Interplay of ecology and evolution under changing environmental conditions

Evolution experiments with heterotrophic bacteria

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Academic Dissertation

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Without deviation from the norm, progress is not possible.

– Frank Zappa
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I  Temporal changes in species interactions in simple aquatic bacterial communities

II  Increased survival during famine improves fitness of bacteria in a pulsed-resource environment

III  Resource availability and competition shape the evolution of survival and growth ability in a bacterial community

IV  Temporal variability in detritus resource maintains diversity of bacterial communities
This thesis is based on the following articles, which are referred to in the text by their Roman numerals:


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Abstract

Organisms interact continuously with their environment. They change the quality of the environment by consuming the resources or otherwise modifying the conditions. Changes in environmental conditions in turn affect fundamentally the dynamics of populations and communities. This two-directional interaction between organisms and environment can also be an important evolutionary force.

In my thesis I have studied how changes in environmental conditions affect organisms on different levels of biological hierarchy ranging from individuals to communities. I have focused on the ecological and evolutionary effects of temporal changes in resource availability. Intermediate resource fluctuations are predicted to promote community diversity. Furthermore, rare fluctuations in resource availability together with inter- or intraspecific competition can shape the evolution of life-history traits. The questions of my thesis were addressed using experimental evolution techniques and heterotrophic bacteria.

The results of my thesis exemplify a system where the interaction between organisms and their environment is dynamic, changing, and reciprocal. I found support for the view that temporal environmental variation promotes diversity within communities. However, also other factors contributed to the species coexistence such as spatial heterogeneity due to bacterial growth as biofilms, and evolutionary changes in life-history traits. I monitored the evolution of fitness related traits (mortality, growth rate, biomass and biofilm production) over hundreds of bacterial generations. When bacteria experienced rare, seasonal changes in their resource availability, the evolutionary response was decreased mortality during resource scarcity. The growth rate increased over evolutionary time in one of my study species, when it experienced interspecific competition and resource fluctuations. In addition, my results show that bacteria modify their habitat, and these changes alter the between species interaction from antagonistic to positive. The temporal changes in species interaction type can partly explain the long-term coexistence of the study species.

Altogether, the results of my thesis show that biological interactions can be complex even in a relatively simple system. Changes in environmental conditions affect population dynamics and coexistence of species, and in turn, the activity of organisms changes the environmental conditions – and evolution adds complexity in these dynamics.
Tiivistelmä


Väitöskirjani tulokset ovat esimerkki siitä, miten toisiinsa kietoutuneita biologiset vuorovaikutukset ovat. Muutos ympäristössä heijastuu eliöiden toimintaan ja eliöyhteisön toiminta taas vuorostaan muokkaa ympäristöä – ja evoluutio muokkaa edelleen tätä vuorovaikutusta.
Summary

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1 Introduction

This thesis contributes to a long tradition of competition research with a modern way of incorporating the possibility of evolution to the ecological dynamics. I have studied experimentally how the life-history traits of bacteria evolve when they face changes in environmental conditions and simultaneously competition within or between species (II, III). I show that the species interaction can change due the habitat modification activity of organisms from competition to mutualism or commensalism (I). In the final chapter of the thesis I exemplify how environmental variation can promote species diversity within communities (IV). My work bridges several biological disciplines: the research questions arise from ecology and evolutionary biology, methods mostly from microbiology. Before going into details of the experiments and results, I will shortly introduce the relevant research fields, including historical snapshots from the early years of evolutionary biology and ecology.

1.1 Tick tock says the eco-evo clock

Historically in biological research ecological and evolutionary phenomena are thought to take place on separate timescales: evolution over several hundreds or thousands of generations, ecological interactions at more rapid pace (Slobodkin, 1961). This dichotomy dates back to Darwin who deduced that evolutionary changes would in most instances be slow and thus, impossible to observe directly (Darwin, 1859). He concluded that we could witness evolutionary change by comparing related organisms that are present now to those preserved in fossil records. The direct observation of evolutionary change is challenging, if we wish to study the evolution of organisms with a relatively long generation time compared to the generation time of the observer (or to the length of the research grant period). But by choosing rapidly reproducing organisms for experiments, it is possible to eyewitness evolution in action.

The idea of utilizing short-lived, small organisms to study evolution experimentally is not new: it actually dates back to late 19th century, when Dallinger used self-made microcosms to test if microbes were able to adapt to increasing temperatures (summarized by Bell, 2008, Chap.3). His test was most successful: the microbial community evolved to tolerate higher and higher temperatures. During recent decades microbial model systems have proven to be a versatile tool to study evolution (reviewed by Elena & Lenski, 2003; Jessup et al., 2004; Colegrave & Buckling, 2005; Buckling et al., 2009; Kawecki et al., 2012). Microbes, especially some bacteria and viruses, have another advantage over larger organisms besides rapid reproduction: they can be stored indefinitely in suspended animation to form a living library of evolutionary history.
For this thesis I have created “a fossil bed in a deep-freeze” (Lenski & Travisano, 1994) and done time-travels with bacteria to see how environmental conditions shape the evolution of resource dependent traits that are potentially important for fitness.

Dallinger’s experiments exemplified how change in environmental conditions causes evolutionary response and fitness increase. The common view is that ecological interactions and environmental conditions can cause selection and eventually evolutionary changes in organisms. However, an increasing body of evidence supports the possibility of rapid evolution, where evolutionary changes affect ecological dynamics (Hairston et al., 2005). In fact, dividing evolutionary and ecological dynamics to separate timescales has always been contradictory: ecology has had an intellectual connection to evolution since the birth of the evolutionary theory. It was Darwin who put forth the idea that the struggle for life – of which resource competition is an essential part – is an important selective agent, and one of the main factors causing the overwhelming diversity we see in nature (Darwin, 1859, Chap. 3).

Before further synchronizing the clocks of ecological and evolutionary dynamics, I will concentrate on the importance of competition in evolution. If as the famous saying goes, “nothing in biology makes sense except in the light of evolution” (Dobzhansky, 1973), it may well be that without theoretical and experimental research on competition, the light of evolution would be dimly lit.

1.2 Competition – or co-operation?

The world we live in is not infinite; the supply of energy and materials for survival, growth and reproduction. Often organisms have simultaneous need for a finite resource. Thus, competitive interactions between biological entities are inevitable. Grover (1997) defines competition as “mutually negative effects between populations or individuals”. According to another textbook definition competition is “...an interaction between individuals, brought about by a shared requirement for a resource in limited supply, and leading to reduction in the survivorship, growth and/or reproduction of at least some of the competing individuals concerned” (Begon et al., 1996, p.214). The crucial point in these definitions is fitness reduction: for an interaction to be competitive, there needs to be reduction at least in one fitness component of at least one of the interacting entities. Otherwise the definition is very flexible: it can be applied on several hierarchical levels and it does not specify what the resource exactly is.

According to a general definition resource is something organisms are able to consume, and something which when consumed becomes unavailable to other organisms (Begon et al., 1996; Grover, 1997). Resource can be e.g. food, light, water, a breeding partner, or space for growth and reproduction. Resource can be categorized as biotic or abiotic (Begon et al., 1996; Grover, 1997). Biotic resources can reproduce like prey species in predator-prey systems, or vegetation in plant-herbivore systems (Begon et al., 1996). Abiotic resources, such as mineral nutrients, do not reproduce (Grover, 1997). In natural systems this distinction is not always as clear-cut as it sounds, but it is essential in theoretical considerations and mathematical models describing the effects of competitive interactions on population dynamics or coexistence (Volterra, 1926; Armstrong & McGehee, 1980; Grover, 1997).
Theoretical work on competition has flourished since the early decades of 20th century (Lotka, 1924; Volterra, 1926). The experimental testing of theoretical predictions of Lotka and Volterra motivated Gause in his groundbreaking competition experiments (Gause, 1934a; b; Gause & Witt, 1935). Gause tested the terms of the long-term coexistence of protists living on bacterial resource, method not so different from those I have used in this thesis, and showed that two species utilizing the same limiting resource cannot indefinitely co-exist, but in time the weaker competitor goes extinct. His work has been crucial in developing the theory on competitive exclusion (Hardin, 1960). Thereafter, a vast amount of research has been done to show if and when competitive exclusion occurs (e.g. Crombie, 1947; Park, 1954; Taylor & Williams, 1975; Tilman, 1977; Gurevitch et al., 1992), and to further develop the theory on competitive interactions (e.g. Brian, 1956; MacArthur & Levins, 1967; Ayala, 1969, 1971; Stewart & Levin, 1973; Levins, 1979; Armstrong & McGehee, 1980; Tilman, 1982; Abrams, 1983; Wilson & Yoshimura, 1994; Chesson, 2000a; b).

