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Disease dynamics, invasion and biological control of environmentally growing pathogens

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ACADEMIC DISSERTATION

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Contributions

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IM = Ilona Merikanto

JL = Jouni Laakso

VK = Veijo Kaitala

Abstract

Many existing and emerging microbial infectious diseases are caused by environmentally growing opportunist pathogens. These pathogens are, contrary to obligatory pathogens, able to survive and replicate in the outside-host environment as free-living microbes that use within-host growth as an alternative replication strategy. This disease class has eco-evolutionary implications in natural populations and causes a serious health and economical threat to humans, our food production and to wildlife. Because of the ability of environmentally growing opportunists to survive and replicate independently of hosts, these diseases are hard to eradicate with conventional methods. The conditions that favor or disfavor environmental opportunism are still poorly understood. Better understanding of the dynamics of these diseases is needed in order to develop proper control methods against them.

In this thesis I have developed novel epidemiological models to describe the disease dynamics of environmentally growing pathogens. These models modify the traditional Susceptible-Infected host (SI-model) framework by combining it to the outside-host community of an environmentally growing pathogen. I have considered how the environmental growth of the pathogens and the antagonistic ecological interactions these pathogens face in the outside-host environment, such as competition, predation and parasitism, affect the disease dynamics, invasion of novel pathogens and biological control of environmentally growing infectious diseases. The analyses show that the disease dynamics of environmentally growing pathogens differ from obligatory pathogens. Importantly, ability to grow in the outside-host environment promotes disease outbreaks and can lead to the extinction of the host, which is untypical in the case of obligatory pathogens. Antagonistic interactions the pathogen faces in the outside-host environment can on the other hand limit disease outbreaks and prevent extinction of the hosts that would otherwise occur due to the disease.

Lack of available hosts, or treating the hosts with antimicrobials does not eradicate environmentally growing opportunist disease, unless the outside-host growth is diminished drastically due to lack of outside-host resources, low competitive ability or susceptibility to predators and parasites. I conclude that the eradication can be accomplished 1) by increasing the outside-host competition, 2) through predation of

pathogens, or 3) through viral infections in pathogens. Particularly, increasing predation pressure of pathogens can be an efficient biological control method against already established environmentally growing pathogens. Increasing outside-host competition, on the other hand, can prevent invasion of novel diseases. Infecting the outside-host bacterial pathogens with phages shows also promising disease control results depending on the efficiency of the phage and the trade-off between pathogenicity and phage resistance. The theoretical framework presented in this thesis gives novel insight into environmentally growing diseases and how they could be controlled.

1 Introduction

Infectious diseases are one of the major eco-evolutionary drivers of natural population dynamics, regulating host populations, affecting host life history and behaviour and possibly influencing species trophic relations. Infectious diseases cause a severe health threat to wildlife, food production and humans. Infectious diseases pose also a major economical burden through health expenses and production losses. As an extreme case, infectious diseases can promote the extinction of the host population. The spread of diseases and the evolution of virulence has been sought to understand trough mathematical models. There is however a large infectious disease group which has been neglected in the traditional disease theory. Traditional microbial disease theory has mainly focused on the interaction between the pathogen and the host and considered environment as a reservoir for passive microbial stages or referred environment as a transmission stage in vectors (Anderson and May 1981; Read 1994; Frank 1996; Levin 1996; Keeling and Rohani 2008). This theoretical framework is only suitable when considering obligatory disease agents that do not reproduce in the outside-host environment, e.g. in soil or aquatic environment. Therefore, traditional disease theory disregards a large group of pathogens that can alternate between within-host and outside-host reproduction strategy (Casadevall 2008; Veneault-Fourrey and Martin 2011; Brown et al. 2012). In my thesis, I will refer to this pathogen class as environmentally growing opportunists.

The ability of environmentally growing opportunist to replicate in the environment in the absence of hosts and the ecological interactions these pathogens face in the outside-host environment can influence on the evolution of virulence as well as the ability of an environmentally growing opportunist to cause disease outbreaks (Casadevall 2008; Veneault-Fourrey and Martin 2011; Brown et al. 2012). Thus the disease dynamics of obligatory and environmentally growing pathogens likely differ. In this thesis, I present novel theoretical framework for environmentally growing opportunist pathogens by coupling the ecology in the outside-host environment to traditional SI-model framework. My aim in this thesis is to describe disease dynamics and invasion of these diseases as well as consider biological control methods against them.

1.1 Environmentally growing opportunist pathogens

Environmentally growing opportunist pathogens differ from obligatory pathogens in their ability to survive and replicate e.g. as saprotrophs in the outside-host environment. Obligatory pathogens on the other hand are dependent on their hosts for survival and replication. Environmentally growing opportunists thus use within-host replication more of an alternative reproduction strategy, and they are often not as host specific as obligatory pathogens (Casadevall 2008; Veneault-Fourrey and Martin 2011; Brown et al. 2012). Environmentally growing opportunist pathogens do not differ from obligatory pathogens in their ecology only by their ability to grow in the outside-host environment: In the environment, these pathogens also actively face multiple ecological interactions with other free-living organisms, such as competition for resources, predation and parasitism, and are exposed to abiotic environmental variation (Pascual and Dunne 2005), which is often stronger than the variation experienced in the homeostatically regulated hosts. Figure 1 presents a simplified food web that environmentally growing pathogens are associated with.

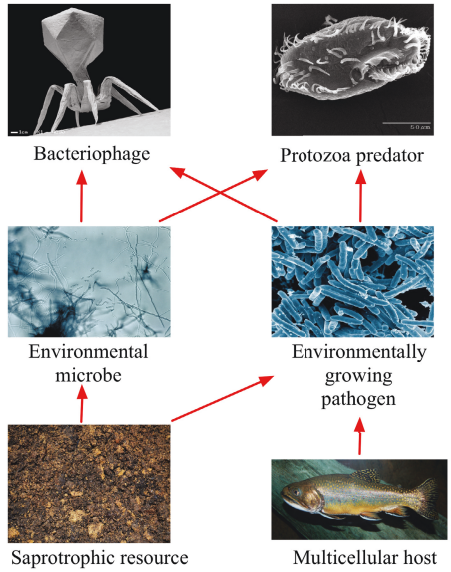

Figure 1. A simplified food web of environmentally growing opportunist pathogens. Arrows show the trophic relations in the system. In the outside-host environment the environmental microbes compete with environmental opportunists for resources.

Many common pathogens are environmentally growing opportunists. These pathogens include for instance bacterial species from aquatic and soil environments as well as from rhizosphere, such as *Vibrio cholera*, *Pseudomonas aeruginosa, Legionella pneumophila, Listeria monocytogenes, Cryptococcus neoformans* and many species from genus *Mycobacterium, Flavobacterium* and *Serratia,* as well as fungal and protozoa pathogens (Grimont and Grimont 1978; Friedman et al. 2002; Leclerc et al. 2002; Berg et al. 2005; Hall-Stoodley and Stoodley 2005; Hilbi et al. 2007; Casadevall 2008; Rahman et al. 2008; Soto et al. 2008; Freitag et al. 2009; Kunttu et al. 2009; Mahlen 2011; Trivedi et al. 2011; Brown et al. 2012; Fisher et al. 2012). In Table 1 I give examples of few well-known environmentally growing opportunist pathogens. I discuss some of these further in different parts of the introduction, with the main focus in the fish columnaris disease that has been utilized in the parameterization of the models presented in this thesis.

Opportunist infections are often associated with immunocompromised hosts, such as *Corynebacterium equi, L. pneumophila* or *P. aeruginosa*, whereas e.g. *Salmonella* spp. and *F. columnare* infect also healthy hosts (Von Graevenitz 1977; Parker and Prince 2011; Declercq et al. 2013). For instance, fish columnaris disease caused by *F. columnare* causes major economical losses in freshwater fisheries all over the world resulting possible death of the whole fish population in a rearing tank (Wagner et al. 2002; Suomalainen et al. 2005; Kunttu et al. 2009; Pulkkinen et al. 2010; Declercq et al. 2013). Opportunistic fungal infections might appear, depending on the species, on both healthy and immunocompromised hosts (Vennewald and Wollina 2005; Harrison et al. 2012). Normal skin microflora e.g. *Propionibacterium acnes* and some species of *Micrococcus* genus on the other hand have been suggested to cause opportunistic infections, such as acne, as the normal microflora community or immune system is suppressed (Smith et al. 1999; Perry and Lambert 2011; Dekio et al. 2012). There are multiple factors that determine whether a host will get infected, such as the condition of the host, infectious dose and the pathogenicity and virulence of the disease agent (Schmid-Hempel 2011). Hosts might differ in their ability to mount an efficient immune response against a disease agent (referred to as immunocompetence), for instance due to genetic differences between individuals or nutrition status. Hosts can also face differences in disease risk due to e.g. behavioural differences or hierarchical position (Schmid-Hempel 2011). With the term pathogenicity I refer to the ability of a disease agent to cause an infection in a particular

encountered host species. With the term virulence I on the other hand refer to the level of harm the infection causes the host. Virulence might not solely depend on the properties of the pathogen but also on the condition and genetics of the host. Both the condition of the host and pathogenicity determine the amount of infectious agents needed to infect the host and make it symptomatic (Loker and Hofkin 2015). For simplicity, this thesis will however disregard the heterogenicity among the hosts in the theoretical framework presented here. Next, I shall shortly discuss the factors that influence the evolution of virulence and pathogenicity of environmentally growing opportunists.

