; t_0, x_0)$.*

The so-called Picard's theorem [8] states that any evolution law given by an ordinary differential equation defined by a (maybe non-autonomous) vector field which is (locally) Lipschitz with respect the space variables (and parameters) defines uniquely an evolutionary process. Note that all the laws considered in this work are differentiable, hence the evolutionary process is also differentiable with respect to initial conditions and parameters.

Let $\Phi\colon D \longrightarrow \mathbb{R}^n$ be an evolutionary process induced by the vector field $f\colon \Omega \longrightarrow \mathbb{R}^n$ defined in the open set $\Omega \subset \mathbb{R} \times \mathbb{R}^n$, which is considered to be the (enlarged) phase space where the evolution takes place. Then, for every $(t_0, x_0) \in \Omega$, $\Phi(.; t_0, x_0)\colon I(t_0, x_0) \longrightarrow \mathbb{R}^n$ is the solution of the initial value problem $\dot{x} = f(t, x)$, $x(t_0) = x_0$.

4.1.1 Integration methods

There exist several analytical methods to find solutions to the initial value problems mentioned in the previous section. However, the reality is that the differential equations which describe such problems usually can not be solved analytically and numerical methods must be used to integrate them.

In this section we will describe a computationally feasible method to find a solution on the initial value problem:

$$\begin{cases} \frac{d}{dt}\, x(t) = f(t, x(t)), \\ x(a) = x_0 \end{cases}$$

where x(t) is the solution we want to find, $\frac{d}{dt}$ denotes the derivative with respect t, and $f\colon [a,b] \times \mathbb{R}^m \longrightarrow \mathbb{R}^m$ the restriction of the vector field defined in $\Omega = I\times \subseteq R \times R^n$ to the interval $[a,b] \subset I$.

As said, to solve this problem we will use numerical integration methods. The method we will use in this project is Runge-Kutta-Fehlberg 4-5, a one-step method that obtains a new value of the orbit using only the previous point. We will divide the interval [a, b] in N + 1 parts, each of these subintervals will be noted as (t_n, x_n), t_n determined by N, x(t_n) the exact value of the orbit and x_n its approximation. We denote step h to the distance of variable t between the current point and the previous one. It will be noted as $h_n = t_{n+1} - t_n$.

The most important part of these type of algorithms is to choose wisely the step h. There are two main errors that can be committed when choosing h. On the one hand, h can be too short and it will imply on an increase of the rounding errors and the computational effort. On the other hand, choosing a step too big means a lost in precision and an increase of the error.

Runge-Kutta general form

The Runge-Kutta methods are a set of iterative methods developed by mathematicians C. Runge and M. W. Kutta around 1900. There are two main terms to define when studying these methods. The first one is the number m of implementations of the function that will be done every iteration. The second is the order p related to the error between one value in the orbit and the one that follows.

Butcher [7] states that if a Runge-Kutta method with m steps and order p one has $m \geq p$ and, if p $\geq$ 5, then m $\geq$ p + 1.

The general form of the Runge-Kutta method of m steps can be written as follow:

$$x_{n+1} = x_n + h \sum_{i=1}^{m} b_i \, \kappa_i,$$

where

$$\kappa_i = f(t_n + c_i h, x_n + h \sum_{j=1}^{i} a_{i,j} \, \kappa_j) \ .$$

A. Iserles proved in [12] that it can be assumed that:

$$c_i = \sum_{j=1}^{m} a_{i,j}.$$

The set of independent variables of these methods are usually expressed through-

out the tables of Butcher:

$$\begin{array}{c|cccc} c_1 & a_{1,1} & a_{1,2} & ... & a_{1,m} \\ c_2 & a_{2,1} & a_{2,2} & ... & a_{2,m} \\ . & . & . & & . \\ . & . & . & & . \\ . & . & . & & . \\ c_m & a_{m,1} & a_{m,2} & ... & a_{m,m} \\ \hline & b_1 & b_2 & ... & b_m \end{array}$$

The order of these methods is determined by the local truncation error when approximating $x(t_{n+1})$. The truncation error ε_{n+1} of a Runge-Kutta method in t = t_{n+1} is defined as follow:

$$\varepsilon_{n+1} = ||x(t_{n+1}) - x_{n+1}||$$

Runge-Kutta-Fehlberg (RKF)

This family of methods combine two Runge-Kutta approximations to choose an optimal value for h in each step of integration to guarantee a small enough truncation error. We will consider methods RK4 and RK5. Note that the cost of the algorithm does not increase since when calculating the values of RK5, we obtain all needed values for method RK4. This version of **RKF** is commonly noted as **RK45**. The general idea of this method is that, given $x(t_n)$ and a specific step h_n, we calculate $\hat{x}_{n+1}$ using RK4 and $\bar{x}_{n+1}$ using RK5 and fix a certain tolerance *tol* for the truncation error. If $||\hat{x}_{n+1} - \bar{x}_{n+1}|| < tol$ we accept $\bar{x}_{n+1}$, otherwise we recalculate h_n. The procedure to choose h is then repeated at each integration step. The error estimation of both approximations

$$||\hat{x}_{n+1} - \bar{x}_{n+1}|| = \left|\left| \frac{1}{360}\,\kappa_1 - \frac{128}{4275}\,\kappa_3 - \frac{2197}{7524}\,\kappa_4 + \frac{1}{50}\,\kappa_5 + \frac{2}{55}\,\kappa_6 \right|\right|$$

is used to calculate the optimal next step, for example as

$$|h_n| = 0.9\ |h|\ \sqrt[5]{\frac{tol}{||\hat{x}_{n+1} - \bar{x}_{n+1}||}}\ ,$$

where 0.9 is a security factor to guarantee the upper bound on the local truncation error. The Butcher table for $RK45$ is

$$\begin{array}{c|cccccc}
0 & 0 & & & & & \\
\frac{1}{4} & \frac{1}{4} & & & & & \\
\frac{3}{8} & \frac{3}{32} & \frac{9}{32} & & & & \\
\frac{12}{13} & \frac{1932}{2197} & \frac{-7200}{2197} & \frac{7296}{2197} & & & \\
1 & \frac{439}{216} & -8 & \frac{3680}{513} & \frac{-845}{4104} & & \\
\frac{1}{2} & \frac{-8}{27} & 2 & \frac{3544}{2565} & \frac{1859}{4104} & \frac{-11}{40} & \\
\hline
 & \frac{25}{216} & 0 & \frac{1408}{2565} & \frac{2197}{4104} & \frac{-1}{5} & \\
 & \frac{16}{135} & 0 & \frac{6656}{12825} & \frac{28561}{56430} & \frac{-9}{50} & \frac{2}{55}
\end{array}$$

In this project we use the **RK45** method implemented in python available in [10] to obtain the numerical results Sections 5.2.5, 5.3.4 and 5.4.

4.1.2 Variability with respect to the initial conditions

Now that we know how to find the solutions of the initial value problems explained in Section 4.1, we discuss how to estimate the variance of these solutions when the initial conditions change. Note that the law considered in this project to describe the evolution of *Alexandrium minutum* is differentiable, hence, the associated evolutionary process is differentiable with respect to the initial condition.

We must bear in mind that in microbiology, and other branches of science, the observation of experimental results is not entirely accurate. In essence, the methods used to carry out these observations have some measurement error as well as human error. The upcoming sections will give us an estimation of the propagation of the measurement error. Now, given the following initial value problem:

$$\begin{cases} \frac{d}{dt}\, x(t, x_0) = f(t, x(t, x_0)) \\ x(t_0, x_0) = x_0 \end{cases}, \tag{4.1}$$

the first variance equation along the solution $x(t, x_0)$ of Section 4.1 with respect to the initial condition x_0 is given by the linear Cauchy Problem:

$$\begin{cases} \frac{d}{dt}\, J(t) = D_x f(t, x(t, x_0))\; J(t) \\ J(t_0) = 1 \end{cases}, \tag{4.2}$$

where

$$J(t) = \frac{\partial x}{\partial x_0}(t, x_0) \ .$$

One can use the **RK45** method described in Section 4.1.1 to compute both $x(t, x_0)$ and $\frac{\partial x}{\partial x_0}(t, x_0)$ simultaneously by solving the following system of equations:

$$\begin{cases} \frac{d}{dt} x(t, x_0) = f(t, x(t, x_0)) \\ \frac{d}{dt} J(t) = D_x f(t, x(t, x_0))\ J(t) \end{cases} \quad \begin{cases} x(t_0, x_0) = x_0 \\ J(t_0) = 1 \end{cases} \ , \tag{4.3}$$

This method is used in Sections 5.2.5 and 5.4.

