Modelling the spread of *Sphaerothecum destruens*, a generalist fungal pathogen

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A thesis submitted in partial fulfilment of the requirements of Bournemouth University for the degree of Doctor of Philosophy

May 2016

Acknowledgements

My deepest gratitude goes to my parents Najeeb and Rihab, who supported me and encouraged me from the moment I was born. Thank you for your trust in me and your unconditional love. Your support means the world to me and I can't thank you enough for all you have done for me.

Thank you to my supervisors Demetra Andreou, Rob Britton and Rudy Gozlan for their guidance over the last three years. Your advice has encouraged me to improve my abilities and to believe in myself. Thank you for pushing me to aim higher, whether it was deciding which journal to submit manuscripts to, or what jobs I could go for. I would also like to thank Rick Stafford for his help with the Bayesian models, and my viva examiners Richard Stillman and Andy Fenton for their feedback and advice.

Special thanks to Benjamin Roche, who got stuck with me in second year and patiently taught me everything about modelling. I could not have made it this far without you Benjamin.

Thank you to all my friends near and far who made the past three years worth it. None of you let me doubt myself for a second, and for that I am grateful.

To the one who puts up with my rants with patience, love and the occasional dose of sarcasm. Danny, thank you for making me smile every day.

Abstract

Humans have altered the global landscape with agriculture, urban development and international trade, and the incidence of emerging infectious diseases (EIDs) has increased as a result. Pathogens can emerge in new areas as a direct result of global transport trade or indirectly due to climate–mediated shifts in parasite geographic range. These pathogens can cause considerable ecological and economic damage, as they accelerate biodiversity loss and threaten global food security. Furthermore, the inherent characteristics of pathogens allow their rapid evolution, intensifying their potential threat. Fungi and fungal-like pathogens are an increasing component of EIDs with highly opportunistic features. One group of these pathogens on the animal-fungal boundary is the Mesomycetozoea, a group which has raised ecological concerns for a range of susceptible host species including birds, amphibians and mammals. To be able to mitigate the impacts of these pathogens effectively, their dynamics and drivers must be better understood.

Using the only Mesomycetozoea fungal species that has been cultured to date, the generalist pathogen *Sphaerothecum destruens*, empirical data on infectivity and pathogen life cycle were used in several epidemiological models to explore how a fungal-like generalist is transmitted within different host communities. First, a single host system was created using the available empirical data. This demonstrated that multiple saturation functions were needed to parameterise the model accurately, and identified incubation and recovery rates as drivers of epidemics. The parameter values obtained from the single-host models enabled the characterisation of mathematical relationships between different parameters, a task which can be difficult in epidemiology. Following this, a multi-host model was used to examine pathogen establishment in different communities. The roles of community structure and composition were explored, including the influence of competitive interactions between host species. Host density, proximity between communities, the competitive interactions between species, and the persistence of free-living pathogen propagules were identified as important factors in disease emergence and community survival. Environmental transmission was a key pathway for pathogen establishment. Finally, the evolution of *S. destruens*' virulence was explored in different conditions and for various transmission strategies. Direct contact and environmental uptake rates were key determinants of pathogen evolutionary stable strategy. Host eradication and selective restocking were evaluated as disease management techniques, examining the advantages and possible repercussions of each approach in light of the pathogen's ecological and evolutionary dynamics.

Mathematical models have been crucial in expanding ecological and epidemiological knowledge in other pathogens, allowing the exploration of diverse conditions and hypotheses about disease dynamics. The reliability of the results and their applicability were greatly enhanced by the inclusion of empirical data, giving this research substantial advantage in the robustness of model outputs. This work provided new insights on how fungal and fungal-like pathogens are transmitted and the risks of establishment in different populations, and can be applied to similar emerging pathogens, especially those that are fungi and fungal-like.

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Glossary

Adaptive dynamics: modelling techniques combining population dynamics, evolutionary dynamics and game theory that were designed to better understand how small mutations can lead to evolutionary change in a particular trait over time (Brannstrom et al. 2013).

Amplification effect: instances where increased species diversity leads to higher pathogen transmission (Mihaljevic et al. 2014).

Biodiversity: throughout this thesis, the term biodiversity is used to refer to species richness (i.e. the number of species in a community), unless otherwise stated (Gotelli and Colwell 2001).

Convergence stable: an evolutionary strategy that can be approached by small mutations in the trait value, which if achieved cannot be invaded by other mutants (Osnas and Dobson 2010).

Dilution effect: where increased species diversity leads to lower (i.e. diluted) pathogen transmission, as the pathogen encounters non-suitable "dead-end" hosts (Keesing et al. 2010).

Emerging infectious diseases: infections which are new in a population, or previously existing infections that are increasing in prevalence or across a wider area at a rapid rate (Morse 1995).

Evolutionary branching: this occurs when a population becomes polymorphic for a particular trait due to disruptive selection, as there is more than one evolutionarily successful strategy (Geritz et al. 1998, Miller et al. 2005).

Evolutionary stable strategy: a trait value that cannot be invaded by any new mutants in the population, as it confers the highest fitness (Osnas and Dobson 2010). A resident trait should be able to resist invasion by any rare mutant if it is at the ESS (Geritz et al. 1998).

Generalist: a pathogen which can infect multiple host species (Peeler et al. 2011).

Healthy carrier: a host that can transmit a pathogen at low levels to other species, without suffering any adverse effects of infection themselves (Spikmans et al. 2013).

Invasive: a non-native species which successfully establishes a niche in new environments and can have negative effects on native species (Leung et al. 2002).

Paratenic: a pathogen host that can be infected but is not required to complete the pathogen's life cycle (Britton 2013).

Reservoir: a host or environment which maintains the presence of the pathogen over a long time period and can allow pathogen re-emergence (Codeco 2001, Cronin et al. 2010).

Saprobic: a life stage of some fungal pathogens which can survive on non-living matter. In some cases this life stage can lead to the extinction of the host species (Mitchell et al. 2008, Rowley et al. 2013).

Singular strategy: in adaptive dynamics, this is a value of a trait which yields a fitness gradient of 0. It can be a convergence stable strategy or lead to evolutionary branching, based on the evolutionary landscape (Boots et al. 2012).

Systemic: a pathogen which can spread throughout the entire host body, usually through the bloodstream (Arkush et al. 2003).

Vector: an organism that carries a pathogen and transmits it to hosts widely (e.g. biting insects) (Roche et al. 2013, Morand et al. 2014).

Virulence: the negative affect a pathogen has on its host. In this thesis, unless otherwise stated, virulence refers to the host mortality caused by infection (Ebert and Bull 2003).

Zoonosis: a disease that is transmitted between animals and humans (Mills 2006).

Chapter 1
General introduction

Summary

This chapter introduces the research topic of the thesis, the epidemiology and biology of the emerging generalist pathogen *Sphaerothecum destruens*. Firstly, the life history and characteristics of generalist pathogens are described in the context of their impacts on ecosystems. Pathogen effects on community organisation and structure are then explored, with an additional section on the role of invasive species in pathogen introduction, followed by a deeper examination of fungal and fungal-like infections. As three types of models are created in this thesis, including several epidemiological models, a Bayesian model, and an evolutionary model, an introduction to epidemiological modelling techniques is provided. Finally, the details of *S. destruens* life history and the datasets used in this work are outlined.

1.1 Generalist pathogens

Forty per cent of all known species are parasitic and form crucial components of food webs (Dobson et al. 2008, Lafferty et al. 2008). Most emerging infectious diseases (EIDs) are pathogens that have broad host ranges, i.e. they are generalist pathogens (Woolhouse and Gowtage-Sequeria 2005). Their capacity to adapt to and infect multiple host species makes them a substantial threat to biodiversity, conservation, and global health (Marcogliese 2007, Jones et al. 2008, Torgerson and Macpherson 2011, Roche et al. 2013). Indeed, the majority of pathogens infecting livestock and carnivores are generalists (Haydon et al. 2002), with 58% of human pathogens being generalist pathogens transmitted from animals (Woolhouse and Gowtage-Sequeria 2005). The number of zoonoses, i.e. wildlife infections spilling over into human populations, is also increasing worldwide (Swaddle and Calos 2008, Roche et al. 2012). Whilst a better understanding of how these pathogens are spreading is important for preventing their emergence, there is still a range of particular characteristics which make the study of these pathogens challenging. These include infection of alternate hosts or vectors, transmission through multiple pathways, and persistence at a low prevalence in animal populations or habitats without clinical signs, i.e. in environmental reservoirs. For example, in marine systems, generalist pathogens can use multiple paratenic hosts, allowing them to

invade new food webs and change species abundance across multiple trophic levels (Marcogliese 2007).

The phylogeny of a host species can usually give some indication of its susceptibility to a particular pathogen (Roche et al. 2015). True generalists can, however, survive across multiple families and are not inhibited by specific host availability, existing at various levels of virulence in different species (Desdevises et al. 2002). Other generalists transmit to their final host through vectors, which can increase their range of transmission and potential host range (Prugnolle et al. 2005, Rauch et al. 2005, Roche et al. 2013). In this context, vectors are defined as organisms that can transmit the pathogen to its final host without becoming infected themselves. The spread of West Nile virus in multiple hosts and broad geographic ranges via mosquito vectors exemplifies this life history strategy and the threat it poses (Allan et al. 2009). Whether they can transmit through vectors or alternate hosts, there is a growing threat of generalist pathogens in novel habitats. Generalists that could emerge and thrive in new environments are likely to have direct lifecycles or be vector-borne (Dobson 2004), but as their interactions with novel species are not known it is difficult to characterise their risks of emergence.

For many generalist pathogens, transmission occurs through direct physical contact with an infected host or through host ingestion of free-living infectious propagules in the environment (Roche et al. 2009, 2011). Many emerged pathogens are able to use both pathways, which can act together to different extents, leading to changes in pathogen virulence that can be difficult to predict; direct transmission is associated with fast-acting infections due to exposure to high pathogen loads in infected hosts, while environmental transmission occurs on a comparatively slower timescale by chronic exposure to free-living pathogen propagules (Roche et al. 2011). The persistence of free-living infectious propagules in the environment increases the probability of transmission to additional hosts, as opposed to direct contact with an infected individual. A case study of infectious pancreatic necrosis in fishes of the Salmonidae family (hereafter referred to as salmonids) demonstrated that the

pathogen could be detected at considerable distances from the infected population (McAllister and Bebak 1997). Furthermore, pathogens with saprobic stages, such as fungal *Allomyces* species, can remain in the environment independently of host survival, potentially leading to host extinction if they emerge in favourable conditions (Mitchell et al. 2008). Infection route (direct versus environmental) in turn influences host adaptation, altering the host response to infection and potentially how the epidemic progresses (Martins et al. 2013). Systemic infections (e.g. throughout the bloodstream) have been shown to have more virulent effects than infection through ingestion (Woolhouse et al. 2001, Martins et al. 2013). Thus, the use of multiple modes of transmission in some generalist pathogens further highlights why it is difficult to characterise their risks.

One important obstacle associated with controlling generalist pathogens is their persistence in disease reservoirs (hereafter referred to simply as reservoirs), i.e. populations or environments that carry the pathogen indefinitely until the habitat or community changes in a way which provides an opportunity for the pathogen to emerge (Haydon et al. 2002, Briggs et al. 2010). The Ebola virus has been shown to persist asymptomatically in three species of fruit bat, among other species (Leroy et al. 2005). Reservoirs of *Lyme borreliosis*, the bacterium that causes Lyme disease, include the white-footed mouse *Peromyscus leucopus* and white-tailed deer, *Odocoileus virginianus* (Duffy et al. 1994, LoGiudice et al. 2003). Reservoirs of disease significantly increase the chance of recurring epidemics, which have had significant consequences for human populations, such as common and severe infections of Lyme disease (Ostfeld and Keesing 2000), and widespread mortality events in the case of the Ebola virus (Siettos et al. 2015, Webb et al. 2015). A study comparing incidences of Lyme disease with populations of white-tailed deer found that lower reservoir abundances were correlated with lower disease prevalence (Duffy et al. 1994). Regulating the pathogen in the reservoir population could thus be key to controlling the pathogen in susceptible hosts (Haydon et al. 2002, Cronin et al. 2010), so examining the role of reservoir hosts and environments in disease transmission can be important. However, because reservoirs often carry the pathogen

asymptomatically, this aspect of disease management is a challenge to study, especially when multiple reservoirs can exist.

Thus, generalist pathogens possess several characteristics that make forecasting their disease dynamics difficult, especially in multi-host systems. The use of alternate hosts or vectors, multiple pathways of transmission, and persistence in reservoirs are all factors which must be included in studies of generalist pathogen emergence.

1.2 Community effects on disease transmission

Community-level interactions can influence pathogen transmission between hosts in a particular habitat (Roche et al. 2009). Interactions within and between species (both host and non-host), and their relationship with the environment can alter the outcome of an epidemic in terms of population decline and recovery. Reciprocally, infections can also change community composition with potentially severe consequences (e.g. significantly shifting trophic interactions; Britton 2013), resulting in a cycle of community-level effects (Altizer et al. 2003). For this reason, many infectious disease outbreaks in the wild occur cyclically as conditions continuously change (Codeco 2001, Morgan et al. 2006). Depending on a pathogen's characteristics, community structure can affect disease emergence, and vice versa. Factors including biodiversity, population density, and species interactions such as predator-prey relationships must be closely considered for their influence on pathogen transmission.

Species diversity within a community can influence both the rate of pathogen spread and the infection pathway taken by the parasite (Johnson and Thieltges 2010). As the world undergoes a biodiversity crisis that has been termed the sixth mass extinction, the repercussions of this global biodiversity loss for disease emergence has been studied extensively (Barnosky et al. 2011). A recent review of EIDs in Asia and the Pacific revealed that biodiversity loss was correlated with an increase in zoonotic and vector-borne epidemics (Morand et al. 2014). Often, higher biodiversity (specifically

greater species richness) creates a ‘dilution effect:’ pathogens are more likely to come into contact with unsuitable ‘dead end’ hosts that they cannot infect or are resistant, halting their transmission to new hosts (Johnson and Thieltges 2010). These wasted contacts can mitigate a pathogen’s effects on the population as a whole (Dobson 2004, Keesing et al. 2010). Higher community biodiversity can also result in wasted contacts by vectors (e.g. mosquitos biting non-host species), again demonstrating the dilution effect. For example, there are fewer human cases of West Nile virus in areas with a higher diversity of avian vector hosts (Swaddle and Calos 2008).

Spatial transmission models reveal that for a sustained epidemic to occur, susceptible and infected individuals need to be closely grouped for a given length of time (Riley 2007). The susceptibility of host species within a community can thus determine the outcome of an epidemic, as each species can contribute differently to a pathogen’s transmission (Fenton et al. 2015). Furthermore, susceptible species in a densely populated community are at a higher risk of extinction from disease due to high transmission levels compared to those in populations of low densities (Keesing et al. 2010). For example, fish stocks in aquaculture sites are often very densely populated in small, confined areas, creating a favourable environment for pathogen emergence (Pulkkinen et al. 2010). The physiological stresses incurred in hosts by these densely populated conditions can trigger infectious disease outbreaks (Ortuño et al. 2001, Gozlan et al. 2006), making infectious disease the leading cause of mortalities in fish culture sites, with potential threats to global food security (Leung and Bates 2013).

When the effects of biodiversity and population density are examined simultaneously, new dynamics can emerge. In density-dependent disease transmission, high species abundance-richness ratios were correlated with higher levels of infection, termed an amplification effect (Mihaljevic et al. 2014). This amplification is effectively the opposite of the dilution effect, and occurs when higher biodiversity actually increases the risk of infection (Keesing et al. 2006). A higher community density comprising several host species is predicted to favour the

spread of a generalist pathogen due to multiple species being used as alternate hosts, possibly confounding the effects of biodiversity loss on pathogen transmission (Lootvoet et al. 2013). Furthermore, an increase in vector species richness (the number of different vectors in a given community) can increase the probability of an epidemic of West Nile virus (Roche et al. 2013). In wild ecosystems, whilst host species are rarely densely populated, higher species richness (when compared to captive habitats) increases the risk of a generalist pathogen being transmitted to novel hosts. This demonstrates a further caveat for areas of high biodiversity that contain threatened species, as often epidemics only become apparent after high mortalities and obviously deteriorating conditions (Thompson et al. 2010, Tompkins et al. 2011). Thus, population densities and species composition can significantly alter pathogen dynamics in wild situations.

Pathogens can influence community structure in several ways (Miki et al. 2011; Fig. 1.1). In most cases, juveniles are more susceptible to infection than mature adults, and their increased mortality can lead to a significant shift in population age structure (Bergmann et al. 2003). In some cases, community changes can alter behaviour in a host-parasite system, such as host selection by the parasite (Keesing et al. 2010). The role of pathogens in community composition extends beyond the interactions between host and vector species, to the potentially active role of non-host species. In addition to the clinical effects of an infection, pathogens can affect community dynamics such as predator-prey interactions and inter-specific competition (Hudson et al. 2006, Marcogliese and Pietrock 2011). Infected hosts may be unable to compete for resources with conspecifics and other species, leading to large-scale structural changes in the population (Gozlan et al. 2006, Auger et al. 2009). While species-specific pathogens may promote species co-existence in communities as they can control the population growth of a particular host species, generalist pathogens tend to have destabilising effects on community structure, as they potentially impact multiple hosts across different trophic levels, and to varying degrees (Fenton and Brockhurst 2007). This is exemplified by the spread of the amphibian chytrid fungus *Batrochochytrium dendrobatidis*, contributing to more than 100 species extinctions

in the last two decades with severe ecosystem-level consequences (Hudson et al. 2006, Fisher et al. 2012).

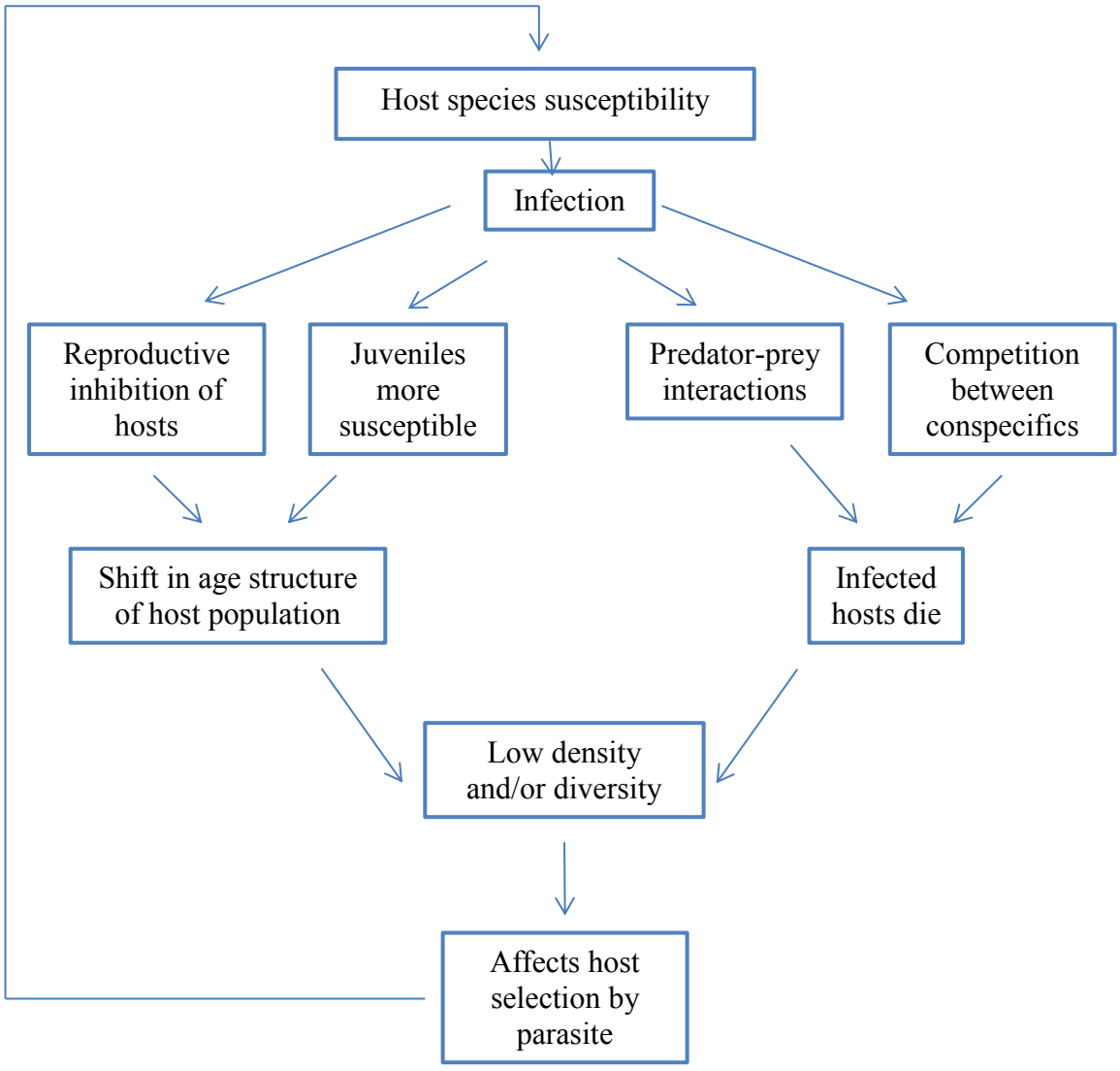

Figure 1.1. Multiple ways by which infection can lead to changes in an affected community, and their consequences (Bergmann et al. 2003; Hudson et al. 2006; Gozlan et al. 2006; Fenton and Brockhurst 2007; Miki et al. 2011; Fenton et al 2015).

In the majority of cases, a pathogen is an integral part of the community food web, using it to reach an intermediate or final host (Johnson et al. 2010). The inclusion of parasites in topological food web studies was a critical step forward in the

understanding of community dynamics (Marcogliese and Cone 1997, Lafferty et al. 2006). Predating on parasites has several possible outcomes. In some cases such as chytridiomycosis, spore predation leads to a lower rate of infection by the parasite. Invertebrates consume *B. dendrobatidis* spores in the surrounding water, decreasing the prevalence of infection (Gleason et al. 2008, Schmeller et al. 2013). However, invertebrate populations can also act as intermediate hosts or carriers of disease (Cáceres et al. 2009), and hosts predating on infected invertebrates could lead to a rise in community disease prevalence. For example, salmonid fishes can experience a rise in proliferative kidney disease (PKD) due to an increase in invertebrates in the system (Okamura et al. 2011). Thus, a particular life stage of a pathogen can have multiple roles in a community food web (e.g. a pathogen can be a prey species while also proceeding to the next life stage in an intermediate host) and this aspect must be considered for each specific pathogen.

Pathogen dynamics are thus affected by biodiversity, species abundance, and community interactions such as predation. In turn, biodiversity and population density can alter the outcome of epidemics by affecting pathogens, vectors and hosts. As biodiversity levels change worldwide, the current understanding of disease dynamics will be reshaped, especially for generalist pathogens. Thus, a community approach is needed to effectively study EIDs, as community composition and population dynamics are intertwined and can be considered as equally important to the epidemiology of a disease.

1.3 Disease introduction through introduced species

Species introductions are an important aspect of biodiversity changes and occur through issues including habitat changes (e.g. temperature increase or flooding) and the intentional or accidental transport of species from their native range to new areas for trade, resource production, conservation, ornamental purposes, or as pets (Murray and Peeler 2005, Gozlan et al. 2006, Farrer et al. 2011). There are a growing number of accidental species introductions worldwide, especially in freshwater ecosystems, where they often occur through escapes from aquaculture or with ballast water, or via

transporting fish stocks in support of fisheries (Gozlan et al. 2010b; Okamura and Feist 2011). Introduced species can change the population dynamics in the new ecosystem via several mechanisms and processes, including predation (Vredenburg 2004), species hybridisation (Mooney and Cleland 2001, Biedrzycka et al. 2012), competition (Gurnell et al. 2004) and disease introduction (Peeler et al. 2011).

A major consequence of introduced species often arises from the introduction of novel pathogens to an ecosystem (Murray and Peeler 2005; Crowl et al. 2008; Poulin et al. 2011). There is a risk that introduced free-living species may introduce their parasite fauna, but enemy release suggests only a small proportion might actually be co-introduced (Keane and Crawley 2002, Torchin et al. 2003). However, where the co-introduced parasites are able to spill-over into native hosts, there are considerable disease risks, for example due to a lack of co-evolutionary processes (Sheath et al. 2015). Pathogen spillback can also occur when an introduced species is a competent host of a native parasite, thus transmitting it even more widely to native hosts (Kelly et al. 2009). Pathogen emergence can also occur through species recovery programmes such as re-introduction of endangered species and captive breeding, demonstrated in some cases of chytridiomycosis emergence (Walker et al. 2008). The increasing incidence of zoonotic epidemics highlights the growing threat of introducing species to non-native environments (Wilcox and Gubler 2005, Morand and Krasnov 2008).

In addition to species transport, introductions of non-native species are also often associated with habitat changes, which can modify (increasing or decreasing) the host range of a parasite or affect the parasite and host distribution within this range (Okamura and Feist 2011; Poulin et al. 2011). As habitats continue to degrade and change as a result of human-mediated activities, this aspect of species introduction and resulting disease emergence requires closer examination. Climate change is also altering the geographic range of species, introducing parasites into novel areas or alternatively limiting their expansion (Karvonen et al. 2010). For example, in China, the invasive snail *Pomacea canaliculata* has become an intermediate host of *Angiostrongylus cantonensis*, a parasitic lungworm that infects humans and has led to the emergence of eosinophilic meningitis in several regions (Lv et al. 2009), with

predictive models indicating that as climate change progresses, the range of the parasite will expand as the invasive snail species' geographic range expands, leading to more widespread emergence of the disease (Lv et al. 2011). In the emergence of salmonid PKD, a temperature increase caused a rise in invertebrate populations which led to higher infection levels (Okamura et al. 2011). Incidences of West Nile Virus have also increased in the last decade, partially due to higher temperatures (Brault 2009). Shifts in the geographic range of species will likely increase in occurrence as global temperatures increase.

Some introduced species, such as topmouth gudgeon *Pseudorasbora parva* (Fig. 1.2), are successful invaders as a result of their tolerance to a wide range of environments. This is a small bodied (< 10cm) fish of the Cyprinidae family (hereafter referred to as cyprinids) that is native to the eastern part of China, Korea and Japan (Pinder et al. 2005) and are the model host of this research. Their broad habitat use, combined with their early maturity and multiple batch spawning, has facilitated their invasion of Europe, with wild populations present in Great Britain (Britton et al. 2007; Gozlan et al. 2010a). Empirical work has demonstrated that *P. parva* are healthy carriers of the fungal–like pathogen *S. destruens*, transmitting it to susceptible species with no associated mortality or decline in their condition (Gozlan et al. 2005). For example, populations of *P. parva* in the Netherlands had a 67- 74 % prevalence of *S. destruens* infections, yet showed no clinical signs (Spikmans et al. 2013). As one of the 10 most invasive species in Europe, *P. parva* is a good model of how species invasion facilitates disease transmission (Britton and Gozlan 2013). In the context of *S. destruens* epidemiology, *P. parva* can be seen as both a vector and a reservoir host, with this thesis examining its role as an invasive host in fungal disease (*S. destruens*) establishment and dynamics.

Figure 1.2. Topmouth gudgeon *Pseudorasbora parva* live for around four years (Gozlan et al. 2010a). They mature at one year of age and have multiple spawning events annually (Pinder and Gozlan 2003). Picture credit Akos Harka (http://www.carpathianbasinspecies.eu/9_Fishes_%28Pisces%29/Species/Pseudorasbora_parva/Pseudorasbora%20parva_logo_0.html).

1.4 Fungal and fungal-like pathogens

Typically, emerging fungal pathogens are generalists within humid and aquatic habitats (Harvell 1999, Jones et al. 2008, Frick et al. 2010, Fisher et al. 2012). Fungi have recently been responsible for infectious outbreaks worldwide which have led to high biodiversity and economic costs (e.g. Elston et al. 1986, Murray and Peeler 2005, Gozlan et al. 2006, Fisher et al. 2012, Ercan et al. 2015). The global emergence of these pathogens and subsequent decline of numerous species has highlighted that fungi and fungal-like pathogens must be considered as a serious threat to conservation efforts and even economic growth (Sarmiento-Ramírez et al. 2014). A newly emerged class on the animal-fungal boundary, the Mesomycetozoea, includes species that can infect mammals, fish, birds and amphibians (Rowley et al. 2013, Gozlan et al. 2014). The model pathogen in this thesis, *S. destruens*, is a member of this taxonomic class. Here the challenges associated with studying fungal pathogens are discussed, including the difficulty of their monitoring, their broad host ranges, and their environmental persistence.

Some microparasitic infections like fungi can be difficult to observe and detect, especially in wild ecosystems (Gozlan et al. 2006, Okamura et al. 2011, Gozlan 2012). This is due to a variety of factors, including their maintenance in low visibility habitats (e.g. in soil), the ability to stay dormant and spread easily (especially in aquatic systems), and their variable life history strategies (Mitchell et

al. 2008, Crowther et al. 2014, Sarmiento-Ramírez et al. 2014). These features make fungi inherently difficult to observe in natural systems, leaving substantial gaps in the accurate characterisation of their impacts.

Studying fungal pathogens is challenging due their presence in multiple hosts, which is amplified by their opportunistic nature (Sarmiento-Ramírez et al. 2014). One example is *Fusarium*, a genus of fungi that infects thousands of plant and animal species (Sharon and Shlezinger 2013). The chytrid fungus *B. dendrobatidis* can infect over 500 species of amphibians, and was recently also detected in crayfish populations (*Procambarus* spp. and *Orconectes virilis*) in the USA (Fisher et al. 2012, McMahon et al. 2013). Other well-known fungal pathogens include the oomycete fungus *Aphanomyces astaci*, which has caused crayfish plague throughout Europe (Holdich et al. 2009, Strand et al. 2014), and the ascomycete fungus *Geomyces destructans*, which is responsible for White Nose Disease in many bat species (Foley et al. 2011). The virulence of fungal pathogens, i.e. how much they negatively affect their host, varies between species, which impacts their transmission within an ecosystem (Fisher et al. 2012). An important epidemiological question is how varying host susceptibility within a community affects fungal pathogen transmission. Answering this question would assist in discerning which geographic areas and species may be most at risk of emerging fungal infections.

Fungal parasites also often have a free-living stage which allows them to survive in the environment (Andreou et al. 2009, Fisher et al. 2012). This means that fungal pathogens can use both direct and environmental transmission, which can alter the progression of an epidemic. For example, water-borne transmission determines disease prevalence in avian influenza and maintains it in the environment (Roche et al. 2009). Free-living stages (spores or zoospores for fungal pathogens) may have a density-dependent effect on the severity of an epidemic, affecting pathogen load in susceptible and reservoir hosts (Ebert et al. 2000, Carey et al. 2006, Briggs et al. 2010). The effect of infective agent concentration on disease progression has been investigated in disease systems such as influenza viruses in birds or cholera in

humans (Codeco 2001, Roche et al. 2009). However, in an aquatic study system, fish hosts are constantly exposed to the infecting environment, which could alter some important processes of the epidemiological system. Furthermore, the presence of a free-living or resting pathogen stage extends the time span and spatial range available for pathogen transmission, and can even drive a host population to extinction (Marcogliese 2007, Fisher et al. 2012). Consequently, this thesis explores whether events in the course of infection were solely dependent on the initial infective dose or were also affected by subsequent spore levels in the environment.

Thus, fungal pathogens are increasing in prevalence due to their opportunistic features and tolerance to extreme conditions. This increasing trend makes understanding fungal pathogen dynamics a conservation priority (Mitchell et al. 2008, Johnson and Thieltges 2010, Thrush et al. 2011, Fisher et al. 2012, Roche et al. 2012, 2013). Their ability to persist in the environment provides an interesting platform to study the role of pathogen load in environmental transmission, especially for aquatic systems. Understanding the roles of host range and free-living propagules in detail will provide insights into how to control the spread of fungal diseases, and possibly indicate more effective detection methods in the wild.

1.5 Epidemiological models

Epidemiological models assist the detection, tracing, and in some cases, the control of infectious disease outbreaks (Dobson 2004). It is often difficult or impossible to empirically track the spread of a pathogen, especially in the wild (Okamura et al. 2011). Predictive models help address gaps in epidemiological knowledge by simulating real situations and running possible scenarios mathematically, given available data for parameterisation. In some cases, such as foot and mouth disease in the UK, models have also helped determine disease management policy (Hollingsworth 2009). There are many existing epidemiological models for emerging pathogens, notably the Fisher et al. (2012) model of the chytrid fungus *B. dendrobatidis*, which highlighted the emergence of fungal pathogens. Epidemiological and spatial models have also been created for White Nose

Syndrome (WNS) to help determine the most effective methods of controlling pathogen spread and protecting bat diversity (Blehert et al. 2009, Foley et al. 2011). Recently, mathematical models were a significant component of the response to the Ebola virus outbreak in West Africa (Butler 2014, Lofgren et al. 2014, Webb et al. 2015). There are several types of models that aid the understanding of disease dynamics and were used in this thesis, including ecological epidemiology models (SIR simulations and Bayesian belief networks) and an evolutionary model. Here, the ecological modelling approaches used in Chapters 4 and 5 are discussed, followed by an examination of their limitations.

SIR models

Epidemiological models that investigate disease dynamics can be difficult to develop empirically. The first recorded epidemiological models using the now well-known SIR model system were developed by Kermack and McKendrick in 1927 (Monteiro et al. 2007). Anderson and May's models in the early 1980's investigated how population dynamics affect host and parasite evolution, and set the modern foundation of epidemiological models. This epidemiological model divides the population into categories based on their stage of infection: susceptible (S), infected (I), and recovered (R) hosts. The rate of change of the population from one stage to the next is measured by differential equations that mathematically represent birth and death rates, transmission and virulence (Fig. 1.3). Over the years, variations of this model have been developed to portray the dynamics of different host-parasite systems, for example SIS or SI models, in which infected hosts do not gain immunity after infection but either become susceptible again or die, respectively. In this thesis, the primary system used was an SEIR system, in which hosts can become exposed for an incubation period before becoming infectious. This will be discussed in detail in Chapter 2.

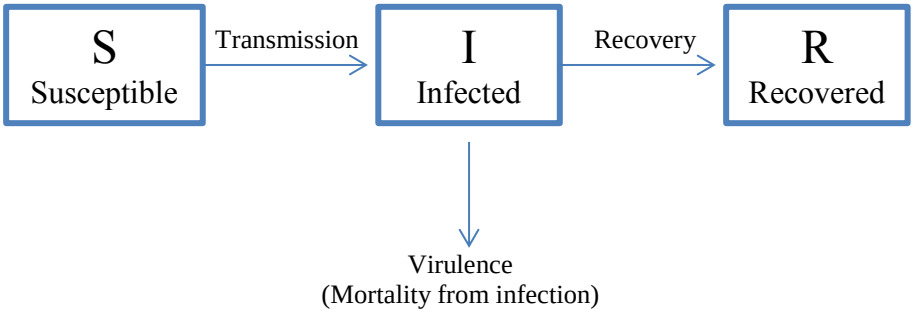

Figure 1.3. In the Susceptible-Infected-Recovered (SIR) system, individuals move from being susceptible to being infected at a given rate of transmission. Infected individuals can recover at a given rate or die from the infection.

Bayesian models

Bayesian models incorporate uncertainty into their frameworks and operate on the probability of events occurring within a set of variables and a set of known interactions (Cooper 1990), and have been used for applications ranging from ecology (Wikle 2003, Stafford et al. 2013) to artificial intelligence (Mittal and Kassim 2007). A major advantage of the Bayesian approach is its application in data-poor conditions, as the uncertainty is inherently incorporated into its probabilistic inferences (Anderson 1998). This is advantageous for examining species interactions in aquatic environments, which can be difficult to measure experimentally (Stafford et al. 2015). While for some questions, the compartmental SIR model is required to determine parameter values and a timescale of the changes within a community, the added advantage of a Bayesian belief network model was included in Chapter 5 to determine the probability of various outcomes.

Model parameterisation

Many epidemiological models rely on surveillance data or user-supplied information (e.g. reporting an infection to a health care professional), and for that reason the data may be underreported or incomplete (Focks et al. 1995, Keeling and Rohani 2008). The prevalence of wildlife diseases is often estimated via sampling an affected area, which can also lead to under or over-reporting bias in the observations the model relies on (Heffernan et al. 2005, Rasmussen et al. 2011). For many diseases, including high-profile infections such as malaria or dengue fever, these data are then

used to estimate parameter values for incubation or infectious periods through maximum-likelihood fitting procedures (Luz et al. 2003, Smith et al. 2008). These procedures use different techniques to calculate what the most likely parameter values are given the available data (Dempster et al. 1997). Infectivity experiments provided the basis of the models in this thesis, creating a significant advantage when parameterising the models as they resulted in detailed, complete and controlled data. This eliminated reporting bias in the observations of host mortality and enabled the specification of initial conditions in the models, resulting in reliable outputs and parameter estimations for several host species.

Knowledge about parasite dynamics between a single host and single pathogen has limited applications (Dobson 2004), with a community approach necessary for a more accurate depiction of transmission and virulence of generalist pathogens. The necessary data are not easily obtained via experiments; ecological interactions, specifically the dynamics involved in disease transmission, can be difficult to imitate and/ or control in experimental conditions. The exact duration of pathogen incubation, the number of infectious agents in an individual host, and whether a host is resistant or recovered, are all examples of uncertainty in pathogen outbreaks. For this reason, all models require certain assumptions to run, which may not be numerically accurate in a real biological system. This can be a major limitation of models, as the assumptions can alter predictions. However, by using data wherever possible, assumptions can be calibrated with empirical evidence. Furthermore, the use of sensitivity analyses helps determine the influence of each assumption or selected parameter on the results, establishing a range of uncertainty in model outputs. As long as the limitations of the datasets are clear and included in the model analyses, then models are tools with high utility in epidemiology and ecology.

1.6 The model species: *Sphaerothecum destruens*

In the 1980's, the first reports of the rosette agent *S. destruens* arose in relation to farmed populations of Chinook salmon *Oncorhynchus tshawytcha* and Atlantic

salmon *Salmo salar* in the USA (Elston et al. 1986, Hedrick et al. 1989). Since then, there have been reports of *S. destruens* in several additional hosts in many countries (Table 1.1). *S. destruens* is an intracellular parasite, closely related to other generalist pathogens such as *Dermocystidium spp.* (affecting fish) and *Rhinosporidium seeberi* (affecting mammals and birds) from the class Mesomycetozoea (Rowley et al. 2013). While this class has fungal characteristics in its life history and transmission strategies, ribosomal DNA (18 S rDNA) sequencing data confirmed its location at the divergence between animals and fungi (Mendoza et al. 2002). For this reason, it is referred to throughout the thesis as 'fungal-like'. This monophyletic class of Mesomycetozoea combines several animal parasites which are difficult to culture in laboratory conditions (Mendoza et al. 2002, Rowley et al. 2013). The only species of this group with available detailed infection data is *S. destruens* (Fig. 1.4), which is the model pathogen of this thesis.

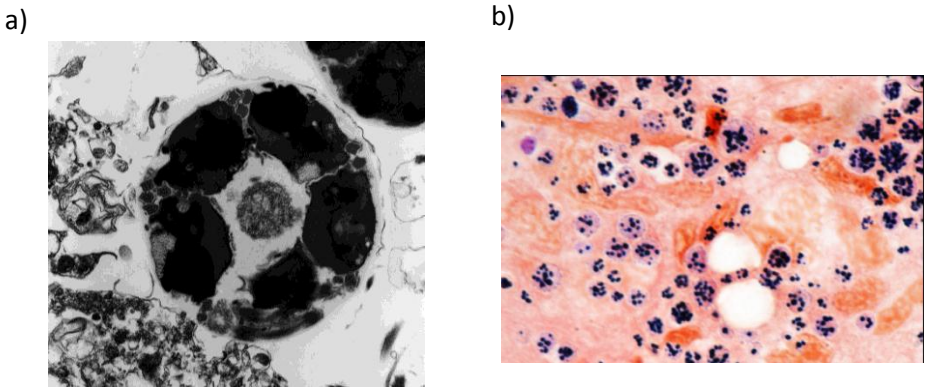

Figure 1.4. *Sphaerothecum destruens* rosettes in Atlantic salmon *Salmo salar* cells (Picture (a) by Kristen Arkush, picture (b) by Paley et al. (2012)).

A broad generalist infecting over 15 known species of commercially and ecologically important fishes, *S. destruens* has a direct lifecycle that involves unicellular spores and in freshwater, motile zoospores (Arkush et al. 2003, Andreou et al. 2011, Ercan et al. 2015). Infection with *S. destruens* occurs through ingestion, gut penetration, and gill or skin attachment (Gozlan et al. 2009). It multiplies within host cells, resulting in a slow-growing infection in various organs. The spores are transmitted to other organs and into the water through bile, urine, and intestinal epithelium, where in freshwater they undergo zoosporulation and can be transmitted to other individuals

(Fig. 1.5) (Gozlan et al. 2009). *Sphaerothecum destruens* is also carried by the highly invasive fish species *P. parva* which shows a high tolerance to the parasite and acts as a disease reservoir (Gozlan et al. 2005, Section 1.3). The life history characteristics of *S. destruens* are representative of the growing threat of fungal generalist pathogens, and are exceedingly relevant in a rapidly changing global environment as climate change will likely expand its geographic range to new habitats.

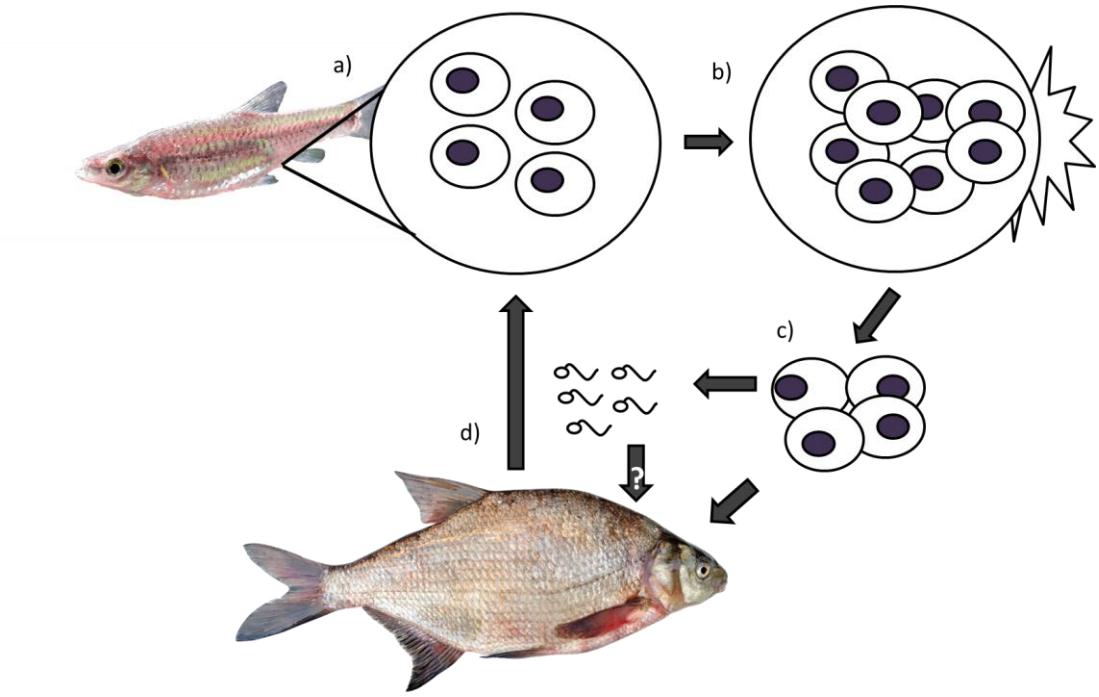

Figure 1.5. Lifecycle of *Sphaerothecum destruens*. a) Spores multiply within host cells until cell death; b) Spores spread within the host and are released into the water through urine, bile, or gut epithelium; c) In freshwater, each spore can divide into up to 5 uniflagellate zoospores and survive for several days depending on the water temperature. Infection occurs directly by contacting infected hosts, or indirectly by ingesting the spores, attachment to the gills or skin, or gut penetration. Photo R. E. Gozlan, Interbrics Seafood Limited.

Table 1.1. Known hosts of *Sphaerothecum destruens*, prevalence and details of infection.

Host species	Infection details	Reference
Atlantic salmon *Salmo salar*	Chronic mortality with 75% prevalence, systemic infection, lesions in kidneys and liver	Hedrick et al. (1989)
Chinook salmon *Oncorhynchus tshawytscha*	Over 95% mortality, systemic disease, especially in spleen and kidney	Elston et al. (1986), Harrell et al. (1986)
Coho salmon *Oncorhynchus kisutch*	98% of experimental population infected, widespread disseminated infection	Arkush et al. (1998)
Rainbow trout *Oncorhynchus mykiss*	42.5% of experimental population infected, less severe infection than salmon	Arkush et al. (1998)
Brown trout *Salmo trutta*	43.3% of experimental population infected, less severe infection than salmon	Arkush et al. (1998)
Brook trout *Salvelinus fontinalis*	Only 2.6% of experimental population infected, possible role as a resistant carrier	Arkush et al. (1998)
Sunbleak *Leucaspius delineatus*	96% population decline over 3 seasons, total inhibition of spawning	Gozlan et al. (2005)
Fathead minnow *Pimephales promelas*	Loss of condition, 60% mortality over 4 months, inhibition of spawning	Gozlan et al. (2005)
Bream *Abramis brama*	53% mortality over 23 days, infection found in kidneys, liver, intestines	Andreou et al. (2012)
Roach *Rutilus rutilus*	37% mortality over 50 days, low prevalence detected in liver, kidneys, intestines	Andreou et al. (2012)
Common carp *Cyprinus carpio*	8% mortality over 3 months, infection found in intestines	Andreou et al. (2012)
Topmouth gudgeon *Pseudorasbora parva*	Resistant host, 33% prevalence found in Turkey, 67-74% prevalence in the Netherlands	Gozlan et al. (2005); Spikmans et al. 2013; Ercan et al. 2015
Oxynoemacheilus sp. (not yet described)	46% mean prevalence in multiple organs, across 2 years of sampling, declines in wild population	Ercan et al. 2015
Smyrna chub *Petroleuciscus smyrnaeus*	50% mean prevalence in multiple organs, across 2 years of sampling, declines in wild population	Ercan et al. 2015
Aegean chub *Squalius fellowesii*	40% mean prevalence in multiple organs, across 2 years of sampling, declines in wild population	Ercan et al. 2015
European sea bass *Dicentrarchus labrax*	44% mean prevalence in multiple organs, across 2 years of sampling	Ercan et al. 2015
Pumpkinseed *Lepomis gibbosus*	45% mean prevalence in multiple organs, across 2 years of sampling	Ercan et al. 2015

The observed life stages of *S. destruens* indicate that it can be transmitted through direct contact or through environmental transmission (with either spores or zoospores), creating a complex study system. Furthermore, there are records of two manifestations of *S. destruens* infection: a nodular form and a disseminated form, which may be correlated to the host immune status; disseminated spores suggest that the host is more susceptible, or equally the pathogen is more virulent in that host (Gozlan et al. 2009). In contrast, the nodular form suggests that the host can contain the infection within their body more effectively (Arkush et al. 1998). The infection can be observed in multiple organs including the kidneys, liver, heart, spleen, and gonads (Mendonca and Arkush 2004). The production of free-living spores and zoospores, and their temperature dependent survival, has been explored experimentally by observing the number of propagules produced at a range of temperatures between 4 and 30°C (Andreou et al. 2009). Thus, *S. destruens* is a generalist pathogen that uses two modes of transmission (direct and environmental), offering a suitable study system of fungal outbreaks.

Based on evidence from experimental infections, *S. destruens* in the wild is predicted to cause chronic population decline rather than severe mortality peaks in host populations (Gozlan et al. 2005, Andreou et al. 2012, Ercan et al. 2015). This is often the case for other fish infections in the wild such as salmon PKD, making their detection difficult (Okamura et al. 2011). Topmouth gudgeon have been established as a resistant host of *S. destruens* and can act as reservoirs or healthy carriers of the disease (Gozlan et al. 2005). As they are highly invasive in Europe and thrive in a wide range of environments, the threat of *S. destruens* emergence in European freshwaters is likely to be high (Ercan et al. 2015). This system enabled the exploration of both the effects of a resistant host on disease transmission and the added stressor of an introduced species in an ecosystem.

Several experimental infection trials on a range of species in the salmonid and cyprinid families provided detailed data (see Section 1.7) about the progression of infection and clinical signs in hosts with different susceptibilities. These data were

used in this thesis to understand the dynamics that drive fungal epidemics in different communities. The models developed in this thesis created a framework for predicting the potential spread of *S. destruens* to new communities and hosts. The unique perspective that *S. destruens* provides is its combination of all aspects of fungal epidemics that may play a role in disease progression, including the perspective of biological invasions.

1.7 Datasets

The datasets used to develop models in this thesis were from multiple published experimental infectivity trials of *S. destruens,* which are described in Table 1.2 and can be found in Appendix 1. All animal procedures described in this thesis followed strict guidelines set forward by the UK Home Office and were performed in accordance with UK Home Office Regulations. The projects were approved by the Bournemouth University ethics committee and performed under Home Office project licence number PPL80/1979.

The key difference between the datasets was the method of infection. In experiments that used bath immersion (Datasets 1 and 2), the host tank systems were flooded with infectious propagules, so hosts were susceptible at Time 0 with extremely high infection load in the environment. Conversely, experiments which used intraperitoneal injection as an infection method (Dataset 2) began with all hosts infected at Time 0, since spores were already in the hosts' bodies. In the experimental cohabitation between *P. parva* and sunbleak *Leucaspius delineatus* (Dataset 3) the infection method was exposure to naturally released spores from *P. parva*, providing the most natural infection method and initial dose despite being a closed system. Together the datasets provided the models with different initial conditions, which allowed parameter calibration and the comparison of multiple scenarios. Furthermore, data on spore lifespan and zoosporulation rates were used to determine propagule lifespan (Dataset 4). Spore and zoospore numbers were monitored daily in sterile water for the number of spores and zoospores surviving each day and their duration of activity (i.e. lifespan).

Table 1.2. A description of the datasets used in the thesis.

	Dataset 1	Dataset 2	Dataset 3	Dataset 4
Reference	Andreou, D., Arkush, K.D., Guégan, J.-F. & Gozlan, R.E. (2012) Introduced pathogens and native freshwater biodiversity: a case study of *Sphaerothecum destruens*. *PloS one*: **7**, e36998.	Paley, R. K., Andreou, D., Bateman, K. S. & Feist, S. W. (2012) Isolation and culture of *Sphaerothecum destruens* from Sunbleak (*Leucaspius delineatus*) in the UK and pathogenicity experiments in Atlantic salmon (*Salmo salar*). *Parasitology:* **139**, 904–14.	Gozlan, R.E., St-Hilaire, S., Feist, S., Martin, P., & Kent, M.L. (2005) Biodiversity: disease threat to European fish. *Nature* **435**, 1046.	Andreou, D, Gozlan, R E, & Paley, R (2009) Temperature influence on production and longevity of *Sphaerothecum destruens* zoospores. 95(6): 1539-41.
Species used and sample size n	1 year old common bream *Abramis brama*, roach *Rutilus rutilus*, common carp *Cyprinus carpio* (n= 60 for each species)	Sunbleak *Leucaspius delineatus*, Atlantic salmon *Salmo salar* (n = 45 for each species)	Topmouth gudgeon *Pseudorasbora parva* (n = 40), sunbleak *Leucaspius delineatus* (n = 80)	NA
Duration of experiment	11 months	3 months	6 months	1 month
Method of infection	Bath immersion (three 4h exposures)	Intraperitoneal injection, bath immersion (one 4h exposure)	Cohabitation	NA
Initial dose	Each exposure contained 8.6 x 10^4 spores/ml	1 x 10^7 spores (injection); 1.3 x 10^5 zoospores/ml (bath immersion)	Unknown	*S. destruens* spores isolated from *L. delineatus* cells were incubated in fresh water at 4, 15, 25, and 30° Celsius.
Tank sizes	70 L	30 L	35 and 70 L	NA
Included in Chapters:	2- 5	2- 5	3-5	2-5

1.8 List of chapters

The research aim of this thesis was to model the epidemiology of a fungal-like pathogen *S. destruens* within the context of host invasion and bi-modal transmission. To predict infectious disease outbreaks in a range of sensitive hosts and communities, pathogen transmission was characterised in light of several biotic and abiotic factors, and then examined through an evolutionary perspective.

Chapter 2

The objective was to quantify the most important and sensitive epidemiological parameters relating to *S. destruens* within five susceptible species and determine the importance of direct contact vs. environmental transmission in fungal disease transmission.

Chapter 3

The objectives were to 1) develop a two-host model which included the pathogen's healthy carrier, and 2) hypothesise and test parameter relationships using estimated parameter ranges to inform general assumptions about the pathogen's disease dynamics.

Chapter 4

The primary objective was to develop a multi-host model in order to characterise how pathogen transmission is affected by host community composition, including species diversity and population density. Furthermore, eradication of the healthy carrier was tested as a disease management technique.

Chapter 5

The objective was to introduce competitive interactions (predation and resource competition) between host species in the multi-host model, to examine community resilience to the introduction of disease. This model could be generalised to any species assemblage.

Chapter 6

The objective was to examine how pathogen virulence is influenced by multiple transmission pathways and community susceptibility, using adaptive dynamics techniques. The findings were generalised to the evolution of fungal pathogens in different communities.

Chapter 7

The objective was to discuss the main findings of this thesis in a wider context to devise empirically based recommendations for managing the spread of emerging fungal diseases.

Chapter 2

The development of a single-host SEIR model for *Sphaerothecum destruens* for five host species

This chapter has been published as: Al-Shorbaji, F. N. et al. The alternate role of direct and environmental transmission in fungal infectious disease in wildlife: threats for biodiversity conservation. *Sci. Rep*. **5**, 10368; doi: 10.1038/srep10368 (2015).

Summary

Emerging fungal pathogens have substantial consequences for infected hosts, as revealed by the global decline of amphibian species from the chytrid fungus *B. dendrobatidis.* Here, based on the outcomes of a set of infection trials of *S. destruens*, a single host epidemiological model was designed. Parameterisation of the model based on the empirical data was followed by sensitivity analyses on estimated parameter values. A high level of dependence on direct transmission in crowded, confined environments was demonstrated. Furthermore, the results established that incubation rate and length of infection dictated the epidemic dynamics of fungal disease. These results shed light on the risks associated with farming conditions and highlight the additional risk posed by invasive species that are highly abundant and can act as infectious reservoir hosts.

2.1 Introduction

Generalist pathogens are increasingly causing significant worldwide declines in a wide range of host species leading to considerable changes in their communities (Anderson and May 1979, Poulin 1997, Roche et al. 2012). As generalist pathogens can transmit to multiple hosts, their virulence is less evolutionarily constrained than specialist disease agents (Woolhouse et al. 2001). In the absence of what would otherwise be strong selective pressures, their outbreaks in the environment often lead to significant losses of species, especially if the pathogen has a free-living stage that can survive outside its hosts in the environment (McCallum and Dobson 1995, Holt et al. 2003, Gozlan et al. 2006, Okamura and Feist 2011).

Free-living infectious stages (such as spores and zoospores) lengthen the timespan of transmission for a pathogen, enabling contact with greater numbers of susceptible hosts (Mitchell et al. 2008). This is consistent with the "curse of the Pharaoh" or "sit and wait" hypothesis, which predicts that free-living infectious stages whose lifespan exceeds the infection time of their hosts are not constrained by virulence, enabling their persistence at high levels of virulence and continued transmission to further susceptible hosts (Ewald 1994, Bonhoeffer et al. 1996, Gandon 1998, Walther and Ewald 2004, Roche et al. 2011). Furthermore, where high proportions of susceptible

hosts are present in a community, the resultant high mortality rates are associated with significant modifications to trophic structure and ecosystem function (Whiles et al. 2009, Fisher et al. 2012). These modifications include species dependent on depleted populations that struggle to survive, or tolerant hosts thriving in the new community as they can transmit the infectious agent without any clinical signs of disease or mortality.

In this chapter, the most influential epidemiological parameters in a fungal pathogen's outbreak were characterised using *S. destruens* as a model system (Section 1.6). This was achieved by combining a unique set of infectivity trials (Section 1.7) and a custom single host epidemiological model. Specifically, the role of alternate transmission methods (direct and environmental) in disease outbreaks and the effect of infection and incubation duration were examined. These parameters are central to the epidemiological understanding of disease dynamics and control.

2.2 Materials and Methods

2.2.1 Model species

A detailed description of *S. destruens* can be found in Section 1.6.

2.2.2 Infectivity trials

Three fish hosts of socio-economic importance, common bream *Abramis brama*, roach *Rutilus rutilus* and common carp *Cyprinus carpio*, were challenged with *S. destruens* infections via bath immersion (Table 1.2). Infection could have occurred at the time of exposure, or subsequently, due to direct and indirect transmission. Mortalities were recorded daily for 11 months post exposure with the resulting mortalities in the exposed groups: 53% in *A. brama*, 37% in *R. rutilus*, and 8% in *C. carpio*. Infection by *S. destruens* was confirmed using PCR detection methods in fish tissue samples and histological examination of the fish tissues. In a separate experiment, *L. delineatus* and Atlantic salmon *Salmo salar* were exposed to *S. destruens* via intraperitoneal injection as well as bath immersion in two separate exposure trials for 84 days (Table 1.2). Up to 33% and 90% mortality was observed

in injected *L. delineatus* and *S. salar* respectively, and 13% and 7% mortality in bath immersion trials in *L. delineatus* and *S. salar*, respectively. The datasets for the five species were used to calibrate and define the parameters and initial conditions of the model, with the aim of creating a model that could be used across all exposure scenarios.