Table 1. Examples of environmental opportunist diseases.

Pathogen	Environment	Hosts	Disease	Reference
Bacillus anthracis	Soil bacteria	Animals	Anthrax	(Beyer and Turnbull 2009; Turner et al. 2013)
Burkholderia cepacia complex (Bcc)	Soil and rhizosphere bacteria	Plants, Animals, Human	Bacterial soft-rot disease, Lung inflammation	(Govan et al. 1996; Coenye and Vandamme 2003; Berg et al. 2005; Barak and Schroeder 2012)
Burkholderia pseudomallei	Soil, rhizosphere and aquatic bacteria	Human	Melioidosis	(Berg et al. 2005; Jabbar and Currie 2013)
Clostridium tetani	Soil bacteria	Animals	Tetanus	(Levin 1996; Afshar et al. 2011)
Cryptococcus neoformans	Soil fungus	Human, Cat	Cryptococcosis	(Casadevall 2008; Trivedi et al. 2011)
Flavobacterium columnare	Aquatic bacteria	Freshwater fishes	Columnaris disease	(Kunttu et al. 2009; Pulkkinen et al. 2010; Declercq et al. 2013)
Flavobacterium psychrophilum	Aquatic bacteria	Salmonids	Cold-Water Disease, Rainbow Trout Fry Syndrome	(Madetoja et al. 2000)
Francisella tularensis	Soil and aquatic bacteria	Mammals	Tularaemia	(Thelaus et al. 2009)
Fusarium solani	Soil fungus	Sea turtles	Hatch failure, Juvenile weakness	(Sarmiento-Ramírez et al. 2010)
Legionella pneumophila	Aquatic bacteria	Human	Lung inflammation	(Friedman et al. 2002; Leclerc etal. 2002)
Listeria monocytogenes	Soil and aquatic bacteria	Plants, Animals	Gastrointestinal infections	(Freitag et al. 2009; Toledo-Arana et al. 2009)
Mycobacterium fortuitum	Soil and aquatic bacteria	Human	Infections	(Hilbi et al. 2007)
Mycobacterium marinum	Aquatic bacteria	Animals	Tuberculosis (fish and amphibian), lung inflammation (human)	(Hilbi et al. 2007)
Mycobacterium tuberculosis	Aquatic bacteria	Human	Tuberculosis	(Hilbi et al. 2007; Ahmad 2010)
Pseudomonas aeruginosa	Aquatic and rhizosphere bacteria	Human	Lung inflammation	(Miller and Bassler 2001; Berg et al. 2005; Hall-Stoodley and Stoodley 2005; Kumar et al. 2013)
Pseudomonas veronii	Rhizosphere bacteria	Human	Intestinal inflammatory pseudotumour	(Cheuk et al. 2000)
Salmonella spp.	Soil and rhizosphere bacteria	Animals, Human	Gastrointestinal infections	(Santamaria and Toranzos 2003; Berg et al. 2005)
Serratia entomophila	Soil bacteria	Grass grub	Larvae mortality	(Godfray et al. 1999)
Serratia marcescens	Soil, rhizosphere and aquatic bacteria	Plants, Insects, Vertebrates	Urinary track infections, central nervous system diseases, pneumonia and other respiratory diseases, wound infections	(Grimont and Grimont 1978; Berg et al. 2005; Tan et al. 2006; Mahlen 2011)
Stenotrophomonas maltophilia	Soil, rhizosphere and aquatic bacteria	Plants, Human	Blood-stream infections, pneumonia	(Berg et al. 1996; Berg et al. 2005; Looney et al. 2009)
Verticillium dahliae	Soil fungus	Plants	Verticillium wilt	(Maas 1998; Tjamos et al. 2000; Klosterman et al. 2011)
Vibrio cholerae	Aquatic bacteria	Human	Cholera	(Chakraborty et al. 2000; Hall-Stoodley and Stoodley 2005; Murugaiah 2011)

1.2 Evolution of virulence and pathogenicity among environmentally growing pathogens

Traditional epidemiological theory often assumes a trade-off between virulence and transmission, which results from obligatory disease relationship between host survival and pathogen growth (Ebert and Bull 2003). In other words, increased mortality to infection also reduces the fitness of the obligatory pathogen that is dependent on the host for survival and reproduction. Therefore, according to the transmission-virulence trade-off hypothesis virulence should be optimized to maximize the number of secondary infections produced by the primary infection (R_0>1, where R_0 indicates the number of secondary infections) (Read 1994; Frank 1996; Levin 1996; Walther and Ewald 2004). Environmental opportunists are, in contrast to obligatory pathogens, at least partly free from this trade-off as they can survive and replicate independently of hosts. For instance, in the case of columnaris disease the death of a host causes little harm to the fitness of the pathogen as the release rate (within-host growth) is in fact greater from a dead host as compared to a living one (Kunttu et al. 2009). Evolution of high virulence may thus be promoted among environmental opportunists. This seems to be the case at least concerning columnaris disease, where more virulent strains have emerged and become more common in fish farms during the last two decades (Pulkkinen et al. 2010). Long survival time without replication in the environment, for instance as resting spores, has also been predicted to promote development of high virulence (Day 2002; Kamo and Boots 2004; Walther and Ewald 2004; Caraco and Wang 2008; Roche et al. 2011; Boldin and Kisdi 2012). Other theories of the evolution of high virulence consider host-dependent mechanisms, which include short-sighted within-host strain competition and utilization of multiple hosts (Frank 1996; Levin 1996; Lipsitch and Moxon 1997; Gandon and Poulin 2004; Bell et al. 2006). Other host-dependent mechanism include for instance the relation between life cycles of the host and the parasite, and whether the disease dynamics are governed by selective sweeps or the red queen effect (Schmid-Hempel 2011).

Ecological interactions, such as competition between microbes in the outside-host environment are also likely to influence the evolution of pathogenicity among environmental opportunist pathogens (Casadevall 2008; Ehrlich et al. 2008; Friman et al. 2009; Mikonranta et al. 2012). Environmental opportunism seems a successful strategy, as

replication is possible in both the within-host and outside-host environment. Environmentally growing opportunists are able to escape environmental competition, predation and parasitism to resource rich within-host environment. Yet, even though many environmental bacteria in the soil and water harbour virulence genes and also gain them through horizontal gene transfer between microbes, they only seldom are pathogenic (Pallen and Wren 2007; Casadevall 2008; Persson et al. 2009; Wang and Behr 2014). Environmental opportunism is not cost free, as there is often a trade-off between survival and growth in the outside-host environment and being able to infect or replicate in the within-host environment (Casadevall 2008; Freitag et al. 2009; Friman et al. 2009; Mikonranta et al. 2012). For instance, more virulent *L. monocytogenes* strains are weaker competitors in the outside-host environment than less virulent strains, as the more virulent strain allocates energy on genes enabling the virulence. This reduces their ability to face multiple challenges in the outside-host environment (Freitag et al. 2009). Also, a trade-off between the ability to defend against predation by protozoa and virulence has been observed in *S. marcescens* (Friman et al. 2009; Mikonranta et al. 2012). This raises a question should environmental opportunism be promoted in a situation where environmental competition, predation or parasitism is relaxed or there is an abundance of potential hosts available. It could be that specialization towards free-living organism or towards obligatory pathogenicity might otherwise be more beneficial.

