Observational and model evidence for and against the dilution effect: examples from pathogens and parasites of wild rodents

by

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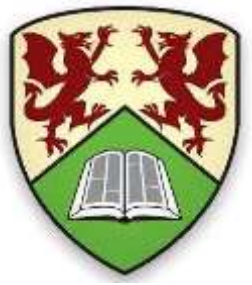

A thesis submitted to Aberystwyth University in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

Institute of Biological, Environmental and Rural Sciences
Aberystwyth University

September 2018

Word Count of thesis: DECLARATION	70000
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There is no self-awareness in ecosystems, no language, no consciousness, and no culture; and therefore no justice and democracy; but also no greed or dishonesty.

—*Fritjof Capra, The web of life*

These, then, are some of the basic principles of ecology – interdependence, recycling, partnership, flexibility, diversity, and, as a consequence of all those, sustainability. [...] the survival of humanity will depend on our ecological literacy, on our ability to understand these principles of ecology and live accordingly.

—*Fritjof Capra, The web of life*

Acknowledgements

Firstly, I would like to express my sincere gratitude to my first supervisor Dr Claire Risley and my second supervisor Dr Joe Ironside for their continuous support through my PhD and for their advice, patience, and motivation, especially during the difficult time of the writing up. Additionally, I would like to thank Dr Kim Kenobi, without who I could not have made it through the modelling work (and through these four years). His infinite support and kindness made the impossible possible: I managed to learn how to deal with the terrifying "R". He is honestly one of the best human being I have ever met, and I am so grateful he dedicated time to my research.

Besides my supervisors, I really want to thank all the people in IBERS that provided invaluable help at every stage of my PhD journey. In particular, Dr Jo Hamilton, who gave me essential advice on macroparasite analysis, Dr Joseph Jackson, who provided useful information to design the live-trapping study, and Gareth, Rori, Julie, and Colin who fulfilled all my requests for field and lab work equipment, reducing substantially my stress levels. Outside IBERS, I am profoundly grateful to Dr Michael Kosoy, Dr Richard Wall, Dr Swaid Abdullah, and Dr Stuart Siddell for their suggestions and for providing positive controls for my PCRs.

I also have to thank all the people involved in my field and lab work. First of all, Paul Culyer and his staff at the Stackpole Estate: I could not think of nicer, more supportive, and more professional people, and also of a more beautiful place to spend time outdoors. It was a fantastic experience and I am immensely grateful to all of you (especially Jon, Sam, and Alex). Secondly, the Skomer staff, Dr Tim Healing, and all the volunteers who were an invaluable help with the data collection on the island. And finally, I owe a huge thank to all the undergraduates who helped me in the field and in the lab (Aaron, Alice, Alex, Bridget, Emily, Hannah, Jacques, Karolina, Katie H., Katie W., Michaela, Nicole, Peter), and among those a "very special mention" goes to Scarlett, Joe and Tina.

They were great research assistants, and I could not have accomplished anything without them.

So many brilliant people were crucial in these PhD years. In Aberystwyth, I found an amazing community of friends and colleagues. They are all precious to me and I feel we shared so much life. Thanks to Adil, Ally, Amy, Christine, Claire, Gaspare, Lizzy, Marton, Mathilde, Max, Nathan, Paolo, Pippa, Sara, Tamsin, and last but definitely not least the great, old, wise, fatherly Niall (I will always owe you Niall, I hope you won't ever need an organ or something because I will have to donate it to you considering my life debt!!).

Thanks to my two Francesca, I am so grateful to Aberystwyth for meeting you. You are truly wonderful. Thanks to the acroyoga people, especially for meeting Bronya: thank you for the fantastic time spent away from the office. Thanks to all the Aber Organic Food Coop for supporting my ideas and me. Thanks to Julia and Rob for sorting out my living arrangements in a very difficult moment of my PhD journey.

Here in Wales, I also found an "adoptive" family who welcomed me and made such a difference in my PhD experience. Thank you Alex, Jenny, Leslie, David, Sam, Jacob, and Isaac.

Although they were not in Aberystwyth, I have to thank all my friends spread around the world because they are always by my side, listening, moaning, travelling, dreaming, and partying. Thank you Carmen, Claudia, Cristina, Gianluca, Laura, Ottavia, Mara, Salvatore, Teresa, Anna, Matia (and Gemmina) and so many others for taking care of me and for letting me taking care of you. And thank you my friend for supporting me during the time I was desperately looking for a PhD, and for encouraging me to embark in this one, and for make me regret my decision more than a few times.

In conclusion, by far the most important acknowledgement goes to my family, in particular to my parents, to whom I owe everything. They have been the greatest support and inspiration to me, and the best life companions I could have ever desired, and there is no need to add any redundant words. Thank you.

Abstract

In disease ecology, the relationship between biodiversity and pathogen transmission is still under investigation. In particular, the dilution effect, namely that higher biodiversity decreases disease transmission, is currently the most debated eco-epidemiological theory in the context of multi-host pathogen systems. Mechanisms of dilution include transmission and encounter reduction, and susceptible host regulation.

This study integrated empirical data and mathematical modelling to investigate the transmission of parasites and pathogens in Welsh wild rodent communities, as rodents are considered an ideal system to study multi-host parasite/pathogen transmission in the eco-epidemiological context. Rodents were live-trapped and faecal samples and ecto-parasites were screened for parasites and pathogens. Field data were used, where relevant, to parameterise models of infection that investigated the effects of parameter variation and community composition on pathogens with different transmission modes. The final aims were to provide additional knowledge on Welsh rodent communities, to identify rodent-borne parasites/pathogens circulating in the sampling area, and to improve understanding of local transmission dynamics, testing the dilution effect through eco-epidemiological modelling.

The main results from the parasite and pathogen screening were: a. the observation of host heterogeneity in ecto-parasite and macroparasite prevalence and burden, with different host species contributing in different ways to the transmission pool; b. the isolation of *Anaplasma phagocytophilum* and *Babesia microti* in ixodid ticks; c. *Bartonella* spp. were isolated in fleas, *B. rochalimae,* notably, for the first time in the UK.

The directly transmitted pathogen model outputs confirmed that reduced (or "diluted") infection prevalence might not represent a true dilution effect to some hosts, since prevalence could decrease simultaneously with the increase of infectious individuals. The model was effective in recognising susceptible host regulation via inter-specific competition and predation as the most important dilution mechanism.

Modelling the two similar but different host-tick-pathogen systems showed that the parameters affecting the juvenile stages of the ticks were the ones most affecting pathogen transmission: crucial information to develop targeted control strategies. In the system with the more generalist vector, *Ixodes ricinus*, dilution effect was more significant and more dilution mechanisms were observed. The key parameters regulating transmission were also different between the two systems, but the dilution was observed only with regards to infectious hosts, as more complex communities led to amplification of infectious nymphs, representing amplified human disease risk.

With regards to the flea-borne *Bartonella*, force of infection and proportion of hosts transmitting vertically were the parameters most affecting transmission and degree of the dilution, which occurred through the mechanism of regulation of susceptible hosts, providing evidence that community composition was crucial to the dynamics of pathogen transmission. The average flea burden of infested hosts was another important parameter, which was estimated from empirical data, demonstrating the importance of field data collection. Finally, in each system, the parameters most affecting pathogen transmission, were also the most uncertain.

This study supported the idea that the dilution effect is not a universal principle, but it can be observed under certain conditions considering the appropriate epidemiological metric. Nonetheless, in the context of pathogen emergence risk factors were identified, especially alteration of biological communities caused by human disturbance. Hence, it may be more sensible to investigate local pathogen dynamics, gather data, and develop specific control measures instead of trying to find a one-fits-all disease-diversity relationship. In conclusion, the eco-epidemiological approach, overcoming boundaries between disciplines, is crucial to investigate and control wildlife pathogens, to conserve biodiversity, and reduce human disease risk.

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Chapter 1

General introduction

1.1 The ecosystemic approach in disease ecology

Disease ecology – the "ecological study of host-pathogen interactions within the context of their environment and evolution" (Kilpatrick and Altizer, 2010) – is gaining increasing attention due to the current context of rapid biodiversity decline and environmental change, which have been hypothesised to play a crucial role in the increasing of emerging/re-emerging diseases. Before the Nineties, this discipline mainly focused on the population scale impact of parasites and epidemics (e.g. Rinderpest in Africa), but, in the last few decades, investigations targeted within-host processes (due to advances in areas such as immunology) and community and ecosystem scale processes (Tompkins *et al.*, 2011).

Disease ecology, in a conservation perspective, is essential because parasites and pathogens can shape species communities (Roche and Guégan, 2011), and host-pathogen relationship affects co-evolution, altering species persistence (Decaestecker *et al.*, 2013). Antagonistic co-evolution might boost host genetic diversity, at the host community level and within host-species, increasing survival through the accumulation of resistance alleles (Decaestecker *et al.*, 2013). Therefore, in order to undertake effective conservation strategies, it is vital to preserve also evolutionary processes at the host-pathogen interface. Understanding existing and emerging pathogen dynamics may be crucial to balance the risks diseases pose to endangered species and the evolutionary processes that are necessary to maintain viable wildlife populations, especially

now that human pressures are affecting changes in pathogen biology (Altizer *et al.*, 2003).

According to De Castro and Bolker (2005) and Smith *et al.* (2009a) disease mediated extinctions have been underestimated: pathogens are not only directly responsible for extinctions but they are likely to affect population fitness negatively, and to cause more severe outcomes in small, isolated and less genetically variable populations (Pedersen *et al.*, 2007; Smith *et al.*, 2009a). Furthermore, many disease agents are not subject to population density thresholds and they can survive in very small host populations (Lloyd-Smith *et al.*, 2005), or they can persist in a reservoir host, causing a major impact in other, more susceptible hosts (Smith *et al.*, 2009a). The drivers of these phenomena appear to be: - habitat loss and alterations that restrict species movement, increase individual contact rates and so the chance of a disease spreading, deplete food resources and decrease genetic variability; - climate change that affects, directly or indirectly, pathogen survival, transmission, host susceptibility, and hosts' and vectors' abundance and distribution (Harvell *et al.*, 2002); - increase in domestic-wildlife species contact that enhances the transmission of domestic animals diseases to wildlife; - increase of invasive species that introduce new pathogens in native species (Smith *et al.*, 2009a).

Regarding human zoonotic risk, the ecosystemic approach in disease ecology has revealed that environmental modifications and wildlife communities' alteration induced by anthropogenic disturbance might determine an intensification of the emergence of zoonoses (Pongsiri *et al.*, 2009). For example, overfishing of mollusc-eating fish in Lake Malawi has resulted in a greater number of *Bulinus* sp. gastropods (intermediate host) and the subsequent spread of schistosomiasis (Stauffer *et al.*, 2006). Nevertheless, the causal mechanisms are not fully understood and the complexity of the human-natural systems in which hosts, pathogens, and vectors interact makes it extremely difficult to assess general patterns of disease emergence or epidemics (Wilcox and Gubler, 2005).

In the mentioned example, biodiversity is considered to provide, as an "ecosystem service", protection from disease transmission. Ecosystems have been recognised to absorb and recycle nutrients, produce biomass, food, water, and to realise many other functions that are assumed to be useful for humans, and are so called "ecosystem services" (Millennium Ecosystem Assessment Program, 2005). Human pressures, such as habitat fragmentation, alteration, and loss, reduce biodiversity directly and indirectly (Fahrig, 2003), and this in turn "reduces the efficiency by which ecological communities capture biologically essential resources, produce biomass, decompose and recycle biologically essential nutrients" (Cardinale *et al.*, 2012). Biodiversity amplifies the stability of ecosystem functions, even if the impact of it on each ecosystem process is nonlinear, and it seems to be influenced by the loss of key species, which exhibit specific functional traits with major effects on ecosystems (Chapin *et al.*, 2000; Tilman, 2000). However, some researchers have highlighted the potential for ecosystems to create "disservices" (e.g. von Doehren and Haase, 2015), and in particular the likelihood of increasing the emergence of new zoonotic diseases (Dunn *et al.*, 2010; Jones *et al.,* 2008a). If this is true then conservation efforts may be threatened by concerns about disease emergence (Ostfeld and Keesing, 2017).

In order to analyse the connection between biodiversity and human zoonotic disease emergence, Ostfeld and Keesing (2017) evaluated evidence of the three assumptions in the suggested causal chain linking high vertebrate diversity with the probability of emergence of infectious diseases. The three assumptions are:

1. The more species of vertebrates there are in a specific location, the more total vertebrate-borne pathogen species are present there. To satisfy this assumption, each vertebrate species has to harbour at least some unique pathogens; otherwise, if most pathogens are shared among hosts, the total number of host species is less important.
2. The more pathogen species occur in a specific area, the higher the percentage of potentially zoonotic species. For this assumption to be true, zoonotic pathogens have to be almost equally distributed across all

vertebrate pathogens. In fact, if zoonotic pathogens are predominant in a restricted range of host taxa, then the total number of host species becomes less important.

3. The more potentially zoonotic pathogens are present in an area, the more human disease can be expected.

Regarding the first statement, the relationship between pathogens and hosts has been found to be not linear, and, in most cases, the increase of host species did not lead to an increase in species richness of pathogens (e.g. Dobson *et al.*, 2008). Nonetheless, the relationship between vertebrates and viruses and bacteria diversity, which are the dominant groups of emerging infectious zoonoses, is generally unknown (Jones *et al.*, 2008a). Ultimately, other sources of non-linearity in the relationship can be host sharing and differences in species–area accumulation curves of hosts and pathogens.

In the second case, no formal examination of the correlation between total vertebrates pathogen richness and zoonotic (potential or actual) pathogens has been performed, but it is recognised that some host taxa are much more likely to harbour zoonotic pathogens (Han *et al.*, 2015). According to Han *et al.* (2016), rodents, and secondarily carnivores, are the taxa more frequently involved in zoonotic transmission, and the ones with higher zoonotic reservoir potential. In particular, they identify 244 species of rodents hosting 85 unique zoonotic pathogens. Also, communities usually consist of few highly abundant species and an increasing number of more rare species (Magurran, 2004), so, when moving from less to more diverse vertebrate communities, it is possible to notice an accumulation of rare species. Therefore, if rare species were involved in zoonotic transmission, then the second assumption would be supported; however, the above mentioned study suggests that the more abundant and ubiquitous vertebrate species, e.g. rodents, are more often responsible for zoonoses and more suitable to act as reservoir hosts for zoonotic pathogens (see section 1.2.1). Beyond doubt, in order to understand the link between vertebrate and zoonotic pathogen diversity, it is also important to understand

community composition/structure and the relative abundance of species along different gradients of diversity (Ostfeld and Keesing, 2017).

Dunn *et al.* (2010) examined one aspect related to the third assumption; focusing on some zoonotic diseases they investigated whether the status (endemic, sporadic, or non-endemic) of the diseases was correlated, in each country, with the total species richness of human pathogens (bacteria, viruses, helminths, and protists). The results were that pathogen richness was positively correlated to endemicity; however, per capita spending on health care was the strongest negative predictor, while minimum actual evapotranspiration the strongest positive predictor, of endemicity of the chosen diseases. In fact, studies suggest that interaction between pathogens can lower disease severity through mutual interference, and that pathogen diversity is not adequate as a metric of epidemiological impact, which should be measured using prevalence of infection (Johnson *et al.*, 2015a). Finally, Han *et al.* (2016) did not find any correlation between zoonotic host diversity and zoonotic pathogen diversity, and discovered that despite higher zoonotic host diversity at lower latitudes, the diversity of zoonotic pathogens was rather evenly distributed across latitudes.

Considering the above, it is difficult to draw a definitive conclusion due to the complexity of host-parasite interaction and community composition, but an area of agreement between scientists is that human disturbance is progressively increasing both risk of transmission and/or human exposure to actual or potential zoonotic pathogens. For example, pathogen spillover from wildlife to humans is occurring more often in disturbed habitats and in cases of increased exposure through specific human activities, such as hunting, laboratory work, veterinary practice, etc. (Johnson *et al.*, 2015b). Nevertheless, a spillover event (i.e. when a pathogen jumps from one species to another) is just the first step needed for a disease to "emerge". And evidence has been found to corroborate the hypothesis that high biodiversity inhibits the other steps required for disease emergence (establishment, spread, and impact on hosts) (Keesing *et al.*, 2010; Ostfeld and Keesing, 2012; Johnson *et al.* 2015b).

The ecosystemic approach, and the application of principles of community ecology, is therefore essential in understanding wildlife disease ecology, since infectious diseases are often the result of multi-species interactions (Johnson *et al.*, 2015b). Research in disease ecology typically involves one of the following levels: a. within- host, concerning the interactions between host immune system and parasites; b. between-host, focusing on parasite spread in host populations; c. regional/biogeographical scales, using methods from macro-ecology.

The processes at those levels and in between the levels are hard to interpret only from observational data so methodologies such as field experiments and eco-epidemiological modelling are increasingly used to shed light on the nexus between biodiversity and disease transmission.

In conclusion, "the degree to which biodiversity will regulate infection by a particular parasite depends on: the degree to which host assembly is deterministic; whether the parasite is niche or dispersal limited; how the increase in richness affect host and vector abundance" (Johnson *et al.*, 2015b).

1.2 Dilution effect: definitions, mechanisms, examples, and critique

In the previous section it was emphasised how crucial it is to consider the whole community when investigating wildlife disease transmission, especially when interested in making predictions for wildlife conservation or human zoonotic risk. In this section, dilution effect (one of the most prominent theory in disease ecology) will be discussed, and examples from previous studies will be provided to describe it.

Several studies in wildlife disease ecology, especially regarding vector-borne diseases, supported the hypothesis that higher biodiversity may reduce disease prevalence in the most competent host, reducing disease transmission and disease risk to humans (Keesing *et al.*, 2010) through what has been called the dilution effect (Ostfeld and Keesing, 2000). That is, an increase in host diversity, altering the proportion of the most competent host population, results in lower disease prevalence (Schmidt and Ostfeld, 2001). This phenomenon has been theorised to be an ecosystem service, negatively affected by biodiversity loss (Keesing *et al.*, 2010).

Ostfeld and Keesing (2000) are among the first to focus explicitly on the dilution effect, coining the term, in the context of Lyme disease in USA. They presented a conceptual model of how Lyme disease (caused by the spirochaete *Borrelia burgdorferi* through the bite of an ixodid tick) risk for humans is reduced in regions with higher host species richness and evenness. In North America, where the most competent reservoir host of *B. burgdorferi* is the white-footed mouse (*Peromyscus leucopus*), Ostfeld and Keesing (2000) showed that the prevalence of infection in ticks was lower in areas where the density and/or the proportion of the most competent host were altered by the presence of other, less competent, host species. Non-competent Lyme disease host-species, providing alternative blood meals for ixodid ticks, “dilute the power of the white-footed mouse to infect ticks by causing more ticks to feed on inefficient

disease reservoirs" (Ostfeld and Keesing, 2000). Nevertheless, in this case study, the species richness of ground-dwelling birds, competent hosts for the spirochaete and the tick, was positively correlated with per capita Lyme disease cases, proving that prevalence of vectors might increase with increasing host-diversity when they acquire disease agents effectively from many hosts. The concept of the dilution effect was explored further and modelled mathematically by Schmidt and Ostfeld (2001), using the same Lyme disease system. Using an empirically based model, they inferred the role of additional non-competent host-species in order to quantify the diluting effect. They used simulations to assemble host communities, which had different species richness, species evenness, and net interactions between alternative hosts and mice. They found that increasing species richness - but not evenness - reduced disease risk, and the greater the abundance of less competent reservoir species, the stronger the dilution effect. The assumed conditions to be met for the dilution to occur were: -the presence of a generalist vector; - variable reservoir competence among hosts; - absence (or low degree) of vertical transmission in vectors; - the species most likely to persist when diversity declines support a greater abundance of pathogen and/or vector (conversely, those in more diverse environments tend to be less competent hosts); - the species most likely to occur when diversity increases diminish contact rates between most competent hosts and pathogens or their abundance (Ostfeld and Keesing, 2012; Schmidt and Ostfeld, 2001). Ostfeld and Keesing (2012) demonstrated that there are different levels of competence among hosts in the majority of host-pathogen interactions, and there is evidence that the last two conditions are also frequently met (Johnson and Thieltges, 2010; Ostfeld and Keesing, 2000; Pongsiri *et al.*, 2009), but their generality has not been fully established yet.

In plants, amphibians and rodents, it has been shown that communities might have nested patterns of species composition along diversity gradients (i.e. some species tend to be more abundant and ubiquitous, while the rarer species are progressively lost when diversity decreases), and there might also be a positive correlation between resilience and host competence (Cronin *et al.*, 2010; Johnson *et al.*, 2012b; Previtali *et al.*, 2012; see section 1.2.1). On the other hand,

the dilution effect is expected not to occur in the case of highly specialized pathogens or vectors, and to be nullified where a single host does not allow the pathogen to be maintained and transmitted (Ostfeld and Keesing, 2012).

The potential mechanisms underlying dilution are shown in Box 1.1.

Box 1.1. The dilution effect mechanisms theorised in the influential paper by Keesing *et al.* (2006).

a. Reduction in space use (e.g. sympatric species), and consequent encounter reduction between susceptible and infected individuals.

b. Reduction of the probability of transmission because of species sharing the same vector but unable, or less competent, to infect it.

c. Reduction of the number of susceptible hosts (e.g. by predation).

d. Increasing of the recovery rate of infected individuals.

e. Increasing of the mortality rate of infected individuals.

Additional mechanisms are not excluded, and, in sum, the dilution effect is intended as the net effect of species diversity reducing disease risk by any of a variety of mechanisms (Keesing *et al.*, 2006).

Lyme disease is not the only example in support of this theory. Ezenwa *et al.* (2006) found that non-passerine species richness was negatively correlated with both mosquito and human West Nile Virus (WNV) infection rates, although there was no significant relationship between passerine species richness and measures of infection risk. These results, in alignment with the dilution effect hypothesis, suggest that non-passerine diversity might decrease WNV growth in mosquitoes, minimizing human disease risk. Furthermore, in the context of WNV, it has been found that northern cardinals and other birds of the family Mimidae act as infection "supersuppressors" attracting mosquitoes at the critical time of the year providing protection against human spillover and further evidence of dilution (Levine *et al.*, 2016).

Kedem *et al.* (2014), in a natural gradient of rodent-species richness, examined the possible mechanisms mediating the relationship between host-species

richness and microbial prevalence, along with microbe specificity. They identified two dominant flea-borne bacterial lineages (host-specific bacteria, and host-opportunistic bacteria), and observed that host-species richness affected prevalence of these bacteria directly and indirectly. The host-specific bacteria were less abundant in richer host communities, probably because of the increased frequency and density of the less suitable host-species. Regarding the opportunistic lineage, host-species richness had direct and indirect effects, largely mediated by modifications of flea densities and by the presence of the other bacterial lineage. The results provided evidence that host-species richness is associated with multiple elements of community structure, including density and proportion of the different host-species, total host densities, vector densities, and microbial co-infection rates. Also, they supported the mechanisms described in Keesing *et al.* (2006), transmission reduction, encounter reduction, susceptible host regulation, infected-host mortality, vector regulation, and co-infection, confirming that these are not mutually exclusive.

In another study about rodent-borne diseases, experimental large mammal defaunation was carried out in a savannah ecosystem in East Africa to test how this would have affected rodent populations, and infection prevalence of *Bartonella* spp. in rodents (host-species) and fleas (vector species) (Young *et al.*, 2014). Results described no increase in infection prevalence in rodents or vectors, but bacterial abundance doubled, doubling also the density of infected hosts and fleas; these findings reinforce the ideas that large wildlife defaunation increases landscape-level disease transmission through the decrease of susceptible host regulation, and that there is an host/vector density response in biodiversity-disease relationships. In fact, investigations on the effects of an introduced species on native host-parasite interactions in a rodent community in Ireland showed that the flea-borne *Bartonella* spp. prevalence in the native wood mouse (*Apodemus sylvaticus*) populations declined significantly in response to the introduction of bank voles (*Myodes glareolus*), even if the infection was detected only in wood mice (Telfer *et al.*, 2005).

Dilution has not been suggested only in vector-borne diseases; in the case of Hantavirus Pulmonary Syndrome (HPS) and other diseases directly transmitted by a broad range of Hantaviruses, small mammal diversity (primary reservoir hosts) was negatively associated with human disease risk in different locations (Clay *et al.*, 2009a; Ruedas *et al.*, 2004; Suzán *et al.*, 2009; Yahnke *et al.*, 2001).

Venesky *et al.* (2014) showed a dilution effect with both laboratory and field data, and demonstrated that dilution and amplification are strongly related to definite host traits. They were among the first researchers linking manipulative experiments with the analysis of field data on species richness, host identity, spatial autocorrelation and disease prevalence, in order to ascertain causality and ecological relevance. They used an amphibian-*Batrachochytrium dendrobatidis* system, due to the global pathogenic importance of this fungus, to test the capacity of different amphibian species to filter fungal spores from the water column, and the consistency between laboratory results and field patterns of amphibian species richness, host identity and disease prevalence. Results demonstrated that spores declined as a function of tadpole diversity; in particular, dilution was enhanced by the presence of specific species, which were seen to be capable to filter, and consequently remove, a remarkable quantity of spores from the aquatic environment. These findings suggest that species identity is a key metric to evaluate pathogen dilution, and community composition influences the magnitude of the dilution effect.

In order to investigate community composition in the context of dilution effect, Johnson *et al.* (2012a) performed wetland surveys of host community structure and linked them with mechanistic experiments on a multi-host parasite system. They discovered that snail host communities were strongly nested: the competent hosts for the selected parasite (the trematode *Ribeiroia ondatrae*) prevailed in low-richness assemblages, whereas unsuitable host species increased with the increase of assemblage diversity. Further, density of the most competent host was negatively associated with snail species richness.

Orlofske *et al.* (2012) expanded the experiments above, including not only host-species, but also other constituents of the community under investigation (e.g.

predators, sympatric species, etc.) to identify the various mechanisms altering parasite transmission. In brief, presence of either predators or alternative hosts remarkably reduced infection in the most competent host, suggesting the importance of evaluating the complex dynamics of the entire community when estimating the effects of diversity on pathogen prevalence.

In Britain, an investigation on long term (1992-2000) data about amphibian mortality highlighted that frog mortality due to Ranavirus was associated with anthropogenic abiotic alteration of the habitat, in particular, disease prevalence was positively correlated with frog population density and negatively correlated with the presence of alternative hosts (North *et al.*, 2015).

Another element to take into account to understand these effects is the spatial scale, which can affect the results of different studies. A review on zoonotic risk of Lyme disease showed that opposite results have been obtained when considering different spatial scales (Wood and Lafferty, 2013). Considering a landscape scale (urban to suburban to rural), strong evidence for a positive link between forestation and/or biodiversity and Lyme disease risk was found, whereas the traditional, local, perspective might lead to opposite predictions (Wood and Lafferty, 2013). At the host scale dilution is supported considering that the ability of a pathogen to establish itself in a host population is negatively correlated with the number of genotypes in the population, and the infection disappears more quickly in a more genetically diverse population (Lively, 2010). Similarly, a negative relationship between diversity and disease is observed in the context of microbiomes (Hanski, 2014); for instance, healthy corals have been found to sustain more diverse microbial communities than those affected by white plague disease (Sunagawa *et al.*, 2009).

To summarise, from these examples, it can be theorised that dilution is expected in a disease system when host species have different degree of competence in maintaining and transmitting the focal pathogen, and encounters between susceptible and infectious hosts (or vectors) are dominant in lower diversity communities. The second condition implies that most competent host species tend to persist as biodiversity is lost (nested community structure), and are

common across a wide range of community assemblages (Johnson *et al.*, 2015a). The research has been further expanded including in the picture the diversity of the system beyond host species, namely including ecological relationships such as predation, competition and co-infection. These components of diversity may affect disease transmission in more complex ways; interactions with non-host species can influence host species abundance/distribution and community structure and so, indirectly, disease transmission and the potential dilution effect (Buhnerkempe *et al.*, 2015). In conclusion, species that are more likely to be present in more diverse assemblages, in order to exhibit dilution power, need to reduce one or more of the following: abundance, susceptibility or tolerance of competent species; encounters between competent species and pathogen; encounters between competent species and vectors; overall competence of the host community; abundance of the pathogen; abundance of vectors (Johnson *et al.*, 2015a). So, the research focus is shifted from species richness to species identity and to functional groups and community composition.

Despite evidence suggesting that dilution conditions exist in a wide variety of ecological systems, and observations of dilution effect having been recorded for micro and macroparasites, in both aquatic and terrestrial systems, and in various types of host communities, researchers are still debating the question. In fact, support to the opposing theory, amplification effect – i.e. increased host diversity increases disease transmission – can also be found in the scientific literature.

Randolph and Dobson (2012), in a very influential review paper, revised the dilution effect hypothesis, stating that this can be actually relevant to simple host-pathogen systems, but that it can rarely apply to complex systems, typical of many zoonoses and vector-borne diseases. The first pitfall found was that, generally, research in support of a dilution effect did not distinguish between functional diversity (e.g. species identity, specific host features), species richness, or species evenness. In addition, they reviewed the theoretical and empirical evidence in favour of the hypothesis and concluded that dilution is

rarely supported, even amongst vector-borne diseases, which are the most studied in the dilution framework, because most of the studies considered only the prevalence and not the abundance of infected vectors. Their final conclusion was that a dilution or amplification effect depends specifically on community composition rather than biodiversity per se. Cardinale *et al.* (2012) extensively reviewed literature about ecosystem services and concluded that there is still no strong support for the dilution effect; it is not clear how general the dilution effect is and what exactly it means, e.g. reduction of zoonotic risk, reduction of spillovers, reduction of pathogen establishment probability (Wolfe *et al.*, 2007). The concept that higher biodiversity reduces infectious disease prevalence is also criticised by Salkeld *et al.* (2013), whose extensive meta-analysis suggests that the relationship between biodiversity and zoonotic disease risk is idiosyncratic. While a dilution effect would be appealing because of the positive implications in wildlife conservation and public health, their results show a highly heterogeneous relationship between host diversity and disease, mainly determined by specific local conditions and by the ecology of hosts, pathogens, and vectors. According to Salkeld *et al.* (2013), controlled field experiments with removal of specific hosts or vectors are necessary to understand, locally, disease ecology and the relationship between all the species in the system, and what are the influences on disease transmission (and biodiversity) of other factors such as rainfall, anthropogenic disturbance, human behaviour, latitude, etc.

In addition, other studies involving mathematical modelling and/or collecting data from the field (also field experiments) did not get results in favour of the dilution hypothesis. A case of amplification has been provided in the system grouse-hare-deer-louping ill virus (transmitted by ticks) in Britain. The system with the three hosts, instead of one or two, enabled disease persistence and amplified prevalence, increasing abundance of the vector (Gilbert *et al.*, 2001). Other studies found that higher host species diversity corresponded to a decreased prevalence but also to an increased infectious population, reinforcing the idea that empirical observations of dilution might be a misinterpretation of field data (Roche *et al.*, 2012). States *et al.* (2014) compared *Borrelia burgdorferi*

infection prevalence in *Ixodes scapularis* nymphs, density of infected nymphs, and *Borrelia burgdorferi* genotype diversity at different sites and observed that nymphal infection prevalence was similar across sites where the most competent host dominated small mammal community and sites with higher species richness and evenness. Further, the density of *Borrelia burgdorferi* infected nymphs, in contrast with the dilution hypothesis, was higher in the more diverse sites. An amplification effect was also recognised by Thoma *et al.* (2014), who observed a positive effect of biodiversity on Puumala Virus (PUUV) prevalence in Central Europe, a finding that contrasts what has been documented for other Hantaviruses elsewhere (e.g. SNV, Choclo Virus, Laguna Negra Virus, and Bayou virus). It has been hypothesised that the particular composition of the small mammal community was responsible for this result. Central European small mammal communities are naturally less diverse than American ones, so American Hantaviruses might have several reservoir species and transmission might occur between species (Peixoto and Abramson, 2006), whereas it appears that only the bank vole is the reservoir species of PUUV (Thoma *et al.*, 2014).

In conclusion, although there is theoretical and experimental support for the dilution effect, the likelihood that biodiversity can provide protection from human zoonotic diseases it is still controversial (Wood *et al.*, 2014), and it is the variability in community composition that significantly affects the intensity of pathogen transmission (Roche *et al.*, 2012). Indeed, there are still open questions regarding the subject, and, as mentioned before, the current research focuses are: identifying diluting species traits and combining community ecology principles with disease ecology (Johnson *et al.*, 2015b), and linking anthropogenic change with its effects on biodiversity and disease emergence (Hosseini *et al.*, 2017). Also, from the pathogens' perspective, in order to assess the generality of dilution and predict disease emergence, it is important to understand the factors that determine host generalism, since identifying pathogens that are likely to have still unknown additional hosts can be a tool in evaluating transmission risk (Walker *et al.*, 2017).

1.2.1 Reservoir competence and species resilience

The reservoir host of an infectious disease is the source of infection and is able, alone, to sustain the pathogen in a population, while reservoir community refers to the minimum set of hosts who are necessary to sustain the pathogen in the population (Nishiura *et al.*, 2009). The reservoir potential is considered to be a function of numerous epidemiological parameters: host susceptibility, host infectivity, and duration of infectiousness (Huang *et al.*, 2013). This concept has been linked to numerous species life history traits such as life span, body size and reproductive rate and it has been hypothesised that species with faster life histories, investing less in immunological defence, are more competent reservoir hosts (Previtali *et al.*, 2012). Species with faster life histories are predicted to invest more in innate immune responses, which include non-specific defences against a wide variety of pathogens, while long-lived species are predicted to invest more in adaptive immune responses, highly pathogen-specific (Previtali *et al.*, 2012). These predictions have been tested in a rodent community inhabiting deciduous forests in north-eastern United States and the results were consistent with the pace-of-life immune-defence hypothesis (Previtali *et al.*, 2012). Other evidence of this relationship has been provided by the positive relationship between natural antibody levels and incubation period in bird and mammal species, suggesting that longer developmental times are associated with an improved adaptive immune system (Lee *et al.*, 2008). However, Huang *et al.* (2013) did not find any significant relationship between incubation/gestation period and reservoir competence. In fact, Huang *et al.* (2013) used several life-history traits (body mass, incubation/gestation time, and clutch/litter size), surrogates of other characteristics linked to immune response such as metabolic rate, to test the relationship between these and reservoir competence. Their results showed that life history traits could explain some interspecific variation in reservoir competence, and in particular, body mass was a strong predictor of the reservoir competence in Lyme disease. Further, fast-lived amphibian species were found to be more prone to be

infected by a trematode (*Ribeiroia ondatrae*), acting as amplifying hosts in the community (Johnson *et al.*, 2012a).

Based on these results, it is expected that species with a high reservoir competence are more likely to be those that are more widespread and more resilient. In fact, species with faster life histories usually have lower energetic requirements and higher reproductive rates, which increase their survival likelihood in disturbed, low diversity habitats (Cardillo, 2003). This is confirmed by the studies linking species traits and extinction risk. In general, species with slower life histories are less resilient; also, species that needs larger home ranges are subjected to a higher extirpation risk (Joseph *et al.*, 2013). Species at higher trophic levels are also more prone to local extirpations due to their demography and, in some cases, direct human impact (Duffy, 2003). Thus, assuming the nested nature of communities (Almeida-Neto *et al.*, 2007; Wright *et al.*, 1998), it appears that the species lost in the less diverse assemblages are also those less responsible for disease transmission, also determining a higher abundance of more competent species in low diversity systems due to release from competition or predation (Joseph *et al.*, 2013). This might be one of the conditions that underlie the dilution effect together with what was stated in the previous sections.

An example is provided by the amphibian survey in 345 wetlands performed by Johnson *et al.* (2013) in California (USA). The amphibian community composition of the wetlands changed in a non-random fashion, with the highly competent host species for the trematode *Ribeiroia ondatrae* dominating the less diverse specie assemblages, whereas more resistant host species were progressively more common in more diverse communities. Therefore, amphibian species richness strongly diluted parasite transmission, with a 78.4% decline in realised transmission in more diverse assemblages. The study reveals a consistent link between species richness and community competence, emphasising the importance of the community-based approach in the investigation of infectious diseases.

Parasite evolution and in particular local adaptation of pathogens has also been suggested as a possible mechanism for dilution, since the selective pressure of losing hosts during community disassembly may lead the parasites to evolve and infect the most abundant or widespread hosts, boosting the negative relationship between extirpation risk and host competence (Ostfeld and Keesing, 2000).

1.3 Disease ecology in the current context of anthropogenic change

In the context of disease ecology, there is general agreement that the variability of results obtained by different studies investigating the relationship between biodiversity and pathogen transmission/emergence is due to specific species traits, community disassembly/assembly rules, and epidemiological metrics considered. The majority of the studies focuses on how biodiversity affects disease dynamics and how this is related to human disease risk and spillover; however, this seems to be in tight connection to anthropogenic change, which appears to be a major cause of disease emergence (Hosseini *et al.*, 2017). Not taking into consideration habitat disturbance may lead to biased conclusions, which do not account for functional diversity, and for the processes that convert hazards (pathogens) into risks (disease outbreaks) (Hosseini *et al.*, 2017). For example, it has been suggested that human and wildlife emerging infectious diseases (EIDs), such as amphibian chytridiomycosis, Nipah virus disease, and West Nile Virus (WNV), are essentially caused by the new ecological conditions produced by human environmental disturbance, creating a vicious circle of negative impact on biodiversity (Daszak *et al.*, 2001). Also, it has been hypothesised that biodiversity, being the natural reservoir for infectious diseases, may be considered the hazard (i.e. disease emergence), but it does not necessarily represent the risk since biodiversity hotspots are not often disease emergence hotspots, while emergence is more often related to human related factors (Hosseini *et al.*, 2017; Jones *et al.*, 2008). An example could be the Ebola virus, which circulates in wildlife and can be considered a hazard. However, the risk of an outbreak in the human population is related to various risk factors, mostly related to human activities (e.g. bushmeat consumption, forest fragmentation and access to deep forest areas, etc.) that affect the likelihood and the magnitude of the actual risk (Ezenwa *et al.*, 2015).

Humans determine environmental changes at different levels, on a global scale, climate change affects host and vector distribution, influencing vector-borne diseases (e.g. increased temperatures at higher latitudes/elevations allow parasites and vectors to expand their range) (Mills *et al.*, 2010), but also directly transmitted diseases, as in the case of Hantaviruses (Dearing and Dizney, 2010). Similarly, urbanization alters disease transmission routes, and may increase zoonotic risk, for example altering species behaviour. Urban and peri-urban resource availability facilitates contacts between humans and urban-adapted wildlife species, and between wildlife and domestic species, which can also be non-native species, boosting pathogen circulation and adaptation in new hosts (Bradley and Altizer, 2007). Moreover, human-modified landscapes may impact wildlife susceptibility to diseases through contaminants, pollutant, zooanthroponoses (Messenger *et al.*, 2014), and stress increase (Brearley *et al.*, 2013). The links between habitat modifications (e.g. matrix hostility, loss of connectivity), physiological stress and disease susceptibility are not completely clear yet, but it has been established that elevated stress levels decrease immunity, and are likely to reduce recovery rates (Brearley *et al.*, 2013). Also, the amount, type and scale of disturbance may change the outcomes in terms of wildlife disease transmission and human disease risk (Gottdenker *et al.*, 2014). An exemplar case is the Puumala virus (PUUV) in Belgium, where human infection risk is higher in partially disturbed landscapes than in deforested areas (Linard *et al.*, 2007; Tersago *et al.*, 2008). In contrast, small mammals' seroprevalence of Hantaviruses in Panama was higher in disturbed and edge habitats than in forested areas (Suzán *et al.*, 2008), and a negative relationship between Sin Nombre virus (SNV) prevalence in deer mice and disturbance was found in North America (Lehmer *et al.*, 2014). The last finding was not determined by the higher density of the most competent host, but by a reduced long-term survival of deer mice, including infected individuals, along a gradient of disturbance, emphasising the difficulty of predicting prevalence patterns due to the complex interconnections among population demography and habitat quality. The change in land use and the increase of connectivity through global travel, trade and introduction of non-native species can create novel species

associations and, indirectly, alter associations of hosts, parasites and vectors (Rogalski *et al.*, 2017). Young *et al.* (2016) provide a very comprehensive summary of the potential effects of the introduction of alien species on native communities and pathogen richness and prevalence. For example, an invasive species can dilute or amplify a native pathogen depending on its susceptibility and competence to transmit it; in alternative, invasive species might carry invasive pathogens that might spread in the native host community. Also, introduced pathogens can adapt altering their life cycle, evolving specialisation or generalism, and modifying other features such as longevity, reproductive strategy, host breadth, etc. (Cable *et al.*, 2017).

Human pressures are directly and indirectly selecting for new pathogens (Hulme, 2014); large-scale changes to the environment caused by human are affecting the evolution of wildlife pathogens, leading to evolutionary shifts in parasite characters through their effects on parasite survival and reproduction (Vander Wal *et al.*, 2014). A remarkable example comes from the analysis of the genetics and evolution of oral transmission in *Toxoplasma gondii*, which indicate that the parasite started to bypass sexual recombination in favour of oral transmission relatively recently, in coincidence with human agricultural expansion (Su *et al.*, 2003). Therefore, human actions influence host-parasite co-evolutionary and eco-evolutionary dynamics at the same time, making it difficult to interpret the observations from the field (Rogalski *et al.*, 2017).

Lambin *et al.* (2010) identified general principles of landscape epidemiology using eight case studies from Europe and West Africa (e.g. WNV in Senegal, PUUV in Belgium, malaria in the Camargue), with the aim of distinguishing and estimating the effects of land changes on emerging/re-emerging vector-borne diseases and/or zoonoses. They concluded that variation in disease risk does not only depend on the presence and extension of a critical habitat, but also on its spatial configuration, e.g. connectivity, which is crucial for vector and/or host distribution. Landscape, in terms of land cover and land use (management, ownership, human behaviour), might be considered as a proxy for specific associations of reservoir hosts and vectors, and together with climate might

regulate the emergence and the spatial concentration/diffusion of infection risk. In the context of disease transmission, they advocated for a more dynamic view of landscapes that includes social and ecological processes, spatial and temporal interactions between habitats, human activities, hosts and vectors. The case studies showed that spatial variations in disease risk were regulated by three groups of factors: a. biology of vectors, hosts and pathogens with their pathogenic cycle; b. ecosystem processes at a landscape scale, including ecosystem structure, connectivity, configuration, climate, species interactions; c. human factors, such as land use, human behaviour, socio-economic and cultural conditions.

In this complex scenario, the focus in disease ecology is on functional diversity, and so, the investigation of host or pathogen traits that make them more likely to be involved in disease emergence, that make a host more susceptible to an outbreak, or that may determine host capability of diluting or amplifying certain pathogens (Ostfeld and LoGiudice, 2003; Venesky *et al.*, 2014). It appears that the most effective metric to measure biodiversity in relation to disease transmission may be species identity rather than species richness, and, as mentioned in the previous sections, the loss of particular species, even the order in which they are lost, might radically change disease transmission (Venesky *et al.*, 2014). Hence, the knowledge required to fully understand the relationship between biodiversity and pathogen prevalence includes the functional role played by each species and the sequence with which species are added to or lost from communities in nature (Keesing *et al.*, 2010; Ostfeld and LoGiudice, 2003). This knowledge is rarely available, and despite the examples provided in section 1.2.1, a general validation for the theory that the most resilient species are also the most competent reservoir hosts is still lacking. As it is knowledge is still lacking about the functional consequences of a decline in biodiversity in a whole community (multiple trophic groups) (Soliveres *et al.*, 2016).

Ostfeld and LoGiudice (2003) tried to address this lack of knowledge using simulation models to assess the effect of the sequence of species loss from vertebrate communities on human exposure risk to Lyme disease. They

simulated plausible sequences of species loss from landscapes exposed to anthropogenic disturbance, such as deforestation and fragmentation, removing species according to specific criteria: a. decreasing order of body mass; b. decreasing order of home-range size; c. from highest to lowest trophic level; d. in the estimated order described for Midwestern U.S.A. mammals in forest patches included in an agricultural matrix; e. random. Results differed for each simulation, with the randomized sequences of species loss resulting in a decrease in disease risk, in contradiction with results from non-randomized sequences, in fact highlighting the significance of both species identity and the order in which species are lost. Murray *et al.* (2015) suggested that "faunal convergence among regions, which might arise as a result of non-random biodiversity loss…could coincide with convergence in infectious disease assemblages and, in turn, disease risks."