Competition research has tried to disentangle how resource availability affects biological diversity. MacArthur and Levins (1967) extended the competitive exclusion theory, and formalized the idea that competition for common resource leads either to coexistence due to niche differentiation, or to the eventual exclusion of inferior competitors. Thus, resource competition can cause selection for niche differentiation. For example, in heterogeneous environment where many resources are available simultaneously, some species may evolve to be generalists, able to utilize several substitutable resources, and other species may specialize on one or very few substrates (MacArthur & Levins 1967). Obviously, the premise is that no organism can be a jack-of-all-trades and master of all; in other words, trade-offs limit the evolution of competitively superior generalist (reviewed by Stearns 1989; Roff & Fairbairn 2007). While the concept of a trade-off between costly traits is common in biological thinking, it is good to bear in mind that “trade-offs must be demonstrated, not assumed” (Futuyma & Moreno, 1988). Trade-offs between fitness related traits are not the only mechanism that prevents the evolution of superior organism. For example Whitlock (1996) argues the negative correlation may not be found between different traits but the trade-off exists at the level of evolutionary rate, because narrow niche breadth is likely to evolve more quickly than phenotypic plasticity or ability to utilize broad niche. Even though the concept of trade-offs is easy to grasp, the existence of trade-offs is challenging to demonstrate experimentally (Stearns, 1989), though examples do exist (e.g. Schluter, 1994; King et al., 2004; Novak et al., 2006; Buckling et al., 2007; Friman et al., 2008; Porter & Rice, 2013; Torres-Barceló et al., 2013).

Competition can be divided to different subcategories. Three categories are based on the competition mechanism: exploitation, interference and apparent competition. The first two are relevant in my thesis. In exploitation competition competing entities use a common limiting resource, and the negative effects of competition are due to reductions in resource levels (Park, 1954; Brian, 1956). The intensity of exploitation competition is connected to the resource availability and demand. Interference competition arises from direct interactions between competitors where one competitor tries to prevent others from gaining access to the common resource (Brian, 1956). In contrast to the exploitation competition, the intensity of interference competition can be
high even if the resource is not limiting. Apparent competition focuses on systems of three or more species, most commonly predator-prey systems, where two prey species have antagonistic effect on each other when they share common predator (Holt, 1977). Alternating between two prey species allows predator population and also predation pressure to increase, which then causes adverse effects on both prey species. Similar system has also been termed competition for enemy free space (Jeffries & Lawton, 1984). The bacteria in my experiments potentially have overlap in resource preferences and the system does not include predators. Thus, the competition stems from resource availability and can be both exploitation and interference competition.

Though for the most part of my thesis I concentrate on competitive interactions, also other interactions are potentially common and important in microbial communities. Different categories of interaction types are presented in Table 1. In natural microbial communities mutualistic and commensalistic associations between species can be common (Paerl & Pinckney, 1996; Schink, 2002; Little et al., 2008, but see Foster & Bell, 2012). Unicellular organisms are limited in their metabolic capacity, and the biogeochemical cycle is most likely dependent on the interactions within microbial communities (Paerl & Pinckney, 1996). For example in aquatic environments aerobic and anaerobic microbes can benefit from each other: the ability to tolerate molecular oxygen \((O_2)\) may limit the survival and growth of prokaryotes whereas eukaryotic microalgae may be sensitive to anaerobic metabolism (Paerl & Pinckney, 1996). The synergistic interactions within microbial communities can make growth, reproduction, or biogeochemical cycling more efficient, and as a result enhance biomass production in aquatic environments (Paerl & Pinckney, 1996). Furthermore, as pointed out by Gross (2008) there are several examples on positive interactions between species, and he exemplifies how the positive interactions between competitors can enable high diversity within communities. On the other hand, Little et al. (2008) question the existence of purely commensal microbial interactions. They reckon that researchers have difficulties in determining the costs and benefits of microbial associations, and thus also fail to determine the interaction type correctly (Little et al., 2008).

Table 1. Interaction between species (or individuals within species) can be divided to several categories depending on the costs and benefits each species receives.

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Whether the interaction between organisms is beneficial or harmful is often difficult to measure, especially on natural or semi-natural conditions (Laska & Wootton, 1998). Determining the type and intensity of biotic interactions is further complicated if organisms change the conditions of their growth environment so that this habitat modification (Jones et al., 1994) or niche construction (Odling-Smeee et al., 2003) alters also interactions between species. The method I have used to measure the net-effect of biotic interactions (I) is based on the idea that environment mediates the interactions between bacteria, and the instantaneous growth rate is an indicator of the environment quality. In short: bacteria are allowed to grow in a given environment, then they are removed, and then the growth rate of the test bacteria in the consumed resource environment is used as an estimate of the quality of the environment.

One of the limitations of my approach is that it is possible to monitor the growth rate of only one species at a time. If the interaction would require the actual presence of other community members, one option would be to measure species growth individually and in a community. The difference between biomass produced in a community and the sum of biomass produced by species in monocultures indicates the net-effect of interspecific interactions (Freilich et al., 2011; Foster & Bell, 2012). A schematic illustration of this approach is presented in Figure 1. Based on this approach, Foster and Bell (2012) suggested that the interactions between species in natural bacterial communities are likely not cooperative, but competitive. Freilich et al. (2011) used metabolic modelling of species interactions which indicated that the interactions between coexisting bacteria are most often competitive or unidirectionally beneficial. Furthermore, the probability of competition between species increases monotonically when the degree of resource overlap increases (Freilich et al., 2011). In contrast, the relationship between the likelihood of cooperation and resource overlap is unimodal: An intermediate resource overlap maximizes the probability of cooperative interaction between bacterial species (Freilich et al., 2011). However, if the antagonistic interactions have a significant decreasing effect on the productivity of the population, measuring the net-effect can mask the positive interactions between species, which still can be relevant for species coexistence (Gross, 2008).

### 1.3 Evolution when environmental conditions vary

Environmental variation can have profound effects on all levels of biological hierarchy ranging from genes to communities and ecosystem functioning. Environmental variation is commonly categorized as either spatial or temporal, and further to fine- or coarse-grained (MacArthur & Levins, 1964) in relation to e.g. the generation time of organisms (Kassen, 2002). In this thesis I have concentrated on the effects of temporal environmental variation in resource availability on diversity within communities and on evolutionary changes over prolonged time periods. In laboratory conditions it is easier to manipulate environmental conditions to vary either temporally or spatially, but in natural systems both variation types may occur simultaneously. Still, the spatial component of environmental variation cannot be completely excluded, as for example the bacterial biofilms form spatial heterogeneity to microcosms and can enable diversity within populations (Rainey & Travisano, 1998) and communities (Hansen et al., 2007).
A generalization based on theory is that in constant environments a specialist strategy is more likely to evolve, and in temporally varying environments selection leads to the evolution of generalists (Futuyma & Moreno, 1988). Seasonal environmental fluctuations and stochastic environmental variation are predicted to select generalist-genotypes that are able to survive under alternating conditions (e.g. Levins, 1968; Roughgarden, 1972; Slatkin & Lande, 1976; Lynch & Gabriel, 1987). Theoretical work suggests that environmental fluctuations select individuals with an increased tolerance over the most frequently experienced condition, as their tolerance curve width evolves to match the width of environmental fluctuations (Levins, 1968; Lynch & Gabriel, 1987; Kassen, 2002). There is experimental support for the general prediction that specialists are more likely to evolve in constant, generalists in a variable environment. For example Escherichia coli bacteria evolved a specialist strategy in a constant temperature and generalist in

