Master of Science Thesis

The effect of environmental fluctuations on the invasion success of bacterial invader *Serratia marcescens*

Emmi Räsänen

University of Jyväskylä

The Department of Biological and Environmental Science

Ecology and Evolutionary Biology

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UNIVERSITY OF JYVÄSKYLÄ, Faculty of Mathematics and Science

Department of Biological and Environmental Science
Ecology and Evolutionary Biology

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ABSTRACT

The global climate change is presumed to increase the amount of fluctuations in the environmental conditions. According to the theory, this could increase the amount of species invasion into new areas if fluctuations affect the ecological and evolutionary processes that make species successful as invaders, and native communities and their environments more susceptible to invasions. Disturbed environments are assumed to be more prone to invasions and the fluctuations in invasive species' home range could pre-adapt them to tolerate similar conditions elsewhere. Under fluctuating conditions, natural selection could potentially favor traits like generalism, which is profitable in adaptation to wide range of conditions. These attributes could lead to better competitive ability of the invader against the native species, especially if the native species are mal-adapted to tolerate fluctuating conditions. Moreover, the distant relatedness between the invasive species and its native competitors is hypothesized to reduce their competition for the same resources and so increase the success of the invader. I tested these theories of how fast environmental fluctuations and relatedness of species could affect the invasion success, with my aim to find effects that would be generalizable over the species. In this study, the invasion success meant the ability of the invader population to competitively displace the population of its competitor species. Bacterial species that had evolved in stable or fluctuating temperature were competed against the dominant bacterial invader *Serratia marcescens*, which had also evolved in stable or fluctuating temperature and the invasions were initiated in environments with similarly stable or fluctuating temperature. In addition, the competitor species were differently related to the invader. My results indicated strong species-specific effects on invasion success, which could be due to the more intense competition detected between closely related species. For most of the species, the rapid temperature fluctuations during invasion made invasions more successful. Unexpectedly, the evolution in the fluctuating environment did not significantly enhance the success of *S. marcescens.* Instead, my study showed that under fast fluctuations, natural selection could select for generalist genotypes, which invade better also in suboptimal environments. I found the superiority of *S. marcescens* also when its competitor species had mal-adapted to tolerate thermal fluctuations, but this result was strongly affected by one species. Overall, my results indicate that in the future, the traits of the invader, the attributes of its native competitors and the environmental conditions during invasion need to be considered together when predicting the success of the invasive species under fluctuating conditions.

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TIIVISTELMÄ

Ilmastonmuutoksen on odotettu lisäävän ympäristöolosuhteissa tapahtuvien vaihteluiden määrää. Teorian mukaan nämä vaihtelut voisivat lisätä vieraslajien levittäytymistä uusille alueille, mikäli ne vaikuttavat ekologisiin ja evolutiivisiin prosesseihin, jotka tekevät lajeista menestyksekkäitä vieraslajeja sekä paikallisista eliöyhteisöistä ja ympäristöistä alttiimpia levittäytymiselle. Häiriöherkkien alueiden on oletettu olevan otollisia levittäytymiselle, minkä lisäksi vieraslajit ovat voineet valmiiksi sopeutua sietämään vaihtelevia olosuhteita alkuperäisalueellaan. Nopeasti vaihtelevissa ympäristöolosuhteissa luonnonvalinta voi suosia ominaisuuksia, kuten generalismia, joka auttaa eliöitä sopeutumaan monenlaisiin ympäristöihin. Nämä ominaisuudet voivat johtaa vieraslajin parempaan kilpailukykyyn alkuperäislajeja vastaan, varsinkin jos alkuperäislajit eivät ole sopeutuneet sietämään ympäristön vaihtelua. Lisäksi vieraslajien on oletettu menestyvän paremmin niiden ollessa kaukaisempaa sukua alkuperäislajeille ja sen vuoksi kilpailevan vähemmän samoista resursseista. Tässä tutkimuksessa tarkoituksenani oli testata edellä mainittuja teorioita ja löytää ilmiöitä, jotka olisivat yleistettävissä kaikille tutkimuslajeille. Tutkimuksessani levittäytymismenestys tarkoitti vieraslajipopulaation kykyä syrjäyttää sitä vastaan kilpailevan lajin populaatio. Bakteerilajeja, joiden populaatiot olivat kehittyneet joko tasaisessa tai vaihtelevassa lämpötilassa kilpailutettiin *Serratia marcescens* vieraslajibakteeria vastaan, jonka populaatiot olivat myös kehittyneet joko tasaisessa tai vaihtelevassa lämpötilassa, ja lämpötila levittäytymisen aikana oli samalla tavoin joko tasainen tai vaihteleva. Lisäksi kilpailijalajit olivat vaihtelevissa määrin sukua vieraslajille. Kokeen tulokset osoittautuivat herkiksi kilpailijalajin identiteetille, mikä saattoi johtua voimakkaammasta kilpailusta läheistä sukua olevien lajien välillä. Useimpien lajien tapauksessa vaihteleva lämpötila levittäytymisen aikana kasvatti vieraslajin menestystä. Yllättäen vieraslajin kehitys vaihtelevassa lämpötilassa ei parantanut merkitsevästi sen levittäytymismenestystä. Sen sijaan vaihtelevassa lämpötilassa luonnonvalinta voisi mahdollisesti valita generalisteja, jotka menestyvät hyvin myös epäoptimaalisissa olosuhteissa. *S. marcescens* menestyi parhaiten myös silloin, kun sen kilpailijalaji ei ollut sopeutunut sietämään lämpötilavaihtelua, mutta tähän tulokseen yhdellä kilpailijalajeista oli vahva vaikutus. Tulosteni perusteella tulisi tulevaisuudessa sekä vieraslajin että sen paikallisten kilpailijoiden ja levittäytymisen aikaisen ympäristön ominaisuudet huomioida yhdessä, kun halutaan ennustaa vieraslajien menestystä vaihtelevissa olosuhteissa.

Contents

1. INTRODUCTION

The current climate change is presumed to increase not only the temperature globally, but also the amount of fluctuations in environmental conditions (IPCC; Stocker *et al.* 2014). Fluctuating conditions create selection pressures, which could select for traits that are profitable in adaptation to fast climate change (Levins 1968). The global warming has already enhanced the spread of many invasive species (Dukes & Mooney 1999, Clements & Ditommaso 2011), but it is also possible that the evolution under fluctuating conditions could contribute to the species' ability to invade new areas (Lee & Gelembiuk 2008, Saarinen *et al.* 2017). Invasive species are known to be a problem in many invaded ecosystems, and they can, for example, competitively displace native species (Mooney & Cleland 2001). This calls for studies to predict the success of invasive species under future changing climatic conditions. The major questions are: how fluctuations affect the ecological and evolutionary processes that make (1) species successful as invaders, (2) native communities less resistant to invasions and (3) novel environments more susceptible to invasions.

