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Mathematical models of environmental opportunist pathogen dynamics

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May 19, 2015

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ACADEMIC DISSERTATION

To be presented for public examination with the permission of the Faculty of Biological and Environmental Sciences of the University of Helsinki in Auditorium 5, B–building in Viikki (Latokartanonkaari 7) on Friday 29th of May, at 12 o'clock noon

Helsinki 2015

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ISBN 978-951-51-1257-6 (paperback)

ISBN 978-951-51-1258-3 (PDF)

http://ethesis.helsinki.fi

Unigrafia, Helsinki 2015

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I A mechanistic underpinning for sigmoid dose-dependent infection. Anttila, J., Mikonranta, L., Ketola, T., Kaitala, V., Laakso, J. and Ruokolainen, L. *In review in Oikos.*

II Loss of competition in the outside host environment generates outbreaks of environmental opportunist pathogens. Anttila, J., Ruokolainen, L., Kaitala, V. and Laakso, J. *PLoS ONE. 2013. 8(8):e71621.*

III Environmental variation enables invasions of environmental opportunist pathogens. Anttila, J., Laakso, J. and Ruokolainen, L. *Manuscript.*

IIII Environmental variation generates environmental opportunist pathogen outbreaks. Anttila, J., Kaitala, V., Laakso, J. and Ruokolainen, L. *Manuscript.*

Contributions:

	I	II	III	IIII
Original idea	JA	JL	JL	LR
Model construction	JA	JA	JA	JA
Model analysis	JA, LR, VK	JA, LR	JA, LR	JA, LR
Experimental work	LM, TK	-	-	-
Data analysis	JA, LR	-	-	-
Manuscript	JA, JL, LR, VK, TK, LM	JA, JL, LR, VK	JA, JL, LR	JA, JL, LR VK

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verum pone moras et studium lucri,
nigrorumque memor, dum licet, ignium
misce stultitiam consilis brevem:
dulce est desipere in loco.

— Horatius, Odes IIII.13

Abstract

Environmental opportunist pathogens are a class of organisms that are able to both infect multicellular hosts and grow in the outside-host environment as free-living organisms. Environmental opportunism differs from obligate parasitism in that direct host-to-host contact is not necessary for disease transmission and that there are environmental pathogen reservoirs which in suitable conditions act as sources of infection. Because of this, environmental opportunist pathogens form a persistent threat to human health, livestock, and wildlife, and cannot be eradicated by treating hosts. Three well-known examples of pathogens of this class are *Vibrio cholerae*, *Flavobacterium columnare*, and *Bacillus anthracis*, all of which cause sporadic outbreaks. Between infections, these pathogens are subject to multiple biotic and abiotic environmental pressures in the outside-host environment. While environmental opportunist pathogens are not dependent on live hosts for transmission and thus benefit from increased virulence, balancing between the two environments, within-host and outside-host, might incur trade-offs and thus limitations to their spread.

In this thesis I have developed mathematical models of environmental opportunist pathogen dynamics and studied the effects of environmental variation and outside-host interactions on patterns of pathogen outbreaks. The studies included in the thesis address (i) the origin of a sigmoidal dose-dependent infectivity response, (ii) the effect of competition in the outside-host environment on opportunist pathogen outbreaks, (iii) the effect of environmental variation on environmental opportunist dynamics, and (iiii) how environmental variation enables invasions of emerging opportunist pathogen strains. The modelling approach has enabled identification of factors such as alleviation of competitive pressure and certain kinds of environmental variation as outside-host environmental factors that promote outbreaks. Additionally, modelling results can be used to suggest control strategies to reduce the probability of environmental opportunist pathogen outbreaks.

1. Mathematical modelling in ecology and epidemiology

1.1 A brief historical overview

Dynamic models describe changes of states in time. States that are of interest could be, in ecology for example the densities of certain species and in epidemiology the densities of a host species in different states of health or infection. While dynamic models provide a trajectory for the evolution of states from a given set of initial states and parameter values, this is only rarely directly useful in a predictive sense. This is because most models are crude simplifications that are limited in scope to contain a mathematical description of only the most relevant features of a certain phenomenon.

Simple dynamics models are in the development of a theoretical understanding of the systems and phenomena modelled. This understanding is put to test when a model is developed and assumptions are made, and then either reinforced or made questionable when the model output qualitatively matches observed behaviour or bear no resemblance with reality whatsoever. Models that sensibly reproduce some dynamical properties of nature can be further used to explore conditions or situations that are difficult to directly observe, and propose hypotheses with the formal and concise language of mathematics.

The change of a quantity in time is readily expressed by a differential equation, i.e. an equation that relates an unknown function with its derivative. In a dynamic model this describes the change of a state, e.g. population density, as a function of time. While the mathematics of differential equations was developed already in 1666 and 1674 independently by Isaac Newton and Gottfried Leibniz and since adopted as 'the language of physics', widespread use in sciences concerned with populations of interacting entities started much later. The era of mathematical ecology began in 1925 when Alfred J. Lotka published his work 'Elements of Physical Biology' (Lotka, 1925).

For this, mathematical modelling in ecology, evolution, and epidemiology owes much to the development of chemical reaction kinetics through the ideas of Waage & Guldberg (1864). Their celebrated 'law of mass action' relates the reaction rate of two interacting molecules to a rate constant and the densities of the two molecules. By similar reasoning, this idea has been since utilised in dynamic models of interactions between much larger entities, despite the fact that entities such as large animals do not move about by diffusion or react by collisions.

Many models assume other, more complicated mathematical forms for interac-

tion terms on basis of reasoning or empirical observations. The best example in ecological theory is probably the holling disk equation used to describe predator–prey interaction (Holling, 1959). While these forms are often as such useful, how these more complicated functional forms arise is an interesting question. Attempts to derive them from 'first principles', i.e. simpler models that only contain 'law of mass action' interaction terms can shed light to the mechanistic understanding of the interactions.

1.2 Combining ecology and epidemiology

1.2.1 Introducing environmental opportunist pathogens

This Thesis presents dynamic models of environmental opportunist pathogens. Environmental opportunist pathogens are organisms that are able to both live and grow freely in the environment and enter and exploit a host body. These pathogens presented in more detail in section 2 below. The main focus is on bacterial pathogens, but fungal and other microbial or even multicellular pathogens of this class exist.

Environmental opportunist pathogens combine two different worlds: ecological dynamics in the environment, and epidemiological dynamics through entering and infecting hosts. Some pathogens stop here. They harm their host, possibly killing it, but have no means of exiting the dead host and thus the host is a dead end to the pathogen (Adiba *et al.*, 2010). In a more interesting scenario the pathogens not only infect and harm the host, but reproduce inside it and can return to the environment after killing the host or by continuous shedding. In this case the infection is not purely coincidental (Brown *et al.*, 2012), but can lead to selection of virulence factors and evolution of host–parasite interaction.