Figure 1. The net-effect of species interactions can be estimated by comparing how much biomass species produce in monocultures vs. when grown together. For example when species Red or Yellow grows alone in a given environment, each produces some species-specific amount of biomass. If species do not interact, the biomass produced together equals the sum of alone produced biomass of each species. Also the species fitness is the same in monocultures and in co-growth. If species compete for resources, they produce more biomass in monocultures than when grown together, and the co-growth decreases fitness. If species cooperate, they produce more biomass together than alone, and have also higher fitness. The net-effect comparison cannot separate between mutualism and commensalism, and it can mask situations where interaction affects one species negatively and the other species positively, or if the benefits of co-growth are smaller than antagonistic effects. Also other resource dependent measures, such as growth rate, could be used to estimate the interaction type between species. (Modified based on Fig. 1 in Freilich et al., 2011, and Fig 1. in Foster and Bell, 2012)
variable temperatures (Bennett et al., 1992). Similar results were obtained with *Chlamydomonas reinhardtii* in response to variation in light conditions (Kassen & Bell, 1998).

The frequency of temporal environmental variation in relation to the generation time of organisms is predicted to affect the evolution of the level of specialization. A generalist strategy may evolve if environmental changes occur within generation (Lynch & Gabriel, 1987), or depending on the underlying assumptions also if the frequency of environmental change is longer than the generation time (Levins, 1968). Also, when the frequency of environmental change is longer than the generation time, organism may experience the environment as constant, and the outcome of evolution is not a generalist strategy, but repeated specialization on the prevailing conditions (Kassen, 2002). For example bacteriophage øX174 specialized repeatedly to an alternating host (*E. coli*, *Salmonella enterica*) instead of evolving to be a generalist able to infect both host species (Crill et al., 2000). However, most experimental evidence point to the direction that generalist evolve when environmental conditions fluctuate (reviewed by Kassen, 2002).

In my experiments I exposed bacteria to environmental variation where resources become available in pulses. Resource pulses are by definition rare temporal events of increased resource availability that have short duration and large magnitude (Yang et al., 2008). Resource pulses are common both in terrestrial and aquatic environments, and may lead to conditions where resource abundance is followed by prolonged periods of resource scarcity (Yang et al., 2008). Resource pulses can have substantial effects on population growth rates, productivity, community composition (Grover, 1997; Chesson & Huntly, 1997), and species interactions (Chesson et al., 2004). For example seasonal algal blooms are caused by sudden increase in nutrient availability (Anderson et al., 2002). The serial transfer procedure in evolutionary ecology experiments using microbial model systems mimics the conditions of seasonal environment where resource abundance is followed by a period of resource scarcity (Rainey et al., 2000). The evolutionary response to seasonal, rare resource pulses can be both an increase in survival through the interpulse period or an increased ability to utilize the resource abundance right after the pulse (Yang et al., 2008).

Organisms themselves cause another form of environmental variation that is relevant for my thesis. Bacteria secrete several types of extracellular molecules including metabolic by-products, enzymes, and molecules targeted for e.g. cell-to-cell communication or growth inhibition. The evolution of cross-feeding interaction a.k.a. the ability to utilize metabolic by-products of other organisms is repeatedly reported in bacterial systems both within species in *E. coli* monocultures (Rosenzweig et al., 1994; Vasi et al., 1994; Treves et al., 1998; Wintermute & Silver, 2010) and between species within bacterial communities (Lawrence et al., 2012). Bacteria can use extracellular molecules, especially growth inhibitors, in interference competition within or between species (De Freitas & Fredrickson, 1978; Campos & Wahl, 2009). Bacteria secrete various small molecules with low mass for different purposes. When the cell density increases also the concentration of extracellular molecules increases, and eventually reaches a threshold after which cells are able to detect the signal. This quorum sensing enables bacteria to detect the population density in their proximity (Miller & Bassler,
2001; Camilli & Bassler, 2006; Keller & Surette, 2006). How much extracellular molecules are secreted varies depending on e.g. resource availability, community composition, population size, and growth mode (individual cells vs. biofilms). By secreting extracellular molecules, bacteria can actively modify their environmental conditions. This habitat modification can affect species interactions and evolutionary dynamics.

1.4 Competition and coexistence in variable environment

In the previous chapter I discussed how environmental variation could affect the interactions between species and cause evolutionary changes in organisms. Another theme in my thesis is the effects of environmental variation on ecological interactions within populations and communities. Environmental variation is predicted to fundamentally affect competitive interactions and community composition (Hutchinson, 1961; Levins, 1979; Chesson & Huntly, 1997; Ranta et al., 2006). Much of the discussion on the effects of environmental variation has focused on whether and how variation enables species coexistence and maintains species diversity (Hutchinson, 1961; Stewart & Levin, 1973; Armstrong & McGehee, 1980; Chesson & Huntly, 1997; Chesson, 2000b).

From the competitive exclusion principle follows that resource availability sets upper limit to species diversity. Still, even relatively simple environments commonly harbour diverse and complex communities. Hutchinson (1953, 1961) was one of the first to note on the controversy between observed species diversity in nature and what could be predicted based on resource competition theory in plankton communities. He suggested that environmental variation in relation to the generation time of organisms influences the community dynamics, and may prevent competitive exclusion. Especially, when the time required for competitive exclusion is equal to the frequency of environmental change, the equilibrium conditions where competitive exclusion could occur are never met and thus, environmental variation enables diversity within plankton communities.

Following Hutchinson’s work, Connell formulated the intermediate disturbance hypothesis, which predicts that intermediate variation produces most diverse communities (Connell, 1978). The diversity is high, because neither constant environment specialists nor rapid colonizers are able to dominate in environment with intermediate environmental disturbances. However, this has been relatively difficult to demonstrate experimentally (but see e.g. Kassen et al., 2000, and review in Grover, 1997). Just recently Fox (2013) argued that the underlying logic in the intermediate disturbance hypothesis is flawed, and the mechanisms that cause diversity peak at intermediate disturbance levels are different than what they are claimed to be. Whether or not Fox’s suggestion for abandoning intermediate disturbance hypothesis will be generally accepted, the prevailing view is that environmental heterogeneity potentially enables the coexistence of species.

Environmental variation enables species coexistence also if species differ in niche preferences, for example one species has growth advantage when resources are scarce and another when resources are abundant (Tilman, 1982; Grover, 1997). The same applies for diversity within species: the maintenance of genetic variation is possible, if environmental conditions vary and individuals differ in their response to these
conditions and in niche preferences (Rainey et al., 2000). Experiments on microbial models have been used to test what maintains diversity within species and within communities. Rainey et al. identify ecological factors that maintain or enable polymorphism within microbial populations: an ecological opportunity or a vacant niche, specialization and concomitant trade-offs in fitness related traits, and finally a negative frequency-dependent selection, where rare genotypes are favoured (Rainey et al., 2000 and references therein). These same mechanisms work also on a community level and can enable species diversity (Begon et al., 1996; Grover, 1997).

Diversity maintaining mechanisms are often classified as variation-independent or variation-dependent (reviewed by Chesson, 2000b). I mentioned niche differentiation above as a mechanism that promotes diversity when environmental conditions vary. However, niche differentiation, or its more limited case resource partitioning, is often given as an example of variation-independent mechanism that maintains diversity (Grover, 1997; Chesson, 2000b). Both are true: environment has to be heterogeneous in order to have various niches, but the environmental conditions need not to change spatially or temporally. However, spatial or temporal variation can create new niches, and thus also promote diversity through niche differentiation. In comparison, the variation-dependent mechanisms would not work in heterogeneous but stable environment. The variation-dependent mechanisms are (1) the storage effect, where competing species experience spatial or temporal differences in growth rates (Chesson, 1994), and (2) relative non-linearity, where species-specific responses to resource availability differ (functional response) and the availability of resources fluctuates (Armstrong & McGehee, 1980; Chesson, 2000b).