2.2.3 Susceptible-Exposed-Infectious-Recovered model (SEIR)

The pathogen-host system was modelled using an adaptation of the SIR framework (Anderson and May 1992) (Fig. 2.1). To calibrate the model more accurately to the observed data, a closed system was assumed which disregarded natural birth and death rates of the population. Mortality that occurred in the control tanks, where hosts were sham exposed to the pathogen, was factored out of the experimental mortalities before analysis.

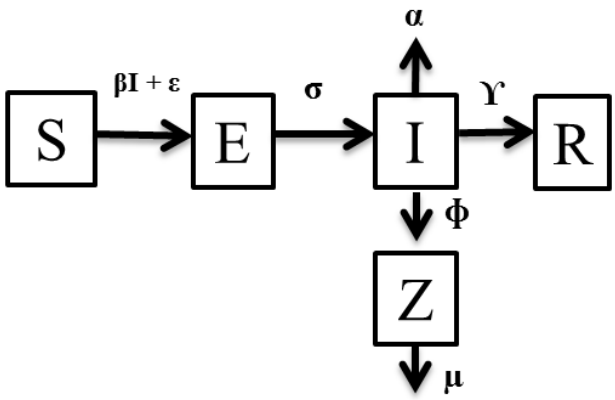

Figure 2.1. A visual representation of the SEIR model categories. Susceptible individuals (S) are exposed to infection at rate β (direct transmission) + ε (environmental transmission). After the exposed state (E), individuals become infectious (I) at rate σ. Infectious individuals either die as a result of disease at rate α, or recover (R) at rate ɣ. Infected individuals release spores (Z) at rate ϕ, including zoosporulation, which have a collective mortality rate of μ.

A series of differential equations determined the rate of change of the population between compartments corresponding to stages of infection. Susceptible individuals (S) become infected (Eq. 2.1) through direct transmission such as contact (β) with infectious individuals (I) as well as through environmental transmission from the water column (ε) through spores or zoospores (Z):

$$\frac{dS}{dt} = -\beta SI - \varepsilon\left(\frac{Z}{Z + k_e}\right)S$$

Eq. 2.1

The role of spores and zoospores in this host-parasite system is unquestionably important, as infection occurs through contact with and ingestion of these spores. The constant k_e is the number of spores required for a 50% probability of infection, as has been done for other pathogens such as *Vibrio cholera* (Codeco 2001) or avian influenza viruses (Roche et al. 2009). For these systems, epidemiological parameters such as recovery rate are not dose-dependent, as hosts have a fixed contact point with the environment (e.g. drinking from an infected water source). However, in this study system hosts are constantly exposed to the environmental free-living stages of the pathogen (spores and zoospores) which can potentially alter some important processes of the epidemiological system. A higher level of spores in the water may accelerate the progress of infection, as spores accumulate in the host more rapidly. Thus, this can accelerate the incubation and mortality rates. Moreover, this assumption makes sense considering that this group of pathogens has shown a dose-dependent disease progression (Carey et al. 2006). The necessity of this assumption was tested for each host species.

Epidemiological models on such systems are rare. Thus, the epidemiological model was fitted to experimental data by considering both the presence and absence of dose dependent disease progression for each epidemiological parameter following infection. For datasets that use bath immersion as an infection method, elevated spore levels lead to the saturation functions behaving as linear functions. Thus, the addition of the saturation function did not help or harm the model fit. However, for a host infected via intraperitoneal injection with low initial free-living spore concentration, the saturation functions improved the model fit over models that did not include the saturation functions (Table 2.1, Fig. 2.2). For that reason, saturation functions were included for all the parameters, to allow a good fit across all species and all experimental datasets. The results demonstrated that dose-dependent disease progression is important for fitting the model to the experimental data.

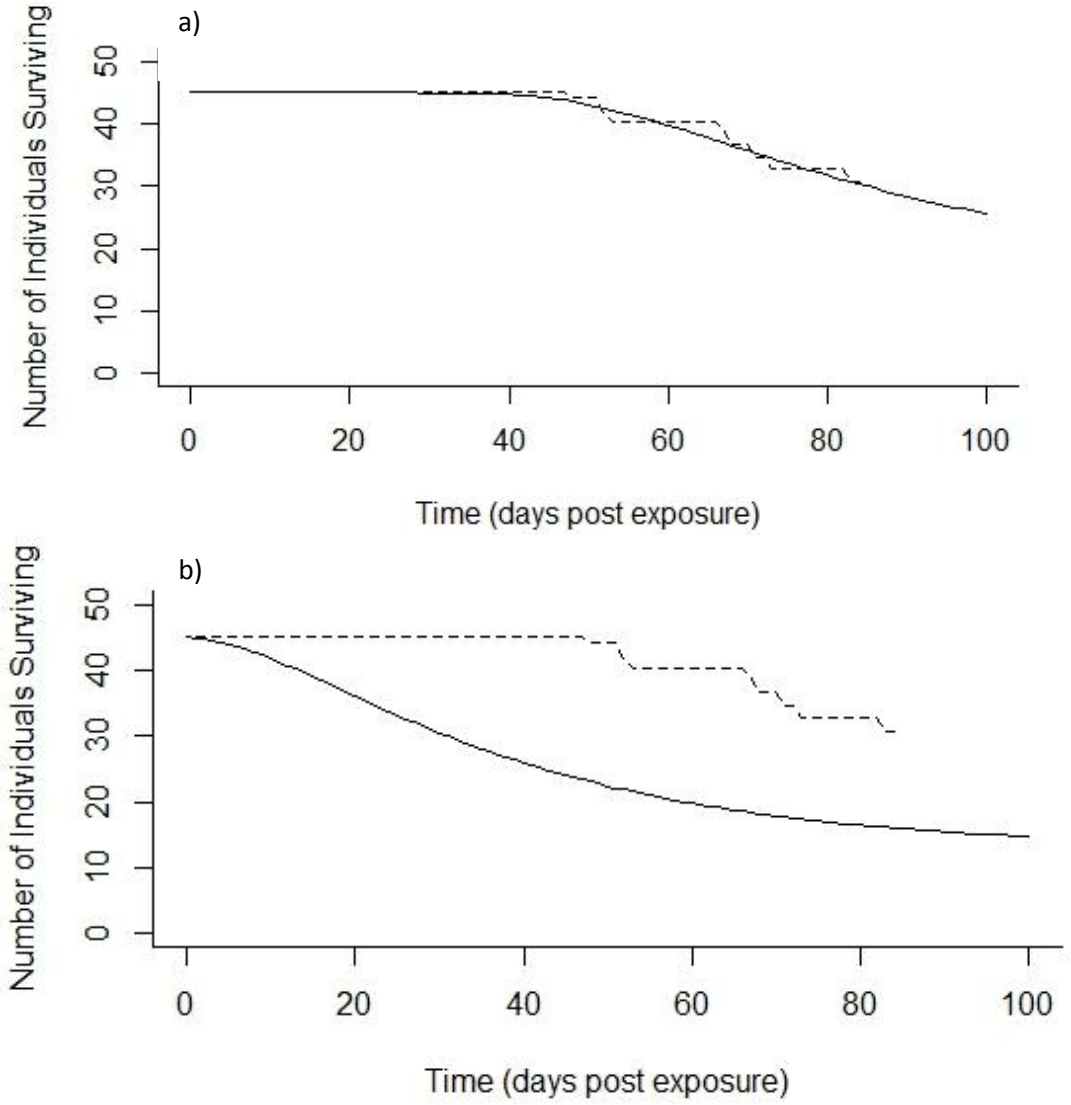

Figure 2.2. The generalised model including all the saturation functions (a) is the best fit for sunbleak *Leucaspius delineatus* data (dashed line). When the model only includes saturation for environmental transmission of spores (ε), it does not fit the observed data (b).

Table 2.1. Akaike's information criterion (AIC) for models that include or exclude parameter saturation functions. Saturation functions are needed for all parameters in the course of infection for *Leucaspius delineatus* (dAIC ≅ 2) while a saturation function is required only for infection for the other host species.

Saturation function included for:	AIC				
	Abramis brama	*Rutilus rutilus*	*Cyprinus carpio*	*Leucaspius delineatus*	*Salmo salar*
ε	431.544 (MSE= 63.78)	433.19 (MSE= 64.84)	226.29 (MSE= 8.19)	No convergence	317.70 (MSE= 37.9)
ε, σ	433.06 (MSE= 63.47)	435.44 (MSE= 65)	237.27 (MSE= 8.96)	363.99 (MSE = 61.50)	334.54 (MSE= 45.32)
ε, σ ,ɣ	435.56 (MSE= 63.79)	437.13 (MSE= 64.80)	229.06 (MSE= 8.09)	367.59 (MSE = 62.68)	339.33 (MSE= 45.32)
ε, σ, ɣ, α	436.73 (MSE= 63.26)	439.75 (MSE= 65.20)	237.29 (MSE= 8.61)	362.07 (MSE = 57.31)	298.25 (MSE= 27.89)

Thus becoming infectious, recovered or dead was correlated to the concentration of spores and zoospores in the environment, as a dose-dependent progression of infection was assumed. The threshold values k_s, k_g, and k_a were introduced for σ, ɣ, and α respectively to reflect this dose-dependent response and better represent the slow growing and chronic nature of the infection in the wild (Ebert et al. 2000). Exposed (E) individuals (Eq. 2.2) were infected but not yet infectious (they are not releasing spores) and became infectious (I) after an incubation period of $1/\sigma*(Z/(Z+k_s))$ days:

$$\frac{dE}{dt} = \beta SI + \varepsilon\left(\frac{Z}{Z+k_e}\right)S - \sigma\left(\frac{Z}{Z+k_s}\right)E \qquad \text{Eq. 2.2}$$

Infectious individuals either experienced mortality as a result of disease at rate $\alpha*(Z/(Z+k_a))$, or can recover (R) at rate $ɣ*(Z/(Z+k_g))$, corresponding to the length of infection (Eq. 2.3 and 2.4):

$$\frac{dI}{dt} = \sigma\left(\frac{Z}{Z+k_s}\right)E - \gamma\left(\frac{Z}{Z+k_g}\right)I - \alpha\left(\frac{Z}{Z+k_a}\right)I \qquad \text{Eq. 2.3}$$

$$\frac{dR}{dt} = \gamma\left(\frac{Z}{Z+k_g}\right)I \qquad \text{Eq. 2.4}$$

In the model assumptions, recovered individuals could not be re-infected. The experimental design dictated that all individuals came into contact with the pathogen. Individuals that did not die either overcame the infection without noticeable signs of deterioration or were naturally resistant. After the experiments were complete, hosts were dissected and tissue samples tested for *S. destruens* presence. It was impossible to differentiate between recovered hosts and naturally immune hosts at the end of the experiment.

Spores are shed into the water from infected individuals at a rate of ϕ per day, and can divide into 5-7 zoospores (additively, they constitute Z) and survive for $1/\mu$ days (Eq. 2.5). Zoospore release rates and spore survival were determined experimentally under sterile conditions (Andreou et al. 2009). These values were comparable to ones reported from close relatives to *S. destruens* (Mitchell et al. 2008). There is currently no experimental way to determine spore or zoospore numbers in non-sterile water, so these empirically determined values were used.

$$\frac{dZ}{dt} = \phi I - \mu Z \qquad \text{Eq. 2.5}$$

2.2.4 Parameter sensitivity

The most appropriate model parameters were determined by establishing a biologically realistic range for parameter values and systematically testing the model output against the observed data for the best fit. Mean squared error (MSE) was used

to determine model accuracy and the best fit model, measuring the difference between model output and observed values (Wallach and Goffinet 1989). Direct and environmental transmission (β and ε, respectively), and mortality rate due to infection (α) were optimised in R (Version 0.97.551 © 2009-2012) using the optim() function in the *lattice* package (Sarkar 2008). A Nelder-Mead simplex algorithm was run for a maximum of 10^5 iterations, terminating if the MSE could not be reduced further (Barton and Ivey 1991). Every model estimation started with ± 25% random variation in the seed values of the optimisation function, to test a range of possible seed values and uncover any local maxima, thus incorporating uncertainty in the starting values and ensuring that the global maximum was identified. This resulted in an estimated set of parameter values resulting in the lowest MSE, and the iteration with the lowest MSE overall was selected as the best fit model.

The sensitivity of a parameter correlates to its influence on the model output; a highly sensitive parameter could change the results significantly when changed by a small amount, while a less sensitive parameter can be varied across a wide range and have no impact on the model output. Thus, after each parameter value was estimated, the parameter was individually varied to determine which values led to a significant change in MSE of the model (measured as ± 5%). This was done to examine the precision of the parameter estimation across the models, and established a range of plausible values for each parameter. This sensitivity analysis increased the robustness of the model estimations. It must be noted that the model MSE could deviate by more than 5% when multiple maximal or the minimal values for all parameters are input into the model.

It was expected that parameter sensitivity would differ based on the method of infection, as each type of experimental setup could constrict certain parameters. The nature of the experimental setups for *A. brama*, *C. carpio*, and *R. rutilus* was predicted to restrict incubation and recovery while leaving parameters related to spore release and transmission with a wider range of possible values, as the system was saturated with infectious propagules. However, for *L. delineatus* and *S. salar*, there were two methods of infection: bath immersion and intra-peritoneal injection (Paley et al. 2012; Appendix 1). In the injection experiments, the parameters that were predicted to be restricted in the model were shedding rate (i.e. spore release)

and threshold levels of infection, as the starting spore level in the tanks was 0. By using both datasets for *L. delineatus* and *S. salar*, combining the parameters from both experimental methods was expected to provide a more precise estimation across all parameters.

To examine the role of direct transmission in crowded environments β was set to 0 to observe how the epidemic would progress in the absence of direct transmission.

2.3 Results

2.3.1 Model parameterization

Infections in *A. brama* were rapid with a minimum length of 10 days of infection from first exposure and an epidemic occurring within 7 days (Fig. 2.3). They also had higher transmission rates (β = 0.15-0.99, ε =0.12-0.7) than the other host species. This is supported by the results of experimental infections, where *A. brama* were highly susceptible to *S. destruens* infection and represented the most sensitive host. During these experimental challenges to the pathogen, all mortalities occurred within 23 days of the last exposure to the pathogen, suggesting both a short incubation rate and high transmission rate between infected hosts. Although the infection rate was lower than for *A. brama*, *R. rutilus* were also highly susceptible to *S. destruens* infection, with a minimum infection time of 26 days after the first exposure to the first mortality (Fig. 2.3).

Transmission parameters for *R. rutilus* optimised to 0.08-0.1 (β), and environmental transmission optimised to 0.003-0.007. In contrast, *C. carpio* were not as sensitive to infection as the other species, despite being tested at the same life stage (Fig. 2.3). They experienced a significantly longer timescale of infection, with the first mortality occurring 55 days post first exposure to *S. destruens* spores. Overall, *C. carpio* appeared to be more resistant to the pathogen (only 8% of the treatment group experienced mortality and survivors had no *S. destruens* DNA detected 11 months post exposure), indicating a full recovery or a natural immunity to the pathogen. The parameterization reflected these observations, with direct and indirect

transmission values at 0.0155-0.0170 and 0.0008-0.0012 respectively. Mortality from infection (α = 0.017-0.025) was significantly lower than that of *A. brama* and *R. rutilus*.

In *L. delineatus*, the model output was sensitive to other parameters, due to the different experimental setup. Incubation (0.23-0.233), recovery rate (0.14-0.17) and spore related parameters (including threshold levels for each stage of infection) had narrow parameter value ranges (Fig. 2.4). The same was true for *S. salar* (Fig. 2.5) which had a lower estimated rate of spore release than cyprinid species (45-100 compared with 350+ for other species). This was consistent with histo-pathological studies of *S. destruens* in salmonid species, which revealed the infection was more contained in nodules rather than disseminated throughout the host body (Arkush et al. 2003, Paley et al. 2012), thus limiting the shedding of spores from salmonid hosts. All parameter ranges are listed in Table 2.2.

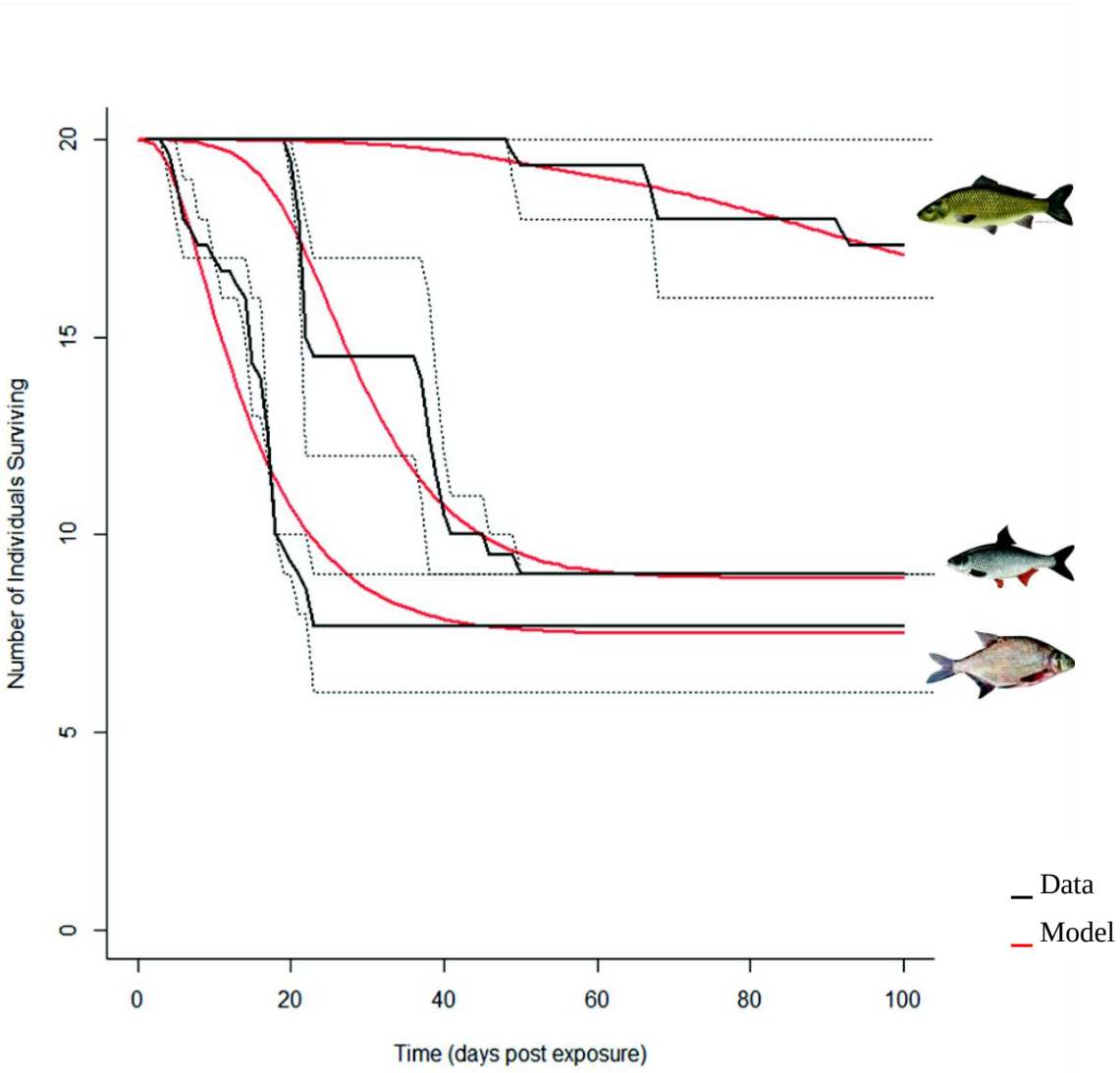

Figure 2.3. The model output for an outbreak of *Sphaerothecum destruens* in juvenile bream *Abramis brama*, roach *Rutilus rutilus* and carp *Cyprinus carpio*. The model output was compared with observed data from Andreou et al. (2012) for *A. brama* (lower), *R. rutilus* (middle) and *C. carpio* (top). The minimum and maximum values of the three replicate samples are shown as dashed lines. Images are all in the public domain as copyrights have expired:

http://commons.wikimedia.org/wiki/File:Braxen,_Iduns_kokbok.jpg,
http://commons.wikimedia.org/wiki/File:Rutilus_rutilus5.jpg,
http://commons.wikimedia.org/wiki/File:Cyprinus_carpio3.jpg

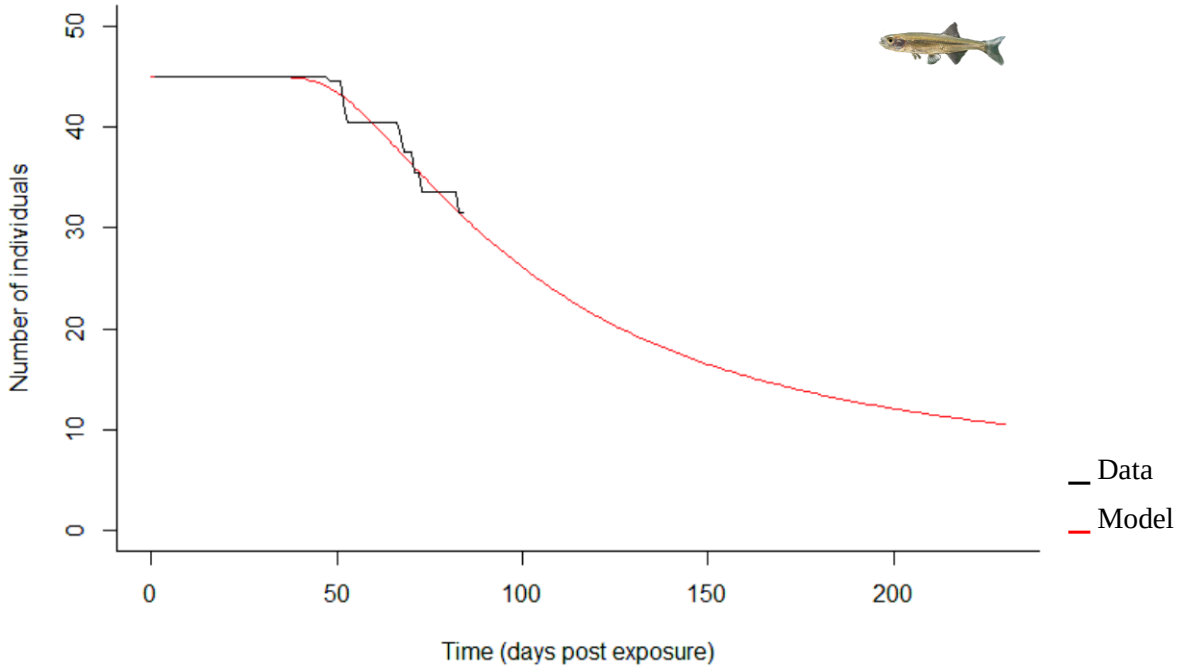

Figure 2.4. Surviving population of *Leucaspius delineatus* when exposed to *Sphaerothecum destruens*. The model output has been fitted to Paley et al. (2012) published data and projected for 250 days, to observe how the epidemic would progress. Image credit R.E. Gozlan.

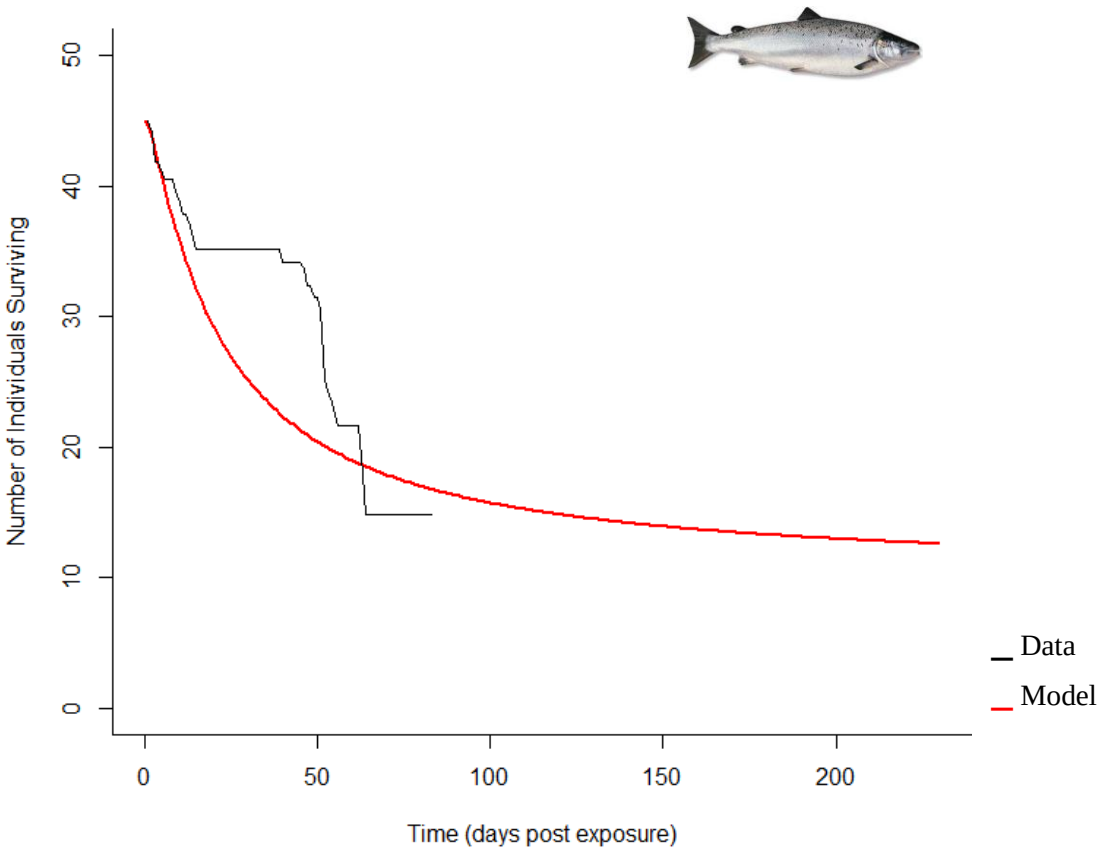

Figure 2.5. Surviving population of *Salmo salar*, a sensitive host to *Sphaerothecum destruens* exposed to its infectious propagules via intraperitoneal injection (Paley et al. 2012). The best fit for the single-host model is shown in red and extended for 200 days. Image credit: http://nw08.american.edu/~vconn/seafood/images/salmon.jpg.

The fit of the *S. salar* single host model was not as precise as the fit of the models for the cyprinid species. In the experiment, multiple mortalities occurred on several days and were followed by periods of up to two weeks where no mortalities occurred. This affected the model fit, as the model used mean rates of mortality and infection, leading to gradual population decline. However, as the start and end points of the fitted model were close to the observed data, this model was considered sufficiently accurate. Furthermore, this was the best fit model that could be achieved across all parameter combinations.

Table 2.2. Parameter ranges for each susceptible host species. Values within these ranges led to model fits that were ± 5% of the optimal MSE.[1]

Parameter (per day rate)	*A. brama*	*C. carpio*	*R. rutilus*	*L. delineatus*	*S. salar*
Direct transmission (β)	0.15 - 0.99	0.0155 - 0.017	0.08 - 0.1	0.015 - 0.02	0.008 - 0.016
Mortality from infection (α)	0.165 - 0.18	0.017 - 0.025	0.129 - 0.133	0.22 - 0.3	0.06 - 0.08
Environmental transmission (ε)	0.12 - 0.7	0.0008 - 0.0012	0.003 - 0.007	0.0074 - 0.008	0.004 - 0.008
Incubation rate (σ)	0.09 - 0.16	0.013 - 0.0805	0.095 - 0.11	0.23 - 0.233	0.04 - 0.11
Recovery rate (γ)	0.095 - 0.105	0.065 - 0.072	0.099 - 0.101	0.14 - 0.17	0.02 - 0.04
Spore release (ϕ)	0 - 10000	350 - 650	0 - 4000	330 - 350	45 - 100
Spore mortality (μ)	0.15 - 0.3	0.18 - 0.205	0.18 - 0.21	0.48 - 0.53	0.48 - 0.55
Ke*	0 - $1*10^8$	0 - $1*10^6$	0 - $6*10^7$	500 - 50000	0 - $1*10^7$
Ks*	0 - 45000	5000 - 11000	0 - 15000	6800 - 7200	2000 - $3*10^6$
Ka*	0 - 25000	8000 - 12000	6000 - 11000	3000 - 10000	10000 - $1*10^6$
Kg*	0 - 50000	4000 - 10000	6000 - 15000	6500 - 10000	5000 - $1*10^6$

[1] Biological definition of K values: the number of spores ingested to achieve a 50% probability of the infection progressing to the next stage. Each subscript corresponds to the category of infection (e = environmental transmission, s = incubation, a = mortality from infection, g = recovery).

2.3.2 Sensitivity analysis

The outcome of the model was most sensitive to the rate of recovery (γ) (alternatively duration of infection $1/\gamma$). The incubation rate of the pathogen (σ) also greatly affected the duration of an epidemic (Fig. 2.6). The rate of mortality from infection (α) was important in the progression of an epidemic in *A. brama* and *R. rutilus*, two highly susceptible species. When direct transmission was set to 0, the parameter estimates for environmental transmission increased. However, it was expected that this increase was largely due to the confines of the data and model structure. The relationship between the transmission parameters will be discussed in more detail in the following chapter. Direct and environmental transmission operate at different timescales so are independent of one another; while direct transmission requires contact between infected individuals, environmental transmission occurs on a longer timescale that is dependent on the lifetime of infectious propagules. Transmission is predicted to also be affected by population density, although to different degrees for direct and environmental transmission. The estimated levels of environmental transmission in the model were not sufficient to cause the observed outbreaks in every species, and direct transmission was necessary. It was expected that direct transmission plays an important role in these conditions (i.e. a closed system with high density), and this result supported that.

Other parameters only substantially affected the model output when they were altered by magnitudes of 10 to 1000. For example, there was very little sensitivity for threshold values (K) in the single host models based on bath immersion experiments. However, the models for *L. delineatus* showed sensitivity to these parameters due to the incorporation of alternate infection methods in the model parameterisation. Therefore, the saturation thresholds for all susceptible species were kept consistent with *L. delineatus* values determined from the single host and cohabitation models. This was done for simplicity and consistency across species.

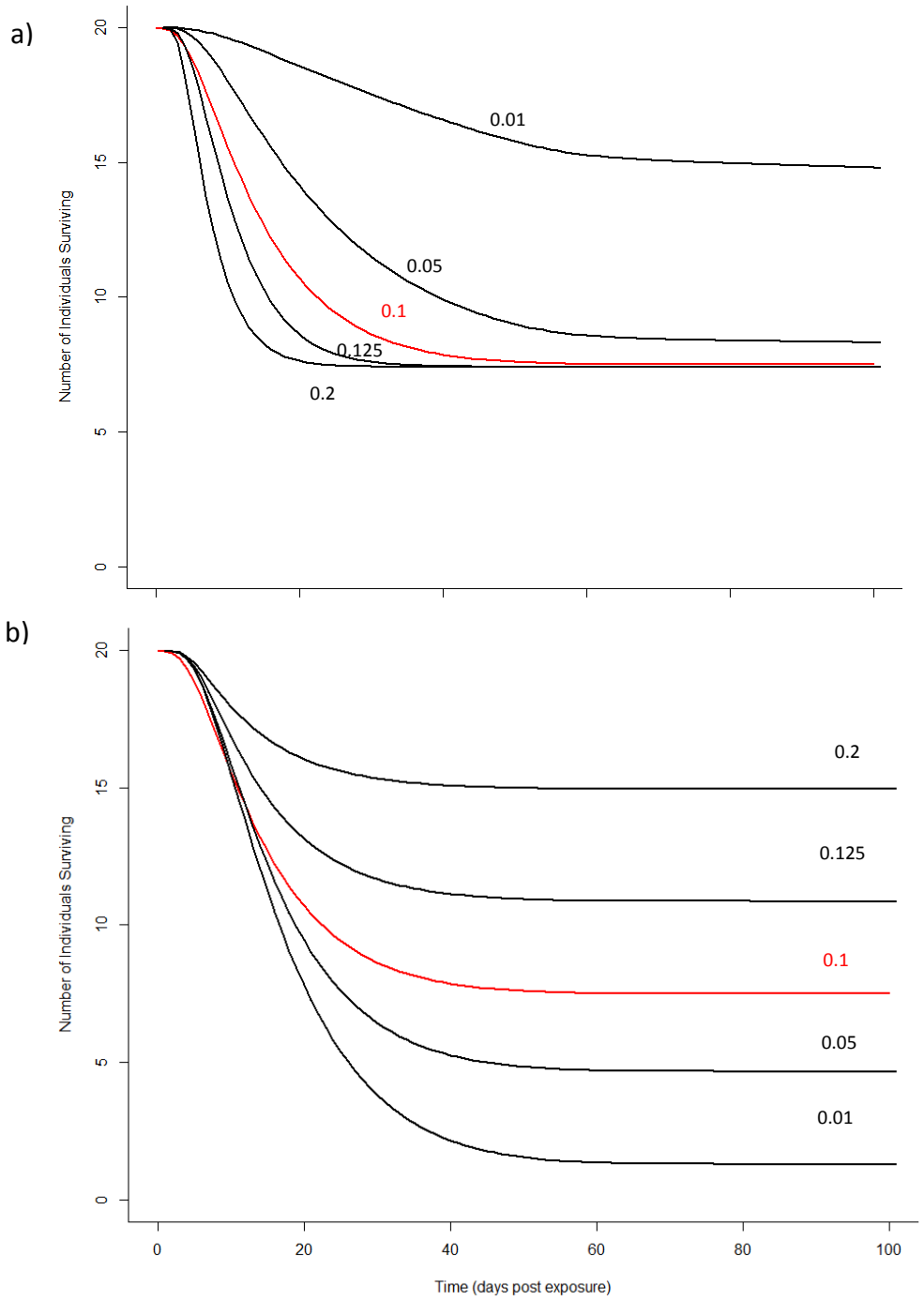

Figure 2.6. Sensitivity analysis of incubation and recovery parameters. Changing the parameter values of σ (incubation) (a) and γ (recovery) (b) from the original common bream *Abramis brama* model values of 0.1 (red) reveals how largely they affect the outcome of the *Sphaerothecum destruens* epidemic. For σ, values lower than 0.1, indicating a longer time of incubation, mitigate the epidemic. Higher values of σ accelerate the progression of the epidemic; however the epidemics plateau at the same level as other values. In contrast, lower values of γ (tested at the same levels as σ) (indicating longer time to recovery) reduced population survival.

2.4 Discussion

Direct transmission through contact between infectious and susceptible individuals (such as in farming conditions or breeding aggregations) was predicted to play a strong role in the development of the epidemic. The results have also identified that the incubation rate (σ) and length of infection (γ) are the two key parameters influencing the severity of *S. destruens* outbreaks. Altering these parameters greatly affected transmission and thus the progression of the epidemic (Monteiro et al. 2007) with the incubation rate largely impacting the speed of the epidemic. At the community level, species with a long infection time (low γ) that are also tolerant to the parasite (low α) could act as reservoirs by shedding low levels of infectious spores over a longer time period, potentially leading to a sustained disease outbreak. This has direct implications for the management of future fungal outbreaks. The density of infectious propagules may alter the incubation rate and length of infection, modulating the severities of observed infections, and will be explored in Chapters 4 and 5.

Fungal spores can survive in the environment outside their hosts for several days, increasing the probability of contact with sensitive hosts (Andreou et al. 2009). For example, the saprobic free-living stages of the chytrid fungus have been shown to be a main driver of epidemics of this pathogen (Mitchell et al. 2008). The documented variation in the number and longevity of *S. destruens*' spores and zoospores may have played a minimal role in driving the epidemic in these closed systems, as the experimental numbers used were well above infection threshold levels. However, this variation could play a more significant role for environmental transmission in natural systems where host contact rates and water flow vary. The high levels of transmission and susceptibility observed here confirmed the risk posed by *S. destruens* of spreading to new areas. This risk is further reinforced by the existence of *P. parva* as a healthy carrier that is highly invasive, has a broad thermal tolerance and can disperse rapidly (Gozlan et al. 2005, Andreou et al. 2009). As a generalist parasite, *S. destruens* shows a range of infectivity of hosts and an increased species richness can in effect lower the prevalence of the disease in that environment (Roche et al. 2012). Determining which community composition can best constrain pathogen emergence and prevent a widespread outbreak has important implications for

controlling disease risk and ultimately for species conservation. Currently, *A. brama* is both the most sensitive to and the highest transmitter of the disease, which may affect communities to varying degrees based on their structure (Johnson and Thieltges 2010, Roche et al. 2012).

Ecosystems are rapidly changing and will continue to do so as a result of global changes, such as significant alterations in land-use, species introductions and habitat destruction (Okamura and Feist 2011, Peeler and Feist 2011). These all directly or indirectly affect the environmental transmission and contact frequencies between potential hosts, with a knock-on effect on the evolutionary bi-stability of the pathogen's transmission patterns (Karvonen et al. 2010, Roche et al. 2011, Leung and Bates 2013). Although creating this evidence-based model has established an important baseline for understanding disease dynamics in fungal parasites, a more comprehensive community level network model requires development in order to fully understand the dynamics and potential impacts of such pathogens on host populations and biodiversity.

Chapter 3
Epidemiological parameter estimation

Summary

The single host models in Chapter 2 provided empirically based species-specific parameter ranges for *S. destruens* in five susceptible species. In the first part of this chapter, an additional dataset was used to create a two host model which included *L. delineatus* and *P. parva*, the pathogen's healthy carrier. Single host models provide limited insights into community disease dynamics compared to multi-host models, which incorporate species interactions. In order to build multi-host epidemiological models for *S. destruens* and explore the effects of this generalist pathogen in different habitats (Chapters 4 and 5), the precision of the parameters obtained from the two host model was determined through sensitivity analyses in the first part of the chapter. In the second part, hypotheses about various parameter relationships (including transmission, incubation, recovery, and spore release) were tested using the single and two host models' parameter estimates to establish general assumptions for parameter values across multiple species and expand the multi-host model's usefulness. Incubation, recovery, and mortality rates displayed consistent patterns between species and those relationships were used to determine parameter values in models built in later chapters. Spore release (i.e. pathogen shedding rate) was a sensitive parameter for *L. delineatus* across the different experiments, and thus spore release values for the remaining hosts were varied to reflect the pattern observed in this species. For parameters linked to pathogen transmission, modal values from the single-host and two host model outputs were selected wherever possible.

3.1 Introduction

Experimental infectivity studies of *S. destruens* provided an important baseline of empirically derived parameters for the pathogen's disease dynamics in susceptible cyprinids and one salmonid (Chapter 2). While single host models are crucial for providing this baseline, their applicability to real systems and conditions can be limited, as species rarely exist in isolation. In addition, generalist pathogens such as *S. destruens* can behave differently in each host species, and transmission can be affected by community disease dynamics. In order to characterise community-level pathogen transmission it was thus necessary to create multi-host models that simulate

real-world conditions. Epidemiological models are developed at the community level with assemblages of different host species in Chapters 4-6, which in turn require correct parameterisation (Table 3.1) in order to have reliable outputs. Parameterisation is thus an integral stage of creating models capable of accurate prediction, as it set the framework of assumptions that underpinned model construction.

The datasets used to calibrate models in Chapter 2 were from *S. destruens* infectivity trials of common bream *A. brama*, roach *R. rutilus*, common carp *C. carpio*, sunbleak *L. delineatus*, and Atlantic salmon *S. salar* (Section 1.7). In the first part of this chapter, an additional model was created for healthy carrier topmouth gudgeon *P. parva* (in a two host setting with *L. delineatus*, Section 1.7, Appendix 1). Combined, all the selected species represent a wide range of susceptibility to *S. destruens*, and are found in typical European temperate freshwater environments (Gozlan et al. 2005, Andreou et al. 2012). Combining and comparing the model outputs for five susceptible species broadened the baseline of parameters that the single host models provided, and allowed a more complete characterisation of the disease dynamics of this generalist pathogen. Furthermore, the epidemiological dynamics of the healthy carrier could be examined. This then enabled the design of a generalised multi-host model for any species assemblage, and helped establish evolutionary trade-offs between parameters in Chapter 6.

Table 3.1. Epidemiological parameters requiring parameterisation in the multi-host model.

Parameter	Description (rates per day)
α	Mortality from infection (virulence)
γ	Recovery rate
σ	Incubation rate
β	Direct transmission rate
ε	Environmental transmission rate
ϕ	Propagule release rate (shedding rate)

It is also necessary to consider possible biases the data imposed on conclusions and model development in order to use them successfully in further analyses of pathogen transmission. The sensitivity of parameter estimations was tested in order to determine the uncertainty of the selected values (i.e. a wide range of equally possible values indicated a higher level of uncertainty in the parameter estimation). This was important in determining the reliability of model outputs, and for establishing general parameter relationships to use in multi-host models. As such, several objectives were defined for this chapter.

Part 1: Incorporating an additional dataset and determining parameter sensitivity across models

- To create a two host model using data from a cohabitation experiment between *P. parva* and *L. delineatus*, allowing model calibration for both species with a different method of infection (compared to bath immersion and intra-peritoneal injection, see Chapter 2).
- To run a sensitivity analysis to refine the possible value range of each parameter (mortality from infection, incubation, recovery, spore release, transmission) for each species, and thus establish the reliability of the model outputs.

Part 2: Estimating relationships between parameters to create large species assemblages

- Hypotheses on correlations between parameters were formulated and tested based on the model estimations, in order to generalise parameter relationships across multiple host species.

Part 1: Incorporating an additional dataset and determining parameter sensitivity across models

3.2 Methods

3.2.1 Developing the two host model

Using the SEIR framework defined for single species, direct interspecies transmission (β) between species *i* and *j* was added, and the number of propagules in the environment (Z) was calculated as the sum of the propagules shed by both hosts (Φ), which survived for 1/μ days. Thus, the two host model represented the added direct and environmental infection pressure from two species rather than one (Fig. 3.1).

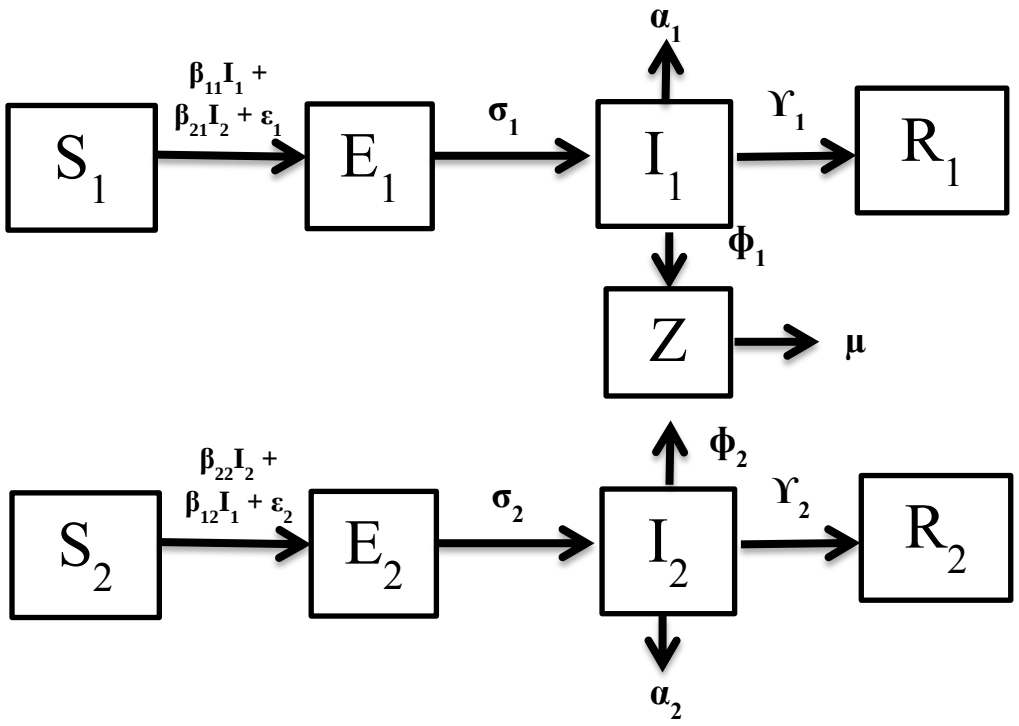

Figure 3.1. A visual representation of the dynamics included in the two-host SEIR model.

The epidemiological parameters estimated for the highly sensitive *L. delineatus* model (Section 2.3) were inputted into the two host model that also included the healthy carrier *P. parva*. To establish parameter values for *P. parva*, the epidemiological parameters for both species were calibrated with experimental cohabitation data of these two species (Gozlan et al. 2005; Appendix 1). Parameters

(incubation, recovery, transmission and spore release) for *P. parva* were estimated by optimising the model to the lowest MSE, similarly to the single host model (Section 2.2). Initial disease prevalence in *P. parva* was set at 100% and systematically reduced by 10% until a best fit was achieved, i.e. it more accurately characterised the initial conditions of the models. This resulted in a set of parameter value ranges for each epidemiological parameter in both species.

3.3 Results and Discussion

3.3.1 Two host model

When 100% of *P. parva* were assumed to be infected, *L. delineatus* mortalities were marginally higher than the observed experimental data (2 sample t-test: $t = 1.74$, d.f. = 464, $p = 0.083$). The best model fit was achieved using 10% prevalence in *P. parva*, a conservative estimate when compared with values of 67-74% found in Dutch populations (Spikmans et al. 2013) and 25-100% in Turkish populations (Ercan et al. 2015). However, given that the pathogen was not detected through PCR in *P. parva* used in the cohabitation experiment (n = 40), this estimate was considered realistic for the model parameterisation. The effect of initial pathogen prevalence on pathogen establishment was further explored in Chapter 4. A non-zero recovery rate for *P. parva* resulted in a better fit to the experimental data of *L. delineatus* mortality, suggesting that healthy carrier recovery and/or immunity plays a role in *S. destruens* disease dynamics (Fig. 3.2). In the cohabitation model, mortality due to infection (α) of *L. delineatus* was initially set to 0.3, as estimated in the single host model. This led to a poor fit for the cohabitation data so a range of mortality rates for *L. delineatus* from 0 to 1 was tested, representing the possible range of pathogen virulence in the susceptible host.

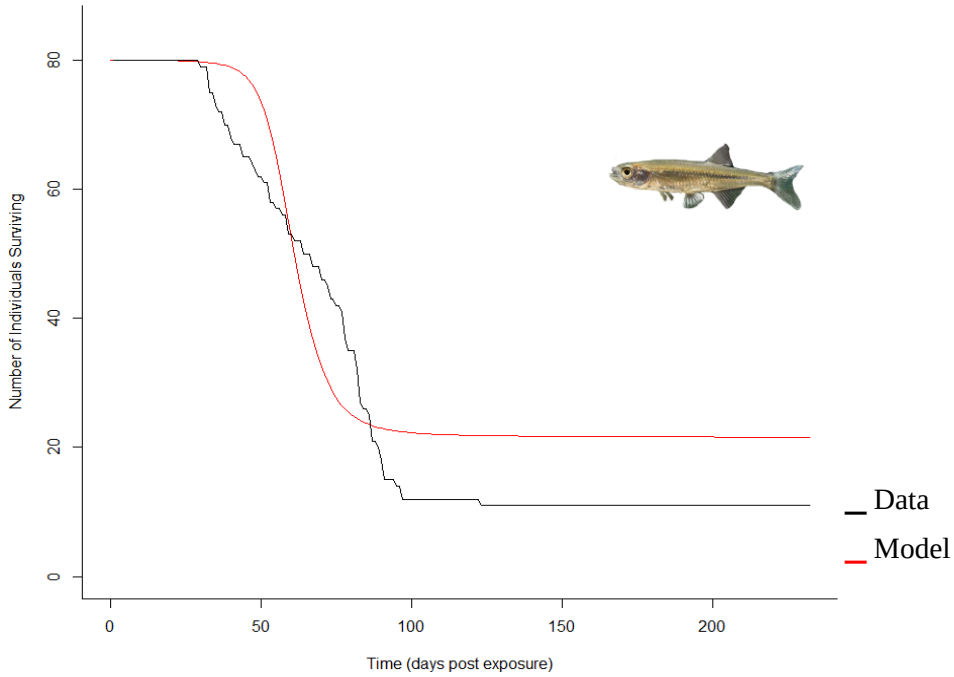

Figure 3.2. Surviving population of susceptible host *Leucaspius delineatus* when cohabited with a healthy carrier *Pseudorasbora parva*. The two host model (red) was calibrated using experimental cohabitation data by Gozlan et al. (2005) shown in black. The mortalities shown represent only *L. delineatus* mortalities. Here, the initial infection prevalence in *P. parva* was set at 10% and the mortality rate from infection for *L. delineatus* was set to 0.3.

The best fit was achieved by setting α of *L. delineatus* to 0.8 (Fig. 3.3), which was significantly higher than the single host model estimate. In the control tanks of the *L. delineatus* single host experiment (Paley et al. 2012), 6% mortality (considered natural mortality, or mortality due to sham exposure to the pathogen) was observed in the absence of *S. destruens* over the duration of the experiment. When this was incorporated into the two-host model, it explained the discrepancy between estimated *L. delineatus* mortality from infection rate in the single host and cohabitation model. In subsequent chapters, a natural birth and death rate were incorporated into the model parameters for each host (discussed in Chapter 4).

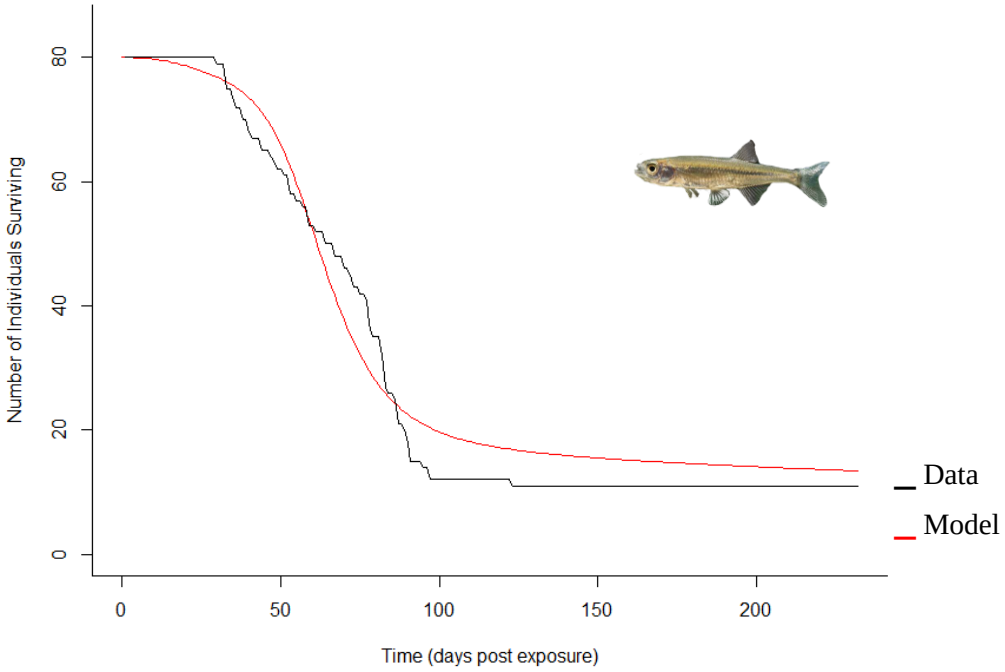

Figure 3.3. Surviving population of susceptible host *Leucaspius delineatus* when cohabited with *Pseudorasbora parva.* The model (red) is a better fit to the data when mortality from infection is increased to 0.8, significantly higher than the single host experiments estimated (0.3). The mortalities shown represent only *L. delineatus* mortalities.

3.3.2 Parameter sensitivity

In the cohabitation experiment, *L. delineatus* ingested spores naturally released from *P. parva*. If both species had unknown parameter values, the complication of an additional host in the model was expected to lead to several sets of equally possible results. However, the advantage of using multiple datasets enabled the incorporation of *L. delineatus* single host model values into the two host model, which allowed the parameterisation of *P. parva*. As *P. parva* behave as healthy carriers, their epidemiological parameters would have been difficult to observe in single-host experiments. Furthermore, the parameter ranges for *L. delineatus* were refined in the two-host model compared to the single host models.

At this stage, each experimental dataset had a parameterised model with a range of possible values (Table 2.2 in the previous chapter). Finalised parameter values were selected for the multi-host models (Table 3.2) based on estimated or modal values

from the parameter ranges. Thus, the combined datasets provided a range of possible values for each parameter and for each species, which represented the uncertainty in parameter estimation. This then allowed the exploration of relationships between parameters, to generate broad assumptions for use in multi-host models in the following section.

Table 3.2. Finalised parameter values for each species in the multi-host model

Parameters	*Pseudorasbora parva*	*Leucaspius delineatus*	*Rutilus rutilus*	*Cyprinus carpio*	*Salmo salar*
Direct transmission (β)	0.011	0.016	0.08	0.016	0.016
Environmental transmission (ε)	0.0007	0.0007	0.0007	0.0007	0.0007
Incubation (σ)	0.072	0.23	0.095	0.013	0.042
Recovery (γ)	0.108	0.14	0.082	0.065	0.037
Mortality from infection (α)	0	0.3	0.13	0.017	0.06
Spore release (ϕ)	52	120	165	120	90

3.4 A note on model selection and optimisation

The incorporation of multiple saturation functions in the SEIR system resulted in the best fit models across all the available datasets, as demonstrated by the model AIC values (Chapter 2). Within each model, the optimisation approach used was a quasi-Newton BFGS method to establish the minima of the function (Schmidt et al. 2009); parameters were given range constraints which were determined by biological knowledge of the study system, and the function began running using the selected seed values of each parameter. The fitting procedure then varied the parameter values incrementally until the best fit was achieved at the minimum MSE (Touretzky 1996).

Many studies use more complex optimisation procedures to determine the best fit parameters. Among these approaches are maximum likelihood estimation (Wallinga and Teunis 2004, Obadia et al. 2012) via different methods such as genetic algorithms (Whitley 1994) or particle filtering (Crisan and Doucet 2002). Maximum likelihood estimation (MLE) is a highly useful and popular method of parameter estimation where a sample of observations can be used to calculate the likelihood of different parameter values resulting in the best fit model (Dempster et al. 1997, Myung 2003).

Particle filtering (also called the sequential Monte Carlo method) uses Monte Carlo algorithms to find approximate optimal estimations based on repeated random samples (Carpenter et al. 1999), and is useful for systems with partial or incomplete observations (Rasmussen et al. 2011). Genetic algorithms calculate the most likely parameter values based on the principles of natural selection; in each iteration, parameter values are randomly selected from a given set of points within a search space, and the most "successful" values then evolve in the next iteration, until an optimal estimation is reached (Touretzky 1996). This is a useful method for stochastic or non-linear parameter estimation, especially for complex systems with many potential contributing factors (Congdon 2000).

The common aspect between these methods is that they are used for systems with incomplete, noisy data, or data with several unknown contributing factors. However, the data in this thesis was highly controlled and consisted of detailed experimental data, so these approaches were not necessary for parameter estimation. The experimental data provided a fully observable process at a fine scale resolution for the duration of the trials with extremely simple dynamics. Minimising the MSE across a large number of iterations showed similar results to maximum likelihood, by selecting parameter values that resulted in the best model fit i.e. values that were the most likely to accurately fit the data.

Part 2: Estimating relationships between parameters to create large species assemblages

The main aim of this section was to test whether the estimated parameters could help formulate broad patterns for this pathogen across different host species, in order to model large species assemblages. These broad patterns are often difficult to quantitatively define for generalist pathogens, as they can behave differently across their host range. Here, the availability of detailed empirical datasets across multiple species and infection methods was a significant advantage in modelling *S. destruens* disease dynamics. Combining the set of parameter estimations for the five susceptible species (which ranged from low to high susceptibility) provided a unique opportunity to attempt a generalisation of the pathogen's disease dynamics in multiple hosts. By examining the species-specific results in combination, correlations between parameters could be observed. This enabled the quantification of how epidemiological parameters correspond to one another, which could help characterise patterns in a multi-host model of pathogen dynamics that could be fit to any species assemblage. Thus, the model could be used to predict pathogen effects in multiple ecosystems, as well as ascertain the contribution of each species to a disease outbreak. Even more importantly, characterising parameter relationships and trade-offs can aid in predicting pathogen evolution, a key aspect in epidemiological studies which will be explored in detail in Chapter 6.

3.5 Methods

Relationships between different epidemiological parameters were hypothesised from previous studies on similar pathogens. Specifically, the parameter ranges (Table 2.2) for mortality from infection (i.e. virulence), incubation, recovery, spore release and transmission were examined between susceptible species for consistent values or patterns. This allowed the comparison of the results to published literature, and established parameter relationships. The following hypotheses were formulated and tested:

Hypothesis 1: Incubation rate and mortality from infection. The incubation rate (σ) was the inverse of the duration of incubation. A positive correlation between

virulence (α) and incubation rate was predicted, under the assumption that a shorter incubation time (therefore a larger σ) led to more rapidly acting infections and thus higher mortalities (Daszak et al. 1999, Carey et al. 2006).

Hypothesis 2: Incubation rate and recovery rate. Given that these two parameters were shown to be highly sensitive in the single host models in Chapter 2, a potential relationship between incubation and recovery rate (γ) was tested. It was predicted that they would be positively correlated, based on the hypothesis that a faster progression of infection would lead to a shorter infectious period.

Hypothesis 3: Direct and environmental transmission. The two transmission parameters (direct β and environmental ε) operated at different timescales that were independent of one another in this system. Direct transmission required contact between infected individuals whereas environmental transmission occurred over a longer timescale that was dependent on the lifetime of free-living infectious propagules (Roche et al. 2012). Thus, direct transmission would require many pathogen propagules to increase transmission, while environmental transmission would more likely select for long–lived propagules. Studies have shown a trade-off between propagule production and propagule persistence, with pathogens selecting for high reproduction with a short-life span, or producing few but long-lived propagules (De Paepe and Taddei 2006, Roche et al. 2011). Therefore, it is expected that if direct transmission increased, environmental transmission would decrease, and vice versa. The relationship between the two modes of transmission was tested in the datasets.

