FACULTEIT
BIO-INGENIEURSWETENSCHAPPEN

HOW DO TEMPERATURE-NITRATE INTERACTIONS SHAPE THE SENSITIVITY OF A BROWN SEAWEED TO GLOBAL WARMING?

9046

Laura Van Waerebeek

Promotor: Prof. dr. ir. Jan Baetens
Promotor: Prof. dr. Olivier De Clerck
Tutor: Soria Delva

Master thesis presented for the achievement of the degree “Master of Science in the industrial sciences: biochemie”

Year: 2019 - 2020

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Acknowledgement

Writing a thesis is part of every student's curriculum to receive their master's degree. It takes a lot of time and energy, so being surrounded with people supporting me is really important. Writing this thesis has challenged me in many ways. Although I was not a fan of data processing at the start of this year, the covid-19 outbreak caused me to delve into this data modelling and improve this skill. Maybe even more important, I eventually started to like it. I am grateful that I have been assigned this subject and that I have been able to delve into it, even though I was not able to finish my main experiment.
In this way, I want to thank a few people who helped me completing this thesis :

First of all, I want to adress this acknowledgement to both of my promotors Jan Baetens and Olivier De Clerck for giving me the opportunity to delve into this very interesting topic. In particular, I want to thank Jan Baetens for the guidance through these exceptionally difficult times, by organizing multiple skype meetings.

Secondly, I want to thank my tutor, Soria Delva. She is the one who taught me everything and assisted me with advice about conducting my experiments. When I was struggling, she showed understanding and always helped to find a solution to make it work. In this way, I also want to apologize for the many e-mails and questions I've sended you, but I'm sincerely very thankful you've answered them all, even though it was on weekends or holidays.

I also want to thank everyone in the lab for helping me finding the right materials and answering my questions. Hereby, special thanks to Quinten Bafort for taking on the role as supervisor when Soria was not around.

Finally, I also want to thank my friends and family for supporting me when I was having a hard time.

Preambule

The aim of the original study was to determine how temperature-nitrate concentrations would affect the growth of *Dictyota dichotoma.* During the first semester, some pilot studies were conducted in preparation of the final experiment. Due to the COVID-19 outbreak, I was not able to start and complete my main experiment. Therefore, I had no results. As an alternative, a dataset was given to analyze. However, this dataset came from another experiment. I really struggled with creating structure in my thesis, due to the difference between the pilot studies, in preparation to the original experiment, and the given dataset, which was about another study.

Abstract

Marine ecosystems and in particular, seaweed communities, will be affected by the ongoing global climate change. Through this study, an attempt is made to gain insight on how seaweed populations will react to this global warming. Specifically, six populations of the seaweed species *Dictyota dichotoma* were subjected to a range of different temperatures in a common-garden experiment, to analyze their thermal response of growth. Hereby, it was noted that the populations grown in colder areas showed a tendency to perform better in a future climate, while the populations collected in warmer regions seem to be more vulnerable to climate warming. Based on small differences in thermal growth optimum between the populations, there is a possibility that populations show a tendency for evolutionary adaptation. However, this cannot be confirmed due to the lack of distinction between transgenerational plasticity and genetic adaptation in this experiment.

Keywords: *Climate change, seaweed, thermal response*

Table of content

List of abbreviations

Abbreviation	Description
SW	Natural Seawater
ASW	Artificial Seawater
TM	Tropic Marin
CO2	Carbon dioxide
N_2O	Nitrous oxide
CH_4	Methane
IPCC	Intergovernmental Panel on Climate Change
RCP	Representative Concentration Pathways
TSR	Temperature size rule
HTL	High trophic levels
PUFA	Poly unsaturated fatty acids
ALA	Alpha-linolenic acid
LA	Linoleic acid
AICc	Second order Akaike information criterion
RMSE	Root mean square error
MAE	Mean absolute error
ME	Mean error
SSE	Sum of squared error

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Introduction

Global climate change is already affecting the distribution and composition of seaweed species (Poloczanska et al., 2013). In general, widespread shifts towards the poles and deeper into the ocean are occurring, resulting in a reorganisation of local comunities (Parmesan & Yohe, 2003). Shifts in seasonal life cycle events as well as a reduced body size, are already known as consequences of this global climate change (Poloczanska et al., 2013; Daufresne et al., 2009). Seaweeds harbor an incredible biodiversity and they have already proven their economical and ecological potential (Harley et al., 2012). Research on how those seaweed communities will be affected by global climate change will therefore be very useful. Two important drivers of biological processes, are temperature and nutrient availability (Thomas et al., 2017). Those two parameters will directly be affected by global warming. In the ocean, the sea surface temperature is rising (Reay et al., 2007), resulting in an increased stratification of the water column. Hereby, nutrient limitation can occur (Speight, 2018). Understanding how seaweed species respond to temperature and nutrient availability is of main importance to understand the impact of global warming. The interaction of both temperature and nitrate concentration on the growth of *Dictyota dichotoma*, was going to be studied during this thesis, using an interaction double-exponential model. (Thomas et al., 2017). By conducting this experiment, following hypotheses would be studied: (i) whether or not nutrient limitation influences T_{opt} of a species and (ii) whether or not temperature-nutrient interactions on growth performance are more explicit than when only one factor is taken into account. However, a reorganisation of the proposed content had to be done, since the main experiment could not be performed due to the COVID-19 outbreak.

Another response that recently received increasing attention, is the possibility for evolutionary adaptation in marine ecosystems. Changes in phenotypes through times are due to phenotypic plasticity or potential evolutionairy adaptation. Direct evidence on evolutionary adaptation is rare. Most of the times, potential for phenotypic evolution is based on indirect approaches, like common garden experiments or reciprocal transplant approaches (Reusch, 2013). As an alternative for the main experiment, the growth results of *Dictyota dichotoma* populations coming from different locations, grown at different temperatures in a common-garden experiment, were discussed. Hereby, an attempt was made to (i) investigate if there is a difference in termal growth response between the populations (ii) and to define the vulnerability of *Dictyota dichotoma* populations to global climate change.

1 Literature study

1.1 Climate change

The atmosphere contains several gasses that are capable of absorbing infrared radiation emitted by the Earth and retain heat near the surface. This is known as the greenhouse effect (Darkwah et al., 2018). The most important greenhouse gasses are carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and fluorinated gasses (Darkwah et al., 2018). Over the years, the concentration of carbon dioxide and other greenhouse gasses has increased, which is mainly caused by human activity and the growing industries. Due to this rising concentration, more heat is captured, and our climate is warming up (Stefan, 2018). Between pre-industrial times and recent years, human activities have already caused a global warming of 1.0°C. If this global warming continues at the current rate, a global warming of 1.5°C will be reached during 2030-2052 (Leung et al., 2019).

1.1.1 Abiotic effects of climate change on the oceans

More than 80% of the heat added to the global climate system has been absorbed by the oceans. Although the ocean's thermal capacity has caused a slower warming of the ocean surface layer than air temperatures, the average temperature in the upper layer of the oceans has increased over the past 100 years (Reay et al., 2007). This warming of the upper layer will drive greater stratification of the water column, especially in coastal waters were the thermocline will become an even more powerful boundary. According to IPCC scenario RCP 8.5 (Adger & Coauthors, 2007), which represents a scenario of comparatively high greenhouse gas emissions, the sea surface temperature would rise up to 1.5°C by 2050 and 3.2°C by 2100, relative to the sea surface temperatures of 1870-1899. On the other hand, scenario RCP 2.6, which assumes high technological development reducing the greenhouse gas emissions by nearly 90%, predicts a global sea surface temperature rise of 0.8°C by 2050 and 1.2°C by 2100 as represented in Figure 1 (Genner et al., 2017).

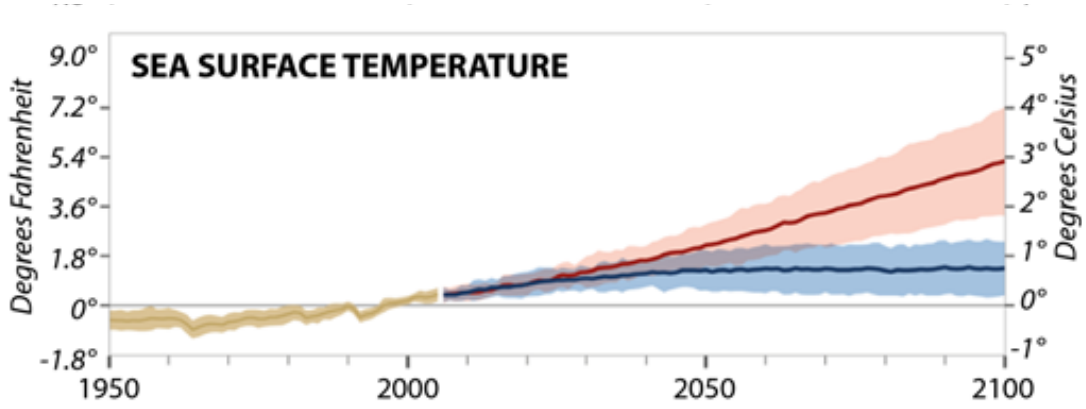


FIGURE 1: SIMULATED INCREASE IN TEMPERATURE ACCORDING TO RCP 8.5 (RED) AND RCP 2.6 (BLUE) (GENNER ET AL., 2017).

Rising ocean temperatures will also have an influence on the availability of nutrients, through an increase of water column stratification. When there is natural stratification, the varying dense layers will be able to mix due to wind upwelling and downwelling. This downwelling causes mixing of the upper surface layer with colder layers at greater depth. As a result of this natural stratification, nutrients are able to reach varying depths (Capotondi et al., 2012). In general, increased stratification will reduce upper ocean nutrient levels by trapping nutrients in deeper layers (Dave & Lozier, 2013). Nitrogen, phosphorus and iron are three of the main nutrients essential for the growth of many organisms (Bindoff et al., 2019). Relative to 2006-2015, the nitrate concentration in the upper 100m is predicted to decline by 9-14% by 2081-2100 under IPCC scenario RCP 8.5. Under scenario RCP2.6 a decline of 1.5-6% is predicted, in response to increased stratification (Figure 2). These predictions are based on medium confidence due to limited evidence (Bindoff et al., 2019). From earth system model simulations, which rely on global climate models providing simulations of the Earth's presence, past and future climate (NCAR & UCAR.), iron concentrations are projected to increase partly due to a decreased consumption in regions of declining nitrate (Misumi et al., 2014).

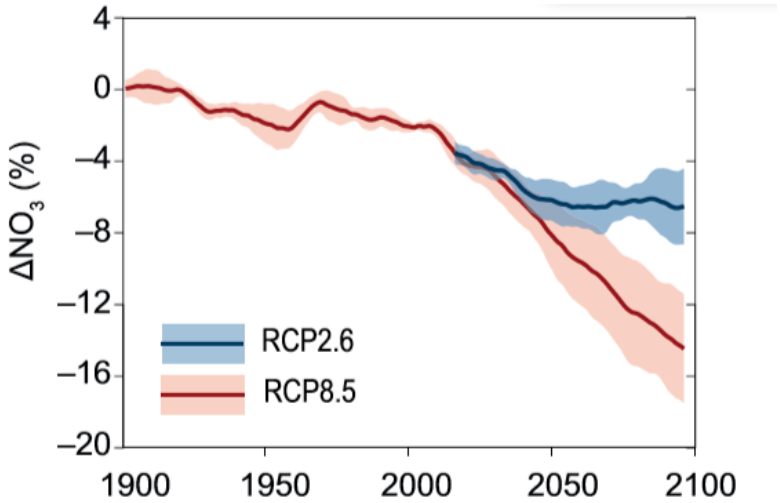


FIGURE 2: NITRATE CONCENTRATION OF THE UPPER 100M, SIMULATED REGARDING GLOBAL CHANGE OVER THE PERIOD 1800-2100 (BINDOFF ET AL., 2019).

Many other environmental variables, like the amount of sea-ice, dissolved oxygen concentration, and ocean acidity, will vary according to temperature. Hereby, temperature change will affect those variables indirectly as well (Genner et al., 2017). In addition, rising temperatures and a subsequent melting of glaciers are causing a global rise in sea level. Over the past 25 years, the total sea level has increased with approximately seven centimeters. It is expected that this rate will accelerate in the future as the melting of glaciers will increase (Nerem et al., 2018).

