Master's Thesis

The speed of environmental change affects the likelihood of evolutionary rescue in *Serratia marcescens*

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As climate change accelerates and habitats free from anthropogenic impacts diminish, populations are forced to adapt quickly. If a population does not have suitable phenotypic plasticity or lacks the ability to migrate, genetic adaptation is the sole way to avert extinction. Evolutionary rescue (ER) is a phenomenon, in which a population that has experienced a population decline due to changed environmental conditions, is able to recover through standing genetic variation or mutation. I tested how the evolutionary background and the speed of environmental change affect populations' ability to recover through ER by exposing clonal populations of *Serratia marcescens* spp. DB11 to two different rates of steady temperature increase. Prior to this experiment the clones were subjected to three different thermal treatments resulting in different evolutionary backgrounds. The thermal treatments were stopped once both treatments reached the pre-defined maximum temperatures of 39.5 and 40.0 °C. The results indicate that a slower environmental change allows populations to grow and recover more efficiently when exposed to environmental extremes resulting in a higher likelihood for ER. Moreover, the evolutionary background has no significant effect on growth or the likelihood of ER, which emphasises the importance that the speed of environmental change has on adaptation. These results underline the importance of understanding how the speed of environmental change affects populations' genetic adaptation, and what kind of consequences climate change could have unless it is slowed down.

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Ilmastonmuutoksen kiihtyessä ja ihmisen aiheuttamien ympäristövaikutusten laajentuessa lajien on sopeuduttava yhä nopeammin. Jos populaatiolla ei ole fenotyyppistä plastisuutta tai mahdollisuutta siirtyä uudelle alueelle, sen on kyettävä hyödyntämään jo olemassa olevaa geneettistä materiaalia tai tuotettava uusia kelpoisuutta lisääviä mutaatioita. Tässä tutkimuksessa tarkoitukseni oli testata, miten populaation evolutiivinen tausta ja ympäristönmuutoksen nopeus vaikuttavat populaation kykyyn selviytyä muuttuvissa ympäristöolosuhteissa. Altistin tasaisessa ja vaihtelevassa lämpötilassa kasvatettuja *Serratia marcescens* spp. DB11 –klooneja kahdelle eri lämpökäsittelylle (hidas ja nopea) tarkastellakseni vaikuttiko aiempi lämpökäsittely populaatioiden kykyyn selviytyä äärilämpötilassa. Kumpikin lämpökäsittely päättyi ennalta määriteltyihin maksimilämpötiloihin (39,5 ja 40,0 °C). Kokeen tulokset osoittavat, että lämpötilan hidas muutos mahdollistaa populaation paremman kasvun äärilämpötilassa ja lisää selviytymisen mahdollisuutta. Evolutiivisella taustalla ei ole merkittävää vaikutusta populaatiokokoon eikä selviytymiseen. Tulosteni perusteella nopeat muutokset ympäristöolosuhteissa rajoittavat lajien kykyä sopeutua, ja tämän takia ilmastonmuutoksen aiheuttamilla sään ääri-ilmiöillä ja habitaattien katoamisella on huomattavia vaikutuksia populaatioiden geneettiseen sopeutumiskykyyn, ellei ilmastonmuutosta pystytä hidastamaan.

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1 INTRODUCTION

Most populations inhabit environments that are constantly changing, thus they are adapted to change (Meyers & Bull 2002). However, human-induced global environmental changes are more severe than naturally occurring changes (Barnosky et al. 2011, Ceballos et al. 2015), therefore resulting in populations being exposed to increasing amounts of stress (Sih et al. 2011). In particular, changes in global temperatures can result in cascading effects across ecosystems (Xu et al. 2009, Butt et al. 2015, IPCC; Hoegh-Guldberg et al. 2018), because temperature is a vital part of species' physiological and biochemical functions and a majority of Earth's species are ectothermic (Angilletta Jr. et al. 2006) and thus, vulnerable to ambient temperatures. Unless populations have phenotypic plasticity, are able to relocate to more habitable areas, or have the ability to genetically adapt *in situ*, their numbers will decline and they will be pushed towards extinction (McAllister et al. 1992, Thomas et al. 2004, Zwaan et al. 2008, Bell & Gonzalez 2009, Chevin et al. 2010, Lachapelle & Bell 2012). As habitats free from anthropogenic impacts diminish and species are exposed to extreme environmental conditions that they may have no phenotypic plasticity for (Gomulkiewicz & Holt 1995, Bell & Collins 2008), genetic adaptation may be the only way to avert extinction (Orr & Unckless 2008, Bellard et al. 2012).

Evolutionary rescue (ER) arises when a population recovers from a decline because of genetic adaptation (Bell & Collins 2008, Bell 2012, Gonzalez et al. 2013). ER can occur in populations that have been negatively affected by environmental change, and when a population is able to survive either by using standing genetic variation or producing beneficial mutations that increase the population's fitness (Gomulkiewicz & Holt 1995, Gonzalez et al. 2013, Killeen et al. 2017). As global climatic events become more unpredictable (Grant 2017) and environmental deterioration isolates populations from each other (Schiffers et al. 2012), it is

important to understand how anthropogenic changes will impact Earth's biodiversity (Gonzalez et al. 2013, Lindsey et al. 2013). Moreover, ER experiments produce more information on how individual populations can survive by producing beneficial evolutionary responses in rapidly changing environmental conditions (Pauls et al. 2013, McDermott 2019) and how the speed of environmental change affects the likelihood of ER (Lindsey et al. 2013).

