Infection in ecosystems:

data, models and effects

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Infection in ecosystems: data, models and effects

Infectie in ecosystemen: data, modellen en effecten

(met een samenvatting in het Nederlands)

Infekcije u ekosistemima: podaci, modeli i uticaji

(sa rezimeom na srpskom)

Proefschrift

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Chapter 1

Introduction

1.1. General introduction

Imagine the African Savannah. Buffalos, zebras and wildebeest are grazing at dawn. Between the bushes, some wildebeest have died and lions are fighting over their carcasses, while hyenas, jackals and vultures are waiting for the moment when they will be able to snatch the leftovers of the lion's food. Many wildebeest are dehydrated and weakened from severe drought this year. The wildebeest are also infected with a *Babesia* parasite, and they carry many ticks that transmit it, but the lions are not aware of this. Some of the lions are themselves infected with Canine Distemper Virus, lowering their resistance to other infections, and perhaps making them tired and slow and changing their hunting behaviour. In fact, this combination of circumstances is probably the reason why lions have an easy feast this morning. What the lions also do not know is that they are susceptible to the wildebeest's infection; several lions that get infected while eating will end up dying from the combined infection. In addition, the hyena can get infected by being in contact with other hyenas or lions, as well as by eating the infected wildebeest. At the same time in this image, some of the zebra carry the anthrax bacteria, some of the buffaloes carry the foot and mouth disease virus, and all of them have various species of worm parasites. Several of these have life stages that occur in the soil or on the grass. All of this goes unseen. It is very easy to neglect infectious agents since they are so small and many times not even visible to our eyes. However, taking into consideration the fact that

Figure 1.1. Typical savanna food web

probably every species in the world is a host for at least one species of infectious agent, we can imagine how profound the effects of these agents should be on our ecosystems.

1.2. Infectious agents in food webs and ecosystems

Infectious agents are a diverse group of organisms that interact directly with their host and indirectly with non-host species through ecological interaction. Herewith that may affect the structure, functioning and stability of ecological communities. Direct interaction with the host can result in subclinical or clinical disease in infected individuals, possibly changing their behaviour or the way they contribute to the dynamics of other species for example because such a species is a predator, a prey or a competitor of the host species. Infected prey may be easier to catch, or may be less nutritious. Infected predators may have a reduced ability to hunt and catch prey. Competitors may take advantage of food or habitat.

Infectious agents received relatively little explicit attention in the research of food webs and ecosystems for a long time. It is only in the last two decades, that ideas on the effects of infectious agents, and their potential importance in food webs, became more prominent. The need to incorporate infectious agents into food web analysis came mostly from ecologists who provided empirical data and observations on patterns in ecosystem behaviour (Amundsen *et al.* 2009, Huxham *et al.* 1995, Kuris *et al.* 2008, Memmott *et al.* 2000, Thompson *et al.* 2004) and introduced the idea that parasites should be added to

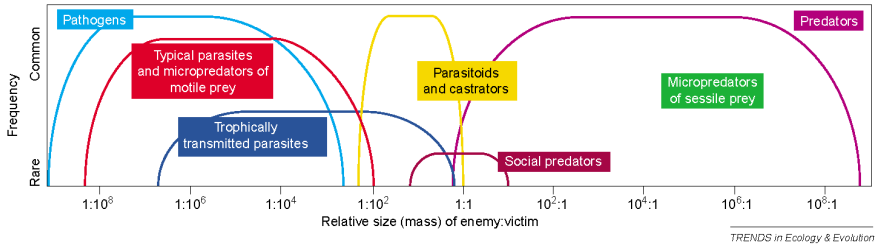


Figure 1.2. Conceptual diagram of the relative size/mass for several different trophic strategies. The horizontal axis represents the relative mass of individual (or clonal) natural enemies to (individual) victims along a log scale. Colored lines indicate locations along this axis where different strategies tend to lie. Adopted from ("Trophic strategies, animal diversity and body size", Lafferty and Kuris 2002).

ecological communities to better understand the complexity of food webs and the related food web dynamics and stability (Arias-González and Morand 2006, Beckerman and Petchey 2009, Byers 2009, Edeline *et al.* 2008, Getz 2009, Lafferty *et al.* 2006a, 2008b, Marcogliese and Cone 1997). The potential reasons for neglecting the importance of infectious agents and difficulties of including them into food webs and ecosystems are their size, diversity and complexity of life stages.

Infectious agents represent living organisms that live on or in other organisms (their hosts) for most of their life while benefiting from their nutrients (Poulin and Morand 2000). Their size can vary from really small (viruses) to sizes comparable to that of their host (Figure 1.2). Furthermore, It is impossible to estimate the number of parasitic species: many hosts species are unstudied or under-sampled (Poulin and Morand 2000), while the discovery of cryptic types of parasitic species increases the uncertainty on exact diversity numbers (Dobson *et al.* 2008). Infectious agents can broadly be distinguished into microparasites (viruses, bacteria, fungi, protozoa), macroparasites (nematodes, trematodes and cestodes), ectoparasites (fleas and ticks), and parasitic castrators and parasitoids (Figure 1.2 & 1.3, Kuris and Lafferty 2000, Lafferty and Kuris 2002).

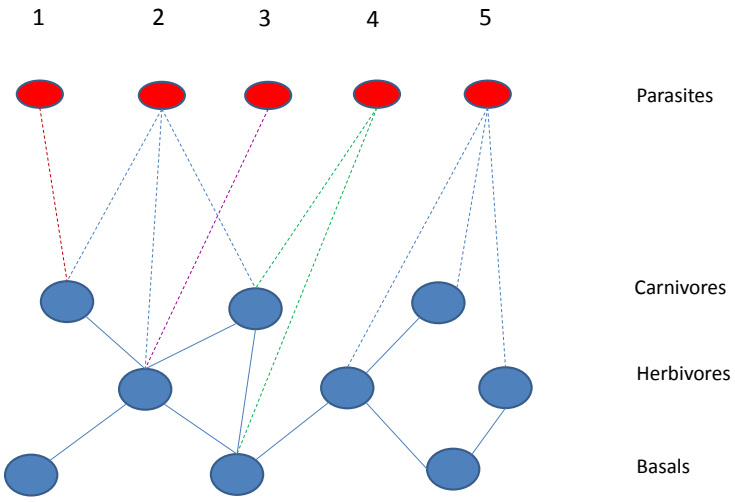


Figure 1.3. Diversity of parasites and type of links 1) Host specific (I & III) or general (II & IV, V) 2) Microparasites (I), macroparasites (II & V), parasitoids (III), parasitic castrator (IV)

Many infectious agents, especially macroparasites have complex life cycles and need more than one host in order to complete it. A typical example of a complex life cycle is that of the digenean trematode, involving three transmission steps. First, the eggs released from adult worms in the definitive host hatch into miracidia. Second, this life stage of the parasite then finds a suitable mollusc as the first intermediate host, which next releases free-living short-lived cercariae into its environment. Cercariae locate a suitable second intermediate host where they transform to metacercariae which must be ingested together with the second intermediate host by an appropriate definitive host for the life cycle to be completed. However, many of these trematodes have life cycles that are reduced by having less life stages (Poulin and Cribb 2002).

Transmission of infectious agents in their ecological communities can be direct, realized between infectious and susceptible individuals of one host species (within-species transmission), between individuals of different host species (between-species transmission) or by feeding on another infected individual (trophic transmission). It can also be indirect, and species can get infected for example through water or soil contamination, or other parts of the environment. Finally, some infectious agents need additional species, like mosquitoes and ticks, for transmission to occur (vector transmission), where these trans-

mitting species can contain essential steps necessary for the completion of the biological life cycle of the agent.

Despite the data that are becoming available, and ecosystem phenomena that have been described, the role of infectious agents inside of ecological communities is still not clear. Infectious diseaseagents are moderately to highly host specific. Simultaneously, all species are hosts to a range of infectious agents and the same species can exhibit a different part of that range in different ecosystems (Sukhdeo 2012).

In food webs, represented as diagrams of species' trophic (consumer-resource) interactions, infectious agents were for a long time considered as another type of consumer (Raffel *et al.* 2008). The most important difference compared to typical consumer is because they attack one victim/host per life stage (Dobson and Hudson 1986). On the other hand, many parasites can act as a resource for other living species in the food web (Johnson *et al.* 2010, Thieltges *et al.* 2013), which represents a direct trophic interaction of infectious agents. Another trophic interaction of infectious agents is when they are trophically transmitted.

Non-trophic interactions of infectious agents in food webs are represented through their influence on a host's vital rates and behaviour (Kéfi *et al.* 2012). These changes translate from infected individuals to the whole population of host species, and through that to the community as a whole. Infectious agents shape the abundance of host species, they alter feeding relationships inside of food web and they can also lead even to functional extinction of species (Poulin 1999). Especially important is the impact that infectious agents can have on competition of other species in the community (Hatcher *et al.* 2006, Lafferty *et al.* 2008c).

As infectious agents are ubiquitous in nature, all food webs can be considered to be influenced by their presence and dynamics. It has been shown that infectious agents dominate food web links: on average around 75% of the total number of links in food webs are parasite-host links (Lafferty *et al.* 2006a). Also, collective biomass of certain groups of parasites in food webs can exceed biomass of other species in the system (Kuris *et al.* 2008). There has been a growing awareness focused on collecting of infectious agent data that will ultimately allow more insight into the ways in which infectious agents interact with food web structure and dynamics. This allows researchers to develop mathematical models and to better understand the importance of infectious agents for structure, functioning and stability of ecological communities.

1.3. Data collection

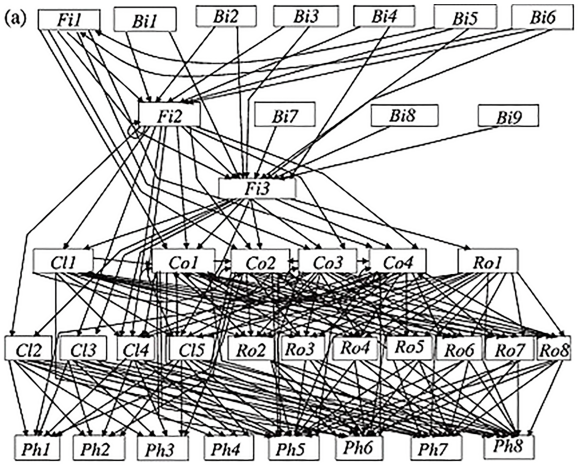
The GlobalWeb database (http://globalwebdb.com) contains around 60 data sets of food webs with parasites. These are the result of assembled food webs from published data over the period of last 90 years. They consist of binary interaction matrices where 1 stands for presence and 0 for absence of interaction between two species in the ecological community. However, in order to better understand the food webs, we need more detailed biological data and information (Cohen *et al.* 1993).

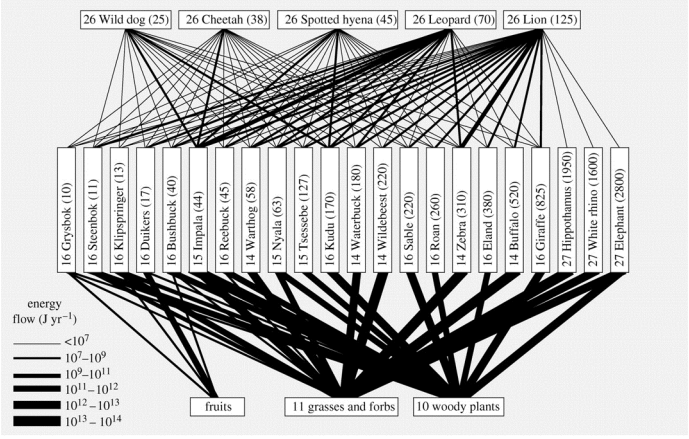
Studying the effects of infectious agents in food webs usually involves measurements of individual size and abundance of each of the taxa at a certain location. It is necessary to detect interactions and their frequencies if possible. In food webs, this calls for studying a diet composition of species that are consumers, and measurement of their resource preferences. Additional observations and laboratory experiments are needed to quantify vital rates of species (eg. mortality and growth). All individuals of species are then dissected for identification of potential infectious agents. Also, field or laboratory experiments are needed to measure the influence of the infectious agent on the behaviour, well being and infection-related morbidity/mortality of their host.

There have been several published data sets in last 5 years which contain more information on the type of parasite-host links, biomass, abundances and phylogeny of the community species (Hechinger *et al.* 2011b, Mouritsen *et al.* 2011, Preston *et al.* 2012, Thieltges *et al.* 2011, Zander *et al.* 2011). Most of the data is gathered via consistent sampling throughout certain time periods, focusing on 'realized' links. Additionally, these food webs can include links that manifest in other locations or at other times. Data on these 'possible' links needs to come from additional published sources (Lafferty *et al.* 2006a).

1.4. Modelling of food-webs with parasites

Ecologists model food webs in different ways: as topological, bioenergetics, and interaction webs (Figure 1.4), increasing in their demand for data (e.g. Paine 1980). Species in topological webs are represented as nodes while feeding relationships are represented as undirected (what do you mean with undirected; mostly it are 'arrows' pointing towards the consumers) links. Bioenergetics webs include quantified nodes (in biomass) and links that represent flows of energy and matter. The interaction web is even more detailed with directed interactions quantified in per capita interaction strength going from consumer to





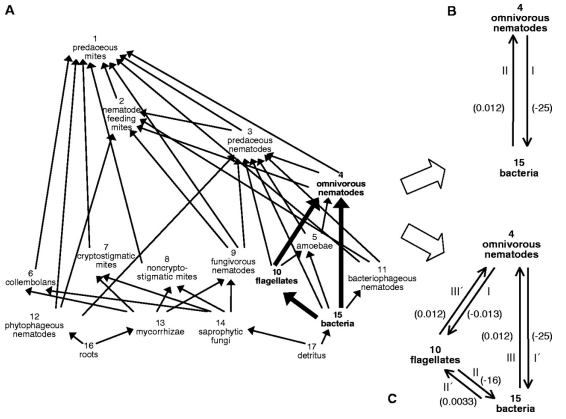


Figure 1.4. Examples of model food webs: topological (Amundsen *et al.* 2009), bioenergetics (Olff *et al.* 2009), and interaction webs (Neutel *et al.* 2002).

resource (negative interaction), and from resource to consumer (positive interaction). There are several ways to define and approach interaction strength (Berlow *et al.* 2004). To quantify feeding rates and interaction strengths, one needs biomass estimates as well as empirical data of the key physiological traits of the species involved, such as the efficiency with which biomass/energy from a resource is converted into new biomass of a consumer (de Ruiter *et al.* 1995). An empirical approach to interaction strength is the conduction of manipulation and press perturbation experiments (e.g. Paine 1980). For construction of theoretical food web models, researchers usually describe the relations by networks and the dynamics of species' abundance or biomass by a system of linked ordinary differential equations and derive from these the per capita interaction strength sensu May (May 1972).

Food web models describe how the abundances of all species in a community change as a function of time and as a result of trophic interactions, and they are often described by Lotka-Volterra-like equations. In these equations, a predator's growth rate is regulated by the prey's abundance, while the prey's growth rate is regulated by a food supply and the predators abundance (Pimm 2002). Researchers developed many models for assembling theoretical food webs that follow certain processes recognized in nature. Such processes may simply be rules of 'who eats whome' (Allesina and Pascual 2008, Cattin *et al.* 2004, Cohen *et al.* 1990, Williams and Martinez 2000) or they can explicitly incorporate biological assumptions on the nature of the interaction (Berlow *et al.* 2009, Lewis and Law 2007, Loeuille and Loreau 2005, Petchey *et al.* 2008, Rossberg *et al.* 2006). However, these models include only trophic and competitive types of interactions. Recently, researchers recognized that other types of interactions should be included in food web models (Kéfi *et al.* 2012, Mougi and Kondoh 2012). One of the proposed ways is to use a network consisting of multiple layers, where every layer represents a different type of interaction (Pilosof *et al.* 2015b).

One of the main problems in studying food webs with infectious agents from a theoretical perspective is to decide how infectious agents should be included in the food web models described above. Simple models of food chains, rather than webs, consisting of an infectious agent, one consumer-resource interaction were useful for ecologists to question the impact of infectious agents on survival and reproduction or behaviour of host (Fenton and Rands 2006, Han *et al.* 2001, Haque and Venturino 2006, Hethcote *et al.* 2004, Hilker *et al.* 2007, 2009, Kooi *et al.* 2011, Malchow *et al.* 2005, 2008, Venturino 1994, 1995, 2002). However, impacts of infectious agents may go beyond single trophic interaction, but can spread in the larger food web that they are part of. More recently, several authors have developed approaches that include infectious agents in a broader food web context

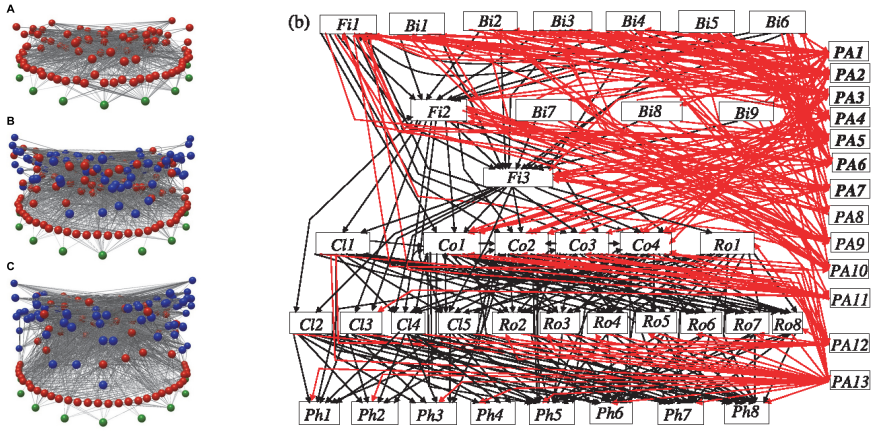


Figure 1.5. Examples of the ways in which authors included parasites to food webs: a) Three-dimensional visualization of real food webs with parasites using data from the Carpinteria Salt Marsh Web (Dunne *et al.* 2013); b) Pelagic food web of the subarctic lake Takvatn with parasite-related links included (Amundsen *et al.* 2009).

of ecological communities (Lafferty *et al.* 2015, McQuaid and Britton 2014, Roberts and Heesterbeek 2013, Warren *et al.* 2010).

Current topological studies on food webs with infectious agents are divided in two general categories (Sukhdeo 2012); 1) studies that include infectious agents into food web diagrams as separate nodes and incorporate them explicitly into the community matrix (Byers 2009, Dunne *et al.* 2013, Huxham *et al.* 1995, Lafferty *et al.* 2008c) as sub-matrices separate from the main matrix (Amundsen *et al.* 2009, Lafferty *et al.* 2006b); 2) studies that include infectious agents as separate sub-webs (Mouillot *et al.* 2008, Pocock *et al.* 2012, Poulin 2010, Vázquez *et al.* 2005). Examples presented in Figure 1.5.

Hechinger (Hechinger 2013, Hechinger *et al.* 2011a) made an important step for inclusion of infectious agents into food web models by developing a scaling framework for parasite within-host abundance, biomass and energy flux. He developed equations which serve a purpose of estimating parasite's impact on hosts in terms of energy flux.

1.5. Overview

This thesis focuses on understanding the influence of infectious agents in food webs and ecosystems. It consists of three parts, which give examples of data collection and

modeling, and of the community-level effects that infectious agents could have. Part I (Chapters 2 and 3) shows the results of field research on macroparasites of top predators, carried out during a period of four years in Serbia. The research provides an illustration of diversity of parasites and their hosts in real-life systems and illustrates the work involved in obtaining such data.

Chapter 2 gives an overview of the first findings and prevalence of hearthworm (*Driofiralia immitis*) in several free ranging wild carnivores from Serbia. The research includes the diversity of canid (golden jackal, fox, wolf), felid (wild cat), and mustelid species (beech marten, stone marten, European polecat, badger and otters), potential hosts of this type of parasite.

Chapter 3 focuses on the different parasites that can be found in one host species. The study is focused on diversity of gastrointestinal helminth species in large sample of jackals. The diversity of parasites and its prevalence is compared between six locations in Serbia, as well as in between six countries (Serbia, Bulgaria, Greece, Iran, Tajikistan and Uzbekistan). In this study we show that parasites can be highly general with high distribution range as well as specific and rare.

Part II focuses on an overview of existing empirical research on infectious agents impact on different levels of biological organization (Chapter 4), and their diversity, types and functional roles (Chapter 5). We introduce a new indirect approach of modeling infectious agents in food webs and analyse an example of the simplest consumer-resource model with an infected resource.

Chapter 4 provides a broad range of infectious agent's influence in food webs and ecosystems. We systematically classify the effects of infection agents on energy flow, community interactions, diversity and ecosystems covering a broad range of infectious agents in a broad range of host species. Further, we discuss a concept of new indirect approach of modelling infectious agents in food webs that concentrates on the ways infectious agents affect the existing links across host and non-host nodes, by influencing the strength of consumer-resource interaction.

Chapter 5 we further deepen the knowledge of infectious agents in ecosystems by cataloging their diversity, types and functional roles. We explain the specific relationship of infectious agents and their hosts that can have aspects of both trophic and non-trophic interaction. By looking at a simple model of a microparasite in a very basic Lotka-Volterra consumer-resource system, we give an example of an indirect approach previously introduced (Chapter 4). Additionally, our model studies the potential differences of infectious

agents in different type of ecosystems, from aquatic to terrestrial.

Part III (Chapters 6, 7 and 8) focuses on the quantification of infectious agents impacts in food webs. Chapter 6 explores the impact of infectious agents in ecosystems, by using an indirect approach where infectious disease agents are represented through the influence on the life history of their hosts. By decreasing and increasing host's mortality, we mimicked the effect of changes in infection prevalence in host species and were able to calculate the effects on the structure and stability of the ecological community as a whole.

Chapter 7 is continuation of work done in Chapter 5 and Chapter 6. We wanted to find out if infectious agents could influence the stability of the system thought their effect on the hosts. We again use an indirect approach, but we question the effect of infectious agents in ecosystems thought change of intrinsic growth rate like in Chapter 6, but also change in the behavior of the hosts species like in Chapter 5. The influence of infectious agent's change in behavior is quantified through its effect on prey preference and production efficiency of predator and prey hosts.

Chapter 8 represents a spatial multiplex-based framework for modelling multi-host parasite transmission considering multiple diffusion mechanisms. We consider trophic and non-trophic (host-parasite) interactions between nodes representing species populations embedded in a given environment. Each node has an identity, i.e. predator, prey or parasite vector, and it is represented according to its given frequency. The resulting multiplex is composed of two distinct layers, which are both spatially embedded. Our model was inspired by the multiple transmission routes of *Trypanosoma cruzi*, the etiological agent of Chagas disease.

Chapter 2

Levels of infection of intestinal helminth species in the golden jackal *Canis aureus* from Serbia

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Abstract

During the past decade, golden jackal populations have substantially increased, yet little is known of their potential for transmitting parasites within animal and human hosts. In the present study, between 2005 and 2010, 447 jackals from six localities in Serbia were examined for intestinal parasites. Two species of trematodes (*Alaria alata, Pseudamphistomum truncatum*), three nematodes (*Toxocara canis, Ancylostoma caninum, Gongylonema sp.*), and seven cestodes (*Taenia pisiformis, Taenia hydatigena, Multiceps multiceps, Multiceps serialis, Mesocestoides lineatus, Mesocestoides litteratus, Dipylidium caninum*) were identified. *Pseudamphistomum truncatum* and *M. serialis* species were recorded for the first time. The overall prevalence of parasitic infection was 10.3%. No significant differences were found in the prevalence of infection between males and females ($P > 0.817$), between localities ($P > 0.502$), or with regard to annual cycles ($P > 0.502$). In the infected jackal population, 65% harboured multiple infections and one individual was a host to five different types of parasite species, the highest number of parasites we recorded in a single host. These findings indicate that although the prevalence of gastrointestinal helminths in the jackal population in Serbia is significantly lower than expected from earlier studies, further monitoring is required given the jackals rapid population increase.

2.1. Introduction

The golden jackal (*Canis aureus Linnaeus 1758*) is a canid of medium size with one of the widest distribution ranges in the world, a range that is still expanding (Demeter and Spassov 1993, Kryštufek *et al.* 1997, Mitchell-Jones *et al.* 1999). In the past few decades, it has undergone several changes, especially in Europe, where the stretch of the Danube through ex-Yugoslavia and Romania has traditionally been described as the northern border of residential populations (Kryštufek *et al.* 1997). However, the present distribution of the jackal additionally comprises parts of central Europe (Arnold *et al.* 2012, Mitchell-Jones *et al.* 1999). Apart from Greece, where the jackal population has decreased (Giannatos, 2004, Giannatos *et al.*, 2005), European populations are both rapidly increasing and widening their ranges (Arnold *et al.* 2012, Kryštufek *et al.* 1997). In Serbia, jackals neared extinction due to extensive poisonings organized after World War II, initially aimed at controlling the size of the wolf population and lessening the damage they caused to domestic animals. Only two relic populations survived in Srem and in eastern Serbia (Milenković 1983, 1987). At the beginning of the 1980s, the species started to spread quickly and to increase in number (Savić *et al.* 1995), which resulted in the fusion of the two relic populations and the widening of their range. This range now covers more than half of the territory of modern Serbia (Ćirović *et al.* 2008, Zachos *et al.* 2009).

The increasing jackal range in Europe was not automatically followed by detailed parasitological research. Only two studies in Europe, namely in Bulgaria and Greece, have been undertaken on the intestinal helminths of the golden jackal, and these are based on small numbers of hosts (Bulgaria $n = 13$, Greece $n = 5$) examined (Papadopoulos *et al.* 1997, Trifonov *et al.* 1970). More work has been done in Asia, where comprehensive studies, particularly in Iran, showed high prevalences in jackals, ranging from 66.7 to 100% (Dalimi and Mobedi 1992, Dalimi *et al.* 2006, Meshgi *et al.* 2009, Sadighian 1969). In central Asia, 13 species of intestinal helminths were identified in Tajikistan and 8 species in Uzbekistan, but, again, data were limited (Heptner and Naumov 1967). In Israel, two helminth species, *Ancylostoma caninum* and *Dipylidium caninum*, were identified using faecal flotation techniques (Shamir *et al.* 2001). High prevalences of *Toxocara leonina*, *A. caninum* and *Mesocestoides lineatus* were found in Iran (Dalimi and Mobedi 1992, Dalimi *et al.* 2006, Meshgi *et al.* 2009, Sadighian 1969) and also *Uncinaria stenocephala* and *Taenia hydatigena* have been found in Europe (Papadopoulos *et al.* 1997, Trifonov *et al.* 1970).

However, these studieswere limited by the small number of jackals examined. The aim of the present investigation, therefore, was to identify species of gastrointestinal helminths in a large sample of hosts and to evaluate the potential role of the golden jackal in enhancing transmission of these helminths in Serbia and Europe.

2.2. Material and methods

2.2.1. Collection and examination of jackals

In cooperation with local hunting associations, the carcasses of all available, legally hunted jackals were collected in six locations in Serbia between 2005 and 2010. This collection was conducted throughout the main hunting season, the winter period from December until February, when jackals are sexually mature and therefore presumed to be adults. In total, 447 animals (239 males and 208 females) were collected: 40 from Negotin (MGRS 34T FP29), 162 from Veliko Gradište (MGRS 34T EQ45), 49 from Velika Plana (MGRS 34T EQ00), 119 from Smederevo (MGRS 34T DQ75), 39 from Svilajnac (MGRS 34T EP19) and 38 from Surčin (MGRS 34T DQ46) (Figure 2.1). The date of collection, location and sex of each jackal were noted. After morphometric analysis of each individual, the stomach and intestine were removed in the field and immediately frozen at -20°C. For safety reasons, the material was additionally frozen at -80°C for 3 days and then thawed at room temperature prior to parasitological investigation in the laboratory of the Scientific Institute of Veterinary Medicine of Serbia.

The analysis of the gastrointestinal helminths was part of wider research on the jackal's diet (Ćirović *et al.* 2009). For this reason, the stomach was first scrutinized for food contents. Parasites found in the stomach were removed and stored in 70% ethanol until eventual determination. The intestines were opened with a longitudinal cut and the entire intestinal contents were collected and washed with a water jet over a 200 μm sieve. All visible parasites were transferred to Petri dishes. Furthermore, to detect the presence of small parasites embedded in the mucosa, intestinal walls were scraped with a wooden spatula or a glass microscope slide. All helminths found were first washed in warm water and then fixed. Nematodes were fixed in 70% ethanol, while cestodes and trematodes were fixed in a mixture composed of 5% formalin, 85% ethanol and 10% glacial acetic acid. Nematodes were studied in depression slides using lactophenol wet mounts. Cestodes and trematodes were stained with acetic carmine, and after dehydration were mounted with Canada balsam. Scolices of taeniids were severed and mounted

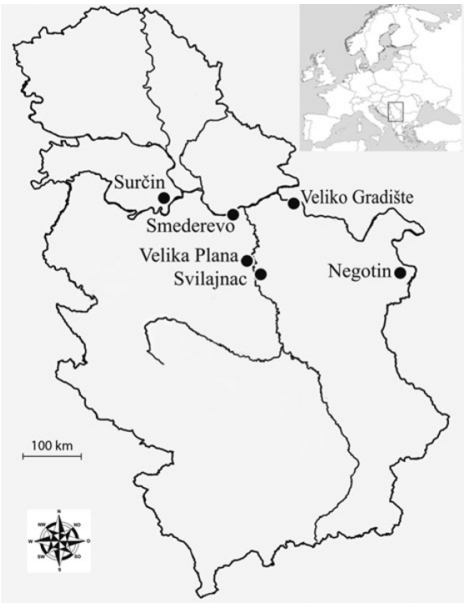

Figure 2.1. The collection sites of golden jackals examined from Serbia between 2005 and 2010.

in lactophenol; sufficient pressure was applied to the coverglass to cause the rostellar hooks to lie flat. Identification was based on the number, size, shape and arrangement of rostellar hooks.

All helminths were identified using the relevant keys (Kozlov 1977, Soulsby 1982), counted and deposited in the collections of the Scientific Institute of Veterinary Medicine of Serbia and the Faculty of Biology of the University of Belgrade.

2.2.2. Data analysis

Prevalence (P) and mean intensity (MI) were calculated according to Bush *et al.* 1997. For each intestinal helminth, prevalences were noted for each locality and for the entire sample of jackals. In addition, prevalence was calculated according to annual cycles (2005-2010) and host sex. The G-test was used for statistical evaluation of differences between the six localities (Sokal and Rohlf 1995) in addition to the number of infected/uninfected hosts for each year. The chi-square test was used to compare the prevalence of intestinal helminths in male and female hosts. The mean intensity was calculated for the entire host sample and at each locality. Data were analysed using Statistica 5.1

(Statsoft, Tulsa, Oklahoma, USA) with the level of significance being $P < 0.05$.

2.3. Results

Up to 46 of 447 jackals (10.3%) were infected with at least one helminth species and a total of 12 species were identified, namely two trematodes, *Alaria alata* and *Pseudamphistomum truncatum*, three nematodes, *Toxocara canis, Ancylostoma caninum* and *Gongylonema sp*. and seven cestodes, *Taenia pisiformis, Taenia hydatigena, Multiceps multiceps, Multiceps serialis, Mesocestoides lineatus, Mesocestoides litteratus* and *Dipylidium caninum* (Table 2.1).

The highest prevalence value was recorded at Smederevo (15.1%), followed by Veliko Gradište (10%), Negotin (10%), Velika Plana (6.1%), Surčin (7.9%) and Svilajnac (5.1%). These differences, which varied from 5.3% in 2007 to 15% in 2008, were not significant ($P > 0.9999$), relative to locality. Similarly, no significant differences in prevalence were found between years ($P > 0.502$) nor relative to host sex ($P > 0.817$). The smallest number of infected jackals (4 from 75 examined) was recorded in 2007, while the highest number (11 from 102 examined) was recorded in the last year - 2010. The most frequent parasites found in this study were the cestodes *M. lineatus* (found in 26 individuals) and *M. litteratus* (found in 21 individuals), with the respective prevalences of 5.8 and 4.7% and mean intensities 69.73 ± 9.38 and 64.33 ± 15.14. These two species also had the widest distribution range, having been recorded at all six locations.

Generally, all cestodes had a relatively higher prevalence in comparison to nematodes and trematodes. *Toxocara canis* was the nematode with the highest prevalence (1.6%) and a mean intensity of 7.86 2.14, while among trematodes *A. alata* had the highest prevalence (0.9%) with a mean intensity of 19 3. *Pseudamphistomum truncatum* and *A. caninum* were the only two parasites recorded in one animal only. With the exception of *Gongylonema sp.*, which were found in the stomach, all parasites were found in the intestine. Regarding the diversity of parasites, the highest number of species (10) was recorded at Veliko Gradište and Smederevo, while the smallest number of parasite species (4) was recorded at Velika Plana, Svilajnac and Surčin. While 34.8% (16) of the infections were monospecific, the majority (65.2%, 30) of individuals had multiple infections. Within the group with multiple infections: 37% (17) of jackals had two species of helminths, 21% (10) had three, while 4% (2) hosted four species. Only one animal had five species of parasites in its intestine, the highest number of recorded parasites per

Table 2.1. The prevalence (P%) and mean intensity (MI ± SE) of gastrointestinal helminths in the golden jackal *Canis aureus* from six collection sites in Serbia; N Âij number of hosts examined.

	Negotin N=40		Veliko Gradište N=162		Velika Plana N=49		Smederevo N=119		Svilajnac N=39		Surčin N=38		Total N=447	
Helminth species	P	MI	P	MI	P	MI	P	MI	P	MI	P	MI	P	MI
Class Trematoda														
Alaria alata	2.5	16	0.6	12	-	-	0.8	19	2.6	29	-	-	0.9	19±3.63
Pseudamphistomum truncatum	-	-	0.6	2	-	-	-	-	-	-	-	-	0.2	2
Class Nematoda														
Toxocara canis	-	-	1.8	8±5.57	4.1	8±1	1.7	7.5±1.5	-	-	-	-	1.6	7.86±2.14
Gonglyonema sp.	-	-	-	-	2	1	-	-	-	-	2.6	1	0.4	1
Ancylostoma caninum	-	-	-	-	-	-	0.8	2	-	-	-	-	0.2	2
Class Cestoda														
Taenia pisiformis	5	8±1	0.6	17	-	-	3.4	11.5±3.33	2.6	2	-	-	1.8	10.12±2.17
Taenia hydatigena	-	-	1.8	4.7±2.19	-	-	0.8	1	-	-	-	-	0.9	3.75±1.80
Multiceps multiceps	2.5	3	1.8	3.7±1.2	-	-	2.5	2.3±0.33	-	-	-	-	1.6	3±0.53
Multiceps serialis	-	-	1.8	3	-	-	0.8	2	-	-	-	-	1.1	2.75±0.25
Mesocestoides lineatus	10	133.5±23.04	3.7	75±19.57	2	87	10.1	54.7±10.30	2.6	31	5.3	27±20	5.8	69.73±9.38
Mesocestoides litteratus	7.5	41±12.05	4.9	102.5±36.24	4.1	43±20	5	39.5±6.44	2.6	64	2.6	21	4.7	64.33±15.14
Dipylidium caninum	-	-	1.2	5±1	-	-	3.4	4.7±1.11	-	-	2.6	5	1.6	4.86±0.63

jackal in Europe. Most of the analysed animals were hosts to cestodes only (76%, 35), while 10% (5) were hosts to nematodes only. Four animals were hosts to both cestodes and trematodes, two animals were hosts to both cestodes and nematodes, and one animal was infected with both nematodes and trematodes. No single animal was infected with trematodes only. The findings of *P. truncatum* and *M. serialis* represent the first record of these parasites in jackals.

2.4. Discussion

The present analysis has shown that jackals in Serbia are hosts to 12 species of intestinal helminths. According to the available literature, 32 species of intestinal helminths have been recorded across the entire distribution range (Table 2.2). Of the 12 species found in this research, *I. truncatum* and *M. serialis* have never been found infecting jackals before, and *M. lineatus* and *D. caninum* are recorded for the first time in jackal populations in Europe. Acanthocephalan species were not found in this study nor in other studies in Europe (Papadopoulos *et al.* 1997, Trifonov *et al.* 1970) (Table 2.2), although they have been found in Iran and Tajikistan (Dalimi *et al.* 2006, Heptner and Naumov 1967, Meshgi *et al.* 2009, Sadighian 1969).

The total prevalence of gastrointestinal parasites in Serbia was significantly low (Table 2.1) at only 10.3%. All previous studies have indicated a much higher helminth infection of jackals (Dalimi and Mobedi 1992, Dalimi *et al.* 2006, Meshgi *et al.* 2009, Papadopoulos *et al.* 1997, Sadighian 1969, Trifonov *et al.* 1970). Similarly, the prevalence of each helminth species was considerably lower than in previous studies.

These differences could be partly explained by seasonal variations in prevalence, which is well known in canids (see Saeed *et al.* 2006). The low infection rate we found in Serbia could be explained by differences in sampling seasons between this study and other studies, since our study included specimens only from the winter, while studies from Greece, Bulgaria and Iran included the entire year. Detecting whether the diversity of intestinal helminths also shows seasonal variation is left for future research. The other

Table 2.2. The occurrence (+) of intestinal helminths in the golden jackal from parts of Europe including Bulgaria (Trifonov *et al.* 1970), Iran (Dalimi and Mobedi 1992, Dalimi *et al.* 2006, Meshgi *et al.* 2009, Sadighian 1969), Greece (Papadopoulos *et al.* 1997), Tajikistan (Heptner and Naumov 1967), Uzbekistan (Heptner and Naumov 1967) and Serbia. *N* = number of hosts examined.

	Bulgaria N=13	Iran N=10-100	Greece N=5	Tajikistan N=?	Uzbekistan N=?	Serbia N=447
Trematoda						
Alaria alata	+		+			+
Alaria canis		+				
Pseudamphistomum truncatum						+
Ascocotyle sinoecum	+					
Echinochasmus schwartzi		+				
Nematoda						
Toxocara canis		+	+	+	+	+
Toxocara leonina	+	+		+		
Uncinaria stenocephala	+	+	+	+	+	
Ancylostoma caninum		+	+	+	+	+
Rictularia cahirensis		+			+	
Rictularia affinis					+	
Strongyloides stercoralis		+				
Trichocephalus vulpis		+				
Oxynema crassispiculum		+				
Cestoda						
Taenia pisiformis	+	+	+	+		+
Taenia hydatigena	+	+		+		+
Taenia ovis		+		+		
Hydatigena taeniaeformis		+		+	+	
Taenia endothoracicus		+				
Multiceps multiceps	+	+				
Multiceps serialis						+
Mesocestoides lineatus		+		+	+	+
Mesocestoides litteratus	+					+
Dipylidium caninum		+		+	+	+
Diplopylidium nolleri		+				
Joyeuxiella pasqualei		+				
Sparganum mansoni				+		
Diphyllobothrium mansonoides				+		
Echinoccocus granulosus	+	+				
Acanthocephala						
Macracanthorhynchus hirudinaceus		+				
Macracanthorhynchus catulinus				+		
Oncicola canis sjoukje		+				

possible reason for the differences in prevalence and diversity between other studies and ours is that the jackal population in Serbia has developed only recently. It is known that species introduced to a new area are usually less heavily parasitized, with a lower prevalence than the original population from which they have descended (Torchin *et al.* 2003).

When the diversity of intestinal helminths between Serbian and Iranian populations is compared, only seven species are mutual, and all of them are common parasites of canid species. The difference in diversity of intestinal parasites in the two countries could be explained by the rule that, in some host species, similarities in parasite communities decay exponentially with increasing distance (Poulin 2003). The data in table 2.2 show that the diversity of intestinal parasites in Serbia differs from both Bulgaria and Greece despite their close proximity. Note that these studies considered only a small number of hosts and more data are therefore required in order to come to a proper conclusion.

In Serbia, as in most other countries of south-eastern Europe, the jackal is the second most numerous wild canid after the red fox. Almost all intestinal helminths recorded in the jackal are also found in the red fox, with only a nematode from the genus Gongylonema not yet found in the red fox (Pavlović 1994). Like the jackal, the red fox is an autochthonous canid of medium size, and the niches of the two animals overlap to a great extent (Lanszki *et al.* 2010, 2006). From an ecological point of view, this could explain the high number of common intestinal parasites of these two species.

Considering the constant spreading of the jackal's range and the permanent increase of its populations both in Serbia and throughout south-eastern Europe (Arnold *et al.* 2012), it seems that this animal is likely to have a great parasitological significance, both for animal and human health, as a reservoir of specific parasites. For example, *T. canis, M. lineatus and D. caninum* are transmittable to humans and other canids. Of these, *T. canis* could be the most important as it is reported to be a common parasite in domestic and wild canids worldwide. In light of this, the next (necessary) step should be better monitoring of jackal population dynamics, as well as the prevalence of their parasites, throughout Serbia and the whole of its European range.