Finally, besides changes in the aforementioned ocean properties, another phenomenon can be observed, which is linked to the increased absorption of carbon dioxide by the oceans. Approximately 50% of the CO_2 caused by human activity remains in the atmosphere, 20% is taken up by the terrestrial biosphere, and the remaining 30% is absorbed by the oceans (L.Sabine et al., 2004). The increased emission of CO_2 causes the CO_2 uptake by the oceans to rise, subsequently leading to an increase in the concentration of free H^+ and a decrease in pH (Feely et al., 2004). This phenomenon has been termed ocean acidification, and its underlying chemical processes are represented in Figure 3 (Feely et al., 2004). Since the pre-industrial period, ocean acidification has led to an overall decrease of 0.1 pH units (Doney et al., 2009)

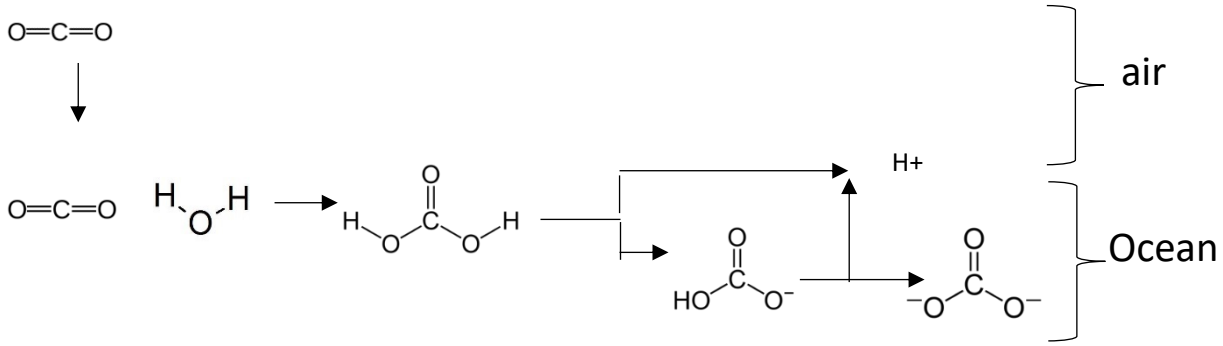


FIGURE 3: ACIDIFICATION PROCESS CAUSED BY INCREASED CO_2 UPTAKE BY THE OCEANS (FEELY ET AL, 2004).

1.1.2 Impact of global climatic changes on marine ecosystems

Climate change is causing changes in chemical and physical properties of the ocean resulting in various consequences for marine ecosystems (Brierley & Kingsford, 2009). Due to the size and complexity of the oceans, but also the difficulty to collect marine samples, the knowledge of how climate change is affecting marine ecosystems lags behind that of terrestrial ecosystems (Rosenzweig et al., 2008). In general, there are three well-known universal ecological responses to global warming in aquatic systems (Daufresne et al., 2009). These will be discussed here in more detail.

Range shifts

The most noticeable impact of global warming is the widespread shift in biological systems (Poloczanska et al., 2016). Warming temperatures causing range shifts of marine species, have been observed across all ocean regions (Poloczanska et al., 2013). In general, it is shown that marine species will migrate towards the poles (Burrows et al., 2011). Studies of Sirenko et al., 2007 and Mueter et al., 2008 show the increased northward distribution of invertebrates and fish in the Bering Sea. Additionally, in response to complex patterns of shifting isotherms and geographical barriers, it is expected that there will be some east-west distribution shifts and shifts towards the equator as well (Burrows et al., 2011). Shifts deeper into the oceans will be the result of species taking refuge in cooler, deeper waters or when there is no latitudinal shift possible (Pinsky et al., 2013). In general, those shifts will result in a reorganization of local communities when species are added or removed and as interactions among species change (Wootton et al., 2008). For example, cold-water arctic species that are unable to adapt quickly to the rising temperatures will decrease in abundance, eventually resulting in a loss of biodiversity (Bluhm et al., 2011; Węsławski et al., 2011; Somero, 2011) (Figure 4).

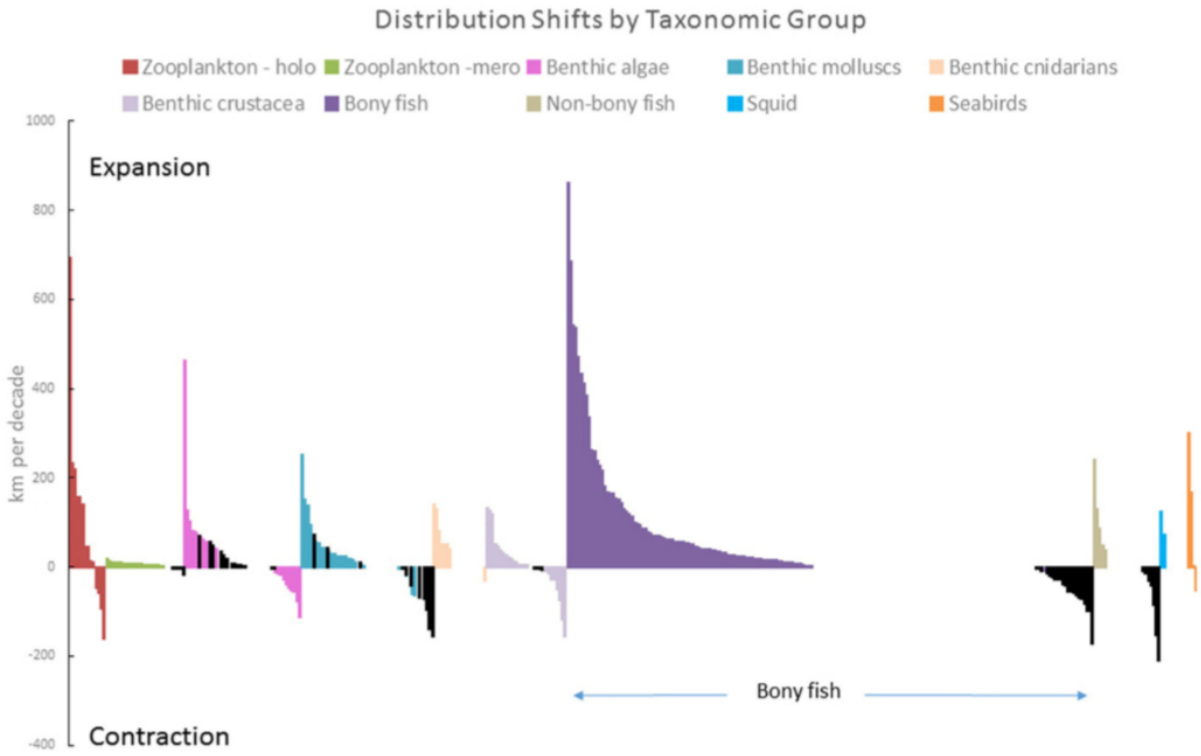


FIGURE 4: SHIFTS IN SPECIES DISTRIBUTION BY TAXONOMIC GROUPS (POLOCZANSKA ET AL, 2013).

Phenology shifts

A second well known response to global warming is a change in phenology (Poloczanska et al., 2016). Phenology can be described as repeated seasonal life cycle events (Edwards, 2016). Those phenological phases will reflect the complicated environmental changes on organisms and ecosystems in a measurable form. Over the years, phenological time series have been recorded and serve now as a valuable source for the investigation of climate change, because temperature will be the trigger for seasonal behavior in several marine organisms (Ahas & Aasa, 2006). In the central North Sea, the plankton community responds to climate change with variation among species (Edwards & Richardson, 2004). On the one hand, either an advance or a stasis in peak abundance was observed for spring-and-summer blooming species. On the other hand, in autumn-and winter-peaking species, delays were observed (Poloczanska et al., 2016). Fish eggs, 12.9 days per decade, and larvae, 9.5 days per decade, showed the greatest advancements. In migratory species, changes in phenology have also been observed. For example, in the North-east Atlantic, tuna will arrive earlier at productive feeding grounds (Poloczanska et al., 2016). Based on the collected timings on the first eggs of seabirds, several species showed advances up to 8.4 days per decade (Wanless et al., 2009). Figure 5 illustrates a general impression of the phenological shifts caused by climate warming.

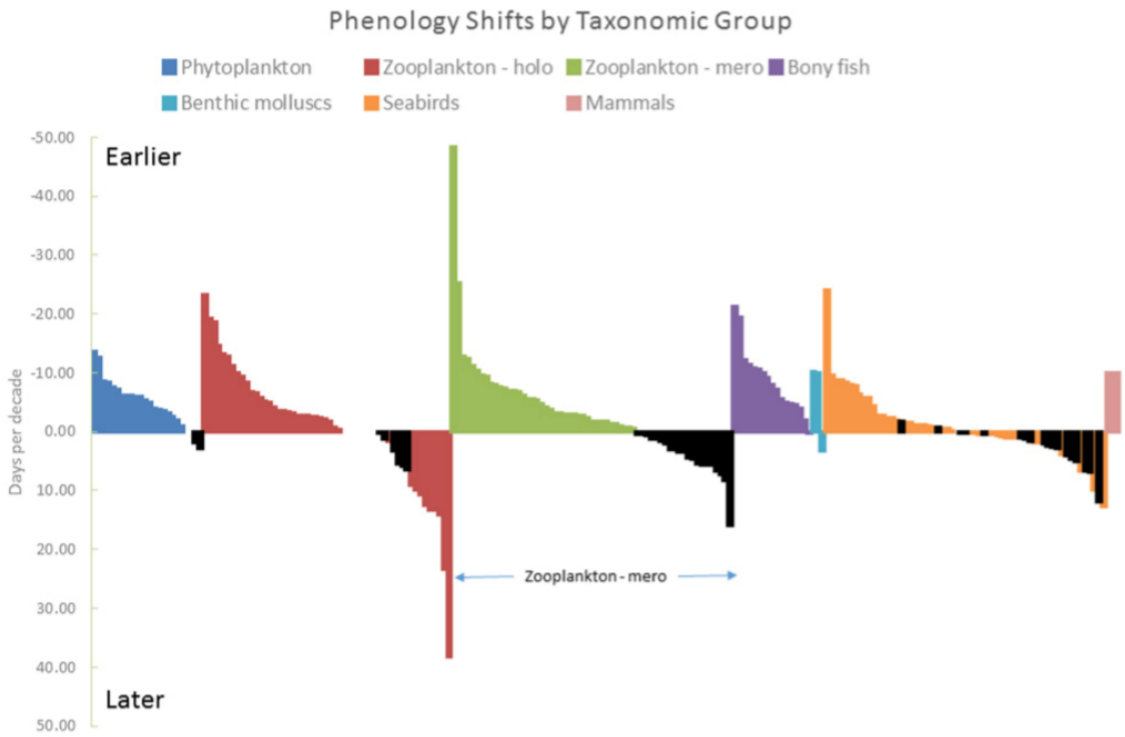


FIGURE 5: OBSERVED PHENOLOGY SHIFTS BY TAXONOMIC GROUPS (POLOCZANSKA ET AL., 2013).

Phenological shifts will be variable across ocean regions and taxonomic groups. In addition, the phenomenon of an earlier arrival of spring caused by climate change can be observed in numerous ecosystems, not only in aquatic systems (Asch, 2015). The timing of insect appearance and migratory bird arrival, as well as flowering and leaf unfolding dates, are some of the many processes that are affected by climate change (Parmesan & Yohe, 2003).