The theory of coincidental evolution of virulence proposes that pathogenicity rises when traits that evolved to benefit survival and growth in the outside-host environment also coincidentally enable pathogenicity (Read 1994; Frank 1996; Brown et al. 2012). Let us assume that the ability to infect a host would not be a dead-end for the novel pathogen, but would allow replication in the host and transmission either to a new host or back to environment. If this was the story behind every new pathogen, should not pathogenicity increase among all free-living microbes, as there would be no cost of being an opportunist? It has been suggested that coincidental evolution of pathogenicity has probably occurred in cases where bacteria have developed the ability to avoid predation by amoeba. Amoebae resemble macrophages in phagocytosis and killing mechanisms of bacteria. Thus the mechanisms that have allowed bacteria to survive inside amoeba and utilize amoebal resources for growth enable them to evade immune defences by mammalian macrophages (Matz and Kjelleberg 2005; Hilbi et al. 2007; Derengowski et al.

2013; Erken et al. 2013). For example, it has been suggested that the survival and growth of *L. pneumophila* inside amoeba has enabled it to infect and replicate inside inside human alveolar macrophages and epithelial cells (Hoffmann et al. 2014). However, considering the abundance of non-pathogenic free-living microbes in our environment in the light of commonness of horizontal gene transfer among microbes and high densities of potential hosts in food production or urban areas, trade-offs likely exist between traits affecting survival and growth in the outside-host environment and infectivity and replication within-host. Host immunity of course at least partly explains why many of the free-living microbes are not infectious. However, the resource conditions and developmental pressures differ between within-host and outside-host environment and can also explain why the majority of the microbial diversity is not pathogenic. Expression of different genes might be needed in the outside-host environment as compared to within-host environment. For instance, gene and regulatory RNA expression in *L. monocytogenes* differs drastically in the outside-host and within-host environment (Toledo-Arana et al. 2009). In many cases, free-living microbes have larger genome as compared to environmental opportunists, while genome reduction due to adaption to within-host life is emphasized in obligatory bacteria (Toft and Andersson 2010**)**. This phenomenon, which however is not universal, has been suggested to reflect the relative stability of within-host environment as compared to outside-host environment. Therefore, it has also been thought that switching from obligatory pathogen back to purely free-living microbe is unlikely as the outside-host environment contains more challenges for successful growth than within-host environment due to multiple antagonistic interactions and the loss of metabolic functions also limit utilization of outside-host resources (Casadevall 2008).

Environmentally growing opportunists could be seen as a missing link between obligatory pathogens and purely free-living microbes. Adaptation to within-host lifestyle could promote the evolution of environmentally growing opportunists towards obligate pathogens. It would be interesting to consider which situations promote this transition towards obligatory pathogenicity, commensalism or mutualism. On the other hand, opportunism could be a stable strategy. In this thesis, I concentrate in taking the initial steps in understanding the invasion of novel environmentally growing opportunists and their disease dynamics. However, situations where obligatorism is favoured are also discussed in the Results and discussion section of this thesis.

Human activities have at least in part facilitated the emergence of new environmentally growing opportunist diseases. In food production, cultivating species in high densities facilitates transmission and thus favours emerging disease agents. Furthermore, promoting loss of genetic diversity, especially on population level, reduces the capacity of the innate immune system to cope with various pathogens. Lowered genetic diversity of hosts also enables environmental opportunists to adapt easier to within-host environment (Mennerat et al. 2010). Rearing fishes in high densities and lowered genetic diversity of the host population have been for instance suggested to promote evolution of virulence in *F. columnare* in freshwater fisheries (Pulkkinen et al. 2010). Furthermore, the density of people living in cities and global mobility of people, animals and trade goods has increased especially in recent decades favouring the spread of diseases (Patz et al. 2000; Murray and Peeler 2005; Fisher et al. 2012). Urbanization has also created more demands on proper sanitation conditions. For instance, *V. cholera* can be found in aquatic environments all around the world although cholera outbreaks usually occur in countries where water sanitation is poor (Mandal et al. 2011; Murugaiah 2011; Lutz et al. 2013). Usage of antimicrobials also increase susceptibility to environmentally growing opportunist diseases by reducing normal microflora, which would inhibit colonization of environmental opportunists, such as *P. aeruginosa*, as well as promote development of antibiotic resistant bacterial strains (Von Graevenitz 1977; Alanis 2005; Ehrlich et al. 2008; Oliveira et al. 2012). Furthermore, some environmental opportunists may benefit from climate warming (Harvell et al. 2002; Pulkkinen et al. 2010; Murugaiah 2011; Fisher et al. 2012). For example, climate warming is, at least partly, responsible for the increased and more severe disease epidemics of columnaris disease (Pulkkinen et al. 2010). There is thus a high demand for novel managing methods against environmental opportunist diseases. In order to find suitable control methods a better understanding of the dynamics and invasion conditions of these diseases is needed.

1.3 Management of environmentally growing pathogens

Traditional methods in disease management have included attention to good sanitation and hygiene, use of quarantine procedures as well as development of drugs and vaccines.

Eradication of environmentally growing diseases by using antimicrobial treatments is, however, often unsuccessful. Antimicrobials are in many cases targeted against the pathogen population living in the within-host environment and not the pathogen population in the outside-host environment (Alanis 2005). Thus, antimicrobial treatments do not eradicate disease agents from the environment even though all the hosts would be treated and are able to manage poorly environmentally growing diseases. This has been seen in the case of cholera and columnaris disease (Rahman et al. 2008; Pulkkinen et al. 2010). In some cases, such as columnaris disease, antimicrobial treatments are also unsuccessful in treating sick individuals when infected hosts cease feeding and these drugs are administered in food mixtures (Pulkkinen et al. 2010; Oliveira et al. 2012). Furthermore, intensive use of antimicrobials has led to development of multidrug resistant strains, and the use of disinfectants in the environment potentially lessens outside-host competition in favour of the opportunist pathogen. Hospitals are ideal places for spread of resistant strains due to intensive administration of drugs and low diversity of harmless microbes. In hospitals, immunocompromised patients can act as hotspots for multiple infections, which also enable increased horizontal transfer of resistant genes among bacteria. In intensive farming, fertilization with manure and polluting environments with wastes containing antimicrobials also promote the spread of antibiotic resistance via horizontal gene transfer among aquatic and soil microbes (Davies and Davies 2010; Jechalke et al. 2013; Jechalke et al. 2014). Better methods than the use of antimicrobials to manage environmentally growing opportunist diseases are thus needed.

Methods that reduce the pathogen population size in the environment also reduce possibilities for environmentally growing opportunist disease outbreaks. Such methods could serve as a first hand disease control. Particularly, intensifying the antagonistic pressures posed by competitors, predators or parasitism in the outside-host environment could limit the outside-host growth of pathogens. There is empirical evidence showing that increasing the antagonistic pressures in the outside-host environment comes with a trade-off cost for the pathogen promoting reduced virulence (Skurnik and Strauch 2006; Friman et al. 2009; Mikonranta et al. 2012; Laanto et al. 2012; Vasanthakrishnan et al. 2015). For instance, competition between the pathogens and the environmental microbial community can, at least in theory, prevent disease outbreaks (Anttila et al. 2013). The Increased diversity of the bacterial community in the outside-host environment might,

however, increase horizontal gene transfer of virulence factors across bacteria species. For instance, virulence of *M. tuberculosis* seems to have been acquired through horizontal gene transfer from many different bacteria. This has contributed to the evolution of this pathogen from an environmental bacteria to an environmental opportunist that is capable of infecting humans (Wang and Behr 2014).

Lytic bacteriophages (Abedon 2008) have however raised interest as a biological control method against pathogens, which is addressed as phage therapy (Levin and Bull 2004). Phage therapy has been discussed as an alternative method for antimicrobials in the treatment of hosts as phages only target specific bacteria cells. In this thesis I study how targeting the outside-host pathogen population with phages could be utilized in the biological control of environmentally growing pathogens (chapter **IV**).

The trouble with using phages in long-term biological control is that, as towards antimicrobials, bacteria can rapidly develop resistance against bacteriophages (Oliveira et al. 2012). Yet, in some cases, there can be a trade-off between virulence and the ability to resist phages (Skurnik and Strauch 2006). Thus, while phage therapy in this scenario would not lower the densities of an environmentally growing opportunist, it can reduce infections if the opportunists allocate resources in the defence against bacteriophages rather than allocating them in infectivity and growth in the host. Experiments have indicated that this kind of trade-off between virulence and resistance to phages seems to exist at least in the case of *F.columnare* (Laanto et al. 2012). Phage therapy experiments have resulted both promising results as well as some failures (Skurnik and Strauch 2006; Oliveira et al. 2012). When using phage therapy as a biological control method, phage cocktails targeting multiple pathogen strains can be more efficient method than using a single phage. Phages can be highly strain specific and the same environment can harbour multiple strains of the same pathogen, as in the case of columnaris disease in fish tanks (Suomalainen et al. 2005; Oliveira et al. 2012). This, of course, can make phage therapy quite troublesome to apply successfully in practise.