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Chapter 3

First findings and prevalence of adult heartworms (*Dirofilaria immitis*) in wild carnivores from Serbia

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Abstract

Heartworm *(Dirofilaria immitis)* is a parasitic roundworm that causes a zoonotic disease known as dirofilariosis. Little is known about the role of wild carnivores serving as reservoirs in nature. Therefore, we examined 738 hearts and lungs of free ranging wild carnivores from Serbia to determine the presence of adult heartworms. During the period 2009–2013, the prevalence in golden jackals (*Canis aureus*) was 7.32 %, in red foxes (Vulpes vulpes) 1.55 %, in wolves (*Canis lupus*) 1.43%, and in wild cats (*Felis silvestris*) 7.69 %. No adult heartworm specimens were found in beech martens (*Martes foina*), stone martens (*Martes martes*), European polecats (*Mustela putorius*), badgers (*Meles meles*) or otter (*Lutra lutra*). The highest recorded prevalence was in 2013 (7.30%) and the lowest in 2012 (1.6 %). In jackals, the prevalence was higher in males (10 %) than in females (4.06 %), while in foxes the prevalence was 1.75 % in males and 1.26 % in females. The most infected host was a wolf in which 37 adult specimens were found. Because of the potentially significant role in the life cycle of *D. immitis*, populations of wild carnivores in Europe should be further examined and tested for heartworm infections.

3.1. Introduction

Dirofilaria immitis is a widespread filarial nematode which can be found in temperate, subtropical and tropical regions of the world. It is one of several species of the genus *Dirofilaria* which cause zoonotic infections (Orihel and Eberhard 1998). Together with *Dirofilaria repens*, *Dirofilaria immitis* shows poor host specificity and it is likely to infect various mammal species (Barriga 1982). Canine heartworm disease is a lifetearing, severe condition. Canids and felids, domestic and wild, function as its main reservoirs. It is known that heartworm larvae are transmitted by cuculid mosquitoes (*Culex, Aedes, Anopheles, Culiseta spp.*) to the host animals (Bargues *et al.* 2006, Vezzani *et al.* 2011). First preadult worms can be found in the host's right ventricle and pulmonary artery 70–85 days after infection. The prepatent period for *D. immitis* is 6–9 months (Anderson 2000). This canine heartworm causes canine and feline cardiopulmonary dirofilariosis and it is also responsible for human pulmonary dirofilariosis (Muro *et al.* 1999, Orihel and Eberhard 1998). The number of cases of this disease in humans is probably underestimated because these nodules and granulomas are often discovered accidentally (Muro *et al.* 1999) and sometimes confused with lung cancer (Gómez-Merino *et al.* 2002, Mulanovich *et al.* 2008) because of radiographic similarity. At least 372 cases of human pulmonary dirofilariosis have been reported worldwide of which 32 were in Europe (Simón *et al.* 2012), but very few are definitely diagnosed by molecular methods. However, this vector–borne disease is poorly investigated in wild animals especially in Europe where limited data is available. In the case that wild canids should be a source for infection of domestic dogs, the disease should find "new" epidemiological chain and should spread in the country.

Aside from the studies of microfilaremia in domestic dogs (Tasić *et al.* 2008, 2012), no data are available about the prevalence of *D. immitis* in wild carnivores from Serbia. The aim of this study is to present the first findings and prevalence of *D. immitis* in several free–ranging carnivores from Serbia including canid, felid, and mustelid species.

3.2. Material and methods

During the period 2009–2013, 738 hearts and lungs of wild carnivores have been examined: 437 jackals (*Canis aureus*), 193 red foxes (*Vulpes vulpes*), 70 wolves (*Canis lupus*), 13 wild cats (*Felis silvestris*), 14 beech martens (*Martes foina*), 4 stone martens

(*Martes martes*), 4 European polecats (*Mustela putorius*), 2 badgers (*Meles meles*) and 1 otter (*Lutra lutra*). The number of examined animals per year varied from 105 (in 2009) to 215 (in 2010). The animals (or just their hearts and lungs) were collected from various regions of Serbia, more specifically in the vicinity of 95 settlements. Material for this research was obtained in cooperation with local hunting clubs and as road-kill. Except for road kill, all the animals were legally shot during hunting seasons. Therefore, most of the animals were collected during the winter season (December-February), in total 616. Other seasons are represented with a much smaller sample size: 57 samples were collected in spring, 17 in summer, and 48 in autumn. We kept records of all of the animals' sex and most of their body parameters such as body mass, body length with head, tail length, hind foot length, ear length, and height at the shoulders. The organs were stored at -20°C prior to dissection. The examination was conducted in the laboratory of the Faculty of Biology, University of Belgrade. Due to the poor condition of most carcasses, the blood was not tested for the presence of microfilariae. Great care and attention went into cutting open the hearts, together with the pulmonary arteries and the lungs in order to keep the adult worms intact and prepared for visual examination. Whole parasites and fragments were preserved in 96% ethanol.

The chi-square test was used to compare the heartworm prevalence between gender, years, and seasons. Because of the sample size of infected animals, differences between males and females were calculated only for jackals and foxes. Differences between years and seasons were calculated for all collected samples. Data were analyzed using Statistica 5.1 (Statsoft, Tulsa, Oklahoma, USA) with the level of significance being $p<0.05$.

3.3. Results

The overall prevalence during the examination period was 5.01%. The prevalence of adult *D. immitis* in golden jackals was 7.32%, in red foxes 1.55%, in wolves 1.43%, and in wild cats 7.69%. The relation between the number of infected animals per year is not statistically significant (χ^2=12.66). Reviewing by years based on the total sample, the highest prevalence (7.3%) was recorded in 2013 when the adult heartworms were detected in 10 out of 137 animals and the lowest in 2012 when the prevalence was 1.6% (2 positive out of 125 examined). In 2009, the prevalence was 2.86% (3 positive out of 105 examined animals), in 2010 was 6.51% (14 positive out of 215 examined), and in 2011 it was 5.13% (8 positive out of 156 examined; Table 1). Statistical significance (χ^2=1.1) was observed in the number of infected animals between seasons. Most of the

infected animals (31) were from the winter season, while three were from spring and three from autumn. Regarding sex, no statistical significance was observed in jackals (χ^2=5.62) unlike in foxes (χ^2=0.07). The prevalence in golden jackals was 4.06% in females (8 positive out of 197 examined females) and 10% in males (24 positive out of 240 examined males), while the prevalence in foxes was much lower with 1.26% in females (1 positive out of 79 examined females) and 1.75% in males (2 positive out of 114 examined males). In beech martens, stone martens, European polecats, badgers, and in otters, no adult heartworm specimen was found. The minimal intensity of infection in golden jackals was 1 and the maximal was 6 (median=2). In red foxes, minimal intensity of infection was 1 and the maximal was 7 (median=1). The intensity of infection for the wild cat and wolf was 2 and 37, respectively.

Adult heartworms were found in 32 jackals, most of them (*n*=22) originated from settlements which are located along the Velika Morava River (by downstream order: Trnovče (*n*= 3), Miloševac (*n*=2), Lozovik (*n*=1), Lipe (*n*=12), Šalinac (n=2), Kulič (*n*=2)). From the localities along the lower course of Danube River, we found *D. immitis* adults present in six specimens (by downstream order: Zatonje (*n*=4), Sirakovo (*n*=1), Kladovo (*n*=1). Three infected specimens originated from Titel, which is located on the Tisa River and one from Ogar which is located near the Sava River (Figure 3.1). Adult heartworms were found in foxes in the vicinity of the settlements Boljevci and Dobanovci, which are located near the Sava River and in the settlement of Srbovo, which is located near the Danube River (Figure 3.1).

Heartworms were found in a single wild cat, in the vicinity of Sefkerin which is located on the Tamiš River. Heartworms were also found in a wolf on Željin Mountain (Fig. 3.1).

3.4. Discussion

Apart from the findings in jackals, foxes, and wild cats which were all in river valleys, the only finding of a heartworm in a wolf was on a mountainous area in central Serbia. The animal was shot on Željin Mountain; with the closest settlement Kozinci, at 1,000 m.a.s.l.

Data on mosquito species, abundances, densities, as well as the presence of *Dirofilaria* in Serbia south of the Sava and Danube Rivers is limited. One of the studies from Serbia showed an overall *D. immitis* seroprevalence rate of 22.9% in 2009 in dogs from two areas: Pančevo and Veliko Gradište (Tasić *et al.* 2012). From these 2 areas in the same

Table 3.1. Number of positive/negative/prevalence per host and by years

Year/host	Canis aureus			Vulpes vulpes			Canis lupus			Felis silvestris			Martes martes			Martes foina			Mustela putorius			Meles meles			Lutra lutra			Total		
	+	-	P (%)	+	-	P (%)	+	-	P (%)	+	-	P (%)	+	-	P (%)	+	-	P (%)	+	-	P (%)	+	-	P (%)	+	-	P (%)	+	-	P (%)
2009	2	95	2.06		3					1		100		3											1			3	102	2.86
2010	12	120	9.09	2	60	3.23		13			4			1			2						1					14	201	6.51
2011	7	70	9.09	1	42	2.33		18			4						11			3								8	148	5.13
2012	2	58	3.33		37			24			2						1			1								2	123	1.6
2013	9	62	12.68		48		1	14	6.67		2												1					10	127	7.3
Total	32	405	7.32	3	190	1.55	1	69	1.43	1	12	7.69	0	4	0	0	14	0	0	4	0	0	2	0	0	1	0	37	701	5.01

+/- Number of animals positive/negative to *Dirofilaria immitis* adults presence

P (%) Prevalence

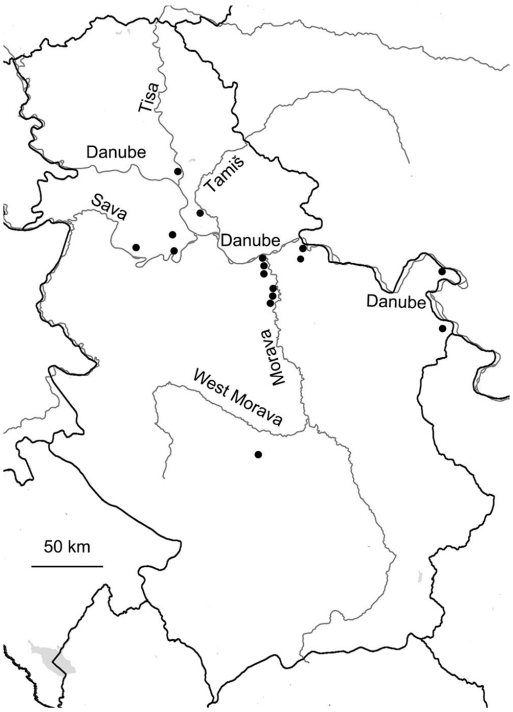

Figure 3.1. The collection sites of golden jackals examined from Serbia between 2005 and 2010.

year (2009), we checked 38 golden jackals, 2 pine martens, and 1 wild cat. The wild cat was the only animal that had adult heartworms present.

According to the study conducted in Vojvodina (the province in northern part of Serbia) during the period 2007–2009 (Vujić *et al.* 2010), most of the mosquitoes (73%) collected were *Culex pipiens complex, Anopheles maculipennis complex*, and *Aedes vexans* which are known as species whose vector capacities for *D. immitis* have already been demonstrated (Bargues *et al.* 2006, Simón *et al.* 2012, Vezzani *et al.* 2011). Their dynamics were mostly influenced by the water level of the Danube, Sava, and Tisa Rivers.

Apart from the reported cases of human dirofilariosis in Serbia caused by *D. repens* (Džamić *et al.* 2009), so far there has been no report on human dirofilariosis caused by *D. immitis*. However, there is always a potential risk that some cases are misidentified (Gómez-Merino *et al.* 2002, Mulanovich *et al.* 2008).

There are only a few reports on dirofilariosis in wolves in Europe. To the best of our

knowledge, this is the fourth published report of a free-ranging wolf which was infected with *D. immitis* adults in Europe, after findings in Spain, Italy, and Bulgaria (Georgieva *et al.* 2001, Pascucci *et al.* 2007, Segovia *et al.* 2001). Aside from the fact that free-ranging wolves are poorly investigated on heartworm presence, the low prevalence in wolves could be explained by different habitat preferences between this host and the vector species. In Serbia, wolves are mostly restricted to mountainous parts of the country (Milenković 1997) where weather conditions are not favorable for mosquito development. However, wolf packs have large home ranges in southern, central, and eastern Europe spanning from 80 to 240 km^2 (Kusak *et al.* 2005, Okarma *et al.* 1998).

Apart from wolves, foxes are already recognized as a potential important reservoir of dirofilariosis in nature (Magi *et al.* 2008). When compared to other European studies based in Spain, Portugal, Italy, and Bulgaria (where the reported prevalence is from 1.7% up to 36%) where a similar number of animals was investigated, (Eira *et al.* 2006, Georgieva *et al.* 2001, Gortázar *et al.* 1994, Kirkova *et al.* 2011, Magi *et al.* 2008), the prevalence of adult *D. immitis* specimens in Serbia is lower.

Recently, the only two studies in Europe concerning the prevalence of *D. immitis* in jackals were conducted in Bulgaria (Georgieva *et al.* 2001, Kirkova *et al.* 2011) on a much smaller sample size. The result of this study on the prevalence (7.32 %) of the heartworm in jackals is similar to the values reported in Bulgaria (9.6 and 5.5 %, respectively).

The life cycle of *D. immitis* in cats differs from the one in dogs and they generally do not produce microfilariae. So far, dirofilariosis in domestic and feral cats has only been reported in Europe. This is the first report of adult heartworm presence in a free ranging wild cat in Europe.

3.5. Conclusion

Although the presented data are about adult worms, it is likely that jackals, wolves, and foxes, even in this last species microfilaremia is quite infrequent, could act as reservoir of infection. Moreover, if we consider the actual significance of the obtained results, we should speculate that probably the infection is much more prevalent in domestic dogs than until now expected in Serbia. Furthermore, numerous studies show increasing numbers of foxes living in urban areas and jackals approaching settlements for food (Ćirović *et al.* 2014, Contesse *et al.* 2004), and as potential reservoirs, they can increase the risk for dirofilariosis for domestic dogs. Further research on the jackal is needed because this

species is widening its distribution range in Europe and its local density is increasing (Arnold *et al.* 2012, Šálek *et al.* 2014), thus it could play a significant role in spreading the dirofilariosis in nonendemic areas.

In view of these findings, research on heartworm infections in populations of wild carnivores in Europe should be extended since they could be a significant part of *D. immitis* lifecycle.

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Chapter 4

Infectious disease agents mediate interaction in food webs and ecosystems

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Abstract

Infectious agents are part of food webs and ecosystems via the relationship with their host species that, in turn, interact with both hosts and non-hosts. Through these interactions, infectious agents influence food webs in terms of structure, functioning and stability. The present literature shows a broad range of impacts of infectious agents on food webs, and by cataloguing that range, we worked towards defining the various mechanisms and their specific effects. To explore the impact, a direct approach is to study changes in food-web properties with infectious agents as separate species in the web, acting as additional nodes, with links to their host species. An indirect approach concentrates not on adding new nodes and links, but on the ways that infectious agents affect the existing links across host and non-host nodes, by influencing the 'quality' of consumer–resource interaction as it depends on the epidemiological state host involved. Both approaches are natural from an ecological point of view, but the indirect approach may connect more straightforwardly to commonly used tools in infectious disease dynamics.

4.1. Introduction

The concept of a food web in community ecology provides a conceptual framework to study and understand relationships between species (Begon *et al.* 2006, de Ruiter *et al.* 2005, Levin *et al.* 2009, McCann 2011, Moore and de Ruiter 2012, Odum and Barrett 1971, Pimm 1982, 2002, Polis and Winemiller 1996). Species that infect other species have received relatively little attention in these studies, but in recent years this has been changing (promoted notably by Lafferty *et al.* 2006a,b, 2008b). Here, we discuss approaches for studying infectious agents as part of the food-web framework.

Food webs can typically be thought of in three different ways (Figure 4.1), with increasing detail in data required (de Ruiter *et al.* 1995, Hunt *et al.* 1987, Lafferty *et al.* 2006a, O'Neill 1969). First, in the form of diagrams or networks, where organisms (species or functional groups of species) are represented in the form of nodes, and where feeding relationships between consumers and resources are represented as links (topological webs). Second, as flows of energy and matter (bioenergetics webs). Third, in terms of interaction strengths across the species links (interaction webs)–that is, combining biomass estimates, usually at some (assumed) steady state, and empirical data on key physiological traits of the species (such as lifespan, energy-conversion efficiencies and diet preferences). Many food-web studies have shown that interaction strengths are strongly patterned (de Ruiter *et al.* 1995, Emmerson and Raffaelli 2004, McCann and Yodzis 1994, McCann 2011), and that both distribution of interaction strengths and the topological structure of the web are important for stability in ecosystems de Ruiter *et al.* 1995, Neutel *et al.* 2002.

The initial papers highlighting the *need* to incorporate infectious agents into food-web analysis are largely concentrated on empirical work on parasites in aquatic systems. Papers have mostly either provided empirical data (Amundsen *et al.* 2009, Huxham *et al.* 1995, Kuris *et al.* 2008, Lafferty *et al.* 2006a, Thompson *et al.* 2004) or highlighted the need to consider parasites in our efforts to understand food webs and ecosystems (Arias-González and Morand 2006, Beckerman and Petchey 2009, Byers 2009, Edeline *et al.* 2008, Getz 2009, Lafferty *et al.* 2006a,b, 2008b). As parasites in aquatic systems have, as a rule, a life cycle with one or more obligatory free-living stages (either different sequential manifestations of the same parasite individual or offspring of an individual, produced inside a host), the parasite as a species is (at least partly) free living and quantifiable (e.g. in terms of biomass). Consequently, it is natural to take a direct approach and incorporate an infectious species as a (special type of consumer) node in the web,with

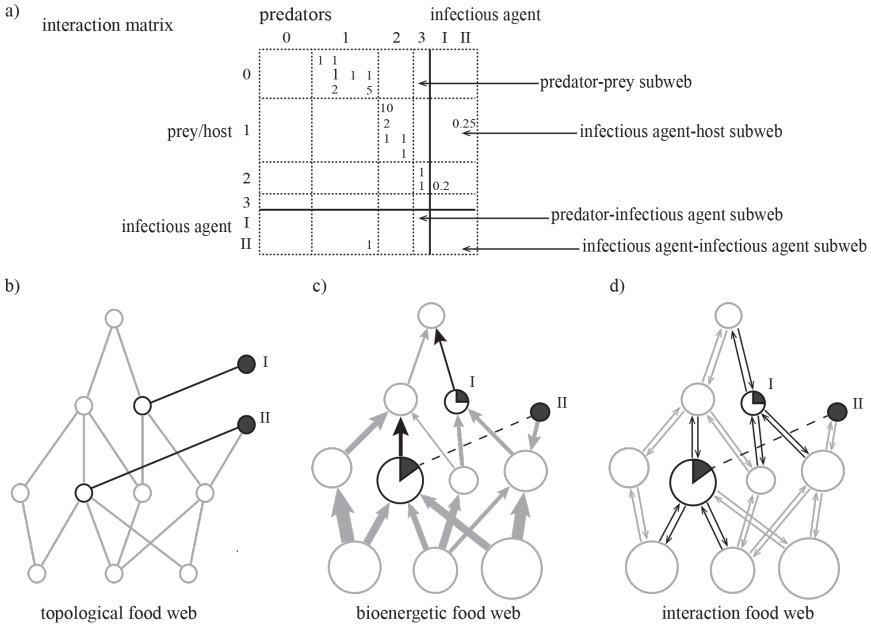

Figure 4.1. Illustrations of topological, bioenergetic and interaction food webs (for an imaginary system). (*a*) Interaction matrix for the imaginary web (with species ordered from left to right in each trophic level) and its four subwebs (e.g. Lafferty *et al.* 2006b). (*b-d*) These subwebs are included in different way into different types of food webs: in black, a pathogen (I) and a parasite (II). (*b*) Infectious agents can be directly included in the food web (here illustrated for the topological food web only) through new nodes (black filled, infectious agent; black open, host species; grey open, non-host species) and links (grey, predator-prey; black, infectious agent-host); in this case, we would use a binary form for the interaction matrix. (*c,d*) The bioenergetic and interaction food webs here illustrate the indirect inclusion only. The black part of a circle represents the infected proportion of the population of that host species, and black arrows are examples of energy flow/interaction strength affected; the dashed line highlights that the free-living stages and the within-host stages of a parasite represent the same biological species, with separate bookkeeping for the different stages.

links to the host species it uses as resource, with possible free-living stages ingested as 'prey' (Figure 4.1b). Infectious agents potentially change the topological properties of the host and non-host species' network, thus generating insight into their effects on the foodweb. One could also incorporate infectious agents indirectly by thinking of them as living inside their hosts and influencing the energy flow and interaction strengths of the existing consumer-resource links in the host/non-host network (Figure 4.1c,d). The main part of this review (4.2) is devoted to a systematic classification of mutual influences of infectious agents on energy flow and interaction strength in food webs, covering a range of parasites and pathogens, in a broad range of host species. Such classifications may

well reveal unexpected differences between apparently closely related infectious agents, and notably also show similarities between (apparently) unrelated infectious agents that become clear only at the ecosystem level. Such a focus improves on the pairwise 'one agent-one host' interactions in many epidemiological studies, also in those related to wildlife, which may so far have 'hidden' these aspects. In 4.3, we briefly return to the various ways in which to extend the theoretical food-web framework to address effects of infectious agents, for future understanding of the many examples that have been documented.

4.2. Infectious agents and interaction strengths: a catalogue of examples

We systematically group (mostly empirical) papers studying effects of infectious agents in ecosystems and on food-web interaction. Other reviews of such studies have been published, focusing mainly on parasites (Hatcher *et al.* 2012). As a classification principle, interaction between infectious agents and the ecosystem is represented at various levels of biological integration. The studies we review have specifically highlighted these interactions, but in fact, because of their ubiquitous nature, infectious agents possibly impact on life-history traits, behaviour, feeding or other individual-level aspects, and thus influence, to some extent, all ecological quantification. It would be rare, if not impossible, to obtain field data from an infection-free system.

4.2.1. Infectious agents and energy (flow and biomass)

Infectious agents can lead to increased or decreased energy flow through food webs by affecting feeding rates, growth, mortality, fecundity, behaviour and other properties of individuals.

Most infectious agents force a host to redirect parts of its energy, assimilated for biomass production and maintenance, towards investment in immune response (Anderson and May 1979, Lafferty *et al.* 2006b). Parasitoids and parasitic castrators use their host's energy directly for their own maintenance and production. Infectious agents also affect growth rates at the host level by changing food consumption, or by affecting the assimilation and production efficiency of the consumed food (Otto *et al.* 2007). One possible effect is altering feeding behaviour (e.g. infected hosts eat less/more or change the size

or species of prey). An example of the former is the herbivorous snail *Littorina littorea*, which is frequently parasitized by the trematode *Cryptocotyle lingua*; uninfected snails consumed 40% more ephemeral macroalgal biomass than infected snails in the laboratory, possibly because the digestive system of infected snails is compromised by *C. lingua* infection (Wood *et al.* 2007). Another example is the tapeworm, which causes infected sticklebacks, *Gasterosteus aculeatus*, to eat smaller prey (Bergersen 1996). Similar examples exist in hosts from terrestrial ecosystems. Avian malaria causes *Hawaiian honeycreepers* (Drepanidinae) to consume less food and consequently to lose body weight (Atkinson *et al.* 2000). Gastrointestinal parasites and many other infectious disease agents can reduce the ability of a host to absorb nutrients, altering digestive efficiency and compromising the host's nutritional status (Koski and Scott 2001).

Infectious agents may also change consumption in the opposite way, causing infected individuals to eat more. One example is the parasitic castrator trematode *Cercaria batillariae* in mud snails, *Batillaria cumingi*, which increases the food consumption of its hosts for its own energy needs and induces gigantism (Byers 2000, Miura *et al.* 2006). Similarly, air-breathing freshwater snails *Physa acuta* infected with the parasitic castrator trematode *Posthodiplostomum minimum* grazed more rapidly than uninfected snails (Bernot and Lamberti 2008). This is even more interesting when we consider that biomass of a parasitically castrated host population can exceed the biomass of the uninfected host population (Kuris *et al.* 2008).

Infectious agents can affect biomass of the host population through lower fecundity and castration. Some infectious agents can increase survival and growth rate, but reduce fecundity. An example is the fungus *Atkinsonella hypoxylon*, which infects ramets of the grass *Danthonia spicata* (Clay (2006)). An example of castration is the previously described trematode *C. batillariae*, which castrates its host so that after infection snails can only produce larval trematodes (Miura *et al.* 2006).

In any interaction between two trophic levels, a change in energy flow due to the presence of an infectious agent at one level may influence the other level. Examples include infections in prey resulting in additional predation driven by unusual behaviour in infected individuals (Kuris 2005, Lafferty and Morris 1996, Seppälä *et al.* 2004, Thomas *et al.* 2005). Changes in grouse behaviour can result from intestinal helminth parasites, which may contribute to higher mortality through increased predation by mammalian predators (Isomursu *et al.* 2008). The behaviour- changing effects of the pathogen *Toxoplasma gondii* have been well documented: infected rodents behave in ways that make them more vulnerable to predation, thus increasing the odds for the pathogen to reach cats, to

complete its life cycle (Vyas *et al.* 2007). Infection in prey that induces mortality may also increase energy available to consumers. This was observed in 1994 in Serengeti National Park, when severe drought in 1993 and a *Babesia sp.* infection in Cape buffalo led to increased numbers of buffalo carcasses available to be consumed by lions. The lions, partly immune-compromised by an outbreak of canine distemper virus (CDV), became additionally infected with *Babesia sp.*, through ixodid ticks with a broad host range, possibly causing substantial mortality by this combination of factors (Munson *et al.* 2008). A similar combination, involving plague in prairie dogs and CDV in black-footed ferrets, possibly led to the decline in the wild of the black-footed ferret (Williams *et al.* 1988), where plague in prairie dogs currently appears to frustrate re-introduction programmes (Matchett *et al.* 2010).

Parasites may also be consumed directly as an energy resource without infecting their predator. Such predation on parasites is a natural process that happens in many communities and ecosystems, and can occur in at least three ways: as concomitant predation of parasites with prey; predation on living stages; and grooming (Johnson *et al.* 2010). An example of this is that 16-408 ticks per bird were found in the stomach contents of captured oxpeckers (Bezuidenhout and Stutterheim 1980, Samish *et al.* 2004, Van Someren 1951). We discuss another grooming example related to ticks and opossums below. A different mechanism is the mistletoe, a parasite that has fruits available year round, flowers with abundant nectar and foliage rich in nutrients (Watson 2001). This parasite is an important food source for many species of birds and mammals (Press and Phoenix 2005). Furthermore, cercariae (larval forms of trematodes) that do not find suitable hosts within a certain time, and thus die, contribute to the detrital pools in aquatic ecosystems (Bernot and Lamberti 2008) or become food for non- host species of fish (Kaplan *et al.* 2009).

Between ecosystems, infectious agents may also contribute indirectly to changes in energy flow. For example, the Dutch elm disease fungus increases energy flow between the terrestrial and aquatic ecosystems through increased mortality of hosts, which contributes to coarse woody debris in streams (Peterken and Mountford 1998) Similarly, nematomorph parasitoids change the behaviour of crickets, which then enter streams and become prey for trout (Sato *et al.* 2012).

The importance of the functional role of the host in the ecosystem can be seen, for example, with detrivores. Helminth parasites may alter behaviour post-infection of this functional group and decrease consumption of detritus, which further enhances energy flow through communities and ecosystems (Hernandez and Sukhdeo 2008).

Pathogens are abundant in aquatic environments. The oceans contain an estimated 1030

virus particles, with 1023 infections occurring each second (Suttle 2007). Influences of viruses in such ecosystems are still underexplored, but several examples exist that show these influences to be varied and complex. For example, viruses infecting primary producers (phytoplankton) in marine ecosystems can have a substantial negative effect on productivity (Suttle *et al.* 1990). In this respect, a fascinating system is the use of resource chloroplasts to gain energy from photosynthesis within the consumer's cells by the solar-powered sea slug, *Elysia chlorotica*. The synchronized sudden death of slugs appears to be connected to a release of viral particles from the chloroplasts, suggesting that these parasitoid viruses play a role in regulating the life history of these consumers through infection of the resource (Pierce *et al.* 1999, Rohwer and Thurber 2009).

4.2.2. Infectious agents and species (biodiversity)

At the species level, the term biodiversity is frequently used to account for species richness, described as the number of species of a particular taxon or life form that characterizes a particular biological community, habitat or ecosystem (Levin *et al.* 2009). Biodiversity of food web/ecosystem is influenced by infectious agents directly through their own diversity, as well as through their influence on host and non–host diversity. Diversity of infectious agents is difficult to assess, and complicated further by the discovery of cryptic species (Dobson *et al.* 2008). Infectious agents can have great influence if they are introduced to a new ecosystem. Examples include spill-over from terrestrial to marine ecosystems, such as toxoplasmosis of sea otters, aspergillosis of sea fans and many others (Burge *et al.* 2013, Harvell *et al.* 1999). Usually, introductions of agents are connected with introductions of infected hosts. Apart from transmission among the newly imported hosts, sometimes the infectious agent is also transmitted to a native species that may be even more susceptible. One of the most well-studied examples is the replacement of red squirrel with grey squirrel, possibly mediated by parapoxvirus, which causes higher mortality in red squirrel (Rushton *et al.* 2001).

Although infectious agents are considered to be one of the main factors directing species extinctions in natural ecosystems, research has shown that of 833 known species extinctions in the last 500 years (Smith *et al.* 2006) only 31 were known to be partly owing to infectious disease agents. It is very rare for an infectious disease to be listed as the single factor that contributed to extinction of a particular species (Smith *et al.* 2006). For example, the chytrid fungus *Batrachochytrium dendrobatidis* in combination with environmental factors has led to the extinction of dozens of frog species in tropical regions in recent decades (Berger *et al.* 1998, Fisher *et al.* 2012).

4.2.3. Infectious agents and community (interactions)

Infectious agents in communities interact with their hosts, but also mediate negative and positive interactions between host and non-host species. Within hosts, parasites and pathogens form interaction networks, and may modify each other's dynamics (Jolles *et al.* 2008, Munson *et al.* 2008, Telfer *et al.* 2010). More fieldwork is needed to elucidate such interactions between infectious agents, but effects may be difficult to disentangle. For example, Maas *et al.* 2012 looked at more than 600 lions in Kruger Park, searching for possible synergistic effects between bovine tuberculosis and feline immunodeficiency virus, potentially mimicking the influence of tuberculosis and HIV established in humans, but found no evidence for a similar relation in lions.

Community-level changes are frequently forced by decline in a community's keystone population and affect predator-prey, competitive, mutualistic and other community interactions. As examples of other parasite-mediated interactions are well reviewed elsewhere (Daskin and Alford 2012, Hatcher *et al.* 2006, Lafferty *et al.* 2008a), we restricted our interest to infectious agents that mediate predator-prey interactions.

Results of predator-prey interaction mediated by infection depend on the trophic position of the infected species. First, infection may occur at a low trophic level and influence consumers at upper trophic levels. Second, infection may influence species at mid-trophic level, possibly leading to changes in species populations of the same functional group, or propagate up and down trophic levels and produce trophic cascades. Effects may include prey switches by predators, when one prey species is reduced in abundance because of an infectious disease, leading to decreased interaction with that particular prey. Examples of infection-mediated predator- prey interactions are widespread in various ecosystems. In an aquatic ecosystem, an outbreak of unidentified infectious (possibly bacterial) agent in a keystone herbivore, the sea urchin (*Diadema antilarum*), induces high mortality. Because of its controlling effects of algal abundance (Carpenter 1990), the loss of *Diadema* from coral reef systems where they had previously been abundant encouraged growth of their main resource, benthic algae; this was characterized as a main ecological phase shift from a coral-dominated system to an algal-dominated system (Hughes 1994). Similarly, in a terrestrial system, anthrax, during the wet season, fatally infects zebras, springboks, wildebeest and oryx, as well as, during the dry season, elephants. As a consequence, infected carcasses are available for vultures, hyenas, lions and blackbacked jackals year round. Decline in populations of major herbivore species such as zebras and elephants force cascading effects in the ecosystem (Getz 2009, 2011). These types of change are moreover affected by outbreaks in the top predator population. An example is canine

parvovirus (CPV) in wolves, which induced a shift from top-down to bottom-up control of moose population dynamics: as CPV decimated the wolf population, moose growth rate is regulated by bottom-up effects and climate (Wilmers *et al.* 2006).

Infectious agents may also indirectly influence predator-prey interaction and drive community composition, altering behaviour of the host. As already stated, nematomorph parasitoids affect the behaviour of crickets, causing them to enter streams to become available as prey for trout. This indirectly influences trout, causing them to eat fewer benthic invertebrates, thus inducing an increase of benthic algae and a decrease in the rate of leaf breakdown (Sato *et al.* 2012).

4.2.4. Infectious agents and ecosystem (physical characteristics, ecosystem engineering)

Infectious agents sometimes influence physical characteristics of the ecosystem to which they belong, and the term 'ecosystem engineering' has been used as a metaphor to describe these effects. This phenomenon was first defined by Jones *et al.* (1994, 1997) to represent direct or indirect control of resource availability mediated by an organism's ability to cause physical state changes in abiotic or biotic materials. This definition includes space among the resources an organism can use for growth, maintenance and (re)production. Diverse examples of infectious agents as ecosystem engineers, with either direct or indirect influence, have been described (Hatcher and Dunn 2011, Thomas *et al.* 1999).

Among the infectious agents that are themselves ecosystem engineers would be parasitic plants such as mistletoes. They change physical characteristics of an ecosystem (e.g. by providing nests for many animals). In southwest Oregon, mistletoe (*Arceuthobium douglasii*) brooms provide nests for the northern spotted owl (Marshall *et al.* 2003). Likewise, the Dutch elm disease fungus affects forest structure by changing the amount of standing material and creating canopy gaps that alter microclimate; tree defoliation increases the amount of light reaching the bottom and promotes herb and scrub growth; dead trees contribute to coarse woody debris in streams and decreased availability of nest sites (Hanula 1996, Peterken and Mountford 1998). In the same way, the fungus *Phytophthora cinnamomi* converted large areas of eucalyptus forest to monocot-dominated open savannah, eliminating nest sites and food for many animals (Weste and Marks 1987).

Infections may also have indirect impacts on the ecosystem. For instance, rinderpest

outbreaks in the 1960s caused a decline of ungulates in Maasai Mara National Park. The lack of herbivores facilitated an increase in dry-grass fires, which led to a significant decrease in acacia trees, an important part of the ecosystem (Dublin 1991). These trees provide shade for ungulates, as well as nest sites for raptors, owls, vultures and a variety of other bird species (Tews *et al.* 2004). Holdo *et al.* (2009) found the opposite effect in Serengeti National Park, after rinderpest was eradicated there as a strong regulator of wildebeest. Similarly, rabbits, after being introduced to Great Britain in the eleventh century as a domestic animal, were by the 1950s sufficiently abundant in the wild that they were preventing regeneration of woody plants in many habitats. This led to a habitat transition from a forest-dominated to a grassland-dominated ecosystem. Introduction of the myxoma virus led to decline of the rabbit population and the re-establishment of forest after 20 years (Dobson and Crawley 1994).

One can also find these examples in aquatic ecosystems. Larval trematodes that encyst the foot of the cockle *Austrovenus stutchburyi* reduce the burrowing ability of cockle, making them easier prey for host birds, and the shells of stranded cockles create habitat for a rich and distinctive epibiont community (Mouritsen and Poulin 2010, Thomas *et al.* 1998). The impact of cockles on the benthic community is governed by reduced sediment disturbance, increased surface structural complexity and availability of larval trematodes as an additional food resource. The shells of dead cockles are so abundant that they offer important new habitat in the mudflat ecology.

4.2.5. Effects of different levels of biological integration on infectious agents

Infectious agents are also influenced by energy flow, biodiversity, community structure and abiotic characteristics of an ecosystem.

With regard to biomass and energy, for example, hantavirus prevalence in small rodent host species increases as a result of boosts in primary food production in mast years (Clement *et al.* 2009, Jonsson *et al.* 2010), and a similar effect is observed for plague prevalence in small rodent host species when climate (notably rainfall) induces boosts of primary producers, leading to host population growth (Samia *et al.* 2011), and subsequent plague outbreaks (Davis *et al.* 2008a). Susceptibility to *Metschnikowia bicuspidata* infection, its evolution and the sizes of outbreaks among host individuals of *Daphnia dentifera* (an interaction to be discussed in more detail below) is influenced by the level of ecosystem productivity in lakes and the quality of the algal resource for the *Daphnia*

(Duffy *et al.* 2012, Hall *et al.* 2008).

Perhaps the most hotly debated effect concerns the influence of community biodiversity on the infectious agent. Biodiversity and the ratio of suitable (competent) and unsuitable (incompetent) hosts direct the survival of infectious disease agents in food webs (Johnson and Thieltges 2010). This diversity involves vectors and (other) hosts of infectious agents, as well as non-hosts.

Vector diversity, as the research on transmission of vector-borne diseases shows, is relevant because the potential for infectious agents to persist increases in multi-species host populations. For example, for fleas transmitting the plague bacterium among rodents, the presence of multiple vector species able to infest multiple susceptible host species creates a more connected host network (Eisen *et al.* 2012). In West Nile virus transmission, there might be season-dependent shifts in feeding behaviour of the mosquito vector species, shifting from (virus-competent) birds to (less competent) mammals (Kilpatrick *et al.* 2006).

Whether individual species are suitable/unsuitable as hosts will determine the ability of the infectious agent to become established in a community (Leung and Poulin 2008). For example, certain plant pathogens showed reduced prevalence but also reduced diversity in host species that are threatened, compared with non–threatened host species (Gibson *et al.* 2010).

Indirect influences on infectious agents can result from increased diversity of predator species, which may change the behaviour of prey. For instance, the deer mouse spends more time in shelters as the number of predators increases, thus decreasing infection spread (Dizney and Ruedas 2009).

Transmission of an agent may significantly increase after loss of non-host species: prevalence of Sin Nombre virus rose 2–14% with decline of diversity (Keesing *et al.* 2009). Lyme disease is transmitted by blacklegged ticks, but Virginia opossums can predate on 83-96% of the ticks that attach to them and engorge, and loss of this weakly competent host species has led to increased Lyme prevalence in ticks that switch to feeding on mice, which are both strongly competent and weakly grooming (Keesing *et al.* 2009).

Although the influence of biodiversity on prevalence in specific host species is no longer questioned, there is considerable debate concerning the generality of the effect, what factors determine whether the relationship is positive or negative, and what the mechanisms and causes are (Ostfeld *et al.* 2008, Randolph and Dobson 2012). With respect to

the latter, mechanisms often referred to are either transmission- and/or contact-related. But increased bird biodiversity did not reduce transmission or reduce encounters between mosquitoes infected with West Nile virus and competent hosts, for instance, even though a negative relationship was found between bird diversity and human incidence (Swaddle and Calos 2008).

One known large influence of community diversity on the infectious agents is through extinction or decline of key species for highly host-specific agents. If their population sizes are under threshold, the agents cannot persist (Lloyd-Smith *et al.* 2009). For example, extinction of five North American carnivore species is predicted to lead to extinction of 56 parasite species (Dunn *et al.* 2009).

Community structure affects infectious agents also through the behavioural and social characteristics of hosts. This is seen in the behaviour that some species of host develop to avoid infectious agents (e.g. mammals smelling infected faeces and avoiding contaminated areas Hutchings *et al.* 2006). Various social behaviours like mating strategies, social avoidance, group size and group isolation have different consequences for transmission (Loehle 1995). For instance, mating behaviour may increase host susceptibility: male field crickets Gryllus lineaticeps produce chirped songs to attract mates, and the parasitoid fly *Ormia ochracea* uses this song to locate them 9Beckers and Wagner 2012. Furthermore, species that live in high-density populations facilitate transmission because of the frequency of contacts between individuals (Beldomenico *et al.* 2008). Increased frequency of contacts can be induced not only in social groups of the same species but also between different species that share a place of foraging, water or shelter (Craft *et al.* 2008). Thus, for example, different rodent species may become infected with the plague bacterium by indirect contact because they frequent the same burrows (Collinge *et al.* 2005).

A particularly interesting interaction between *Daphnia dentifera*, one of its invertebrate predators (*Chaoborus midge* larvae) and a yeast parasitoid (*Metschnikowia bicuspidata*) (Duffy *et al.* 2011) is an example of association of community and ecosystem influence. Here, the predator produces a chemical compound that has two effects. The direct effect is that it induces growth of the *Daphnia* prey individuals. Owing to this growth in size, these individuals become more susceptible to the fungus, in the sense that bigger individuals filter a larger volume of water, and therefore ingest larger numbers of spores. A second indirect effect of this is that, once infected, larger individuals increase transmission rates, as they produce more spores than would infect *Daphnia* of a normal size.

4.3. Consequences for thinking about infectious agents in food webs

The examples above illustrate both the breadth and depth of various types of interaction between infectious agents and food webs/ecosystems. This motivates the need for a theoretical framework, as an additional tool to generate robust insight into the mechanisms behind, and consequences of, this interaction, and to explore possible generic principles.

Table 4.1. Selected examples from the review.