In summary, greater biodiversity may increase the hazard of emerging infectious diseases, due to the potential presence of a larger number of pathogens, but this is not always the case and it has been found that biodiversity is not predictive of disease risk at all scales or in all systems (Hosseini *et al.*, 2017). Human disturbance often decreases biodiversity in a non-random fashion (Lambin *et al.*, 2010), determining loss of functional diversity from the system and usually the persistence of host species responsible for amplifying pathogens (Keesing *et al.*, 2010). Further, when human activities increase in areas with higher biodiversity, pathogen introductions/spillover or increased human exposure may lead to disease emergence that can mistakenly be linked with the high diversity of the area; biodiversity conservation may therefore reduce disease transmission and human disease risk directly preserving the functional diversity of the system and also reducing contact between humans and wildlife (Hosseini *et al.*, 2017).

1.4 Epidemiological modelling as a tool in disease ecology

It emerges, from what was said above, that the study of wildlife disease transmission in the context of community ecology is very complex and requires a multidisciplinary approach. In this study, the emphasis will be placed mainly on multi-host multi-parasite systems, which are dominant in wildlife, and on the effects of external factors on epidemiological outputs, e.g. the relevant interactions in wild populations that affect disease persistence and transmission, or the circumstances under which they occur (Tompkins *et al.*, 2011). Studies where natural populations may be used in laboratory experiments, or experimental studies of wild populations are strongly advocated; however, also eco-epidemiological modelling has been extensively, and successfully, used in wildlife disease ecology (e.g. Clay *et al.*, 2009b; Clay *et al.*, 2014; Keesing *et al.*, 2006; Ostfeld and Keesing, 2000; Ostfeld and LoGiudice, 2003; Roche *et al.*, 2012; Schmidt and Ostfeld, 2001). Mathematical modelling is an invaluable tool since the processes of systematically refining model assumptions, interpreting variables, and estimating parameters are crucial in explaining the observed patterns, predicting the future course of outbreaks, and evaluating control strategies (Choisy *et al.*, 2007; Keeling and Rohani, 2007; Heesterbeek *et al.*, 2015).

Historically, the modelling approach to host-pathogen or host-parasite interactions started in the context of human infections with the model of smallpox by Bernoulli (1760) (McCallum, 2015). Subsequently, the approach used for human infections was applied also in the context of livestock and wildlife; the fundamental modelling framework for micro and macroparasites was developed by Anderson and May (Anderson and May, 1978, 1979; May and Anderson, 1978, 1979), who elaborated the Susceptible – Exposed – Infected – Recovered (SEIR) compartmental framework.

Modelling wildlife diseases presents different challenges compared to humans or livestock, since modellers usually face a lack of data; in addition, wildlife population is not constant and can be influenced by various factors, and the transmission mode may often depend on population density and be somewhere in between frequency and density dependent (Smith *et al.*, 2009b). Models can be used to make predictions about wildlife (e.g. predict population-level epidemic dynamics from individual-level), to understand epidemiological trends, or understand the impact of control measures (e.g. vaccination) (Keeling and Rohani, 2007). In general, models are "wrong" by definition, because they make assumptions to simplify the real system under investigation; however, models can be developed in a way that they capture the essential features of a system, balancing between accuracy, transparency, and flexibility (Keeling and Rohani, 2007). Accuracy means reproducing the observed data and reliably predicting future dynamics, and it is vital for predictive models (Johnson *et al.*, 2015a). These models, according to the chosen aim, may require a qualitative or quantitative fit: a qualitative fit is suitable to gain insights into disease dynamics, whilst quantitative fit is necessary if the aim is, for example, to check the future effects of a control measure (Keeling and Rohani, 2007). Transparency is the ability to understand how the model components affect disease dynamics and interact with each other; this is an essential feature for models used for better understanding dynamics (Johnson *et al.*, 2015a). Finally, flexibility is a measure of how easy it is to adapt the model to different situations, and it is crucial in the case of evaluating control policies or predicting future disease in a changing environment (Johnson *et al.*, 2015a). In summary, the two key attributes of a good model are: a. the appropriateness to the purpose, following the principle of parsimony, and with the right balance of accuracy, transparency, and flexibility; b. parameterisation based on available empirical data.

In the context of wildlife diseases, most studies involving mathematical models have been used to describe single-host single-pathogen systems, despite these not being common in reality; or, when more species have been included in the systems, these are usually host to the same pathogen and they do not interact

with each other (Roberts and Heesterbeek, 2013). There is a disproportionate number of studies investigating only epidemiological interactions. In the context of multi-species systems, it would be more appropriate to include also ecological interactions, such as consumer-resource relations and competition (Roberts and Heesterbeek, 2013). Indeed, interactions with non-host species in the same community affect infectious disease dynamics in host-species; thus, multi-species eco-epidemiological modelling approaches should aim to fulfil these criteria: a. include features of both frequency and density dependent transmission; b. perform a community assembly that is neither entirely additive nor substitutive (i.e. saturating) (Mihaljevic *et al.*, 2014); c. describe variable patterns of community disassembly depending on biodiversity loss drivers (e.g. habitat destruction, direct exploitation, etc.); d. include host-parasite specificity as a function of host diversity (Johnson *et al.*, 2015b).

1.5 The model for studying multi-host parasite transmission: wild rodent communities

Wild rodent communities are an ideal system for studying multi-host parasite transmission in a community context for numerous reasons.

Firstly, rodents harbour a remarkable proportion of zoonotic parasites (22.5 %) (Cleaveland *et al.*, 2001), and rodent-borne diseases are currently a real risk for human health (Meerburg *et al.*, 2009). Indeed, rodents represent, or are part of, the zoonotic reservoir for Hantaviruses (e.g. PUUV, HPS), *Leptospira* spp. (leptospirosis), *Yersina pestis* (bubonic plague), and *Borrelia burgdorferi* (Lyme disease). Rodents also possess also a higher zoonotic potential than other taxa regarding viruses, and in particular vector-borne viruses, which pose a high zoonotic risk (Olival *et al.*, 2017). Also, Han *et al.* (2015), observed that rodents have a much higher probability of harbouring undiscovered zoonotic pathogens based on traits, which, in general, make them ideal reservoir species, and are: early sexual maturity, high reproductive rate, large litters, short gestation periods, rapid postnatal growth rate, small body size. Understanding rodent-associated transmission is therefore important from an applied disease-control perspective, especially because they represent one of the most resilient taxa and are predicted to rise as a consequence of defaunation of large mammals (Young *et al.*, 2014).

Furthermore, rodent communities represent ideal natural study systems, even when considering pathogens with no zoonotic or conservation importance. Usually, more than one species share the same habitat, occupying slightly different ecological niches; they are small in size and their restricted, but overlapping, home ranges mean that individuals, and so the population, can be relatively easily surveyed, characterised and manipulated (Wolton and Flowerdew, 1985).

Finally, rodent communities harbour a large variety of endemic pathogens, which have been also used as models of human infections (e.g. *Bartonella* spp.,

Birtles *et al.*, 1994; Herpesvirus, Knowles *et al.*, 2012), and the study of these in the field, under natural conditions, may provide useful additional information to the lab-based results. They are also ideal to investigate multi-host parasite transmission because individual rodents often carry more than one pathogen at the same time, and these pathogens seem to infect multiple rodent species (Begon *et al.*, 1999; Telfer *et al.*, 2007c; Paziewska *et al.*, 2012).

In the United Kingdom, the ground dwelling rodent community is dominated by wood mice (*Apodemus sylvaticus*), bank voles (*Myodes glareolus*), and field voles (*Microtus agrestis*); these species are abundant, widespread, and often occur in sympatry (Crawley, 1970; Greenwood, 1978), despite some habitat preferences. These species are considered the main reservoir hosts for a variety of microparasites, such as Gammaherpesvirus (Knowles *et al.*, 2012), Cowpox virus (Crouch *et al.*, 1995) and *Bartonella* spp. (Birtles *et al.*, 1994), macroparasites, such as intestinal helminths (Behnke *et al.*, 1999), and ectoparasites (Whitaker, 2007). Nevertheless, it appears that the role of each species in transmission dynamics of generalist infectious agents is different. Begon *et al.* (1999), using long-term longitudinal time-series epidemiological data of cowpox infection in bank voles and wood mice, investigated which transmission processes best explained the infection dynamics of the virus in each host species population. The striking result was that, although prevalence of infection in both species that occurred in the same environment was high, the transmission between species was negligible. Subsequently, a different study found that bank voles might have a role in the initial invasion of cowpox virus into small wood mouse populations (Begon *et al.*, 2003). Another example regarding the analysis of the reservoir role of different species is provided by Telfer *et al.* (2007c). They found that the prevalence of *Bartonella birtlesii* in wood mice was positively correlated to bank vole density, suggesting again that bank voles might have an essential role in between-species transmission to maintain the bacteria in the population of the other species.

1.6 Outline of the research

The Welsh ground-dwelling wild rodent community (see Appendix IV for a brief description of the study species and their images) was chosen as the target host-species community for this research in the context of dilution effect.

As illustrated in the previous section, wild rodent communities are ideal systems to investigate multi-host parasite and pathogen systems, therefore Welsh rodent communities were selected since virtually no information about rodent distribution, population density, species assemblages, and pathogen/parasite presence was available in Wales. By contrast, rodent population dynamics and parasite and pathogen screening have been performed in other British regions (e.g. Begon *et al.*, 2009; Bown *et al.*, 2003; Mallorie and Flowerdew, 1994; Marsh and Harris, 2000), but mostly focusing on one single rodent species, field vole (*Microtus agrestis*) (e.g. Burthe *et al.*, 2008; Smith *et al.*, 2006; Turner *et al.*, 2014), while, in this study, multi-species data across different habitats were gathered.

Rodents were live-trapped, and biological samples - faeces and ectoparasites – were collected. Data collected in the field were used, where relevant, to parameterise eco-epidemiological models of infection. Biological samples were screened for pathogens differing in types of transmission and ecology. The dilution effect was investigated in different host-pathogen or host-vector-pathogen systems analysing the effects of parameter variation and community composition, in terms of host and non-host species.

Through the modelling work, the following were examined: - whether prevalence or other epidemiological metrics were affected by community composition; - whether there were diluter and/or amplifier species and their functional role in the community; - whether the dilution effect occurred and which were the mechanisms involved; - which were the key parameters affecting pathogen transmission. According to the suggestions by Johnson *et al.* (2015b), this research gathered more empirical and laboratory data,

investigated the influence of different features of diversity on pathogen transmission, including functional diversity (e.g. predators, non-reservoir hosts), and analysed how the relationship between host diversity, parasite diversity and human/wildlife disease risk vary among different systems.

The final aims were to provide additional knowledge on Welsh rodent communities' population dynamics, to identify rodent-borne parasites and pathogens circulating in an area where human/wildlife disease risk was not assessed, and to improve understanding of wildlife pathogen local transmission dynamics.

This knowledge is particularly significant in Wales for the following reasons:

- the country is predominantly rural, and human settlements are in close proximity with wildlife;
- agriculture and tourism are the main economic sectors, so humans are in close contact with livestock, and significantly use the countryside for recreational activities;
- a very widespread farming system involves grazing of open areas, so livestock and wildlife extensively interact and might share parasites and pathogens;
- conservation programmes are in place, e.g. pine marten (small rodents specialist predator) reintroduction, for which this information are extremely relevant;
- UK-wide projects are currently on going to monitor vectors and vector-borne pathogens, but these mainly focus on pets and human zoonotic risk, overlooking wildlife (e.g. Big Tick Project and Big Flea Project by MSD Animal Health).

The research findings might contribute developing improved policies and/or management actions to conserve biodiversity and reduce human zoonotic risk.

Chapter 2

Rodent community characterisation and analysis of population dynamics

2.1 Introduction

Small rodent communities are ideal systems for multi-host parasite transmission study, as discussed in section 1.5. Ground dwelling rodents were therefore chosen as the target community in this eco-epidemiological study.

Small rodents have also important roles in ecosystem functioning; they are primary and secondary consumers, seeds and mycorrhizal fungi dispersers (Moore *et al.*, 2007), seed consumers, and prey of several vertebrate predators (Whitaker and Hamilton, 1998). Further, they are sensitive to habitat modifications, although they are found in a wide range of habitats (including anthropologically modified areas), and are able to influence their own environment (Breitbach *et al.*, 2012); for example, a dominance of seed predator species can decrease forest regeneration (Struhsaker, 1997). Moreover, they represent a massive prey biomass for their predators, therefore their density has a strong impact on predator species' abundance and diversity (Salamolard *et al.*, 2000); owls, weasels, and stoats may fail to breed due to low rodent density (King, 1985).

Considering the above, it appears clear that small rodents have significant interactions with other species, and can be considered keystone species (Rayfield *et al.*, 2009), assuming that, in an ecosystem, these are the species that have considerable more interactions with other species (Jordàn, 2009). Hence, it is worthwhile focussing on small rodents not only from the epidemiological, but

also from the ecological point of view, facilitating the investigation of how ecological relationships impact disease transmission.

Nonetheless, there are few studies assessing small rodent diversity and population dynamics, especially on a local scale (Breitbach *et al.*, 2012). For instance, within forests where fruiting trees predominate, the community will be dominated by seed predators (e.g. *Apodemus* spp.), whereas in open forests, with low canopy cover and high herbaceous ground cover, the community will be characterized by a higher presence of herbivorous species such as *Myodes glareolus* (Suchomel *et al.*, 2012).

Consequently, the first step to acquire knowledge on a population is to assess the status of populations (Cowlishaw and Dunbar, 2000), and so evaluate spatio-temporal abundance and distribution (Chapman *et al.*, 1999). This is especially important for small rodents populations that fluctuate across years and seasons; in Britain small rodents do not have the same regular multi annual fluctuations or drastic summer declines like in Fennoscandia, but certainly they display seasonal variation in numbers (Lambin *et al.*, 2000). In order to capture these variations, and estimate population densities the most common method for small rodents is to use capture-mark-recapture data obtained from live-trapping grids (Krebs *et al.*, 2011).

In the present study live-trapping ground dwelling wild rodents was performed in different locations in Wales to collect information on community composition, to estimate population densities, growth rates, and parameters (e.g. contact rates) for models of infection incorporating ecological and epidemiological relationships, and investigate spatial distribution of the captured individuals. In addition, the fieldwork was used to collect biological samples for pathogen screening. Description of field sites and trapping methodologies are provided in the next sections.

2.2 Methods

2.2.1 Study sites

The study sites included six locations in Wales (see map, figure 2.1), each one having one trapping grid, located to cover different habitats and different potential rodent assemblages. Three trapping grids were placed in the Pembrokeshire Coast National Park (PCNP) in the Stackpole Estate (owned by the National Trust), two trapping locations were located in an area managed by the Forestry Commission (now Natural Resources Wales) in the Rheidol Valley near Cwmystwyth (Ceredigion), and one site was set on Skomer Island (an island off the coast of Pembrokeshire managed by the Wildlife Trust of South and West Wales); the pilot study was performed in a privately managed woodland in Capel Bangor (Ceredigion) (Fig. 2.1).

Here, the characteristics of each trapping location will be described, while the details about live-trapping will be discussed in the next section.

In the Stackpole Estate live-trapping of small rodents was carried out during September/October 2015, May/June 2016, September/October 2016, and May/June 2017. Three trapping grids were set: two in different patches of woodland (MPW and WW) and one in a grassland area (MPG) (Fig. 2.2).

The Mere Pool Woodland, where the MPW trapping site was located, is constituted by a forested area of around 5.5 ha, and it is contiguous with other habitats such as scrub, species-rich unimproved grassland, and *Phragmites* dominated swamp. The mixed deciduous woodland is dominated by the *Fraxinus excelsior*, *Acer campestre*, *Mercurialis perennis* community (W8 in the British National Vegetation Classification - NVC), with a sub-community characterised by *Geranium robertianum* (W8e of the NVC). The canopy is discontinuous, while the shrub layer is very dense and dominated by *Fraxinus* regeneration and *Corylus avellana* (Castle and Mileto, 2002).

The site MPG is found in the same area of the estate, where the grassland was characterised by low cover of fescues with *Sagina nodosa*, *Erigeron acer* and a rich lower plant flora. The closest NVC fit would appear to be SD12, *Carex arenaria - Festuca ovina - Agrostis capillaris* dune grassland; however, there is absence of *Ammophila arenaria* and calcifuges such as *Galium saxatile*. Other associates tend to be robust plants such as *Ononis repens*, *Eupatorium cannabinum*, and *Torilis japonica* or scramblers such as *Rubia peregrina*, and *Lonicera periclymneum*. *Rubus caesius*, and *Rosa pimpinellifolia* scrubs are present, as bracken dominated areas (NVC Survey of Stackpole Estate, 2012 provided by National Trust Officer Paul Culyer).

Last woodland site (WW) was situated in a patch of 25.3 ha forested area that grades to scrub along southern and eastern edges, where is mostly open to sheep grazing. The canopy is dominated by *Acer pseudoplatanus* with some *Quercus ilex*, while the shrub layer is generally quite sparse, constituted by *Acer* regeneration. Other species present, especially along the edges are: *Ulmus glabra*, *Sambucus nigra*, and *Prunus spinosa*. The field layer, in the area where the trapping grid was located, included mostly *Rubus fruticosus*, *Urtica dioica*, and *Hedera elix*; this area was also occasionally grazed by sheep and steers.

In the modelling section, rodent and non-rodent species constituted the full community assembled to investigate the effects of non-host species on disease transmission, so the list of mammals recorded on the Estate is shown in Table 2.1.

The trapping grid on Skomer was placed in the typical Skomer vole habitat (*Myodes glareolus skomerensis*) present on the island, the bracken forest (*Pteridium aquilinum*), specifically in a high-density site approximately 300 m south of North Pond (GR094724) (Healing, 1984). The area was dominated by bracken and brambles (*Rubus fruticosus*), but bluebells (*Hyacinthoindes non-scripta*), and wood sage (*Teucrium scorodonia*) were also present. Worthy of note is that on the island there are no terrestrial predators, and, with regards to mammals, voles share their habitat with common shrews, wood mice, and

rabbits. The island was surveyed for three consecutive years (2015-2017) during late August.

Table 2.1. Wild Mammals (excluding bat species) recorded at Stackpole Estate.

Common Name	***Scientific Name***	**Common Name**	***Scientific Name***	**Common Name**	***Scientific Name***
American Mink	*Mustela vison*	Fox	*Vulpes vulpes*	Polecat	*Mustela putorius*
Badger	*Meles meles*	Grey Seal	*Halichoerus grypus*	Pygmy Shrew	*Sorex minutus*
Bank Vole	*Myodes glareolus*	Grey Squirrel	*Sciurus carolinensis*	Rabbit	*Oryctolagus cuniculus*
Brown Rat	*Rattus norvegicus*	Hedgehog	*Erinaceus europaeus*	Stoat	*Mustela erminea*
Common Shrew	*Sorex araneus*	House Mouse	*Mus musculus*	Water Shrew	*Neomys fodiens*
Feral Ferret	*Mustela furo*	Mole	*Talpa europaea*	Weasel	*Mustela nivalis*
Field Vole	*Microtus agrestis*	Otter	*Lutra lutra*	Wood Mouse	*Apodemus sylvaticus*

In Ceredigion, the woodland site selected for live trapping included a mixed species noble fir (*Abies procera*; non-native species) plantation with few young oaks, birch, beech or hazel in amongst the conifer (Fig. 2.3). The ground flora was well-developed and included common heather and bilberry. Some edges of the woodland patch graded in to scrub dominated by *Sambucus nigra*, *Prunus spinosa*, and *Rubus fruticosus*. In the same wide area, the second site was constituted by an old clear-cut area that was experiencing unmanaged regrowth assuming features of scrubland dominated by *Ulex europaeus* and *Rubus fruticosus* (Fig. 2.3). These sites were sampled, like Stackpole sites, in September/October 2015, May/June 2016, September/October 2016, and May/June 2017. During the sampling period, in the same area, a project for the reintroduction of pine martens was carried out and it has been estimated that the area, intended as the whole site (more than 1000 ha), was visited by 5 individuals in 2015, decreased to 2 in 2016 and 2017. The mammals recorded in the area are listed in Table 2.2.

Table 2.2. Wild Mammals (excluding bat species) recorded in the Forestry Commission area; data from personal communications of other surveyors of the area and from the WWBIC (West Wales Biodiversity Information Centre) database.

Common Name	***Scientific name***
Badger	*Meles meles*
Bank vole	*Myodes glareolus*
Brown hare	*Lepus europaeus*
Common Shrew	*Sorex araneus*
Grey Squirrel	*Sciurus carolinensis*
Pine Marten	*Martes martes*
Red Fox	*Vulpes vulpes*
Wood mouse	*Apodemus sylvaticus*

The site of the pilot study, performed in June 2015 (Fig. 2.3), in Capel Bangor, was a combination of a semi-deciduous natural forest and an old unmanaged conifer plantation. The forest was characterised by a mixture of oak species (*Quercus robur* and/or *Q. petraea*) and birch (*Betula pendula* and/or *B. pubescens*). The ground flora had the typical acidophile species and well-developed lower plant like *Pteridium aquilinum* and *Rubus fruticosus.* Also, there was a fragmentary occurrence of the *Tilio-Acerion* woodland type, while the coniferous patch was dominated by the non-native Sitka sprouce (*Picea sitchensis*). There was a record of mammal sightings for the area and it is showed in Table 2.3.

Table 2.3. Wild Mammals (excluding bat species) recorded in the private woodland surveyed in Capel Bangor; data from personal communications of the owner and from the WWBIC (West Wales Biodiversity Information Centre) database.

Common Name	***Scientific name***
Badger	*Meles meles*
Bank vole	*Myodes glareolus*
Common shrew	*Sorex araneus*
Grey Squirrel	*Sciurus carolinensis*
Polecat	*Mustela putorius*
Red fox	*Vulpes vulpes*
Wood mouse	*Apodemus sylvaticus*

Fig. 2.1. Map of Wales showing the regions where the field sites were located. The sampling areas are shown in red and the figure number refers to the enlarged map displaying the exact location of the trapping grids.

Fig. 2.2. Trapping sites at Stackpole Estate. 1: Mere Pool Valley Woodland (MPW); 2: Mere Pool Valley grassland (MPG); 3: Warren woodland (WW).

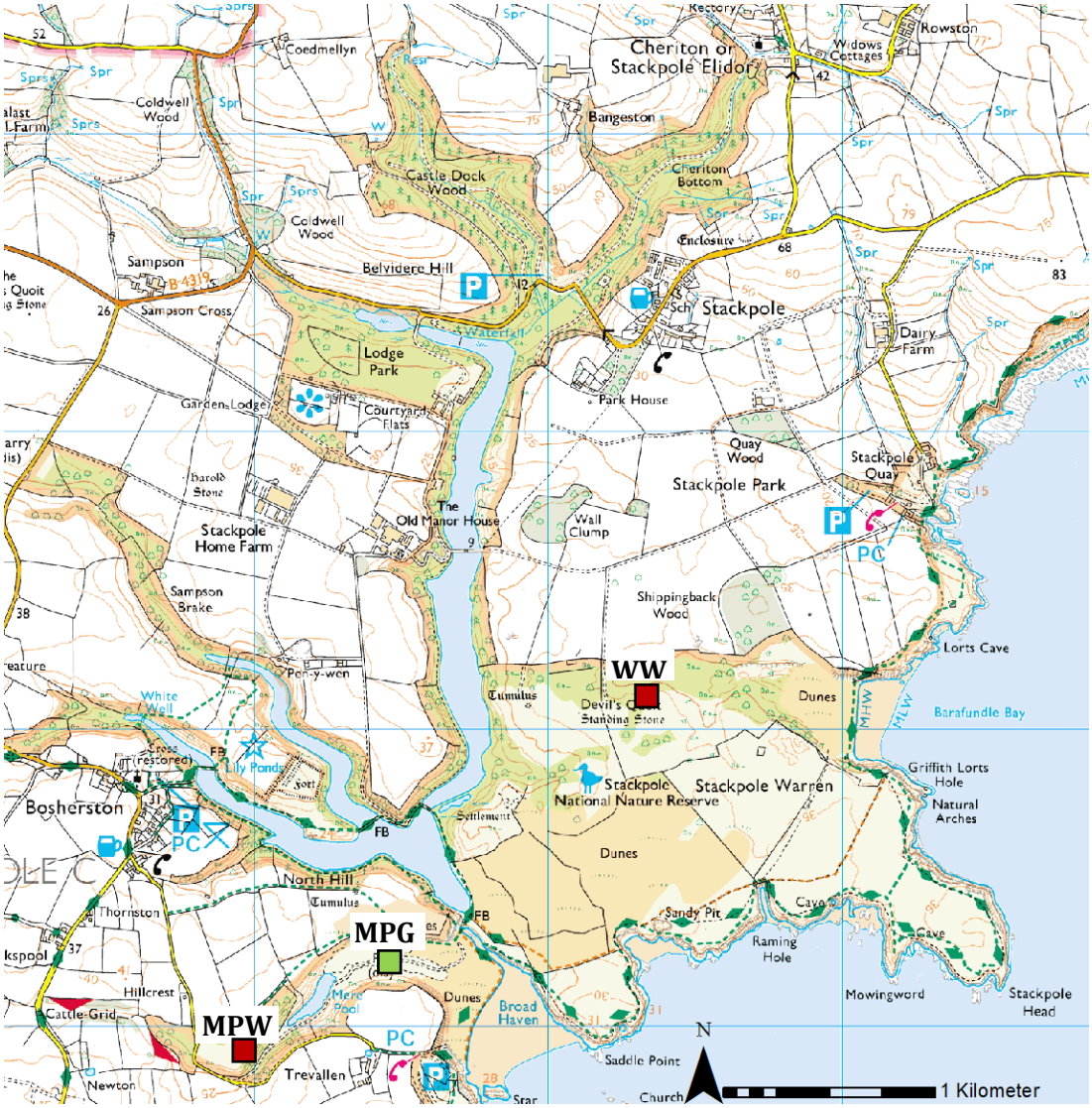
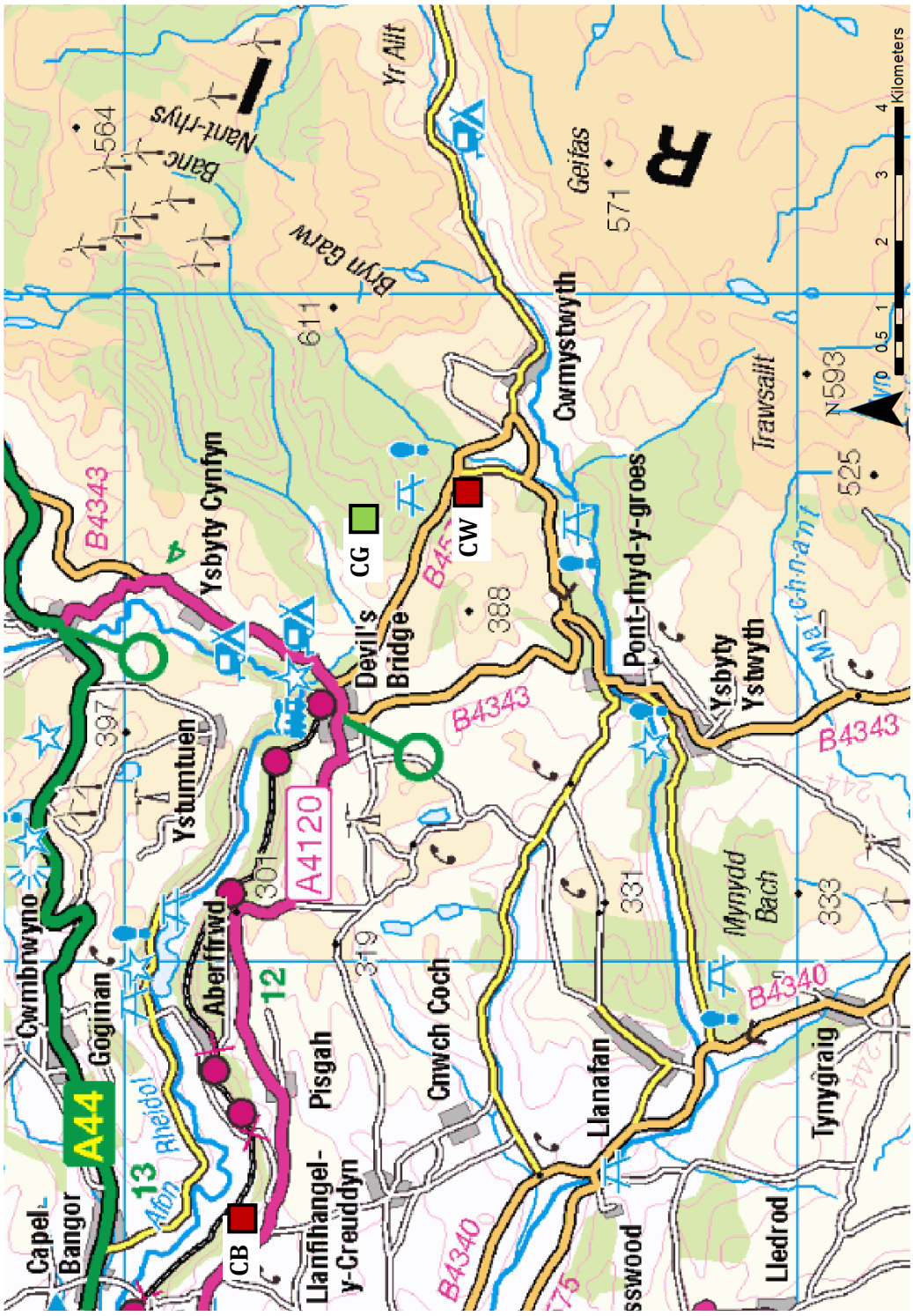


Fig. 2.3. Trapping sites in Ceredigion. CB: Capel Bangor woodland, pilot study (red); CG: grassland, clear-cut (green); CW: conifer plantation (red).



2.2.2 Rodent live-trapping

A regular sized trapping grid was used to investigate ground dwelling small rodent communities in the chosen sites. Compared to linear transects, grids are better in providing spatial resolution to estimate population density, home-ranges, and dispersion (Pearson and Ruggiero 2003). The configuration of the grids was square to diminish the perimeter/area ratio in order to reduce the edge-effect (Gurnell and Flowerdew, 2006), with two traps each grid point (trapping station). The distance between stations was set according to the habitat as suggested by Gurnell and Flowerdew (2006): 15 m distance in woodlands, 5/10 m in grasslands.

In each location, the grid comprised 36 trapping stations (6x6) with one Longworth and one Sherman trap each station, in order to diminish sampling error and yield better estimate of species composition (Anthony *et al.*, 2005; Lambin and MacKinnon, 1997). Therefore, the area covered in the woodland locations was 0.56 ha, in the grasslands 0.12 ha (the distance chosen was 7 m), while in Skomer 0.25 ha (the grid layout was already in place with a distance of 10 m between stations). The traps were set up with appropriate bedding material (hay or straw) and food (rolled oats, carrot chunks, dried mealworms) to comply with animal welfare policies, research ethics regulations, and to decrease mortality (Powell and Proulx, 2003).Traps were also provided with shrew holes, since shrew species are protected under Schedule 6 of the Wildlife and Countryside Act (1981) and a licence is required to trap them. Each trapping grid was apart well above the 250 m suggested to avoid pseudoreplicates (Fauteux *et al.*, 2013).

The live-trapping was performed during two different seasons to estimate individual densities of the pre-breeding recruitment population (May-June) and post-breeding peak population (September-October). The two seasons will be referred as, respectively, spring, and autumn. The sampling was carried out from autumn 2015 to spring 2017.

Each trapping occasion consisted in four consecutive days and nights, with the first day/night being pre-baiting, i.e. the traps were locked open to get the animals accustomed with the new feature in the environment (Jones *et al.*, 1996). So, the traps were checked twice a day for three consecutive days (early morning and sunset) for a total of six checks. Traps were not a permanent feature of the environment and were removed between each trapping session. After each session all the traps were washed and disinfected with Virkon® or autoclaved to avoid cross-contamination between sites or seasons.

Each individual captured was identified at species level, sexed, assigned to an age class according to size/reproductive status – juvenile, sub-adult, adult according to Telfer *et al.* (2002) –, weighed, individually marked by fur clipping, and finally released. On first capture only, biological samples were collected from each individual. Adult individuals were considered reproductively active. Faeces were collected directly from the animal or from the trap tunnel and stored in a sample tube at -80°C. Before being stored in the freezer samples were at ambient temperature maximum for 3 hours, considering the maximum duration of the trap checking session and the transport. Ticks and fleas were collected from each individual and stored at -18°C in sample tubes filled with RNAlater, in order to preserve nucleic acids, or at -80°C without RNAlater, to allow further molecular investigations (more information on sample collection and storage in Chapter 3 and 4). Also, during the sampling period, information about temperature, weather, or extreme conditions that could potentially affect the sampling were collected.

The described methodology, in the chosen sites, was aimed to trap mice and vole species; in particular, considering the locations and the trap size the target species were represented by: *Apodemus* spp., *Mus musculus*, *Myodes glareolus* (*Myodes glareolus skomerensis* on Skomer), *Microtus agrestis* (Sibbald *et al.*, 2006).

2.2.3 Data Analyses

2.2.3.1 Rodents body mass estimation

Body mass in grams, estimated during trapping with a spring scale (100 g • 1 g), was used to compare individuals of different species, sexes, age classes, and captured in different seasons. These data were collected to estimate some allometric parameters to be included in the models of infection. The data were analysed using independent-samples t-test after checking for normality; all the analyses were performed in R (R Core Team, 2016).

2.2.3.2 Population density estimation

Individual density of each species in each site, and for each trapping occasion was estimated with the POPAN algorithm (Schwarz and Arnason, 1996) within the software MARK (White and Burnham, 1999), assuming, during the trapping session, open population, constant survival, and constant capture probability. Goodness-of-fit was tested with the RELEASE suite within the same software. Schwarz and Arnason (1996) developed a likelihood function for the open-population capture-recapture (Jolly-Seber) experiment, which consisted in a generalization to the usual Jolly-Seber representation that models births using a multinomial distribution from a super-population.

Similarly to Mallorie and Flowerdew (1994), for each species, a linear regression was carried out between log-transformed density and the log-transformed density in the following season to identify any density-dependent delayed effect on population abundance.

2.2.3.3 Seasonal growth rate estimation

The growth rate, r, was estimated for each species according to the formula proposed by Lambin *et al.* (2000):

$r_i = log_{10}N_{i(t)} - log_{10}N_{i(t-1)}$

where *N* is the population density of species *i*. The operation was repeated for each season, in order to have a growth rate representing breeding and non-breeding seasons. Considering the trapping design, the growth rate representing the change of the population from the autumn to the following spring is considered the growth during the non-breeding season, while the rate of change during the breeding season is considered from spring to the following autumn.

Growth rates were also estimated allometrically, according to Bolzoni *et al.* (2008), and all the values obtained were compared with the ones found in literature to establish the most appropriate values to include as parameters in the modelling work. The growth rates estimated for different seasons were included, in order to include seasonality in modelling rodent species population.

According to Huitu *et al.* (2004), a linear regression was performed between growth rate and log-transformed population density in order to identify whether individual density had a direct or delayed effect on growth rate.

2.2.3.4 Intra and inter-specific contact rate estimation

A contact was defined as two individuals caught in the same trap or in the adjacent trap in the same trapping session (Grear *et al.*, 2009; Grear *et al.*, 2013; Perkins *et al.*, 2009; VanderWaal *et al.*, 2013). Two types of contacts were estimated, intra and interspecific, for each species and each trapping session. In addition, the contacts were also estimated, for each session, by sex, age class, and breeding status. The total contacts were averaged by day and were used for

the analyses reported in this section, but also were used in the modelling of directly transmitted diseases (see Chapter 5).

According to Clay *et al.* (2009b) and Springer *et al.* (2017), statistical analyses were performed to investigate potential differences in intra and interspecific contact rates between seasons, sites, and species. Since the distribution of the data was not normal (Anderson-Darling test, $p < 0.01$), the statistical test chosen was permutation, in particular, the two-sample Fisher-Pitman permutation test, and the K-sample Fisher-Pitman permutation test when comparing more than two samples. The analyses were carried out in R using the package *coin* (R Core Team, 2016).

A finer grained analysis was completed using the intra and interspecific contacts estimated for each species and for each sex, age class, and breeding condition. Using chi-square test, it was examined whether each of the categories listed above did realise significantly more contacts. Also, as suggested by Clay *et al.* (2009b), weight was used in a linear regression to investigate its potential role as explanatory variable for intra and interspecific contacts. Linear regressions were also used to highlight any potential relationship between contacts and population density. All the statistical analyses were performed in R (R Core Team, 2016).

2.3 Results

2.3.1 Rodent community composition

During the entire period of fieldwork, the species recovered were *Apodemus sylvaticus* (wood mouse), *Myodes glareolus* (bank vole), and very few *Microtus agrestis* (field vole); on Skomer, *Myodes glareolus* was present with the subspecies *Myodes glareolus skomerensis* (Skomer vole). See Appendix IV for a brief description of the species and their images.

During the 4968 trap-nights, 680 unique individuals were caught, 258 bank voles, 183 Skomer voles, 230 wood mice, and 9 field voles, for a total of 1195 captures (including recaptures). The individuals were balanced in terms of sex with an overall male to female ratio of 1.08. Excluding Skomer, where the voles occur in very high density, and massively exceeded wood mice, the ratio wood mice: bank voles was 0.86. The age class far more represented in sampled bank voles was sub-adult (52.64%), namely individuals that present adult pelage and are heavier than juveniles, but are not reproductively active (no descended testes in males, or perforated vagina in females). By contrast, in wood mice, both adults and sub-adults were almost evenly captured (sub-adults 43.18%, adults 48.64%). Juveniles constituted a very small percentage of the total individuals in every site but Skomer, where they accounted for the 22.40% of the individuals trapped; almost all the juveniles were captured in the post-breeding peak season (late summer/early autumn).

In all the woodland sites and on Skomer the community was constituted by two species, wood mouse and bank vole (or Skomer vole), while the grassland comprised only wood mice, and the clear-cut site presented a three species assemblage including also filed voles. In every trapping grid, including on Skomer Island, the traps presented signs of shrew visits, such as bedding and food carried out through the shrew hole and distinctive scats in the trap tunnel.

2.3.2 Rodents body mass

The species, excluding Skomer voles, did not differ significantly in weight ($F = 1.07$, $p = 0.34$), while Skomer voles were significantly heavier than all the other species ($F = 47.96$, $p < 0.001$) (Table 2.4). Combining data of all captured individuals, average body mass did not differ significantly between males and females (Table 2.5). This result was consistent even when the data were analysed by species, but, on Skomer, female voles showed a tendency to be slightly heavier than males (Table 2.5; $p = 0.1$), while the opposite trend was true regarding bank voles (Table 2.5; $p = 0.06$).

Juveniles, in all species, weighted significantly less than adults and sub-adults ($F = 125.5$, $p < 0.001$). Adults and sub-adults pooled together of all species, excluding Skomer voles (which were sampled in only one season) were lighter in late summer/early autumn with an average weight of 17.82 (SE 0.28), while in spring the average body mass was 23.35 (SE 0.36) ($t = -11.07$, $p < 0.001$).

Table 2.4. Average body mass and standard error of all unique individuals captured during the study. In brackets the sample size.

Species	Body mass (g)	St.Er
Bank vole	19.30 (258)	0.34
Field vole	22.22 (9)	1.93
Skomer vole	26.33 (183)	0.59
Wood mouse	19.58 (230)	0.43

Table 2.5. Results of the independent samples t-test related to gender of all unique individuals captured during the study divided by species. n: sample size; St.Er: standard error; df: degrees of freedom.

Species	n	Sex	Body mass (g)	St.Er	df	t	p
Pooled species	320 345	F M	21.65 21.08	0.43 0.36	622.31	-1.00	0.32
Bank vole	139 125	F M	18.69 19.98	0.49 0.45	245.80	1.89	0.06
Field vole	3 6	F M	26.33 20.17	2.90 2.18	4.34	-1.70	0.16
Skomer vole	104 85	F M	27.15 25.27	0.82 0.78	175.23	-1.61	0.10
Wood mouse	88 144	F M	19.29 19.70	0.65 0.57	178.12	0.46	0.65

2.3.3 Population density

Population density of the all the species (excluding Skomer voles) during the sampling seasons is displayed in Fig. 2.4. Inter-seasonal fluctuations are evident, and in general it is noticeable higher density in the post-breeding peak season (autumn). Looking at Fig. 2.5, it is reasonable to hypothesise that populations may be subjected to yearly fluctuations.

In general, density (N) was not related to the previous season density (pooled data excluding Skomer). Nevertheless, when the same analysis was carried out by species, wood mouse density was slightly negatively related to the previous season density ($\log N_t = 2.38 - 0.6 * \log N_{t-1}$, $r^2 = 0.26$, $p = 0.07$), while density of bank voles showed a slightly positive relationship with the previous season density ($\log N_t = 0.24 + 0.85 * \log N_{t-1}$, $r^2 = 0.43$, $p = 0.05$).

Regarding Skomer, wood mice were in very low numbers to give a reliable population estimate; Skomer voles were present at very high densities each sampling year, but the traps available were too low and the grid did saturate, so also in this case the estimation, which heavily relies on recaptures, were not precise. Nonetheless, the values were 262 (ind/ha) in 2015, 504 (ind/ha) in 2016, and 356 (ind/ha) in 2017.

Fig. 2.4. Average population density (number of individuals per hectare ± standard error) of sampled species across sampling seasons (excluding Skomer voles). Wood mouse: solid line; Bank vole: dotted line; Field vole: dashed line.

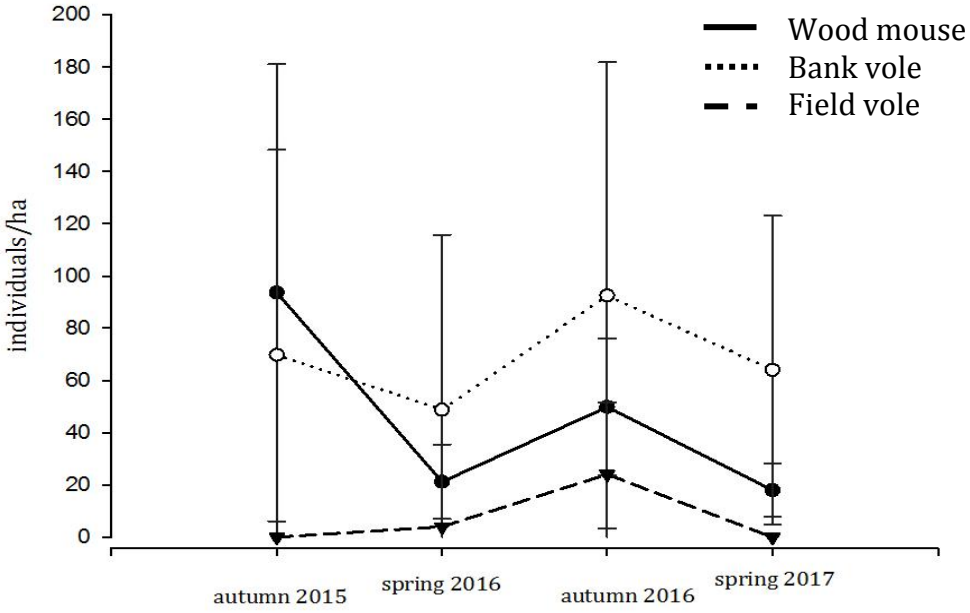
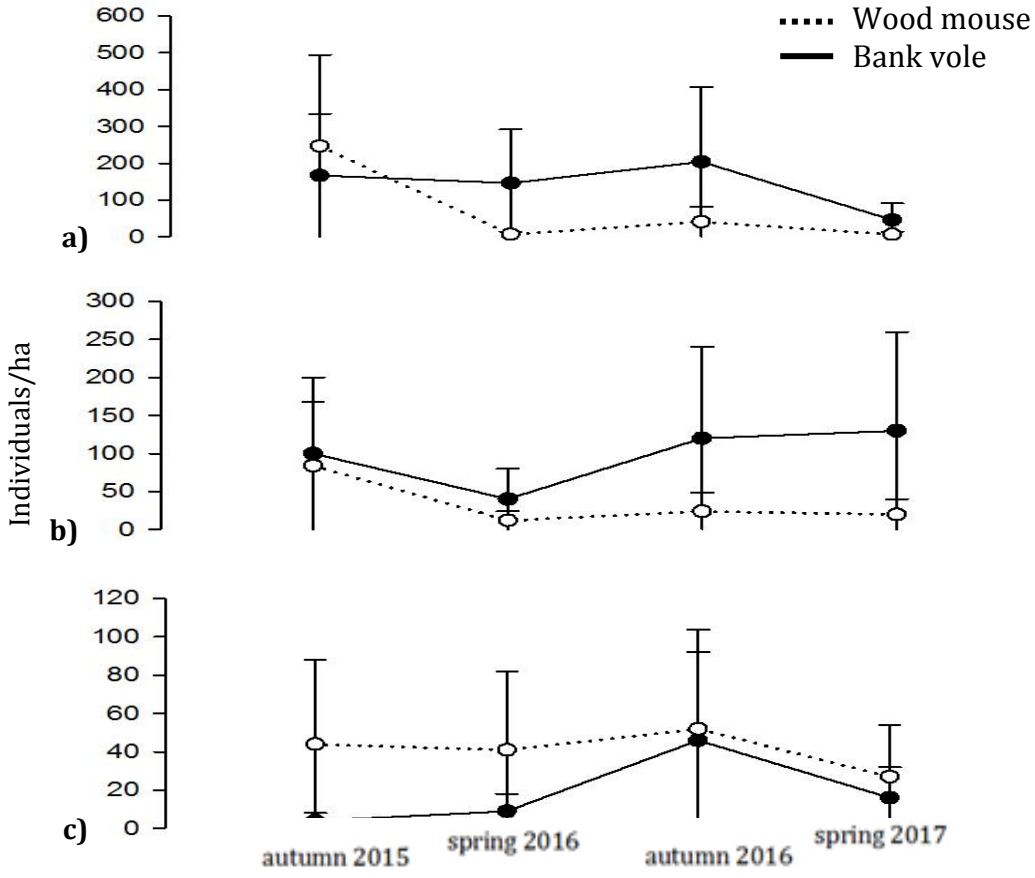


Fig. 2.5. Population density (number of individuals per hectare ± standard error) of sampled species across sampling seasons. a) site MPW; b) site CW; c) site WW. Wood mouse: dotted line; Bank vole: solid line.