Hypothesis 4: Transmission rates and mortality from infection. Both transmission parameters were tested against virulence, as enhanced transmission could increase virulence (Ewald 1994, Pulkkinen et al. 2010). Higher transmission was predicted to correlate with a faster progression of the infection, as the number of propagules in a host would accumulate more rapidly. As the host ingests infectious propagules at a higher rate, these could multiply faster within the host body and cause mortality earlier. Here, it was further assumed that host population density affected the rate of direct transmission (β), with higher direct transmission rates in more dense host populations (Wood et al. 2010).

Hypothesis 5: Spore release and environmental transmission. Environmental transmission (ε) occurred through the water column via contact with infectious propagules. It was hypothesised that as the number of infectious propagules in the water increased, the probability that they would come into contact with new susceptible hosts would increase, until an uptake threshold was reached. Thus, a positive logistic correlation between environmental transmission ε and rate of spore release ϕ could be expected.

Hypothesis 6: Spore release and mortality from infection. Often, high mortality rates are correlated with a higher infection load (Carey et al. 2006, Briggs et al. 2010). If this is true, a higher number of spores should be released by more highly infected (or more susceptible) hosts (DiRenzo et al. 2014). Here, host body size was also considered, as larger species may take longer to accumulate the pathogen in relation to host biomass, but can release a greater number of spores than smaller susceptible species. A positive correlation was thus hypothesised between spore release and virulence, with larger body sizes releasing higher numbers of infectious propagules.

Standardised major axis (SMA) regression is widely used in ecology to measure how various factors are correlated with one another when there is uncertainty in both parameters (Warton et al. 2012). Here, median parameter values for each susceptible species were used as data points to fit SMA regression models (using the *smatr* R package) for different parameter combinations according to the hypotheses, and the corresponding minimum and maximum values of each parameter range were incorporated into the figures as uncertainty in the selected values. Minimum and maximum parameter values which were considered outliers were investigated with regards to their leverage on the regression line (Appendix 2). Points with high leverage were significantly different from the independent variable mean (i.e. high or low values of x) and influenced the trend line if they also had a high residual. Valid regression lines were used to establish relationships between parameters across species. For parameters that did not have valid regression lines, the data points were examined for modal values between species, or alternatively parameter values were selected based on epidemiological literature on similar pathogens.

3.6 Results and Discussion

Hypothesis 1: Incubation rate (σ) and mortality from infection (α)

The data for *S. destruens* across species supported the relationship predicted in Hypothesis 1 (Fig. 3.4), demonstrating a significant positive correlation between mortality from infection (α) and incubation rate (σ).

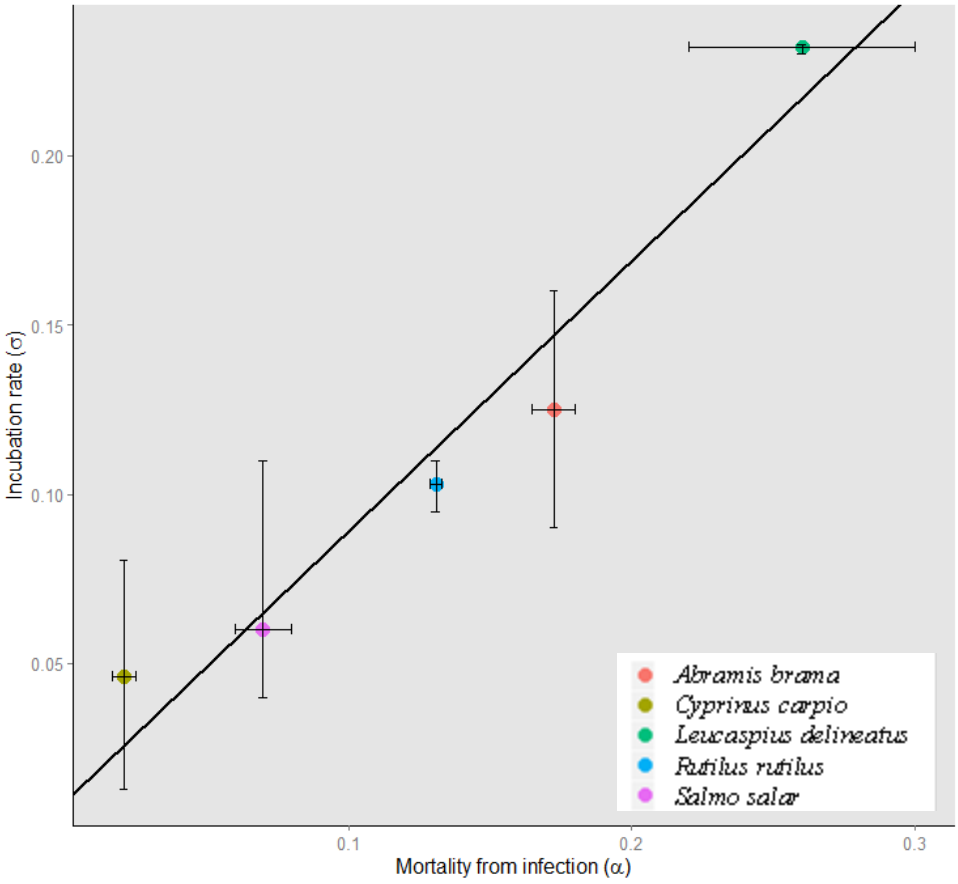

Figure 3.4. The median values of incubation rate for each species (σ) plotted against the median values of mortality from infection (α) for each species, with the minimum and maximum values of each range incorporated as uncertainty. There was a significant positive linear correlation between mortality (α) and incubation rate (σ) (SMA: $\sigma = 0.79\alpha + 0.009$, $p < 0.001$, $R^2 = 0.94$).

Hypothesis 2: Recovery rate (γ) and incubation rate (σ)

The data revealed that there was a significant linear relationship between incubation and recovery rate (Hypothesis 2; Fig. 3.5). These estimated parameter relationships were used in subsequent models.

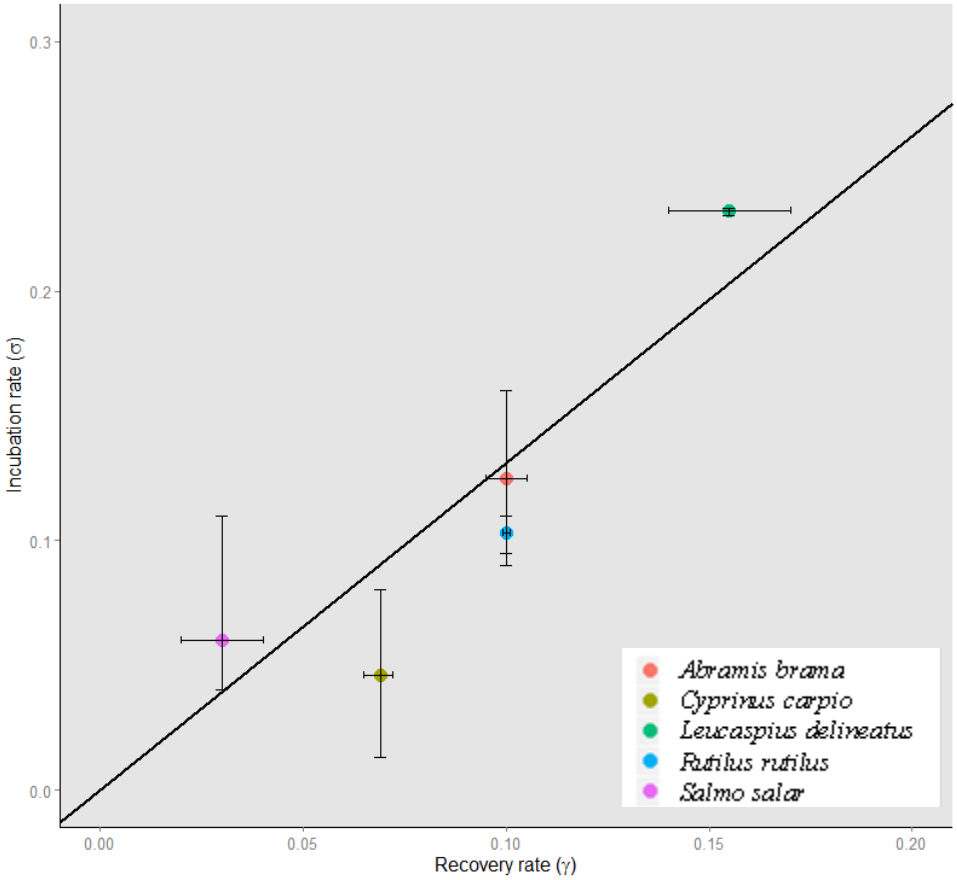

Figure 3.5. The median values of incubation rate for each species (σ) plotted against the median values of recovery rate (γ) for each species, with the minimum and maximum values of each range incorporated as uncertainty. There was a significant linear correlation between recovery rate and incubation rate (SMA: $\sigma = 1.31\gamma$, $p < 0.001$, $R^2 = 0.95$).

The relationships found between incubation rate and mortality from infection, and incubation and recovery rate suggested that there could be a positive correlation between recovery rate and mortality from infection. In practice it is problematic to disentangle the respective effects of mortality from infection and recovery, as both are ways to end the period of infection. Virulent infections can act more rapidly than less virulent infections, with hosts either recovering or dying within a shorter time (van Baalen 1998). For example, the amphibian chytrid fungus, *B. dendrobatidis,*

which is a relatively similar pathogen to *S. destruens*, has a short duration of infection (i.e. high recovery rate) and a high mortality rate (Daszak et al. 1999). Alternatively, other pathogen systems demonstrate an inverse relationship between mortality from infection and recovery rate (Anderson and May 1982). Based on the data, the median parameter values across the susceptible species supported the presence of a positive correlation between recovery rate and virulence (SMA: $\gamma = 0.49\alpha + 0.026$, $p = 0.056$, $R^2 = 0.75$), but due to the reasons stated above this correlation is not necessarily a causal relationship.

Hypotheses 3 and 4: Transmission

Initially, it appeared that the data was consistent with a positive correlation (possibly linear or logistic) between the two modes of transmission (Hypothesis 3; Fig. 3.6a). However, the apparent correlation between environmental and direct transmission was most likely an artefact of the model system (see Chapter 2). An examination of the residuals revealed outliers in the data and their corresponding leverage (Appendix 2) indicated they significantly influenced the model fit, compromising its validity. The outlier species (*A. brama*) was then excluded to examine whether any patterns emerged, but there was still no apparent relationship between direct and environmental transmission (Fig. 3.6b). There was also no significant mathematical relationship between either mode of transmission and mortality from infection after *A. brama* was excluded (Hypothesis 4; Fig. 3.7).

Given that the parameter ranges for *A. brama* were outliers for transmission and spore release, this species was excluded from further analyses to avoid any large skew in the results. The broad parameter ranges for *A. brama* were most likely because of the high mortality rates in the experimental trials compared to the other species, which could have limited the precision in parameter values. While *L. delineatus* was also a susceptible species with high mortalities, its parameter ranges could be more precisely determined by the inclusion of additional datasets in the model parameterisation (the intra-peritoneal injection and cohabitation trials). This was not an option for *A. brama* as it was included in only one dataset.

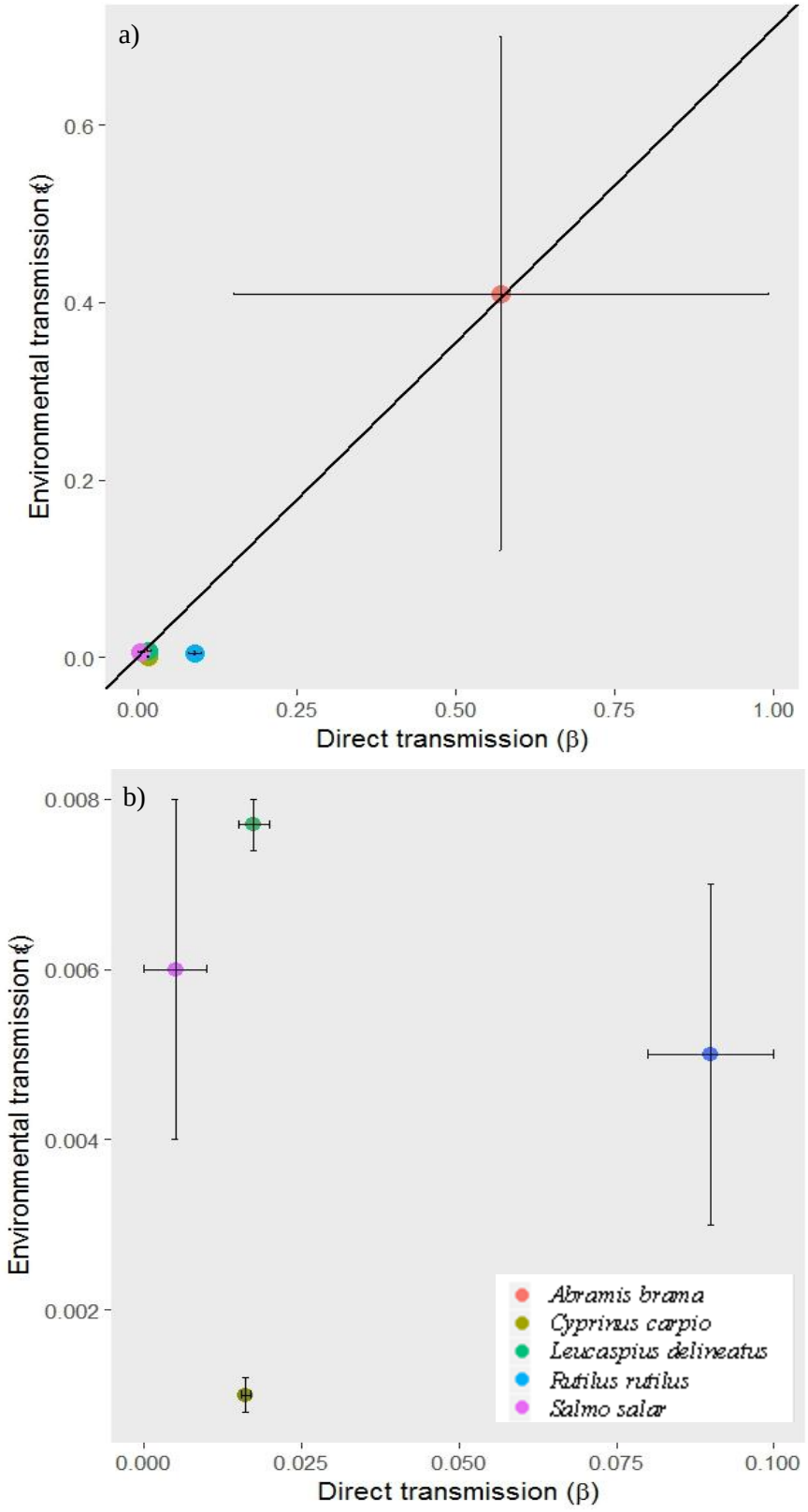

Figure 3.6. The median values for direct and environmental transmission with uncertainty incorporated, including *Abramis brama* (a) and excluding this species (b). There was a significant linear relationship between the two parameters when *A. brama* was included (SMA: $\varepsilon = 0.71\beta$, $p < 0.001$, $R^2 = 0.97$), which was likely an artefact of the system (Appendix 2). When *A. brama* was removed from the analysis, there was no significant relationship between the transmission parameters (SMA: $\varepsilon = 0.118\beta$, $p = 0.27$, $R^2 = 0.38$).

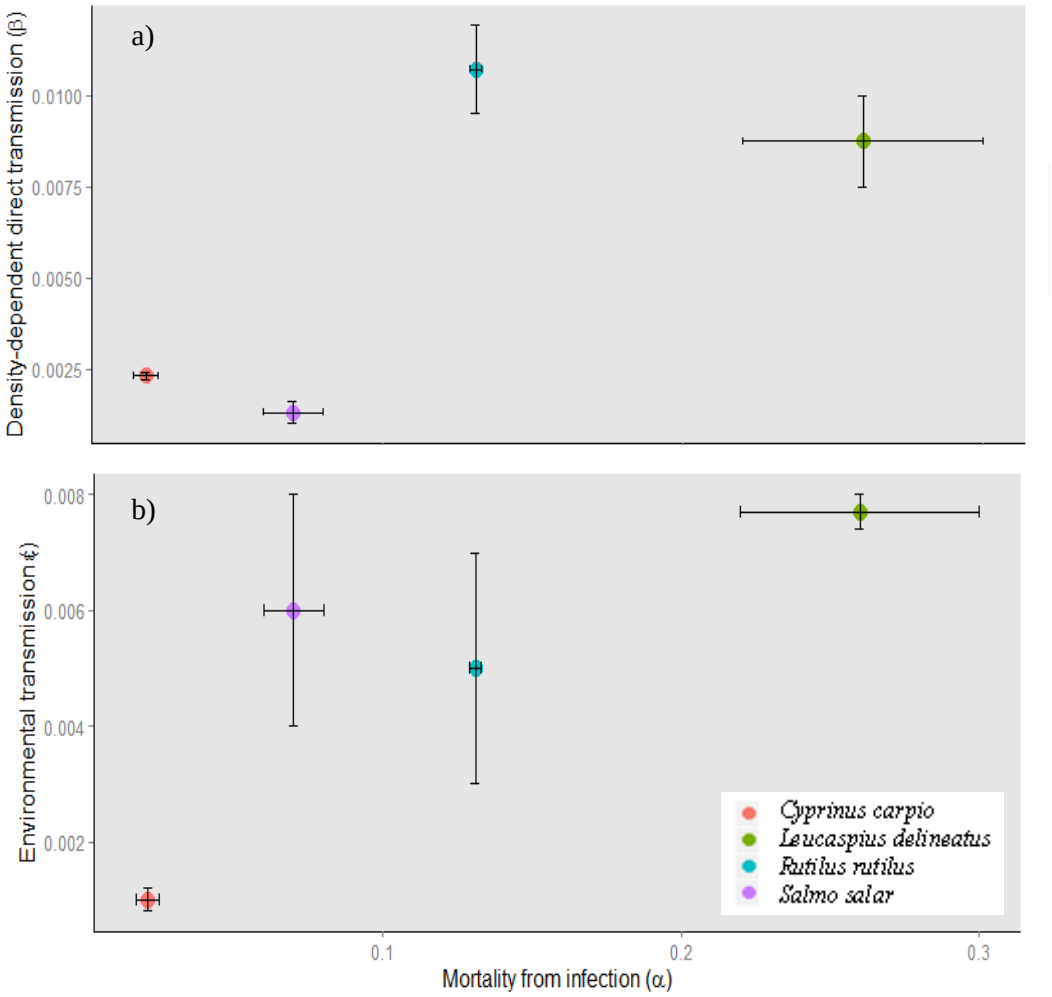

Figure 3.7. The median values of the transmission parameters (with population density incorporated into calculations of direct transmission) plotted against mortality from infection, including the minimum and maximum values of the ranges shown as uncertainty. No relationship was found between direct transmission (a) and mortality from infection (SMA: $\beta = 0.045\alpha + 0.0003$, $p = 0.28$, $R^2 = 0.51$), or environmental transmission and mortality from infection (b) (SMA: $\varepsilon = 0.028\alpha + 0.0016$, $p = 0.17$, $R^2 = .67$). *Abramis brama* had significantly higher uncertainty in the transmission parameters than the remaining species, so was excluded from this analysis.

Transforming the data to uncover potential log-log relationships between transmission parameters also led to non-significant results. Due to the difficulty in establishing mathematical relationships for transmission, it was necessary to determine whether both transmission pathways played an important role in pathogen dynamics or if one could be disregarded in the multi-host model. The pathogen's basic reproductive number R_0 (defined as how many additional infections resulted from one infectious individual) based on Roche et al. (2011) was used to determine

the respective impact of direct and environmental transmission on total pathogen transmission. The R_0 system used was not designed with the inclusion of multiple saturation functions, yielding numerically inaccurate results. A reproductive number including the saturation functions would have complicated the results unnecessarily, especially as infectious propagule levels were inflated in the bath immersion experimental settings. However, Equation 3.7 still provided an approximated framework through which the relative contribution of each mode of transmission could be investigated:

$$R_0 = \frac{N * \chi * \beta}{\gamma + m + \alpha} + \frac{N * \varepsilon * \phi}{\mu * k_{\varepsilon} * [\gamma + m + \alpha]} \qquad \text{Eq. 3.7}$$

where N was host population size, χ was direct contact rate, β was the rate of direct transmission given a contact, and the remaining parameters were as defined previously (Table 3.1, Roche et al. 2011). The first term correlates to the contribution of direct transmission, and the second term correlates to environmental transmission. Host contact rate was set at 1 for calibration with the experimental trials, in which all hosts were guaranteed contact with one another in the tanks. The results (Table 3.3) demonstrated that in the experimental conditions direct transmission contributed to the host infections ($R_0 > 1$) and could not be excluded. Note that in natural conditions, it would be expected that direct transmission would decrease compared to a closed system, as hosts directly contact one another less frequently (with the exception of aquaculture). Conversely, it was expected that environmental transmission would play a larger role in natural settings, thus both modes of transmission were maintained in the multi-host models. Environmental transmission could not be excluded, as in experimental conditions both donor host *P. parva* and receiver host *L. delineatus* were physically separated (Gozlan et al. 2005) but environmental transmission still led to a severe *S. destruens* outbreak in *L. delineatus*. The relative contributions of direct and environmental transmission were examined to determine if there was a correlation between these values (Fig. 3.8).

Table 3.3. Basic reproductive number of *Sphaerothecum destruens* in different species, with the separate influence of direct and environmental transmission in the closed experimental systems.

	Cyprinus carpio	*Rutilus rutilus*	*Leucaspius delineatus*	*Salmo salar*
R_0 *direct*	3.42	6.91	1.7	5.4
R_0 *environmental*	0.0442	0.065	0.0556	0.122

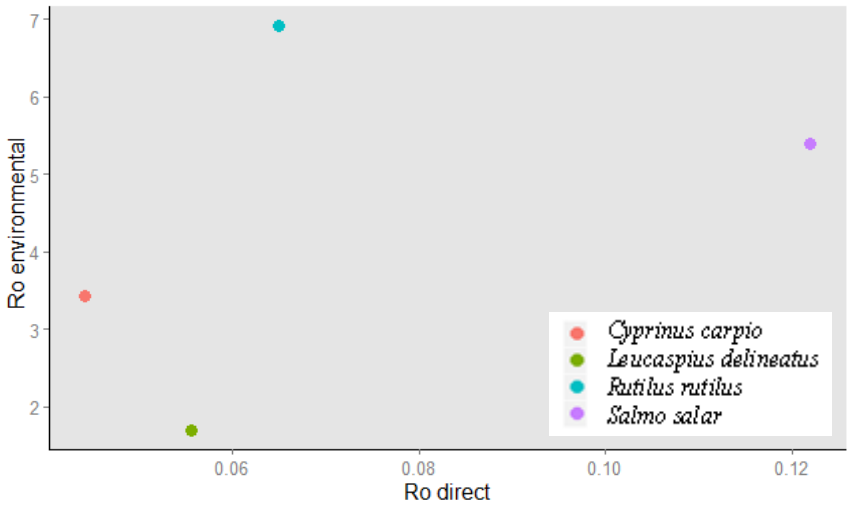

Figure 3.8. A comparison of the relative contributions of direct and environmental transmission in different hosts. There was no significant correlation between the two parameters (lm: $R_{env} = 0.0067 *(R_{direct}) + 0.043$, $p = 0.56$, $R^2 = 0.21$).

As no mathematical trends between species were supported by the available data for transmission parameters, values for direct and environmental transmission in the multi-host models were determined by selecting modal values within the parameter ranges. The direct transmission value for *L. delineatus*, *C. carpio*, and *S. salar* was set at 0.016. For *R. rutilus*, the minimum value of its range (0.08) was selected, as it was the nearest value to the majority of host species. For environmental transmission, 0.007 was selected for host species. Transmission pathways and pathogen reproductive number were discussed in more detail in Chapter 6, where pathogen evolution was investigated.

Hypothesis 5: Environmental transmission (ε) and spore release (ϕ)

In the bath immersion trials, the host environment was saturated with spores in a small, closed space, rendering the relationship between environmental transmission and spore release difficult to determine empirically. There was no valid correlation found in the data between environmental transmission and the rate of spore release (Hypothesis 5), and a logistic growth function could not be fitted to the data (Fig. 3.9).

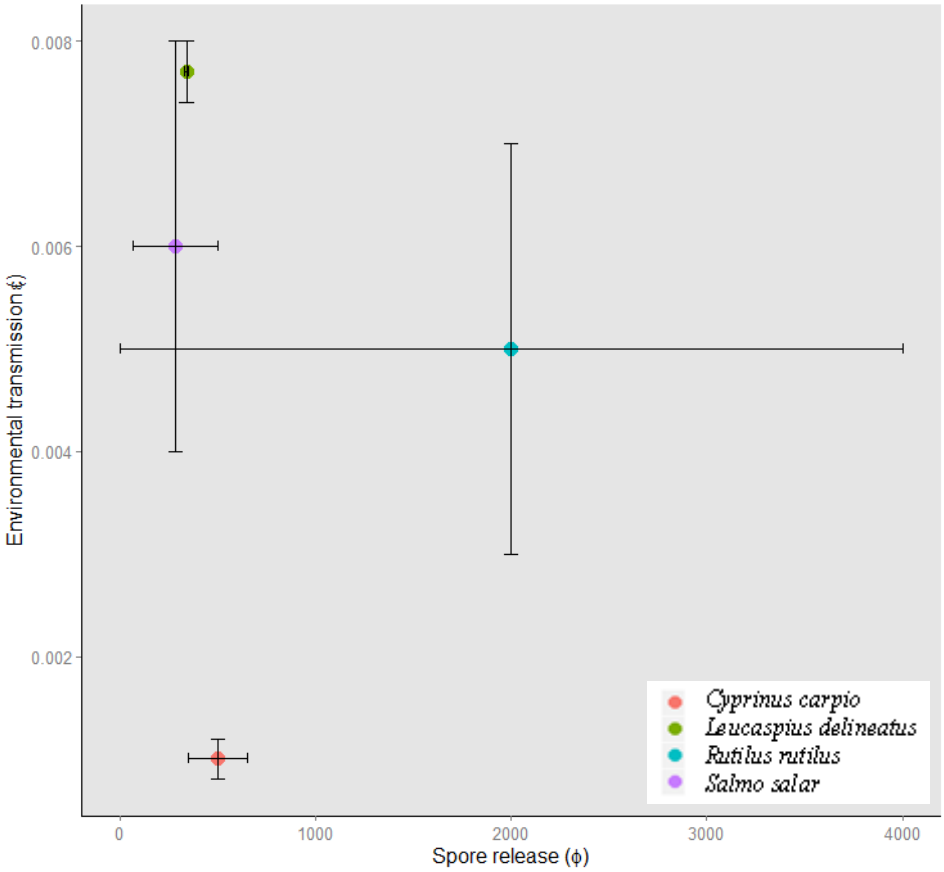

Figure 3.9. The median values of spore release (ϕ) against the median values of environmental transmission (ε) for each species, with minimum and maximum values shown as uncertainty. There was no significant correlation between environmental transmission and release of spores based on the available data (SMA: $\varepsilon = 52^{-6}\phi$, $p = 0.25$, $R^2 = 0.41$).

Hypothesis 6: Spore release

Spore release was tested against mortality from infection, but there were no significant correlations between the two parameters (Fig. 3.10). Body mass (w) from each species (recorded in the experiments) and mortality from infection were incorporated into a linear mixed effects model to test Hypothesis 6. No significant correlations between body size, spore release or mortality were observed (Fig. 3.11).

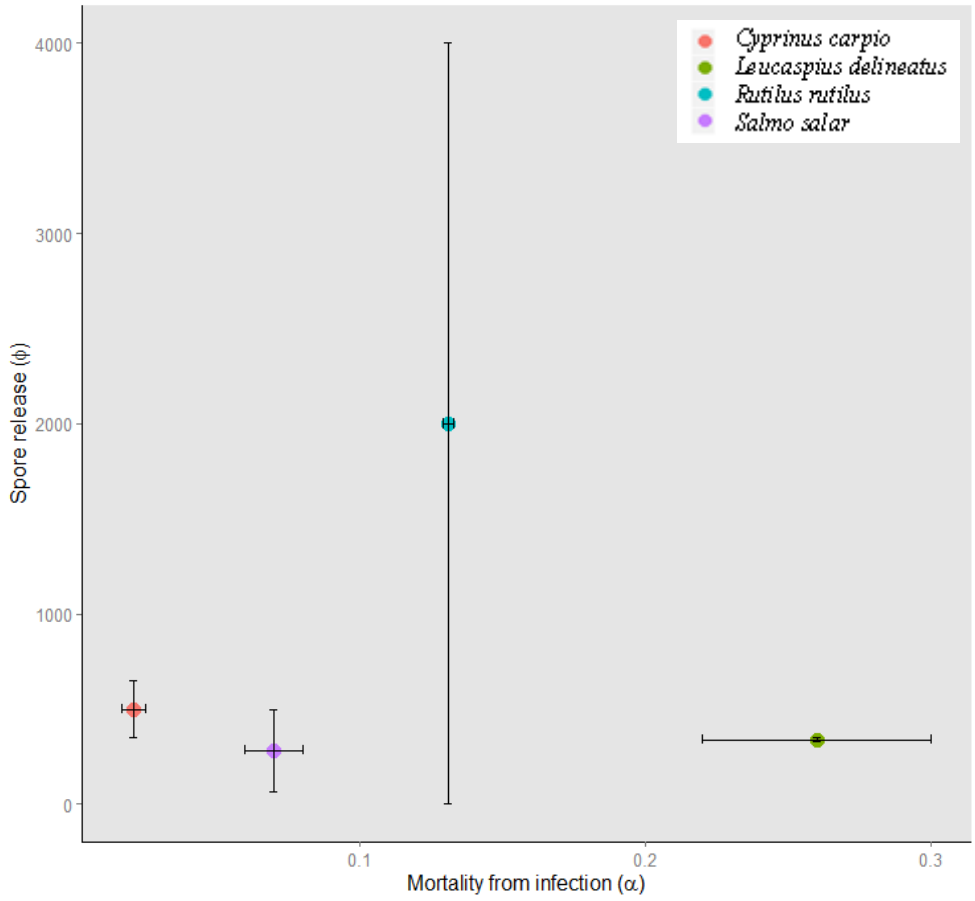

Figure 3.10. Median mortality from infection (α) values for each species plotted against the median spore release (φ) values for each species. The data was fitted to a mixed effects model that included mortality from infection and body mass as variables (SMA: $\varphi = 379.2(\alpha) + 9484.7(w) - 2609.6$, $p = 0.28$, $R^2 = 0.36$). No significant correlation between the parameters was observed.

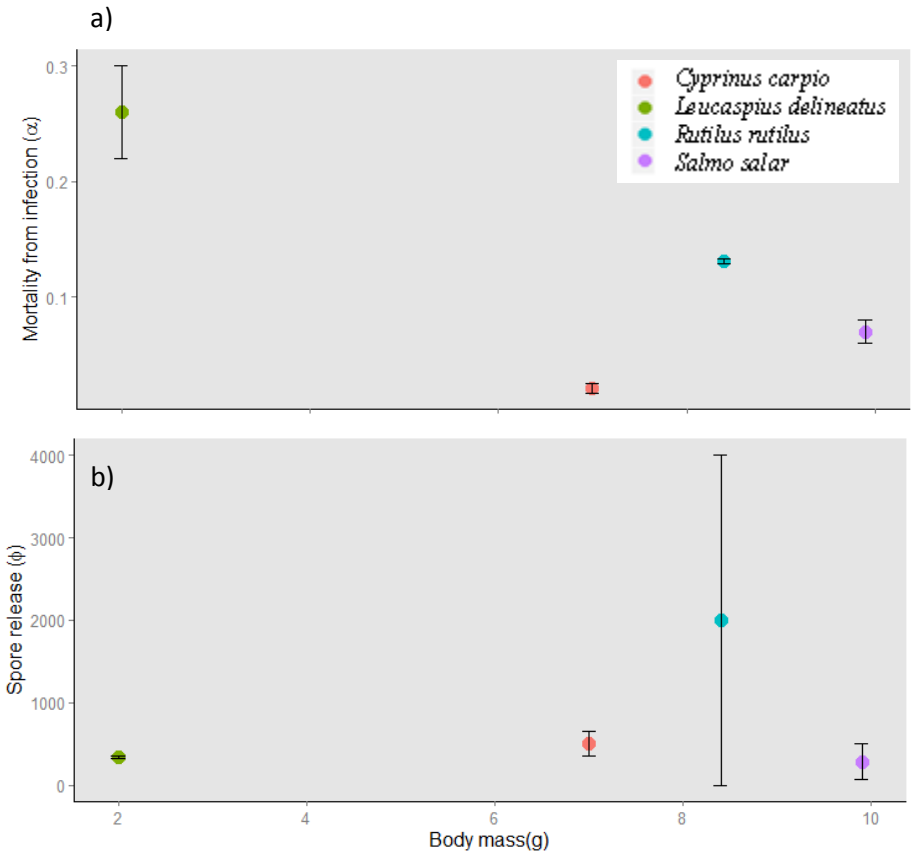

Figure 3.11. A comparison of body mass and (a) mortality from infection and (b) spore release. There was no correlation between body mass and mortality (ordinary least squares regression (OLS): $\alpha = -0.023(w) + 0.28$, $p = 0.22$, $R^2 = 0.61$) or between body mass and spore release for cyprinid species (OLS: $\phi = 303(w) + 70$, $p = 0.71$, $R^2 = 0.086$).

Due to no evident correlations between body size, spore release or mortality from infection, a number of assumptions were made for the rate of spore release:

1. It was assumed that there would be a positive correlation between body size and the number of spores released per day (ϕ). This was based on modelling work by Rudnev et al. (2006) and DeLeo and Dobson (1996), which showed that larger body sizes can accumulate higher infection loads, until the peak of infection is reached.

2. In the wild, the probability of spore ingestion by hosts is reduced compared to captive habitats, due to uncontrolled factors such as water currents, ingestion by non-host species, or attachment to sediments (Schmeller et al. 2013, Searle

et al. 2013). In each following chapter, the magnitude of spore release was varied to represent specific tested scenarios.

3. The cohabitation experiment, although still a closed system, more closely represented natural conditions than the single host experiments due to the method of infection. The experimental cohabitation did not artificially flood the system with spores, but only included spores naturally released from *P. parva*. In the two host model, the rate of spore release for *L. delineatus* found in the single host model (350 per day) resulted in significantly faster mortalities than those observed. Thus, this parameter was re-estimated in the two host model, testing for a value between 10 and 500. The value that resulted in the lowest MSE for the two host model output was 120 spores per day, 34% of the *L. delineatus* value in the single host model. This adjustment was thus applied to the remaining species' spore release values in the multi-host models to simulate more naturally occurring infections.

The datasets provided invaluable points of reference for model parameterisation, and allowed relationships between parameters to be hypothesised and tested (Table 3.4).

Table 3.4. Relationships between parameters for the multi-host models.

Parameter	Relationship (assumed/estimated)
α	Estimated from data
σ	Assumed to correlate with α ($\sigma = 0.79\alpha + 0.009$)
γ	Assumed to correlate with σ ($\gamma = \sigma / 1.31$)
β	Estimated from data (modal value)
ε	Estimated from data (modal value). In later chapters, this value was reduced to account for larger habitats (as this value was representative of a small tank).
Φ	Estimated from *L. delineatus* values and assumption extended to all susceptible species. Reduced in later chapters to account for different ecological conditions.
μ	Estimated from Andreou et al. (2009)

3.7 Conclusions

The formulation of quantifiable assumptions about parameter relationships is often difficult to achieve for generalist pathogens, as they behave differently in different hosts and environments. However, for recovery and incubation rates, the data supported a strong relationship between parameters, which was consistent among species. In instances where there were no identifiable relationships, appropriate values were selected based on the available information, wherever possible. This was achieved by examining overlapping parameter ranges between species, and selecting the modal value for direct and environmental transmission. Parameters corroborated between single and cohabitation experiments provided reliable values to calibrate the spore release values. In subsequent chapters some parameters were modified to account for the various scenarios tested, which was discussed when it occurred.

While the available data was crucial for model parameterisation, estimated parameter values may be inflated due to the experimental setups, which must be taken into account when considering the model outputs. The experiments were designed to cause a maximum number of infections, rather than characterising the natural pattern of pathogen transmission a host would experience in the wild. It is important to note, however, that experiments which answer all the necessary questions to fit models are often expensive, time consuming and, more importantly, not necessarily logistically feasible. Nonetheless, this work demonstrates how previous studies can provide an important baseline for models of pathogen transmission, and enabled the formulation of multi-host models with reliable parameter estimates.

Chapter 4
Factors associated with generalist pathogen establishment in animal communities, and the consequences of host eradication on disease epidemiology

This chapter has been published as: Al-Shorbaji F N, Roche B, Gozlan RE, Britton JR, and Andreou D. (2016) The consequences of reservoir host eradication on disease epidemiology in animal communities. *Emerging Microbes and Infections*, 5 (5): e46.

Summary

Non-native species have often been linked with introduction of novel pathogens that spill-over into native communities and the amplification of the prevalence of native parasites. In the case of introduced generalist pathogens, their disease epidemiology in the extant communities remains poorly understood. Here, *S. destruens* was used to characterise the biological drivers responsible for disease emergence in temperate fish communities. A range of factors relating to both the pathogen and the surrounding host communities were used in a multi-host SEIR model to test how these factors affected disease epidemiology. These included: (1) physical proximity to the introduced healthy carrier (*P. parva*) and the densities of susceptible native host species; (2) the initial pathogen prevalence in the introduced healthy carrier; (3) inter-specific connectedness (i.e. contact between species); (4) the time-lag between the introduction of the healthy carrier and its eradication; and (5) the role of species background mortality on the resilience of the local community. These were modelled across 23 combinations and the results indicated that the spill-over of pathogen propagules via environmental transmission resulted in rapid establishment in adjacent fish communities (< 1 year). Although disease dynamics were initially driven by environmental transmission in these communities, once sufficient numbers of native hosts were infected, the disease dynamics were driven by intra-species transmission. Subsequent eradication of the introduced healthy carrier, irrespective of its timing (after 1, 2 or 3 years), had limited impact on the long-term disease dynamics among local fish communities. These outputs reinforced the importance of rapid detection and eradication of non-native species, in particular when such species are identified as healthy carriers of a generalist pathogen.

4.1 Introduction

Introductions of non-native species into new regions are accelerating globally due to human-mediated activities including trade, food production and pest control (Gozlan et al. 2010a). Non-native species can act as drivers of disease emergence (Gozlan et al. 2006, Peeler et al. 2011) and can cause irreversible consequences for ecosystem functioning and services (Sanders et al. 2003, Pejchar and Mooney 2009). Although

'enemy release' processes suggest many pathogens will be lost during the introduction process, a small proportion do get released (Liu and Stiling 2006, Sheath et al. 2015) and have the potential to spill-over into novel hosts and lead to disease emergence (Power and Mitchell 2004, Section 1.3). Recent examples of emergence of introduced diseases include White Nose Syndrome in bats (Blehert et al. 2009, Foley et al. 2011), snake fungal disease (Sutherland et al. 2014), and the chytrid fungus in amphibians (Garner et al. 2006, Mitchell et al. 2008, Fisher et al. 2012). Whilst pathogen spill-over is closely associated with introduced species, it can also result from captive situations where species are farmed; aquaculture has been identified as a major source of new parasites and an amplifier of extant ones (Murray and Peeler 2005, Pulkkinen et al. 2010, Johansen et al. 2011).

After their introduction into animal communities, non-native generalist pathogens tend to have a higher probability of establishment than specialists as a result of a greater number of suitable hosts (Altizer et al. 2003, Power and Mitchell 2004, Section 1.1). Furthermore, pathogens with environmentally transmitted propagules are also able to persist in the environment for prolonged periods prior to transmission, increasing their dispersal ability and infection of new populations (Mitchell et al. 2008, Fisher et al. 2012). This persistence has been observed in multiple high-profile pathogen systems, including cholera (Codeco 2001) and avian influenza virus (Roche et al. 2009). Following introduction, new host populations provide additional opportunities for the development of persistent reservoirs of pathogen populations (Poulin et al. 2011) and thus can lead to subsequent episodes of disease emergence (Murray and Peeler 2005, Rimstad 2011). For example, animal reservoirs of human African trypanosomiasis (sleeping sickness) are expected to facilitate disease re-emergence in human populations even after the infection has been eradicated in the human population (Funk et al. 2013). Nevertheless, there are several community and physical factors that influence the ability of pathogens to spread and establish in neighbouring communities. These include the species composition, abundance and susceptibility of new hosts, the interactions between these new host species, and the initial pathogen prevalence (i.e. the infection pressure) (Reno 1998, Holt et al. 2003, Peeler and Feist 2011, Section 1.2). Species composition, host abundance and pathogen prevalence are well characterised factors

in disease emergence, although not always considered in combination (Bell et al. 2011, Mihaljevic et al. 2014, Fenton et al. 2015). It is expected that host background mortality could also influence the outcome of infectious outbreaks (Williams and Day 2001, Mennerat et al. 2010). Host populations with high background mortality, due to their life history or a pre-existing infection, are predicted to be less able to recover following an epidemic (Mennerat et al. 2010, Peeler and Feist 2011). How these factors interact to influence subsequent pathogen establishment and dynamics, and whether their effects are additive or synergistic, is poorly understood.

Management of pest species and diseases in spatially restricted systems, such as lakes, ponds and aquaculture systems, often involves chemical treatments to eradicate hosts in order to avoid future pathogen emergence (Mardones et al. 2009, Wallace et al. 2009). In the case of host-specific pathogens, eradication can eliminate reservoir or intermediate hosts or vectors of the disease (Haydon et al. 2002), as shown by malaria transmission rates being reduced via control of mosquito populations (Kelly-Hope et al. 2008). Generalist parasites can be more difficult to eradicate given their potential presence in larger numbers of host populations, resulting in eradication having to target multiple species. Moreover, for generalist pathogens with environmentally transmitted propagules, there is a higher likelihood that the parasite also rapidly establishes in adjacent communities soon after its introduction (Roche et al. 2009). Recent experimental work on environmentally transmitted fish pathogens including *Flavobacterium columnare* supported this hypothesis, finding evidence of infections in communities downstream of infected hatcheries (Jakaitis 2014). The epidemiological complexity of generalist pathogens is difficult to characterise based solely on empirical data, and instead could be better understood using predictive epidemiological models that can be developed using existing data and be applied to predict outcomes of specific scenarios (Bonhoeffer et al. 1996, Day and Prince 2007).

The aim of this chapter was thus to examine potential factors affecting the establishment of a generalist pathogen following its environmental introduction from an adjacent source population, and to predict the consequences of its eradication

from the source population on the adjacent community. Through modifying the epidemiological model developed in Chapters 2 and 3, the objectives were to investigate how disease establishment and its dynamics in the local communities were influenced by: (1) their physical proximity to the introduced healthy carrier and the densities of susceptible native host species; (2) the initial pathogen prevalence in the introduced healthy carrier; (3) inter-specific connectedness (i.e. contact between species); (4) the time-lag between the introduction of the healthy carrier and its eradication; and (5) the role of species background mortality on the resilience of the local community. The pathogen *S. destruens* and its healthy carrier *P. parva* were used as the model system.

4.2 Materials and Methods

4.2.1 Host-pathogen model system

Epidemiological parameters have been calibrated for several species including common carp *C. carpio* (low susceptibility), roach *R. rutilus* (medium to high susceptibility), and sunbleak *L. delineatus* (high susceptibility). These species were selected for the model as they are representative of the fish assemblages for which *S. destruens* epidemiological data exists.

In this chapter, *P. parva* will be referred to as the introduced healthy carrier. In the UK, *P. parva* invasion has been minimised via eradication of lentic populations to prevent their dispersal into lotic environments (Britton et al. 2011). Each eradication operation involved the application of the piscicide rotenone and to date have all been effective at extirpating *P. parva*. The programme of *P. parva* eradication in the UK was used to model the removal of the introduced healthy carrier population. For the purposes of this study, it was assumed that all introduced hosts were eradicated and no infectious propagules reached local communities post-eradication.

4.2.2 Multi-host SEIR model

An epidemiological model was developed for *S. destruens* by extending the single-host SEIR model previously developed (Chapter 2) to a multi-species context (Roche

et al. 2012, 2013) with the addition of demographic rates (birth *b* and natural mortality *m*) and inter-species contact rates. All the assumptions relevant to epidemiological processes at a species level, especially the dose-dependent transitions between categories, were discussed in Section 2.2.3.

In this model, susceptible individuals of Species *i* could become infected by contacting infectious spores (Eq. 4.1) through direct contact (β) with infectious hosts from species *i* or *j*, or indirectly through ingesting free-living spores $\varepsilon_i(Z/(Z+k_{ei}))$. Upon infection, an individual of species *i* became exposed (E_i), meaning it has been infected but is not yet infectious (Eq. 4.2). They moved to the Infectious class (I_i), where they could release spores and transmit the pathogen to other hosts (Eq. 4.3), through a rate corresponding to the inverse of the pathogen incubation period in species *I*, $\sigma_i(Z/(Z+k_{si}))$. Infectious individuals remained infectious until they died (with rate $\alpha_i(Z/(Z+k_{ai}))$ or recovered (R_i,) with rate $\gamma_i(Z/(Z+k_{gi}))$ (Eq. 4.4). In the experimental trials (Andreou et al. 2012), it was impossible to distinguish between hosts that were immune or recovered, so no distinction was made in the model as well. Therefore, it was assumed that recovered individuals could not be re-infected. Finally, the number of infectious propagules (Z) in the environment was the sum of infectious propagules released by each infectious individual per day (Φ). Zoospore concentration decreased with clearance rate μ (Eq. 4.5). All the equations were fully described below:

$$\frac{dS_i}{dt} = b_i N_i - S_i \sum_{i}^{n} \beta_{ji} I_j - \varepsilon_i \left(\frac{Z}{Z + k_{ei}} \right) S_i - m_i S_i \qquad \text{Eq. 4.1}$$

$$\frac{dE_i}{dt} = S_i \sum_{i=1}^{n} \beta_{ji} I_j + \varepsilon_i \left(\frac{Z}{Z + k_{ei}} \right) S_i - \sigma_i \left(\frac{Z}{Z + k_{si}} \right) E_i - m_i E_i \qquad \text{Eq. 4.2}$$

$$\frac{dI_i}{dt} = \sigma_i \left(\frac{Z}{Z + k_{s_i}} \right) E_i - \gamma_i \left(\frac{Z}{Z + k_{gi}} \right) I_i - \alpha_i \left(\frac{Z}{Z + k_{ai}} \right) I_i - m_i I_i \qquad \text{Eq. 4.3}$$

$$\frac{dR_i}{dt} = \gamma_i \left(\frac{Z}{Z + k_{g_i}} \right) I_i - m_i R_i \qquad \text{Eq.4.4}$$

$$\frac{dZ}{dt} = \sum_{1=i}^{n} \phi_i I_i - \mu Z \qquad \text{Eq. 4.5}$$

4.2.3 Parameter estimation

For each species, epidemiological parameter values (direct (β) and environmental transmission (ε), incubation rate (σ), recovery rate (γ), infection threshold levels (K), and mortality from infection (α)) were based on previously determined estimates (Chapter 3). Conversely, the magnitude of interspecific direct transmission (β_{ji}) was explored in this chapter while density-dependent birth (*b*) and natural mortality rates (*m*) were estimated based on existing population data (www.fishbase.org). Spore release (ϕ) values were also based on estimates in Chapter 3, and were further reduced by two thirds to simulate loss of spores due to water currents, attachment to sediments and predation (Searle et al. 2013). All parameter ranges are shown in Table 4.1.

Spore mortality rate (μ) was estimated based on experimental data on spores in sterile water (Andreou et al. 2009). The estimation in Chapters 2 and 3 represented a significantly shorter lifespan, due to experimental setups that used a continuous filtering system of the tanks (therefore the spores were rapidly flushed from the system). Here, the selected value was based on cold water temperatures (4-10°C).

No competition between host species was included, but a population carrying capacity for each species was introduced to represent intra-specific competition, under the assumption that smaller species were more abundant than larger species (Anderson-Teixeira et al. 2009). Initially, carrying capacity (K) for each species was

calculated according to De Leo and Dobson's (1996) allometric calculation of body weight *w* per km^2:

$$K = 16.2w^{-0.70} \quad \text{Eq. 4.6}$$

However, given the average body sizes found for each species (www.fishbase.org) this resulted in very small populations of *C. carpio* and *R. rutilus* to include in the model (Table 4.2). Thus, the overall assumption that smaller species have higher carrying capacities was maintained, but the specific values for each species were estimated based on knowledge and experience of UK fish surveys and population sizes (J.R. Britton, personal communication). The values used in the model are significantly higher than those found in wild populations, but reflect heavily stocked angling habitats. Furthermore, the population size of *P. parva* was estimated at a higher level to reflect their highly invasive nature and life history (Gozlan et al. 2010b). These magnified values were used to uncover the role of population density in disease transmission more clearly, as smaller populations showed no significant differences between outputs. These results were also highly relevant for heavily stocked aquaculture facilities.

Table 4.1. Parameter values for all species, for communities in close proximity (CP) and relatively far proximity (FP) from the introduced healthy carrier.

Parameter (per day)	*Leucaspius delineatus*	*Cyprinus carpio*	*Rutilus rutilus*	*Pseudorasbora parva*
β (direct transmission)	0.015-0.020	0.016-0.017	0.080-0.100	0.011
α (mortality from infection)	0.220-0.300	0.017-0.025	0.129-0.130	0
ε (environmental transmission)	0.0007	0.0007	0.0007	0.0007
σ (incubation rate)	0.230-0.233	0.013-0.081	0.095-0.11	0.072
γ (recovery rate)	0.140-0.170	0.065-0.072	0.099-0.101	0.108
K_e (threshold for infection)	30,000	30,000	30,000	30,000
K_a (threshold for mortality)	3,000	3,000	3,000	3,000
K_g(threshold for recovery)	6,500	6,500	6,500	6,500
K_s (threshold for incubation)	7,200	7,200	7,200	7,200
μ (spore mortality)	0.071	0.071	0.071	0.071
ϕ (spore release)	40	40	55	13 (CP); 0.050 (FP)
b (birth)	0.010	0.011	0.004	0.010
m (natural mortality; low level)	0.000430	0.000140	0.000170	0.000430
Population carrying capacity	1500 (low); 3000 (high)	500 (low); 1000 (high)	800 (low); 1600 (high)	3000 (low); 6000 (high)

Table 4.2. Population carrying capacity values for each species in the model.

	Cyprinus carpio	*Rutilus rutilus*	*Leucaspius delineatus*	*Pseudorasbora parva*
Common body mass (g)	400	195	2	5
K (DeLeo and Dobson 1996)	30	51	1255	661
K (low density values selected for model)	500	800	1500	3000

4.2.4 Modelled scenarios

Environmental pathogen transmission was modelled in a fish community adjacent to an introduced *P. parva* population infected with *S. destruens*, which will be referred to as the local community and the source population respectively. The introduced healthy carrier population represented a source that could have been an aquaculture facility, a reservoir upstream of a dam, or a fishing lake/pond. The local community included a species with low susceptibility to *S. destruens* (*C. carpio)*, medium susceptibility (*R. rutilus*), and high susceptibility (*L. delineatus*). Each of the hypotheses listed below was tested using multiple combinations of parameter values (Fig. 4.1) to investigate disease establishment and potential recovery of species following eradication of the healthy carrier in the source population.

- Scenario 1. The geographical distance between the healthy carrier and local community was investigated for its influence on the force of infection (with more infectious propagules reaching communities that are closer to the healthy carrier and vice versa). As such, communities in close proximity to the source population were subjected to 25% of *P. parva* produced spores ($\phi/4$), and communities further away to only 0.1% of produced spores ($\phi/1000$).
- Scenario 2. The influence of disease prevalence in the introduced healthy carrier was tested as a driver of disease emergence in the local community using 10% and 40% initial prevalence levels, which were based on Chapter 3

and estimates of high prevalence of the amphibian chytrid fungus in the wild (Bell et al. 2011).

- Scenario 3. The impact of source and local population density on disease dynamics was tested as a factor influencing disease dynamics. Low-density populations were 50% of the initial abundance values used as high densities.
- Scenario 4. The influence of community connectedness (i.e. how often species interacted with each other) was examined via interspecies direct transmission. In communities with a high degree of connectedness, interspecies direct transmission values were set at 0.01, marginally lower than intraspecific values. In communities where species did not interact with each other regularly, this interspecies transmission value was set at 0.0001, a value that indicated isolation between species.
- Scenario 5. The healthy carrier's population was eradicated at 1, 2, or 3 years post introduction to test the effect of earlier vs. later eradication. This was achieved by setting the introduced healthy carrier population and the propagules they produced to 0 at a given time of eradication. This reflected the method used to eradicate *P. parva*, as it is assumed the rotenone would eliminate both host and pathogen from the habitat, and thus ensured that no infectious propagules would contact the local community post eradication.
- Scenario 6. The local community's level of background mortality was tested for its effect on community response to introduced infection. All the scenarios (n = 23) were repeated separately with a level of natural mortality 10 times the equilibrium value estimated from observed data, for all species (Table 1, www.fishbase.org). This represented communities that were experiencing significantly high levels of stress and thus could display a compromised immune response (i.e. overcrowded fish farms that were already infected with a different pathogen; (Ortuño et al. 2001, Magnadóttir 2006). This allowed the implicit testing of the impact of stress and multiple infections on the local community's response to introduced infection.

Multiple scenarios were run in all relevant combinations (detailed in Fig. 4.1), to explore the additive effects (or not) of these factors. In each scenario, the local community was monitored for a total of 5,000 days (approximately 14 years),

allowing the observation of long-term patterns. All simulations were run in R (Version 0.97.551 © 2009-2014). The Shannon index was calculated for each community to observe changes in species diversity using the *vegan* package. Outputs of each species' abundance over time were compared for significant differences between scenarios using Welch 2 sample t-tests and Kruskal-Wallis tests in the R *stats* package Version 2.15.3.

4.3 Results

Influence of geographical distance between the introduced healthy carrier and local community, and the effect of population density

Greater geographical distance between the introduced healthy carrier and the local community led to significantly delayed mortalities from infection in the local community (Fig. 4.2, 2 sample t-test for the first 300 days post-introduction: $t = -16.9$, d.f. = 1913, $p < 0.001$). In communities where 0.1% of the spores from the *P. parva* reached the local community at 10% prevalence (scenarios 16 to 21, see Fig. 4.1), the epidemic was delayed by 1-2 years compared to communities at close proximity to the source population, as spores required a longer time to accumulate within susceptible hosts. Over the course of the simulation, there was no significant difference in total mortality between geographically distant and near communities (2 sample t-test: $t = -0.948$, d.f. = 80000, $p > 0.05$). In all figures, eradication at 2 years was not shown for clarity. Overall, high-density populations maintained higher species diversity for a longer duration compared to low-density populations (2 sample t-test: $t = 78.9$, d.f. = 16428, $p < 0.001$), as evidenced by the community Shannon diversity index over time (Fig. 4.2e, f; Appendix 3).

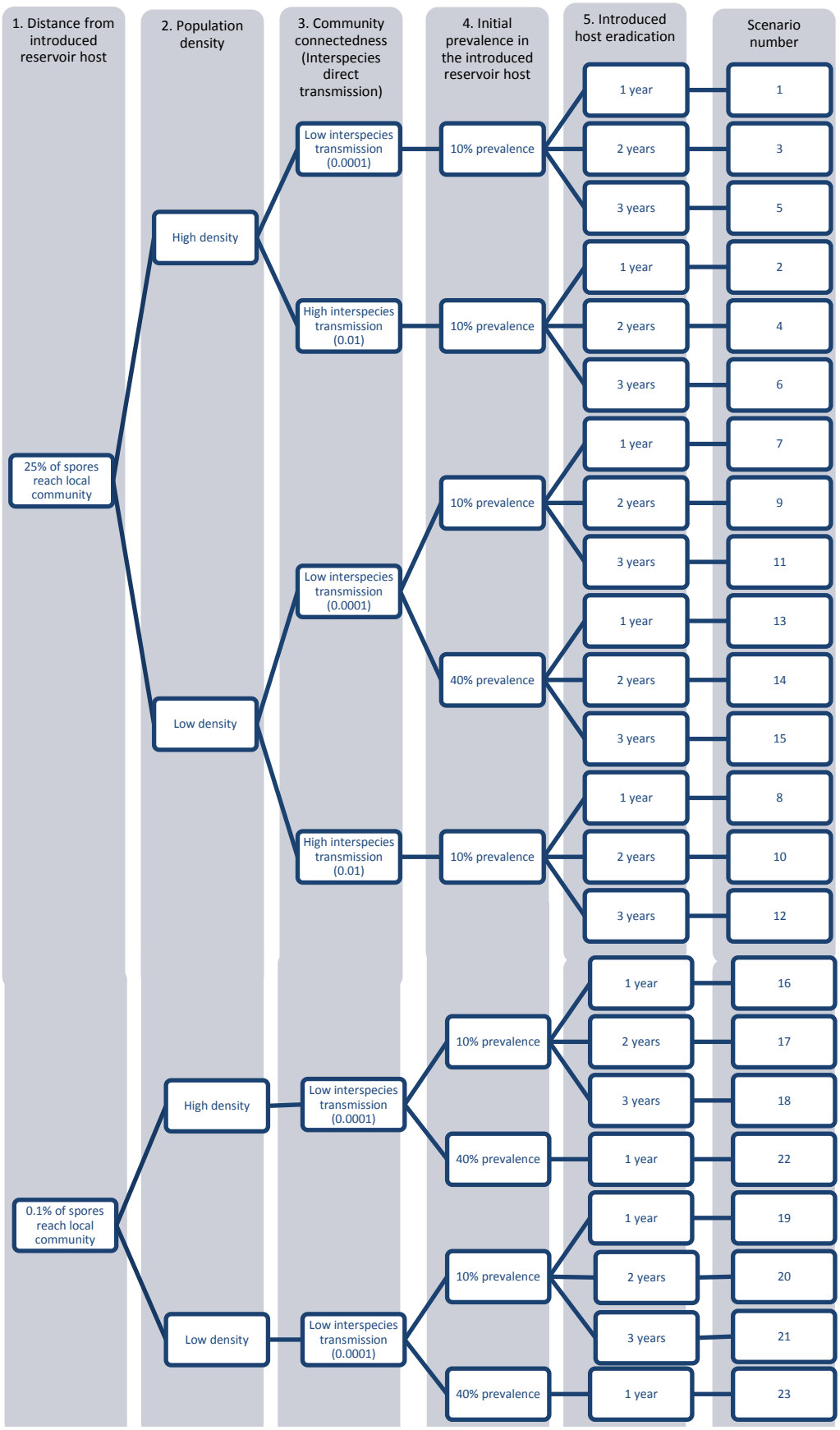

Figure 4.1. Scenarios testing various community factors on the establishment of a generalist pathogen via an introduced host. Columns 1-5 represent the main factors tested, with the specific scenarios numbered. Furthermore, the role of high background mortality (Scenario 6) was tested by running all the scenarios again with 10x the equilibrium mortality value.