The intensity of competitive interactions may vary when environmental conditions vary. Variation in resource availability may change the competitive ranking of species, which is an important component of variation-dependent species co-existence (Chesson & Huntly, 1997). For example species that are superior competitors during resource abundance may be inferior when resources are low. If then resource availability varies, and species do not outcompete each other before environmental change occurs, the alternating competitive ranking of species enables coexistence. Whether species compete only with individuals of the same species or also with other species is also important for species coexistence: as long as the within species competition is stronger than between species, species are likely to coexist (Chesson & Huntly, 1997; Chesson, 2000b).

The underlying mechanisms that cause and maintain diversity within populations and communities are likely to depend on organisms and specific environmental conditions. Often it is challenging to answer even the basic (but crucial) questions, such as: What are the important environmental components? What actually is the limiting resource? How many resources are available in environment at any given time? As organisms evolve, their resource preferences may change, the biotic interactions can change, and these changes may in turn increase or decrease diversity within populations or communities.

1.5 Evolution on ecological timescale

As I have presented in the previous sections, the concept of competition is deeply rooted in modern ecological thinking and is
considered as one of the major forces causing evolutionary changes. Still, even though the importance of ecological interactions and environmental conditions on evolution has been acknowledged for a long time, the possibility that evolution could affect the current ecological dynamics has not been fully implemented in ecological research.

Experimental evidence shows that evolutionary changes can be rapid and relevant in ecological dynamics. According to Hairston et al. (2005) character evolution can be considered rapid when evolutionary changes affect also ecological dynamics. Hairston and his colleagues list examples on systems with rapid evolutionary dynamics. First is a microbial predator-prey community, where rapid evolution in prey changes the predator-prey dynamics, and evolution affects population dynamics (Yoshida et al., 2003). Second example is from a lake ecosystem, where changes in environmental conditions cause increase in the amount of primary producers (cyanobacteria), which is followed by a rapid evolutionary response in consumer species (Daphnia sp.). Thus, evolution alters community interactions (Hairston et al., 1999). The third example comes from another lake ecosystems, where evolutionary changes in Daphnia growth rate and body phosphorus are predicted to affect the nutrient limitation of phytoplankton production, biomass, and species composition, and thus potentially have a cascade effect on ecosystem processes (Elser et al., 2000). In each of these cases the observations are more accurately interpreted when the possibility of evolutionary change is included to the explanatory factors.

Hutchinson (1965) phrased the well-known saying how ecosystem is the theatre where evolutionary play takes place. But how do the environmental conditions affect the play and what if the players change the props? Advance in experimental evolution has made it possible to set up the theatre and choose actors. Even though strictly speaking evolution is not directed, and there is place for happenstance in the evolutionary play, some principles of evolutionary process are becoming clearer due to experimental evolution.

The basic protocol in evolutionary ecology experiments is that a microbial population or a community consisting of several species is grown in chosen environmental conditions, samples are taken at several time steps, and the characteristics and the performance of evolved strains are tested and compared to their ancestors. This approach has several advantages: environmental conditions are controllable, large populations can be used in experiments, replication is relatively easy, long time-series lasting hundreds or thousands of generations can be acquired in a relatively short time, and for many species advanced molecular tools are available to tinker with the genetics. Microbial model systems are “The Beagle in a bottle” as figuratively named by Buckling et al. (2009) in their review on the utility of microbes in evolutionary ecology research.

The early evolution experiments with microbes demonstrated periodic selective sweeps consistent with the competitive exclusion principle: in simple environments, containing only a single resource, the dominant genotype in a population is repeatedly replaced with a fitter descendant (e.g. Novick & Szilard, 1950; Atwood et al., 1951). When the environmental complexity is increased, the common outcome of evolution is adaptive radiation. Sympatric
adaptive radiation due to resource competition is predicted to result in a community consisting of ecologically differentiated genotypes adapted to different resource components (MacLean et al., 2005). A cumulative body of experimental evidence shows that competition for resources causes adaptive radiation and often also increased diversity (e.g. Helling et al., 1987; Schluter, 1994; Rainey & Travisano, 1998; Friesen et al., 2004; MacLean et al., 2005; Maharjan et al., 2006; Massin & Gonzalez, 2006, reviews by Lenski & Travisano, 1994; Travisano & Rainey, 2000; Rainey et al., 2000).

In heterogeneous environments the common outcome of evolution is a range of specialists able to coexist (Rainey & Travisano, 1998; Barrett et al., 2005). In simple environments bacteria evolve to utilize different strategies that enable coexistence: resource partitioning, cross-feeding interactions (Helling et al., 1987; Treves et al., 1998; Rozen & Lenski, 2000), or cannibalism (Rozen et al., 2009). The cross-feeding interaction has been reported to evolve in nutrient-limited or starvation conditions in E. coli (Helling et al., 1987; Turner et al., 1996; Treves et al., 1998; Vasi & Lenski, 1999; Finkel & Kolter, 1999). The evolved strains can be superior competitors in nutritionally poor conditions, and inferior to their ancestor when resources are abundant (Vasi & Lenski, 1999), but not necessarily. Escherichia coli strains evolved in nutrient poor conditions can survive in mixed populations with their ancestor, and when resource levels are diminished the evolved strains take over the population (Zambrano et al., 1993). Monitoring the evolutionary changes of E. coli in nutrient limited conditions has also revealed that the stationary phase in population growth of bacteria can be evolutionarily highly dynamic with new genotypes emerging repeatedly (Finkel & Kolter, 1999).

The examples I have presented on the effects of environmental conditions on evolution demonstrate how interconnected the ecological and evolutionary dynamics can be. Both biotic and abiotic environmental factors can shape the evolution within populations and communities. The biotic environmental factor relevant in my thesis is interactions between organisms, e.g. intra- and interspecific competition. Other biotic factors are predation, parasitism, or demographic changes within population (Levins, 1968; Meyers & Bull, 2002). For the purpose of this thesis I have defined non-renewable resources as part of the abiotic environment. By definition abiotic environment contains for example physical and climatic factors (Levins, 1968; Meyers & Bull, 2002). Most often abiotic and biotic environmental factors are interconnected and dynamic. Abiotic factors affect interactions between organisms, and the activity of organisms changes abiotic environmental conditions (Jones et al., 1994; Odling-Smee et al., 2003; Hastings et al., 2007). Furthermore, both biotic and abiotic environmental factors can vary in time and/or space (Levins, 1968; Meyers & Bull, 2002). In the eco-evolutionary processes environmental factors are working together with selection within communities. For example, species interactions could impede or facilitate the evolution on abiotic conditions when species exist together (Chesson, 2000b; Lankau, 2011; Lawrence et al., 2012). So far few experimental evidence point to the direction that abiotic selection can be both diminished or strengthened in the presence of competitor species (Collins, 2011; Lawrence et al., 2012).

2 Aims of the thesis

In this thesis I explore the combined effects of environmental factors (resource fluctuations) and biotic interactions
(competition) on environment mediated interactions (I), on the evolution of life-history traits (II, III), and on species diversity (IV) in bacterial populations and communities.

In the first chapter I explore how the activity of bacteria alters the growth conditions in a system where bacteria grow on non-renewable detritus resource. Biotic and abiotic environment are interconnected: bacteria consume resources and also modify their environment by secreting extracellular enzymes and other molecules. In turn, these environmental changes affect organisms. I test what happens within a weeklong growth period in batch cultures. Besides resource depletion, does the quality of the environment change and how is it reflected to the growth dynamics of individual bacterial species (I)?