Previously, ER has been studied by exposing populations to stress at varying rates of change in environmental conditions, e.g. salinity (Bell & Gonzalez 2009), light (Bell 2013), toxins (Lindsey et al. 2013) and temperature (Killeen et al. 2017). In addition, some have identified what kind of changes the exposure to varying conditions has on phenotype and genotype frequency (Lindsey et al. 2013, Killeen et al. 2017), and how the type of reproduction (sexual or asexual) affects the likelihood of producing beneficial genotypes (Lachapelle & Bell 2012). Whereas asexual reproduction results in genetically identical offspring and mutation is required to produce genetic variation, sexual reproduction results in offspring with novel genotypes by mixing existing genetic variation (Lachapelle & Bell 2012, Bell 2013). These studies conclude that fast evolutionary responses to environmental changes can stop a rapid decline in population size and result in a population recovery.

As mentioned before, populations live in dynamic environments which affects selection (Meyers & Bull 2002), hence the reason why it is important to consider how the evolutionary background affects adaptation when studying ER. Populations may have prior experience from environmental disturbance, and it can give a competitive advantage when exposed to similar conditions again (Lee & Gelembiuk 2008, Gonzalez & Bell 2013). However, it has also been suggested that previous exposure to stressful conditions may not select for a better fitness when exposed to the stressor again, and selection may be more focused on traits that are less directly correlated with fitness (Travisano et al. 1995). Additionally, evolutionary effects

could be so subtle in significance that large ecological effects can override them greatly as mentioned in Saarinen et al. (2019).

The purpose of this thesis was to study how the speed of environmental change and populations' evolutionary background affect the likelihood of ER. To test this, clonal populations of *Serratia marcescens* spp. DB11 bacteria were exposed to stress by subjecting populations to steady increase in temperature at two different rates (slow and fast increase) to detect if the speed of environmental change and the evolutionary background have an impact on the ability to avert extinction and recover through ER. Previously, these clones were reared in three different thermal treatments that resulted in different evolutionary backgrounds (Ketola & Saarinen 2015). The hypotheses were 1) the slower the environmental change the more likely is a population able to reach a higher biomass yield and recover through ER, and 2) evolutionary background affects the biomass yield and the likelihood of ER. The hypotheses were based on the ideas that were put forward by Lee & Gelembiuk (2008) and Ketola et al. (2013).

2 MATERIALS AND METHODS

2.1 Study species

For this experiment, I used clonal populations of *Serratia marcescens* spp. DB11 obtained from an experiment by Ketola & Saarinen (2015). *S. marcescens* belongs to the family of *Enterobacteriaceae*, and it is a gram-negative, non-sporulating opportunistic pathogen, and it has a generation time of 26 minutes at 32 °C (Jennison 1935). It generally lives in temperatures between 5 to 40 °C, and it is between 0.5-0.8 by 0.9-2.0 μm in size. The bacterium is globally distributed, and it has been identified as a cause of hospital infections (Hejazi & Falkiner 1997) as well as a significant insect pathogen (Flyg et al 1980). I chose this species because of its

tendency to grow well in varying temperatures and because there were samples from different evolutionary backgrounds available. Additionally, using an asexual species allowed me to strictly observe how previous (ancestral) adaptation and mutation can affect fitness (Elena & Lenski 2003).

2.2 Evolutionary background

The bacterial clones that I used in this work originate from an experimental evolution experiment (Ketola & Saarinen 2015). This experiment started from a single clone, and that clone was reared in 31 °C for two weeks to adjust it for growing conditions, and then seeded to three Bioscreen C® (Oy Growth Curves Ab, Ltd, Helsinki, Finland) 100-well spectrophotometer plates with ten populations in each plate. These three plates were exposed to three different thermal regimes, which of one was at constant 31 °C, one at smooth fluctuating (30 min. intervals) temperatures between 24, 31 and 38 °C and one at abrupt fluctuating (45 min. intervals) temperatures between 24 and 38 °C. Each of these thermal regimes lasted for two months and consisted of 27 renewals in total (renewals every 48 h) and the estimated generation time was ca. 5.32 generations per renewal totalling in 143.64 generations.

2.3 Defining maximum thermal tolerance

Before the initial experiment, I measured *S. marcescens* spp. DB11 populations for their thermal maximum limit by measuring their biomass growth in different temperatures using temperature-controlled spectrophotometers (OD 600 nm wavelength, Bioscreen C® Oy Growth Curves Ab, Ltd, Helsinki, Finland). For this, I used Bioscreen 100 plates by filling five wells with 400 µl of NB medium [NB medium: 10 g of nutrient broth powder (Difco, Becton & Dickinson, Sparks, MD) and 2.5 g of yeast extract (Difco) in 1 l of sterile ddH2O] and adding 10 µl of high density bacteria inoculum into the NB medium. The NB medium was preheated in

the spectrophotometers for two hours before pipetting the populations into the wells. During this time, the populations were kept in a thermal cabinet (Lab Companion, ILP-12, Jeio Tech, Seoul, Korea) in a 15 ml centrifuge tube (Sarstedt, Numbrecht, Germany) containing 2 ml of NB medium at approximately 39 °C to avoid abrupt temperature changes when pipetting into the Bioscreen plates. I observed the growth development for a duration of 24 hours, after which I estimated the growth based on the growth curves. I performed four different measurements in temperatures of 39, 40, 41 and 42 °C using two spectrophotometers, so that on the first day, I tested temperatures of 39 and 40 °C, and on the second day temperatures of 41 and 42 °C. The measurements indicated that the maximum temperature, at which the bacteria managed could grow in, was approx. 40.0 °C. The temperatures 41 and 42 °C showed high lethality.