Effect of the initial pathogen prevalence in the healthy carrier population

For communities at a short geographical distance to the healthy carrier population (scenarios 1 to 15), higher initial pathogen prevalence in the healthy carrier did not significantly accelerate the rate of infection in the local community as high numbers of infectious propagules were already in contact with the susceptible species. However, pathogen prevalence in the healthy carrier significantly influenced population decline in the community geographically further away from the source population (scenarios 16, 19, 22, 23; Fig. 4.3). Higher initial pathogen prevalence in the healthy carrier population led to accelerated mortalities from infection when compared with low prevalence (Fig. 4.3a-d). The effect of initial pathogen prevalence was more pronounced in low-density populations, but was still significant in both high and low-density local communities (2 sample t-test (high-density): $t = 2.94$, d.f. = 50008, $p < 0.05$; 2 sample t-test (low-density): $t = 14.27$, d.f. = 49912, $p < 0.001$). This was expected, as higher initial prevalence would result in faster population growth of the pathogen, and thus a more rapid occurrence of secondary infections. This indicated that the abundance of infectious propagules in the environment drove community infection (i.e. mortality) in susceptible hosts (Fig. 4.3e-f). This was further tested by decreasing the infectious propagule lifespan to five days (the minimum duration of activity observed by Andreou et al. 2009). Decreasing propagule survival delayed mortalities in the local community by 6-7 years, and reduced the total number of environmental propagules (Fig. 4.4).

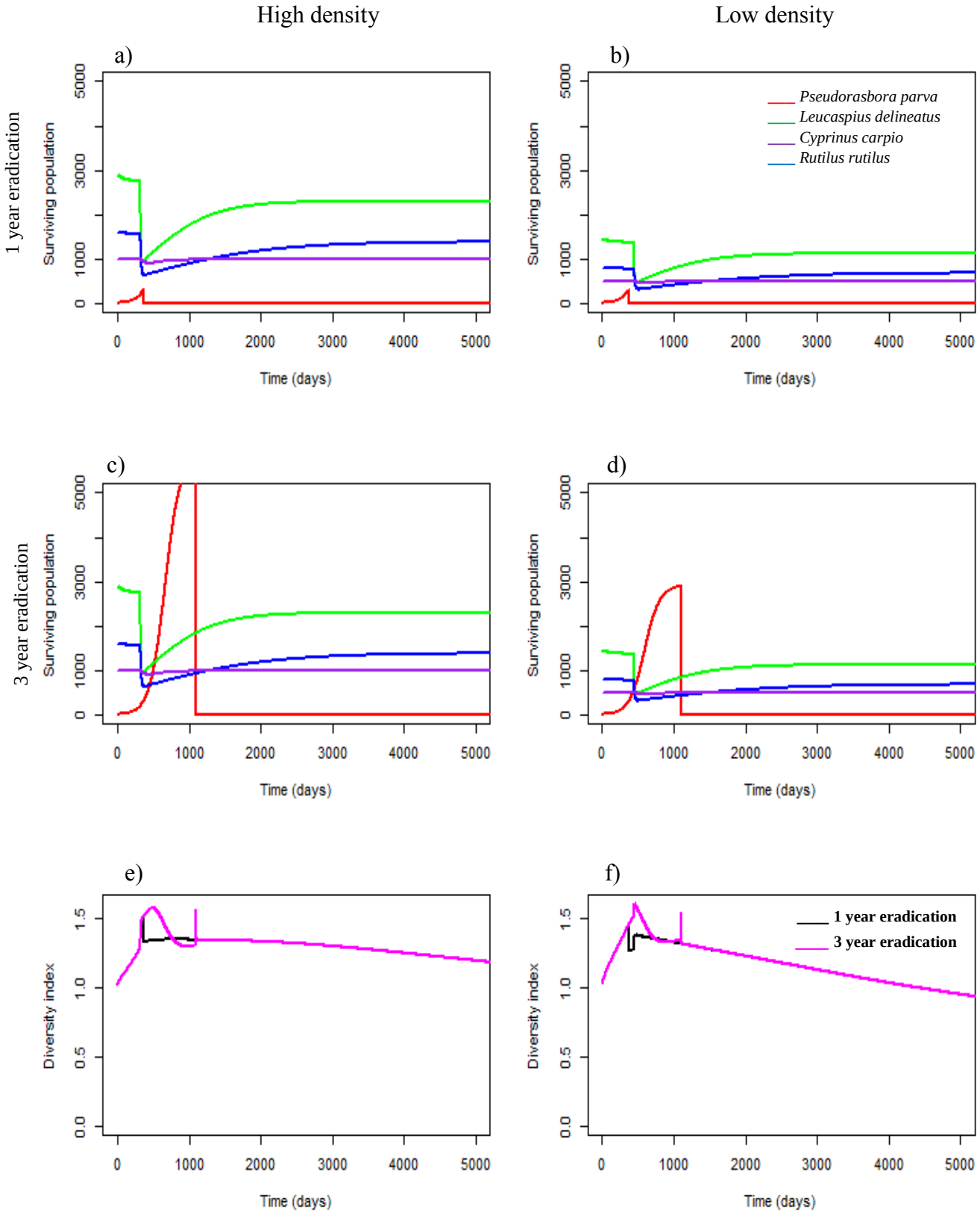

Figure 4.2. The abundance of each species in geographically distant communities at 1 and 3 year eradication of *Pseudorasbora parva* (a-d) and the corresponding Shannon diversity indices (e-f). Here, the role of population density (Scenario 2) and eradication time (Scenario 5) were tested. Higher population density accelerated the epidemic for *Leucaspius delineatus*, *Rutilus rutilus*, and *Cyprinus carpio* compared with lower density. The Shannon biodiversity index of each community at different times of eradication of *P. parva* demonstrated that high-density communities maintained higher biodiversity.

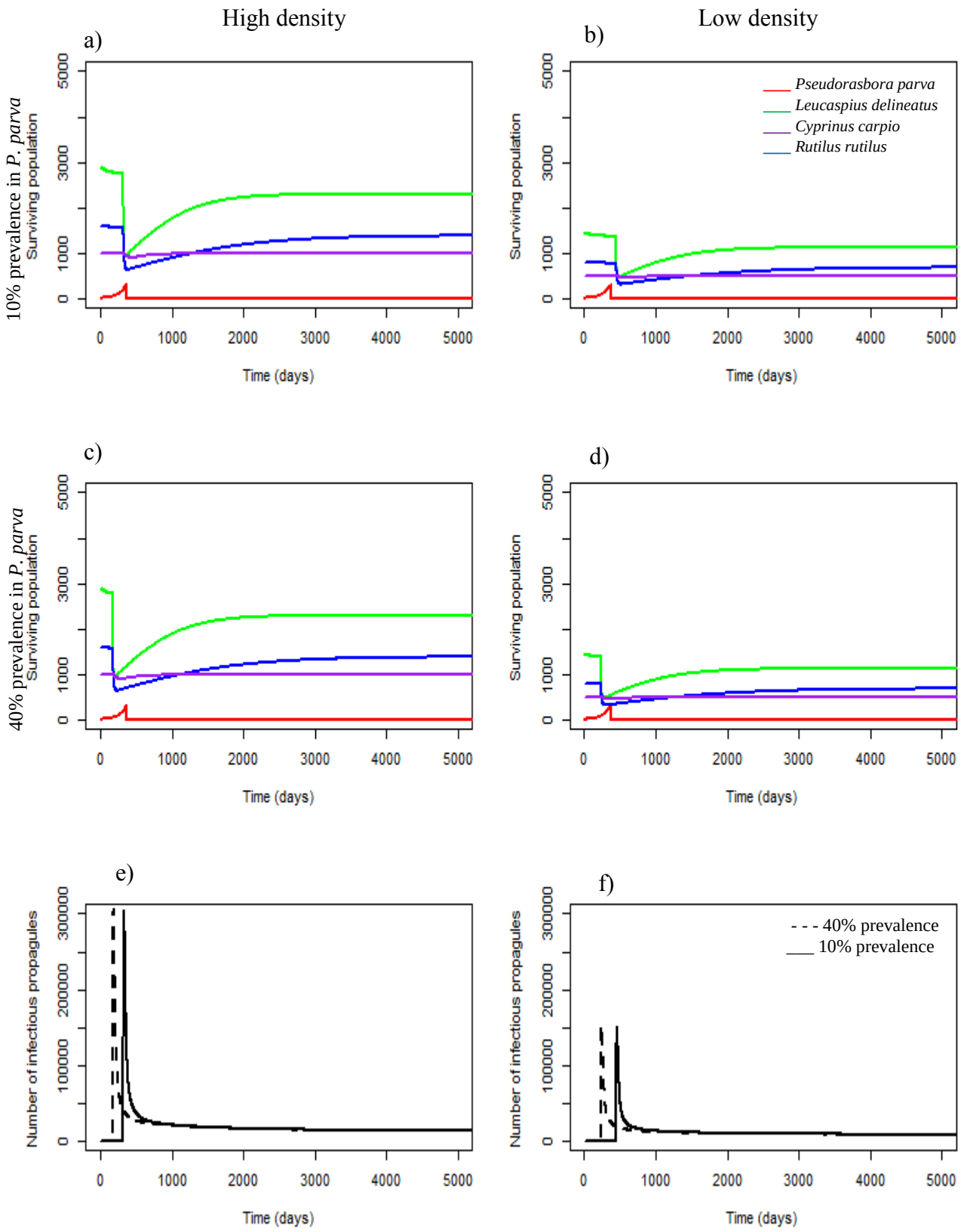

Figure 4.3. The abundance of each species (a-d) and corresponding *Sphaerothecum destruens* infectious propagule levels (e-f) over time at 10% (a, b) and 40% (c, d) initial prevalence of infection in *Pseudorasbora parva*. Here, the role of initial pathogen prevalence in the introduced host (Scenario 4) was tested. High initial prevalence accelerated the decline of *Leucaspius delineatus*, *Rutilus rutilus*, and *Cyprinus carpio* compared with 10% prevalence, although the local community declined to similar levels in both cases. The role of population density (Scenario 2) was also tested, demonstrating that high population density was correlated with higher levels of spores in the environment accumulating at a more rapid pace (e) compared to lower population densities (f).

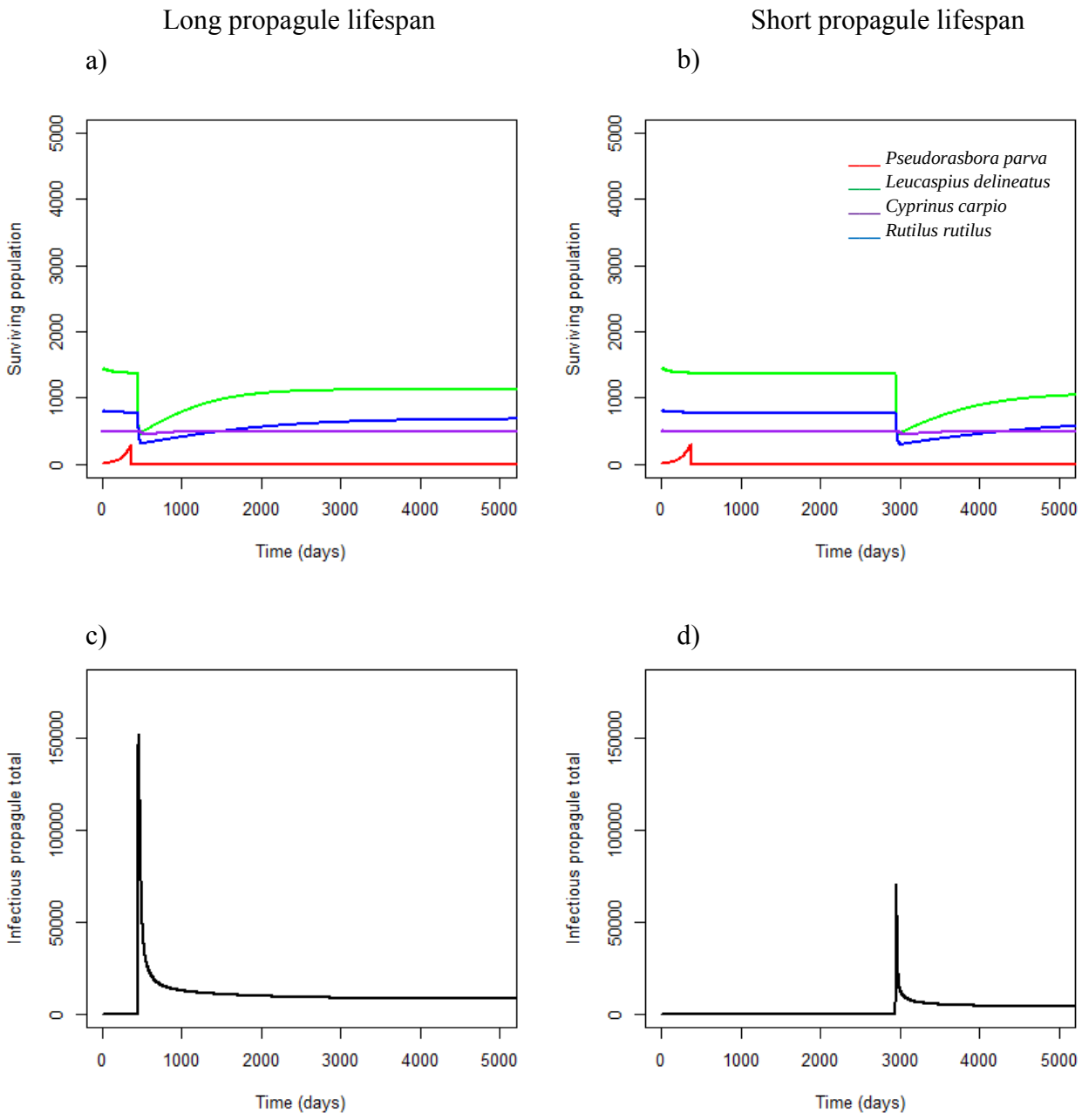

Figure 4.4. The effect of propagule lifespan on the local community's level of mortality. Longer propagule lifespan (14 days) accelerated mortality within the local community (a) compared to shorter propagule lifespan (5 days) (b), and resulted in higher levels of propagules in the environment (c). Conversely, short propagule lifespan resulted in lower propagule levels in the environment (d).

Effect of healthy carrier eradication time and geographical distance between communities

When the local community contacted 25% of infectious propagules from the introduced healthy carrier (scenarios 1-12), the disease established in the local community within a year (Fig. 4.5). All local communities recovered following the disease outbreak, irrespective of eradication time, although to lower levels compared to their starting population size. Following the initial environmental transmission from the introduced healthy carrier to the local community, the disease dynamics in the local community were largely driven by intraspecific transmission. In *C. carpio* and *L. delineatus*, later eradication times (2, 3 years) led to higher mortalities in the 14 year period observed, especially in low-density communities (Kruskal-Wallis test (*C. carpio*): chi-squared = 53.61, $p < 0.001$; (*L. delineatus*): chi-squared = 9.47, $p < 0.05$). However, over the timespan of 14 years *C. carpio* and *L. delineatus* achieved a similar equilibrium population size regardless of eradication time in each scenario.

Eradication of *P. parva* at different times resulted in identical peaks of infectious propagule levels, although the peaks were higher in high-density compared with low-density populations (Fig. 4.6). This suggested that the timing of eradication did not mitigate the local community's overall infection levels once the initial environmental introduction had occurred. Furthermore, in both high and low densities, after the outbreaks occurred the level of infectious propagules was maintained above 0, suggesting that the infection persisted in local communities at low levels. To demonstrate the key role of environmental transmission, the model excluded it (by setting ε to 0) (Fig. 4.7), which prevented an epidemic from occurring in the local community.

Effect of connectedness between species

Examining scenarios with 10% prevalence (1-12) in terms of their Shannon diversity index revealed that direct inter-specific transmission had no significant effect on local community mortality (2 sample t-test: $t = -0.04$, d.f. = 20000, $p > 0.05$).

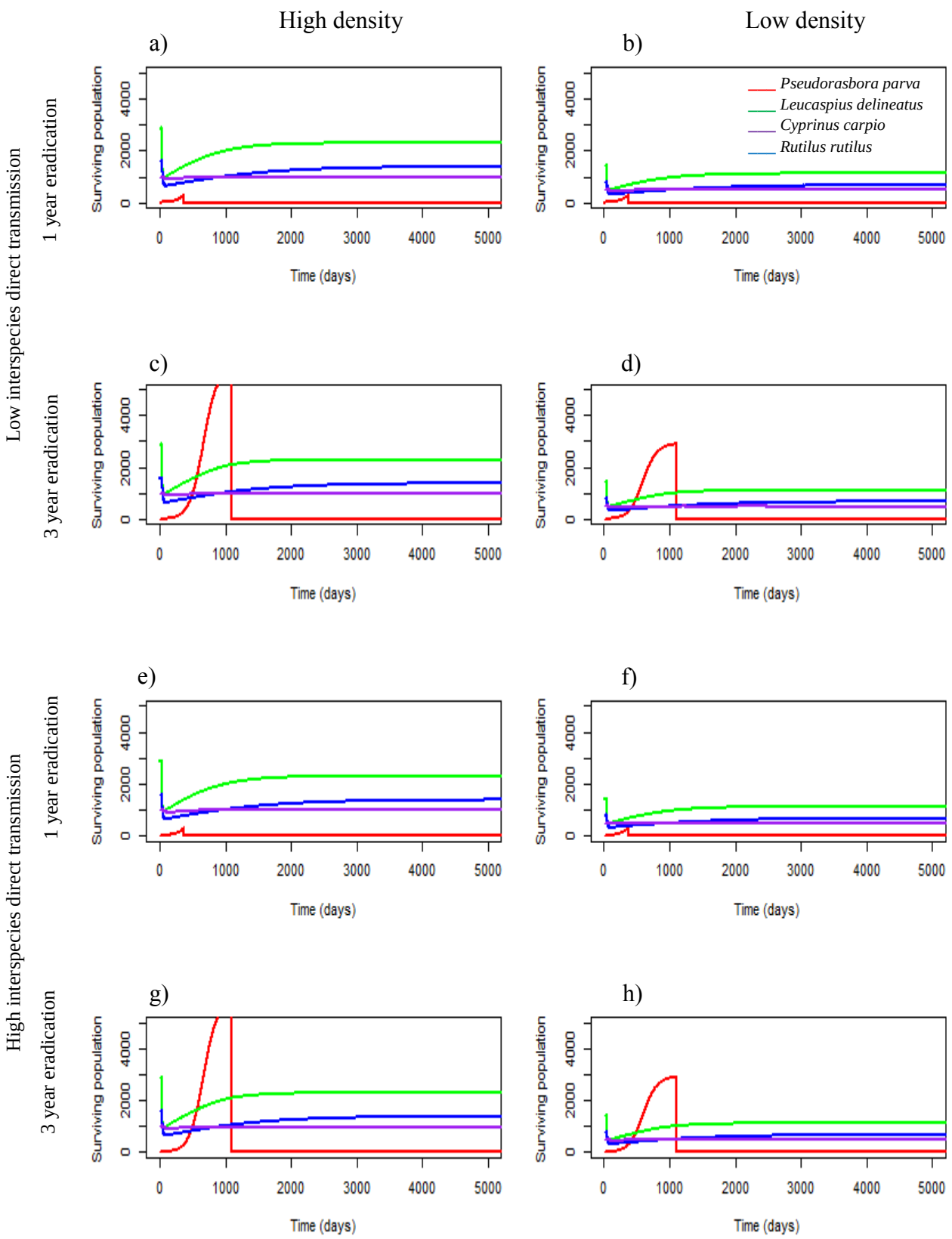

Figure 4.5. The abundance of each species at 1 and 3 year eradication of *Pseudorasbora parva* at high and low densities, and at high and low direct interspecies transmission. Here, the role of interspecies connectedness (Scenario 3) and eradication time (Scenario 5) were tested. For all tested scenarios, there was no significant effect of eradication time on *Rutilus rutilus* abundance; however there was a strong effect for *Leucaspius delineatus* and *Cyprinus carpio* abundance. Interspecies transmission had no significant effect on disease mortalities in local communities.

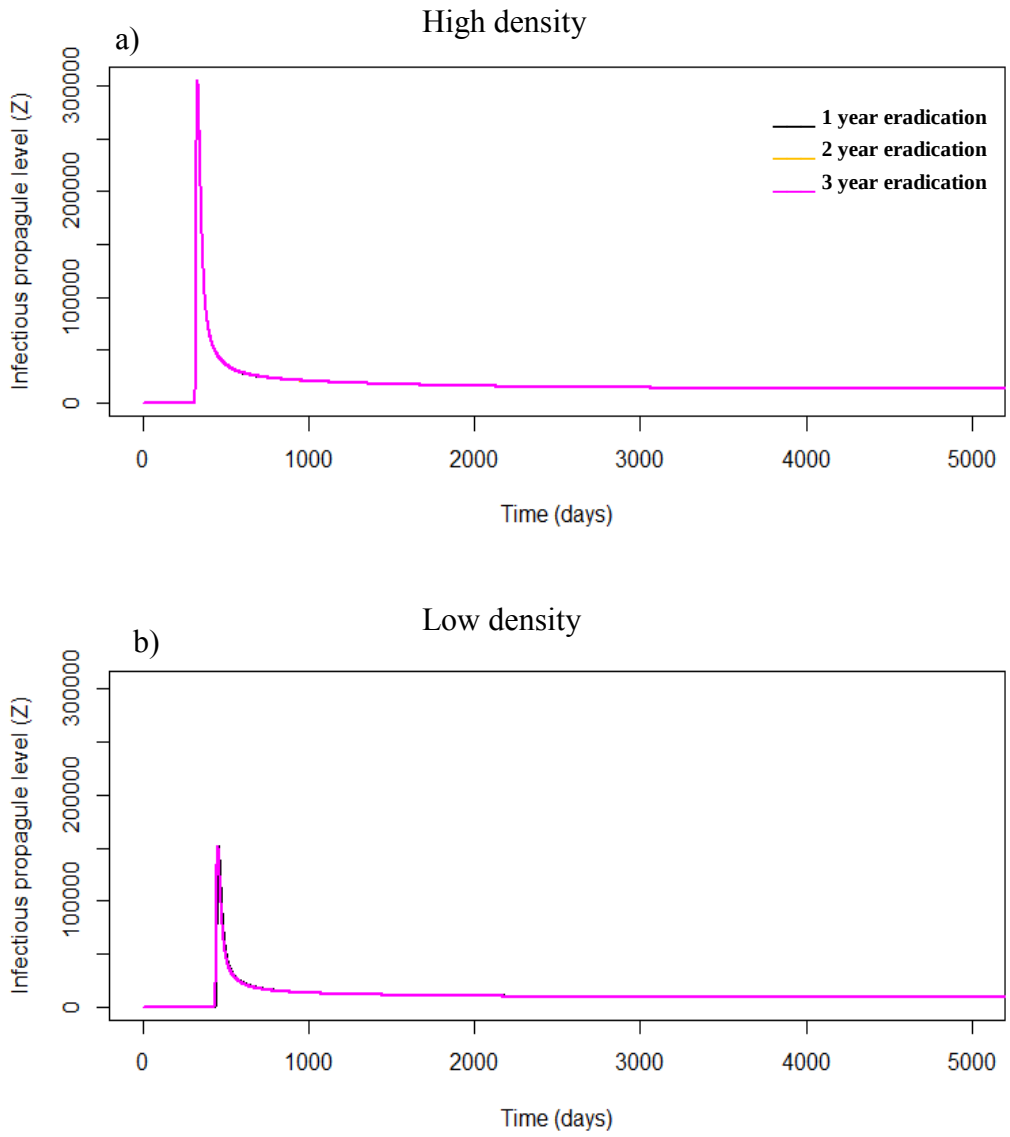

Figure 4.6. The number of infectious propagules in a community, at 1, 2 and 3 year eradication of *Pseudorasbora parva* in high and low-density communities. Here, the role of eradication time (Scenario 5) was examined more closely. In both high (a) and low (b) density communities, eradication time did not affect the number of infectious propagules in the system. Overall, low-density populations had lower propagule numbers compared to high-density populations.

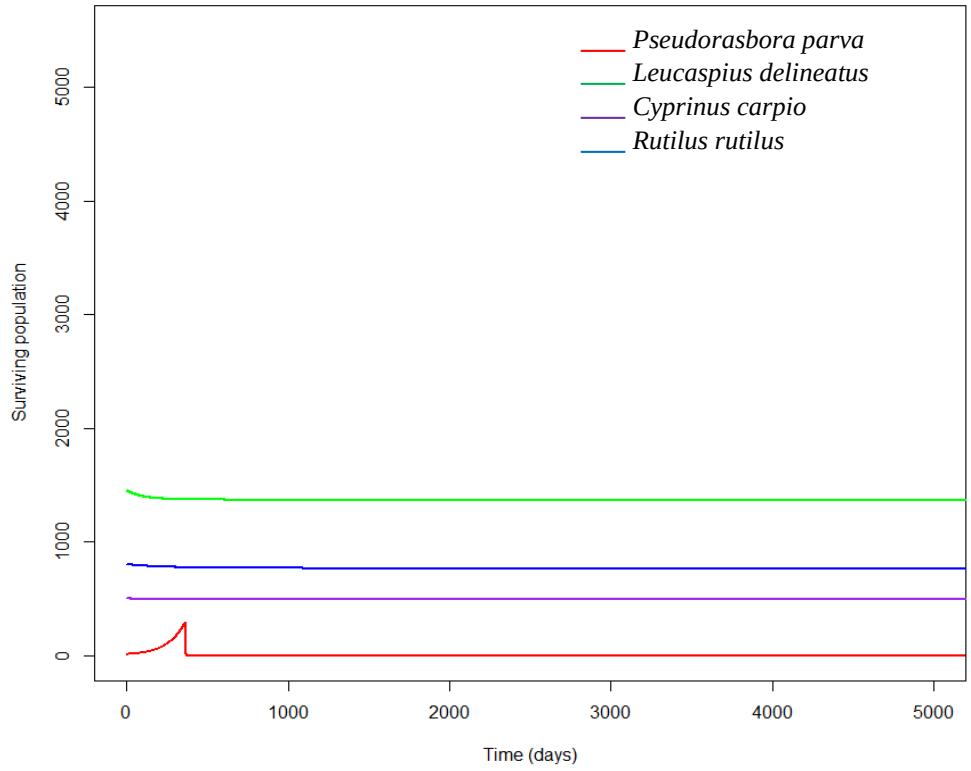

Figure 4.7. Excluding environmental transmission from *Sphaerothecum destruens*' transmission strategies prevented an epidemic from occurring in the local community, demonstrating the importance of environmental transmission in the pathogen's dispersal.

Effect of high background mortality on the resilience of the local community

In scenarios with high background mortality, there was an initial population decline observed in the local community due to the magnified background mortality rate. Following this, each community experienced a further population decline due to infection with *S. destruens* (Fig. 4.8). The timing of this second decline was dependent on the initial population density of the local community; high-density populations led to significantly faster epidemics than low-density populations (2 sample t-test: $t = -26.14$, d.f. = 9710, $p < 0.001$).

Here, direct transmission between species affected the starting time of infection, although there was no significant effects on final species abundance (2 sample t-test: $t = 0.5$, d.f. = 50 007, $p > 0.05$). Individuals could die before they began to release significant numbers of infectious propagules, so the relative contribution of direct transmission to pathogen expansion increased as the contribution of environmental transmission decreased. In high-density populations, the first infection following

introduction was delayed by 11-17 days when direct interspecies transmission was low. This delay was 14-21 days in low-density populations. Following the outbreak, the level of infectious propagules remained above 0 in the local community, indicating that the infection could persist at low levels. Furthermore, susceptible species such as *R. rutilus* and *L. delineatus* remained at severely depleted population sizes, with no population growth observed. Less susceptible species such as *C. carpio* recovered to equilibrium population size.

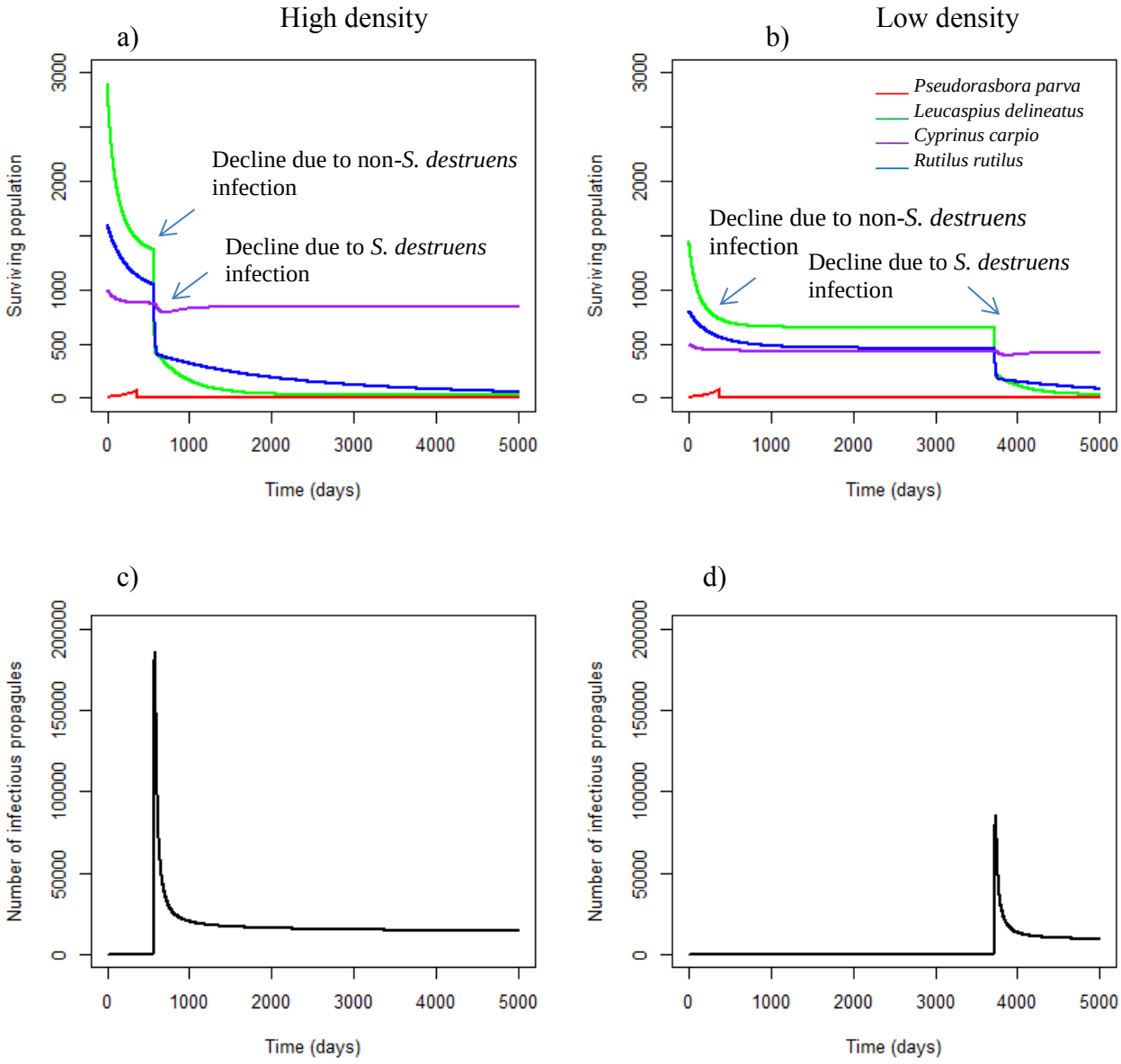

Figure 4.8. The effect of high background mortality on community recovery in high (a) and low (b) density communities, and the peaks in infectious propagule levels due to *Sphaerothecum destruens* outbreaks (c, d). The initial decline in species' abundance is a result of increased natural mortality (simulating an additional infection), followed by a decline from *S. destruens* infection at different times in high and low densities. Here, Scenario 6 was tested, demonstrating that high background mortality can prevent susceptible species (*Leucaspius delineatus* and *Rutilus rutilus*) in the local community from recovering after infection.

4.4 Discussion

The release of environmentally transmitted propagules of a generalist pathogen such as *S. destruens* for less than a year was predicted to be sufficient for its establishment in local host communities. The disease dynamics in local communities were initially driven by environmental transmission of the parasite from the healthy carrier population. The results demonstrated that without the presence of environmental transmission, the *S. destruens* epidemic would not have occurred in the local community. However, once there were multiple infected hosts in the local communities, the epidemic was no longer mediated by spill-over from the source population, but among susceptible host populations in the local community. In the long-term, both *L. delineatus* and *C. carpio* declined to similar levels in all eradication scenarios. These patterns were consistent in highly conservative scenarios where only a small proportion (0.1%) of the infectious propagules reached the local community. High background mortality in the local community (e.g. due to multiple infections) prevented susceptible species' populations from recovering to sustainable levels. Overall, high-density populations maintained higher levels of species diversity for a longer duration compared with low-density populations. The main results were summarised in Fig. 4.9.

If *S. destruens* was present in natural systems, the predicted pattern of mortality suggests an initial rapid decline in the local communities after the introduction of the pathogen. The timing of this decline was dependent on the lifetime of the infectious propagules, which has important implications: as the lifetime of infectious propagules is dependent on temperature, changing climates could significantly impact the geographic range and prevalence of this infection in the environment. Following the initial decline, impacted populations would then recover and stabilise at a lower population density than their initial levels. In scenarios with high background mortality levels (i.e. hosts with pre-existing infections), the results indicated that susceptible species could not recover. This has important implications for community resilience, suggesting that if infection was already present in the community and *S. destruens* was introduced, susceptible species could not recover to

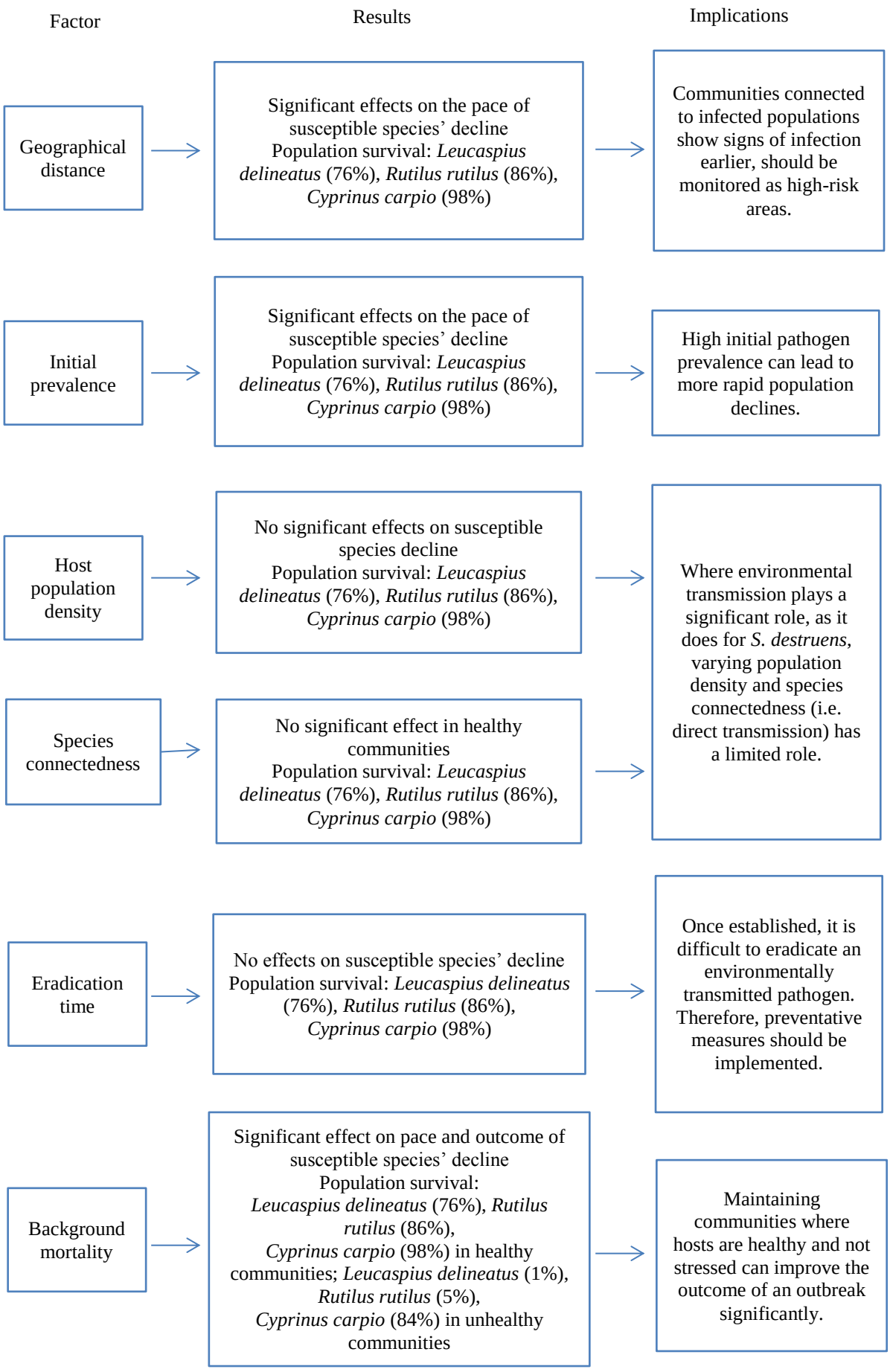

Figure 4.9. A summary of model results, showing the surviving percentage of the initial populations.

sustainable levels. Furthermore, surviving populations maintained the infection at a low prevalence, becoming reservoirs of infection themselves (Peeler et al. 2011). The populations were sustained below the necessary density required for an infectious disease outbreak (i.e. remained below the epidemic threshold (McCallum 2001)). However, if a pathogen can persist in multiple reservoir hosts, re-emergence of disease could occur if the populations cross the epidemic threshold, irrespective of eradication of the initial introduced host.

These risks are associated with pathogens with mixed transmission modes such as *S. destruens*, which operate under complex dynamics. Direct and environmental transmission act across two different time-frames; direct transmission indicates rapid infections driven by host contact rate, while environmental transmission takes place on a longer time scale with spores accumulating in the environment over time, leading to chronic pathogen exposure (Roche et al. 2011). In this system, environmental transmission played a key role in the dispersal of the pathogen between communities, facilitating the pathogen's initial emergence (Rohani et al. 2009). Following this, direct transmission of virulent pathogens in the local community caused rapid declines in susceptible species' populations. After the mortalities occurred, infectious propagules remained in the environment at low levels, creating the potential for pathogen re-emergence (Morens et al. 2004). If the pathogen only used direct transmission, no spill-over would have occurred into the local community as pathogen dispersal would have been inhibited in time. Conversely, if the pathogen only used environmental transmission, the outcome would have been chronic decline due to exposure to low environmental levels. Because *S. destruens* can use both methods of transmission, both high host mortalities and the potential for pathogen re-emergence from the environment are possible.

The continued persistence of a generalist pathogen in the environment and in local host communities could have substantial consequences for aquaculture and recreational fisheries (Elston et al. 2008, Walker and Mohan 2009). As demonstrated here, eradication of the healthy carrier is unlikely to eliminate the pathogen if it has

already dispersed. The lag time between detection and eradication of an introduced species can be considerable due to issues of resources, legislative and policy requirements, and political will (Britton et al. 2011). For example, *P. parva* was first detected in UK aquaculture in the mid-1980s and in the wild in the mid-1990s, but the first eradication of a wild population was in 2005 (Britton and Brazier 2006). Following eradication of free-living hosts, a common procedure is then to re-stock with new fish in order to restore high population densities in both recreational and commercial settings (Cowx 1994). However, this work suggests that this would not prevent the re-emergence of the pathogen or even its continued spread into new systems, as the pathogen was maintained at low levels in reservoirs after the outbreak. In the shrimp farming industry, pathogen re-emergence from disease reservoirs has occurred multiple times after populations were restocked with new individuals (Walker and Mohan 2009). This has severe public health and financial consequences, particularly as the conditions in which many aquaculture facilities operate (i.e. extremely high densities) can lead not only to emergence, but to an increase in pathogen virulence (Mennerat et al. 2010, Pulkkinen et al. 2010). Thus, in disease management terms the benefit of eradicating the free-living species and/or subsequent restocking might be limited.

To date, *P. parva* has been introduced to numerous sites in Europe and North Africa, with several links to major river catchments (Gozlan et al. 2010b). If these introduced populations carry *S. destruens* even at a low prevalence, they pose a potentially serious threat to native fish communities in surrounding water bodies. Here, it was predicted that environmental transmission was sufficient for pathogen establishment in neighbouring communities, and could lead to multiple reservoirs of infection. The bi-modal transmission of the pathogen increased the risk of pathogen emergence and persistence, which has profound long-term implications for the ecology and management of affected populations. The results and recommendations from this work can also be applied to other generalist pathogen systems with bi-modal transmission, such as *B. dendrobatidis*, and provide a new level of consideration in eradication protocols. For these pathogens, rapid diagnosis and if possible eradication is important to minimise mortalities, as this study demonstrated that even one year following introduction is sufficient for pathogen establishment.

For terrestrial systems, it is expected that eradication would have a more significant impact on disease emergence, because hosts are not embedded in their environment as they are in aquatic systems, and the pathogen may not be established in an environmental reservoir. Developing tools such as eDNA detection or filtering procedures (Strand et al. 2014) following animal transport can potentially prevent the initial emergence of a pathogen like *S. destruens*. In freshwater habitats, pathogens can also spread downstream and lead to native species decline, as predicted in this work and demonstrated empirically (Jakaitis 2014), which can have substantial economic and ecological consequences.

This work stressed the importance of using preventative measures against pathogen expansion and establishment, particularly for chronic generalist diseases, as reactive measures such as eradication may not work. Relevant measures can include emerging multi-host pathogens on notifiable parasite lists, especially if effective surveillance systems and response protocols are developed that facilitate early detection and the initiation of rapid actions to prevent their dispersal to adjacent communities. There are currently eight notifiable fish diseases in the UK, including *Gyrodactylus salaris*, a macro-parasite which causes highly virulent infections in Atlantic salmon *Salmo salar* (Johnsen and Jensen 1986, DEFRA/CEFAS 2014). Studies on the spread of this infection between populations in countries like Norway have provided a strong basis for the successful development and application of management strategies in the UK, such as heightened surveillance, development of early warning systems and, should an outbreak occur, monitoring of dispersal in the wild (e.g. Bartley et al. 2006; Peeler et al. 2007). Furthermore, this work has demonstrated that maintaining healthy populations in general is critical for population recovery. This example demonstrates the utility of using existing knowledge of a pathogen to design and implement robust management programmes to minimise the chance of disease outbreaks, and the actions needed to limit their potential spread. Such approaches could have strong application to the management of more novel and generalist pathogens such as *S. destruens*.

Chapter 5
The influence of host competition on community resilience to disease outbreaks

Summary

Outbreaks of generalist pathogens are influenced by host community structure, including population density, host diversity, and competitive species interactions, but these effects are not fully understood. The role of competition and predation on host community resilience to disease outbreaks was assessed using two independent methods: an epidemiological multi-host SEIR model and a Bayesian belief network, which predicted the likelihood of population decline based on assumed relationships between species. The introduction of *S. destruens* through *P. parva* was simulated in a community comprised of species in multiple trophic levels, mimicking realistic ecosystems that are affected by this pathogen. Epidemiological parameters, including mortality from infection, pathogen incubation rate, and host recovery rate, were set to different simulation based values in each species and varied in secondary consumers to observe top-down effects of infection on the survival of a host community. Model predictions suggested that high virulence and rapid infections of secondary consumers would lead to more significant declines in community biodiversity than chronic infections with low mortality rates. Secondary consumers were predicted to inhibit the population growth of *P. parva* through predation. However, the results demonstrated that *S. destruens* aided in *P. parva* establishment by reducing the competitive abilities of the resident species. After introduction, the pathogen remained in the environment due to its persistence in host populations and long-lived propagules. These results were complemented by a Bayesian belief network, which used broad assumptions of competitive interactions and predicted an additive effect of infection and host competition on resident species' decline, which was mitigated when resident secondary consumers were resistant to infection. The persistence of pathogen propagules in the environment has strong consequences for pathogen management, as predation of the introduced host was not sufficient to effectively and sustainably control the spread of the disease, except at very high levels.

5.1 Introduction

Shifts in food web structure can arise due to parasites altering the symmetry of competition between species and affecting density-dependent dynamics (Dobson et al. 2008, Tompkins et al. 2011). The consequences of infection by a particular parasite can vary according to its host specificity and the community structure of potential free-living hosts, and range from species-specific mortalities to community-wide disease outbreaks. Generalist pathogens can cause major shifts in biodiversity and food web structure through infecting a broad range of species across different trophic levels (Fenton and Brockhurst 2007, Thompson et al. 2010, Peeler et al. 2011). The emergence of a generalist pathogen can potentially alter the structure and demography of host populations, with cascading effects on interactions between populations at the community level (Britton 2013). Parasites can also affect host behaviours, further impacting interspecific interactions (Barber et al. 2000, Parris and Cornelius 2004). Empirical work suggests that generalist pathogens emerge in some host communities but not in others (Fisher et al. 2009, Olson et al. 2013, Ercan et al. 2015), implying that host community structure may influence the resilience of ecosystems to pathogen emergence. Nevertheless, current understandings of how host community structure impacts generalist pathogen emergence remains poor.

Parasites can significantly affect the structure of food webs (Lafferty et al. 2008, Amundsen et al. 2009, Poulin and Leung 2011), especially when associated with species introductions. For example, the introduction of rinderpest virus to wild African ungulates in the Serengeti completely altered the ecosystem, as it reduced populations of mid-trophic level species by 80% (Lafferty et al. 2008). This then affected carnivore populations and changed the vegetation, leading to an increase in fires and habitat destruction (Hudson et al. 2006). Amphibian declines due to the chytrid fungus *B. dendrobatidis* have changed primary production and phytoplankton populations in an ecosystem, which had consequences for consumers such as insects and effectively disrupted ecosystem stability (Whiles et al. 2006, Sime-Ngando 2012). Furthermore, pathogens have been shown to cause increased predation of their infected hosts through the process of parasite increased trophic transmission (PITT), which can lead to significant changes in community organisation as infected

populations at lower trophic levels are increasingly preyed upon (Lafferty 1999, Barber et al. 2000). As the increasing global incidence of *B. dendrobatidis* has demonstrated (Fisher et al. 2009, Olson et al. 2013), fungal and fungal-like pathogens are particularly threatening to biodiversity and ecosystem function (Fisher et al. 2012, Ercan et al. 2015). This is particularly true for fungal pathogens introduced via non-native hosts, as there may be additional community level changes caused by the introduction of a non-native species (Britton 2013).

Some characteristics of an infection can further affect the response of the host community. For example, it is possible that chronic infections would keep the susceptible host populations permanently depleted, compared to rapidly acting infections which would be cleared from the population faster (Jolles et al. 2005). Alternatively, chronic infections could allow the population to recover over a longer time, as there would be no severe peaks in mortality (Lachish et al. 2011). Furthermore, the role of resistant species in community resilience is an important question in the context of disease management; if the pathogen is transmitted trophically and infected hosts are predated on by a resistant species, this could limit the spread of the pathogen to new hosts, as the consumer is effectively a transmission "dead-end" for the pathogen (Barber et al. 2000, Roche et al. 2012). In this instance, a case can be made for using selective restocking (i.e. enhancing the population density of a particular species in a community) as a potential disease management strategy, as increasing the proportion of a non-susceptible species to the community could limit pathogen transmission (Roche et al. 2012, Searle et al. 2013). Thus, it is important to consider the characteristics of the infection and the susceptibility of host species when examining community resilience.

Through the use of mathematical models, this chapter tested how host community composition and competitive interactions influenced the resilience of a community to an introduced generalist pathogen infection. The model species were *S. destruens* and its healthy carrier *P. parva*. Several fish species of the families cyprinidae (e.g. *A. brama, C. carpio, R. rutilus*) and salmonidae (e.g. *S. salar, Oncorhynchus mykiss, Salmo trutta*) have experienced elevated mortality as a result of infection (Arkush et

al. 1998, Andreou et al. 2012, Paley et al. 2012). The aims of the multi-host epidemiological and Bayesian models were to: 1) predict the effects of pathogen introduction on host population survival in a community comprising several susceptible fish species, and 2) test how some competitive food web interactions affected the fish community's resilience to the emergence of *S. destruens* and its healthy carrier. To do so, mortality from infection, pathogen incubation rate, and recovery rate in hosts at high trophic levels were varied.

5.2 Materials and Methods

5.2.1 Host-pathogen model system

S. destruens was described in detail in Section 1.6.

5.2.2 Community assemblage

Theoretical communities were assembled to include interspecific competition and a range of disease resistance. Included species were selected due to the availability of data on their competitive interactions and susceptibility to *S. destruens* infection. The communities included two secondary consumer species (based on brown trout *S. trutta* and rainbow trout *O. mykiss*), two prey species (*L. delineatus* and *P. parva*, the introduced healthy carrier) and two additional species with high and low disease resistance (*C. carpio* and *R. rutilus*, respectively). The latter two species were not considered as prey or predators, but were in direct competition with the designated prey species and as such were referred to as 'competing species' (Fig. 5.1). Such a community structure mimicked wild communities that could become infected with *S. destruens* (Gozlan et al. 2005, Ercan et al. 2015). Note that it is considered unlikely this particular species assemblage would exist in sympatry in the wild, especially the co-existence of the salmonid species with species such as *C. carpio*. However, for the purpose of this model it was useful as it was supported by empirical data to create general conclusions. Species that were present in the community before the introduction of *P. parva* were referred to as resident species. Parameter values were selected based on previous empirical studies of *S. destruens* and similar pathogens (Appendix 4).

5.2.3 Multi-host ecological models

Two complementary methods, a SEIR simulation and a Bayesian belief network (herein referred to as simply the Bayesian framework), were used to model host communities where a healthy carrier and generalist pathogen were introduced. While the SEIR model predicted the timeline and level of population decline by simulating a pathogen outbreak, the Bayesian framework predicted the likelihood of that decline occurring given different initial conditions and species interactions. Together, the two methods provide both a temporal perspective of community changes (SEIR), as well as the likelihood of these changes to occur (Bayesian). The Bayesian framework was run independently of the SEIR simulation model, and the results of the two methods were compared.

Firstly, changes in species' abundance over time were simulated using empirically derived parameter values for demography (birth rate, natural mortality and population carrying capacity), competition (predation and resource competition) and infection (epidemiological parameters, see Appendix 4 for details). Mortality from infection, incubation, and recovery rates of secondary consumers were simulated across a range of values to model different host community responses and predict possible disease outbreaks. In the Bayesian framework, species' interactions were included to determine likelihood estimations of the overall effects of infection and competition on species' abundance. The likelihood of the secondary consumers population decline was varied to explore the effects on the remainder of the community. This complemented the SEIR simulation model by providing a framework which included uncertainty.

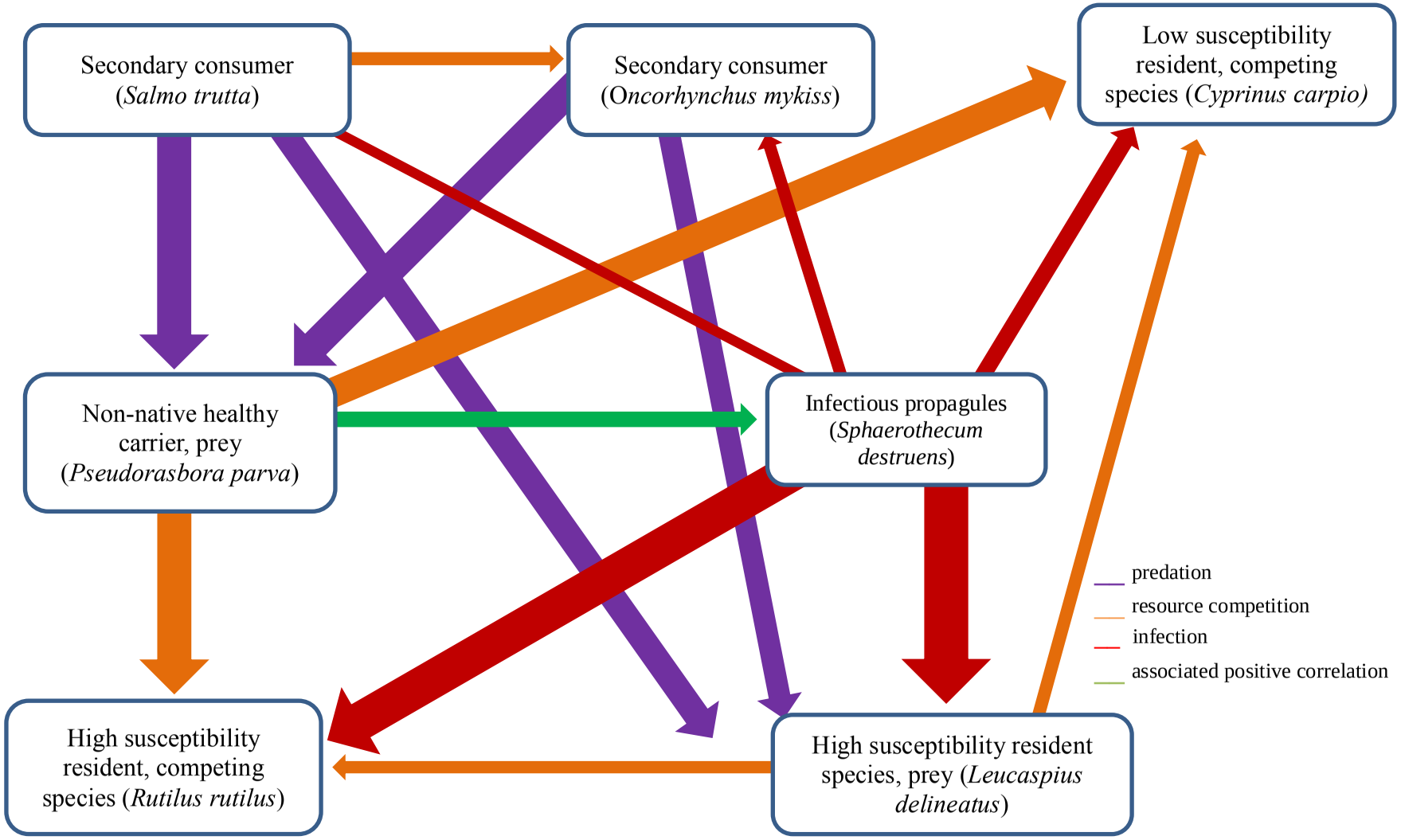

Figure 5.1. The interspecies interactions included in the community models. The green arrow indicates a correlated increase of infectious propagules as the healthy carrier density increases (as they introduce the pathogen). Red arrows indicate an inverse relationship between infectious propagules and species density due to infection. Similarly, orange arrows indicate declines due to resource competition as one species out-competes the other, and purple arrows indicate predation between the connected species. The thickness of the arrows signifies the strength of the interaction.