The aim of the second chapter is twofold: First, I test if the long-term coexistence over hundreds of generations is possible for two species with contrasting growth strategies in an environment where new resources become available in rare pulses. Second, I ask do the resource fluctuations or the biotic interactions or their combination exert evolutionary changes in life-history parameters? This question of combined effects of biotic and abiotic environment on the evolution is addressed also in the third chapter. The evolutionary response is monitored on bacterial populations (II) and individual bacterial clones (III) to see if the level of scrutiny affects the observed patterns and detection of possible trade-offs between measured characteristics.

In the final chapter of my thesis, I explore how the frequency of environmental variation affects diversity within bacterial communities (IV). Besides testing the intermediate disturbance hypothesis, I monitor how the environmental resource fluctuations are reflected to the growth rate of the whole bacterial community (IV).

3 Materials and methods

3.1 Study organisms

In Chapters I-III the study organisms are two bacterial species Serratia marcescens (from American Type Culture Collection strain ATCC 13880) and Novosphingobium capsuleatum (ATCC 14666). These bacteria are heterotrophic, gram-negative, rod shaped, and do not form spores. Both prefer aerobic growth conditions, but S. marcescens is able to survive and grow also in anaerobic conditions. The main reasons for using this species pair is that both grow readily on hay extract medium and differ in growth responses: on average N. capsuleatum grows faster than S. marcescens on low concentration whereas S. marcescens grows faster on intermediate and high concentrations. Differences in functional responses should enable the long-term coexistence of this species pair. In the evolution experiments species are likely to experience different selection due to existing differences in ancestral strains. The practical reason for choosing these species is that they can be separated based on colony morphology: S. marcescens forms white, pink or red colonies, whereas N. capsuleatum forms yellow colonies when grown on Nutrient Broth agar plates.

Serratia marcescens belongs to the family of Enterobacteriaceae (Grimont & Grimont, 1978; Krieg & Holt, 1984). It is a cosmopolite species and opportunistic pathogen able to infect wide variety of hosts including plants, insects, fishes and mammals (Tan, 2002; Grimont & Grimont, 2006). It is a nosocomial pathogen capable of causing e.g. pneumonia,
intravenous catheter-associated infections, and endocarditis in humans (Van Houdt et al., 2007). It can secrete exoenzymes that break down the resource particles and enhance nutrient uptake (Benedik & Strych, 1998). As these exoenzymes can be beneficial for co-existing species, there is potential for commensalism or mutualism in resource use in communities where *S. marcescens* is included.

*Novosphingobium capsulatum* belongs to the family of Sphingomonadaceae (Leifson, 1962; Yabuuchi et al., 1990; Takeuchi et al., 2001). It has been found from for example soils, rose tree roots, clinical specimens, and stocked distilled water (Takeuchi et al., 2001). The type strain of *N. capsulatum* is able to assimilate *e.g.* D-cellulbiose, fumarate, and gluconate [detailed list in (Tiirila et al., 2005)]. Though *N. capsulatum* is generally considered non-pathogenic, it has been found to cause severe symptoms and mortality in white grub (*Brahmina coriacea*) larvae and it is a potential biological control for pest insects (Sharma et al., 2012).

Six more bacterial species were used to test the effect of temporal resource variation to species diversity within communities (IV). All chosen species grow readily on the Nutrient Broth agar and hay extract medium. The altogether eight heterotrophic bacterial species were randomly divided to two four-species communities using the criteria that species were distinguishable based on colony colour and morphology on agar plates. One community included *Novosphingobium capsulatum*, *Bacillus megaterium* (ATCC 14581), *Budowica aquatica* (ATCC 51341), and *Pseudomonas putida* (ATCC 12633), and the other *Serratia marcescens*, *Bacillus cereus* (ATCC 14579), *Comamonas aquatica* (ATCC 11330), and *Cupriavidus necator* (ATCC 17698).

Both *Bacillus* species (family Bacillaceae) are gram-positive, rod-shaped, able to form spores, and prefer aerobic growth conditions. *Bacillus cereus* is considered an opportunistic pathogen, and some *B. cereus* strains cause *e.g.* gastrointestinal infections (Kotiranta et al., 2000). *Bacillus megaterium* is non-pathogenic and it is found in various habitats *e.g.* seawater, sediment, and dried food. *Bacillus megaterium* is commonly used in biotechnology and industry [for a review see (Vary et al., 2007)]. *Budowica aquatica* is a gram-negative, rod shaped, facultative anaerobe, and belongs to the family of Enterobacteriaceae (Aldová et al., 1983). It can be found in *e.g.* surface and sewage waters (Schubert & Groeger-Söhn, 1998). *Comamonas aquatica* is a gram-negative, rod-shaped aerobe, and belongs to the family Comamonadaceae (Wauters, 2003). *Pseudomonas putida* is a gram-negative, rod-shaped, flagellated aerobe, and belongs to family Pseudomonadaceae. It is very common in most soil and water habitats, able to live even in heavy metal or organic carbon polluted soils and sediments (Wu et al., 2011). Due to versatile metabolic abilities and non-pathogenicity, *P. putida* is potentially useful in bioremediation. *Cupriavidus necator* is a gram-negative, cocccoid, rod-shaped bacterium, and belongs to β-proteobacteria. ATCC 17698 is originally isolated from garden pond sludge. *C. necator* strain ATCC 43291 found in soil is a non-obligate predator of other gram-positive and -negative bacteria (Makkar & Casida, 1987), but to my knowledge the *C. necator* ATCC 17698 strain is not predatory.

All species differ in their functional response to the hay extract medium (Figure 2). The estimated maximum growth rate (*r*max) and the half saturation constant (*Ks*) of each species (Table 1 in IV) are the empirical coefficients of the Monod function, a saturating function that describes the growth
of microorganisms on limiting substrate (Monod, 1949; Grover, 2000). The parameters were calculated based on growth measurements of each species at concentrations ranging from 0.1 to 1gL⁻¹ of hay extract (IV).

3.2 Environmental conditions

In all experiments bacteria experienced temporal variation in resource availability. Bacteria were grown in batch cultures (I), in microcosms with rare, pulsed resource renewal (II, III) or with fluctuating periodic resource flow (IV). In all of the long-term cultures the resource medium was phosphate buffered cereal leaf extract (1g of hay extract in 1l of water, equalling dry weight 2.15mgl⁻¹). The microcosms were polycarbonate cell culture Erlenmeyer flasks with membrane-filtered caps (250ml, Corning) (Figure 3). Bacteria were also grown on 10gl⁻¹ Nutrient Broth 10% agar plates e.g. prior to inoculation to hay extract medium, or for population size estimates based on serial dilution.

In the 13 weeks long experiment with two bacterial species (II, III) resources were renewed weekly, which caused a pulse-like resource inflow. Two pulse amplitudes were used in the renewal: either 99.9% or 70% of the total volume (150ml) was replaced with a fresh growth medium. Bacteria grew either alone or together, so I could compare the effect of intra- vs. interspecific competition in both environmental regimes. All treatments (6) had three replicates (total 18 microcosms). Bacteria samples from each weekly resource renewal were stored for biomass measurements and for analysis of population size and evolutionary change in fitness related traits. In the weeklong experiment (I) the growth conditions were similar as in the long-term experiment.
During the week sterile-filtered samples of the growth medium were taken at several time-steps.

In the flow through microcosms programmable peristaltic pumps regulated the resource flow individually for each of the 30 microcosms (Figure 4). Bacterial communities experienced different levels of resource fluctuations in microcosms for 28 days. Resource flow was constant, intermediate, or slow. The resource flow was controlled hourly. In the constant environment resource flow was 1.4mlh⁻¹. In the environment with intermediate fluctuations 27.5h period of resource flow of 2.8mlh⁻¹ was followed by equal time of no resource flow. The slow fluctuation treatment had similar resource flow rate as intermediate fluctuations only the fluctuations and the period without resource inflow both lasted 84h. The five replicate time-series within each treatment were started at a random phase. First new resource was pumped in the microcosms and immediately thereafter an equal volume was pumped out, so that the total volume of each microcosm remained constant throughout the experiment. The resource outflow causes mortality in populations and resource inflow increases growth if resource availability is limiting the growth. Living bacterial samples from the microcosms communities were taken every 3rd day.