In contrast to parasitism by bacteriophages, predation by protozoa is more efficient in reducing bacteria densities. Indeed, it has been described as the forerunner causing bacteria mortality (Fenchel 1980; Finlay and Esteban 1998; Jürgens and Matz 2002; Sherr and Sherr 2002; Menon et al. 2003). Development of resistance towards a predator is also not as probable as development of resistance towards phages as the outside-host

environment usually harbours high variety of potential predators targeting the same microbial species, and because the feeding of predators rely on less specific prey recognition mechanisms (Zhang et al. 2014). Although bacteria have developed mechanisms to escape predation, these defence mechanisms have been shown, at least in some cases, to be traded off with virulence (Friman et al. 2009; Mikonranta et al. 2012). Predation by ciliate and rotifer protozoa has shown promising results in reducing the densities of environmentally growing pathogens in outside-host environment, consequently reducing infections, in case of such as bacteria *Salmonella typhimurium* and *Klebsiella pneumonia* and fungi *Batrachochytrium dendrobatidis* (Mallory et al. 1983; Schmeller et al. 2014).

2 Aims of the thesis

The aim of this thesis is to develop novel epidemiological models suitable for microbial pathogens that also grow and interact in the outside-host environment. The focus is on bacterial pathogens. The parameterization example of the models is based on fish columnaris disease, although the models can also be utilized in studies of other environmentally growing bacterial, fungal or protozoan diseases. Overall, these models seek to be the first step in understanding the disease dynamics (**I**, **II**, **III**, **IV**), invasion (**II**) and biological control (**III**, **IV**) of environmentally growing opportunist pathogens.

Chapter **I** presents a basic epidemiological model for environmentally growing pathogens. It considers how the pathogen's ability to grow independently of hosts in the outside-host environment alters the traditional SI disease dynamics. Chapter **I** explores the conditions when the density dependent environmental growth promotes disease outbreaks and/or when it increases the likelihood for host extinction due to the disease. The model in chapter **I** also seeks to reveal how does the growth rate of the hosts and pathogen virulence, outside-host mortality and release rate influence the disease dynamics of environmentally growing pathogens.

As microbes in the environment are rarely alone, in the chapter **II** I add environmental competition between a non-pathogenic microbe and environmentally growing pathogen to the model presented in chapter **I**. Chapter **II** has two aims: First, chapter **II** seeks to understand the situations in which a novel environmentally growing opportunist pathogen is able to successfully invade when it is competing with a superior non-pathogenic strain in the outside-host environment. The second aim is to understand how the competition between the pathogenic and non-pathogenic strain in the outside-host environment influences on the long-term disease dynamics of environmentally growing opportunists.

Chapter **III** aims first to understand the influence of outside-host predation on the long-term disease dynamics of environmentally growing pathogens. It also studies the impact of a biological control method by continuously adding pathogen predators to the system and thus increasing the predation pressure on the pathogen to achieve the local eradication of the disease.

Chapter **IV** considers outside-host phage therapy as a biological control method against environmentally growing opportunist diseases. Here, outside-host phage therapy is

carried out by introducing lytic viruses to the outside-host environment to infect the pathogen population there. First I seek to quantify how efficient the phage should be in infecting the pathogens to eradicate the disease successfully. Secondly, different scenarios are presented to address the issue of resistance towards phages by considering different trade-off possibilities between pathogen infectivity and phage resistance. These scenarios seek to reveal situations when outside-host phage therapy can be successful in eradicating the disease.

3 Methods

3.1 Models

The models presented in this thesis all consist of deterministic continuous time models coupling three or more differential equations. The intent of the models is to combine SI model to the outside-host ecology of an environmentally growing pathogen. Most of the models and their parameterization are based on the characteristics of columnaris disease. Chapter **II** also presents supplementary model versions applicable to more benign environmentally growing opportunist diseases. Here, I outline the intensions behind the model structure. For the specific model equations, the reader is kindly referred to the respective chapters of the thesis.

3.1.1 Basic model for environmentally growing pathogens

Chapter **I** present a basic epidemiological disease model for environmentally growing pathogens in its simplest form (figure 3.1). This model consists of three differential equations describing the changes in time (t) in the densities of susceptible (S) and infected hosts (I), and pathogens in the environment outside-host (P):

$$\frac{dS}{dt} = r_s(1-S)S - \beta PS - \mu_{SI}S \qquad (1)$$

$$\frac{dI}{dt} = \beta PS - (\alpha + \mu_{SI})I \qquad (2)$$

$$\frac{dP}{dt} = \Lambda\alpha I + r_p(1-f_pP)P - \mu_P P \qquad (3).$$

The separate equation for the pathogen population in the outside-host environment (3) includes host-independent density dependent growth of the pathogen population ($r_P(1-f_PP)$, where r_P stands for pathogen outside-host growth rate and f_P for the negative influence of pathogen population density on its growth). The equations for susceptible (1) and infected hosts (2) are based on SI model G of Anderson & May (1981). The

susceptible hosts have a density dependent logistic growth, $r_S(1\text{-}S)$, but the model assumes that the infected hosts are unable to grow or compete for resources with susceptible hosts. This assumption is suitable for host-sterilizing and highly virulent pathogenic diseases, such as columnaris disease (Pulkkinen et al. 2010). The model assumes similar density independent background mortality for the susceptible hosts and infected hosts due to other causes than the disease (μ_{SI}). Furthermore, the population of the infected hosts can decrease due to mortality to the disease (α). The pathogen population in the outside-host environment decreases depending on a density independent death rate (μ_P). Other parameters are explained in the figure 3.1.

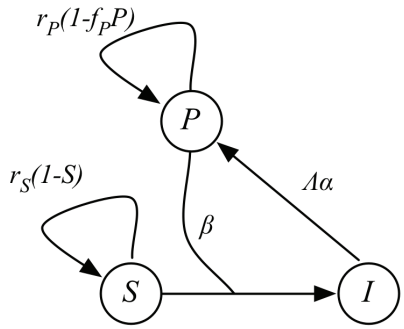

Figure 3.1. Schematic drawing of the model developed in Chapter **I**. The susceptible hosts (S) are infected through environmental transmission (β) of the pathogens (P). Pathogens are released at a rate Λ to the environment from the infected hosts (I) upon their death due to the infection (α). The release upon the death of an infected host in contrast to continuous release from the infected hosts is a simplification in the model. This assumption is based on columnaris disease in which the release rate of novel pathogens from live hosts is considerably lower than the release rate from dead hosts (Kunttu et al. 2009). Also, the pathogens are only released from the hosts as they die to infection but not if the hosts die for instance due to predation. This assumption is again based on columnaris disease, which is an ectoparasite and is not known to survive in the gastrointestinal tract (Declercq et al. 2013).

3.1.2 Outside-host competition model

The model presented in Chapter **II** adds a non-pathogenic competitor in the outside-host environment (B) to the basic model presented in Chapter **I** (figure 3.2). This allows the

model to explore how a novel pathogenic strain is able to invade in the presence of a superior non-pathogenic competitor in the outside-host environment. Also, this model can be used to predict the influence of outside-host competition on the long-term dynamics of environmentally growing opportunist disease.

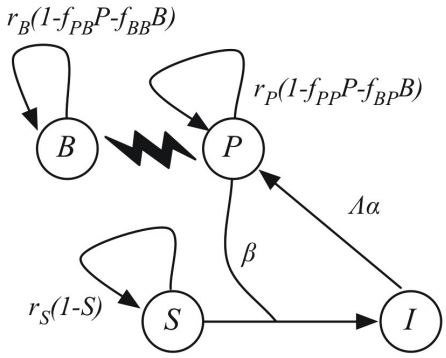

Figure 3.2. The model developed in Chapter **II** introduces an outside-host competitor (B) of the pathogen (P) to the model presented in Chapter **I**. The competitor is assumed to be a free-living non-pathogenic microbe that is competing for resources in the outside-host environment with the pathogen (indicated with the serrated line). Both the pathogen and the competitor have their own density dependent outside-host growth (f_{PP} for the pathogen and f_{BB} for the competitor), where the density dependence can differ between the competitors. The growth of the pathogen and its competitor also induce negative impacts on each other determined by competition coefficients f_{PB} and f_{BP}). For the other parameters see figure 3.1.