4.1.3 Error propagation

Once described how to calculate the variational of a differential equation with respect the initial condition, we will talk about the Taylor expansion and how it's going to be useful in the upcoming sections to estimate the propagated observational initial error.

Let f be a (n+1)-derivable function in an interval I. Then for all $a, x \in$ I, we have:

$$f(x) = \sum_{j=0}^{n} \frac{f^{(j)}(a)}{j!}\ (x-a)^j + R_n(x) \ , \tag{4.4}$$

where

$$R_n(x) = f(x) - \sum_{j=0}^{n} \frac{f^{(j)}(a)}{j!}\ (x-a)^j \ .$$

The Lagrange Mean Value Theorem guarantees the existence of $c \in \langle x, a \rangle$ such that:

$$R_n(x) = \frac{f^{(n+1)}(c)}{(n+1)!}\ (x-a)^{n+1} \ .$$

In essence, given the Cauchy Problem in Eq. (4.1), where x_0 is the observed initial value and let $\hat{x}_0$ be the actual initial value (unknown), using Taylor's expansion of order 1, one can conclude

$$x(t, \hat{x}_0) = x(t, x_0) + \varepsilon\ \frac{\partial x(t, x_0)}{\partial x_0} + o(\varepsilon^2) \ , \tag{4.5}$$

where ε is an upper bound of observation error estimation.

4.2 Fitting Linear and non-linear models

In the course of this project we will use the mathematical models described in Section 2.2.2 to give an expression of a population of *Alexandrium minutum*'s growth rate during the exponential phase as a function of some environmental variables of our selection. Each of these methods contain parameters of which nothing is known about a priory. For this reason we need to fit the models into our experimental data in order to find the values of these parameters that best approach our case. If the model to fit is linear, the algorithm to use is Least-Square method. Unfortunately, this method is not useful for non-linear models, in such case one can use the so-called Levenberg-Marquardt algorithm.

4.2.1 Linear models. The Least squares problem

Given a parameter vector $\mathbf{p} \in \mathbf{R^n}$, a control vector $\mathbf{y} \in \mathbf{R^m}$, n generating functions $f = (f_0, ..., f_{n-1})^T$ and an estimated measurement vector $\hat{\mathbf{x}} \in \mathbf{R^m}$, a linear model is a relation of the form $\hat{\mathbf{x}} = p_0\, f_0(\mathbf{y}) + p_1\, f_1(\mathbf{y}) + ... + p_{n-1}\, f_{n-1}(\mathbf{y}) = < \mathbf{p}, f(\mathbf{y}) >$. This relation induces the following over-determined $m \times n$ system, $F\mathbf{p} = \hat{\mathbf{x}}$:

$$\begin{pmatrix} f_0(y_0) & f_1(y_0) & ... & f_{n-1}(y_0) \\ f_0(y_1) & f_1(y_1) & ... & f_{n-1}(y_1) \\ . & . & & . \\ . & . & & . \\ . & . & & . \\ f_0(y_{m-1}) & f_1(y_{m-1}) & ... & f_{n-1}(y_{m-1}) \end{pmatrix} \begin{pmatrix} p_0 \\ p_1 \\ . \\ . \\ . \\ p_{n-1} \end{pmatrix} = \hat{\mathbf{x}}\,. \tag{4.6}$$

The goal is to find a vector parameter $\mathbf{p}$ which minimizes the quadratic error $||\hat{\mathbf{x}} - F\,\mathbf{p}||_2^2$. In particular, the solution of this least-squares problem is the solution of the $n \times n$ linear system of normal equations $F^T F\,\mathbf{p} = F^T\,\hat{\mathbf{x}}$.

4.2.2 The Levenberg Marquardt algorithm

Let f be a function which maps a parameter vector $\mathbf{p} \in \mathbf{R^m}$ to an estimated measurement vector $\hat{\mathbf{x}} = f(\mathbf{p})$, $\hat{\mathbf{x}} \in \mathbf{R^n}$. Given an initial parameter estimate p_0 and a measured vector $\mathbf{x}$, the Levenberg-Marquardt algorithm is an iterative method which minimizes the squared distance $\epsilon^T \epsilon$ with $\epsilon = ||x - \hat{x}||$. In essence, the method consists on solving the Least-Squares problem given by the following normal equation:

$$\mathbf{J}^T\,\mathbf{J}\,\delta_p = \mathbf{J}^T\,\epsilon\,, \tag{4.7}$$

where J is the Jacobian martix $\frac{\partial f}{\partial \mathbf{p}}$ and δ_p is a stepsize which minimizes the residual error $||\mathbf{x} - f(\mathbf{p} + \delta_p)||$. The Levenberg-Marquardt algorithm solves a slight variation of 4.7, known as the augmented normal equations

$$\mathbf{N}\delta_p = \mathbf{J}^T \epsilon \ ,$$

where $\mathbf{N}$ is identical to $\mathbf{J}^T \mathbf{J}$ except for the diagonal elements, which are given by a so-called *damping term* $\tau > 0$ such that $\mathbf{N}_{ii} = \tau + [\mathbf{J}^T \mathbf{J}]_{ii}$. In each iteration of the algorithm one has to evaluate whether $\mathbf{p} + \delta_p$ reduces the residual error. If it does, $\mathbf{p} + \delta_p$ is accepted and the process repeats for a decreased *damping term* τ, otherwise the *damping term* is increased and the normal equations are recalculated.

4.3 Hermite Interpolation

At the end of this project we study how the specific growth rate of a population of *Alexandrium minutum* is affected by the variation of the environmental variables, and to do so we first need to describe these variables as functions of time. Doing such thing can be a hard task sometimes because all the information we have about these functions are a discrete set of values. For this reason, we use a method of interpolation to find polynomials which equals these functions in the values that are known (this numerical method is applied in Section 5.3).

In numerical analysis, Hermite interpolation is a generalization of Lagrange interpolation. While Lagrange allows computing a polynomial of degree less than n that takes the same value at n given points as a given function, Hermite computes a polynomial of degree less than mn such that the polynomial and its $m-1$ first derivatives have the same values at n given points as the function and its $m-1$ first derivatives.

Existence and uniqueness
Given a closed interval $[a, b]$ and $x_0, ..., x_m \in [a, b]$, with $x_0 < x_1 < ... < x_m$ a function $f \in C^r[a, b]$, $r > 0$ for which it's only known $f^j(x_i)$ for $j <= n_i$ and $i <= m$. There exists one unique polynomial of degree n, with $n = \sum_{i=0}^{m} n_i \ - 1$, such that

$$f^j(x_i) = P^j(x_i) \ .$$

Numerical calculation
The calculation of Hermite's polynomial of degree n can be made by using a generalized Newton divided differences procedure, considering a set of m points $\{x\}_0^m$, the values these points take in the function $\{y\}_0^{m-1}$ with their respectives n_j

derivatives ($j = 0, ..., m$).
Consider the table of points

$$\begin{array}{ccc} (x_0, f_0^{(0)}) & , \ldots , & (x_0, f_0^{(n_0-1)}) \\ (x_1, f_1^{(0)}) & , \ldots , & (x_1, f_1^{(n_1-1)}) \\ . & & . \\ . & & . \\ . & & . \\ (x_m, f_m^{(0)}) & , \ldots , & (x_m, f_m^{(n_m-1)}) \end{array}$$

Hence, the Hermite interpolated polynomial of degree n can be written as:

$$P_n(x) = f[\hat{x}_0] + \sum_{i=0}^{n} f[\hat{x}_0, ..., \hat{x}_{i+1}] \prod_{j \leq i} (x - \hat{x}_j) \ , \tag{4.8}$$

where

$$f[\hat{x}_i, ..., \hat{x}_{i+j}] = \begin{cases} \frac{f[\hat{x}_{i+1}, ..., \hat{x}_{i+j}] - f[\hat{x}_i, ..., \hat{x}_{i+j-1}]}{\hat{x}_{i+j} - \hat{x}_i}, & \text{if } \hat{x}_i \neq \hat{x}_{i+j} \\ \frac{f_l^j(x_l)}{j!} & \text{if } \hat{x}_i = \hat{x}_{i+j} \text{ for l such that } x_l = \hat{x}_i \end{cases} .$$

Chapter 5

Results

5.1 Growth rate

In this section, we will test the secondary models presented in section 2.2.2 that relate the specific growth rate μ with the environmental variables S, I and T.