Infectious agent/disease	Host	Type of infectious agent	Type of ecosystem	Reference
Infectious agents influencing different levels of biological integration				
trematode	snail	parasitic castrator	aquatic	Wood *et al.* 2007
tapeworm	fish	parasite	aquatic	Bergersen 1996
malaria	birds	pathogen	terrestrial	Atkinson *et al.* 2000
CDV + *Babesia*	lion	pathogen	terrestrial	Munson *et al.* 2008
mistletoe	tree	parasite	terrestrial	Samish *et al.* 2004
nematomorph parasitoid	crickets	parasitoid	terrestrial	Sato *et al.* 2012
toxoplasmosis	sea otter	pathogen	aquatic	Burge *et al.* 2013, Harvell *et al.* 1999
parapoxvirus	squirrels	pathogen	terrestrial	Rushton *et al.* 2001
fungus	frog	pathogen	terrestrial	Berger *et al.* 1998, Fisher *et al.* 2012
unknown (suspected bacterial)	sea urchin	pathogen	aquatic	Carpenter 1990, Hughes 1994
CPV	wolves	pathogen	terrestrial	Wilmers *et al.* 2006
fungus	eucalyptus tree	pathogen	terrestrial	Weste and Marks 1987
rinderpest	wildebeest	pathogen	terrestrial	Holdo *et al.* 2009
trematode	cockle	trophically transmitted	aquatic	Thomas *et al.* 1998
Biological integration influencing infectious agents				
hantavirus	rodent	pathogen	terrestrial	Clement *et al.* 2009
plague	rodent	pathogen	terrestrial	Johnson and Thieltges 2010
West Nile virus	birds	pathogen	terrestrial	Eisen *et al.* 2012
Borrelia burgdorferi	rodent	pathogen	terrestrial	Dizney and Ruedas 2009
parasitoid fly	cricket	parasitoid	terrestrial	Loehle 1995
yeast	*Daphnia*	parasitoid	aquatic	Collinge *et al.* 2005

Many of the examples (Table 4.1) involve effects on energy flow and interaction strength, and it is possible that studying infectious agents indirectly (i.e. via the way they influence interaction strength) is an effective approach to such insight. This is especially relevant for pathogens.

Conceptual research on effects of infectious disease agents in food webs has so far been mainly directed at a direct topological approach by explicitly incorporating the agents, notably parasites, as species in the web of host and non-host interaction. Lafferty *et al.* (2006b) distinguish various sorts of links, such as parasite-parasite, parasite-host and predator-prey, also making a useful distinction between 'possible' and 'realized' links. They show that doing a careful accounting of such different links clarifies the large influ-

ence that parasites can have when included as species in the topological web.

Many pathogens hardly have individual biomass (although the total biomass of pathogens as species is underexplored and may be substantial) and generally have no free-living stage, and their transmission is not explicitly considered, but is assumed to occur inside populations of a host species that is presented by one node. Here, an approach where the agent is incorporated indirectly, through its effect on hosts, may be fruitful. Consider, as an example, a system with a consumer species and a resource species, with a pathogen that can infect the consumer in an immunizing infection. Instead of treating all consumer individuals the same, as one would do when studying this interaction in the absence of the pathogen, we now differentiate the consumer individuals by epidemiological state, differentiating susceptible, infectious and recovered/immune consumers of this species in the most basic case. The interaction strength quantifying the link between a particular class of consumers and the resource will now depend on the epidemiological state of those consumers. Similar reasoning also applies to the inclusion of parasites, using parasite load as epidemiological state (Diekmann *et al.* 2013), or parasitoids.

Which approach is most feasible will depend on the food web and infectious agent studied, the type of question one wants to address, and the level of detail available in data. The direct approach allows one to explore infectious agents as biological species- for example, exploring the ecological influence of a new species of infectious agent, as did Dunne *et al.* (2013), the influence of parasite mortality on biomass redistribution in the web, the distribution of infectious species over trophic levels and the role of infectious species in maintaining biodiversity. The resulting changes in topological structure and ecosystem stability deserve substantial future attention. If one is interested in questions that require quantification of within- and between-host species spread of infection, the indirect approach may be a more natural point of departure. Examples are questions on the evolution of virulence, jumps to/emergence in new host species, the ability to invade a given ecosystem, persistence, effects of control strategies and changes in prevalence over various host species in the web. Viewing interactions between hosts and non-hosts as being mediated by the (dynamic) epidemiological status of the individuals involved is close to the established methodology in epidemic theory of infectious diseases, thus suggesting a feasible theoretical framework combining community ecology and epidemiology (Roberts and Heesterbeek 2013).

Extending and combining epidemiological and ecological theoretical frameworks of analysis would allow us to understand the observed types of behaviour at the ecosystem level and to explain them in terms of lower (e.g. one consumer-one resource, species or even

individual) level interactions, mechanisms and processes. Both fields have considerably increased their methodology in recent decades and are able to address very complex phenomena with low-dimensional models- for example, in the case of population dynamics of structured populations, emerging behaviour from complexity in ecosystems or infectious agents in highly heterogeneous structured populations of hosts, including heterogeneity in contacts, modelled in networks. These approaches have shown that infectious agents are able to both stabilize and destabilize predator-prey interaction, mediate coexistence of resources and consumers, affect spatial patterning of populations, as well as have regulatory and other conservation consequences (Beltrami and Carroll 1994, Hadeler and Freedman 1989, Malchow *et al.* 2008, Oliveira and Hilker 2010, Roberts and Heesterbeek 2013, Venturino 1994). Such studies in the mathematical biology literature, often aiming at insight for generic systems, and mostly focusing on just two interacting species (apart from the infectious agent), have indeed mostly taken the approach of differentiating between epidemiological states in a consumer or a resource, or in both. There are a number of studies where models are analysed for specific systems to interpret empirical data (for example, thework of Hall *et al.* 2008 and Duffy *et al.* 2012, and other papers by these authors mentioned therein). The studies so far have hardly integrated epidemiology and food-web ecology by thinking in terms of interaction strength or energy flow (with the work of Getz on anthrax being one of the exceptions Getz 2011).

If we are to understand observed patterns as reviewed in this paper, and ultimately predict what repercussions changes to ecosystems may have with regard to infectious disease prevalence and distribution, including emergence in human populations that may result, then developing such theories for realistic systems is essential.

4.4. Acknowledgements

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Chapter 5

Linking ecology and epidemiology: the case of infected resource

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Abstract

In nature, ecological communities exist as a result of different interactions between species determining structure, functioning and stability. Empirical as well as theoretical studies are mostly focused on trophic (consumer-resource) interactions and non-trophic interactions separately. Recently, in theoretical and field work, studies started to explore combinations of these interaction types, notably looking at the way infectious disease agents affect consumer- resource relationships in food-webs. Here, we illustrate such influence by looking at a simple model of a microparasite in a very basic Lotka-Volterra consumer-resource system. We show that even in this simplest of settings one can see a diverse range of subtle changes in system behaviour if one lets the main trophic parameters for both host and non-host species be influences by non-trophic interaction.

5.1. Introduction

Interspecific interactions in ecological communities are the main mechanisms that determine structure, functioning and stability of ecosystems (Allesina and Tang 2012, May 1972, 1973, Mougi and Kondoh 2014, Neutel *et al.* 2002). These interactions can be qualitatively positive, negative or neutral, and pairs of these interactions between two species may be of opposite sign (e.g., trophic, parasitic) or of equivalent sign (e.g., mutualistic, competitive). Most of the research on ecological interactions has focused on feeding relations (Begon *et al.* 2006, Levin *et al.* 2009, McCann 2011, Moore and de Ruiter 2012, Odum and Barrett 1971, Pimm 1982), but in recent studies of ecological communities this was extended to parasitic (Huxham *et al.* 1995, Kuris *et al.* 2008, Lafferty *et al.* 2006b, Thompson *et al.* 2004) and non-parasitic non-trophic relations (Fontaine *et al.* 2011, Kéfi *et al.* 2012, Mougi and Kondoh 2012, Sauve *et al.* 2014, Thebault and Fontaine 2010), see also the chapter by Kéfi et al. 2016).

In this chapter, we focus on parasitic relations and notably on the question how trophic interactions and infectious agents mutually influence each other. Here, we will refer to the combined classes of infectious species as parasites (see next section for details). The impact of parasites in an ecological community can be quantified through their direct influence on the food web structure, as well more indirectly through the way they influence physiological traits of host species and trophic relations of the host and non-host species (Kéfi *et al.* 2012, Selakovic *et al.* 2014). In this chapter we first briefly discuss the diversity of parasitic interactions, their relationships with host and non-host species, as well as their effects on a simple consumer-resource relationship consisting of one host and one non-host species. The largest part of the chapter is devoted to exploring a basic model, to show how intricately ecological and epidemiological effects are interwoven, even in the simplest possible ecosystem consisting of two species. Even though this model is basic in the sense that it low- dimensional and not meant to realistically represent any particular system, the analysis does hint at broader ecological insight, for example into possible differences between terrestrial and aquatic ecosystems based on parasitic interaction. The simple analysis highlights the need to study the link between ecology and infectious disease epidemiology in more realistic models.

5.1.1. Parasitic interactions, diversity, types, functional roles and modelling

The relationship between parasites and their hosts can have aspects of both trophic and non- trophic interactions. Parasites are in essence consumers of resources, but they are different from typical consumers in several ways. For example, while a typical consumer has more than one victim during its life, parasites as a rule have only one victim per life stage (Lafferty and Kuris 2002). Also, parasites do not necessarily kill or fully consume their victims. Parasites may also act as prey in a food web, and can be seen as part of trophic interaction in this way as well (Johnson *et al.* 2010, Marcogliese and Cone 1997, Thieltges *et al.* 2013). Inclusion of 47 parasites in an aquatic food web, for example, gave rise to 1093 new interactions of parasites that were prey for other species (Lafferty 2013). Parasitic interaction can directly or indirectly influence attributes of species in ecological networks, comparable to other non-trophic interactions. The non-trophic interaction that affects attributes of nodes (hosts), and in that way influences the consumer-resource relation, is called 'interaction modification' (Kéfi *et al.* 2012). The attributes could have a direct or indirect influence on the behaviour of hosts and non-hosts, handling time of prey, prey preference, assimilation efficiency, conversion efficiency, mortality, reproduction and growth, and they are common for the different classes of parasites.

Parasites are diverse but the magnitude of this diversity is unknown and it is impossible to estimate the number of species (Dobson *et al.* 2008). The main characteristic is that they use a host individual's energy for growth, reproduction and survival. They have, however, very different life histories and sizes. We distinguish microparasites (viruses, bacteria, fungi, protozoa), macroparasites (endo-parasites such as helminthes), ectoparasites (fleas and ticks), parasitic castrators and parasitoids (Kuris and Lafferty 2000, Lafferty and Kuris 2002). At one end of the size spectrum, viruses vary in length 30-200 nm. For example, rabies virus has a length of 180nm (Baer 1991). At the other side of the spectrum, tapeworms vary from 1mm to several meters, like Diphyllobothrium (Faust *et al.* 1968). Furthermore, size and mass of a parasite compared to their hosts are very diverse and depends on type of a parasite. While most microparasites have ratios between $1{:}10^8$ and $1{:}10^2$, parasitoids and parasitic castrators are sometimes of mass and size comparable to those of their host (Lafferty and Kuris 2002).

Ecto-parasites affect their hosts through energy drain by sucking their blood and by activation of a host's immune response with their saliva. This drain of energy can produce subtle subclinical responses, even when these parasites do not by themselves cause disease in their hosts. However, ticks and fleas can also transmit other parasite species,

notably microparasites, initiating infection inside of the host that can lead to strong clinical effects, including substantial morbidity impairing normal ecological functioning, and mortality. In Ngorongoro Conservation park in 2000 and 2001 there occurred significant mortality among buffaloes, wildebeests, lions and rhinoceros that had showed infection with *Babesia* species transmitted by ticks (Munson *et al.* 2008, Nijhof *et al.* 2003). But parasites carried by ecto-parasites can also cause only subtle sub-clinical effects in host species to which they have strongly adapted.

Subclinical and clinical effects of parasites impact on the overall fitness of the host. Microparasites and macroparasites often negatively influence the fitness of the host, while parasitic castrators and parasitoids reduce fitness of the host to zero (Kuris and Lafferty 2000, Lafferty and Kuris 2002) Fitness reduction (e.g., reduced growth and reproduction) of the host originates from the effect of the parasites on the host's ability to feed and on the efficiency of using ingested food for the maintenance and production (Anderson and May 1979, Lafferty *et al.* 2006b). Workers of the bumblebee, *Bombus terrestris*, challenged with lipopolysaccharides in order to induce their immune system under starvation, reduced survival by 50 to 70% (Moret and Schmid-Hempel 2000). Further, multiple parasite infections in North American red squirrels *Tamiasciurus hudsonicus* negatively impact reproductive success due to allocation of the energy towards immune response (Gooderham and Schulte-Hostedde 2011). Similarly, experimental removal of ectoparasites (mainly fleas) in Columbian ground squirrels (*Spermophilus columbianus*) led to an increase of female body condition (Neuhaus 2003). Parasitic castrators and parasitoids extend this effect even more utilizing almost completely the host's energy that is assimilated through trophic interaction directly for its own reproduction and growth, leading to zero reproduction or death for the host itself (Hechinger *et al.* 2008, Kuris and Lafferty 2000, Lafferty and Kuris 2002, 2009).

Parasites can have many different functional roles (Poulin 1999, Selakovic *et al.* 2014). The difference in the susceptibility of possible hosts, gives to the parasite a role in shaping the population abundance of the host species, thereby affecting the other types of non-trophic interactions. The difference in susceptibility to the malarial parasite *Plasmodium azurophilum* of two species of lizards in the Caribbean plays an important role in their coexistence (Schall 1992). Some species of parasite affect their host by changing the host's behavioural and physical characteristics and by altering the feeding relationship of the host with its consumers and predators (Moore 2002). An experiment of three-spined sticklebacks (*Gasterosteus aculeatus*) that received the same amount of uninfected prey and prey infected with *Pomphorhynchus laevis* showed significant difference in the predation rate on infected individuals due to parasite's impact on colour and behaviour

(Bakker *et al.* 1997). Sometimes parasites lead host species to functional extinction, an example of which will be given below. Many additional interesting examples from the literature that illustrate other functional roles of parasites have been reviewed elsewhere (Selakovic *et al.* 2014).

The above motivates a closer look at how in ecological theory, parasites of all types can be integrated, and what then can be learned from studying the combined ecological and epidemiological dynamics. There is a large and rich literature on infectious disease dynamics and its mathematical and computational tools and models (see Diekmann *et al.* 2013 and Heesterbeek *et al.* 2015, for recent overviews). Mostly, this literature has developed around combinations of one parasite species and one host species. Broadly speaking, there are two modelling approaches depending on the nature of the parasite. If the extent of infection in a host individual and its effects on the hosts' life history can be quantified at the level of the parasite and is influenced by, or even depends on, re-infection, models are in terms of the "degree" of infection (for example, number of hosts carrying n parasites, or the mean parasite load of infected individuals or the environment). These models typically relate to macroparasites. Typically, such parasites and models involve distinct stages in the life cycle, related to different host species or free-living in the environment. If the course of infection and its effects on the host are a more or less autonomous process from first successful exposure, models are in terms of generalized and uniform epidemiological states for host individuals (for example, susceptible, latently infected, infectious, immune). Such models typically relate to microparasites.

Work relating to multiple host species interacting with a single parasite species has emerged, but initially ignoring relations within and between host species that were not linked to infection..Only in recent years has there been more substantial effort to regard parasites in systems of multiple host species that also interact ecologically. There is a growing literature, with studies ranging from specific models to more general theory (see Roberts and Heesterbeek 2013 and the references given there).

In addition to the distinction in approaches in epidemiological models for macro- and microparasites, the added ecological dimension introduces another choice to be made: infectious disease agents can be studied directly or indirectly (Selakovic *et al.* 2014). In the direct approach, parasites are studied as species in food webs, represented by nodes in the web with links to species that are their hosts. In an indirect approach, parasites are studied through their effects on hosts, for example by recognizing different epidemiological states for host individuals of the host species involved, or by recognizing individual hosts with different dynamic infection levels. The indirect approach would

combine well with existing epidemiological modelling frameworks for both macro- and microparasites. The direct approach could combine with macroparasitic epidemiological models, especially for systems with a free-living stage of the parasite.

Here, we aim to give an idea of the intricate way in which ecological and epidemiological processes interact in determining dynamic behaviour, using an indirect approach. We do so by studying a model for a simple situation for which the ecological dynamics, in the absence of a parasite, are well known. To motivate our model and analysis, we give a few examples of infection in the resource species and its effect on the consumer- resource relationship as an introduction to our next section where we discuss the influence of infection in the resource to the interaction with the consumer. Gerbils, *Gerbillus andersoni*, affected by the higher abundance of the fleas (*Synosternus cleopatrae*) than in nature had higher rates of body mass lost than non-parasitized control individuals (Hawlena *et al.* 2006). This was probably due to their reduced attention to forage (Raveh *et al.* 2011). The loss of body mass in gerbils influences their consumers by their need to use more energy to catch additional prey, but on the other side it makes prey more available because of the lack of attention to detect a predator. Further, nematomorph parasite infects cricket and changes its behavior leading crickets to enter streams and become a new prey connection for the touts changing the strength of their neighboring interactions in the trophic network (Sato *et al.* 2012), where we see the indirect impact of the rest of the trophic network community. Infection in resource can lead to functional extinction of consumer species: the Asian chestnut fungus effectively extirpated the American chestnut from eastern US forests, causing the apparent extinction of several phytofagus insects (Anagnostakis 1987).

We do not claim realism in our model that allows insight into specific disease systems, but at a general level we can discuss the bidirectional influence between a parasite and a C-R relation in different types of ecosystems in terms of energy flow, mirroring Rip and McCann 2011 who looked at non-parasitic systems. We explore the influence of non-trophic parasitic behaviour going from aquatic to terrestrial ecosystems. Although terrestrial and aquatic parasites influence their hosts and non-hosts in similar ways, there are distinctions between terrestrial and aquatic environments that influence, for example, parasite biodiversity. Only nine animal phyla are found in terrestrial ecosystems compared to 34 in aquatic ecosystems. This indicates that biodiversity of hosts and parasites may be higher in aquatic ecosystems (McCallum *et al.* 2004). Further, there are differences in types of parasites which appear in the two ecosystems. Parasitoids are relatively common for terrestrial ecosystems (Godfray 1994), while the opposite applies for parasitic castrators (Kuris 1974). The differences between the two environments and their

parasites extends to ways of transmission. Rates of spread of infection in marine ecosystems are higher than those observed in terrestrial ecosystems (McCallum *et al.* 2003). Also, vertical transmission is very rare in aquatic ecosystems, as well as vector transmission of the diseases (although there are some examples: fireworms spreading *Vibrio sp.* among corals).

5.2. Including a simple microparasite affecting feeding behaviour in a simple consumer-resource relationship

Taking into consideration the above examples illustrating how different types of parasites affect their hosts, we analysed a simple Lotka-Volterra consumer-resource model. Mathematically more sophisticated, and ecologically more realistic, models have been studied, but not in a detailed way exploring the interplay between parameters typically involved in the ecology and in the epidemiology. Our aim is not to provide maximal realism, but to explore the interplay in system satisfying the minimum requirements to make it non-trivial.

A simple Lotka-Volterra system, for a consumer interacting with a resource, is used for a broader discussion on stability and energy flux. We use the notation in Rip and McCann 2011, who analyze the stability of the simple system without parasites, and concentrate on 'relative energy ratio' (defined below). They regard the largest real part of the eigenvalues of the Jacobian evaluated at the steady state where resource (R) and consumer (C) co-exist. The system is given by

$$\begin{aligned} \frac{dR}{dt} &= rR\Big(1 - \frac{R}{k}\Big) - aCR \\ \frac{dC}{dt} &= eaCR - mC \end{aligned} \tag{5.1}$$

with resource growth rate r (biomass time^{-1}), resource carrying capacity k (biomass), consumption coefficient a (biomass^{-1} time^{-1}), conversion efficiency e (dimensionless), and consumer mortality m (time^{-1}). The consumer is assumed not to have alternative sources of food. Rip & McCann take $r = k = 1$ for convenience and we will do

the same. The dynamics around the non-trivial steady state (R^*, C^*) are governed by the value of a combined parameter that we shall denote by $d := m/(ea)$, i.e. d (unit: biomass) denotes the C-isocline (see Figure 5.1, left graph). We mirror Rip & McCann and call the 'predation rate' ea between the consumer and the resource relative to the consumer loss rate the relative energy ratio ($ea/m = 1/d$) (note that Rip & McCann use the word "flux", but we prefer to avoid that because it suggests units time^{-1}). They argue that aquatic ecosystems have a higher relative energy ratio, a high herbivore/plant ratio and more variable population dynamics, compared to terrestrial ecosystems. Therefore, low values of d would relate more to the behaviour of an aquatic ecosystem and unstable dynamics, while high values of d would relate more to the behaviour of a terrestrial system with low herbivore/plant ratio and stable population dynamics.

The steady state of the simple model (R^*, C^*) = (d, $1/a$ - d/a) is stable when it exists, i.e. for 0 < d < 1. Because for this simple system the eigenvalues of the Jacobian evaluated at the steady state can be given analytically, one can easily show that the largest real part (and hence the stability) depends on d in a way described in Figure 5.1, right graph. The Jacobian is given by

$$J = \begin{pmatrix} -d & -ad \\ e(1-d) & 0 \end{pmatrix} \tag{5.2}$$

If 0 < d < $4ae/(1+4ae)$, the eigenvalues are complex and the largest real part is linearly decreasing in d; for $4ae/(1+4ae)$ < d < 1, the eigenvalues are real and a non-linear increasing function of d. So, although the non-trivial steady state is stable where it exists, the return time to equilibrium, as provisionally measured by the absolute value of the largest negative real part of the eigenvalues, is a non-linear function of the combined parameter d, describing the ecological balance for the consumer of death and recruitment via resource consumption.

This simple system, with clear behaviour, is an interesting null model to explore the influence of infectious agents on consumer resource interaction. We now regard a non-lethal parasite for which only the resource species is a host. We study the dynamics and stability of the parasite-resource-consumer system. In this specific case we model consumer-resource- microparasite interaction. One could model the epidemiology in many different ways but we choose to keep things simple as an initial exploration and allow some analytic tractability. The consumer-resource-microparasite system we study is as follows:

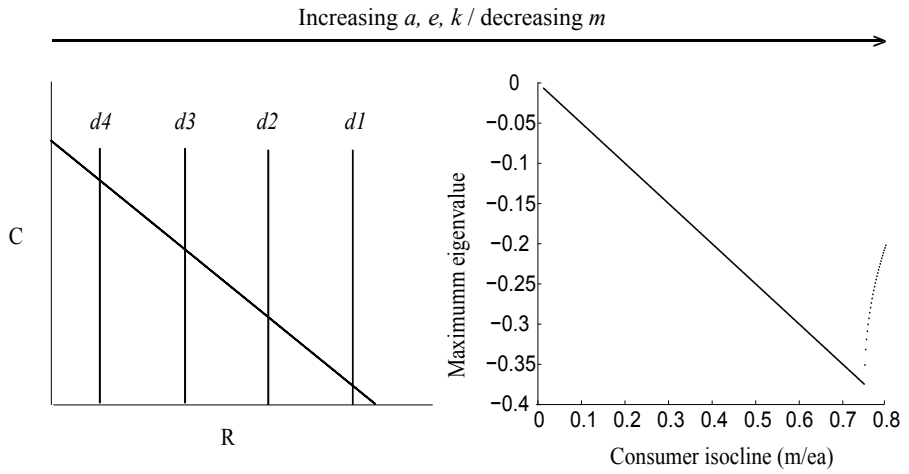


Figure 5.1. Simple Lotka Volterra model analyzed as in Rip and McCann 2011 (a) Increasing d, i.e. decreasing the relative energy ratio as defined below equation (5.1) in the text, shifts the consumer isocline (vertical line) relative to the resource isocline from right to left ($d1 \rightarrow d4$). (b) Shifting the consumer isocline to the left, increases the maximum eigenvalue (decreases stability)

$$\begin{aligned} \frac{dR_s}{dt} &= rR_s\Big(1-\frac{R_s}{k}\Big) - aCR_s - \beta R_s R_i \\ \frac{dC}{dt} &= eaR_sC + qepaR_iC - mC \\ \frac{dR_i}{dt} &= \beta R_s R_i - paR_iC \end{aligned} \tag{5.3}$$

Here, R_s denotes the susceptible resource population and R_i the infected (= infectious) resource population. The transmission rate is denoted by β (time^{-1}). It is the probability per unit of time for one susceptible individual to become infected, i.e. the infection pressure that one infectious individual exerts on susceptibles. The dimensionless factors $p > 0$ and $q > 0$ describe the influence that infection has on the consumption coefficient and conversion efficiency, respectively.

It is important to first note that now we have two different biological points of view for stability. There is ecological stability and epidemiological stability. Ecological stability refers to the balance in the system in terms of intra- and interspecies interaction in the absence of infection; epidemiological stability refers to the balance in the system in terms

of the parasite and its hosts. In the absence of the parasite (R_i = 0), system (5.3) is equal to the Lotka-Volterra system (1) in the (R_s ,C)-plane. The non-trivial steady state (R_s^*, C^*, 0) of system (3) is ecologically stable in that plane. The first question is when this steady state is also epidemiologically stable, i.e., able to withstand invasion by the infectious agent of the resource. For situations where the agent is able to invade, i.e. where the steady state (R_s^* , C^*, 0) is epidemiologically unstable, one can then ask the next question under which conditions an endemic steady state is stable, where susceptible and infected resources and consumers all coexist, and how this stability depends on the values of the ecological and epidemiological parameters.

In Roberts and Heesterbeek 2013, the invasion problem is studied for systems where any number of host and non-host species can ecologically interact in a food web, and where a microparasite interacts epidemiologically with its host species. If the uninfected states for all species are listed first (characterizing individuals of non-host species as being always in the uninfected state), followed by the infected states in the same order of species, a general system has a Jacobian matrix of the following form

$$J = \begin{pmatrix} A & B \\ D & T \end{pmatrix}$$

where matrix A is the ecological community matrix, i.e. the Jacobian of the reduced system when the parasite is absent, and where matrix T is the epidemiological matrix describing transmission among the host species. When J is evaluated at a given steady state, the eigenvalues of J as usual determine the stability of that steady state. In the case of invasion of an infectious agent (i.e., when looking at the infection-free steady state), we have that matrix D is the zero matrix, and the eigenvalue problem decouples in the eigenvalues of the community matrix A, now fully governing ecological stability of the infection-free steady state, and the eigenvalues of the epidemiological matrix T, governing the epidemiological stability of the infection-free steady state (Roberts and Heesterbeek 2013). The characteristic equation of J is then the product of the characteristic equations of A and T.

In system (5.3), where we assume for convenience that $r = 1$ and $k = 1$, the Jacobian at the infection-free steady state (R_s^*, C^* , 0) = (d, $(1 - d)/a$, 0) is given by

$$J = \begin{pmatrix} -d & -ad & -\beta d \\ e(1-d) & 0 & pqe(1-d) \\ 0 & 0 & \beta d - p(1-d) \end{pmatrix}$$

and we see that the ecological stability is governed by matrix (5.2), as expected, and that the epidemiological stability is governed by the one-dimensional matrix $T = \beta d - p(1-d)$. Hence, the infection-free steady state is epidemiologically stable as long as $\beta d - p(1-d) < 0$, or when $R_0 < 1$, where

$$R_0 := \frac{\beta d}{p(1-d)} \tag{5.4}$$

is the basic reproduction number of the infection system. The basic reproduction number is the average number of new cases of an infection caused by a typical infected individual in a fully susceptible population of hosts in steady state (see Diekmann *et al.* 2013).

Note that R_0 in (5.4) is a combination of ecological and epidemiological parameters because a non-host species (the consumer) influences through ecological interaction, epidemiologically relevant aspects of resource individuals (in this case their life expectancy). The biological interpretation is that an infected resource individual is expected to produce βd new cases per unit of time during its infectious period with expected length $1/p(1-d)$. The latter is 1 divided by the probability per unit of time of dying (i.e. by being eaten by a consumer in our model), in the steady state at invasion of the parasite. When $R_0 > 1$, the steady state is epidemiologically unstable and the agent can invade. In Figure 5.2, we show curves in a feasible part of the (β,d)-plane where $R_0 = 1$, for various values of p. We see that, for parasites of limited infectiousness, successful invasion needs more severe ecological effects (smaller values for p) and higher values for the resource steady state, compared to parasites that induce high infectiousness. Increasing the severity of the ecological effect of the parasite on the consumer-resource interaction (i.e., decreasing the value of p) increases the area of the (β,d)- parameter space where the parasite can invade. In ecological terms, if p is small, consumers eat a relatively small proportion of the infected resource population that can hence contact a relatively larger part of the susceptible resource population, leading to more transmission.

Upon successful invasion, the system moves away from the state (R_s^*, C^*, 0), which is ecologically stable in the (R,C)-plane, and moves into the three-dimensional space with variables: susceptible resource, consumer, and infectious resource. The system can then

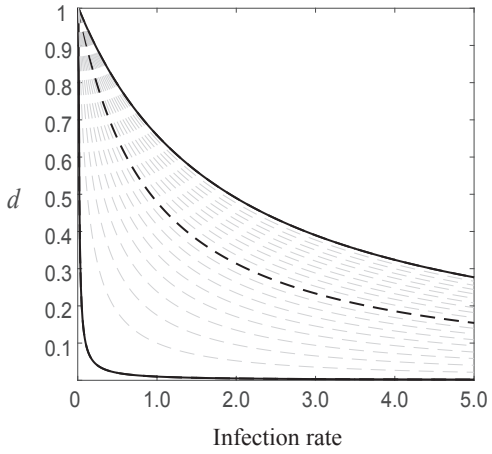


Figure 5.2. The ability of the parasite to invade the consumer-resource system, as a function of infection rate β , the steady state population size of the resource in the absence of infection, and for a range of values for the influence of the parasite on the feeding of the host (p; three specific values indicated). For a given value of p, the parasite is able to invade the system ($R_0 > 1$) for (β,d)- combinations above the corresponding line.

converge to the steady state (R_s^*, C^*, R_i^*) given by

$$R_s^* := \frac{pq - \beta d}{pq + (q-1)\beta}$$
$$C^* := \frac{\beta R_s^*}{pa}, \quad R_i^* := \frac{d}{pq} - \frac{R_s^*}{pq}$$

where resource and consumer co-exist and the parasite is endemic (endemic steady state). Some algebra shows that the endemic steady state is feasible, i.e. exists in the sense that all three variables are non-negative, if β takes a value in the interval

$$\frac{p(1-d)}{d} =: \beta_1 < \beta < \beta_2 := \frac{pq}{d} \tag{5.5}$$

In this range we will, in the next section, numerically explore the interaction between ecology and epidemiology for the stability of the co-existence of the susceptible resource, the infectious resource and the consumer population.

5.3. Numerical exploration of the stability of coexistence in model (5.3)

The analysis of model (5.3) shows how microparasite which does not produce mortality but only influences the behaviour of its host, affects simple consumer-resource systems in different types of environment. In a series of figures, we explore how the stability of the endemic steady state with co-existing consumer, resource and microparasite changes if we vary epidemiological aspects (the infection transmission rate β and the effect of the parasite on the consumption coefficient and the conversion efficiency) and ecological aspects (notably the resource population size d in steady state in the absence of infection, varying between 0.4 and 1 as a result of a mortality variation between 0.3 and 0.75 in steps of 0.05). In table 5.1, we give 10 different regimes of values for the combined parameter d that are explored. By varying d we simulate 10 different 'types of environment', and for each of these we vary the infection transmission rate over a continuous range. Within those combinations for every fixed type of environment (d) and infection transmission rate (β), we additionally vary two other parameters, the conversion efficiency (qe) and the consumption coefficient (pa). We first vary the conversion efficiency by increasing the value of q between 0 and 1 in steps of 0.1, Finally, for every (d, β, qe)-combination we vary the consumption coefficient (pa) by changing the parameter p between 0 and 2 in steps of 0.1 to simulate the effect of parasite on the feeding behaviour of its host, allowing for decreased (p<1) or increased (p>1) consumption of the infected resource by the consumer. We produce graphs of the largest real part of the eigenvalues of the Jacobian matrix, evaluated at endemic steady state, with positive values implying an unstable steady state, and negative values implying stability.

In Figure 5.3, we show stability in the (β, d)-plane, for a range of values for the epidemiological effects p and q. The curves in the left panel of Figure 3a indicate where the endemic steady exists and is stable, with various shades of grey indicating the size of the largest real part. Darker shades denote smaller values of the real part of the dominant eigenvalues, and tell us when the system is more resilient to perturbations (a higher return time to equilibrium). White in that figure indicates that either the steady state does not exist in that range of parameter space, or that the steady state is unstable. The results show that the reason an endemic steady state does not exist, for a given combination of parameters (d, qe, pa), depends on the value for the infection rate: at low values for the infection rate the parasite is not able to invade the system under the given conditions, whereas at high values for the infection rate the susceptible resource and consumer

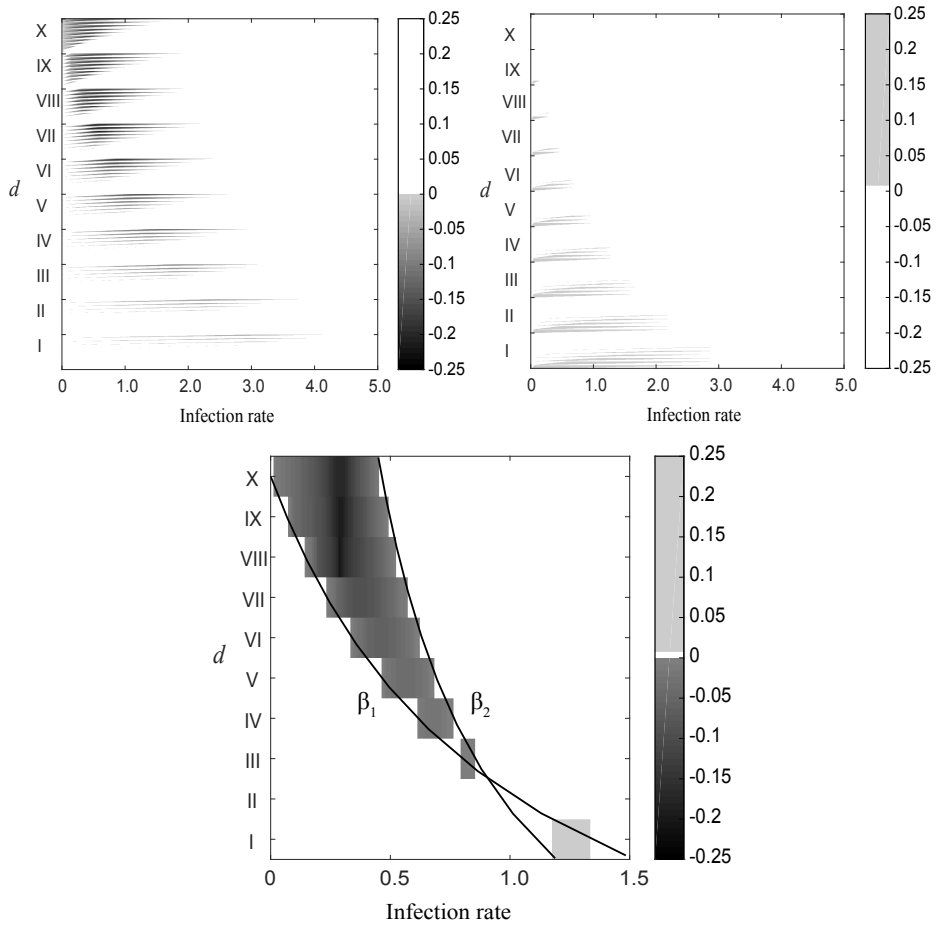


Figure 5.3. Stability analyses of the model (R_s^*, C^*, R_i^*). a) Stability in the (β, d)-plane, for a range of values for the epidemiological effects p and q. The left panel indicates where the endemic steady state exists and is stable, and the right panel indicates where is unstable. b) Endemic steady state of the model exists only between β_1 and β_2 given in (5). In this simulation of the toy model we took the values of rates of change for assimilation efficiency (p=0.9) and conversion efficiency (q= 0.5).

interaction cannot sustain the high infection pressure. In Figure 5.3a, right panel, the curves in shade of grey indicate where the steady state exists and is unstable, so here 'white' means: the steady state does not exist or is stable). The perturbation of any of the parameters that produce an unstable steady state will easily lead to extinction in either infectious resource or susceptible resource and consumer. Figure 5.3b shows that the endemic steady state exists only in the β interval given by (5.5). In a series of additional

Table 5.1. Parameter regimes used for the numerical exploration of model (5.3)

Regime	I	II	III	IV	V	VI	VII	VIII	IX	X
d	0.4	0.467	0.533	0.6	0.667	0.733	0.8	0.867	0.933	1

figures, we examined the model without any impact of q and p parameters, with an effect of the disease only on q and only on p separately, as well as we compared the effect of p<1 and p>1 on the stability of the system (Appendix, additional figures).

The stability analyses of the model without any effect of the parasite on conversion efficiency and consumption coefficient of consumer (p= q = 1) shows smaller ranges for the infection rate where the species can coexist, compared to the model where these influences are included. That implies the importance of the non-trophic parasitic influences on their hosts and non-hosts for the stability of the system to allow a wider ecological range of interaction conditions suitable for coexistence. The influence of the conversion efficiency parameter and the consumption coefficient parameter separately, showed different effects: when we keep system without change in consumption coefficient parameter (p=1), lower values of conversion efficiency drive the system sometimes to stable and sometimes to unstable behaviour, while when we keep system without change in conversion efficiency (q=1) lower values of consumption coefficient always lead the system to stability.

If we assume that C-R interaction is affected by the microparasite through both ways of influence (p and q), but we are interested in the importance of only the effect of microparasite on the consumption coefficient, we get other interesting insights. The model shows that greater consumption ($p > 1$) of the infected prey gives greater stability to the system and that coexistence of all species can occur with higher infection rates under this condition.

Further, the effects of microparasite on the C-R interaction in different types of environment are examined using the parameter d as in Rip & McCann (2011). Following their argumentation, we interpret that going from small to large values of d, means that the ecosystem that is modelled changes from 'aquatic' to 'terrestrial'. The stability of the C-R interaction with the infection in the resource if it exists is more often stable in the terrestrial ecosystems, whereas in the aquatic ecosystems it more frequently is unstable. The non-trophic influence of parasites on their hosts and on non-hosts is different in different types of ecosystem. Lowering the conversion efficiency leads to instability in the aquatic ecosystems while in a more terrestrial ecosystem it does not have a destabilizing

Table 5.2. Results of percentage of coexistence and stable/unstable distribution ranges of infection rates that support consumer-resource-microparasite coexistence in different types of environment (regimes of predation). Distribution range of infectious rate depends on the consumption coefficient and conversion efficiency parameters.

d	% of coexistence	% of stable	% of unstable
I	12,9 (β[0,01-4,34])	23,9 (β[0,02-4,34])	76,1 (β[0,01-2,86])
II	10,5 (β[0,01-3,72])	35,5 (β[0,13-3,72])	64,5 (β[0,01-2,18])
III	9,0 (β[0,01-3,25])	48,7 (β[0,01-3,25])	51,3 (β[0,01-1,67])
IV	8,0 (β[0,01-2,89])	61,9 (β[0,01-2,89])	38,1 (β[0,01-1,27])
V	7,6 (β[0,01-2,60])	73,9 (β[0,01-2,60])	26,1 (β[0,01-0,95])
VI	7,5 (β[0,01-2,37])	83,7 (β[0,01-2,37])	16,3 (β[0,01-0,69])
VII	7,6 (β[0,01-2,17])	91,2 (β[0,01-2,17])	8,8 (β[0,01-0,47])
VIII	7,9 (β[0,01-2,00])	95,9 (β[0,01-2,00])	4,1 (β[0,01-0,29])
IX	8,3 (β[0,01-1,89])	98,6 (β[0,01-1,89])	1,4 (β[0,01-0,13])
X	8,7 (β[0,01-1,73])	100 (β[0,01-1,73])	0

effect. A higher consumption coefficient (pa) in the aquatic ecosystem sustained higher infection rates compared to the more terrestrial ones.

The coexistence of consumer-resource-microparasite interaction and the stability distributions of this interaction in different types of ecosystem are presented in Table 5.2 and Figure 5.4. In the table, we show the percentage of the coexistence and infection rates that are supported, as well as the stability distributions with infection rates in different regimes of the steady state susceptible resource population in the absence of the parasite, d (or 1/(relative energy ratio)). Figure 5.4 shows the stability distributions for each of the ten regimes of d (the left panel) and is comparable to Figure 5.1 where stability decreases if we move the consumer isocline (d) to the left. Further, the right panel of Figure 5.4 singles out one regime for d (regime V) to better observe the influence of the parasite-related parameters (p and q) on the stability of the interaction. The table and figure suggest that every type of C-R relationship and thus the type of the ecosystem has a specific range of infection rates that can be sustained, and a specific range of parasite induced characteristics of resource and consumer that allow co- existence in a stable ecosystem of all three species. Additionally, in the table we observe that the ecosystems that support the largest ranges for the infection rate and the feeding influences of parasites are the ones with lower values for d, i.e. the ones with higher relative energy ratios, which we interpret as aquatic ecosystems.