2.3.4 Seasonal growth rate

Non-breeding season growth rates were mostly negative, or much lower than breeding season values in both species: bank voles and wood mice (Fig. 2.6 and Fig. 2.7). It was impossible to estimate growth rate for field voles due to the very low number of individuals trapped, and, regarding Skomer voles, the analysis was not performed because of data being collected only in one season. Too few bank voles were trapped in site CG and no individuals were captured in site MPG, thus it was not possible to estimate growth rates.

The general trends were very similar between the two species, but it appeared that wood mice are subject to greater population decrease during non-breeding seasons.

However, non-breeding seasons average growth rates were not significantly different between species ($t = 1.41$, $p = 0.18$), and so were breeding season averages ($t = 0.16$, $p = 0.88$) (Fig. 2.8 and Table 2.6). These values, estimated form empirical data, were much lower than the ones obtained allometrically according to Bolzoni *et al.* (2008) (Fig. 2.8 and Table 2.6).

Considering both species pooled data, population density (N) had a direct positive effect on growth rate (r): $r = -1.22 + 0.73 * \log_N$, $r^2 = 0.30$, $p < 0.01$. However, analysing the data separately indicated that this relationship was significant only for wood mice ($r = -1.61 + 1.08 * \log_N$, $r^2 = 0.57$, $p < 0.01$). In addition, growth rate in one species was not explained by opposite species density.

Previous season individual density showed a delayed negative effect on growth rate in both species (Fig. 2.9 and Fig. 2.10), but in wood mice this effect was stronger (wood mouse: $r_t = 1.49 - 1.12 * \log N_{t-1}$, $r^2 = 0.65$, $p < 0.001$; bank vole: $r_t = 0.89 - 0.52 * \log N_{t-1}$, $r^2 = 0.52$, $p = 0.03$).

Fig. 2.6. Growth rate estimated for bank voles using the formula from Lambin *et al.* (2000). On the x axis the different sites. Black: non-breeding season 2015/16; white: breeding season 2016; grey: non-breeding season 2016/17.

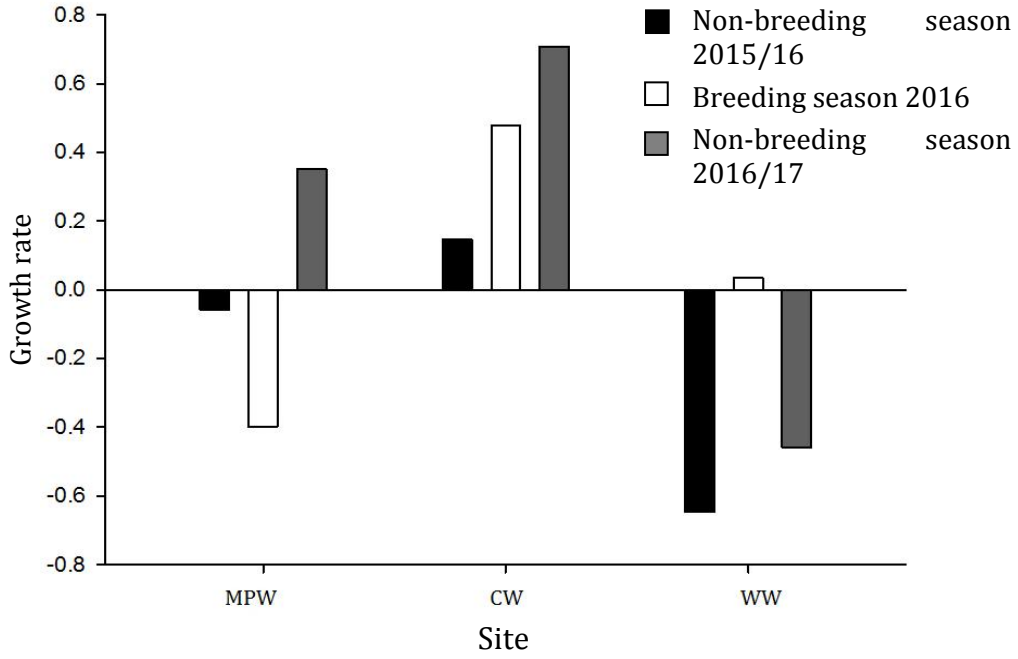


Fig. 2.7. Growth rate estimated for wood mice using the formula from Lambin *et al.* (2000). On the x axis the different sites. Black: non-breeding season 2015/16; white: breeding season 2016; grey: non-breeding season 2016/17.

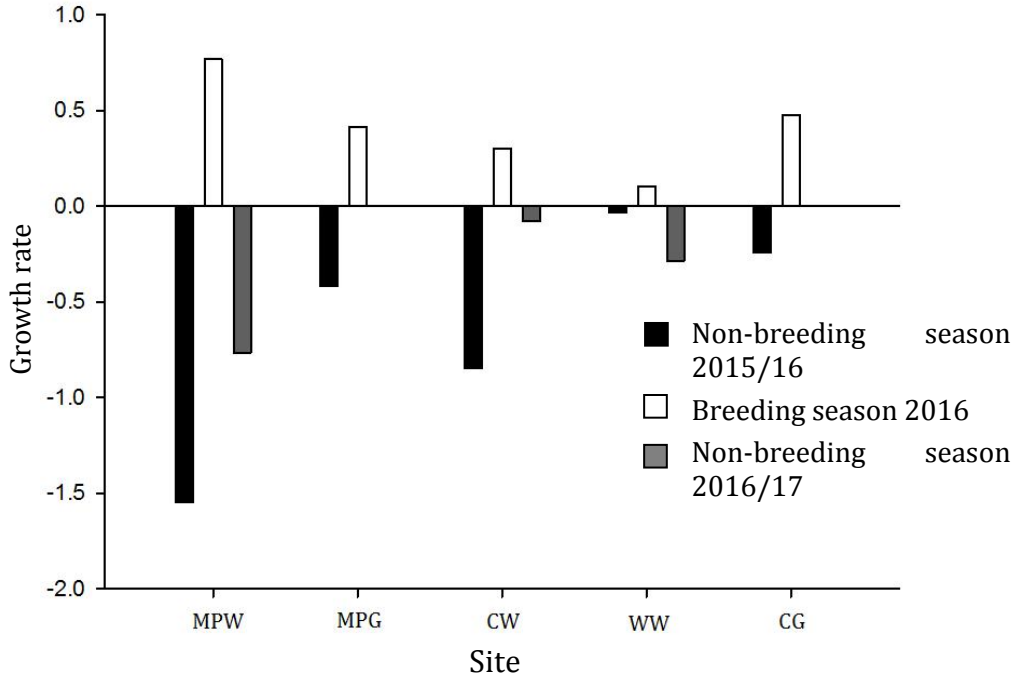


Fig. 2.8. Comparison between average seasonal growth rates and allometric estimation. Error bars represent standard deviation. Black: bank vole; grey: wood mouse.

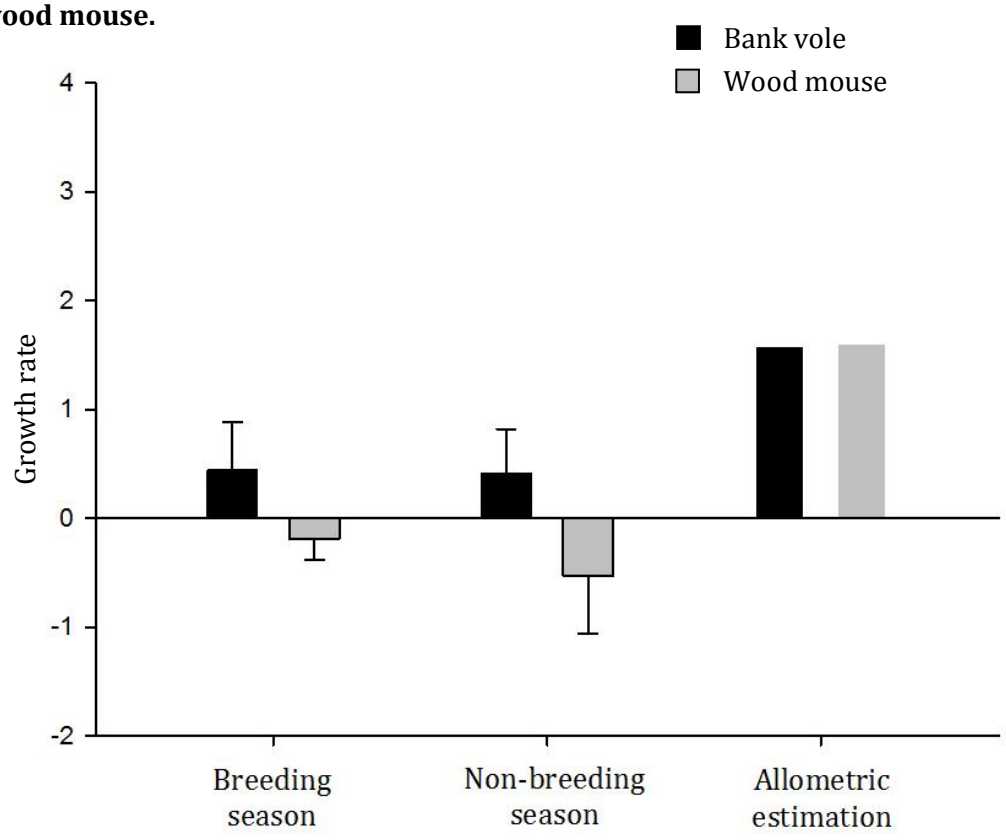


Fig. 2.9. Relationship between seasonal growth rate and individual density of the previous season in wood mice. Density was log-transformed. See text for the line equation.

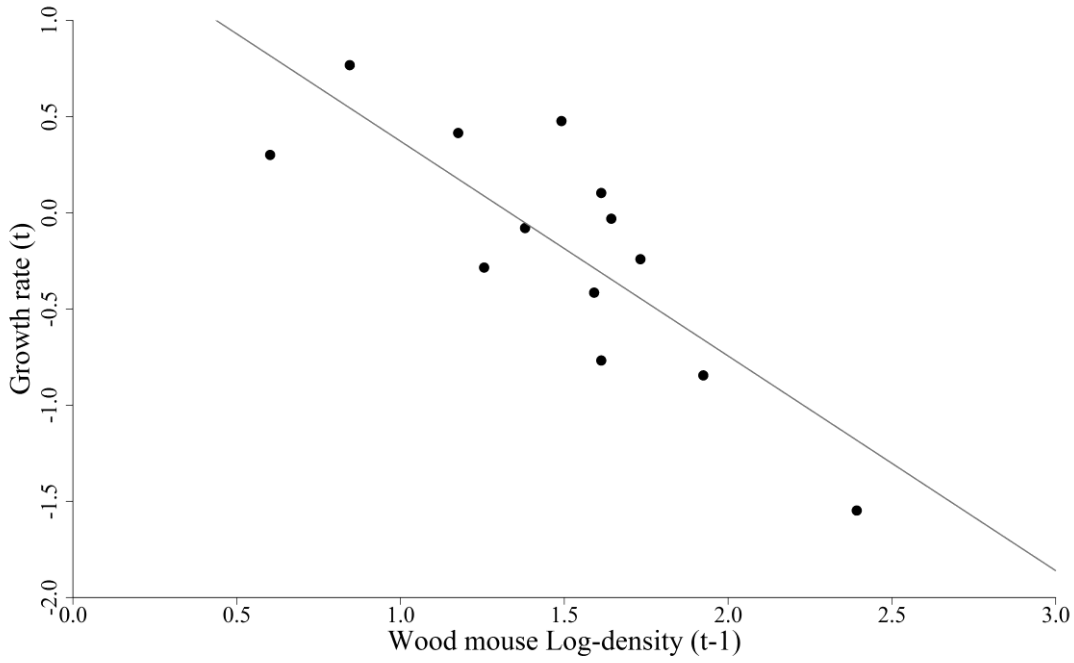


Fig. 2.10. Relationship between seasonal growth rate and individual density of the previous season in bank voles. Density was log-transformed. See text for the line equation.

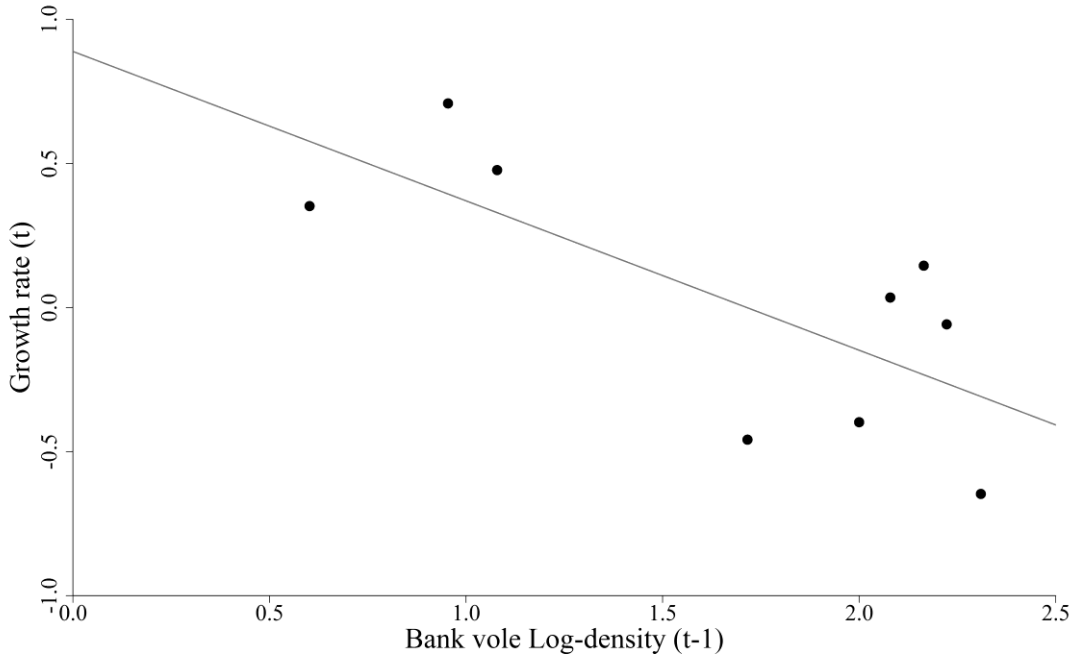


Table 2.6. Growth rate values averaged by season for each species, and values obtained by allometric estimation according to Bolzoni *et al.* (2008); the body mass used for the estimation was the average of adult and sub-adult individuals. Sample size in brackets. St.Er: standard error.

Species	Season	Mean	St.Er
Bank vole	Breeding	0.444 (3)	0.163
Wood mouse	Breeding	0.413 (5)	0.151
Bank vole	Non-breeding	-0.196 (6)	0.141
Wood mouse	Non-breeding	-0.526 (8)	0.207
Bank vole	Allometric	1.590	
Wood mouse	Allometric	1.615	

2.3.5 Intra and inter-specific contact rate

The frequency distribution of unique contacts per individual was non-normal, but highly aggregated, indicating that a small number of individuals were responsible for a large proportion of the contacts. The overdispersion parameter of the negative binomial regression fitting the data was 5.18 for intra-specific contacts, and 1.73 inter-specific contacts (pooled data excluding Skomer voles); therefore, inter-specific contacts resulted more aggregated than intra-specific.

The average daily individual contact rates estimated for each species are showed in Fig. 2.11. No significant difference was found in contact rates within species, while bank voles performed more intra-specific contacts than wood mice ($Z = 2.84$, $p = 0.004$), and wood mice slightly more inter-specific contacts than bank voles ($Z = -1.66$, $p = 0.09$).

The analysis performed by season did not reveal any significant difference in either intra or inter-specific contacts (pooled data excluding Skomer voles due to the high-density bias); the difference was not significant when data were analysed by species (Fig. 2.12). There was no significant difference between sites in intra-specific contacts (Fig. 2.13), while the daily average of inter-specific contacts differed between sites ($Z = 9.33$, $p = 0.05$) (Fig. 2.13).

Inter-specific contacts in bank voles were positively associated with the intra-specific contacts in wood mice ($y = 0.22 + 0.41 * x$, $r^2 = 0.51$, $p < 0.01$), and the opposite relationship was also significant ($y = 0.45 + 0.39 * x$, $r^2 = 0.85$, $p < 0.001$) (Fig. 2.14).

The contact rates were also analysed in relation to individual characteristics: sex, age class, and reproductive status. Females realised more intra-specific contacts ($Z = 3.41$, p-value < 0.001), but only when the data were analysed for all the species together. In general, juveniles were found to perform significantly more intra-specific contacts that the other age classes ($Z = 9.99$, $p < 0.001$), and this was significant also in bank voles ($Z = 9.55$, $p < 0.01$). In addition, juveniles

performed less inter-specific contacts than adults and sub-adults ($Z = 8.89$, $p = 0.01$), but when the data were analysed by species this was true only in bank voles ($Z = 7.56$, $p = 0.02$). Reproduction status seemed not to have any influence on intra or inter-specific contact rates.

A negative binomial regression was employed to investigate the effect of body mass on contact rates. The model using pooled data did not show any significance, but the data considered separately revealed that among wood mice heavier individuals realised less intra-specific contacts ($p = 0.01$). In contrast, weight only affected inter-specific contacts in bank voles; the regression showed that heavier individuals performed less inter-specific contacts ($p = 0.04$). Both models were tested for goodness-of-fit and both showed a good fit ($p > 0.05$). The addition of other variables to the basic model, including just body mass as explanatory variable, significantly decreased the fit.

Intra-specific contacts were positively related with population density regarding pooled data ($r^2 = 0.70$, $p < 0.001$) and bank voles ($r^2 = 0.82$, $p < 0.001$), but this relationship was just slightly significant for wood mice ($r^2 = 0.16$, $p = 0.07$), and not significant for field voles (Fig. 2.15). The models were all significantly different from each other. Population density of one species did not affect intra-specific contacts of the other species.

Population density did not show the same association with inter-specific contacts, but a higher proportion of inter-specific contacts was performed at lower individual densities (pooled data excluding Skomer voles: $y = 0.33 – 0.001*x$, $r^2 = 0.15$, $p = 0.02$), although the proportion of variance explained by the model was low.

Analysing the relationship between contacts and densities of the opposite species, the only significant relationship was found between wood mice inter-specific contacts and bank vole density ($r^2 = 0.84$, $p < 0.001$) (Fig. 2.16).

Fig. 2.11. Average number of daily individual contacts realised by each species during the trapping period. Error bars represent standard deviation. Black: intra-specific contacts; white: inter-specific contacts.

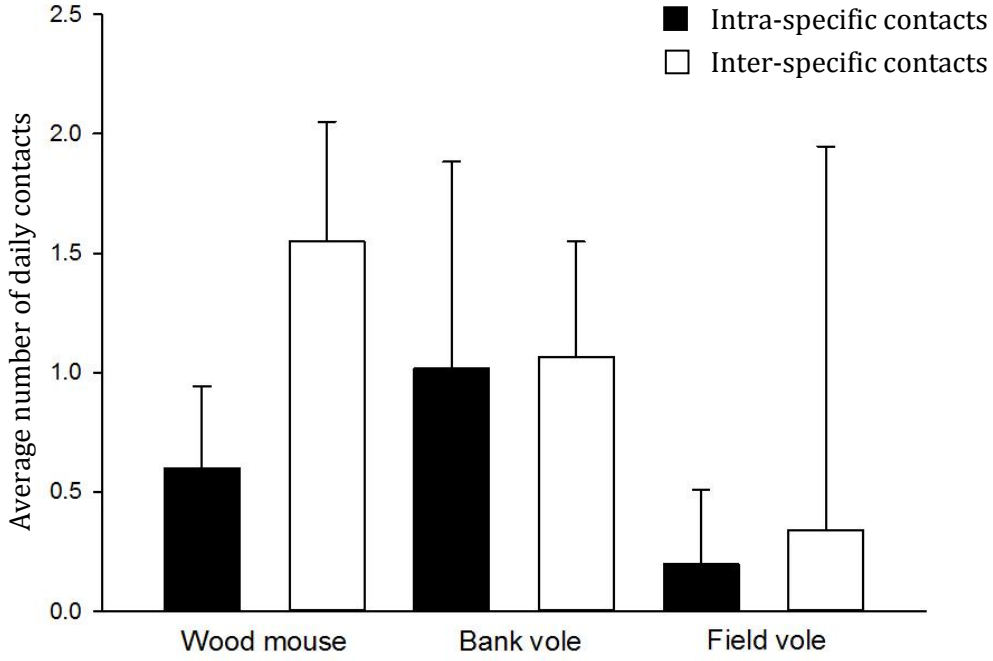


Fig. 2.12. Box and whiskers plot of daily individual contact rates estimated for each of the two different seasons of trapping (autumn: post-breeding peak; spring: pre-breeding recruitment). Pooled data from all the sites excluding Skomer. White: bank vole; light grey: field vole; dark grey: wood mouse. a) Intra-specific contacts; b) Inter-specific contacts.

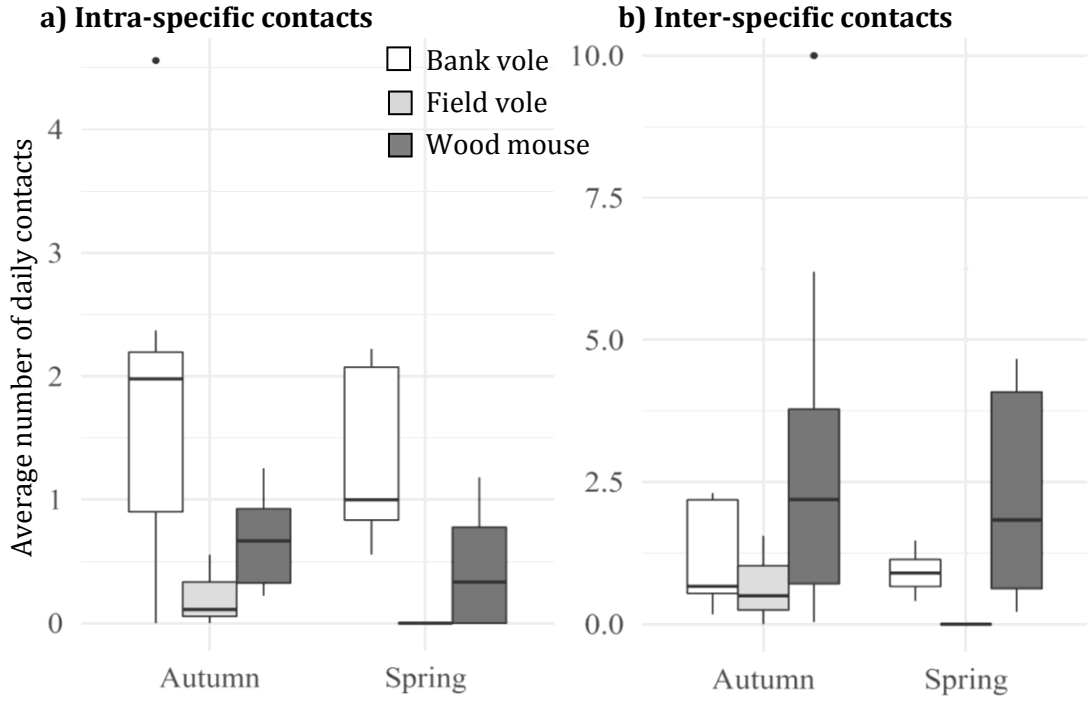


Fig. 2.13. Box and whiskers plot of daily individual contact rates estimated for each trapping site (data pooled by species). Different letters represent significance (p <0.05). White: intraspecific contact rate; grey: interspecific contact rate.

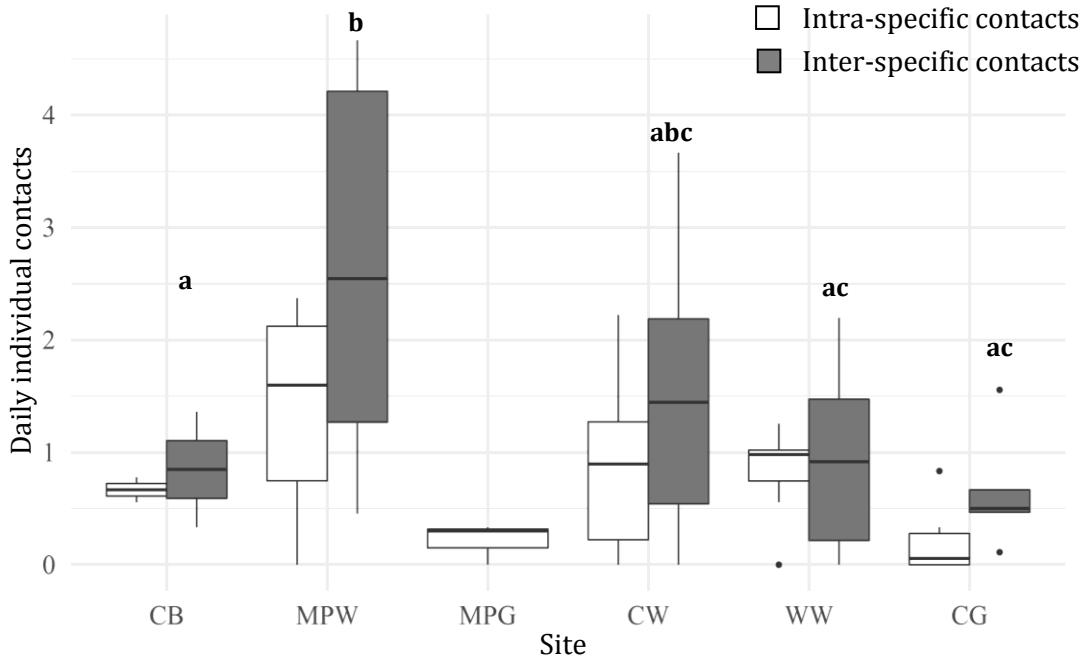


Fig. 2.14. Relationship between daily average inter-specific and intra-specific contacts in bank voles and wood mice. Each data point represents a trapping session. Squares: bank vole; triangles: wood mouse. Solid line: regression line of the relationship between bank vole inter-specific contacts and wood mouse intra-specific contacts. Dashed line: regression line of the relationship between wood mouse inter-specific contacts and bank vole intra-specific contacts. See text for line equations.

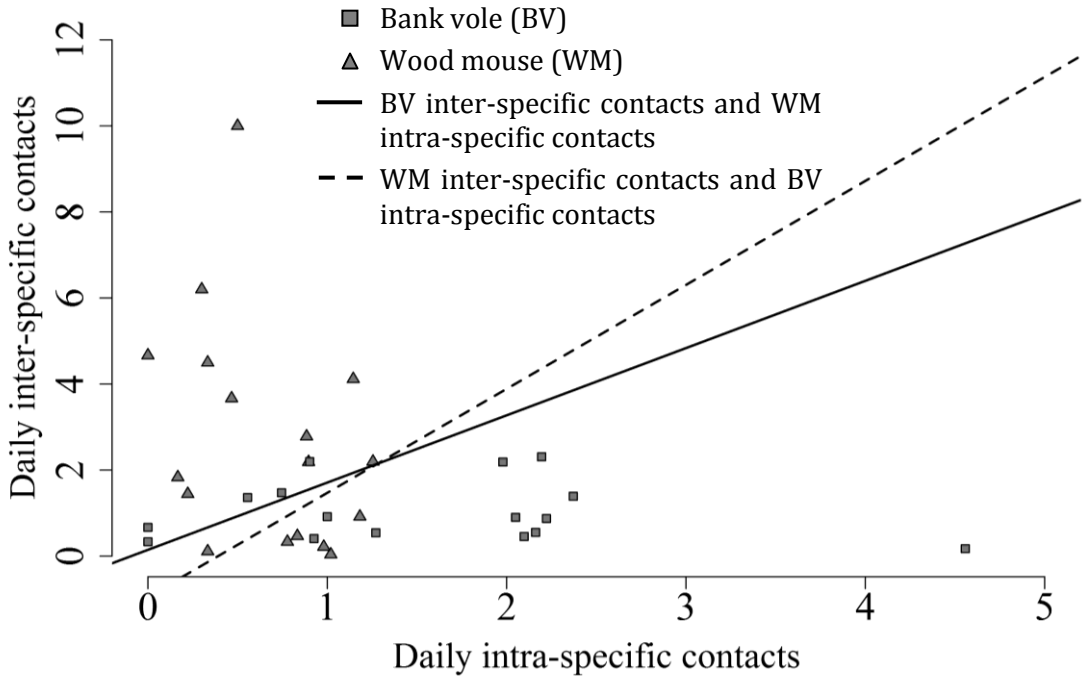


Fig. 2.15. Relationship between daily average intra-specific contacts and individual density. Each data point represents a trapping session. Squares: bank vole; triangles: wood mouse. Solid line: regression line (pooled species). Dashed line: regression line for bank voles. Dotted line: regression line for wood mice.

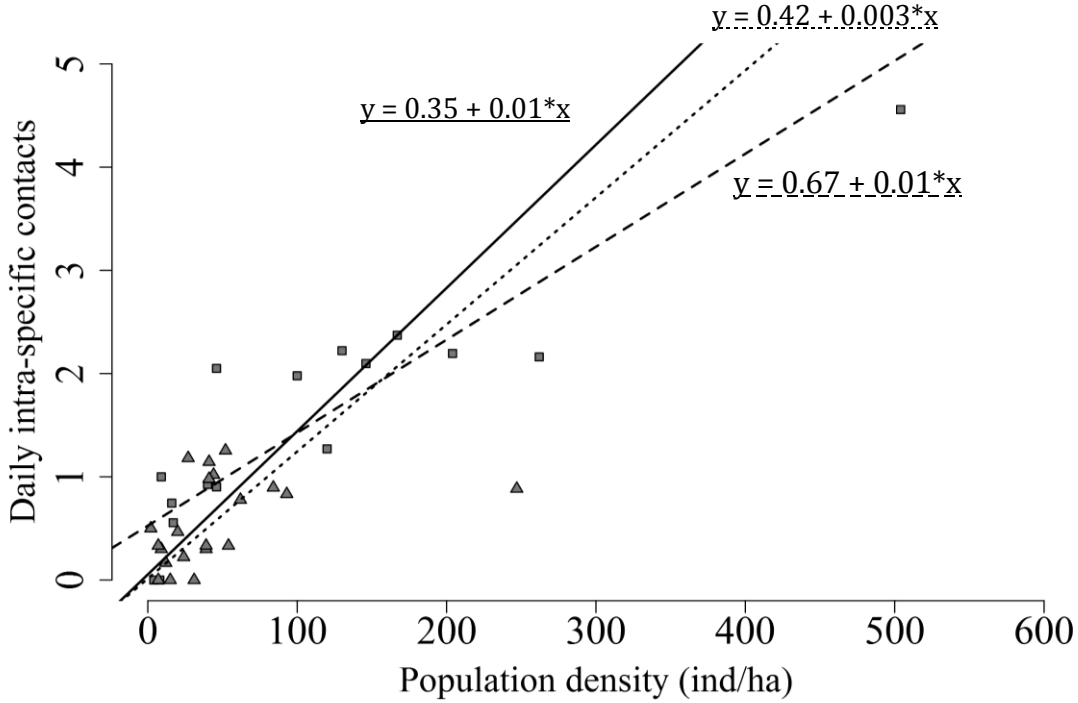
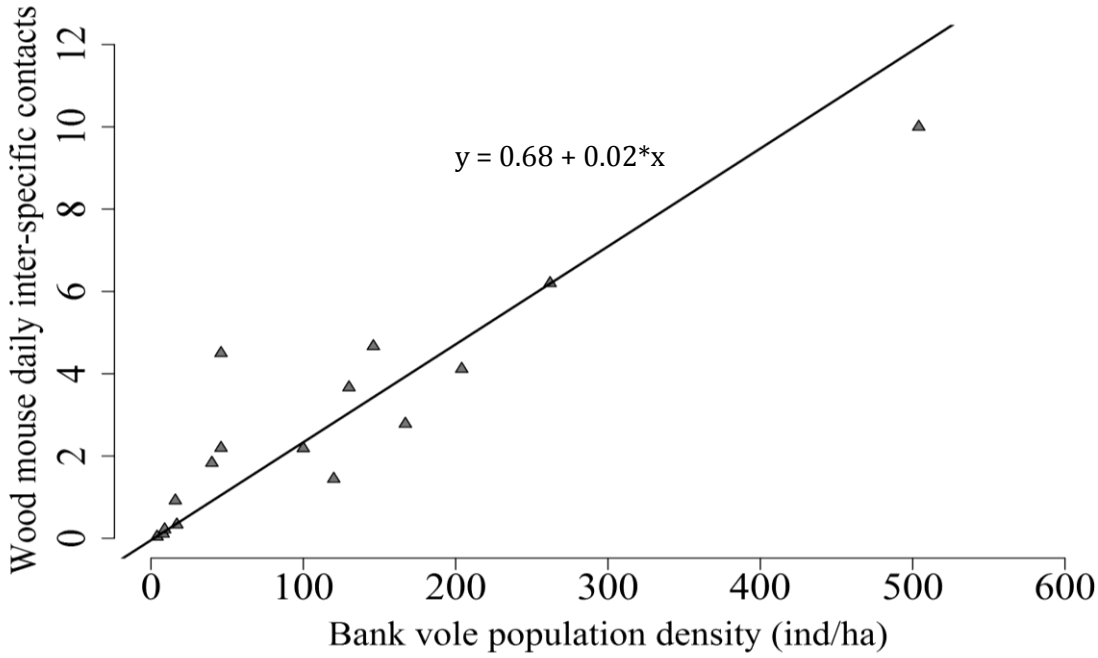


Fig. 2.16. Relationship between daily average wood mice inter-specific contacts and bank vole individual density. Each data point represents a trapping session.



2.4 Discussion

2.4.1 Rodent community composition

The species captured reflected the expected community assemblages in the habitat chosen, but, despite the vicinity to human settlement/activities of some sites, there were no house mice trapped (*Mus musculus*). In the grassland sites, were it was expected a greater abundance of field voles, mostly wood mice were trapped, while field voles (*Microtus agrestis*) was present at very low densities only in the clear-cut site. It is reported that field voles may go through more marked population fluctuations compared to other small rodent species in Britain (Lambin *et al.*, 2000), so the result may be due to true low density during the sampling period in the selected sites.

Wood mice (*Apodemus sylvaticus*) were found in all the sites sampled, reflecting the extreme generalism of the species, which has been recorded in all types of woodlands, hedgerows, open areas, and agricultural fields (Millán de la Peña *et al.*, 2003). No yellow-necked wood mice (*Apodemus flavicollis*) were trapped, although present in south Wales its distribution is restricted to ancient woodlands and it is very localised (Marsh and Harris, 2000).

All the sites, with the exception of one grassland, revealed the presence of bank voles, which are very widespread in UK. The species often occurs in the same habitat of wood mice, but the microhabitat preferences are slightly different and they usually minimise the niche overlap, depending more on forested areas (Torre and Arrizabalaga, 2008). Also, they influence each other's density and distribution (Fasola and Canova, 2000), and *M. glareolus* exhibit less dispersal abilities and smaller home-ranges than mice species (Kozakiewicz *et al.*, 1999).

The Skomer vole (*Myodes glareolus skomerensis*) is a distinct island subspecies that has recently evolved after probably accidental introduction to the island (Hare, 2009). It is larger than the mainland bank vole, and also presents some

differences in terms of behaviour, social organisation, and ecology (Corbet, 1964). These are due to the adaptation to the generally high-density life in the patches of bracken forests, and the absence of terrestrial predators (Fullagar *et al.*, 1962; Adler and Levins, 1994). Skomer voles have a breeding season generally lasting from May to September and the period of study (end of August/beginning of September) motivated the high percentage of juveniles captured in this site.

Wood mice and bank voles, in Great Britain, are thought to reproduce from March/April to October, but the two species are not fully synchronous (Mallorie and Flowerdew, 1994; Huitu *et al.*, 2004), therefore this might explain the differences in percentage of adults and sub-adults. The high percentage of sub-adults found in bank vole populations may reflect an earlier breeding peak than wood mice.

2.4.2 Rodents body mass

Skomer voles were, as expected, significantly heavier than all sampled species, due to the "island syndrome", a term used to summarise all the systematic differences in morphology, demography, reproduction, and behaviour that rodents show after isolation and adaptation on island habitats (Adler and Levins, 1994). Also, on Skomer, females appeared to be slightly heavier than males, and this may be caused by the decrease of male adult mass as the breeding season progress for the maturation of young males. In fact, the sampling was carried out towards the end of the breeding season, when individuals born at the beginning of it already left the nest, and entered the sub-adult, or adult population. However, female body mass varies according to reproductive state so the data needs to be interpreted with caution. Also, there

was no difference in body weight between females and males on mainland species.

In all the species juveniles were significantly lighter than other age classes, but there was no difference between adults and sub-adults. Therefore, for each species, the average body mass of adult and sub-adult individuals was the value chosen to estimate allometric parameters (Bolzoni *et al.*, 2008) for the models of infection (see Chapter 5), including the growth rate showed in Fig. 2.8.

Finally, all adults and sub-adults were found to be lighter in the post-breeding recruitment season (autumn), reflecting the difference in demography between the two seasons of sampling. The spring sampling season therefore captured the pre-breeding recruitment population, mostly represented by heavier, older individuals, conversely, in autumn, the population also comprised younger and lighter individuals born earlier in the season. This confirmed that the sampling strategy was effective in capturing two different moments in populations' fluctuations.

2.4.3 Population density

The high individual density of Skomer voles, although the estimation lacked in precision, was in agreement with previous reported values (Fullagar *et al.*. 1962; Harris *et al.*, 1995; Healing *et al.*, 1983; Healing, personal communication; Loughran, 2013). The values recorded on Skomer at different times have always been higher, up to four times, than the mainland bank vole populations (Healing, 1984). Skomer populations also seem to have intra-annual cycles, with minimum densities occurring in late spring, before breeding, and peaks in autumn, by the end of the breeding season. Therefore, considering the time of sampling, it is reasonable to think that the estimates in this study represented almost the population peak.

It has been reported that these voles also exhibit multiannual cycles, but the amplitude or the determinants of the cycles are not fully understood (Healing, 1984; Loughran, 2013). Skomer has no terrestrial predators, so the only predator pressure comes from avian predators (barn owls, short-eared owls, kestrels); this together with density-dependent dynamics, (e.g. breeding suppression, decrease of dispersal), and density-independent factors (e.g. weather, resource availability) might regulate Skomer voles cycles (Loughran, 2013).

Mainland species, as expected, displayed higher density in autumn, when there is recruitment of new individuals born earlier in the breeding season, compared to the spring values, which represented the population at the beginning of the breeding season. In Britain, bank voles are known to exhibit annual cycles of abundance with a winter/spring decline and a progressively increase in numbers towards the autumn (Alibhai and Gipps, 1985). This was well documented by the densities found in every site of the study; however, it was impossible to speculate about a potential multiannual cycle.

Small rodents in northern Fennoscandia have regular multiannual cycles, which seem to be dampened along a latitudinal gradient towards the south due to the greater diversity of generalist predators and alternative preys, the decrease of snow cover, and the increase of habitat heterogeneity (Hanski *et al.*, 1993; Hanski *et al.*, 2001; Hanski and Henttonen, 1996; Sundell *et al.*, 2004; Turchin and Hanski, 1997). In Britain there have been different findings, Lambin *et al.* (2000) described, in field voles, cyclic dynamics very similar to those reported from Fennoscandia, but in bank voles these characteristic multiannual cycles were never reported (Petty, 1999; Sundell *et al.*, 2012). This difference may be due to the lack of long-term studies, which failed to identify the cycles, or to the unexpected population regulation of multispecies assemblages and the local predatory dynamics (Hanski and Henttonen, 1996; Hanski *et al.*, 2001; Sundell *et al.*, 2004). This lack of synchrony between rodent species has been compared to the area of Fennoscandia around the 60°N, where forest inhabiting bank

voles tend to have more stable populations than field voles inhabiting grasslands or clear-cuts (Hansson and Henttonen, 1985; Lambin *et al.*, 2000).

Ultimately, the factors affecting population density are likely to be a combination of first order effects, such as predation and competition, as well as secondary effects connected to food resources, influenced by weather and vegetation cover (Gorini *et al.*, 2012; Lima *et al.*, 2006; Sundell, 2006). In addition, disease processes and parasitism may also influence behaviour, social organisation, and survival, contributing to population dynamics (Altizer *et al.*, 2006; Begon *et al.*, 2009; Cavanagh *et al.*, 2004; Telfer *et al.*, 2002).

Density in bank voles and wood mice showed different relationship with previous season density, highlighting the complexity of density-dependent processes in different populations. However, the lack of long-term data and the low significance of the regression models make impossible to draw any conclusion. In fact, in a microtine population in England the density-dependent lag was found to be between 1.5 and 2 years (Lambin *et al.*, 2000), but the lack of data did not allow testing this pattern in the current study. Evidence support that delayed density-dependent effects on recruitment have strong effect on rodent population (Aars and Ims, 2002). In particular, densities during non-breeding season have been found to determine the onset time of breeding season, playing a role in population cycles (Ergon *et al.*, 2011; Smith *et al.*, 2006).

2.4.4 Seasonal growth rate

Seasonal growth rate clearly showed the difference between non-breeding and breeding season, confirming the intra-annual fluctuations remarked by density estimates. The values were in agreement with other studies on rodent species (Careau *et al.*, 2013; Merritt *et al.*, 2001; Huitu *et al.*, 2004; Lambin *et al.*, 2000).

The difference between wood mice and bank voles was not significant in both seasons, but this may be due to the small size of the data set; in fact, looking at the graph it seems that there is a trend for wood mice to have a more distinct reduction in population during non-breeding season. There is no knowledge about a greater overwinter mortality of wood mice compared to bank voles, but this pattern may be determined by the asynchrony of population fluctuations, so that the two species might have been in different phases of their intra and inter-annual cycle (Hanski *et al.*, 2001; Mallorie and Flowerdew, 1994; Sundell *et al.*, 2004).

The values found empirically were much lower than the allometric estimate suggested by Bolzoni *et al.* (2008), who used growth rate and other allometrically estimated parameters to parameterise a model for microparasites infection in a range of host differing in body size. This will be taken in account in the modelling section (Chapter 5), where the growth rates derived from population estimates will be used as parameters in the models of infection of various pathogens.