5.1.1 Specific growth rate and Salinity - Modifying Ratkowsky Model

The relationship between the Specific growth rate and salinity is similar to the one defined by the Ratkowsky model, yet it can be seen in Figure 5.1 that the curve described has a quadratic form in a central interval while it experiments an exponential drop out of it.

To describe this relationship we assumed that there exists a certain salinity value, S_{opt}, at which μ_{max} is reached. Also, we must consider the minimum and maximum salinity values, S_{min}, S_{max} resp., for which population growth actually exists. Hence, $S_{min} \leq S_{opt} \leq S_{max}$.
In addition, when μ_{max} is reached, we can assume that the osmotic pressure inside and outside the cell are equal and that it is not necessary to waste energy on active transportation related to salinity. Under these assumptions made, we consider an interval of S centered at S_{opt} on which the cell is able to control its cytoplasm composition with no additional effort. Hence, inside of this interval the curvature has a quadratic form, specified by parameter b.

Once surpassed the boundaries of the central interval, the osmotic regulation systems are overwhelmed and the cell has difficulty maintaining its internal composition properly. This effect is mathematically reflected on both exponential terms, specified by parameters c_{max} and c_{min} in the following relation that we propose:

$$\mu(S) = \mu_{max}\left(1 - b\left(\frac{S - S_{opt}}{S_{opt}}\right)^2\right)\left(1 - e^{c_{min}\ (S_{min} - S)}\right)^2\left(1 - e^{c_{max}\ (S - S_{max})}\right)^2. \quad (5.1)$$

Now, we must fix the parameter b at a specific value to ensure $\mu(S) \geq 0\ \forall S \in [S_{min}, S_{max}]$. One has

$$\mu(S) \geq 0 \iff 1 - b\left(\frac{S - S_{opt}}{S_{opt}}\right)^2 \geq 0\,. \quad (5.2)$$

Note that S_{opt} is unknown and hence the maximum M_S of $\left(\frac{S - S_{opt}}{S_{opt}}\right)^2$ for $S \in [S_{min}, S_{max}]$ takes different values depending on its position inside of the interval $[S_{min}, S_{max}]$:

- If $S_{opt} \geq \frac{S_{max} + S_{min}}{2}$, then $M_S = \left(\frac{S_{min} - S_{opt}}{S_{opt}}\right)^2$.
- If $S_{opt} < \frac{S_{max} + S_{min}}{2}$, then $M_S = \left(\frac{S_{max} - S_{opt}}{S_{opt}}\right)^2$.

If we consider

$$b_\infty = \frac{1}{max\{\left(\frac{S_{max} - S_{opt}}{S_{opt}}\right)^2, \left(\frac{S_{min} - S_{opt}}{S_{opt}}\right)^2\}}\,,$$

then, for $b = b_\infty$ one can guarantee (5.2), and (5.1) can be written as follows:

$$\mu(S) = \mu_{max}\left(1 - b_\infty\left(\frac{S - S_{opt}}{S_{opt}}\right)^2\right)\left(1 - e^{c_{min}\,(S_{min} - S)}\right)^2\left(1 - e^{c_{max}\ (S - S_{max})}\right)^2. \quad (5.3)$$

Now we will test the model by fitting it into a set of experimental results from table 3.2 at top right. Since the model includes a lot of parameters we will fix some of them to decrease the degree of freedom; in particular $c_{min} = 1$, $c_{max} = 0.5$, $S_{min} = 0$. Due to the fact that it is not a linear model, we will use the Levenberg-Marquardt algorithm (Section 4.2.2) gnuplot implementation to fit the experimental data.

Reference	$\mu_{max} \pm SE$ day^{-1}	$S_{opt} \pm SE$ (p.s.u)	$S_{max} \pm SE$ (p.s.u)
Hwang 2000	0.1731 ± 0.0063	18.92 ± 0.58	39.25 ± 0.70

Table 5.1: Fitted parameters of 5.3 to data from Table 3.2.

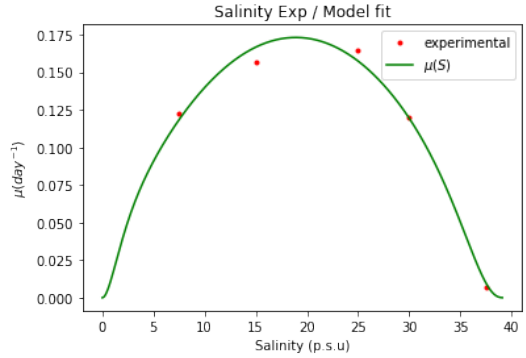


Figure 5.1: Relationship between salinity and *Alexandrium minutum* Specific growth rate $\mu(S)$. The green curve corresponds to (5.3) with fitted paramteres in Table 5.1 while red dots are the values of experimental data from Table 3.2.

5.1.2 Specific growth rate and Irradiance - Blackman Model

To find the relationship between μ and irradiance we will take the experimental data from table 3.2 and fit those points to the Blackman model (2.9), using the Least-Squares method (Section 4.2.1). Unfortunatelly, the only empirical experiments found that study the relationship between irradiance and μ only tested 3 different values for light intensity and therefore the error analysis is inaccurate. One can appreciate in Figure 5.2 that growth is limited by a certain irradiance I_k.

μ_{max} (d^{-1})	I_k $(mmol\ photon\, m^{-2}s^{-2})$
0.1799 ± 0.021	129.15 ± 22.12

Table 5.2: Fitted parameters of (2.9) to data from Table 3.2 at top right.

5.1.3 Specific growth rate and Temperature - Ratkowsky Model

To test the Ratkowsky model defined in (2.8) we will use two datasets. The first one, found in Table 3.2, has tested 5 different temperature values and it will work to confirm the relationship described by Ratkowsky. The second one, from table 3.2 at top left, with only 3 different temperature values, is not enough to provide reliable fits for the parameters a and b, yet it will be useful later when we want to relate the three environmental variables with μ and test the results comparing

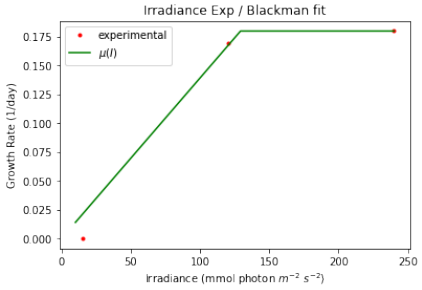


Figure 5.2: Relationship between irradiance and *Alexandrium minutum* Specific growth rate

to the data found in Table 3.2. Both fits are done using the Least-Squares method (Section 4.2.1).

Experiment 1 - Schmidt [6]

Repeating the process for the salinity relationship with μ we have fixed the parameters $T_{min} = 0$ and $c = 0.5$ to decrease the degrees of freedom and, therefore, uncertainty.

Reference	T_{max} (^{o}C)	b $(d\ (^oC)^{-1})$
schmidt [6]	25.25 ± 0.2690	0.03180 ± 0.00053

Table 5.3: Fitted parameters of (2.8) with data in Table (5.1.3).

Experiment 2 - Hwang2000 [3]

Repeating the process for the salinity relationship with μ we have fixed the parameters $T_{min} = 5$ and $c = 0.5$ to decrease the degrees of freedom and, therefore, uncertainty.

Reference	T_{max} (^{o}C)	b $(d\ (^oC)^{-1})$
Hwang 2000 [3]	31.45 ± 1.39	0.02324 ± 0.007638

Table 5.4: Fitted parameters of (2.8) with data in Table 3.2 at top left.

Maximums and minimums

It is easy to see in figure 5.3 that the minimums of $\mu(T)$ are in $T \in \{T_{min}, T_{max}\}$

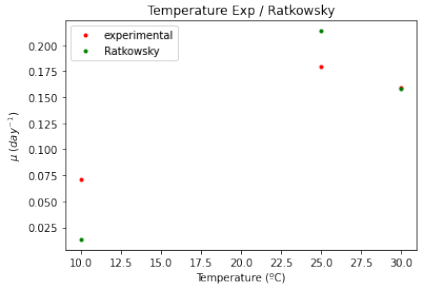


Figure 5.3: Relationship between temperature and *Alexandrium minutum* Specific growth rate's square root using Ratkowsky's model

when the growth rate is zero and the maximum is located somewhere near T_{max} before the fast exponential drop. To obtain the maximum, one has to solve for

$$\frac{d\mu}{dT}(T) = 2\,b^2\,(T - T_{min})\,\left(1 - e^{a(T-T_{max})}\right)\left(1 - e^{a(T-T_{max})}\,(1 + 2\,a\,(T - T_{min}))\right) = 0\,.$$

Note that $a,\ T_{min},\ T_{max},\ b$ are fixed values (they are determined by other environmental variables and intrinsic properties of the cell which we are considering to be fixed). In the figure 5.4 it can be seen how the function starts positive and decreases up to negative values, crossing 0 at one particular point.