3.3 Measured variables

To detect the evolutionary changes that potentially affect the overall fitness of bacteria I tested the performance of ancestral and evolved bacteria from the long-term experiment in separate fitness assays (II, III). In the fitness assays the population size of bacteria was monitored at several time steps within a week. From the obtained time-series I calculated fitness-correlated traits.

Figure 3. Microcosms where S. marcescens and N. capsulatum were grown (I-III). (Picture © J. Korhonen)
Growth rate (II, III) and timelag before reaching the maximal growth rate (II) are used as indicators of fitness during resource abundance. End population size (II), yield (III), and mortality (II, III) describe the response to low resource conditions. As an additional fitness estimate I measured biofilm production (III), which in S. marcescens is an adaptation against stressors, such as antibiotics or predators (Matz & Kjelleberg, 2005; Friman et al., 2008).

In the multispecies experiment the population size of each species and total biomass of the bacterial community were monitored every 3rd day throughout 28d long the experiment (IV). Shannon diversity index was calculated based on the population sizes (IV). Population size was measured based on the counts of colony forming units (CFU) from serially diluted bacterial samples grown on agar plates (II, IV). Population biomass was measured as the turbidity of liquid samples, where optical densities (OD) were measured on regular short time-intervals using a Bioscreen C spectrophotometer (Growth Curves AB Ltd, Finland) (I-IV). The CFU counts can be used as an estimate on the number of living bacterial cells in a given sample, based on an approximation that each colony originates from an individual cell. The total biomass measured as OD includes also dead biomass. However, especially in the short-term measurements, the accumulation of dead biomass was most likely insignificant, and thus did not influence the growth rate estimates.

When time-series were based on CFU-counts (II), the maximum growth rate was obtained by fitting a linear regression to the logarithm of the population size data of each replicate population during the 0–50h of growth. The time lag was the period from the start of the measurement to the mid-point of the time-window used in the fitting of the regression line. Maximum population size was the highest population size within the weeklong measurement. The mortality was calculated.
as the difference between the maximum population size and the population size at the end of the cycle divided by the maximum population size.

In cases where time-series were based on OD-measurements (I, III, IV), the parameter estimates were done as follows. Growth rates were calculated as the slope of the linear regression of natural logarithms of the background-corrected optical densities versus time. The maximum instantaneous growth rate was the point where the slope of the linear regression reached its highest value. Yield equals maximum biomass. As mortality estimate I used biomass reduction after the maximum biomass was reached (III). Total mortality was calculated as maximum biomass minus end biomass, and proportional mortality as total mortality divided by the maximum biomass.

The biofilm production of bacterial clones during a weeklong fitness assay (III) was measured using crystal violet stain protocol (O’Toole & Kolter, 1998; Friman et al., 2008). At the end of the week, the biofilm was stained with crystal violet, and dissolved in 96% ethanol. The absorbance of the solution was measured with a spectrophotometer and used as an estimate of the amount of biofilm.

3.4 Bioassay for measuring changes in resource availability

To track how qualitative changes in environment affect the growth rate of bacteria, the consumed resource medium was filter-sterilized (0.22µm filters Millex MCE 33mm 50S), and then bacteria were re-introduced to the filtrate and short-term growth rate calculated. Calculation was based on OD measurements in similar fashion as in Chapters III and IV. Growth rate in filtrate is here used as an estimate of the biologically meaningful resource availability. I used the described bioassay to track temporal changes in resource quality within a week in batch cultures (I) and to track resource availability in bacterial communities during 28d long experiment (IV). Similar method is used in applied microbiology in research-related applications (Arrieta-Escobar & Belin, 1982; Kato et al., 2005; Andersson et al., 2008), in microbial ecology to test the survival of microbes on different substrates (Jannasch, 1968) or to detect cross-feeding interactions (Helling et al., 1987), and in evolutionary ecology to test the role of resource modification in evolution of within species diversity (Luckinbill, 1978; Spencer et al., 2007; Rozen et al., 2009) or to test how resource environment mediates species interactions (Gause et al., 1934; Lawrence et al., 2012).

3.5 Statistical tools

The General Linear Mixed Model (GLMM) procedure was used to model how time spent in the long-term experiment and treatments affected measured traits in bacterial populations (II) or clones (III), when bacteria were exposed to resource pulses. Also the change in clonal variation (SD) was modelled using GLMM (III). GLMM was constructed separately for each species and each measured trait. Student’s two tailed t-test with corrections for unequal sample sizes (formula 7.5, p. 191 in [Ranta et al., 2002]) and Bonferroni corrected p-values was used for the pair-wise comparison of evolved bacterial clones from different treatments to their ancestor (III).

A repeated measures ANOVA (RMANOVA) was used to model the effect of consumer and test species identity and consumption time on the growth rate of the test species in filtered, conditioned growth medium (I).
model included growth rate as a response variable, sampling time as a repeated within subject factor, and consumer species and test species identity and their two-way interaction as between subject factors. The measurements in unconsumed medium were excluded from the RMANOVA. Univariate ANOVA was used for comparing a) growth in filtrates from different sampling points and treatments to the growth in unconsumed medium, b) the effect of one vs. two-consumer species treatment on growth in filtrate (I). The effect of sample handling on unconsumed medium (the effect filtering and freezing), and comparison of growth rates between test species in unconsumed medium were analyzed using 2-tailed t-test (I). Two-way ANOVA was used to test the effect of resource fluctuation treatment on biomass coefficient of variation, and RMANOVA to test the effect of time and treatments on population size, biomass, species number, Shannon diversity index (H’), species evenness, and growth rates from the resource bioassays (IV). When the sphericity assumption of RMANOVA was violated, Greenhouse–Geisser corrected F values were used. Population densities and biomasses were log transformed for statistical analyses.

All statistical analyses were performed using SPSS v.12 (IV) or v16 (I-III) (SPSS Inc., Chicago IL).

4 Results and discussion

4.1 Environment mediates species interactions

I found, that besides consuming available resources, bacteria modify their resource environment (I). This habitat modification activity is reflected to bacterial growth rates and may enable commensalism or mutualism in resource use. Though in most times the growth rate in consumed medium was lower than in fresh medium, I measured also higher growth rates of both bacterial species in consumed medium. These high growth rates in consumed resource could result from the secretion of extracellular enzymes, or the accumulation of nutritionally valuable secondary metabolites.

In the simplest case the bacterial growth dynamics in a batch culture are expected to follow a resource consumer model with saturating Monod-type growth responses under resource-pulse conditions (Grover, 1997). In this scenario the population should first follow logistic-like growth and then an exponential decline due to resource depletion. According to the bacterial growth rates in consumed resource medium, the resource availability changed also qualitatively, not just quantitatively. Quantitative change would simply mean decrease in resource availability. I argue that the qualitative change enables other interaction types than resource competition.

My results show, that during the first ten hours of growth in batch cultures both species N. capsulatum and S. marcescens modify the resource so that it facilitates the growth of S. marcescens (Fig. 4A in I). The hay extract medium is a complex resource including recalcitrant particles such as lignin and cellulose. Extracellular enzymes can expedite resource uptake by breaking up these substrates. Of the two species, at least S. marcescens can produce exoenzymes (Benedik & Strych, 1998). Enzymes are actively produced by bacterial cell to enhance its own nutrient uptake. However, bacteria have limited means in restricting the enzymes from being exploited by other individuals of the same or other species. Enzyme production is one aspect of microbial life, which enables a wide array of interactions ranging from interference and
exploitation competition to co-operation and cheating (West et al., 2006).