2.4 Evolutionary rescue experiment

To test how the speed of environmental change and the evolutionary background affect populations' ability to reach a higher biomass yield, I subjected populations to two different rates of temperature increase. The environmental treatments were conducted with temperature-controlled Bioscreen C® spectrophotometers (Oy Growth Curves Ab, Ltd, Helsinki, Finland) to detect if the speed of temperature change (fast or slow) and the evolutionary background affect biomass yield and the likelihood of ER.

To initiate the experiment, I used cryoreplication to obtain 90 *S. marcescens* spp. DB11 populations that had been kept in –80 °C, in 80 % glycerol solution (Ketola & Saarinen 2015). In cryoreplication sterilised steel pins are pressed into frozen sample wells to obtain populations. This method enables both obtaining populations and maintaining samples in storage for further use (Duetz et al. 2000). I grew the populations in NB medium for 24 hours before initiating each treatment to ensure a high enough bacterial density. The samples had been pre-randomized so that the

wells on the original 100-well spectrophotometer plate (Bioscreen C® Oy Growth Curves Ab, Ltd, Helsinki, Finland) contained bacteria populations (N = 90) from the three different thermal treatments in random order (Ketola & Saarinen 2015).

I grew the populations in NB medium in rising temperatures in the spectrophotometers and subjected the populations either to slow or fast thermal treatment (Fig. 1). Both spectrophotometers had a starting temperature of 31 °C and final temperature of 39 °C (defined in the pre-experiment). The duration for both treatments was nine days. In the slow treatment, the populations were first subjected to a daily 1 °C increase in temperature for the first nine days (temperatures increased from 31 to 39 °C) after which the temperature was raised to maximum temperatures of 39.5 °C in one spectrophotometer and 40.0 °C in the other. In the fast treatment, the populations were first subjected to a steady temperature of 31 °C for five days after which the temperature increased 2 °C daily after which the temperature was raised to maximum temperatures of 39.5 °C in one spectrophotometer and 40.0 °C in the other. During the thermal treatment and the exposure to maximum temperatures, I renewed the resources daily by moving populations to new 100-well plates containing fresh NB medium. Both treatments were run simultaneously, and all populations had the same time to evolve. During the experiment, bacterial biomass growth was observed every five minutes.

In the experiment, I renewed the Bioscreen plates at daily (24 h) intervals using the following protocol: 1) I pipetted 400 µl of NB medium into two new 100-well plates to warm up the NB to a desired temperature (to avoid heat shock); 2) I aborted the ongoing spectrophotometer measurements and took out both the plates containing the populations (two plates) and the plates containing only NB (two plates); 3) I pipetted (ThermoFisher Scientific Matrix™ eQualizer 125 µl 12 Channel Electronic Pipette, Waltham, MA USA) 12.5 µl of bacteria inoculum from the previous days' plates into the warm plates that contained only NB medium, so that each well was inoculated from the corresponding well on the old plate; 4) I placed both the newly

pipetted NB plates and the bacteria plates with new resources into the spectrophotometers and adjusted the temperatures accordingly.

To avoid possible bias caused by the spectrophotometers, I rotated the fast and slow treatments between the two spectrophotometers daily (spectrophotometers were labelled previously with the letters A and B). I also marked each row of wells after I had pipetted the populations from the old plates into new plates to avoid possible pipetting errors. To check for possible contamination, I pipetted populations samples from 100-well plates into DNAse methyl green agar plates to confirm that the bacterial growth was *S. marcescens* (Smith et al. 1969). The test showed that there was no contamination. In addition, I placed samples in a freezer in –80 °C for later usage (e.g. for detecting the possible mutations that the thermal treatment may have caused).