1. SEIR simulation model

The multi-host SEIR model categorised each section of a host population based on their infection status (susceptible (S), exposed (E), infected (I) and recovered (R)). Differential equations described the rate of change of a host population from one category to the next. In addition to epidemiological parameters, a competition coefficient (*a)* between each Species *i* and *j* was incorporated, which represented the interspecies interactions (predation and resource competition). Susceptible individuals of Species *i* could become infected through direct contact with an infectious individual (β_i) or by ingesting infectious propagules Z via environmental transmission ($\varepsilon_i(Z/Z+k_{ei})$) (Eq. 5.1). Density-dependent birth (*b*) and natural mortality (*m*) of the population were also included.

$$\frac{dS_i}{dt} = b_i N_i \left(1 - \frac{N_i}{K_i}\right) - S_i \sum_{i}^{n} \beta_{ji} I_j - \varepsilon_i \left(\frac{Z}{Z + k_{ei}}\right) S_i - \sum_{j=1} a_{ji} S_i N_j - m_i S_i$$

Eq. 5.1

When susceptible individuals ingested enough spores, they moved into the exposed (E) category (Eq. 5.2) and remained in this category for the incubation period of the pathogen ($1/\sigma_i\ (Z/Z+k_{si})$).

$$\frac{dE_i}{dt} = S_i \sum_{i=1}^{n} \beta_{ji} I_j + \varepsilon_i \left(\frac{Z}{Z + k_{\varepsilon i}}\right) S_i - \sigma_i \left(\frac{Z}{Z + k_{si}}\right) E_i - \sum_{j=1} a_{ji} E_i N_j - m_i E_i$$

Eq. 5.2

Following the incubation period, individuals became infectious (I) and released spores. Within the infectious category, individuals either experienced mortality from the disease ($\alpha_i(Z/Z+k_{ai})$) or recovered ($\gamma_i(Z/Z+k_{gi})$) (Eq. 5.3). Recovered individuals could not be re-infected (Eq. 5.4). See Section 2.2.3 for details of why saturation functions were used for multiple parameters.

$$\frac{dI_i}{dt} = \sigma_i \left(\frac{Z}{Z + k_{s_i}} \right) E_i - \gamma_i \left(\frac{Z}{Z + k_{gi}} \right) I_i - \alpha_i \left(\frac{Z}{Z + k_{ai}} \right) I_i - \sum_{j=1} a_{ji} I_i N_j - m_i I_i$$

Eq. 5.3

$$\frac{dR_i}{dt} = \gamma_i \left(\frac{Z}{Z + k_{g_i}} \right) I_i - \sum_{j=1} a_{ji} R_i N_j - m_i R_i$$

Eq. 5.4

The number of infectious propagules in the environment Z was dependent on the rate of release of spores from each infected host per day (ϕ_i), and the mortality rate μ of the spores (Eq. 5.5). This mortality rate was based on experimental work on the longevity of *S. destruens* spores and zoospores (Andreou et al. 2009), and varies according to temperature.

$$\frac{dZ}{dt} = \sum_{1=i}^{n} \phi_i I_i - \mu Z$$

Eq. 5.5

A population carrying capacity for each species was introduced under the assumption that smaller species were more abundant than larger species (Froese and Pauly 2015). This represented intraspecific competition within the community, as birth rates declined after reaching carrying capacity (Britton et al. 2008). Birth and natural mortality rates were based on species data from www.fishbase.org. Selected parameter values with detailed explanations are in Appendix 4.

2. Bayesian belief network

Bayesian belief networks operate through conditional dependencies between different variables; the outcome of each variable (in this case, a species' population) depends on its connections with other variables and their connections with each other (Jie et al. 1997). This generates a probability over whether a species' population will decline, based on previous knowledge of their interactions. A Bayesian belief network incorporating all species was used (using the belief network model developed by Stafford et. al (2015) and as described below), which included the assumed interspecific interactions (Fig. 5.1). The model used VBA (Visual Basic for

Applications) programming in Microsoft Excel to describe the probable increase or decrease in species abundance based on their predicted interactions, given the introduction of the pathogen.

Within this framework, the probability of Species A's population size increasing ($P(A_i)$) or decreasing ($P(A_d)$) was first assigned prior values, where $P(A_i) + P(A_d) = 1$. For each species, these values were based on known changes in the community. In this instance, the only certain disturbance to the community was the introduction of the healthy carrier and pathogen. Thus, the prior probability of population size increase was only changed in the introduced species, with the remaining species' prior values kept at 0.5.

Posterior probability estimates of increasing or decreasing species abundance were incorporated into the Bayesian framework using published data on competitive interactions within the theoretical species assemblage (Table 5.1). Each interaction was reciprocal, although not necessarily symmetrical between species, and all interactions were independent of one another. The model required inputs for all the probabilities of Species B increasing/decreasing, given that Species A was increasing/decreasing. Thus the intermediate probability of Species B increasing given interacting Species A was:

$$P(B_i|A) = [P(B_i|A_i) * P(A_i) + P(B_i|A_d) * P(A_d)] \quad \text{Eq. 5.6}$$

Following this, the posterior probability of increasing/decreasing for each species was calculated in two ways, one which included certainty provided by prior information (Eq. 5.7) and one that excluded prior values if there were more certain values (i.e. further away from 0.5) for species interactions (Eq. 5.8):

$$Post(B_i) = P(B_i) + |1 - P(B_i)| * \left[\sum_{1-n} P(B_i) * \frac{P(B_i|A)-0.5}{n}\right] \quad \text{Eq. 5.7}$$

$$Post(B_i) = \sum_{1-n} \frac{P(B_i|A)}{n} \quad \text{Eq. 5.8}$$

where n is the number of interactions with Species B. The final posterior value was the value furthest from 0.5 (the value with the highest probability of occurring). The

new posterior probability was then inserted into the model as the new prior probability, with two iterations representing the number of trophic levels in the system (i.e. secondary consumers eat prey species). The number of iterations is set to the maximum number of connections in the system, to incorporate the effects of trophic cascades. In this instance, two iterations were sufficient to reflect the effects of infection on the lower trophic level.

Table 5.1. Top-down species interaction parameters considered for the Bayesian model. Grey cells indicate no interaction between species. Numbers represent the probability of a species in a column decreasing, given that the species in the row is increasing.

	P. parva	*L. delineatus*	*R. rutilus*	*C. carpio*	*O. mykiss*	*S. trutta*	*S. destruens*
P. parva (healthy carrier, prey species)		0.55	0.8	0.7			0.2
L. delineatus (susceptible host, prey species)	0.55		0.6	0.6			
R. rutilus (susceptible host)				0.55			
C. carpio (susceptible host)			0.55				
O. mykiss (secondary consumer)	0.8	0.8				0.6	
S. trutta (secondary consumer)	0.8	0.8			0.4		
S. destruens (pathogen)		0.9	0.8	0.6	0.55	0.55	

5.2.4 Modelled scenarios

The focus of the simulation models was on the secondary consumer species to ascertain how infection of species at high trophic levels can affect the remainder of the resident community; aspects of epidemiology that were varied included the incubation rate of the pathogen, the mortality rate from infection, and the recovery rate of the host (Appendix 4). Incubation rate and mortality from infection were

positively correlated with one another in *S. destruens* epidemiology, with further evidence of a positive correlation between incubation rate and recovery rate (Chapter 3). Firstly, the three epidemiological parameters were changed independently of one another in secondary consumers, to test the effect of each parameter individually on overall community survival, as the parameters may be unlinked in some pathogen systems. Following this, the mortality rate and the pathogen incubation rate were mathematically coupled based on empirical data and varied along a range of values, against a range of possible values for recovery rate. Finally, the three parameters were correlated with each other to quantify the effects of acute versus chronic infections on community resilience. Changing the parameter values simultaneously provided a more complete perspective on pathogen dynamics in this particular system. This allowed the exploration of how disease resistance at high trophic levels could influence the whole community. Furthermore, competition coefficients were altered to test the effect of increased predation pressure on the survival of the introduced host and pathogen. Species' abundance in the model was monitored for approximately 14 years, to examine long-term population trends. All models were run in R (Version 2.15.1) and differences in model output were tested for statistical significance using Welch 2-sample t-tests in the R *stats* package. The Shannon diversity index of the different fish communities was calculated in R in the *vegan* package.

The Bayesian belief network was tested using three variations in the initial assumptions: (1) competitive interactions were included, but infection was excluded to determine the effects of competition on species abundance; (2) infection was included and the secondary consumers were assumed to be resistant to infection; and (3) infection was included and the secondary consumers were assumed to be susceptible to infection (and thus had a higher probability of decline).

5.3 Results

SEIR simulation: Secondary consumer species can mitigate the impacts of invasion and infection

The interspecific interactions were modelled in 1) the absence of the introduced healthy carrier; 2) the absence of the pathogen (i.e. 0% prevalence); and 3) the presence of both healthy carrier and pathogen. The resident community remained at a stable equilibrium without the introduced species, as expected (Fig. 5.2a). Without infection, *P. parva* was predicted to establish a stable population within 2-3 years of introduction (Fig. 5.2b). Neither secondary consumer species showed any significant changes in population size in the absence of infection, but their presence and predatory position in the food web limited the population growth of the healthy carrier *P. parva* compared to communities with no predation (2 sample t-test- $t = 10.71$, d.f. = 5969, $p < 0.0001$). The remaining resident species demonstrated minor declines to lower stable levels, due to competition with *P. parva*.

The simulations then focused on the impact of *S. destruens* in the community. Each epidemiological parameter (incubation rate, mortality from infection, and recovery rate) was varied individually to determine its precise effect on community resilience. When the secondary consumer species had high mortality from infection, predating on *P. parva* controlled its population growth (as it did in the absence of infection) but did not prevent pathogen establishment. All resident species were predicted to experience population declines within one year of introduction (Fig. 5.3a). *Leucaspius delineatus, S. trutta,* and *O. mykiss* were predicted to recover after an initial decline due to infection, although to lower levels than previously. However, *C. carpio* and *R. rutilus* continued to decline for the duration of the simulation. These declines were attributed to competition with the introduced species and continuing infections within the resident species (2-23% prevalence).

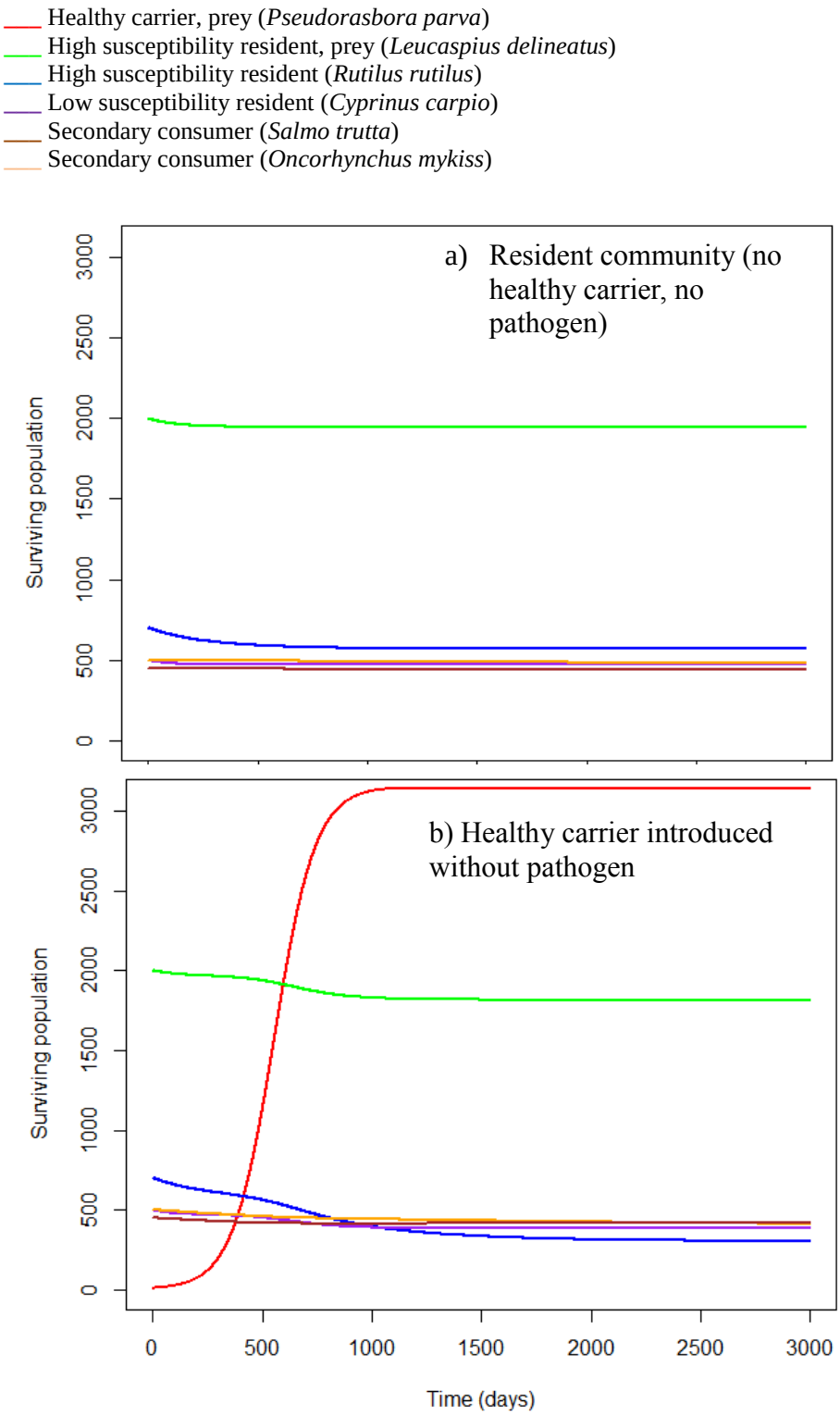

Figure 5.2. Species abundance in a community without any disturbance (a) and in a community where a healthy carrier was introduced, but not the pathogen. The community remained stable without any disturbances, but when the healthy carrier was introduced, minor declines were observed in *Leucaspius delineatus*, *Rutilus rutilus*, and *Cyprinus carpio*.

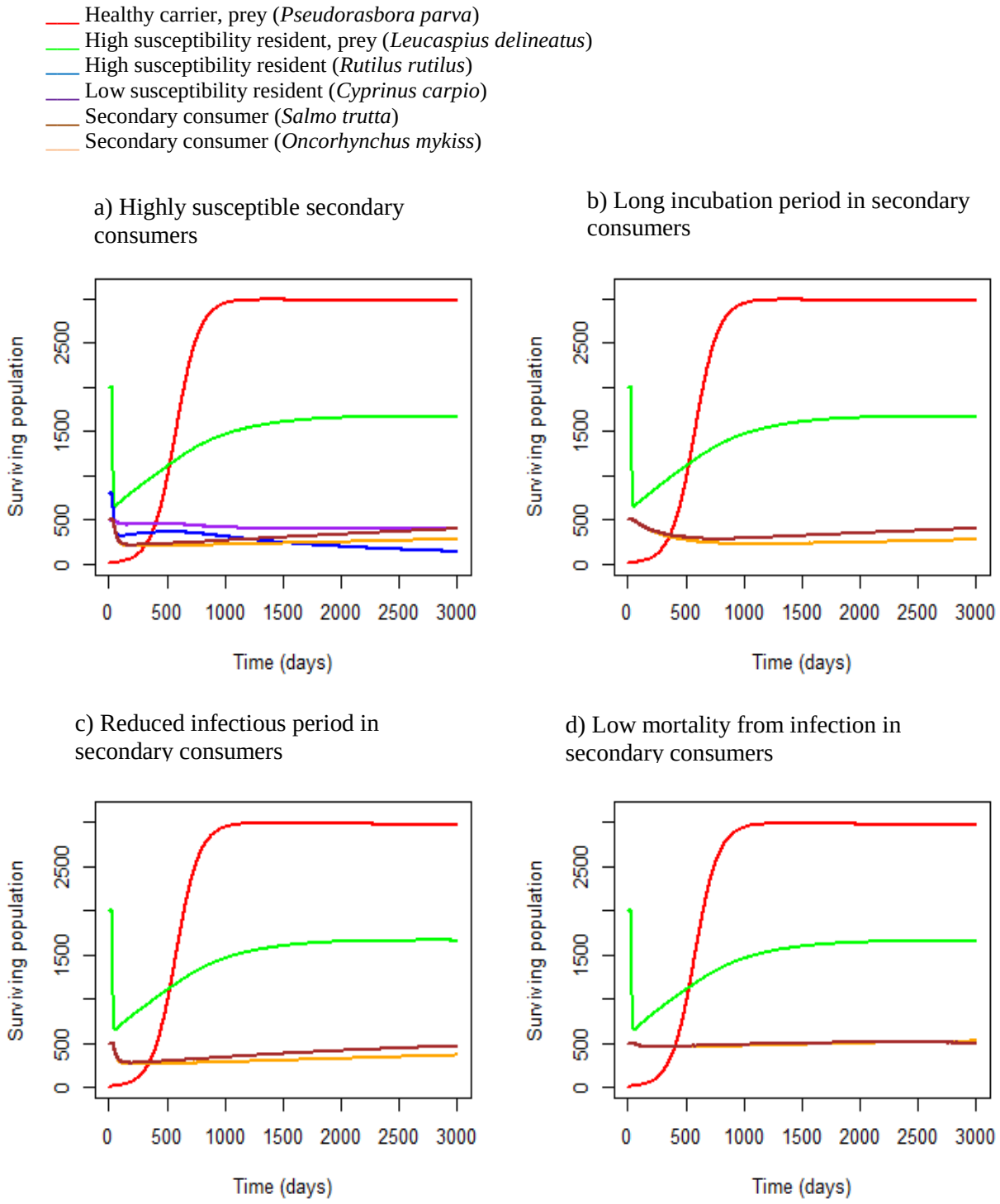

Figure 5.3. Species abundance after *Pseudorasbora parva* and *Sphaerothecum destruens* were introduced to a community, when: a) the community included highly susceptible secondary consumer species, which were able to control the population growth of *P. parva*. All resident species experienced an initial population decline due to infection, followed by population recovery in the secondary consumers and *Leucaspius delineatus*, and further chronic decline in *Cyprinus carpio* and *Rutilus rutilus* (not shown in the remaining plots); b) Secondary consumer species had slow-growing infections which delayed their initial decline from infection. The remaining species displayed similar trends as (a); c) The infectious period of the consumers was reduced, which led to higher populations of all resident species; d) The consumers' mortality from infection was reduced which led to higher populations of the consumers and *L. delineatus*, while also reducing the population size of *P. parva*.

The three epidemiological parameters were then varied one at a time from the original values to test the effect of each parameter on disease dynamics in the community. When secondary consumers had high mortality from infection but slow-growing infections, their initial decline took place more slowly (over approximately two years) with significantly higher population size in *O. mykiss* compared with rapidly growing infections (Fig. 5.3b; 2 sample t-test- *S. trutta*: $t = 0.419$, d.f. = 6000, $p > 0.05$; *O. mykiss*: $t = 15.91$, d.f. = 4873, $p < 0.0001$). Reducing the infectious period of the consumers led to higher populations of all resident species (Fig. 5.3c), while decreasing their mortality from infection led to larger populations of the two consumers and *L. delineatus* compared to other scenarios (Fig. 5.3d; 2 sample t-test: $t = -11.97$, d.f. = 5983, $p < 0.0001$). Furthermore, this reduced the *P. parva* population (2 sample t-test: $t = 7.15$, d.f. = 5977, $p < 0.0001$). The infectious propagule levels in the environment indicated the timing of the initial outbreak and revealed that *S. destruens* was expected to persist at low levels, indicating that chronic infection was possible (Fig. 5.4).

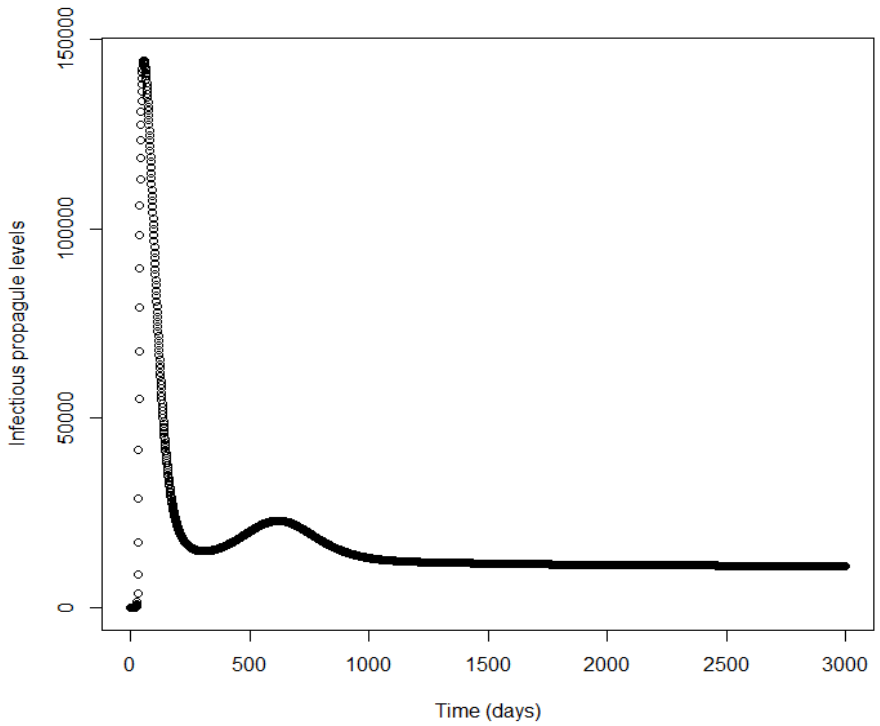

Figure 5.4. Environmental spore levels for the duration of the simulation. A peak in environmental spore levels was observed at the start of the simulation, which coincided with an initial population decline in all resident species' populations. Low levels of environmental spores were present throughout the simulation.

Following the individual parameter changes, the model was run for a range of parameter values incorporating a positive correlation between mortality from infection and incubation rate. Fish community diversity was lowest when mortality from infection was high (with a short incubation rate) and recovery rate was low (i.e. a long infectious period) (Fig. 5.5). There was a sharp decline in species richness when the consumers' mortality from infection surpassed a threshold ($\alpha = 0.1$). Beyond this threshold, species richness did not recover to its initial levels after 10 years, across all parameter combinations.

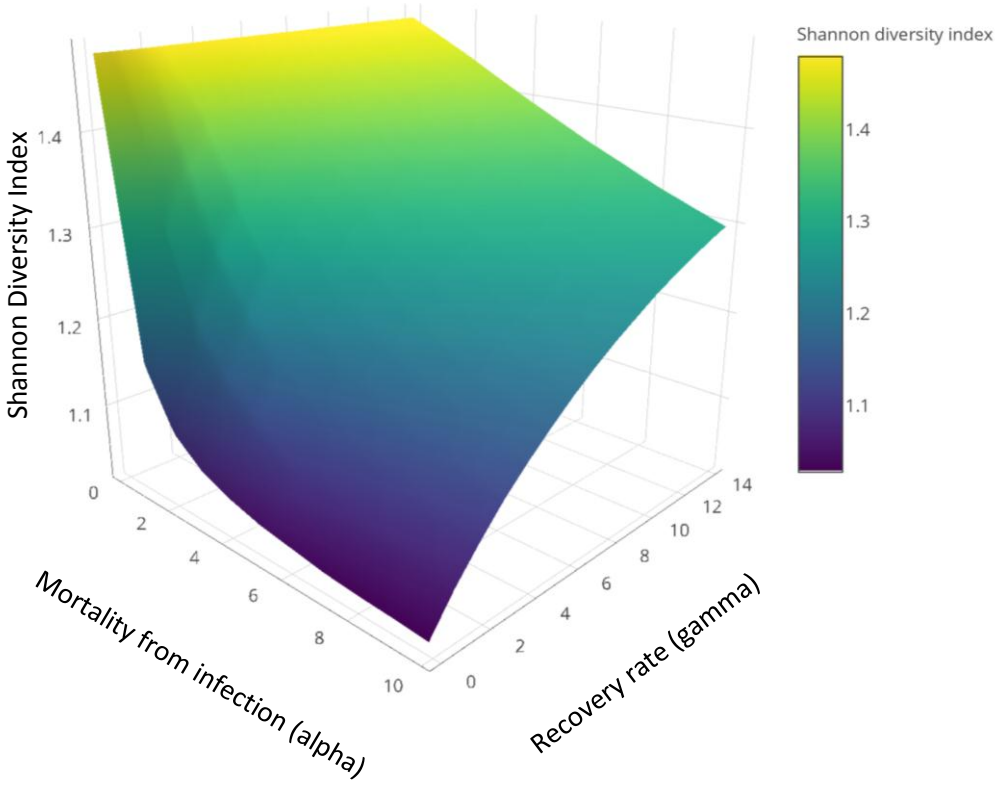

Figure 5.5. The Shannon diversity index of the resident fish community 10 years after healthy carrier introduction, across a range of mortality from infection and recovery rates of secondary consumer species. Low mortality from infection maintained high diversity across all values of recovery. Higher recovery rates in secondary consumers helped maintain high community diversity further. The results demonstrated that species introduction can have a long-term effect on community diversity, and resistant species at high trophic levels can sustain higher levels of diversity after a disturbance.

The likely dynamics in this system were that incubation, recovery, and mortality rates were positively correlated. Low virulence, chronic infections were able to sustain higher community diversity than high virulence, acute infections (2 sample t-test: $t = 24.12$, d.f. = 7299, $p < 0.001$, Fig. 5.6). This suggested that resistant secondary consumers could help maintain the diversity of the entire community at higher levels.

High predation pressure on *P. parva* (a value of 10^{-6} compared to the lowest value of 10^{-9}) was predicted to prevent their establishment (Fig. 5.7). Uninfected consumers were predicted to reduce the population of *P. parva* more than infected consumers, indicating that infection inhibited their competitive ability. However, in a realistic ecological setting the consumers could also have predated on *L. delineatus* to the same degree, which was predicted to lead to a population crash. In terms of *S. destruens*, very few infections occurred initially, and there were no severe outbreaks; the pathogen was contained below threshold levels.

Bayesian model: Competition between species is still an important determinant of resilience to disturbances, even in the absence of infection

In the absence of infection, competition still played a significant role in species decline when a competing healthy carrier was introduced into a resident community (Fig. 5.8). The most significant divergence between decline due to only competition and decline due to both competition and infection was in the resident prey species, *L. delineatus*; when the secondary consumers were resistant (i.e. low likelihood of decline in the presence of the pathogen), infection caused population decline to be 8% more probable compared to the absence of infection. This decline was 26% more probable when the secondary consumers were also susceptible to infection (i.e. highly likely to decline in the presence of the pathogen).

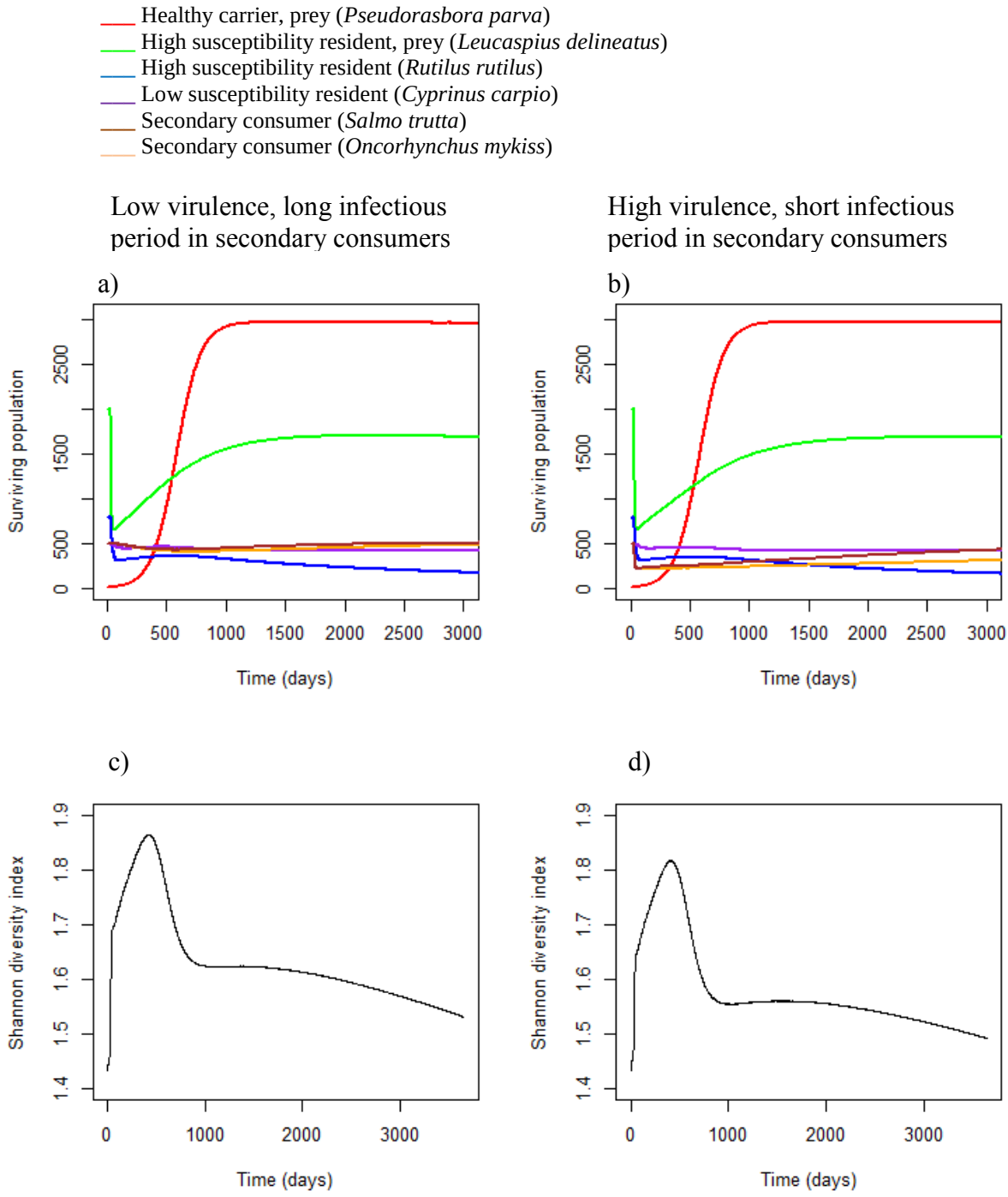

Figure 5.6. Species abundance after a healthy carrier and a generalist pathogen were introduced to a community, when: a) Secondary consumer species had low mortality from infection and a long infectious period; b) Secondary consumer species high mortality from infection and a short infectious period. Shannon diversity indices were calculated for both simulations shown in a) and b), and were displayed in c) and d), respectively. Lower virulence and longer infectious periods helped maintain higher community biodiversity over time (c) compared to high virulence, short infectious periods (d).

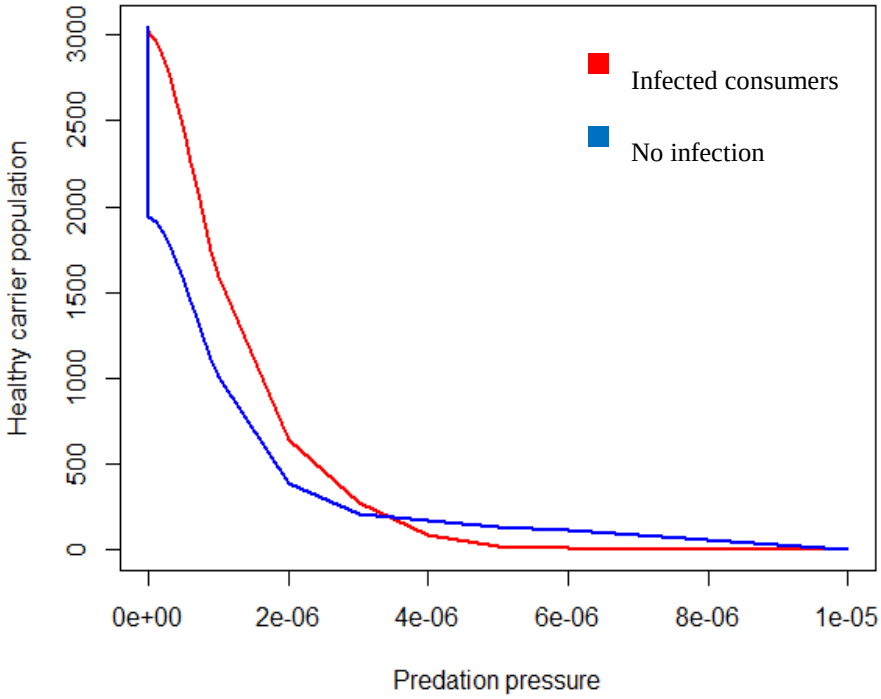

Figure 5.7. Healthy carrier abundance 10 years post-introduction under a range of predation pressures. High predation prevented the introduced species from establishing both in the presence of infection (blue) and the absence of infection (orange). Uninfected consumers were able to reduce the population of the healthy carrier more than infected consumers (2 sample t-test: t = -1.78, d.f. = 34, p = 0.083).

For communities with resistant secondary consumers, the inclusion of infection did not have any significant effects on secondary consumers and the probabilities of the resident species' decline were similar in the presence and absence of infection. However, for susceptible secondary consumers, the additive effects of competition and infection led to a higher likelihood of population decline (especially for the outcompeted *O. mykiss*) compared to the absence of infection (5% and 18%, respectively). This decline was predicted to affect the survival of the other species. While *L. delineatus* was predicted to decline further (potentially due to higher infection pressure), *C. carpio* and *R. rutilus* were less affected (potentially due to a reduced level of competition with *L. delineatus*).

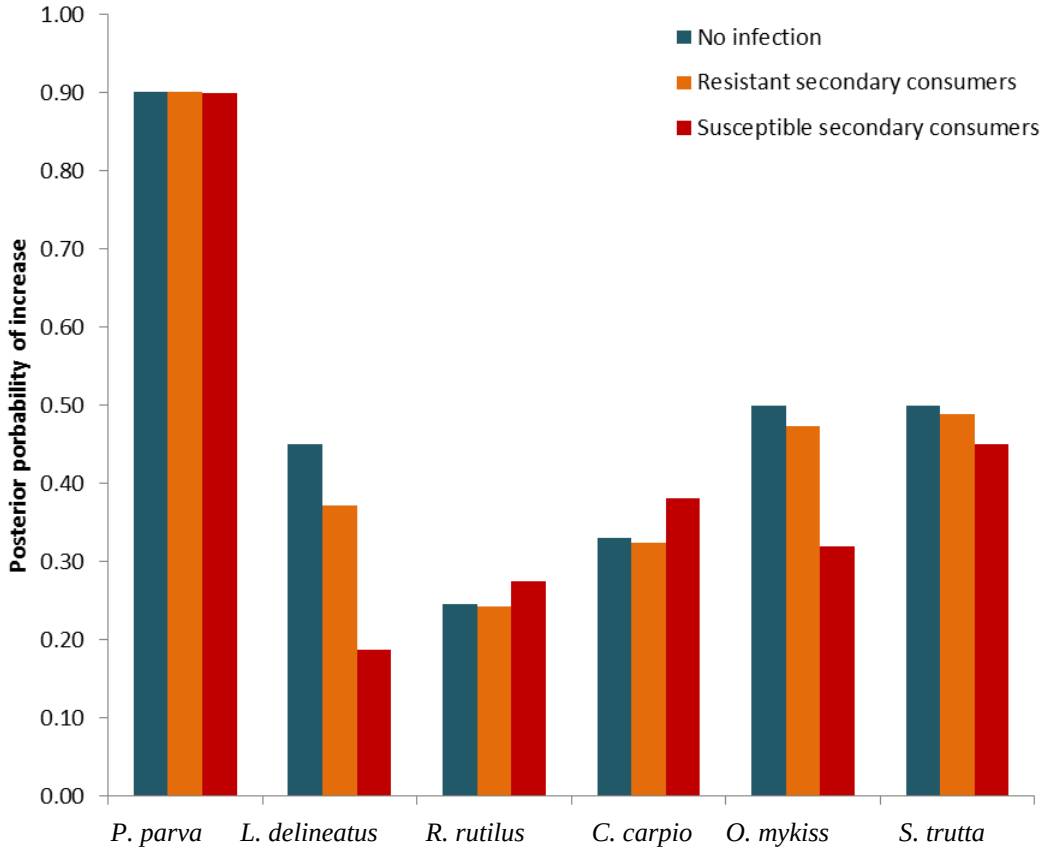

Figure 5.8. The probability of each species' population density increasing in the presence and absence of infection, when the secondary consumers were resistant to infection (orange) or susceptible to infection (red) using a Bayesian framework of species interactions. This was compared with the absence of infection (blue).

5.4 Discussion

The SEIR simulation model predicted that secondary consumer species could inhibit the population growth of an introduced healthy carrier, but would not prevent the spread of infection. High predation pressure on the introduced species did suppress its population growth and contained the pathogen, but also potentially caused the extinction of resident species occupying similar trophic niches. When epidemiological parameters were varied individually, long incubation periods still led to secondary consumer species' decline in the model but with a greater lag-phase than rapidly acting infections. Short infectious periods in species at high trophic levels were correlated with higher population sizes of all resident species. When the three epidemiological parameters were correlated, chronic infections of secondary consumers helped to maintain higher long-term community diversity than acute

infections. Thus, biotic resistance through predation could alleviate the consequences of healthy carrier introduction (Britton 2013) and to a certain degree contained the pathogen, but also had significant consequences for resident community structure.

A comparison of the two modelling methods revealed that the results obtained by each were similar in content, although to a different level of detail (Fig. 5.9). While the SEIR simulation model provided a temporal perspective that quantified changes in each species' population over time, the Bayesian framework incorporated uncertainty into the model, resulting in the likelihood of a given species' population increasing or decreasing in general. The output data from the SEIR model allowed deeper exploration into the pathogen's disease dynamics, by varying specific aspects of its epidemiology (e.g. changing only incubation rate). However, the interactions between specific components of epidemiology are not certain, so the predictions made in the Bayesian framework were also helpful as the general conditions of host resistance could be altered. Thus, both methods were useful in their own respect, and this work demonstrated that they can be used in tandem to create a more complete understanding of pathogen dynamics.

The initial introduction of the healthy carrier and generalist pathogen reduced the abundance of all susceptible resident species below the threshold needed for a second epidemic to occur. The expected population decline of susceptible resident species is also consistent with experimental studies, where the increased biomass of the healthy carrier significantly decreased the competitive ability of the other species (Britton 2012). However, some susceptible host populations were predicted to continuously decline due to chronic exposure to the low levels of pathogen propagules in the environment. Thus, resident species were predicted to decline due to a synergistic effect of infection and competition, highlighting potential risks of disease introduction to species at lower trophic levels. A synergistic effect of competition and infection has been shown to lead to decline in otherwise stable populations, even for predatory species. The decline of the red squirrel *Sciurus vulgaris* in the UK is thought to be a combination of direct competition with grey squirrels and the presence of a parapoxvirus, even at low prevalence (Tompkins et al. 2003).

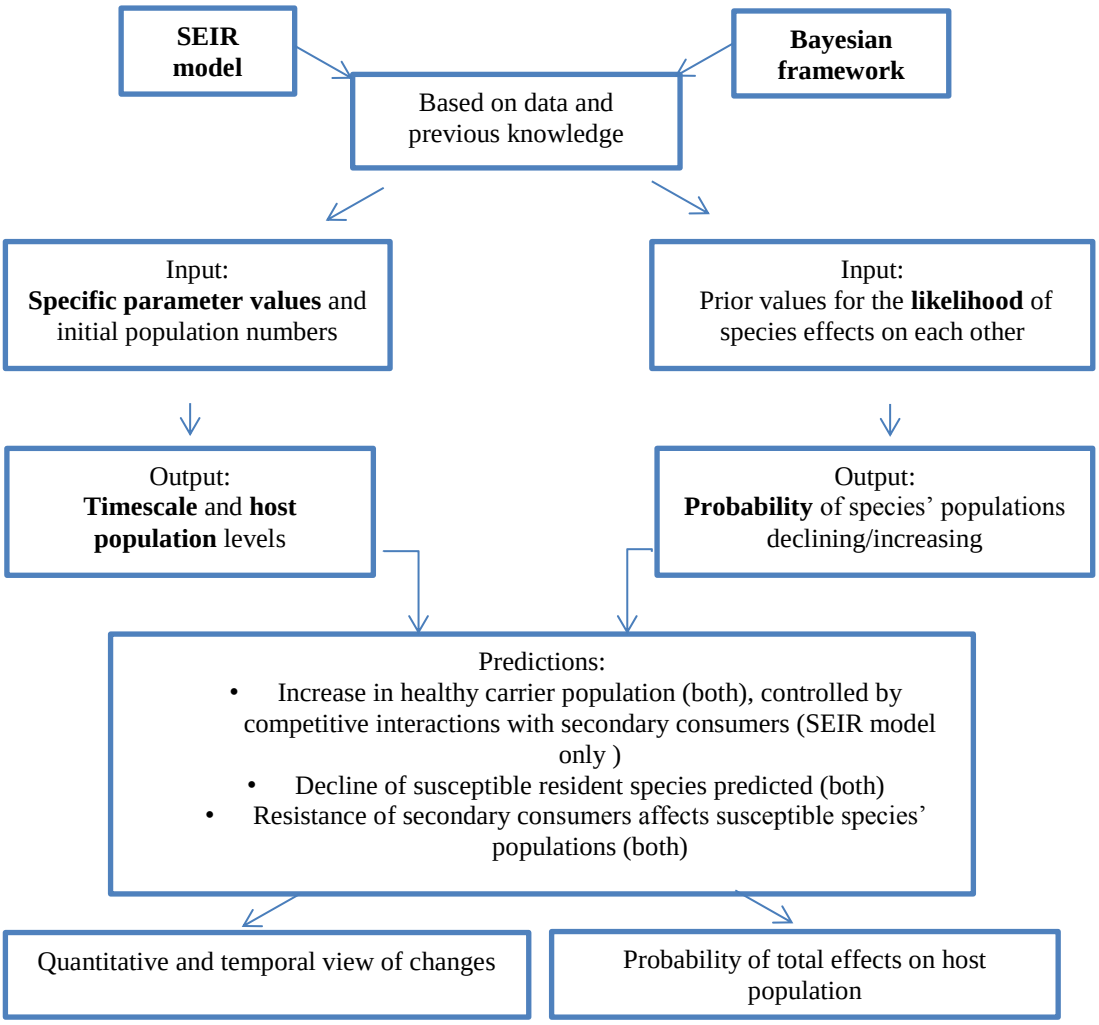

Figure 5.9. Schematic comparison of the two modelling methods used and the results obtained. Some parallels are clear between the two methods, but each gives a different type of conclusion.

Where the community is comprised of susceptible hosts, high host diversity can amplify the spread of generalist pathogen infection (Mihaljevic et al. 2014). However, increasing the diversity of a host community to include non-competent hosts has successfully limited the spread of Lyme disease (Ostfeld and Keesing 2011) and rodent-borne haemorrhagic fevers (Mills 2006). This dilution effect caused by resistant or dead-end hosts has been used to limit the spread of infection and create herd immunity (Johnson and Thieltges 2010). In this work, higher disease resistance helped maintain the community diversity. Previous work has demonstrated that where hosts (or the pathogen itself) are predated on by other species, disease prevalence can decrease (Roy and Holt 2008, Searle et al. 2013). For example, there

is some evidence that *B. dendrobatidis* outbreaks are correlated with low levels of macro-invertebrates, which usually prey on the chytrid spores (Schmeller et al. 2013, Strauss and Smith 2013). Here, the infection persisted in the environment and in susceptible host populations, which became reservoirs of infection except at very high levels of predation. This could explain why disease outbreaks are observed in some communities and not others, highlighting the importance of looking at the specific community composition, and not only at biodiversity levels, when studying infectious disease outbreaks. In terms of disease dynamics, this demonstrated that the host community composition has to be examined through both competition and susceptibility perspectives in order to ascertain the likelihood of an epidemic, and to predict if the host community can recover from an epidemic.

A biocontrol approach through predation has already been tested in this model system, demonstrating that *P. parva* populations could be reduced by perch *Perca fluviatilis* (a secondary consumer species) (Davies and Britton 2015). However, this increased the risk of negative top-down effects on ecosystem function (Eby et al. 2006). Predation as a form of biocontrol has been explored in a number of systems (Maharaj et al. 1992, Snyder and Wise 1999, Symondson et al. 2002), but often there are unforeseen limitations and repercussions, such as non-target species declining due to direct predation or knock-on effects resulting from the shifting community structure (Knapp et al. 2001, Louda et al. 2003). Unwanted effects of species removal on the remaining species in the community have been observed in several systems (Zavaleta et al. 2001, Bergstrom et al. 2009), and can be difficult to control retrospectively. In this system, higher predation could eliminate the introduced host, but could also increase predation of resident species and potentially cause their extirpation. This elevated predation pressure could also lead to consumer species increasing to unsustainable levels, further disrupting the functioning of the ecosystem. In the context of disease eradication, if the pathogen is already established in several host species or in the environment, removing one host species will not eliminate the pathogen (Wobeser 2002), but could actually contribute to disease re-emergence. Thus, as shown by this work, this management approach may not be effective in mitigating the effects of the pathogen. The findings and methodology in this work can be applied to multiple systems to establish the risks of

pathogen emergence and more importantly, predict a given community's resilience to infectious outbreaks.

Chapter 6
The evolutionary dynamics of generalist pathogens with multiple modes of transmission

Summary

The evolution of virulence in generalist pathogens could be influenced by both multiple host species and modes of transmission. Generalist pathogens' transmission is less constrained by host extinction compared to specialist pathogens as they can persist in multiple hosts. As such, they can face different selective pressures based on community composition. Evolutionary Stable Strategies (ESSs) for the pathogen were examined under different ecological conditions, testing the effect of host susceptibility to the pathogen and direct and environmental contact rates on pathogen virulence. The results demonstrated that multiple modes of transmission significantly affect the evolutionary stable (ES) virulence of a pathogen. High direct contact rates led to lower pathogen virulence, and high environmental contact rates led to evolutionary bi-stability in the pathogen, with potentially co-existing low and high virulence strains. At intermediate levels of direct and environmental contact rates, higher ES virulence was predicted in the model. Community susceptibility affected the predicted patterns of virulence. With *S. destruens*, these patterns have different implications in different habitat types. For example, in aquaculture, high direct contact rates between hosts could keep pathogen virulence down, although this effect may not occur if the system is continuously restocked with susceptible hosts. In contrast, in the wild, environmental contact rates may be comparatively higher than direct contact rates, and this could lead to the emergence of two pathogen strains at low and high virulence.

6.1 Introduction

Generalist pathogens have remained a major interest of epidemiology for over 40 years, and increasing numbers of studies are incorporating the effects of multiple hosts on pathogen transmission (Anderson et al. 1986, Rigaud et al. 2010, Osnas and Dobson 2012, Mihaljevic et al. 2014). This continuing interest is due to several remaining challenges in the study of generalists, including the difficulty in monitoring them (especially in wild populations), the incomplete understanding of some generalists' life history, and their potential emergence in novel hosts (Power and Mitchell 2004, Thompson et al. 2009, Rowley et al. 2013). The growing incidence of generalist zoonoses, exemplified by the prevalence of Chagas disease

(WHO 2002, Coura and Viñas 2010) or West Nile Virus (Allan et al. 2009, Anthony et al. 2014), demonstrates how important it is to fully understand generalist pathogen transmission and virulence in order to better prepare for new instances of emergence. For both epidemiologists and public health officials, this understanding is also critical for managing the pathogen effectively and in the long-term (Lootvoet et al. 2013), to avoid management approaches that may inadvertently cause more virulent strains to evolve (Dieckmann et al. 2005, Coors and De Meester 2011).

A major barrier to completely understanding generalist pathogen disease dynamics is that the traditional trade-off between virulence and transmission does not apply to generalists, as they can exist in multiple hosts at varying levels of virulence (Osnas and Dobson 2010), and some persist in environmental reservoirs (Merikanto et al. 2014). In contrast, specialist pathogens are highly adapted to a particular host or a few closely related hosts, evolving in predictable environments. Therefore, their dynamics are relatively stable (Sasal et al. 1999). Generalists tend to persist in unstable or heterogeneous environments and they increase pathogen persistence and transmission between populations (Frank 1996, McMahon et al. 2013). For this reason, their disease dynamics are constantly changing and are difficult to predict. These generalist attributes have been established for several emerging pathogens, including *B. dendrobatidis* and *S. destruens* (Fisher et al. 2012, Ercan et al. 2015), and result in a number of unknown variables that may contribute to the pathogenicity of a generalist.

Historically, studies have focused on pathogen dynamics in one host, but more papers are examining how pathogen evolutionary dynamics change in multiple host systems (Regoes et al. 2000, Dobson 2004, Böhm et al. 2009, Rigaud et al. 2010, Osnas and Dobson 2012). These have demonstrated that where a pathogen can infect multiple hosts, various virulence strategies can evolve based on between species transmission levels, the timing of transmission, and resulting host mortality, for example (Osnas and Dobson 2012, Mihaljevic et al. 2014). In practice, it is difficult to quantify transmission between host species, so the effect of host quality or host shifting on pathogen evolution is not fully understood (Rigaud et al. 2010). It is

predicted that community susceptibility could influence pathogen virulence, with lower susceptibility communities leading to pathogens with higher virulence at ESS, compared to highly susceptible communities. The rationale for this is that communities with a higher level of resistance or immunity to a pathogen can act as agents of selection for higher virulence (Mackinnon and Read 2004). These combined selective pressures can lead to two potential outcomes as the pathogen encounters different hosts. One outcome is the specialisation of different pathogen strains in different host species and therefore virulence (and/or transmission) can increase (Antonovics et al. 2013). Alternatively, generalism can occur and pathogen virulence (and/or transmission) can vary across host species (Woolhouse et al. 2001, Leggett et al. 2013). Therefore, although it introduces complex dynamics to the system, it is crucial to consider multi-host assemblages when studying pathogen evolution.

Furthermore, some generalists transmit to their hosts via alternate infection pathways, e.g. *Vibrio cholerae* (Codeco 2001) and avian influenza virus (Roche et al. 2009), adding another layer of complexity to their disease dynamics. The inclusion of multiple transmission modes into evolutionary studies has been a relatively new development in the field of epidemiology, showing that multiple ESSs can exist for these pathogens, dependent on transmission pathway (Dunn and Smith 2001, Roche et al. 2011). As each pathway operates through a different mechanism, and is thus under different selective pressures, pathogen transmission routes can determine its virulence (Williams and Day 2001, Martins et al. 2013). Direct transmission occurs through contact between infected and susceptible hosts, initiating a fast progression of the disease as susceptible hosts contract high numbers of propagules from the infected host, while environmental transmission takes place by the ingestion of free-living infectious propagules, which would accumulate over a slower timescale (Tien and Earn 2010, Roche et al. 2011).

The duration of transmission can lead to different evolutionary outcomes for the pathogen (Osnas and Dobson 2010). Environmental transmission via long-lived propagules can lead to high virulence when the pathogen is introduced, because the

pathogen is not dependent on host survival to reproduce (Bonhoeffer et al. 1996, Walther and Ewald 2004). Furthermore, free-living pathogens may need to replicate in a hostile external environment with high levels of competition with other pathogens and other environmental stressors, leading to the evolution of higher replication rates and virulence (Merikanto et al. 2014). For pathogens with high environmental transmission that can also use direct transmission, evolutionary bi-stability at high and low virulence has been predicted in a one host system (Roche et al. 2011), a hypothesis which was tested here in a two host system. Conversely, for direct transmission to occur the host needs to remain alive and able to come in contact with new hosts (except for pathogens that transmit after host death, see Day 2002), and therefore high virulence can limit the pathogen's ability to transmit (Ewald 1994, Galvani 2003); thus lower pathogen virulence can be predicted for pathogens with high levels of direct transmission (Clayton and Tompkins 1994). However, it is predicted that high-density communities would lead to the evolution of higher virulence as contact rates between hosts would increase. For example, high densities in fish farms led to changes in *Flavobacterium columnare* life history, leading to more virulent infections in salmon (Pulkkinen et al. 2010). Therefore, pathogens in susceptible communities with high direct contact rate were predicted to evolve higher virulence compared to pathogens evolving in communities with low direct contact rates.

Here, both multiple hosts and multiple modes of transmission were included to understand what drives the virulence of generalists, using conclusions derived from previous models of *S. destruens*. Firstly, a multi-host SIR model was created which could vary the initial susceptibility of a host community, to test its effects on pathogen transmission and ES virulence. Secondly, a model for pathogen reproductive number was derived based on the SIR system. The relative contribution of direct and environmental transmission to pathogen reproductive success was explored across a range of virulence and community susceptibility, determining the success of each transmission pathway. Finally, using adaptive dynamics techniques the conditions in which a mutant strain could invade a resident strategy were explored. The role of ecological parameters such as direct and environmental contact rates in pathogen evolution was tested.

6.2 Methods

6.2.1 The epidemiological model

A multi-host epidemiological model was created to explore the evolutionary dynamics of a generalist fungal-like pathogen, based on *S. destruens* and its hosts as a model system. The SIR model included two pathways of transmission (direct and environmental) but removed the exposed stage of infection and multiple saturation functions used in previous chapters. This modification simplified the model system in order to better explore the pertinent questions of evolutionary dynamics in more detail, while remaining applicable to multiple systems.

Susceptible individuals of Species *j* could become infected with pathogen strain *i* through density-dependent direct transmission or environmental transmission through spore uptake (Fig. 6.1; Eq. 6.1). Here, χ represented the direct contact rate among individuals, and ρ was the contact rate with the environment (i.e. uptake rate). One saturating function was included in this model for the rate of environmental transmission: K_j represented the threshold value for environmental transmission (i.e. the number of spores that needed to be ingested for a 50% probability of infection). Its value was kept consistent with previous chapters. A density-independent birth rate (v) and a natural mortality rate (m) were included for each species. Infected individuals (I) either died from infection (at rate α) or recovered (R) at rate γ (Eq. 6.2 and 6.3). Infected individuals released ϕ spores per day which died at rate μ (Eq. 6.4).

$$\frac{dS_j{}^i}{dt} = v - \sum_{j=1}^{n}\left[\chi_j S_j{}^i I_j{}^i \beta_j{}^i + \rho_j{}^i S_j{}^i \left(\frac{Z^i}{Z^i + K_j{}^i}\right)\right] - m_j S_j{}^i \quad \text{Eq.6.1}$$

$$\frac{dI_j{}^i}{dt} = \sum_{j=1}^{n}\left[\chi_j S_j{}^i I_j{}^i \beta_j{}^i + \rho_j{}^i S_j{}^i \left(\frac{Z^i}{Z^i + K_j{}^i}\right)\right] - (m_j + \alpha_j{}^i + \gamma_j{}^i) I_j{}^i \quad \text{Eq. 6.2}$$

$$\frac{dR_j{}^i}{dt} = \gamma_j{}^i I_j{}^i - m_j R_j{}^i \quad \text{Eq. 6.3}$$

$$\frac{dZ^i}{dt} = \sum_{j=1}^{n} \sum_{i=1}^{n} \left[\phi_j{}^i I_j{}^i \right] - \mu^i Z^i \qquad \text{Eq.6.4}$$

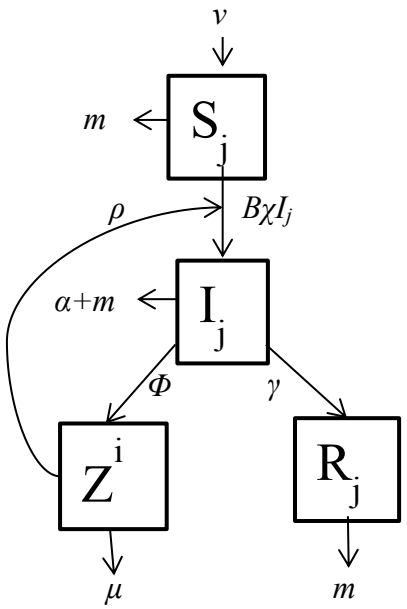

Figure 6.1. Schematic outline of the multi-host SIR model in a given Species j infected with pathogen strain i.

Parameters were consistent with previous chapters, unless otherwise specified in Table 6.1.

6.2.2 The basic reproductive number

A pathogen's basic reproductive number (R_0) is one of the most important values in epidemiology, and it quantifies the fitness of a pathogen in a population comprised of only susceptible individuals (Heffernan et al. 2005). It is defined as the number of secondary infections one infected host can cause (Heesterbeek 2002), and represents how successful a particular pathogen strain is under given conditions (e.g. host community composition). This number provides crucial insights into the risks of pathogen emergence in a system (Antia et al. 2003), and the consequences of emergence (i.e. how many hosts could become infected).

Table 6.1. Epidemiological parameters used in the multi-host evolutionary model.

Parameter	Description (rates per day)
v	Birth rate independent of population density
χ	Contact rate between individuals
β	Direct transmission rate
ρ	Contact rate with the environment
Z	Number of infectious propagules in the environment
K	Threshold value for 50% probability of infection in strain i
m	Natural mortality rate of host species
α	Virulence
γ	Recovery rate
ϕ	Propagule release rate (i.e. shedding rate)
μ	Propagule persistence in the environment

If the basic reproductive number of a pathogen is greater than 1, it will emerge in a system. For this SIR model, the R_0 of pathogen strain *i* in two hosts (N1 and N2) was calculated as:

$$
\begin{aligned}
{R_0}^i = {} & \frac{{\beta_1}^i \chi_1 N_1}{2({\gamma_1}^i + m_1 + {\alpha_1}^i)} + \frac{{\beta_2}^i \chi_2 N_2}{2({\gamma_2}^i + m_2 + {\alpha_2}^i)} \\
& + \left\{ \frac{({\beta_{12}}^i \chi_{12} {\beta_{21}}^i \chi_{21} - {\beta_1}^i \chi_1 {\beta_2}^i \chi_2) N_1 N_2}{({\gamma_1}^i + m_1 + {\alpha_1}^i)({\gamma_2}^i + m_2 + {\alpha_2}^i)} \right. \\
& \left. + \left[\frac{{\beta_1}^i \chi_1 N_1}{2({\gamma_1}^i + m_1 + {\alpha_1}^i)} + \frac{{\beta_2}^i \chi_2 N_2}{2({\gamma_2}^i + m_2 + {\alpha_2}^i)} \right]^2 \right\}^{\frac{1}{2}} \\
& + N_1 \rho_1 \left[\frac{{\phi_1}^i}{{\mu_1}^i K_1 ({\gamma_1}^i + m_1 + {\alpha_1}^i)} + \frac{{\phi_2}^i}{{\mu_2}^i K_2 ({\gamma_2}^i + m_2 + {\alpha_2}^i)} \right] \\
& + N_2 \rho_2 \left[\frac{{\phi_1}^i}{{\mu_1}^i K_1 ({\gamma_1}^i + m_1 + {\alpha_1}^i)} + \frac{{\phi_2}^i}{{\mu_2}^i K_2 ({\gamma_2}^i + m_2 + {\alpha_2}^i)} \right]
\end{aligned}
$$

Eq. 6.5,

where β_{12} was the rate of interspecies direct transmission from Species 1 to 2, and vice versa. The first three terms calculated the contribution of direct transmission to the pathogen's reproductive number, and were based on Gandon (2004). Each host species could be differently affected by a pathogen strain, with different levels of mortality, recovery, and transmission, so the contribution of each host to total pathogen reproductive success needed to be considered. The contribution of environmental transmission from one host to total pathogen reproductive success has been demonstrated by adding environmental transmission to direct transmission, incorporating the infection threshold level, propagule persistence, and shedding of spores (Rohani et al. 2009, Breban et al. 2010). In a multi-host system, each host species can shed different numbers of infectious propagules, and can have different levels of spore uptake based on their susceptibility. This affects the pathogen's reproductive number. Therefore, the contribution of each host must be included discretely and then added together. Here, the host specific uptake of spores, pathogen persistence and shedding rate were included through the sum of the two species' parameters in the last two terms of Eq. 6.5. This represented the additional contribution of environmental transmission to pathogen reproductive success from each host species, incorporating the propagule longevity of the pathogen as well as the uptake rate in each host species. More resistant host communities are predicted to have lower R_0 values.