1.2.2 Ecological tools

One defining characteristic of environmental opportunist pathogens is that they are able to exhibit growth in their environmental free-living state. As such, they are also part of an environmental community that could consist of other strains or phenotypes of the same species, and other species, microbial or otherwise. Interactions with these species could be competitive, predatory, parasitic, or mutualistic, and exchange of genetic material between different bacterial strains or species could also occur. These growth and interaction dynamics can be modelled with the classical mathematical ecology toolbox (Kot, 2001), e.g. logistic (Verhulst, 1838) or chemostat (Monod, 1950) growth models, Lotka–Volterra competition models (Volterra, 1926; Lotka, 1932), and resource–consumer, or more specifically, predator–prey models (Rosenzweig & MacArthur, 1963). Competition for limited resources can be cosidered one of the most profound interactions and microbial communities especially are shaped heavily by different ways of competing since migration to new resources is not possible to them in the large scale (Hibbing *et al.*, 2010).

1.2.3 Epidemiological tools

Classical epidemiological models, starting from the work of Kermack & McKendrick (1927), consider infectious diseases that are transmitted by host-to-host contact. Such models are suitable for pathogens that are obligate parasites and have a very short life span outside a host body. This allows for major simplifications as the models can be fully centred on host dynamics. The classical epidemiological models assign the host individuals into compartments of susceptible (S) and infectious (I). Additional compartments of recovered or removed (R) and exposed (E) can be included to more realistically model diseases that grant immunity upon recovery or for which there is a significant incubation period, respectively. Host dynamics consist of a description of transitions between these states. Simple epidemiological models with small variations have been widely and succesfully applied to many actual case studies (Brauer *et al.*, 2008).

The formation of immunity is an important factor in epidemiological dynamics because it affects the stability of host–pathogen interactions and the time-scale and characteristic patterns of epidemics. The duration of immunity after recovery varies to a large extent. For example measles in humans conveys a lifelong immunity (Krugman *et al.*, 1965), pertussis conveys immunity that lasts for 4–20 years (Wendelboe *et al.*, 2005), and respiratory syncytial virus (Hall *et al.*, 1991) and many venereal diseases such as chlamydia and gonorrhea convey only partial or no immunity at all.

1.2.4 Infectivity response

There has been much debate on the appropriate infectivity response, i.e. the shape of the function linking the encounters with infectious individuals to the probability of getting an infection (McCallum *et al.*, 2001). The simplest and most common is obtained by multiplying the densities (numbers per unit area) of infectious individuals and susceptible hosts with a rate constant (most often appears as βSI) as described by the law of mass action. This term is often called bilinear, i.e. linear in S and linear in I, or just simply linear infectivity response.

In models of environmental pathogens infection is the main reaction that combines the ecological outside-host community to the epidemiological host dynamics. Thus, how this is incorporated in the model is extremely important. Transmission of pathogens from environment to host and from host to host are not entirely similar. In host-to-host transmission, the amount of pathogens transmitted in a contact is not considered important. Each contact with an infectious individual can be thought of having a good chance of infecting a susceptible individual (otherwise why call an individual infectious). In environment-to-host transmission the contacts are between susceptible host and the infective particles, i.e. pathogens. A contact does not necessarily have a good chance of causing an infection, and contacts can be much more frequent. In this sense, alternatives to the 'law of mass action' term should be considered.

While the simplest mathematical forms of infectivity response are often a good first approximation in direct host-to-host transmission, there are a number of reasons why other forms of infectivity re-

sponse might produce behaviour that is qualitatively more realistic. Departing from the linear form, an increase in pathogen encounters could give rise to an overproportionate or underproportionate increase in the probability of infection, leading to a convex or a concave shape of infectivity response, respectively. Underproportionate increases are backed by empirical studies on parasite dose-response (Regoes *et al.*, 2003), and understandable, as one might reason that an increase in pathogen encounters might increase the probability of infection less when the rate of encounters is already large.

When the number of encounters per time unit is small, overproportionate increases in probability of infection with increasing pathogen encounters are likely. Most multicellular animals come into contact with potential pathogens from the environment regularly and their immune system most likely takes care of the intruders. The concept of an infectious dose is relevant most notably in bacterial diseases which are transmitted through ingestion (foodborne or waterborne). While it has been shown that there is no completely safe dose of such bacteria, the probability of any symptoms given a dose smaller than a certain infective dose is very small. For example for toxigenic and non-toxigenic *V. cholerae* these are in the order of 10^4 and 10^6, respectively (Kothary & Babu, 2001).

The mechanism behind the convex shape of the infectivity response with doses below an infective dose in bacterial pathogens could have two origins. Firstly, many bacteria require co-operative effort to defeat the immune system and exploit host tissue. Secondly, the immune system effort to fight off the intruders might get saturated when facing a large quantity of infective particles. There are several studies concerning the co-operative effort of bacteria in infection process. For example, it has been shown that *V. cholerae* expresses virulence factors only when the density of cells is high enough (Zhu *et al.*, 2002). Another important example of co-operative effort in infection process is the formation of biofilm, which makes the bacterial cells inaccessible to the immune system but can not be produced in sufficient quantity by only a few cells (Hall-Stoodley & Stoodley, 2005; Hoiby *et al.*, 2011).

Combining the reasoning behind both overproportionate and underproportionate relationships between pathogen density and probability of infection leads to a sigmoidally shaped relationship which is convex at low pathogen densities and concave at high pathogen densities. In addition to being theoretically sound, the sigmoidal infectivity response is supported by empirical work (Glynn *et al.*, 1994; McLean & Bostock, 2000, **I**).

1.2.5 Environmental variation

Ecological and epidemiological patterns of environmental pathogens are not explained solely by species interactions as the abiotic environmental conditions vary. This variability is recognised as a key driver in many ecological systems (Halley, 1996; Ruokolainen *et al.*, 2009). In addition, not all biotic interactions can be feasibly included in a model as such, and to some degree the minor interactions can be thought of as adding to the environmental variability. Environmental variation can have a large effect on environmental opportunist pathogen dynamics because a large part of their

life cycle can be spent between infections.