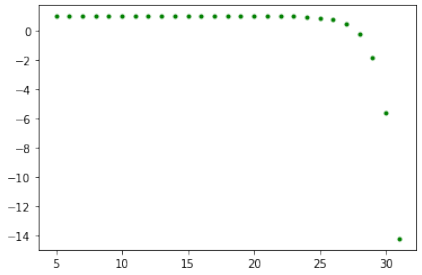


Figure 5.4: Plot of $1 - e^{a(T-T_{max})}\ (1 + a\,(T - T_{min}))$ when $T \in (T_{min}, T_{max})$.

For $T \in (T_{min}, T_{max})$, $\frac{d\mu}{dT}(T) = 0$ reduces to solve

$$f(T) = 1 - e^{a(T-T_{max})}\ (1 + a\,(T - T_{min})) = 0 \tag{5.4}$$

It is easy to see that there exists a unique temperature value such that the condition (5.4) is hold. In essence, since $0 < a < 1$,

$$f(T_{min}) = 1 - e^{a\,(T_{min}-T_{max})} > 0, \qquad f(T_{max}) = -a\,(T_{max} - T_{min}) < 0\,,$$

and by the Bolzano's theorem, there exists T such that $f(T) = 0$.

Moreover, since $f' = -a\, e^{(T-T_{max})}\,(2 + a\,(T - T_{min})) < 0\ \forall T \in (T_{min}, T_{max})$ we have that f is strictly decreasing. Hence, the point T such that $f(T) = 0$ is unique. To find the solution of (5.4) we apply a fix point simple iteration scheme, defined by the iteration function

$$g(T) = -\frac{\ln\left(1 + a\ (T - T_{min})\right)}{a} + T_{max}\ ,$$

which is a local contraction. We start the iteration at $T = T_{max}$. Using the parameters adjusted in 5.1.3, with $\varepsilon = 10^{-8}$ the maximum for $\mu(T)$ is $T = 27.76\ ^{o}$C, which can be confirmed in figures 5.4 and 5.3.

5.2 Multi-factorial growth rate

In this section we want to give a multi-factorial expression of μ based on the expressions found in section 5.1 which relates μ with each of the environmental variables S, I and T.

The process will be the following: multiply the two expressions to give a general expression of μ depending on these variables, make some statements about the parameters to keep consistency, use experimental data to test the calculations and finally use the resulting expression of growth rate $\mu(S, I, T)$ in Baranyi-Roberts (2.3) and compare with experimental data.
To test our analytical calculations we used as data sources the results shown in table 3.2 of three experiments that study the effect of these variables on a population's growth. The control conditions of these experiments are:

$$S = 15,\ T = 25,\ I = 120\ .$$

5.2.1 Salinity and Temperature

We will give an expression of the specific growth rate using the curves $\mu(S)$ and $\mu(T)$ described by (5.3) and (2.8):

$$\begin{cases} \mu(T) = b_T\ (T - T_{min})^2\ \left(1 - e^{a_T\ (T - T_{max})}\right)^2 \\ \mu(S) = \mu_{S,max}\ \left(1 - b_\infty\ \left(\frac{S - S_{opt}}{S_{opt}}\right)^2\right)\ \left(1 - e^{c_{min}\ (S_{min} - S)}\right)^2\ \left(1 - e^{c_{max}(S - S_{max})}\right)^2 \end{cases} \tag{5.5}$$

Let $M = ||\mu(T)||_{\infty,[T_{min},T_{max}]}$ and $N = ||\mu(S)||_{\infty,[S_{min},S_{max}]}$. We consider

$$\psi(T) = \frac{\mu(T)}{M}, \qquad \rho(S) = \frac{\mu(S)}{N},$$

and define

$$\mu(S,T) = \mu_{max}\psi(T)\rho(S)\ ,$$

for a suitable parameter μ_{max} to be determined. By definition in (5.3), we know that for $T = 25$ we have $\mu(S_{opt}) = \mu_{max|T=25}$. Then, one considers

$$\mu_{max} = \frac{\mu_{max|T=25}\, M\, N\, b_T}{\mu(T=25)} \in [0,1]\,. \tag{5.6}$$

We represent the corresponding surface in Figure 5.5. Using the data in table 3.2, we have $\mu_{|T=25}(18.92) = 0.1731\, day^{-1}$, and according to (5.6), $\mu_{max} = 0.1793\, day^{-1}$. Hence, the parameters of the surface fixed in order to match the conditions shown in Table 3.2 are shown in Table 5.2.1.

Parameter	**Value**
S_{min}	0
S_{opt}	18.92
S_{max}	39.25
c_{min}	1
c_{max}	0.5
μ_{max}	0.1793
T_{min}	5
T_{max}	31.96
a_T	0.5

Table 5.5: Fixed parameters to plot surface $\mu(S,T)$.

By construction, the critical points of $\mu(S,T)$ are given by the Cartesian product of the critical points of $\mu(S)$ and $\mu(T)$. Hence, the maximum (S_{opt}, T), where T is the maximum of $\mu(T)$, and the minimums (S_{min}, T_{min}), (S_{max}, T_{min}), (S_{min}, T_{max}) and (S_{max}, T_{max}). See Figure 5.5 at the top left for a representation of the surface $\mu(S,T)$.

5.2.2 Irradiance and Temperature

To study the relationship between growth with I and T we will consider salinity to be fixed by a certain S_0 and use Blackman (2.9) and Ratkowsky (2.8) model to

give and expression of $\mu(I,T)$ as independent variables, setting some statements about the parameters involved to ensure consistency with other expressions of the growth rate. Consider

$$\begin{cases} \mu(T) = b_T\ (T - T_{min})^2\ \left(1 - e^{a_T(T-T_{max})}\right)^2, \\ \\ \mu(I) = \begin{cases} \mu_{I,max}\ \frac{I}{I_k}, & \text{If } I < I_k \\ \mu_{I,max} & \text{otherwise} \end{cases} \end{cases} . \tag{5.7}$$

Combining both expressions as done before for $\mu(S,T)$, we get:

$$\mu(I,T) = \begin{cases} \lambda\ (T - T_{min})^2\ \left(1 - e^{a_T\ (T-T_{max})}\right)^2\ \frac{I}{I_k}, & \text{If } I < I_k \\ \lambda\ (T - T_{min})^2\ \left(1 - e^{a_T\ (T-T_{max})}\right)^2 & \text{otherwise} \end{cases} , \tag{5.8}$$

where λ is determined from the relation

$$\mu(S = 15, T = 25) = \lambda\ f(25)\ \frac{120}{129} \iff \lambda = \frac{129\ \mu(S = 15, T = 25)}{120\ f(25)} .$$

In Figure 5.5 one can find the surface $\mu(I,T)$ at the top right. The parameters used have been collected from Tables 5.4 and 5.2 or calculated according to the conditions shown in Table 3.2.

Parameter	**Value**
T_{min}	5
T_{max}	31.96
a_T	0.5
I_k	129
λ	$4.768 \cdot 10^{-4}$

Table 5.6: Fixed parmeters to plot $\mu(I,T)$.

One has the critical points given by the Cartesian product of the critical points of $\mu(I)$ and $\mu(T)$. Hence, the minimums $(0, T_{min})$, $(0, T_{max})$. Note that the image of the surface's upper bound is reached at (I,T) for $I >= I_k$ and T the maximum for Ratkowsky (see Figure 5.5 for a representation of the surface and the critical points).