The basic reproductive number does not incorporate immune or recovered individuals in the system i.e. the entire population is considered susceptible. This is useful in short-term epidemiological studies of pathogen invasion in a novel habitat. From an evolutionary point of view, the effective reproductive number, $\hat{R}_0^{\,i}$, which is the pathogen's reproductive number in a population where strain *i* is at endemic equilibrium, examines the long-term invasion success of a mutant strain strategy (Bettencourt and Ribeiro 2008). It is calculated by replacing N1 and N2 in Eq. 6.5 with S1 and S2, respectively. A pathogen with $\hat{R}_0^{\,i} > 1$, whatever *i* is, will outcompete all other strategies and will therefore be an ESS. Conversely, if $\hat{R}_0^{\,i} < 1$ for some pathogen strain *i*, this strategy can be competitively excluded by other

strategies, and thus will be not a global ESS. If $\hat{R}_0^{\,i} > 1$ when the current strategy is in the vicinity of *i*, but $\hat{R}_0^{\,i} < 1$ for very different strategies, then *i* can be a local ESS.

Depending on the pathogen's life history and the environmental conditions, several ESSs could exist (Roche et al. 2011). A mutant pathogen strain could invade a resident strain at equilibrium if the mutant had a $R_0 > 1$. Over time, pathogen strains in the population should evolve to the fitness maxima.

6.2.3 Model assumptions

Before examining the evolutionary success of different pathogen life history strategies, it was necessary to establish trade-offs between epidemiological parameters, as parasite traits are correlated with each other in ways that can constrain pathogen evolution (Gandon 2004, Osnas and Dobson 2010). This was performed in Chapter 3 for an *S. destruens* pathogen strain which was kept consistent throughout Chapters 4 and 5, but here several assumptions were relaxed and pathogen evolution was explored across multiple life history strategies. Relationships were thus hypothesised for direct transmission (β), recovery rate (γ), spore release (ϕ), and the persistence of spores in the environment (μ) against virulence (α), similarly to the relationships described in Roche et al. (2011).

Low susceptibility species were assumed to have low direct transmission levels, with transmission levels increasing as susceptibility to the pathogen increased (Eq. 6.6, Fig 6.2a). The single host models developed in Chapter 2 also revealed a linear correlation between the duration of infection (or recovery rate γ) and virulence α (see Chapter 3 for a detailed discussion) (Eq. 6.7, Fig 6.2b). Over a short time scale, spore longevity in *S. destruens* is driven by temperature, but here this factor was excluded as the model focused on long-term evolutionary changes. It was assumed that higher numbers of propagules were released as virulence increased, until a maximum number per day was released in heavily infected individuals (Eq. 6.8, Fig 6.2c). Furthermore, a trade-off was predicted to exist between propagule production and

persistence (De Paepe and Taddei 2006, Roche et al. 2011). The trade-off between spore 'quality' and 'quantity' led to the assumption that when low numbers of spores were released per day they would persist for longer compared to high numbers of spores released per day. These assumptions allowed the existence of two environmental transmission strategies by the pathogen. As the rate of environmental uptake is reliant on propagule production and persistence, it is predicted that there is a higher uptake level for a pathogen with many, short-lived propagules or conversely for a pathogen with few, long-lived propagules (Fig. 6.3) Thus, spore lifespan (1/μ) was limited by virulence (Eq. 6.9, Fig 6.2d). The use of a Gompertz function in the propagule production trade-off yielded a curve with a slow rate of growth at the beginning and end with a steeper gradient at intermediate levels.

$$\beta(\alpha) = a(1 - e^{-b\alpha}) \quad \text{Eq. 6.6}$$

$$\gamma(\alpha) = c\alpha + d \quad \text{Eq. 6.7}$$

$$\phi(\alpha) = g + he^{-le^{-p\alpha}} \quad \text{Eq. 6.8}$$

$$\mu(\alpha) = q_0 + q(1 - e^{-u\alpha}) \quad \text{Eq. 6.9}$$

Where *a, b, c, d, g, h, l, p, q*, and *u* are constants, calculated to be as similar as possible to the results obtained from the experimental data. In combination, these trade-offs represented a pathogen that could utilise both direct and environmental transmission, with the costs of each transmission pathway incorporated. This allowed the exploration of multiple transmission strategies for the pathogen.

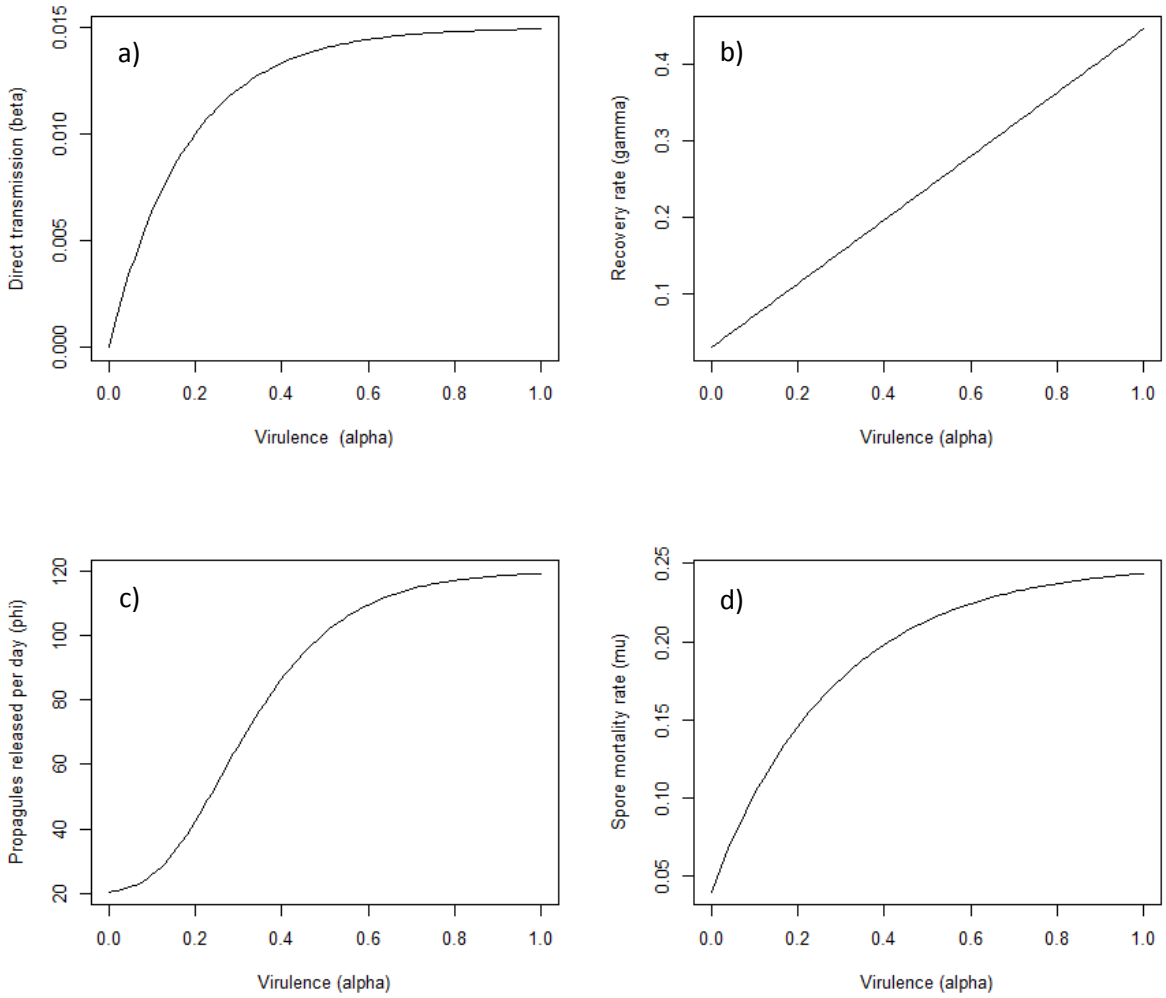

Figure 6.2. The trade-offs between a) direct transmission; b) recovery rate; c) propagule release rate; and d) spore mortality in the SIR system and the pathogen's virulence (α), calibrated with *Sphaerothecum destruens* data. Trade-off values for each parameter:

$\beta(\alpha) = 0.45(1 - e^{-5.5\alpha})$; $\gamma(\alpha) = 0.497\alpha + 0.026$; $\phi(\alpha) = 20 + 100e^{-5.5e^{-6.5\alpha}}$; $\mu(\alpha) = 0.04 + 0.21(1 - e^{-3.5\alpha})$.

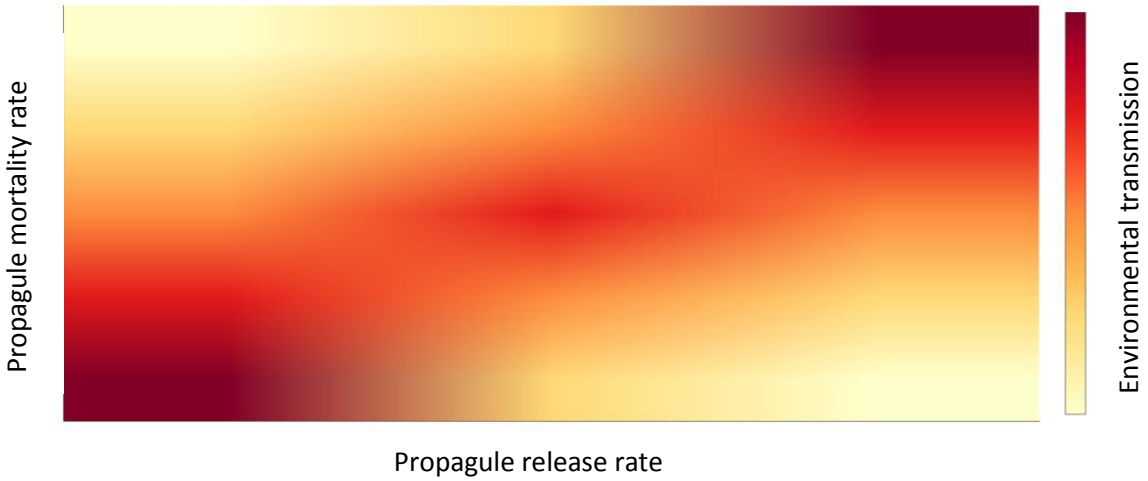

Figure 6.3. It was predicted that environmental transmission would be highest (red) for pathogens with few, long-lived propagules or many, short-lived propagules, due higher propagule persistence in these conditions. This assumption was based on the trade-off described by De Paepe and Taddei (2006).

6.2.4 Hypotheses testing

The multi-host SIR system developed in Section 6.2.2 was used to explore: (1) the epidemiological impact of pathogen virulence in different communities; and (2) the evolutionary success of pathogens under different ecological constraints (direct and environmental contact rates).

Specifically, the effects of community susceptibility and virulence on pathogen reproductive success were examined. The trade-offs in Section 6.2.3 were incorporated into a two-host SIR system where pathogen virulence in Species 1 was set as α (which was run for a range of values from 0.01-1). The evolutionary virulence of the pathogen was the value of α, and that was reflected in the mortality from infection in Species 1. However, as with many generalists, virulence can vary across host species due to a variety of factors such as host response, and different host species can experience higher levels of mortality from the same pathogen. Here, the impact of differential host response on pathogen evolutionary virulence was explored by including an additional host which experienced a higher level of mortality from a pathogen with virulence α. Thus, while the evolutionary virulence of the pathogen was α, Species 2 experienced 25% higher mortality from infection than Species 1 (α *1.25). The total population density of the two-host community

was kept constant, but the ratio of hosts from Species 1 to Species 2 was varied, changing the average level of susceptibility (mortality from infection) within the community from low to high, and thus testing the effects of community susceptibility on pathogen evolution. The pathogen was introduced to the SIR system and the epidemiological R_0 was calculated, considering the relative contribution of direct and environmental transmission to pathogen $\hat{R}_0$. In doing so, it was possible to explore the influence of virulence on the pathogen's reproductive success in different community structures.

Until this point, the ecological parameters (direct contact rate and environmental contact rate) were kept constant. However, changes in ecological conditions can alter pathogen transmission strategies and influence virulence. In order to characterise a pathogen's ES virulence in different transmission pathways, three pathogen transmission strategies were modelled in communities with a range of direct contact rates, using adaptive dynamics techniques. The three transmission strategies were designed so that environmental uptake rate was dependent on propagule persistence, and direct contact rate between hosts was varied from very low (0.0001) to intermediate (0.2) and finally to high (0.8). Environmental uptake rate was kept at an intermediate level. Mutant pathogen strains were introduced to equilibrium populations and their invasion success was examined through pair-wise invasion plots. Following this, a range of direct contact rates (from 0 – 0.8) and environmental uptake rates (from 10^{-7}-10^{-2}) were simultaneously varied in the two host system, to further explore how ecological parameters can influence ES virulence in different communities.

6.3 Results

Community susceptibility and pathogen virulence effects on R_0

When direct and environmental contact rates were kept constant, pathogen R_0 was greater for high and low susceptibility communities, with some communities displaying two maxima in R_0 at low and high virulence (Fig. 6.4a). The relative contribution of each mode of transmission was examined to determine these peaks

more clearly. Direct transmission had the highest R_0 values at low pathogen virulence (α = 0.12), in both low and high susceptibility communities, while remaining comparatively low at medium susceptibility (Fig. 6.4b). The contribution of environmental transmission to pathogen success was highest for pathogens at very low and at high virulence (α = 0.04 and 0.56) with the highest overall levels in highly susceptible communities (Fig. 6.4c). These findings demonstrated that there were various possible fitness peaks for the pathogen, which would be dependent on the transmission mode adopted by the pathogen strain.

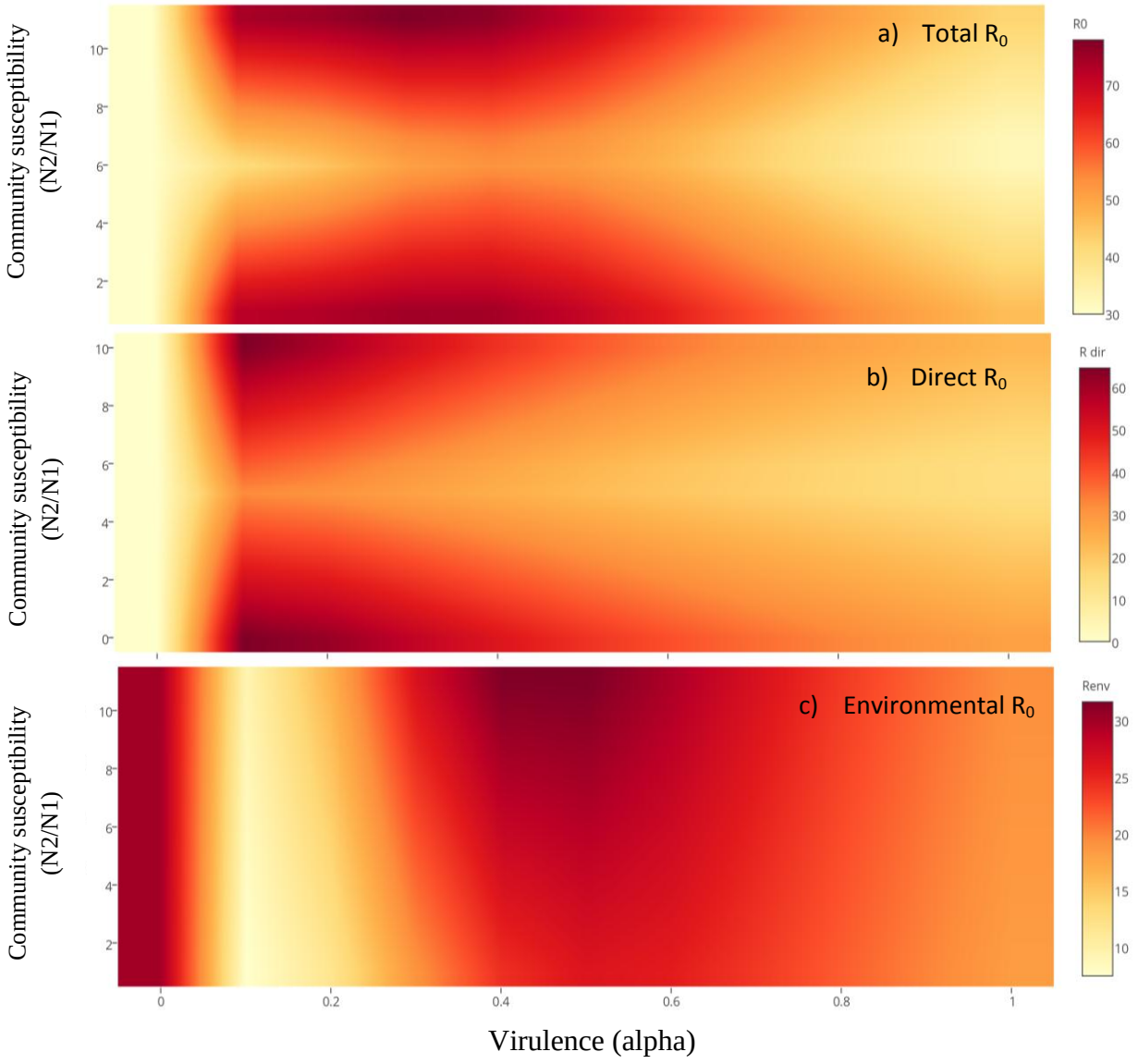

Figure 6.4. a) The basic reproductive number (R_0) of a pathogen strain across a range of community susceptibility and pathogen virulence (α). For some communities, two peaks in R_0 can be observed at different levels of pathogen virulence; b) The contribution of direct transmission to pathogen R_0 across a range of community susceptibility and virulence demonstrated that direct transmission was highest at low to intermediate pathogen virulence;

c) The contribution of environmental transmission to pathogen R_0 across a range of community susceptibility and virulence demonstrated that environmental transmission was highest at low and high pathogen virulence, as predicted by the trade-offs.

The evolutionary stable virulence of different pathogen transmission strategies

The invasion exponent of mutant strains was calculated for different pathogen transmission strategies varying direct contact rate (see Methods). Pathogens in communities with high direct contact rates had a low ES virulence of 0.12 (Fig. 6.5a). Mutant strains introduced in this population would evolve to this level of virulence as it was the most successful (i.e. maximal R_0), and was also convergence stable. Conversely, when direct contact rates were very low, there were two virulence strategies that yielded maximal pathogen success (α = 0.53, 0.1). This indicated that a mutant strain introduced in this population could evolve to be more virulent or less virulent to reach an ESS. For mutants introduced at α = 0.001 - 0.4, invading strains both above and below the resident strain were simultaneously possible (Fig. 6.5b). Thus, this strategy yielded two convergence stable strategies, but they were not evolutionary stable, indicating that evolutionary branching or bi-stability could occur. When the rate of direct contact was intermediate (χ = 0.2), the maximal pathogen success was at a higher virulence of α = 0.37, yielding a convergence and evolutionary stable strategy (Fig. 6.5c).

The role of ecological parameters in pathogen transmission strategy and success

The values for environmental uptake rate (ρ) and direct contact rate (χ) were then simultaneously varied in low, medium and high susceptibility communities to determine their combined effects on ES virulence and the resulting number of possible stable strategies (Fig. 6.6). At low levels of direct and environmental contact rates, no ESSs emerged for the pathogen across all communities, as all pathogen reproductive numbers were <1. As the environmental uptake rate increased, two ESSs emerged (α = 0.1, 0.6) in all communities, as demonstrated in Fig. 6.4. At intermediate direct contact rate and low environmental uptake rate, one ESS was observed at low virulence (α = 0.2) in both high and low susceptibility communities. For medium susceptibility communities, this ES increased to 0.3 and then declined to 0.2 at higher direct contact rates. Overall, for intermediate levels of direct and

environmental contact higher virulence ESSs of α = 0.5 and 0.4 emerged. When environmental uptake rates were high, the pathogen demonstrated evolutionary bi-stability in all communities, across all direct contact rates. Thus, there were four general evolutionary outcomes observed: no ESSs, an ESS at intermediate virulence, an ESS at higher virulence, and a bi-stable strategy with high and low virulence.

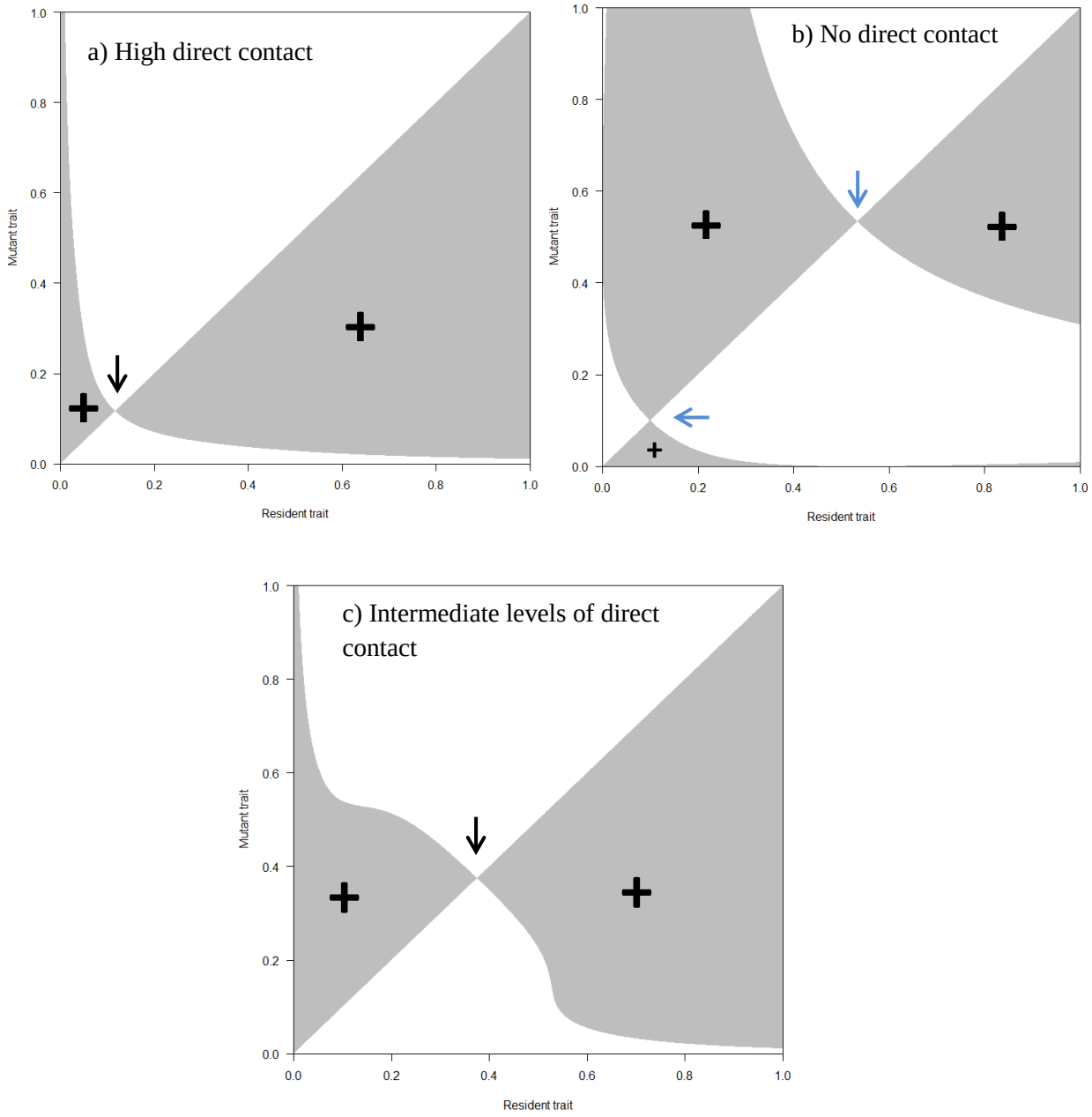

Figure 6.5. Pairwise invasion plots indicating where a mutant strain could invade at equilibrium for: a) A pathogen with high direct contact rates (χ = 0.8); b) A pathogen with low direct contact rates (χ = 0.0001); and c) A pathogen with intermediate direct contact rate (χ = 0.2). Areas in grey indicate where a mutant strain could invade. Black arrows indicated ESSs of the pathogen, and blue arrows indicated convergence stable strategies. For pathogens with very low direct contact rates, two strategies can co-exist (b).

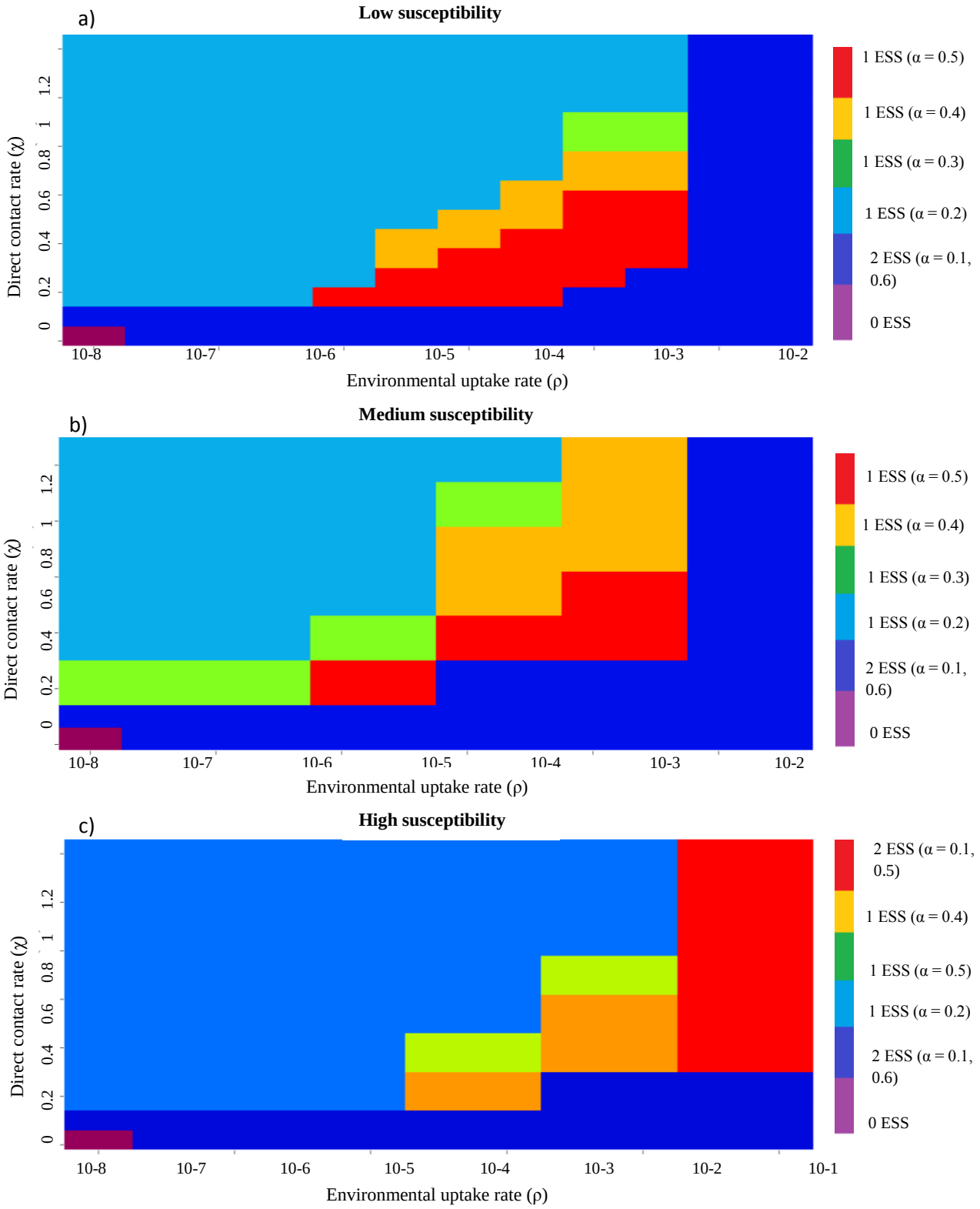

Figure 6.6. A summary of the effects of environmental uptake rate (ρ) and direct contact rate (χ) on the evolutionary stable virulence strategy (α) of a pathogen, and how many strategies were possible. This was examined for a) low, b) medium, and c) high susceptibility communities. Four outcomes are possible: no ESS at low contact rates, 1 ESS at low virulence, 1 ESS at high virulence, and 2 ESSs possibly co-existing at high and low virulence.

For conditions where bi-stability was predicted, multiple pathogen strains were introduced into a two host system, and pathogen prevalence was observed in both hosts over time to determine if a particular strain became specialised in either host (Fig. 6.7). As predicted in Fig. 6.5, a low virulence and a high virulence strain persisted in both hosts and the environment. There was no significant difference between pathogen prevalence in each host species, with the low virulence strain persisting at higher levels in both hosts.

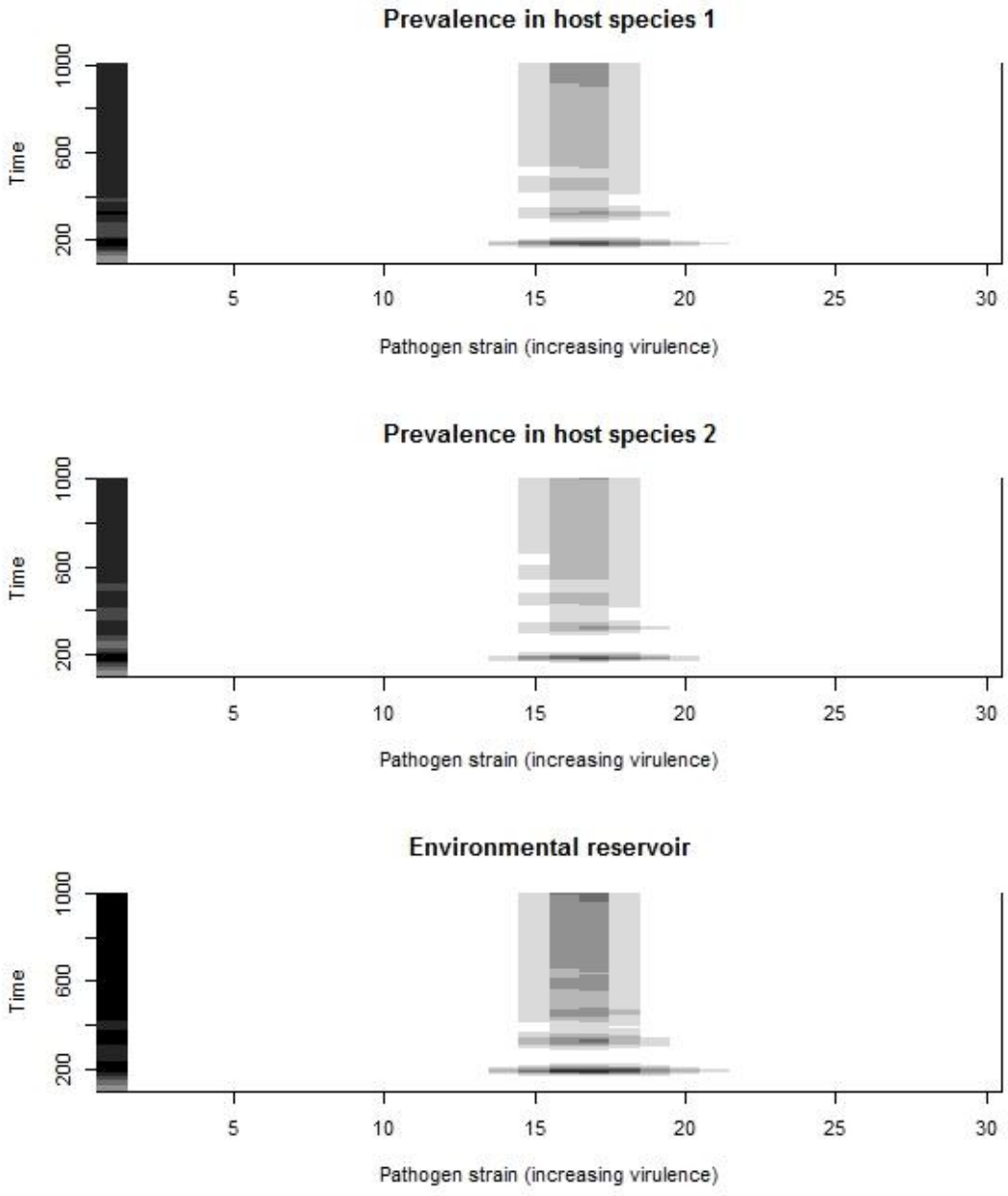

Figure 6.7. The prevalence of different pathogen strains in two host species and in the environment over time (darker colours signify higher prevalence). Pathogen strains were introduced into the two host SIR system at a range of virulence levels (0-1) in conditions where evolutionary bi-stability could emerge, and monitored until equilibrium. A low virulence and a high virulence strain persisted in both hosts (a & b), and in the environmental reservoir (c) as predicted.

6.4 Discussion

In conditions with high levels of direct contact between hosts, pathogen virulence remained relatively low, overriding the influence of environmental uptake on pathogen virulence except at high levels of environmental uptake. This was contrary to the prediction that high direct contact would lead to high pathogen virulence. However, higher virulence did evolve at intermediate levels of direct and environmental contact. When the direct contact rate was low relative to environmental uptake, there were two possible pathogen ESSs: one at low virulence and one at high virulence. Evolutionary branching into high and low virulence strains has been observed in other pathogen systems, such as avian influenza virus (Mutinelli et al. 2009, Rohani et al. 2009), *F. columnare* (Decostere et al. 1999), and cholera (King et al. 2008). These results demonstrated that community structure dictates the evolution of pathogen virulence (Dieckmann et al. 2005), in terms of host composition and contact rates between species.

For both direct and environmental transmission pathways, the trade-offs between propagule persistence, host recovery and direct transmission created optimal virulence strategies where pathogen reproductive number peaked; environmental transmission was highest at low and high pathogen virulence, while direct transmission was highest at low pathogen virulence. Higher community susceptibility led to higher pathogen reproductive success (R_0) given constant environmental parameters, although the ESS of the pathogen's virulence remained consistent across communities. Nonetheless, it has been demonstrated that increases in R_0 can lead to subsequent changes in pathogen virulence (Antia et al. 2003). As shown by previous studies, transmission pathway was the strongest determinant of pathogen virulence (Roche et al. 2011, Martins et al. 2013). Manipulating the direct and environmental contact rates for the pathogen led to several potential ESSs for the pathogen, including low and high virulence, as well as evolutionary bi-stability.

The impact of direct contact rate on pathogen virulence has significant implications for the management of *S. destruens* and similar pathogens. When a generalist

pathogen is introduced into an area with naïve hosts, higher virulence is predicted as host densities and thus contact rates are high, and host defences are likely undeveloped (Daszak et al. 1999, Hawley et al. 2013). This is true for infections introduced into naïve wild habitats and also for fish farms (Pulkkinen et al. 2010). In the wild, susceptible populations would become depleted, potentially reducing direct contact rate, while environmental uptake could remain at relatively high levels (depending on the habitat). In this instance, evolutionary bi-stability of the pathogen into two strains is possible. In farmed conditions, depleted populations are often restocked with new (potentially susceptible) hosts, keeping direct contact rates and pathogen reproductive numbers elevated. In this case, while pathogen reproductive numbers would remain high, adding more susceptible hosts to the population (and thus keeping direct contact rates high) could keep pathogen ES virulence down (Mackinnon and Read 2004), which was demonstrated in the current model. However, if the host population is continuously supplemented high-density populations can actually lead to increases in pathogen virulence, as pathogen transmission is no longer constrained by host mortality (Phillips and Puschendorf 2013). As shown in this work, if direct contact rates are at an intermediate level, ES virulence could increase. For these reasons, virulence management remains debated in the epidemiological community, as unknown factors can influence the virulence of the pathogen (van Baalen 2002, Alizon et al. 2009).

In the case of *S. destruens*, the release of infectious propagules from infected hosts guarantees that there is an environmental component to pathogen transmission, so an evolutionary bi-stability event is possible in conditions where direct contact between hosts is low. Water-borne transmission is a key route for many emerging pathogens (including the chytrid fungus *B. dendrobatidis*) and understanding the role of the environmental infection pathway is crucial for predicting future cases of emergence (Johnson and Speare 2003, Karanis et al. 2007, Mian et al. 2009). The lifespan of *S. destruens* spores declines with temperature (Andreou et al. 2009), suggesting that few, long-lived propagules at low temperatures would facilitate higher environmental uptake levels in colder months, as well as increase the risk of disease spread downstream. This would be further exacerbated by any flooding that may occur due to precipitation. A recent study in Turkey found higher prevalence of *S. destruens*

infection in colder months (Ercan et al. 2015), supporting this hypothesis. It is thus expected that evolutionary bi-stability of *S. destruens* virulence is more likely in colder habitats.

In environments where hosts are sparse or widely distributed (e.g. a river or large lake), pathogens may evolve greater propagule longevity in order to come in contact with naïve hosts, which has been shown to lead to higher virulence (Boots and Sasaki 1999). Long-lived propagules of environmentally transmitted pathogens have been shown to cause cyclical mortality patterns in host populations (McCallum 2001) or even host extinction (Mitchell et al. 2008). Furthermore, pathogen persistence in the environment is a major contributing factor to pathogen genetic diversity which can lead to unpredictable dynamics in wild populations (Roche et al. 2014). These conclusions are highly relevant for *S. destruens*, as environmental propagules could lead to new infections as they travel downstream and encounter naïve hosts, or to the re-emergence of infection when the susceptible host population has recovered over the infection threshold.

This work did not include the co-evolution of host defences, which would play a large role in pathogen evolution. Host susceptibility can affect the transmission of the pathogen, as observed in this work, as well as the host response to infection (Best et al. 2009, Boots et al. 2012). This in turn can alter how the pathogen evolves, for example higher host immune responses can lead to more virulent pathogens (Sasaki and Godfray 1999, Gandon and Michalakis 2000, Mackinnon and Read 2004). Furthermore, the presence of multiple infections can influence both host and pathogen evolution (Rigaud et al. 2010), via mechanisms such as competition between pathogens and the activation of the host immune response (Pedersen and Fenton 2007). This model also excluded the latent stage of infection, as its importance has already been demonstrated in other systems with relation to pathogen virulence (Ebert 1994, Day 2002a, Osnas and Dobson 2010). These studies have demonstrated that the timing of pathogen transmission can be a critical component of optimal virulence, with longer latent periods leading to higher ES virulence (Osnas and Dobson 2010).

The current model incorporated multiple modes of transmission, multiple hosts, and the trade-off between pathogen production and persistence, which has been overlooked in many studies (De Paepe and Taddei 2006). Overall, the results support previous findings that pathogen transmission pathways are significant determinants of virulence, with implications for pathogen management. The environmental component of *S. destruens* transmission is predicted to be a significant contributor to its virulence in wild habitats, such that a non-virulent and a virulent strain could evolve. In constantly changing freshwater environments with varied species composition, *S. destruens* is predicted to remain a generalist, with lower virulence in high-density habitats and potentially bi-stable strains in low-density habitats. In contrast, high-density, homogeneous populations like those found in fish farms could lead to the specialisation of *S. destruens* strains on particular hosts. In this instance, virulence could be predicted to increase due to the continuous restocking of hosts, as observed in other systems (Pulkkinen et al. 2013). Although virulence management requires a more in-depth examination into the effect of all possible contributing factors to virulence within each host community, this work has provided a framework that describes the important elements in generalist pathogen spread via multiple modes of transmission.

Chapter 7
Discussion and Conclusion

Summary

The major conclusions of each chapter are reviewed and placed in a wider context of current ecological and epidemiological modelling, disease management protocols, and in the study of host-pathogen evolution. Firstly, the models are critically analysed with a discussion of current models of similar pathogens, with the work highlighting the advantages and disadvantages of different experimental methods with regards to model parameterisation. It emphasized gaps in epidemiological knowledge on fungal pathogens, and suggests further experimental work to determine infection loads and to quantify infection threshold doses more precisely. Secondly, the multi-host models allowed the determination of several biotic and abiotic drivers of disease emergence and persistence, and the results are used to formulate a risk analysis framework for *S. destruens*. Different management strategies are discussed based on predictions from the multi-host and evolutionary models. Finally, the implications of this work are discussed for knowledge about generalist fungal pathogens, and future directions of how these models can be used on a wider scale to inform epidemiologists and policy makers about the risks of pathogen emergence.

7.1 Summary of results

Chapter 2: The development of a single host SEIR model for Sphaerothecum destruens

Single host epidemiological models were created for four cyprinid species (*A. brama, R. rutilus, C. carpio,* and *L. delineatus*) and one salmonid species (*S. salar*) infected with *S. destruens*, and were parameterised using empirical data. These models formed the basis of the SEIR system in this thesis, and were unique because they adopted saturation functions for multiple parameters (transmission, mortality, incubation, and recovery) compared to traditional models which only use saturation functions for environmental transmission. Sensitivity analyses revealed that this inclusion was necessary to fit the models to all available experimental datasets accurately. Thus, parameter values for five susceptible species were established, and formed a novel SEIR model for this pathogen system.

Chapter 3: Epidemiological parameter estimation

An additional experimental dataset was parameterised in a two-host model, expanding the baseline of data to include the healthy carrier (*P. parva*). The range of uncertainty for each parameter was established across all models, establishing the precision of the parameter values. Relationships and trade-offs between parameters were hypothesised and critically tested using the results from the single host parameter ranges across all species. These relationships were used to build multi-host models in Chapters 4 and 5 that could be used for any species assemblage, and furthermore calibrated parameter trade-offs in the evolutionary model in Chapter 6. This chapter was crucial for the formulation of multi-host models, and quantifying parameter relationships.

Chapter 4: Factors associated with generalist pathogen establishment in animal communities, and the consequences of host eradication on disease epidemiology

A multi-host SEIR model was created to examine several biotic and abiotic factors affecting pathogen establishment, and tested eradication as a management protocol for mitigating the effects of an introduced pathogen. Once introduced, the pathogen was established in communities within a year following its introduction, and the timing of eradication did not prevent disease emergence. Populations with low background mortality (i.e. healthy host communities) recovered to sustainable levels, but susceptible populations with high background mortalities were extirpated after pathogen introduction. The timing of epidemics was determined by the level of spores in the environment, demonstrating that environmental transmission is a critical pathway for *S. destruens* establishment in new communities. High-density populations maintained higher levels of diversity over time after pathogen introduction, compared to low-density populations. Although healthy host communities could recover, they maintained the infection at a low prevalence in the environment and in susceptible hosts, indicating that disease re-emergence could occur in the future under different conditions (e.g. an increase in host population density, or the introduction of a new susceptible host). The results highlighted an important gap in disease management strategies, as eradicating the initial infective population did not prevent pathogen dispersal or persistence. Thus, viable

management approaches need to better address pathogen environmental transmission pathways.

Chapter 5: The influence of host competitive interactions on community resilience-implications for disease control

A further level of complexity was added to the multi-host model by incorporating resource competition between species and trophic levels to include predation. Models included both an SEIR simulation model and a Bayesian belief network, which were independently used to determine the role of ecological interactions in community resilience against infection. Combining these two methods strengthened the overall conclusions derived from the models and provided a more complete framework of studying disease dynamics. The role of infection in species at high trophic levels was examined in detail by including two secondary consumer species in the community assemblage and varying their incubation rate, recovery rate, and mortality from infection. The model indicated that resistant species at high trophic levels could mitigate the effects of the introduced host but had limited effects on the introduced pathogen. High predation pressure on the introduced species was predicted to prevent its establishment as well as the pathogen's, but had severe consequences for the remainder of the resident community.

Chapter 6: Exploring the evolutionary dynamics of pathogens with multiple modes of transmission

A generalised model of pathogen transmission in a multi-host SIR system was created to examine pathogen evolution. Multiple trade-offs between parameters (direct transmission, spore persistence, recovery rate, and virulence) were established based on the results from Chapter 3 and epidemiological literature, and bi-modal transmission was modelled across different levels of virulence. Pathogen reproductive success was measured in a two host system using a model of pathogen reproductive number, testing the effects of community susceptibility and transmission pathway. Community susceptibility was shown to be a factor in determining the reproductive success of the pathogen. Using adaptive dynamics, the

potential long-term evolution of evolutionary stable strategies for *S. destruens* was explored given various transmission strategies. *S. destruens* strains in hosts with high levels of direct contact were found to evolve to lower virulence, while pathogens with low direct contact could potentially develop evolutionary bi-stability with a low virulence and a high virulence strain.

7.2 Placing the research in the context of epidemiology

Data from different sources were used in parallel to create a unified epidemiological model that could be used for any species assemblage. The incorporation of multiple datasets which used different infection methods was a novel approach to creating generalised epidemiological models, as it determined the most appropriate model design based on data availability (i.e. using multiple saturation functions). Model calibration with empirical data strengthened the reliability of the outputs and provided an opportunity to quantify relationships between epidemiological parameters such as mortality, incubation and recovery rates. The models also helped determine remaining gaps in knowledge for fungal pathogens, including the exact threshold dose for infection, the susceptibilities of hosts in natural environments, and the difference in susceptibility across host lifespan. Identifying these gaps has helped inform future experimental setups. Here, the limitations of this approach were also considered.

The combined datasets represented three methods of infection (bath immersion, intra-peritoneal injection, and natural release of spores from the healthy carrier), which are commonly used in infection experiments (Murray et al. 1992, Harmache et al. 2006, Locke et al. 2010). These experiments test host susceptibility and thus host systems are often immersed in or injected with high doses of a pathogen. While this high infection pressure ensures a maximum number of initial infections (Bowden et al. 2002), it does not necessarily represent the spread of the infection in more natural systems, where the pathogen would unlikely exist at such high concentrations. For example, in rivers and lakes, fish hosts live in larger systems (compared to aquaria) with lower contact rates, whereas in farmed conditions the habitat could be more

similar to the experimental conditions (i.e. a closed system with high-density populations). This high initial dose leads to some uncertainty and more importantly bias in the model results, which is likely to be a common issue for models based on infectivity experiments.

In single host experiments, hosts were exposed initially to high levels of the pathogen and then left to recover, whereas in the cohabitation experiment susceptible hosts were continuously exposed to low levels of the pathogen for an extended period, potentially facilitating a higher number of infections overall. In a study of the amphibian chytrid fungus in boreal toads *Bufo boreas*, increasing the duration of exposure to the pathogen led to an increase in mortality (Carey et al. 2006). The chronic decline observed in the cohabitation experiment compared with the single host experiments also supported the prediction made in Chapter 2 that each stage of infection required reaching a spore threshold until either mortality or recovery. Furthermore, the results indicated that pathogen load determined the time to mortality, highlighting the role of infectious propagules in determining the pace of the infection.

As the parameterisation of models was based on a set of mortality values in relatively short-term experimental settings, the use of these models over longer time periods may lead to biased results. Stochastic influences in the environment (which were not incorporated) and evolution of the pathogen and hosts would play a role in determining the outcome of infection in a community (Galvani 2003, Morgan et al. 2006). As the conditions of the community changed (e.g. temperature, resource availability, contact rate, etc.), the conditions in the model would also change, affecting the predicted outcomes of the model. This is a limitation of all models and predictive capability will vary across time if the model framework is not dynamic enough, especially for natural ecosystems (Fielding and Bell 1997). To create an accurate long-term model that included all potential influencing factors, information on the system needs to be consistently gathered over time and inputted back into the model for re-calibration. Even then, stochastic and seasonal influences could alter the model outcomes (Greenman et al. 2004, Bolzoni et al. 2008). For this reason, long-

term predictions of models (especially when conditions can change rapidly) must be treated with caution. Nonetheless, the community approach used in the models provided valuable insights into the specific questions that were put forth in the thesis. It demonstrated how community interactions like predation and resource competition could limit pathogen transmission and host mortality. Furthermore, host community susceptibility affected pathogen reproductive number, and ecological parameters like contact rates between hosts and the environment were shown to be crucial factors in determining pathogen virulence.

Some epidemiological parameters were constricted more than others based on the method of infection and species susceptibility. For example, *A. brama* had significantly higher uncertainty in parameter values compared to other species under the same experimental conditions and was therefore excluded from the multi-host models. This was likely an additive effect of the bath immersion initial conditions and their high susceptibility to *S. destruens*; unlike *L. delineatus* (another highly susceptible host), there were no additional datasets to recalibrate the model parameters more precisely. The incorporation of three datasets for *L. delineatus* demonstrated how combining multiple datasets calibrated the model more effectively and precisely overall. In terms of model calibration, the most accurate infection method was the cohabitation experiment, as susceptible hosts were infected naturally through shedding of spores. However, if the other datasets were not available this would have inhibited parameterisation as there were multiple species in the system. The models utilised experimental data gathered for various objectives which did not include quantifying transmission parameters. The experimental trials focussed on determining host susceptibility. However, the use of this data for the models emphasised the flexibility of the models in using such existing data, and demonstrated that reliable models could be formulated using data collected for different objectives. The conclusions from the models also provide a platform to create an experimental design in which model parameters could be determined with a higher level of precision. A potential infection method that could aid in model parameterisation would be to inject one host with the pathogen, place it with uninfected hosts, and then observe the time taken for signs of infection to appear in

other hosts (Fig 7.1). This experimental design would allow the maximum number of parameters to be measured via a single experiment.

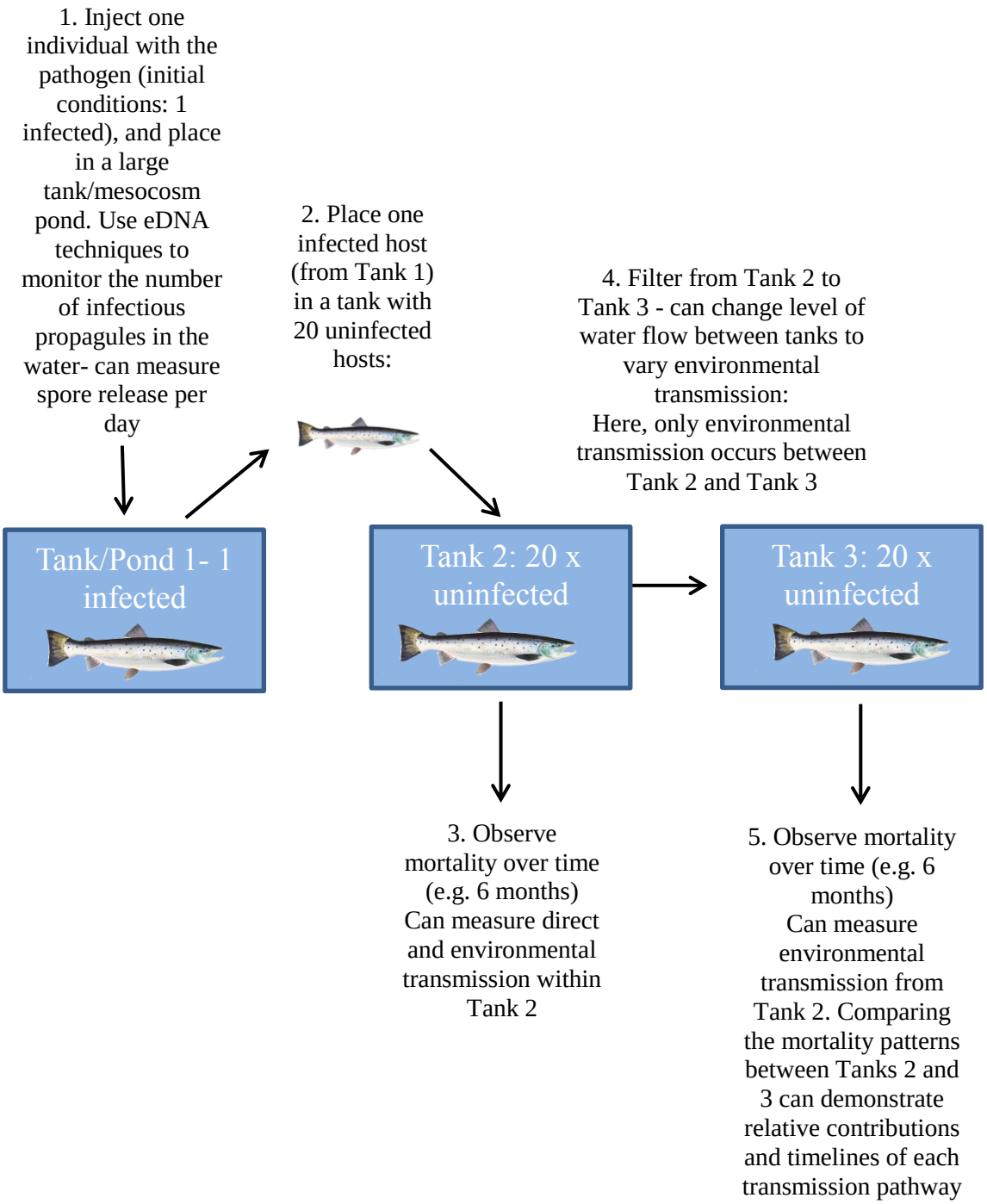

Figure 7.1. Experimental design which allows more precise model parameterisation of spore release and transmission. Image credit: http://nw08.american.edu/~vconn/seafood/images/salmon.jpg.

Beyond experimental design, several important questions about the epidemiology of fungal diseases were addressed in this thesis. There is evidence that both generalism and environmental survival increase pathogen persistence and facilitate easier disease transfer between populations (Codeco 2001, McMahon et al. 2013). One important conclusion from this work that supported this hypothesis related to the importance of bi-modal transmission for *S. destruens*, and how environmental transmission is crucial for pathogen expansion and establishment in new communities (Simberloff 2009, Tien and Earn 2010, Roche et al. 2011, Chapter 4). The environmental persistence of infectious propagules has been demonstrated in systems such as *Vibrio cholerae* (Codeco 2001) and *Cryptosporidium*, a protozoan infecting humans (King and Monis 2007). Here, it was shown to be critical for *S. destruens* outbreaks, indicating that pathogen environmental persistence should be an integral component in response protocols. These factors were previously uncharacterised in this system, and this knowledge will be useful for determining appropriate disease protocols in this and in similar systems.

There are remaining knowledge gaps pertaining to *S. destruens* specifically and fungal pathogens generally. Firstly, a more precise threshold dose for infection (K) has yet to be determined. Although the *L. delineatus* models gave an approximate range for K values, a more accurate quantification could help in determining the risk of an outbreak occurring as well as establish an epidemic timeline. Furthermore, work is needed on the susceptibility of hosts in natural environments. While the experimental work gives valuable insight into the dynamics of pathogen transmission, there are numerous additional factors that may contribute to pathogen transmission in the wild. Among them are the host contact rate and propagule persistence in different aquatic environments. These factors are critical determinants of whether an outbreak is predicted to occur and to what extent hosts may be affected. Also, many host species experience varying levels of susceptibility to infection throughout their lifetime (Sol et al. 2000). Understanding the risks of host infection and mortality at different life stages can inform the models more accurately, with the incorporation of population age structure into the framework - if one life stage is more or less resistant than another, the effects of an epidemic can vary

(Castillo-Chavez et al. 1989). These questions which have been highlighted by this work, have led to subsequent experiments in this system (Appendix 5).

Currently, epidemiological models are incorporating increasing ecological complexity into their frameworks in order to have outputs which are applicable in the real world. Competitive interactions such as predation were included in the models to test how species introductions can affect community resilience and response to infection. Other studies are examining similar aspects of ecology-driven epidemiology, by including competitive interactions and stochasticity in models over evolutionary timescales, leading to novel conclusions about pathogen evolution (e.g. Dorigatti et al. 2013, Farahpour et al. 2015, Haldar et al. 2015). Other work has examined how the introduction of new species into food webs affects the biodiversity and stability of a given community over long periods of time (Allhoff et al. 2015) which has applications in evolutionary epidemiological settings. Conversely, real-time modelling of the Ebola virus outbreak using current infection data was a critical component of predicting clusters of disease and minimising the risks and damage caused by the virus (Siettos et al. 2015, Webb et al. 2015). Thus, the inclusion of ecological factors and evolutionary outcomes, as well as the input of empirical data, are on the frontier of current models of epidemiology. Expanding disease dynamics beyond the interactions between host and parasite is the future of epidemiological modelling, and the use of empirical data in these interdisciplinary models is a key step. The models in this thesis have included several of these novel directions, creating highly relevant conclusions.