Reduced body size

Another recently described ecological response to global warming, is a decline in body size (Daufresne et al., 2009; Forster et al., 2012; Cheung et al., 2013). This response, based on the temperature-size rule (TSR), is found in diverse organisms. The TSR describes the phenotypic plastic response of species' size to temperature (Wootton et al., 2008). Unfortunately, temperature influence on organism size remains poorly understood (Kingsolver & Huey, 2008). A potentially important driver of the TSR of animals, is the oxygen supply (Forster et al., 2012). Based on data simulated by PISCES-APECOSM from 1850-2100, regarding the RCP 8.5 business as usual scenario, the maximum body-size of high trophic levels (HTL) organisms is expected to decrease by 9–10.5% by the end of the century. This decrease will depend on the community. The HTL biomass includes the total pelagic biomass (Lefort et al., 2015). The migratory community is predicted to show a decrease in size of 1.3 cm in 2080–2100 compared to 1985–2005. The mesopelagic community is predicted to show a higher decrease than the migratory community namely a reduction of 2.1 cm. The epipelagic community is the most affected, with an expected decline of 5 cm in its mean maximum body-size (Lefort et al., 2015; Jones et al., 2013).

Adaptive responses

Despite the aformentioned effects of climate change, marine animals and plants show potential for adaptive evolution (Reusch, 2013). Unfortunately, few multigenerational experimental approaches have been carried out to explore the potential of genetically adaption to climate change. However, in the fish world, there are several examples about potentially evolutionary and plastic responses (Crozier & Hutchings, 2013). In marine plants, the potential for adaptive evolution was mostly studied with respect to temperature regimes, either using common garden or reciprocal transplant approaches (Reusch, 2013). An example is the study of Winters et al., 2011, using the seagrass *Zostera marina*. Hereby, evidence has been revealed for thermal adaptation of the northern versus southern population, regarding their photophysiology. Direct assessment of genetic changes in the genome is another way to show evolutionary adaptation (Travisano & Shaw, 2013).

1.2 Seaweeds

Seaweeds can be described as the assemblage of macroscopic, multicellular marine green, red and brown algae although at some point in their life cycle, they will be unicellular as spores or zygotes (Maggs & Callow, 2003). Evolutionarily, seaweeds are rather diverse. In general, they can be divided into three phyla, based on their thallus color. The phylum *Phaeophyta* includes brown algae, the phylum Rhodophyta represents the red algae, and the phylum *Chlorophyta* refers to green algae (Maggs & Callow, 2003). However, in addition to their pigmentation, they differ in biochemical and ultrastructural features. Those differences originate through various processes during their evolution. As a consequence, they are classified into other kingdoms. Hereby, green and red algae are situated in the kingdom *Plantae* and brown algae in the kingdom *Chromista* (Guiry, 2020).

1.2.1 Effects of climate change on seaweeds

Seaweeds are structuring agents that harbor an incredible biodiversity. They are known to be vulnerable to chemical as well as to physical changes in the marine environment. Changes in ocean properties as a result of climate change have an impact on seaweed performance via an increased stress as well as changes in resource availability (Harley et al., 2012).

Temperature effects

Temperature determines the performance of seaweeds at the fundamental levels of enzymatic processes and metabolic functions (Ahas & Aasa, 2006). Like any other organism, seaweeds have an optimal growth temperature (Breeman, 1988). There are two factors that play an important role in the impact of disruptive stress on an organism. Disruptive stress includes heat stress and cold stress and they both damage seaweeds (Davison & Pearson, 1996). First of all, the exposure time to disruptive stress is very relevant. Organisms are able to cope with strong temperature stress for several hours, if afterwards, they return to their optimal conditions to recover. When they are exposed for multiple weeks or to even more stressful conditions, physiological dysfunction becomes severe and will most likely result in cell death (Wiencke et al., 1994 ; Eggert, 2012).

On cellular level, disruptive stress will mostly affect proteins and membrane-associated processes (Los & Murata, 2004; Eggert, 2012). Under higher temperatures, proteins face loss of stability resulting in decreased enzyme activity or inactivation, while higher temperatures will cause fluidization of membranes and disintegrate the lipid bilayer (Los & Murata, 2004). This damage, in addition to other changes, will result in a reduced photosynthesis and carbon assimilation, causing a slower growth and eventually lead to mortality (Davison & Pearson, 1996). Despite all those negative effects, seaweeds are able to evolve increased tolerance or activation of recovery mechanisms. This increased tolerance becomes possible through an

increased production of heat shock proteins by environmental stress (Sørensen et al., 2003), an increased proportion of PUFA's to tolerate cold stress, and the availability of anti-oxidants and compatible solutes (Murata & Los, 1997)

Temperature as well as nutrients are both nonlinear drivers of biological processes. Little is known about their interactive effects on growth (but see Thomas et al., 2017). A study of Gerard, 1997 shows that, kelps who were able to accumulate nitrogen, were able to tolerate periods with heat stress while those who could not accumulate nitrogen, experienced negative growth rates. Interactions among temperature and other abiotic stressors like pH were studied as well (Harley et al., 2012). For example, the negative effect of an increased temperature on the growth rate of a tropical warm-temperate coralline algae was greater under a reduced pH (Anthony et al., 2008).

Temperature- response curves show a characteristic shape (Bulté & Blouin-demers, 2006), as represented in Figure 6. Growth rate as well as photosynthetic rate increase with temperature, plateau at maximum level and decrease rapidly after reaching the upper critical temperature (Eggert, 2012).

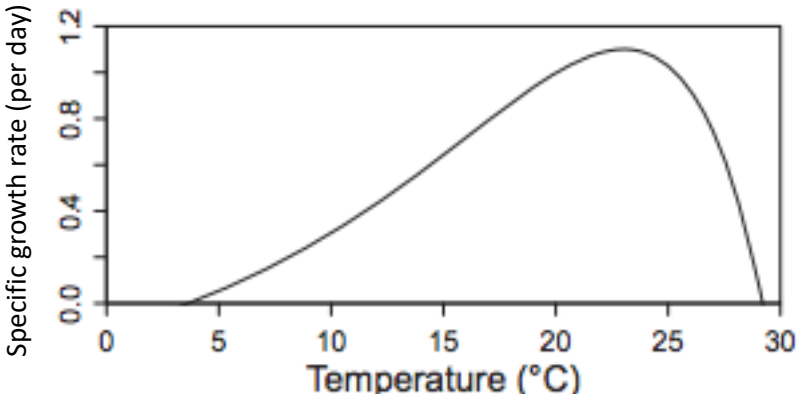


FIGURE 6: VISUALIZATION OF A THERMAL RESPONSE CURVE (THOMAS ET AL., 2017).

Nutrient effects

Seaweed growth is limited by the availability of nutrients, such as nitrogen and phosphorus (Lapointe, 1989). In general, nutrient requirements can be divided into three categories, being macronutrients (N, P, C, ..), trace elements (Zn, Fe, ..), and vitamins (Harrison & Hurd, 2001). Phosphorus will be used in energy transfer and will serve as a structural element. Nitrogen will be of major metabolic importance in compounds, such as amino-acids and purines (Lobban & Harrison, 1994). Among the possible limiting nutrients, nitrogen limits most of the time the growth. Phosphorus will be the second. Rising temperatures caused by climate warming will also have an influence on the availability of nutrients. Specifically, they will cause increased stratification of the water column, resulting in a decrease of nutrient availability for many seaweeds (Speight & Speight, 2018). On the other hand, there is an increased load of nutrients

running into coastal waters, creating eutrophication of those waters. Factors causing eutrophication are a combination of multiple human induced stressors (Justic et al., 2009). This excess of nutrients causes harmful algal blooms, reduced water quality, hypoxia and loss of natural resources (Justic et al., 2009).

Two equations are used to describe the relationship between growth rate of an individual and nutrient concentration (Lobban & Harrison, 1994). Only few cases in macroalgae use the Monod equation, using external nutrient concentration, to describe this relationship (Lobban & Harrison, 1994). The Droop equation is most recommended. Here, the growth rate is related to the intracellular nutrient concentration (Sommer, 1991). Figure 7 represents a visualization of the Droop equation. This equation describes a rectangular hyperbola with an asymptote equal to the maximum specific growth rate. Growth rate increases with an increasing nutrient concentration up until a steady state is reached (Lobban & Harrison, 1994).

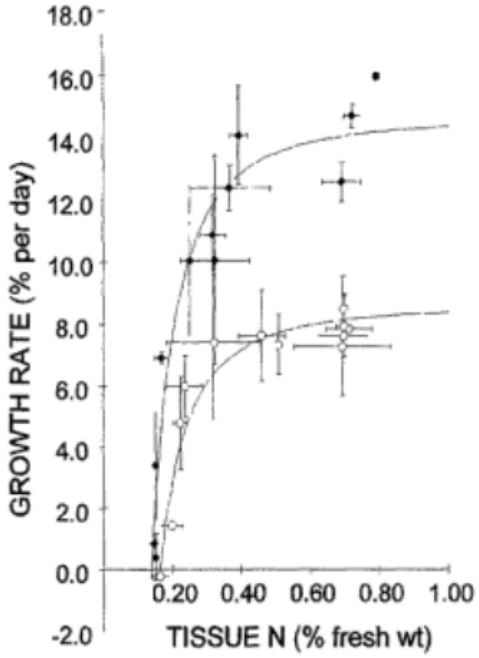


FIGURE 7: GROWTH RATE AS A FUNCTION OF TISSUE NITROGEN ACCORDING TO THE DROOP EQUATION (LOBBAN & HARRISON., 1994).

TABLE 1: REPRESENTATION OF THE MONOD-EQUATION AND DROOP-EQUATION (LOBBAN & HARRISON., 1994).

Monod-equation	**Droop-equation**
$\mu = \mu_m \frac{S}{K_s + S}$	$\mu = \mu'_m(1 - \frac{q_0}{q})$
K_s: half saturation constant growth S: substrate concentration	q = cell quota q_0= subsistence cell quota

1.2.2 Importance of seaweeds

Ecological potential

Seaweeds are important parts of marine ecosystems and form the base of many marine food webs (Zi-Min Hu, 2016). They serve as a safe harbor for marine organisms like fish, where they can hide for predators (Vandendriessche et al., 2007). Seaweeds retain sediment and dampen waves (Hasselström et al., 2018). Among seaweeds, several complex biotic interactions occur (Lobban & Harrison, 1994). Herbivory is an example of marine organisms using algal communities as a food source (Lobban & Harrison, 1994). In many communities, herbivores and algae co-exist (Vasquez et al., 1984). Seaweed communities are also very suitable as spawning habitats. *Cololabis saira* and *Hyporhampus sajori* use floating seaweeds as a spawning habitat just as larvae and juveniles of *Seriola Lalandi* and *Trachurus symmetricus* will use it as a nursery habitat.

Economical potential

Various studies have shown the economic potential of algae in many different ways. In general, algae can be used as a food source, as biofilters to remove pollutants and nutrients from wastewater, as bioindicators, and for the production of useful compounds such as metabolites (Sulaiman et al., 2016). One of those useful compounds that algae can produce, are diterpenes (Vallim et al., 2005). Those compounds show antifouling, anti-oxidant and cytotoxic activities, as well as antitumor and antiviral activities, which make them interesting for pharmaceutical use (Vallim et al., 2005). Besides producing diterpenes, algae also constitute a source of polyunsaturated fatty acids (PUFAs) (Harwood & Guschina, 2009; Li et al., 2002). While PUFAs are vital for the human metabolism, mammals are not capable of synthesizing certain essential forms, like α-linolenic acid (ALA) and linoleic acid (LA) (Harwood & Guschina, 2009). Therefore, they rely on their diet to obtain these substances. They are also very useful in the removal of nutrients, like nitrogen and phosphorus, out of the water. Seaweeds are capable to store nutrients as well. In that way, eutrophication can be combated (Fei, 2004). In addition to nitrogen and phosphorus, they are also able to store CO_2 so they will contribute to the reduction of ocean acidification (Lau et al., 1995; Fei, 2004)

1.2.3 General characteristics of brown algae

Brown algae are part of the phyla Phaeophyta. The brown color is the result of the xanthophyll pigment fucoxanthin (Sheath & Wehr, 2003). The majority of brown algae lives in marine environments, where they fulfill an important role providing habitat or food (Guiry, 2020). Growth is achieved by divisions in a single apical cell or a row of apical cells. The most common and simplest form is a branched, filamentous thallus. This branching will appear when the apical cell divides and produces new apical cells. Worldwide, about 1500-2000 species of brown algae are known, including the largest and fastest growing seaweed species (Maggs & Callow, 2003).