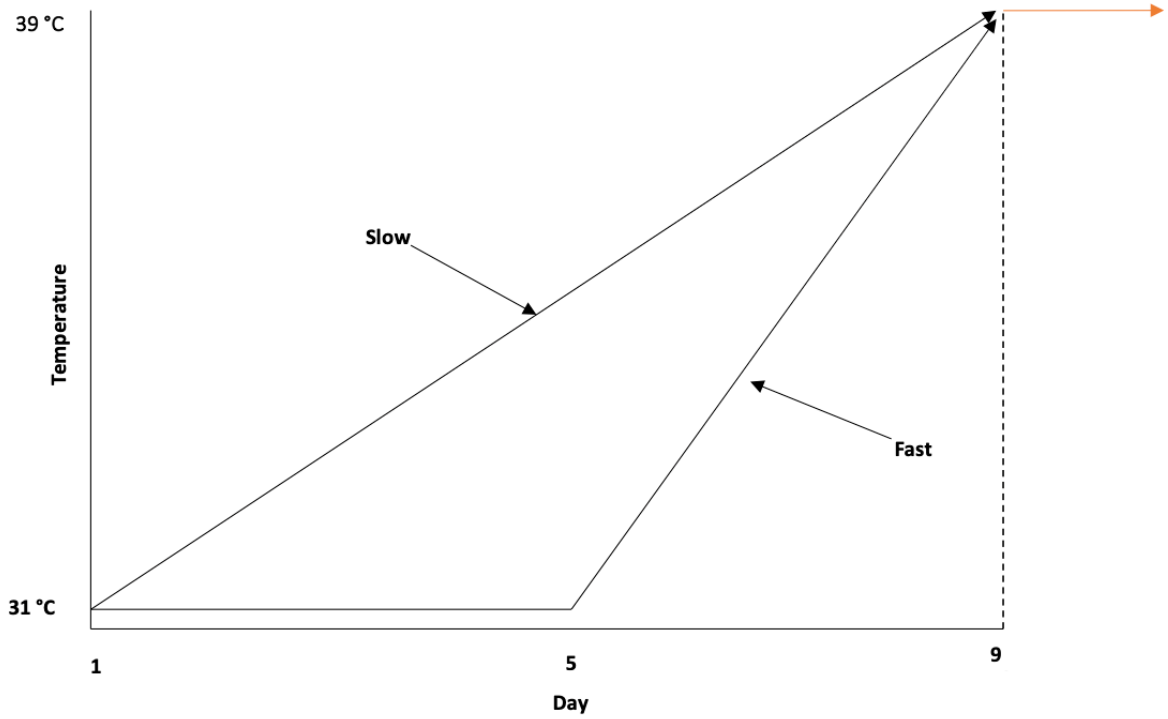

Figure 1. The rate of temperature increase from 31 °C to 39 °C in both thermal treatments. Once both treatments reached the temperature of 39 °C the temperatures were increased to maximum temperatures of 39.5 and 40.0 °C for additional four days (indicated with the single arrow on the top right corner).

To evaluate how the strains that had undergone different rates of environmental change perform at extreme temperatures, I conducted growth measurements at two extreme environments by exposing the populations to extreme temperatures of 39.5 and 40.0 °C. Two plates were made from the slow treatment and two plates from the fast treatment, so that one plate from each treatment was placed in 39.5 and 40 °C. Two ending temperatures were chosen to increase capturing ER around the maximal thermal limit. After the four days, with daily renewals of resources at these temperatures, I lowered the temperatures back to the starting temperature of 31 °C for two days to observe if the populations that presented no or lower growth had become extinct or if they had just become dormant due to inhospitable thermal conditions (Jones & Lennon 2010). From this data I used the highest recorded OD as maximum biomass yield (response variable) which was used to measure the ability to grow and use the provided resource efficiently. Whilst ER is the extinction risk, the amount of biomass tells quite directly the changes to it.

2.5 Data analysis

The raw Bioscreen C® (Oy Growth Curves Ab, Ltd, Helsinki, Finland) growth measurement data was first processed with RStudio (RStudio Team 2015, version 3.6.1), and the growth development for each population was observed. I used optical density (OD 600nm wavelength) and changes to it to measure population performance at extreme temperatures. Maximum biomass yield (maximum OD) was used to define how efficiently the populations were able to use resources at given temperatures. I detected the maximum biomass yield from the raw OD data using a 20 data point sliding window over a 24-hour period for each measurement day and fitted a linear regression of time against OD. After processing the raw data, I filtered the data with Microsoft Excel (2020, version 16.35) and selected out the wells that contained water (pre-randomisation).

I ran a two-part statistical analysis to test the effects that the speed of temperature change and the evolutionary background had on populations' ability to reach a higher biomass yield and on the likelihood of ER. Both parts were done using the linear mixed-effects models lme4 package (version 1.1-21) in RStudio (RStudio Team 2015, version 3.6.1) (Bates et al. 2015).

In the first part, I used general linear mixed model (Grueber et al. 2011) to test if the speed of environmental change (two levels: fast and slow) and the evolutionary background (three different treatments by Ketola & Saarinen 2015) had an effect on the maximum biomass yield. For this I used the biomass yield data from the first day of exposure to maximum temperatures. I set the maximum biomass yield as the response factor and the evolutionary background, the thermal treatment and their interaction as the fixed factors controlling for the maximum biomass yield. I set the inoculum size as a continuous covariate to control the deviations in starting population size (the size could have an effect on selection; Collins & de Meaux 2009). I set the population of origin as the random factor to control for the non-independency of observations because the measured clones come from the same population.

In the second part, I used generalized linear mixed model (Bolker et al. 2009) with logit link to model the effects that the speed of temperature change and the evolutionary background had on the likelihood ER. Rescue was defined based on the OD data: threshold for ER was set at 0.5 OD (populations that reached OD above 0.5 showed growth, whereas populations below 0.5 showed none and were extinct). I set extinction as the response factor and the evolutionary background and the thermal treatment as the fixed factors controlling for the likelihood of extinction or ER. The interaction between the thermal treatment and the evolutionary background was not included in this analysis as the interaction was not significant. I set the inoculum size as a continuous covariate to control the deviations in starting

population size and the population of origin as the random factor to control for the non-independency of observations.