Individual density had a direct positive effect on growth rate, especially for wood mice, but this effect may be just an artefact due to the short time scale of the data; density usually has a direct or, more often delayed, negative effect on population growth (Aars and Ims 2002; Merritt *et al.* 2001; Smith *et al.* 2006).

However, according to the delayed-density effect, previous season individual density showed a negative relationship with growth rate in both species, confirming that intra-specific density-dependent factors are primary determinants of population dynamics (Burthe *et al.*, 2010; Huitu *et al.*, 2004; Lambin *et al.*, 2000; Smith *et al.*, 2006).

2.4.5 Intra and inter-specific contact rate

The importance of social structure in epidemiology has been largely demonstrated in humans and wildlife, since the way individuals interact affect the spread of an infectious disease in a population and the probability of individuals to be infected (White *et al.*, 2015; Silk *et al.*, 2017). Contact network analyses are used to quantify individual-level and population-level patterns of social behaviour and their relationship with epidemiological data, and this information are invaluable tools for statistical and epidemiological modelling of host–pathogen systems (Silk *et al.*, 2017).

A comprehensive analysis of the contact network of the communities sampled was beyond the aim of the study, but the estimation of the daily individual contact rates is a simple and easy way to explore contact distributions, describe contact heterogeneity, and investigate shared space (Grear *et al.*, 2009; Perkins *et al.*, 2009; VanderWaal *et al.*, 2013). Although, this methodology may miss information about quality of contacts, which has been found to be more suitable than frequency in explaining patterns of disease transmission (Clay *et al.*, 2009b; White *et al.*, 2015). Nevertheless, in a comparison of methods to quantify contact networks using radio telemetry and capture–mark–recapture data, Perkins *et al.* (2009) found that both methods of data collection produced similar contact distributions for a population of yellow-necked mice, but capture-mark-recapture may underestimate the numbers of contacts. Additionally, the method used in the study is applicable to investigate directly and indirectly transmitted diseases, being a surrogate of shared space use, which also underlies social and transmission networks (Sih *et al.*, 2017).

In order to maximise the information about shared space use, identify heterogeneities, and potential superspreaders, individual daily contacts were estimated for each species and for each class of individuals (classified by sex, age, reproductive status, weight).

The analysis of individual daily contacts revealed that the distribution of both intra and inter-specific contacts was highly aggregated, meaning that a small number of individuals were responsible for a large proportion of interactions. This was also found in deer mice, in an investigation of transmission of Sin Nombre Virus in USA (Clay *et al.*, 2009b).

It appeared that for each species considered (bank vole, field vole, and wood mouse) there was no difference between intra and inter-specific contacts. However, bank voles performed more intra-specific contacts than wood mice, while wood mice tent to interact more with other species. This might be related to differences in population density, space use, and the greater dispersal abilities of wood mice, which have larger home ranges and are able to exploit a higher range of habitats (Douglass *et al.*, 1992; Geuse *et al.*, 1985; Kikkawa, 1964).

However, contacts are not static, and social interactions are likely to vary in different conditions (e.g. breeding and non-breeding), as confirmed also in other species such as mouse lemurs, cattle, raccoons (Chen *et al.*, 2014; Hirsch *et al.*, 2016; Springer *et al.*, 2017). The results showed no variation of contacts across seasons, but inter-specific contacts were significantly different across sites, reflecting different species assemblages, habitat use, and relative densities.

With regards to individual characteristics, females and juveniles were found to realise more intra-specific contacts, as expected due to the higher rate of male dispersal, and the typical female territoriality in rodent species (Dobson, 1982; Douglass *et al.*, 1992; Wolff, 1993). This might have implication in disease transmission, when considering the demography and the social structure of the target populations. In addition, in wood mice weight was negatively associated with intra-specific contacts, but in bank voles this was negatively associated with inter-specific contacts. So, older, heavier wood mice may be more mobile and have more inter-specific transmission potential, while heavier bank voles might have more intra-specific transmission potential; body weight was found positively associated with number of contacts and transmission potential by

Clay *et al.* (2009b). According to the analysis on body mass, heaviest individuals could not be distinguished in terms of sex or reproductive status.

Inter-specific contacts in both species were positively associated with intra-specific contacts of the alternative species, and this can be interpreted in light of the relationship found between contacts and individual density. In fact, in both species intraspecific contacts increased with density, consequently it is likely that an increase in density of either species boosted inter-specific contacts.

There was no significance between density and inter-specific contacts, but considering their proportion, this was inversely related to individual density, although this result needs to be interpreted with caution because the model did not have a good fit. This might mean that there was a decrease in proportion of inter-specific contacts when the population increased due to aggregation and limited movements to avoid aggressive interactions and overlap of territories (Bogdziewicz *et al.*, 2016; Hestbeck, 1982). However, it also seemed that inter-specific contacts in wood mice increased with the increase of bank vole density, suggesting that interaction between mice and voles was mainly determined by an increase in abundance of the second species.

In conclusion, it is clear that there is a relationship between density and contacts of both types, but this is highly complex, and often non-linear. In fact, it has been found that in wildlife contacts and density have a linear density-dependent relationship at low densities, while the relationship progresses towards a frequency-dependent one when density increases (Davis *et al.*, 2015; Smith *et al.*, 2009b).

Chapter 3

Characterisation of ecto-parasite infestation in Welsh wild rodents

3.1 Introduction

Ecto-parasites are a group of diverse parasitic organisms that live on the outside of their host's body for a variable length of time (Hersh *et al.*, 2014a). These can affect host body condition and physiology (Hawlena *et al.*, 2006; Lourenco and Palmeirim, 2007; Heylen and Matthysen, 2008), reproduction (Neuhaus, 2003; Fitze *et al.*, 2004; Hillegass *et al.*, 2010), and behaviour (Brown and Brown, 1992; Raveh *et al.*, 2011). Ecto-parasites can influence host survival and population dynamics, depending on factors such as host and parasite(s) taxonomic identities, duration, and intensity of infestation; however, these effects are variable and not uniform across all individuals in a particular population (Brown *et al.*, 1995; Krkosek *et al.*, 2007; Devevey and Christe, 2009). In fact, ecto-parasite infestation, or burden, varies considerably among individuals, and usually there is a high level of aggregation, also determined by host individual characteristics (Anderson and May, 1978; Brunner and Ostfeld, 2008). Higher burdens have been found in males due to their size or hormonal profile (Gorrell and Schulte-Hostedde, 2008; Devevey and Brisson, 2012), but this pattern has multiple exceptions (Kiffner *et al.*, 2013; Krasnov *et al.*, 2005). Intensity of infestation can also be the result of environmental aggregation (e.g. due to microhabitat conditions; Calabrese *et al.*, 2011). These parasites can also affect their hosts by transmitting a wide range of diseases; in fact, they have been found to be vectors of a large number of pathogens of different taxa, including humans. In particular, among this disparate group of parasites, ticks and fleas are very

frequently involved in disease transmission in small mammals (Bitam *et al.*, 2010; Espinaze *et al.*, 2016).

Ticks are arachnids of the order Ixodida, and are obligate ecto-parasites feeding on the blood of a wide variety of vertebrates (Klompen *et al.*, 1996). The target community of the study, small rodents, act as hosts for different species of hard ticks (family Ixodidae). Ixodid ticks have a three-host life cycle (larva, nymph, and adult), during which rodents represent host for all or just some life stages, depending on the tick species (Paziewska *et al.*, 2010), and their abundance is determined by abiotic factors (e.g. habitat, climate), but also by the host community composition (Gray, 2008). In the past, ticks were thought to be specialist parasites, being specifically adapted to feed on a particular host (Hoogstraal and Kim, 1985), but more recently it has been discovered that they exhibit prevalent host generalism (Espinaze *et al.*, 2016). Hence, understanding tick-host associations, and how multiple host species regulate tick dynamics, is very important to comprehend ticks ecology, and predict patters of tick distribution, especially in the context of tick and tick-borne disease management and control (Cumming and Van Vuuren, 2006). Further, generalism may increase the chance of disease transmission and favour spillover events in a larger number of host species (Power and Mitchell, 2004), that is why ticks (and tick-borne diseases, see Chapter 4) are ideal multi-host parasites to be investigated in the context of disease ecology (Dobson, 2014).

Among rodents, ixodid ticks have not been found to negatively affect host survival, and tick burden has also been positively associated with survival in white-footed mice (*Peromyscus leucopus*) (Hersh *et al.*, 2014), probably because the most heavily parasitised individuals were also the most likely to survive (Perkins *et al.*, 2003). Prevalence and intensity of infestation of ticks in small rodents have been extensively investigated and different patterns of species preferences have been found (e.g. Hussein, 1980; Paziewska *et al.*, 2010). Also sex seemed to affect infestation, in particular, heavier males were usually found to have higher burdens (e.g. Perkins *et al.*, 2003; Harrison *et al.*, 2010). Finally, Brunner and Ostfeld (2008) noticed that rodent density was negatively related to

tick burdens, but this has not been found to be always true, most likely because, when the number of questing ticks is so high, this nullifies the rodent density-dependent effect on tick burden (Paziewska *et al.*, 2010).

The second ecto-parasite taxon of interest is fleas (Insecta, Siphonaptera), which are small, laterally flattened, wingless insects; they are holometabolous insects with a life cycle that, from egg to adult, comprises several larval stages and a pupal stage (Bitam *et al.*, 2010). They feed on blood on many higher vertebrates, preferring small burrowing mammals, and alternate between periods occurring on the host body and periods occurring in the host burrow (or nest) (Krasnov *et al.*, 2002). As vectors, they harbour a large number of pathogens, of which the majority are still understudied, but can represent a serious threat in terms of emerging diseases (see Chapter 4) (Bitam *et al.*, 2010). In general, flea biology and ecology have not been fully understood yet, especially flea-host relationship is still under investigation, but latest research revealed that species compositions of flea species host spectra were determined by an interaction between species phylogeny and traits (Krasnov *et al.*, 2015; Krasnov *et al.*, 2016). Compared to ticks, fewer studies concerned flea prevalence patterns, and the existing ones revealed an extreme complexity in host-habitat-parasite associations (Krasnov *et al.*, 2002), making the collection of data about local prevalence and abundance of fleas an invaluable tool in uncover host-parasite dynamics and flea role as disease vectors (Kowalski *et al.*, 2015).

As for ticks, rodent host species characteristics and population dynamics seemed to influence prevalence and intensity of infestation of fleas. Host density and flea burden or prevalence relationship has been found to be different in different host species-flea species association, exhibiting linear or asymptotic shapes (Krasnov *et al.*, 2002; Krasnov *et al.*, 2015; McCauley *et al.*, 2008; Young *et al.*, 2014). No universal rule can be drawn by examining host gender preferences of fleas (Kiffner *et al.*, 2014), or preferences for a specific host age-class (Hawlena *et al.*, 2005). For example, Kowalski *et al.* (2015), in Poland, found male-biased parasitism in *Apodemus agrarius* and *Myodes glareolus*, but not in *A. flavicollis*, and this was due to the relationship with body mass only in *A. agrarius*. Another

study observed both male and female biased parasitism, and, in particular, the pattern changed across seasons, suggesting that flea host gender preference involves many factors related not only to the host (e.g. size, immunity, spatial behaviour) (Krasnov *et al.*, 2005), but also to the parasite (Krasnov and Matthee, 2010).

In this study, tick and flea patterns of prevalence and intensity of infestation were investigated among the Welsh rodent communities sampled (see Chapter 2); in the next section details are provided about methodology of parasite collection and identification.

Additionally, identification of ecto-parasites was performed both morphologically and molecularly. Morphological identification based on phenotypic traits is not always possible due to specimen degradation, complexity of pathognomonic features, or lack of expertise and/or local ID keys, although it is economic and convenient (Ernieenor *et al.*, 2017; Marrelli *et al.*, 2007). Thus, it is useful to develop a different characterization method in order to differentiate species, and subspecies, which is also reliable and convenient (Pagel Van Zee *et al.*, 2007). Molecular approaches, based on mitochondrial (mt) and ribosomal DNA (rDNA) fragments, have been proven to be an efficient tool for tick identification, and at the same time estimate genetic variation and discriminate the closely-related species (e.g. Chitimia *et al.*, 2010; Lv *et al.*, 2013; Lv *et al.*, 2014). Polymerase chain reaction (PCR) amplification of specific genome regions, sequence analysis of the amplicons obtained, and alignment of the data with reference sequence have been successfully employed to distinguish arthropod species, including ticks (Casati *et al.*, 2008; Che Lah *et al.*, 2016; Zahler *et al.*, 1995).

Among genome regions, the cytochrome oxidase subunit I (COI) is the most commonly used marker for identification and barcoding, especially invertebrates (Deagle *et al.*, 2014). This gene has been considered ideal due to the higher mutation rate, maternal inheritance and haploid nature of the mtDNA encoded, and widely used for tick and flea phylogenetic analyses (e.g. Ćakić *et al.*, 2014; Chitimia *et al.*, 2010; Ernieenor *et al.*, 2017; Lawrence *et al.*, 2015; Márquez, 2015). However, COI gene is also more frequently used because no other regions

can be found in taxonomically verified databases, but often it has been proven not to be the best barcoding marker choice (Deagle *et al.*, 2014). It has been suggested that markers should be study-specific, and that several barcode markers should be routinely used in molecular identification studies (Deagle *et al.*, 2014; De Barba *et al.*, 2014). Thus, genotypic identification of the ecto-parasites collected was performed amplifying not only the COI gene, but also a different fragment of DNA, 16S for ticks and 18S for fleas, which was proven effective in the taxa considered, with the aim to contribute to the advancement of ticks and fleas metabarcoding techniques. The 16S gene was chosen because successfully used in UK to identify *Ixodes trianguliceps* (Bown *et al.*, 2006), which was by far the most represented species in the tick pool according to the morphological identification. Regarding the fleas, 18S gene was selected because traditionally used in flea phylogenetic analyses (e.g. Whiting *et al.*, 1997; Whiting, 2001; Whiting *et al.*, 2008), so greater information was available also for comparing the results of this study.

3.2 Methods

3.2.1 Ecto-parasite collection

Ecto-parasites, namely ticks and fleas, were collected from small rodents sampled during live-trapping performed from autumn 2015 to spring 2017. Seven sites in Ceredigion and Pembrokeshire (Wales) were selected and a regular sized trapping grid (6x6) was used to capture ground dwelling rodents in two seasons (spring and autumn) (see Chapter 2 for more details about field methods). Each individual was screened at its first capture for ecto-parasites, while no ticks and fleas were collected during recaptures.

Ticks were collected, after visual inspection, with fine point forceps mainly from the cephalic area of the animals. In fact, ticks mostly occur in the anterior third of small rodents, and in particular on the ears (Hussein, 1980; Randolph, 1975a). Fleas were collected according to McCauley *et al.* (2008) and Young *et al.* (2014). Each individual was held over an open, deep and transparent, plastic bag and then combed for 10 strokes with a flea comb; all the fleas recovered from both, the bag and the comb, were collected.

Specimens were stored at -18°C in sample tubes filled with RNAlater, or at -80°C without RNAlater, in order to preserve nucleic acids and allow molecular investigations. Before storing the samples in the freezer, usually the maximum amount of time the samples spent at ambient temperature was 3 hours (considering the trap checking session time and the transport); however, most of the time, the parasites not preserved in RNAlater were still alive at the moment of freezing. RNAlater was used because proven to be effective in preserving nucleic acids for 24 hours at 37°C, 1 week at 25°C, 1 month at 4°C, or indefinitely at -20°C (Drakulovski *et al.*, 2013). The RNAlater solution was prepared according to De Wit *et al.* (2012).

3.2.2 Morphological identification

Frozen samples were incubated with Dietrich's fixative solution (equal parts of 96% ethanol, glycerol, and double distilled water) overnight in the fridge in 2 ml sample tubes. The morning after incubation the batch of samples were identified under a high magnification microscope mounted on a cavity slide with a drop of Dietrich's solution, and assigned a new sample code for molecular analyses. The fixative solution improved the visualisation of the specimen and protected it from degradation during the identification process. The identification was performed at species level for ticks according to Hillyard (1996), and Snow (1978) for larval samples; life stage and sex of the adults was also recorded. Fleas were identified, when possible, at subspecies level according to Whitacker (2007).

3.2.3 DNA extraction and PCR for molecular identification

After morphological identification, the samples were washed for 1 hour in 70% Ethanol, 1 hour in 50% Ethanol, 1 hour in 30% Ethanol, 1 hour in 10% Ethanol, and 1 hour in double distilled water, and placed in a new 0.5 ml sample tube (Ash *et al.*, 2017; Harris *et al.*, 2009). In this tube the specimens were prepared for DNA extraction through alkaline digestion (Bown *et al.*, 2003). Each sample was macerated with a pipette tip and incubated overnight at 56°C with 20 µl of Proteinase K (Qiagen, Germany) and 80 µl of PBS solution (ThermoFisher, UK) to increase DNA yield. After incubation, 0.5 ml of 1.25% ammonia solution was added to the sample (1 ml for engorged adult female ticks), which was then heated at 100°C for 20 minutes, and centrifuged at 17000 rpm for 5 minutes. Finally, the lid of the tube was opened and the sample was heated at 100°C until half of the volume was evaporated. Alternatively, for some samples DNA was extracted with the tissue protocol of Qiagen Mini Kit (Qiagen, Germany) to

compare DNA yields between methodologies. The DNA sample obtained was used for the following PCR reactions and kept in the freezer for storage at -18°C.

Tick samples were employed in two different PCR reactions with two different sets of primers targeting two regions of DNA. The first PCR targeted a region of ~710 bp of the COI gene, using the primers LCO-1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO-2198 (5'-TAAACTTCAGGGTG ACCAAAAAATCA-3') from Folmer *et al.* (1994). The amplification program consisted of a total of 55 cycles: denaturing at 95 °C for 30 s, annealing at 45°C for 1 min, and extension at 72°C for 45 sec, with an initial denaturation at 95°C for 3 min, and a final elongation at 72°C for 2 min. The reaction mix of 10 µl consisted of 2 µl of DNA template, 0.5 µl of each primer, 5 µl of Biomix (Bioline, UK), and 2 µl of nuclease free water. This reaction mix was used for all the PCR reactions described in this section. In addition, the samples were subjected to a reaction targeting a segment of the 16S gene of ~460 bp, using the forward primer 16S+1 (5'-CTGCTCAATGATTTTTTAAATTGCTGTGG-3') and the reverse primer 16S-1 (5'-CCGGTCTGAACTCAGATCAAGT-3'), according to Black and Piesman (1994) and Bown *et al.* (2006). The conditions of this reaction were: denaturing at 95 °C for 20 sec, annealing at 55°C for 20 sec, and extension at 72°C for 50 sec (35 cycles), with an initial denaturation at 95°C for 2 min, and a final elongation at 72°C for 5 min.

DNA samples obtained from fleas were used in a PCR targeting the COI gene, as described for the ticks, while the second reaction targeted a portion of around ~450 bp of the 18S gene. The primers were designed from those reported in Whiting (2001) and Whiting (2001): Con-Dia-F (5'-ATGCATGTCTCAGTGCAAGC-3') and Con-Dia-R (5'-AGCTTTTTAACCGCAACAAC-3'). The amplification reaction comprised an initial denaturation step at 94°C for 2 min, 35 cycles of denaturing at 94°C for 1 min, annealing at 50°C for 1 min, extension at 72°C for 90 sec, and finally an elongation step at 72°C for 10 min.

In each case the positivity of the amplification was confirmed by electrophoresis in a 1% agarose gel. PCR products were then stored frozen at -18°C.

Amplified DNA was purified using SureClean Plus (Bioline, UK) according to the manufacturer protocol, and then the samples, consisting of 1 µl forward primer, 1 µl purified DNA, and 4 µl nuclease free water, were sequenced with an AB3500 DNA sequencer (Applied Biosystems).

3.2.4 Sequence alignment and phylogenetic analysis

Sequences obtained from tick and flea specimens were compared to sequences deposited in GenBank using the BLAST feature of NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul *et al.*, 1990). Considering tick samples, 15 sequences of the length of 390 bp representing the COI fragment, and 56 sequences of the length of 253 bp representing the 16S fragment were imported in MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets (Kumar *et al.*, 2015). Appropriate sequences downloaded from GenBank were added to the data sets to represent out-groups, and some sequences of the species expected in the data set were also added and imported together with the sequences obtained in this study. Multiple sequences alignment was performed by ClustalW, grouping the sequences according to the gene considered. The sequences for the analysis were chosen according to their quality, short sequences or with a low quality chromatogram were excluded. The same software was used for phylogenetic analyses; phylogenetic trees were constructed by the Maximum Likelihood method based on best fitting model for each set of sequences (i.e. lowest BIC score), and bootstrap test of 1000 replicates. Regarding fleas, 13 sequences of length 356 bp representing the COI gene, and 59 sequences of 276 bp for the 18S gene were selected for phylogenetic analysis. The same selection criteria and methodologies illustrated for the tick data were applied.

3.2.5 Statistical analyses

Patterns in prevalence of ecto-parasites in rodent populations (namely the proportion of individuals found hosting ecto-parasites) were analysed utilising generalised linear models, fitting a Poisson distribution. It was investigated whether there were significant differences among host species, host age classes, host gender, sampling sites, and sampling season. Generalised linear modelling was also used to investigate the relationship between average ecto-parasite prevalence and host population density. Differences in prevalence among different species/life stages of ticks and fleas were examined with Pearson's Chi-square test for independence, in order to identify any significant difference of frequency among host species, sampling season. Further, intensity of infestation, i.e. mean number of fleas on each individual host, patterns were investigated with non-parametric Wilcoxon rank test or the non-parametric Kruskal-Wallis test to identify differences among host species, host age classes, host gender, sampling sites, and sampling season. These statistical tests were chosen according to the guidelines provided by Alexander (2012) regarding analysis of skewed data, in particular parasite counts and prevalence, and to similar published studies (e.g. Paziewska *et al.*, 2010), in order to present comparable results. Finally, the relationship between mean $\log_{10}$ parasite burden and $\log_{10}$ variance (Taylor's power law) (Taylor, 1961) was considered. All the analyses were performed in R (R Core Team, 2016).

3.3 Results

3.3.1 Prevalence and intensity of infestation of ecto-parasites

3.3.1.1 Ticks

In total, 225 ixodid ticks were collected from 120 rodent individuals, the 16.28% of total individuals sampled, of two species: *Apodemus sylvaticus* (wood mouse) and *Myodes glareolus* (bank vole) (Fig. 3.1).

Across all the individuals sampled (pooled species) during the entire study, total infestation prevalence was 15.99%. Prevalence was higher in bank voles (18.14%) than wood mice (16.09%) ($p < 0.01$), and site CW (coniferous woodland in Ceredigion) showed, overall, a higher prevalence of ticks (32.60%) ($p = 0.004$) compared to the other sites. No difference in prevalence was found between different age classes, but males exhibited a higher rate of infestation (21.74%) compared to females (13.17%) ($p < 0.01$). Ticks were more prevalent in spring (27.18%) rather than in autumn (12.87%) (pooled data excluding Skomer because sampling occurred only in one season) ($p < 0.01$).

Bank voles were more heavily parasitised than wood mice ($H = 11.859$, $p = 0.02$), and, overall, intensity of infestation was higher in males than females ($W = 50390$, $p < 0.01$), but the analysis performed by species revealed that this was true only for bank voles ($W = 20308$, $p < 0.01$) (Table 3.1). Average number of ticks per individual was also higher in spring than in autumn ($W = 51550$, $p < 0.01$) (Fig. 3.2).

Regarding tick species, *Ixodes trianguliceps* was, by far, the most represented in the sample set, being the most frequent species recovered on both host species (Table 3.2 and Fig. 3.3). This result was also confirmed by molecular identification (see section 3.3.1.1). Analysis of the life stages (pooled data) revealed that adults (essentially adult females, as very few males were collected) were more abundant in spring, while larvae and nymphs were more abundant in autumn ($p < 0.01$)

(Fig. 3.4 and Fig. 3.5), also different tick life stages were not differently distributed across host species ($p > 0.05$).

The plot displaying Taylor's power law relationship for ticks attached to the two host-species (Fig. 3.6) showed that tick distribution on bank voles and wood mice was different, being more clustered on the first host (slope > 1).

Finally, tick prevalence was negatively associated with wood mouse density, but the fit of the model was quite poor (Prevalence = $-139.02 - 89.33 * N_{BV} - 78.05 * N_{WM}$, $R^2 = 0.17$, $p = 0.02$), while there was no significant relationship with bank vole individual density.

Table 3.1. Average number of ticks per individual rodent. St.Dev: standard deviation; M: males; F: females.

Host species	Sex	Mean	St.Dev
Bank vole	M+F	0.38	1.36
	F	0.19	0.48
	M	0.23	0.55
Wood mouse	M+F	0.20	0.52
	F	0.28	1.28
	M	0.51	1.47

Table 3.2. Prevalence of tick species occurring on the sampled rodents according to morphological identification. In brackets sample size. Unknown species were specimen collected, but degraded to be identified by phenotypic features.

Species	**Prevalence (%)**
Ixodes acuminatus	1.78 (4)
Ixodes hexagonus	1.33 (3)
Ixodes ricinus	4.44 (10)
Ixodes trianguliceps	84.00 (189)
Ripicephalus sanguineus	0.44 (1)
Unknown	8.00 (18)

Fig. 3.1. Histogram representing frequency distribution of ticks on rodent populations. Dashed lines represent the median of non-zero values, which is 1 for all species. White: bank vole; grey: field vole; black: wood mouse.

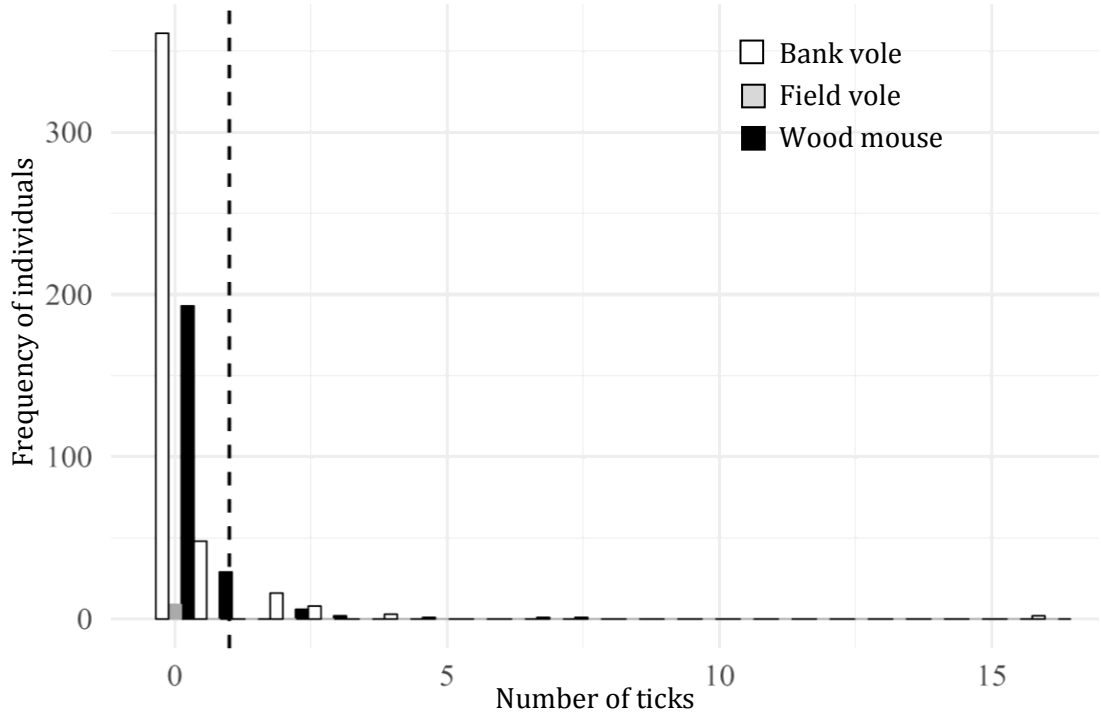


Fig. 3.2. Average number of ticks per individual rodent across sampling seasons. Error bars represent standard error. Solid line: pooled species; dotted line: bank vole; dashed line: wood mouse.

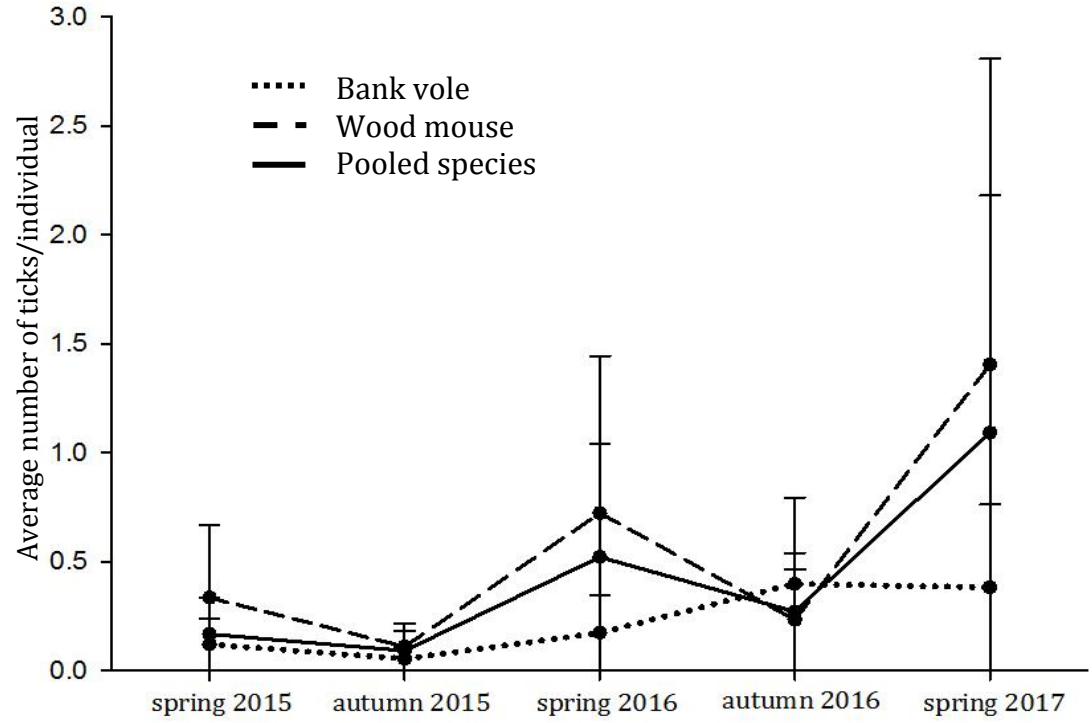


Fig. 3.3. Prevalence of tick species occurring on the sampled rodents according to morphological identification. Unknown species were specimens collected but too morphologically degraded to be identified by phenotypic features. Black: bank vole; grey: wood mouse.

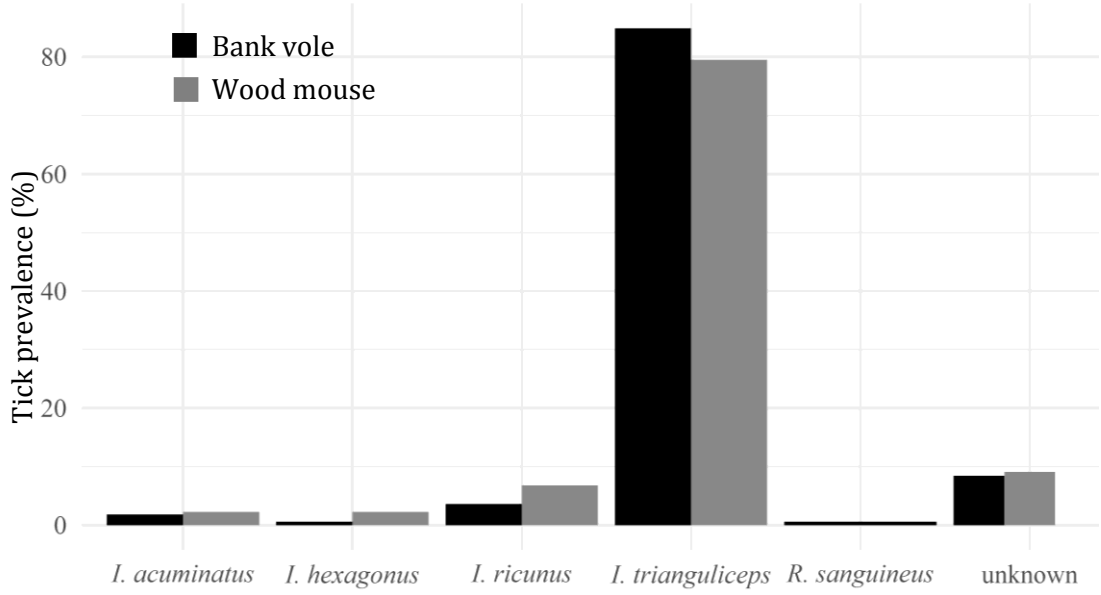


Fig. 3.4 . Percentage of tick stages found in the two different sampling seasons. Percentage was calculated on the total number of ticks collected in each of the two seasons (bars do not reach 100% because of degraded specimens of which life stage was unknown). Light grey: larvae; dark grey: nymphs; black: adults.

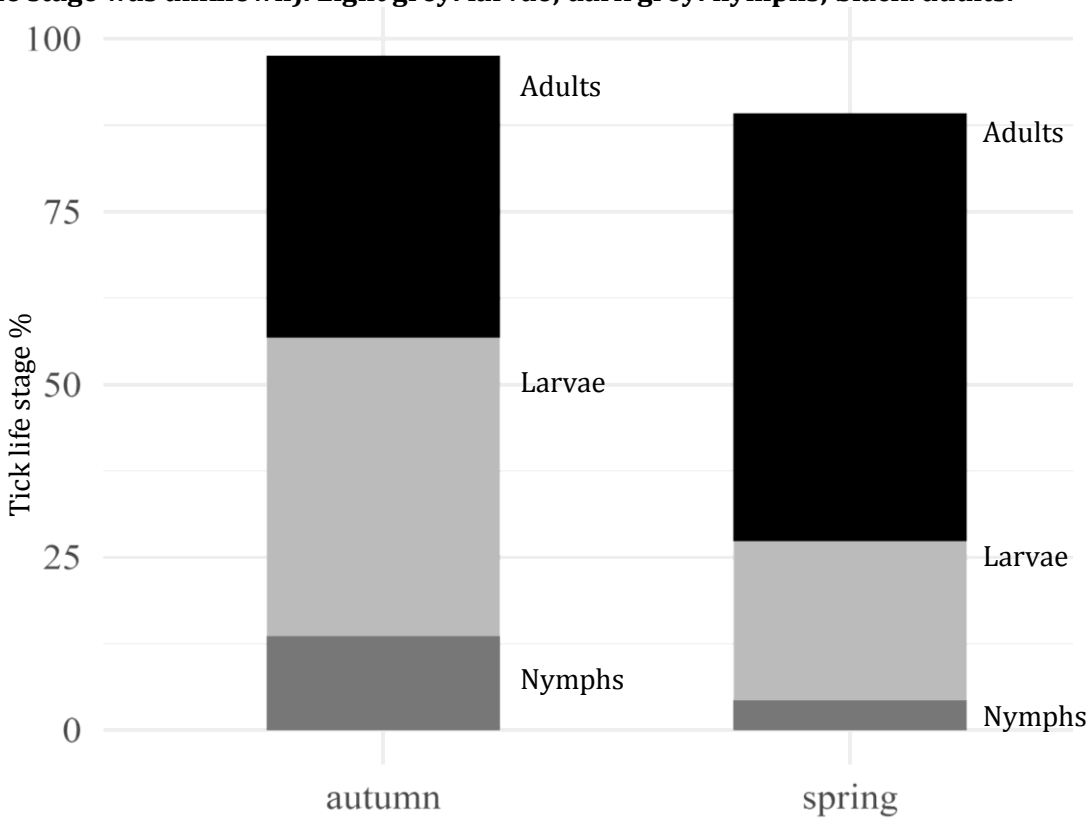


Fig. 3.5. Percentage of tick life stages across sampling seasons. Percentage was calculated on the total number of ticks collected in each sampling season. Solid line: adults; dashed line: nymphs; dotted line: larvae.

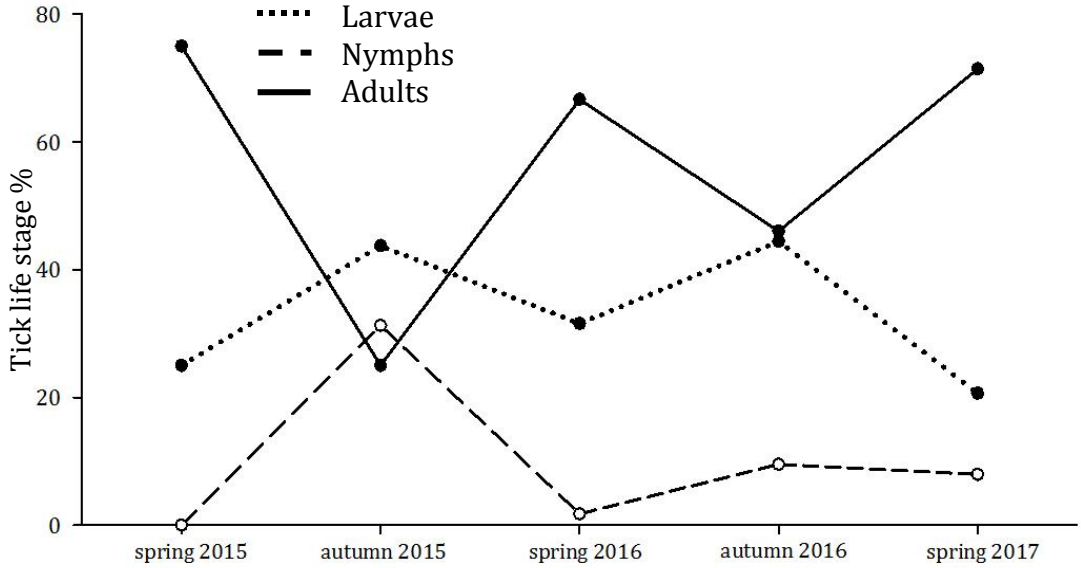
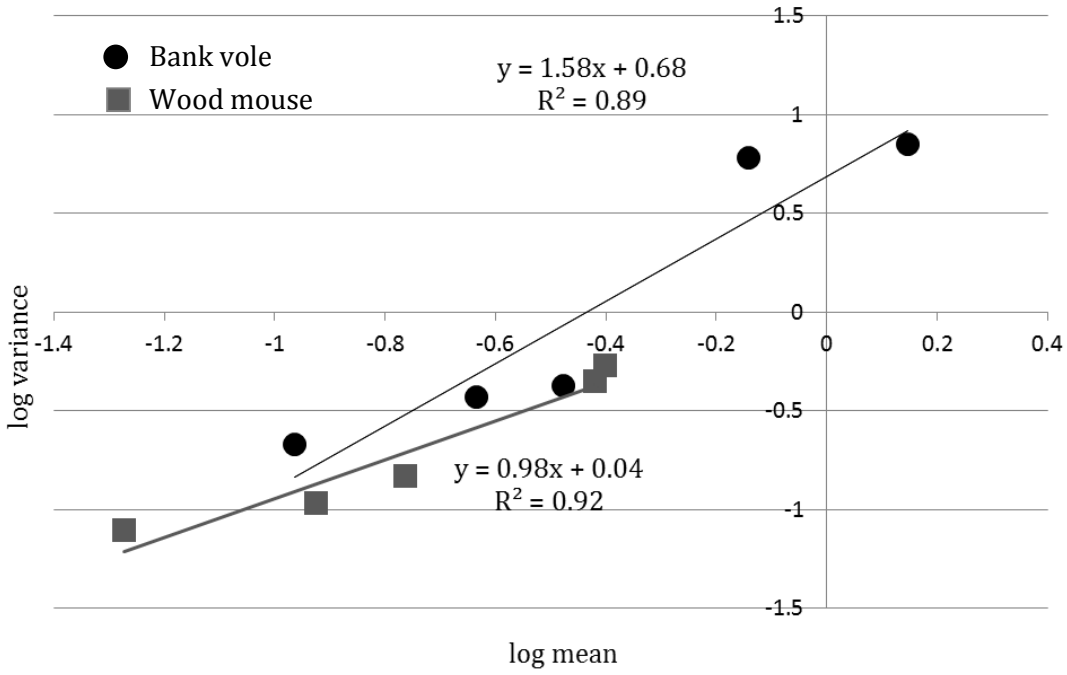


Fig. 3.6. Taylor's power law relationship for ticks. Plot of log mean infestation versus log variance for all tick species. Each data point represents a trapping session. Circles: bank vole; squares: wood mouse.



3.3.1.2 Fleas

Overall, 100 fleas were collected from 71 individuals, including all the rodent species trapped (i.e. bank vole, field vole, and wood mouse); these represented the 9.63% of the population sampled (Fig. 3.7).

Flea total prevalence was 8.70%, and there was no difference in prevalence between seasons, sites, or host characteristics like sex or age class. However, wood mouse displayed significantly lower prevalence than the other species ($p < 0.01$) (Table 3.3).

Also regarding fleas, the intensity of infestation was higher in bank voles ($H = 29.181$, $p < 0.01$) (Table 3.3), which displayed a slightly higher flea burden in spring ($W = 17408$, $p = 0.09$) (Fig. 3.8). No significant difference was found among sexes or age classes. Similarly, no significant patterns of infestation were exhibited by wood mice.

In total, 13 species of fleas were morphologically identified, but two specimens were identified only at genus level (Table 3.4). Prevalence varied across these species, and values resulted to be significantly different ($p < 0.01$). In addition, *Ctenophtalmus* sp., *Megabothris* sp., and *Hystrichopsylla* sp. were more prevalent in autumn ($p < 0.05$) (flea species were grouped by genus for this analysis) (Fig. 3.9).

Taylor's power law relationship for fleas (Fig. 3.10) represented Poisson-distributed data (slope = 1), being the fleas mostly occurring singularly on the hosts.

Further, flea prevalence was negatively associated with wood mouse density, but, as before, the model did not show a good fit ($Prevalence = -126.27 - 99.68 * N_{BV} - 78.74 * N_{WM}$, $R^2 = 0.13$, $p = 0.05$).

Table 3.3. Average number of ticks per individual rodent and prevalence of infestation. St.Dev: standard deviation.

Host species	Mean	St.Dev	Prevalence (%)
Bank vole	0.19	0.63	12.24
Field vole	055	1.01	33.33
Wood mouse	0.03	0.17	3.04

Table 3.4. Prevalence of flea species occurring on the sampled rodents according to morphological identification. In brackets sample size. Unknown species were specimen collected, but degraded to be identified by phenotypic features.

Species	Prevalence (%)
Amalareus penicilliger	4.00 (4)
Ctenophthalmus (Ctenophthalmus) nobilis nobilis	26.00 (26)
Ctenophthalmus (Ctenophthalmus) nobilis vulgaris	12.00 (12)
Ctenophthalmus sp.	1.00 (1)
Doratopsylla dasycnema dasycnema	1.00 (1)
Hystrichopsylla talpae talpae	14.00 (14)
Leptopsylla (Leptopsylla) segnis	1.00 (1)
Megabothris (Gebiella) turbidus	17.00 (17)
Megabothris (Megabothris) walkeri	5.00 (5)
Megabothris sp.	1.00 (1)
Nosopsyllus (Nosopsyllus) fasciatus	1.00 (1)
Nosopsyllus londiniensis	2.00 (2)
Peromyscopsylla spectabilis	2.00 (2)
Rhadinopsylla (Actenophthalmus) pentacantha	2.00 (2)
Typhloceras poppei poppei	1.00 (1)
Unknown	10.00 (10)

Fig. 3.7. Histogram representing frequency distribution of fleas on rodent populations. Dashed lines represent the median of non-zero values, which is 1 for all species. White: bank vole; grey: field vole; black: wood mouse.