1.2.4 General characteristics of dictyota dichotoma

Phaeophyceae is a class of the phylum *Phaeophyta*. This class contains the species *Dictyota dichotoma*, which is a common species of algae (Sheath & Wehr, 2003).

Natural habitat

Dictyota dichotoma is a common and widespread brown algal species, present in the North-East Atlantic from the Canary Islands and Mediterranean Sea to southern Norway. In addition, the species has also been found in Argentina and South- Africa, as a result of introductions from European populations in these regions (Steen et al., 2019).

Reproduction

Dictyota dichotoma is able to reproduce both sexually and asexually and is a haplodiplont organism with isomorphic ploidy phases (Steen et al., 2019). A haplodiplontic organism has both multicellular haploid and diploid stages (Steen et al., 2019). During their sexual life cycle, there will be a union of male sperm (male gametophyte) and a female egg (female gametophyte), resulting in a diploid zygote. The zygote will divide mitotically and create a new sporophyte. In turn, this sporophyte will produce tetraspores, scattered on its surface, which will give rise to new haploid gametophytes (Lobban & Harrison,1994). The regulation of vegetative growth and sexual reproduction in laboratory conditions makes this species a promising model organism for a wide range of studies. Under laboratory conditions, the minimum time needed to complete the life cycle of *Dictyota dichotoma* is approximately two months (Bogaert et al., 2016).

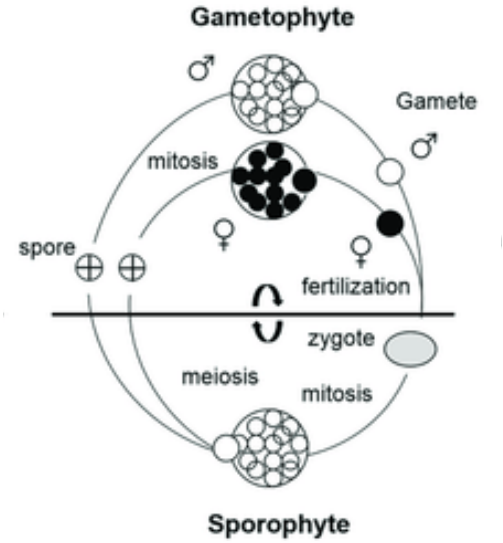


FIGURE 8: SEXUAL LIFE CYCLE OF *DICTYOTA DICHOTOMA* (de Bettignies et al., 2018).

In addition, certain abiotic factors can be used to induce fertility. For example, it has been proven that sporogenesis in young germlings can be caused by nutrient depletion or by using red light. However, by continuous incubation of *Dictyota dichotoma* at *8°C,* fertility was completely inhibited (Bogaert et al., 2016).

Dictyota dichotoma can also spread by fragmentation (i.e. asexual reproduction). Hereby, the adult sporophyte will split into fragments. These fragments will eventually develop into full-grown individuals which are genetically identical to their parents. The main disadvantage of this process is the lack of genetic diversity. Hereby, they are more vulnerable to parasites and diseases as well as to changing environmental conditions (Mouritsen, 2013).

2 Material and methods

2.1 Pilot studies and final experiment

To ensure that the main experiment would succeed, a few pilot studies were performed in advance. By conducting those experiments, several parameters could be determined which were applied in the final experiment. Figure 9 represents the link between each of these pilot studies. Specifically, in the "growth medium experiment" we evaluated the growth of *Dictyota dichotoma* in artificial versus natural culture media. The medium that yielded the best growth results would have been used in the final experiment as well as in the second trial of the pilot study "maternal provisioning". The aim of the "growth model experiment" was to identify the growth model that describes best the increase in surface area of the algae over time. This model was subsequently used to determine and compare the growth rate in the "growth medium experiment" and would have been applied in the final experiment to calculate the growth rate of the algal individuals. The produced sporophytes would be the source for gametophyte germlings, used in the final experiment.

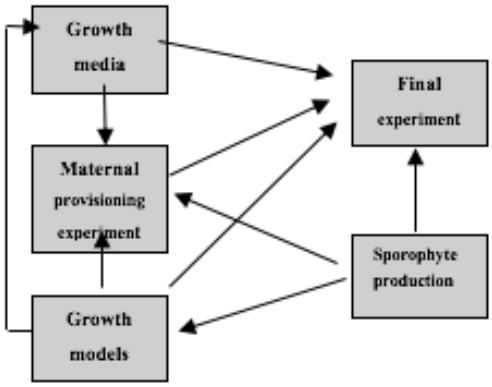


FIGURE 9: SCHEMATIC OVERVIEW OF THE PILOT STUDIES AND THE FINAL EXPERIMENT.

2.2 Pilot studies

2.2.1 Culture conditions

Experiments were carried out using laboratory cultured sporophyte germlings derived from gametophytes of populations in Goes (The Netherlands) and Noirmoutier (France) or laboratory cultured gametophyte germlings derived from sporophytes of Tenerife. Cultures from different populations were used, depending on the availability of germlings, and algae in general, at the start of an experiment. During each experiment, algae were cultured in petri-dishes, which were placed in temperature- controlled incubators under light intensities ranging from 20 to 30 µmol photons m^{-2} s^{-1} .

2.2.2 Sporophyte production

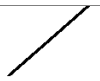
The production of sporophytes has its main purpose in the final experiment. When the sporophyte becomes fertile it releases spores, which would have been used in the final experiment to test the effect of temperature and nutrient concentrations on the growth rate of *D. dichotoma.* In the final experiment, we would use spores instead of adult material, due to their small size. Otherwise, a large volume of medium would be required in order to keep the nutrient concentration constant.

To obtain sporophytes, a fertile male and female gametophyte were placed together in a petri-dish, where they released sperm and egg cells. For the final experiment, all gametophytes were lab cultured and were originally derived from sporophytes of Goes. This was repeated five times using different gametophyte couples. The resulting zygotes were allowed to grow and develop into adult sporophytes in crystallising dishes (100ml). For the pilot studies "evaluation of growth models" and "maternal provisioning", sporophyte germlings were derived by crossing lab cultured gametophytes of Noirmoutier. Both were stored at a temperature of 20°C

2.2.3 Evaluation of growth models

Two sets of 10 two-week-old sporophyte germlings, which were the result of a single crossing between two *D. dichotoma* specimens as mentioned in section 2.2.2, were cultivated for 23 days in petri-dishes, under two different temperatures, being 22°C and 25 °C. Two different temperatures were chosen to find out whether the most suitable growth model depends on the temperature or not. The medium, natural seawater (SW) + 10ml/L of the nutrient solution mPES (West & McBride, 1999), was refreshed weekly to avoid nutrient limitation. Every two to three days, a photograph of each germling was taken using a microscope. Following this, the surface area of each algal individual was determined using the open-source image processing programs imageJ (version d1.47, Wayne Rasband) and GIMP (version 2.8, The GIMP team). For the model fitting and selection, suitable models proposed in the literature were collected. Based on the paper published by Paine et al. (2012), seven empirical equations describing different growth models, were tested (TABLE 2able 2). The function nlsLM of package "minpack.lm" including the method "LM" algorithm was used in R version 3.6.3 for model fitting. The best model was chosen based on the goodness of fit, by comparing the corresponding second order Akaike criterion (AICc), root mean square error (RMSE), mean absolute error (MAE) and mean error (ME) for each algal individual per growth model. To calculate AICc values, the package AICcmodavg was used in R version 3.6.3. AICc is an indicator for model performance and is designed to compare different models. For each individual, the most suitable growth model was determined by a trade-off between the lowest AICc on the one hand, and the smallest error (RMSE, MAE and ME) on the other hand. Per temperature, it was counted how many times each model was the most suitable. Finally, the two most suitable growth models were fitted on one individual at each temperature.

TABLE 2: THE SEVEN TESTED GROWTH MODELS IN THE PILOT STUDY " EVALUATION OF GROWTH MODELS" AND THEIR CORRESPONDING EQUATION. THE MODELS ARE FIRST-ORDER DIFFERENTIAL EQUATIONS. THE PARAMETER Mo REPRESENTS THE INITIAL BIOMASS, R THE ABSOLUTE INCREASE IN BIOMASS PER TIME UNIT AND THE PARAMETERS K AND L REPRESENT UPPER AND LOWER ASYMPTOTES $P=K-L-Mo$ (PAINE ET AL., 2012).

Model	Equation	Form
linear	$\mathrm{M_0} + \mathrm{rt}$	
exponential	$\mathrm{M_0}e^{rt}$	
Power law	$(\mathrm{M_0}^{1-\beta} + rt(1-\beta))^{\frac{1}{1-\beta}}$	
monomolecular	$K - e^{-rt}(K - \mathrm{M_0})$	
Three-parameter logistic	$\frac{\mathrm{M_0}K}{\mathrm{M_0} + e^{-rt}(K - \mathrm{M_0})}$	
Four- parameter logistic	$L + \frac{\mathrm{M_0}(K-L)}{\mathrm{M_0} + Pe^{-rt}}$	
Gompertz	$K(\frac{Mo}{K})\, e^{-rt}$	

2.2.4 Evaluation of growth media

The purpose of this experiment was to determine the optimal growth medium for *D. dichotoma*. Three media were tested, being autoclaved artificial seawater (ASW) (Appendix 5), autoclaved "Tropic Marin", and filtered, autoclaved seawater. To each medium, 10mL/L mPES was added to provide enough nutrients. Each medium had twelve replicates so a total of 36 four-week-old gametophyte germlings derived from sporophytes sampled in Tenerife, were used. This experiment ran for three weeks in an incubator set at 22°C. Every week, the medium was refreshed. Algal individuals were photographed at the beginning and at the end of the experiment and their surface areas were determined, in the same way as outlined in Section 2.2.3. Based on the outcome of the growth model experiment for the individuals grown at 22°C, the exponential growth model was used. The P-value of a Shapiro-Wilk test was greater than 0.05 so the growth data were normally distributed. According to a P-value smaller than 0.05 for the Levene's test, equal variances could not be assumed so a non-parametric Kruskal-Wallis test was used to see whether the growth difference between the media was significant or not.

2.2.5 Maternal provisioning

In order to conduct reliable growth measurements when growing algae at different nutrient concentrations, nutrient reserves received from the parental algae must be consumed first. The purpose of this experiment was to determine how long this process takes. Two sets of 10 sporophyte germlings, derived as described in Section 2.2.2, were used for this experiment. Ten sporophyte germlings were cultivated in ASW to which 10ml/L mPES was added. The other ten were cultivated in ASW without the addition of mPES. The individuals grown in medium without mPES, will have at some point too little nutrients left to continue their growth, resulting in a growth difference relative to the individuals cultivated in SW + mPES. By using a micropipette, the sporophyte germlings were isolated from the culture, immediately after fertilization. To avoid contamination of the ASW-medium with nutrients originating from the SW while isolating the germlings, those germlings were immersed two times in ASW before putting them in their final medium (West & McBride, 1999). Every day, a photograph of each germling was taken by using a camera that was attached to a microscope. For the statistical part, multiple one-sided two sample t-tests were conducted, using SPSS version statistics25, to test if the surface-area of the individuals in ASW+ mPES was significantly higher than that in ASW without mPES for each day. Secondly, the most suitable growth model out of the nonlinear asymptotic growth models described by Paine et al. (2012) (monomolecular, three-parameter logistic, four-parameter logistic and Gompertz) was identified for the data derived from the individuals grown without mPES. The use of a non-linear asymptotic growth model allows to determine the parameter estimate *K*, which represents the upper horizontal asymptote of the growth function. In that way, the moment upon which the growth of the individuals cultivated in medium without mPES approaches zero could be determined. Model fitting was done in the same way as described in Section 2.2.3. Hereafter, the most suitable model was fit on the data and visualized using package ggplot2 in R version 3.6.3.

2.3 Final experiment

In the final experiment, the influence of nutrient availability and temperature on the growth rate of *Dictyota dichotoma*, would have been studied. Gametophyte germlings, lab cultured derived from sporophytes of Goes as described in Section 2.2.2, would have been cultivated in petri-dishes at several temperatures and nutrient concentrations. Taking into account the result of the growth media experiment, the used medium would have been natural SW + mPES. Growth of each germling would have been monitored over the course of the experiment. Unfortunately, it was not possible to start this experiment, due to the COVID-19 outbreak.