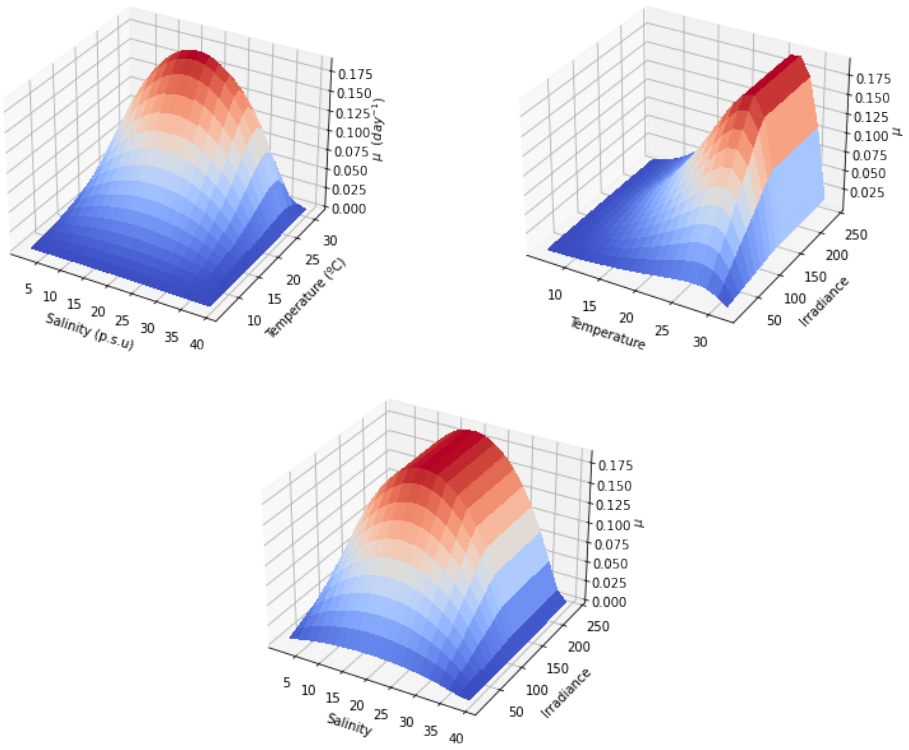


Figure 5.5: Surface of μ(S, T) generated by the curves $\mu(S)$, $\mu(T)$ (top left), surface of μ(I, T) generated by the curves $\mu(I)$, $\mu(T)$ (top right) and surface of μ(I, T) generated by the curves $\mu(S)$, $\mu(I)$ (bottom).

5.2.3 Irradiance and Salinity

To study the relationship between growth with S and I we will consider temperature to be fixed at a certain T_0 and use the models described in (5.3), (2.9) resp.) to give and expression of μ with (S, I) as independent variables, setting some statements about the parameters involved to ensure consistency with other expressions of the growth rate. Consider

$$\begin{cases} \mu(S) = \mu_{S,max}\left(1 - b_\infty\left(\frac{S-S_{opt}}{S_{opt}}\right)^2\right)\left(1 - e^{c_{min}\ (S_{min}-S)}\right)^2\left(1 - e^{c_{max}(S-S_{max})}\right)^2, \\ \mu(I) = \begin{cases} \mu_{I,max}\ \frac{I}{I_k}, & \text{If } I < I_k \\ \mu_{I,max} & \text{otherwise} \end{cases} \end{cases}.$$

We want to find a value τ such that

$$\mu(S,I) = \begin{cases} \tau\ g(S)\ \frac{I}{I_k}, & \text{If } I < I_k \\ \tau\ g(S), & \text{otherwise} \end{cases}, \tag{5.9}$$

being

$$g(S) = \left(1 - b_\infty \left(\frac{S - S_{opt}}{S_{opt}}\right)^2\right) \left(1 - e^{c_{min}\ (S_{min} - S)}\right)^2 \left(1 - e^{c_{max}(S - S_{max})}\right)^2 .$$

Using the results obtained in Section (5.2.1) and the parameters in Table 5.2, to determine τ we impose that

$$\mu(S = 15, T = 25) = \tau\ g(15)\ \frac{120}{129} \iff \tau = \frac{129\ \mu(S = 15, T = 25)}{120\ g(15)} .$$

With the function described above, we represent the resulting surface $\mu(S,I)$ in Figure 5.5 at bottom. The parameters used have been collected from Tables 5.1 and 5.2 or calculated according to the conditions shown in Table 3.2.

Parameter	Value
S_{min}	0
S_{max}	39.25
S_{opt}	18.92
I_k	129
τ	0.1861
c_{min}	1
c_{max}	0.5

Table 5.7: Fixed parameters to plot $\mu(S,I)$

One has the critical points of $\mu(S,I)$ given by the product of the critical points of (5.3) and (2.9). Hence, the minimums $(S_{min}, 0)$, $(S_{max}, 0)$. Note that the image of the surface's upper bound is reached at (S_{opt}, I) for $I >= I_k$ (see figure 5.5 for a representation of the surface and the critical points).

5.2.4 Irradiance, Temperature & Salinity

Now we want to use the three surface found above to define a 4-dimensional surface described by Irradiance, Temperature and Salinity. Let $\mu(S,I,T)$ be the

final surface, $\mu(S,I)$, $\mu(I,T)$ and $\mu(S,T)$ the equations found in (5.9), (5.8) and (5.2.1) respectively. Let

$$f(T) = (T - T_{min})^2 \left(1 - e^{a_T(T-T_{max})}\right)^2, \qquad g(S) = \left(1 - \frac{1}{(\frac{S-S_{opt}}{S_{opt}})^2_{\infty}} \left(\frac{S - S_{opt}}{S_{opt}}\right)^2\right).$$

We want to find $\delta > 0$ such that

$$\mu(S,I,T) = \begin{cases} \delta\ f(T)\ g(S)\ \frac{I}{I_k} & \text{If } I < I_k \\ \delta\ f(T)\ g(S) & \text{otherwise} \end{cases}. \tag{5.10}$$

In particular, using the results obtained in Section 5.2.1 and the parameters adjusted in table 5.2, to determine δ we impose the following condition

$$\mu(S = 15, T = 25) = \delta\ f(25)\ g(15)\ \frac{120}{129} \iff \delta = \frac{129\,\mu(S = 15, T = 25)}{120\, f(25)\, g(15)}\ .$$

One has the critical points given by the product of the critical points of $\mu(S)$. $\mu(T)$, $\mu(I)$. Hence, the minimums are $(0, I, T)$, $(S, 0, T)$, $(S, I, 5)$ for all S, I, T, and the maximum is $(18.92, I, T_0)$, where T_0 is the maximum for (2.8) and $I >= 129$.

5.2.5 Numerical tests

We use the expression (5.10) in the Baranyi-Roberts model (2.3) and compare it to the old version of the model where μ is given by (2.1). The Baranyi-Roberts differential equation with (5.10) is written as:

$$\frac{dN(t)}{dt} = \mu(S,I,T)\ \frac{e^{bt}}{1 + ae^{bt}} \left(1 - \frac{N(t)}{K}\right) N(t)\ . \tag{5.11}$$

First of all, we need to find proper values for a and b. To do so, we will use the least-squares method (Section 4.2.1) to fit (5.11) into the values from the table 3.2. The parameters, with the respective standard deviation are:

$$a = -0.3003 \pm 0.02647,\ b = 0.01953 \pm 0.0040$$

With a and b fixed, we want to compare the resulting $N(t; t_0, N_0)$ with the experimental observations from table 3.2 and the old version of Baranyi-Roberts. As it can be observed in figure 3.1, the exponential phase starts on the fourth day of experiment and ends in the 20th. Therefore, using equation (2.1) and table 3.2 , we have:

$$\mu = \frac{\ln(165062) - \ln(7390)}{20 - 4} = 0.1941\ day^{-1}\,.$$

Assume that the sampling error of the initial value N_0 plus the possible deviations due to the digitization of the experimental data are fitted by a certain $\varepsilon > 0$. Using **RK45** (section 4.1.1) to solve the system of equations :

$$\begin{cases} \frac{dN(t)}{dt} = \mu(S,I,T)\frac{e^{bt}}{1+ae^{bt}}\left(1-\frac{N(t)}{K}\right)N(t) \\ \frac{dJ(t)}{dt} = \mu(S,I,T)\frac{e^{bt}}{1+ae^{bt}}\left(1-2\,\frac{N(t)}{K}\right)J(t) \end{cases} \quad \begin{cases} N(t_0) = N_0 \\ J(t_0) = 0 \end{cases}, \tag{5.12}$$

where

$$J(t) = \frac{\partial N(t)}{\partial N_0}\,.$$

Note that the experimental data from Table 3.2 is contained in the interval $N(t) \pm \varepsilon\,\frac{\partial N(t)}{\partial N_0}$ during the lag and exponential phases. In figure 5.6 one can appreciate how the classic version of Baranyi-Roberts (where $\mu = 0.1941$ is given by (2.1)) is out of the interval $N(t) \pm \varepsilon\,\frac{\partial N(t)}{\partial N_0}$ during the lag and exponential phases, which means that calculating the growth rate from the relation of *Alexandrium minutum* with its environment is a more accurate approach than estimating the growth rate using (2.1).