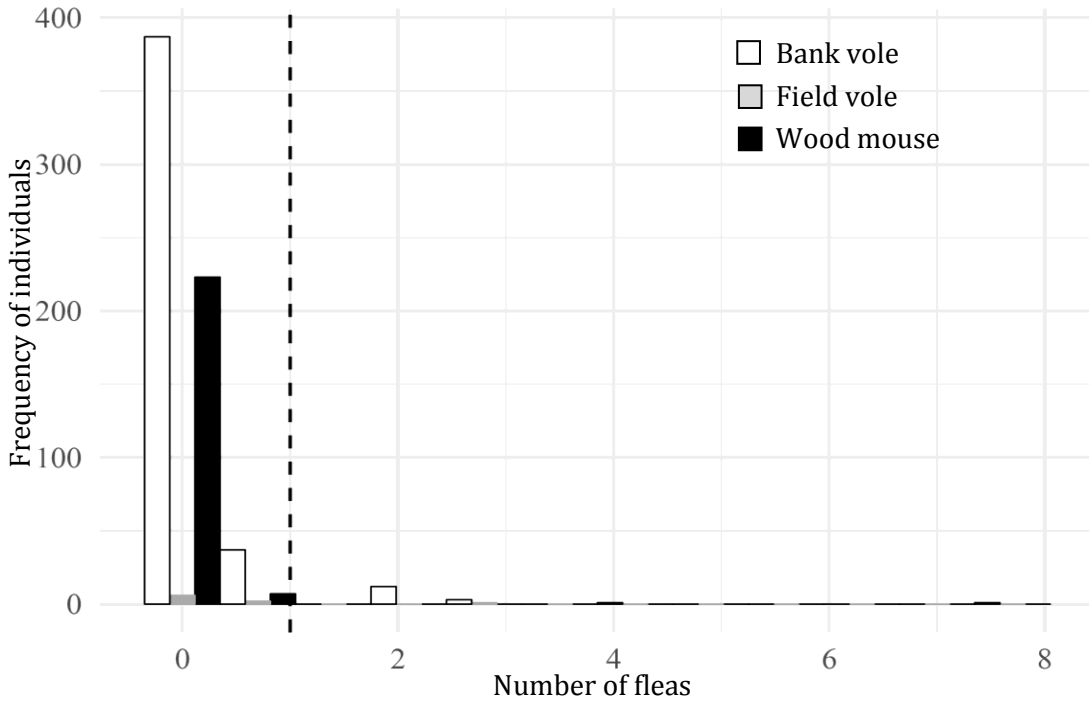


Fig. 3.8. Average number of fleas per individual rodent across sampling seasons. Error bars represent standard error. Solid line: pooled species; dotted line: bank vole; dashed line: wood mouse.

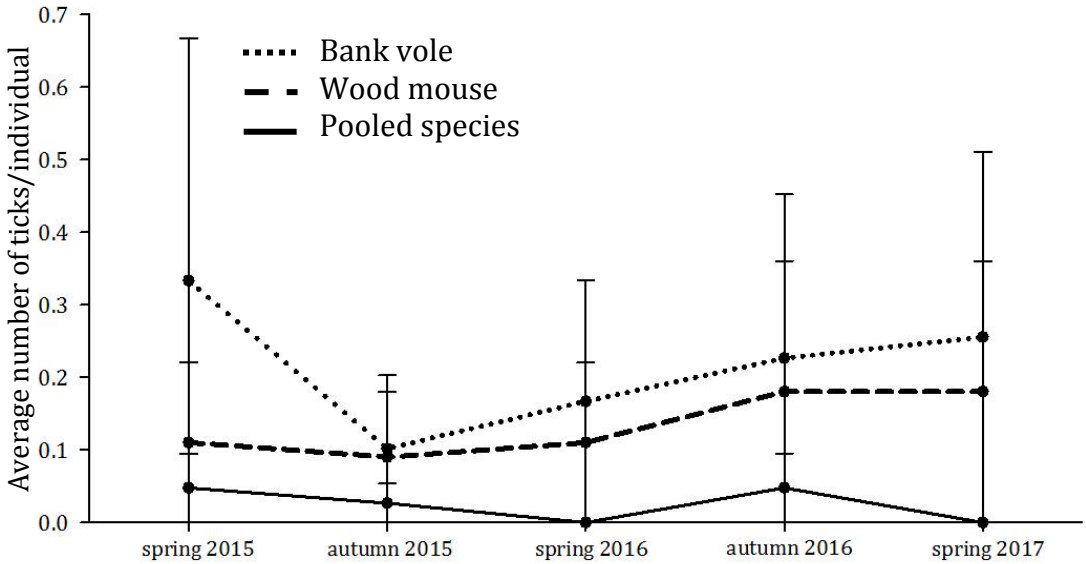


Fig. 3.9. Percentage of flea species found in the two different sampling seasons. Percentage was calculated on the total number of fleas collected in each of the two seasons.

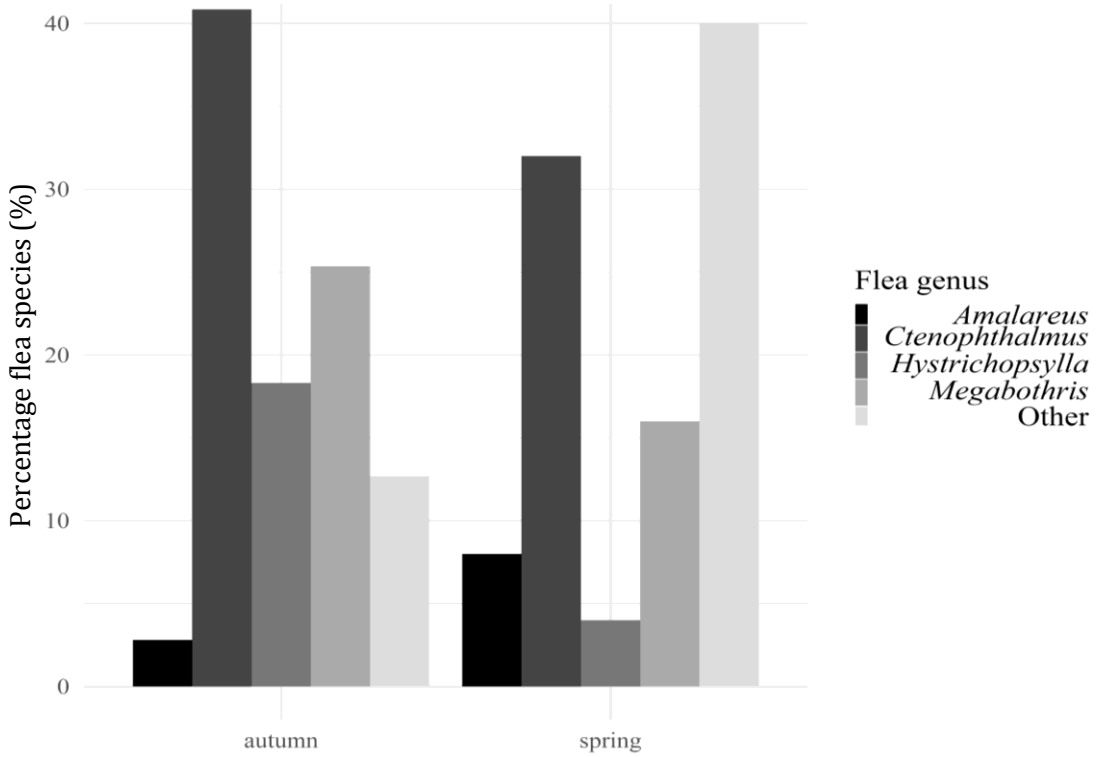
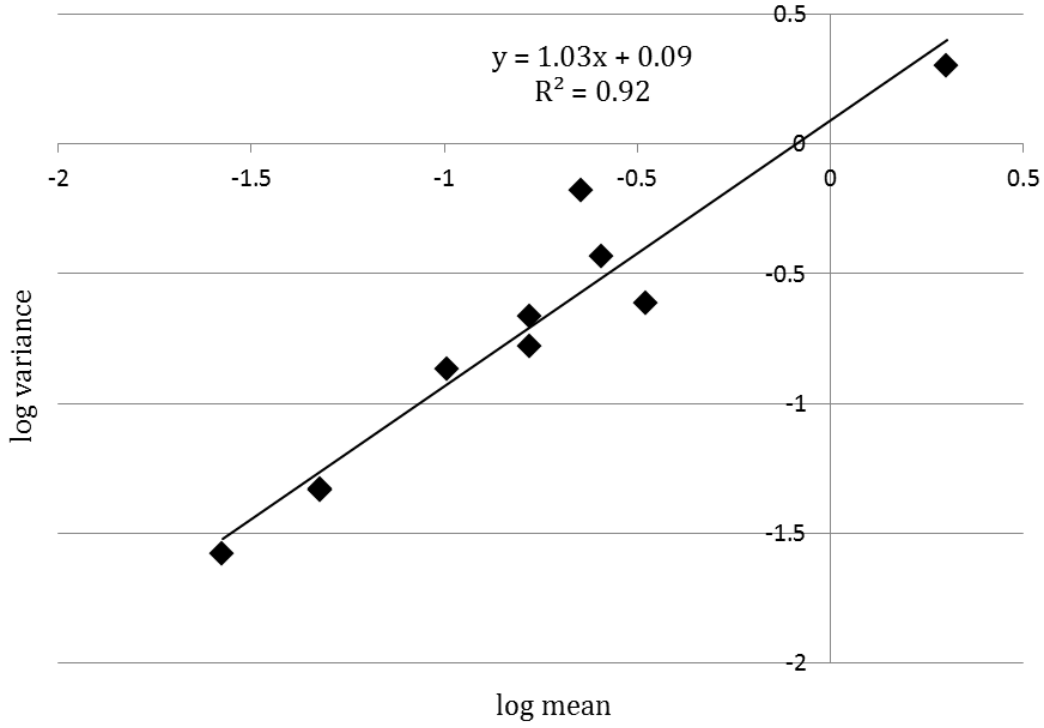


Fig. 3.10. Taylor's power law relationship for fleas. Plot of log mean infestation versus log variance for all flea species. Each data point represents a trapping session (data pooled for all host species).



3.3.2 Phylogenetic analysis

3.3.1.1 Ticks

The sequences obtained from tick specimens were compared with the ones deposited in GenBank to confirm morphological identification, and to verify whether there were sequences available for the species sampled. When using the sequences representing the segment of COI gene, the identification at species level was not possible because the search results returned identity ~90% with several different species of the *Ixodes* genus. In addition, there were no sequences in the GenBank database for the COI gene of *Ixodes trianguliceps*, namely the most represented species in the study. On the other hand, the sequences representing the 16S gene fragment matched the *I. trianguliceps* sequences in the GenBank database (identity ranging from 99% to 100%), confirming that the vast majority of the samples were actually of this species, and seven samples were actually phenotypically misidentified. In particular, four samples were morphologically identified as *I. ricinus*, and three as *I. hexagonus*, but they were, according to the molecular identification, *I. trianguliceps*.

After selecting the best sequences, the COI gene based phylogenetic tree was created using 15 sequences from the sampled ticks of the length of 390 bp (Fig. 3.11). The COI fragment was really effective in separating the *I. trianguliceps* clade from other Ixodes species; however, this clade seemed also split, with two samples probably representing a different *I. trianguliceps* subspecies (they were found on the same host species but in two different sites). Some samples identified morphologically as a different species were included in the *I. trianguliceps* clade, but the high bootstrap values and the match with sequences in GenBank database, makes molecular identification more likely to be correct.

Better sequences were obtained amplifying the 16S gene, thus 56 sequences of the length of 253 bp were included in the phylogenetic tree (Fig. 3.12). In this case the separation among the out-groups, and the clades including *Ixodes* species was clear, but the *I. trianguliceps* clade is split in different sub-groups not matching any pattern regarding host species or location. However,

bootstrap values of these branches were quite low, excluding one representing some *I. trianguliceps* samples collected from a shrew (included only in the phylogenetic data analysis).

Fig. 3.11. Phylogenetic tree of the partial COI gene sequences from ticks constructed by Maximum Likelihood method based on the Tamura 3-parameter model (Tamura, 1992) and 1000 bootstrap replicates. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed (50% majority rule consensus tree). Numbers indicate bootstrap values reported as percentages. Accession number is displayed for the sequences downloaded from GenBank. Species names represent morphological identification. *: sample not matching morphological identification.

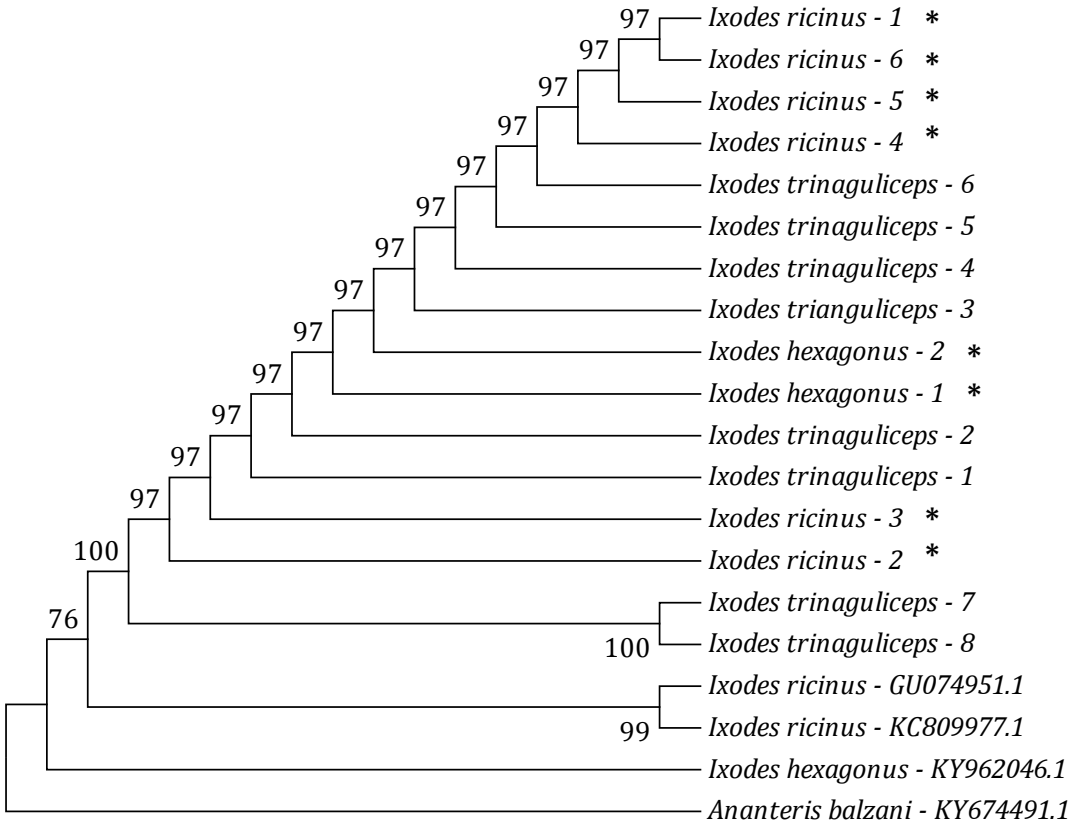
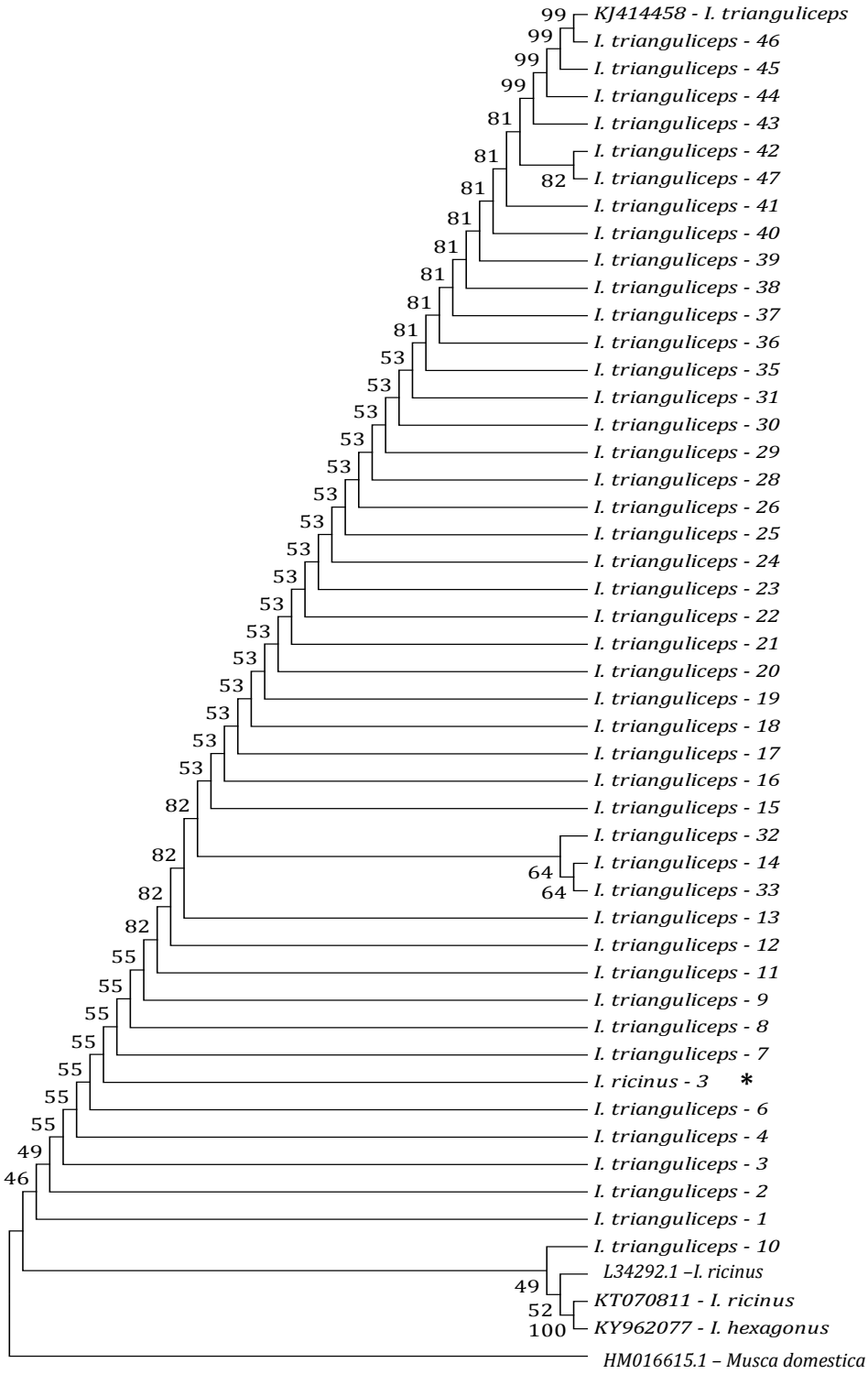


Fig. 3.12. Phylogenetic tree of the partial 16S gene sequences from ticks constructed by Maximum Likelihood method based on the Tamura 3-parameter model (Tamura, 1992) and 1000 bootstrap replicates. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed (50% majority rule consensus tree). Numbers indicate bootstrap values reported as percentages. Accession number is displayed for the sequences downloaded from GenBank. Species names represent morphological identification. *: sample not matching morphological identification.



3.3.1.2 Fleas

Flea sequences were identified on GenBank, but it was impossible to verify morphological identification in that way because sequences representing both genes were unable to provide definitive results. Identity >90% was found for a wide range of flea species of different genera and families, always including the genus of the species identified phenotypically. Four species were likely to be morphologically misidentified, since the BLAST search gave high matching scores with another species, and this result was confirmed by phylogenetic analyses. In particular, one sample identified as *Ctenophtalmus nobilis nobilis*, and two as *Nosopsyllus londiniensis*, were found to be *Amalareus penicilliger penicilliger*, while one sample identified as *A. penicilliger penicilliger* resulted *C. nobilis* instead.

The phylogenetic tree of the partial COI gene included 13 sequences of length 356 bp and it is shown in Fig. 3.13. The COI gene was able to separate different species in different clades, and this, in addition with the BLAST results, confirmed that the morphological identification of the above-mentioned specimens was incorrect. Interestingly, the *C. nobilis* clade was split, probably representing the two subspecies *C. nobilis nobilis*, and *C. nobilis vulgaris*, which are extremely challenging to distinguish only with morphology, and impossible even in female specimens.

Finally, 59 sequences (276 bp) were obtained for a section of the 18S gene, and the phylogenetic tree realised is displayed in Fig. 3.14. Overall, the tree lacked resolution, as confirmed by the lower bootstrap support on clades within the tree. Different species were clustered together, and *C. nobilis* specimens from Skomer voles were clustered together, but in different clades.

Fig. 3.13. Phylogenetic tree of the partial COI gene sequences from fleas constructed by Maximum Likelihood method based on the General Time Reversible model (Nei and Kumar, 2000) and 1000 bootstrap replicates. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed (50% majority rule consensus tree). Numbers indicate bootstrap values reported as percentages. Accession number is displayed for the sequences downloaded from GenBank. Species names represent morphological identification. *: sample not matching morphological identification.

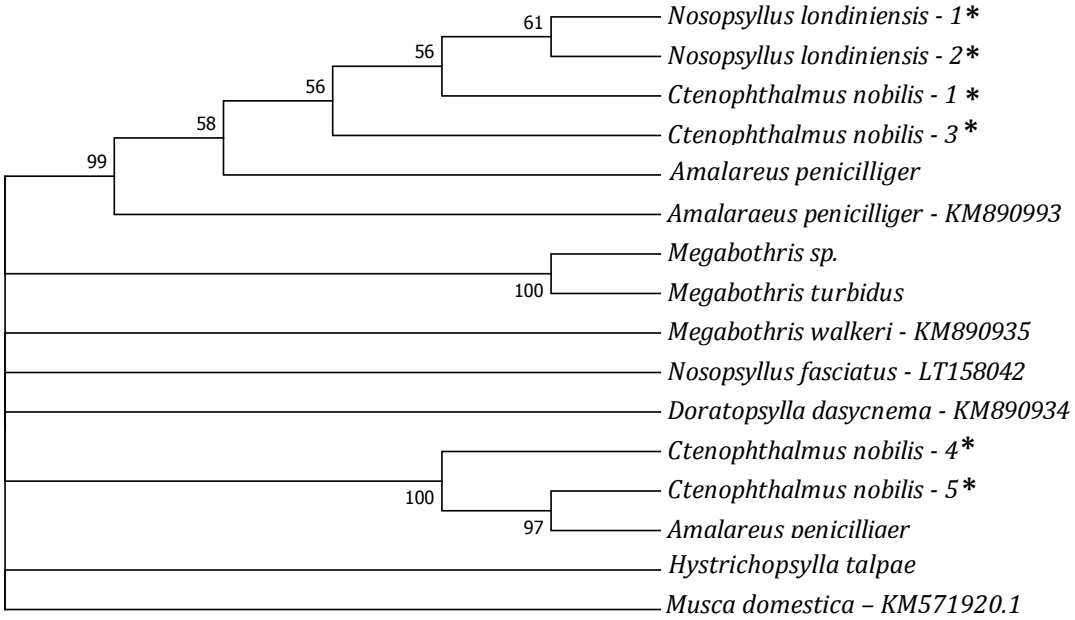
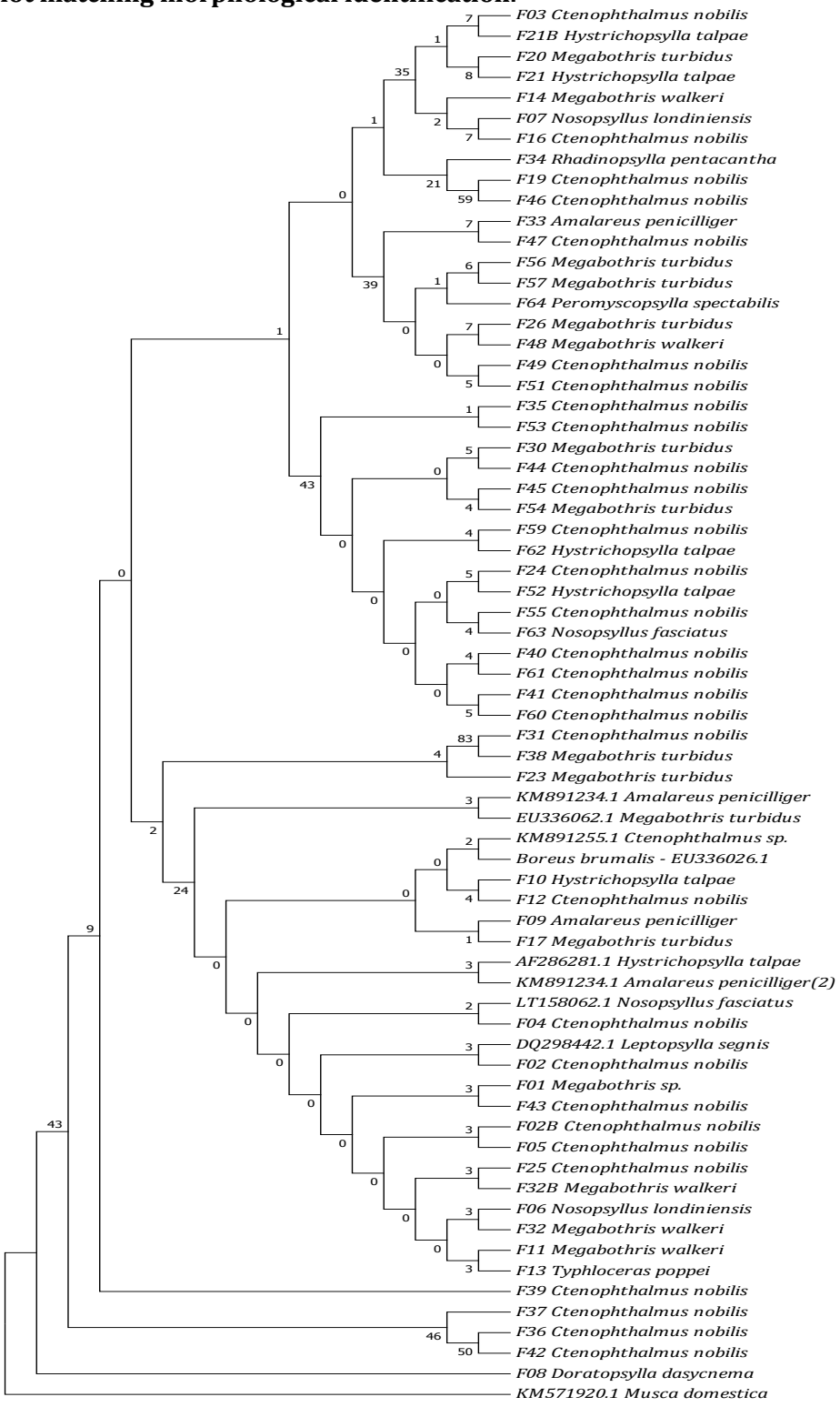


Fig. 3.14. Phylogenetic tree of the partial 18S gene sequences from fleas constructed by Maximum Likelihood method based on Jukes-Cantor model (Jukes and Cantor, 1969) and 1000 bootstrap replicates. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed (50% majority rule consensus tree). Numbers indicate bootstrap values reported as percentages. Accession number is displayed for the sequences downloaded from GenBank. Species names represent morphological identification. *: sample not matching morphological identification.



3.4 Discussion

3.4.1 Factors affecting prevalence and intensity of infestation of ecto-parasites

The numbers of ecto-parasites recovered from rodents were in general agreement with other studies in which collection was made on living, unanesthetised individuals (e.g. Paziewska *et al.*, 2010; Randolph, 1975a). Although collection of ecto-parasites from live individuals may underestimate the actual parasite burden compared to anesthetised or euthanised animals, it has been shown that this method gives an accurate estimation of total ecto-parasite loads (Mooring and McKenzie 1995); further, the sampling method used for fleas has been proven to be a reliable indicator of flea population size (Krasnov *et al.*, 2004).

The proportion of the population parasitised by ticks and fleas was very small, supporting the "20/80 Rule" (see Perkins *et al.*, 2003; Woolhouse *et al.*, 1997), which suggests that usually a small proportion of individuals in a population (20%) is responsible for a certain phenomenon, in this case sustaining the ecto-parasite population, and potentially transmitting the related pathogens. According to the results, the percentage of the population carrying either ticks or fleas was lower than the 20%, and the population carrying both ecto-parasites was even lower, being 2.98% (only 22 individuals). Consequently, it was interesting to investigate patterns of prevalence and intensity of infestation to identify potential categories of individuals more likely to be involved in vector-borne disease transmission.

In general, bank voles were more parasitised by ticks and fleas than wood mice in terms of prevalence and also parasite burden. This finding is in agreement with Hussein (1980), which found microtine rodents more heavily parasitised by *Ixodes trianguliceps* than murine species in north-western England. In addition, the low level of general infestation found in this study was similar to the findings of Rudolph (1975a), who also reports similar burdens for *I. trianguliceps* in small

mammal species in England. In this study, *I. trianguliceps* accounted for 87% of the ticks collected, with only three cases of two different species of ticks co-feeding on the same individual. The higher prevalence in bank voles may be determined by the overall dominance of *I. trianguliceps*, since, when *I. ricinus* has been found to be the dominant tick species, wood mice were the most parasitised hosts (e.g. Gray *et al.*, 1999, Ireland; Kurtenbach *et al.*, 1995, Germany). Regarding fleas, host preferences have been recorded in Kenya, where *Saccostomus mearnsi* (Mearn's pouched mouse), among other small rodents, was recorded to account for > 95% of the fleas collected over 2 years (Young *et al.*, 2014). Bank voles were more parasitised than wood mice also in Ireland, where they represented an introduced species, although the two species shared the same flea species assemblage (Telfer *et al.*, 2005). Nonetheless, no conclusions can be drawn on the parasite host preferences, since an important part of the ground dwelling small mammal community, shrew species, was not sampled, but these species are known to share the same ecto-parasite species, and represented a highly prevalent population (Bray *et al.*, 2007; Mysterud *et al.*, 2015; Randolph, 1975a).

Commonly, patterns of parasites prevalence and infestation have not been related to a species per se, but its density, home range, social structure, and behaviour (Krasnov *et al.*, 2002). In this study, bank vole data showed that gender and season were the factors influencing ecto-parasite burden. Both, ticks and fleas were more prevalent and abundant in spring, and among males.

According to tick life cycle, mostly dependent on temperature and humidity, ticks were expected to be more abundant in spring; in fact, in the UK, they peak in spring and decline over the summer to have another, smaller peak, in autumn (Dobson *et al.*, 2011; Randolph, 1975b; Randolph *et al.*, 2002; Randolph, 2004). In spring, a higher *I. trianguliceps* burden was also found in Norway, and the finding was not related to rodent demography; however, *I. ricinus* displayed the opposite trend, being more abundant in autumn (Mysterud *et al.*, 2015). This may suggest a sort of niche segregation between the two species that could not be investigated in this study because of the scarcity of *I. ricinus* collected.

Nonetheless, this phenomenon may be extremely important in the context of disease transmission (e.g. host-parasite-pathogen association, infection seasonality), and it would be interesting to investigate sites where different species of ticks can be found in allopatry.

Fleas are also influenced by climate, and the effect of seasonality have been reported in temperate and tropical areas (e.g. McCauley *et al.*, 2008, Kenya; Harris *et al.*, 2009, Poland). In Kenya, prevalence and intensity of infestation of the genus *Xenopsylla* were at their lowest after the rainy season, but other work in East Africa demonstrated that temperature, rainfall, and humidity determined different responses in *Xenopsylla* species, and in different locations (McCauley *et al.*, 2008). In fact, not all flea species have the same reproductive strategy. In Poland, it has been observed that *Hystrichopsylla orientalis* is univoltine, with adults emerging between June and August and undergoing a decline during autumn and winter, while *Ctenophthalmus agyrtes* and *Megabothris turbidus* are bivoltine, emerging in March, and again in July/August (Harris *et al.*, 2009). This species-specific behaviour, together with climate variables may explain the seasonality of the current results. Bank vole flea burden was higher in spring, but when analyses were carried out by flea genus, *Hystrichopsylla*, *Ctenophthalmus*, and *Megabothris* were more prevalent in autumn. This suggests that UK flea species may also adopt different reproductive strategies, which may be dependent by the climatic variables, but also by taxonomic characteristics. Interestingly, Telfer *et al.* (2007a) noticed that in autumn the flea community was more diverse, and dominated by *Peromyscopsylla spectabilis*, *H. talpae talpae*, and *C. nobilis*, while in this study spring flea community was more diverse, dominated by singletons. Nevertheless, as mentioned before, *H. talpae*, *Ctenophthalmus* sp., and *Megabothris* sp. were significantly more prevalent in autumn, suggesting that these species might have a similar reproductive seasonality across British Islands. This might influence investigations on flea diversity, host-flea assemblages, and flea-borne disease prevalence, since studies of rodent ectoparasites in temperate areas are usually suspended during winter, or are not continuous during the year, likely missing considerable information about flea diversity. In terms of flea diversity and host-flea assemblages, this study found

results in agreement with other field studies carried out in British Islands (e.g. Withenshaw *et al.*, 2016; Telfer *et al.*, 2007a), but a higher number of species were recovered.

Another factor affecting ecto-parasite burden was sex, with more males carrying a higher number of ticks and fleas. Male gender-biased parasitism has been extensively reported across different taxa of parasites, but this pattern has also multiple exceptions due to the fact that parasites might choose the most convenient host: lower immune response, better trophic resource, higher probability of encounter, higher probability of transmission (Christe *et al.*, 2007). Schalk and Forbes (1997) found that male biased parasitism was mainly recorded across arthropods and due to the lower host immune response caused by androgens. Among small rodents, individuals more likely to survive, namely heavier males, were reported to carry higher tick burdens (e.g. Perkins *et al.*, 2003; Harrison *et al.*, 2010), and examples of male biased parasitism in rodent fleas exist too (e.g. Smith *et al.*, 2005). However, this subject is still debated for different taxa; for example, no consistent patterns of the effect of host sex and body mass on rodent flea abundance was found in different biomes (Kiffner *et al.*, 2013). The effect of sex was found to be related to host species ranging behaviour, or mediated by host body mass, so in host species with opposite sexual dimorphism female were more parasitised (Kiffner *et al.*, 2013; Kiffner *et al.*, 2014). In Siberian chipmunks, *I. ricinus* male biased infestation was inconsistent, being connected to the season of sampling and the season of birth of the host (Le Coeur *et al.*, 2015). In Negev desert, male and female biased parasitism was observed among rodent fleas, and this varied with season as well (Krasnov *et al.*, 2005). Therefore, tick and flea host gender preference can involve factors related both to the host and the parasite.

In conclusion, host preference may be a compromise between host suitability (Brunner and Ostfeld, 2008), host habitat preferences (home range in an area with a favourable microhabitat) (Randolph, 2004), host dispersal abilities, and abundance in different habitats (Boyard *et al.*, 2008). This complex mechanism might explain the finding of higher tick prevalence in the site CW, the coniferous

woodland in Ceredigion; in fact, no different rodent community was found there, and no factors usually increasing tick abundance, such as grazing species presence, characterised the site. It may be just possible that the lush understorey, dominated by common heather and bilberry, represented a better microhabitat for the ticks, which are recognised to be favourably influenced by higher levels of humidity (Berger *et al.*, 2014; Dobson *et al.*, 2011; Randolph, 2004).

Ticks and fleas were distributed differently across host species, as showed by the Taylor's power law plots. This relationship defines clustering, with the slope of the line representing a specific distribution of the organism per unit area (Taylor, 1961). Slope values > 1 indicate clustering, while slope = 1 indicates Poisson-distributed data, so the less steep is the slope the less overdispersed are the data. Ticks, especially on bank voles, were more clustered than fleas, confirming the behavioural, ecological, and spatial differences of these two organisms parasitising the same rodent populations.

Both tick and flea prevalence were negatively associated with wood mouse density, but the fit of the model was low, while there was no significant relationship with bank vole density. Host-density has been suggested to be positively correlated to ecto-parasite abundance/prevalence, but changes in host density may have unclear effects on parasite populations; for example, in ticks numerical responses may be biased by the difference between real (total tick population) and visible (questing individuals) tick population (Dobson, 2014). This relationship may be also complicated by delayed density dependent effects, as Telfer *et al.* (2007a) observed in rodent-flea dynamics in England; they recorded higher flea infestations the year after a peak of host density, but lower infestations during phases of high host density. Krasnov *et al.* (2002), investigating the flea, *Xenopsylla dipodilli*, infesting the gerbil *Gerbillus dasyurus*, also found a negative relationship at high, but not at low host densities. The explanation proposed was that at high rodent densities, there are more transient individuals that do not have access to burrows and are therefore less likely to be infested. In this study no clear relationship was found between ecto-parasites and host density. The only finding was a not well-supported negative relationship between wood mice

density and ecto-parasite prevalence. This may be due to the fact that wood mice were significantly less parasitised (or at least less parasites were recovered), or may indicate that the species dilutes tick and flea prevalence, although, this not necessarily mean a decrease in total parasite abundance. Fleas are known to spend most of the time in the burrows that are shared among all ground dwelling small mammal species, and ticks quest on the vegetation, available for any host, so there is a high percentage of parasitic population that could be in the environment, or on non-sampled hosts. The finding may also reflect the actual absence of relationship between host density and ecto-parasites, as supported by other studies (e.g. McCauley *et al.*, 2008; Stanko *et al.*, 2002; Stanko *et al.*, 2006) Several mechanisms have been suggested to explain the decoupling of host density and parasite intensity/prevalence. For instance, host grooming behaviour, and consequent parasite mortality (Fichet-Calvert *et al.*, 2003; Krasnov *et al.*, 2006; Stanko *et al.*, 2002; Whiteman and Parker, 2004), or parasite characteristics such as transmissibility, life history, and exposure to intra/inter-specific competition (Krasnov *et al.*, 2005; Stanko *et al.*, 2006).

To summarise, it seems clear that is challenging to identify a species, or a category, which universally represent a higher risk in terms of vector-borne disease transmission; therefore, in order to better understand disease dynamics, it is essential to appreciate local host-vector interaction dynamics.

3.4.2 Effectiveness of molecular approach in ecto-parasite species identification

Ecto-parasite samples proved to be difficult to analyse molecularly. The DNA yield from extraction varied greatly from different samples and the extraction via commercial kit yield remarkably less DNA than alkaline digestion. The quality of DNA obtained was also very variable, but overall primers targeting smaller fractions were more effective in PCR reactions. This phenomenon is not

uncommon with this type of samples, in which the large amount of chitinous structures and other chemical compounds interfere with DNA extraction and amplification (Sándor, personal communication). In insects, the large amount of polyphenol bound proteins, created by the enzymes (phenol-oxidases) in the cuticle, represent the most abundant contaminants in extracted DNA samples, but the mechanism by which phenolic compounds inhibit DNA-polymerase is not clear yet, they might bind DNA itself reducing PCR reactions (Arakane *et al.*, 2005; Koonjul *et al.*, 1999). Further, inhibitors of PCR amplification are present in engorged ticks, which might be not completely removed even after a correct DNA extraction (Schwartz *et al.*, 1997b). These reasons are likely to explain the not always successful molecular work on the ecto-parasite samples, and as a result a limited amount of good quality sequences, of which the length might have affected the resolution of phylogenetic analysis.

Despite these difficulties, alternatives to morphological identification of arthropod vectors, which can be difficult because lack of expertise, or degraded specimens, have been developed and are still under development (e.g. DNA barcoding, MALDI-TOF MS) (Diarra *et al.*, 2017). The most common technique is sequencing gene from ribosomal sub-units (e.g. 12S, 16S, 18S), or the cytochrome oxidase subunit 1 (COI) (Yssouf *et al.*, 2016), but there is currently no universal PCR protocol, and debate about the best pairs of primers is still undergoing (Ernieenor *et al.*, 2017). Further, when DNA sequences are used for a BLAST search, it is assumed that sequences in GenBank database are correct and the database is comprehensive enough to allow species identification, but often this is not the case (Bridge *et al.*, 2003; Song *et al.*, 2008). However, molecular markers have been successfully used for arthropod species identification and phylogenetic analyses, including ticks and fleas (Ernieenor *et al.*, 2017; Che Lah *et al.*, 2016; Schmidt *et al.*, 2015; Whiting, 2001; Whiting *et al.*, 2008). Therefore, in this study both morphological and molecular analyses were performed to compare the two methodologies, assess the matching of the obtained sequences with those in the GenBank database, and compare the effectiveness of the widely used COI fragment with other molecular markers used in ticks and fleas.

Tick specimens represented almost entirely *Ixodes trianguliceps*, which was effectively identified morphologically, except for seven samples. COI sequences were not present on GenBank for *I. trianguliceps*, while all the sequences were effectively matched with sequences obtained amplifying the 16S ribosomal sub-unit. This reveals that there are still gaps in tick molecular studies, and an extensive collection of sequences it is still not available. In fact, all the 16S sequences from *I. trianguliceps* were from a single study about this tick species in UK (Bown *et al.*, 2006). However, the phylogenetic tree built according to the COI sequences was much more informative than the one constructed with the 16S; and it confirmed that the mismatch of the seven sequences with GenBank database was actually true, since the sequences were correctly clustered within the *I. trianguliceps* clade with a very high bootstrapping support. However, the clade seemed to split, with two samples probably representing a different species or subspecies. In order confirm this, further analyses are required, also because all the samples were collected from same host species, but the two split ones differed in location between each other, so it is arduous to form any hypothesis. The second phylogenetic tree clearly separated the *Ixodes* genus from the out-group, and clustered the different species together, but probably longer sequences, and so better resolution, was needed to identify any pattern in the *I. trianguliceps* clade. Interestingly, four *I. trianguliceps* collected from a shrew clustered together on a separate branch of the clade, suggesting some genetic variability of the same species parasitising a phylogenetically different host (shrews are not part of the rodent order Rodentia, but they are included in the Eulipotyphla order). Although this finding needs further analyses to be confirmed, this may be likely since shrews have been often found highly parasitised (Bray *et al.*, 2007; Mysterud *et al.*, 2015; Randolph, 1975a), and *I. trianguliceps* may have evolved specific adaptations to such host. For example, *I. scapularis* and *Amblyomma americanus* have been reported to express different saliva proteins when stimulated to start feeding on different hosts (Tirloni *et al.*, 2017). Different races of the same species are also found in *I. uriae* (seabird tick), which displays different population genetic structure according to the parasitised host (McCoy *et al.*, 2003). Finally, these results confirmed that 16S

gene gives less phylogenetic resolution than COI, due to its slower mutation rate, but can be used as complementary to COI, when COI fails to produce reliable results, as 16S sequences are generally, and in particular also in this study, of better quality (Lv *et al.*, 2014).

Collected fleas displayed a much higher species variability compared to ticks, and morphological and molecular identification was more challenging. The absence of comparator sequences on GenBank, for the species of interest, made impossible the validation of the phenotypic identification through the BLAST search. However, three species that were morphologically identified as *Nosopsyllus londiniensis* and *Ctenophtalmus nobilis* were subsequently identified as *Amalareus penicilliger* thank to the comparison between GenBank results and the phylogenetic tree constructed with the sequences obtained from the COI segment. The phylogenetic classification was reliable since the species were all clustered as *A. penicilliger*, while, according to the morphological classification they would have been in different families or sub-families (*A. penicilliger* and *N. londiniensis* are included in the Ceratophyllidae family, but they are classified in different sub-families; *C. nobilis* is comprised in the Ctenophthalmidae family). Similarly, one sample identified as *A. penicilliger* was reliably identified as *Ctenophtalmus nobilis* thank to the analysis performed with the COI gene. As for the ticks, the tree constructed with the COI gene was more informative. The 18S gene tree lacked resolution, different species were clustered together, and there was no separation with regards to location or host species. Again, 18S typically shows lower mutation rates than COI therefore has less informative sites than COI (Hebert *et al.*, 2003). In this case, the combination of phenotypic and genetic approaches was essential to determine species identification, and allow the characterisation of flea community. Fleas are widespread vectors of emerging and re-emerging infectious diseases (Bitam *et al.*, 2010), therefore more effort should be put into developing a reliable identification method for eco-epidemiologists (without an entomological background) to support their investigation of host-parasite-pathogen dynamics. According to the results, COI, the most used fragment of mtDNA for barcoding, might still represent the best choice, but more research is needed especially for obscure taxa, such as

Siphonaptera, where the combination of morphological and molecular approaches is still required, particularly when focusing on the pathogen they potentially harbour (Lawrence *et al.*, 2015). Also, the BLAST search alone was mostly insufficient to provide definitive information on species identification, so more work is necessary to increase the number of sequences and their quality on GenBank. As GenBank relies on direct submissions from individuals, the volume of new information translates also into error accumulation, so more control and accuracy is necessary to be considered as a fully reliable tool for species identification (Shen *et al.*, 2013).