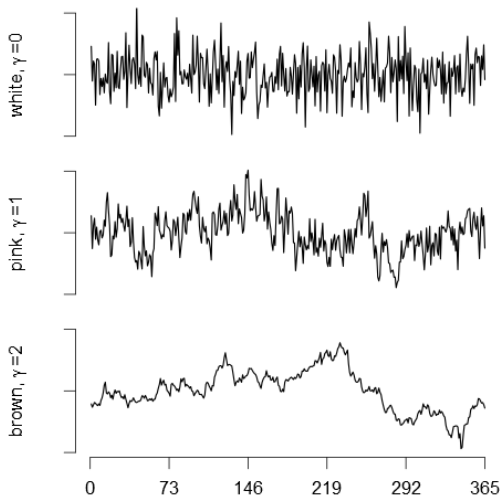

Figure 1.1: Stochastic variations at different levels of autocorrelation, i.e. 'colour'.

The two most important environmentally varying abiotic factors are temperature and rainfall, both of which have clear annual patterns but vary randomly on a shorter timescale. Indeed, environmental variation is most often partly predictable, i.e. deterministic, due to diurnal and seasonal fluctuations, and partly unpredictable, i.e. stochastic. Most studies have been conducted using mathematically simple variation such as sinusoidal variation (Nisbet & Gurney, 1982) or white gaussian noise, i.e. uncorrelated normally distributed random numbers (Roughgarden, 1975). However, variation that best resembles natural evironmental variation is obtained by generating stochastic noise with a certain kind of autocorrelation structure, or spectrum or 'colour' (Halley, 1996). It is also important to consider the ecology of constituent species with respect to the time scale of environmental fluctuations (Ruokolainen *et al.*, 2009).

1.3 Model analysis by numerical simulation

The tradition of mathematical modelling in ecology, evolution, and epidemiology was developed on pen and paper long before the advent of computational resources. For this reason, old literature presents mainly simplified models and analyses of their equilibria in different conditions. Because of this simplicity some very general results can be derived for these models. For example, with reasoning based on a very crude predator–prey model Vito Volterra was able to give a perfect argument to the question of predatory fish density increase during the war years as posed by Umberto D'Ancona (1954). Even though the model proposed by Volterra is considered 'structurally unstable', i.e. having unrealistic stable state properties, by modern mathematical ecologists it is of seminal importance even in the modern day ecology (Kot, 2001).

Traditionally, the main objective in modelling methodology has been to derive concise closed-form results by the means of mathematical analysis, and this calls for numerous simplifications. The assumptions of such simplified models can be restrictive in terms of application. In models based on differential equations adding complexity such as additional state variables, additional terms, or nonlinearities in model structure, can quickly make analytical work on model equilibria unfeasible so that either the expressions for equilibria are so complex that no general results can be stated based on them, or equilibria can not be

obtained at all.

Including more realism requires a different approach in model analysis. In the past few decades, some of the focus has shifted to analysing models by numerically simulating the model equations. While some generality is easily lost and outcomes cannot be expressed concisely in the form of simple equations, this approach opens doors to exploring models that capture more of the complexities and intractability inherent in natural processes (Sharma & Annila, 2007).

A model that needs to combine many different elements, such as the combined ecologial and epidemiological dynamics explained in section 1.2 above, cannot be easily simplified without losing some essential features of the actual system. For the most part, this thesis work resorts to numerical methods for model analysis.

2. Environmental opportunist pathogens

2.1 In general

Environmental opportunist pathogens are a class of organisms that are able to both persist in the outside-host environment as free-living organisms and invade and exploit multicellular hosts. Such pathogens, emerging from the environment, are a persistent threat to humans, food production, and wildlife. Some well known examples of this class, all of which can cause severe outbreaks, are: *Vibrio cholerae* in humans, *Flavobacterium columnare* at fish farms, and *Bacillus anthracis* in livestock and wild animals. These examples are discussed in more detail below. A few other environmental bacterial species, e.g. *Serratia marcescens*, are similary able to infect a wide variety of hosts, but do not cause outbreaks and have less impact in general. The white nose syndrome in bats is a fungal example of an environmental opportunist pathogen. This soil inhabiting fungus infects bats during hibernation, dramatically reducing winter survival.

Environmentally transmitted, but not free-living, pathogens have been studied to a larger extent. Although not true environmental opportunists, they differ from classical host-to-host transmitted pathogens in disease dynamics, and also in the evolution of virulence (Bonhoeffer *et al.*, 1996; Day, 2002). In host-to-host transmission the host is required to stay alive to make contacts with susceptible hosts, but in transmission via the environment this constraint is removed. Thus in environmentally transmitted diseases evolution should favour higher virulence. Moreover, if the pathogen can consume a large part of the dead host body, it benefits greatly from increased virulence (Godfray *et al.*, 1999; Kunttu *et al.*, 2009). Indeed, many diseases caused by environmentally transmitted diseases can be lethal (Walther & Ewald, 2004). Quite a few models of environmentally transmitted pathogens exist, looking either at the disease dynamics (Breban *et al.*, 2009;

Rohani *et al.*, 2009) or evolution of virulence (Day, 2002; Boldin & Kisdi, 2012) in this context.

Environmental opportunist pathogens have an added difficulty in terms of control strategies. They cannot be completely eradicated by treating hosts as they can be re-introduced to a susceptible host population from the environmental reservoir. From modelling point of view, adding pathogen growth terms in the environment to the equations complicates the models considerably. This has, however, been done by a few authors, especially in models of cholera epidemics (Codeco, 2001; Jensen *et al.*, 2006; Bertuzzo *et al.*, 2010), but also otherwise (Joh *et al.*, 2009; Merikanto *et al.*, 2012).

2.2 Well-known examples

2.2.1 *Flavobacterium columnare*

F. columnare is a fish pathogen that causes major economical losses in commercial fisheries (Pulkkinen *et al.*, 2010). While fish infected by *F. columnare* can be found in natural waters, it can cause massive outbreaks only in high-density cultured environments. The infection caused by *F. columnare* is called columnaris disease and it especially affects young fish in summer when water temperature increases (Kunttu *et al.*, 2012; Pulkkinen *et al.*, 2010). The increase in water temperature is linked to bacterial growth rate increase, and additionally can be a stress factor in salmonid fish. The columnaris disease has very high mortality and the bacterium is saprotrophic, i.e. able to consume the dead fish host tissue, leading to massive bacterial shedding to the surrounding waters (Kunttu *et al.*, 2009).

Interestingly, *F. columnare* has three colony morphotypes: rough, rhizoid and soft (Kunttu *et al.*, 2011). The cells in rhizoid morphotype are infective, motile, and have a certain filament-like cell structure. The motility of the rhizoid morphotype is enabled by a surface protein that is a target to phage viruses. The cells in a rough or soft morphotype do not produce this protein and are thus immune to the phage viruses, but are immotile and non-infective. The rhizoid morphotype is also associated with secretion of chondroitinase enzymes that degrade host connective tissues (Suomalainen *et al.*, 2006).