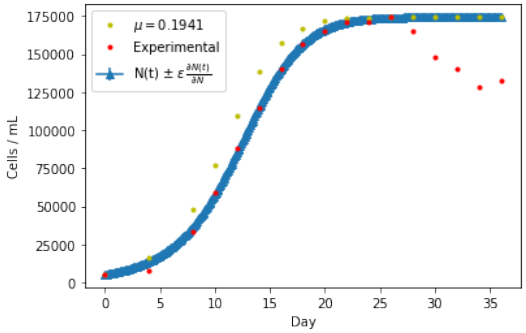


Figure 5.6: Comparison of the two versions of Baranyi-Roberts with a and b fitted and $S = 15$ p.s.u, $T = 25\,^{o}$C and $I = 120\ mmol\ photon\ m^{-2}\,s^{-2}$ and $\varepsilon = 100$. Yellow dots correspond to the evolutionary process given by calculating mu as (2.1) and the red dots correspond to the evolutionary process given by $\mu(S,I,T)$.

5.3 Variability of environmental conditions

The purpose in this section is to study the variation day-to-day of the environmental conditions used to describe the specific growth rate μ.

5.3.1 Irradiance (I)

We will consider two scenarios: a population of *Alexandrium minutum* grown in their natural habitat (coastal sea) and one in a laboratory culture.

Laboratory culture

In this scenario, the irradiance given to the culture has a pattern 12:12, which means the culture is given a constant irradiance I_0 for the first 12 hrs of the day while the other 12 hours receives an irradiance of 0. We consider a smooth transition from I_0 to 0 taking place in 2 ε units of time. Hence, irradiance's variation over a day can be expressed as follows:

$$I(t) = \begin{cases} I, & t \in [\varepsilon, 12-\varepsilon) \\ f(t), & t \in [12-\varepsilon, 12+\varepsilon] \\ 0, & t \in (12+\varepsilon, 24-\varepsilon) \\ g(t), & t \in [24-\varepsilon, 24+\varepsilon] \end{cases}, \tag{5.13}$$

where $f, g \in C^1$, and extended periodically with period 24.

Concerning f, we impose $f(12-\varepsilon) = I,\ f'(12-\varepsilon) = 0\, f(12+\varepsilon) = 0,\ f'(12-\varepsilon) = 0\, f(12+\varepsilon)$ and Hermite interpolation provides the degree 4 polynomial of degree 4 which approaches the function f.

$$P(t) = I - \frac{I}{4\,\varepsilon^2}\,(t-(12-\varepsilon))^2 + \frac{I}{8\,\varepsilon^3}\,(t-(12-\varepsilon))^2(t-(12+\varepsilon))\,. \tag{5.14}$$

Analogously, when the lights are turning on, if we consider the button to be pressed at time $t = t_0 - \varepsilon$ (and therefore, it's totally pressed by time $t = t_0 + \varepsilon$), the polynomial that describes this smooth increase is the following:

$$Q(t) = \frac{I}{4\,\varepsilon^2}\,(t-(t_0-\varepsilon))^2 - \frac{I}{8\,\varepsilon^3}\,(t-(t_0-\varepsilon))^2(t-(t_0+\varepsilon))\,. \tag{5.15}$$

To summarize, the variation of irradiance over a day is expressed by

$$I(t) = \begin{cases} \frac{I}{4\,\varepsilon^2}\,(t+\varepsilon)^2 - \frac{I}{8\,\varepsilon^3}\,(t+\varepsilon)^2(t-\varepsilon), & t \in [0, \varepsilon] \\ I, & t \in (\varepsilon, 12-\varepsilon) \\ I - \frac{I}{4\,\varepsilon^2}\,(t-(12-\varepsilon))^2 + \frac{I}{8\,\varepsilon^3}\,(t-(12-\varepsilon))^2(t-(12+\varepsilon)), & t \in [12-\varepsilon, 12+\varepsilon] \\ 0, & t \in (12+\varepsilon, 24-\varepsilon) \\ \frac{I}{4\,\varepsilon^2}\,(t-(t_0-\varepsilon))^2 - \frac{I}{8\,\varepsilon^3}\,(t-(t_0-\varepsilon))^2(t-(t_0+\varepsilon)), & t \in [24-\varepsilon, 24) \end{cases}. \tag{5.16}$$

The Figure 5.8 illustrates an example of this function with an irradiance $I = 0.5$ and $\varepsilon = 0.1$.

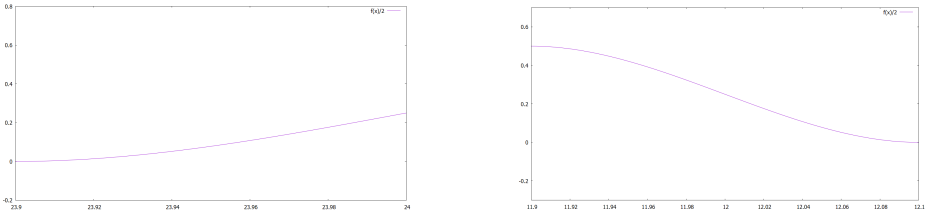


Figure 5.7: Smooth increase (left) and smooth decrease (right) when light is turned on at hour $24 - \varepsilon$ and turned off at hour $12 - \varepsilon$, respectively, with $\varepsilon = 0.1$.

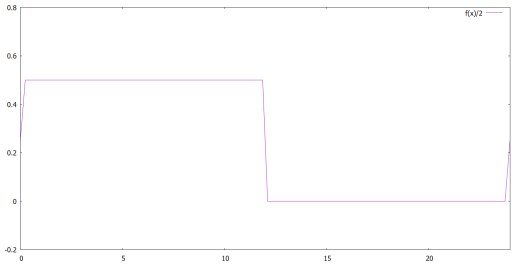
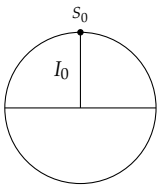


Figure 5.8: $I(t)$ evaluated in $t \in [0, 24)$ for $I = 0.5$ and $\varepsilon = 0.1$.

Natural habitat

We will take as assumptions that other uncontrolled environmental variables such as humidity, air temperature or any other phenomena that can vary irradiance, remain constant over *Alexandrium minutum*'s lifespan and we will consider only the intra-day variability of I as a function of the sun's position and the angle it forms with the population of *Alexandrium minutum*.



Let S_0 be the sun's position when it forms a $\frac{\pi}{2}$ angle with the observer, i.e *Alexandrium minutum*, and consider the maximum irradiance it receives during the day , I_0, which is reached at that specific point. If we consider the irradiance to only depend on the angle $\theta \in [0, 2\pi]$ that forms the sun's position with the

observer, the function f_{I_0} that describes I is the following:

$$f_{I_0}(\theta) = \begin{cases} I_0 \, sin(\theta) & , \theta \in [0, \pi) \\ 0 & , \theta \in [\pi, 2\pi) \end{cases} . \tag{5.17}$$

Note that the variability of θ over a day is pretty simple to parameterize by t (assuming 12 hrs of sunlight):

$$\theta(t) = \frac{t2\pi}{24} = \frac{t\pi}{12} .$$

Hence,

$$f_{I_0}(t) = \begin{cases} I_0 \, sin(\frac{t\pi}{12}) & , t \in [0, 12) \\ 0 & , t \in [12, 24) \end{cases} . \tag{5.18}$$

5.3.2 Temperature (T)

Due to the fact that sea surface temperature moves in a range of 2-3 C during the course of the day and the lifespan of a population of *Alexandrium minutum* is short (around 30 days), we will assume that the effects of the temperature variations in the growth rate are insignificant and therefore consider T to be constant over the lifespan of *Alexandrium minutum*.