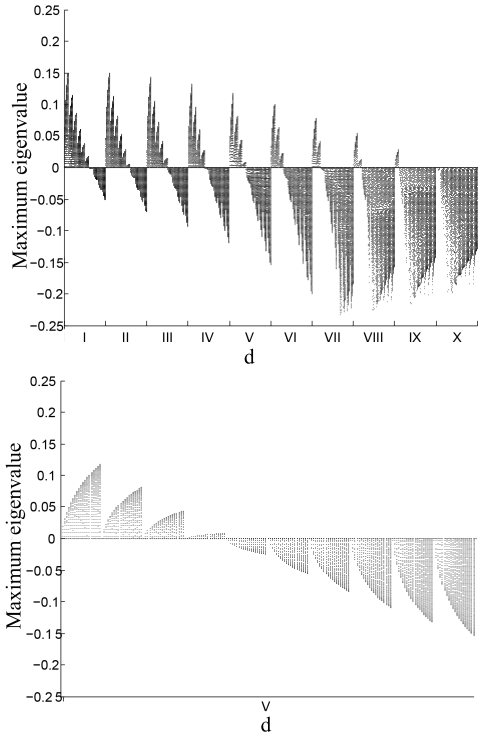


Figure 5.4. Distribution of stable (-) /unstable (+) values in different regimes of d. Every dot represents the real part of the dominant eigenvalue for certain combination of parameters (β, d, qe, pa). Left panel: all regimes for d, ; right panel: zooming in on regime V (contains steps of qe parameter which incorporate steps of pa parameter that with certain infectious rate gives maximum eigenvalue of the consumer-resource-microparasite system)

5.4. Discussion

The research on the inclusion of other than feeding-type interactions in ecological communities, and how these other interactions affect the functioning, structure and stability of the ecosystems, developed fast in recent years (Allesina and Tang 2012, Fontaine *et al.* 2011, Kéfi *et al.* 2012, McQuaid and Britton 2014, Mougi and Kondoh 2012, 2014, Sauve *et al.* 2014, Thebault and Fontaine 2010). Parasitic interaction is one of the first types that got recognized as important in this respect (Dunne *et al.* 2013, Huxham *et al.* 1995, Kuris *et al.* 2008, Lafferty 1992, 2013, Lafferty and Kuris 2002, Lafferty *et al.* 2006b, McQuaid and Britton 2014, Poulin 1994, 1999, Sukhdeo 2012, Thompson *et al.* 2004). One of the proposed ways for studying this particular type of interaction is through the

effects parasites have on hosts and on the non-host species their hosts interact with -as non-trophic interaction. This way of incorporating parasites describes their influence via the effect the parasite has on the (physiological/epidemiological) state and behaviour of host and non-host species in their ecological network. This is an indirect approach (Kéfi *et al.* 2012, Selakovic *et al.* 2014), compared to approaches where parasite species are described as biological species, represented directly via their own node in an ecological network where they are linked to nodes representing their host species. Many food web studies have shown that interaction strengths in food webs are strongly patterned (McCann 2011), and that both distribution of interaction strengths and the topological structure are important for the stability in ecosystems (Allesina and Pascual 2008, Neutel *et al.* 2002). The indirect way of inclusion could be a method to add more accuracy to these analyses by including non-trophic interactions as a real world simulation.

Similar C-R models have already discussed the effect of parasites on the host behaviour and benefits of consumers foraging on a parasitized resource (Lafferty 1992), as well as the behavioural effect of trophically transmitted parasites on the dynamics of the consumer-resource relationship (Fenton and Rands 2006). Here we go one step further with a preliminary interpretation of these effects comparing different types of ecosystem and the range of parasitic influence that are supported by these environments. However, our approach is limited to a model that is very general and we use it only for an initial theoretical discussion, and to highlight phenomena that could occur in relation to stability and co- existence. Moreover, we have concentrated on microparasites that affect only feeding-related behaviour of their host without a direct disease-induced effect on the mortality of the host. The advantage of a simple model is that the mutual influence between ecology and epidemiology can be more easily explored. These effects may also occur in more realistic settings, and notably food webs should be explored, rather than the simple two-species food chain in this initial analysis, and for a broader range of parasite types and their influences on hosts.

We included the knowledge about the influence of infectious agents on their hosts and non-hosts interactions to the simple consumer-resource interaction using the idea of relative energy ratio (sensu Rip and McCann 2011, who call it relative energy flux). The idea is that any biological trait that increases the relative energy ratio (predation rate of the consumer relative to its loss term) makes the CR biomass ratio top heavy and the system less stable. Rip & McCann examine terrestrial vs aquatic ecosystems and predict that aquatic ecosystems tend to have a higher relative energy ratio and decreased stability relative to terrestrial ecosystems. Our analysis shows how the same idea relates to microparasites in such a setting. One can imagine that every combination of paramet-

ers we explored is one type of parasite which influences the C-R relationship in its own specific way. The cross-ecosystem analysis shows that C-R interactions with parasites in different environments are stable within certain ranges of parasitic influence on its host, and therefore for smaller or larger sets of potential parasites. Our analysis suggests that aquatic-like systems, in the above sense, support broader ranges of parasites compared to the aquatic ones. This agrees with the observation of a higher biodiversity of parasites and their hosts in aquatic ecosystems (McCallum *et al.* 2004). For example, oceans contain an estimated 10 30 virus particles, with 10 23 infections occurring each second (Suttle 2007). Our analysis also shows that aquatic ecosystems with parasites are more unstable for coexistence of susceptible and infectious resource sub-populations and consumers, compared to terrestrial systems. This conclusion agrees with the discussion on cross-ecosystem stability from Rip and McCann 2011, for the pure CR-case without parasites.

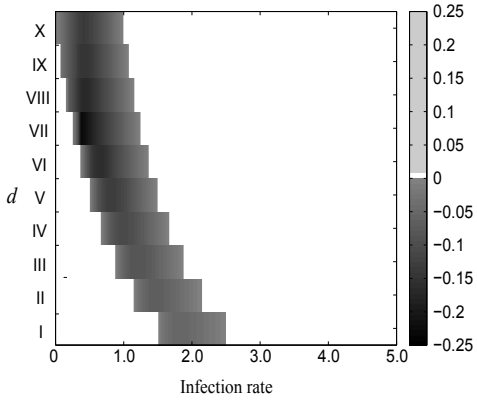
Indirect inclusion of parasites confirms several general insights. Consumer-resource interaction with infection can be stable over broad ranges of values for epidemiological parameters and of influence on ecological processes. These ranges depend on the ecological characteristics of the CR system, and parasites can extend the ecological range of coexistence. The analysis gives a clear idea of the importance of non-trophic interaction via parasites in ecosystems. Although our model is basic, it does capture the essentials. In the introduction section we discussed many examples of how different parasites affect their hosts from direct energy drain to indirect change in feeding interaction between resource and consumer. For example, infected resource individuals may be caught less easily by a consumer or more easily. If part of the resource population is infected,, consumers spend either more time in search of a suitable prey, or find infected prey using less energy than in the absence of the parasite. Once caught, infected resource individuals may also affect consumers by reduced feeding value. These trait-mediated effects of parasites can be described with conversion efficiency or consumption coefficient parameters in Lotka-Volterra models. Our results show that including non-trophic influences of parasites increases the stability range and coexistence of the consumer-resource-parasite system compared to the system without non-trophic influence of parasite on its host and non-host. For instance, greater consumption of the infected resource increases stability of the system and supports higher infection rates. With a higher consumption of infected resource consumers control the infection spread in their resource in our basic setting (in line with the healthy herds hypothesis- Packer *et al.* 2003).

The next step is to expand this theoretical model to a food web that includes many connected C-R relationships, and to explore trait-mediated parasitic impacts on energy flow,

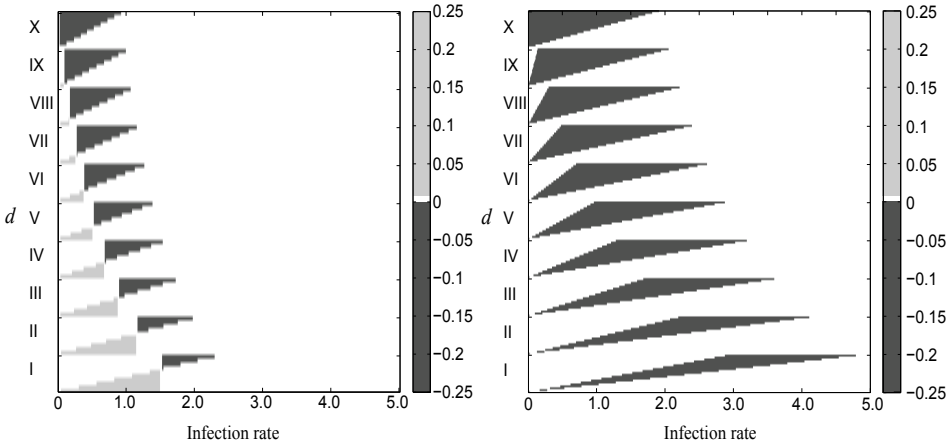
strength of interactions and stability in different types of ecosystem. Parasites included in food webs in indirect way can increase and decrease the strength of interaction between neighbouring species. It would be interesting to see consequences of such influences even when they are very weak, as such weak links have been shown to play a role in ecosystem stability (Neutel *et al.* 2002). Because one can hardly observe or measure ecosystems without parasites playing a role (as every living species is a host to probably several types of parasite (Rossiter 2013), it may be that interaction strengths are importantly moderated by the omnipresence of parasites and that these parasites, even though very weak effects on individuals, do play a major role in shaping stability and structure in real ecosystems.

Supplementary Information

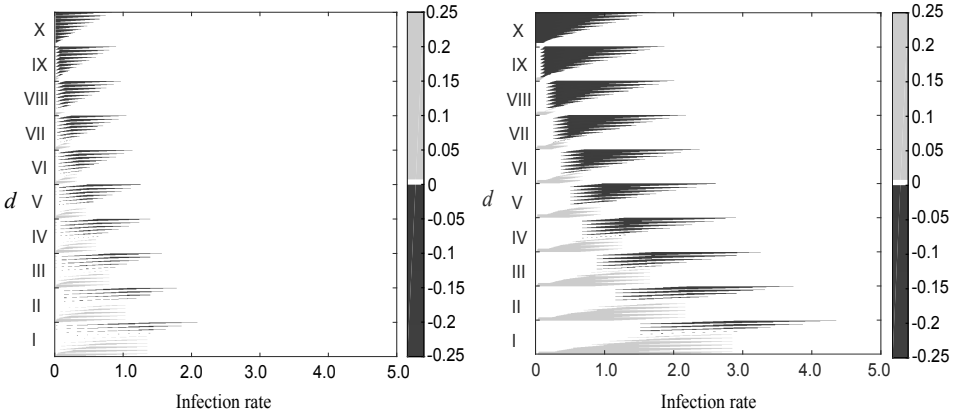
I The stability analyses of the model without impact of epidemiological parameter p on consumption coefficient (a) of the consumer and epidemiological parameter q on conversion efficiency (e) of the consumer.



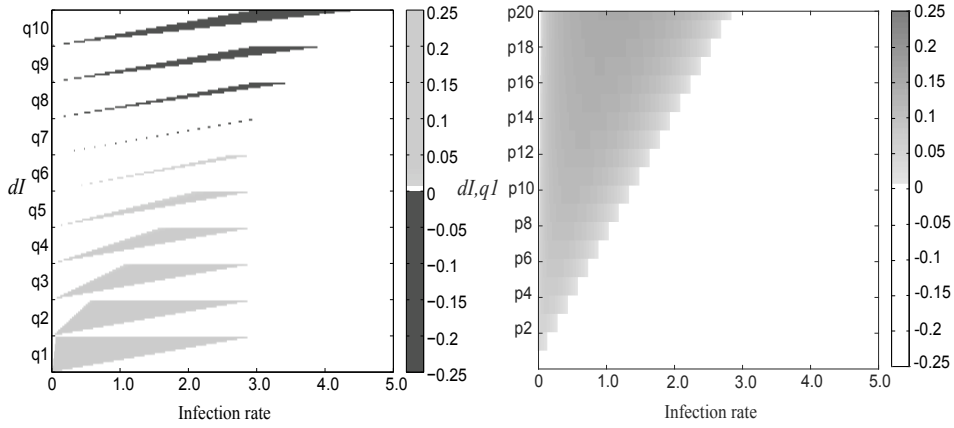
II Stability analyses of infection impact on only conversion efficiency q (when the p parameter is fixed) and only consumption coefficient p (q parameter is fixed).



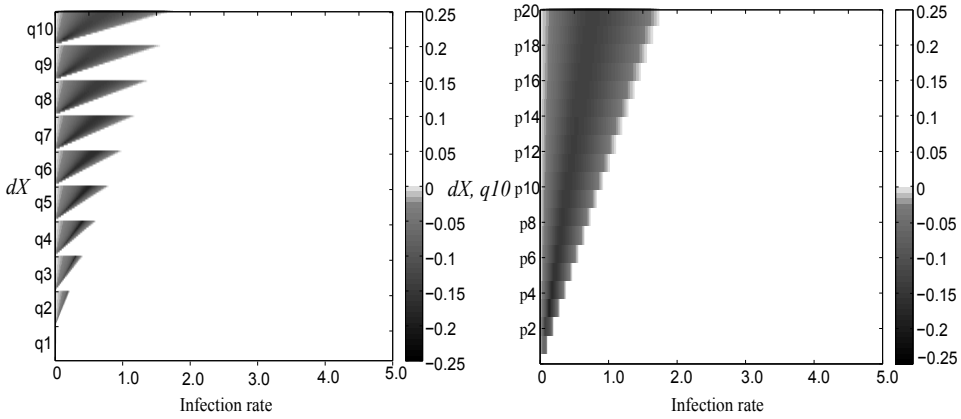
III Stability analyses for infection impact on consumption coefficient parameter. Left panel shows the stability analyses with combination of parameters d, qe and $a^*(p<1)$, while right panel shows the stability analyses with combination of parameters d, qe, and $a^*(p>1)$.



IV Stability analyses of the toy model fixed only on dI where we show the influence of parameters p and q in this situation of energy flux that is compared to energy flux with higher turnover rates as in aquatic ecosystems.



V Stability analyses of the toy model fixed only on dX where we show the influence of parameters p and q in this situation of energy flux that is compared to energy flux of terrestrial ecosystems.



Chapter 6

The response of ecological communities to increased and decreased infection prevalence at different trophic levels

Sanja Selaković, Torbjörn Säterberg, Bo Ebenman, Hans Heesterbeek

submitted

Abstract

By altering vital rates of their hosts, infectious agents affect interactions of species in ecosystems. It has been shown that infectious agents can endanger as well as promote coexistence of species in ecological communities, but the outcomes and mechanisms involved remain poorly understood. We take a new indirect approach in investigating wider community effects of changes in infection prevalence at different trophic levels. By increasing or decreasing mortality rates in selected species, we mimic waxing and waning outbreaks of infection in those species. We find that even relatively small changes in infection-induced mortality of a given species can cause large decline in the densities of others. Furthermore, primary producers are more sensitive to change in the infection prevalence than top predators. Overall, our study shows that infectious agents are likely to be major players in ecosystems and both infection outbreaks and infection decrease can lead to profound system changes.

6.1. Introduction

Parasites and pathogens, which we shall collectively refer to as infectious (disease) agents, are diverse groups of organisms that are an integral part of ecological communities. Not only do they represent a significant part of biodiversity and biomass of ecosystems (Kuris *et al.* 2008, Lafferty *et al.* 2008c, Poulin 1999), but they also shape and influence the diversity, structure, functioning and stability of communities directly and indirectly in many ways (Selakovic *et al.* 2014, Sukhdeo 2012). Directly, they affect the behaviour and life-history traits, such as mortality and reproduction, of hosts. Owing to interdependences among species these direct effects of the infectious agents on their hosts further indirectly affect the ecological community at large through impacts on the abundance and dynamics of other host and non-host species Lafferty *et al.* 2008c, Poulin 1994, 1999). Indeed, a number of studies show that the direct effects of infectious disease agents on host species can lead to large declines in the abundance, even extinctions, of other species in ecological communities (Anderson 1965, Carpenter 1990, Dobson and Crawley 1994, Duffy and Sivars-Becker 2007, Getz 2009, Holdo *et al.* 2009, Hollings *et al.* 2013, Sato *et al.* 2012, Thomas *et al.* 1998, Weste and Marks 1987). For instance, infection outbreaks affecting keystone species can lead to extinction cascades (Dobson and Crawley 1994, Tansley and Adamson 1925). Here reduced abundance of the host species might trigger a top-down trophic cascade or disrupt predator-mediated coexistence. There are also studies showing that parasites and pathogens can mitigate coexistence of potentially competing species and hence promote biodiversity (Bagchi *et al.* 2014). Here, increased abundance of the host might lead to overexploitation of prey or competitive exclusion of species at the same trophic level. Thus, infection outbreak as well as decrease in infection can potentially cause large changes in the structure of ecological communities. Indeed, increased infection prevalence might lead to functional/ecological extinction (Säterberg *et al.* 2013) of a host species while decreased infection prevalence can potentially turn a host species into a natural invader (Carey *et al.* 2012), that is, promote functional invasions.

Thus, increased as well as decreased infection prevalence can influence the species composition of ecological communities. However, the nature and extent of this influence has not been systematically explored. Interesting questions are, for example, whether increased infection prevalence of a host species will have larger community impact than decreased infection prevalence, and how the answer will depend on the trophic position of the host species directly affected. To address these issues, and to more systematically and quantitatively explore the potential impact of infectious disease agents on ecological

communities, we use an indirect approach, as opposed to a more direct approach where infectious agents are included as separate species in a food web with links to their hosts (Selakovic *et al.* 2014). This indirect approach considers the effect of infectious disease agents through their impact on a focal host species' life-history traits, in our case host infection-induced mortality rate. We analytically derive the changes in additional mortality rate, necessary to cause changes in species' equilibrium densities to such a degree that quasi-extinction of other species in the community results, where quasi-extinction is defined here as a reduction in density of a species by 90%. Here, increasing and decreasing mortality rate mimic waxing and waning of infection prevalence, respectively, in the focal host species. We also explore to what extent the risk of a host species to go functionally extinct or invasive, following a change in its infection prevalence (infection prevalence), depends on its trophic level.

6.2. Methods

We generate 1000 feasible (i.e., with all species having positive abundance) and locally stable pyramidal food webs consisting of 12 species, using the approach in (Kaneryd *et al.* 2012). These food webs have three trophic levels; primary producers (basal species), herbivores and carnivores. The number of consumer-resource links, L, in the systems is based on empirically observed connectances, C (defined as $L/S2$, where S is the number of species in the web) (Digel *et al.* 2011, Dunne *et al.* 2002). The links are randomly distributed with two restrictions: consumers must have at least one prey and carnivores must have at least one herbivore prey. We analyse two scenarios: one where consumer species show strong preference for one of their prey species ('specialists') and one where consumers show equal preference for each of their prey species ('generalists').

We describe community dynamics by generalized Lotka-Volterra equations (see Kaneryd *et al.* 2012 and Supplementary information for details and parameterization):

$$\frac{dN}{dt} = N_i(r_i + \sum_j \alpha_{ij} N_j) \tag{6.1}$$

where N_i is the density of species i, r_i is the intrinsic growth rate of species i, and α_{ij} is the per capita interaction strength that species j exerts on species i. The per capita interaction strengths, α_{ij}, represent different types of interactions: interspecific

competition if both j and i are basal species, trophic interactions if j is a consumer (resource) and i its resource (consumer) and intraspecific competition if $i = j$. In matrix notation:

$$\frac{dN_i}{dt} = N'(r + AN) \tag{6.2}$$

where N' is a matrix with population densities on the diagonal and zeros elsewhere, N is a vector of population densities, r is the vector of per capita growth rates and A is the community interaction matrix with elements α_{ij} .

We follow Säterberg *et al.* (2013) in the analysis of this system. The interior equilibrium $\hat{N}$ is given by:

$$\hat{N} = -A^{-1}r \tag{6.3}$$

where A^{-1} is the inverse interaction matrix. To increase or decrease the mortality rate of a focal species j (mimicking change in infection prevalence in that species), we decrease or increase its intrinsic growth rate, respectively, with an amount ε_j :

$$r_j{}' = r_j + j \tag{6.4}$$

keeping the growth rates of all other species unchanged. The new equilibrium for any species i, given a change in the mortality rate of focal species j, is:

$$\hat{N_i}{}' = \hat{N}_i - \varepsilon_j \gamma_{ij} \tag{6.5}$$

where γ_{ij} is the (i, j)'th element of A^{-1} . Now, setting $\hat{N_i}{}' = p\hat{N}_i$, where $0 \leq p < 1$, in eq. (6.5) gives $\varepsilon_j(i) = \hat{N}_i(1 - p)/\gamma_{ij}$. This is the change in the intrinsic growth rate of species j that would lead to a $(1-p)$-proportional decrease in the equilibrium abundance

of species i. We wish to find the largest negative (closest to zero) and smallest positive ε_j for each species j. To find this, we calculate $\varepsilon_j(i)$ for all species i and compute the maximum negative value and minimum positive value, respectively:

$$\varepsilon_j^{inc} = max_i(\varepsilon_j(i) : \varepsilon_j(i) < 0) \tag{6.6}$$

$$\varepsilon_j^{dec} = max_i(\varepsilon_j(i) : \varepsilon_j(i) > 0) \tag{6.7}$$

Thus, ε^{inc} is the smallest increase in focal species j's mortality rate that will lead to a $(1-p)$- proportional decrease in the equilibrium abundance of a species in the community. Likewise ε^{dec} is the smallest decrease in focal species j's mortality rate that will lead to a $(1-p)$-proportional decrease in the equilibrium abundance of one species in the community.

To investigate potential community-wide effects of changes in infection prevalence, we derive for each species j, the smallest change (positive as well as negative) in its intrinsic growth rate, r_j , needed to cause a given proportional decrease (90% or 50%; interpreted here as "quasi-extinction") in the equilibrium abundance of any species in the ecological community. The analytically derived changes in intrinsic growth rate are evaluated relative to the initial, baseline intrinsic growth rate of the focal species. Additionally, for each focal species, we record the ratio of smallest decrease and smallest increase in its intrinsic growth rate needed to cause a quasi-extinction. For consumer species the intrinsic growth rate is an intrinsic mortality rate, while for the primary producers the intrinsic growth rate is the intrinsic birth rate minus the intrinsic mortality rate. A decrease in the infection prevalence of a focal species leads to a decrease in its intrinsic mortality rate and hence to an increase in its intrinsic growth rate. Cases where the magnitude of the decrease in intrinsic mortality rate is larger than the initial intrinsic mortality rate are not biologically feasible, since the fraction of the initial intrinsic mortality rate that is due to parasites cannot be larger than one. Moreover, such cases would lead to positive intrinsic growth rate of consumer species and a positive growth rate of consumers without prey is not feasible. These special cases only occurred for herbivore focal species. We limit our analyses to the feasible cases, that is, cases where the magnitude of decrease in intrinsic mortality rate is smaller than the initial intrinsic mortality rate. We excluded around 50%

and 40% related to herbivore focal species, for the scenarios leading to a 90% and 50% change, respectively.

In addition to the theoretical analyses, we explore patterns of infection of species at different trophic levels in six natural aquatic systems using data sets available online (Hechinger *et al.* 2011b, Mouritsen *et al.* 2011, Preston *et al.* 2012, Thieltges *et al.* 2011). First, we generate food webs based on the free-living, non-parasitic species only. This means that links representing predation on free- living non-feeding parasite stages are excluded. Next, we calculate the trophic positions (trophic heights) using the 'PreyAveragedTrophicLevel' function (Williams and Martinez 2004), implemented in the R package *'Cheddar'* (Hudson *et al.* 2013). We assign each of species to one of the following trophic categories (levels): basal (B), herbivore (H) and carnivore (C). All species with trophic position equal to, or higher than, 2 are assigned to the carnivore trophic level. Finally, we use information on infectious agent-host links to quantify the diversity of agents per host species at each trophic level. The following types of infectious agent-host links are included: parasitic castrators, pathogens, macroparasites, parasitoids, trophically transmitted parasites and trophic transmissions (for the definition of the different infectious agent links see (Hechinger *et al.* 2011b).

6.3. Results

We present the results for the case where quasi-extinction of a particular species is interpreted as a 90% change in its density, i.e. $p = 0.1$. Additional results for the case $p = 0.5$ are given in the Appendix. Overall, increased as well as decreased focal host mortality rate frequently leads to quasi- extinction of other species in the community. Communities are less robust to an infection-induced change in the mortality rate of primary producers than to similar changes in herbivores or carnivores. Furthermore, for herbivore and carnivore focal species, the decrease in the mortality rate needed to cause a quasi-extinction in the community is smaller than the increase in mortality rate needed, while the opposite is found for primary producers.

Pattern of quasi-extinctions: Quasi-extinctions of focal species themselves are more frequent in food webs with specialist consumers than in webs with generalist consumers (Fig. 6.1 A and C).

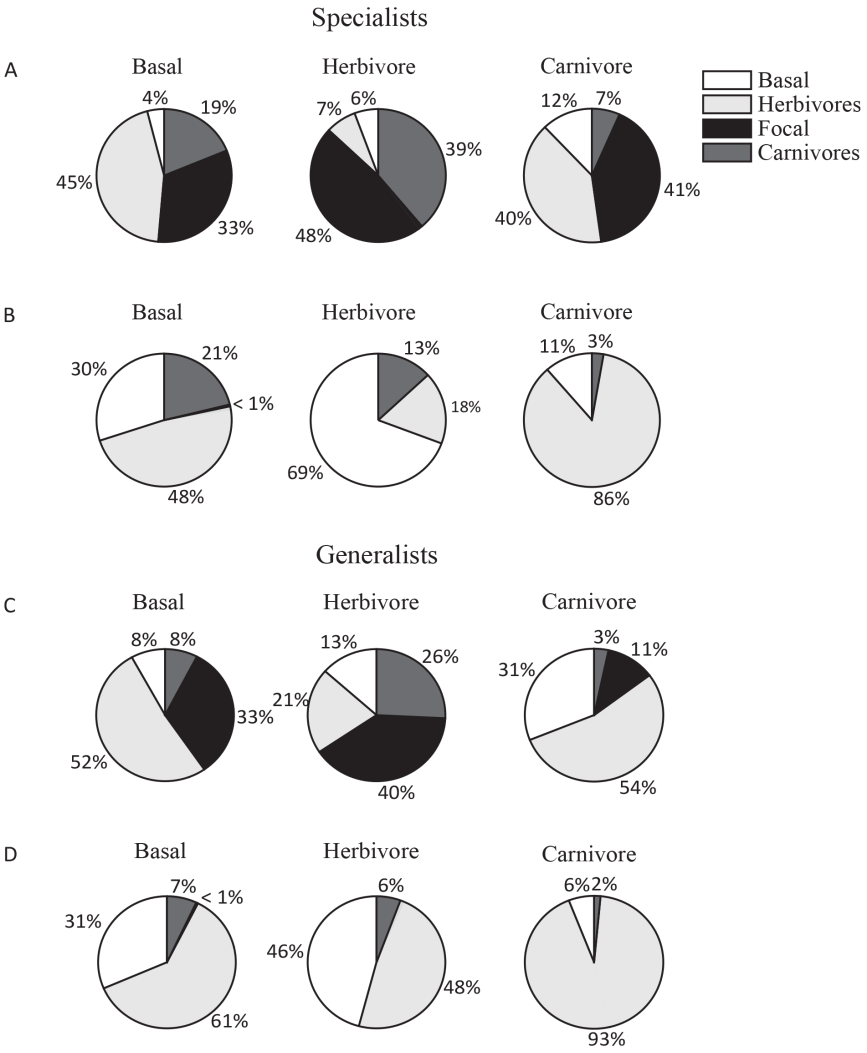


Figure 6.1. The proportion of quasi-extinctions (p=0.1) affecting basal species, herbivores, carnivores or the focal species themselves following an infection-induced change in mortality rate of basal, herbivore and carnivore focal species, respectively. A) Increased mortality of the focal species, and B) decreased mortality of the focal species, both in communities with specialist consumers. C) Increased mortality of the focal species, and D) decreased mortality of the focal species, both in communities with generalist consumers.

Infection-induced mortality increase in basal focal species most frequently leads to quasi-extinctions of herbivore species, increases in herbivores mainly affect carnivore species, and increases in carnivores most frequently lead to quasi-extinctions of herbivores. Thus, increased mortality rate (increased infection prevalence) has bottom-up effects at basal and herbivore trophic levels, and top-down effect at the carnivore level. Species at the same trophic level as the focal species are less affected. As can be expected, focal species almost never go quasi-extinct following a decrease in their own mortality rate (Fig. 6.1 B and D), although this did happen in a few cases for basal focal species. Decreased mortality rates in basal species lead most frequently to quasi-extinctions in herbivores, decrease mortality rates in herbivores lead to quasi-extinction of mostly basal species, and decreased mortality rate in carnivores lead mostly to quasi-extinction of herbivore species. To summarize, decreased infection-induced mortality in a species usually leads to quasi-extinction of a species at the trophic level below it, except, of course, in the case of basal focal species.

Increased versus decreased infection prevalence: The change in mortality rate needed to produce a quasi-extinction in the community is shown in figure 6.2. Overall, communities with generalist consumers are less robust to changes in the mortality rate of focal species, than communities with specialist consumers. For basal focal species, relatively small increases in mortality rate lead to quasi-extinctions. For focal species at the herbivore and carnivore trophic levels, relatively small decreases in the mortality rate lead to quasi-extinctions, while the increase in mortality rate necessary to cause a quasi-extinction is higher than the initial, baseline, intrinsic growth rate. Comparing the increase and decrease in mortality rate in focal species needed to cause a quasi- extinc-

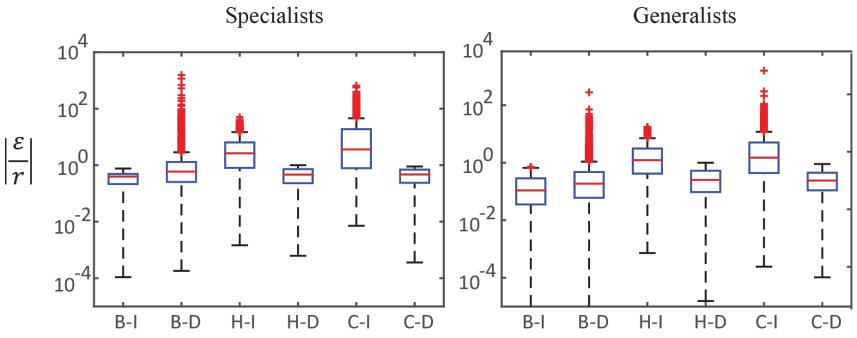


Figure 6.2. Boxplots showing the relative increase (I) and decrease (D) in mortality rate, $||$, for basal (B), herbivore (H) and carnivore (C) species, respectively, needed to cause quasi-extinction in the community.

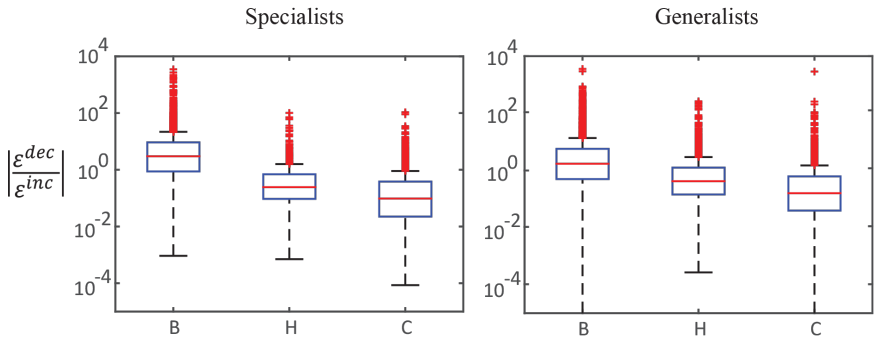


Figure 6.3. Boxplots comparing the increase and decrease of mortality rate, needed to cause quasi-extinction in the community, for basal (B), herbivore (H) and carnivore (C) species.

tion we find that in basal focal species decrease of mortality rate needed to be larger than the increase of mortality rate, while for herbivore and carnivore focal species the increase in mortality rate needed to be larger than the decrease in mortality rate (Fig. 6.3).

In light of these theoretically derived results it is interesting to find that among species in natural food webs carnivores are hosts to a relatively large number of parasite species, compared to basal species (Fig. 6.4; for additional information and data see Figure 6.8, Table 6.1 in Supplementary information).

6.4. Discussion

The large majority, perhaps all, free-living species are likely to be hosts of at least one infectious disease agent (Rossiter 2013). It has been shown that infectious agents can endanger as well as promote coexistence of species in ecological communities (e.g. Bagchi *et al.* 2014, Dobson and Crawley 1994), but when to expect one or the other outcome, and what mechanisms are involved, remains poorly understood. Using an indirect analytical approach we find that increased infection prevalence as well as decreased infection prevalence in a focal host species can lead to major declines in the abundance-so called quasi-extinctions-of other species in the community. The pattern in these quasi-extinctions can gives insight into the mechanisms involved.

We find that increased mortality in basal and herbivore focal species frequently causes quasi- extinction of species at a higher trophic level -an example of a bottom-up cascade. Increased mortality in carnivore focal species frequently causes quasi-extinctions

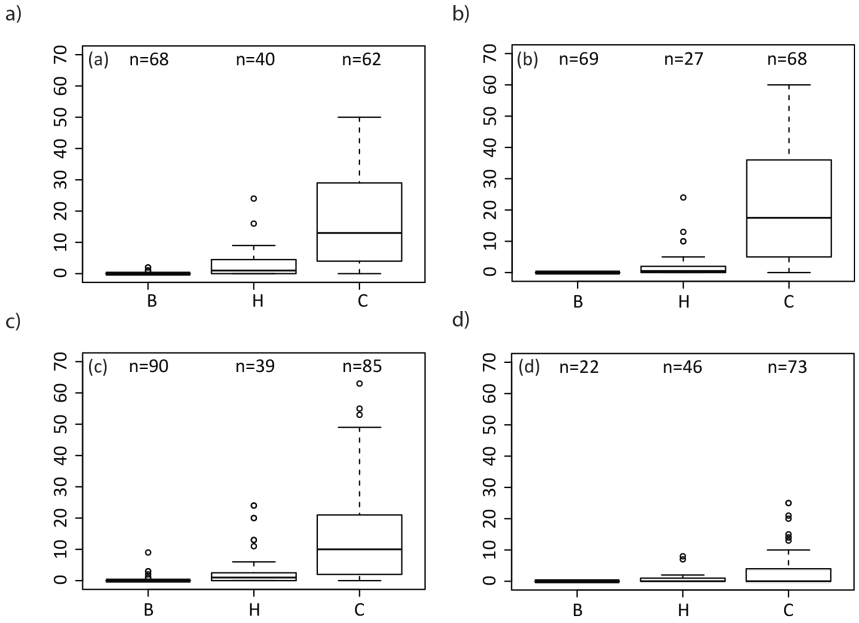


Figure 6.4. The number of parasitic species per host species (S) at different trophic levels (B - basal species; H - herbivores; C - carnivores), in four natural aquatic ecosystems (a - CSM, b - BSQ, c - EPB, d - Otago).

of herbivore species, most likely as a consequence of increased intensity of competition among herbivores species for shared resources. Decreased carnivore abundance leads to decreased predation pressure on herbivores, which in turn increases the intensity of competition among the herbivores in a disruption of predator-mediated coexistence. Similar patterns have been found in recent studies focusing on true extinctions of other species, rather than quasi-extinctions, following increased mortality rate of a focal species (Säterberg *et al.* 2013, Sellman *et al.* 2015).

Likewise, decreased infection prevalence in focal species triggers quasi-extinctions of other species in the community. In particular, even relatively small decreases in the mortality rates of herbivore and carnivore focal species can lead to major reductions in the abundance of other species. Here, decreased mortality rate in herbivores and carnivores triggers quasi-extinctions of species at lower trophic levels-the mechanisms involved being top-down cascades and disruption of predator- mediated coexistence. The most frequent outcome of decreased mortality in a basal focal species is quasi-extinction of a herbivore species. This is a somewhat counter-intuitive result-the most likely explanation is that an increase in the abundance of the focal basal species causes a decrease

in the abundance of other competing basal species with negative consequences for their herbivores (see Bagchi et al. 2014).

The response of ecological communities to increased and decreased infection prevalence is also likely to depend on patterns in the strength of interactions among species. The distribution of interaction strengths in ecological communities has important consequences for their stability (Allesina and Tang 2012, Borrvall *et al.* 2000, Christianou and Ebenman 2005, de Ruiter *et al.* 1995, Emmerson and Raffaelli 2004, Kokkoris *et al.* 2002, McCann *et al.* 1998, Neutel *et al.* 2002, Tang *et al.* 2014). We find that communities where consumers show strong preference for one of their prey species (specialists) are more robust to increases in infection-induced mortality rate of focal species, than communities where consumers show equal preference for each of their prey species (generalists). A possible reason for this is that in communities with specialist consumers most consumer-resource links will be relatively weak. If consumer-resource links are weak, a perturbation affecting one species (such as an outbreak of an infection involving mortality of that host) should be less likely to propagate in the food web (see also Sellman *et al.* 2015).

Here, we have focused on the situation where infectious disease agents influence the mortality rate of their hosts and how this in turn may affect the abundances of other species in the community. However, parasites and pathogens may affect their hosts in many different ways, and infection does not always lead to (clinical) disease in each host species, or to additional mortality as direct result of disease. For instance, some types of infectious agent will mainly affect the feeding behaviour of their hosts and thereby change the strengths with which the host species interact with the species to which they are linked in the food web. Such changes can have substantial effects on the stability of the food web as a whole (Berg *et al.* 2011). Even changes in the strength of weak links might have consequences at the community level, as it has been shown that weak links in long loops of resource-consumer interactions are important for local stability (Neutel *et al.* 2002).

Our analysis of the response of ecological communities to increased and decreased infection prevalence of a given species is based on an indirect approach. Infectious agents are implicitly considered through their effect on the mortality rates of their hosts and we assume that there is a direct positive relationship between the infection prevalence and mortality rate of a given host species. One could either interpret this as the effect of a specific infectious agent with a very narrow host range that has clinical effects leading to mortality in the focal species, or as the combined effect of all infectious agents for which that focal species acts as a host. Because the relation with infection prevalence is

indirect in our analysis, one might argue that the changes in mortality for focal species may also be caused by other factors, for example environmental, climate change, and the effects we see would therefore not necessarily be those of infection change. However, for each analysis we assume that the change in mortality only acts on the focal species, keeping the growth rates of all other species in the ecosystem at their original level. This precludes at least more generic changes that could underlie the change in mortality. Another aspect of our approach is that each calculation is based on a single focal species. Many infectious agents will have a wider host range in the same community, but we have focused this initial exploration on highly specialised infectious agents. This leaves the more general case unexplored. Finally, we do not explicitly treat parasites and pathogens dynamically in the interaction network. In other words, the infection dynamics in the target host species is not modelled explicitly. This holds not only for transmission, but also for dynamics of possible free-living stage (in case of parasites), within-host dynamics and disease severity. We therefore also ignore parasite-density dependent mortality. Including infection dynamics would involve a description of the different (epidemiological) states for target host individuals and the infectious agent, and would involve making explicit assumptions on the type of agent, the type of host species and various characteristics of the transmission process and epidemiology of that particular host-agent combination (Diekmann *et al.* 2013). These are, however, clearly next steps that need to be taken. An advantage of our current initial indirect exploration is that results can be achieved based on analytical derivations in setting that at least regards full food webs. A disadvantage is that it is unclear how the results will be influenced by infection dynamics, multiple agents, multiple hosts, and infection-related feedback. It is clear, however, that food webs and ecosystems are complex systems. Hence intuition for what will happen in wider network cannot be easily obtained by looking at single nodes or pairs of linked nodes. So various factors that one could assume to be counteracting or dampening, when viewed in singe species or in two-species food chains, could average out the effects we observe, or instead reinforce them, in full food-web settings. We argue that it makes good sense, in light of the complexity, to analyse the problem in smaller steps, but starting from an actual food-web view.

To conclude, our study shows that parasites and pathogens are likely to be major players in many ecological communities. Increased as well as decreased infection prevalence of a host species can lead to large declines in the abundances- quasi-extinctions-of other species in a community. Such quasi-extinctions can cause stability loss in the community, potentially giving rise to further extinctions (Säterberg *et al.* 2013, Sellman *et al.* 2015). In addition, we found that top predators in the ecosystem not only harbour more different species of infectious agent, an idea that was already well-known (Chen *et al.* 2008,

Dobson *et al.* 2008), but that the ecosystem is also more robust to changes in top predator mortality due to infection, in the sense of the occurrence of quasi- extinctions in the community. We conclude that changes in the prevalence of infectious agents in specific species might have more profound and more indirect consequences than previously thought. An exploration of such wider consequences of infectious disease outbreaks and local infection eliminations is an essential next step in the understanding of structure, stability biodiversity and functioning of ecosystems.