3 RESULTS

The results showed that the speed of environmental change had an effect on populations' ability to reach a higher biomass at extreme temperatures ($P < 0.001$ in 39.5 °C, $P < 0.01$ in 40.0 °C) (Fig. 2). At the maximum temperature of 39.5 °C, the *S. marcescens* populations (N = 90) that experienced a slower temperature increase were able to reach a higher biomass yield (OD) than the populations that experienced a faster temperature increase (Slow: average biomass yield = 0.190, SE = 0.004; Fast: average biomass yield = 0.158, SE = 0.002; Wald's $\chi_2 = 21.955$, $P < 0.001$).

At the maximum temperature of 40.0 °C the results were similar, yet the magnitude of difference was smaller. The populations (N = 90) that experienced a slower temperature increase reached a higher biomass yield than the populations that were exposed to faster temperature increase (Slow: average biomass yield = 0.153, SE = 0.002; Fast: average biomass yield = 0.146, SE = 0.002; Wald's $\chi_2 = 10.487$, $P < 0.001$).

When observing the effect of evolutionary background on the biomass yield, there was no significant relationship in either of the maximum temperatures between the maximum biomass yield and the evolutionary background (39.5 °C: Wald's $\chi_2 = 0.024$, $P = 0.988$; 40.0 °C: Wald's $\chi_2 = 0.155$, $P = 0.925$) or the combination of evolutionary background and thermal treatment (39.5 °C: Wald's $\chi_2 = 1.000$, $P = 0.607$; 40.0 °C: Wald's $\chi_2 = 0.913$, $P = 0.634$). The starting inoculum size had a significant effect on the ability to reach a higher biomass in both 39.5 °C (Wald's $\chi_2 = 290.742$, $P < 0.001$) and in 40.0 °C (Wald's $\chi_2 = 314.258$, $P < 0.0001$).

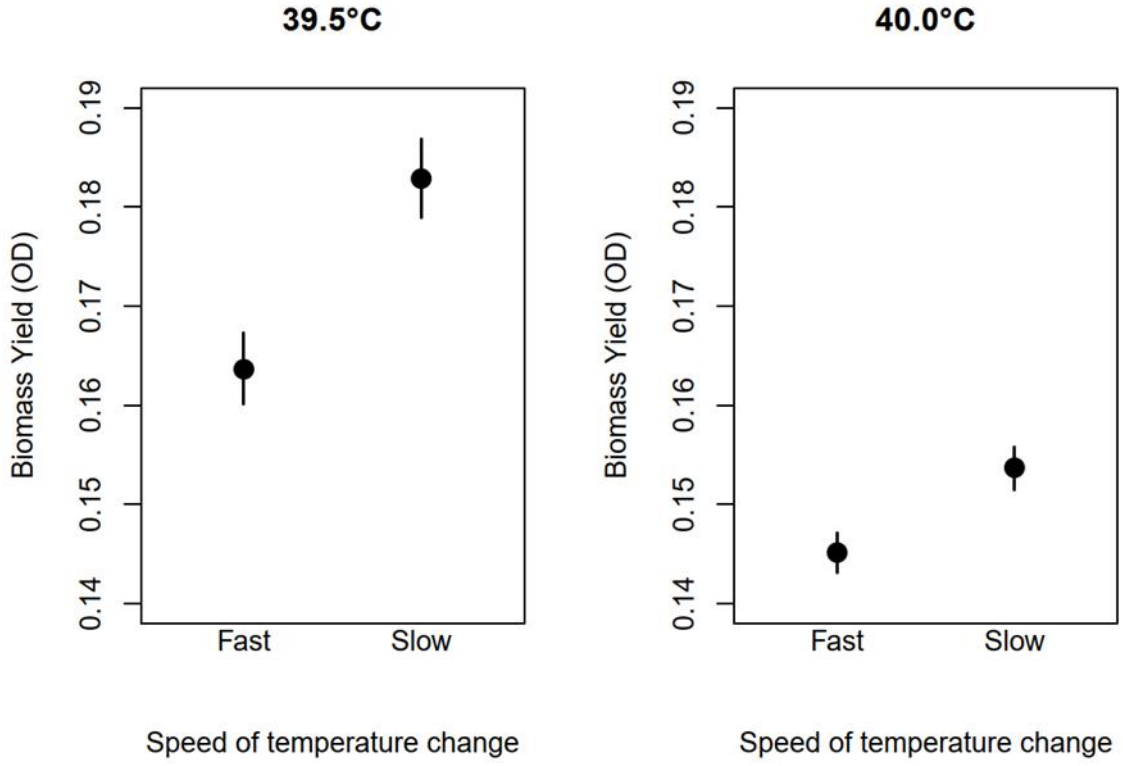

Figure 2. The effects that the speed of environmental change had on *S. marcescens* populations' biomass yield ($OD_{600\ nm}$) when exposed to maximum temperatures of 39.5 and 40.0 °C after the initial nine-day treatment. The dots correspond to the estimated marginal means of the general linear mixed model and the vertical bars the standard error of the mean.