After the initial growth promoting change in the resource quality I detected another peak at growth rates. *Serratia marcescens* modified the resource medium so that *N. capsulatum* was able to grow fast in resource consumed for 30–50h (Fig. 4B in I). This again may result from the enzyme production of *S. marcescens*, or alternatively *N. capsulatum* is able to use metabolic waste products of the other species. This type of cross-feeding interaction has been reported in multispecies bacterial community (Lawrence et al., 2012), and it may promote diversity within bacterial populations (Treves et al., 1998; Finkel, 2006; Rozen et al., 2009). Even though *N. capsulatum* had smaller population size than *S. marcescens* when grown together (Fig. 2 in I), the dynamic interaction in resource use may have contributed to the long-term coexistence of these species (II).

Altogether my results on the bacterial growth dynamics in consumed resource medium show that the bacteria change their environment, and these changes could switch the interaction between species from competition to mutualism or commensalism. However, the growth rates in consumed medium do not reflect the observed population dynamics when species actually grow together. When I compare the population time series of both species from the long-term experiment where bacteria grew alone or together, it is evident that both *S. marcescens* and *N. capsulatum* produce smaller population sizes in co-growth than in monocultures (Figure 5). Following the logic presented in Figure 1, this indicates that competition dominates the interaction between these species, especially after the fast growth phase during the interpulse period. The interaction does not change through evolutionary time, even though based on the growth rates in consumed resource medium *N. capsulatum* could benefit from the presence of *S. marcescens*. However, the net effect can mask the positive interaction between species. If two species compete but also have positive interaction so that the competitively dominant species causes increase in growth rate or alternatively decrease in mortality of the inferior species, the positive effects may enable species coexistence (Gross, 2008). Between my study species, this is a potential scenario: based on the population sizes *S. marcescens* was the dominant competitor, and based on the growth rates in consumed medium *N. capsulatum* benefits from *S. marcescens*. Thus, although the net effect of species interactions was decrease in population size, which indicates that competition dominates the interaction between *S. marcescens* and *N. capsulatum*, species can still have also positive interactions.

### 4.2 Competition and resource availability shape evolutionary dynamics

My results on evolutionary dynamics of two bacterial species show, that the evolutionary response to rare resource fluctuations was species-specific, relatively fast, and was affected by both the biotic interactions, and the magnitude of the resource pulses (II, III). Most changes in fitness related traits were evident already after one resource pulse cycle. The evolutionary changes were dominated by the survival through the interpulse period when resources are scarce.

*Increased survival*

The general response of bacteria to rare resource pulses was decreased mortality during low resource conditions. Mortality decreased after one pulse-interpulse cycle,
and the improvement was substantial especially when measured at population level (Fig. 2 in II), but also in S. marcescens clones (Fig. 3 in III). These results partially support my hypothesis that the interpulse periods select for increased survival (Table 2).

My hypothesis was that if the resource pulse amplitude is small, selection favours economical resource utilization strategy and increased survival, whereas in large resource pulse environment the ability to grow quickly during the period of resource abundance could provide a competitive edge. The evolution of increased survival has been reported before in bacteria that have been exposed to temporal resource fluctuations (Vasi et al., 1994; Novak et al., 2006; Finkel, 2006; Rozen et al., 2009). These and my results highlight the significance of survival during the resource deprivation phase in environments where new resources become available rarely.

Figure 5. Population sizes of the ancestor (1\textsuperscript{st} week) and the evolved (2\textsuperscript{nd} and 13\textsuperscript{th} week) Serratia marcescens (upper panel) and Novosphingobium capsulatum (lower panel). Both species grow to larger population sizes in monocultures (alone) than when grown together (co-growth), and thus competition dominates species interaction (see Figure 1). (Data are from Fig. 1 in II, note the difference in scale on y-axis.)
Trade-offs
I measured also other characteristics than survival that are beneficial in pulsed resource environment (growth rate immediately after the pulse) or can be used as fitness estimates (end population size, yield, and biofilm formation). Bacteria did not have decreased performance in any of these characteristics, except *S. marcescens* clones had slower growth rate than their ancestor. All of the measured traits demand resources and in theory there could be trade-offs between e.g. fast growth rate and survival (Roff & Fairbairn, 2007).

Based on my findings, the evolutionary increase in survival does not necessarily cause a decline in growth performance. I found no indication on a trade-off between measured traits on population level (II), whereas fitness measurements of bacterial clones revealed a potential trade-off between survival and growth rate in *S. marcescens* (III).

Effects of resource availability and competition
My results show that in general the mortality of *S. marcescens* was higher and end population sizes of both species smaller when species experienced interspecific competition. Thus, competition obviously took its toll.

I also found evidence on the combined effect of resource fluctuations and competition: the growth rate of *N. capsulatum* clones increased when they had co-evolved with *S. marcescens*, especially if they had experienced also large resource fluctuations (Fig. 1B in III). However, when the same evolved bacterial strains were grown in batch cultures with *S. marcescens*, the growth rate of evolved *N. capsulatum* did not differ from the ancestor (Fig. 5B in II). The interspecific competition in environmental conditions where a large amount of resource becomes periodically available selects for fast growth during resource abundance, but the presence of the competitor species prevents the growth at maximal speed.

The only indication on the effect of within species competition is the increased biofilm production of *S. marcescens* clones in monocultures (Fig. 4A in III). Biofilms create a new spatial niche, and may be a way to avoid competition within the free water (Davey & O’Toole, 2000). However, selection caused by the serial transfer method should have favoured free-swimming bacterial cells. Still, evolved bacterial clones produced as much or more biofilm as their ancestors (Fig. 4 in III). Evidently, the transfer method did not exclude biofilm-forming cells from the population.

Based on the difference in the growth strategies of these species the opportunist *S. marcescens* should be competitively superior during resource abundance whereas the gleaner *N. capsulatum* during resource scarcity. Thus, it is not surprising that competition affected different traits in these species. Depending on the direction of selection caused by competition and environmental factors, competition could potentially speed up or slow down evolution (Osmond et al., 2013). As a conclusion, interspecific competition may have both hindered adaptation to low resource conditions (mortality of *S. marcescens*) and hasten response to resource abundance (growth rate of *N. capsulatum*).

4.3 Resource fluctuations maintain diversity
Two bacterial species with contrasting growth strategies were able to coexist in the pulsed resource environment (II), but of the
two multispecies communities that experienced various degrees of environmental fluctuations, all four species coexisted only in one community (IV). Several mechanisms can enable the coexistence of species in temporarily variable environment. For example in temporally fluctuating environment, the coexistence of species with overlap in resource preferences is possible, if species have different functional response to resource availability (relative non-linearity, Armstrong & McGehee, 1980; Chesson, 2000b), or due to the storage effect, where species are able to enter a refuge (dormant stage, seed banks, long-lived adults) during adverse environmental conditions and grow again when conditions are favourable (Chesson, 1994). From these mechanisms, the relative non-linearity was more important in the two-species system, whereas both mechanisms contributed to the coexistence in four species community. In addition, also variation independent mechanisms affected the species coexistence.

Coexistence of two species
In the previous sections I reasoned that the two bacterial species in my experiments compete with each other, but can also benefit from each other. When S. marcescens and N. capsulatum were reared together in an environment with rare resource pulses species coexisted throughout the 13 weeks long experiment (II). The growth environment (hay extract medium) was a mixture of several chemical compounds with varying nutritional value, and thus, enabled species to specialize on different resource compounds. Serratia marcescens and N. capsulatum differ in their functional response to hay extract medium, but they also have overlap in resource use.

Based on the functional responses to hay extract medium, I expected that S. marcescens would have a competitive advantage during resource abundance immediately after the resource pulse, while at the late phase of the interpulse the competitive ranking would change in favour of N. capsulatum. Contrary to what I expected, S. marcescens was competitively superior also during the interpulse. The competitive superiority of S. marcescens most likely results from an evolutionary response to both competition and resource fluctuations.