Bacteriophages are abundant in natural waters (Abedon, 2008) making the phage-resistance highly important for *F. columnare* cells in the outside-host environment. Occasionally, given the opportunity to enter a fish host, the cells benefit much more from the virulence factors of the rhizoid morphotype and are less likely to encounter phages. Some *F. columnare* strains have been observed to switch between the morphotypes quickly, further facilitating responses to changing environment (Laanto *et al.*, 2012; Sundberg *et al.*, 2014).

2.2.2 *Vibrio cholerae*

Cholera is perhapt the most studied environmental opportunist pathogen because of the severe epidemics it can cause in humans. Although not common any

more in developed countries, in the past decade cholera has caused epidemics in Haiti and various parts of Africa (WHO, 2013). *V. cholerae* is found naturally in brackish water and estuaries, and the epidemics are linked to environmental factors (Colwell & Huq, 1994; Lipp *et al.*, 2002; Koelle *et al.*, 2005; Ruiz-Moreno *et al.*, 2007; de Magny *et al.*, 2008).

The strains of *V. cholerae* vary in infectivity ranging from completely non-pathogenic to highly infective isolates from infected hosts (Faruque *et al.*, 1998). The *V. cholerae* cells shed from hosts are termed 'hyperinfective' and are thought to be necessary for epidemic spread (Merrell *et al.*, 2002; Hartley *et al.*, 2006). Much of the virulence is linked to a temperate bacteriophage which contains genes for toxin production and can be transmitted horizontally (Faruque *et al.*, 1998). The ability to form aggregates through biofilm production is also thought to be important in virulence and transmission (Faruque *et al.*, 2006).

2.2.3 *Bacillus anthracis*

The causative agent of anthrax, *B. anthracis*, is a soil bacterium that can infect a wide range of animal hosts, affecting wildlife, livestock, and occasionally humans (WHO, 2008). *B. anthracis* has a rather limited capability to growth in soil conditions, and thus can be considered almost an obligate pathogen. Environmental conditions, however, have an impact on its life-cycle as it can form spores that persist in the environment for long periods of time. Both spore formation and germination require a certain temperature and humidity (WHO, 2008), and thus the incidence of anthrax in wildlife is heavily dependent on temperature and rainfall (Turner *et al.*, 2013).

B. anthracis can infect through various routes and the infection can be acquired by contact with either vegetative cells or spores. Nevertheless, direct transmission in host-to-host contact is belived to be of minor importance, and during an outbreak most infections occur by contact with bacteria shed from diseased carcasses (Beyer & Turnbull, 2009). A dose in the order of 10^4 cells is required for infection via the oral route or inhalation (WHO, 2008).

The virulent strains of *B. anthracis* carry two plasmids encoding for virulence factors (Guignot *et al.*, 1997). One encodes for a thick capsule that helps in immune system evasion, and the other encodes for a toxin.

2.3 Susceptibility to environment and biological interactions

An epidemiologically important characteristic of all the well-known examples, *F. columnare*, *V. cholerae*, and *B. anthracis* presented above is that they most often cause disease outbreaks that can be severe in terms of mortality and spread in the local host population. Rarely do these pathogens cause an endemic state where the disease is prevalent in a population for a long period of time. The classical epidemiological models of obligate pathogens are clearly not adequate

for the purpose of describing this characteristic.

Environmental opportunist pathogens are susceptible to environmental variation, as explained in section 1.2.5 above. Furthermore, the pathogenic species is rarely alone. Competition for shared resources is most likely to reduce the density of the free-living potential pathogen. Moreover, the factors and traits required for host tissue utilisation, immune system evasion, and other means to be pathogenic, are often traded off with some other capability. In *F. columnaris* the virulence factors are traded off with phage resistance, and in *V. cholerae* toxigenic strains most likely have a metabolic cost associated with toxin production. Virulence factors have also been linked to certain nutrient use and predatory defence capabilities (Matz *et al.*, 2005).

The contrasting life styles might explain why, apart from the examples presented here, there are in fact quite few opportunist pathogens that plague the several multicellular host species available. Since the requirements for living free in the environment or within a host body are quite different, it is sensible to assume that for the most of the time the pathogenic forms have reduced competitive ability, making them more susceptible to competitive pressure in the outside host environment. However, many bacteria have a capacity to develop traits, including virulence factors, by rearragements and recruitment of pre-existing genetic material (Ochman & Moran, 2001) or by plasmid transfer (Dionisio *et al.*, 2002) leading at times to rapid adaptation to new conditions.

Despite the fact that the requirements for successful growth inside a host and outside in the environment are different, the variability within a host, especially temporally, is much weaker. Once an organism has acquired the ability to enter and survive within a certain location of a host body, this can be thought of as a refuge from an environment that could contain highly variable hazards, including predators, competitors, toxins, and harsh abiotic conditions. Furthermore, if host tissue can be utilised for reproduction, it is possible to turn the scenario around and view the host as a reservoir in which these facultatively free-living organisms can live through harsh environmental conditions. In this way, environmental opportunist pathogens with their ability escape into and exploit hosts, may have a significant but scarcely understood effect upon environmental microbial communities.

3. Aims of the study

The main aim of this thesis work is to develop mathematical models to study the ecology and epidemiology of environmental opportunist pathogens. Research questions of particular interest are:

- How can the sigmoidally shaped relationship between pathogen density and rate of infections be derived mathematically? The derivation will give insight to the mechanism behind a sigmoidal infectivity response. Chapter **I** presents the derivation and arguments for its use for increased realism in the epidemiological modelling framework. In chapters **II**–**IIII** the models utilise a sigmoidal infectivity response, and in chapters **II** and **III** the results are compared to a classical linear infectivity response.

- What is the explanation to the outbreak nature of environmental opportunist pathogens? Many known environmental opportunist pathogens cause outbreaks that can be severe, but usually die out after an infection peak. Chapter **II** presents a model that can produce cyclic outbreak dynamics. Sporadic outbreak patterns can be triggered by environmental variation, as shown in chapters **III** and **IIII**. Related to this question, chapter **III** looks also into how fast growing environmental pathogenic strains emerge.

- How environmental effects such as competitive interactions and temporal variation targeted at an environmental opportunist pathogen affect epidemiological host dynamics? Additionally, how the ability to gain reproductive output from infected hosts affect the ecology of environmental opportunist pathogens? Chapter **II** looks into the effect of outside-host competitive pressure on the environmental opportunist pathogen. Chapter **III** looks at the effect of environmental variation on a pathogenic strain that has its growth limited by between-strain competition. Chapter **IIII** looks into environmental variation affecting different parts of an eco-epidemiological system, and the role of immunity in environmental pathogen outbreak dynamics.