The most recent literature on *S. destruens* has uncovered a high prevalence of infection (up to 100%) in several additional susceptible hosts (Ercan et al. 2015), demonstrating its relevance as a model pathogen system and validating the predictions made by the models in this thesis. However, these elevated mortalities have not been observed in continental European habitats, despite the widespread presence of *P. parva*. This could be a result of low *S. destruens* prevalence in European *P. parva* populations (e.g. below 5-10%). Alternatively, the comparatively low temperatures across Europe compared to Turkey could facilitate the evolution of

S. destruens to produce few, long-lived propagules. This could increase the contribution of the environmental transmission pathway, which has two implications. Firstly, this pattern of transmission supports the chronic decline of host populations, which may be difficult to observe especially in the wild. Secondly, high environmental transmission can lead to the emergence of pathogen bi-stability, which means that an avirulent strain could persist in those populations. However, population declines have not yet been observed in farmed or angling populations either, where higher direct contact rates between hosts would be expected to cause high mortalities. Again, this could be due to a low prevalence of *S. destruens* in those populations. It could also be due to strict animal transport guidelines that limit the accidental introduction of the disease and healthy carrier. In habitats where *P. parva* introduction has occurred, for example in the UK, native species decline has been observed but subsequent eradication of the introduced species has limited the threat of infections (Britton et al. 2009). Environmental dispersal of the infectious propagules has not been monitored or reported, and may be limited by the attachment of spores to sediments or predation by zooplankton (Schmeller et al. 2013, Searle et al. 2013).

Despite some limitations in the certainty of the parameter values, the models provided important insights into the disease dynamics of *S. destruens* in multiple settings. Environmental transmission and propagule persistence were shown to be important drivers of disease emergence and pathogen evolution in this system. The ecological multi-host models were applicable to any species assemblage and allowed the observation of population trends under different conditions. In the final data chapter, the knowledge obtained from the datasets about parameter relationships was used to expand the ecological framework by adding an evolutionary dimension, with applications in multiple pathogen systems.

7.3 Disease management perspectives and the effects of temperature on disease emergence

The most important overall conclusion derived from the models was that disease emergence must be considered through a complete ecological perspective,

incorporating environmental conditions, competitive interactions between species, and host resistance to infection. The multi-host models established important risk factors associated with pathogen emergence and tested two disease management strategies, eradication and predation of the introduced healthy carrier. By establishing the influence of ecological and physical factors on epidemiology, the models could impact how management approaches are designed and inform risk analysis frameworks for emerging pathogens (Arthur et al. 2009, Akoll et al. 2012). Here, the risk factors for *S. destruens* emergence were discussed, including the roles of community composition and ecological conditions in relation to pathogen life history, followed by a critical analysis of the disease management techniques tested. The conclusions were combined to formulate a detailed qualitative risk analysis for *S. destruens*.

The introduction of a non-native species is a significant risk factor for disease emergence, and the invasion success of some introduced species and subsequent changes in their new geographic range can be due to their accompanying parasites (Garner et al. 2006, Gozlan et al. 2006, Hudson et al. 2006). Species introductions have facilitated outbreaks of disease worldwide, with pathogens like West Nile Virus, the amphibian chytrid fungus, and avian influenza being high-profile examples (Garner et al. 2006, Allan et al. 2009, Brito et al. 2012). This was demonstrated strongly in the multi-host models, as the introduced species caused disease outbreaks and population decline in the native species even at low (10%) initial infection prevalence. Given that *S. destruens* prevalence was shown to be significantly higher in several other *P. parva* populations (67-74% in the Netherlands and 25-100% in Turkey; Spikmans et al. 2013; Ercan et al. 2015, respectively), the results suggested high risks of disease outbreak in susceptible populations in the event of *P. parva* introduction.

A key risk factor was that host populations with high background mortality could not recover after a disease outbreak, with susceptible species becoming extinct within ten years of pathogen introduction. This emphasised that maintaining healthy ecosystems where potential hosts are not stressed (i.e. not overpopulated, with low infection

levels overall) is crucial for both biodiversity conservation and economic sustainability of fisheries (Barton and Iwama 1991, Costanza and Mageau 1999). Other driving factors determined through the community models included the population density of host species as well as species composition within a community. While high-density populations led to higher numbers of infected individuals, they maintained their initial levels of species richness for longer than low-density populations. However, as this work demonstrated, species susceptibility within the ecosystem must be taken into account; communities containing populations with more resistant species were predicted to maintain their structure for longer than susceptible communities. Furthermore, higher species diversity is predicted to lower the proportion of infected individuals in the community (Roche et al. 2012).

These results have several implications. In the case of artificial systems such as fish farms, population density is likely to be the most significant contributing factor to disease emergence, as potential hosts are kept in closed conditions, allowing the pathogen to cross the epidemic threshold. The high number of infections predicted in high-density populations suggests that fish farms would suffer severe consequences, as already shown in Turkey (Ercan et al. 2015). Furthermore, it is likely that there would be low species diversity in farms, even mono-culture in some cases, further exacerbating the effects of infection (Pulkkinen et al. 2010). In the wild, it is expected that shoaling species would experience higher levels of mortality compared to solitary species, as hosts would be closely grouped in large numbers, enabling both direct and environmental transmission. Solitary species or those at low densities would more likely demonstrate chronic decline from environmental transmission. However, the stochastic nature of wild communities can cause disease emergence to occur in some instances and not others, driven by the species composition and the susceptibility of the community, and importantly, by the environmental infection pressure experienced by the communities. Stochasticity has been shown to affect the outcome of disease outbreaks (Rasmussen et al. 2011), and is likely to have stronger effects in constantly changing unpredictable environments such as vulnerable freshwater habitats, e.g. those stressed by drought or pollution. However, the models developed here are still highly useful as they demonstrated several possible outcomes

given shifting parameters, although the model predictions are more reliable for stable environments, as per all models.

The persistence of free-living pathogen propagules was revealed as a crucial driver in fungal epidemics and disease re-emergence, and further emphasized the role of environmental transmission in pathogen expansion. As propagule life span is dependent on temperature as well as pathogen virulence, there is a seasonal component to *S. destruens* disease dynamics where the risk of environmental transmission is higher. This seasonality has been demonstrated in several fish populations in Turkey (Ercan et al. 2015). In the case of *S. destruens*, propagules survive longest at colder temperatures (Andreou et al. 2009), indicating that the risk of emergence from environmental transmission could be higher in winter months. Seasonal variations are also responsible for cyclical epidemics in other systems (Faruque et al. 2005, Koelle et al. 2005, Lello et al. 2008), for example in cholera outbreaks (Faruque et al. 2005, Morgan et al. 2006) and in fungal outbreaks in sea turtle species *Caretta caretta* (Sarmiento-Ramírez et al. 2014). Seasonality affects the risks of environmental transmission, but also species behaviour, birth rates, and geographic range can vary across the year (Greenman et al. 2004, Bolzoni et al. 2008). On a larger scale, environmental changes such as temperature increases due to climate change have been demonstrated to affect disease prevalence and progression (Dobson 2004, Rohr et al. 2011, Leung and Bates 2013). Temperature increases have caused more virulent infections of *F. columnare* in salmonid fish farms (Karvonen et al. 2010). For *S. destruens*, higher virulence could be expected in colder habitats, where propagules survive longer at low temperatures, a hypothesis which should be tested empirically.

Pathogen virulence can also be affected by host demography (Ebert et al. 1997, Choo et al. 2003). For example, reproductive hosts can be more susceptible to infection (Kortet et al. 2003), which could have long-term cascading effects on the community. Chytrid fungus transmission has been shown to increase during seasons of host reproduction (Lips et al. 2006), and reproductive *L. delineatus* have shown a higher prevalence of *S. destruens* infection than non-reproductive hosts (Andreou et

al. 2011). This could have substantial consequences for infected host populations, as a more virulent infection limits their ability to recover to sustainable levels. Cohabitation between *P. parva* and *L. delineatus* has also demonstrated an inhibition of spawning in the latter species, with further implications for conservation (Gozlan et al. 2005).

Eradication is a common way of eliminating non-native species (Myers et al. 2000), and the effects of this process on environmental disease transmission into adjacent animal communities were explored. The models demonstrated that eradication could remove the initial introduced host (i.e. the source of infection) and that a healthy ecosystem could recover from infection even after a three year delay between species introduction and eradication. However, in this particular system, unless eradication is guaranteed to remove all *P. parva* individuals, it can recover to high population levels (Davies and Britton 2015). In other scenarios, *P. parva* occupy the same habitat as the susceptible resident populations, and the eradication protocol would also remove smaller individuals of the resident species (Britton and Brazier 2006). Given the significant effects that eradication has on the entire ecosystem's population structure and density, this management practice is only considered a last resort.

When competitive interactions were added into the multi-host system, the models demonstrated that high predation pressure controlled the population of the introduced host and thus the initial source of the pathogen. It has been shown that in some cases, the lack of natural predators in the invaded community helped *P. parva* establish (Beyer et al. 2007, Britton et al. 2007). Increased predation as a form of biological resistance indicated a potential management strategy, such as using selective restocking of resistant secondary consumers in affected habitats. In a recent experiment, adding perch *Perca fluviatilis* to experimental ponds containing high densities of *P. parva* led to the invasive species population declining significantly due to predation and then remaining at low levels (Davies and Britton 2015). This was more effective than cropping the *P. parva* population, as in that instance the remaining individuals compensated for the decline in population by increasing their reproduction. In order to consider selective restocking as an appropriate disease

management protocol in this system as well as a biocontrol method, the susceptibility of *P. fluviatilis* to *S. destruens* must first be established, as it has shown sensitivity to a related pathogen in the Mesomycetozoea class (Pekkarinen and Lotman 2003). This is to avoid inadvertently increasing pathogen transmission through PITT (Lafferty 1999). There is recent evidence that PITT does occur in *S. destruens* hosts, as infection of European sea bass *Dicentrarchus labrax* in brackish water could have only occurred by predation of infected *P. parva* (Ercan et al. 2015). As the results in Chapter 5 demonstrated, predator susceptibility was predicted to alter the response of the community significantly, with more resistant hosts maintaining the host populations at sustainable sizes for a longer time compared to susceptible predators. However, once the infection became established in the community, high predation of the introduced host did not eliminate it.

There are possible repercussions that should be considered in each management case before undertaking a biocontrol approach (Louda et al. 2003). A significant concern is the occurrence of negative "top-down" effects, where the addition of secondary consumers to an ecosystem can either lead to higher species richness at high trophic levels, or to reduced numbers of native predators (Eby et al. 2006). This has significant and possibly irreversible impacts on the abundance of prey species (Symondson et al. 2002), creating a cascade effect that can change the entire community. This was observed in Chapter 5 as resident (non-target) prey species became extirpated at high predation levels. In the above example of using *P. fluviatilis* to manage *P. parva* populations, the generalist nature of *P. fluviatilis* predation can affect other species adversely if its population density is enhanced (Packer et al. 2003). Furthermore, there is evidence that in some systems higher predation threat is associated with host responses that increase their susceptibility to infection, leading to unforeseen effects on host populations (Packer et al. 2003, Duffy et al. 2011). Using natural competitive interactions as infectious disease management and biocontrol is a potential long-term management approach, but should be considered on a case by case basis, incorporating the ecological consequences of disease outbreak and closely monitored over time (Britton et al. 2011).

A successful method in decreasing chytrid fungus prevalence in affected populations is to use *Daphnia* sp. predation of spores to decrease spore levels in the environment, thus decreasing infection pressure on susceptible hosts (Schmeller et al. 2013, Searle et al. 2013). This method targets the pathogen rather than the affected hosts, and may be effective for *S. destruens*. However, it must first be established whether *S. destruens* survive predation by *Daphnia* sp., which are common in freshwater habitats. If spores survive predation this will reveal a new pathway of *S. destruens* infection, as susceptible hosts could prey on infected *Daphnia* and become infected themselves (an example of PITT). However, if spores do not survive, this could indicate a viable, cost-effective management approach that would help eliminate the infectious propagules (see Appendix 5).

Thus, the roles of population density, species composition, and temperature were inherently tested in the models, demonstrating that the geographic distance between host populations, natural host mortality, and competitive interactions between species are critical determinants of how a community responds to pathogen introduction. Information about the role of biotic and abiotic drivers of disease emergence has previously been used to create invaluable risk analysis frameworks for various pathogens, such as for managing *G. salaris* in *S. salar* (Peeler et al. 2007) and other aquatic pathogens (Murray and Peeler 2005; Bartley et al. 2006; Rödder et al. 2009; Poulin et al. 2011; Akoll et al. 2012). Risk analyses provide managers and policy makers with a contingency plan for all scenarios and steps to minimise risk factors in emergence. Furthermore, they emphasize remaining gaps in knowledge for different pathogens and set forth correct responses to disease emergence (Eisenberg et al. 2002). Here, a qualitative risk analysis for *S. destruens* was created, based on the Covello-Merkhofer (1993) model (Fig. 7.2). This framework can be applied to any UK aquatic habitat, and outlines the most important factors that must be considered to avoid outbreaks, as well as responses to outbreaks. While this framework was designed for *S. destruens*, it is applicable to aquatic pathogens with similar life histories and transmission pathways.

1. HAZARD IDENTIFICATION

- ✓ Restocking fish
  - Is disease present in transporting country/location?
  - Risk of *P. parva* presence?
  - If yes to either question, there is a significantly higher risk of pathogen introduction
- ✓ Existing *P. parva* populations
  - Wetter months have increased risk of disease spread (due to flooding)
  - Lower than 15°C water temperatures can lengthen propagule lifespan and thus transmission
- ✓ Reports of previous *S. destruens* outbreaks
  - Can occur even after eradication protocols
  - Area should be considered at risk for 3- 6 months

2. RISK ASSESSMENT

- ✓ Release assessment
  - Colonisation strategy
    - ✓ Direct transmission:
      - Population density (host contact rate)—higher density is associated with higher direct transmission.
        - High risk areas include fish farms or heavily stocked lakes. Shoaling species are more at risk than solitary species
      - Composition of hosts (susceptibility and competitive interactions)
      - Level of background mortality (general health of the population)
    - ✓ Environmental transmission
      - Temperature (seasonality- propagules survive longer in colder temperatures, but peak around 15 degrees C.
      - Habitat (e.g. flowing water increases risk of pathogen spread more than still water, but has a lower risk of disease emergence as propagules are less likely to accumulate to high levels)
    - ✓ Healthy carrier
    - ✓ Reservoirs of infection (environmental and host)
      - Change in the population density or conditions can cause pathogen re-emergence)
      - Host range in the UK: see Table 7.1
- ✓ Exposure assessment
  - Prevalence in habitats
    - Test regularly by sampling fish or water- e.g. qPCR method (Strand et al. 2014)
  - Transmission rates (established from models for each species)
- ✓ Consequence assessment
  - Fish mortalities (Table 7.1)
    - Consider the costs for aquaculture, angling, and conservation

3. RISK MANAGEMENT

- ✓ Ensure fish stocks do not have *P. parva* that can be transported to new locations
- ✓ Establish secure barriers between *P. parva* populations and other habitats
- ✓ If the pathogen is introduced, keep host populations low after outbreaks until disease is fully removed (confirmation through PCR)
- ✓ Test the water of exposed habitats for the pathogen every 6 months if the area is semi-natural/flowing, and every 2-3 months in aquaculture
- ✓ Regular health checks- can test the water without killing hosts to sample (every 3 months in high risk areas, yearly in low risk areas)
- ✓ Clean equipment used in any infected areas (e.g. nets)
- ✓ Report incidences of flooding near affected areas
- ✓ Potential management: enhance populations of resistant predatory species
- ✓ Potential management: add Daphnia to habitat to prey on environmental spores
- ✓ Potential management: remove species that have a high contribution to transmission (determined via models)- (Woolhouse et al. 1997, Fenton et al. 2015)

4. RISK COMMUNICATION

Who needs to be informed?

- ✓ Anglers
- ✓ Aquaculture and fisheries staff
- ✓ Notify Environment Agency or person in charge of the facility if positive samples are found

Figure 7.2. Key risk factors associated with the emergence of *Sphaerothecum destruens*, and the recommended management response.

Table 7.1. Known *Sphaerothecum destruens* hosts in the UK, with an indication of their importance for economic and conservation purposes and a note of whether they are solitary or shoaling species (an indication of the risk of outbreaks).

Species name	Aquaculture	Angling	Conservation status/function in the ecosystem	Shoaling
Leucaspius delineatus	No	No	Threatened in Europe, primary consumer	Yes
Salmo salar	Yes	Yes	Link between freshwater and marine ecosystems, secondary consumers	No
Abramis brama	No	Yes	Primary consumer	Yes
Rutilus rutilus	No	Yes	Primary consumer (control algal blooms)	Yes
Pimephales promelas	No	No	Primary consumer	Yes
Scardinius erythropthalmus	No	Yes	Primary consumer	Yes
Cyprinus carpio	Yes	Yes	Primary consumer	Yes
Oncorhynchus mykiss	Yes	Yes	Secondary consumers	Yes
Salmo trutta	Yes	Yes	Secondary consumers	Yes

7.4 Evolutionary perspectives

It is important to understand how an emerging pathogen can evolve in order to predict the potential consequences of emergence in different habitats. Pathogens rarely cause extinctions (Lips et al. 2006, McMahon et al. 2013), but they can seriously damage ecosystem functioning and lead to significant economic losses if they affect livestock or aquaculture. Even if a pathogen reaches endemic equilibrium in a community, demographic stochasticity prevents populations from remaining at equilibrium (Champagnat et al. 2006) and ecological changes can lead to abrupt evolutionary changes in the pathogen (Ferriere and Legendre 2013). Given the increasing effects of climate change, evolutionary epidemiology is a critical component in understanding emerging pathogen dynamics. The adaptive dynamics framework in Chapter 6 characterised possible pathogen life history strategies to predict the evolutionary success of pathogen strains using different transmission pathways, and examined the combined effects of virulence and community susceptibility on pathogen reproductive success. Here, the key results are discussed in the context of current literature on host-pathogen evolution, and the wider implications outlined.

Generalist pathogens are complex to study because their presence in multiple hosts can lead to alternate equilibrium states based on the host community (Hassell and May 1986). An important conclusion derived from this research was the importance of bi-modal transmission in generalist pathogen evolutionary success, and how this can be affected by the host community. The structure of host communities create vastly different selective pressures on both host and pathogen, leading to potentially different outcomes (Mennerat et al. 2010). In communities with high direct contact rates, *S. destruens* was expected to evolve to lower virulence. Direct transmission often requires active hosts to contact susceptible individuals, therefore it could be expected that a lower virulence would evolve, as previous theoretical models have shown (Day 2002a). However, high densities of hosts (and therefore high direct transmission) can also lead to higher virulence when a pathogen is introduced (Phillips and Puschendorf 2013). Conversely, low direct contact rates relative to environmental uptake rate led to the evolution of both high and low virulence *S.*

destruens strains. This was indicative of an evolutionary bi-stability between the two strains, with the potential for further evolutionary branching of the pathogen (Dieckmann and Doebeli 1999). The 'sit and wait' hypothesis predicts that environmental transmission (e.g. via spore production) can lead to more virulent pathogens, as shown in Chapter 6 (Walther and Ewald 2004, Dieckmann et al. 2005, Merikanto et al. 2014). When host survival is not crucial for pathogen transmission (often the case with environmental transmission), a pathogen can also evolve to produce toxins to the host if transmission can occur after host mortality (Day 2002b). Higher spore load is also associated with higher virulence within a host (Ebert 1994). However, optimal virulence can become independent of propagule lifespan if the infection is at equilibrium, and the death rate of infected population high compared to propagules (Bonhoeffer et al. 1996, Gandon 1998).

A key factor in pathogen virulence is the role of increased host background mortality (either due to multiple infections or the host's life history). While Chapter 4 examined the ecological outcomes expected, here the expected evolutionary outcomes were considered. The presence of multiple pathogens within a host and in the environment can increase the host immune response and level of competition between parasitic species (Lello et al. 2004, Rigaud et al. 2010, Merikanto et al. 2014). This can consequently lead to increases in pathogen virulence (Frank 1996, Choisy and de Roode 2010), highlighting why maintaining healthy populations is important. Furthermore, the introduction of non-virulent pathogen strains with virulent strains can lead to pathogen recombination, which has formed a "hyper – virulent" strain of *B. dendrobatidis* (Farrer et al. 2011). However, if host mortality is naturally high, lower virulence may evolve in order to increase pathogen transmission during the host's life time (Williams and Day 2001). Virulence is also affected by host growth rate; in naturally high mortality hosts, rapid growth results in low pathogen virulence, while slow growth results in high pathogen virulence (Koella and Restif 2001).

The presence of multiple infections has other implications for host immunity and host-pathogen evolution and it is extremely important to consider the co-evolutionary

process between hosts and pathogens (Altizer et al. 2003). Host resistance in response to infection prevents a host population from going extinct (Duffy and Sivars-Becker 2007, Penczykowski et al. 2011), depending on the time delay between infection and host immune response (Fenton et al. 2006). However, pathogen strains in immune hosts tend to develop higher virulence (Mackinnon and Read 2004), creating a co-evolutionary arms race between pathogens and hosts (Koella and Restif 2001, Best et al. 2009). A common example of this race is the development of antibiotic resistance in response to treatment (Kelly-Hope et al. 2008), or vaccines leading to higher pathogen virulence (Galvani 2003).

Habitat changes can alter pathogen transmission and virulence (Magalon et al. 2010, Poulin et al. 2011). It is expected that predictable environments would facilitate the evolution of more specialist pathogens, as the host resources are guaranteed - while unpredictable environments could lead to the maintenance of generalism in order to survive among new host species (Sasal et al. 1999). When the results are translated to real world situations, pathogens in farms are predicted to evolve very differently to pathogens in the wild. The most significant difference between farmed and wild habitats is the population density of potential host species (and thus the direct contact rate between hosts). For example, in the UK the farmed populations of *O. mykiss* can range from 20-80 kg m^{-3}, while in the wild this would be around 10 fish per m^2 (Peeler and Feist 2011). The high population densities in farming conditions are predicted to lead to higher levels of mortality compared to low densities, due to increased pathogen transmission, for example as observed with the ISA virus in salmonid fishes (Antia et al. 2003, Murray and Peeler 2005, Fisher et al. 2012). High fish densities are also linked with decreased host condition due to increased stress and poor water quality, therefore lowering host resistance (Montero et al. 1998, Peeler and Feist 2011). Finally, farmed fish are reared with the aim of rapid growth, often resulting in genetically homogenous populations which become highly suitable hosts for virulent pathogens (Mennerat et al. 2010, Peeler and Feist 2011).

In aquatic ecosystems, unstable conditions and contact rates can lead to unpredictable bi-stability in pathogen virulence (Roche et al. 2011). There has been

evidence of rapid evolution in response to pathogens in freshwater systems, which have been introduced as a result of climate change, invasive species, and eutrophication, among other pathways (Penczykowski et al. 2011). Host resistance is likely to develop more slowly in the wild as there is a trade-off between developing resistance and avoiding predator threats. Models have demonstrated that intermediate levels of predation maximise host defence and resistance (Toor and Best 2015). Therefore, vulnerability to external pressures like predation can affect the outcome of an epidemic (Choo et al. 2003). However, even small changes in host resistance can lead to significant evolution in pathogen virulence, in order to increase transmission (Best et al. 2009).

It is crucial to fully understand how anthropogenic actions are changing interspecies interactions, as this can have huge implications for disease dynamics (Mooney and Cleland 2001, Wood et al. 2010). For example, deforestation has forced bat species carrying Nipah and Hendra viruses to areas where there is livestock, leading to infections in these naïve hosts (Dobson 2004). Agriculture may have increased the transmission of *Toxoplasmosis gondii* by providing naïve hosts and new pathways for pathogen transmission (Altizer et al. 2003). The consequences of pathogen spill-over for pathogen evolution must be considered (Morgan et al. 2006), as virulence could increase as a result of host switching to a naïve species (Daszak et al. 1999, Peeler and Feist 2011). This also applies to pathogens introduced to new locations via non-native hosts; higher virulence has been demonstrated along the chytrid fungus' invasion front in Central America (Phillips and Puschendorf 2013), as well in eastern oysters *Crassostrea virginica* infected with *Haplosporidium nelsoni* (Burreson et al. 2000). In these examples, not only are the new host communities naïve, but they are also at high densities (since they have not been previously affected).

These factors raise an important question: can pathogen virulence be managed? For several years, virulence management has been attempted as a disease control approach (Ebert and Bull 2003, Dieckmann et al. 2005), but this can have serious consequences if the pathogen's dynamics are not fully understood. For example,

there are concerns that management approaches can lead to increased pathogen virulence and/or transmission (Mennerat et al. 2010). Selective restocking needs to be considered for each case study, as the addition of a partially resistant species can lead to increased pathogen virulence (Ebert et al. 1997). Adding susceptible individuals to a population can potentially keep pathogen virulence down, but host mortalities are expected to continue, as was evidenced during attempts at managing the myxoma virus (Mackinnon and Read 2004). The virulence of the pathogen decreased after contact with naïve hosts but eventually increased in response to the development of host resistance (Best and Kerr 2000). A low stocking density may help limit the advantages of higher pathogen virulence (Murray and Peeler 2005). However, while treating or removing susceptible individuals can limit a current epidemic, it cannot prevent re-emergence from occurring for environmentally transmitted pathogens that can survive outside their hosts (Merikanto et al. 2014).

The time scale of the multi-host models (10-15 years) provided sufficient time for significant mutations to occur in the pathogen, so the evolutionary aspect of epidemiology is critical for long-term disease management. A critical next step is to develop sampling and testing techniques that can anticipate virulence changes, for example by examining host use across geographical distance (Phillips and Puschendorf 2013). The pathogen's encounter rate of different species may help predict alternate host use (Lootvoet et al. 2013). Furthermore, pathogens often infect more closely related hosts than not, which could also help predict potential hosts (Ebert 1994). Understanding these driving forces behind pathogen virulence, from host community composition to pathogen transmission strategy, can not only help mitigate pathogen effects, but also provides a framework for pre-empting changes to higher virulence.

7.5 Conclusions and recommendations

This thesis provided insights on how fungal pathogens can spread and evolve in different communities, formulating hypotheses that could be tested empirically. The drivers of disease in this system were determined and the knowledge was used to

create a risk analysis framework which could be applied to any UK water body, which can help contain and prevent future outbreaks. The most important preventative step in the case of EIDs is consistent pathogen monitoring in areas with elevated risks of emergence. In the case of *S. destruens*, it is crucial that monitoring extends to adjacent communities, given the importance of environmental transmission in pathogen expansion. Long-term monitoring of fish health is the only empirical way to determine whether the disease is causing chronic declines or changing patterns in species richness. The frequency of monitoring should depend on how high the risk of emergence is in that given habitat (recommendations are included in the risk analysis).

The models developed here can be used for any community assemblage to model disease establishment, as well as to test eradication and selective restocking as management strategies. The entire range of transmission, susceptibility, and various species interactions can be included, forming a completely generalised multi-host ecological model. The inclusion of evolutionary dynamics in the final chapter enabled the characterisation of how this pathogen can change over time. There are increasing reports of *S. destruens*, and if not pre-emptively contained it could spread and cause widespread damage- economically and environmentally. As one of several fungal and fungal-like pathogens that are increasing in prevalence, determining the ecological and evolutionary dynamics that drive its emergence can be useful for similar pathogens.

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Appendices

Appendix 1

The datasets used in the thesis to parameterise the models. All datasets are from published literature and cited in the text where appropriate.

Dataset 1 (Andreou et al 2012)

Abramis brama

Days	Number of Mortalities	Number of Mortalities -Tank2	Number of Mortalities -Tank 3	Cumulative Mortalities Tank 1	Cumulative Mortalities Tank 2	Cumulative Mortalities Tank 3	Survivors T1	Survivors T2	SurvivorsT3	Average Surviving Individuals	Min	Max
1	0	0	0	0	0	0	20	20	20	20	20	20
2	0	0	0	0	0	0	20	20	20	20	20	20
3	0	0	0	0	0	0	20	20	20	20	20	20
4	0	1	0	0	1	0	20	19	20	19.67	19	20
5	0	1	1	0	2	1	20	18	19	19	18	20
6	1	0	2	1	2	3	19	18	17	18	17	19
7	0	1	0	1	3	3	19	17	17	17. 67	17	19
8	1	0	0	2	3	3	18	17	17	17. 33	17	18
9	0	0	0	2	3	3	18	17	17	17. 33	17	18
10	1	0	0	3	3	3	17	17	17	17	17	17
11	0	1	0	3	4	3	17	16	17	16. 67	16	17
12	0	0	0	3	4	3	17	16	17	16. 67	16	17
13	1	0	0	4	4	3	16	16	17	16. 33	16	17
14	1	0	0	5	4	3	15	16	17	16	15	17
15	1	3	1	6	7	4	14	13	16	14. 33	13	16
16	1	0	0	7	7	4	13	13	16	14	13	16

17	0	0	4	7	7	8	13	13	12	12. 67	12	13
18	3	3	2	10	10	10	10	10	10	10	10	10
19	0	1	0	10	11	10	10	9	10	9. 67	9	10
20	1	0	0	11	11	10	9	9	10	9. 33	9	10
21	1	0	0	12	11	10	8	9	10	9	8	10
22	0	1	0	12	12	10	8	8	10	8. 67	8	10
23	2	0	1	14	12	11	6	8	9	7. 67	6	9
24	0	0	0	14	12	11	6	8	9	7. 67	6	9
25	0	0	0	14	12	11	6	8	9	7. 67	6	9
26	0	0	0	14	12	11	6	8	9	7. 67	6	9
27	0	0	0	14	12	11	6	8	9	7. 67	6	9
28	0	0	0	14	12	11	6	8	9	7. 67	6	9
29	0	0	0	14	12	11	6	8	9	7. 67	6	9
30	0	0	0	14	12	11	6	8	9	7. 67	6	9
31	0	0	0	14	12	11	6	8	9	7. 67	6	9
32	0	0	0	14	12	11	6	8	9	7. 67	6	9
33	0	0	0	14	12	11	6	8	9	7.67	6	9
34	0	0	0	14	12	11	6	8	9	7.67	6	9
35	0	0	0	14	12	11	6	8	9	7. 67	6	9
36	0	0	0	14	12	11	6	8	9	7. 67	6	9
37	0	0	0	14	12	11	6	8	9	7. 67	6	9
38	0	0	0	14	12	11	6	8	9	7.67	6	9
39	0	0	0	14	12	11	6	8	9	7. 67	6	9
40	0	0	0	14	12	11	6	8	9	7. 67	6	9
41	0	0	0	14	12	11	6	8	9	7. 67	6	9
42	0	0	0	14	12	11	6	8	9	7. 67	6	9
43	0	0	0	14	12	11	6	8	9	7. 67	6	9
44	0	0	0	14	12	11	6	8	9	7. 67	6	9
45	0	0	0	14	12	11	6	8	9	7.67	6	9

46	0	0	0	14	12	11	6	8	9	7. 67	6	9
47	0	0	0	14	12	11	6	8	9	7. 67	6	9
48	0	0	0	14	12	11	6	8	9	7. 67	6	9
49	0	0	0	14	12	11	6	8	9	7. 67	6	9
50	0	0	0	14	12	11	6	8	9	7. 67	6	9
51	0	0	0	14	12	11	6	8	9	7. 67	6	9
52	0	0	0	14	12	11	6	8	9	7.67	6	9
53	0	0	0	14	12	11	6	8	9	7. 67	6	9
54	0	0	0	14	12	11	6	8	9	7. 67	6	9
55	0	0	0	14	12	11	6	8	9	7. 67	6	9
56	0	0	0	14	12	11	6	8	9	7.67	6	9
57	0	0	0	14	12	11	6	8	9	7. 67	6	9
58	0	0	0	14	12	11	6	8	9	7. 67	6	9
59	0	0	0	14	12	11	6	8	9	7. 67	6	9
60	0	0	0	14	12	11	6	8	9	7. 67	6	9
61	0	0	0	14	12	11	6	8	9	7. 67	6	9
62	0	0	0	14	12	11	6	8	9	7.67	6	9
63	0	0	0	14	12	11	6	8	9	7. 67	6	9
64	0	0	0	14	12	11	6	8	9	7. 67	6	9
65	0	0	0	14	12	11	6	8	9	7. 67	6	9
66	0	0	0	14	12	11	6	8	9	7. 67	6	9
67	0	0	0	14	12	11	6	8	9	7. 67	6	9
68	0	0	0	14	12	11	6	8	9	7. 67	6	9
69	0	0	0	14	12	11	6	8	9	7. 67	6	9
70	0	0	0	14	12	11	6	8	9	7. 67	6	9
71	0	0	0	14	12	11	6	8	9	7.67	6	9
72	0	0	0	14	12	11	6	8	9	7. 67	6	9
73	0	0	0	14	12	11	6	8	9	7. 67	6	9
74	0	0	0	14	12	11	6	8	9	7. 67	6	9

75	0	0	0	14	12	11	6	8	9	7. 67	6	9
76	0	0	0	14	12	11	6	8	9	7. 67	6	9
77	0	0	0	14	12	11	6	8	9	7. 67	6	9
78	0	0	0	14	12	11	6	8	9	7. 67	6	9
79	0	0	0	14	12	11	6	8	9	7.67	6	9
80	0	0	0	14	12	11	6	8	9	7. 67	6	9
81	0	0	0	14	12	11	6	8	9	7. 67	6	9
82	0	0	0	14	12	11	6	8	9	7. 67	6	9
83	0	0	0	14	12	11	6	8	9	7. 67	6	9
84	0	0	0	14	12	11	6	8	9	7. 67	6	9
85	0	0	0	14	12	11	6	8	9	7.67	6	9
86	0	0	0	14	12	11	6	8	9	7. 67	6	9
87	0	0	0	14	12	11	6	8	9	7. 67	6	9
88	0	0	0	14	12	11	6	8	9	7. 67	6	9
89	0	0	0	14	12	11	6	8	9	7.67	6	9
90	0	0	0	14	12	11	6	8	9	7. 67	6	9
91	0	0	0	14	12	11	6	8	9	7.67	6	9
92	0	0	0	14	12	11	6	8	9	7.67	6	9
93	0	0	0	14	12	11	6	8	9	7. 67	6	9
94	0	0	0	14	12	11	6	8	9	7. 67	6	9
95	0	0	0	14	12	11	6	8	9	7. 67	6	9
96	0	0	0	14	12	11	6	8	9	7.67	6	9
97	0	0	0	14	12	11	6	8	9	7.67	6	9
98	0	0	0	14	12	11	6	8	9	7. 67	6	9
99	0	0	0	14	12	11	6	8	9	7. 67	6	9
100	0	0	0	14	12	11	6	8	9	7.67	6	9
101	0	0	0	14	12	11	6	8	9	7. 67	6	9
102	0	0	0	14	12	11	6	8	9	7. 67	6	9
103	0	0	0	14	12	11	6	8	9	7. 67	6	9

104	0	0	0	14	12	11	6	8	9	7.67	6	9
105	0	0	0	14	12	11	6	8	9	7. 67	6	9
106	0	0	0	14	12	11	6	8	9	7. 67	6	9
107	0	0	0	14	12	11	6	8	9	7. 67	6	9
108	0	0	0	14	12	11	6	8	9	7. 67	6	9
109	0	0	0	14	12	11	6	8	9	7. 67	6	9
110	0	0	0	14	12	11	6	8	9	7. 67	6	9
111	0	0	0	14	12	11	6	8	9	7. 67	6	9
112	0	0	0	14	12	11	6	8	9	7. 67	6	9
113	0	0	0	14	12	11	6	8	9	7. 67	6	9
114	0	0	0	14	12	11	6	8	9	7. 67	6	9
115	0	0	0	14	12	11	6	8	9	7. 67	6	9
116	0	0	0	14	12	11	6	8	9	7. 67	6	9
117	0	0	0	14	12	11	6	8	9	7. 67	6	9
118	0	0	0	14	12	11	6	8	9	7. 67	6	9
119	0	0	0	14	12	11	6	8	9	7. 67	6	9
120	0	0	0	14	12	11	6	8	9	7. 67	6	9
121	0	0	0	14	12	11	6	8	9	7. 67	6	9
122	0	0	0	14	12	11	6	8	9	7. 67	6	9
123	0	0	0	14	12	11	6	8	9	7. 67	6	9
124	0	0	0	14	12	11	6	8	9	7. 67	6	9
125	0	0	0	14	12	11	6	8	9	7. 67	6	9
126	0	0	0	14	12	11	6	8	9	7. 67	6	9
127	0	0	0	14	12	11	6	8	9	7. 67	6	9
128	0	0	0	14	12	11	6	8	9	7. 67	6	9
129	0	0	0	14	12	11	6	8	9	7.67	6	9
130	0	0	0	14	12	11	6	8	9	7.67	6	9
131	0	0	0	14	12	11	6	8	9	7.67	6	9
132	0	0	0	14	12	11	6	8	9	7. 67	6	9

133	0	0	0	14	12	11	6	8	9	7. 67	6	9
134	0	0	0	14	12	11	6	8	9	7. 67	6	9
135	0	0	0	14	12	11	6	8	9	7. 67	6	9
136	0	0	0	14	12	11	6	8	9	7. 67	6	9
137	0	0	0	14	12	11	6	8	9	7. 67	6	9
138	0	0	0	14	12	11	6	8	9	7. 67	6	9
139	0	0	0	14	12	11	6	8	9	7. 67	6	9
140	0	0	0	14	12	11	6	8	9	7. 67	6	9
141	0	0	0	14	12	11	6	8	9	7. 67	6	9
142	0	0	0	14	12	11	6	8	9	7. 67	6	9
143	0	0	0	14	12	11	6	8	9	7. 67	6	9
144	0	0	0	14	12	11	6	8	9	7. 67	6	9
145	0	0	0	14	12	11	6	8	9	7. 67	6	9
146	0	0	0	14	12	11	6	8	9	7. 67	6	9
147	0	0	0	14	12	11	6	8	9	7. 67	6	9
148	0	0	0	14	12	11	6	8	9	7. 67	6	9
149	0	0	0	14	12	11	6	8	9	7. 67	6	9
150	0	0	0	14	12	11	6	8	9	7. 67	6	9
151	0	0	0	14	12	11	6	8	9	7. 67	6	9
152	0	0	0	14	12	11	6	8	9	7. 67	6	9
153	0	0	0	14	12	11	6	8	9	7. 67	6	9
154	0	0	0	14	12	11	6	8	9	7. 67	6	9
155	0	0	0	14	12	11	6	8	9	7. 67	6	9
156	0	0	0	14	12	11	6	8	9	7. 67	6	9
157	0	0	0	14	12	11	6	8	9	7. 67	6	9
158	0	0	0	14	12	11	6	8	9	7. 67	6	9
159	0	0	0	14	12	11	6	8	9	7. 67	6	9
160	0	0	0	14	12	11	6	8	9	7. 67	6	9
161	0	0	0	14	12	11	6	8	9	7. 67	6	9

162	0	0	0	14	12	11	6	8	9	7. 67	6	9
163	0	0	0	14	12	11	6	8	9	7. 67	6	9
164	0	0	0	14	12	11	6	8	9	7. 67	6	9
165	0	0	0	14	12	11	6	8	9	7. 67	6	9
166	0	0	0	14	12	11	6	8	9	7. 67	6	9
167	0	0	0	14	12	11	6	8	9	7. 67	6	9
168	0	0	0	14	12	11	6	8	9	7. 67	6	9
169	0	0	0	14	12	11	6	8	9	7. 67	6	9
170	0	0	0	14	12	11	6	8	9	7. 67	6	9
171	0	0	0	14	12	11	6	8	9	7. 67	6	9
172	0	0	0	14	12	11	6	8	9	7. 67	6	9
173	0	0	0	14	12	11	6	8	9	7. 67	6	9
174	0	0	0	14	12	11	6	8	9	7. 67	6	9
175	0	0	0	14	12	11	6	8	9	7. 67	6	9
176	0	0	0	14	12	11	6	8	9	7. 67	6	9
177	0	0	0	14	12	11	6	8	9	7. 67	6	9
178	0	0	0	14	12	11	6	8	9	7. 67	6	9
179	0	0	0	14	12	11	6	8	9	7. 67	6	9
180	0	0	0	14	12	11	6	8	9	7. 67	6	9

Cyprinus carpio

Days	Number of Mortalities Tank1	Number of Mortalities Tank 2	Number of Mortalities -Tank 3	Cumulative Mortalities Tank 1	Cumulative Mortalities Tank 2	Cumulative Mortalities Tank 3	SurvivorsT1	Survivors T2	SurvivorsT3	Average Surviving Individuals	Min	Max
1	0	0	0	0	0	0	20	20	20	20	20	20
2	0	0	0	0	0	0	20	20	20	20	20	20

3	0	0	0	0	0	0	20	20	20	20	20	20
4	0	0	0	0	0	0	20	20	20	20	20	20
5	0	0	0	0	0	0	20	20	20	20	20	20
6	0	0	0	0	0	0	20	20	20	20	20	20
7	0	0	0	0	0	0	20	20	20	20	20	20
8	0	0	0	0	0	0	20	20	20	20	20	20
9	0	0	0	0	0	0	20	20	20	20	20	20
10	0	0	0	0	0	0	20	20	20	20	20	20
11	0	0	0	0	0	0	20	20	20	20	20	20
12	0	0	0	0	0	0	20	20	20	20	20	20
13	0	0	0	0	0	0	20	20	20	20	20	20
14	0	0	0	0	0	0	20	20	20	20	20	20
15	0	0	0	0	0	0	20	20	20	20	20	20
16	0	0	0	0	0	0	20	20	20	20	20	20
17	0	0	0	0	0	0	20	20	20	20	20	20
18	0	0	0	0	0	0	20	20	20	20	20	20
19	0	0	0	0	0	0	20	20	20	20	20	20
20	0	0	0	0	0	0	20	20	20	20	20	20
21	0	0	0	0	0	0	20	20	20	20	20	20
22	0	0	0	0	0	0	20	20	20	20	20	20
23	0	0	0	0	0	0	20	20	20	20	20	20
24	0	0	0	0	0	0	20	20	20	20	20	20
25	0	0	0	0	0	0	20	20	20	20	20	20
26	0	0	0	0	0	0	20	20	20	20	20	20
27	0	0	0	0	0	0	20	20	20	20	20	20
28	0	0	0	0	0	0	20	20	20	20	20	20
29	0	0	0	0	0	0	20	20	20	20	20	20
30	0	0	0	0	0	0	20	20	20	20	20	20
31	0	0	0	0	0	0	20	20	20	20	20	20

32	0	0	0	0	0	0	20	20	20	20	20	20
33	0	0	0	0	0	0	20	20	20	20	20	20
34	0	0	0	0	0	0	20	20	20	20	20	20
35	0	0	0	0	0	0	20	20	20	20	20	20
36	0	0	0	0	0	0	20	20	20	20	20	20
37	0	0	0	0	0	0	20	20	20	20	20	20
38	0	0	0	0	0	0	20	20	20	20	20	20
39	0	0	0	0	0	0	20	20	20	20	20	20
40	0	0	0	0	0	0	20	20	20	20	20	20
41	0	0	0	0	0	0	20	20	20	20	20	20
42	0	0	0	0	0	0	20	20	20	20	20	20
43	0	0	0	0	0	0	20	20	20	20	20	20
44	0	0	0	0	0	0	20	20	20	20	20	20
45	0	0	0	0	0	0	20	20	20	20	20	20
46	0	0	0	0	0	0	20	20	20	20	20	20
47	0	0	0	0	0	0	20	20	20	20	20	20
48	0	0	0	0	0	0	20	20	20	20	20	20
49	0	1	0	0	1	0	20	19	20	19. 67	19	20
50	0	0	0	0	1	0	20	18	20	19. 33	18	20
51	0	0	0	0	0	0	20	18	20	19. 33	18	20
52	0	0	0	0	0	0	20	18	20	19. 33	18	20
53	0	0	0	0	0	0	20	18	20	19. 33	18	20
54	0	0	0	0	0	0	20	18	20	19. 33	18	20
55	0	0	0	0	0	0	20	18	20	19. 33	18	20
56	0	0	0	0	0	0	20	18	20	19. 33	18	20
57	0	0	0	0	0	0	20	18	20	19. 33	18	20
58	0	0	0	0	0	0	20	18	20	19. 33	18	20
59	0	0	0	0	0	0	20	18	20	19. 33	18	20
60	0	0	0	0	0	0	20	18	20	19. 33	18	20

61	0	0	0	0	0	0	20	18	20	19.33	18	20
62	0	0	0	0	0	0	20	18	20	19. 33	18	20
63	0	0	0	0	0	0	20	18	20	19. 33	18	20
64	0	0	0	0	0	0	20	18	20	19. 33	18	20
65	0	0	0	0	0	0	20	18	20	19. 33	18	20
66	0	0	0	0	0	0	20	18	20	19. 33	18	20
67	0	0	2	0	0	2	20	18	18	18. 67	18	20
68	0	0	0	0	0	2	20	18	16	18	16	20
69	0	0	0	0	0	0	20	18	16	18	16	20
70	0	0	0	0	0	0	20	18	16	18	16	20
71	0	0	0	0	0	0	20	18	16	18	16	20
72	0	0	0	0	0	0	20	18	16	18	16	20
73	0	0	0	0	0	0	20	18	16	18	16	20
74	0	0	0	0	0	0	20	18	16	18	16	20
75	0	0	0	0	0	0	20	18	16	18	16	20
76	0	0	0	0	0	0	20	18	16	18	16	20
77	0	0	0	0	0	0	20	18	16	18	16	20
78	0	0	0	0	0	0	20	18	16	18	16	20
79	0	0	0	0	0	0	20	18	16	18	16	20
80	0	0	0	0	0	0	20	18	16	18	16	20
81	0	0	0	0	0	0	20	18	16	18	16	20
82	0	0	0	0	0	0	20	18	16	18	16	20
83	0	0	0	0	0	0	20	18	16	18	16	20
84	0	0	0	0	0	0	20	18	16	18	16	20
85	0	0	0	0	0	0	20	18	16	18	16	20
86	0	0	0	0	0	0	20	18	16	18	16	20
87	0	0	0	0	0	0	20	18	16	18	16	20
88	0	0	0	0	0	0	20	18	16	18	16	20
89	0	0	0	0	0	0	20	18	16	18	16	20

90	0	0	0	0	0	0	20	18	16	18	16	20
91	0	0	0	0	0	0	20	18	16	18	16	20
92	0	1	0	0	1	0	20	17	16	17.67	16	20
93	0	0	0	0	1	0	20	16	16	17. 33	16	20
94	0	0	0	0	0	0	20	16	16	17. 33	16	20
95	0	0	0	0	0	0	20	16	16	17. 33	16	20
96	0	0	0	0	0	0	20	16	16	17. 33	16	20
97	0	0	0	0	0	0	20	16	16	17. 33	16	20
98	0	0	0	0	0	0	20	16	16	17. 33	16	20
99	0	0	0	0	0	0	20	16	16	17. 33	16	20
100	0	0	0	0	0	0	20	16	16	17. 33	16	20
101	0	0	0	0	0	0	20	16	16	17. 33	16	20
102	0	0	0	0	0	0	20	16	16	17. 33	16	20
103	0	0	0	0	0	0	20	16	16	17. 33	16	20
104	0	0	0	0	0	0	20	16	16	17. 33	16	20
105	0	0	0	0	0	0	20	16	16	17. 33	16	20
106	0	0	0	0	0	0	20	16	16	17. 33	16	20
107	0	0	0	0	0	0	20	16	16	17. 33	16	20
108	0	0	0	0	0	0	20	16	16	17. 33	16	20
109	0	0	0	0	0	0	20	16	16	17. 33	16	20
110	0	0	0	0	0	0	20	16	16	17. 33	16	20
111	0	0	0	0	0	0	20	16	16	17. 33	16	20
112	0	0	0	0	0	0	20	16	16	17. 33	16	20
113	0	0	0	0	0	0	20	16	16	17. 33	16	20
114	0	0	0	0	0	0	20	16	16	17. 33	16	20
115	0	0	0	0	0	0	20	16	16	17. 33	16	20
116	0	0	0	0	0	0	20	16	16	17. 33	16	20
117	0	0	0	0	0	0	20	16	16	17. 33	16	20
118	0	0	0	0	0	0	20	16	16	17. 33	16	20

119	0	0	0	0	0	0	20	16	16	17. 33	16	20
120	0	0	0	0	0	0	20	16	16	17. 33	16	20
121	0	0	0	0	0	0	20	16	16	17. 33	16	20
122	0	0	0	0	0	0	20	16	16	17. 33	16	20
123	0	0	0	0	0	0	20	16	16	17. 33	16	20
124	0	0	0	0	0	0	20	16	16	17. 33	16	20
125	0	0	0	0	0	0	20	16	16	17. 33	16	20
126	0	0	0	0	0	0	20	16	16	17. 33	16	20
127	0	0	0	0	0	0	20	16	16	17. 33	16	20
128	0	0	0	0	0	0	20	16	16	17. 33	16	20
129	0	0	0	0	0	0	20	16	16	17. 33	16	20
130	0	0	0	0	0	0	20	16	16	17. 33	16	20
131	0	0	0	0	0	0	20	16	16	17. 33	16	20
132	0	0	0	0	0	0	20	16	16	17. 33	16	20
133	0	0	0	0	0	0	20	16	16	17. 33	16	20
134	0	0	0	0	0	0	20	16	16	17. 33	16	20
135	0	0	0	0	0	0	20	16	16	17. 33	16	20
136	0	0	0	0	0	0	20	16	16	17. 33	16	20
137	0	0	0	0	0	0	20	16	16	17. 33	16	20
138	0	0	0	0	0	0	20	16	16	17. 33	16	20
139	0	0	0	0	0	0	20	16	16	17. 33	16	20
140	0	0	0	0	0	0	20	16	16	17. 33	16	20
141	0	0	0	0	0	0	20	16	16	17. 33	16	20
142	0	0	0	0	0	0	20	16	16	17. 33	16	20
143	0	0	0	0	0	0	20	16	16	17. 33	16	20
144	0	0	0	0	0	0	20	16	16	17. 33	16	20
145	0	0	0	0	0	0	20	16	16	17. 33	16	20
146	0	0	0	0	0	0	20	16	16	17. 33	16	20
147	0	0	0	0	0	0	20	16	16	17. 33	16	20

148	0	0	0	0	0	0	20	16	16	17. 33	16	20
149	0	0	0	0	0	0	20	16	16	17. 33	16	20
150	0	0	0	0	0	0	20	16	16	17. 33	16	20
151	0	0	0	0	0	0	20	16	16	17. 33	16	20
152	0	0	0	0	0	0	20	16	16	17. 33	16	20
153	0	0	0	0	0	0	20	16	16	17. 33	16	20
154	0	0	0	0	0	0	20	16	16	17. 33	16	20
155	0	0	0	0	0	0	20	16	16	17. 33	16	20
156	0	0	0	0	0	0	20	16	16	17. 33	16	20
157	0	0	0	0	0	0	20	16	16	17. 33	16	20
158	0	0	0	0	0	0	20	16	16	17. 33	16	20
159	0	0	0	0	0	0	20	16	16	17. 33	16	20
160	0	0	0	0	0	0	20	16	16	17. 33	16	20
161	0	0	0	0	0	0	20	16	16	17. 33	16	20
162	0	0	0	0	0	0	20	16	16	17. 33	16	20
163	0	0	0	0	0	0	20	16	16	17. 33	16	20
164	0	0	0	0	0	0	20	16	16	17. 33	16	20
165	0	0	0	0	0	0	20	16	16	17. 33	16	20
166	0	0	0	0	0	0	20	16	16	17. 33	16	20
167	0	0	0	0	0	0	20	16	16	17. 33	16	20
168	0	0	0	0	0	0	20	16	16	17. 33	16	20
169	0	0	0	0	0	0	20	16	16	17. 33	16	20
170	0	0	0	0	0	0	20	16	16	17. 33	16	20
171	0	0	0	0	0	0	20	16	16	17. 33	16	20
172	0	0	0	0	0	0	20	16	16	17. 33	16	20
173	0	0	0	0	0	0	20	16	16	17. 33	16	20
174	0	0	0	0	0	0	20	16	16	17. 33	16	20
175	0	0	0	0	0	0	20	16	16	17. 33	16	20
176	0	0	0	0	0	0	20	16	16	17. 33	16	20

177	0	0	0	0	0	0	20	16	16	17. 33	16	20
178	0	0	0	0	0	0	20	16	16	17. 33	16	20
179	0	0	0	0	0	0	20	16	16	17. 33	16	20
180	0	0	0	0	0	0	20	16	16	17. 33	16	20
181	0	0	0	0	0	0	20	16	16	17. 33	16	20
182	0	0	0	0	0	0	20	16	16	17. 33	16	20
183	0	0	0	0	0	0	20	16	16	17. 33	16	20
184	0	1	0	0	1	0	20	15	16	17	15	20
185	0	0	0	0	1	0	20	14	16	16. 67	14	20
186	0	0	0	0	0	0	20	14	16	16. 67	14	20

Rutilus rutilus

Days	Number of Mortalities -Tank1	Number of Mortalities -Tank2	Number of Mortalities -Tank 3	Cumulative Mortalities Tank 1	Cumulative Mortalities Tank 2	Cumulative Mortalities Tank 3	SurvivorsT1	Survivors T2	SurvivorsT3	Average Surviving Individuals	Min	Max
1	0	0	0	0	0	0	20	20	20	20	20	20
2	0	0	0	0	0	0	20	20	20	20	20	20
3	0	0	0	0	0	0	20	20	20	20	20	20
4	0	0	0	0	0	0	20	20	20	20	20	20
5	0	0	0	0	0	0	20	20	20	20	20	20
6	0	0	0	0	0	0	20	20	20	20	20	20
7	0	0	0	0	0	0	20	20	20	20	20	20
8	0	0	0	0	0	0	20	20	20	20	20	20
9	0	0	0	0	0	0	20	20	20	20	20	20
10	0	0	0	0	0	0	20	20	20	20	20	20
11	0	0	0	0	0	0	20	20	20	20	20	20

12	0	0	0	0	0	0	20	20	20	20	20	20
13	0	0	0	0	0	0	20	20	20	20	20	20
14	0	0	0	0	0	0	20	20	20	20	20	20
15	0	0	0	0	0	0	20	20	20	20	20	20
16	0	0	0	0	0	0	20	20	20	20	20	20
17	0	0	0	0	0	0	20	20	20	20	20	20
18	0	0	0	0	0	0	20	20	20	20	20	20
19	0	0	0	0	0	0	20	20	20	20	20	20
20	0	0	1	0	0	1	20	20	19	19.5	19	20
21	3	0	0	3	0	1	17	20	19	18	17	19
22	5	0	1	8	0	2	12	20	18	15	12	18
23	0	0	1	8	0	3	12	20	17	14.5	12	17
24	0	0	0	8	0	3	12	20	17	14.5	12	17
25	0	0	0	8	0	3	12	20	17	14.5	12	17
26	0	0	0	8	0	3	12	20	17	14.5	12	17
27	0	0	0	8	0	3	12	20	17	14.5	12	17
28	0	0	0	8	0	3	12	20	17	14.5	12	17
29	0	0	0	8	0	3	12	20	17	14.5	12	17
30	0	0	0	8	0	3	12	20	17	14.5	12	17
31	0	0	0	8	0	3	12	20	17	14.5	12	17
32	0	0	0	8	0	3	12	20	17	14.5	12	17
33	0	0	0	8	0	3	12	20	17	14.5	12	17
34	0	0	0	8	0	3	12	20	17	14.5	12	17
35	0	0	0	8	0	3	12	20	17	14.5	12	17
36	0	0	0	8	0	3	12	20	17	14.5	12	17
37	1	0	0	9	0	3	11	20	17	14	11	17
38	2	0	1	11	0	4	9	20	16	12.5	9	16
39	0	0	2	11	0	6	9	20	14	11.5	9	14
40	0	0	2	11	0	8	9	20	12	10.5	9	12

41	0	0	1	11	0	9	9	20	11	10	9	11
42	0	0	0	11	0	9	9	20	11	10	9	11
43	0	0	0	11	0	9	9	20	11	10	9	11
44	0	0	0	11	0	9	9	20	11	10	9	11
45	0	0	0	11	0	9	9	20	11	10	9	11
46	0	0	1	11	0	10	9	20	10	9.5	9	10
47	0	0	0	11	0	10	9	20	10	9.5	9	10
48	0	0	0	11	0	10	9	20	10	9.5	9	10
49	0	0	0	11	0	10	9	20	10	9.5	9	10
50	0	0	1	11	0	11	9	20	9	9	9	9
51	0	0	0	11	0	11	9	20	9	9	9	9
52	0	0	0	11	0	11	9	20	9	9	9	9
53	0	0	0	11	0	11	9	20	9	9	9	9
54	0	0	0	11	0	11	9	20	9	9	9	9
55	0	0	0	11	0	11	9	20	9	9	9	9
56	0	0	0	11	0	11	9	20	9	9	9	9
57	0	0	0	11	0	11	9	20	9	9	9	9
58	0	0	0	11	0	11	9	20	9	9	9	9
59	0	0	0	11	0	11	9	20	9	9	9	9
60	0	0	0	11	0	11	9	20	9	9	9	9
61	0	0	0	11	0	11	9	20	9	9	9	9
62	0	0	0	11	0	11	9	20	9	9	9	9
63	0	0	0	11	0	11	9	20	9	9	9	9
64	0	0	0	11	0	11	9	20	9	9	9	9
65	0	0	0	11	0	11	9	20	9	9	9	9
66	0	0	0	11	0	11	9	20	9	9	9	9
67	0	0	0	11	0	11	9	20	9	9	9	9
68	0	0	0	11	0	11	9	20	9	9	9	9
69	0	0	0	11	0	11	9	20	9	9	9	9

70	0	0	0	11	0	11	9	20	9	9	9	9
71	0	0	0	11	0	11	9	20	9	9	9	9
72	0	0	0	11	0	11	9	20	9	9	9	9
73	0	0	0	11	0	11	9	20	9	9	9	9
74	0	0	0	11	0	11	9	20	9	9	9	9
75	0	0	0	11	0	11	9	20	9	9	9	9
76	0	0	0	11	0	11	9	20	9	9	9	9
77	0	0	0	11	0	11	9	20	9	9	9	9
78	0	0	0	11	0	11	9	20	9	9	9	9
79	0	0	0	11	0	11	9	20	9	9	9	9
80	0	0	0	11	0	11	9	20	9	9	9	9
81	0	0	0	11	0	11	9	20	9	9	9	9
82	0	0	0	11	0	11	9	20	9	9	9	9
83	0	0	0	11	0	11	9	20	9	9	9	9
84	0	0	0	11	0	11	9	20	9	9	9	9
85	0	0	0	11	0	11	9	20	9	9	9	9
86	0	0	0	11	0	11	9	20	9	9	9	9
87	0	0	0	11	0	11	9	20	9	9	9	9
88	0	0	0	11	0	11	9	20	9	9	9	9
89	0	0	0	11	0	11	9	20	9	9	9	9
90	0	0	0	11	0	11	9	20	9	9	9	9
91	0	0	0	11	0	11	9	20	9	9	9	9
92	0	0	0	11	0	11	9	20	9	9	9	9
93	0	0	0	11	0	11	9	20	9	9	9	9
94	0	0	0	11	0	11	9	20	9	9	9	9
95	0	0	0	11	0	11	9	20	9	9	9	9
96	0	0	0	11	0	11	9	20	9	9	9	9
97	0	0	0	11	0	11	9	20	9	9	9	9
98	0	0	0	11	0	11	9	20	9	9	9	9

99	0	0	0	11	0	11	9	20	9	9	9	9
100	0	0	0	11	0	11	9	20	9	9	9	9
101	0	0	0	11	0	11	9	20	9	9	9	9
102	0	0	0	11	0	11	9	20	9	9	9	9
103	0	0	0	11	0	11	9	20	9	9	9	9
104	0	0	0	11	0	11	9	20	9	9	9	9
105	0	0	0	11	0	11	9	20	9	9	9	9
106	0	0	0	11	0	11	9	20	9	9	9	9
107	0	0	0	11	0	11	9	20	9	9	9	9
108	0	0	0	11	0	11	9	20	9	9	9	9
109	0	0	0	11	0	11	9	20	9	9	9	9
110	0	0	0	11	0	11	9	20	9	9	9	9
111	0	0	0	11	0	11	9	20	9	9	9	9

Dataset 2 (Paley et al 2012)

Numbers shown in the table are the percentage of survivors in the experiment for both methods of infection (bath immersion and intra-peritoneal injection).