The model described in figure 3.2 is again suitable for environmentally transmitted highly virulent pathogens that sterilize and are able to cause the death of a host, as the release from the dead host is more substantial than the release from the living hosts. However, chapter **II** also presents supplementary model versions that consider i) a possibility of both direct and environmental transmission of the pathogen (figure 3.3), ii) resource competition between the susceptible and infected hosts (figure 3.4), iii) ability of the infected hosts to recover from the disease (figure 3.5), and iv) continuous release of the pathogens from the infected hosts instead of release upon death to infection (figure 3.6).

Lastly, chapter **II** presents a model version for an infection type that sterilizes the infected host but is not lethal in any situation (the model presented in Supplement S5).

Also for this model, the continuous release of novel pathogens from the infected hosts applies instead of release of the pathogen upon death of the infected due to the disease. However, the infected hosts are not able to recover from the infection in this model version therefore describing a scenario where the disease is tolerated instead of eradicated.

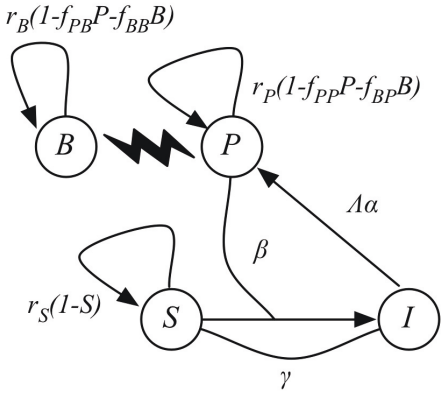

Figure 3.3. The model version in Chapter **II** (Supplement S1) describing infection with both environmental and direct transmission between the hosts (γ). For the other parameters see figure 3.1 and 3.2.

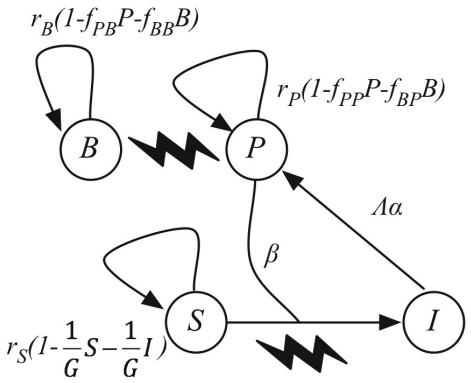

Figure 3.4. The model version in Chapter **II** (Supplement S2) describing competition between the infected and susceptible hosts (indicated with the serrated line). For simplicity the susceptible and infected hosts are assumed to have the same competitive ability, which is determined by the environmental carrying capacity of the hosts (*G*). The resource competition however only affects the growth rate of the susceptible host population, as the infected hosts are unable to reproduce due to the infection. For the other parameters see figure 3.1 and 3.2.

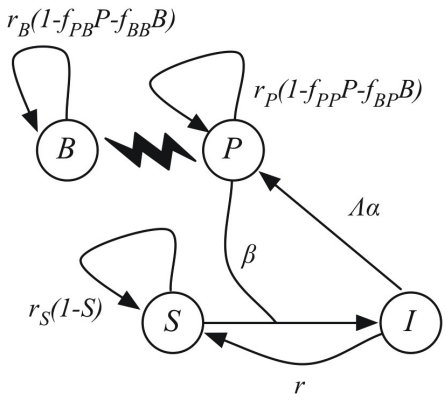

Figure 3.5. The model version in Chapter **II** (Supplement S3) that considers the ability of the infected hosts to recover from the disease (r). For the other parameters see figure 3.1 and 3.2.

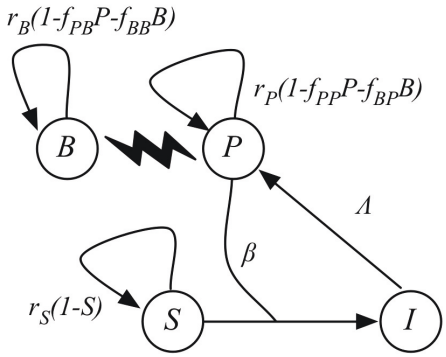

Figure 3.6. The model version in Chapter **II** (Supplement S4) that considers continuous release of novel pathogens from the infected living hosts (Λ) instead of release only upon the death of and infected to the disease ($\Lambda\alpha$). Here the α indicates the virulence of the pathogen. For the other parameters see figure 3.1 and 3.2.

3.1.3 Pathogen predation in the outside-host environment

The model developed in Chapter **III** considers how outside-host predation of the pathogen influences the long-term environmentally growing opportunist disease dynamics (figure 3.7). The model consists of four differential equations describing the changes in time (t) in the densities of susceptible hosts (S), infected hosts (I), pathogens (P) and pathogen

predators in the environment outside-host (Z). The model thus combines SI dynamics based on model G of Anderson and May (1981) and prey-predator model with predator saturation. The predator population grows depending on how efficient the predator is in hunting down pathogens (a) and utilizing them to predator growth (r_Z) as well as on the predator´s half saturation constant (c). The predator population is depleted by a background mortality rate (μ_Z). This model is suitable in describing e.g. protozoa predators. The model assumes a single prey species for the predator. The second part of the Chapter **III** also considers how outside-host predation could function as a biological control method against environmentally growing pathogens by continuously adding predators to the system at a certain inflow rate.

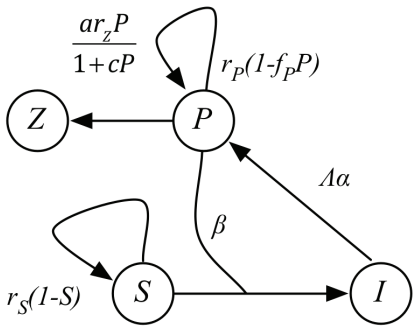

Figure 3.7. Schematic drawing of the model developed in Chapter **III**. This model introduces an outside-host predator of the pathogen to the model developed in Chapter **I**.

3.1.4 Viral infection of the pathogen in the outside-host environment

Chapter **IV** presents three different model versions considering lytic phage therapy in the outside-host environment as a biological control method against environmentally growing opportunist diseases. Here, viral infections of the pathogen are considered in the outside-host environment coupled with the basic model presented in Chapter **I**. The first model version developed in this chapter consists of four differential equations describing the changes in time (t) in the densities of susceptible hosts (S), infected hosts (I), pathogens (P) and bacteriophages in the environment outside-host (F). The model thus combines SI dynamics based on model G of Anderson and May (1981) with lytic viral infection of the outside-host pathogen population (figure 3.8). In addition to background mortality rate

(μ_P), pathogens die as they are infected by a phage. The model assumes that the lysis of the infected pathogen cells is instant. The same parameter thus indicates the mortality rate to phage infection in pathogens and to phage transmission rate (β_F). The population of phages increases as novel phages are released upon the lysis of the pathogen cell (Λ_F). The phage population decreases depending on a certain background decay rate (μ_F).

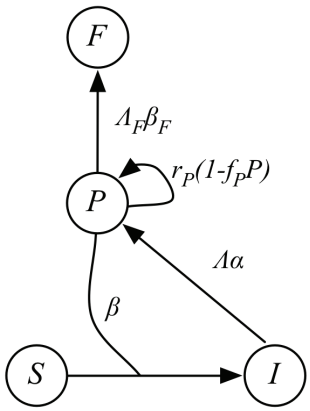

Figure 3.8. Schematic drawing of the first model version developed in Chapter **IV**. This model introduces an outside-host phage of the pathogen (*F*) to the model developed in Chapter **I**.

The second model version considers the ability of the pathogens to develop phage resistance (*m*) against the lytic viral infection (figure 3.9). The model assumes a trade-off between the ability to defend against phages and the ability to cause infections. In this model version the ability to defend against phages totally inhibits the ability to cause infections, so that mutant phage resistant pathogens turn into non-pathogenic free-living microbes. This kind of total phage resistance-infectivity trade-off is suitable for certain pathogenic strains, such as the most virulent strains causing columanris disease (Laanto et al. 2012). The second model version in Chapter **IV** thus consists of five differential equations considering the influence of a phage resistant non-pathogenic competitor in the outside-host environment to the disease dynamics. The non-pathogenic and pathogenic strains compete in the outside-host environment. The density dependent effect of the competitor and the pathogen on each other´s growth is assumed to be equal in the model.

The third model version assumes that the trade-off between the ability to infect hosts and resistance towards phages does not totally prevent pathogenicity even though resistance towards phages lowers infectivity (figure 3.10). This applies to for instance *F. columnare* strain (Os06) which is a more benign form of columnaris disease agent (Laanto et al. 2012). The model thus consists of six differential equations with a separate equation for hosts infected with a phage resistant pathogen strain (for equations, the reader is kindly referred to Chapter **IV**).