5.3.3 Salinity (S)

Salinity is a very important component that affects the physical and chemical properties of seawater. It determines the temperature of oceans and their surroundings, pressure, density, freezing point, insolation, evaporation, humidity, and oceanic currents flow. It influences seawater movements and the habitat of marine life. Ocean Salinity is affected by several factors. These are evaporation, rainfall, river water influx, ocean currents, atmospheric pressure, wind direction, and global warming.

With all these factors, the geographical location and the surroundings are key to determine the variation of salinity in the sea.

As per *Alexandrium minutum*, which is normally located in the coastal sea and given the short lifespan of a population, the variability of salinity in that period of time can be neglected since it is impossible to control all parameters that determine the variation of salinity.

5.3.4 Numerical tests

Let $I(h)$ be the irradiance described as a function of time, whether in the natural habitat (5.18) or in a laboratory (5.16). Consider $S = S_0$, $I = I_0$ and $T = T_0$ to be fixed. The new function for growth rate, expressed as a continuous function of time, is described as follow:

$$\mu_{(S_0,T_0,I_0)}(h) = \begin{cases} \delta\, f_T\, g_S\, \frac{I(h)}{I_k} & \text{If } I(h) < I_k \\ \delta\, f_T\, g_S & \text{otherwise} \end{cases}, \tag{5.19}$$

where $f_T = f(T_0)$, $g_S = g(S_0)$ and $f(T)$, $g(S)$ are defined in Section 5.2.4.

Our purpose is to substitute the constant μ in **BR** (2.3) by $\mu_{(S_0,T_0,I_0)}(h)$ into our model and re-compute the solution of the Cauchy Problem. However, note that **BR** (2.3) is the evlution law of *Alexandrium minutum* with days as the evolution variable, yet $\mu_{(S,T)}(h)$ is a function of the total hours. Therefore, we need to do a variable change in the evolution law (2.3).
Before substituting μ by $\mu_{(S_0,T_0,I_0)}(h)$ we proceed to do the variable change. Consider the **BR** model:

$$\frac{dN(t)}{dt} = \mu\, \frac{e^{bt}}{1 + ae^{bt}} \left(1 - \frac{N(t)}{K}\right) N(t), \tag{5.20}$$

We must do the following variable change:

$$24\, t = h \iff 24\, dt = dh \iff dt = \frac{dh}{24}$$

Applying this change into (5.20) and substituting μ by $\mu_{(S_0,T_0,I_0)}(h)$:

$$\frac{dN(h)}{dh}\, 24 = \mu_{(S_0,T_0,I_0)}(h)\, \frac{e^{b\frac{h}{24}}}{1 + ae^{b\frac{h}{24}}} \left(1 - \frac{N(h)}{K}\right) N(h)\ . \tag{5.21}$$

Note that the growth rate is the inverse of the amount of time each cell needs to assimilate the resources needed in order to increase its biomass up to the volume at which cell division happens. Moreover, μ describes the average inverse of days each cell needs to divide. On the other hand, $\mu_{(S_0,T_0,I_0)}(h)$ gives the inverse of hours each cell needs to divide, considering also the 12 hrs of the day at which growth does not exists because there are no resources (in this case, irradiance). Hence, to give an expression of the growth rate in hours that is equivalent to the growth rate in days, we must multiply $\mu_{(S_0,T_0,I_0)}(h)$ by 2, because we must only consider the time a cell needs to assimilate the resources if and only if there are resources, and there is irradiance only for 12 hours out of 24 every day. Thus, the new **BR** model expressed in hours has this form:

$$\frac{dN(h)}{dh} = \frac{\mu_{(S_0,T_0,I_0)}(h)}{12} \frac{e^{b\frac{h}{24}}}{1+ae^{b\frac{h}{24}}} \left(1-\frac{N(h)}{K}\right) N(h) \ . \tag{5.22}$$

With the evolution law (5.22) defined, we can use the parameters fitted in Section 5.2.5 and a **RK45** implementation in Python [10] to compute the solution of the Cauchy Problem induced by (5.22), with $N(h_0) = N_0$. Moreover, (5.22) is differentiable with respect N_0, therefore we can calculate $\frac{\partial N(h)}{\partial N_0}$ and estimate the propagation of the error (Section 4.1.3) in the evolution process due to deviations in N_0.

The solution of **BR** (5.22) with $I(h)$ as (5.16) is plotted in Figure 5.9. Comparing with the experimental data from Table 3.2 and the solution of 5.22 with growth rate calculated as (2.1), one can appreciate the precision of the new model in replicating the evolution of the cell concentration given by Table 3.2 during the exponential and lag phase. Moreover, Figure 5.9 shows how the variance of $N(h)$ is almost negligible before reaching the stationary phase.

Analogously, the solution in a natural habitat, which irradiance function is given by in (5.18) is plotted in Figure 5.9 in comparison with the experimental data from Table 3.2 and the solution of 5.22 with growth rate calculated as (2.1). Note that the light cycle corresponding to the evolutionary process plotted in 5.10 does not correspond to the light cycle applied in experiment 3.2, yet it is interesting to see how similar both curves are. This is due to the fact that the average amount of irradiance the culture would receive with a light cycle of (5.18) is the same as the culture regarding the experimental data 3.2 received. In addition, Figure 5.10 shows how the variance of $N(h)$ is almost negligible during lag and exponential phase. We have $I_0 = 240$, imposing the average irradiance the population of *microalgae* receives to be 120 and thus emulate the control conditions presented in Table 3.2.

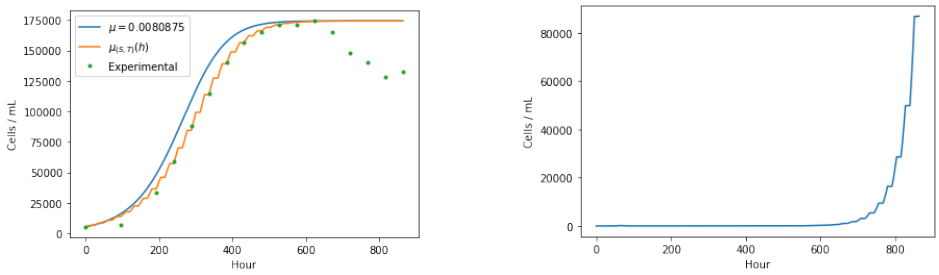


Figure 5.9: Comparison of **BR** (5.22) when the growth rate is given by (2.1) versus $\mu_{(S_0,T_0,I_0)}(h)$, with $I(h)$ given by (5.16) (left) and variation of $N(h)$ with respect to initial condition N_0 (right) with $S_0 = 15$ p.s.u, $T_0 = 25\ ^oC$, $I_0 = 120$ *mmol photon* $m^{-2}\, s^{-2}$ and a, b are parameters fitted in 5.2.5.

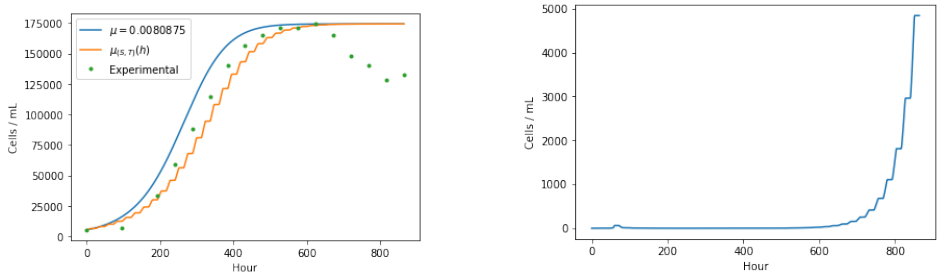


Figure 5.10: Comparison of **BR** (5.22) when the growth rate is given by (2.1) versus $\mu_{(S_0,T_0,I_0)}(h)$, with $I(h)$ given by (5.18) (left) and variation of $N(h)$ with respect to initial condition N_0 (right) with $S_0 = 15$ p.s.u, $T_0 = 25\ ^oC$, $I_0 = 120$ *mmol photon* $m^{-2}\,s^{-2}$ and a, b are parameters fitted in 5.2.5.

5.4 Continuous culture

We consider now the effect of a continuous culture (Section 2.3.2) in a population of *Alexandrium minutum* depending on the dilution factor applied.
Hence, in equation (5.23) we add to the previous **BR** model a term modelling the effect of the dilution factor.

$$\frac{dN_c(h)}{dh} = \mu_{(S_0,T_0,I_0)}(h)\frac{e^{bh}}{1+ae^{bh}}\left(1-\frac{N_c(h)}{K}\right)N_c(h) - D\ N_c(h)\ . \tag{5.23}$$

The dilution factor in the sea is due to the tides, rainfalls and other environmental phenomena. To model dilution's variance over time can be a hard task, due to the large amount of factors involved and, sometimes, such variance is negligible. Thus, we are considering the dilution factor D as a constant.