Fluctuations can create environmental stochasticity, which facilitates species invasions (Davis 2009). Disturbed environments are shown to be more prone to invasions than non-disturbed, since disturbances cause repercussions in native species' population sizes and release resources for invaders to exploit (Burke & Grime 1996, Davis *et al.* 2000, Elton 1958 cited in Davis 2009, Liu *et al.* 2012). In addition, invasive species have been suggested to arise from areas that are heterogeneous and disposed to disturbances (Baker 1974, Lee & Gelembiuk 2008, Foucaud *et al.* 2010, Hufbauer *et al.* 2013). For example, if the species has evolved in a disturbed environment, it might have coincidental pre-adaptations which increase its invasion success in the new environment with similar conditions (Bock 1959, Lee & Gelembiuk 2008, Hamilton *et al.* 2015). Human-altered environments are becoming universal, and if species can adapt to the type and intensity of their disturbances, they could become successful as invaders worldwide. This scenario is known as the anthropogenically induced adaptation to invade hypothesis (AIAI; Hufbauer *et al.* 2012).

Species evolution under environmental fluctuations, which are fast in relation to their generation time, might select for characteristics, such as generalism and phenotypic plasticity that make them subsequently successful as invaders (Levins 1968, Lynch & Gabriel 1987, Kassen 2002, Meyers *et al.* 2005, Lee & Gelembiuk 2008, Duncan *et al.* 2011, Condon *et al.* 2014, Ketola *et al.* 2013). These qualities can increase their ability to tolerate a wide range of conditions; for example, the adaptation to fluctuating temperature by thermal generalism would allow species to prosper in various environments under climate change (Zerebecki *et al.* 2011). The previous studies have shown that generalist genotypes could even have an equal or a superior performance to that of the specialists that have adapted to the prevailing stable environment (Reboud & Bell 1997, Duncan *et al.* 2011, Condon *et al.* 2014). Thus, evolution in fluctuating conditions could potentially generate universally good invaders that are successful in both stable and fluctuating environments (Ketola *et al.* 2013). These findings are against the theory of the Jack-of-all-trades is a master of none hypothesis, which states that there should be a cost for being a generalist, due to which generalists would have intermediate fitness across optimal environments (Lynch & Gabriel 1987, Richards *et al.* 2006). This means that when the conditions during the invasion are stable, the native species with local adaptation to stable conditions should have competitive advantage over the fluctuation-adapted invaders (Marvier *et al.* 2004).

The generalist strategies and preadaptation to fluctuating conditions can lead to the better competitive ability of the invader against native species (Lee & Gelembiuk 2008). This is true especially if the native species have not adapted to the prevailing fluctuating conditions (Kassen & Bell 1998, Duncan *et al.* 2011). Thus, human activities can make environments novel for native species and there could be costs for specialist species of being locally adapted (Sax & Brown 2000, Duncan *et al.* 2011). The lack of adaptation to fluctuations in native species could then make communities less resistant against invasions and increase the risk of extinctions due to the competition with the invader. It has also been argued that the increased fluctuations in temperature could pose even a greater risk to species than the increase of mean temperature (Vasseur *et al.* 2014). On the other hand, if the native species are also pre-adapted to tolerate fluctuations or prevailing conditions in general, the invader might not have competitive advantage due to adaptation to fluctuations (Saarinen *et al.* 2017).

The theories of the resistance of the native communities to invasions are based on the competitive exclusion theory, and there is good experimental evidence for this principle (Hardin 1960, MacArthur & Levins 1967, Gause 1934 cited in Begon *et al.* 2006, Strauss *et al.* 2006, Violle *et al.* 2011). Native species within a community compete for resources with the invader, which may hinder invasive species from establishing, particularly if they share fully similar niche requirements (Fargione & Tilman 2005, Violle *et al.* 2011). Based on the competition-relatedness hypothesis, distant relatedness between invasive and native species should increase the success of an invader, as they will compete less intensely due to having more different ecological niches (Darwin 1859, Cahill *et al.* 2008). This is also known as Darwin's naturalization hypothesis, which has been tested with different species and methods, leading to varying results (Ricciardi & Mottiar 2006, Cahill *et al.* 2008, Jiang *et al.* 2010, Burns & Strauss 2011, Tingley *et al.* 2011, Violle *et al.* 2011, Ferreira *et al.* 2012, Narwani *et al.* 2013, Godoy *et al.* 2014). In theory, the naturalization hypothesis could hold true, since invasive species usually come from distant locations and lack common evolutionary history with their local community (Darwin 1859, Cox 2004).

Conversely to competition-relatedness hypothesis, it has been suggested that the close relatedness could increase the invasion success if the similarity of the resource requirements of the invasive and the native species make novel environment more suitable for the invader (Darwin 1859, Davis 2009). This positive effect could potentially outweigh the negative effect of more intense competition between close relatives (Duncan & Williams 2002, Park & Potter 2013). In addition, distantly related species can be ecologically more similar, if they have adapted to similar kinds of environmental conditions due to convergent evolution (MacArthur & Levins 1967). Altogether, the degree to which competition and relatedness are affecting the success of invasive species is a subject that is still under study (Wiens & Graham 2005, Alexandrou *et al.* 2015).

The aim of my study was to test how fast temperature fluctuations affect the invasion success. In order to investigate the multifactorial nature of invasions, I ran an experimental design to test together the effects of the environmental conditions during the invasion, and the environmental conditions during the evolutionary histories of both the invasive and the native species on the success of the invader. I used several bacterial species that had evolved either in stable or fluctuating temperature conditions and implemented the artificial invasions in similarly stable or fluctuating temperature conditions (Saarinen 2016). In order to investigate also the effect of relatedness between competing species on invasion success, I had an environmental pathogen *Serratia marcescens* as an invader competing against five differently related bacterial species in bicultures. My interest was to find effects that would be generalizable over all the study species.

In this study, the invasion success meant the ability of the invader population to competitively displace the population of the competitor species that received the invasion. My study question was how the rapid temperature fluctuations in the environment during invasion, and in the historical environments of both the invader and its competitor species, with the effect of the phylogenetic relatedness of these species, affect the invasion success. I hypothesized that the invasion success will increase if:

1) The disturbed environments are more susceptible to invasions, due to which the temperature fluctuations during invasion would promote the success of the invader (Figure 1).

2) The invasive species' evolution under fluctuating thermal conditions and its pre-adaptation to fluctuating environments lead to populations with a greater competitive ability and propensity to invade (Figure 1).

3) The competitor species has not evolved under fluctuating thermal conditions and is mal-adapted to fluctuating environments, which affect its competitive ability and make the population less resistant to invasion (Figure 1).

4) The more distantly related competitor species will compete less intensely with the invader due to having more different ecological niches, making the competitor species' population less resistant to invasion.