4. Materials and Methods

4.1 Models of environmental opportunist pathogens

4.1.1 Chapter I

All chapters utilise a dynamic model described by two or more coupled differential equations. This section gives an outline of the model ideas and intentions behind the model structures. For the actual model equations the reader is kindly referred to the respective chapters.

In chapter **I** the intent is to develop a model from which one can derive a sigmoidal infectivity response. A suitable model was found to be one in which each encounter with a pathogen has a fixed probability of increasing the hosts exposure level, whereas immune system operates at a constant rate to decrease the exposure level. A host can become infected only after a certain required amount of exposure levels.

A procedure known as time-scaling (Edelstein-Keshet, 2005) can simplify the model (figure 4.1) by making the assumption that exposure and immune system events settle to an equilibrium much faster than actual infections form. This allows one to express the relationship between pathogen density and infectivity in a single sigmoidally shaped function Furthermore, the model assumes the pathogen density is high enough to remain unchanged by encounters with the hosts.

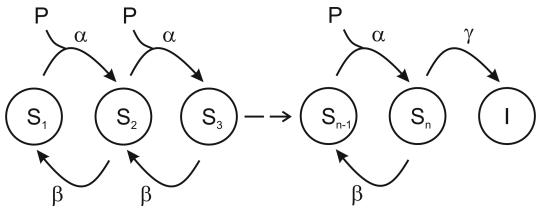

Figure 4.1: Schematic drawing of the model used in **I**. The susceptible hosts S_i with different indices i represent hosts with different level of exposure to pathogens P. Each ecounter (reaction α) increases the level of exposure $S_i \rightarrow S_{i+1}$. Host immune system reduces the level of exposure at a fixed rate (reaction β). If the host reaches a certain level of exposure S_n it can become infected (reaction γ) with a rate that is independent of pathogen density. Assuming that pathogen encounters α and immune system function β are fast processes compared to actual infection γ the rate of infections formed is a sigmoidal function of pathogen density (**II**).

4.1.2 Chapter II

Traditionally, parasitism and resource–consumer interactions are treated separately in ecological theory. However, the models used in **II**–**IIII** may be thought of as resource–consumer models with a microbial consumer that is able to utilise a multicellular host resource in addition to being able to support itself in low densities with other resources in its environment.

Chapter **II** investigates the effect of competitive interactions in the environment to environmental opportunist pathogen dynamics (figure 4.2). The model thus

introduces one or more species to the environment to compete for resources with the pathogen. As a simplification, the competing species are assumed to have the same competitive ability. The pathogen in this model is assumed to be relatively lethal, conveying no immunity, and saprotrophic, matching well with the traits of *F. columnare* described in section 2.2.1 above.

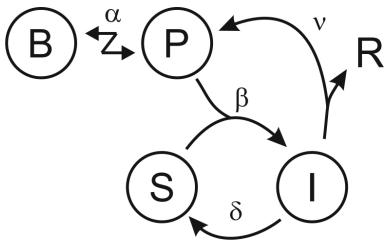

Figure 4.2: Schematic drawing of the model presented in **II**. One or more non-pathogenic species B compete with a pathogenic species P. Pathogenic species P can infect a susceptible host S which becomes an infected host I, An infected host I can recover or die. The death of an infected host I releases more pathogens P. The reactions presented above are: competition α, infection β, recovery δ, and infection mortality ν. In addition, the model contains growth and death reactions for hosts and the outside host community.

4.1.3 Chapter III

Models in chapters **III** and **IIII** add environmental variation. The chapter **III** starts from an idea that potentially pathogenic strains with the ability for fast growth are produced constantly at a low frequency, but can not compete with the parent strain when the environment is stable. The role of environmental variation in enabling pathogen outbreaks is then investigated.

The model assumes a pathogen that causes some mortality, but is only released in the environment by continuous shedding from infected hosts. Additionally, the disease conveys a transient immunity upon recovery. As such, the pathogen traits are mostly similar to *V. cholerae* described in section 2.2.2 above.

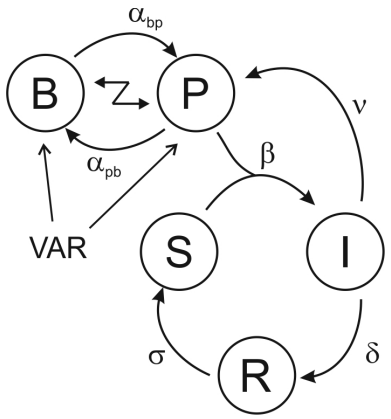

Figure 4.3: Schematic drawing of the model presented in **III**. Benign environmental strain B and opportunist pathogen strain P can transform into one another. P is assumed to be capable of fast growth, but B is assumed to be more efficient competitor, readily outcompeting P in a stable environment. Pathogenic strain P can infect a susceptible host S which becomes an infected host I. An infected host I can recover or die. Infected hosts both shed pathogens continuously to the environment. A recovered host R gains a transient immunity which is lost in time and the host is returned to the susceptible class. In this model environmental variation affects both strains B and P in the same way by varying the carrying capacity in the environment. The reactions presented above are: mutation α, infection β, recovery δ, immunity loss σ, andcontinuous shed λ. In addition, the model contains growth and death reactions for hosts and the environmental strains.

4.1.4 Chapter IIII

Chapter **IIII** looks into the effects of environmental variation in more detail. The model contains a single pathogenic environmental species coupled to host dynamics. A transient immunity is included and the effect of different durations of immunity is studied. In this chapter environmental variation has two targets and its effect is studied separately and to both targets at the same

time. As the first option, the variation targets the pathogen in such a way that it affects both how its rate of growth and its carrying capacity in the environment. As the second option, the variation affects infective dose of a sigmoidal infectivity response, or, put more simply, the susceptibility of the host to the pathogen.

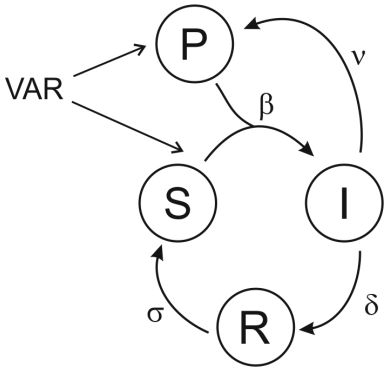

Figure 4.4: Schematic drawing of the model presented in **IIII**. Environmental opportunist pathogen P can infect a susceptible host S which becomes an infected host I. An infected host I can recover or die. Infected hosts both shed pathogens continuously and release pathogens in the event of death. A recovered host R gains a transient immunity which is lost in time and the host is returned to the susceptible class. In this model environmental variation can affect either pathogen growth, infectivity threshold, or both. The reactions presented above are: infection β, recovery δ, immunity loss σ, continuous shed λ, and infection mortality ν. In addition, the model contains growth and death reactions for hosts and the pathogen.