Supplementary Information

6.4.1. Appendix S1

Community structure Communities were pyramidal in shape, i.e. the number of species decreases with increasing trophic level. The community size S, i.e. the number of species in a community, was fixed (S = 12) and the species were distributed over three trophic levels: one half of the species were basal species (B) at trophic level 1, one third of the species were herbivore species (H) at trophic level 2 and one sixth of the species were top consumers (C) at trophic level 3. The number of consumer-resource links L in the systems was based on empirically observed connectances (expressed as L/S^2) and the links were randomly assigned with two restrictions: consumers must have at least one prey and top consumers must have at least one herbivore prey. We chose L such that connectance varied between 0.08-0.3, which is in the range observed in natural food webs (Dunne et al. 2002; Digel et al. 2011). Parameter values For species at the first trophic level (basal species) intrinsic rates of change, r_i, were set to 1. Intrinsic rates of change, r_i , for consumers were drawn from the uniform distribution [-0.01 0] and sorted such that species at higher trophic levels have larger values (closer to zero). Interspecific competition among basal species, α_{ij}, was drawn from a uniform distribution [-0.2 -0.1]. The degree of self-regulation (intraspecific competition), α_{ii}, was set to -1 for basal species and assumed absent in consumer species. For consumer resource interactions, α_{ij}, were parametrized as follows:

$$\alpha_{ij} = -h_{ij}$$

$$\alpha_{ji} = -\alpha_{ij} e_{ij}$$

Here k is a constant drawn from a uniform distribution [0 1]; e_{ij} is a conversion efficiency representing the proportion of consumed biomass that is converted into new consumer biomass - this parameter is set to 0.2 for a resource on an adjacent trophic level and to 0.02 for omnivorous links; h_{ij} represents the preference of consumer j for resource i. We explore two prey preference scenarios:

1) Specialist consumers showing strong preference for one of their prey species:

$$h_{ij} = 0.9 \quad or \quad h_{ij} = \frac{0.1}{(number_{prey} - 1)}$$

2) Generalist consumers showing equal preference for each of their prey species:

$$h_{ij} = \frac{1}{(number_{prey} - 1)}$$

6.4.2. Additional analysis (p=0.5)

Results of the smallest negative and positive change in the mortality rate that will lead to a $(1p)$- proportional decrease in the equilibrium abundance of any species in the ecological community, when p=0.5.

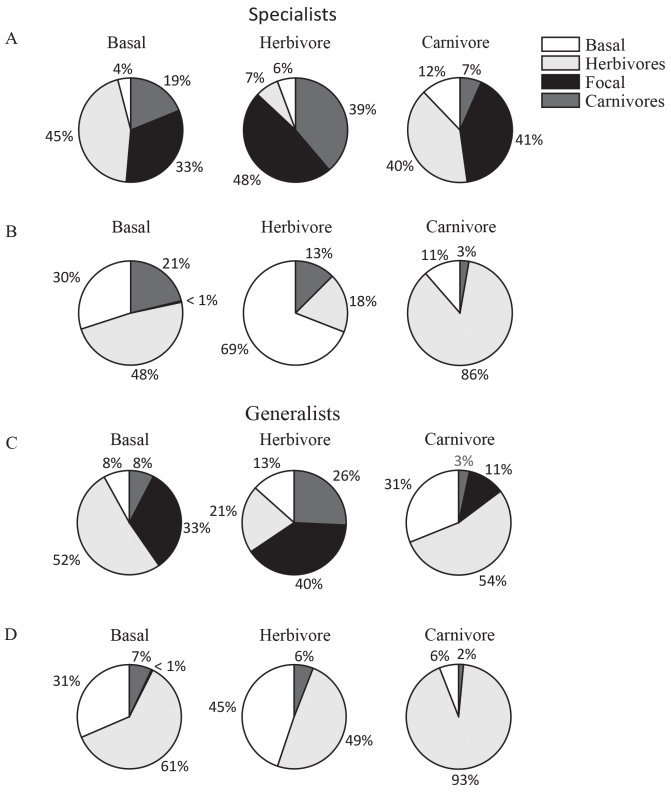


Figure 6.5. The proportion of quasi-extinctions affecting basal species, herbivores, carnivores or the focal species themselves following an infection-induced change in mortality rate of basal, herbivore and carnivore focal species, respectively. A) Increased mortality of the focal species, and B) decreased mortality of the focal species, both in communities with specialist consumers. C) Increased mortality of the focal species, and D) decreased mortality of the focal species, both in communities with generalist consumers.

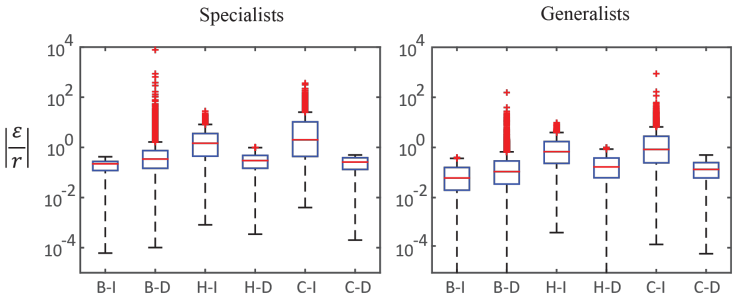


Figure 6.6. Boxplots showing the relative increase of mortality or infection prevalence (I) and decrease of mortality or infection prevalence (D) in the growth rate of basal (B), herbivore (H) and carnivore (C) species, respectively, needed to cause quasi-extinction (p=0.5) in the community.

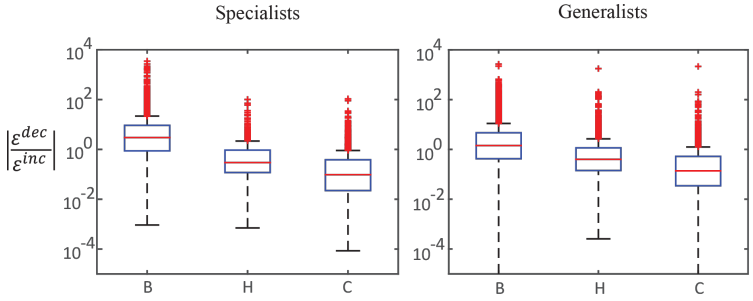


Figure 6.7. Boxplots comparing the increase and decrease of mortality/infection prevalence needed to cause quasi-extinction (p=0.5) in the community, for basal (B), herbivore (H) and carnivore (C) species.

6.4.3. Empirical data

We explored six data sets that are available online: Carpinteria Salt Marsh- CSM, Estero de Punta Banda- EPB, Bahia Falsa in Bahia San Quintín- BSQ (Hechinger et al. 2011), Quick pond-QP (Preston et al. 2012), Otago data set (Mouritsen et al. 2011), Sylt data set (Thieltges et al. 2011). Four of the data sets are presented in the main text. The two remaining data sets (Sylt and Quick Pond) presented here have small number of herbivore species which might be due to under-sampling.

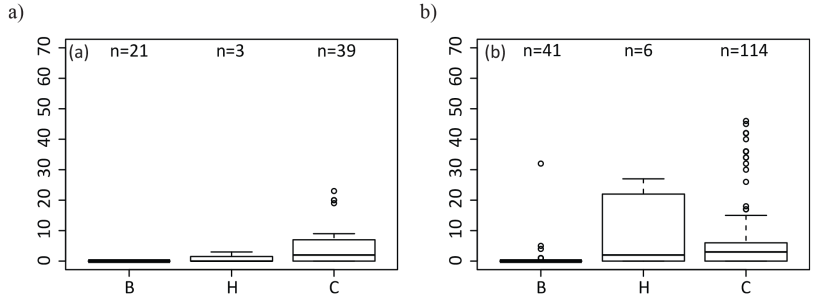


Figure 6.8. Number of parasitic species per host species at different trophic levels (B - basal species, H - herbivores, C - carnivores) in additional two aquatic ecosystems (a-QP and b-Sylt).

Table 6.1. Analysis of six real food webs with parasites

Dataset	TL	S	PC	Pt	Mp	Po	Tp	Tt	∑ parasites	∑ total sp	par/sp
CSM	B	69	0	0	7	1	0	0	8	77	0.1159
CSM	H	27	20	4	4	0	57	10	95	122	3.5185
CSM	C	68	2	58	500	0	108	516	1184	1252	17.4118
BSQ	B	68	0	0	0	0	0	0	0	68	0
BSQ	H	40	22	2	4	0	59	0	87	127	2.175
BSQ	C	62	1	53	579	1	91	615	1340	1402	21.6129
EPB	B	90	0	8	8	3	8	7	34	124	0.3777
EPB	H	39	20	3	10	2	76	22	133	172	3.4102
EPB	C	85	7	60	538	1	100	552	1258	1343	14.8
QP	B	21	0	0	0	0	0	0	0	21	0
QP	H	3	0	0	2	0	0	1	3	6	0.75
QP	C	39	7	24	51	0	54	38	174	213	4.4615
Otago	B	22	0	0	0	0	0	0	0	22	0
Otago	H	46	12	0	3	0	16	1	32	78	0.6956
Otago	C	73	2	0	97	0	43	103	245	318	3.3562
Sylt	B	41	0	0	0	0	0	0	0	41	0
Sylt	H	6	0	0	4	0	8	4	16	22	2.6667
Sylt	C	114	26	0	388	0	131	385	930	1044	8.1579

*TL- trophic level, S-number of non-parasitic species, PC- parasitic castrator, Pt- pathogens, Mp-macro-parasites , Po- parasitoids , Tp- trophically transmitted parasites, Tt- trophic transmission

Chapter 7

Sub-clinical and clinical effects of infectious agents on food web stability

Sanja Selaković and Hans Heesterbeek

work in progress

Abstract

Infectious agents affect behaviour and vital rates of their hosts, influencing the interactions between species in the community and in that way potentially change the stability of the ecosystem. Empirical examples show a variety of ways in which different types of infectious agents can affect their hosts. We take an indirect approach in investigating wider community effects of these influences on hosts at different trophic levels. By decreasing and increasing resource preferences of consumers, conversion efficiencies and growth rates, we mimic subclinical and clinical influence of infection in the community. We find that the different ways in which infectious agents affects their hosts can make the food web they are part of both more and less stable, as measured by the size of the largest real part of the eigenvalues of the community matrix. Influence of infection on resource preference of consumers has more impact on the change of stability than the effect of infection on conversion efficiencies of consumers. We find that different types of influence of infectious agents on a focal species create similar patterns in the way in which stability changes. Subclinical and clinical effects of infectious agents in focal species of hosts, more frequently lead to increase of stability of the community than to decrease. The study suggests that infectious agents may be important for the stability of ecosystems.

7.1. Introduction

By affecting behaviour and intrinsic growth rate of its hosts, infectious agents change the interaction strength between species of ecological communities (Selakovic *et al.* 2014). These changes in interaction strength between community species lead to changes in the overall stability of the system. The concept of stability, and especially the way in which the food web structure has evolved as a buffer to deal with disturbance, is one of the main concepts in food web theory (McCann 2011). The concept of interaction strength is interpreted in different ways (Berlow *et al.* 2004, Laska and Wootton 1998). We approach it as a quantification of the positive per capita effect of a resource on a consumer, and the negative per capita effect of a consumer on a resource. Interaction strengths are the elements of the community matrix (or mathematically speaking: Jacobian matrix) (May 1973) obtained from a description of food web dynamics using a system of ordinary differential equations, and defined as the partial derivatives near an equilibrium state. The largest real eigenvalue of the Jacobian matrix can be negative or positive, and that tells us if system in that particular equilibrium is stable or unstable.

What we know about different types of infectious agents is that they affect hosts in many clinical and sub-clinical ways affecting their behaviour, feeding, reproduction and mortality (see Table 7.1). All of these ways in which infectious agents affect their hosts influence the interactions between species in the community. Effects of the infectious agents on the host species are sometimes large, but they can also be subtle. Empirical examples of these situations were described in Chapters 3 & 4 of this PhD-thesis.

Table 7.1. The ways infectious agents affect their hosts shown through their effect on parameters of a Lotka-Volterra model (I- increase, D- decrease, N-neutral)

Consumer affected	Host that is infected with parasite			
	Microparasites	Macroparasites	Parasitoids	Parasitic castrators
hjij(resource preference)	D	D	I	I
aij (attack rate)	N/I	N/I	I	I
T(handling time)	N/D	N/D	N/D	N/D
e (conversion eff)	D	D	D	D
reproduction	N/D	N	D	D
mortality	N/I	N	I	N

For some combinations of infectious agent and host species there are mostly sub-clinical effects in infected host individuals, for example affecting (feeding) behaviour and in that way change the interaction strengths with species to which they are directly linked (e.g. infected host individuals have less energy to search for food than healthy individuals, because part of the energy is needed to fight the infection, or they can be less easily caught by their consumers). Even if these changes are small (or they affect only a small proportion of the population), these could have substantial effects on the stability of the food web as a whole because it has been shown that even weak interaction strengths in long loops of consumer-resource interaction are important for stability (Neutel *et al.* 2002).

Clinical and subclinical influence of infectious agents on the stability of a food web have not been systematically explored. It is clear that in complex systems, of which food webs are examples, intuition about what happens in a network is difficult to derive by studying nodes or pairs or linked nodes in isolation. When studying infectious agents and stability at food-web level, interesting questions are, for example, which type of subclinical effect has the biggest impact on stability, or whether the stability of the system always changes in the same direction if species of certain trophic levels are affected by the infection. To address these issues, and to more systematically and quantitatively explore the potential impact of infectious disease agents on stability of ecological communities, we use an indirect approach and mimic the infectious agents' impact on stability of the ecological communities through their effect on behaviour, physiology or growth rates of a focal species in a simulated collection of realistic food webs. We are doing this by focusing only on changes in parameters governing resource preference, conversion efficiency, growth and mortality (see Table 7.1). We explore potential effects through different scenarios that depend on which focal species is affected (consumer, resource, trophic level) and whether or not species that are directly connected with infected species are allowed to compensate for the change in interaction strength between species in the community, for example by changing their feeding preference. We explore patters of different impact on the stability of the community, given the different choices that are made.

7.2. Methods

We generated 1000 feasible (i.e., with all species having positive equilibrium biomass) and locally stable pyramidal food webs of 12 species, using the approach from Kaneryd

et al. 2012. The food webs have three trophic levels; species are basal (1-6, B), herbivorous (7-10, H) or carnivorous (11-12, C). We only regard food webs where consumer species are 'specialists' (i.e., they have a strong preference for one type of resource, compared to others that they consume with equal, but small, preference, see the explanation in Chapter 6). We assume dynamics described by a generalized Lotka-Volterra system (Chapter 6).

The interior equilibrium of the generalized Lotka-Volterra system is given by:

$$\hat{N} = A^{-1}r \tag{7.1}$$

where $\hat{N}$ is the vector of equilibrium densities of all species, A^{-1} is the inverse interaction matrix and r is the vector of the intrinsic growth rates of the species. For a more detailed description used to simulate food webs with quantified equilibrium abundances, interaction matrices and growth rates, we refer to Chapter 6. The interaction matrix A (May 1974) depends on the parameters h_{ij} representing the preference of consumer j for resource i (i.e., the fraction of species i in the total diet of species j), and conversion efficiency e_{ij} (i.e. the proportion of consumed biomass converted into new consumer biomass). We use the interaction matrix **A** and the vector of species equilibrium densities $\hat{N}$ to calculate a new community Jacobian matrix **J** at that equilibrium (Berlow et al. 2004). The largest real part of the eigenvalues of **J** is denoted by λ_{max} , and $\lambda_0(n)$ is defined as the largest real part for the simulated food web with ordinal number n, where 0 denotes that this is in the baseline situation without infection-induced change. The largest real part of the Jacobian matrix gives us information on the stability of system; system is stable if λ_{max}<0 or unstable if λ_{max}>0.

We use an auxiliary parameter q to investigate the influence of infectious agents on the stability of the food webs. The effect of parameter q on prey preference and conversion efficiency simulates the possible behavioural change, with respect to food gathering and physiological ability to use the energy from the consumed food into its own growth, in (sub-)clinically infected species. For intrinsic growth rate, the parameter q simulates the possible change in growth of infected host populations. An increasing value of q can mimic a situation where the infection has an increasing and decreasing influence on the focal species, respectively, compared to the baseline situation. Here, increase can be interpreted as infection prevalence that rises compared to the baseline, and decrease may reflect an outbreak that is fading out compared to the baseline situation. We vary the

parameter q from 0 to 0.95 in steps of 0.05, and calculate (depending on the scenario studied, see below:

I. Increase and decrease of resource preference (h_{ij})

$$h'_{ij} = h_{ij} \pm (h_{ij} * q) \tag{7.2}$$

II. decrease of conversion efficiency (e_{ij})

$$e'_{ij} = e_{ij} - (e_{ij} * q) \tag{7.3}$$

III. increase and decrease of growth rate (r_{ij})

$$r'_{ij} = r_{ij} \pm (r_{ij} * q) \tag{7.4}$$

in one species at the time (called the focal species), keeping the parameter values for the other species unchanged. After changing the value of q, we allow the system to reach a new equilibrium. We recalculate the new interaction matrix A' (in case of I. and II.), equilibrium values $\hat{N}'_i$ for every species i, and the measure of stability λ'_{max}, the largest real part of the associated Jacobian community matrix J'.

For every focal species i and a food web n, we calculate a string of new $\lambda'_{max}(i, q)$. $\lambda'_{max}(i, q)$ represents a new stability equilibrium system reached after the infectious agent affected the focal species i and produced a change q compared to the baseline situation. Cases where the magnitude of the change in parameter q would lead to a non-feasible new equilibrium density in any of the community species were excluded and labelled NF ("Not feasible"). Here, we are interested in cases where the influence of the infectious agent in focal species produces feasible equilibria.

We distinguish seven distinct scenarios depending on the way the infectious agent influences the focal species and the role of the focal species in consumer-resource interaction:

A. Decreased predation by a focal consumer due to infection,

B. Increased predation by a focal consumer due to infection,

C. Decreased predation on a focal resource species due to infection, the consumers of that focal resource do not compensate for the lack of resource,

D. Increased predation on a focal resource species due to infection, the consumers of that focal resource do not compensate for the lack of resource,

E. Decreased predation on a focal resource due to infection, the consumers of that focal resource fully compensate for the lack of resource,

F. Mortality increase in the focal species,

G. Mortality decrease in the focal species.

In the study of the behavioural change in focal resource species we simulate two types of scenarios; 1) consumers are not able to compensate for a change in behaviour of the resource (C&D) or 2) consumers are able to compensate such a change (E). The second scenario implies the ability of consumer to shift its prey preference to its other resource species that are not infected. In our set up, this implies that every change in a row of the interaction matrix leads to changes in columns (Figure 1). Compensation required in consumption is assumed to be divided equally over all other resources that different consumers of the infected resource have as their sources of food.

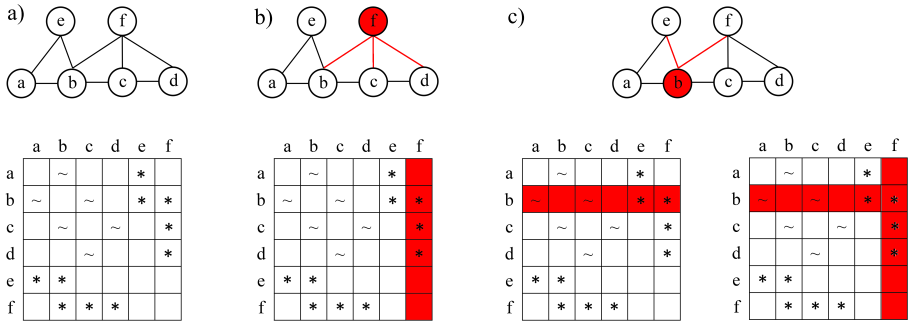


Figure 7.1. Illustration using an imaginary food web and its interaction matrix including consumer-resource ($*$) and competitive ($\sim$) types of interactions (a). The infected host (focal) species is marked red. b) If the focal species is a consumer, the interaction with its resource will change (A&B scenarios); c) If the focal species is a resource, consumers either do not compensate for a reduction in the availability of the focal resource (C&D, on the left) or fully compensate for that (E, on the right).

In total we have seven scenarios (A-G) and for every scenario we study 1000 food webs of 12 species with different levels of the infectious agents' influence on behaviour, physiology or growth rate of focal species i, as governed by the parameter q. Firstly, we

give examples with selected scenarios and single, representative, food webs. Secondly, we search for patterns of stability change due to infection. We use strings of community stability given the infectious agents' influence at level q ($\lambda'_{max}(i,q)$) to look for possible patterns of stability change under different scenarios (Table 7.2).

Table 7.2. Example of one (i,q) string

q	0	0.05	0.1	0.15	0.2	...	0.65	0.7	0.75	0.8	0.85	0.9
$\lambda_{(i,q)}$	λ_0	$\lambda_{0.05}$	$\lambda_{0.1}$	$\lambda_{0.15}$	$\lambda_{0.2}$	...	$\lambda_{0.65}$	$\lambda_{0.7}$	$\lambda_{0.75}$	λ_{end}	NF	NF

In preliminary work, we looked at the (i,q) strings of stability in different focal species and scenarios, and we distinguished eight dominant patterns of stability change by visual check. To distinguish pattern in $\lambda(i,q)$ string of a focal species, we use λ_0 which represents the maximal real part of the eigenvalues of the system in the baseline steady state, and λ_{end} which is the maximal real part for the value of the largest value of q before the influence of the infectious agent on its host leads to extinction of a species in the system (see Table 2) (i.e., for larger values of q, the system has a non-feasible steady state where not all 12 species are present). The position of λ_{end} possibly changes for every focal species and simulated food web. Furthermore, we identify $min(\lambda)$ and $max(\lambda)$ as the smallest and the largest values in a $\lambda(i,q)$ string between λ_0 and λ_{end}. We then look at eight dominant patterns of stability and look at the frequency in which they occur for the different scenarios, over all focal species and simulated food webs:

I. Constant stability

$$\lambda_0 = max(\lambda) = min(\lambda)$$

II. Decrease of stability

$$\lambda_0 = min(\lambda) \quad \& \quad \lambda_{end} = max(\lambda)$$

III. Decrease of stability followed by the small increase

$$\lambda_0 = min(\lambda) \quad \& \quad \lambda_{end} \neq max(\lambda)$$

IV. Decrease of stability followed by the increase of stability bigger than baseline steady state stability

$$\lambda_0 \neq max(\lambda) \quad \& \quad \lambda_{end} = min(\lambda)$$

V. Increase of stability

$$\lambda_0 = max(\lambda) \quad \& \quad \lambda_{end} = min(\lambda)$$

VI. Increase of stability followed by the small decrease

$$\lambda_0 = max(\lambda) \quad \& \quad \lambda_{end} \neq min(\lambda)$$

VII. Increase of stability followed by the increase of stability bigger than baseline steady state stability

$$\lambda_0 \neq min(\lambda) \quad \& \quad \lambda_{end} = max(\lambda)$$

VIII. other types of behaviour.

We analyse the frequency of patterns of stability change at different trophic levels in different scenarios by looking at the ratio of the number of times each pattern type occurs per total number of species at basal, herbivore or carnivore trophic levels. Additionally, for every value of q in the different scenarios and trophic levels, we analyse if (i, q) is equal, bigger, smaller than λ_0, or whether no feasible equilibrium exists for that value of q. This serves to quantify the frequency of stability being constant, decreased or increased compared to the frequency of extinctions (NF values) with increasing values of q for focal species at basal, herbivore or carnivore trophic levels in different scenarios.

7.3. Results

The potential impacts of infectious agents on behaviour, physiology and growth rates of its host lead to increase or decrease of the system's stability, compared to the baseline situation (q = 0), which is usually followed by the extinction of a species in the system. We found seven dominant patterns of stability change as a consequence of the impact of infectious agents on a focal species; the eight pattern is a remainder category. Although we can find these stability patterns in every trophic level and scenario, the frequency of stability change for the different values of q shows that increase in stability is dominant.

In Figure 7.2 we give an example of stability change in one food web for scenarios A

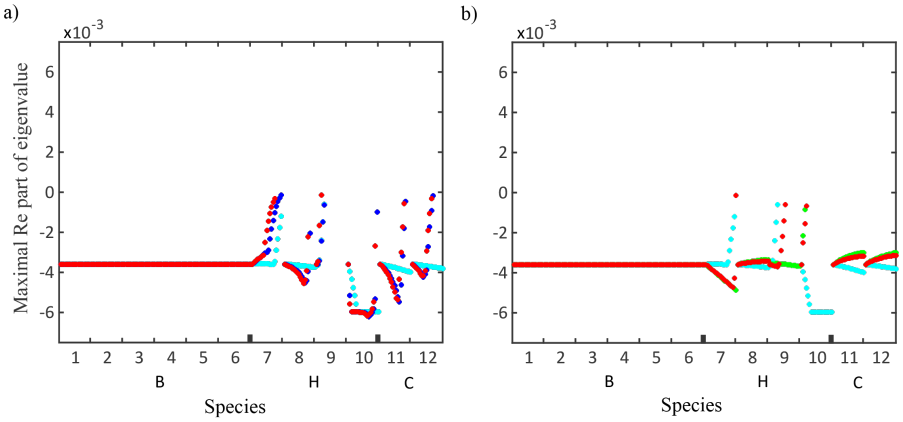


Figure 7.2. Stability change in one selected food web as a function of focal species 1-12, and increasing value of q (0-0.95, with steps of 0.05) for each species. Depicted are effects of changing the resource preference (decreased in the left panel (blue points) and increased in the right panel (green points)), conversion efficiency (cyan points), and the combined influence of both parameters (red points), with a) scenario A, b) scenario B.

and B where a consumer species is affected by the infection. We show effects of q on resource preference, conversion efficiency separately and their combined effect. To illustrate the way to read the figure, focus on e.g. species 7 and the effect of resource preference. Each point within the interval marked with '7', represents the largest real part of the eigenvalues for the system with increasing values for q. Decreased resource preference (scenario A, Figure 7.2a) leads to decrease of stability, while increased resource preference (scenario B, Figure 7.2b) leads to increased stability (i.e. largest real part becomes more negative) until, for some intermediate value of q, stability peaks after which stability decreases. However, other species (e.g. species 8, 9, 10, 11 or 12, Figure 7.2a) do not show the same trend. The stability changes due to the effect of q on resource preference and conversion efficiency can have the same direction (e.g. species 7, Figure 7.2a) or an opposite direction (e.g. species 10, 11 and 12, Figure 7.2b). Furthermore, the stability of the combined effect on both resource preference and conversion efficiency follows the direction of stability change by the infectious agent's influence on resource preference.

Figure 7.3a shows the stability change of seven different scenarios in one food web. We show only the stability change of the combined effect of resource preference and conversion efficiency for the different scenarios. The stability has different directions of change for different focal spices and scenarios. It can increase, decrease or change the direction for a range of steps in the value of q. Figure 3b shows four different food webs

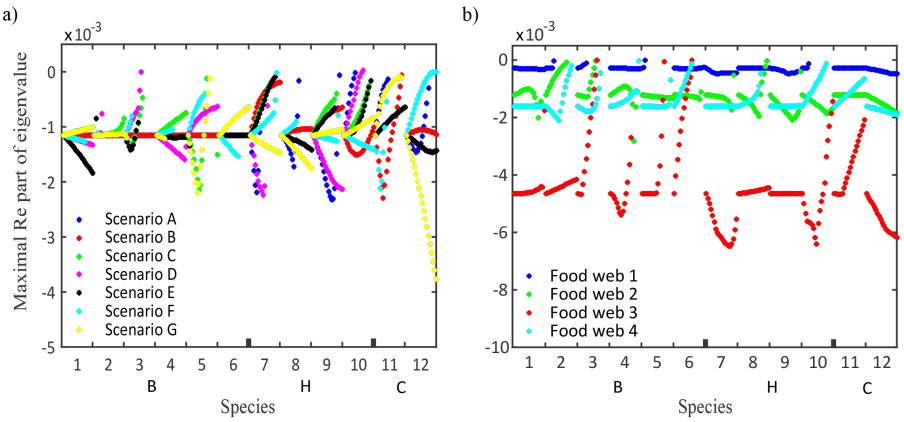


Figure 7.3. The diversity of the potential influence of the infectious agent's impact on behaviour and growth in focal species in a) one selected food web with the seven different scenarios, b) four different selected food webs with the scenario C.

for scenario C. If we compare 'food web 1' and 'food web 3', the initial baseline stability levels of the food webs are different. Under the effect of an infectious agent, the food web that was originally more stable ('food web 3'), shows bigger changes in stability in most of the focal species than the food web that was originally less stable ('food web 1').

Dominant patterns of stability change are shown in Figure 7.4. By visual inspection of stability changes in different food webs and different scenarios, we distinguished eight types that show repetition of specific trends of stability change for every focal species affected by the q parameter (seven broad patterns and one remainder category). The main patterns of stability change that can be distinguished are constant stability (I), decrease of stability (II-IV) and increase of stability (V-VII). However, these types have subtypes where direction of stability change can shift from decrease to increase (IV) or from increase to decrease (VII) of stability. Stability can experience even discontinuous with an increase of q parameter or multiple changes of direction (VIIIa, VIIIb and VIIIc, respectively).

Frequency of the stability patterns over all simulated food webs for different scenarios is presented in Figure 7.5. We show a frequency of the eight dominant patterns of stability change among the total number of focal species, grouped by trophic level (for convenience denoted by basal, herbivorous or carnivorous). The results show that we can find all eight types of stability patterns at every trophic level and specific scenario (A-G).

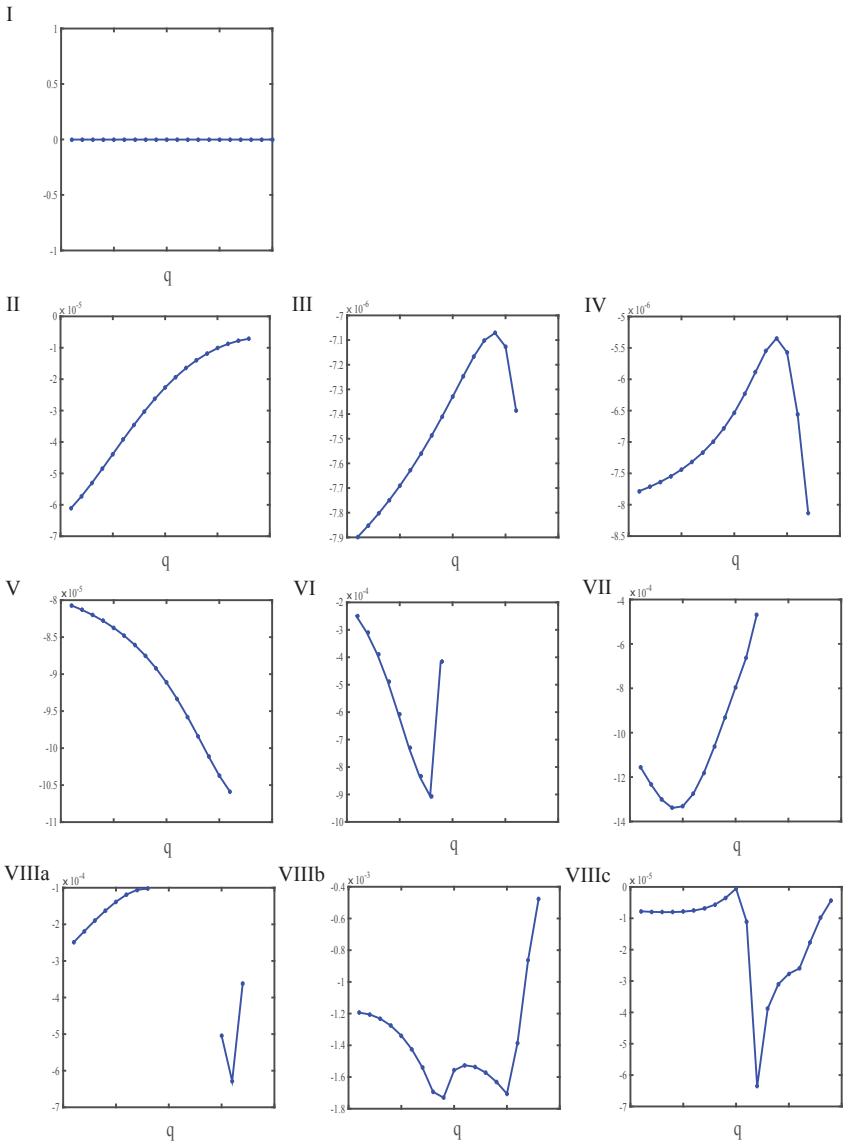


Figure 7.4. Examples of types in stability change due to behaviour or growth change in focal species. I-constant, II-decrease, III-decrease followed by the increase smaller than the baseline situation, IV-decrease followed by the increase bigger than the baseline, V-increase, VI-increase followed by the decrease smaller than the baseline, VII-increase followed by decrease bigger than the baseline, VIII-other types of behaviour (a-discontinuous, b,c-multiple changes of direction).

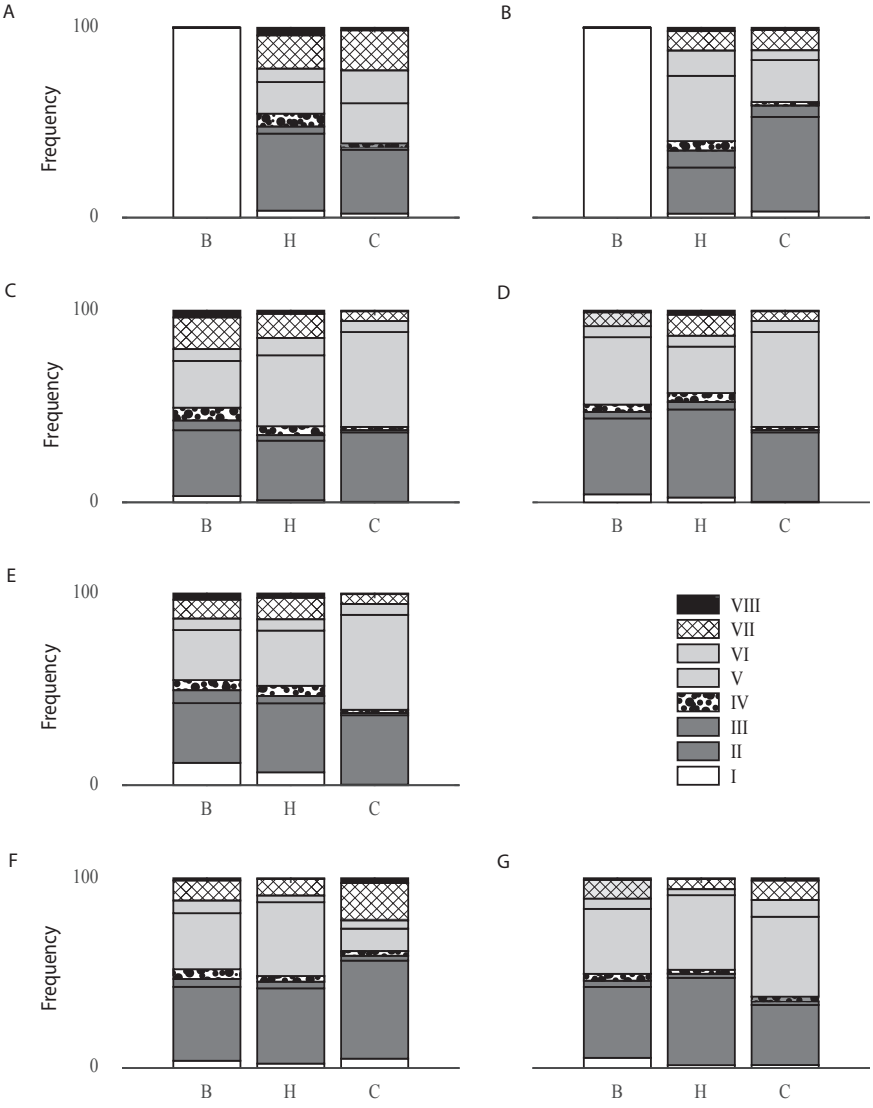


Figure 7.5. Frequency of the eight types of stability change for the seven scenarios. Panel A- decreased predation of the consumer due to infection, B- increased predation of consumer due to infection, C- decreased predation of the resource species due to infection, consumer does not compensates for reduced availability or quality of the resource, D- increased predation of the resource species due to infection, consumer does not compensates for reduced availability of the resource, E- decreases predation of the resource due to infection, consumer compensates for reduced availability of the resource, F- mortality increase in focal species, G-mortality decrease in focal species.

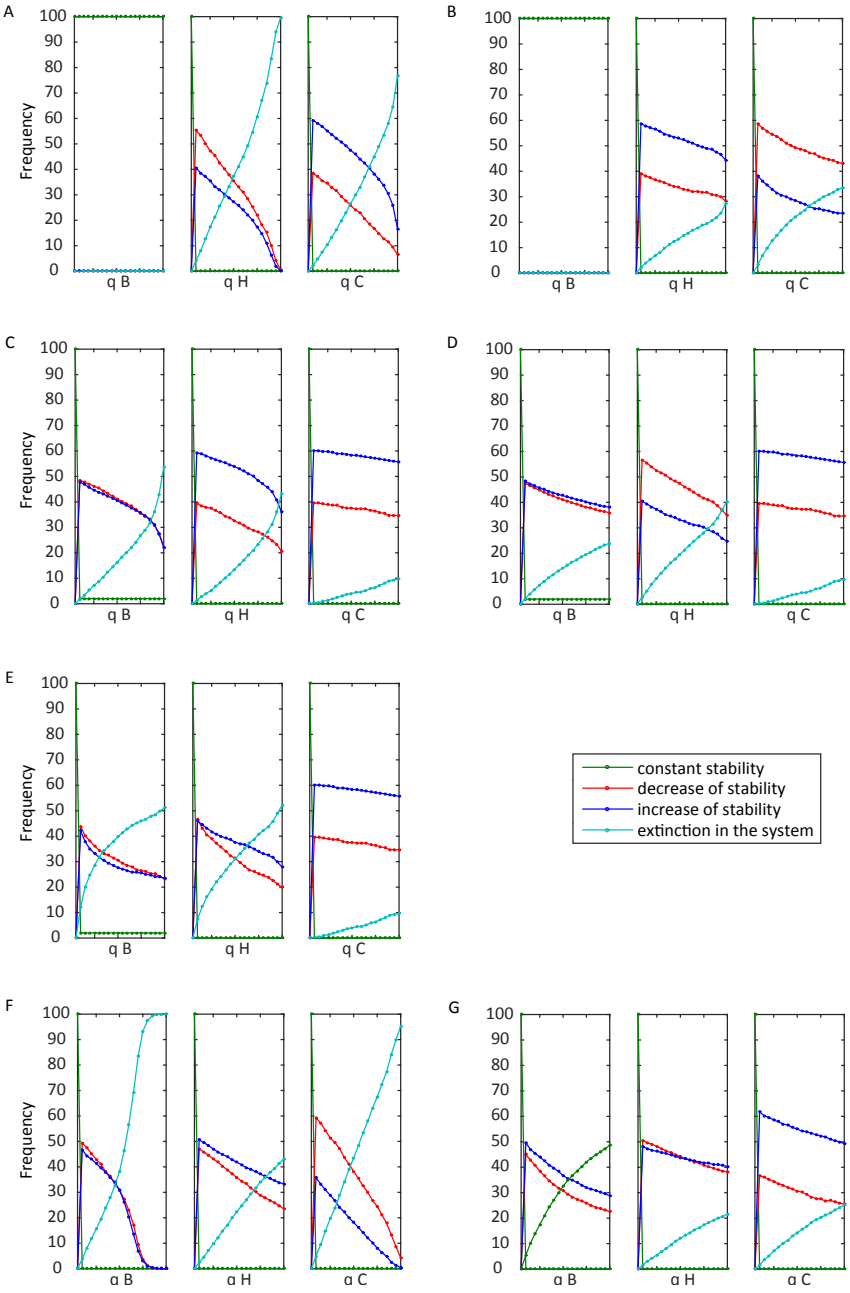


Figure 7.6. Frequency of stability (constant, decrease, increased) or extinctions, over a range of q (0-95%, in steps of 5%) in the seven scenarios.

In scenarios A and B behaviour of consumers is affected. Constant stability is dominant at the basal level as a result of our assumption that infectious agents cannot affect behaviour of basal species. Decrease of stability (dark grey) is more frequent than increase of stability (light grey) in herbivores compared to carnivores in scenario A. The opposite is true for scenario B. The stability of the system decreases more frequently if the focal species is a carnivore. Increasing followed by decreasing stability (VII) has high frequency.

Scenarios C, D and E represent results of the effect of infection in resource species. Infection can influence focal resource species to become less available or have lower quality for their consumers. In scenario C, consumers of that infected resource species are not compensating for that reduction. Scenario E that studies characteristics when consumers switch their resource preference and increase consumption of other resource species that are not infected, shows almost the same results as scenario C.

The last two scenarios (F and G) study the effect of decreased or increased growth rate on the stability of the system. The infectious agent's effect on the decrease of growth rate in carnivores more frequently leads to decrease of stability. Interestingly, one of the most frequent patterns of stability is type VII where stability increases, peaks and then decreases.

Frequency of stability change (constant, decrease, increased) and extinctions, calculated for a total number of basal, herbivorous or carnivorous species at every step for the value of q in seven scenarios (A-G), is presented in Figure 7.6. The increase of the stability (blue line) is dominant compared to decrease of stability (red line) in the majority of trophic levels and scenarios; one type of stability change (increase or decrease) prevails. In rare case there is a switch of dominating type of stability change with an increase of q (e.g. herbivores in scenario G). Note the increase of the frequency of extinctions in the systems due to increasing value of q. Smaller changes in the growth rate of focal species increases stability, while bigger changes lead to stability decrease.