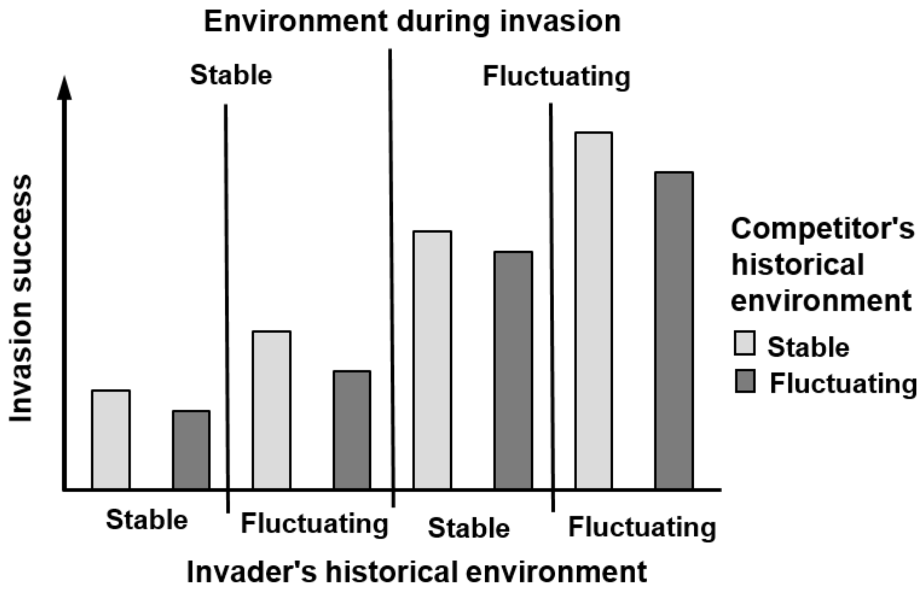

Figure 1. The expected effects of the temperature fluctuations on the invasion success. The invasion success was assumed to be higher when the environment during the invasion was fluctuating, when the invader has experienced fluctuating conditions in its historical environment and when the competitor species has not experienced fluctuating conditions in its historical environment.

2. DATA AND METHODS

2.1. Study species

In this experiment, I had 6 study species, all originally obtained from ATCC® (American Type Culture Collection): *Enterobacter aerogenes* ATCC® 13048™, *Serratia marcescens ssp. marcescens* ATCC® 13880™, *Escherichia coli* ATCC® 11775™, *Pseudomonas putida* ATCC® 12633™, *Pseudomonas fluorescens* ATCC® 13525™ and *Novosphingobium capsulatum* (ATCC® 14666™). Bacterial species were chosen based on their abilities to grow in the same medium and to tolerate the rapidly fluctuating temperature range (20 °C–30 °C–40 °C) of the experiment. Originally, the clones were acquired for the experiment described in Saarinen 2016. Before my experiment, the strains had evolved 2.5 months in stable (30 °C) or fluctuating (20 °C–30 °C–40 °C, at 2 h intervals) temperature conditions. The temperature fluctuations were fast, occurring within the generation times of all study species. The stable temperature was near the optimal temperature for all the bacterial species, when the maximum growth rate and yield were measured (Saarinen 2016). *S. marcescens* was chosen as an invader because it is known to invade and dominate the populations of other study species. *S. marcescens* is also easy to identify when growing on the blue-green agar plates (Smith *et al.* 1969). The phylogenetic distance of the study species is presented in the neighbor-joining tree based on 16S ribosomal RNA gene (Figure 2).

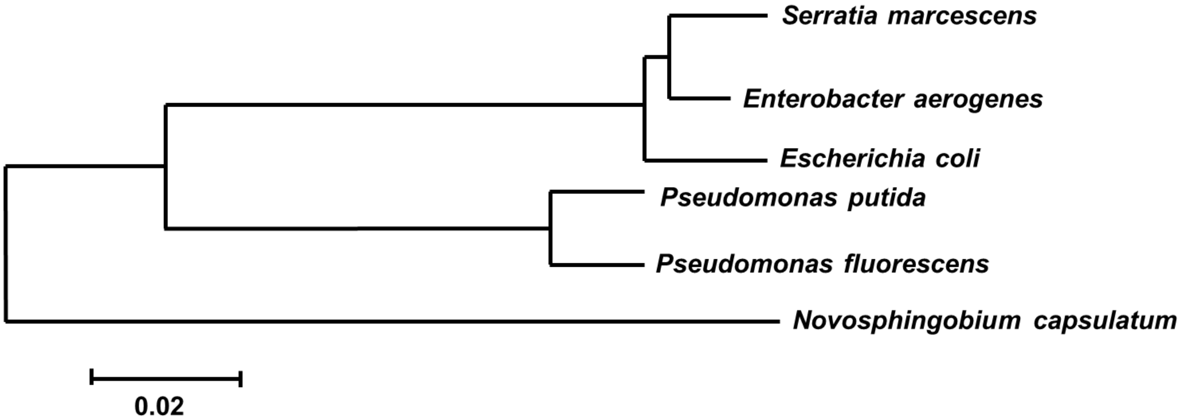

Figure 2. The estimated phylogeny of the study species. The neighbor-joining tree is based on 16S ribosomal RNA gene and the scale bar represents the number of nucleotide substitutions per site.

2.2. Invasion experiment

My study design allowed me to separate the effects of the environment during invasion, the historical environment of the invader and the historical environment of the competitor species (stable vs. fluctuating temperature in all cases) on the invasion success of *S. marcescens*. In this experiment, the invasion success means *S. marcescens* clones' ability to competitively displace the population of one of its competitor species receiving the invasion i.e. the proportion of the *S. marcescens* colonies from the total colony count. The invader population that had evolved in either stable or fluctuating environment invaded the competitor species' population that had also evolved in either stable or fluctuating environment. I implemented the invasion experiment in 2 environments, one with stable (30 °C) and the other with fluctuating (20 °C–30 °C–40 °C, at 2 h intervals) temperature. These environments matched the conditions during bacterial evolution in stable and fluctuating environments (Saarinen 2016).

In a previous study (Saarinen 2016 II), 10 populations of each of the 6 study species were allowed to evolve separately for 2.5 months at stable (30 °C) and in rapidly fluctuating (20 °C–30 °C–40 °C) temperature environments. After the evolution treatment, 4 clones were isolated from each of the populations and frozen at -80 °C (1:1 in 80% glycerol). My invasion experiment was initiated by using 8 of these independently evolved replicate populations (n = 8). 1 clone of each of the *S. marcescens* population was chosen randomly to compete with 1 clone of each of the population of its 5 competitor species in biculture. Furthermore, the competitor species had different phylogenetic distance to *S. marcescens*, which allowed me to investigate the effect of relatedness between competing species on invasion success (Figure 2). The genetic distances extracted from the phylogeny were used as a proxy for the niche complementarity and the intensity of the competition between two study species. The 16S ribosomal RNA gene sequences were obtained from the NCBI GeneBank nucleotide sequences database and the phylogeny was constructed in MEGA version 5 (Saarinen 2016).

In this study, experimental microcosms were 15 ml centrifuge tubes (Sarstedt, Numbrecht, Germany) containing 3 ml of sterile Nutrient Broth medium (10 g of nutrient broth powder (Difco, Becton & Dickinson, Sparks, MD) and 1.25 g of yeast extract (Difco) in 1 l of sterile ddH2O). I initiated artificial invasions with asymmetric starting conditions for the competition by pipetting 2 µl of *S. marcescens* to all of the 320 tubes with 48 µl of one of its competitor species. Half of the tubes were put in stable (30 °C) and half in fluctuating (20 °C–30 °C–40 °C, at 2 h intervals) temperature environment (thermal cabinets: Lab Companion, ILP-12; Jeio Tech, Seoul, Korea) and the tube caps were kept loose to ensure gas exchange. I allowed the species to compete a total of 3 days, after I sampled 500 µl of bacterial suspension from each tube into cryotubes containing 500 µl of 80 % glycerol and stored them at -80 °C for later analysis.