Finally, ecto-parasite population genetic studies are essential to address questions that cannot be addressed just with ecological methods. For example, genetic variation can be studied at a temporal (e.g. Dharmarajan *et al.*, 2009), or at spatial scale (Paulauskas *et al.*, 2006). Genetic variability can be investigated with regards to host-preferences, especially interesting when considering generalist parasites like ticks, and the formation of host specific races within a species (McCoy *et al.*, 2003). All of this information represent critical insights to better understand host-parasite interactions, and consequently pathogen transmission (Araya-Anchetta *et al.*, 2015).

Chapter 4

Screening of directly-transmitted and vector-borne pathogens and parasites

4.1 Introduction

Sampled rodent communities were screened for several parasites and pathogens. The high potential of rodents as reservoirs for wildlife and human pathogens was discussed in Chapter 1, so faecal samples collected from the trapped individuals and their ecto-parasites were analysed to identify presence and prevalence of parasites and pathogens with different types of transmission modes. All the pathogens and parasites screened pose an epidemiological challenge because of the ability to infect multiple hosts, although generalism dynamics are still to be clarified (Webster *et al.*, 2017). Host species of a particular pathogen may differ in abundance, exposure and susceptibility, so it is likely that each species does not contribute equally to parasite transmission (Altizer *et al.*, 2003). Certain species may contribute disproportionately to transmission, representing a "key host", responsible for the persistence of the pathogen in the population (Streicker *et al.*, 2013). Therefore, parasites and pathogens known to be generalists, but described to have differential affinity for different host species and host characteristics, were selected in the study.

4.1.1 Directly transmitted pathogens

In particular, the faecal samples were examined to detect Herpesvirus, *Escherichia coli*, *Mycobacterium microti*, and helminths. The significance of each of these pathogens will be discussed below.

4.1.1.1 Herpesvirus

The most documented Herpesvirus infecting wild rodent populations has been recognised as Murid Herpesvirus 4, which is a Gammaherpesvirus and infects respiratory and immune system cells (Blasdell *et al.*, 2003). It has been reported to be present in UK, where its prevalence has been found higher in wood mice than bank voles, and not related to any seasonal or demographic pattern; although, it was more prevalent in heavier males, which were probably subjected to reactivation of latent infections (Telfer *et al.*, 2007b).

4.1.1.2 *E. coli*

E. coli, is one of the most abundant bacteria associated with human and animal stool, but some strains are extremely pathogenic (e.g. shiga toxin-producing strains - STEC), and livestock and wildlife may act as reservoir (Hughes *et al.*, 2009). In birds, the circulation of strains producing pathogenic toxins was associated with season, probably due to seasonal risk factors, such as diet or dispersion patterns (Hughes *et al.*, 2009). Peri-urban rodent faecal samples, in Madagascar, were found to be almost three times more likely to carry *E. coli* than livestock, containing strains also found in human faeces (Bublitz *et al.*, 2014). However, in Europe, wildlife is known to harbour a much wider range of

strains compared to humans, and wild rodents displayed very low prevalence in several investigations (e.g. Healing *et al.*, 1980; Swiecicka *et al.*, 2003).

4.1.1.3 *Mycobacterium microti*

Mycobacterium microti, the causative agents of vole tuberculosis, is a member of the *M. tuberculosis* complex (Brosch *et al.*, 2002; van Soolingen *et al.*, 1998) and causes chronic, endemic infection in different species of wild British rodents, altering their population dynamics (Burthe *et al.*, 2008; Cavanagh *et al.*, 2002; Kipar *et al.*, 2013; Turner *et al.*, 2014). Tuberculosis in voles causes clinical pathology, but only in the later stages of the infection; it has a slow development with externally visible cutaneous lesions recognisable only at a very late stage (Burthe *et al.*, 2008; Cavanagh *et al.*, 2004; Kipar *et al.*, 2013). Post-mortem examination and culture from tissues of infected organs (e.g. lungs, spleen, liver, mesenteric lymph nodes) indicated much higher prevalence than previously estimated only on external signs (Cavanagh *et al.*, 2002). *M. microti* has also been involved in infections in human subjects (Horstkotte *et al.*, 2001; Niemann *et al.*, 2000) and domestic animals (Emmanuel *et al.*, 2007; Rüfenacht *et al.*, 2011).

4.1.1.4 Helminths

Helminths were also investigated in fresh faecal samples to characterise macroparasite communities in different species or categories (e.g. adults vs. juveniles, males vs. females). The targeted macroparasites were included in two phyla, Nematoda and Platyhelminthes. Nematodes are round worms that go through several stages of larval development shedding their cuticle; the last larval stage represents the immature adult (Taylor *et al.*, 2007). In the direct life

cycle, the first two larval stages are free living stages and infection occurs with the ingestion of the third larval stage, but it can sometimes occur through skin penetration (Taylor *et al.*, 2007). Some nematodes have an indirect life cycle, namely they have an intermediate host where the first two larval stages develop, and so the infection of the final host (where the worm will reproduce) is usually caused by the ingestion of the intermediate host (but it can be inoculation of the larva if the intermediate host is a blood sucking arthropod) (Taylor *et al.*, 2007).

Platyhelminthes, i.e. flat worms, are divided in two classes, Trematoda and Cestoda. The first class includes parasites having both direct and indirect life cycles, but the trematodes found in small mammals (as final hosts) belong to the subclass Digenea and all have an indirect life cycle, where the larval stages develop in a molluscan intermediate host (Taylor *et al.*, 2007). Similarly, cestodes have an indirect life cycle, with one or more intermediate hosts (where the larval form develops), and a final host which get infected ingesting the larva (inside the intermediate host) and excrete parasite eggs through the faeces (Taylor *et al.*, 2007).

According to Walker *et al.* (2017) data collected from live animals underestimate macroparasite prevalence and abundance, but host-parasite associations, and trends such as gender-biased parasitism or taxonomic clusters can still be detected. Analysing host heterogeneities among rodents and shrews for 11 species of multi-host parasites (including helminths and coccidia), Streicker *et al.* (2013) recorded that the magnitude of host heterogeneity varied considerably across parasites, suggesting a continuum of host specialisation.

4.1.2 Vector-borne pathogens

4.1.2.1 Tick-borne pathogens

Ticks were screened for the following vector-borne pathogens, *Anaplasma phagocytophilum* (bacterium of the order of Rickettsiales), *Babesia microti* (intraerythrocytic protozoan), and *Borrelia burgdorferi* s.l. (spirochete bacterium).

The tick-borne pathogens selected represent more or less recognised zoonotic threats (Gray, 2006; Homer *et al.*, 2000), with *B. burgdorferi*, causative agent of Lyme disease, being one the most widespread and well-studied zoonotic tick-borne pathogen in temperate regions of North America, Europe, and Asia (Dantas-Torres *et al.*, 2012; Kilpatrick *et al.*, 2017a). In UK, these have rodent species as main reservoir hosts and can be transmitted by different species of ticks, in particular *Ixodes ricinus* and *I. trianguliceps* (Bown *et al.*, 2003; Bown *et al.*, 2006). Ixodid ticks can be simultaneously infected by these organisms, for example *I. scapularis* has been reported to be commonly co-infected with different pathogen associations, suggesting that pathogens facilitate or limit other pathogens infection (Adelson *et al.*, 2004; Hersh *et al.*, 2014b). Among rodents, these infections have different levels of prevalence, but do not seem to affect survival (Bown *et al.*, 2008), or to be correlated with rodent tick diversity (Foley and Piovia-Scott, 2014). However, Ostfeld *et al.* (2014) found that reservoir competence for *B. burgdorferi* s.l., *B. microti*, and *A. phagocytophilum* were associated with attributes of particular species; for example, *B. burgdorferi* competence seemed mainly positively correlated with population density, while *B. microti* competence was negatively associated with body mass.

4.1.2.2 Flea-borne pathogens

Rodent fleas have been recognised as vectors for numerous species of bartonellae (Bitam *et al.*, 2010; Tsai *et al.*, 2011). In Britain, the flea *Ctenophthalmus nobilis* collected from bank voles was confirmed to be an efficient vector for *B. taylorii* and *B. grahamii* (Bown *et al.*, 2004), and five species have been confirmed to circulate in woodland rodent communities (Birtles *et al.*, 2001; Telfer *et al.*, 2007). Small mammals have demonstrated a high *Bartonella* prevalence, but infections do not result in clinical disease (Kosoy *et al.*, 1997; Telfer *et al.*, 2010). Also, diversity of *Bartonella* species in ecto-parasites seems to be much wider than species detected in hosts (Tsai *et al.*, 2011).

Pathogen screening might also reveal patters of co-infection; in fact, several genera of microparasites have frequently been found infecting the same individual (e.g. Healing, 1981). Infection risk for a microparasite has been reported to be significantly positively or negatively correlated with the infection by other microparasites. For instance, *B. microti* infection was positively correlated with anaplasmosis, but negatively correlated with *Bartonella* infection, likewise individuals infected by *A. phagocytophilum* were significantly less at risk of *Bartonella* infections (Telfer *et al.*, 2010). In addition, helminth co-infections affect magnitude of infection and parasite species assemblages, and have an impact on microparasite infections, altering host vulnerability (Budischak *et al.*, 2015). Finally, some pathogens are assumed to infect multiple hosts, but between-species transmission among sympatric hosts may not be inevitable, therefore genetic analyses of generalist pathogens are also essential to determine whether host species are actually infected by the same organisms (e.g. different strains) (Withenshaw *et al.*, 2016).

4.2 Methods

4.2.1 Extraction and amplification of pathogen DNA

Faecal samples and ecto-parasites were collected from individual rodents as illustrated in the Methods section of Chapter 2 and 3. DNA was extracted from ecto-parasites as described in the Methods section of Chapter 3, while total DNA was extracted from faecal samples using QIAamp DNA Stool Mini Kit (Qiagen, UK) according to manufacturer protocol. Eluted DNA samples were used for PCR reactions and kept in the freezer for storage at -18°C. In total 358 faecal samples were analysed from the two highest density sites, Skomer and Mere Pool Valley woodland in Stackpole (site MPW). These were collected from autumn 2015 to autumn 2016; 299 samples were from bank voles (including Skomer voles), and 59 samples from wood mice.

Herpesvirus detection consisted of a nested PCR amplification targeting the highly conserved DNA polymerase (DPOL) gene of Herpesviruses using the consensus primer sets (ILK, DFA, TGV, KG1, and IYG), according to Vandevanter *et al.* (1996) and Zheng *et al.* (2016). The first reaction included the primers DFA (5'-GAYTTYGCNAGYYTNTAYCC-3'), ILK (5'-TCCTGGACAAGCAGCARNYSGC NMTNAA-3'), and KG1 (5'-GTCTTGCTCACCAGNTCNACNCCYTT-3'), while the second reaction comprised TGV (5'-TGTAACTCGGTGTAYGGNTTYACNGGNGT-3') and IYG (5'-CACAGAGTCCGTRTCNCCRTADAT-3'). The reaction mix of 10 µl consisted of 2 µl of DNA template, 0.5 µl of each primer, 5 µl of Biomix (Bioline, UK), and 1.5 µl of nuclease free water (2 µl in the second reaction). The amplification program, in both reactions, comprised 45 cycles: denaturing at 95°C for 20 sec, annealing at 46°C for 1 min, and extension at 72°C for 30 sec, with an initial denaturation step at 95°C for 12 min, and a final elongation at 72°C for 10 min. In the second reaction 2 µl of the product from the first reaction was included as template. As positive control was added "Clinical Virology

Multiplex I: Immunodeficiency panel working reagent for Nucleic Acid Amplification Tests (NAT)" from NIBSC, according to manufacturer instructions. The expected size of the DNA amplified was between ~160 and ~232 bp.

Escherichia coli primers were selected according to Wang *et al.* (1996) (ECO-1 5'-GACCTCGGTTTAGTTCACAGA-3' and ECO-2 5'-CACACGCTGACGCTGACCA-3'), as they were able to amplify a wide range of *E. coli* strains from different species, targeting the *malB* promoter gene. The reaction mix of 10 µl included 2 µl of DNA template, 0.5 µl of each primer, 5 µl of Biomix (Bioline, UK), and 2 µl of nuclease free water. This reaction mix was used for all the following PCRs described in this section. The amplification program consisted in an initial denaturation of 15 sec at 94°C, 55 cycles of denaturing at 94°C for 3 sec, annealing at 50°C for 1 min, and extension at 72°C for 35 sec, and a final elongation step of 2 min at 72°C. The expected product size was ~585 bp. After a trial PCR, and the sequencing of a positive sample, this was added to the reaction as positive control.

In order to identify more strains of *Mycobacterium microti* two sets of primers were used targeting the flanking regions of the RD1^{mic} gene (RD1micFl-Fw 5'-GCAGTGCAAAGGTGCAGATA-3' and RD1micFl-Rv 5'-GATTGAGACACTTGCCACGA -3'), and the internal region of the same gene (RD1micInt-Fw 5'-TCCGTACCTTTCCGACTATC-3' and RD1micInt-Rv 5'CGGGAAGGTGTTATCTCCTC-3') (Brosch *et al.*, 2002; Smith *et al.*, 2009c). In both reactions the amplification program included 90 sec at 95°C, 35 cycles of denaturing at 95°C for 30 sec, annealing at 58°C for 1 min, and extension at 72°C for 4 min, and a final elongation of 3 min at 72°C. Strains with an intact RD1^{mic} gene were expected to generate products of ~642 bp with the internal primer and flanking primer, while strains with RD1^{mic} deleted were expected to generate a product of ~360 bp with the flanking primers only (Smith *et al.*, 2009c).

A nested PCR targeting the 16S rDNA was performed to identify *A. phagocytophilum* (Massung *et al.*, 1998). First reaction included the primers ge10r (5'-TTCCGTTAAGAAGGATCTAATCTCC-3') and ge3a (5'-CACATGCAAGTC GAACGGATTATTC-3'), and second reaction ge9f (5'-AACGGATTATTCTTTATAGC

TTGCT-3') and ge2 (5'-GGCAGTATTAAAAGCAGCTCCAGG-3'). The final expected product size was ~546 bp, and the two amplification programs comprised an initial denaturation at 95°C for 2 min, 40 cycles (30 in the second PCR) of denaturing at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 1 min, and a final elongation step at 72°C for 3 min.

Primers and protocol to identify *Babesia microti* were selected according to Bown *et al.* (2008), who used primers specific to strains circulating in northern England (KebabF 5'-GAATTTCTGCCTTGTCATTAATC-3' and KebabR 5'-GTAAATACTGGAAGATAGTAAGG-3'), with an expected final product size of ~240 bp. The program included 55 cycles: denaturing at 95°C for 20 sec, annealing at 50°C for 50 sec, extension at 72°C for 30 sec; in addition, 2 min initial denaturation at 95°C, and 2 min final elongation at 72°C were performed. Additionally, in order to test whether other strains were circulating at the study sites another PCR reaction targeting the 16S rDNA gene with the primers BAB-1 (5'-CTTAGTATAAGCTTTTATACAGC-3') and BAB-4 (5'-ATAGGTCAGAAACTTGAA TGATACA-3') (Schwartz *et al.*, 1997a) was conducted with the following amplification program: initial denaturation at 94°C for 2 min, 35 cycles of denaturing at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 2 in, and final elongation at 72°C for 2 min (Adelson *et al.*, 2004; Duh *et al.*, 2001; Persing *et al.*, 1992). Expected product size was ~238 bp.

The 23S rDNA gene was targeted for *B. burgdorferi* PCR amplification (Bb23Sf 5'-CGAGTCTTAAAAGGGCGATTTAGT-3' and Bb23Sr 5'-GCTTCAGCCTGGCCATAA ATAG-3') (Courtney *et al.*, 2004). The amplification protocol consisted of an initial denaturation at 95°C for 10 min, 55 cycles of denaturing at 95°C for 15 sec, annealing at 50°C for 1 min, extension at 72°C for 1 min, and final elongation for 2 min at 72°C.

All the tick samples tested for *A. phagocytophilum*, *B. microti*, and *B. burgdorferi* were pooled according to site, season, and host of collection because low pathogen prevalence was expected. Positive controls for these organisms were obtained from Prof Richard Wall (University of Bristol).

Finally, DNA from bartonellae was amplified with the methodology described by Roux and Raoult (1995), and successfully employed by Telfer *et al.* (2005) to distinguish *Bartonella* species. A *Bartonella* genus-specific PCR assay that amplifies a fragment of the 16S–23S intergenic spacer region (ISR) consisted of a semi-nested PCR (first reaction big-F 5'-TTGATAAGCGTGAGGTC-3' and big-R 5'-TCCCAGCTGAGCTACG-3'; second reaction reverse primer substituted by bog-R 5'-TGCAAAGCAGGTGCTCTCCCA-3'). Both reactions programs were as follows: initial denaturation 3 min at 96°C, 40 cycles of denaturing at 96°C for 10 sec, annealing at 55°C for 10 sec, extension at 72°C for 50 sec, and final elongation at 72°C for 5 min. The use of PCRs targeting the ISR exploits recognized inter-*Bartonella* species hypervariability, and so amplification products obtained from different species are of different sizes (Roux and Raoult, 1995). In addition, another reaction targeting the ssrA gene of *Bartonella* was performed according to Diaz *et al.* (2012). The primers, designed to have a final product of ~253 bp, were ssrA-F (5'-GCTATGGTAATAAATGGACAATGAAATAA-3') and ssrA-R (5'-GCTTCTGTTGCCAGGTG-3'). The protocol included 2 min at 95°C as initial denaturation step, 55 cycles: denaturing at 95°C for 15 sec, annealing at 50°C for 1 min, extension at 72°C for 30 sec, and a final elongation step of 2 min at 72°C. Positive controls for bartonellae were obtained from Dr Michael Kosoy (Centre for Disease Control and Prevention, Ft. Collins, USA).

For each PCR described, the presence of amplified DNA was confirmed by electrophoresis in a 1% agarose gel, with the exception of the *Bartonella* targeting the ISRs, as a 3% agarose gel was employed. PCR products were then stored frozen at -18°C.

Amplified DNA was purified using SureClean Plus (Bioline, UK) according to the manufacturer protocol, and then the samples, consisting of 1 µl forward primer, 1 µl purified DNA, and 4 µl nuclease free water, were sequenced with AB3500 DNA sequencer (Applied Biosystems). Sequences obtained were compared to sequences deposited in GenBank using the BLAST feature of NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul *et al.*, 1990). In the case of the positive samples for *B. microti* and bartonellae, the sequences were imported in

MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets (Kumar *et al.*, 2015). Appropriate sequences downloaded from GenBank were added to the data sets to represent out-groups, and sequences of the species expected in the data set were also added and imported together with the sequences obtained in this study. Multiple sequences alignment was performed by ClustalW with the same software. The sequences for the analysis were chosen according to their quality, short sequences or with a low quality chromatogram were excluded. MEGA was also used for phylogenetic analyses; phylogenetic trees were constructed by the Maximum Likelihood method based on best fitting model for each set of sequences (i.e. lowest BIC score), and bootstrap test of 1000 replicates.

4.2.2 Faecal egg count (FEC) for helminths

Fresh faecal samples were collected during trapping sessions in spring 2016 and autumn 2016 from site MPW, MPG, CW, and WW. The same day of collection, the gastrointestinal parasite burden was determined based on faecal egg count (FEC), a non-invasive method widely used in ecological studies (Biedrzycka and Kloch, 2016; Sommer, 2005). Faecal flotation was performed using saturated salt solution, and the count of parasite eggs was estimated using a Modified McMaster technique (Dunn and Keymer, 1986). For each sample, 0.01 g were weighed, placed in a mixing tube with 2 ml of flotation solution and 5 metal beads and shacked for 30 seconds until the faecal material was broken down. This solution was filtered with a very fine sieve in a new tube, and immediately pipetted into the chambers of a McMaster slide until completely filled. After 30 minutes the chambers were examined under high magnification microscope, and all egg types were counted. The number of eggs was calculated

per 1 g of faeces. Eggs were identified taxonomically at genus level, or at class level with regards to trematodes.

4.2.3 Data analysis

According to Alexander (2012), prevalence data were analysed using logistic regression and non-parametric Wilcoxon signed rank test (or the non-parametric Kruskal-Wallis test in case of more than two groups).

Helminths descriptive statistics included arithmetic mean and median to investigate individual burden intensity, and geometric mean to investigate parasite distribution across infected individuals (Alexander, 2012). Macroparasite burden was compared using negative binomial regression, and the same test was used to identify whether season, species, sex, age class, site, co-infection with another parasite, or the interaction among these factors explained patterns of infection loads. Further, using the methodology illustrated in Streicker *et al.* (2013), the relative host-species contribution to transmission was estimated for helminths. Briefly, π_i is the relative contribution of host species *i* to the parasite total transmission and it is proportional to the product of that species abundance, infection and shedding asymmetries.

$$\pi_i = \frac{H_i p_i \lambda_i}{\sum_{j=1}^{N} H_i p_i \lambda_i}$$

where H_i is the total number of individuals of species *i* infected or not, p_i is the proportion of infected (prevalence) in species *i*, λ is the number of *per capita* eggs shaded by individuals of species *i*, and N the number of host species in the community. The equation used to estimate the host-species contribution relative to the average in the host community was:

$$\pi_i = \frac{\theta_{Ai}\theta_{Ii}\theta_{Si}}{N}$$

where θ_A represents the degree of asymmetry in host abundance, θ_I the degree of asymmetry in host prevalence, and θ_S the degree of asymmetry in eggs shedding.

$$\theta_{Ai} = \frac{H_i}{\overline{H}}$$

where $\overline{H}$ is the average number of individuals per host species in the whole community.

$$\theta_{Ii} = \frac{p_i}{\overline{p}}$$

where $\overline{p}$ is the total number of infected individuals in the community divided by the total number of individuals (i.e. the average prevalence of infection in the community, regardless of species identity).

$$\theta_{Si} = \frac{\lambda_i}{\overline{\lambda}}$$

where $\overline{\lambda}$ is the total number of infective stages shed by all infected individuals in the community divided by the total number of infected hosts (i.e. the average per capita rate of shedding in the whole community, regardless of species identity). For host species *i* to be a key host, $\pi_i > T$ (T = threshold value), at least one of these asymmetries must considerably exceed 1. In other words, it either needs to be much more abundant than other hosts in the community, and/or be infected more than expected (e.g. be more exposed to the parasite, or more susceptible), and/or shed more infective stages. This equation shows that a host-species can make an asymmetric (disproportionate) contribution to the total infectious pool by being super-abundant, super-infected, and/or a super-shedder.

All the analyses were performed with the software R (R Core Team, 2016).

4.3 Results

4.3.1 Directly transmitted pathogens

Total Herpesvirus prevalence was 1.12% (4 individuals), but only bank voles were found positive, therefore wood mouse prevalence was 0%, while bank vole was 1.34%. All the positive samples were collected from Skomer, representing two adult females and two adult males. The sequences, of length ~150 bp showed high similarity (>90%) with human alphaherpesvirus 3 varicella-zoster (3 samples), and human alphaherpesvirus 2 herpes simplex (1 sample).

Only six individuals (1.67%) were found to have *E. coli* in the faeces, of which one was an adult male wood mouse from site MPW, and 5 bank voles (2 females from Skomer, and 2 females and 1 male from site MPW), therefore the relative species-specific prevalence was 1.69% and 1.67% respectively. All the positive samples were collected in autumn. The identification of the sequences via the BLAST search revealed that most of the strains were associated with human strains; in particular, two displayed high homology with the strain LF82 (associated with Crohn's disease), and three were highly similar to the innocuous laboratory strain K12. Finally, the sequence retrieved from the wood mouse sample did not show high similarity with any particular strain.

There was no *M. microti* DNA found in the faecal samples analysed.

Analysis of the confidence of freedom for prevalence results are displayed in Appendix II.

4.3.2 Helminths detected by FEC

Total sample size for the FEC was 212, of these individuals 69 were found not being parasitised at all (no eggs counted in the McMaster slide grid).

Two phyla were represented in the samples, Nematodes and Platyhelminthes, and Cestoidea was the most represented class among the latter, with very few individuals carrying Trematode eggs. Amongst Nematodes, *Trichuris* sp., *Enterobius* sp., *Necator* sp., and *Ascaris* sp. were recovered in the samples analysed, while *Hymenolepis* sp., *Taenia* sp., and *Dypilidium* sp. were the Cestoideans observed.

Considering pooled data, Cestoidean burden was higher than Nematoda ($W = 7313.5$, $p < 0.01$) (Fig. 4.1). According to the negative binomial regression Nematode burden was negatively associated with intensity of infection of Cestoidea ($p = 0.03$), while the opposite relationship was not significant (Fig. 4.2). Nematode infection was higher in bank voles than wood mice ($p < 0.01$), while this was not true regarding Cestoidean. Also, Nematode burden was significantly higher in spring (both species pooled data, $p < 0.01$), while the opposite (higher burden in autumn) was observed for Cestoda ($p < 0.01$) (Fig. 4.3). According to the negative binomial distribution model, Nematode burden was not explained by sex, but this covariate was significant when season was added to the model, resulting in males being more parasitised in spring ($p < 0.05$). Juveniles were less parasitised and "sub-adult" was the age class with highest intensity of infestation ($p < 0.5$). Finally, intensity of infection in site WW was significantly higher than other sites. Cestoda infestation, in the population sampled, was higher in females ($p < 0.05$), and this relationship was stronger in autumn ($p < 0.01$). Age class was also associated with patterns of parasite burden, with juveniles and sub-adults displaying higher parasites loads ($p < 0.01$). With regards to parasite genera, *Taenia* burden was higher in wood mice ($p < 0.05$), while *Dypilidium* and trematodes were only recorded in bank voles (Fig. 4.4). Finally, prevalence of *E. coli* (data available only for site MPW)

was not significant in explaining intensity of infestation for both nematodes and cestodes.

Nematode prevalence (the proportion of infected) was higher in bank voles ($p < 0.05$), and in spring (both species pooled data, $p < 0.05$), but sex and age class were not significant (Table 4.1). In this case cestode prevalence was not significant in predicting Nematoda prevalence. On the other hand, cestode prevalence was not explained by season, host-species, sex, age class, or nematodes prevalence (Table 4.1). For a summary of descriptive statistics see Table 4.2 and Table 4.3.

Regarding host heterogeneities in transmission, the wood mouse represented the super-spreader host-species for cestodes and the bank vole for nematodes; wood mouse asymmetry was mainly in the number of eggs shed (super-shedder), while bank voles had high values for all the three components of asymmetry (Fig. 4.5). Analysing the data by parasite genus revealed that wood mice contributed disproportionally to the transmission of *Ascaris*, *Enterobius*, and *Taenia*, representing always a super-shedder species. On the contrary, bank vole asymmetry was mostly represented by host abundance and prevalence, or by all three components. The result showed that bank vole was a key host-species for *Hymenolepis*, *Necator*, and *Trichuris*.

Fig. 4.1. Box and whiskers plot representing nematodes (white) and cestodes (grey) intensity of infection (log transformed). Individuals not infected are not shown.

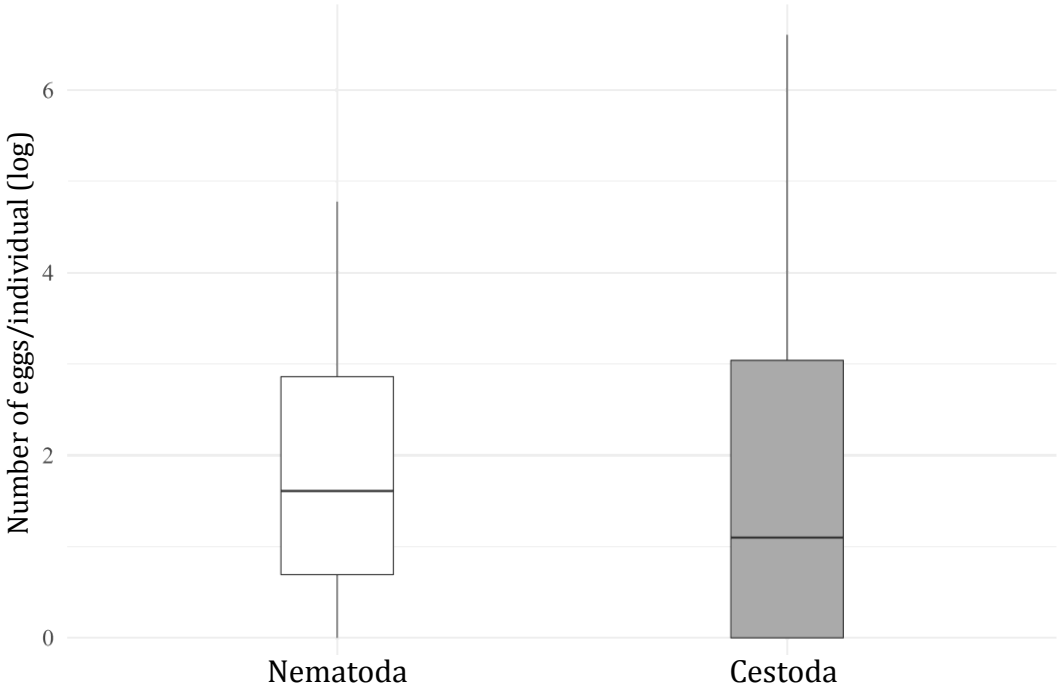


Fig. 4.2. Box and whiskers plot representing the relationship between nematodes and cestodes intensity of infection. Individuals not infected are not shown.

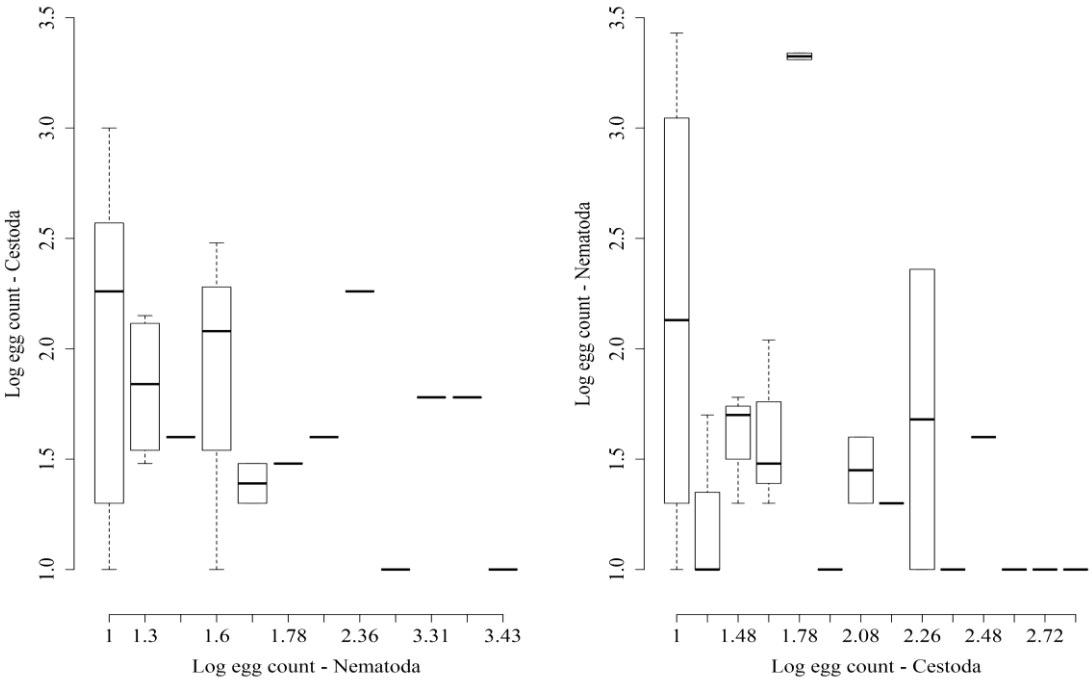


Fig. 4.3. Box and whiskers plot representing nematodes (white) and cestodes (grey) intensity of infection (log transformed) in the two seasons of sampling. Individuals not infected are not shown.

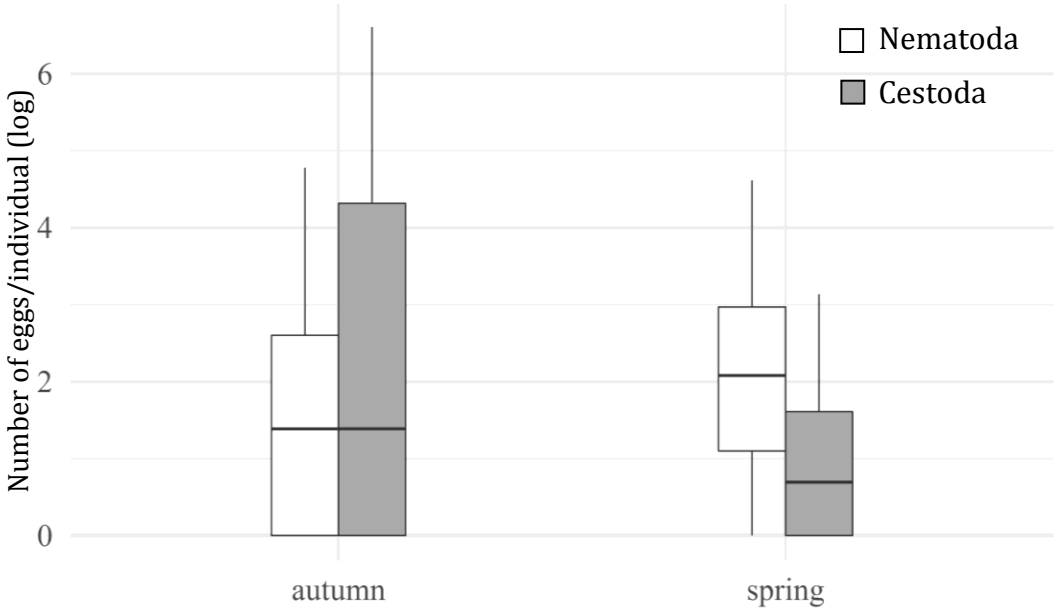


Fig. 4.4. Box and whiskers plot of parasites intensity of infection (log transformed) in bank voles (BV) and wood mice (WM). Nematodes (grey scale) and cestodes (green scale) are displayed by genus. Individuals not infected are not shown. Dots represent outliers.

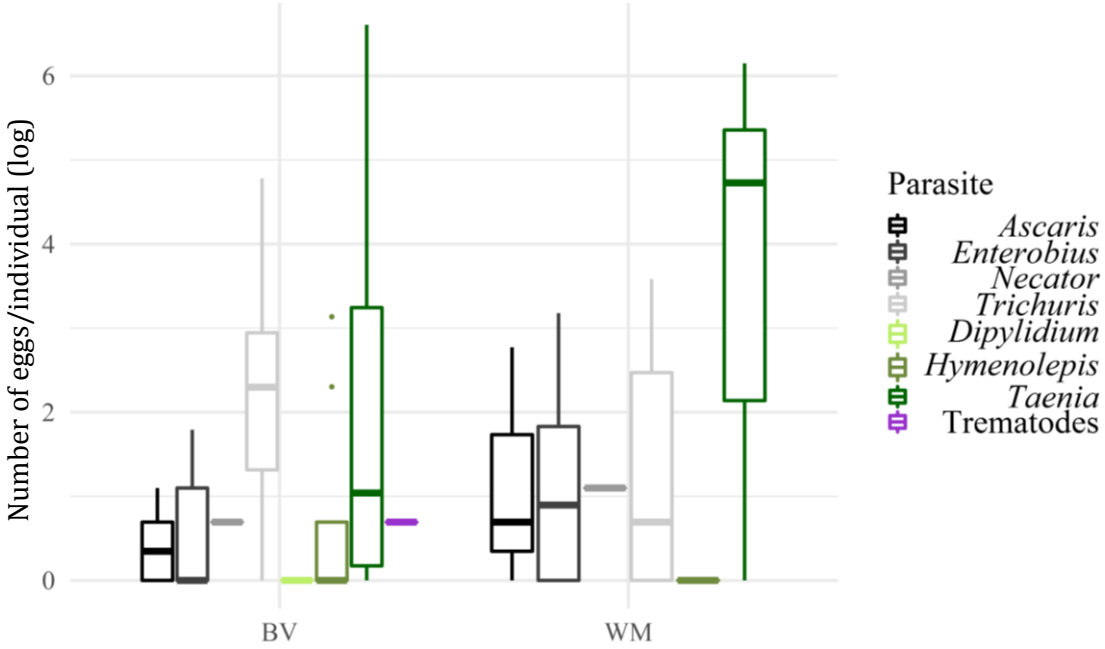


Fig. 4.5. Contributions of three sources of host heterogeneity for the parasites by class and by genus. Label values represent the proportion to the total contribution of infective stages produced by each host species (π). Key host species for each parasite: π > 0.5. a) Nematoda; b) Cestoda; c) *Ascaris*; d) *Enterobius*; e) *Hymenolepis*; f) *Necator*; g) *Taenia*; h) *Trichuris*.

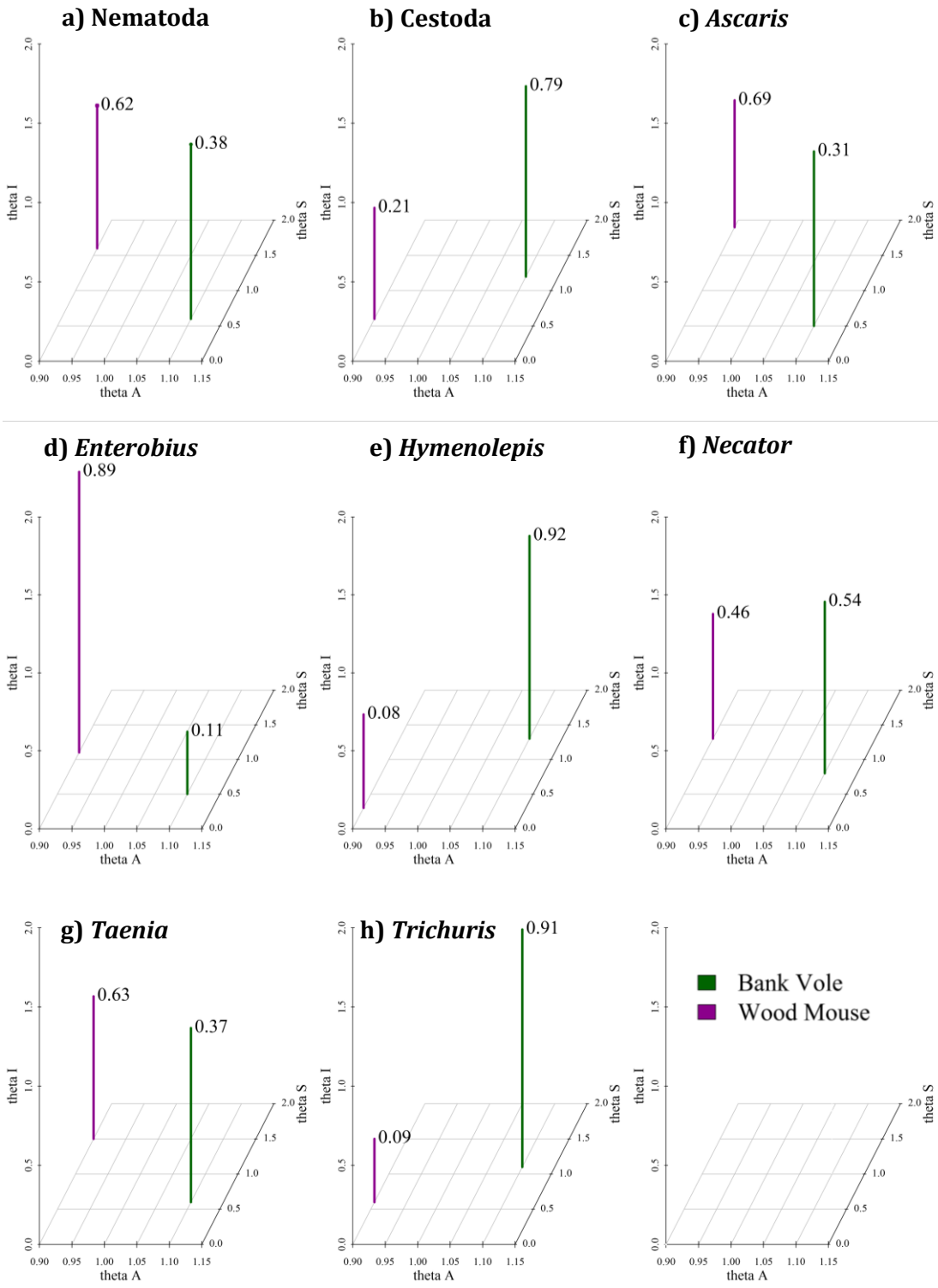


Table 4.1. Proportion of infected (prevalence) by host-species and season of sampling. BV: bank vole; FV: field vole; WM: wood mouse.

Season	Species	Nematoda	Cestoda
Autumn	BV	0.54	0.25
	FV	0.00	0.43
	WM	0.24	0.26
Spring	BV	0.80	0.30
	WM	0.84	0.12

Table 4.2. Nematoda: descriptive statistics. BV: bank vole; FV: field vole; WM: wood mouse; F: female; M: male; A: adult; S = sub-adult; J = juvenile; St.Dev: standard deviation.

Season	Species	Sex	Age	Mean	St. Dev	Median	Geometric Mean
Autumn	BV	F	A	3.43	4.39	1.00	3.10
Autumn	BV	F	J	0.20	0.45	0.00	1.00
Autumn	BV	F	S	2.89	6.38	0.00	3.25
Autumn	BV	M	A	26.36	36.35	9.50	19.92
Autumn	BV	M	J	0.00	0.00	0.00	na
Autumn	BV	M	S	4.60	8.31	1.00	4.75
Autumn	FV	F	A	0.00	0.00	0.00	na
Autumn	FV	F	J	0.00	na	0.00	na
Autumn	FV	M	A	0.00	na	0.00	na
Autumn	FV	M	S	0.00	0.00	0.00	na
Autumn	WM	F	A	6.33	15.03	0.00	6.08
Autumn	WM	F	J	0.00	na	0.00	na
Autumn	WM	F	S	0.44	0.73	0.00	1.26
Autumn	WM	M	A	1.00	2.26	0.00	2.88
Autumn	WM	M	S	1.22	4.70	0.00	2.71
Spring	BV	F	A	10.21	10.35	7.50	6.25
Spring	BV	F	J	0.00	0.00	0.00	na
Spring	BV	F	S	14.90	16.95	11.50	11.81
Spring	BV	M	A	18.67	31.92	8.00	9.32
Spring	BV	M	S	12.21	17.35	5.00	9.69
Spring	WM	F	A	11.50	14.85	11.50	4.69
Spring	WM	F	S	4.17	5.98	2.00	2.86
Spring	WM	M	A	9.08	10.78	4.00	6.53
Spring	WM	M	S	0.00	na	0.00	4.58

Table 4.3. Cestoda: descriptive statistics. BV: bank vole; FV: field vole; WM: wood mouse; F: female; M: male; A: adult; S = sub-adult; J = juvenile; St.Dev: standard deviation.