The speed of environmental change had an effect on populations' ability to avert extinction (Wald's χ_2 =1.993, P < 0.0001), whereas the evolutionary background had no effect (Wald's χ_2 = 15.839, P = 0.369). The populations that were subjected to the slow treatment were more likely to avert extinction and recover through ER (35.5%, Table 1), whereas the populations that were subjected to the fast treatment had a smaller chance on surviving (8%, Table 1). This analysis was done only for the populations that were exposed to the maximum temperature of 39.5 °C, as almost all populations went extinct at the maximum temperature of 40.0 °C. The evolutionary background, the speed of environmental change and their interaction had very little effect on the likelihood of ER and only the speed of environmental change was significant (P < 0.0001). The population of origin (random effect) had very little effect on both the maximum biomass yield (σ_2 < 0.00003, SD = 0.005) and

the likelihood of ER (σ^2 = 1.526, SD = 1.235) and did not differ greatly from other factors.

Table 1. The results for the generalized linear mixed model with log link for the likelihood of ER after the initial thermal treatments at the maximum temperature of 39.5 °C. The limit for survival was set at 0.5 OD (600nm) as populations that were below the size 0.5 OD went extinct and showed no or very little (declining) growth. Confidence level 95%.

Treatment	Est.	SE	Lower CL	Upper CL
Fast	8.0%	0.616	3.3%	18.1%
Slow	35.5%	0.585	2.2%	52.0%

When observing biomass development during the slow and fast thermal treatments, there were no clear differences between treatments (Appendix 1). In both treatments the populations were able to grow efficiently and use the provided resource. The growth curves indicate that the highest biomass yield was reached at temperatures between 33 and 35 °C.

4 DISCUSSION

As the effects of anthropogenic change and global warming accelerate, species are forced to find ever faster ways to adapt (Barnosky et al. 2011, Ceballos et al. 2015). I tested the theory of evolutionary rescue (ER) and studied if the speed of environmental change has an effect on population growth (biomass yield) and populations' ability to recover through ER, and if the evolutionary background affects the ability to adapt. Firstly, the main result of this study suggests that the speed of change sets limits to adaptation, putting more emphasis on the rate at which a population is able to produce beneficial adaptations in changing environmental conditions, than on the evolutionary background of organisms (Lindsey et al. 2013). The *S. marcescens* populations that experienced a slower temperature increase were able to use resources better and gain a higher biomass yield (Fig. 2). These populations were also more likely to recover from a population decline and recover through ER when exposed to maximum temperatures. The populations that were exposed to a faster temperature increase suffered a faster decline at higher temperatures and were less likely to recover through ER, and thus more likely of going extinct. The results confirm the first hypothesis and support the theory that a slower environmental change increases the likelihood of ER, thus enabling species to recover from a population decline by selecting individuals with a higher stress-tolerance (Gomulkiewicz & Holt 1995, Bell & Gonzalez 2009, Kirkpatrick & Peischl 2013).

Secondly, both populations were able to grow efficiently in both fast and slow thermal treatments, but when reaching the thermal maximums of 39.5 and 40.0 °C, the populations that experienced the slow treatment grew better (Fig. 2) and were more likely to recover through ER. Additionally, exposure to 40.0 °C was lethal to most populations and the likelihood of ER was very low. This indicates that the change could have occurred too fast and the populations did not have enough time to adapt, and that the surviving populations were able to produce beneficial

evolutionary responses and reach ER (Lindsey et al. 2013). Moreover, if the temperature increase is too severe and higher than the population's thermal maximum, even a slow gradual change may not result in beneficial genetic adaptations and rescue if the change exceeds population's persistence (Gonzalez et al. 2013). These results align with previous studies (Bell & Collins 2008, Bell 2012, Kirkpatrick & Peischl 2013, Ramsayer et al. 2013, Killeen et al. 2017) and were consistent with the theory of ER (Gomulkiewicz & Holt 1995, Bell & Collins 2008, Gonzalez et al. 2013).

The speed of environmental change, together with demographic effects, have an impact on populations' ability to survive in a changing environment, as it exerts selective pressure on populations (Perron et al. 2008). Intermediate levels of exposure to a stressor can select for mutations that promote adaptation in new environmental conditions (Samani & Bell 2010) allowing the populations to adapt gradually (Bell 2012). This could explain why the *S. marcescens* populations that experienced the slow temperature increase reached higher biomass yields and were more likely to recover through ER. On the other hand, sudden harsh environmental conditions and continuous environmental variability can reduce population's average fitness and the number of individuals resulting in a population decline, thus affecting the likelihood of producing beneficial genetic adaptations (Gomulkiewicz & Holt 1995, Lindsey et al. 2013, Killeen et al. 2017). This could explain why the populations that experienced the fast temperature increase were more likely to go extinct. Moreover, for a beneficial mutation to be selected in a population, environmental conditions need to remain relatively constant for a certain period of time (Hao et al. 2015), yet in the wild climate change affected environmental fluctuations rarely remain stable, therefore reducing fitness constantly and forcing populations to adapt at accelerating rates (Bell & Gonzalez 2011, Bellard et al. 2012). In addition, environmental fluctuations that enable temporary amelioration may relax selection for beneficial genotypes and interfere

with ER (Hao et al. 2015), hence resulting in an evolutionary trap in which populations fail to adapt to change (Ferriere & Legendre 2013).