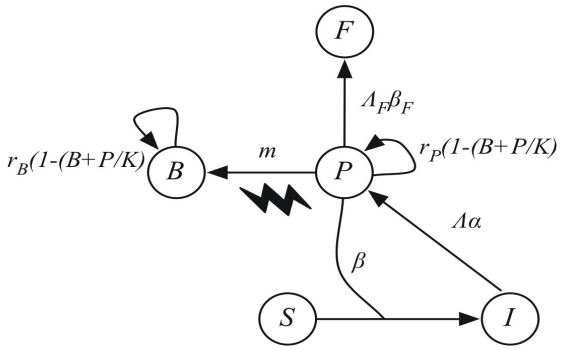

Figure 3.9. Schematic drawing of the second model version developed in Chapter **IV**. This model adds a phage resistant non-pathogenic competitor (*B*) to the first model version in this chapter. Pathogens turn at a certain rate (*m*) to non-pathogenic environmental microbes as they gain phage resistance.

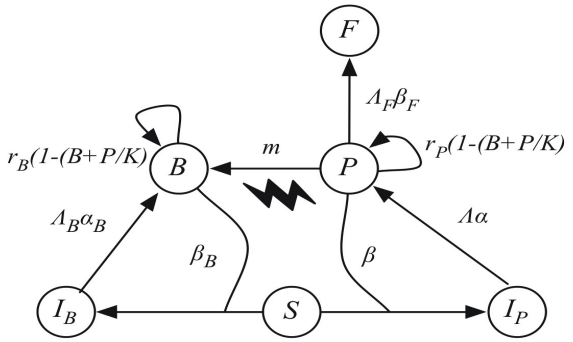

Figure 3.10. Schematic drawing of the third model version developed in Chapter **IV**. This model adds hosts infected with phage resistant pathogen strain (I_B) to the second model version in this chapter (**IV**). The phage resistant pathogens have their own environmental transmission rate (β_B), release rate (Λ_B) and virulence (α_B), of which the transmission rate is in the analyses assumed to be lower than the transmission rate of the non-resistant pathogen.

3.2 Parameterization of the models

The models developed in this thesis allow wide range of parameterization depending on the disease of interest. In this thesis, the parameterizations of the models for numerical simulations were chosen to be most suitable for environmentally growing opportunist bacterial pathogens and for the most part for multicellular hosts with relatively high annual fecundity, such as fish. In general, columnaris disease in fishes was kept in mind when choosing the parameter space for the numerical simulations. However, also wider parameter range for host fecundity as well as pathogen outside-host growth ability and virulence were analysed in models **I** and **II**. In **III** and **IV** the parameterization of the infection type and the host on the other hand was aimed more suitable for relatively virulent environmental opportunist diseases targeting hosts with relatively high fecundity. The parameterizations in these models were set to describe an infection scenario where in the absence of biological control the disease would drive the host close to extinction (**III**) or result the extinction of the host (**IV**). Wide parameter range was explored in numerical simulations for certain variables, which can vary strongly or are hard to estimate in practice, such as competitive interactions (**II**). Furthermore, predator and phage efficiencies were assumed to vary over a quite wide parameter range in **III**-**IV** in order to analyse the effective biological control result.

3.3 Analyses of the models

The dynamics of the models were analysed using analytical solutions when possible and numerical simulations. Equilibrium solutions were analysed by local stability analysis in all the models in **I-IV**. Stability analyses of the equilibrium solutions of the community were used to study the local stability of the models in **I-IV**. The invasion success of the novel pathogens was analysed at the equilibrium in the absence of the disease (**II**). The invasion success of the pathogen predators (**III**) and phages (**IV**) was analysed at the equilibrium in the absence predators or phages, respectively. In the stability analyses different combinations of two parameters were analysed at the time. Both parameters in question were given several different values from the value range used in the stability

analyses. Invasion of the novel pathogen or introduction of an antagonist relation was concluded to be successful when the equilibrium solution was locally unstable. Otherwise, the invasion of the pathogen, predator or phage was unsuccessful.

The long-term dynamics of the models were analysed with numerical simulations. In the long-term dynamics, bifurcation diagrams were obtained by scoring the minimum and maximum values of population fluctuations after removing the initial transient (**I-IV**). The simulation length was varied in the chapters from 350 days to 70 000 days in order to uncover the long-term dynamics of the models. In the bifurcation diagrams, the values of different variables were varied one at the time (between 20 to 30 evenly distributed values from the used value range) in order to evaluate the effect of the parameters. MATLAB (versions 2011b-2014b, solvers ODE45 and ODE15s) was used to perform the numerical simulations.

4 Results and discussion

4.1 The role of outside-host growth on epidemiology

Environmentally growing pathogens can produce a range of dynamics from stable equilibrium to cyclic outbreaks of the disease (**I-IV**). The positive equilibrium of the susceptible and infected hosts is always locally stable and the instabilities of the system are caused by the pathogen, specifically due to the outside-host growth of the pathogen (**I**). The destabilizing effect of outside-host pathogen growth depends on the outside-host mortality of the pathogen, which acts as a pathogen population sink when it exceeds the pathogen population growth rate. The destabilizing effect of outside-host growth is seen when it is lower or close to outside-host mortality rate. Once outside-host growth rate increases, host population quickly goes extinct. Higher susceptible host growth on the other hand enables stable coexistence of hosts and pathogens also on higher outside-host growth rates. The results from chapters **I** and **II** suggest that the level of outside-host growth that does not drive the hosts extinct promotes cyclic dynamics. Also increased release rate of the pathogens from the infected, which correspond the pathogen within-host growth rate, has a destabilising effect on the disease dynamics by increasing the pathogen population size in the environment and thus promoting infections.

The strength of the density dependence of the outside-host growth influences the frequency and the amplitude of cyclic behaviour of the system (**I**). Increasing the density dependence of the outside-host growth has a stabilizing effect on the disease dynamics. The density dependence of the outside-host growth determines the strength of intraspecific competition and thus limits pathogen population growth. The size of the pathogen population is directly related to the disease outbreaks – the bigger the outside-host pathogen population is, the higher is the infection risk for susceptible hosts. Increased virulence also acts indirectly as a density dependent mechanism on pathogen population and has a stabilizing effect on the disease dynamics. Higher virulence speeds up the release of pathogens from infected hosts as they die due to the disease and thus increases intraspecific competition among pathogens in the outside-host environment. Also in traditional SI-models, increased host mortality due to the disease has a stabilizing effect to

host-pathogen dynamics when the pathogens are efficiently regulating host growth (Anderson and May 1978a). In our model analyses, however, the stabilizing effect of increased virulence comes from indirectly increased intraspecific competition among pathogens in the outside-host environment that regulates the pathogen outside-host growth. Also the increased outside-host mortality of the pathogens has a stabilizing effect on the disease dynamics by lowering the growth of the pathogen outside-host population.

Increased outside-host growth of the pathogen can drive the host extinct depending on the growth rate of the host. Extinction of the host however does not necessarily result the extinction of the pathogen if the pathogen is able to grow in the environment independently of the hosts. The environmental growth thus facilitates future disease outbreaks once the susceptible hosts become available again. Limiting the pathogen outside-host growth is thus crucial for disease prevention. Solely treating the hosts does not eradicate the disease agents from the environment and therefore fails to prevent future disease outbreaks. This has been seen for instance in the disease control attempts of cholera and columnaris disease with antibiotics (Rahman et al. 2008; Pulkkinen et al. 2010). The ability to grow independently of hosts might also promote development of higher virulence, as the cost of virulence does not directly limit future population growth even though it might have stabilizing effects on disease dynamics.

4.2 Invasion of novel environmentally growing pathogens

Many routes to pathogenicity have been suggested, such as pathogens rising form normal microflora, through phoresy or through prey survival inside a predator (Schmid-Hempel 2011). It is logical to assume that environmentally growing opportunist pathogens emerge from free-living microbes as they gain mutations allowing them to infect potential hosts when given the opportunity. Many free-living aquatic and soil bacteria contain virulence factors, but do not always act as pathogens – in other words they do not always express these virulence factors (Pallen and Wren 2007; Casadevall 2008; Persson et al. 2009). Environmental opportunism would seem to be beneficial strategy as it enables utilization of both outside-host and within-host resources and would thus give a competitive advantage in the outside-host environment. However, many of the environmental microbes

generally do not cause diseases. What explains specialization to only outside-host environment could be the empirically proven trade-offs between survival and growth in the outside-host environment and virulence (Freitag et al. 2009; Friman et al. 2009; Mikonranta et al. 2012). Another cause of the prevalent specialization could be efficient host immune system. Yet, novel environmentally growing pathogens emerge from time to time and also many well-known pathogens that have ridiculed humans and our food production for a long time can reproduce in the outside-host environment (Casadevall 2008; Veneault-Fourrey and Martin 2011; Brown et al. 2012).