Days	*Leucaspius delineatus*		*Salmo salar*	
	Injection	Bath	Injection	Bath
1	100	100	100	100
2	100	100	98	100
3	100	100	93	100
4	100	100	93	100
5	100	100	91	100
6	100	100	90	98
7	100	100	90	96
8	100	100	90	96
9	100	100	88	96
10	100	100	86	96
11	100	100	84	96
12	100	100	84	96
13	100	100	82	96
14	100	98	80	96
15	100	98	78	96
16	100	94	78	96
17	100	94	78	96
18	100	91	78	96
19	100	91	78	96
20	100	91	78	96
21	100	91	78	96
22	100	91	78	96

23	100	91	78	96
24	100	91	78	96
25	100	91	78	96
26	100	91	78	96
27	100	91	78	96
28	100	91	78	96
29	100	91	78	96
30	100	91	78	96
31	100	91	78	96
32	100	91	78	96
33	100	91	78	96
34	100	91	78	96
35	100	91	78	96
36	100	91	78	96
37	100	91	78	96
38	100	91	78	96
39	100	91	78	96
40	100	91	76	96
41	100	91	76	96
42	100	91	76	96
43	100	89	76	96
44	100	89	76	96
45	100	89	76	96
46	100	89	75	96
47	100	89	72	96
48	100	89	72	96
49	98	89	70	96

50	98	87	70	96
51	98	87	68	96
52	98	87	56	96
53	90	87	54	96
54	8.7	87	52	96
55	87	87	50	96
56	87	87	48	96
57	87	87	48	96
58	87	87	48	96
59	87	87	48	96
60	87	87	48	96
61	87	87	48	96
62	87	87	48	93
63	87	87	40	93
64	87	87	33	93
65	83	87	33	93
66	78	87	33	93
67	78	87	33	93
68	78	87	33	93
69	78	87	33	93
70	78	87	33	93
71	75	87	33	93
72	70	87	33	93
73	70	87	33	93
74	70	87	33	93
75	70	87	33	93
76	70	87	33	93

77	70	87	33	93
78	70	87	33	93
79	70	87	33	93
80	70	87	33	93
81	70	87	33	93
82	67	87	33	93
83	67	87	33	93

Dataset 3 (Gozlan et al 2005)

The cohabitation experiments between *Leucaspius delineatus* and *Pseudorasbora parva.* Below is the raw data for *L. delineatus* mortality from two years of experiments.

	Number	Replicates
Large tanks	40	*2
Small tanks	20	*4

2003						2004				
	Day		SYSTEM 1		SYSTEM 2			SYSTEM 1		SYSTEM 2
26/04/2003		TANK	FL	TANK	FL	29/04/2004	TANK	FL	TANK	FL
26/04/2003	1	Tank1	56	Tank1	60	29/04/2004	Tank1	48	Tank1	55
26/04/2003	2	Tank1	58	Tank1	62	29/04/2004	Tank1	60	Tank1	57
26/04/2003	3	Tank1	56	Tank1	61	29/04/2004	Tank1	61	Tank1	57
26/04/2003	4	Tank1	60	Tank1	50	29/04/2004	Tank1	62	Tank1	59

26/04/2003	5	Tank1	61	Tank1	61	29/04/2004	Tank1	60	Tank1	57
26/04/2003	6	Tank1	52	Tank1	62	29/04/2004	Tank1	63	Tank1	58
26/04/2003	7	Tank1	66	Tank1	55	29/04/2004	Tank1	58	Tank1	58
26/04/2003	8	Tank1	61	Tank1	57	29/04/2004	Tank1	45	Tank1	59
26/04/2003	9	Tank1	49	Tank1	62	29/04/2004	Tank1	62	Tank1	57
26/04/2003	10	Tank1	57	Tank1	60	29/04/2004	Tank1	50	Tank1	51
26/04/2003	11	Tank1	50	Tank1	50	29/04/2004	Tank1	64	Tank1	60
26/04/2003	12	Tank1	50	Tank1	49	29/04/2004	Tank1	63	Tank1	59
26/04/2003	13	Tank1	47	Tank1	58	29/04/2004	Tank1	60	Tank1	48
26/04/2003	14	Tank1	63	Tank1	50	29/04/2004	Tank1	59	Tank1	46

26/04/2003	15	Tank1	58	Tank1	60	29/04/2004	Tank1	68	Tank1	58
26/04/2003	16	Tank1	57	Tank1	60	29/04/2004	Tank1	67	Tank1	60
26/04/2003	17	Tank1	61	Tank1	51	29/04/2004	Tank1	62	Tank1	47
26/04/2003	18	Tank1	58	Tank1	51	29/04/2004	Tank1	67	Tank1	51
26/04/2003	19	Tank1	60	Tank1	55	29/04/2004	Tank1	62	Tank1	60
26/04/2003	20	Tank1	51	Tank1	60	29/04/2004	Tank1	60	Tank1	60
26/04/2003	21	Tank2	56	Tank1	63	29/04/2004	Tank2	67	Tank1	59
26/04/2003	22	Tank2	61	Tank1	48	29/04/2004	Tank2	61	Tank1	57
26/04/2003	23	Tank2	58	Tank1	58	29/04/2004	Tank2	57	Tank1	51
26/04/2003	24	Tank2	55	Tank1	51	29/04/2004	Tank2	58	Tank1	60

26/04/2003	25	Tank2	46	Tank1	60	29/04/2004	Tank2	57	Tank1	59
26/04/2003	26	Tank2	49	Tank1	56	29/04/2004	Tank2	49	Tank1	60
26/04/2003	27	Tank2	47	Tank1	56	29/04/2004	Tank2	48	Tank1	59
26/04/2003	28	Tank2	60	Tank1	58	29/04/2004	Tank2	52	Tank1	63
26/04/2003	29	Tank2	60	Tank1	62	29/04/2004	Tank2	57	Tank1	50
26/04/2003	30	Tank2	61	Tank1	60	29/04/2004	Tank2	58	Tank1	64
26/04/2003	31	Tank2	60	Tank1	59	29/04/2004	Tank2	65	Tank1	60
26/04/2003	32	Tank2	60	Tank1	60	29/04/2004	Tank2	65	Tank1	61
26/04/2003	33	Tank2	64	Tank1	59	29/04/2004	Tank2	60	Tank1	60
26/04/2003	34	Tank2	60	Tank1	60	29/04/2004	Tank2	60	Tank1	58

26/04/2003	35	Tank2	65	Tank1	50	29/04/2004	Tank2	56	Tank1	54
26/04/2003	36	Tank2	55	Tank1	59	29/04/2004	Tank2	60	Tank1	58
26/04/2003	37	Tank2	60	Tank1	55	29/04/2004	Tank2	65	Tank1	60
26/04/2003	38	Tank2	61	Tank1	48	29/04/2004	Tank2	59	Tank1	60
26/04/2003	39	Tank2	56	Tank1	54	29/04/2004	Tank2	59	Tank1	63
26/04/2003	40	Tank2	58	Tank1	58	29/04/2004	Tank2	49	Tank1	56
26/04/2003	41	Tank3	49	Tank2	48	29/04/2004	Tank3	60	Tank2	57
26/04/2003	42	Tank3	52	Tank2	45	29/04/2004	Tank3	64	Tank2	57
26/04/2003	43	Tank3	47	Tank2	48	29/04/2004	Tank3	50	Tank2	63
26/04/2003	44	Tank3	58	Tank2	65	29/04/2004	Tank3	55	Tank2	55

26/04/2003	45	Tank3	45	Tank2	67	29/04/2004	Tank3	54	Tank2	56
26/04/2003	46	Tank3	63	Tank2	65	29/04/2004	Tank3	61	Tank2	51
26/04/2003	47	Tank3	62	Tank2	65	29/04/2004	Tank3	60	Tank2	49
26/04/2003	48	Tank3	60	Tank2	65	29/04/2004	Tank3	47	Tank2	54
26/04/2003	49	Tank3	60	Tank2	67	29/04/2004	Tank3	51	Tank2	58
26/04/2003	50	Tank3	58	Tank2	60	29/04/2004	Tank3	56	Tank2	50
26/04/2003	51	Tank3	64	Tank2	62	29/04/2004	Tank3	58	Tank2	57
26/04/2003	52	Tank3	58	Tank2	49	29/04/2004	Tank3	66	Tank2	61
26/04/2003	53	Tank3	50	Tank2	61	29/04/2004	Tank3	63	Tank2	57
26/04/2003	54	Tank3	52	Tank2	56	29/04/2004	Tank3	63	Tank2	60

26/04/2003	55	Tank3	56	Tank2	65	29/04/2004	Tank3	60	Tank2	58
26/04/2003	56	Tank3	55	Tank2	51	29/04/2004	Tank3	58	Tank2	50
26/04/2003	57	Tank3	46	Tank2	58	29/04/2004	Tank3	59	Tank2	49
26/04/2003	58	Tank3	48	Tank2	50	29/04/2004	Tank3	52	Tank2	57
26/04/2003	59	Tank3	44	Tank2	51	29/04/2004	Tank3	62	Tank2	57
26/04/2003	60	Tank3	55	Tank2	51	29/04/2004	Tank3	62	Tank2	64
26/04/2003	61	Tank4	56	Tank2	53	29/04/2004	Tank4	64	Tank2	63
26/04/2003	62	Tank4	62	Tank2	60	29/04/2004	Tank4	47	Tank2	55
26/04/2003	63	Tank4	63	Tank2	64	29/04/2004	Tank4	47	Tank2	63
26/04/2003	64	Tank4	64	Tank2	56	29/04/2004	Tank4	59	Tank2	61

26/04/2003	65	Tank4	61	Tank2	60	29/04/2004	Tank4	58	Tank2	65
26/04/2003	66	Tank4	60	Tank2	65	29/04/2004	Tank4	56	Tank2	57
26/04/2003	67	Tank4	58	Tank2	64	29/04/2004	Tank4	48	Tank2	62
26/04/2003	68	Tank4	62	Tank2	50	29/04/2004	Tank4	49	Tank2	60
26/04/2003	69	Tank4	58	Tank2	57	29/04/2004	Tank4	61	Tank2	60
26/04/2003	70	Tank4	59	Tank2	60	29/04/2004	Tank4	61	Tank2	64
26/04/2003	71	Tank4	52	Tank2	61	29/04/2004	Tank4	60	Tank2	47
26/04/2003	72	Tank4	50	Tank2	60	29/04/2004	Tank4	60	Tank2	50
26/04/2003	73	Tank4	57	Tank2	55	29/04/2004	Tank4	63	Tank2	52
26/04/2003	74	Tank4	56	Tank2	51	29/04/2004	Tank4	62	Tank2	58

26/04/2003	75	Tank4	58	Tank2	62	29/04/2004	Tank4	60	Tank2	63
26/04/2003	76	Tank4	57	Tank2	51	29/04/2004	Tank4	66	Tank2	52
26/04/2003	77	Tank4	48	Tank2	57	29/04/2004	Tank4	44	Tank2	58
26/04/2003	78	Tank4	50	Tank2	56	29/04/2004	Tank4	56	Tank2	51
26/04/2003	79	Tank4	62	Tank2	50	29/04/2004	Tank4	57	Tank2	48
26/04/2003	80	Tank4	66	Tank2	65	29/04/2004	Tank4	52	Tank2	57

Dataset 4 (Andreou et al 2009)

The zoospore experiments where *Sphaerothecum destruens* spores were incubated in fresh water at different temperatures and observed for signs of motility and zoosporulation. The observations included the number of zoospores observed, their motility, and the number of spores observed. Measurements were taken from both cell-associated and cell-free spores.

Temperature	4	4	4	4	4	4	4
Day	0	1	2	3	4	5	6
Mean ZP1	0	0	0	47.3	33.5	NA	NA
Mean ZP2	0	0	0	NA	NA	57	240
Average 1 and 2	0	0	0	47.3	33.5	57	240
SD ZP	0	0	0	NA	NA	NA	NA
Mean Motile ZP1	0	0	0	14.5	2	NA	NA
Mean Motile ZP2	0	0	0	NA	NA	0.3	13
Average 1 and 2	0	0	0	14.5	2	0.3	13
SD motile ZP	0	0	0	NA	NA	NA	NA
Mean Spore1	0	55.3	32.5	3	13.3	NA	NA
Mean Spore2	0	55.3	32.5	NA	NA	9.8	5.5
Average 1 and 2	0	55.3	32.5	3	13.3	9.8	5.5
SD spore	0	0	0	NA	NA	NA	NA
Mean ZP1.C	0	0	0	94.5	67	NA	NA
Mean ZP2.C	0	0	0	NA	NA	114	480
Average 1 and 2	0	0	0	94.5	67	114	480
SD ZP cell	0	0	0	NA	NA	NA	NA
Mean Motile ZP1.C	0	0	0	29	4	NA	NA
Mean Motile ZP2.C	0	0	0	NA	NA	0.5	26
Average 1 and 2	0	0	0	29	4	0.6	26
SD motile ZP cell	0	0	0	NA	NA	NA	NA
Mean Spores 1.C	0	110.5	65	6	26.5	NA	NA
Mean Spores 2.C	0	110.5	65	NA	NA	19.5	11
Average 1 and 2	0	110.5	65	6	26.6	19.6	11
SD spores cell	0	0	0	NA	NA	NA	NA

4	4	4	4	4	4	4	4	4	4	4	4
18	17	16	15	14	13	12	11	10	9	8	7
430.5	364.5	375.5	269.3	382.3	NA	NA	385.3	427.3	328.3	199	113.3
NA	NA	577.5	479.3	573.8	714.5	464.8	NA	NA	416.5	354	321.5
430.5	364.5	476.5	374.3	478	714.5	464.8	385.3	427.3	372.4	276.5	217.4
NA	NA	142.8	148.5	135.4	NA	NA	NA	NA	62.4	109.6	147.3
7.5	5.3	6.3	16.3	2.8	NA	NA	8	9.3	5.3	7.3	5.8
NA	NA	2.3	2.3	15	29.8	8.8	NA	NA	4	2.8	11.3
7.5	5.3	4.3	9.3	8.9	29.8	8.8	4	9.3	4.6	5	8.5
NA	NA	2.8	9.9	8.7	NA	NA	NA	NA	0.9	3.2	3.9
9.5	9.5	11.3	9.3	12.5	NA	NA	8.5	7	10	12.3	9
NA	NA	3.5	6.5	5.5	17	9.3	NA	NA	6.5	2.5	15.5
9.5	9.5	7.4	7.9	9	17	9.3	8.5	7	8.3	7.4	12.3
NA	NA	5.5	1.9	4.9	NA	NA	NA	NA	2.5	6.9	4.6
861	729	751	538.5	764.5	NA	NA	770.5	854.5	656.5	398	226.5
NA	NA	1155	958.5	1147.5	1429	929.5	NA	NA	833	708	643
861	729	953	748.5	956	1429	929.5	770.5	854.5	744.8	553	434.8
NA	NA	285.7	297	270.8	NA	NA	NA	NA	124.8	219.2	294.5
15	10.5	12.5	32.5	5.5	NA	NA	16	18.5	10.5	14.5	11.5
NA	NA	4.5	4.5	30	59.5	17.5	NA	NA	8	5.5	22.5
15	10.6	8.5	18.5	17.8	59.6	17.6	8	18.6	9.3	10	17
NA	NA	5.7	19.8	17.3	NA	NA	NA	NA	1.8	6.4	7.8
19	19	22.5	18.5	25	NA	NA	17	14	20	24.5	18
NA	NA	7	13	11	34	18.5	NA	NA	13	5	31
19	19	14.8	15.8	18	34	18.6	17	14	16.5	14.8	24.5
NA	NA	11	3.9	9.9	NA	NA	NA	NA	4.9	13.8	9.2

15	15	4	4	4	4	4	4	4	4	4	4
1	0	28	27	26	25	24	23	22	21	20	19
37	0	NA	NA	NA	374.3	217.5	305.5	284.3	NA	NA	
21.75	20.8	528.8	420.5	453.3	NA	NA	464.3	434.8	566	640.3	NA
29.4	10.4	528.8	420.5	453.3	374.3	217.5	384.9	359.5	566	640.3	NA
10.8	14.7	NA	NA	NA	NA	NA	112.3	106.4	NA	NA	NA
1	0	NA	NA	NA	1.8	1.3	1	1	NA	NA	NA
0.25	0.8	0.8	1.8	2.3	NA	NA	1.3	1.3	1.5	2.8	NA
0.6	0.4	0.8	1.8	2.3	1.8	1.3	1.1	1.1	1.5	2.8	NA
0.530	0.5	NA	NA	NA	NA	NA	0.2	0.2	NA	NA	NA
23.75	49.5	NA	NA	NA	16.3	11.3	16.5	5.3	NA	NA	NA
16.5	14	8.5	9	13.5	NA	NA	10	9.8	25.8	6.3	NA
20.1	31.8	8.5	9	13.5	16.3	11.3	13.3	7.5	25.8	6.3	NA
5.1	25.1	NA	NA	NA	NA	NA	4.6	3.2	NA	NA	NA
41.5	0	NA	NA	NA	748.5	435	611	568.5	NA	NA	NA
0	0	1057.5	841	906.5	NA	NA	928.5	869.5	1132	1280.5	NA
20.8	0	1057.5	841	906.5	748.5	435	769.8	719	1132	1280.5	NA
29.3	0	NA	NA	NA	NA	NA	224.5	212.8	NA	NA	NA
1.5	0	NA	NA	NA	3.5	2.5	2	2	NA	NA	NA
0	0	1.5	3.5	4.5	NA	NA	2.5	2.5	3	5.5	NA
0.8	0	1.6	3.6	4.6	3.6	2.6	2.3	2.3	3	5.5	NA
1.1	0	NA	NA	NA	NA	NA	0.4	0.4	NA	NA	NA
28	0	NA	NA	NA	32.5	22.5	33	10.5	NA	NA	NA
99	0	17	18	27	NA	NA	20	19.5	51.5	12.5	NA
63.5	0	17	18	27	32.6	22.6	26.5	15	51.6	12.6	NA
50.2	0	NA	NA	NA	NA	NA	9.2	6.4	NA	NA	NA

15	15	15	15	15	15	15	15	15	15	15	15
13	12	11	10	9	8	7	6	5	4	3	2
700.5	NA	NA	1114	641.8	865	858.5	857.5	NA	NA	472.5	189.8
662.5	968.3	871.5	NA	NA	575.3	659	762.5	678.5	746.5	NA	NA
681.5	484.1	871.5	1114	641	720.1	758.8	810	678.5	746.5	472.5	189.8
26.9	NA	NA	NA	NA	204.9	141.1	67.2	NA	NA	NA	NA
1.5	NA	NA	5.25	7.75	13.5	11	10.5	NA	NA	16.3	12.5
12.3	21.8	4.5	NA	NA	5.75	5.25	7	8.8	8.8	NA	NA
6.9	21.8	4.5	5.3	7.8	9.6	8.1	8.8	8.8	8.8	16.3	12.5
7.6	NA	NA	NA	NA	5.5	4.1	2.5	NA	NA	NA	NA
4.8	NA	NA	13.5	5.25	8	12.3	10.3	NA	NA	5.5	7
5.3	9.75	3.5	NA	NA	5	4.3	21.8	3.8	3.5	NA	NA
5	9.8	3.5	13.5	5.3	6.5	8.3	16	3.8	3.5	5.5	7
0.4	NA	NA	NA	NA	2.121	5.7	8.1	NA	NA	NA	NA
1936.5	1743	NA	NA	1150.5	1318	1525	1357	1493	NA	NA	43.5
NA	NA	2228	1283.5	1730	1717	1715	NA	NA	945	379.5	74
1936.5	1743	2228	1283.5	1440.3	1517.5	1620	1357	1493	945	379.5	58.8
NA	NA	NA	NA	409.8	282.1	134.4	NA	NA	NA	NA	21.6
43.5	9	NA	NA	11.5	10.5	14	17.5	17.5	NA	NA	0.5
NA	NA	10.5	15.5	27	22	21	NA	NA	32.5	25	2
43.5	9	10.5	15.5	19.3	16.3	17.5	17.5	17.5	32.5	25	1.3
NA	NA	NA	NA	11	8.1	4.9	NA	NA	NA	NA	1.1
19.5	7	NA	NA	10	8.5	43.5	7.5	7	NA	NA	33
NA	NA	27	10.5	16	24.5	20.5	NA	NA	11	14	47.5
19.5	7	27	10.5	13	16.5	32	7.5	7	11	14	40.3
NA	NA	NA	NA	4.2	11.3	16.3	NA	NA	NA	NA	10.3

25	25	25	25	25	25	25	15	15	15	15	15
6	5	4	3	2	1	0	18	17	16	15	14
		869.3	462.8	678	231	0	NA	902.3	871.3	933	975.3
516	664.5	NA	NA	504.3	333	0	NA	NA	NA	694.3	727.8
516	664.5	869.3	462.8	591.1	282	0	NA	902.3	871.3	813.6	851.5
NA	NA	NA	NA	122.9	72.1	0	NA	NA	NA	168.8	175.0
NA	NA	20	123	46.75	31.25	0	NA	2.25	4.8	16.8	12
1	10	NA	NA	7.75	7	0	NA	NA	NA	0.8	13.3
0.5	5	10	6.1	27.3	19.1	0	NA	2.3	4.8	8.8	12.6
NA	NA	NA	NA	27.6	17.1	0	NA	NA	NA	11.3	0.9
NA	NA	10.5	7.5	11.5	6	0	NA	8.8	3.3	10.5	12.3
1.8	14	NA	NA	2.5	44.8	0	NA	NA	NA	1.75	1.75
1.8	14	10.5	7.5	7	25.4	0	NA	8.8	3.3	6.1	7
NA	NA	NA	NA	6.4	27.4	0	NA	NA	NA	6.2	7.4
NA	NA	869.3	462.8	678	231	0	NA	NA	1388.5	1455.5	1325
516	664.5	NA	NA	504.3	333	0	1804.5	1742.5	1866	1950.5	1401
516	664.5	869.3	462.8	591.1	282	0	1804.5	1742.5	1627.3	1703	1363
NA	NA	NA	NA	122.9	72.1	0	NA	NA	337.6	350	53.7
NA	NA	20	12.3	46.8	31.3	0	NA	NA	1.5	26.5	24.5
1	10	NA	NA	7.8	7	0	4.5	9.5	33.5	24	3
1	10	20	12.3	27.3	19.1	0	4.5	9.5	17.5	25.3	13.8
NA	NA	NA	NA	27.6	17.1	0	NA	NA	22.6	1.8	15.2
NA	NA	10.5	7.5	11.5	6	0	NA	NA	3.5	3.5	10.5
1.8	14	NA	NA	2.5	44.8	0	17.5	6.5	21	24.5	9.5
1.8	14	10.5	7.5	7	25.4	0	17.5	6.5	12.3	14	10
NA	NA	NA	NA	6.4	27.4	0	NA	NA	12.4	14.8	0.7

30	30	30	30	30	30	25	25	25	25	25
5	4	3	2	1	0	11	10	9	8	7
NA	367.3	314	688	201.3	0	380.3	619.3	596.5	752	1465
297.8	NA	NA	554.5	130.8	0	3041.8	NA	NA	539	485
297.8	367.3	314	621.3	166	0	1711	619.3	596.5	645.5	975
NA	NA	NA	94.4	49.9	0	1882	NA	NA	150.6	693
NA	6.3	13.5	24.3	23	0	0	0	0.25	0	0.3
7.3	NA	NA	6.8	9.75	0	26.3	NA	NA	0	0.5
7.3	6.3	13.5	15.5	16.4	0	13.1	0	0.1	0	0.4
NA	NA	NA	12.4	9.4	0	18.6	NA	NA	0	0.2
NA	5.3	20.3	61.3	28.5	0	3.5	2.5	6	9	14
4.5	NA	NA	46.8	13.8	0	67.8	NA	NA	0.3	4.5
4.5	5.3	20.3	54	21.1	0	35.6	2.5	6	4.6	9.3
NA	NA	NA	10.3	10.4	0	45.4	NA	NA	6.2	6.7
NA	367.3	314	688	201.3	0	380.3	619.3	596.5	752	1465
297.8	NA	NA	554.5	130.8	0	NA	NA	NA	539	485
297.8	367.3	314	621.3	166	0	380.3	619.3	596.5	645.5	975
NA	NA	NA	94.4	49.9	0	NA	NA	NA	150.6	693
NA	6.3	13.5	24.3	23	0	0	0	0.3	0	0.3
7.3	NA	NA	6.8	9.8	0	NA	NA	NA	0	0.5
7.3	6.3	13.5	15.5	16.4	0	0	0	0.3	0	0.4
NA	NA	NA	12.4	9.4	0	NA	NA	NA	0	0.2
NA	5.3	20.3	61.3	28.5	0	3.5	2.5	6	9	14
4.5	NA	NA	46.8	13.8	0	NA	NA	NA	0.3	4.5
4.5	5.3	20.3	54	21.1	0	3.5	2.5	6	4.6	9.3
NA	NA	NA	10.3	10.4	0	NA	NA	NA	6.2	6.7

Appendix 2

Cook's distance

Regression models were run in Chapter 3 (Epidemiological parameter estimation) to determine correlations between parameters. For each proposed relationship, the influence of points with high leverage was examined to ensure that outliers did not bias the regression outcome. This influence was measured by Cook's distance D_i (Eq. A2.1), a key diagnostic for determining the accuracy of linear regressions (Zhu et al. 2012).

$$D_i = \frac{\sum_{j=1}^{n}(Y_j - Y_{j(i)})2}{p*MSE} \qquad \text{Eq. A2.1}$$

where Y_j is the predicted value of the model for observation j, $Y_{j(i)}$ is the predicted value for observation j when observation i was omitted, and p is the number of parameters in the model. Higher Cook's distance is associated with high leverage of residuals, indicating that the regression was not valid. Thus, any regression line with points that had Cook's distance greater than 1 was disregarded. The residuals of each proposed regression and the corresponding Cook's distance calculations are located here.

Hypothesis 1: Incubation and mortality from infection

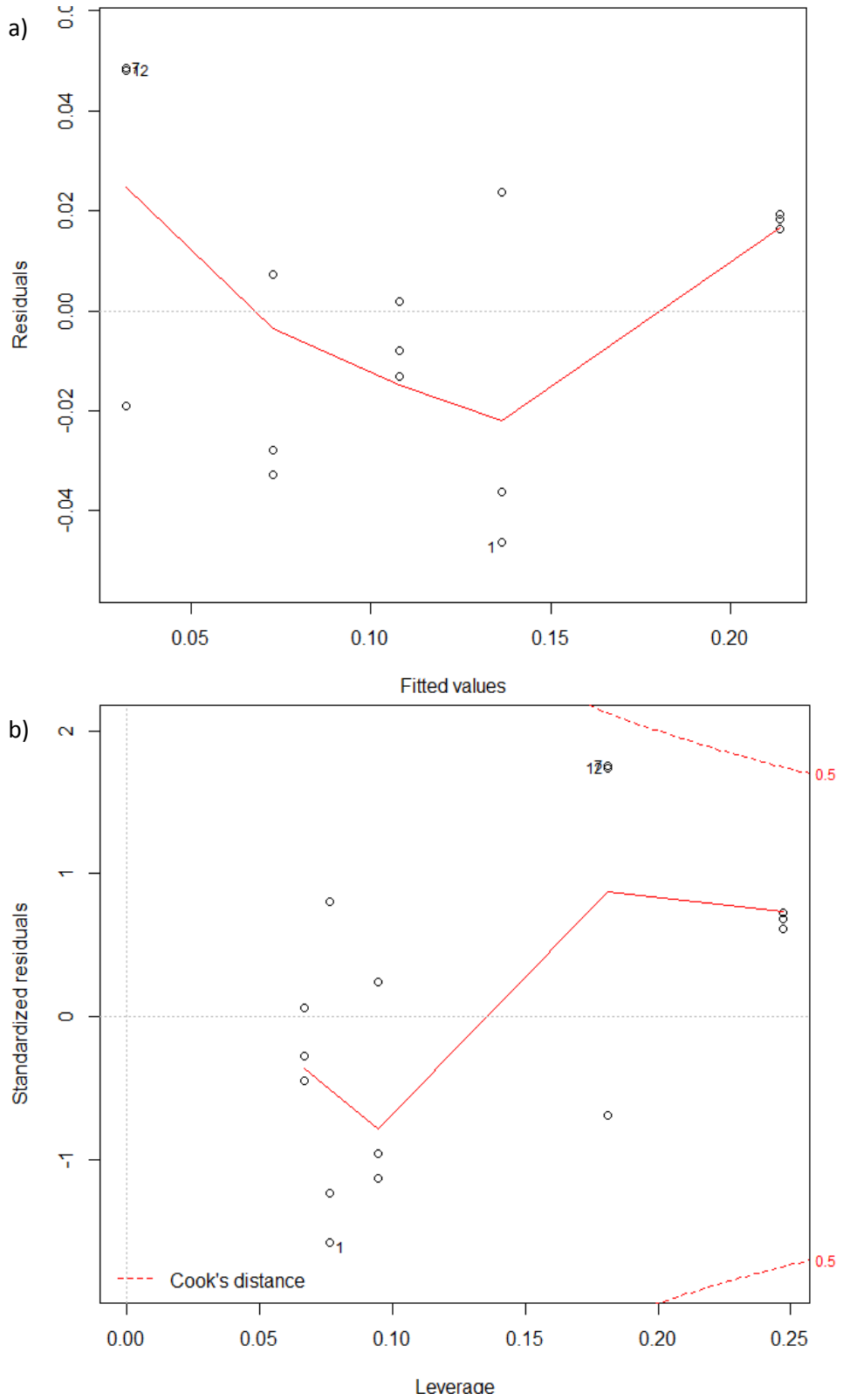


Figure A2.1. The residuals of the linear model between mortality and incubation. There are a number of outliers in the data (a) that could affect the slope of the model. When the leverage of these points was considered (b) the model was not significantly affected (Cook's distance < 0.5).

Hypothesis 2: Incubation rate and recovery rate

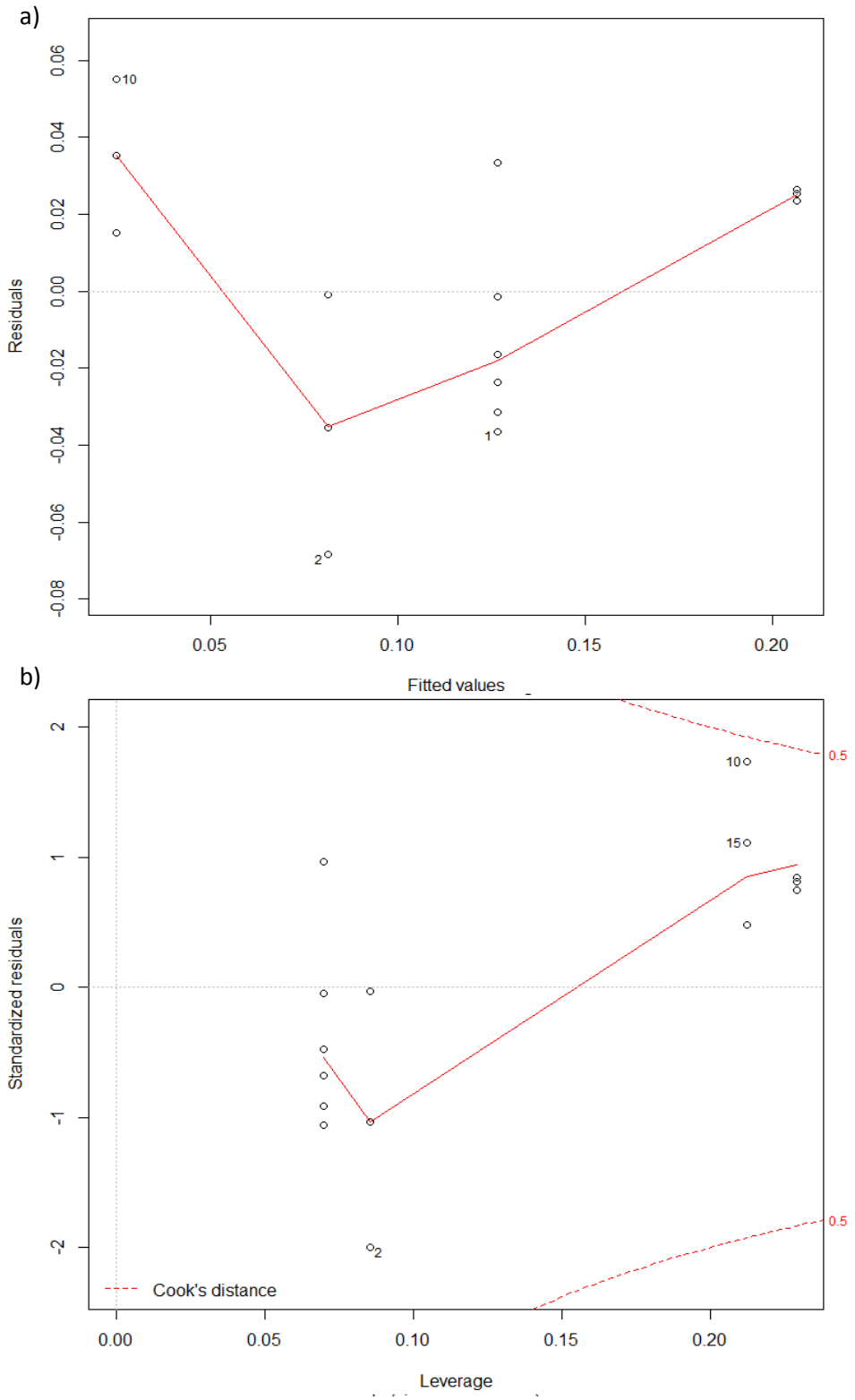


Figure A2.2.The fitted values of the linear model between recovery and incubation rate against the corresponding residuals (a). There were no major outliers and all points had a Cook's distance <0.5 (b). Thus, there was a significant correlation between recovery rate and incubation rate based on the data.

Hypothesis 3: Direct and environmental transmission

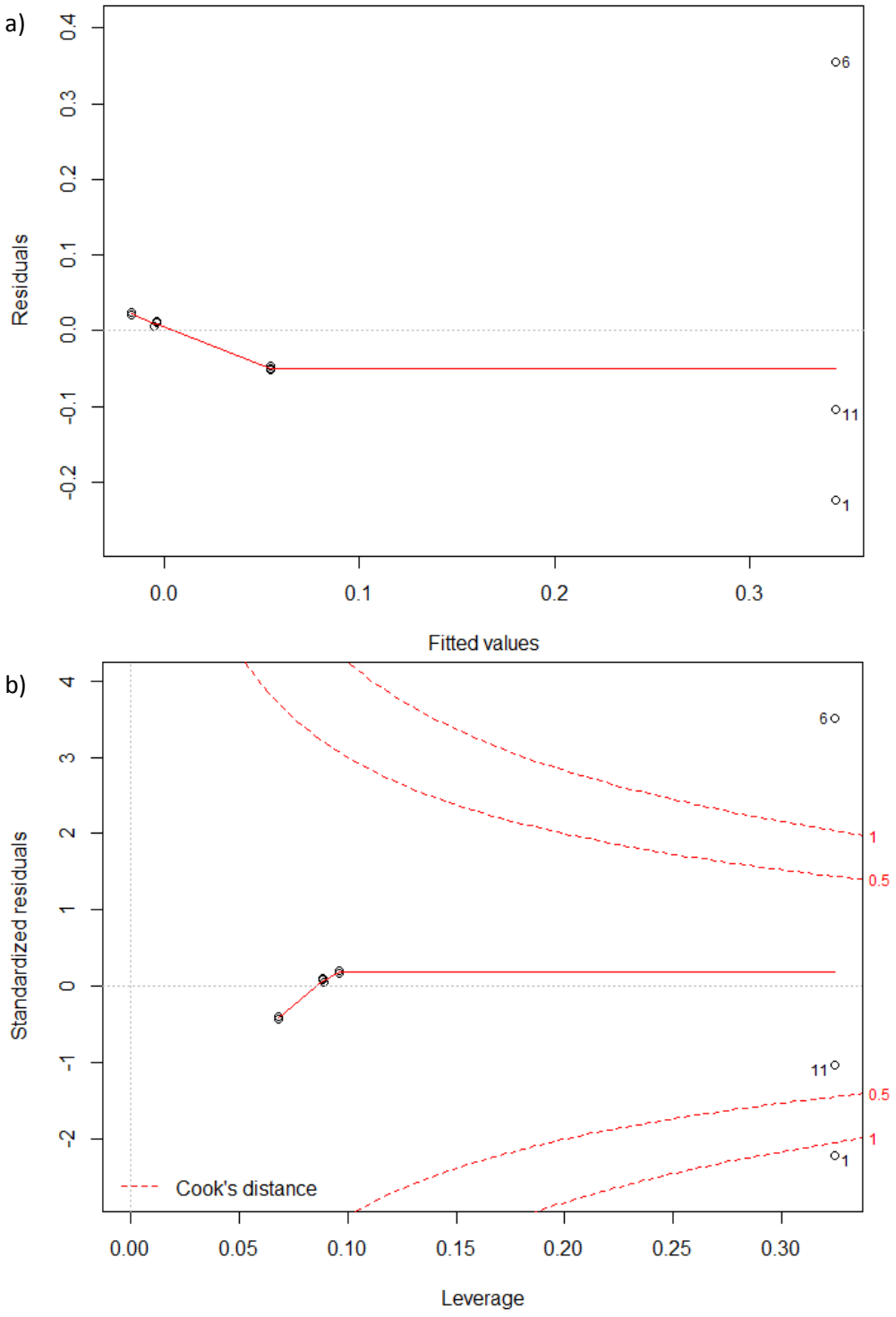


Figure A2.3. Residuals for the direct vs. environmental transmission linear model. The presence of outliers (a) suggested that this correlation should be treated with caution, as those outliers also had high leverage (b) and thus influenced the trend line. The regression was therefore considered invalid.

Hypothesis 5: Environmental transmission and spore shedding rate

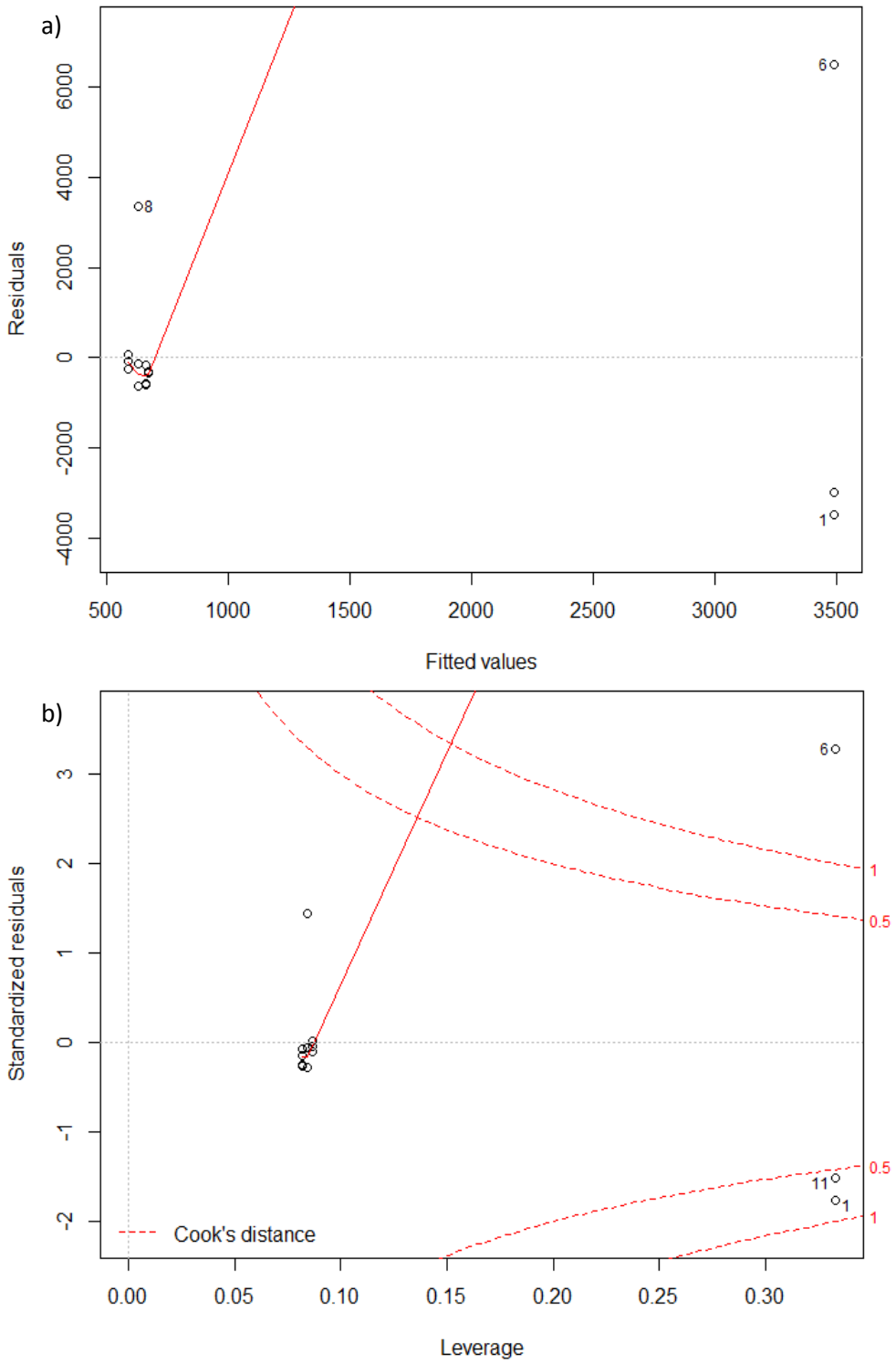


Figure A2.4.The fitted values of the linear model between environmental transmission and spore release against the corresponding residuals. As can be seen from the residuals (a) and the leverage (b) graphs, there were several outliers with high leverage. Thus, there was no significant correlation between environmental transmission and spore release based on the data.

Appendix 3

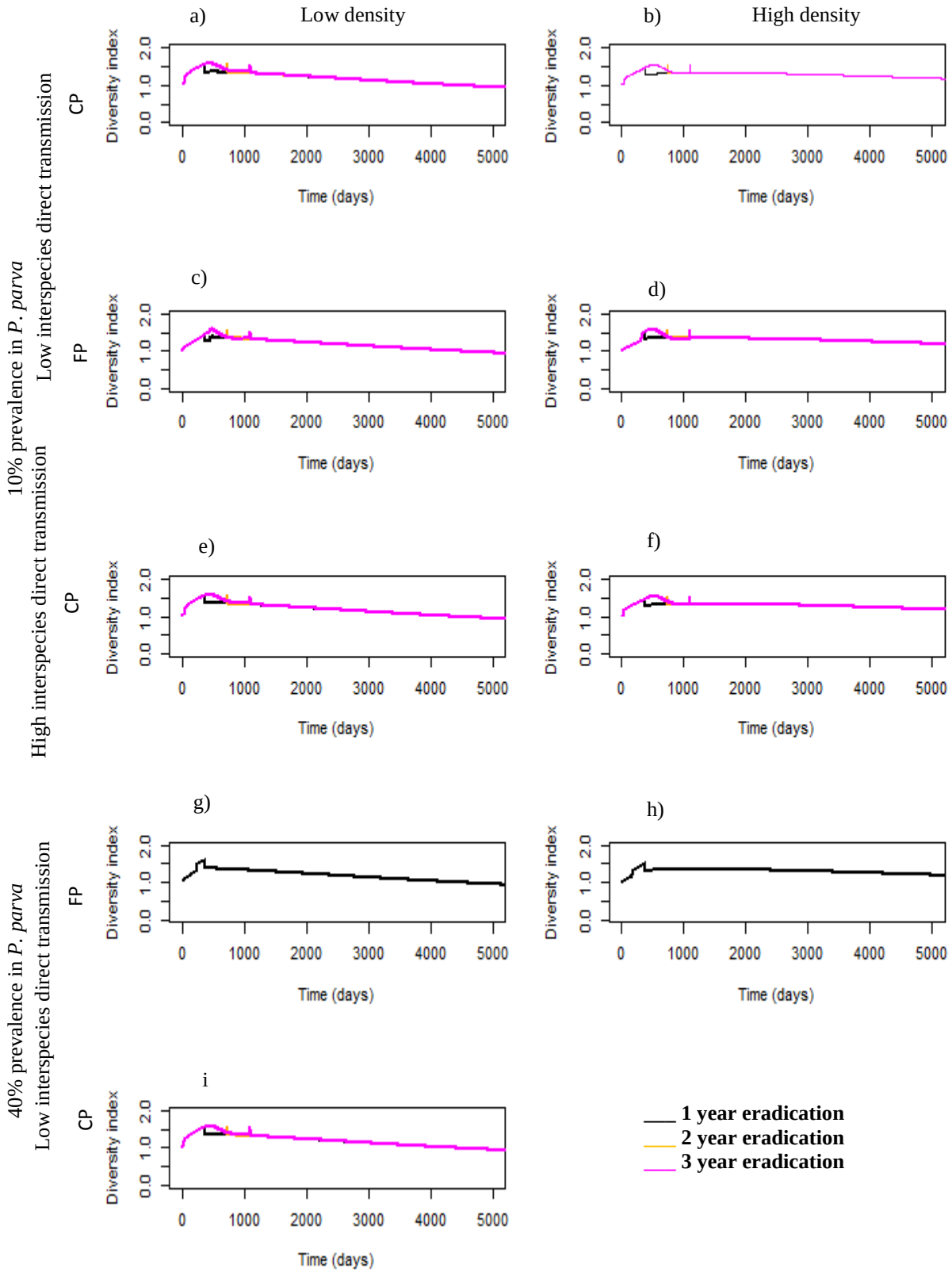


Figure A3.1. The Shannon diversity index of all eradication scenarios over time. High-density populations maintain higher levels of diversity than low density communities. (0.96 vs. 0.68, respectively) The proximity between introduced host and local communities affected the level of biodiversity initially (CP = close proximity; FP = far proximity). However, in the long term, communities at each density declined to similar levels of biodiversity across all scenarios.

Appendix 4

The specific relationships which were assumed to exist between species and the parameters selected for the community model in Chapter 5 were discussed in detail.

Competitive interactions

The secondary consumer species were based on facultative piscivores *Salmo trutta* and *Oncorhynchus mykiss* (Beyer et al 2010). It was assumed that they preyed on both *P. parva* and *L. delineatus*, as the latter two species occupy similar niches. (Note that in the wild, young of the year *R. rutilus* and *C. carpio* would be preyed on by the salmonids, but as the model only included average adult sizes for each species, these species were not considered prey). Given that an overlapping trophic niche has been demonstrated between *P. parva* and *L. delineatus* (Pinder and Gozlan 2003), in the model the two species could compete for space and/or resources when co-existing in a small area, in conjunction with the principle of limiting similarity (Meszéna et al. 2006). The model further assumed that the healthy carrier *P. parva* competed with both *R. rutilus* and *C. carpio* through exploitative competition, although not to extinction (Xie et al. 2001, Britton, Cucherousset, et al. 2011, Britton 2012). Competition for habitat use between the two consumer species was also included (Gatz et al. 1987). The published literature used to create these general assumptions is summarised in Table A4.1.

Table A4.1. A summary of the competitive interactions included in the models, and corresponding reference.

Competitive interaction	Reference
Trout prey on *P. parva* and *L. delineatus*	Pinder and Gozlan 2003; Beyer et al 2007
P. parva competes with *R. rutilus* and *C. carpio* for resources and space at high densities, and has been shown to prey on their eggs	Xie et al 2001; Britton et al 2007; Britton 2012
Species that occupy similar trophic niches compete for resources	Menge & Sutherland 1976; Gatz et al 1987; Meszena et al 2006

Competition coefficient

To estimate realistic values for competition coefficient *a* in the SEIR model, infection was excluded by setting infection prevalence to 0 and competition coefficient values were selected based on the assumptions in Table S1. A matrix of initial arbitrary values for *a* was set and then varied until population trends consistent with the model assumptions were observed (Table A4.2). A sensitivity analysis was run on these values to determine their effect on population dynamics, by adjusting each value by ± 30%. The final values selected for *a* and used in Chapter 5 reflected an equilibrium population that had interspecific competition but no infection, based on the model's general assumptions.

Table A4.2. Values for competition coefficient a in the SEIR multi-host model.

	P. parva	*L. delineatus*	*R. rutilus*	*C. carpio*	*O. mykiss*	*S. trutta*
P. parva	2×10^{-7}	2×10^{-7}	4×10^{-7}	4×10^{-7}	1×10^{-7}	1×10^{-7}
L. delineatus	2×10^{-7}	2×10^{-7}	4×10^{-7}	4×10^{-7}	1×10^{-7}	1×10^{-7}
R. rutilus	1×10^{-7}	1×10^{-7}	2×10^{-7}	2×10^{-8}	1×10^{-10}	1×10^{-10}
C. carpio	2×10^{-8}	2×10^{-8}	2×10^{-8}	2×10^{-7}	1×10^{-10}	1×10^{-10}
O. mykiss	3×10^{-7}	3×10^{-7}	1×10^{-10}	1×10^{-10}	2×10^{-7}	2×10^{-10}
S. trutta	3×10^{-7}	3×10^{-7}	1×10^{-10}	1×10^{-10}	1×10^{-7}	2×10^{-7}

Susceptibility to infection

The epidemiological parameter estimations for all cyprinid species were estimated in Chapters 2 and 3. The parameter values for *S. salar* were used initially for the secondary consumer species and then varied to explore a range of susceptibility of the two species (see Table A4.3). This allowed the exploration of secondary consumer species with differing responses to infection (based on *S. salar*, *O. mykiss*, and *S. trutta*). The number of spores released by infected hosts per day was assumed to be 20% of the values estimated from the single host models, to account for water current and attachment to sediments or non-hosts in natural systems, compared to the closed tank systems in the experimental trials (Searle et al. 2013). For the purpose of this study, these estimations represented of a range of species that varied in disease resistance and in trophic interactions. The parameters may be numerically uncertain, and the particular species assemblage unlikely, but they fit the purpose of the model to simulate a theoretical community and answer questions about community resilience.

Parameter estimation

The secondary consumer species' values were varied in the main text to observe the effects of changing host susceptibility at high trophic levels on community resilience. These variations were only applied to the secondary consumers in order to test how changing the progress of infection in some species within a community affects the abundance of other species. First, the three epidemiological parameters were varied individually to quantify the effect of each one (incubation rate, mortality from infection, and recovery rate) on community survival.

1. Secondary consumer species' epidemiological parameter values were kept consistent with those estimated for *S. salar*, to represent highly susceptible species.
2. To model an extremely slow-growing infection, the incubation time of the infection for both secondary consumer species was lengthened from 0.042 to 0.004.
3. The secondary consumer species' recovery rates were changed from 0.037 to 0.07 to reduce the duration of infection, modelling species that could recover from *S. destruens* infection rapidly once the incubation period had elapsed.
4. To test the effect of reduced host mortality from infection on community resilience, the virulence of the pathogen in the secondary consumer species was reduced to 0.003. This value was selected as it indicated extremely low susceptibility to disease.

Following the individual parameter variations, it was assumed that the epidemiological parameters were correlated with one another. Mortality from infection was varied from 0-0.5, and recovery rate from 0-0.3. Within this examination, the incubation rate of the two hosts was varied according to a relationship with mortality from infection (see Chapter 3). Finally, the combined effects of the three epidemiological parameters on community survival were observed based on the relationships found in Chapter 3, by examining the community Shannon biodiversity index 10 years after species introduction.

Table A4.3. Parameters used in the epidemiological SEIR simulation model.

Parameters	*P. parva*	*L. delineatus*	*R. rutilus*	*C. carpio*	*S. salar*
Spore release (φ)	10	24	33	24	18
Environmental uptake (ε)	0.0007	0.0007	0.0007	0.0007	0.0007
Direct transmission (β)	0.011	0.015	0.080	0.015	0.015
Birth (b)	0.0100	0.0100	0.004	0.011	0.000430
Natural mortality (m)	0.00043	0.00055	0.00017	0.00014	0.000084
Threshold values K (Ke, Ks, Ka, Kg)	30,000; 7,200; 3,000; 9,000	30,000; 7,200; 3,000; 9,000	30,000; 7,200; 3,000; 9,000	30,000; 7,200; 3,000; 9,000	30,000; 7,200; 3,000; 9,000
Incubation (σ)	0.072	0.230	0.095	0.013	0.042
Recovery (γ)	0.108	0.140	0.082	0.065	0.037
Mortality from infection (α)	0	0.300	0.130	0.017	0.06
Spore mortality rate (μ)	0.071	0.071	0.071	0.071	0.071

Appendix 5

Identifying the knowledge gaps related to *S. destruens* and other generalist fungal pathogens enabled the design of experiments that could help further the understanding of this pathogen's dynamics.

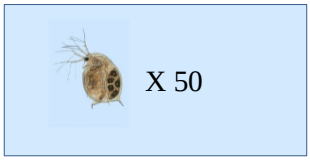
Spores of the amphibian chytrid fungus are predated on by *Daphnia*, resulting in a lower prevalence of infection in that habitat (Schmeller et al. 2013, Searle et al. 2013). Given the similarities between the two pathogens, it is likely that *Daphnia* would also prey on *S. destruens* spores and/or zoospores. If true this can indicate a viable management approach for lowering environmental transmission of *S. destruens* in the wild. However, there are several key differences between the chytrid fungus system and the *S. destruens* system: some hosts of *S. destruens* prey on *Daphnia* (Slusarczyk et al. 2005, Bartosiewicz and Gliwicz 2011); if the infection survives in *Daphnia* this indicates a potentially new method of infection of fish hosts. However, if the infection does not survive in *Daphnia*, this approach could be a useful disease management tool. Furthermore, by establishing how many spores one *Daphnia* can ingest, and then how many *Daphnia* are preyed on by a fish host, the results can lead to a more precise estimation of the infection threshold level for the host. The following experiment was designed to answer these questions (Fig. A5.1).

Step 1

- Put set concentration of *S. destruens* spores in one tank (**10 replicates**)
- Place 50 *Daphnia* in the water
- Measure the concentration of spores in the water at 1, 2, 3 day intervals.

→ Do *Daphnia* consume *S. destruens* spores? At what levels?

→ Can this be a viable management technique?



Step 2

- Extract *Daphnia* from tanks after 3 days, wash gently with sterile water
- Place 100 *Daphnia* into tank with 10 uninfected susceptible fish which prey on *Daphnia* (**3 replicates**)-
  - Measure of high infection pressure
- Place 50 Daphnia into tank with 10 uninfected susceptible fish which prey on *Daphnia* (**3 replicates**)-
  - Measure of low infection pressure
- Monitor fish hosts for signs of infection for up to 3 months- histopathology and PCR

→ Can susceptible fish become infected via this pathway? Can this help determine the infection threshold more precisely?

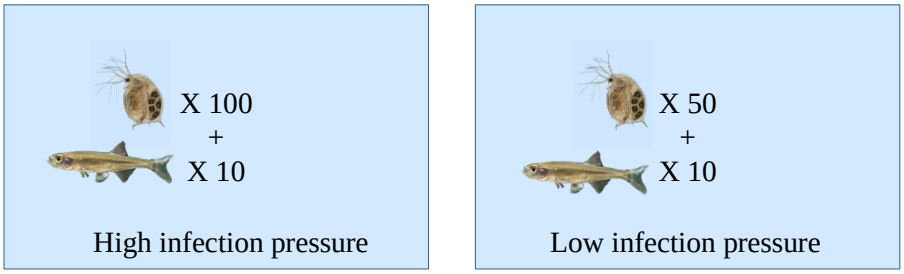


Figure A5.1 Experimental design to demonstrate if *Daphnia* can decrease the prevalence of *Sphaerothecum destruens* spores in a habitat and determine the infection threshold in susceptible hosts more precisely.