Thirdly, when observing how the evolutionary background affected populations' biomass yield and the likelihood of ER, the results show no significant relationship between the evolutionary background and the ability to reach a higher biomass yield at maximum temperatures, or the combination of these two factors. The result does not support the second hypothesis and means that the previous exposure to fluctuating and steady thermal treatments (Ketola & Saarinen 2015) did not give an advantage to any of the populations. This result coincides with previous studies that have theorised that a population's evolutionary background does not necessarily have a large impact on adaptation to changing temperatures or other changing environmental conditions (Travisano et al. 1995, Lee & Gelembiuk 2008, Saarinen et al. 2019). The reasons why the evolutionary background did not affect the biomass yield or the likelihood of ER in this experiment could be that the previous evolutionary treatment, in which the populations were subjected to smooth and fluctuating thermal treatments (Ketola & Saarinen 2015), did not result in beneficial mutations or did not select for certain genotypes that would result in a higher biomass yield in the new conditions (Bell & Gonzalez 2011). In addition, the fluctuations in the previous treatment could have disrupted genetic adaptation, and therefore impede ER (Hao et al. 2015), which could explain why the evolutionary background had a nonsignificant role in this experiment.

Additionally, even though the starting inoculum size was the continuous covariate to control for the deviations between initial population sizes, and not a specific point of interest, it had an effect on the maximum biomass yield in both thermal treatments. The results indicate that a larger population is more likely to survive, as it has more individuals that may hold beneficial genotypes, thus resulting in a smaller risk of extinction and a higher chance of ER (Bell & Gonzalez 2009, Bell 2012, Ramsayer et al. 2013). This also aligns with previous studies, which state that population size is important in the likelihood of ER: a large population is more

likely able to produce beneficial mutations or contain beneficial adaptations inherited from ancestors, whereas a small population is more likely to succumb to extinction due to demographic stochasticity (Bell & Gonzalez 2009, Willi & Hoffmann 2009, Samani & Bell 2010, Ramsayer et al. 2013).

Adapting to changing temperatures is generally harder than any other adaptation, because it affects overall metabolic functions (Angilletta Jr. et al. 2016). Nevertheless, testing the effects of temperature changes on populations is important, as climate change accelerates changing the thermal conditions species are adapted to (Deutsch et al. 2008, Hoffman & Sgrò 2011). To test the overall impacts of climate change on populations and the likelihood of ER, other factors such as CO_2 or salinity, could be studied together with temperature (Bell & Gonzalez 2009, Lauchlan & Nagelkerken 2019). Moreover, studying the impacts of short-term and long-term fluctuating environmental conditions and steady environmental conditions with a combination of factors (e.g. CO_2 and temperature) could provide more information about populations' evolutionary responses to climate change (Ketola & Saarinen 2015, Saarinen et al. 2018, Saarinen et al. 2019). More recently, ER and its connections to non-genetic inheritance have been explored. Evolutionary potential may increase through epigenetic buffering by reducing genetic loss, increasing the amount of novel genetic variation and inheriting phenotypes that have been induced by environmental changes. These factors could result in a higher likelihood of ER (Danchin 2013, O'Dea et al. 2016).

When observing the results of this thesis, it must be remembered that ER also has its restrictions. It only considers standing genetic variation and mutations as the sources for adaptation (Gomulkiewicz & Holt 1995), whereas in the wild most populations experience demographic changes and migration that bring new alleles into the population, which may give the population a better chance of surviving (Hufbauer et al. 2015, McDermott 2019). Nonetheless, as habitat fragmentation increases and climate change accelerates, demographic effects and migration might become less important, which puts emphasis on population recovery that is based

on genetic adaptations, and moreover ER (Gomulkiewicz & Holt 1995). In addition, to get a more thorough understanding of how the *S. marcescens* population size developed after treatment, I could have continued the maximum temperature exposure for multiple days to see if more rescues occur, and if the U-shaped growth curve, that is typical for ER (Bell & Gonzalez 2009, Gonzalez et al. 2013), develops.

To conclude, my aim was to study how the speed of environmental change and the evolutionary background affect populations' ability to recover from an environmental change and if these factors have an impact on ER. My results finding higher chances for ER in conditions in which the environmental change occurs slower correspond to the predictions of ER and emphasise the relationship between the speed of environmental change and populations' ability to survive the change (Gomulkiewicz & Holt 1995, Bell & Collins 2008, Gonzalez et al. 2013). The results show that a slower environmental change gives populations a better persistence and increases the likelihood of surviving in new environmental conditions, whereas fast change diminishes the chances of survival. These results align with previous studies (Bell & Gonzalez 2009, Collins & de Meaux 2009, Bell & Gonzalez 2011, Lindsey et al. 2013, Killeen et al. 2017). Genetic diversity and the ability to produce beneficial evolutionary responses can vary between same species' populations, which emphasises the importance of studying what kind of intraspecific impacts climate change can have (Pauls et al. 2013). Yet, even though ER has the ability to enable a population to avert extinction, climate change still alters its habitat and may narrow its spatial range making the rescue only partial (Schiffers et al. 2012).