7.4. Discussion

Infectious agents are ubiquitous in nature. They dominate food web links (Amundsen *et al.* 2009, Lafferty *et al.* 2006a), shape population dynamics and alter interactions between their host and non-hosts (Hudson *et al.* 2006, Poulin 1999, Selakovic *et al.* 2014). They affect stability of food webs by increasing diversity and complexity of

ecological networks (Dunne *et al.* 2013). The majority of studies explored the effects of infectious agents on structure and stability in food webs by adding them as separate nodes (species) and links (Chen *et al.* 2008, Lafferty *et al.* 2006b). However, from an epidemiological point of view, one can argue that infectious agents are different from typical consumers (Lafferty and Kuris 2002, Sukhdeo 2012). The objective of the current study is to explore the influence of infectious agents on stability of their ecosystem indirectly through the effects they may have on behaviour, physiology and growth of their hosts. We find that the potential effects of infectious agents on interactions of species profoundly affect the stability of the food webs they are part of, and in a wide variety of patterns. These effects on stability can be relatively small or large depending on the initial stability of a system, before a change in infection prevalance, and on the intensity of infectious agent's effect. Stability increases or decreases until one of the species experiences extinction, but in our simulations the system did not shift from stable to unstable while keeping a feasible equilibrium with all species present.

Food webs with a larger maximal negative real part of the eigenvalues at the feasible equilibrium, showed larger sensitivity to an effect of infectious agent. We interpret a larger negative real part here as being "more stable" compared to situations where the eigenvalues have a smaller maximal real part. This is an assumption that is questionable, as in the mathematical sense, a system is either stable or unstable, depending on whether the maximal real part of the eigenvalues is < 0, or > 0, respectively. The changes in maximal real part show, however, that it is important that measures are developed to characterize how stable a system is compared to the same system in a different parameterization or different steady state. Our choice is the most pragmatic for an initial view, but is not founded on mathematical arguments.

The way infectious agents influence their hosts is also important. We decided to explore the effects of resource preference and conversion efficiency. The reason for choosing conversion efficiency comes from the knowledge that many infectious agents probably affect their host by redirecting parts of their energy, which is assimilated for biomass production, maintenance and reproduction, towards investment in immune response. The influence on resource preference is possibly the most important way in which infectious agents affect food webs, since this directly impacts on feeding relations in the web. This occurs in different ways, as explained in (Selakovic *et al.* 2014). Our results indicate that influence of infectious agents on behaviour (here expressed through resource preference) has stronger effect on the stability of the whole system than influence on conversion efficiency. The stability change of the combined effects of conversion efficiency and resource preference showed that the direction of change follows that of effects on resource

preference.

Furthermore, we explored the importance of the role of the focal species in the web (consumer or resource) when considering stability. The results did not show any specific differences. Additionally, they did not show any patterns of stability characteristic for the specific trophic levels of food webs. However, the pattern where stability increases as a result of infection had a slightly bigger frequency in most of the trophic levels and different scenarios. Looking at the change in different focal species at the same trophic level, the analysis showed that very different stability patterns are possible, even under a similar influence of the infectious agent. From this we conclude that changes in stability produced by the infectious agent probably depend on the direct and indirect links of the focal species and the interaction strength of those links.

The patterning of strong and weak links of interaction strengths over all food web links has an important consequences for the stability of food webs (Allesina and Tang 2012, Christianou and Ebenman 2005, de Ruiter *et al.* 1995, Emmerson and Raffaelli 2004, Kokkoris *et al.* 2002, McCann *et al.* 1998, Neutel *et al.* 2002, Tang *et al.* 2014). Infectious agents affect the strength of these interactions, for example by changing the behaviour (e.g. predation), physiology and growth rate. The impact on one species further transfers through the ecological network to species that are not directly connected. Every species is host to several or many infectious agents. Simultaneously, many infectious agents have a wide range of host species. Although we explore the potential influence of infectious agent in only one species at the time, we show that even small effetcs of infectious agents impact significantly on the stability of system. If one imagines a sub-web of infectious agents affecting the food web network, the influence that they have on interactions in ecological communities becomes more meaningful. We showed that sometimes the influence of infectious agents increases and sometimes decreases stability. The overall effect of the sub-web of all infectious agents combined could therefore equalise the stability effect on the food web, but without a thorough analysis of the full complex system it is impossible to trust ”intuition” In addition,the parasite richness is shown to be higher at higher trophic levels (Chapter 6, Chen *et al.* 2008, Dobson *et al.* 2008).

This study is an attempt to better understand the role of infectious agents in food webs and ecological communities. The potential effects of the infectious agents show different consequences for the stability of the system even in the same focal species and role of that species as a consumer or resource. We found eight different patterns that show how stability can change under the influence of infection in one focal species at a time. The

frequencies of stability increase vs decrease were almost equivalent with slightly bigger frequency of increased stability in most of the trophic levels and scenarios, as measured over many simulated food webs. The next step of this study will be to quantify the potential influence of infectious agents on interaction strengths between species in the system. The further questions that we will address are the importance of exact positions of host species in the food web, the importance of relations with their immediate neighbours in the web, and the importance of link weight in the interaction network.

This research on the subclinical and clinical effects of infectious agents shows promising first results and a potential to deepen our knowledge on the roles of infectious agents in food webs and ecosystems. We conclude that even small changes in the behaviour of one species in a food web can increase or decrease the stability. Of course, the same caveats hold with regard to simplifications in our analysis that were discussed in detail in Chapter 6, notably the indirectness of the approach and the neglect of explicit epidemiological dynamics. An exploration of the kind of effect that could be produced by the entire subweb of infectious agents is an essential next step in the understanding the role infectious agents have in food webs.

Chapter 8

Parasite Spreading in Spatial Ecological Multiplex Networks

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submitted

Abstract

Several parasites may be transmitted among their hosts through different mechanisms at the same time and each mechanism may be represented as a network of interactions. For this reason, modelling parasite spreading in ecological systems is still an open challenge. We present a novel spatially-embedded multiplex network framework for modelling multi-host infection spreading through multiple routes of transmission. Multiplex networks are a particular kind of multi-layer networks where the same set of nodes can be connected according to different topologies and mechanisms on each layer. Our model is inspired by *Trypanosoma cruzi*, a parasite transmitted by trophic and vectorial mechanisms. In our ecological multiplex network, nodes represent species populations interacting through a food web and a vectorial contaminative layer at the same time. We modelled Susceptible-Infected dynamics in two different scenarios: a simple theoretical food web and an empirical one. Our simulations in both scenarios show that the infection is more widespread when both transmission mechanisms are considered at the same time and it is minimised when they have similar importance. This indicates that trophic and contaminative transmission may have additive effects in real ecosystems. We also find that the ratio of vectors-to-host in the community (i) crucially influences the infection spread, (ii) regulates a percolating phase transition in the rate of parasite transmission and (iii) increases the infection rate in hosts. Through the study of the multiplex structure and immunisation experiments, we show that the multiplex topology is fundamental in outlining the role that each host species plays in parasite transmission in a given ecosystem. We also show that the multiplex models provide a richer phenomenology in terms of parasite spreading dynamics compared to more limited mono-layer models. Our work opens new horizons and provides new quantitative tools for modelling multi-channel spreading in networked systems.

8.1. Introduction

Pathogens and parasites ("parasites" hereafter) are one of the most widespread and diverse life forms (Dobson *et al.* 2008, Poulin and Morand 2014). Several parasites infect multiple host species (Rigaud *et al.* 2010) and many of these parasites may infect their host using different routes of transmission (Poulin 2011). Multi-host parasites include many zoonoses with complex dynamics that challenge infection control and prevention efforts (Dobson 2004). For instance, several multi-host protozoan parasites of public health concern exhibit more than one mode of transmission: *Toxoplasma gondii* can infect its hosts by fecal-oral transmission, the consumption of an infected prey, and through the placenta (Dubey 2004); *Cryptosporidium* directly infects its hosts via sexual contact or via fecal-oral transmission (Fayer *et al.* 2000); *Trypanosoma cruzi* can be transmitted by insect vectors, the consumption of an infected prey, and also through the placenta (Jansen *et al.* 2015, Noireau *et al.* 2009). This complexity of host types and transmission modes challenges the development of models that account for the different sources of variation. The network approach is a promising alternative because it allows accounting for the individual, species-level and spatial sources of heterogeneity (Barter and Gross 2015a, Craft and Caillaud 2011).

Contact networks can be explicitly used to understand the epidemiological consequences of complex host interaction patterns (Bansal *et al.* 2006, Craft *et al.* 2009, Dalziel *et al.* 2014, Ferrari *et al.* 2006, Keeling 2005, Meyers *et al.* 2005). In a contact network, each individual is represented as a node and each contact that potentially results in transmission between two nodes is represented as an edge (or link). Interactions can also be embedded in space (Craft *et al.* 2009, Davis *et al.* 2008b, 2015) where the probability of interaction between nodes may depend on the distance between them. The number of contacts of a node is called the degree of the node and the degree distribution is a fundamental quantity in network theory (Dalziel *et al.* 2014). All epidemiological models make assumptions about the underlying network of interactions, often without explicitly stating them. For example, classical mean-field models used in epidemiology assume that all the interactions have the same probability of leading to transmission (Anderson *et al.* 1992). Contact network models, however, mathematically formalise this intuitive concept so that epidemiological calculations can explicitly consider complex patterns of interactions (Bansal *et al.* 2007). A different approach consists in considering metapopulation dynamics (Colizza and Vespignani 2008), instead of individual contacts.

Recently, the recognition that real-world networks may include different types of interac-

tions among entities prompted the development of methods that take into account the heterogeneity of interactions as well (Boccaletti *et al.* 2014, Kivelä *et al.* 2014). Examples include multi-modal transportation networks in metropolitan areas (Barthélemy 2011, Lima *et al.* 2015, Morris and Barthelemy 2012), or proteins that interact with each other according to different regulatory mechanism (Cardillo *et al.* 2013, Cozzo *et al.* 2013). Ecological systems are also characterised by multiple types of relationships among biological entities, organised and structured on different temporal and spatial scales (Kéfi *et al.* 2015, Kivelä *et al.* 2014). Such representations can be described as "multiplex networks" (De Domenico *et al.* 2013, Mucha *et al.* 2010, Pilosof *et al.* 2015c, Wasserman and Faust 1994). Multiplex networks are a particular kind of multi-layer networks where the same nodes appear on all the layers but they can be connected differently on each layer. Each multiplex layer contains edges of a given type. In the context of parasites that can be transmitted over multiple transmission channels, multiplex networks can be used to include distinct mechanisms of parasite transmission (Pilosof *et al.* 2015c). This approach encapsulates the heterogeneity in the transmission of real-world diseases and helps us understand how the interplay between different modes of transmission affects infection dynamics in an ecosystem (Buono *et al.* 2014a, Lima *et al.* 2015, Salehi *et al.* 2014).

Descriptions of ecological multiplex networks (Kéfi *et al.* 2015, Pilosof *et al.* 2015c) and studies of infection spreading over multiplex structures (Buono *et al.* 2014a, Gomez *et al.* 2013, Salehi *et al.* 2014) have recently appeared in the literature. Previous approaches have already described the structural characteristic of food webs that include parasites (Lafferty *et al.* 2006b) and tried to incorporate parasites in food webs using network framework (Lafferty *et al.* 2008c). The effect of multiple hosts on parasite spreading dynamics have also been explored in the context of disease risk (Keesing *et al.* 2006), disease emergence in a target host (Fenton and Pedersen 2005), parasite sharing and potential transmission pathways (Pilosof *et al.* 2015d) and also in a multilayer network exploring cross-species transmission (within and between host species) (Pilosof *et al.* 2015a). However, the consideration of real ecological scenarios in the analysis of parasite spreading through multiple transmission mechanisms is still an open problem. We propose a spatial multiplex-based framework to model multi-host parasite transmission through multiple transmission mechanisms. In this framework, each transmission mechanism can be represented in a different layer of the multiplex network structure. Our model is inspired by the complex ecology of *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae) in its multiple host community. *T. cruzi* is a relevant example of a multi-host parasite and the pathogen causing the Chagas disease, a serious infection affecting 6-9 million people (Hotez *et al.* 2008). The main infection route to humans involves the

insect vectors (triatomine kissing bugs), but oral transmission is also recurrent (Shikanai-Yasuda and Carvalho 2012). Vectors get infected when consuming blood meals from an infected host, while host infection occurs through the contact of vector's faeces and the biting wound or a mucosa (stercorarian transmission). In sylvatic hosts the stercorarian transmission may occur when the animal scratches the bite and inadvertently rubs the parasite-contaminated matter into the lesion (Kribs-Zaleta 2006). Infection by the oral route occurs when a mammal host ingests infected triatomine faeces, food contaminated with the parasite or by preying on infected vectors or mammals (Jansen *et al.* 2015).

Preliminary studies (Kribs-Zaleta 2006, 2010a, Pelosse and Kribs-Zaleta 2012) used mean-field methods to model *T. cruzi* transmission among its hosts and vectors. Their results indicate that in a fully connected network with no explicit spatial structure, vectorial and oral transmission effects are additive in maintaining and furthering the spread of the infection (Kribs-Zaleta 2006). We use a Susceptible-Infected (SI) model to describe parasite transmission dynamics in spatially embedded multiplex networks. The multiplex framework helps us understand how infection spread is related to the multiplex structure and what is the epidemiological importance of vectors and hosts in different ecological scenarios. We first investigate the parasite spreading across aggregated parasite-host and trophic interactions. In order to measure the influence of the spatial embedding, we contrast the behaviour of a non-spatial model against one where nodes are embedded in space. We then study a reference spatial multiplex network in order to understand the interplay between the multiplex structure and epidemiological dynamics. In the vectorial transmission layer, vectors are contaminated after interacting with infected hosts and transmit the parasite when interacting with non-infected hosts. In the trophic transmission layer hosts acquire the parasite after feeding on infected vector or host. Finally, we use empirical data of a local *T. cruzi* host community, the ecosystem Selva de Canastra (Rocha *et al.* 2013), to model the dynamics of *T. cruzi* multiple transmission routes on its multiple hosts.

With the multiplex framework we aim to understand the effect of multiplex topology and the relative importance of vectorial and trophic transmission for parasite spreading dynamics. We use multiplex cartography (Battiston *et al.* 2014) to characterize species structural importance in the network and compare scenarios with different relative frequency of vectors. We then explore the speed of parasite spreading depending on the importance of vectorial and trophic transmission in scenarios with different frequency of vectors. Finally, we explore the effect of species structural importance on parasite spreading by simulating immunisation experiments.

8.2. Methods

We model a set of N populations interacting within an ecosystem via a network framework. Our aim is to model the diffusion of a multi-host parasite within the ecosystem. Nodes represent populations and they have identities, i.e. their species types (predator, prey, and vector). We denote with $S = \{s_k\}_{k=1}^{s}$ the set of all the s species types. Each node in the network is of a given species type s_k with frequency f_k, normalised such that $\sum_{k=1}^{s} f_k = 1$.

Given that we do not have enough information about the individual-level patterns of interactions, we will consider the food-webs in terms of interacting populations. We consider nodes as populations that follow the same formalism of individual-based dynamics. Our approach is based on the following assumptions: (i) we consider that the parasite transmission is fast and that all the individuals within a population instantaneously gets infected once transmission occurs (in other words, we do not consider metapopulation dynamics such as considering parasite spreading within the population and dispersal among populations Colizza and Vespignani 2008); (ii) we consider the parasite spreading happening at a much faster rate than any birth-death dynamics (which we do not consider).

We assume that individuals from populations can disperse across the system and potentially interact with other populations, according to a *dispersal layer*. The dispersal layer is an undirected graph with adjacency matrix D, so that $d_{ij} = d_{ji} = 1$ if population i can interact with j and vice-versa. In the following subsections, we define the topology of the dispersal layer as being either an Erdös-Rényi random graph or a random geometric graph. The main difference between the two is that the latter includes the notion that only spatially close enough populations can interact with each other (since on random geometric graphs nodes are embedded in space and linked if closer than a certain threshold distance ρ).

In our model, population interaction can potentially give rise to either (i) trophic interactions (a given species feeding on another one) or (ii) contaminative interactions (a given species of host getting in touch with vectors and transmitting the parasite through blood exchanges). Considering only trophic (or contaminative) interactions gives rise to the trophic (or vectorial) layer. Alternatively, considering both interactions together gives rise to an aggregated layer.

Transmission on a given network layer are allowed according to node identities $\{s_k\}$ and

are defined according to the corresponding $s \times s$ *interaction matrices*, T for the trophic layer, V for the vectorial layer and $A = T \oplus V$ for the aggregated layer, where $\oplus$ indicates the Boolean OR function. There is no direct interaction between populations of the same species type because there is no cannibalism in the trophic layer and also no parasite transmission among vectors in the vectorial layer. This means the main diagonal of all interaction matrices are all 0s. The filtering of the dispersal layer through either T or V or A produces s-partite graphs, i.e. there are no edges between nodes of the same species types. We notice that filtered trophic interactions give rise to a directed network layer while we obtain an undirected vectorial layer from allowed contaminative interactions.

Providing the collection of species types S, the topology of the dispersal layer D, choosing if considering trophic and vectorial layers as separate or aggregated, and defining the corresponding interaction matrices fully determines the model framework. We explore the following models, enlisted in order of presentation:

- a random graph as dispersal layer, with 3 species types and aggregated interactions, called Random Aggregated Network (RAN);
- a random geometric graph as dispersal layer, with 3 species types and aggregated interactions, called Spatial Aggregated Network (SAN);
- a random geometric graph as dispersal layer, with 3 species types, interactions kept separate across a 2-layer multiplex structure, called Spatial Multiplex Network (SMN);
- a random geometric graph model, with 20 species, interactions kept separate across a 2-layer multiplex structure according to ecological empirical interactions. This model is called Spatial Ecological Multiplex Network (SEMN).

We considered both smaller ($N = 1,000$ nodes) and larger networks ($N = 10,000$ nodes) with the same average degree. While the results obtained in both cases were robust to the network size change, the networks with $N = 10,000$ nodes displayed less finite-size effects. Therefore, in the following we present simulation and analytic results for networked ecosystems made of $N = 10,000$ nodes. The average degree of considered networks has been tuned in order to obtain connected dispersal layers, in which there is at least one path connecting each pair of nodes. This minimises statistical biases due to disconnectedness of a non-negligible fraction of populations.

8.2.1. Random aggregated network model

In the random aggregated network model (RAN) nodes have $s = 3$ possible identities, $S = s_1, s_2, s_3 =$ predator,prey,vector with species frequencies f_1, f_2, f_3 respectively. Herbivorous mammals are in general more abundant than carnivorous Damuth (1981) and for sake of simplicity we assume prey populations being double as frequent as predator populations, $f_2 = 2f_1$. Therefore, given that $f_1 + f_2 + f_3 = 1$, one obtains that $f_1 = (1 - f_3)/3$ and $f_2 = 2(1 - f_3)/3$, thus leaving the vector frequency $f_3 = f_v$ as a free parameter of the model. In this model the dispersal layer has the topology of an Erdös-Rényi with probability p_{ER}. Therefore, no space is included in the RAN model. In order to consider fully connected graphs in our simulations and to reduce the effects of degree heterogeneity we fixed a $p_E R$ giving rise to networks with average degree $\langle k_E R \rangle = p_E R \cdot (N - 1) \approx 28.27$. The RAN model filters interactions among predator, prey and vector populations from the dispersal layer according to the interaction matrix A defined as:

$$A = T \oplus V = \begin{pmatrix} 0 & 0 & 0 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \end{pmatrix} \oplus \begin{pmatrix} 0 & 0 & 1 \\ 0 & 0 & 1 \\ 1 & 1 & 0 \end{pmatrix} = \begin{pmatrix} 0 & 0 & 1 \\ 1 & 0 & 1 \\ 1 & 1 & 0 \end{pmatrix}. \tag{8.1}$$

For instance, $t_{21} = 1$ means that $s_2 =$ prey populations are eaten by $s_1 =$ predator populations. Notice that allowed interaction in T are directed (from the eater to the eaten, as usual in food-webs Bueno *et al.* 2003, Pilosof *et al.* 2015c, Ramos 2007) while they are undirected in V, since they represent ecological exchanges of infected fluids between the host and the vector species Rocha *et al.* 2013). The above filtering creates the aggregated single layer of the model, where trophic and contaminative interactions are combined and where parasite diffusion occurs.

8.2.2. Spatial aggregated network model

In the spatial aggregated network model (SAN) the dispersal layer is a random geometric graph (RGG). Therefore, populations are embedded in a space. Nodes are scattered uniformly at random within the 2D space $\Omega = [0, 1]^2$ with periodic boundary conditions, i.e. a toroidal space. As known from previous works (Sattenspiel 2009), the average degree of an RGG is $\langle k_{RGG} \rangle = \pi N \rho^2$. For the sake of comparisons with the RAN model, we chose $\rho = 0.03$, thus having $\langle k_{RGG} \rangle = \langle k_{ER} \rangle = 28.27$. The interaction

matrix A filtering the only aggregated network layer is the same as in the RAN model. Also species types are distributed as in the RAN model.

8.2.3. Spatial multiplex network model

In the spatial multiplex network model (SMN) the dispersal layer is a random geometric graph (RGG) with nodes spatially embedded and species types distributed as in the SAN model. However, we keep trophic and contaminative interactions as distinct on two separate layers. These structured interactions give rise to a multiplex network (Boccaletti *et al.* 2014, Kéfi *et al.* 2015, Kivelä *et al.* 2014), in particular an edge-coloured node-aligned graph where populations are replicated across both layers and no explicit inter-layer edges are considered (De Domenico *et al.* 2013). The interaction matrices filtering the trophic and the vectorial layer are respectively T and V, as defined above in Equation 8.1. A multiplex network visualisation of the SMN model is provided in Figure 8.1.

8.2.4. Spatial ecological multiplex network model

In our last model, the spatial ecological multiplex network (SEMN), the dispersal layer is a random geometric graph (RGG), as in the SAN model. Also, trophic and contaminative interactions are kept separate analogously to the SMN model. In SEMN we used empirical ecological data within the model (Rocha *et al.* 2013). Specifically, we use data from an epidemiological study of *T. cruzi* infection in wild hosts in Southeast Brazil (Rocha *et al.* 2013) to estimate the trophic and vectorial interaction matrices T_{eco} and V_{eco} (see Supplementary Information), considering a total of 20 species. For the trophic interaction matrix T_{eco}, we build a qualitative potential food-web based on the animals diets (Amboni 2007, Bueno *et al.* 2003, Carvalho Neto and Santos 2012, Cavalcanti 2010, Ramos 2007, Reis *et al.* 2011). As there was no species-level classification of the biological vectors present in the area, we considered the vectors as one single species type. We use species prevalence to estimate contaminative interactions in V_{eco} (Rocha *et al.* 2013). We assume that positive parasitological diagnostics for *T. cruzi* could be used as a proxy for vectorial transmission, since only individuals with positive parasitaemia are able to transmit the parasite (Jansen *et al.* 2015). The vectorial layer was constructed based on the assumption that species with positive prevalence in hemocultive transmit the parasite to vectors and that species with positive prevalence in serology can be infected from vectors. The SEMN model has a total of 20 species types: $a = 7$ predators, $b = 12$

prey and 1 vector species. As in the previous models, we assumed that prey populations have double the frequency of predator populations (see RAN model). We considered all the predator and prey species populations having identical frequencies f_{pred} and f_{prey} respectively, such that:

$$af_{pred} + bf_{prey} + f_v = 1 \rightarrow f_{prey} = 2\frac{1 - f_v}{a + 2b} = 2f_{pred}. \tag{8.2}$$

Therefore, by tuning f_v we change also the frequency of predator and prey populations. The SEMN model is the most realistic one of this study since it takes into account spatial embedding, multiplex structure and empirical ecological data.

8.2.5. Parasite transmission dynamics

To simulate the parasite transmission dynamics a node, i.e. a population of a given species type, is endowed with an infected or a susceptible state. We start the simulation by infecting a fraction $\phi_0 = 0.28\%$ of all populations. In the RAN model we infect one node at random and let the infection spread along a random walk on the dispersal layer. We start measuring the infection dynamics after $N\phi_0$ nodes are infected. Similarly, in the other three spatial models, we infect all the nodes in a random circle of radius $r_0 = 0.03$, that is, $\pi N r_0^2 \approx 28.2$ populations become infected at the beginning, on average. Subsequently, the parasite spreading evolves in SMN and SEMN models as follows:

1. A random node i is chosen together with one of its neighbours j on the dispersal layer.
2. The vectorial layer is chosen to be considered for the parasite transmission with probability p_v, which is a measure of the vectorial layer importance. Step 3 is then performed when the vectorial layer is chosen. Otherwise, step 4 takes place.
3. If node i is infected and the edge (i, j) exists in the vectorial layer, node j becomes infected as well (vectorial layer parasite transmission).
4. If node i is infected and the edge (i, j) exists in the trophic layer, node j becomes infected as well (trophic layer parasite transmission).
5. Steps 1-4 are repeated $N = 10^4$ times per each time step, i.e. an average of 1 update per node per time step, until T_{max} time steps are reached.

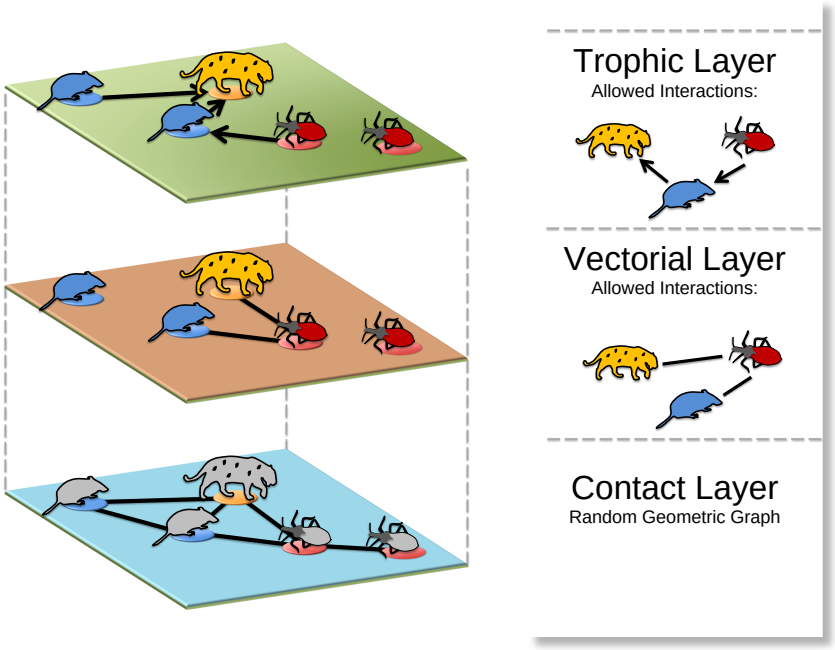

Figure 8.1. Visual representation of our model over the three layers: a trophic layer, a vectorial layer, and their underlying contact layer. Nodes are relative to the three-species example and they are drawn according to their species types, e.g. “predator", “prey" and “vector". Trophic and vectorial layers allow only for specific interactions to be present within the system, according to the species types involved in them. For instance, the allowed interactions in the three-species model are reported on the right. The parasite can spread on both such layers. When a node gets infected in one layer it gets infected on all the others as well. While the dispersal layer induces the other two, it is only the trophic and the vectorial layers that constitute our ecological multiplex networks.

For RAN and SAN models parasite transmission occurs only on the aggregate layer without considering steps 2, 3 and 4. This is equivalent in treating contaminative and trophic interactions in an aggregate, unweighted way. Each population can be randomly chosen at each time step and at the end of the transmission process every node is chosen once, on average. This parasite transmission model is equivalent to an SI model with contact rate $\beta = 1$, where β is the probability for an individual to become infected when exposed to the disease (Sattenspiel 2009). For the sake of simplicity, we assume $\beta = 1$ in both the trophic and vectorial layers and across all the species. Our assumption leads to the disease firstly spreading across the geodesic paths of the multiplex topology (Jeub *et al.* 2015, Sattenspiel 2009) so that our infection process depends solely on the multiplex network structure.

8.2.6. Model parameter values

Let us summarise the main parameters of our models and relative values. In this study we consider networks of $N = 10,000$ populations (nodes) and average degree $\langle k \rangle = 28.27$ for the dispersal layer ($p_{ER} = \langle k \rangle / (N-1)$ for random graphs, $\rho = 0.03$ for RGGs). We chose these parameter values in order to consider fully connected multiplex networks. Let us underline that we consider a multiplex connected component as the set of all nodes that can be reached from each other by considering all edge types of a node De Domenico *et al.* 2014. Given that we have directed edges in the trophic layer, we have to consider the notion of *strongly connected component* on the multiplex topology, i.e. a set of nodes that can be reached from each other considering oriented paths along directed edges of any colour.

The maximum number of time steps $T_{max} = 10^4$ has been numerically tuned in order to let the system reach equilibrium. Each time step considers $N = 10^4$ updates for the parasite spreading dynamics, i.e. an average of 1 update per node per time step. The frequency of vector populations f_v is a free parameter of the model, together with the vectorial layer importance p_v in the SMN and SEMN models.

8.2.7. Immunisation

In order to investigate the role played by predators and prey populations in spreading the parasite we focus on multiplex models (SMN and SEMN models). Using immunisation simulations we study the dynamics of parasite spreading when the same number of either

predator or prey populations have been immunised. An immune node is not susceptible to the parasite. The number of immune nodes is determined per species by specifying the probability of immunisation π_k for each species $k \in S$. To perform the immunisation, populations of species s_k are randomly chosen with probability π_k and are set to be immune.

We consider two immunisation scenarios to investigate the relative role that predator or prey populations have in spreading the parasite. In the first scenario only prey populations are immunised while in the second scenario only predator populations are immunised. For simplicity, the π_k values for all prey and predator populations are set uniformly, however they are chosen in order to immunise the same total number of predators and the same total number of prey. From an ecological point of view, the immunisation simulations answer the following question: given the fictional possibility of vaccinating a limited number $\phi \ll N$ of populations against the parasite, is it more efficient to immunise predator populations or prey ones in order to hinder the parasite spreading?

8.2.8. Multiplex cartography

A multiplex cartography visually represents the role played by a given node across different layers according to its topological features (Battiston *et al.* 2014, Boccaletti *et al.* 2014, Guimera and Amaral 2005). We build on previous literature (Battiston *et al.* 2014, De Domenico *et al.* 2013) by considering a cartography based on the following two measures: the multidegree or overlapping degree o_i and the participation coefficient P_i of node i. As in (Battiston *et al.* 2014, De Domenico *et al.* 2013), the multidegree o_i is defined as the sum of all the degrees of node i across the M multiplex layers:

$$o_i = \sum_{\alpha} k_i^{(\alpha)}. \tag{8.3}$$

where $k_i^{(\alpha)}$ is the degree of node i in the layer $\alpha \in \{1, ..., M\}$. The overlapping degree o_i represents a proxy of the overall local centrality that a node has within the multiplex network. Differently from Battiston *et al.* 2014, we consider o_i rather than its standardised counterpart $z_i = \frac{(o_i - \langle o_i \rangle)}{\sigma(o_i)}$ because our multiplex networks do not display Gaussian-like multidegree distributions. We consider hubs in our multiplex networks as those nodes being in the 95th percentile of the multidegree distribution.

The distribution of the connections over the different layers can be expressed via the

participation coefficient P_i of node i:

$$P_i = \frac{M}{M-1}\left[1 - \sum_{\alpha=1}^{M}\left(\frac{k_i^{(\alpha)}}{o_i}\right)^2\right]. \tag{8.4}$$

P_i ranges between 0 (for nodes that concentrate all their connections in one level only) and 1 (for nodes that distribute connections over all the M layers uniformly). In the following, we visualise our multiplex network cartography by clustering together individual points (each one referring to a given node) into 2D bins, thus obtaining a 2D histogram resembling a heat-map. The binned quantities are the overlapping degree on the y-axis and the participation coefficient on the x-axis.

8.2.9. Infection measures

On a macroscopic scale, we investigate parasite spreading by computing the *global infection time*, defined as the time step at which the largest (in node size) weakly connected component of the multiplex network is infected. Alternatively, the infection time indicates the time step t_{inf} at which the disease infects the most nodes within the network. If $R(t) = N_{inf}(t)/N$ if the ratio of infected populations/nodes at time t, then $\text{Max}_t(R(t)) = R(t_{inf})$.

Infection times represent a global, macroscopic statistics of the parasite spreading. To analyse the evolution of transmission in more detail we use the *parasite ratio increase* $\Delta R(t) := R(t+1) - R(t)$, i.e. the increase of the ratio of infected populations in one time step. The $\Delta R(t)$ is a measure for the rate at which the parasite is spreading within the multiplex network.

In order to capture the spatial features of our SMN and SEMN models we measure also $\langle\lambda\rangle$ defined as the average distance of the infected nodes from the centre of the embedding square $\Omega := [0,1]^2$ (where the infection originates). Given our assumption of uniform spreading of species populations within Ω, it is relatively straightforward to compute an upper bound $\langle\lambda\rangle^*$ for $\langle\lambda\rangle$ as:

$$\langle\lambda\rangle^* = \iint_0^1 \sqrt{(x-\frac{1}{2})^2 + (y-\frac{1}{2})^2}\,dxdy \approx 0.3826. \tag{8.5}$$

$\langle\lambda\rangle^*$ represents the maximum average distance of infected populations from the centre of the embedding space (also the origin of the infection).

8.3. Results

Our results focus on: (i) highlighting the role of spatial correlations on the parasite spreading dynamics, (ii) assessing the differences between aggregated and multiplex models, (iii) highlighting the topological features of our models through cartography (Battiston *et al.* 2014) while relating them to parasite spreading at different values for the vector frequency f_v and importance of vectorial transmission p_v, and (iv) quantifying how different species promote or not parasite spreading by means of immunisation simulations. We first report the results concerning the aggregate models (RAN and SAN), then the three-species reference one (SMN) and the spatial ecological multiplex network (SEMN) as last.

8.3.1. Aggregate network models: the role of space

Comparing the results of the aggregate models RAN and SAN provides quantitative information about the role played by space. In Figure 8.2 (a) we compare the ratio of infected nodes over time for the RAN and SAN models by means of simulations and analytical results. Assuming a mean-field approximation, where every population can be potentially infected by any other one in the system, it is possible to write down the following equations for the infection dynamics:

$$\dot{n}_1 = \left(\frac{f_1 N - n_1}{N}\right)\left(\frac{n_2}{N} + \frac{n_3}{N}\right) \tag{8.6}$$

$$\dot{n}_2 = \left(\frac{f_2 N - n_2}{N}\right)\frac{n_3}{N} \tag{8.7}$$

$$\dot{n}_3 = \left(\frac{f_3 N - n_3}{N}\right)\left(\frac{n_1}{N} + \frac{n_2}{N}\right) \tag{8.8}$$

where $n_k = n_k(t)$ is the number of infected nodes of species type $k \in 1, 2, 3$ at time t.

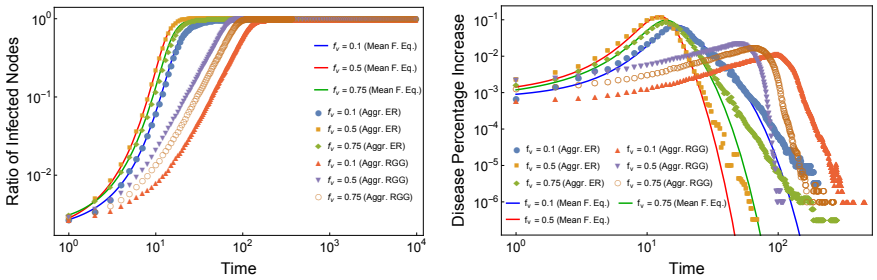

Figure 8.2. (a): Ratio of infected nodes over time for the random aggregate network (RAN) and the spatial aggregate network (SAN) models, at different frequencies f_v of vector populations in the system. (b): Parasite ratio increase of infected nodes over time for the random aggregate network (RAN) and the spatial aggregate network (SAN) models, at different frequencies f_v of vector populations in the system.

Each equation considers how a given susceptible species population can be potentially infected in the model through its edges with other species population types. For instance, let us consider the infection dynamics of predator populations ($k = 1$). At time t, the probability of finding a susceptible predator population in the system is $(Nf_1 - n_1)/N$. However, in all models which consider 3 species, a susceptible predator population can receive the parasite infection either from feeding on infected prey populations (the probability of sampling one is equal to n_2/N) or from being contaminated by an infected vector population (the probability of sampling one is equal to n_3/N). Analogous reasoning leads to the Equations 8.7 and 8.8. Notice that having directed edges leads to prey getting infected only through infected vectors in Equation 8.7.

Even though the mean field approximation does not consider the networked structure of the underlying dispersal layer, Figure 8.2 (a) shows that analytical results from the mean field equations reasonably approximate simulation results on ER random graph topologies (in RAN) at different vector frequencies f_v. Theory and simulations agree in indicating that the infection spreading dynamics reaches its maximum value around 20 time steps in the RAN model. Increasing the vector frequency does not always lead to the infection dynamics reaching its maximum value in less time steps. In fact, when we have $f_v = 0.1$ the ratio of infected nodes reaches its maximum value later than in the $f_v = 0.5$ case, i.e. the global infection time decreases. However, further increasing vector frequency from $f_v = 0.5$ to $f_v = 0.75$ leads to an increase rather than to a reduction in the global infection time.

For completeness, we also show in Figure 8.2 (b) the relative parasite ratio increases indicating the rate of parasite diffusion over time. We notice that the RAN model always

displays a peak over time in the parasite ratio increases. This means that the parasite diffusion initially accelerates and it later slows down since susceptible populations become rarer in the system. Simulations and analytical results for the RAN model also agree in the appearing ordering of these peaks. Here, reaching earlier the maximum ratio of infected nodes means reaching earlier the peak in the parasite ratio increase. This is because we assume that populations of the same species type do not interact with each other (i.e. our networks are $k-$partite graphs). Since infection must always pass through a vector-host-vector path in order to infect other vectors, adding too many vector populations is detrimental for the global infection time.

In the SAN model, when the dispersal layer changes from an ER random graph to an RGG, the infection reaches its maximum spread at a much later stage (around 100 time steps). We observe that inserting spatial correlations makes the mean field approximation unreliable in describing the simulation results. This is because the spatial embedding makes nodes different from each other according to their location in space.

Parasite ratio increases reveal that the RAN model displays also a faster infection spreading dynamics when compared to its spatial counterpart, the SAN model. Interestingly, both the aggregated models display a peak in the evolution of the parasite ratio increases. Overall, the addition of space increases the global infection time and it reduces the parasite spreading rate.

8.3.2. Spatial multiplex network model: the role of trophic and contaminative interactions

The 3-species reference model (SMN) consists of the simplest epidemiological scenario for the multiplex transmission. It is based on the simplest trophic chain in which vectors are consumed by prey populations and prey are consumed by predator populations. In the vectorial layer the vectors contaminate both prey and predator populations, see also Figure 8.1.

In Figures 8.3 (a)-(d), the multiplex cartographies highlight the degree centrality and participation coefficient of each species type at different vector frequencies f_v. Individual nodes are binned according to colour-coded two dimensional tiles so that the resulting plot resembles a heatmap.

When vector populations are rare in the system ($f_v = 0.01$, Figure 8.3 (a)), predators'

participation coefficient is low. This means that predators interactions are concentrated mostly in the trophic layer and predator populations interact mostly with prey populations. Prey populations show a broader range of participation and this indicates that preys interact with predators and vectors on both layers. Vector populations have the highest participation coefficient and are hubs in the multiplex, since their links are uniformly distributed between both layers.

When f_v goes from 0.1, Figure 8.3 (b), to 0.25, Figure 8.3(c), vector populations show a broader range of participation coefficients indicating that their connections are distributed on both layers. Similar behaviour is reported when $f_v = 0.5$ (plot not presented). At vector frequency $f_v = 0.75$, vector populations are the most frequent in the system and each species type occupies a different region in the cartography (Figure 8.3 (d)). Thus, we have: (i) prey populations linked to vectors on both trophic and vectorial layers becoming almost truly multiplex hubs (participation coefficient value close to one and high multidegree), (ii) predator populations with a broad range of participation coefficients, (iii) vector populations with a broader range of participations coefficients but loosely connected to other populations because vectors do not interact with each other.

The multiplex structure in the SMN model allows for the infection to spread either on the vectorial layer (with probability p_v) or on the trophic layer (with probability $1 - p_v$) at each time step (see section 2.5). This interplay leads to the global infection time potentially being a function of the vectorial layer importance p_v. As reported in Figure 8.4 (b), when vector frequency is $f_v = 0.1$, the global infection time has its minimum for $0.4 < p_v < 0.8$. Hence, when the parasite spreads across both trophic and contaminative edges with roughly the same probability, its spreading on the whole multiplex networked ecosystem requires less time. Since the trophic layer in the SMN model is not fully connected and thus the infection cannot reach the entire network, we do not show infection times for $p_v = 0$. On the other hand, we do not consider the $p_v = 1$ case in order to always consider the food-web while focusing on the multiplex structure.