Chapter **II** addresses the issue of invasion of newly emerged environmentally growing pathogenic strain under outside-host competition with a superior non-pathogenic strain. Competition in the outside-host environment prevents invasion of novel pathogens when the competition in the outside-host is strong. Successful invasion in the presence of a superior non-pathogenic competitor requires high infectivity, within-host growth and virulence. Thus, the model analysis shows that novel environmental opportunist pathogens can out-compete superior non-pathogenic competitors in the environment when they are able to gain high enough fitness advantage from the within-host growth. Higher virulence might also be promoted in environmental opportunists as compared to host-specialized obligatory pathogens to give them a competitive advantage in the outside-host environment. Increased mortality of the hosts is traded off with lowered transmission among host-specialized obligatory pathogens according to the virulence-transmission trade-off theory (Read 1994; Frank 1996; Levin 1996; Walther and Ewald 2004). For environmentally growing saprotrophic pathogens that can also replicate and be released from a dead host (Kunttu et al. 2009), increased mortality of the host might not carry the same costs as for obligatory pathogens. Replication in a dead host has actually measured to be higher than replication in a living host among saprotrophic *F. columnare* (Kunttu et al. 2009).

It should be noted that when outside-host mortality of the pathogens or competition is very high, novel pathogens fail to invade. Under strong outside-host competition higher outside-host growth of the pathogens does not help invading pathogens. On the contrary, higher outside-host growth of the pathogens hastens the depletion of available susceptible hosts and subjects novel pathogens further to intra- and inter-competition under already intense competition thus limiting the ability of novel pathogens to invade. Higher

susceptible host growth rate is needed when competition is intensified to gain fitness advantage from the within-host growth in order to ensure successful invasion of the pathogen. Without the fitness advantage from the within-host growth, invasion of novel pathogens would not be possible under outside-host competition with a superior non-pathogenic strain.

The results above are based on a model describing an environmentally transmitted pathogen that is released from the infected upon their death to infection. Other model versions are also considered in chapter **II** (Supplements S1-S5) regarding invasion success of a novel environmentally growing pathogen. Direct transmission of the pathogens between susceptible and infected hosts does not alter the invasion results above. However, when the infected hosts are able to recover from the infection, higher pathogenicity, referring here to environmental transmission rate, within-host growth and virulence, is needed in order to invade as a novel pathogen. The pathogen should increase the within-host replication, as the recovery of a host would be a dead-end for the pathogen. Therefore those pathogens that are able to effectively infect, grow and release from the infected are crucial in preventing the within-host strategy becoming a population sink for the pathogen. In the scenario where infected hosts are able to recover from the infection, also higher virulence promotes invasion of novel pathogen if outside-host competition is weak. Pathogens should thus aim to kill the host before it is able to recover from the disease in order to gain fitness from the within-host. Furthermore, already a low level of outside-host competition is able to prevent invasion in this scenario, as the competitive advantage from the pathogenicity is in this case lower as compared to a situation where the host is not able to recover. Lastly, also lower level of pathogen outside-host growth rate than in a situation where infected hosts are unable to recover is sufficient to prevent invasion. This scenario could thus promote specialization towards within-host life as compared to opportunism among novel environmentally growing pathogens.

Continuous release of the pathogens from the infected as opposed to release upon the death of an infected host alters invasion results presented above only when both competition and virulence is high. In this situation pathogen is not able to invade. High virulence does not benefit continuously released pathogens in contrast to pathogens released upon the death of an infected as it diminishes within-host replication by cutting down the release from the living hosts. Thus lower level of virulence should be promoted

when release of pathogens happens only from the living hosts as compared to pathogens that are released from a dead host. More benign pathogen that does not result the death of an infected host is on the other hand able to invade regardless of the level of outside-host competition, except trivially when pathogenicity is very low. In this situation, when either continuous release rate or environmental transmission is very small, the novel pathogen fails to invade, as it is unable to replicate sufficiently in either outside-host environment or within-host.

The invasion results in chapter **II** indirectly imply that reduced microbial diversity and thus relaxed environmental competition give room for invasion of novel pathogens. Also high densities of available hosts promote emergence novel environment opportunist pathogens. This scenario is common in intense farming. High usage of antimicrobials in food production on the other hand increase leakage of antimicrobials to the environment and affect competition among microbes (Alanis 2005; Davies and Davies 2010; Oliveira et al. 2012) possibly promoting invasion of novel diseases.

Trade-off between the ability to grow in the outside-host environment and within-host might not be as costly for a novel invading pathogen as to already established pathogen because, as described above, low level of outside-host growth increases the changes for successful invasion as compared to higher level of outside-host growth in a situation where novel pathogens have to compete with superior non-pathogenic microbes in the outside-host environment. The influence of outside-host competition on the level of virulence among novel invading pathogens depends on the other hand on the type of pathogen release from the hosts. Outside-host competition promotes high level of virulence among novel environmentally growing pathogens that are released upon the death of an infected. If however, pathogens are released only from the living hosts, lower level of virulence should be promoted in order to gain successful invasion. The results from chapter **II** thus give novel insights into evolution of pathogens from free-living form.

4.3 Influence of competition in the outside-host environment on long-term disease dynamics

The results from the chapter **II** show that strong outside-host competition can prevent the extinction of hosts, which would occur in the absence of environmental microbial competition because of the environmentally growing opportunist disease. Intense outside-host competition between non-pathogenic strain and pathogenic strain can also result the extinction of already established pathogenic strain, completely preventing further disease outbreaks. Furthermore, outside-host competition can produce also cyclic dynamics as well as allow locally stable co-existence of the competitor, the pathogen and the hosts.

When the density dependent intra- and inter-specific environmental growth factors are equal, the pathogen is able to outcompete the competitor with greater outside-host growth rate if it gains sufficient competitive advantage from within-host growth. This requires that the growth rate of the susceptible hosts has to be high and also the ability of the pathogen to grow in the outside-host environment needs to be sufficient.

However, if competitive pressure of the non-pathogenic strain on pathogen´s growth is somewhat stronger than the pathogen competitive ability, the dynamics are cyclic and non-pathogenic competitor density is increasing while populations of hosts and the pathogen are decreasing. As competition pressure inflicted by the non-pathogenic strain intensifies, the pathogen population stabilizes close to zero and the susceptible host population is able to increase in size. When competition pressure inflicted by the non-pathogenic strain on the pathogen is strong, the pathogen goes extinct even though both susceptible host growth rate and pathogen outside-host growth rate would be high. Outside-host competition has been shown to prevent disease outbreaks or drive pathogens extinct also in a sigmoidal infectivity response model for environmentally growing pathogens (Anttila et al. 2013). In the same paper, analyses of the linear infectivity response model was however not able show prevention of disease outbreaks or extinction of the pathogen by increased due to outside-host growth (Anttila et al. 2013). Results from the chapter **II** also show that increasing susceptible host growth rate has a stabilizing effect on disease dynamics, which can also be seen in chapter **I** results, although here the susceptible host growth rate is allowed to reach higher levels and thus the result is more

clear. This result is comparable to traditional SI-dynamics, where reduced host reproduction can be destabilizing (Anderson and May 1978b).

4.4 Pathogen outside-host predation in disease control

Parameter values for the outside-host predation model were chosen to cause stabilization of the susceptible host population close to extinction due to the disease in the absence of pathogen predators. We could thus establish that adding predation pressure of the pathogen into the system profoundly influenced disease dynamics. Outside-host predation of the pathogen promotes cyclic disease dynamics similarly as predicted by classical predation-prey theory (May 1973; Kendall 1998). The cyclic disease outbreaks are produced as outside-host predation of the pathogen acts as a pathogen population sink allowing periodic increase of the susceptible host population. However, there is a threshold for predation pressure under which predators are unable to invade the system unless they are continuously introduced there. This is the case when either predator attack rate or prey conversion rate is very low. Once the predator is able to invade the disease dynamics are cyclic until it is stabilized at high predation pressure level, where the populations of the predator, pathogen and infected are close to extinction. At low to intermediate attack rates that allow invasion of the predator the disease dynamics are purely cyclic. At higher attack rates there are two coexisting attractors (cyclic and locally stable equilibrium). Once the attack rate increases further, the disease dynamics are locally stable allowing co-existence of all the populations in the system. Similar transition from cyclic to either cyclic or locally stable to only locally stable dynamics is seen when the ability to convert prey to predator growth is varied.