The balance between extinction and survival during an environmental change is a battle between demography and evolution: populations may have the ability to evolve rapidly, yet their habitat disappears making adaptation irrelevant and vice versa (Maynard Smith 1989). As climate change accelerates, environmental changes grow more extreme resulting in radical environmental fluctuations that force species either to find ever faster ways to adapt and forcing change on entire

ecosystems (IPCC; Hoegh-Guldberg et al. 2018) or to perish (Trisos et al. 2020). These challenges can result in an evolutionary race of adaptations in which adaptation needs to happen faster than the environment changes (Orr & Unckless 2008). In order to slow down biodiversity loss and stop the cascading effects caused by climate change (Butt et al. 2015, Ceballos et al. 2015), it is vital to secure both genetic and phenotypic diversity (Gomulkiewicz & Holt 1995) and aim for a slower environmental change (Perron et al. 2008, Collins & de Meaux 2009, Bell & Gonzalez 2011, Killeen et al. 2017).

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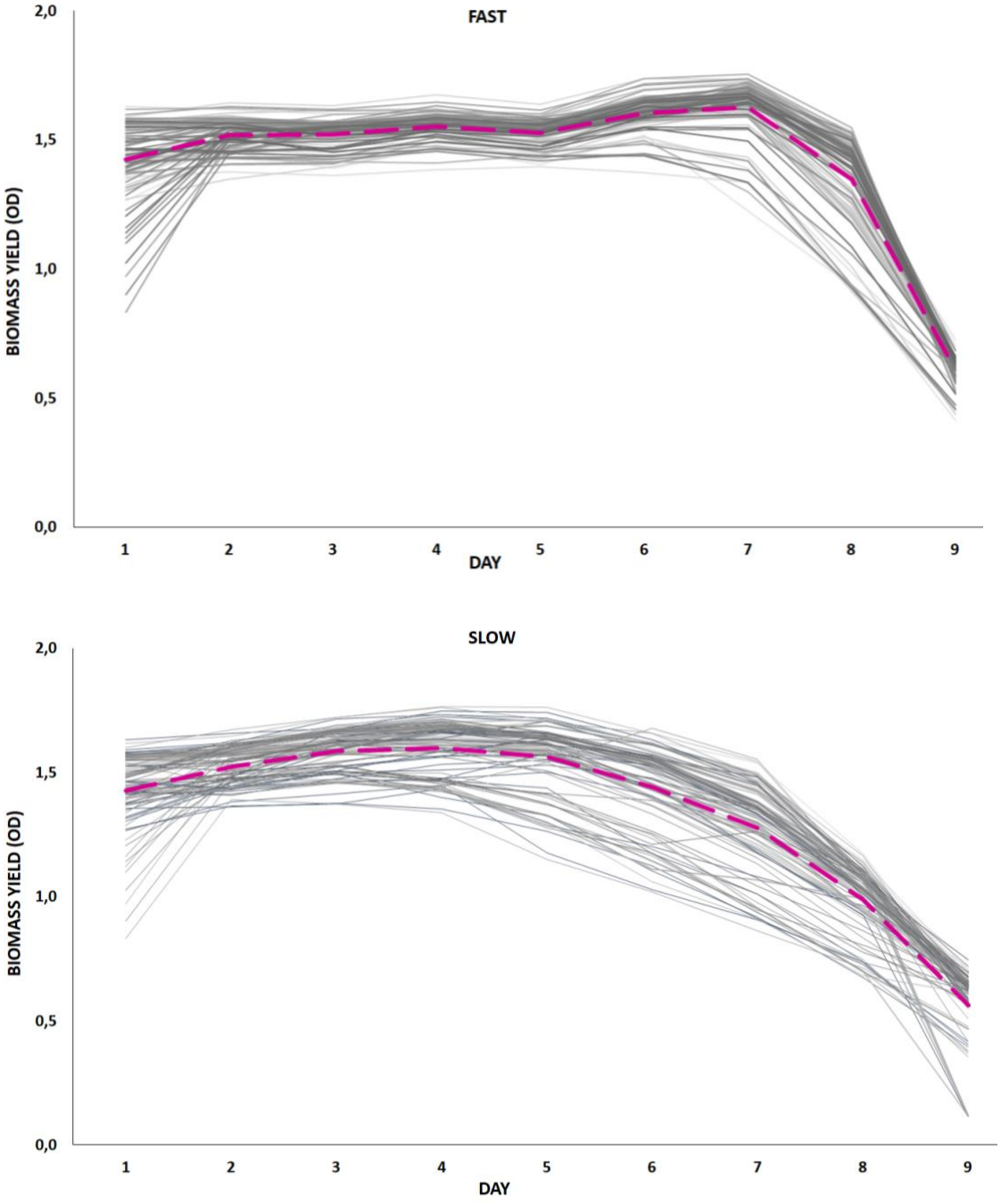
APPENDIX 1. MAXIMUM BIOMASS YIELD DEVELOPMENT DURING THE SLOW AND FAST TREATMENT ACROSS NINE DAYS.



The average maximum yield is indicated with the bold dashed line.