With the evolution law (5.23) defined, we can use the parameters fitted in Section 5.2.5 and the conditions for salinity, temperature and irradiance from Table 3.2 to emulate the control conditions and use **RK45** to compute the solution of the Cauchy Problem induced by (5.23), with $N_c(h_0) = N_0$. Moreover, (5.23) is differentiable with respect to the parameter D, therefore we can calculate $\frac{\partial N_c(h)}{\partial D}$ and estimate the propagation of the error (Section 4.1.3) in the evolution process due to deviations in D. This means to solve the system of equations:

$$\begin{cases} \frac{dN_c(h)}{dh} = \mu_{(S_0,T_0,I_0)}(h)\frac{e^{bh}}{1+ae^{bh}}\left(1-\frac{N_c(h)}{K}\right)N_c(h) - D\ N_c(h) \\ \frac{dJ(h)}{dh} = \mu_{(S_0,T_0,I_0)}(h)\frac{e^{bh}}{1+ae^{bh}}\left(1-2\ \frac{N_c(h)}{K} - D\right)J(h) - N_c(h) \end{cases} \quad \begin{cases} N_c(t_0) = N_0, \\ J(t_0) = 0 \end{cases}, \tag{5.24}$$

where now

$$J(h) = \frac{\partial N_c(h)}{\partial D} \ .$$

We will solve this system for different values of D and see how it affects to the evolution of N over time. Note that during the exponential phase of the culture of Table (3.2) the average growth rate is around $0.18\, day^{-1}$. Hence, since we want to compare the effect of dilution with the control conditions we consider the values

$$\begin{aligned}
D_0 &= 0\, day^{-1} = 0\, hour^{-1} \\
D_1 &= 0.1\, day^{-1} = 0.004167\, hour^{-1} \\
D_2 &= 0.2\, day^{-1} = 0.008333\, hour^{-1} \\
D_3 &= 0.3\, day^{-1} = 0.01250\, hour^{-1} \\
D_4 &= 0.4\, day^{-1} = 0.01667\, hour^{-1}
\end{aligned}$$

The solution with a light cycle of 12:12 and $I(h)$ given by (5.16) is plotted in Figure 5.11. In Figure 5.12 we show the continuous evolutionary process which would take place in the natural habitat, with a light cycle described as (5.18). In both figures one can appreciate small drops in cell concentration during the day. This behaviour corresponds to the hours of the day with no irradiance in which there is not cell division due to lack of resources and the effects of dilution decrease the cell concentration. One can appreciate in both figures how for $D = D_4$ cell concentration never increases. However, dilution does not affect individual cell division itself. Note in Figure (5.11) (right) how the variability of $N_c(h)$ with respect parameter D is negligible for all values of D during exponential and lag phase. Note that the absolute value of the variation of $N_c(h)$ with respect to the parameter D increases exponentially at the stationary phase due to the fact that the *BR* model we are discussing in this project only applies for lag and exponential phase and does not consider stationary phase.

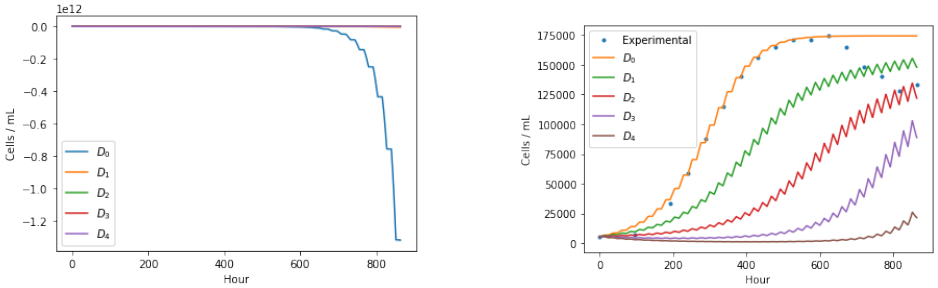


Figure 5.11: Evolutionary process given by continuous culture $N_c(h)$ (left) and variation of $N_c(h)$ (right) with respect parameter D, where D takes the values shown in 5.4 and irradiance is calculated as (5.16).

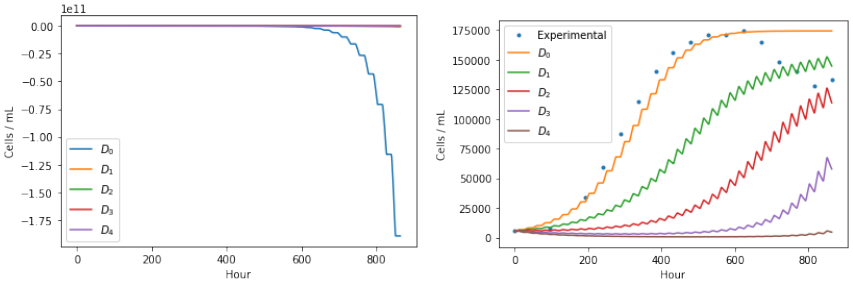


Figure 5.12: Evolutionary process given by continuous culture $N_c(h)$ (left) and variation of $N_c(h)$ (right) with respect parameter D, where D takes the values shown in 5.4 and irradiance is calculated as (5.18).

Chapter 6

Conclusions

In this project we have seen how the evolution law given by the **BR** model serves to describe the evolutionary process described by a population of *Alexandrium minutum* during the lag and exponential phases, either in a laboratory culture or in its natural environment. Ordinary differential equations will enable us to include the parameter corresponding to the maintenance energy into the **BR** model and thus characterize the stationary phase of this evolutionary process.
We used **RK45** to compute the population evolution with great precision, regardless of the complexity of their evolution law, ensuring a virtually negligible computational error.

We can conclude that the relationship between population growth and the environment where the *microalgae* develops can be written as the product of the expressions that relate this growth to each of the environmental variables. Hence, we can assume that the specific growth rate is the inverse of the time each cell needs to assimilate the necessary resources for the cell to divide under optimal environment conditions. Throughout the use of Blackman's model we can assume that irradiance is a growth-limiting substrate for photosynthesis (simillar to the nutrient substrate) and its effect is key to understant how the population evolves along the hours of the day, depending on the level of irradiance that cells receive. Finally, with the study of the continuous culture we have seen how adding dilution in a **BR**-type population model does not affect the rate at which cells divide or their ability to do so, but rather the evolutionary process itself, decreasing cell concentration proportionally to it.

Chapter 7

Perspectives

Now that we have studied how to calculate the optimal conditions for irradiance, salinity and temperature there are many questions we could ask to ourselves about the interaction of this *microalgae* with its environment and how its evolutionary process depends on the type of interactions the specie has. As next steps for this investigation, we propose the following set of experiments:

- This project could not study the effect of different nutrient sources on a population of *Alexandrium minutum*'s specific growth rate due to the fact that the few existing experiments found had unreliable data. For this reason we propose these two experiments:
  - Test which nutrient source is optimal, whether phosphate or nitrate.
  - Assume that the Blackman's model describes the relation between nutrient substrate and growth rate and add this relation to find a new expression of the specific growth rate that includes nutrient and test the results by studying some cultures with different nutrient substrate concentration.
- From a mathematical point of view it would be interesting to study the host-parasite oscillation dynamics given by a combined experiment. This type of relation, along with competitive relations, would be described with a Lotka-Volterra-like model.
- It has been observed how populations of *Alexandrium minutum* migrate to the seabed when cells receive no irradiance and go back to surface with day light. We propose to cultivate a population of *Alexandrium minutum* in a column of liquid to study the effects of migration in the evolutionary process and the migration time.

Appendix. Iterative method to find fixed points in contractile functions

```
import numpy as np

tmin = 5
tmax = 31.45
a = 0.8

def contractive(temp):
    return -np.log(1 + a*(temp - tmin))/a + tmax

#   The parameter temp is the starting point of the iteration
def itera(temp=25.5, eps=1e-8):
    T = temp
    aux = T
    T = contractive(T)
    iter = 0
    while iter < 1000:
        while abs(aux - T) >= eps:
            aux = T
            T = contractive(T)
            iter += 1
        return T
```

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