Increasing the frequency of vector populations does not accelerate parasite spreading in the multiplex network and the faster spreading occurs when $f_v = 0.5$ (8.4). The infection time decreases monotonically with the increase of vectorial layer importance p_v when $f_v = 0.25, 0.5$ or 0.75, but this pattern was not observed when $f_v = 0.1$. This is related to the topology of the allowed interactions in the SMN vectorial layer. In SMN the vectorial layer is undirected and vector populations are connected to both predator and prey populations. The trophic layer has directed interactions and parasite transmission requires at least two steps to spread from vector to predator populations. These topolo-

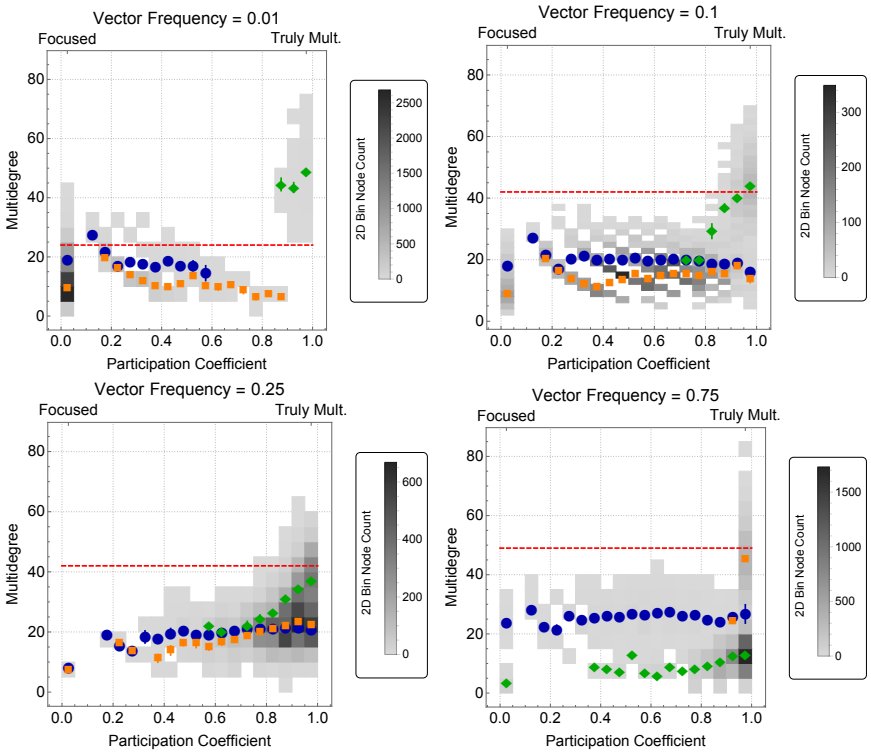

Figure 8.3. Cartographies as 2D histograms for the SMN model for vector frequency $f_v = 0.01$ (a), $f_v = 0.1$ (b), $f_v = 0.25$ (c), and $f_v = 0.75$ (d). The 10000 multiplex nodes are binned in 2D bins, according to their coordinates in the cartography. Bins are colour-coded according to the number of points falling within them: more coloured tiles have the most nodes in them. Coloured dots identify individual species: predators (blue), prey (orange) and vectors (green). Nodes falling above the horizontal red line have degrees above the 95th percentile in the multidegree distribution and they are therefore considered hubs. Error bars represent standard error of the mean.

gical features of the SMN model enables a faster parasite transmission on the vectorial layer rather than on the trophic layer. However, the frequency of different species types also influences parasite transmission in the model. Increasing the vector frequency from $f_v = 0.1$ to 0.25 or even up to 0.5 leads to an overall decrease of the infection times, depending on p_v. This trend changes when vectors are the most frequent species type in the system ($f_v = 0.75$). When the majority of nodes are vector populations the speed of parasite spreading increases in relation to $f_v = 0.5$ because vectors are not directly connected in neather of the layers. Therefore, a smaller number of predator and prey populations constraints parasite transmission to vectors. In Figure 8.4 (b) we also show the infection time for the SAN model represented as dotted lines for the different vector

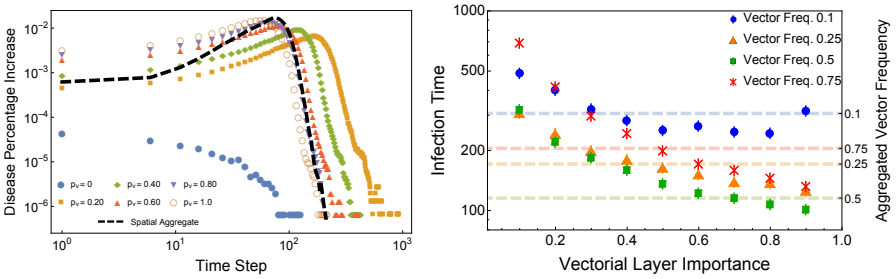

Figure 8.4. (a): global infection rate over time for $f_v = 0.75$ expressing the diffusion speed of the disease over time for SMN model. A qualitatively similar behaviour was observed also for other vector frequencies. (b): global infection time versus vectorial layer importance p_v for different vector frequencies in the SMN model. Dotted lines represent infection time in the SAN model for different vector frequencies. Results in both plots are averages of 100 repetitions.

frequencies. We remember that in the SAN model there is only one aggregated layer where the infection spreads, thus all edges have the same importance. Comparing the infection time of the SAN and SMN models highlights the effect of multiplex structure for parasite spreading dynamics. Independently on the vector frequency, tuning the parasite spreading across trophic and contaminative interactions changes the infection time with respect to the aggregate case.

The speed of parasite spreading across the multiplex structure also reveals interesting patterns. As reported in Figure 8.4 (a) for $f_v = 0.75$, when $p_v > 0$ the parasite transmission initially accelerates within the system ($t < 100$). This behaviour is somehow similar to the one already observed in the SAN model (see Figure 8.2 and the black line in Figure 8.4 (a)). On the other hand, when the infection spreads only on the trophic layer ($p_v = 0$) a qualitatively different behaviour is observed, with no acceleration phase. This is because of the trophic layer topology (see T in the Methods section): the parasite can spread only from vectors to prey and from prey to predator populations. As the infection spreads, it becomes increasingly difficult to infect more populations over time. Vector populations which are susceptible at the beginning will never be infected. The aggregated model (SAN) does not capture this trend since it includes trophic and contaminative interactions mixed together. We observed a consistent behaviour for other vector frequencies $f_v \neq 0.75$. The only difference was in the order of the peaks of parasite spreading rate: the higher p_v the sooner the peak is reached when $f_v > 0.2$. We conjecture that this is because, in environments with many vector populations, the parasite spreads at a faster rate with respect to the trophic layer, so that increasing p_V accelerates the parasite spreading.

We also investigated the infection dynamics for very small values of vector frequencies (Figure 8.5). Simulations indicate that the SMN model displays a critical threshold in the emergence of global epidemics around $f_v = 0.02$. Very small variations in the abundance of vector populations within the simulated ecosystem leads to dramatic changes in the ratio of infected populations after a suitably long relaxation time of $10,000$ time steps (Figure 8.5). We conjecture that this is because vector populations are fundamental in infecting prey populations. Considering the filtering matrices T and V, prey populations can be infected only by interacting with infected vector populations. When vectors are very rare in the system, prey populations (that are quite frequent in the system) get infected at a much slower rate. This bottle-neck translates into a phase transition in the infection rate. Our simulations show that the vectorial layer importance p_v slightly shifts the critical threshold of the phase transition, which occurs across all the different values of p_v (for $p_v = 0$ or $p_v = 1$ plots not reported for clarity). This phase transition marks the beginning of a distinct "phase" of the model ($f_v > 0.02$), for which the parasite percolates throughout the whole system at a faster rate, even when vector frequencies are low. Notice that when $0.02 < f_v < 0.1$, vector populations are multiplex hubs (see (a) and (b) in Figure 8.3), therefore they promote the parasite spreading on both the SMN layers.

As indicated by the grey area in Figure 8.5, the mean distance of infected nodes $\langle\lambda\rangle$ after 10,000 time steps also undergoes a phase transition around $f_v = 0.02$. However, $\langle\lambda\rangle$ converges to its upper bound $\langle\lambda\rangle^*$ at a faster rate compared to the ratio of infected population. Let us consider the case $f_v = 0.04$. The relative ratio of infected nodes is $\approx 70\%$ (see dotted lines in Figure 8.5), variations in the vectorial layer importance provide no evident fluctuations. However, always at $f_v = 0.04$, the mean distance of infected populations from the centre of infection is not 70% of the maximum value, but rather $\langle\lambda\rangle(f_v = 0.04) \approx \langle\lambda\rangle^* \approx 0.384$ (see the grey shape and the dashed black line in Figure 8.5). Therefore, in the same time steps, the infection spreads only across 70% of populations but it covers almost all the distances from the infection origin, in the embedding space. We interpret this as the parasite spreading at a faster rate uniformly over the whole embedding space rather than uniformly across all the considered populations. These different spatial and number diffusion rates are relative to our selected SI dynamics. When the infection probability $\beta = 1$ (as in our case), the infection spreads firstly through geodesics in the network (Jeub *et al.* 2015, Sattenspiel 2009). Having the parasite spreading on geodesics through our spatial multiplex network is compatible with our finding from Figure 8.4: the mean distance of infected nodes from the infection centre saturates faster than compared to the ratio of infected nodes.

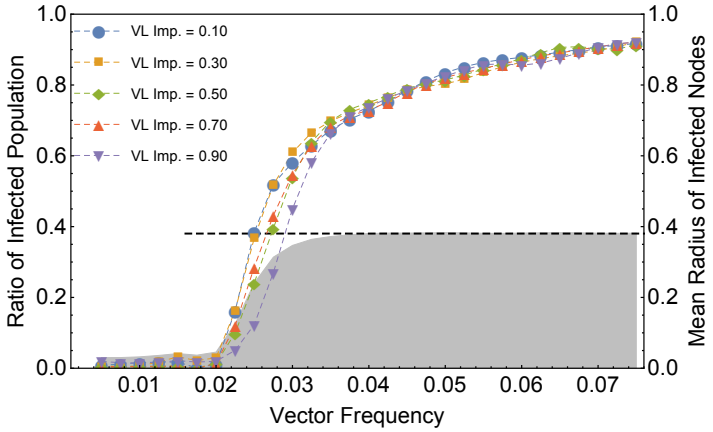

Figure 8.5. Ratio of infected populations after 10^4 steps, sampled at different values of p_v, against vector frequency f_v in the SMN model. When vectors are rare in the system, the system displays a phase transition in the rate of infection. The critical threshold is localised around $f_v \approx 0.02$, for all the values of p_v. The grey shape represents the mean distance of infected population from the origin of the parasite spreading and it is averaged over different p_v values. When $f_v > 0.02$ the infection radius saturates faster than the global percentage of infected populations. All curves are averages of 100 repetitions.

Immunisation scenarios in the SMN model

In order to relate the topological features of each species population in the multiplex to their roles in spreading the parasite across the networked ecosystem, we analyse immunisation scenarios. In the immunisation scenarios a fraction of populations of a given species type (e.g. predators) is immunised against the parasite (see Section 2.7). As reported in the previous section, we found different species having different degree and participation patterns within the SMN model (see the cartographies in Figure 8.3) at high vector frequencies ($f_V = 0.75$). In fact, when $f_v = 0.75$ prey, predator and vector populations occupy different regions in the multiplex cartography. In Figure 8.5 we report the global infection times when the same total number $\phi = 417$ of predator or prey populations is immunised. The chosen ϕ corresponds to immunising half the predator populations in the system. Our results show that immunising prey over predators leads to a greater increase in the system infection times for all values of vectorial layer importance p_v. The better performance of immunising prey over predators is also reflected in the increase of parasite ratio $\Delta R(t)$ (Figure 8.5): immunising preys not only delays a pandemic but also significantly slows down the parasite spreading in the initial accelerating phase (i.e., it lowers the $\Delta R(t)$ when $t < 140$). Even though slowing down the parasite transmission and reaching a pandemic at a later stage might sound equivalent,

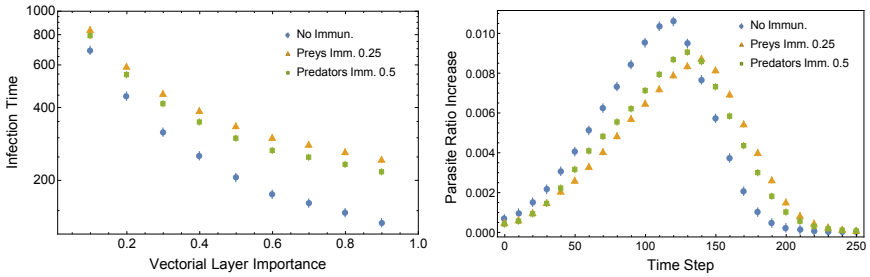

Figure 8.6. (a): global infection time versus vectorial layer importance p_v for different immunisation experiments with $f_v = 0.75$ in the SMN model. No immunisation means that no immunised populations are present in the system while two other dot types represent scenarios in which only prey or predators are immunised, respectively. For immunisation scenarios the same number of populations has been immunised. (b): parasite ratio increase of infected nodes over time for the SMN model for different immunisation scenarios with $f_v = 0.75$. Error bars are computed over 10 independent experiments. Immunising prey is the best choice in terms of both reducing the global infection time and slowing the infection spread over time.

the parasite ratio increase reveals that in the predator immunisation scenario there is a higher diffusion speed in the decelerating infection phase, $t > 140$ (Figure 8.5). Because of this behaviour, we report on both patterns.

This difference could be attributed to the different topology of prey and predator populations in the trophic layer, i.e., the parasite spreads from vector to prey and then from prey to predator populations, so that prey have a higher betweenness in the filtered trophic interactions. Further numerical experiments indicate that this is not the case. Immunisation experiments performed with the same ϕ but with vector frequency $f_v = 0.25$ show that immunising either predators over prey gives statistically equivalent results in terms of both the parasite spreading times and the propagation rates. Therefore, at $f_v = 0.25$ immunising one species type over the other does not change parasite spreading. However, both the $f_v = 0.25$ and the $f_v = 0.75$ instances are relative to the same interaction matrices T and V and to the same number of immunised prey ϕ. Therefore, the relative difference in immunisation performances has to be attributed to the role played by each species within the global network topology. Immunising prey is different from immunising predator populations only when they have different topological patterns within the multiplex network, i.e. they occupy different areas of the multiplex cartography. This evidence points to the meaningfulness of the concept of network cartography for the parasite spreading dynamics: at $f_v = 0.75$ prey populations become truly multiplex hub nodes and assume an important role for parasite spreading, as demonstrated by our immunisation experiments.

8.3.3. Spatial ecological multiplex network model: the role of biodiversity

The SEMN model considers empirical interaction matrices T_{eco} and V_{eco} compared to SMN. Notice that the in V_{eco} the vector contaminates only 7 of the 20 species in the ecosystem, while in SMN it is allowed to contaminate all the other 2 species. In this section we relate the empirical ecological structure to the results for SEMN. The cartographies reported in Figure 8.7 (a-d) represent snapshots of the spatial ecological multiplex network with increasing frequencies of vectors. In all the cartographies there is one predator species that displays a wide variation in the participation coefficient, while the participation coefficients of the other predator species populations is zero. This is because, differently from SMN, the SEMN model has one predator species that can be contaminated by vectorial transmission (see V_{eco} in the Supporting Information), while the other predator species populations have links only on the trophic layer. When vector populations are rare ($f_v = 0.01$), predator and prey populations occupy the same regions of the cartography, as in the SMN model, see Figure 8.7 (a) and (b). A similar case occurs with prey populations, since only half of them have connections on the vectorial layer (see V_{eco} in the Supporting Information). Analogously to the SMN model, increasing the frequency of vectors leads to scenarios where some predator and prey populations display a wide range of participation coefficients. However, at both $f_v = 0.1$ and $f_v = 0.25$ predator populations have a higher multidegree than prey populations. This occurs because predators are at a higher trophic level than prey and thus receive more connections in the trophic layer. Therefore, for values as low as $f_v = 0.1$ the species types show varied and distinct patterns in the cartography. At $f_v = 0.25$, prey populations show an increased participation in the multiplex network as a sign of increased connectivity in the vectorial layer (Figure 8.7 (c)). When vector populations are highly frequent in the system, $f_v = 0.75$, the cartography reveals some extreme patterns: prey species populations that interact with vectors on the vectorial layer display participation coefficient close to 1 while the other prey species show focused interactions (Figure 8.7 (d)). This same pattern was observed between predator species populations that interact with vectors and the predator populations that do not when $f_v = 0.75$ (Figure 8.7 (d)). This was not observed in the SMN model.

The SEMN model also displayed a phase transition in the emergence of a global epidemic, similarly to what happened for the SMN model. However, the different topology of trophic and vectorial layers brought to a slight increase in the critical vector frequency value, from $f_v = 0.02$ (SMN) to $f_v = 0.04$ (SEMN).

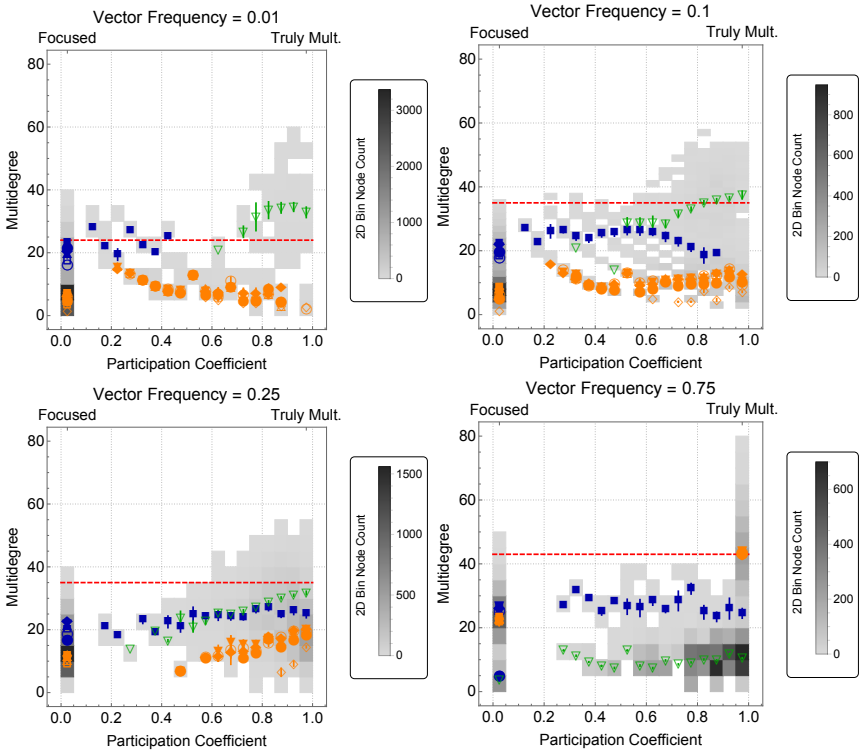

Figure 8.7. Cartographies as 2D histograms for the SEMN model for vector frequency $f_v = 0.01$ (a), $f_v = 0.1$ (b), $f_v = 0.25$ (c), and $f_v = 0.75$ (d). The 10000 multiplex nodes are binned in 2D bins, according to their coordinates in the cartography. Bins are colour-coded according to the number of points falling within them: more coloured tiles have the most nodes in them. Coloured dots identify individual species: predators (blue), prey (orange) and vectors (green). Nodes falling above the horizontal red line have degrees above the 95th percentile in the multidegree distribution and they are therefore considered hubs. Error bars represent standard error of the mean.

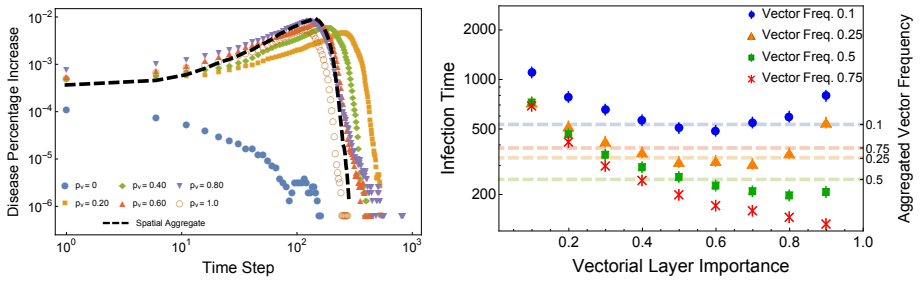

Figure 8.8. (a): global parasite ratio increase over time for $f_v = 0.75$ for SEMN model and different vectorial layer importance. A qualitatively similar behaviour was observed also for other vector frequencies. (b): global infection time versus vectorial layer importance p_v for different vector frequencies in the SEMN model. Dotted lines represent infection time in the SAN model applied to Canastra empirical data for different vector frequencies. Results in both plots are averages of 100 repetitions.

As reported in Figure 8.8 (b), the time required to infect almost all the populations in SEMN is minimised when there is a high frequency of vectors in the environment and a high importance of vectorial layer for parasite transmission. Infection times decrease monotonically when $f_v = 0.5$ and 0.75. However, at vector frequencies $f_v = 0.1$ and 0.25 parasite spreading is optimised when the vectorial layer importance p_v is around 0.6 (8.8 (b)), that is, when vectorial and trophic transmission mechanism have similar importance. Therefore, vectorial and trophic transmission mechanism have an additive effect for parasite spreading only when $f_v < 0.5$. Comparing the results against a spatial aggregate network model using the Canastra matrices (Canastra SAN model) reveals how the multiplex structure can change dramatically the infection time. For instance, when $f_v = 0.1$, the infection time of the Canastra SAN model is halved compared to the SEMN one for $p_V = 0.1$, see also the dashed lines in Figure 8.8 (b). The multiplex structure not always increases the speed of parasite spreading and the multiple dynamics that resulted from the interplay of vectorial layer importance and community composition justifies the value of investigating different transmission routes via multiplexity. Despite the higher connectivity of the trophic layer in the SEMN model, parasite ratio increases behave similarly to the SMN model (8.8 (a)). The parasite spreading propagates much slower on the trophic layer alone than on the full multiplex structure, see the $p_V = 0$ trajectory. Again, considering also contaminative interactions provides qualitatively different dynamics of parasite ratio increases than considering trophic interactions only (8.8 (a)). However, the dynamics of parasite ratio increases in time for the SEMN model are qualitatively similar to the SAN model relative to $p_V > 0$. Increasing the vectorial layer importance accelerates the parasite spreading even though no monotonous relationship is evident from the plots. For $p_v > 0$ the slow-down phase following the increase peaks does not behave

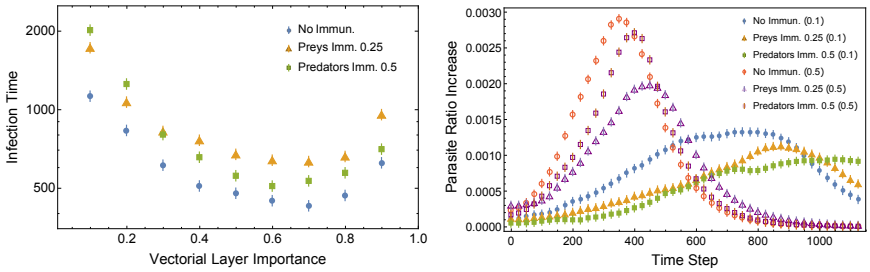

Figure 8.9. (a): global infection time versus vectorial layer importance p_v for different immunisation experiments with $f_v = 0.75$ in the SEMN model. The no immunisation scenario means that no immunised populations are present in the system while other dot types represent scenarios in which only prey or predators are immunised, respectively. For immunisation scenarios the same number of populations has been immunised. (b): parasite ratio increase of infected nodes over time for the SEMN model for different immunisation scenarios with $f_v = 0.75$. Error bars are computed over 10 independent experiments. Differently from the behaviour we observe in the SMN model, immunising prey is less effective than immunising predators in slowing down the disease spread for small p_v values. The opposite scenario happens when $p_v > 0.2$ where immunising prey is more effective than immunising predators, as shown in panel (b) comparing $p_v = 0.1$ and $p_v = 0.8$ immunising scenarios.

independently of p_v (8.8 (a)). Therefore, these peaks cannot be considered good proxies of the infection times in the SEMN model. When the spreading deceleration occurs in different time windows, it sums up differently to the peak times, thus establishing global infection times that are not straightforwardly related to the peak times. For instance, the peak for $p_v = 0.8$ is reached sooner for the $p_v = 0.6$ but the deceleration phase takes longer for $p_v = 0.8$ then for the $p_v = 0.6$ and $p_v = 0.8$ has a higher global infection time compared to $p_v = 0.6$.

Immunisation scenarios in the SEMN model

Unlike the SMN model, the SEMN model has predator and prey populations exhibiting different cartography patterns only at low vector frequencies. Therefore, we investigated immunisation scenarios at $f_v = 0.1$ and $f_v = 0.25$. The results for $f_v = 0.1$ are reported in Figure 8.9 and are analogous to the $f_v = 0.25$ case (plots not shown for brevity).

Both the SMN and the SEMN models are spatially embedded, but SEMN has a higher number of species with interaction patterns based on empirical data. In SEMN, immunising prey over predator populations does not always hamper more the parasite spreading, as it happened in the SMN model. From the cartography in Figure 8.7 (a) one would expect predator populations to play a pivotal role in spreading the parasite, given their

higher multidegree compared to prey populations, on average. However, in the same cartography 6 out of 12 prey species display a higher average participation coefficient compared to 6 out of 7 predator species (with participation coefficient equal to zero). Hence, from the cartography both predator and prey populations could play a central role in promoting the parasite spreading: predators are hubs while prey can spread the infection across both layers. In contrast to the SMN model, it is not possible to make predictions based on the cartography alone. Our immunisation simulations reveal the presence of two scenarios: when the parasite spreads mainly across the trophic layer ($p_v < 0.3$), then immunising the same number $\phi = 346$ of predator over prey populations significantly increases the infection times, (8.9 (a)), and slows down parasite diffusion (Figure 8.9 (b)). This finding relates to the SEMN cartography: predator populations have a high multigree because they are hubs in the trophic layer (here $f_v = 0.1$) and hence promote the parasite spreading through trophic interactions. However, when the vectorial layer importance p_v increases above 0.3, then immunising predator or prey populations does not make noticeable difference. When $p_v > 0.7$ and the parasite spreads mainly through contaminative interactions the most effective immunisation strategy becomes immunising prey populations, since vectors contaminate mostly prey populations in the SEMN model (8.9). Again, this is compatible with the patterns in the multiplex cartography: when p_v is higher, the multiplex structure becomes predominant and the species populations that have higher participation coefficients, such as preys, can promote the infection spread.

8.4. Discussion

It is only recently that network scientists started addressing the multiplex structure of real-world systems such as ecological and epidemiological systems (Boccaletti *et al.* 2014, De Domenico *et al.* 2013, Kivelä *et al.* 2014, Pilosof *et al.* 2015c). They used the multiplex framework for modelling different ecological and epidemiological systems. Multi-layer networks were used in ecological systems to approach different interaction types Fontaine *et al.* (2011), Melián *et al.* (2009) and levels of organisation Barter and Gross (2015b), Belgrano (2005), Scotti *et al.* (2013). More in particular, multiplex networks were used for the first time in Kéfi *et al.* (2015), in order to consider trophic and non-trophic interactions together in a Chilean ecosystem. In epidemiological systems multiplex networks were used to describe parasite spreading with Susceptible-Infected-Susceptible dynamics (Granell *et al.* 2013, Lima *et al.* 2015, Sahneh *et al.* 2013, Sanz *et al.* 2014, Saumell-Mendiola *et al.* 2012), susceptible-infected-recovered dynam-

ics (Buono *et al.* 2014b, Dickison *et al.* 2012, Marceau *et al.* 2011), and multiple types of interactions between random layers (Cozzo *et al.* 2013, Salehi *et al.* 2014, Zhao *et al.* 2014). The modelling of multi-host parasites that are transmitted through multiple mechanisms in the ecosystem can be improved by applying the multiplex framework. We used the multiplex approach to study both a simple predator-prey-vector system as a reference case, and an empirical data from host communities of *T. cruzi* in natural habitat (Canastra). Compared to their aggregate counterparts, both our multiplex network models displayed a richer phenomenology in terms of infection dynamics. Our three-species-system (SMN) as well as our empirical-based model (SEMN) showed that the epidemiological importance of vectors, hosts and parasites might be mapped on the multiplex cartography. Considering the node and link heterogeneity in a spatial context allowed for us to identify percolation thresholds for parasite spreading according to vector frequency. This is particularly interesting because the susceptible-infected dynamics in homogeneous hosts always leads to epidemic waves (in other words, when nodes are not spatially embedded there is no percolation threshold). In addition, we found that multiplex cartography had important implications in parasite spreading dynamics and that parasite transmission depends on: (i) the relative importance of the distinct transmission mechanisms, (ii) the role species play on the overall multiplex structure and (iii) the species relative frequencies in the system.

There is a strong debate in ecology on whether biodiversity reduces or not the risk of infection in host communities (Johnson *et al.* 2013, Keesing *et al.* 2006, Wood *et al.* 2014). In general, the effect of host diversity on parasite transmission depends on the ecological characteristics of hosts and on the mechanism of transmission (Wood *et al.* 2014). The spatial multiplex modelling framework that we propose in this study could be applied to address questions related to the role of multiple host community biodiversity on parasite transmission. In fact, we found that the spatial component has a significant impact on the speed of parasite spreading: spatial correlations slowed the speed of parasite spreading when compared to mean-field approximations. Therefore, considering the spatial structure of host communities in order to infer the importance of different host species for parasite transmission is a fundamental next step in future ecological disease studies (Craft and Caillaud 2011, Pilosof *et al.* 2015c). Percolation thresholds are spatially explicit tipping points that indicate the presence, in some regimes, of non-local correlations within a given system (Davis *et al.* 2008b). For instance, if a network is not strongly connected, then the parasite will not be transmitted to the whole system. In our model the connectivity of the multiplex network was crucially affected by the frequency of different species. For very small frequency of vectors f_v, our model showed a percolation threshold in both the SMN and the Canastra SEMN model. The presence of such phase

transition in the infection rate in an SI dynamics for a non-zero value of f_v is mainly related to (i) the spatial structure and to (ii) directed trophic interactions in the multiplex network. In the SMN model the parasite can percolate through the whole system only if $f_v > 0.02$, while in the Canastra SEMN model the critical vector frequency was found to be around $f_v = 0.04$. No phase transition for $f_v > 0$ was found in the RAN model, where nodes are not spatially embedded. We conjecture that the increase in the percolation threshold from the SMN to the SEMN models might be due to a higher diversity of potential hosts: with more species available there is an increased chance that vectors will interact with animals that do not become infected with the parasite. Interestingly, our theoretically computed frequencies agree with previous findings that even a small frequency of vectors in the ecosystem is sufficient to maintain Chagas disease in a human population (Reithinger *et al.* 2009).

Multiplex cartography (Battiston *et al.* 2014) considers both the relative frequency of each species and the interactions they have in both the trophic and the vectorial layers. Comparisons with aggregated networks revealed that considering trophic and vectorial transmission routes together can change dramatically the parasite spreading dynamics, depending on the relative frequency of vectors in the ecosystem. More in detail, the parasite spreading dynamics depends on the interplay between community species composition and the relative importance of the transmission mechanisms. In fact, when there is homogeneity in species composition (i.e. when the relative frequency of vectors $f_v \sim 0.5$), the lowest infection time is registered when the parasite spreads on both layers at the same time (i.e. for intermediate values of p_v) in both the SMN and the SEMN models. Therefore, our theoretical network models indicate that vectorial and trophic mechanisms of transmission can be additive in sustaining the spread of multi-host parasites such as *T. cruzi*, further agreeing with previous studies (Kribs-Zaleta 2006). In random multiplex networks (Saumell-Mendiola *et al.* 2012) the epidemic process also depends on the strength and nature of the coupling between the layers. In our case the vectorial layer importance p_v can be thought of as an implicit coupling between the layers, quantifying how much the vectorial layer is more important than the trophic layer in spreading the parasite. Previous investigation (Boccaletti *et al.* 2014, De Domenico *et al.* 2013, Kivelä *et al.* 2014, Saumell-Mendiola *et al.* 2012) showed that epidemic dynamics on a multiplex structure can be fundamentally different from the same dynamics on each multiplex layer considered as separate. Our results indicate that multiple mechanisms may speed up parasite spreading, even when the transmission layers are highly structured and differ in their topologies. The multi-layered transmission, which is observed in many parasites with complex life cycles and multiple mechanisms of infection, seems to be a very efficient strategy for spreading in communities of multiple hosts.

In vector-borne diseases, densities of hosts and vectors as well as the ratio of their densities, have strong implications for parasite transmission (Kribs-Zaleta 2010b, Pelosse and Kribs-Zaleta 2012, Ross and Thomson 1911, Velascohernandez 1994). The SMN model shows that higher vector frequencies make the vectorial layer faster in spreading the parasite from vectors to predator and prey populations. This relationship explains why infection times decrease monotonically with increased importance of the vectorial layer. On the other hand, if the vector frequency is low and the parasite spreads only on the trophic layer, it becomes increasingly difficult to infect more populations over time. In this situation the fastest global infection is achieved when both mechanisms of transmission are likewise selected for parasite spreading (there is a minimum in the infection time around $p_v = 0.6$). Moreover, in the Canastra SEMN model, we observe an analogous minimum even with higher vector frequencies. This suggests that global infection time is minimised when both mechanisms of transmission have similar importance in more complex ecological scenarios. Notice that considering both the transmission mechanisms but with one layer much more important than the other (e.g. $p_v = 0.1$) can lead to drastic increases in the infection time. The evolution and maintenance of mutually important multiple routes of transmission may be selected in parasites that infect a high number of host species.

Furthermore, using the multiplex cartography we predict that the relative importance of each mechanism for parasite spreading depends on the host community composition and relative frequency of species. We find that species structural patterns, encapsulated within the multiplex cartography, are a valuable measure to evaluate the importance of each species for parasite spreading. These findings are confirmed by the immunisation simulations. For instance, in the SMN model, a higher frequency of vectors ($f_v > 0.5$) increases prey populations connectivity and therefore their participation in the multiplex topology. We find different results when considering a more realistic ecological scenario. In the SEMN model, predator populations dominated the multiplex topology because of their higher connectivity and higher average multidegree. Immunising prey populations in the reference SMN model dramatically increases global infection time and the rate of disease spreading in the populations. However, in the SMN model immunising prey over predators results in different infection times only when these species occupy distinct regions in the multiplex cartography. This result points to the meaningfulness of the network cartography for understanding the parasite spreading dynamics. In fact, the multiplex cartography shows that prey participate more and have higher degree in the three-species multiplex network and thus could be a better target for immunisation. The immunisation simulations confirm this: immunising prey populations hampers the parasite spreading with respect to immunising the same number of predator populations. In

the Canastra SEMN model, predators are the species type that attain most of their connections in the multiplex network and thus have a higher importance in the cartography. This pattern suggests that the predators are acting as a sink for the parasite and can thus reduce the overall parasite transmission in the SEMN model. This is mainly due to the fact that predators are hubs in the trophic layer and hence show a higher multidegree in the cartography. When the parasite spreads mainly in the trophic layer ($p_v < 0.3$) the immunisation experiments indicate that immunising predators hampers the disease more compared to immunising prey. This is in agreement with empirical studies pointing out the potential importance of predators as parasite bio-accumulators (Jansen *et al.* 2015, Rocha *et al.* 2013). However, prey also display a slightly higher average participation in the Canastra cartography and hence could also play a central role in spreading the parasite. In fact, when the vectorial layer importance p_v is above 0.7, immunising prey populations becomes the most effective immunisation strategy. This is because vectors contaminate mostly prey in the Canastra multiplex network. Again, the roles played by each species in the multiplex cartography depended on the frequency of vectors and is related to their importance for parasite spreading.

It has to be underlined that the main aim of our multiplex model is not to provide a realistic mechanism for the spreading dynamics of *T. cruzi* in wild hosts. Instead, our approach aims at providing a comprehensive framework for investigating the spreading of multi-host parasites across different transmission mechanisms. Additional information should be taken into account if one would want to study the dynamics of *T. cruzi* in wild hosts and Chagas disease epidemiology. For instance, it is known that the stercorarian transmission results in a much higher probability of parasite transmission from host to vector than from vector to host (Rabinovich *et al.* 1990). More realistic models should include these differences via different contact rates on different layers. In addition, host physiological and ecological characteristics influence their probability to transmit *T. cruzi*. A higher proportion of insects in host diets increase host probability of infection (Rabinovich *et al.* 2011, Rocha *et al.* 2013, Roellig *et al.* 2009). Finally, host species that share ecological habitat with vector species are more likely to be exposed to the infection (Jansen *et al.* 2015). Many zoonoses, which are infections naturally transmitted between vertebrate animals and humans, may have multiple hosts and mechanisms of transmission. Examples of zoonoses transmitted to humans by arthropod vectors include Malaria, Leishmaniasis, Chagas disease, West Nile virus, plague and Lyme disease (Schmidt and Ostfeld 2001). The multiplex framework presented here could improve our understanding of the epidemiology and evolution of these parasites and help us elaborate more efficient control strategies for reducing disease incidence in humans. Last but not least, different or additional layers could be included within our multiplex frame-

work to make the model more realistic, such as direct transmission mechanism or the network of human interactions with its socio-ecological characteristics. Outside of the ecological perspective, our spatial multiplex network model could be applied to modelling networked systems made of spatially embedded interacting agents where instead parasite infection there is a given information spreading process.

Author Contributions

A.A., C.S.A., S.S. and M.S. conceived and designed the study, A.A. and A.G. wrote the code, A.A., A.G. and M.S. performed experiments, C.S.A. analysed the empirical data and M.S. analysed the simulation data, A.G. and M.S. conceived the analytical part, C.S.A., S.S. and M.S. discussed the results. All authors wrote the paper and gave final approval for publication.

Supplementary Material

The vectorial and trophic matrices for the Canastra SERN model have been uploaded as supplementary material.

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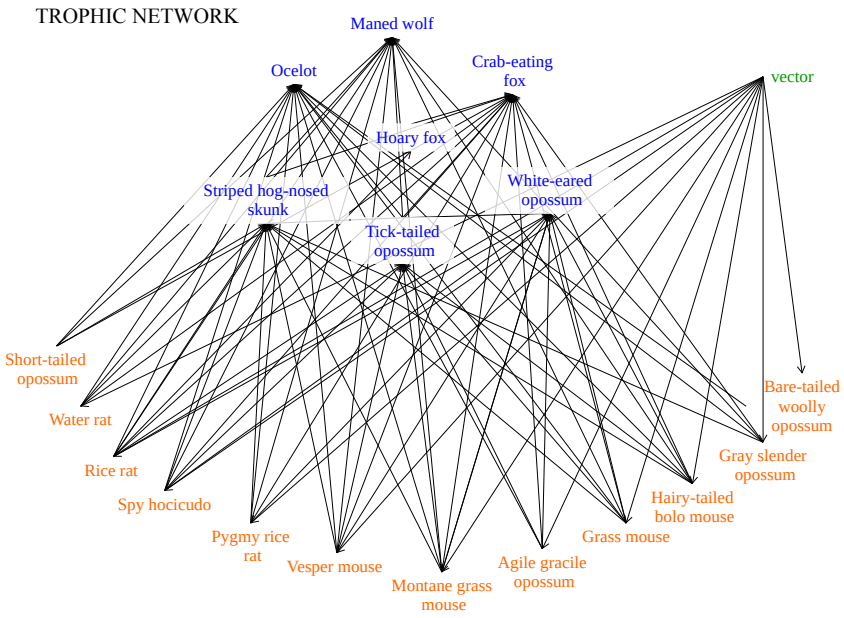
Supplementary Information



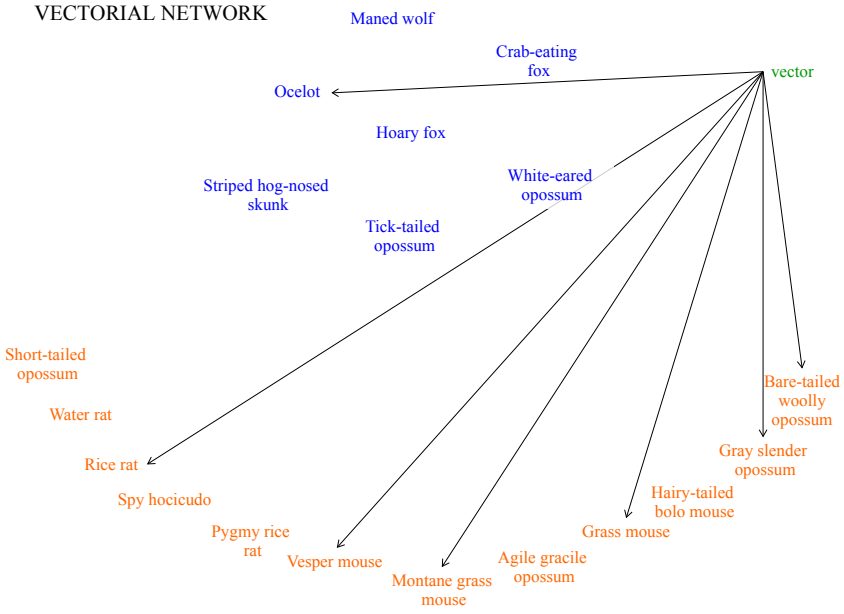


Table 8.1. TROPHIC MATRIX

Common name	**Species**	CHR	LEO	CER	LYC	CON	DID	LUT	CAL	NEC	MON	MAR	OXY	CES	NEC	AKM	AKO	GRA	OLI	CAL	TRI
Maned wolf	*Chrysocyon brachyurus* (CHR)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ocelot	*Leopardus pardalis* (LEO)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Crab-eating fox	*Cerdocyon thous* (CER)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hoary fox	*Lycalopex vetulus* (LYC)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Striped hog-nosed skunk	*Conepatus semistriatus* (CON)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
White-eared opossum	*Didelphis albiventris* (DID)	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thick-tailed opossum	*Lutreolina crassicaudata* (LUT)	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bare-tailed woolly opossum	*Caluromys philander* (CAL)	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Water rat	*Nectomys squamipes* (NEC)	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Short-tailed opossum	*Monodelphis sp* (MON)	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gray slender opossum	*Marmorsops incanus* (MAR)	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spy hocicudo	*Oxymycterus delator* (OXY)	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Rice rat	*Cerradomys subflavus* (CES)	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Hairy-tailed bolo mouse	*Necromys lasiurus* (NEC)	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Montane grass mouse	*Akodon montensis* (AKM)	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Grass mouse	*Akodon sp* (AKO)	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Agile gracile opossum	*Gracilinanus agilis* (GRA)	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pygmy rice rats	*Oligoryzomys spp* (OLI)	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Vesper mouse	*Calomys sp* (CAL)	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Kissing bug	*Triatominae* (TRI)	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0

Table 8.2. VECTORIAL MATRIX (only host-vector interactions are allowed)

Common name	Species	TRI
Maned wolf	Chrysocyon brachyurus (CHR)	0
Ocelot	Leopardus pardalis (LEO)	1
Crab-eating fox	Cerdocyon thous (CER)	0
Hoary fox	Lycalopex vetulus (LYC)	0
Striped hog-nosed skunk	Conepatus semistriatus (CON)	0
White-eared opossum	Didelphis albiventris (DID)	0
Thick-tailed opossum	Lutreolina crassicaudata (LUT)	0
Bare-tailed woolly opossum	Caluromys philander (CAL)	1
Water rat	Nectomys squamipes (NEC)	0
Short-tailed opossum	Monodelphis sp (MON)	0
Gray slender opossum	Marmorsops incanus (MAR)	1
Spy hocicudo	Oxymycterus delator (OXY)	0
Rice rat	Cerradomys subflavus (CES)	1
Hairy-tailed bolo mouse	Necromys lasiurus (NEC)	0
Montane grass mouse	Akodon montensis (AKM)	1
Grass mouse	Akodon sp (AKO)	1
Agile gracile opossum	Gracilinanus agilis (GRA)	0
Pygmy rice rats	Oligoryzomys spp (OLI)	0
Vesper mouse	Calomys sp (CAL)	1
Kissing bug	Triatominae (TRI)	0

Chapter 9

Summarizing Discussion

Awareness of the importance of infectious agents for structure, functioning and stability of natural ecosystems emerged already almost two decades ago. However, the discussion on how exactly infectious agents should be included into scientific thinking about food webs is still ongoing. Data collection of food webs with parasites and pathogens is improving, while modelling tools are still in development. In this thesis we show the initial steps we took in order to better understand the potential impact of infectious agents in food webs and ecological communities. Firstly, we give examples of empirical studies we undertook to collect data on parasites in real ecosystems. Secondly, by extensive literature review we learned about the variety of infectious agents, the diversity of the interactions with their hosts and of their observed impact on a variety of real ecosystems. We developed conceptual ideas on how to model infectious agents in food webs, and provide a simple example of such model study. Thirdly, we quantified the clinical and subclinical effects of infectious agents on food web structure and stability, using an indirect approach by adding infectious agents not as stand-alone species, but through the effects they have on their hosts. Additionally, we show an example of a new approach of multiplex networks that could be used in future for modelling the effects of infectious agents in food webs, explicitly recognizing different types of interaction between infectious agents and its host that occur in complex systems.