2.3. Colony counting

To determine the invasion success of *S. marcescens*, I counted the proportion of the invader colonies in each sample 3 days after the invasion. I plated all the 320 frozen samples in a random order. I used standard dilution series technique, where I pipetted 100 µl of thawed bacterial suspension into 900 µl of sterile ddH2O, and repeated the tenfold dilution 6 times to achieve 10^{-5}- and 10^{-6}-fold dilutions of the original samples. These dilutions allowed me to count separate colonies on agar plates. The discrimination of species, *S. marcescens* or other, was conducted by using Methyl green DNase test agar plates (Becton and Dickinson and Company, Sparks, MD; premade at Tammertutkan maljat, Tampere, Finland). DNase plates enable the separation of *S. marcescens* colonies from the competitor species colonies because only *S. marcescens* can break down DNA enzymatically by secreting DNase (Ketola *et al.* 2016). This appears as a clear halo around the *S. marcescens* colonies on the blue-green agar plates (Smith *et al.* 1969). After 2–3 days of propagation at room temperature, the *S. marcescens* colonies had grown large enough to produce distinct halos. I marked all the colonies on the plates with marker pens and counted the ratio between *S. marcescens* colonies and its competitor specie’s colonies. The entire experiment lasted from May 9th to July 2nd, 2016.

2.3. Data analysis

I tested the effect of environment during invasion, the historical environment of the invader and the historical environment of the competitor species, with the effect of the phylogenetic relatedness of these species, on the invasion success of *S. marcescens*. As a measure of invasion success, I modeled the odds of encountering *S. marcescens* colonies from all bacterial colonies in a DNase agar plate. I had non-normal proportion data and the analysis included random effects, so I analyzed the data with generalized linear mixed model (GLMMs; Bolker *et al.* 2009). I used a binomial error distribution and a logit link, and set the total number of colonies in a plate as a denominator to control for the total number of events in a trial (SPSS version 24.0, IBM-SPSS, Chicago, IL, USA). GLMMs are more suitable for analyzing proportional and binomial data than the arcsine square root transformation, which has long been the recommended procedure in statistics of ecological research (Warton & Hui 2011).

In this experiment, I had 3 fixed factors, the environment during invasion, the historical environment of the invader and the historical environment of the competitor species, which all had 2 levels, stable and fluctuating temperature treatments. I fitted these 3 fixed factors, all their 2-way interactions and the 3-way interaction as explanatory variables. I also fitted the phylogenetic distance between *S. marcescens* and its competitor species as a continuous fixed factor explaining the invasion success. The population of the *S. marcescens*, regardless of its historical environment, and the identity of the competitor species were fitted as random factors. This was done to control for the non-independency of the observations, arising from the fact that some invader clones were measured in 2 environments and against several competitor species.

In addition, I executed sensitivity analyses to separate the species-specific effects on the results of the generalized linear mixed model. The model was re-run 5 times, excluding 1 of the 5 competitor species from the full model in their turn. Based on these analyses, I will mainly discuss the results that were generalizable over the species (Appendix; Tables 1–5, Figures 1–5). The directions of the effects in all GLMMs were concluded from the estimated marginal mean values and their standard errors. The post hoc analyses were corrected for multiple comparisons by using the sequential Bonferroni correction.

3. RESULTS

The results indicated high invasion success of *S. marcescens* (mean odds 72–99 %) 3 days after the invasion. In 3 out of 5 competition treatments (*P. putida*, *P. fluorescens* and *N. capsulatum*), the competitor species was almost competitively displaced and the invasion success of *S. marcescens* was close to 100 % (the proportion of the colonies from the total colony count). Thus, it was less convincing to interpret the effects of the temperature fluctuations from these species because of the lack of variation in invasion success. There was more variation in invasion success when *S. marcescens* was competing against *E. coli* and *E. aerogenes*. Sensitivity analyses indicated that the removal of *E. coli* and *E. aerogenes* affected some factor interactions very strongly (from highly significant $P < 0.001$ to clearly non-significant $P > 0.12$); especially *E. coli* had a disproportionately big effect on the results of the full model. This suggests that the results from the mixed model containing all competitor species could arise only because of the inclusion of these species in the model (Table 1, Appendix; Table 4, Table 5).

For the statistical full model, the factor and factor interactions that were found sensitive to the removal of species are not discussed in detail. Excluding *E. coli* from the model made the effect of the competitor species' historical environment and its interactions with the environment during invasion or the invader's historical environment non-significant (Appendix; Table 4). Excluding data from the *E. coli* changed also the main result as without *E. coli* the invasions by *S. marcescens* were more successful when the environment was fluctuating (est. = 0.980, s.e. = 0.006) (Appendix; Figure 4), rather than a stable environment (est. = 0.958, s.e. = 0.011, $P = 0.022$) (cf. Appendix; Figures 1, 2, 3, 5).

The 3-way interaction showed sensitivity for excluding *E. aerogenes* from the model, becoming non-significant, unlike in the full model (Appendix; Table 5).

For some factors, the analyses showed far less sensitivity to removal of species. Despite the result that removal of *N. capsulatum* rendered the effect of environment during invasion to be tentatively significant (P = 0.074) (Appendix; Table 1), its overall effect on the result of the full model was moderate by reducing the significance only slightly (P = 0.02) (Table 1). Such an effect suggests that the environment during invasion have a statistically significant effect on most of the species. Hence this effect is brought to discussion. In addition, the non-significant effect of the invaders' historical environment did not become significant in any of the analyses, and all the analyses indicated a strong effect of the environment during invasion by the invaders' historical environment interaction (Table 1, Appendix; Tables 1–5).

In the full model, the invasion by *S. marcescens* was more successful when the environment during invasion was stable (est. = 0.960, s.e. = 0.010) (Table 1), rather than fluctuating (est. = 0.956, s.e. = 0.011, P = 0.045). The invaders' historical environment did not have a significant effect on the invasion success (P = 0.140) (Table 1), but the pairwise test indicated to the direction that the invasion success of populations that had evolved in fluctuating environment could be higher (est. = 0.965, s.e. = 0.010) than the populations that had evolved in stable environment (est. = 0.949, s.e. = 0.014, P = 0.166). In the stable environment, the invasion was stronger if the invader had evolved in fluctuating environment (est. = 0.972, s.e. = 0.008) than if the invader had evolved in stable environment (est. = 0.941, s.e. = 0.016, P = 0.025). However, this difference was not found in fluctuating environment, were the invasion success of the invaders that had evolved in fluctuating environment (est. = 0.955, s.e = 0.013) was not higher than the invaders' that had evolved in stable environment (est. = 0.956, s.e. = 0.012, P = 0.899).

The phylogenetic distance had a positive effect on invasion success (t = 4.21, s.e = 3.379, b = 14.22, P = 0.043) (Table 1). The closest relatives of *S. marcescens* were the strongest in hindering its invasion success (Figure 4). This effect is discussed as generalizable over the species, even though it became non-significant after removing the competitor species from the model. In the full model, the random effect of the invaders' population, regardless of its historical environment, was significant (est. = 0.227, s.e. = 0.090, P = 0.012), but the identity of the competitor species was non-significant (est. = 0.279, s.e. = 0.231, P = 0.228). The significance of random factors did not change in the sensitivity analyses, but the discussion will be based on the generalizable effects of the fixed factors.