In order to accomplish complete extinction of the pathogen, predators need to be added to the system continuously in the scenario where predators are pathogen specialists. Chapter **III** results show that even a weak predator can cause the extinction of the pathogen on quite low external introduction rates of the predator. Higher introduction rates are needed the weaker the predator is for accomplishing extinction of the pathogen. Under the extinction threshold of the pathogen the disease dynamics are cyclic, unless the predator is very inefficient. In this case, there is a parameter area allowing for stable co-

existence of all the four populations. However, in this scenario the susceptible hosts are very close to extinction. Paradoxically, when using a very weak predator in biological control, the infected population can increase under the pathogen extinction threshold with increasing predator introduction rate. Here however, the outside-host pathogen population is decreasing as predators are added more to the system. The presence of predators allows susceptible host population to increase as the pathogen population is reduced due to predation. Yet, when the predator is not efficient enough in suppressing the pathogen population, the pathogen population does not decrease fast enough to prevent further infections. Increased growth of the susceptible host population actually offers improved infection possibilities for the remaining pathogen population and we can see an increase in the amount of infections even though the overall pathogen population is decreasing. To ensure successful biological control of the disease the predator efficiency in feeding pathogens and the necessary introduction rate of the predators should always be estimated beforehand for a particular system in question.

To summarize, outside-host predation can be efficient method in disease prevention. Empirical evidence also suggest that presence of pathogen predators in the environment can lower virulence in bacteria due to evolution of growth and virulence related traits and the associated trade-offs (Friman et al. 2009; Mikonranta et al. 2012). Protozoa predation has also been successfully tested against environmentally growing opportunist pathogens, such as *Salmonella typhimurium* and *Klebsiella pneumonia* (Mallory et al. 1983; Schmeller et al. 2014). It has also been shown that presence of pathogen predators in the outside-host environment can prevent invasion of novel pathogenic bacteria strains (Liu et al. 2012). Thus, maintaining efficient top down control in the outside-host food web has a potential to diminish disease outbreaks.

4.5 Outside-host phage therapy in disease control

Using phage therapy in disease treatment has recently evoked a lot of interest since antimicrobial resistance is becoming more common (Skurnik and Strauch 2006; Oliveira et al. 2012). However, the theory on how bacteriophages could be utilized in eradicating environmentally growing pathogens from the environment has thus far been lacking.

Chapter **IV** studies how outside-host parasitism of the pathogen influences environmentally growing disease dynamics and if outside-host phage therapy could be utilized in biological control of these diseases. Three model versions are presented in **IV** considering different phage resistance scenarios.

The first model version (figure 3.8) makes the assumption that the pathogen is not able to develop resistance towards bacteriophages. The aim here is to assess how the presence of pathogen viruses affects disease dynamics in a situation where the pathogen would otherwise drive the host extinct. The results show that the presence of phage with high enough transmission rates successfully prevents the extinction of the host. In this case, the susceptible host population is able to stabilize close to its carrying capacity. Phage with high transmission rate will quickly drive pathogen population close to zero, followed by almost complete eradication of infections among hosts. This happens regardless of phage release rate as long as phage release rate is above zero. Instead, when the phage transmission rate is low, the pathogen will drive the hosts extinct and the dynamics reduces to classical prey-predator dynamics between the pathogen and the phage. All in all, how efficient the phage is in infecting pathogen cells makes a difference to the success of the disease control. Phage with strong ability to infect pathogens is able to almost completely eradicate the disease from the host population.

The first model version (figure 3.8) discussed above is in many cases unrealistic in long-term disease control, as it does not consider the development of resistance against bacteriophages. Previous phage therapy models that have addressed outside-host treatment of cholera with phages disregard the possibility of development of resistance towards bacteriophages and are thus unrealistic when considering long-term dynamics (Jensen et al. 2006; Kong et al. 2014). The development of phage resistance among bacteria is common and usually also quite rapid phenomena (Oliveira et al. 2012). The second model version (figure 3.9) presented in **IV** thus considers the ability of the pathogen to develop phage resistance. Here, the model assumes a strong trade-off between phage resistance and pathogenicity, where development of phage resistance completely inhibits pathogenicity. In this scenario the development of phage resistance can eradicate the disease from the host population completely as phage resistance spreads. This scenario can also produce stable and cyclic dynamics, where all the five populations are present. When transition rate from pathogenic form to resistant non-pathogenic form is low the disease dynamics

between the pathogen and susceptible and infected hosts are cyclic. As transition rate from pathogen to phage resistant non-pathogenic microbe increases, there is a parameter area where the number of infections actually increase similarly as seen in the case of outside-host predation. This can happen as phage with low transmission rate goes extinct and the increased transmission rate from pathogen to phage resistant allows periodic increase of susceptible host population. This in turn enables higher infection potential for the remaining pathogens even though the pathogen population in the outside-host environment is decreasing. Once the transition rate increases further, the disease dynamics stabilize allowing depletion of infections and increase of the susceptible host population until the disease is completely eradicated. This is possible even though the phage transmission rate would be low. However, if phage transmission is very low, the hosts and the phage go extinct, as phage is unable to prevent infections regardless of transition rate from pathogen to phage resistant non-pathogenic microbe. Yet again, knowledge of the biological system in question is necessary in order to predict the outcome for the disease control by phage therapy in practice.

The third model version (figure 3.10) of outside-host phage therapy relaxes the extreme trade-off assumption between phage resistance and pathogenicity by allowing reduced infectivity of the phage resistant pathogen as compared to non-resistant pathogen. A stable coexistence of all populations is possible when phage resistant transmission rate is very low. Otherwise, the phage resistant pathogens with even a low infectivity will cause the extinction of the host and the phage. In the absence of hosts and phages the non-resistant pathogen and the phage resistant pathogen persist in a stable coexistence in the outside-host environment. Here, even a slight change towards infectivity among phage resistant would render phage therapy useless. This scenario would thus result failure of phage therapy in disease control and other disease control methods should be considered, such as intensifying outside-host pathogen predation and microbial competition. In conclusion, the successfulness of the outside-host phage therapy as a biological control method crucially depends on the trade-off scenario between the infectivity and phage resistance as well as the efficiency of the phage in infecting pathogens.

5 Conclusions

There seems to be a trend of increased occurrence of environmentally growing opportunist diseases promoted by industrialization, urbanization and intensified food production both creating hotspots for high densities of potential hosts and providing vast amounts of outside-host nutrients. In this thesis I analyse the disease dynamics and control possibilities of environmentally growing pathogens. These model analyses aim to be the first step in understanding this vast disease class as previous theory on environmentally growing pathogens is mostly lacking.

I propose that limiting the ability of an environmental opportunist pathogen to replicate and survive in both within-host and outside-host is a key to control environmental opportunist disease outbreaks. High usage of nutrients in intensive farming is a problem because it promotes the growth of environmental opportunists in the outside-host environment while high densities of cultivated species make opportunist within-host growth a beneficial replication strategy. For instance, improved removal of dead fishes and fish feces from rearing tanks could limit saprotrophic growth of *F. columnare* in freshwater fisheries.

Also the loss of environmental biodiversity promotes invasion of novel environmentally growing opportunists, and changes the long-term epidemiology. Diverse ecosystem with multiple antagonist interactions, such as microbial competition, pathogen predation and viral infections of the pathogens is essential in order to keep already established environmentally growing pathogens in check. The model analyses suggest that both outside-host predation and phage therapy can give promising outcomes as biological control methods against environmentally growing opportunist diseases, against which antimicrobials are quite useless. With both outside-host predation and with phage therapy, it is essential to know the biological properties of a particular pathogen-antagonist system in order to gain successful local eradication of the disease. Counter intuitively, the presence of very weak predators or bacteriophages can actually promote the number of infections even though the overall pathogen population is decreasing. The outcome of the disease control is also crucially dependent on the trade-off properties between pathogenicity and phage resistance. Continuous external addition of even weak predators to the system with high enough rates can however be a very efficient control method

against these diseases. The same applies to outside-host phage therapy when the phages are at least relatively good in infecting pathogen cells and the possible development of phage resistance comes with a strong trade-off in pathogenicity.

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