4.2 Implementation of environmental variation

The effect of varying environment in model systems can be studied by varying a parameter or several parameters simultaneously. Natural environmental variation shows both deterministic and stochastic components. Sinusoidal deterministic variation can be incorporated in a model in a straightforward way by letting some parameter value fluctuate according to:

$$\theta(t) = \sqrt{2}\ A\ sin\left(\frac{2\pi t}{f}\right) \qquad (4.1)$$

In equation 4.1 f denotes frequency. The only relevant frequency in this context is that of one year, the other options being too fast or too weak to affect the epidemiological dynamics. Amplitude, denoted by A, sets the variance of the time series around the mean. For comparison with stochastic variation, presented below, this is scaled by a factor of $\sqrt{2}$ to obtain a common variance.

Stochastic environmental variation can be implemented in a model system by varying model parameters according to random numbers. The studies **III** and **IIII** that utilise environmental stochasticity generate this variation as so-called $1/f$ noise (Halley, 1996). The generation procedure, as described by Cohen *et al.* (1998), sums together sine waves with frequencies f ranging from 1 to $2/n$ to generate a noise series of n points:

$$\theta(t) = A\ \sum_{f=1}^{n/2} \frac{1}{f^{-\gamma/2}} sin\left(\frac{2\pi t}{n} f + \phi_f\right) \qquad (4.2)$$

The phases ϕ_f are randomly selected between 0 and 2π from uniform distribution. The $1/f$ noise series can be of dif-

ferent frequency distribution or 'colour' akin to light spectrum. This is controlled by the spectral exponent γ. With $\gamma = 0$ the successive points are uncorrelated leading to 'white gaussian noise'. Increasing γ results in positively autocorrelated or 'red' noise and decreasing γ results in negatively autocorrelated or 'blue' noise. 'Reddened' noise is generally thought of being able to capture a wide range of naturally occurring environmental variation (Vasseur & Yodzis, 2004) and autocorrelation with specifically $\gamma = 1$ or 'pink' noise is considered a realistic proxy for environmental noise (Vasseur & Yodzis, 2004).

Stochastic $1/f$ noise was 'injected' to the simulated systems such that a new value of environmental variation was drawn each day of simulated time. Thus, within a single day the environment would stay the same and the autocorrelation structure generated by equation 4.2 represents autocorrelation between subsequent days.

4.3 Parameter range

In model analysis by numerical simulation it is necessary to choose a parameter space, i.e. ranges of parameters in which the model simulations are run. Since the range of biologically relevant parameter values is vast, a single set of parameter values specific for a certain host–pathogen system works to exemplify model behaviour. For the most part, the models in **II**–**IIII** were studied numerically in a parameter range well suited for a bacterial pathogen and a small animal host. With this choice of parameterisation the results of numerical analyses in **II**–**IIII** are as such best suited to understanding a relatively lethal opportunist disease dynamics in wildlife or fish. However, the models were constructed in a general way to allow for different kinds of parameterisations for different purposes.

The species interaction parameters, especially the parameters in the infectivity response, are very hard to estimate based on reasoning alone. For these, several alternatives were tried and the values with interesting dynamical outcomes were examined in detail. In studies **III** and **IIII**, the effect of environmental variation was investigated by setting the interaction parameters such that in a stable system infections prevalence is very low.

5. Results and discussion

5.1 Infectivity response

In models of environmental opportunist pathogens, a linear infectivity response easily results in an endemic situation where the prevalence of the disease is high and stable (**II**, **III**). The reason for this is that pathogens are to some degree always able to infect and gain reproductive output from the hosts, unless the infectivity rate constant is very close to zero (**II**). This effectively prevents host population recovery.

Mechanistically, a sigmoidal infectivity response can be derived from a simple model that requires multiple successive exposure events to pathogens before actual infection (**I**). In this model, each pathogen contact promotes the susceptible hosts level of exposure while immune system works at constant rate to diminish the level of exposure. If the susceptible host reaches a certain level of exposure, it can become infected. This occurs with a rate independent of pathogen density. This model can be time-scaled such that the pathogen encounters and immune system action are assumed to be fast processes that settle in a pre-equilibrium much faster than actual infections form. With the time-scaling, the rate of infections formed becomes a sigmoidal function of pathogen density. The resulting function has the form:

$$f(P) = \gamma \left(\frac{\alpha P}{\beta}\right)^{n-1} \frac{\frac{\alpha P}{\beta} - 1}{\left(\frac{\alpha P}{\beta}\right)^{n} - 1} \quad (5.1)$$

where P is pathogen density, α is encounter rate, β is immune response rate, and γ is infection rate at full exposure after n encounters (figure 4.1). Here it should be noted that the ratio α/β constitutes a single parameter.

While this functional form derived in **I** is sigmoidal, models in **II**–**IIII** use a similar but mathematically more convenient function that allows adjustment of the sigmoidal slope and 50% infective dose independently:

$$f(P) = \frac{\left(\frac{P}{ID_{50}}\right)^{\kappa}}{1 + \left(\frac{P}{ID_{50}}\right)^{\kappa}} \quad (5.2)$$

The overproportionate increase, or convexity, at low densities has a large impact to epidemiological dynamics. Simpler infectivity terms with overproportionality have been shown to cause multiple equilibria and periodic solutions in models (Liu *et al.*, 1986, 1987). Similarly to an Allee effect, small pathogen densities are not viable and cannot effectively infect. Thus, susceptible hosts are safe unless the pathogen density is increased first by some means. This effect has also been shown in obligate pathogen context (Regoes *et al.*, 2002).

5.2 Environment as a source of infections

Environmental opportunist pathogens can cause a disease outbreak in which the infected hosts release pathogens into the environment. This in turn causes more infections, resulting in a cascade of pathogen density increase and more infections. This cascade goes on until the pool of susceptible hosts is depleted. Then, if the environment cannot support a high pathogen density, it will decay until the susceptible host supply is replenished. If the equilibrium pathogen density in the environment (without host) is negative, cyclic pathogen outbreaks may occur (**II**). The mechanism of cyclic outbreaks here is analogous to that in resource–consumer models, i.e. overexploitation of the resource (Rip & McCann, 2011). Since the pathogen consumer density follows the host resource density with a small delay, this forms an alternating pattern of high and low resource and consumer densities. If the environment can support a high enough pathogen density to cause infections, the susceptible host regrowth is prevented and dynamics are stabilised (**II**).