Evolutionary increase in survival during resource scarcity with no apparent trade-off in growth ability kept S. marcescens as the dominant species through the resource pulse and interpulse periods (II). However, S. marcescens was not able to competitively exclude N. capsulatum. Possibly the combination of the evolution of faster growth of N. capsulatum (III) and beneficial cross-species effects in the consumed resource (I) together prevented the exclusion of N. capsulatum.

My interpretation is that the observed long-term coexistence of S. marcescens and N. capsulatum cannot be explained only by the variation-dependent mechanisms such as relative non-linearity. Other variation-independent mechanisms, especially differentiation in resource preferences enabled the coexistence. In addition, the habitat modification (I) and evolutionary changes (II, III) contributed to the species coexistence.

Coexistence of four species
In Chapter IV we tested whether the diversity in bacterial communities would be highest when they experience intermediate resource fluctuations, as predicted by the intermediate disturbance hypothesis (Connell, 1978). Some support for the diversity promoting effect of environmental
variation was found: communities that experienced slow environmental fluctuations had higher Shannon diversity index ($H'$) compared to constant or rapid environmental fluctuations. The Shannon diversity index depends not only on the number of coexisting species, but also the evenness of the species distribution: the more species and the more evenly distributed they are, the higher the $H'$ (Shannon & Weaver, 1964). The population sizes of individual species (Fig. 2 in IV) or total densities in communities (Fig. 3 in IV) did not differ between different resource fluctuation treatments. Thus, it seems that at least in this specific case the resource fluctuations affected more the evenness of species distribution and as a result of that $H'$, whereas the number of coexisting species was relatively robust.

The Monod parameters differed between bacterial species and indicated a potential opportunist–gleaner trade-off in growth strategies (Figure 2). However, results show that species with overlap in functional responses were not always able to coexist. In the Community 1 all four species coexisted throughout the experiment, but in the Community 2 only two species (Fig. 2 in IV). In both communities, species that disappeared or were in very low numbers had overlap in functional response with some other species in the same community. In Community 1 low population density had *B. megaterium*, and in Community 2 below detection level fell densities of *C. terrigena* and *C. necator*. Thus, competitive interactions based on the growth of each species in monoculture could partly explain the observed dynamics. This being said, the problems of this simple explanation for species coexistence have to be acknowledged.

Based on my results, I argue that the predictive power of the Monod type growth responses in explaining community dynamics is poor. Although the dynamics within communities could retrospectively be explained by assuming that species with overlap in functional response cannot coexist, several other factors also contributed to the community dynamics. First, the bacterial species differ in several other aspects than in Monod response. Most of the species are for example strict aerobes, but some are facultative anaerobes (*S. marcescens* and *B. aquatica*). The microcosms were continuously mixed to ensure homogeneous oxygen conditions. Still it is possible, that there were anaerobic conditions on microscale. Second, as I demonstrate in Chapter I, bacteria are able to modify their environment and even increase the biologically meaningful resource availability. The environmental conditions in chemically complex environment which is further changed by the bacterial activity is far from the default growth conditions of the Monod model (Monod, 1949; Grover, 1997). Thus, the simple predictions made based on Monod type growth are not logically sound. In the four species communities the resource availability decreased throughout the experiment based on the growth rates of bacterial community in filtered samples of the growth medium (IV). The environmental fluctuations were not reflected to growth rates in filtrates. This indicates that the bacterial community was able to buffer the effect of environmental variation. Finally, the last destructive sampling revealed that a large fraction of the total bacterial biomass was in the biofilm. Some species absent from the free water were detected in biofilms. The amount of biofilm was similar regardless of the frequency of resource fluctuations. Biofilm growth may have been an important factor in buffering the effects of resource fluctuations.
All things considered, the population dynamics of bacteria in communities were most likely affected by several factors simultaneously: habitat modification, species interactions, fluctuations in resource availability, spatial heterogeneity due to biofilm formation, and potential evolution. Of these, only habitat modification and evolutionary changes were not monitored in the four species communities, and for all the other factors it is possible to find various degrees of support.

**Table 2.** Summary of the main hypothesis of this thesis and support based on results with reference to the following chapters.

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Support based on the results</th>
<th>Chap.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth rates in the consumed resource medium reflect the growth dynamics of bacteria in batch cultures.</td>
<td>Yes, in monocultures, but not when two species grew together. The growth rates in consumed medium indicate positive resource mediated interaction between species, but in co-growth species compete with each other.</td>
<td>I, II</td>
</tr>
<tr>
<td>Growth rate of bacterial community varies depending on the frequency of environmental fluctuations.</td>
<td>No. The growth rate of a community in consumed resource medium was not affected by the frequency of resource fluctuations, indicating that bacteria were able to buffer the environmental variation.</td>
<td>IV</td>
</tr>
<tr>
<td>In a pulsed resource environment, large pulses select for rapid growth rate and smaller pulses for survival.</td>
<td>Possibly. Survival increased as a general response to rare resource pulses. However, the evolutionary response most likely depends more on the characteristics of the species and less on the resource pulse amplitude.</td>
<td>II, III</td>
</tr>
<tr>
<td>There is a trade-off between fitness related traits in temporally fluctuating environment.</td>
<td>Possibly. On the population level, none of the measured traits showed impairment. However, on the clonal level the survival of <em>S. marcescens</em> increased and growth rate decreased.</td>
<td>II, III</td>
</tr>
<tr>
<td>Species with contrasting growth strategies coexist when environmental conditions vary.</td>
<td>Yes, but also variation independent mechanisms such as resource partitioning affected the coexistence.</td>
<td>II, IV</td>
</tr>
<tr>
<td>Intermediate environmental disturbance produces highest diversity.</td>
<td>Yes. The Shannon diversity index was highest in the slowly fluctuating environment. However, also other mechanisms contributed to the species coexistence.</td>
<td>IV</td>
</tr>
</tbody>
</table>
5 Conclusions

What does it take to live long and prosper, when environmental conditions change to unfavourable direction? In my thesis I have focused on this type of fundamental questions of ecology and evolutionary biology. These questions are not trivial: due to current environmental changes, such as climate change or habitat fragmentation, many organisms face a situation where they either need to disperse, or adjust. Phenotypic plasticity enables organisms to respond to the changes in environmental conditions to some extent. However, if changes in phenotype are not enough and dispersal is not possible, organisms must “either adapt or die out” (Bell & Collins, 2008). Experimental evolution provides tools for testing in what premises evolutionary rescue could happen, and what are the constraints of adapting to environmental change (Bell & Collins, 2008; Collins, 2010).

The results of my thesis support the view that the interaction between organisms and their growth environment is dynamic and two-directional. I have shown that both the environment and organisms are able to change, and indeed do change. In batch cultures, the habitat modification altered the growth conditions, enabling further changes in species interactions. Biofilm formation changed the spatial structure of the microcosms, which affected both the species diversity and resource availability within bacterial communities. The survival and the growth rate of bacteria evolved to better match the conditions of pulsed resource environment. The interactions between species both hastened or hindered the adaptation to changes in environmental conditions. The evolutionary response to resource pulses was rapid, indicating that the interaction between environment and organisms is potentially a powerful selective agent.

In evolutionary ecology we often end up working with systems where interactions are loops, where – as in my thesis – some environmental change causes a response in organisms, and organisms in turn change the environmental conditions. Furthermore, the potentially rapid evolution adds complexity to the interactions. Of course, one can always argue that calling evolution rapid is just semantics, and less relevant from the biological point of view. On the other hand, we are still far from the point where evolutionary thinking is fully incorporated in ecology and other biological research disciplines. As long as evolution is deemed a slow process without much relevance to current biological processes, emphasizing that evolution can be rapid and it affects contemporary phenomenon is necessary, not just rhetoric. Evolution plays a part in all levels of biotic world. To resolve future challenges ranging from the evolution of antibiotic resistance to the long-term effects of environmental changes on the diversity of nature, understanding the reciprocal nature of ecology and evolution is essential.
6 Acknowledgements

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7 References


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