2.4 Alternative project: analysis of thermal response data

A dataset was analysed as an alternative for the final experiment. This dataset contains information about the thermal growth response of six *D. dichotoma* populations spread across Europe. Specifically, the following populations were studied: Goes (The Netherlands), Finvarra

(Ireland), Punta del Hidalgo (Tenerife), Bergen (Norway), Cadiz (Spain), and Noirmoutier (France). In each population, fertile sporophytes were sampled and brought back to the laboratory facilities of Ghent University, where they were allowed to release spores. The resulting gametophyte germlings were placed in an antibiotics solution for two weeks, to remove any source of contamination. After those two weeks in antibiotics, they were placed in autoclaved SW + 10mL mPES/ L without antibiotics to recover from this intensive treatment. Following this recovery, a four-week common garden experiment was performed in order to examine the thermal response of growth in each population. To this end, germlings of each population were cultivated under eight different temperatures, being 8°C, 14°C, 18°C, 20°C, 22°C, 24°C, 26°C, and 28°C. The surface area of each individual was measured at the beginning and at the end of this experiment. Based on these measurements the growth rate was calculated using the exponential formula outlined in Section 2.2.3. This work was carried out beforehand by this thesis' tutor, as part of another project.

As part of this master thesis, the thermal growth response of the six different *D. dichotoma* populations was examined. Firstly, four different thermal growth models (Table 3) were fitted on all observations of one population (Bergen), resulting in one thermal growth curve per model, using the function nlsLM of the package "minpack.lm" in R version 3.6.3. The most suitable thermal response model was determined, using the same method as outlined in Section 2.2.3. Hereby, the AICc was used according to following rule: (number of observations/the number of parameters) < 40. In order to compare the growth curves between the different populations, the NLIN procedure of SAS 14.1, was used. Specifically, a full model and a reduced model were created, based on the Blanchard equation, since this was the most suitable thermal growth model. In the full model, all parameters (*Tmax, To, Gmax,* β) are allowed to differ for each population, in contrast to the reduced model where all the parameters are the same for each population. Tmax represents the maximum temperature for growth, Gmax represents the growth at optimum temperature (To) and β is a scaling factor. An Anova test was used to see if there was a significant difference between the reduced and the full model. This was used to find out whether allowing differences in model parameters between populations significantly improved the fit of the model. In the case of a significant difference, a half-reduced model was designed, in which some parameters were allowed to vary between populations and some were kept constant. A visualization of each parameter estimate per population was created using R version 3.6.3 package ggplot2. Based on this plot, the parameters that were kept constant across the populations in the half-reduced model, $Tmax$ and β, were chosen. The remaining parameters (To and $Gmax$) were allowed to differ between populations. Afterwards, an Anova was used to see whether the half-reduced model fits the data equally well as the full model. The aim of this test was to determine if the parameters $Tmax$ and β were the same for each population. If that was the case, the SSE of the half-reduced model would not be significantly different from the SSE of the full model.

TABLE 3: OVERVIEW OF THE FOUR DIFFERENT THERMAL GROWTH MODELS USED IN THIS THESIS AND THEIR CORRESPONDING EQUATION. GMAX REPRESENTS GROWTH AT OPTIMUM TEMPERATURE (*TO*), *TMAX* AND *TMIN* DESCRIBES RESPECTIVELY THE MAXIMUM AND MINIMUM TEMPERATURE FOR GROWTH. $\boldsymbol{\beta}$ IS A SCALING FACTOR.

Model	Equation	Source
Blanchard	$Gmax * (\frac{Tmax-T}{Tmax-To})^{\beta} \exp(-\beta(\frac{Tmax-T}{Tmax-To} - 1))$	(Blanchard et al., 1996)
β-distribution	$Gmax * \left(\frac{Tmax-T}{Tmax-To}\right) * (\frac{T-Tmin}{To-Tmin})^{(\frac{To-Tmin}{Tmax-To})}$	(Yin et al., 1995)
Yan & Hunt	$Gmax * \left(\frac{Tmax-T}{Tmax-To}\right) * (\frac{T}{To})^{\frac{To}{Tmax-To}}$	(Yan& Hunt, 1999)
Yan & Wallace	$Gmax - b(T-To)^2$	(Yan et al., 1996)

3 Results

3.1 Pilot- studies

3.1.1 Sporophyte production

Figure 10 represents a photograph of a sporophyte germling. When a female egg is fertilized, this can be distinguished by an oval shape instead of a round shape. Out of multiple crosses, only five came out successful.

FIGURE 10: VISUALIZATION OF A SPOROPHYTE GERMLING.

TABLE 4: GAMETOPHYTE COUPLES PLACED TOGETHER IN A PETRI-DISH, RESULTING IN SPOROPHYTE GERMLINGS AFTER FERTILIZATION. THESE SPOROPHYTE GERMLINGS DEVELOP IN AN ADULT SPOROPHYTE. 'DATE' REPRESENTS THE DAY AT WHICH FERTILIZATION OCCURRED.

♀	♂	**date**
GOES142	GOES116	12/11/2019
GOES143	GOES109	30/10/2019
GOES148	GOES124	12/11/2019
GOES154	GOES147	25/10/2019
GOES130	GOES126	/

3.1.2 Evaluation of growth models

Two individuals cultivated at 25°C and one individual at 22°C, were removed from the dataset. During the experiment, those algal individuals got curled up resulting in non-reliable surface area measurements. Hereby, a total of nine individuals at 22°C and eight individuals at 25°C, remained at the end of the experiment. According to the corresponding AICc, RMSE, MAE and ME values (Appendix 1 & 2), the most suitable growth model for five out of nine individuals at 22°C, was the exponential growth model. The exponential model was followed by the three-parameter logistic model with three out of nine individuals having this as the most suitable growth model. For the individuals cultivated at 25°C, the majority (six out of eight individuals) had the three-parameter-logistic growth model as the most suitable growth model. The difference between the exponential and three-parameter logistic model (Figure 11), is visually more clear for the individual cultivated at 25°C

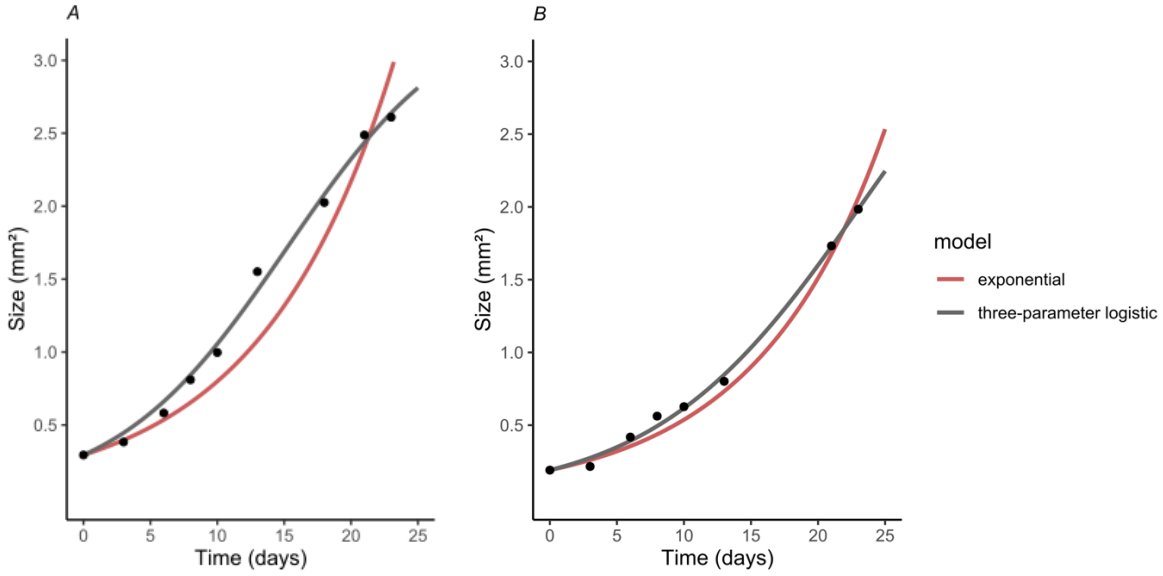


FIGURE 11: THE EXPONENTIAL (RED) AND THE THREE-PARAMETER LOGISTIC GROWTH MODEL (GRAY) ON ONE INDIVIDUAL PER TEMPERATURE. **A**: SPOROPHYTE GERMLING CULTIVATED AT 25°C. **B**: SPOROPHYTE GERMLING CULTIVATED AT 22°C.

3.1.3 Evaluation of growth media

Per growth medium, the growth of the individuals was calculated and visualized in Figure 12. The growth rate of *D. dichotoma* was significantly different between the different media (Kruskal-Wallis test, p= 0.005). Specifically, algae grown in ASW grew significantly slower compared to algae grown in SW (Dunn test, p = 0.019). Remarkable was that most of the algae cultivated in ASW were dying. After 18 days in the medium, the algal individuals in SW looked more healthy and bigger than the individuals in ASW (Figure 13). When the experiment was finished, parameters like pH and salinity were determined (Table 5). No major differences could be observed for those parameters between the media. However, it should be mentioned that the pH and salinity measurements were not accurate, due to the lack of well-functioning equipment.

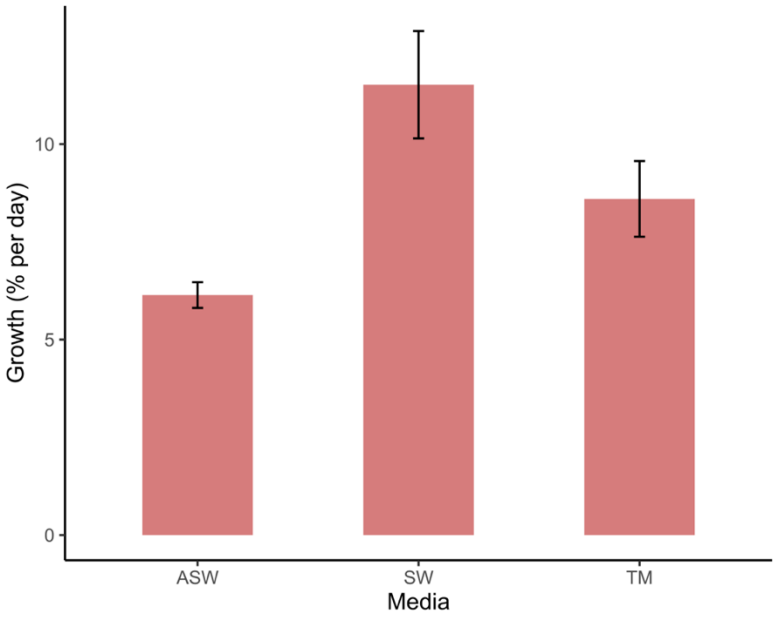


FIGURE 12: RELATIVE GROWTH RATE OF *DICTYOTA DICHOTOMA* IN DIFFERENT CULTURE MEDIA, BEING ARTIFICIAL SEAWATER (ASW), NATURAL SEAWATER (SW), AND TROPIC MARIN MEDIUM (TM). VALUES ARE MEANS ± SE (N=12).

TABLE 5: PH AND SALINITY MEASURED AT THE END OF THE EXPERIMENT FOR SIX DIFFERENT INDIVIDUALS PER MEDIUM.

ASW		**SW**		**TM**	
individual	**pH**	**individual**	**pH**	**individual**	**pH**
TENE25	7,8	**TENE20**	8,1	**TENE18**	7,9
TENE2	7,8	**TENE25**	8,1	**TENE20**	8,1
TENE23	7,8	**TENE23**	8,1	**TENE25**	8,1
TENE4	7,8	**TENE2**	8,1	**TENE22**	8,1
TENE22	7,8	**TENE22**	8,1	**TENE26**	8,0
TENE26	7,8	**TENE18**	8,2	**TENE12**	8,1
salinity	31	**salinity**	33	**salinity**	34

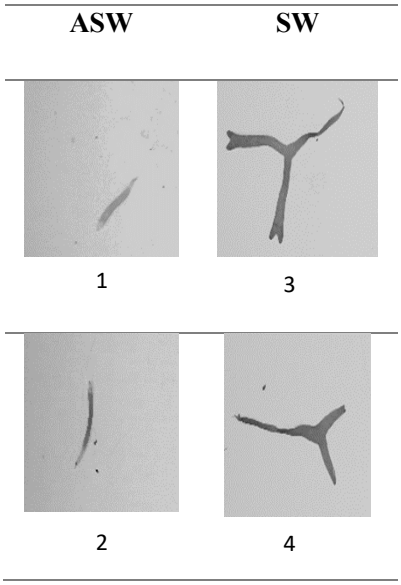


FIGURE 13: HEALTH STATUS OF INDIVIDUALS AFTER 18 DAYS IN THE MEDIUM. PHOTO1-2: MEDIUM = ASW. PHOTO3-4: MEDIUM= SW.