The rests of the results were more sensitive to the exclusion of species and should be used carefully for generalizing purposes. These results indicated that the invasion was more successful if the competitor species had evolved in the stable environment (est. = 0.961, s.e = 0.010) (Table 1), rather than in the fluctuating environment (est. = 0.954, s.e. = 0.012, P = 0.013). Moreover, invasion was more successful if it occurred in the fluctuating environment and the competitor species had evolved in stable environment (est. = 0.964, s.e. = 0.009) than if the competitor had evolved in fluctuating environment (est. = 0.945, s.e. = 0.014, P = 0.007). When the invader had evolved in a stable environment and the competitor species had also evolved in a stable environment (est. = 0.958, s.e = 0.012), the invaders' densities were higher than if the competitor had evolved in a fluctuating environment (est. = 0.939, s.e. = 0.017, P = 0.007). The effect of the 3-way interaction was significant only when the environment during invasion was fluctuating (Table 1, Figure 3). Invasion success was higher when the invader had evolved in the stable environment and the competitor species had also evolved in the stable environment (est. = 0.971, s.e = 0.009) than if the competitor had evolved in the fluctuating environment (est. = 0.935, s.e = 0.018, $P = 0.007$).

Table 1. The results of the generalized linear mixed model testing the effects of the environment during invasion, the historical environment of the invader and the historical environment of the competitor species, with the effect of the phylogenetic relatedness of these species, on the invasion success of *S. marcescens* 3 days after the invasion. The fixed factors are presented in the table and the effects that were generalizable over the species are highlighted in bold. The first degree of freedom was 1 for all factors and factor interactions.

	F	df_2	P
Environment during invasion (E)	5.46	309	0.020
Invader's historical environment (I)	2.45	14	0.140
Competitor's historical environment (C)	11.99	309	0.001
E×I	90.81	309	0.001
E×C	44.32	309	0.001
I×C	27.70	309	0.001
E×I×C	17.60	309	0.001
Phylogenetic distance	11.42	3	0.043

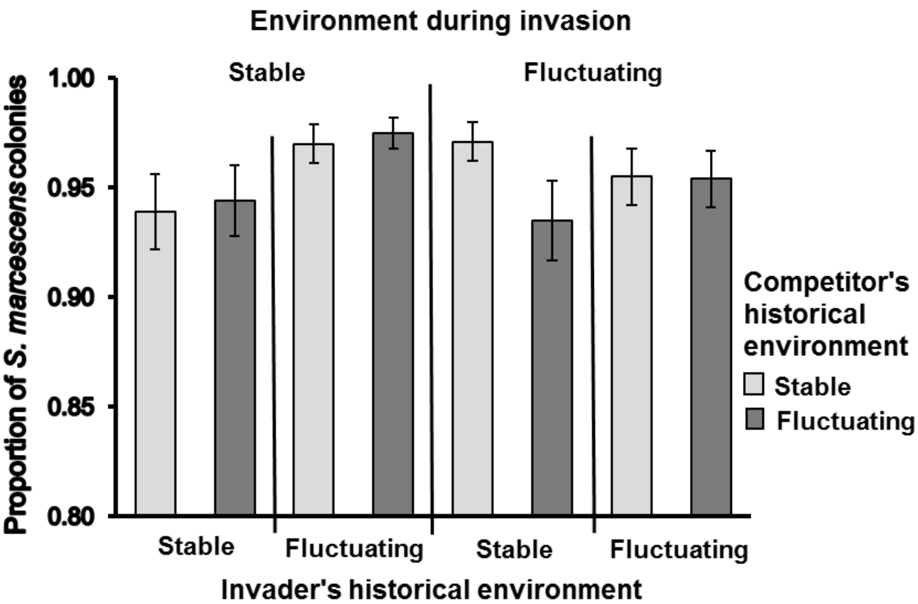

Figure 3. The proportion of *S. marcescens* colonies from the total colony count indicating the invasion success 3 days after the invasion. The invasions took place in stable or fluctuating thermal environment. The invader population that had experienced either stable or fluctuating temperature in its historical environment invaded the competitor species population that had experienced either stable or fluctuating temperature in its historical environment. The bars correspond to the estimated marginal means of the generalized linear mixed model and the error bars reflect ± 1 standard error of the mean.

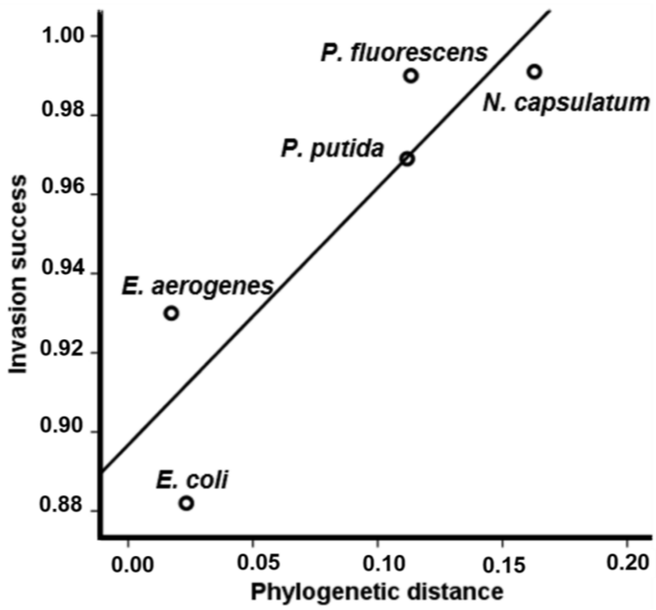

Figure 4. The effect of phylogenetic distance between invader *S. marcescens* and its competitor species explaining the invasion success, i.e. the proportion of the *S. marcescens* colonies from the total colony count. The regression line is estimated from the marginal mean values of the generalized linear mixed model.

4. DISCUSSION

The ongoing climate change is increasing the amount of fluctuations in global temperature (Stocker *et al.* 2014), and despite their potential to amplify species invasions in future, very few studies exist on this subject (Kreyling *et al.* 2008, Lee & Gelembiuk 2008, Ketola *et al.* 2013, Saarinen *et al*. 2017). I tested the theories that have commonly been suggested in literature of how fast environmental fluctuations could affect the success of invasive species. Firstly, the rapid temperature fluctuations could affect the evolutionary processes that lead to populations with a greater competitive ability and propensity to invade in many kinds of environments (Lee & Gelembiuk 2008). In contrast to this theory, I found that the historical environment of the *S. marcescens* had only a tentative effect (P = 0.14) (Table 1) on the invasion success, however, suggesting to the direction that the adaptation to fluctuating environments could improve the invasiveness of species. Under rapidly fluctuating conditions, natural selection should select for generalists with phenotypic plasticity and broad tolerance (Levins 1968, Lynch & Gabriel 1987, Kassen 2002, Meyers *et al.* 2005, Lee & Gelembiuk 2008, Duncan *et al.* 2011, Condon *et al.* 2014). Other studies have found more profound effects of these traits on the higher invasion success of species that have evolved in disturbed or fluctuating environments (Foucaud *et al.* 2010, Ketola *et al.* 2013, Saarinen *et al.* 2017).