9.1. Collecting real data of food webs with infectious agents

Many researchers have invested substantial effort in collecting food web data with parasites and pathogens. Most studies relate to parasites and rarely include pathogens, possibly because they are easier to collect. Despite these efforts, one of the biggest challenges is that many parasitic species still go unrecorded since the numbers of examined hosts are small (Poulin and Morand 2000). One third of helminth parasite species found in birds and mammals manifest in less than 5% of the examined host species. Even the collectors of high resolution data of food webs with parasites admit that they underestimate the numbers of parasites more than they do for free living species (Lafferty *et al.* 2006b). Although methods to estimate numbers of missing species of parasites in collected samples have been developed, they still remain a poor replacement for the actual data. Big currently available data sets of food webs with parasites include 'possible' links defined as links that were detected in other locations or times, but missed (or not observed) in the food web actually sampled. In **Chapter 3** we show that if species of infectious agent and its host are present and connected in one ecosystem, that this does not imply that they will be equally connected in another ecosystem where they are both present. Likewise, despite the fact that species of parasite and their host species are present in both ecosystems, they can have different types of interactions. While in one ecosystem a parasite has a low prevalence and a small effect on its host population, the situation can be quite different in another ecosystem, where parasites have high prevalence and strong effect on their host (e.g. in the later successional stages of ecological communities). The same ingredients, a parasite and a host species, can show very different interaction in different ecosystem contexts, depending on other species present and their interactions.

Furthermore, many species of infectious agents that have a potentially huge impact on the dynamics of populations in food webs receive less attention due to difficulties in collecting data. Pathogen and fungal species are notable examples of those. For example, every second approximately 10^{23} viral infections occur in the ocean (Suttle 2007). Species of the chytrid group of zoosporic fungi infect and kill a wide variety of hosts, including fish, zooplankton, freshwater phytoplankton, algae, and other aquatic fungi. They are recognized as an important driving factor in phytoplankton seasonal successions (Sime-Ngando 2012).

One of the important effects of infectious agents in food webs is their influence on the

physiology and behaviour of their hosts. They influence physiology of the host by redirecting parts of its energy, assimilated for biomass production and maintenance, towards investment in immune response. Behaviour can be affected in many ways e.g. infected individuals can eat less or more, or they may be caught less or more easily by a consumer. We study a number of empirical examples that measured these kinds of effects of infectious agents on their host in **Chapter 4**. We also show that behavioural and physiological change in the host due to parasitic infection can have extensive consequences in natural ecosystems e.g. they can lead to trophic cascades. Laboratory experiments are needed to quantify these kinds of effects of parasites on their hosts in currencies that are recognized in food web theory, e.g. abundance, biomass, feeding rates or death rates.

Other methods that could be relevant in the future with helping to understand the dynamics of food webs influenced by parasites are stoichiometry and molecular techniques. One example of stoichiometry research was shown in (Bernot 2011). They show that trematode infected snails had higher body N:P ratios then uninfected individuals. Additionally, there is an increase of using molecular techniques for more accurately describing trophic linkages in food webs (Wirta *et al.* 2014) and for identification of parasites (Tavares *et al.* 2011).

Current data sets on food webs with parasites and pathogens are described in terms of binary interaction matrices, types of links, life stages, body sizes, abundances, biomass and phylogeny of species in the community. Although these data are usually incomplete, they already give us better understanding on interactions between trophic species or groups in food web and parasitic subweb. Nevertheless, the research could extensively benefit from additional data such as quantified predation matrices and quantified non-trophic interaction matrices. Quantified predation matrices could contain information on 'the intensity of consumption' of infectious agents on their hosts in terms of e.g. increases in mortality rate in the host population due to the infectious agent. Nontrophic interaction matrices could contain quantified non-trophic influences of infectious agents on their hosts, such as possible subclinical influence on behaviour.

9.2. Why is it so difficult to model infectious agents in food webs?

In modelling food webs with parasites, one of the first dilemmas modellers have is the small size of pathogens and parasites compared to their hosts. The fact that infectious

agents are deriving energy from or affecting behaviour of much larger hosts, together with the fact that infectious agents are usually found on higher trophic levels, and that species at high trophic levels are on average larger than species of lower trophic levels (Brose *et al.* 2006, Memmott *et al.* 2000), are all aspects of this issue. The problem appears since most of the models that simulate food webs (without infectious agents), include the size of individuals as one of the criteria to establish trophic hierarchies. Examples are cascade models (Cohen *et al.* 1993, 1990), niche models (Williams and Martinez 2000) and nested hierarchy models (Cattin *et al.* 2004). Leaper and Huxham (Leaper and Huxham 2002) suggest that size criteria used for ordering species prevents modelling parasites in a models previously mentioned, and also Warren *et al.* (2010) propose that the same factor of ordering can be used if parasitic and predatory links are separated and ordered by different rules.

Another problematic piece of the modelling puzzle is the fact that many infectious agents possess several distinct life stages, and that each of these stages usually occurs in different host species. Hence, different stages of infectious agents may affect hosts at different trophic positions again raising the question of trophic position of that infectious agent (Huxham *et al.* 1995). These parasites can have one or more free living stages that are important resource for free-living consumer species, e.g. through active and passive foraging (Johnson *et al.* 2010, Thieltges *et al.* 2013). Thus, they have different types of links in an interaction network. Cirtwill and Stouffer (2015) show that these different types of links are distributed differently in the food web context.

A further issue is how to model transmission of infectious agents in food webs. As transmission between susceptible and infectious individuals of the same species is the minimally required pathway for many agents, taking this into account in food web modelling seems essential to understand dynamical effects. This direct transmission should then be accommodated in the food web approach one is taking. Many infectious agents, however, have multiple ways and means of transmission. **Chapter 8** proposes a new modelling framework that can help to solve that puzzle of including different types of transmission into food web. We give an example by following trophic and vector transmissions in the multi-host system. Furthermore, the transmission of infectious agents may also depend on the type of habitat. Even simple systems of consumer-resourceinfectious agent do not consider the importance of habitat type for modelling transmission. We discussed the differences between aquatic and terrestrial environments in **Chapter 5**, and gave a simple example of stability analysis in a range of habitat types for a simple consumer-resource-microparasite interaction.

Many hosts are simultaneously infected with several types of infectious agents. Species of infectious agents in one host form a web of interactions, where interaction between a pair of infectious agents can be positive or negative (Telfer *et al.* 2010). Interactions between infectious agents modify host susceptibility and heterogeneity between hosts (Cattadori *et al.* 2008). Telfer *et al.* (2010) emphasize the danger of looking at only single host-parasite interactions and the need to better understand the community interactions between infectious agents. **Chapter 6** and **Chapter 7** follow this idea and study the interaction between host species and infectious agent's community.

9.3. Direct vs indirect inclusion of infectious agents in food webs

Many studies include infectious agents as separate nodes to food webs. This way of inclusion of infectious agents in food webs increases linkage density and connectance, which are shown to influence stability of the system (Lafferty *et al.* 2006b). This is not a satisfactory solution for epidemiologists and parasitologists since infectious agents are different from typical consumers e.g. in terms of number of victims (**Chapter 5**, Dobson and Hudson 1986, Lafferty and Kuris 2002, Sukhdeo 2012). Many species of infectious agents should be included into food webs as separate nodes, since they are important energy resource for other species in the community. The effect of infectious agents on their host through draining of their energy and non-trophic impacts of infectious agents on food web dynamics are, however, neglected in this way. We give many examples from nature in **Chapter 4** and **Chapter 5** of infectious agents having other types of interaction with their hosts that are not comparable to typical trophic types of interaction.

We used an indirect way of representing the effects of infectious agents on their hosts by assuming that decrease and increase in mortality of one host species at a time mimics the waxing and waning of an outbreak of infection in that host species. One could argue that these types of influences through mortality could also be caused by other influences than infection, e.g. by climate or hunting of that host species. However, climate would unlikely affect only a single species in an ecological community. Hunting could act specifically on a one species of ecological community of course, but our analysis works as a sensitivity analysis for each individual species in the web. As all species have infectious agents to which they are host (Rossiter 2013), we feel that our indirect approach is a valid initial attempt to gauge effects of infectious outbreaks. A strong simplifying assumption is also that we regard only one host at a time, whereas many pathogens and parasites have

a broader host range in the same ecosystem. In **Chapter 6** and **Chapter 7**, we look at the potential effects of infectious agents in terms of changes in growth rate, physiology and behaviour on one host species at the time. However different epidemiological states for individuals of that species are not modelled as separate dynamic. This way of including infectious agents, without explicit modelling of infectious agents, disregards the transmission process, which may certainly influence the results, as we have argued that transmission is an essential part of the nature of infectious agents. However, we aimed to explore the simplest setting that at least included infectious agents in a full food web description. This type of research gives us an initial point of view about potential roles of infectious agents in food webs. We started this research with an idea that interactions between species evolve to the stable equilibrium. From that point of view, infectious agents are a necessary component of communities that affects food web from within. Food webs that are observed in nature are usually assumed to be in a stable equilibrium. That suggests that the effects of infectious agents are already included inside of these data, and we approach to quantifying the potential additional effect of infectious agents by looking at the food webs and measuring their resilience to perturbations it terms of increased and decreased effect of infectious agents on food web and ecological community. Future analyses would need to take transmission dynamics into account and could then also include density dependence and nonlinear feed back between epidemiology and ecology, which may be very important in gauging effects, notably of parasites.

The total effect of all infectious agents in one host population is assumed to influence that host's mortality in **Chapter 6**. We explore the effect of infectious agents by increasing and decreasing mortality rate of their host in order to find consequences for the rest of the community. Our results showed that outbreak of disease as well as decrease of infection prevalence in focal species can both lead to quasi-extinctions (reduction in density of a species by 90%) of other species. These extinctions can occur within the same or at different trophic levels of the ecological community. Our analysis indicates that natural communities influenced by the decrease of infection prevalence (or local eradication of an infectious agent), can also lead to quasi-extinctions in the ecosystem, perhaps counter to intuition. Decrease of infection prevalence can therefore have negative consequences for the rest of the community. This is a very important for e.g restoration ecologists: one could argue that infectious agents should be restored together with other species if they indeed play an important role in regulating the ecosystem dynamics.

Likewise, in **Chapter 7** we explore the non-trophic subclinical effect of different infectious agents on the behaviour, physiology and growth rate of their hosts by measuring the effect on the stability of the system. These changes in behaviour, physiology and growth

rate are again assumed to be an indirect consequence of combined effect of different infection agents uniquely affecting the single focal host species. We find that the potential effects of infectious agents on interactions of species profoundly affect the stability of the food webs they are part of, and in a wide variety of patterns. We find eight patterns where stability increases, decreases or changes the stability direction. However, the pattern where stability increases as a result of infection had a slightly bigger frequency in most of the trophic levels and different scenarios. Because this is still work in progress, the further questions that we will address are the importance of exact positions of host species in the food web, the importance of relations with their immediate neighbours in the web, and the importance of link weight in the interaction network. This research on the subclinical effect of infectious agents shows promising first results and a potential to deepen our knowledge on the roles of infectious agents in food webs and ecosystems. For the analysis in **Chapter 7**, the same simplifying assumptions as discussed in the previous paragraph with respect to Chapter 6 are relevant, and in future research the effects of relaxing them on our preliminary insight needs to be investigated.

Chapter 8 is another representation of including infectious agents in an indirect way in a food web, but now with a focus on different ways of transmission. We use a multiplex network where the same nodes appear on all the layers, but they can be connected according to different topologies and mechanisms on each layer. We show the application of the multiplex framework to both a simple predator-prey-vector reference case and to real data from host communities of *Tripanosoma cruzi* in tropical forest. In both cases, we find that genuine multiplex measures such as the multiplex cartography are actually capable to predict the ecological role played by different host species in the spread of the infection. Our theoretical network model indicates that parasitic and trophic interactions are additive in sustaining the spread of the *T. cruzi* parasite, further validating previous preliminary studies.

9.4. The role of parasites in the ecosystems

In Chapter 6, the results on mimicking effects of waxing and waning outbreaks of infection in species at the highest trophic level show that these species can tolerate stronger effects of infectious agents on their growth rate than species at lower trophic levels. The analysis also shows that even very small decreases in mimicked infection-induced mortality, assumed to be the effect of a decrease of infection prevalence, can lead to quasi-extinctions in the food web. The results on quantifying the effects of an increase or

decrease of mortality, suggest that we could find higher numbers of infectious agents in upper trophic levels. This aspect of ”carnivore” and ”herbivore” species is noted in the analyses of the real food webs with parasites. The analysis of four real food webs showed that infectious agents are present in every food web and at each trophic level. However, the highest diversity of parasites per host species can be found at the highest trophic levels, as opposed to basal levels, which are sometimes even relatively parasite-sparse. In **Chapter 4** we give examples of infectious agents leading to extinction of their hosts, but we show also that this happens only in combination of other factors, e.g. environmental, that decreased fitness of host species.

9.5. Conclusion

The meaning of terms such as 'infectious agents', 'pathogens' and 'parasites' was always negatively comprehended. There are of course good reasons for this because of the large scale and profound morbidity and mortality that infectious agents cause in many species, decidedly also including humans. Infectious agents were originally neglected in food web research, possibly because of their size and the fact that most live inside other species, both complicating data collection. However, many studies have recently shown that infectious agents could play a much more dominant and different type of role than previously thought. One could imagine a sub-web of infectious agents that is parallel with a food web, where the dynamics of both webs depend on each other and evolve together. Infectious agents interact directly with their hosts and indirectly with non-host species. Direct interaction can result in subclinical or clinical disease in infected individuals, possibly changing the behaviour, physiology, growth and mortality of their hosts, and through that changing the way these species contribute to the ecological community, e.g. in terms of biomass. By the subclinical and clinical influence on their hosts, infectious agents influence interactions between species in ecological communities and affect structure, functioning and stability of the ecosystems. Although more research is needed to quantify these effects, the importance of infectious agents in food webs as major players is already evident. They can therefore play a much more positive, and even essential, role, in addition to their already established negative image. A healthy ecosystem may be one that is rich with infectious agents (Hudson *et al.* 2006).

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Summary

Infectious agents are ubiquitous in nature. They can be broadly distinguished into microparasites (viruses, bacteria, fungi, protozoa), macroparasites (nematodes, trematodes and cestodes), ectoparasites (fleas and ticks), parasitic castrators and parasitoids. Although these types of infectious agents are very different in the way they affect their host, they all tend to live on or in their hosts for most of their life while benefiting from its nutrients. Ecologically speaking, infectious agents are part of food webs (networks of species that have trophic consumer-resource relations), and the way they influence their hosts can have aspects of both trophic and non-trophic interaction. Infectious agents are in essence consumers of resources (their hosts), but they differ from typical consumers in having only one victim per life stage and not necessarily killing or fully consuming their victims. As many other species in the food web, infectious agents may also act as resource in a food web.

The interaction between infectious agents and their hosts can affect the host in different ways; notably interaction can result in subclinical or clinical disease in infected host individuals. Subclinical, infectious agents possibly have an impact on life-history traits, behaviour, feeding or other individual-level aspects of their hosts because of increased energy use. Infected predators may have a reduced ability to hunt and catch prey, while infected prey may be easier to catch or less nutritious. Clinical, infectious agents may increase mortality in their hosts. By changing behaviour or survival of their hosts, infectious agents indirectly influence other species, including non-host species, of their ecological community. These effects could be measured through an infectious agent's influence on energy flow, biodiversity, community interactions and the abiotic part of the ecosystem. In that way infectious agents may affect structure, functioning and stability of ecological communities.

In this thesis we use data collection, data analysis, and mathematical and computational

modelling to study the potential impact of different types of infectious agents in food webs and ecosystems. **Chapters 2** and **3** show results of field research on macroparasites of top predators, carried out during a period of four years in Serbia. The research provides an illustration of the diversity of parasites and their hosts in real life systems and illustrates the work involved in obtaining such data. **Chapter 2** shows different parasites that can be found in single host species (golden jackal, *Canis aureus*). It shows how this host can be infected with different parasite species and have different parasite loads throughout its large spatial distribution range. **Chapter 3** shows the impact of one species of parasite (heartworm, *Driofilaria immitis*) in different carnivore species. Heartworm larvae are transmitted by cuculid mosquitoes (*Culex, Aedes, Anopheles, Culiseta spp.*) to the host animals, where they cause canine and feline cardiopulmonary and human pulmonary dirofilariosis. The study showed that even though hosts species can have overlapping ecological niches, they can have different parasite loads.

Second part of the thesis gives an overview of the direct and indirect ways in which infectious agents affect their hosts and non-hosts species. In **Chapter 4**, we systematically classify the effects of infectious agents on energy flow, community interactions, diversity, and at the ecosystem level, covering a broad range of infectious agents in a broad range of host species. We discuss a concept of new indirect approach of modelling infectious agents in food webs that concentrates on the ways infectious agents affect the existing links across host and non-host nodes, by influencing the strength of consumer-resource interaction. **Chapter 5** further deepens the knowledge of infectious agents in ecosystems by cataloguing their diversity, types and functional roles. We explain the specific relationship of infectious agents and their hosts that can have aspects of both trophic and non-trophic interaction. By looking at a simple model of a microparasite in a very basic Lotka-Volterra consumer-resource system, we give an example of the indirect approach previously introduced (**Chapter 4**), in different type of ecosystems, from aquatic to terrestrial.

The third part of the thesis focuses on understanding, using mathematical and computational modelling, the way infectious agents affect food-webs and ecosystems. We quantified the clinical (**Chapter 6**) and subclinical (**Chapter 7**) impacts of infectious agents on food web structure and stability, using an indirect approach by adding infectious agents not as stand-alone species, but through the effects they have on their hosts. Additionally in **Chapter 8**, we show an example of a new approach based on multiplex networks that could be used in future for modelling the effects of infectious agents in food webs, explicitly recognizing different types of interaction between infectious agents and its hosts that occur in complex systems. Chapter 6 explores the impact of infectious agents in

food webs by using the indirect approach by decreasing and increasing the mortality of species in the web, one at a time. We mimic the effect of changes in infection prevalence in one species and in that way quantify influence of that effect on the structure and stability of the ecological community as a whole. **Chapter 7** explores the influence of infectious agents on stability of the ecosystem through the effect they have on their hosts. We again use our indirect approach, but we now focus on subclinical effects on host species through change in the behaviour of the host species. The influence of an infectious agent's change in behaviour is quantified through its effect on resource preference and conversion efficiency of consumer-resource interaction. **Chapter 8**, finally, represents a spatial multiplex-based framework for modelling multi-host parasite transmission considering two layers of interaction. We consider trophic and non-trophic (host-parasite) interactions between nodes representing species' populations embedded in a given environment. Each node has an identity, i.e. predator, prey or parasite vector, represented by its frequency. The resulting multiplex is composed of two distinct layers, which are both spatially embedded. Our model was inspired by the multiple transmission routes of *Trypanosoma cruzi*, the etiological agent of Chagas disease.

This thesis makes an attempt to shed the light on the importance of infectious agents as potentially major players in food webs. Although the phrases such as, 'infectious agents', 'pathogens' and 'parasites' are usually negatively comprehended, we show that they could have a more positive, and even essential, role for structure and stability of natural ecosystems.

Samenvatting

Veroorzakers van infectieziekten zijn alomtegenwoordig in de natuur. Ze kunnen grofweg worden onderscheiden in microparasieten (virussen, bacteriën, schimmels, protozoa), macroparasieten (nematoden, trematoden en cestoden), ectoparasieten (vlooien en teken), parasitaire castrators en parasitoïden. Hoewel deze infectieuze soorten zeer verschillend zijn in de manier waarop ze invloed hebben op hun gastheer, verblijven ze allemaal op of in hun gastheren voor het grootste deel van hun leven, en profiteren ze van die gastheer. Ecologisch gezien, maken infectieuze soorten deel uit van ecosystemen en voedselwebben (bijvoorbeeld netwerken van soorten die van elkaar leven door predatie), en kan de manier waarop ze hun gastheren beïnvloeden aspecten van zowel trofische (bijvoorbeeld predator-prooi) en non-trofische interactie hebben. Infectieuze soorten zijn in essentie consumenten (de gastheer), maar verschillen van de typische consument omdat een infectieus individu vaak slechts één slachtoffer per levensfase heeft en dit slachtoffer niet noodzakelijkerwijs doodt of volledig "verbruikt". Zoals veel andere soorten in een voedselweb, kan een infectueuze soort ook fungeren als voedselbron in een voedselweb.

De wisselwerking tussen ziekteverwekkers en hun gastheer kan de gastheer op verschillende manieren beïnvloeden; interactie kan met name resulteren in subklinische of klinische ziekte bij geïnfecteerde gastheren. Subklinisch gezien, hebben ziekteverwekkers mogelijk invloed op voortplanting, gedrag, voeding, weerbaarheid bijvoorbeeld als gevolg van de toename van het gebruik van energie. Geïnfecteerde roofdieren kunnen een verminderd vermogen hebben om te jagen en prooi te vangen, terwijl geïnfecteerde prooidieren wellicht makkelijker te vangen zijn of minder voedzaam. Klinisch gezien, kunnen ziekteverwekkers sterfte van hun gastheer veroorzaken of beïnvloeden. Door het veranderen van gedrag of het overleven van hun gastheren, beïnvloeden ziekteverwekkers ook indirect andere soorten, met inbegrip van niet-gastheersoorten in hetzelfde ecosysteem. Deze effecten kunnen in kaart worden gebracht door het bestuderen van de invloed

een ziekteverwekker op de energiestromen in een ecosysteem, de biodiversiteit, interacties tussen de andere soorten in een ecosysteem en voedselweb en het abiotische deel van het ecosysteem. Op allerlei manieren beïnvloeden ziekteverwekkers de structuur, de werking en de stabiliteit van de ecologische gemeenschappen.

In dit proefschrift gebruiken we het verzamelen van gegevens en hun analyse, en met wiskundige en computermodellen om de mogelijke gevolgen van de verschillende soorten van ziekteverwekkers in voedselwebben en ecosystemen te bestuderen. In de **hoofdstukken 2** en **3** worden de resultaten beschreven van veldonderzoek naar macroparasieten van top-predatoren dat gedurende een periode van vier jaar in Servië is uitgevoerd. Het onderzoek kwantificeert de diversiteit van parasieten en hun gastheren in een echte ecosysteem, en illustreert de werkzaamheden die gedaan moeten worden om zulke gegevens te verkrijgen. **Hoofdstuk 2** onderzoekt de parasieten van de jakhals (*Canis aureus*). Het laat zien hoe deze gastheer kan worden besmet met verschillende soorten parasieten e mate van besmetting kan variëren over het ruimtelijke gebied waar de gastheer voor komt. **Hoofdstuk 3** toont de invloed van één soort parasiet (hartworm, *Driofilaria immitis*) in verschillende vleesetende gastheren. Hartwormlarven worden overgebracht door cuculid muggen (*Culex, Aedes, Anopheles, Culiseta spp.*), en veroorzaken bij gastheren als honden, katten en de mens verschillende hart- en longziekten. De studie toonde aan dat, gastheersoorten verschillende mate van besmetting kunnen hebben, ondanks overlap in hun ecologische niche.

In het tweede deel van het proefschrift wordt een overzicht gegeven van de directe en indirecte manieren waarop ziekteverwekkers invloed hebben op hun gastheren en de andere soorten in hun ecosysteem. In **hoofdstuk 4** worden systematisch de invloeden van ziekteverwekkers in kaart gebracht op energiestromen, interacties tussen soorten, de biodiversiteit, en op het ecosysteem als geheel, voor een breed scala aan ziekteverwekkers en een breed scala aan gastheer soorten. Er wordt een nieuwe indirecte benadering besproken voor het modelmatig bestuderen van ziekteverwekkers in voedselwebben. Die benadering richt zich op de effecten die ziekteverwekkers hebben op de sterkte van bestaande interacties tussen de andere soorten in een voedselweb, in plaats van op de ziekteverwekkers zelf. **Hoofdstuk 5** verdiept dit idee verder door het catalogiseren van hun diversiteit, soorten en functionele rol. We leggen de specifieke relatie tussen ziekteverwekkers en hun gastheren uit, die zowel trofische als non-trofische kanten heeft. We illustreren de indirecte benadering (**hoofdstuk 4**) door te kijken naar de invloed van een ziekteverwekker in een zeer eenvoudig Lotka-Volterra model als karikatuur voor interactie tussen een predatorsoort en een prooisoort. We bootsen door keuzes in het model verschillende soorten sterk vereenvoudigde ecosystemen na, van aquatische tot

terrestrische.

Het derde deel van het proefschrift richt zich meer op begrip van de invloed van ziekteverwekkers in een voedselweb met behulp van wiskundige en computermodellen. De klinische (**hoofdstuk 6**) en subklinische (**hoofdstuk 7**) gevolgen worden gekwantificeerd van een ziekteverwekker op voedselweb structuur en stabiliteit. met behulp van de indirecte benadering, maar nu voor grotere, en meer realistische, voedselwebben. In **hoofdstuk 8** tonen we een voorbeeld van een nieuwe aanpak gebaseerd op multiplex netwerken die in de toekomst wellicht kan worden gebruikt om de verschillende typen interactie tussen ziekteverwekkers en gastheren die optreden in complexe systemen expliciet te maken in een model. **Hoofdstuk 6** onderzoekt het effect van ziekteverwekkers in voedselwebben door het verlagen en verhogen van de sterfte van steeds een van de soorten in het web. We bootsen het effect na van veranderingen in infectieprevalentie in één soort en kwantificeren op die manier de invloed op de structuur en stabiliteit van de ecologische gemeenschap als geheel. **Hoofdstuk 7** onderzoekt de invloed van ziekteverwekkers op de stabiliteit van het ecosysteem door het effect ze hebben op hun gastheren. We maken wederom gebruik van onze indirecte benadering, maar concentreren ons nu op subklinische effecten op de gastheer door middel van verandering in het gedrag van die gastheer, met name door middel van het effect op voedsel voorkeur van de gastheersoort en het omzettingsrendement van van de prooien die de gastheer eet. **Hoofdstuk 8** tenslotte is een ruimtelijk multiplex-gebaseerd raamwerk voor het modelleren multigastheer parasiet dynamica met twee lagen van interactie. Wij beschouwen trofische en non-trofische (gastheer-parasiet) interacties tussen knooppunten in het netwerk en modelleren een parasiet die tussen gastheren word overgebracht door een vector (bijvoorbeeld een insect). Elk knooppunt heeft een identiteit, dat wil zeggen roofdier, prooi of vector, vertegenwoordigd door haar frequentie. De resulterende multiplex bestaat uit twee afzonderlijke lagen, die beide ruimtelijk zijn ingebed. Het model is geïnspireerd op de verschillende transmissieroutes van *Trypanosoma cruzi*, de verwekker van de ziekte van Chagas.

Dit proefschrift probeert licht te werpen op het idee dat ziekteverwekkers potentieel belangrijke onderdelen zijn van voedselwebben en ecosystemen. Hoewel de woorden ”ziekteverwekker” en ”parasiet” meestal een negatieve interpretatie hebben, wordt aangetoond dat de soorten die er mee worden aangeduid op het niveau van natuurlijke ecosystemen als geheel wel eens een positievere en zelfs essentiële rol zouden kunnen spelen door hun invloed op structuur en stabiliteit.

Rezime

Infektivni agensi su prisutni svuda u prirodi. U širem smislu razlikujemo mikroparazite (viruse, bakterije, gljivice, protozoe), makroparazite (nematode, trematode i cestode), ektoparazite (buve i krpelje), parazite kastratore i parazitoide. Iako su ove vrste infektivnih agenasa veoma različite u načinu na koji utiču na svog domaćina, sve one teže da žive na ili u svojim domaćinima tokom većine svog života i koriste njegove hranljive materije. Ekološki gledano, infektivni agensi su deo mreža ishrane (mreže vrsta organizama koje imaju trofičke odnose potrošača-resursa), a način na koji utiču na svoje domaćine može imati aspekte kako trofičke tako i ne-trofičke interakcije. Infektivni agensi su u suštini potrošači resursa (svojih domaćina), ali se razlikuju od tipičnih potrošača u tome što imaju samo jednu žrtvu po životoj fazi, I obično ne ubijaju svoje žrtve. Kao i mnoge druge vrste organizama u mrežama ishrane, infektivni agensi mogu takođe biti resurs drugih vrsta.

Interakcija između infektivnih agensa i domaćina može uticati na domaćina na različite načine; interakcija može dovesti do subkliničke ili kliničke bolesti u inficiranim pojedincima domaćina. Subklinički, infekcija može uticati na karakteristike životnih istorija organizama, njihovo ponašanje, ishranu ili druge individualne karakteristike domaćina usled povećane upotrebe njegove energije. Inficirani predatori mogu imati smanjenu sposobnost za lov i hvatanje plena, dok inficirani plen može biti lakše uhvaćen ili manje hranljiv. Klinički, infekcije mogu da povećaju smrtnost svojih domaćina. Uticajem na promenu ponašanja ili preživljavanje svojih domaćina, infektivni agensi posredno utiču na druge vrste organizama, uključujući vrste ne-domaćina, u okviru njihove ekološke zajednice. Ovi efekti se mogu meriti kroz uticaj infektivnih agenasa na protok energije, biodiverzitet, interakcije u okviru ekoloških zajednica i na abiotički deo ekosistema. Na taj način infektivni agensi mogu uticati na strukturu, funkcionisanje i stabilnost ekoloških zajednica.

U ovoj tezi koristimo prikupljanje podataka, analizu podataka, matematičke I računarske modele za proučavanje potencijalnog uticaja različitih vrsta infektivnih agenasa na mreže ishrane i ekosisteme. **Poglavlja 2 i 3** pokazuju rezultate terenskog istraživanja makroparazita u top predatorima, sprovedenog tokom četiri godine u Srbiji. Istraživanje oslikava raznovrsnost parazita i njihovih domaćina u prirodnim sistemima i neophodne korake koje treba izvršiti za dobijanje takvih podataka. **Poglavlje 2** pokazuje različite vrste parazita koji se mogu naći u jednoj vrsti domaćina (šakal, *Canis aureus*). Ovo poglavlje pokazuje kako jedan domaćin može biti inficiran različitim vrstama parazita i imati različito opterećenje parazitima u okviru širokog geografskog rasprostranjenja. **Poglavlje 3** prikazuje uticaj jedne vrste parazita (srčanog crva, *Driofilaria immitis*) u različitim vrstama mesoždera. Larve srčanog crva na domaćine prenose komarci (*Culex, Aedes, Anopheles, Culiseta spp.*), gde izazivaju kardiopulmonarnu bolest kod pasa i mačaka kao i plućnu dirofilariozu kod ljudi. Studija je pokazala da, iako se ekološke niše domaćina preklapaju, oni mogu imati različita opterećenja parazitima.

Drugi deo teze daje pregled načina na koje infektivni agensi direktno i indirektno utiču na svoje domaćine i ne-domaćine. U **poglavlju 4**, sistematski klasifikujemo uticaj infektivnih agenasa na protok energije, interakcije u okviru ekoloških zajednice, biodiverzitet, i abiotički deo ekosistema, pokrivajući široke spektre infektivnih agenasa i vrsta domaćina. Diskutujemo o konceptu novog indirektnog pristupa modelovanja infektivnih agenasa u mrežama ishrane koji je fokusiran na načine na koje infektivni agensi mogu uticati na postojeće veze preko domaćina i ne-domaćina, menjajući snagu interakcije između potrošača i resursa. **Poglavlje 5** dodatno produbljuje znanje o infektivnim agensima u ekosistemima kroz katalogizaciju njihovog diverziteta, tipova i funkcionalnih uloga. U ovom poglavlju smo fokusirani na specifične odnose infektivnih agenasa i njihovih domaćina koji mogu da imaju aspekte kako trofičke tako i ne trofičke interakcije. Kroz jednostavan model microparazita u *Lotka-Volterra* sistemu potrošač-resurs, dajemo primer indirektnog modelovanja koji smo prethodno opisali (**Poglavlje 4**), u različitim tipovima ekosistema, od vodenih do kopnenih.

Treći deo teze se fokusira na razumevanje načina na koji infektivni agensi utiču na mreže ishrane i ekosisteme, koristeći matematičke i računarske modele. Kvantifikujemo klinički (**Poglavlje 6**) i subklinički (**Poglavlje 7**) uticaj infektivnih agenasa na strukturu i stabilnost mreža ishrane, koristeći indirektan pristup dodavanjem infektivnih agenasa kroz efekte koji imaju na svoje domaćine. Osim toga, u **Poglavlju 8**, pokazujemo primer novog pristupa zasnovanog na multipleks mrežama koja bi mogla da se koriste u budućnosti za modelovanje efekata infektivnih agenasa u mrežama ishrane, eksplicitno prepoznavajući različite vrsta interakcija između infektivnih agenasa i njegovih domaćina

koje se dešavaju u kompleksnim sistemima. **Poglavlje 6** istražuje uticaj infektivnih agenasa u mrežama ishrane pomoću indirektnog pristupa, smanjujući i povećavajući smrtnost u jednoj po jednoj vrsti u mreži. Na taj način, mi imitiramo efekat promena u prevalenciji infekcije u jednoj vrsti i na taj način kvantifikujemo uticaj infektivnog agensa na strukturu i stabilnost ekološke zajednice u celini. **Poglavlje 7** istražuje uticaj infektivnih agenasa na stabilnost ekosistema kroz efekat koji imaju na svoje domaćine. Ponovo koristimo indirektni pristup, ali smo sada fokusirani na subkliničke efekte koje infektivni agensi mogu da imaju na vrste domaćina, kroz promenu njihovog ponašanja. Uticaj infektivnog agensa na promenu u ponašanju je kvantifikovan preko svog uticaja na izbor resursa i efikasnost konverzije resursa tokom potrošač-resurs interakcije. **Glava 8** predstavlja prostorni multipleks okvir modelovanja parazita koji se prenose preko više vrsta domaćina kroz dva tipa ekoloških interakcija. Mi uključujemo trofičke i ne-trofičke (domaćin-parazit) interakcije između populacija vrsta koje su ugrađene u datom okruženju. Svaka populacija ima identitet, potrošač, resurs ili parazit vektor, koji je predstavljen određenom frekvencijom. Dobijena multipleks mreža se sastoji od dva različite mreže populacija, koje su prostorno ugrađene. Naš model je inspirisan parazitom *Tripanosoma cruzi* koji ima različite načine prenošenja I koji je etiološki agent Chagas bolesti.

Ova teza je pokušaj da se baci svetlo na značaj infektivnih agenasa kao potencijalno važnih igrača u mrežama ishrane. Iako su fraze kao što su, ”infektivni agensi”, ”patogeni” i ”paraziti” obično negativno shvaćene, mi pokazujemo da bi mogli da imaju pozitivniju, pa čak i suštinsku, ulogu za strukturu i stabilnost prirodnih ekosistema.

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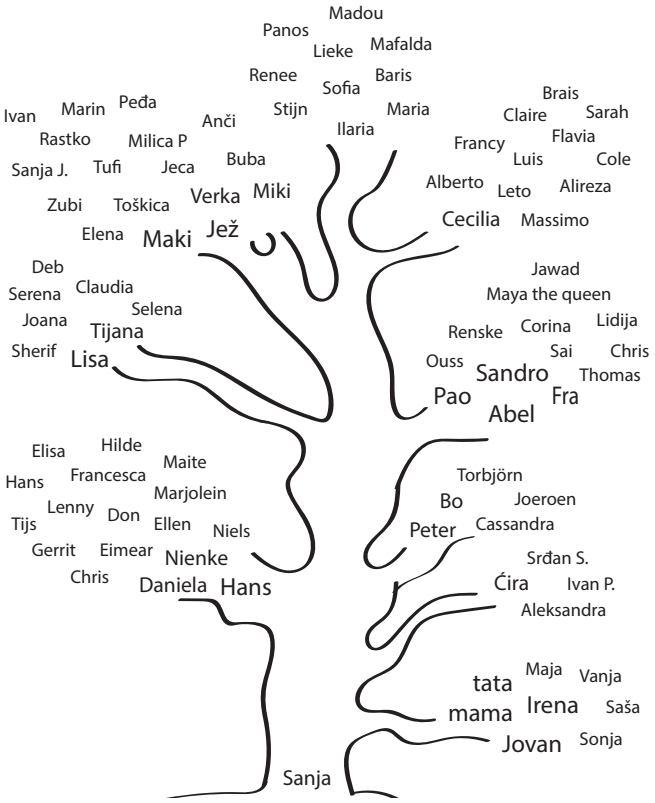

My PhD journey was a great adventure. I got the chance to learn so much, meet a lot of wonderful people, gain new experiences, see the world and grow as a person immensely. Thank you all for being there for me!

Curriculum vitæ

Sanja Selaković was born on 4th of August 1984 in Valjevo, Serbia. After graduating from high school, she went on to study Ecology and Environmental sciences at Faculty for biology, University of Belgrade in 2003. She received her MSc diploma in 2010. During the course of her MSc at the department of Animal ecology, she has worked on projects focused around carnivore species in Serbia such as jackals, wolves and foxes under supervision of Dr. Duško Ćirović. Right after finishing her thesis on fitness of jackals and foxes in Serbia, she started the PhD program at the same department with a focus on laboratory work on macroparasites in carnivore species. In this period she was also involved in teaching activities in animal ecology, biogeography as well as urban ecology courses. Sanja also followed physical theatre curriculum at the Moving Academy of performing Arts from the Netherlands in Serbia during period from 2008-2011. She moved to the Netherlands in July 2011 to start a PhD in modelling of infectious agents in food-webs at the Theoretical Epidemiology Group at Utrecht University, from which the results are presented in this thesis. Her PhD was a part of the Complexity program of NWO that encourages research into complex systems and complex processes. As part of this research program she was trained to work in interdisciplinary group. In 2014, Sanja attended Complex Systems Summer School in Santa Fe, New Mexico where she collaborated with researchers with backgrounds in physics, mathematics, computational science, engineering, social sciences, medicine, ecology and astrophysics. She also collaborated with a group from Department of Physics, Chemistry and Biology, Division of Theoretical Biology, Linköping University in Sweden. She presented her work at several international conferences. Sanja is currently working as a Postdoctoral researcher at the Geoscience department of Utrecht University, where she studies the influence of species on dynamics of estuary systems.

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