3.1.4 Parental provisioning experiment

The normality assumption was verified using a Shapiro-Wilk test, for each day separately. The only data that were not normally distributed, were the data at the fourth day of the individuals in the medium without mPES. The assumption of homogeneity of variance, assessed using a Levene's test, was fulfilled always. According to the one sided two sample t-tests, the difference in surface area between the individuals in mPES and those without mPES, was significant after five days ($p = 0.042$) (Figure 14). After those five days, the amount of nutrients left, were not sufficient to keep up with the growth rate of the individuals grown in addition of mPES. For the second part of the analyses, the three-parameter logistic growth model turned out to be the most suitable. After fitting this growth model on the data, visualized in Figure 15, the parameter K was 0.06697 mm^2. This implies that the growth of the individuals, grown in media without mPES, approaches zero at a size of 0.06697 mm^2. This process takes approximately 15 days.

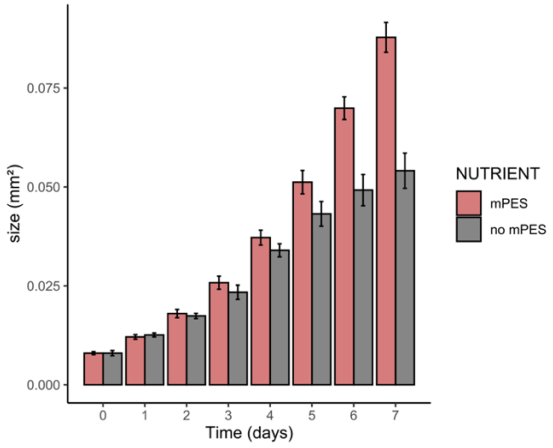


FIGURE 14: BARPLOT OF THE SIZE OF THE INDIVIDUALS PER DAY, CULTIVATED IN MEDIUM WITH MPES (RED) AND WITHOUT MPES (GRAY).

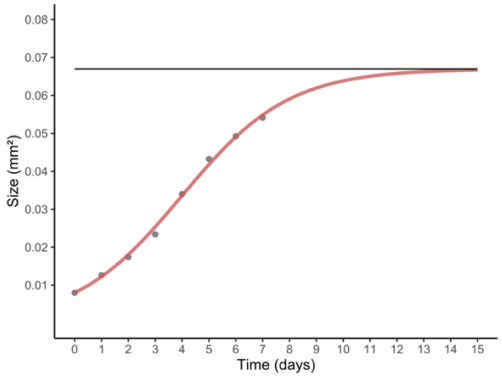


FIGURE 15: VISUALIZATION OF THE FIT OF THE THREE PARAMETER-LOGISTIC GROWTH MODEL ON THE INDIVIDUALS CULTIVATED IN MEDIUM WITHOUT MPES (RED) AND THE CORRESPONDING UPPER ASYMPTOTE K (BLACK).

3.2 Analysis of thermal response data

According to the AICc, RMSE, MAE and ME values, the most suitable thermal growth model for the population 'Bergen', is the Blanchard model (appendix 3). As a result, further analysis is based on this Blanchard model. After fitting a reduced and a full model to the data (Figure 16), those two models could be compared. Based on the sum of squared errors (SSE), the full model (SSE= 4511.5) gives a better fit than the reduced model (SSE= 6650.6). The full model was significantly better than the reduced model ($p < 0.001$), so the populations will generally be different from each other, according to the better fit when the parameters were able to change per population. The same was repeated for the full model and the half-reduced model. The latter (SSE= 4860.6) was already better than the reduced model. However, there was still a significant difference between the half-reduced and the full model ($p < 0.001$). The half-reduced model does not fit the data equally well as the full model. If it would, there could be assumed that there were no big differences for the parameters *Tmax* and β between the populations. Figure 17 shows the parameter estimates per population. The most notable differences here, are the higher Tmax for populations of Finvarra and Noirmoutier and the higher *Gmax* for populations of Cadiz and Tenerife, relative to the others.

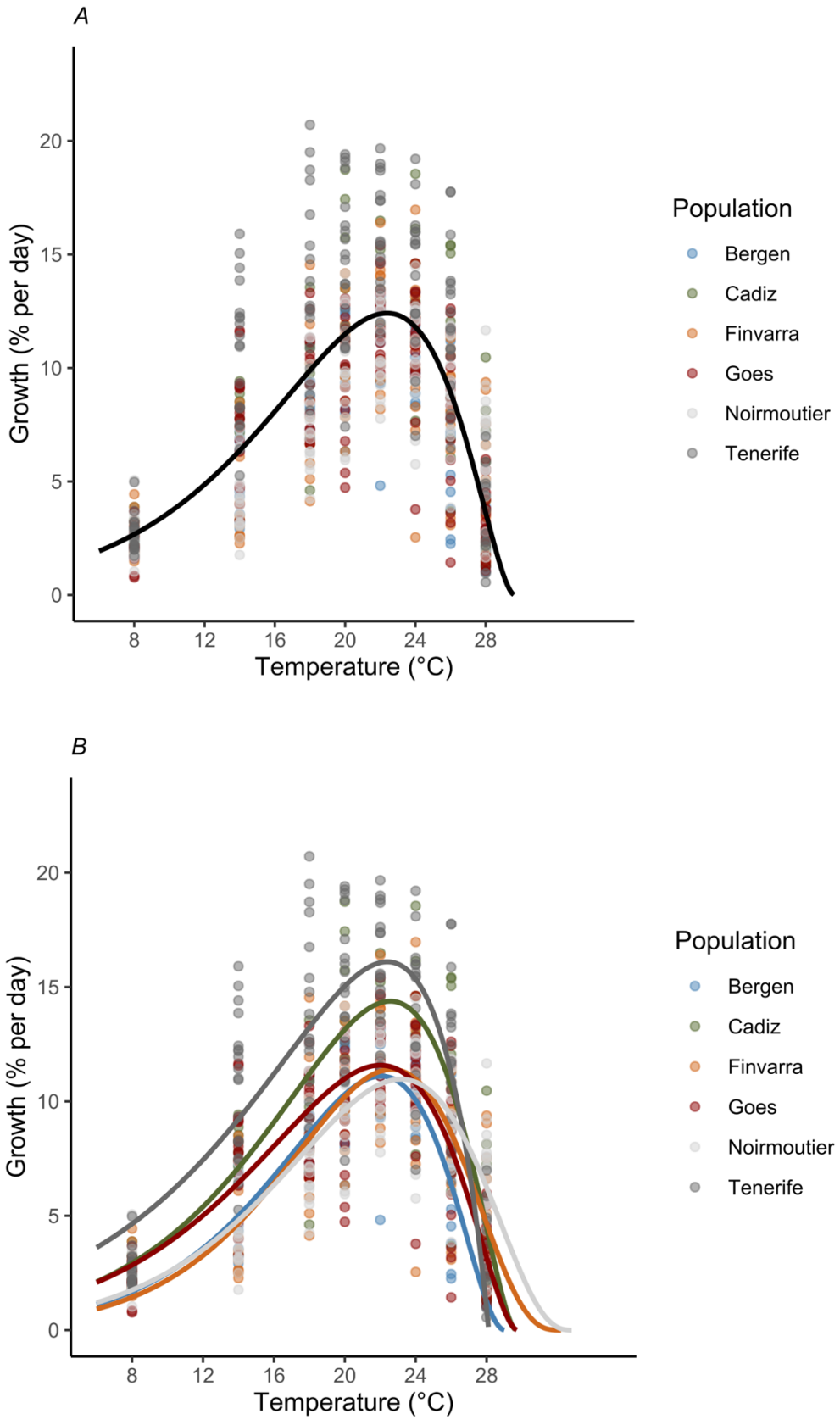


FIGURE 16:PLOT OF **A**: REDUCED MODEL AND **B**: FULL MODEL ON THE SIX DIFFERENT POPULATIONS: BERGEN, CADIZ, FINVARRA, GOES, NOIRMOUTIER AND TENERIFE. THE DOTS REPRESENT THE GROWTH FOR EACH INDIVIDUAL AT A CERTAIN TEMPERATURE PER POPULATION. THE LINES REPRESENT THE THERMAL RESONSE CURVE(S).

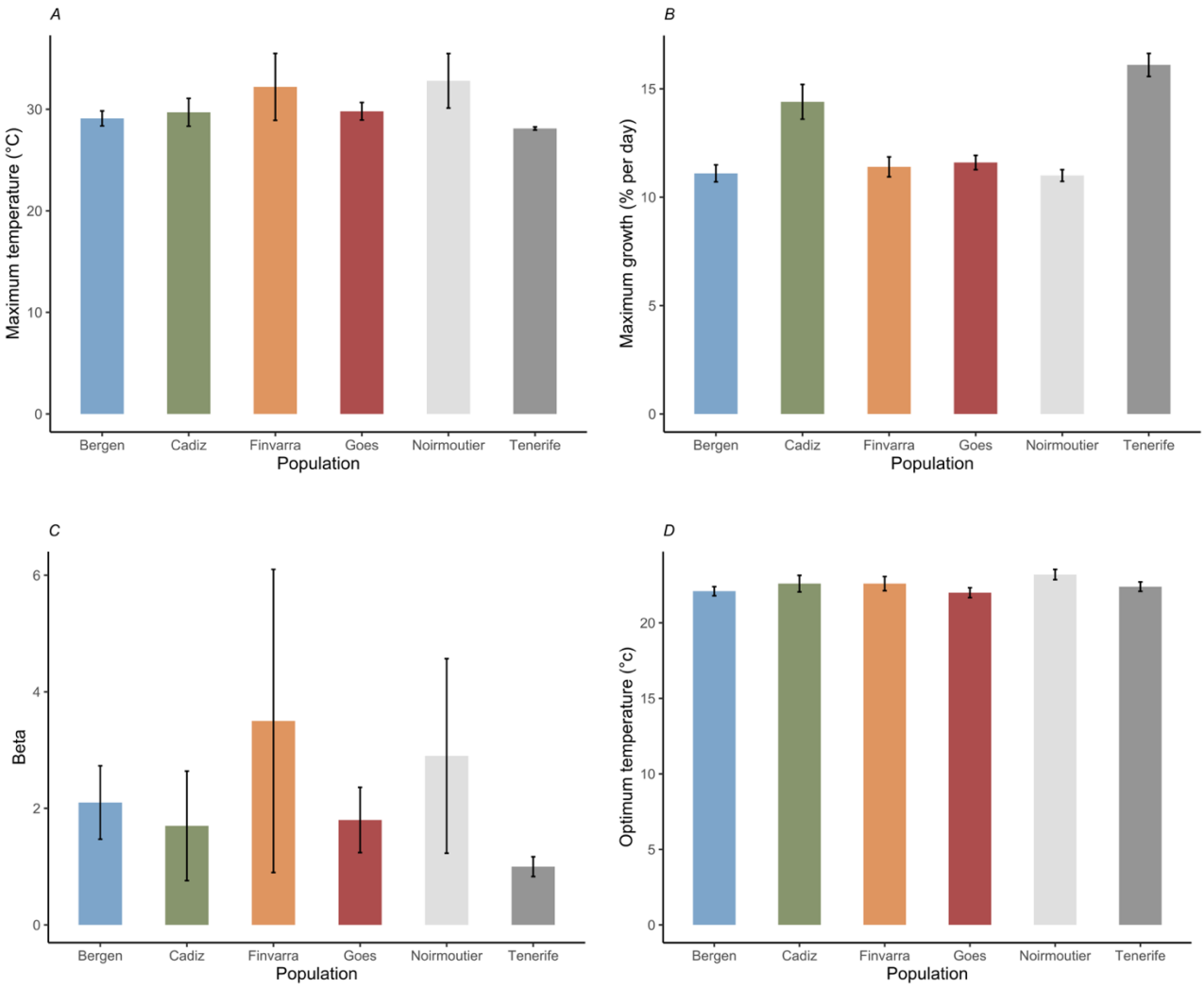


FIGURE 17: BARPLOT OF THE FOUR PARAMETER ESTIMATES OF THE BLANCHARD MODEL VISUALIZED PER POPULATION. A: TMAX, B: GMAX, C: BETA, D: OPTIMUM TEMPERATURE. VALUES INDICATE MEANS ± SE.