Without immunity, the host resource can be overexploited by the pathogen and driven to local extinction. The formation of immunity upon recovery prevents this by making recovered hosts transiently unavailable to the pathogen. If the duration of immunity is short, recovered hosts can lose immunity and be re-infected during the same pathogen outbreak, increasing the severity of the outbreak and potential for oscillations. Longer immunity leads to less severe outbreaks and more stability (**IIII**). This is in contrast with results of Gomes *et al.* (2004) on classical epidemiological models of obligate pathogens where longer immunity provides more potential for damped oscillations and shorter immunity tends to stabilise the system. The key factor here is depletion of susceptible hosts. In the models considered by Gomes *et al.* (2004) infection mortality was not included and, as a simplification, host birth rate was equal to death rate in order to keep total host density constant. Thus susceptible hosts could be depleted only by immunisation. In **IIII** the impact of infection mortality is significant and increases with decresing duration of immunity, leading to more depletion of host supply to the pathogen.

5.3 The importance of outside-host interactions

If we assume that a potential pathogen requires virulence factors that come with an upkeep cost paid in competitive ability, the free-living pathogen is outcompeted in the environment by non-pathogenic species (**II**) or non-pathogenic forms of the same species (**III**). Similar reduction in pathogen viability is achieved by assuming a larger cost in competitive ability and by increasing the number of competing species in the environment (**II**). Together with the threshold effect imposed by the sigmoidal infectivity response, competition can prevent the opportunist pathogen from growing to densities in which it can effectively infect hosts.

However, any event that temporarily reduces the competitive pressure, or otherwise increases the density of the free-living pathogen, can result in a pathogen outbreak. If the pathogen can support its density in the environment with the reproductive output from infected hosts, the host-pathogen interaction can result in a bi-stable system. The system will stay indefinitely in either, a state with infections, or in a state with few infections and low pathogen densities, unless disturbed by e.g. environmental variation (**II**,**III**). In the models, the outcome of the bi-stable system depends on the species densities with which the simulations are initiated, as this determines to which attractor the system falls into.

Since competitive pressure can prevent an environmental opportunist pathogen from reaching a density high enough to cause an outbreak, removal of competitive pressure can result in an outbreak (**II**). Competitive pressure could be reduced, for example, by introducing an unspecific mortality factor such as a disinfectant or an unspecific antibiotic to the outside host community. If the induced mortality is not large enough to kill the pathogen, the pathogen can benefit from reduced competition and escape the environmental conditions by entering hosts. In **II** this results in cyclic pathogen outbreaks.

5.4 Environmental variation and its effect

Environmental variation can temporarily change the opportunist pathogen density in the environment and thus affect host dynamics through increased or decreased infections (**III**,**IIII**). Assuming a linear infectivity response the effect of environmental variation is relatively small (**III**). If the infectivity parameter is set low so that recovery rate from infections is much higher, variation has to be extremely strong for any significant amount of infected hosts to form. On the other hand, if the infectivity parameter is set to a higher level, infections form easily with smaller pathogen densities as well. Then, the system easily reaches a state where the disease prevalence is stable, and can again be qualitatively changed only by very high amplitude variation.

Assuming a sigmoidal infectivity response and starting from a situation in which the pathogen equilibrium density in the environment is very low, the prevalence of infections is very small. Applying environmental variation to this can cause the pathogen density to temporarily increase, resulting in a large increase in host infections and further pathogen density increase, i.e. an outbreak. For this effect there is a threshold on variation amplitude. Small amount of variation is not able to increase pathogen density enough to actually increase the infectivity response to the point where it begins to increase significantly.

The colour of environmental fluctuations has a qualitative influence on the dynamics. For white noise, i.e. fast variation ($\gamma = 0$), the threshold effect with amplitude is the highest, requiring stronger variation for any effect on host dynamics (**III**,**IIII**). This is because fast variation is easily absorbed by the system as the changes are not persistent enough to cause much pathogen density accu-

mulation or loss. With environmental 'reddening', i.e. increase in variation autocorrelation ($\gamma > 0$), the infectivity threshold is crossed with smaller variation amplitudes.

Environmental variation can help a fast growing and potentially pathogenic mutant strain to overcome a parent strain that is a better competitor in the environment (**IIII**). The fast growth rate could be e.g. due to additional capacity to take up nutrients from the environment, which could mean more receptors for a certain nutrient or a wider variety of receptors. This additional capacity is useful only when nutrients are readily available, and would only increase the metabolic cost when nutrients are limited and competition for them is fierce.

The fast growing mutant strain could thrive transiently when some event temporarily increases the available nutrients, or reduces overall population densities so that there would be excess nutrients. Otherwise it would be, for the most part, outcompeted by the parent strain with less metabolic cost. These events can be caused by natural variation in the environment and are apparent in a model system that incorporates environmental variation. If this strain is additionally potentially pathogenic with the ability to exploit a nutrient-rich host body, envrionmental variation could cause an outbreak (**III**).

In the model and parameterisation considered in **III** the system contains a stable attractor with high disease prevalence, i.e. the system can be locked in a persistent endemic state. Variation with intermediate amplitude can cause the system to enter this state. Increasing the variation amplitude makes the system also switch back to the state with no infections and low free-living pathogen density. This results in switching between the two states in a way that qualitatively resembles sporadic outbreaks. For the stochastic case, the range of variation amplitudes that can result in the stable endemic high prevalence state is dependent on noise colour. 'White', i.e. uncorrelated ($\gamma = 0$), variation is the least likely to cause switching back to the infectionless state, whereas 'pink' ($\gamma = 1$) variation is the most likely.

The effect of environmental variation depends on which part of the ecological or epidemiological system is affected. In chapter **IIII** two targets were studied: pathogen growth rate, and infective dose, or more precisely, the ID_{50} parameter of the sigmoidal infectivity response (equation 5.2). The effects of variation are more severe if infective dose is affected. If the infective dose drops, more infections form rapidly. Conversely an increase in infective dose can instantly decrease or prevent infections altogether. This is in contrast with changes in conditions for pathogen growth which only has an effect on the epidemiological dynamics after pathogen density has had time to accumulate or decay.

If two parts of the system are simultaneously targeted by envrionmental variation, the most interesting result is that their effects can cancel one another (**IIII**). Increases in pathogen growth are less likely to cause outbreaks if they are positively correlated with infective dose. For example, higher temperature might cause increased pathogen growth potential in the environment but if at the same time the hosts immune system is activated, it is able to handle more intruders. On the other hand, negatively correlated variation effects could increase disease incidence significantly. For example, increasing water temperature is a

stress factor to salmonid fish, causing a decrease in their immune system function. Coupled with increased pathogen growth, this can lead to an increased risk, which might explain the incidence of *F. columnare* outbreaks mainly during summer in commercial fisheries.