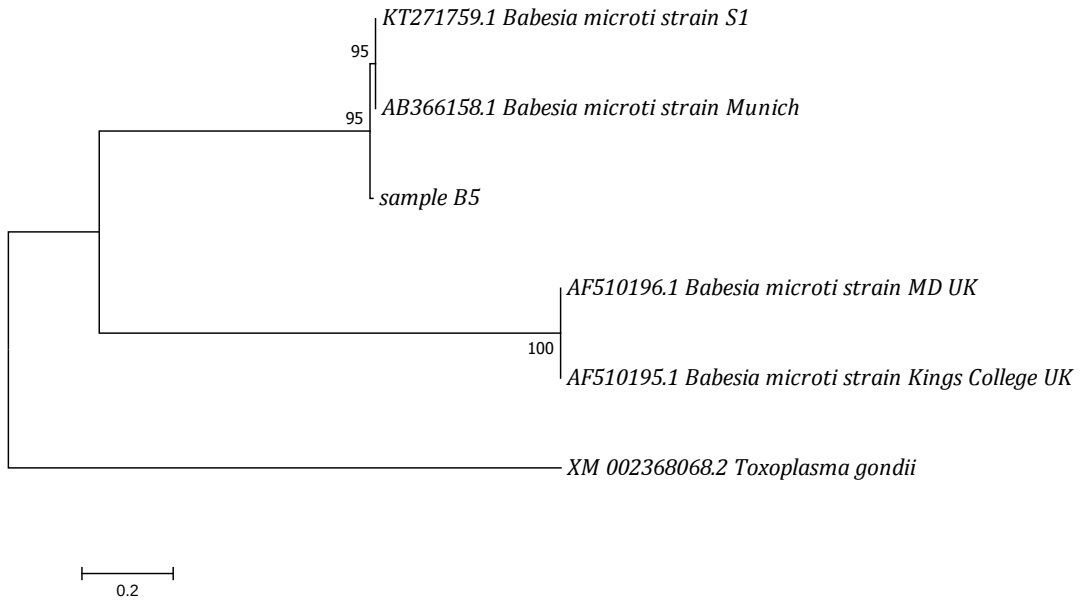
Season	Species	Sex	Age	Mean	St. Dev	Median	Geometric Mean
Autumn	BV	F	A	0.00	0.00	0.00	na
Autumn	BV	F	J	176.80	319.72	12.00	105.44
Autumn	BV	F	S	4.16	15.06	0.00	5.01
Autumn	BV	M	A	0.29	0.61	0.00	1.26
Autumn	BV	M	J	1.00	1.41	1.00	2.00
Autumn	BV	M	S	4.15	12.28	0.00	4.97
Autumn	FV	F	A	0.00	0.00	0.00	na
Autumn	FV	F	J	3.00	na	0.00	3.00
Autumn	FV	M	A	0.00	na	3.00	na
Autumn	FV	M	S	12.50	13.43	0.00	8.12
Autumn	WM	F	A	0.33	0.52	12.50	1.00
Autumn	WM	F	J	0.00	na	0.00	na
Autumn	WM	F	S	91.33	166.96	0.00	57.14
Autumn	WM	M	A	17.92	59.83	0.00	11.24
Autumn	WM	M	S	15.83	43.38	0.00	23.62
Spring	BV	F	A	2.64	6.07	0.00	2.65
Spring	BV	F	J	5.00	7.07	0.50	10.00
Spring	BV	F	S	0.80	1.93	5.00	3.46
Spring	BV	M	A	0.89	1.62	0.00	1.49
Spring	BV	M	S	0.21	0.63	0.00	2.00
Spring	WM	F	A	0.00	0.00	0.00	na
Spring	WM	F	S	1.83	4.49	0.00	11.00
Spring	WM	M	A	0.54	1.33	0.00	3.46
Spring	WM	M	S	0.00	na	0.00	na

4.3.3 Pathogen screening in ticks

Molecular analysis revealed that *Anaplasma phagocytophilum* was present in site MPW (one of the woodland in Stackpole) in the pooled sample of ticks collected from bank voles in spring. The sequence displayed high similarity (id > 99%) with the sequences present in GenBank database for the species.

No positive results were found for *B. microti* (Kielder forest strains), and *B. burgdorferi*. However, *Babesia microti* was present at site CW (conifer woodland in Ceredigion), detected in a sample of ticks collected from bank voles in spring. The sequence recovered showed high homology (id = 97%) with a *B. microti* strain (Munich) from Europe isolated from rodent species and from a human case in Spain. Phylogenetic analyses confirmed that the strain identified was more related to European strains than to other strains from UK (Fig. 4.6).

Fig. 4.6. Phylogenetic tree of the partial 18S gene from *Babesia microti* constructed by Maximum Likelihood method based on Kimura 2-parameter model (Kimura, 1980) and 1000 bootstrap replicates. Numbers indicate bootstrap values reported as percentages. Accession number is displayed for the sequences downloaded from GenBank. The scale bar corresponds to 0.20 change per nucleotide.



4.3.4 Pathogen screening in fleas

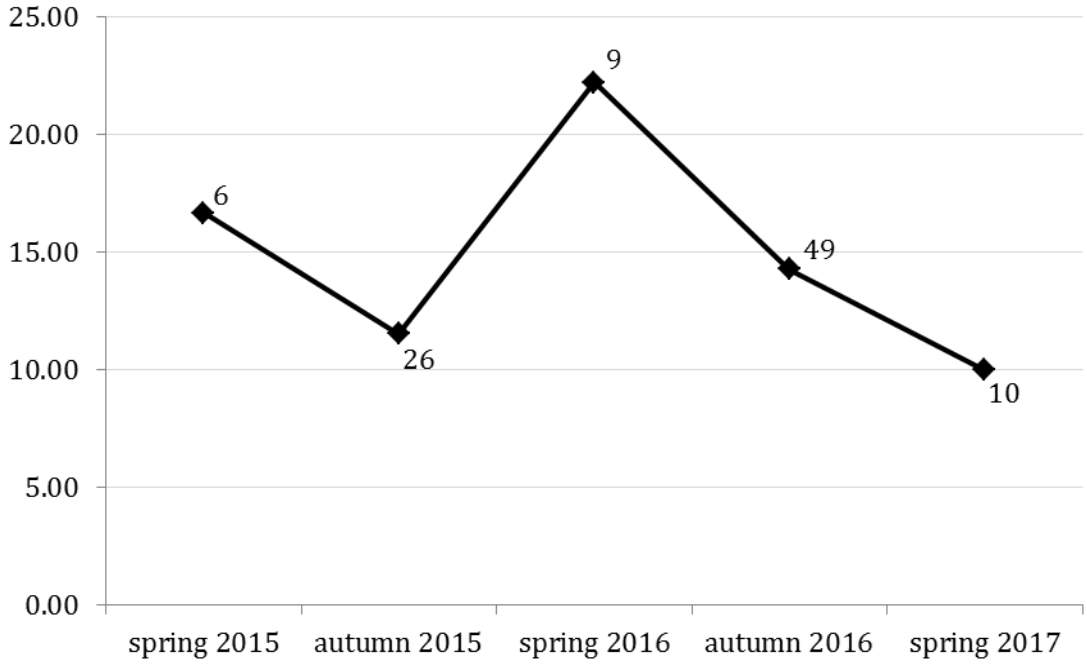
All the fleas collected during trapping sessions (100) were analysed for detecting *Bartonella* species, and the total prevalence was 14.00%. Thus, 14 individual fleas were positive according to the molecular results. All the positive fleas were collected from bank voles, with the exception of one from a field vole and one from a wood mouse. With regard to flea species, the infected fleas were represented by three *Amalaraeus penicilliger*, five *Ctenophthalmus nobilis*, three *Hystrichopsylla talpae*, and three *Megabothris turbidus*. The logistic regression showed that prevalence was not associated with flea species, flea burden, season, trapping session, or site (Fig. 4.7). In fact, positive fleas were collected from almost all study sites. Among bank voles, which hosted the highest number of infected fleas, flea prevalence did not significantly differ among sites, and host individual characteristics (sex, age class) were not associated with flea infection.

Representative sequences among those identified through the BLAST search were chosen to perform a phylogenetic analysis, and investigate *Bartonella* species circulating among the studied populations (Fig. 4.8). The phylogenetic tree showed that at least three species of *Bartonella* were represented in the samples (*B. taylorii*, *B. grahamii*, and a species > 96% similar to *B. rochalimae*). No association was present between *Bartonella* species and flea species.

The ssrA fragment was really effective in separating the *Bartonella* species, as confirmed by the high bootstrap values and the match with sequences in GenBank database.

Fig. 4.7. Plot of all *Bartonella* species prevalence (%) across a) trapping seasons and b) study sites (labels represent sample size).

a)



b)

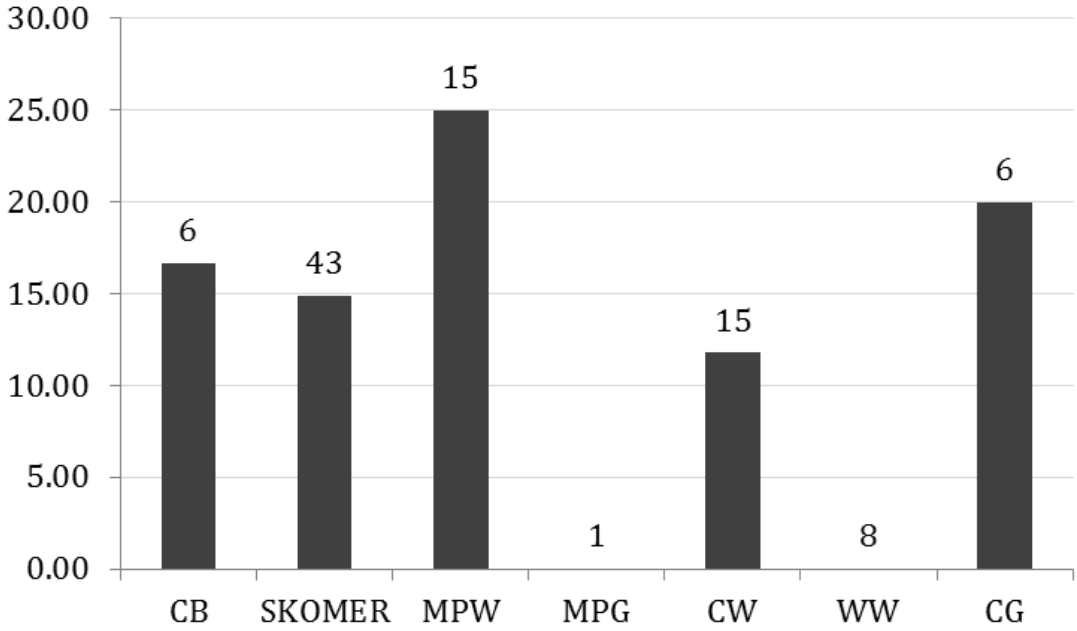
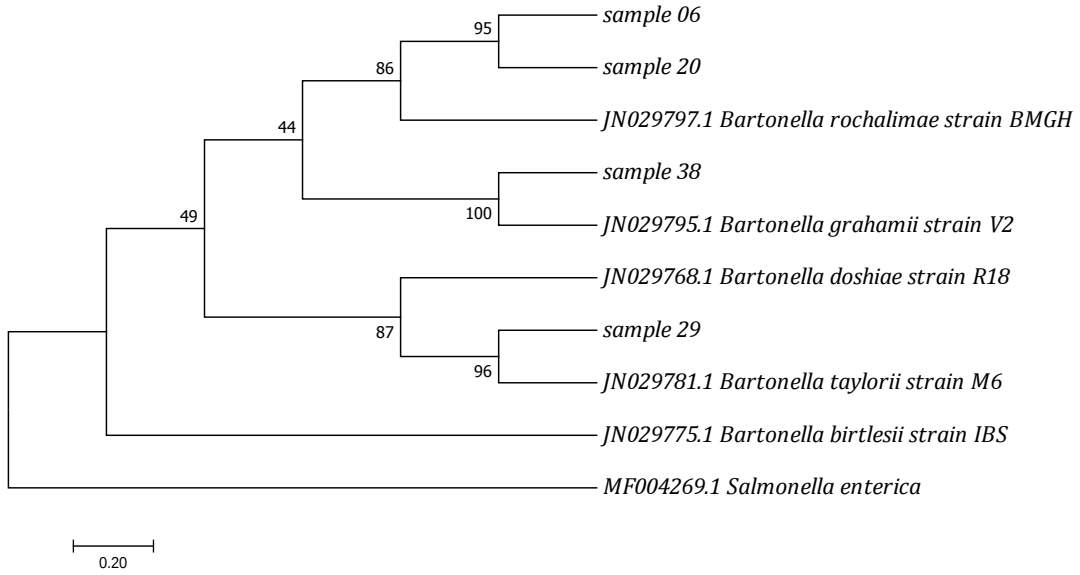


Fig. 4.8. Phylogenetic tree of the partial ssrA gene from *Bartonella* species constructed by Maximum Likelihood method based on Kimura 2-parameter model (Kimura, 1980) and 1000 bootstrap replicates. Numbers indicate bootstrap values reported as percentages. Accession number is displayed for the sequences downloaded from GenBank. The scale bar corresponds to 0.20 change per nucleotide.



4.4 Discussion

4.4.1 Low prevalence of directly transmitted pathogens

MHV-68 (murine Herpesvirus), or MHV-4, was originally isolated from bank voles in Slovakia by Blaskovic *et al.* (1980), when they also isolated other related Herpesviruses (MHV-60 and MHV-72) from bank voles and from wood mice (MHV-76 and MHV-78). The high diversity of Herpesviruses in natural population is also confirmed by other studies (e.g. wood mice in Cheshire: Hughes *et al.*, 2010; wild rodents in China: Zheng *et al.*, 2016), that is why the choice of pan-herpesvirus primers seemed the best strategy to detect Alpha, Beta, and Gammaherpesvirus. This technique, employed by Zheng *et al.* (2016) on wild rodents and shrews rectal swabs in China, revealed a low viral loads in faeces, but a prevalence up to ~8% of a wide variety of Beta and Gammaherpesvirus. The very low prevalence found in this study might be explained by the type of sample tested. In fact, despite individuals testing PCR positive for their entire lifetime when spleens and lungs are tested (Blasdell *et al.*, 2003), these viruses can be latent for long periods, with the consequence of no shedding of viral particles (Nash *et al.*, 2001). Finally, it seems that the wood mouse is the major reservoir host (Telfer *et al.*, 2007b), but no wood mice were found infected in the populations sampled. This finding, together with the similarity to human HVs of the positive samples, may suggest that no MHVs are circulating at the sampling sites, but other strains, potentially originated by rodent-human proximity, are present and have different epidemiological dynamics (e.g. more affinity with bank voles).

E. coli occurrence in the mammalian gut has been found to vary from the more than 90% of gut microbiota in humans to the 56% of gut microbiota in wild mammals (Gordon and Cowling, 2003; Tenaillon *et al.*, 2010). In particular, it has been hypothesised that the presence of *E. coli* in wild mammals is positively

related to exposure to human contamination (Iovine *et al.*, 2015). In rodents, this bacterium has always been recovered at low prevalence (Kozak *et al.*, 2009; Sayah *et al.*, 2005; Swiecicka *et al.*, 2003), supporting the findings of this study. Further, no pathogenic strains are reported to have rodents as reservoir hosts (e.g. Healing *et al.*, 1980; Kilonzo *et al.*, 2013). Therefore, the presence, in the rodent samples analysed, of strains similar to human ones is not surprising, but also needs further investigation at a finer genetic scale. In fact, high levels of genetic diversity have been reported in *E. coli* occurring in wild hosts, and similar features to human strains (e.g. antibiotic susceptibility) has also been found (Swiecicka *et al.*, 2003). The positive samples were all collected in autumn almost exclusively from bank voles, and this may be explained by the relationship between gut microbiota and diet. In spring and summer, bank voles feed mostly on green parts of plants, while in autumn and winter they incorporate more seeds, influencing microbial flora (Ecke *et al.*, 2018; Gebczynska, 1983; Hansson, 1979, 1985). It is likely that different food items determine presence and abundance of *E. coli*, and this would also be supported by the fact that no positive samples were found among Skomer voles, which have a less diverse diet, totally lacking in seeds, due to Skomer habitat features. However, this is a hypothesis that needs further investigation to be confirmed.

No individuals were found infected by *Mycobacterium microti*, and this could be due to the absence of lesions to the gastro-intestinal tract, as found by Burthe *et al.* (2008), Cavanagh *et al.* (2002), and Kipar *et al.* (2013), who recommend the analysis of liver and spleen to obtain a reliable prevalence estimation. Although, Wells (1946) found a high rate of mycobacterium shedding in faeces and urine, combined to frequent lesions to gastro-intestinal and urinary tract; in addition, *M. avium* has been found in sheep faeces, which has been successfully analysed through a molecular approach (Marsh and Whittington, 2001). Ultimately, further investigations are needed to conclude that *M. microti* is absent from the populations sampled.

However, the confidence of freedom analysis (Appendix II) revealed that the prevalence estimated was likely to be reliable, since PCR is a methodology that

is considered highly sensitive and highly specific (Yang and Rothman, 2004). PCR has proved to be highly specific and sensitive in the detection of bacteria in several different types of samples, including faecal swabs (Vaidya *et al.*, 2008; Whyte *et al.*, 2002).

4.4.2 Prevalence, intensity of infection, and host heterogeneity of rodent helminths

Distribution of helminths, having complex life cycles, depends on survival and distribution of definitive and intermediate hosts, and on environmental conditions (Poulin and Morand, 2004). These are usually aggregated, with a small proportion of hosts being majorly parasitised, including nematodes and cestodes (Walker *et al.*, 2017). In the study, 67.45% of the population was found to be parasitised by at least one genus, while 54.24% was infected by nematodes, 25.47% by cestodes and 12.26% by both taxa, confirming that different types of parasites, differing in life cycle, present distinct transmission dynamics. This was corroborated by the preferential distribution in different categories of hosts, and seasonal patterns. Moreover, another factor determining parasite prevalence and intensity is the interaction among parasite species (Behnke, 2008). Cestode burden was found to be generally higher than that of nematodes, and negatively affected nematodes intensity of infection, suggesting that among the population sampled cestodes exerted a higher inter-specific competition, although being less prevalent, probably because of their indirect life cycle. Nevertheless, published experimental data established that interactions between intestinal helminths of rodents were mostly mediated by host immune response, and not by direct effect of one species upon the other (Behnke, 2008). In addition, definitive conclusions cannot be drawn due to sampling methodology. Faecal egg count is recommended when invasive

sampling (e.g. post-mortem) cannot be performed and gives reliable results on a variety of hosts (e.g. Lynsdale *et al.*, 2015; Seivwright *et al.*, 2004). However, it can give biased results due to factors related to parasites (e.g. adult parasite sex ratio, number, and fecundity: Guyatt and Bundy, 1993; Tompkins and Hudson, 1999), or to the hosts (e.g. sex and age: Wood *et al.*, 2013). Helminths also have interactions with gut microbiota, as demonstrated in humans and animals (Berrilli *et al.*, 2012; Kreisinger *et al.*, 2015; Loke and Lim, 2015); nevertheless, in this study, most likely due to the limited amount of data available, no association was found between *E. coli* prevalence and parasite prevalence or intensity of infection.

Nematodes and cestodes seemed to display different seasonality; in fact, burden was higher in spring for the first, while in autumn for the latter, but this result needs to be considered with caution due to the quantity of data available. Nematode genera recorded present a direct life cycle with a phase of maturation in the environment outside the host, and their survival is mainly related to soil moisture and suitable temperature (Haukisalmi *et al.*, 1996; Lewis, 1987). Thus, as it happens in other species (Vlassoff *et al.*, 2001) spring may represent the peak of egg shedding for this type of parasites. On the other hand, cestodes need an intermediate host, so their annual variation is associated with the availability of the intermediate host (Foronda *et al.*, 2011). These differences in life cycle might also explain the higher nematode burden in bank voles, which include a very low proportion of animal matter in the diet (e.g. insects representing intermediate hosts for cestodes), especially in spring (Gebczynska, 1983; Lewis, 1987). Ultimately, parasite characteristics and host ecology both contribute to shape host-parasite associations, and need to be taken in account when considering relative host contribution to the transmission in multi-host parasites. Undoubtedly, the environment, and the overall species community play a role too. In this study, a significantly higher burden of nematodes was found in the WW woodland that was usually grazed by sheep and cows (hosts of several nematode species), and with a lower presence of masting trees, determining higher degree of herbivory and a higher risk of nematode larvae ingestion.

With regards to host individual factors, sex and age class affected parasite distribution. Male-biased infection was found in nematodes, while the opposite was recorded for cestodes. Male-biases have been frequently found in nematodes among rodents and other species (Ferrari *et al.*, 2004; Perkins *et al.*, 2008; Poulin, 1996), but this phenomenon is not always observed across all seasons and locations (Hwang *et al.*, 2010; Krasnov *et al.*, 2012); also, nematode transmission seems to be driven not only by disassortative mixing (Perkins *et al.*, 2008). Interestingly, the less common female-biased parasitism has been previously reported in cestode intensity of infection. For example, Hwang *et al.* (2010), among red-backed voles (*Myodes gapperi*), found higher Cestoda intensity in females, and in particular in juvenile females. Similarly, in birds, cestode infection has been reported to be higher in females than in males (Poulin, 1996). Additionally, experimental findings have shown that in different congenic and syngeneic strains of mice, females become more infected with *Taenia crassiceps* (Huerta *et al.*, 1992; Larralde *et al.*, 1995; Morales-Montor *et al.*, 2015; Sciutto *et al.*, 1991; Terrazas *et al.*, 1998). This differential susceptibility to parasite infection seems to be caused by the different immune-mediated response in males and females, determined by the distinct hormonal configuration (Schalk and Forbes, 1997). In fact, this is confirmed by the often attenuated differences in juvenile individuals, that have less sexually distinctive hormonal profiles (Schalk and Forbes, 1997). However, in this study juveniles were significantly less parasitised by nematodes, while displayed a higher parasite burden compared to adults regarding cestodes. Usually, juveniles are highly parasitised because they lack of acquired immunity (Anderson and Crombie, 1984; Anderson and May, 1985; Dobson *et al.*, 1990), but maternal immunity, host behaviour, and specific host-parasite dynamics may have determined this result. In fact, biased parasitism occurs often, but not always within the same host-parasite association, it might vary spatially or temporally, and be mediated by environmental factors (Krasnov *et al.*, 2012).

Finally, to understand parasite transmission in a multi-host system, it is necessary to be able to identify the contribution of each host to transmission. In some cases the transmission can be sustained by a community of hosts, but, in

other cases, one key host disproportionately drives parasite spread, representing a super-spreader (Fenton *et al.*, 2015; Streicker *et al.*, 2013; Streicker *et al.*, 2015). Identification of the type of contribution of different hosts is essential in designing control strategies, understanding effects of host community change on pathogen transmission, and eco-epidemiological modelling. The result of host heterogeneity analysis revealed that bank voles were key hosts for nematodes, while wood mice were for cestodes, and this was due to different mechanisms. Bank voles, also when the analysis was performed at parasite genus level, contributed to transmission mainly in terms of host abundance and prevalence (i.e. super-abundant and super-infected host), while wood mice represented exclusively super-shedder hosts. Clearly, the methodology might have influenced the results, for example, unequal detection and sampling of host species, or of parasite eggs. However, this demonstrates that even in an apparently simple and common multi-host-parasite system, over a small spatial and temporal scale, host heterogeneities are detectable. Hence, this framework might be adapted and applied to other multi-host-parasite systems, which are ubiquitous and often implicated in disease emergence (Cleaveland *et al.*, 2001; Smith *et al.*, 2009a), for tailoring more effective and ecologically sound wildlife and pathogen management plans to alleviate human and wildlife disease risk (Fenton *et al.*, 2015; Johnson *et al.*, 2015a; Rynkiewicz *et al.*, 2015).

4.4.3 Prevalence of tick-borne pathogens

The three pathogens investigated in tick specimens are considered, in the UK, to have rodent species as primarily reservoir hosts, and they have been recorded in different ixodid tick species, in particular *Ixodes ricinus* and *I. trianguliceps* (Bown *et al.*, 2003; Bown *et al.*, 2006). *Anaplasma phagocytophilum* was

detected only in one site, in ticks recovered on bank voles. This finding is in agreement with Bown *et al.* (2003), who found this species more likely to be infected compared to wood mice, probably due to a higher tick burden (as recorded in this study, see Chapter 3). The sample was constituted by ticks collected in spring (the trapping season including May and June), and previous studies recorded *A. phagocytophilum* only from late spring to late autumn (Chvostáč *et al.*, 2018). No definitive conclusions can be made due to limited data, but it seems that the seasonal patterns was respected, most likely driven by seasonal increases of *I. trianguliceps* adults (this study, Bown *et al.*, 2003). *I. trianguliceps* is not only competent in transmitting this pathogen, but, in Britain, is considered the main reservoir vector, despite the mean numbers of ticks per rodent being low (this study, Bown *et al.*, 2003; Burri *et al.*, 2011). It is likely that the sympatric, and when present more abundant, *I. ricinus* might acquire the infection from rodents and it is responsible for rodent prevalence and human disease risk amplification (Bown *et al.*, 2003). Similarly, in USA, the *A. phagocytophilum*-*I. spinipalpis*-dusky wood rat cycle is maintained by the amplification caused by the sympatric tick *I. pacificus*, which is responsible for the transmission to humans and domestic animals (Castro *et al.*, 2001). The very low presence of *I. ricinus* at the sampling sites, and the short infectious period reported in rodents (short amount of time for the ticks to be infected) (Bown *et al.*, 2003) may explain the low recovery of this pathogen in the analysed samples. In addition, highly prevalent species, such as birds, have been proposed as reservoir hosts in different locations in Europe, since prevalence in rodents has been found consistently low (Baráková *et al.*, 2018; Blaňarová *et al.*, 2014; Bown *et al.*, 2009; Burri *et al.*, 2011).

According to the results, *Borrelia burgdorferi* s.l. did not occur in the ticks collected. *I. trianguliceps* accounted for more than 87% of the pool of ticks, and this species is not considered of major importance in transmitting the spirochete (Kilpatrick *et al.*, 2017a; Stanek *et al.*, 2012). However, the pathogen has been identified in multiple environments, harboured by several species of ixodid ticks (Dantas-Torres *et al.*, 2012). Low prevalence of infection in rodents recorded in several European countries (e.g. Ireland, UK, Slovakia) is considered

part of a growing evidence that small mammals may not be the main reservoir for Lyme disease in Europe (Chvostáč *et al.*, 2018; Gray *et al.*, 1999; Kurtenbach *et al.*, 1998). The reason might be found in the low rates of *I. ricinus* nymphal infestations in rodents, while transmission dynamics may be primarily shaped by abundance and temporal population fluctuations of other species, such as ungulates and birds (Chvostáč *et al.*, 2018; Gray *et al.*, 1999).

Only one tick sample was positive for the protozoan *Babesia microti*, although this has been often recorded in Britain, in *I. trianguliceps* (Bown *et al.*, 2008; Hussein, 1980) and in *I. ricinus* (Abdullah *et al.*, 2018). When the PCR was performed with primers specific to strains from northern England no sample resulted positive, thus it may be possible that the strains circulating at the sampling sites are genetically diverse. In fact, *Babesia* has been found to be a very diverse genus, as several new species have been involved in human cases in geographical locations where the enzootic cycle was known for decades but the human disease risk was neglected (Hunfeld *et al.*, 2008). Yet, low prevalence has been reported in other studies (e.g. Welc-Falęciak *et al.*, 2008), and absence has been previously reported on Skomer island (Healing, 1981). On the other hand, in *B. microti* low prevalence might have been determined by a non-preferential host-vector association. In Europe, high prevalence has been found in *I. ricinus* (Baráková *et al.*, 2018), and in other species of rodents (*Microtus arvalis* and *M. oeconomus*) (Welc-Falęciak *et al.*, 2008), suggesting that the role of different vector and rodent species in maintaining the enzootic cycle of *B. microti* might vary. Moreover, the strain recovered from the positive sample displayed high relatedness to a European strain (Munich) isolated from ticks and rodents in several European countries, and involved in the first human case of *B. microti*-caused babesiosis in Spain (Arsuaga *et al.*, 2016). On the other hand, the phylogenetic tree showed that this strain is highly dissimilar from the British strains found in the GenBank database (MD and King's College strains, Zahler-Rinder *et al.*, unpublished). This finding strengthens the importance of molecular analysis to understand specific distribution and ecology of *B. microti* strains. It seems likely that, in UK, different strains of *B. microti* are circulating,

and these might not only differ with regards to host and vector preferences, but also in terms of zoonotic risk (Gray, 2006).

Finally, another factor to consider in interpreting the results is the interaction between pathogens. For example, *I. scapularis* co-infections with *B. burgdorferi* and *B. microti* occur more often than expected by chance, whereas co-infections with *A. phagocytophilum* and *B. microti* are less common than expected (Diuk-Wasser *et al.*, 2016; Hersh *et al.*, 2014b); although, in rodents, Telfer *et al.* (2010) found that *B. microti* infection was positively correlated with anaplasmosis. This means that factors that regulate rodent species, including competition, predation, and habitat disturbance are likely to affect independent and combined infection prevalence in vectors. Hence, the results found may be explained by true absence/low prevalence of the pathogens, low competence of the rodent and vector populations sampled, negative impact on the pathogens tested of other undetected infections, or a combination of these factors.

4.4.4 *Bartonella* prevalence in the sampled flea community

Bartonella was detected in the most represented flea species in the pool (*Amalaraeus penicilliger*, *Megabothris turbidus*, *Hystrichopsylla talpae*, and *Ctenophthalmus nobilis*). The overall prevalence of 14% was in the range of values found in other field studies (e.g. Abbot *et al.*, 2007; Abreu-Yanes *et al.*, 2018; Stevenson *et al.*, 2003; Withenshaw *et al.*, 2016). However, prevalence recorded in rodents has often been much higher: 62% in small woodland rodents in Britain (Birtles *et al.*, 1994), 76% in cotton rats in Georgia, 76% in white-footed mice in North Carolina (Kosoy *et al.*, 1997), 45–57% in Norway rats in Los Angeles, New Orleans, and Portugal (Ellis *et al.*, 1999), and 44% in rodents in China (Ying *et al.*, 2002). In Ireland, Telfer *et al.* (2005) observed Bartonella infection in a similar flea community to the one sampled in this

study, and reported similar flea species prevalence. Here, amongst the fleas collected *Amalaraeus penicilliger* showed 42.86% prevalence while *Ctenophthalmus nobilis* 12.82%, *Hystrichopsylla talpae* 21.43%, and *Megabothris turbidus* 16.67%. Statistical analyses did not reveal any pattern of association between flea infection and other factors related to fleas or hosts. Indeed, it has been observed that flea prevalence was not directly related to host infection, since dynamics of transmission are driven by the complex distribution of species-specific *Bartonella* strains (Withenshaw *et al.*, 2016). In general, in rodent host species prevalence displays seasonal variations connected with host demography and patterns of acquired immunity to different *Bartonella* species (Kosoy *et al.*, 2004a; Telfer *et al.*, 2007a). However, this could not be tested in this study, and no seasonality was recorded in flea infestation. As expected, *Bartonella* infections were recorded in fleas collected from bank voles, as this represented the dominant species in the rodent community (Bai *et al.*, 2002; Birtles and Harrison, 1994; Gutiérrez *et al.*, 2015; Kosoy *et al.*, 1997); higher prevalence has been observed in highly abundant hosts, and when high flea burden occurs (Cevidanes *et al.*, 2017).

The best sequences, according to the BLAST search, were used for the phylogenetic analysis, but no species-specific prevalence was estimated because the molecular approach adopted was not optimal for fine characterisation of the wide diversity of *Bartonella* strains. However, the sequences chosen to represent the results of molecular analysis were appropriate to reveal that at least three species (or three different groups with high similarity to *B. taylorii*, *B. grahamii*, and *B. rochalimae*) were circulating at the sampling sites. *B. taylorii*, and *B. grahamii* have been widely recorded in rodent fleas in UK (Birtles *et al.*, 2000; Telfer *et al.*, 2007a; Telfer *et al.*, 2007c; Withenshaw *et al.*, 2016), and in other countries (e.g. Špitalská *et al.*, 2017), but, to the best of my knowledge, it is the first time that *B. rochalimae* (or a highly similar species) is described in UK. This species has been previously isolated in different hosts, e.g. foxes in France, raccoons and coyotes in USA (Henn *et al.*, 2009), dogs, foxes, rock hyraxes, and Tristam's jirds in Israel (Marciano *et al.*, 2016). Among rodents, it has been observed in rats (Spain: Abreu-Yanes *et al.*, 2018; USA: Gundi *et al.*,

2012; Taiwan: Lin *et al.*, 2008), in *Apodemus flavicollis* (yellow-necked wood mouse) and *Microtus arvalis* (common vole) in Slovakia, and finally in *Myodes glareolus* (bank vole) in Lithuania (Lipatova *et al.*, 2015) and France (Buffet *et al.*, 2012). In this study, it has been isolated from fleas collected from bank voles (including Skomer voles), confirming its potential distribution across multiple rodent species. Interestingly, it has rarely been isolated from fleas (but see Pérez-Martínez *et al.* (2009) who isolated *B. rochalimae* from *Pulex irritans* from dogs in Chile), so the role of fleas in transmission has not been clarified yet. This result may provide additional evidence that *B. rochalimae* circulates among small rodents, and it is very likely that fleas act as a vector for transmission. Additionally, *B. rochalimae* is involved in human zoonotic transmission (e.g. Eremeeva *et al.* (2007) reported a case of a bacteraemic patient presenting splenomegaly).

In conclusion, the study provides evidence that ground-dwelling rodent fleas from selected sites in Wales harbour several *Bartonella*, including zoonotic species. Further investigation is needed to better understand host-vector-pathogen associations and estimate potential human disease risk. In fact, small rodents and humans often share the same environment, but flea contribution to *Bartonella* transmission, and the degree of human exposure to flea bites are still not fully understood.

Chapter 5

Eco-epidemiological modelling of rodent-borne pathogens

5.1 Introduction

Research into host-pathogen interaction has mostly been done in systems with a simplified ecology (e.g. human and livestock), but the rise of zoonotic emerging diseases has increased the awareness of the ecological context of infectious diseases (Daszak *et al.*, 2001; Taylor *et al.*, 2001), and has drawn attention to the gap between theory and ecological reality (Roche *et al.*, 2012). Also, in the current context of biodiversity decline and environmental change, disease ecologists' main focus is to predict pathogen transmission in impoverished animal communities, and so prevent potential epidemics arising from wildlife (Roche *et al.*, 2013).

The pioneering work by Ostfeld and colleagues regarding Lyme disease demonstrated that species-rich communities, because of the different degrees of host reservoir competence, are associated with lower *Borrelia* prevalence (Keesing *et al.*, 2010; LoGiudice *et al.*, 2003; LoGiudice *et al.*, 2008; Ostfeld and Keesing, 2000). They illustrated this phenomenon with empirical data and modelling techniques, and termed it dilution effect (Ostfeld and Keesing, 2000). A broader description of the theory underlying dilution and further examples are given in Chapter 1. Since then, dilution has been investigated and modelled extensively, but a mechanistic theoretical framework is currently lacking. It has been hypothesised that in multi-host parasite systems the type of transmission determines the outcome of diversity–pathogen relationships (Faust *et al.*, 2017).

One of the few theoretical studies showed that pathogens with frequency-dependent transmission, e.g. vector-borne, are expected to decrease in prevalence as biodiversity increases, i.e. the dilution effect, because the inclusion of species with low reservoir competence represents wasted bites for vectors (Dobson, 2004; Rudolf and Antonovics, 2005). In reality, this has been described in some cases, but the extent to which these findings can be generalised is unclear. By contrast, pathogens characterised by density-dependent transmission are predicted to increase in prevalence as host diversity increases because of the overall increase in the susceptible population (Dobson, 2004). However, in directly transmitted pathogens, both amplification and dilution effects have been observed in the field (Mihaljevic *et al.*, 2014).

As a consequence, it seems that greater ecological sophistication in epidemiological theory is needed to clarify the dilution and amplification mechanisms and the human zoonotic risk, and identify potential determinants of pathogen spillover (Faust *et al.*, 2017; Johnson *et al.*, 2015a; Kilpatrick *et al.*, 2017b). For example, for directly transmitted pathogens, Dobson (2004) assumed that an increase in host-species led to an increase in host abundance resulting in an increase in transmission rate due to increased contact. In reality, the consequences on transmission of introducing a new species to the host community are more complex, and depend also on competitive interactions in the host assemblage (O'Regan *et al.*, 2015; Roche *et al.*, 2012). In addition, the alteration of contact networks due to the modification of relative abundances of host species has to be taken in account. In fact, the proposed mechanism of dilution in the case of directly transmitted pathogen centres on encounter reduction, namely the decrease of contacts between the most susceptible species because of the introduction of less susceptible hosts (Keesing *et al.*, 2006). It is likely that not all the individuals of the host community perform the same types and quantity of contacts (see Chapter 2); therefore, when modelling population-level pathogen dynamics it is vital to take into account contact network features as an alternative to the standard random-mixing (Keeling and Eames, 2005). This can also be extended to vector-borne pathogens, where vector host preferences and host-specific survival rates might determine

different outputs when alternative host species are added to the community (Roche *et al.*, 2013).

In this study, eco-epidemiological models of transmission for directly transmitted, tick-borne, and flea-borne pathogens were developed with the aim of creating a better link between ecology and epidemiology. The proposed framework included a theoretical approach supported by empirical observations, and the assembly of a realistic community of host (small rodents) and non-host species. The objectives were, according to some of the main challenges in multi-host systems indicated by Buhnerkempe *et al.* (2015), to identify potential dilution or amplification effect in different pathogen systems, the potential mechanisms behind dilution/amplification, the importance of between-species transmission, and the parameters most affecting model outputs. Based on the work of Arino *et al.* (2004), Hadeler and Freedman (1989), Malchow *et al.* (2008), and Venturino (1994; 2001; 2002), the models are demographic models accounting for interactions between different populations in which the pathogen spreads among host species. Although direct estimation of ecological relationships is very complicated, a more realistic modelling approach might be useful to identify key species interactions in the context of pathogen transmission, and direct more efficient data collection for parameter estimation. The biggest challenge in integrating ecological and epidemiological modelling is to find the correct balance between the high level of complexity to include, and the necessary simplifications to be made (Buhnerkempe *et al.*, 2015; Malchow *et al.*, 2008). This will improve the understanding of the ecosystem context of wildlife diseases, including zoonoses, which is important because of the concerns over anthropogenic effects on biodiversity with regards to human health and conservation of endangered species (Altizer *et al.*, 2013; Keesing *et al.*, 2010; Millennium Ecosystem Assessment, 2005).

5.2 Directly transmitted pathogen model: effect of competence, competition and predation on the dynamics of a rodent-borne Hantavirus

The study employed a deterministic multi-host compartmental model constituted by a set of differential equations (Eq. 1-11). The results were produced using the function rk4 in the R package deSolve (R Core Team, 2016), which is based on the classical Runge-Kutta 4th order integration. The model was not explicitly spatial; the area, 1 ha, was considered constant since it was constrained by the sampling unit (Begon *et al.*, 2002), so the populations were expressed in individuals/ha and the parameters were scaled accordingly. The dilution effect was tested by assembling a progressively more complex community: host-species, competitor and predator species were added in turn following realistic assembly rules. The structure of the model was chosen to offer a compromise between complexity and parsimony.

The pathogen chosen for the simulation is a Puumala-like (PUUV-like) Hantavirus. Puumala virus (PUUV) is not currently reported in UK, but it is widespread in mainland Europe and poses a zoonotic risk, being the etiologic agent of nephropathia epidemica (Sauvage *et al.*, 2002; Tersago *et al.*, 2009; Vaheri *et al.*, 2013; Voutilainen *et al.*, 2016). Its main reservoir host is *Myodes glareolus* (bank vole), which is very widespread in UK, but it has been reported in other sympatric small rodents as well (Dubois *et al.*, 2017; Laakkonen *et al.*, 2006; Klingström *et al.*, 2002). The virus, among rodents, is transmitted horizontally via direct contacts (e.g. bites) or excretions (aerosolised urine and faeces) (Bernshtein *et al.*, 1999; Kallio *et al.*, 2006), and the infection is chronic and mainly asymptomatic (Bernshtein *et al.*, 1999; Voutilainen *et al.*, 2015). The ability of the virus to survive outside the host is the reason why it has been hypothesised that PUUV or PUUV-like strains might invade UK, especially under conditions of climate change (Bennett *et al.*, 2010; Kallio *et al.*, 2006). In addition, other Hantaviruses have been reported in Britain, and, in particular,

Pounder *et al.* (2013) isolated a circulating hantavirus in a British field vole, which was phylogenetically closely related to PUUV. The epidemiology of this virus has been extensively investigated (Voutilainen *et al.*, 2016), also in an eco-epidemiological framework, and Thoma *et al.* (2014) observed evidence of the amplification effect; so, it is likely that ecological relationships are affecting pathogen transmission dynamics.

Host community was represented by ground-dwelling small rodents, and, in particular, the model was parameterised, where possible, with data collected during live trapping (see Chapter 2). The most represented host species were considered wood mouse and bank vole, with the addition of a third species, field vole when the community was fully assembled. *Sorex* ssp. shrews were added to the community as a sympatric competitor non-host species, since evidence of their presence were found in the sites sampled (Eq. 10). Finally, the predation was introduced with two terms representing generalist (e.g. avian predation) and specialist (mustelids) predation (Eq. 11). Community assembly was performed starting from one host species and then adding in turn a second host species, the competitor species, the predation terms, and finally the third host species.

Rodent and shrew populations were modelled according to the Lotka-Volterra system, namely they followed a logistic growth tending to species-specific carrying capacity and limited by intra-specific density dependent reduction and inter-specific competition (Lotka, 1925; Hanski *et al.*, 1993; Volterra, 1926). Inter-specific competition among rodents, and among rodents and shrews was represented by a density dependent competition term (Huitu *et al.*, 2004; O'Regan *et al.*, 2015; Turchin and Hanski, 1997). Rodent species were considered better competitors than shrew species since growth rates of common shrew populations have been reported to be negatively correlated with total rodent density (Henttonen *et al.*, 1989; Huitu *et al.*, 2004). These species were all predated upon, but rodent species were considered preferential preys (Korpimaki and Norrdahl, 1989; Korpimaki, 1992). Generalist predation was modelled according to the alternative prey hypothesis (Holling type III

functional response), while specialist predator population was modelled according to the Holling type II functional response based on the Rosenzweig-MacArthur model (1963) with no preference among rodents (Elton, 1942; Erlinge, 1975; Hanski and Henttonen, 1996; Holling, 1965; Krebs and Myers, 1974; Turchin and Hanski 1997). Parameters about specialist predation represented *Mustela nivalis* (least weasel), which was the most common and widespread mustelid across sampled sites. For details about the parameter estimation see Section 5.2.1.

The time scale of the simulations was 20 years to allow the eco-epidemic dynamics to reach equilibrium, and the time step was 1 day. The model included four compartments for the host species – Susceptible, Exposed, Infectious, and Recovered (SEIR) – since there is evidence of a latent (but not infectious) period for the pathogen considered (Allen *et al.*, 2006; McCormack and Allen, 2006), and recovery with life-long immunity (Voutilainen *et al.*, 2016) (Eq. 1-4 and Eq. 5-9). The transmission term, or force of infection (Eq. 1b and Eq. 9), was density-dependent and split into contact rate (φ) and reservoir competence (τ). Contacts were not fixed but varied in relation to individual density (see Parameter estimation Section 5.2.1), in order to allow for heterogeneous (non-random) mixing and represent more realistic transmission dynamics according to Begon *et al.* (2002) and McCallum *et al.* (2001). The reservoir competence term summarised individual susceptibility, ability of the pathogen to magnify and persist in the host, and efficiency of transmission; all the individuals of same host species were equally competent, but different species might display different levels of competence. The initial density for each host-species was set at the average value found from field data (Chapter 2) (pooled sites excluding Skomer). The inoculum was a single infectious individual. The equations for the basic single-host model are given below; see Table 5.1 for the list of variables and parameters.

$$\frac{dS}{dt} = rN\left(1 - \frac{N}{K}\right) - \lambda S \qquad \text{Eq. 1a}$$

$$\lambda = \tau\varphi I \qquad \text{Eq. 1b}$$

$$\frac{dE}{dt} = \lambda S - \kappa E \qquad \text{Eq. 2}$$

$$\frac{dI}{dt} = \kappa E - \sigma I \qquad \text{Eq. 3}$$

$$\frac{dR}{dt} = \sigma I \qquad \text{Eq. 4}$$

The following set of equations represents the multi-host model, including the multi-host force of infection, the competitor and specialist populations.

$$\frac{dS_i}{dt} = r_i N_w\left(1 - \frac{\left(N_i - C_{ji}N_j - \ldots C_{nj}N_n - C_{ci}N_c\right)}{K_i}\right) - \lambda_i S_i - \frac{(gS_i^2)}{(S_i^2 + h^2)}$$

$$- \frac{(\alpha_i N_p S_i)}{\left(\Delta_i + N_i + \frac{\Delta_i}{\Delta_j}N_j + \cdots \frac{\Delta_i}{\Delta_n}N_n + \frac{\Delta_i}{\Delta_c}N_c\right)} \qquad \text{Eq. 5}$$

$$\frac{dE_i}{dt} = \lambda_i S_i - \kappa E_i - \frac{(gE_i^2)}{(E_i^2 + h^2)} - \frac{(\alpha_i N_p E_i)}{\left(\Delta_i + N_i + \frac{\Delta_i}{\Delta_j}N_j + \cdots \frac{\Delta_i}{\Delta_n}N_n + \frac{\Delta_i}{\Delta_c}N_c\right)} \qquad \text{Eq. 6}$$

$$\frac{dI_i}{dt} = \kappa E_i - \sigma I_i - \frac{(gI_i^2)}{(I_i^2 + h^2)} - \frac{(\alpha_i N_p I_i)}{\left(\Delta_i + N_i + \frac{\Delta_i}{\Delta_j}N_j + \cdots \frac{\Delta_i}{\Delta_n}N_n + \frac{\Delta_i}{\Delta_c}N_c\right)} \qquad \text{Eq. 7}$$

$$\frac{dR_i}{dt} = \sigma I_i - \frac{(gR_i^2)}{(R_i^2 + h^2)} - \frac{(\alpha_i N_p R_i)}{\left(\Delta_i + N_i + \frac{\Delta_i}{\Delta_j}N_j + \cdots \frac{\Delta_i}{\Delta_n}N_n + \frac{\Delta_i}{\Delta_c}N_c\right)} \qquad \text{Eq. 8}$$

$$\lambda_i = \tau_i \varphi_{ii} I_i + \tau_j \varphi_{ij} I_j + \cdots \tau_n \varphi_{in} I_n \quad \text{Eq. 9}$$

$$\frac{dN_c}{dt} = (\nu_c - \rho_c) N_c \left(1 - \frac{(N_c - C_{ic} N_i - \ldots C_{nc} N_n)}{K_c}\right) - \frac{(g N_c^2)}{(N_c^2 + h^2)} - \frac{(\alpha_c N_p N_c)}{\left(\Delta_c + N_c + \frac{\Delta_c}{\Delta_i} N_i + \cdots \frac{\Delta_c}{\Delta_n} N_n\right)} \quad \text{Eq. 10}$$

$$\frac{dN_p}{dt} = (\nu_p - \rho_p) N_p \left(1 - \frac{q N_p}{\left(N_i + \frac{\Delta_i}{\Delta_j} N_j + \cdots \frac{\Delta_i}{\Delta_n} N_n + \frac{\Delta_i}{\Delta_c} N_c\right)}\right) \quad \text{Eq. 11}$$

Fig. 5.1 represents the graphical visualisation of the multi-host compartmental model consisting of the equations above.