4 Discussion

4.1 Pilot studies

4.1.1 Evaluation of growth models

For the individuals cultivated at 22°C and those grown at 25°C, different optimal growth models were the result. The temperature of 22°C is closer to the optimum thermal condition for *D. dichotoma* than 25°C (Bogaert et al., 2016). A temperature of 25°C may cause thermal stress for the algal individuals. Due to this thermal stress, the individuals cannot keep up a growth rate compared to the growth rate at 22°C, resulting in a different growth curve. The three-parameter logistic growth model is an asymptotic growth model in contrast to the exponential growth model. For the latter, it is expected that at some point, the individual reaches an asymptotic size (Paine et al., 2012). Due to the use of young germlings in this experiment, the non-asymptotic exponential growth model as an outcome makes sense because this growth model can be appropriate in the initial stages (Paine et al., 2012). To support this argument, it would be better to do this experiment with fragments of adult algae as well and see whether the optimal growth model of the young germlings differs from the optimal growth model of the adult fragment or not. However, in general, linear or exponential growth models are too simplistic. The most commonly used asymptotic form is the logistic (Paine et al., 2012).

4.1.2 Evaluation of growth media

Although the growth difference between SW and TM is not significant, visually there can be derived from Figure 14 that *D. dichotoma* grows best in SW+ mPES. For each medium, the pH and salinity were within a range *D. dichotoma* can survive (Di Cioccio et al., 2012). In general, the salinity level of open ocean surface water is approximately between 34 and 37 parts per thousand, although areas with great rainfall off the coast, experience lower salinity levels (Duxbury & Mackenzie, 2018). Therefore, it can be excluded that the growth difference was due to differences or abnormalities in pH or salinity. During the experiment, the algal individuals cultivated in ASW, did not grow as fast as the ones cultivated in SW and TM. Several days later, they stopped growing at all and died. A possible explanation for the dying algae in ASW, was a precipitate in the duran bottle, which appeared after autoclaving. It is possible that this precipitate consists of components that are essential for the growth of the algal individuals. Calcium salts may have precipitated. Although, if this was the case, the salinity level would have decreased as well. During autoclaving, the pH will rise resulting in an increased precipitation. To avoid this, a pH- buffer was added. There is a possibility something went wrong while adding this buffer, resulting in an increased precipitation (Anderson, 2005). Another possibility was that the composition of ASW differed too much from SW or that an essential component was missing. Due to the same amount of mPES added to each medium, nutrient limitation cannot explain the growth difference. In general, natural medium will be preferred over artificial media.

4.1.3 Parental provisioning experiment

The sporophyte germlings, cultivated in medium without the addition of nutrients (mPES), were able to grow for several days, due to maternal provisioning. This maternal provisioning provides nutrients essential for growth and development of young germlings. During the experiment, germlings ran out of those essential nutrients. Without the addition of extra nutrients (mPES), they would eventually have stopped growing. After five days, the difference in surface area between the germlings provided with extra nutrients and those without, became significant. At that time, there were still nutrients left to continue their growth, but the amount was not enough to keep up with the growth rate of the germlings with extra nutrients. After 15 days, the growth rate approaches zero so, at that time, all nutrients are consumed. The time span between the moment at which a significant difference appears between the size and at which the growth approaches zero, is 10 days. During those 10 days, the state of health of the individuals in medium without mPES deteriorates sharply. After 10 days, the individuals will be too poor to continue working with. As a result, we assumed that after five days in nutrient-free medium, the biggest amount of nutrients received from the parental algae are consumed, but the algal individuals are still healthy enough to work with.

A mPES solution contains, besides nutrients, trace metals and vitamins as well (Appendix 4). The germlings cultivated in ASW without mPES, did not receive those metals and vitamins so it cannot be excluded that those elements affected the growth rate as well as the nutrient limitation. To exclude that the absence of trace metals and vitamins caused the reduced growth, A mPES solution should be prepared without nutrients, but containing all the other components. Because of this, the experiment would have been repeated but this could not be done due to the COVID-19 outbreak.

4.2 Analysis of thermal response data

According to the comparisons between the full and the reduced/half-reduced model, differences are expected between the parameters of the six populations, since the full model allows the parameters estimates to depend on the population. In general, the most notable differences are expected between the populations collected from the most southern and most northern regions. This expectation is based on the potential for adaptive evolution to the ambient temperature to which they are exposed. In this experiment, the most northern regions are Bergen and Finvarra. Cadiz and Tenerife are the most southern ones. Goes and Noirmoutier will then be the regions in between.

We will focus the discussion on the two most meaningful parameters, being G_{max} and T_o (Eggert, 2012). By comparing the thermal response curves of six different *D. dichotoma* populations, the possibility of local thermal adaptation is taken into account. Several studies already tried to show the potential for local adaptation among species. Regarding the study of Mohring et al., (2014) where the optimum temperature for different *Ecklonia radiata* populations depended on their location was linked to the possibility of adaptation to local thermal regimes, there is expected that the optimum temperature, belonging to the populations situated in warmer regions, is higher compared to those in more colder regions. According to the studied populations, *D. dichotoma* individuals had an optimum growth temperature ranging from 22,0°C to 23,2°C. There is about a one-degree Celsius difference among the six populations (Figure 17 D). The optimum temperature of Noirmoutier has slightly shifted towards higher temperatures compared to the populations of Goes, Finvarra and Bergen, situated in colder regions. When only those four populations are considered, *D. dichotoma* seems to have the ability to adapt to local thermal regimes. However, what stands out and was less expected, is that the optimum temperature of the populations situated in warmer areas (Tenerife and Cadiz), seems to be equal or less to the optimum temperature of the populations in the colder areas. Due to the lack of an unambiguous relationship between local sea temperature and thermal optima for growth when all six of the populations are considered, there is no direct evidence that all *D. dichotoma* populations show the capacity to adapt to local thermal regimes.

Multiple factors, including acclimation, developmental acclimation, transgenerational plasticity and local adaptation determine the growth of an organism. In this experiment, thermal growth differences due to acclimation can be excluded since all of the individuals were cultivated under the same conditions in a common-garden experiment (Reusch, 2013). Whether those differences are due to adaptation or transgenerational plasticity, cannot be said with certainty. When adaptation is involved, a phenotypic change (growth) is associated with a change in genotype. However when there is transgenerational plasticity, changes are not due to genotypic changes but to changes in transcription patterns, which can change after a few generations (Winters et al., 2011).

To identify which populations will be more vulnerable to global warming, the optimum temperatures per population are compared. The thermal optima for the most northern populations (Finvarra and Bergen) and for the population of Goes (approximately 22°C), are remarkably higher than temperatures they are ever subjected to in their natural habitat (Assis et al., 2018; Tyberghein et al., 2012). This finding can suggest that cool-water populations are able to withstand greater increases in sea surface temperature than the populations located in warmer regions (Mohring et al., 2014). The optimum temperature of the populations in warmer regions, leans much closer to their natural environment. Taking into account a future global

warming, cool-water populations may perform better in a future climate, since they are growing in temperatures below their optimum. Populations in warmer regions will be more vulnerable to a future climate change, because they are already closer to their thermal limit. However, this has to be nuanced by the fact that only one component (temperature change) of climate change is involved in this study. Factors like CO_2 concentration and nutrient availability are also affected by climate change (Harley et al., 2012). These factors will also affect the growth performance of *D. dichotoma* (Thomas et al., 2017). The finding that cool-water populations may perform better in a future climate and be less vulnerable than the populations in warmer regions, is based on a future increase in temperature and cannot be generalized, because the other factors are not taken into account in this study.

For the growth at optimum temperature (G_{max}), there are some notable differences between the populations. Precisely, G_{max} for the populations Cadiz and Tenerife are higher compared to the other populations (Figure 17 B). Those results indicate that for the six tested populations, the growth in warmer regions is higher. However, at temperatures above T_{opt}, the growth of the most southern populations (Cadiz and Tenerife) declines sharply compared to the other populations. This observation suggests again that the populations situated in the colder regions seems to be less vulnerable to climate warming.

5 Conclusion

Seaweeds harbor an incredible biodiversity and form the foundation of many marine foodwebs. Climate change will affect those seaweed communities. To adress the impact of future global warming, the thermal response of the growth rate for different *Dictyota dichotoma* populations was analysed. The results indicate that the optimum temperature differs among the six populations (Goes (The Netherlands), Finvarra (Ireland), Punta del Hidalgo (Tenerife), Bergen (Norway), Cadiz (Spain), and Noirmoutier (France)). The thermal optima for the northern populations (Finvarra, Bergen and Goes) are higher than their natural thermal environment, while the optima for the southern populations are closer to their upper thermal tolerance limits. So, it can be expected that the cold-water populations of *Dictyota dichotoma* may perform better in a future, warmer climate because they are now growing at temperatures below their optimum. The southern populations will probably be more vulnerable to global warming. However, only an increased temperature is taken into account during this experiment so in general, it is impossible to predict the reaction of *D. dichotoma* to future climate change. According to small differences in optimum temperatures among the populations, *D. dichotoma* shows a potential for evolutionary adaptation. However, whether or not the observed changes are due to transgenerational plasticity or evolutionary change, cannot be confirmed through this experiment.

Despite the reorganisation of this thesis due to COVID19, pilot studies had been carried out in preparation of another experiment. It seems useful to use these results when the original experiment is carried out by someone else.

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7 Appendix

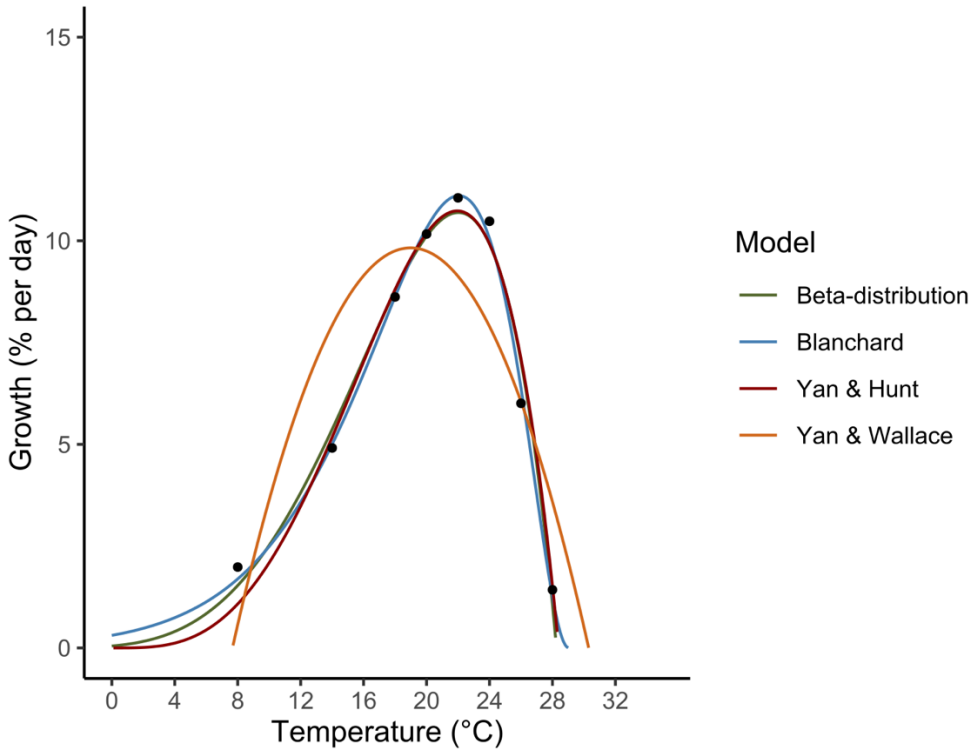
Appendix 1: Growth models and their corresponding AICc, RMSE, MAE and ME per individual at 22°C.