Secondly, the disturbed environments are predicted to enhance the success of the invasive species, if they are pre-adapted to similar conditions in their home ranges (Baker 1974, Lee & Gelembiuk 2008). My results indicated a strong effect of the interaction between the environment during invasion and the invader’s historical environment (Table 1). Contrary to previous findings on invasive species, which show evidence that pre-adaptation of organisms to matching environmental conditions have made them more successful in invading new areas (Ricciardi & MacIsaac 2000, Bossdorf *et al.* 2008, Winkler *et al.* 2008, Foucaud *et al.* 2010, Hamilton *et al.* 2015), my results showed that the adaptation of the invader to fluctuations was more advantageous in the stable environment, rather than in the fluctuating environment (Figure 3). This finding is not in line with the hypotheses of pre-adaptation and anthropogenically induced adaptation to invade (Hufbauer *et al.* 2012, Saarinen *et al.* 2017). From pairwise tests it was clear that the improved invasiveness of *S. marcescens* due to evolutionary background in fluctuating environments was not even tentative when the environment during invasion was fluctuating. Such a result could be explained if the fluctuations cause noise in the estimation of invasion success, and hence make the effect more cryptic in fluctuating environments. Despite this fact, it is noteworthy that the fluctuation-adapted invaders did well in the optimal environment, which is in line with studies of experimental evolution showing that thermal fluctuations can select for a sort of "super generalists" (Ketola *et al.* 2013, Condon *et al.* 2014). These kinds of generalists are better at invading in all conditions, and there can be found only little cost for performance in stable environments (Kassen & Bell 1998, Hughes *et al.* 2007, Duncan *et al.* 2011). Altogether, the microbial organisms have the ability to adapt rapidly, which could make them become successful invaders also in environments dissimilar to those in their native range (Litchman 2010).

Thirdly, the theory and studies suggest that the disturbed and fluctuating environments are more prone to invasions (Burke & Grime 1996, Davis *et al.* 2000, Elton 1958 cited in Davis 2009, Li & Stevens 2012). Unlike I hypothesized, fluctuating temperature during invasion, as such, did not enhance the success of the invader. The invasion of *S. marcescens* was, over all tested species combinations, more successful, when the environment was stable, rather than fluctuating (Figure 3). However, when I explored the results further I found that this effect was caused solely by *E. coli*. If *E. coli* was removed from the analysis, the results showed evidence that the invasion success was higher in the fluctuating environment (Appendix; Figure 4). This finding is consistent with the previous studies made with plants and bacteria (Burke & Grime 1996, Davis *et al.* 2000, Liu *et al.* 2012, Saarinen *et al.* 2017). The reason for the disproportionate effect of *E. coli* could simply be the fact that *S. marcescens* had almost competitively displaced other competitor species in the microcosms 3 days after the invasion. Hence, these species had smaller effects on the invasion success in the full model, overrun by the large effect caused by *E. coli*. On the other hand, there are also some other studies which have not found clear evidence for disturbance to be the most important factor in facilitating invasions (Lozon & MacIsaac 1997).

Moreover, the success of the invader and the consequences for the novel community are highly dependent on the properties of the native competitor species (Davis 2009, Mächler & Altermatt 2012). This was evident from several results that lost their statistical significance if certain species were removed from the analyses (Table 1, Appendix; Table 4, Table 5). The following results were driven largely by *E. coli*; for example, my results indicated that the success of *S. marcescens* was higher when the competitor species had not experienced thermal stress in its historical environment (Figure 3). This result is in line with the hypothesis that the competitors that have not been evolving in fluctuating conditions are weaker in resisting invasions because of the lack of broader tolerance or phenotypic plasticity (Kassen & Bell 1998, Duncan *et al.* 2011, Saarinen *et al.* 2017). In addition, the invasions were more successful when the environment during invasion was fluctuating and competitor species had adapted to stable environment. This is supporting the idea that the mal-adaptation of the native competitor to fluctuating conditions will facilitate invasions (Sax & Brown 2000, Duncan *et al.* 2011). Similarly, the 3-way interaction was non-significant if *E. aerogenes* was removed from the competitor species, but the results of the full model further supported the hypotheses of fluctuating environment during invasion and the mal-adaptation of the competitor species to increase the success of the invader (Saarinen *et al.* 2017).

The strong effects of the *E. coli* and *E. aerogenes* on the invasion success of the *S. marcescens* might be due to their close phylogenetic relatedness, which could make them compete most strongly against the invader (Figure 4). The species-specific effects could be also due to something else in species biology that affects the outcome of the competition. For example, *E. coli* and *E. aerogenes* tolerate higher temperatures of the fluctuations better than other study species (Saarinen 2016). Moreover, bacteria are known to produce extracellular toxins, which negatively affect the growth of their competitor species (Riley & Wertz 2002). Unfortunately, five species pairs, and hence low statistical power, preclude further mapping of these determinants of invasions.

The success of the invasive species can be hindered also by the limiting ecological similarity with the native competitors due to their common ancestry (Darwin 1859). Invasive species, which are distantly related to the native competitors, should be more likely successful as colonists than species that have close congeners in the novel community because of having dissimilar resource requirements (Daehler 2001, Cahill et al. 2008, Violle et al. 2011). In match with the theory, my results showed that distant phylogenetic relatedness between the *S. marcescens* and the competitor species increased invasion success (Table 1, Figure 4). Other studies have also reported that the success of the invasive species can increase due to the more distant phylogenetic relatedness with the native competitors (Strauss *et al.* 2006, Jiang *et al.* 2010, Burns & Strauss 2011, Schaefer *et al.* 2011, Violle *et al.* 2011). In addition, my finding indicates that the phylogenetic distance could be a good predictor of the niche similarity and the intensity of the competition between organisms (Jiang *et al.* 2010, Burns & Strauss 2011, Violle *et al.* 2011). On the other hand, not all studies have found evidence for this prediction, and it should be also noticed that the phylogenetic relatedness is only a potential proxy for niche complementarity of species (Narwani *et al.* 2013, Fritschie *et al.* 2014, Venail *et al.* 2014, Alexandrou *et al.* 2015). For example, the intensity of competition could also be based on fitness differences among species, not only on the differences in their niches (Mayfield & Levine 2010).

Despite the dissimilarities with higher organisms, microbes have been commonly used to study the major theories in biology, which could not be studied with macro organisms (Bennett & Hughes 2009, Buckling *et al.* 2009). In invasion biology, the information from microbial studies can be applied to other invasive organisms due to their similar, enhanced performance traits (Bennett & Hughes 2009, Litchman 2010). In my experiment, *S. marcescens* had high invasion success, which confounded detecting the effects of environmental fluctuations. The found effects on invasion success could have been more pronounced, if I had measured the invasion success already 1 day after initiating the invasion or if the range of temperature fluctuation had been wider. On the other hand, the previous studies that have been made with the same bacterial strains, but have competed *S. marcescens* against multiple species in the same culture and adding the competitor species frequently, did not found as pronounced invasions (Ketola *et al.* 2017, Saarinen *et al.* 2017). It could be argued that my community was too simple as I used one competitor species as “the community”. This is an oversimplification of the nature, where also the biodiversity and the other species interactions than just competition are assumed to affect the resistance to invasions (Davis 2009, Naughton *et al.* 2015). However, the aim of my study design was not to mimic the complexity of natural communities, but to test the theories of competition between individual species on invasion success (Cahill *et al.* 2008, Narwani *et al.* 2013).