Except for sporadicity, periodic variation causes qualitatively the same effects as stochastic $1/f$ noise of a certain corresponding colour. The most significant difference is, however, that scaled to the same variance, periodic variation has much less effect (**IIII**). In other words, variation amplitude needs to be much higher to get the same effect. The reason is that while periodic sinusoidal variation is more persistent than randomly fluctuating noise, the extreme values are not as far from the mean. This indicates that models using periodic variation as a proxy for environmental variation might underestimate the effects because the extreme values might have an effect on the dynamics.

6. Implications

The sensitivity to environmental conditions and species interactions reduce the potential for outbreaks. When outbreaks occur, however, they can be severe because of the high virulence and high output of pathogens from the infected hosts. Prediction of pathogen outbreaks is notoriously difficult and understanding how environmental factors affect the system is necessary for this purpose.

The effect of competition as a limiting factor to environmental pathogen viability can be very large. The chapters **II** and **III** that included competitive interactions assumed that pathogenicity comes with a cost paid in competitive ability. While this assumption might not hold at all times for all pathogens, for the general case it is rational to assume that no organism is a 'jack of all trades'. With the imposed trade-offs, although a multicellular host body is very nutrient rich, the benefits are only realised when hosts are available and can be infected. Otherwise the pathogenicity is most likely a burden and non-pathogenic competitors take over.

As an example, the highly competed low nutrient environment could explain the low prevalence of columnaris disease in natural waters, whereas high host density together with environmental changes and possibly reduction in competition by disinfectants could enable *F. columnare* to cause severe outbreaks in commercial fisheries. Similarly, the virulent *V. cholerae* cells are most likely outcompeted by the non-virulent cells for the most of the time. Only with a suitable opportunity, the more virulent forms can infect the first few hosts, and the cascade of infections follows because of massive shedding of the virulent forms from the hosts.

According to results of this study, introduction of competing species is a viable option for prevention of environmental opportunist pathogen outbreaks. This could be studied in food production, e.g. at fish farms. In larger scale systems, maintaining natural diversity might prevent outbreaks, but not always.

Loss of diversity has been associated with disease for example in gut microbiota (Round & Mazmanian, 2009), and agricultural soil (Nitta, 1991), but has sometimes decreased disease prevalence (Keesing *et al.*, 2010). While there are most likely several factors not accounted for in this thesis work, the mechanism of outbreak prevention by competition could explain the cases where increases in disease prevalence was observed with diversity loss.

Environmental opportunism is a logical stepping-stone between entirely free-living and obligate parasite lifestyles. At some point, (non-viral) parasites have to have evolved from free-living organisms. While fully obligate parasites have large structural differences in comparison with free-living organisms, the potential for opportunistic parasitism can be thought to develop with fewer changes to the free-living organism. This is because many traits that enable host exploitation, such as secretion of proteases and other tissue degrading enzymes or biofilm production, could be relatively simply realised through gene acquisition (Ochman & Moran, 2001). More importantly, these traits are occasionally beneficial in the outside-host environment as well. The evolution towards obligate parasitism from opportunism requires reduction in virulence, development of efficient transmission, and often genome reduction, that might be slower processes.

The emergence of new diseases from bening environmental species is an interesting question that is empirically very hard to study (Jones *et al.*, 2008). Fast growth mutant strains with host tissue exploitation capabilities could quite possibly infect a host if initiated with a dose high enough to outrun the immune system. While the capability for fast growth given the nutrients is perhaps not the most elegant solution to evading host immune system, it has been linked to virulence in several occasions (Gulig & Doyle, 1993; Bull, 1994; Marsh *et al.*, 1994; Paisley *et al.*, 2005).

Looking at this from the viewpoint of the free-living microbe, multicellular hosts are vastly nutrient rich resources that are not readily available for most microbes, as they come and go sporadically and are defended by their immune systems. A specialised microbial species can access the host resource easily, but for a free-living microbe for the most of the time, virulence factors are probably not good investments unless they are beneficial also in some other way.

7. Conclusions

Environmental opportunist pathogens are a fascinating class of organisms that combine two widely different life styles, parasitic and free-living. This thesis hopefully adds to the theoretical understanding of their ecological and epidemiological dynamics, for which there is little previous work.

The results of this thesis work also call for more detailed study of the particular pathogens in their ecological setting. The underlying biological interactions and what parts of the system are affected by environmental variation have a high impact on the epidemiological dynamics. The models presented here could be used to further explore different control methods to reduce the outbreaks in severity and frequency. An intruguing possibility is to exploit the pathogens sensitivity to environmental conditions and susceptibility to biological interactions in the environment.

The mathematical models and the sigmoidal infectivity response developed in this thesis are perhaps not the most concise or elegant mathematically, but strive to include a realistic description, and show why this is necessary.

8. Acknowledgements

This work was performed in the Integrative Ecology Unit, Department of Biosciences, Faculty of Biological and Environmental Sciences at the University of Helsinki. First and foremost I would like to express my deepest gratitude to my superiors Lasse Ruokolainen, Jouni Laakso, and Veijo Kaitala for the support, discussions, and ideas over this period of four years. You gave me just the right amount of freedom and push to make this work and keep me sane. Secondly, I wish to thank everyone in and associated with the group: Heikki, Ilona, Matan, Mikael, Simon, Christina, and Teppo here in Helsinki, and Lotta-Riina, Tarmo, Lauri, Anni-Maria, and others in Jyväskylä. You made all this hard work, and the travels, recreational or work related, so much more enjoyable. I am also grateful to the advisory committee, Eva Kisdi, Anna-Liisa Laine, and Otso Ovaskainen, to the pre-examiners of my thesis, Lutz Becks, and Uno Wennergren, and to my opponent, Per Lundberg, for all the time and interest you have invested in my work. I also wish to thank my past academic mentors during my studies: Raija Laiho, Tuomas Haltia, and Arto Annila. I owe much of my scientific thinking and the will to do research to you.

I wish to thank all my friends and family who have contributed in one way or another to my work or my well being besides the work. Henriikka, you were there when I received the first grant for this work, and you are here now as I am writing these Acknowledgements. I am eternally grateful for everything we have shared. Nino and others at Ruka where I got started with this text, Roni, for friendship and proofreading this text, Markku, for being my long standing partner in crime on the Viikki campus, and Jukka, for being the most effective work stress support group. My family deserves a special thanks for their support and understanding over the years: Rauha, Aaro, Sirpa, Timo, Miika, Taija, and Oiva. You have my heartfelt gratitude, and my sincerest apologies for bringing my work to all the family occasions over the past four years.

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