individu_vector	temp_vector	model_vector	AICC_vector	MAE_vector	RMSE_vector	ME_vector
1	22	linear	12,63486184	0,015112066	0,086558127	-0,004100251
1	22	exponential	5,361564345	0,011354049	0,057785859	0,003399096
1	22	power law	10,11549724	0,011563905	0,057638158	0,004092572
1	22	monomolecular	17,44758932	0,015119265	0,086619352	-0,004018894
1	22	three parameter	10,16156457	0,011354049	0,05778586	0,003399096
1	22	four parameter	16,39831839	0,010485716	0,054774811	1,47262E-09
1	22	Gompertz	10,33628933	0,011081961	0,058349514	0,002791459
2	22	linear	-1,643479927	0,006652526	0,034144575	-0,003269525
2	22	exponential	-3,958751588	0,004939947	0,029544549	0,002002871
2	22	power law	-4,597593907	0,004171604	0,020004787	-0,000873994
2	22	monomolecular	3,985005051	0,006680721	0,034205418	-0,003228149
2	22	three parameter	-7,06149042	0,003629672	0,017149655	-0,001053472
2	22	four parameter	-0,057060596	0,003055898	0,014826581	-2,29703E-11
2	22	Gompertz	-5,260546321	0,004049723	0,019192836	-0,000996293
3	22	linear	9,380153644	0,015848794	0,072240372	-0,005127706
3	22	exponential	-8,842279202	0,0040024	0,026249362	-7,54822E-05
3	22	power law	27,47030424	0,029547564	0,151160284	0,000981878
3	22	monomolecular	14,18902459	0,015849992	0,072275983	-0,005077878
3	22	three parameter	-4,042278945	0,0040024	0,026249362	-7,54821E-05
3	22	four parameter	3,155642362	0,004032557	0,026246331	7,22184E-10
3	22	Gompertz	-3,071235579	0,004364533	0,027704325	-0,000471179
4	22	linear	3,713921476	0,01033942	0,052731256	-0,002636591
4	22	exponential	8,994932971	0,012826757	0,070710771	0,008520931
4	22	power law	6,287991854	0,008610426	0,046597441	-9,53494E-05
4	22	monomolecular	8,539363589	0,010366443	0,052805842	-0,00257916
4	22	three parameter	9,570228817	0,009820169	0,055918324	0,002133208
4	22	four parameter	15,67200546	0,010998993	0,052608606	1,15851E-09
4	22	Gompertz	7,395164761	0,008777999	0,049553616	0,000749313
5	22	linear	8,241798645	0,012482549	0,063334718	-0,005444418
5	22	exponential	-8,066091223	0,00439224	0,022855471	0,001064302
5	22	power law	-2,83883053	0,003918151	0,022329179	0,000342346
5	22	monomolecular	13,8568252	0,012461811	0,063394227	-0,00538015
5	22	three parameter	-2,511212233	0,00427271	0,022791108	0,00083109
5	22	four parameter	6,327335237	0,004169986	0,022097099	4,92751E-09
5	22	Gompertz	-2,795754186	0,00386949	0,022389377	0,000277877
6	22	linear	2,214391519	0,007819879	0,043454773	-0,002274632
6	22	exponential	3,649888101	0,009537159	0,047533714	0,006592919
6	22	power law	2,477396236	0,005532317	0,03112958	0,000908098
6	22	monomolecular	7,84061806	0,007813744	0,043526061	-0,002232515
6	22	three parameter	5,304508852	0,006789776	0,037145886	0,002219056
6	22	four parameter	11,94028795	0,006354031	0,031382672	1,24125E-09
6	22	Gompertz	3,433579983	0,005832583	0,033046642	0,001457721
7	22	linear	-6,326131093	0,004366903	0,025481126	0,000577727
7	22	exponential	8,353193926	0,013029744	0,063777205	0,008430214
7	22	power law	-1,9697081	0,003947527	0,023575652	-0,000359602
7	22	monomolecular	-2,44412971	0,003724366	0,022886864	-0,000474563
7	22	three parameter	-4,37110444	0,00336502	0,020289981	0,000150791
7	22	four parameter	4,92888135	0,003434819	0,020247736	7,2549E-10
7	22	Gompertz	-4,062037283	0,003446644	0,020685726	-0,000251415
8	22	linear	10,54029208	0,016650457	0,07704974	-0,005757511
8	22	exponential	-0,790056268	0,006954459	0,041058069	0,001351005
8	22	power law	3,766780458	0,006338158	0,040507143	0,000420485
8	22	monomolecular	15,35675554	0,016658293	0,077120245	-0,005690525
8	22	three parameter	3,89059778	0,00652124	0,040786741	0,000786546
8	22	four parameter	10,91907141	0,00703442	0,040399921	1,0816E-07
8	22	Gompertz	3,780277618	0,006290751	0,040537528	0,000386837
9	22	linear	2,651263185	0,009503825	0,04465763	-0,004242799
9	22	exponential	-12,63662822	0,003561377	0,017176325	0,001909749
9	22	power law	26,14056523	0,025513392	0,136606776	0,000698378
9	22	monomolecular	8,268945414	0,009516629	0,04470701	-0,004199307
9	22	three parameter	-17,94650215	0,00147623	0,008685586	3,01331E-05
9	22	four parameter	-8,618718087	0,001488965	0,008682574	8,92409E-10
9	22	Gompertz	-18,42793269	0,001495845	0,008428134	-0,000158862

Appendix 2: Growth models and their corresponding AICc, RMSE, MAE and ME per individual at 25°C.

10	25	linear	0,790302328	0,008903883	0,044825855	-0,00453333
10	25	exponential	4,795756717	0,010499799	0,055997684	0,00595874
10	25	power law	-6,392059772	0,004925432	0,023036924	-0,00116107
10	25	monomolecular	5,635508562	0,008962359	0,044938575	-0,004461643
10	25	three parameter	-12,93607314	0,003387302	0,01601526	-0,000944328
10	25	four parameter	-7,845312216	0,002976759	0,014244375	-2,13255E-10
10	25	Gompertz	-8,795528104	0,004191096	0,020157417	-0,001248147
11	25	linear	-11,3793747	0,004485125	0,022798436	-0,001289124
11	25	exponential	10,66070656	0,015412468	0,077566907	0,011725336
11	25	power law	-9,597048172	0,00339501	0,01927952	-6,29321E-05
11	25	monomolecular	-6,452911926	0,004525119	0,022959175	-0,001260137
11	25	three parameter	-0,007178938	0,005614225	0,032845472	0,001694266
11	25	four parameter	5,266807104	0,006016047	0,029512477	5,85704E-10
11	25	Gompertz	-5,624702657	0,003979709	0,024040244	0,000656617
12	25	linear	5,006850374	0,012257096	0,056658259	-0,004256022
12	25	exponential	-21,82511564	0,002631223	0,012760736	0,001203747
12	25	power law	25,64439826	0,027922618	0,136578772	0,000435723
12	25	monomolecular	9,819834586	0,012259849	0,056699144	-0,004212538
12	25	three parameter	-17,31696783	0,002385567	0,012555502	0,000946224
12	25	four parameter	-12,39299434	0,00215464	0,011064182	9,66894E-13
12	25	Gompertz	-17,1876431	0,002236749	0,012646034	0,000553843
13	25	linear	11,25543087	0,016534282	0,080172545	-0,008141303
13	25	exponential	12,54967487	0,016797739	0,086149449	0,010249271
13	25	power law	-2,161223243	0,006376424	0,029140961	-0,001449769
13	25	monomolecular	16,09312433	0,016597046	0,080340609	-0,008037318
13	25	three parameter	-3,346853274	0,005164687	0,027283345	-0,000394494
13	25	four parameter	3,747003759	0,005040646	0,027122933	-4,52744E-10
13	25	Gompertz	-3,78087378	0,005860192	0,02663335	-0,001317646
14	25	linear	6,489082394	0,012993645	0,061521333	-0,006178506
14	25	exponential	12,17720245	0,016237392	0,084385084	0,008653929
14	25	power law	1,736086563	0,007805535	0,036185627	-0,001889562
14	25	monomolecular	11,33731949	0,013061517	0,061686421	-0,006077045
14	25	three parameter	-8,480165093	0,004426726	0,02051369	-0,001787514
14	25	four parameter	-6,640058563	0,003190587	0,015230814	1,25375E-10
14	25	Gompertz	-1,311253828	0,006688685	0,030550019	-0,002133544
15	25	linear	4,940142935	0,01000864	0,051525671	0,002666202
15	25	exponential	14,92238856	0,017679403	0,096155869	0,012332085
15	25	power law	5,862568999	0,007694498	0,038464348	-0,000660276
15	25	monomolecular	4,524422956	0,007030341	0,035378265	-0,000981804
15	25	three parameter	2,083336761	0,005595019	0,030372262	-0,000804563
15	25	four parameter	10,87340361	0,005651557	0,029358309	-3,18564E-10
15	25	Gompertz	2,952081497	0,006042176	0,032066963	-0,00096333
16	25	linear	2,790950253	0,010368242	0,050095551	-0,005365052
16	25	exponential	2,707061415	0,010320494	0,049862624	0,004800432
16	25	power law	-7,045609738	0,004001821	0,022215494	-0,001430979
16	25	monomolecular	7,631206692	0,010413768	0,050207713	-0,005282531
16	25	three parameter	-10,15810357	0,003180012	0,018687851	-0,001156143
16	25	four parameter	-5,303282121	0,003472043	0,016404997	-1,42616E-10
16	25	Gompertz	-8,453173138	0,003582712	0,020544474	-0,001475777
17	25	linear	7,157105668	0,012331153	0,063847434	-0,005815008
17	25	exponential	11,69889842	0,013755673	0,082172296	0,007135297
17	25	power law	6,066953422	0,009042112	0,046028727	-0,002131852
17	25	monomolecular	11,98990073	0,012396505	0,063963866	-0,005744276
17	25	three parameter	0,202212499	0,006493358	0,03322979	-0,002471959
17	25	four parameter	3,812221698	0,005371169	0,027221384	4,91441E-11
17	25	Gompertz	4,160279431	0,008188681	0,041402422	-0,002542912

Appendix 3: Growth-temperature response models fitted on the population “Bergen”.



Appendix 4: Recipe for the preparation of the nutrient solution mPES.

Recipe mPES (1L)

West and McBride, 1999

Component	Mass/Volume	Preparation
$NaNO_3$	3,85g	Weighing in aluminium foil
Na_2 DL-β-glycerophosphate pentahydrate	0,4g	Weighing in aluminium foil
Iron EDTA	100 ml	Graduated cylinder, try to keep EDTA mix sterile
Trace metal solution	200ml	Graduated cylinder, mix well to avoid precipitation
Demi-water	950mL	Add demi-water in the bottle
Autoclave*		Autoclave everything
Vitamin solution	1 tube	Add one tube of vitamin solution, never autoclave this solution!
Demi-water		Add demi-water until you reach 1L

** Prepare a 1L bottle with demi water and put this in the autoclave as well.*

Appendix 5: Recipe for the preparation of ASW.

Artificial seawater after Robinson (1996)		
Component	1L	2L
$NaCl$	26,298 g (450 mM)	52,596 g
$NaHCO_3$	0,210025 g (2,5 mM)	0,42005 g
$MgCl_2.6H_2O$	6,099 g (30 mM)	12,198 g
$MgSO_4.7H_2O$	3,94368 g (16 mM)	7,88736 g
KCl	0,7455 g (10 mM)	1,491 g
$CaCl_2.2H_2O$	1,4702 g (10 mM)	2,9404 g

5 mM Tris: 0,6057 g for 1L
(Tris base)
pH 7.9 or 8.0
autoclaving