To summarize, my aim was to find effects of environmental fluctuations on invasion success that would be generalizable over the species, but there was strong species-specificity in the results. Most of the species showed evidence that the rapid temperature fluctuations during invasion made invasions more successful (Saarinen *et al.* 2017). Surprisingly, the invaders' adaptation to fluctuating conditions did not clearly enhance the success of *S. marcescens.* Instead, my study showed that under rapidly fluctuating temperature, natural selection could possibly select for generalist genotypes, which invade better despite their suboptimal adaptation to current conditions (Duncan *et al.* 2011, Ketola *et al.* 2013). The superiority of *S. marcescens* was found also when its competitor species had mal-adapted to tolerate thermal fluctuations during invasion (Saarinen *et al.* 2017). I conclude that the traits of the invader, the attributes of its native competitors and the environmental conditions during invasion need to be considered together when predicting the success of the invasive species (Davis 2009, Mächler & Altermatt 2012). Moreover, the phylogenetic relatedness could be a useful measure to be used in identifying the species that pose greater risk of successfully invading new communities (Strauss *et al.* 2006). In future, more scientific work is required to predict the species' propensity to invade under novel, fluctuating climatic conditions.

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APPENDIX 1

The results of the sensitivity analyses separating the species-specific effects on the results of the generalized linear mixed model, when 1 of the 5 competitor species were excluded from the model in their turn (Tables 1–5, Figures 1–5). The phylogenetic distance is not presented in tables being non-significant after removing the competitor species from the full model. The first degree of freedom was 1 for all factors and factor interactions.

Table 1. The results of the sensitivity analysis when *N. capsulatum* was excluded from the competitor species in the generalized linear mixed model.

	F	df_2	P
Environment during invasion (E)	3.14	245	0.078
Invader's historical environment (I)	1.62	13	0.225
Competitor's historical environment (C)	10.34	245	0.001
E×I	85.22	245	0.001
E×C	57.88	245	0.001
I×C	33.03	245	0.001
E×I×C	30.36	245	0.001

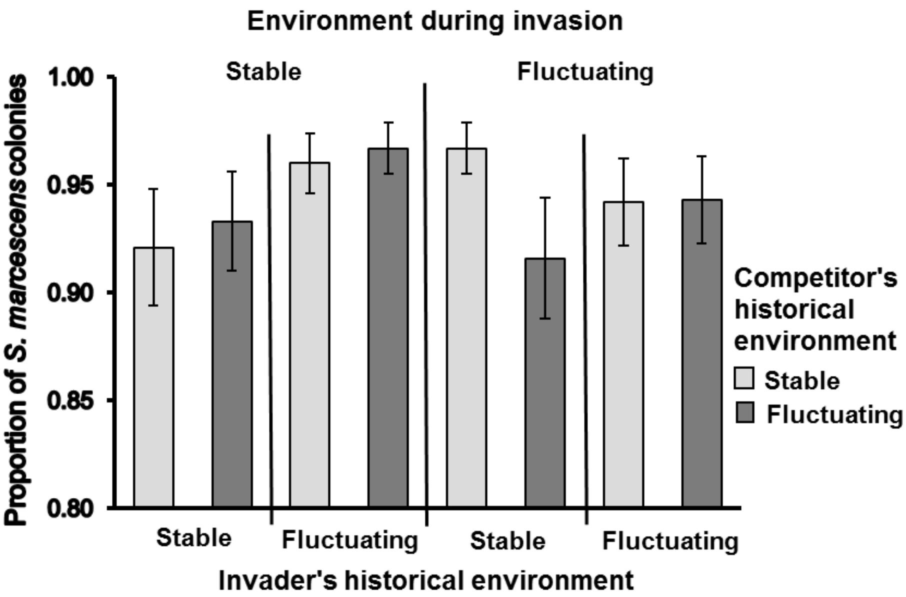

Figure 1. The proportion of *S. marcescens* colonies from the total colony count indicating the invasion success 3 days after the invasion when *N. capsulatum* was excluded from the competitor species in the generalized linear mixed model. The bars correspond to the estimated marginal means and the error bars reflect ± 1 standard error of the mean.

Table 2. The results of the sensitivity analysis when *P. fluorescens* was excluded from the competitor species in the generalized linear mixed model.

	F	df_2	P
Environment during invasion (E)	9.67	247	0.002
Invader's historical environment (I)	2.01	13	0.179
Competitor's historical environment (C)	11.57	247	0.001
E×I	76.58	247	0.001
E×C	60.29	247	0.001
I×C	23.93	247	0.001
E×I×C	28.78	247	0.001

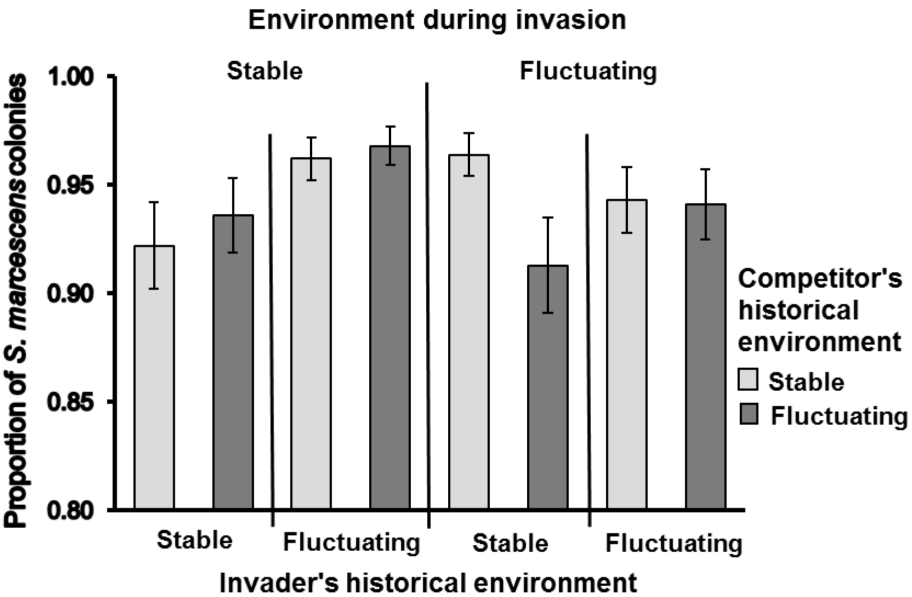

Figure 2. The proportion of *S. marcescens* colonies from the total colony count indicating the invasion success 3 days after the invasion when *P. fluorescens* was excluded from the competitor species in the generalized linear mixed model. The bars correspond to the estimated marginal means and the error bars reflect ± 1 standard error of the mean.

Table 3. The results of the sensitivity analysis when *P. putida* was excluded from the competitor species in the generalized linear mixed model.

	F	df_2	P
Environment during invasion (E)	115.21	245	0.001
Invader's historical environment (I)	2.25	14	0.156
Competitor's historical environment (C)	25.18	245	0.001
E×I	92.27	245	0.001
E×C	33.11	245	0.001
I×C	29.57	245	0.001
E×I×C	7.38	245	0.007

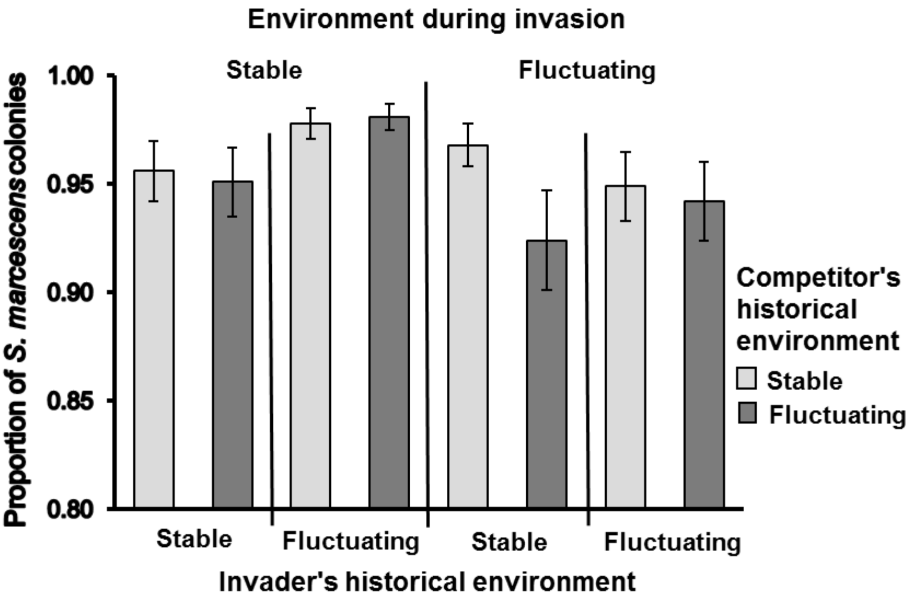

Figure 3. The proportion of *S. marcescens* colonies from the total colony count indicating the invasion success 3 days after the invasion when *P. putida* was excluded from the competitor species in the generalized linear mixed model. The bars correspond to the estimated marginal means and the error bars reflect ± 1 standard error of the mean.

Table 4. The results of the sensitivity analysis when *E. coli* was excluded from the competitor species in the generalized linear mixed model.

	F	df_2	P
Environment during invasion (E)	129.98	245	0.001
Invader's historical environment (I)	3.62	14	0.079
Competitor's historical environment (C)	2.42	245	0.121
E×I	29.89	245	0.001
E×C	1.58	245	0.211
I×C	0.84	245	0.360
E×I×C	19.69	245	0.001

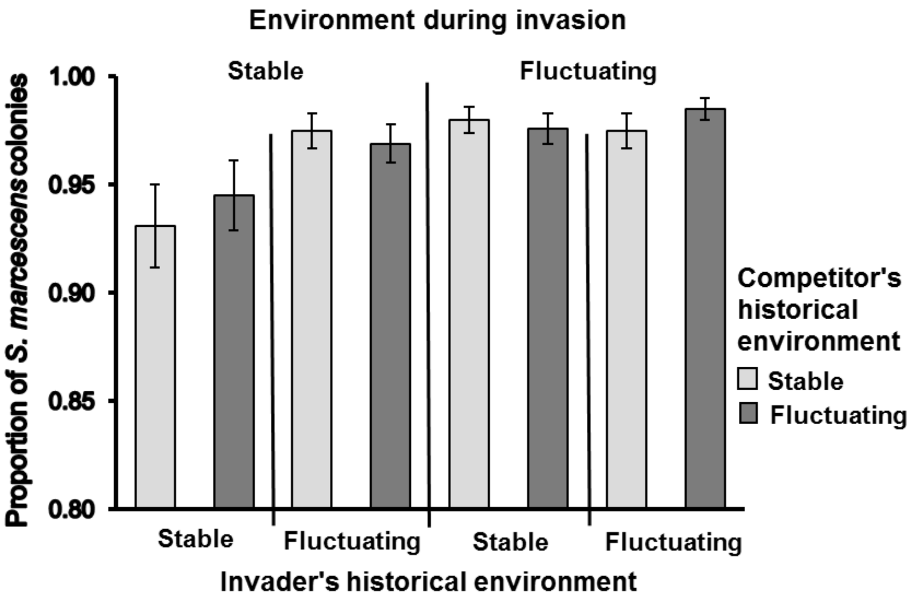

Figure 4. The proportion of *S. marcescens* colonies from the total colony count indicating the invasion success 3 days after the invasion when *E. coli* was excluded from the competitor species in the generalized linear mixed model. The bars correspond to the estimated marginal means and the error bars reflect ± 1 standard error of the mean.

Table 5. The results of the sensitivity analysis when *E. aerogenes* was excluded from the competitor species in the generalized linear mixed model.

	F	df_2	P
Environment during invasion (E)	10.93	245	0.001
Invader's historical environment (I)	1.50	14	0.241
Competitor's historical environment (C)	17.64	245	0.001
E×I	35.15	245	0.001
E×C	25.51	245	0.001
I×C	29.48	245	0.001
E×I×C	0.21	245	0.646

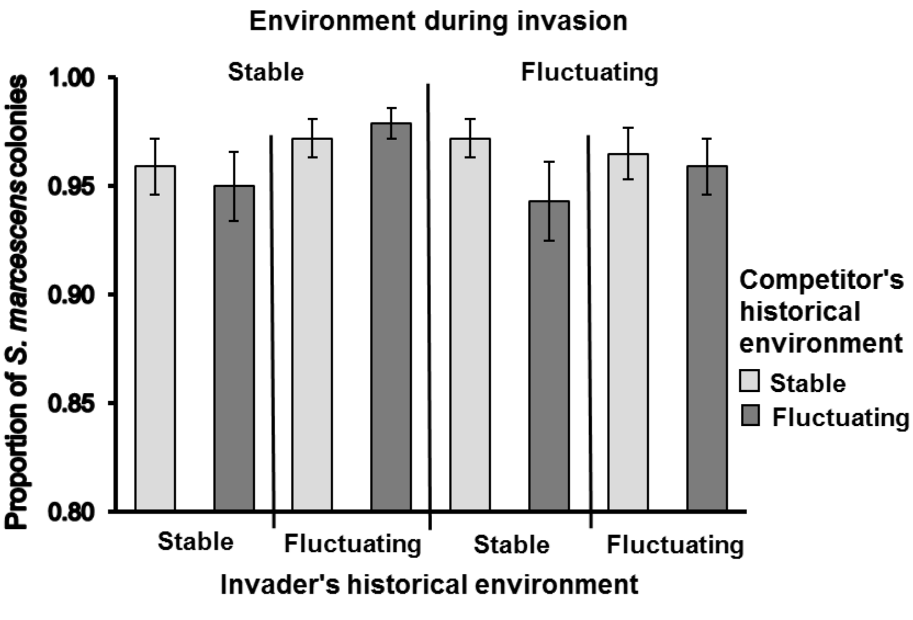

Figure 5. The proportion of *S. marcescens* colonies from the total colony count indicating the invasion success 3 days after the invasion when *E. aerogenes* was excluded from the competitor species in the generalized linear mixed model. The bars correspond to the estimated marginal means and the error bars reflect ± 1 standard error of the mean.