Simulations were performed to test the following hypotheses: a) addition of a less competent host decreases pathogen transmission compared to the single-host scenario; b) inter-specific competition among host-species, affecting the most competent host's relative density, decreases pathogen transmission; c) addition of a non-host competitor and predation decreases pathogen transmission through susceptible host regulation.

Fig. 5.1. Multi-host compartmental model. Boxes represent the four epidemiological compartments in which each host-species population is subdivided: S = susceptible, E = exposed, I = infectious, R = recovered. N (population density) = S+E+I+R. Subscripts represent different species: 1 and 2 = rodent species (host-species); i = non-host competitor (shrew species). λ = force of infection (dependent on competence, infectious, and intra/inter-specific contacts, see Eq. 1b and Eq. 9), r = growth rate (determined by competition and density of rodent and shrew species), c = competition coefficient, N_i = non-host competitor population density, κ = rate at which exposed individuals become infectious (latent period), σ = recovery rate. Arrows indicate the direction of movement of individuals between classes and are labelled by the transition rates. Arrows pointing outside the boxes represent mortality.

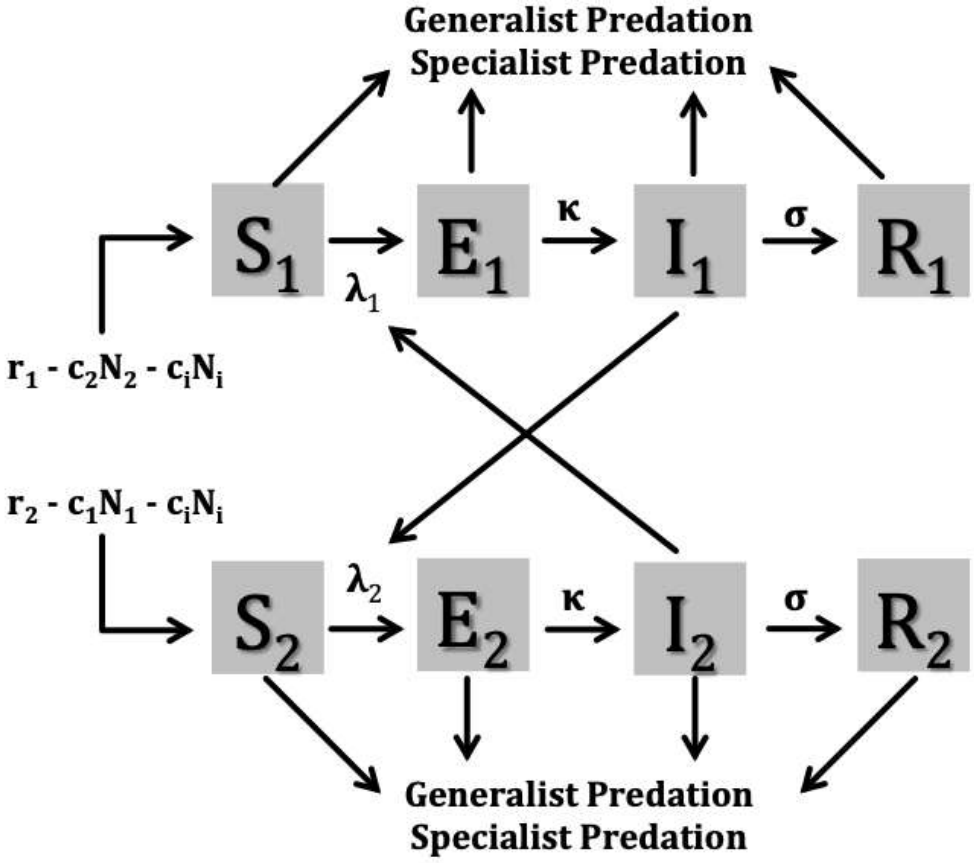


Table 5.1. List of model variables and parameters. For details about parameters estimation see section 5.2.1.

Symbol	Description (source where relevant)	
S	Number of susceptible individuals	
E	Number of exposed individuals	
I	Number of infectious individuals	
R	Number of recovered individuals	
N	Total number of individuals (population size)	
i, j, n	Host-species	
c	Non-host competitor species	
p	Specialist predator	
C	Competition factor	(O'Regan *et al.*, 2015; this study)
g	Saturation rate of generalist predation	(Turchin and Hanski, 1997)
h	Prey density at which generalist predation rate is half of the maximum	(Turchin and Hanski, 1997)
K	Carrying capacity	(Bolzoni *et al.*, 2008; De Leo and Dobson, 1996)
q	Specialist predator-prey ratio constant	(Turchin and Hanski, 1997)
r	Rodent growth rate	(this study)
α	Maximum consumption rate of specialist predator	(Turchin and Hanski, 1997)
Δ	Half-saturation constant (specialist predator)	(Turchin and Hanski, 1997)
κ	Rate at which exposed become infectious	(Wolf, 2004)
λ	Force of infection (Eq. 1b and Eq. 9)	
ν	Birth rate	(Bolzoni *et al.*, 2008; De Leo and Dobson, 1996)
ρ	Death rate	(Bolzoni *et al.*, 2008; De Leo and Dobson, 1996)
σ	Recovery rate	(Allen *et al.*, 2009)
τ	Reservoir competence	(this study)
φ	Contact rate	(this study)

5.2.1 Parameter estimation

All the parameters, listed in Table 5.1 were estimated, where possible, from the data collected in this study (see Chapter 2), and from literature. Wood mouse and bank vole growth rates (r) were calculated from field data according to Lambin *et al.* (2000) (section 2.2.3.3), but using log_e instead of log_{10} because it was to be used directly in the model as growth rate. The values obtained for the breeding and non-breeding seasons were scaled by day, as for the other parameters in the model, and seasonality was included in the model considering the breeding season from March to October, and the non-breeding season November to February (Hörnfeldt, 1994; Stenseth *et al.*, 2002). Due to lack of data, the bank vole growth rate was also used for modelling the field vole population. Carrying capacity for all the species, and the birth and death rates of the non-host species were estimated allometrically with the following formulae: $K = 16.2w^{-0.70}$, $\nu = w^{-0.25}$, $\rho = 0.4w^{-0.25}$ (where w is the mean body mass in g) (Bolzoni *et al.*, 2008; De Leo and Dobson, 1996). The body mass values used were: the average of adults and sub-adults (this study) for each rodent species (Skomer voles were excluded due to their larger size), while adult average values from PanTHERIA database (Jones *et al.*, 2009) were used for the common shrew and the least weasel. The competition factor (C) values were computed algebraically, ranging from 0 (competition absent) to the maximum value for the competing species to co-exist (for example, in the case of competition of species *i* over species *j*, the maximum value is K_j/K_i). Logistic regression was performed between daily contact rates and individual densities found in the field (Chapter 2) to obtain regression equations for intra (φ_{ii}) and inter-specific (φ_{ij}) contact rates so that $\varphi_{ii} = a_1 + b_1N_i$, and $\varphi_{ij} = a_2 + b_2N_j$ (where a_1, b_1, a_2 and b_2 are the coefficients for the regression equations). In this way, both intra and inter-specific contact rate increased with individual density, as revealed by empirical data (Sundell *et al.*, 2012). The range of values for reservoir competence was calculated from the equations, τ_{min} = 0, while τ_{max} was ~0.35 for each host-species ($\tau_{i\,max} = 1/(a_1 + b_1K_i)$ where a_1 and b_1 are the coefficients in the

regression equation of intra-specific contacts and density). The latency period (κ), or rate at which exposed individuals become infectious, for the pathogen considered, was obtained from the value reported by Wolf (2004), while the recovery rate (σ) was calculated with the formula 1/infectious period, using the value from Allen *et al.* (2009). All the parameters relating to predation were estimated with the formulae proposed by Turchin and Hanski (1997), but using empirical data from this study or data relative to UK. The formula for the saturation rate of generalist predation was g = 70*10*L (where L is the latitude of the sampling sites), so the value used in the model was an average of all the sampled sites. In the aforementioned paper, the density at which the generalist predation rate is half of the maximum (h) was estimated by fitting long-term population data, so in this study a sensitivity analysis was performed (see next section). The maximum consumption rate of the specialist predator (α) was estimated using the average weight of adults and sub-adults individuals sampled (all rodent species pooled) and the average weight of British weasels reported in literature (Tapper, 1979). The formula, which is $\alpha = 0.6w_p/w_r + \frac{1}{2}0.6w_p/w_r$ (0.6 g is the weasel average daily intake per gram of body mass according to Gillingham (1984), w_p is the average weasel body mass in g, and w_r is the average rodents body mass in g), includes 50% surplus killing. Half saturation constant (Δ) represents the prey density (rodents/ha) at which the specialist predator consumption rate reaches one-half of the maximum (α) (Turchin and Hanski, 1997). The formula is $\Delta = \frac{\alpha * N_{crit} - \alpha_{crit} * N_{crit}}{\alpha_{crit}}$, where N_{crit} is the critical minimum density of voles below which the weasel is unable to reproduce, and α_{crit} is the consumption rate by an individual weasel when prey density is N_{crit}; these values were estimated according to Tapper (1979). Finally, the specialist predator-prey ratio constant (q) represents the predator population equilibrium in relation to prey numbers. With regard to shrews, α and Δ were estimated separately, since they are lighter and less preferred by predators. Table 5.2 shows all the parameter values (or range of values) used for the simulations and the starting population densities.

Table 5.2. List of starting conditions and parameter values used for the simulations. When a range of values is provided, sensitivity analysis has been performed (see section 5.2.2).

Symbol	Description	Value
N_w	Wood mouse population	49 (ind/ha)
N_b	Bank vole population	75 (ind/ha)
N_f	Field vole population	30 (ind/ha)
N_j	Shrew population	20 (ind/ha)
N_p	Weasel population	3 (ind/ha)
cbw	Competition of wood mouse over bank vole, field vole and shrew respectively (range)	0-1.01
cfw		0-0.95
cjw		0-1.74
cwb	Competition of bank vole over wood mouse, field vole and shrew respectively (range)	0-0.98
cfb		0-0.91
cjb		0-1.71
cwf	Competition of field vole over wood mouse, bank vole and shrew respectively (range)	0-1.05
cbf		0-1.10
cjf		0-1.82
cwj	Competition of shrew over wood mouse, bank vole, and field vole respectively (range)	0-0.57
cbj		0-0.58
cfj		0-0.55
g	Saturation rate of generalist predation	0.49
h	Prey density at which generalist predation rate is half of the maximum (range)	1-67.5
q	Specialist predator-prey ratio constant	56
r_{b+}, r_{b-}	Bank vole growth rate breeding season (+), and non-breeding season (-)	0.007; -0.002
r_{w+}, r_{w-}	Wood mouse growth rate breeding season (+); non-breeding season (-)	0.04; -0.006
α	Maximum rodent consumption rate of specialist predator (range)	1-18
α_s	Maximum shrew consumption rate of specialist predator	7.67
Δ	Half-saturation constant (rodent)	11.31
Δ_s	Half-saturation constant (shrew)	22.62
κ	Rate at which exposed become infectious	0.14
σ	Recovery rate	0.11
τ	Reservoir competence (range)	0 – 0.35

5.2.2 Sensitivity analyses

Sensitivity analysis was performed on parameters of interest for which there was no direct estimation, or a range of plausible values. Firstly, sensitivity analysis was carried out on reservoir competence via systematic sampling (100 intervals were input into the sensitivity between the minimum and the maximum value selected). In fact, multiple rodent species were found to be susceptible to the pathogen and potentially competent to transmit it, although the bank vole is considered the main reservoir host (Kariwa *et al.*, 2009; Klingström *et al.*, 2002; Sauvage *et al.*, 2002). The sensitivity analysis on this parameter was intended to test the dilution mechanism of transmission reduction (Keesing *et al.*, 2006), namely does the variability of competence reduce pathogen prevalence due to the higher proportion of individuals with lower competence?

Sensitivity analysis by Latin hypercube sampling (Iman *et al.*, 1981a,b) (values were randomly chosen between the minimum and the maximum found in the selected sources) was then carried out on competition between host species, and between host species and non-host competitor species, and finally, on generalist and specialist predation, altering values of h and α respectively. In these cases the dilution mechanism tested was susceptible host regulation due to inter-specific competition or predation, to detect the degree and the direction of the impact of these ecological relationships on pathogen transmission, and the potential identification of dilution (or amplification).

As a final point, some of the most relevant rodent-borne pathogens were chosen (Table 5.3), and modelled varying the relative parameters (Table 5.3) to test whether the results obtained modelling PUUV were consistent across a range of different directly transmitted pathogens.

Table 5.3. List of rodent-borne pathogens and relative parameters modelled to compare the results obtained modelling PUUV.

Pathogen	κ	σ	Source	Notes
Cowpox virus	0.14	0.32	(Bennett *et al.*, 1997; Chantrey *et al.*, 1999; Hazel *et al.*, 2000)	Bank vole found more competent
Leptospira spp.	1	0.0014	(Holt *et al.*, 2006)	Virtually no latency and life-long infection
Mouse hepatitis virus (Co-V)	1	0.14	(Navas and Weiss, 2003; Siddell *et al.*, 1983; Weiss and Navas, 2005)	Virtually no latency
Murine Herpesvirus (MuHV-4)	0.071	0.03	(Francois *et al.*, 2010; Sunil-Chandra *et al.*, 1992; Telfer *et al.*, 2007b)	Wood mice found more prevalent
Murine Norovirus (MNV)	0.25	0.05	(Compton, 2008; Goto *et al.*, 2009)	Mostly laboratory data
Mycobacterium microti	0.17	0.0014	(Cavanagh *et al.*, 2004; van Soolingen *et al.*, 1998; Wells, 1946)	Probably life-long infection

5.2.3 Results

Comparing the single-host and the two-host scenarios, keeping reservoir competence (τ) constant and assuming no inter-specific competition, it seemed that the number of infectious was almost double in the second case, while prevalence was very similar between the different scenarios (Fig. 5.2). Wood mouse and bank voles were modelled with species-specific growth rates and carrying capacities, therefore the relative single-host scenarios were different due to their characteristic demography (Fig. 5.2).

Fixing bank vole reservoir competence at 0.06 and varying wood mouse competence (assuming no inter-specific competition), the proportion and number of total infectious at equilibrium were virtually unchanged compared with the two-host scenario with equal reservoir competence (Fig. 5.3).

Fig. 5.4 shows the complete results of the sensitivity analysis on reservoir competence performed on both hosts together. This analysis showed that, in the absence of inter-specific competition, the increase in competence determined a parallel increase in both proportion and total number of infectious, but the shape of the increase was slightly different on the x and y axis due to the demographic differences of the two host species (e.g. different recruitment rate). Also, above ~0.05 (for both species) the maximum transmission occurred and prevalence and number of infectious reached a plateau.

However, when inter-specific competition was added in the two-host scenario, the proportion and actual number of infectious displayed opposite trends. Higher competition decreased the number of infectious, increasing pathogen prevalence (proportion of infectious) (Fig. 5.5). In addition, comparing the equilibrium values of the two-host scenarios with and without inter-specific competition (setting the same reservoir competence values), a low level of competition was enough to decrease the number of infectious (but not prevalence).

Similarly, inter-specific competition with the non-host species led to a decrease in total infectious numbers, and total susceptible population, and an increase in pathogen prevalence (Fig. 5.6). Although sensitivity analysis was performed on four parameters (cwj, cbj, cjw, cjb), representing competition of the non-host with each host species and vice versa, the model was most sensitive to the parameters cbj and cwj, competition of the non-host over the host species.

Variation of predation intensity had a significant effect on the total number of infectious, especially generalist predation (Fig. 5.7b). The parameter corresponding to intensity of generalist predation was allowed to vary more than that for specialist predation, since the realistic range of mustelid density and efficiency in predation was smaller than the realistic range of generalist predation. In this case the plots displaying proportion and number of infectious at equilibrium were markedly different due to the effect of predation on the non-host competitor population, and to the complex effect on host species relative densities (Fig. 5.7). Intensification of specialist predation led to a slight increase in pathogen prevalence, and a less marked decrease of infectious numbers, while generalist predation showed the opposite trend, with the output being more variable (Fig. 5.7).

In Fig. 5.8, proportion of infectious, total number of infectious, and density of host-species are displayed along a gradient of community complexity. The course of the epidemic and the equilibrium values were very similar with regards to pathogen prevalence, with the only exception being the wood mouse single-host scenario (Fig. 5.8 a, Fig. 5.9). By contrast, the number of infectious was more noticeably affected by the community assembly, showing an increase from the single to two-host scenario, and then a progressive decrease due to the addition of host-species inter-specific competition, non-host species, and finally predation (Fig. 5.8b, Fig. 5.9). With the chosen set of values for competition and predation parameters, the latter had a major effect on regulating susceptible host populations (Fig. 5.8c), leading to a more marked effect on the epidemiological dynamics.

Finally, the results obtained using parameters relating to PUUV were confirmed modelling other rodent-borne pathogens (Fig. 5.10, Fig. 5.11). Although very different epidemic courses, when considering the equilibrium values across the gradient of community complexity, proportion and number of infectious followed the same trend found previously, namely little variation of proportion of infectious, and a hump shaped response of infectious numbers (Fig. 5.11).

Fig. 5.2. Comparison between single-host and two-host scenarios. Proportion a) and total number of infectious b) for scenarios including wood mouse only (dotted line), bank vole only (dashed line), and both species with no inter-specific competition (solid line). τ = 0.06; cbw = cwb = 0.

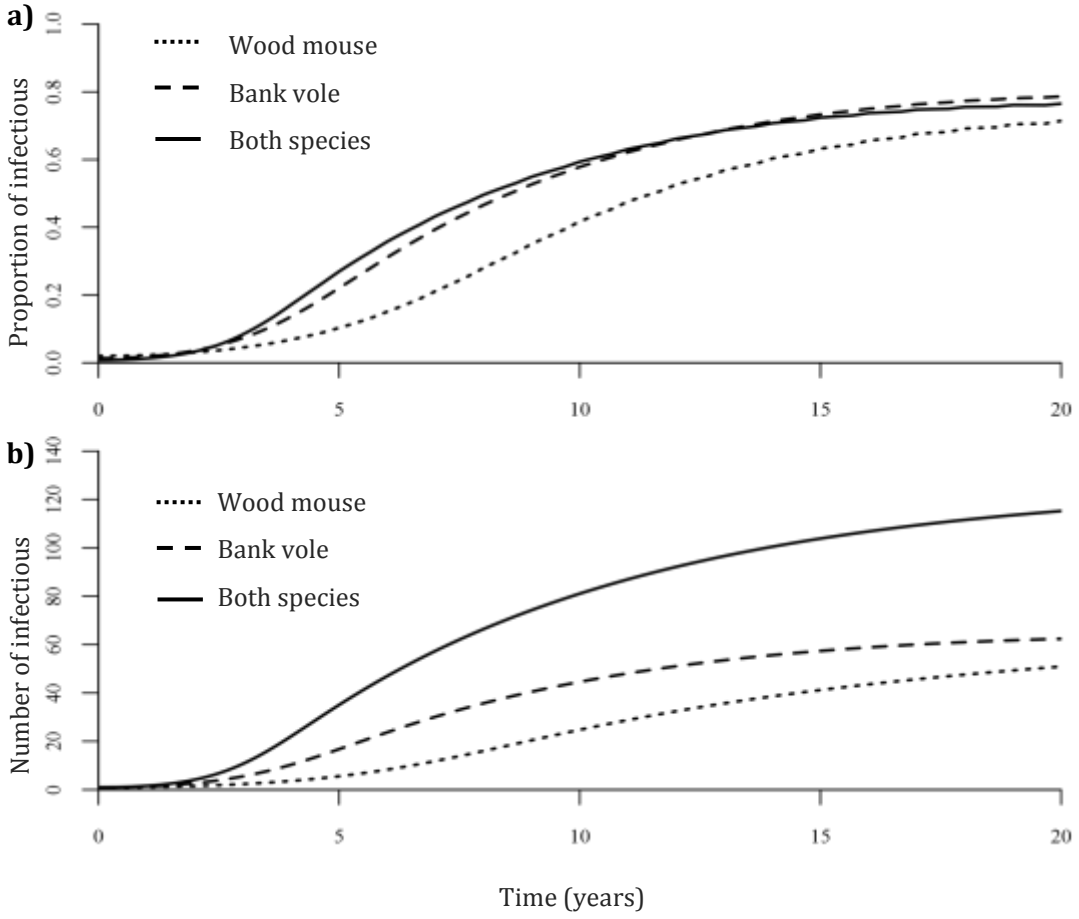


Fig. 5.3. Two-host scenario (wood mouse and bank vole). Equilibrium values for proportion a) and total number of infectious b). On the x-axis τ wood mouse values. Reservoir competence: τ bank vole = 0.06; competition: cbw = cwb = 0.

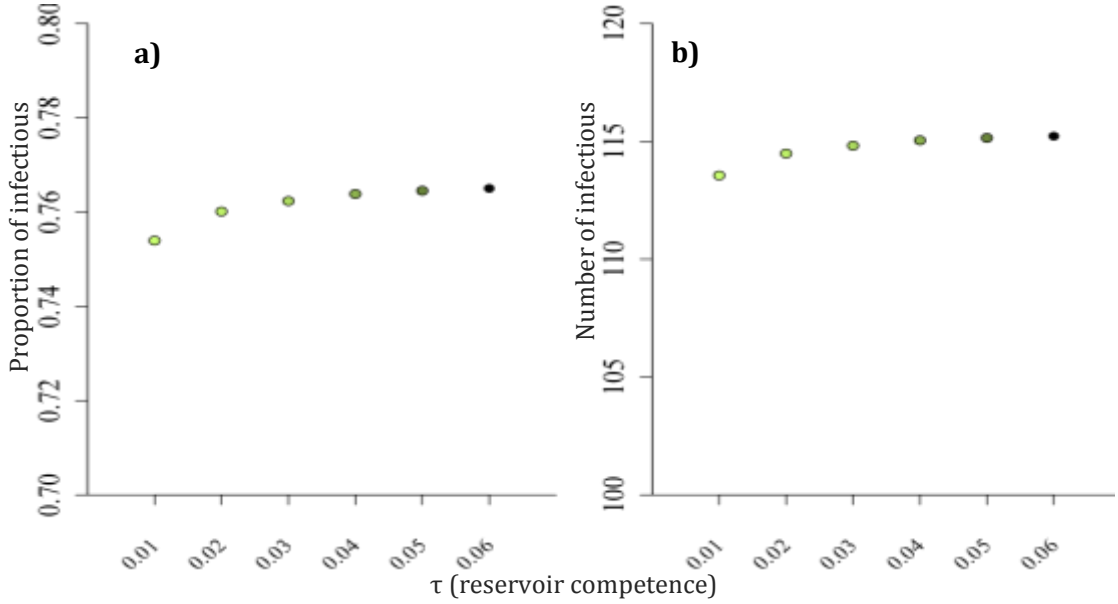
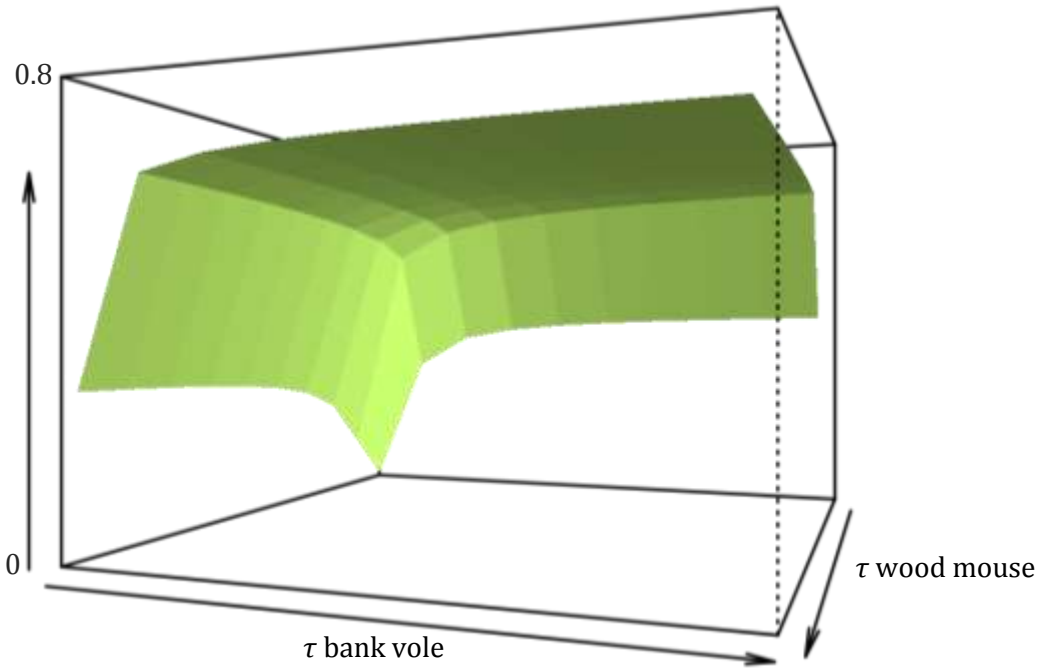


Fig. 5.4. Two-host scenario (wood mouse and bank vole): sensitivity analysis on reservoir competence. Equilibrium values for proportion a) and total number of infectious b) are shown on the z-axis. $0 \leq \tau$ (reservoir competence) ≤ 0.1; competition: cbw = cwb = 0.

a) Proportion of infectious



b) Number of infectious

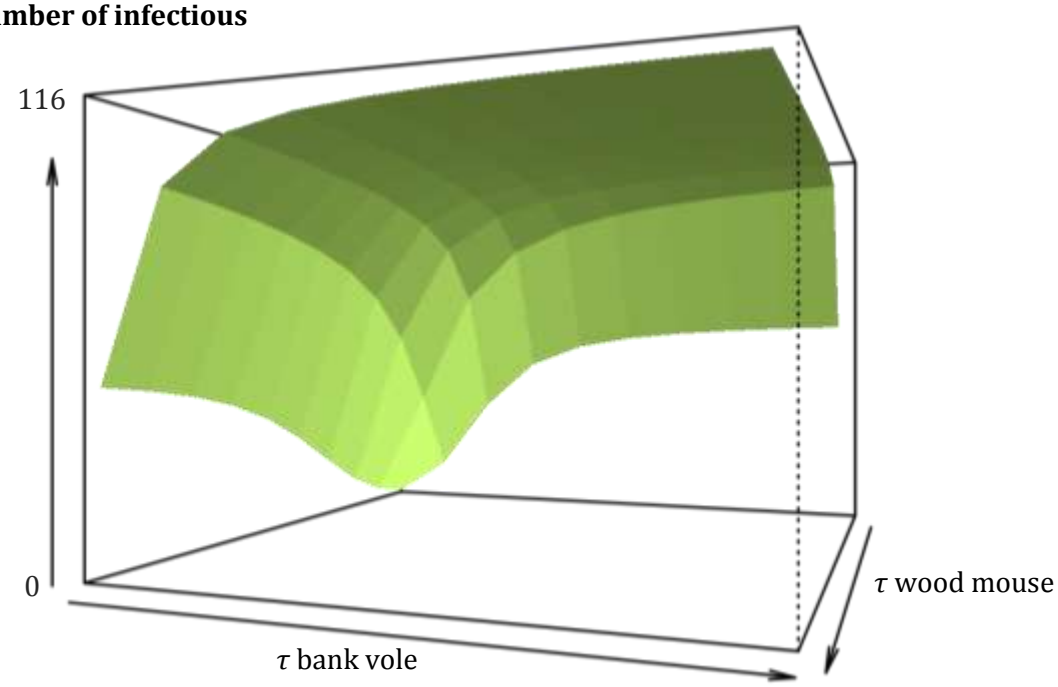
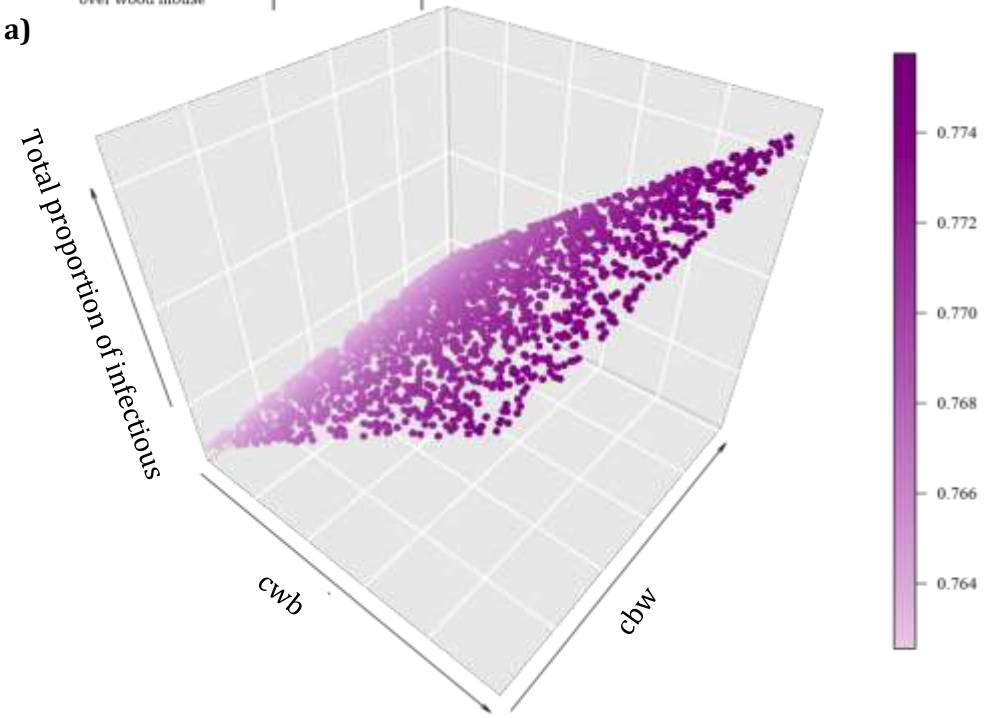


Fig. 5.5. Two-host scenario (wood mouse and bank vole): sensitivity analysis on inter-specific competition. Equilibrium values for proportion a) and total number of infectious b). Latin hypercube sampling 2000 replicates.

Parameter name	Parameter symbol	Value (or range)
Reservoir competence	r bank vole r wood mouse	0.06 0.03
Competition of wood mouse over bank vole	cbw	0-1.01
Competition of bank vole over wood mouse	cwb	0-0.98



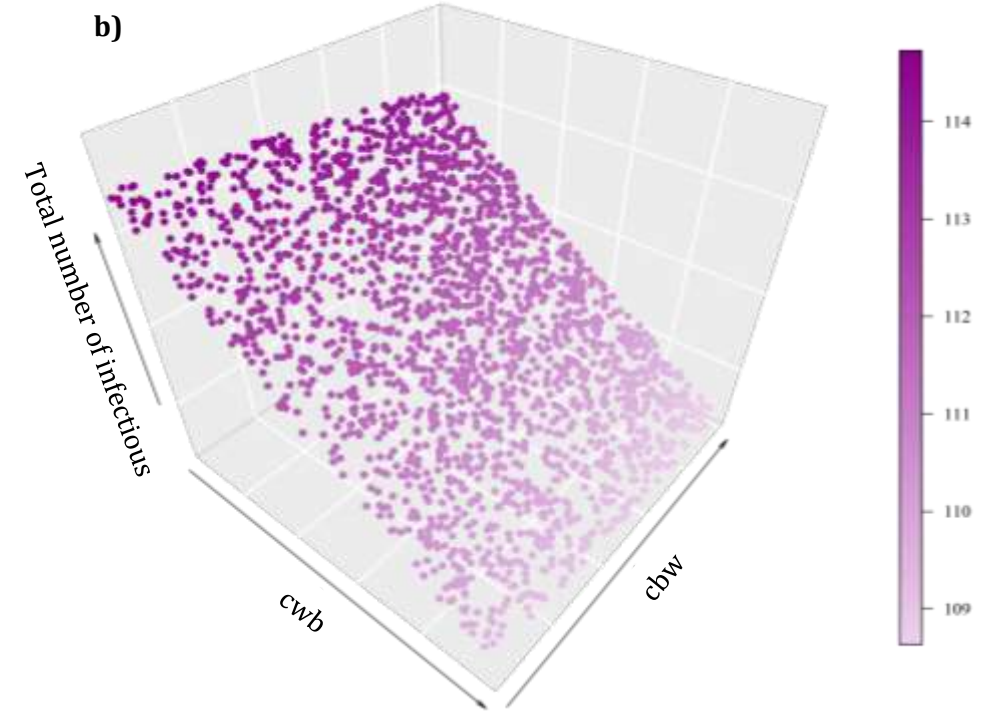


Fig. 5.6. Three species scenario: two hosts (wood mouse and bank vole) and non-host competitor. Sensitivity analysis on non-host inter-specific competition: equilibrium values for proportion a) and total number of infectious b). Latin hypercube sampling 2000 replicates.

Parameter name	Parameter symbol	Value (or range)
Reservoir competence	r bank vole	0.06
	r wood mouse	0.03
Competition of wood mouse over bank vole, and shrew	cbw	0.20
	cjw	0-1.74
Competition of bank vole over wood mouse, and shrew	cwb	0.20
	cjb	0-1.71
Competition of shrew over bank vole, and wood mouse	cbj	0-0.58
	cwj	0-0.57

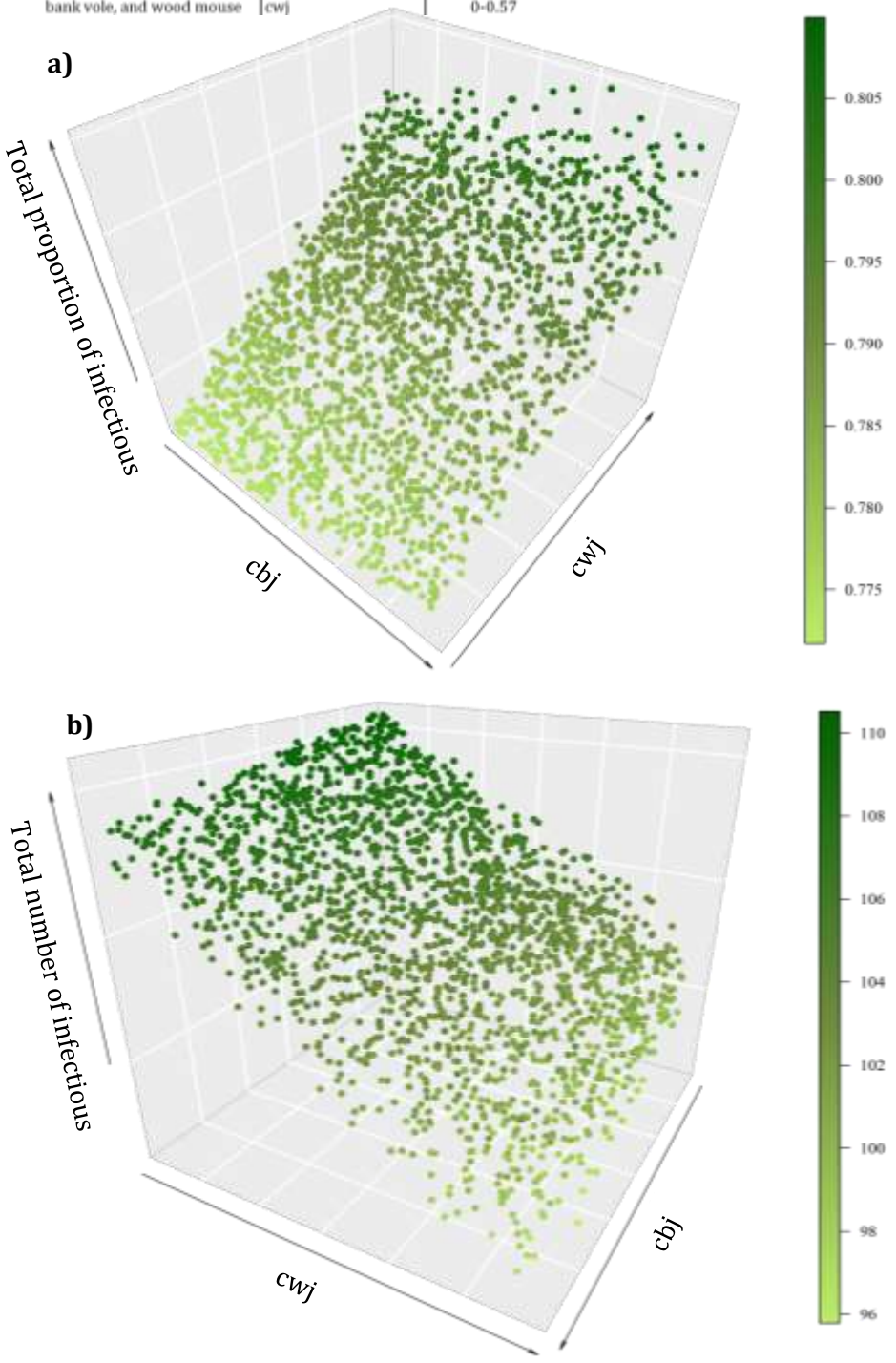
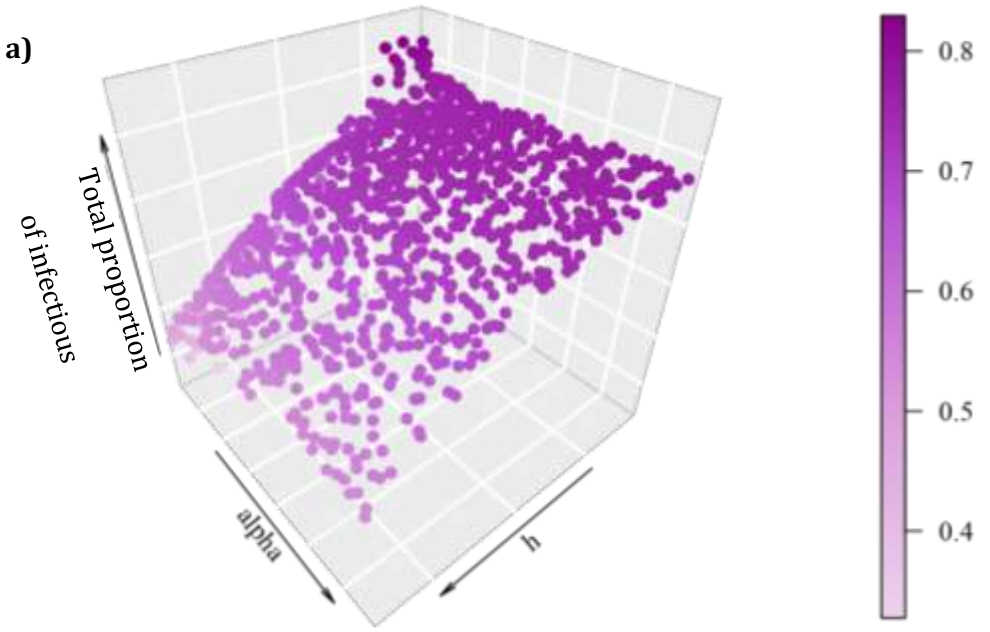


Fig. 5.7. Full community scenario: three hosts (wood mouse, bank vole, and field vole), non-host competitor, generalist and specialist predation. Sensitivity analysis on predation: equilibrium values for proportion a) and total number of infectious b). Latin hypercube sampling 2000 replicates.

Parameter name	Parameter symbol	Value (or range)
Reservoir competence	r bank vole	0.06
	r wood mouse	0.03
	r field vole	0.05
Competition of wood mouse over bank vole, field vole, and shrew	cbw	0.20
	cfw	0.19
	cjw	1.04
Competition of bank vole over wood mouse, field vole, and shrew	cwb	0.20
	cfb	0.18
	cjb	1.03
Competition of field vole over bank vole, wood mouse, and shrew	cbf	0.22
	cwf	0.21
	cjf	1.09
Competition of shrew over bank vole, field vole, and wood mouse	cbj	0.12
	cfj	0.11
	cwj	0.11
Prey density at which generalist predation rate is half of the maximum	h	1-67.5
Maximum rodent consumption rate of specialist predator	α	1-18



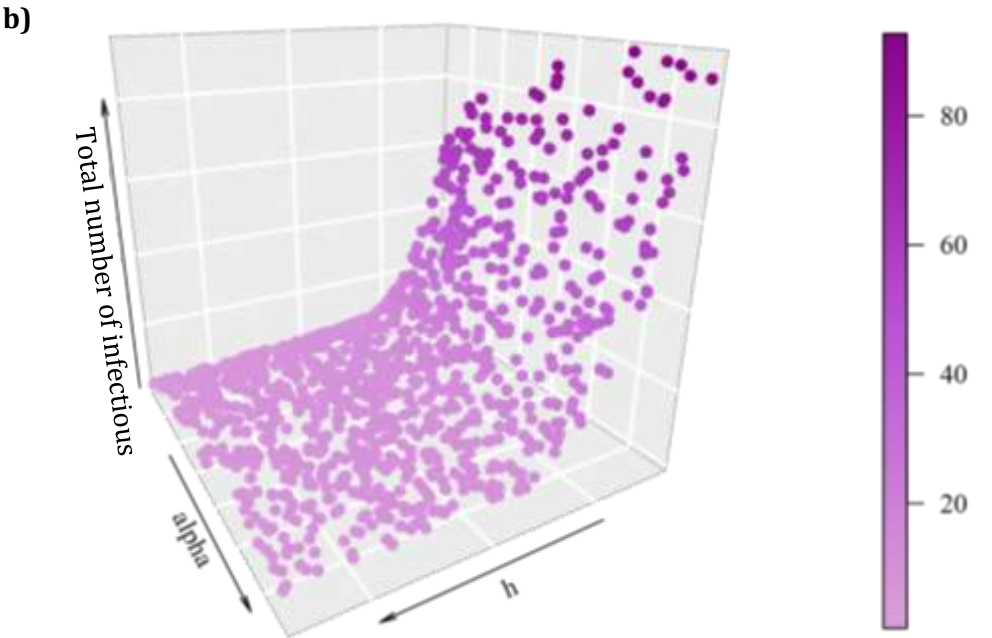


Fig. 5.8. Comparison between different community assemblages. Proportion of infectious a), total number of infectious b), and total rodent population c). Wood mouse only (black dotted line), bank vole only (black dashed line), two-host species with no inter-specific competition (black solid line, parameters as in Fig. 5.2); two-host species with inter-specific competition (purple dot-dashed line, parameters as in Fig. 5.6); two-host species and non-host competitor (purple dotted line, parameters as in Fig. 5.7); full community (purple solid line, h = 9.9, α = 1, other parameters as in Fig. 5.7).

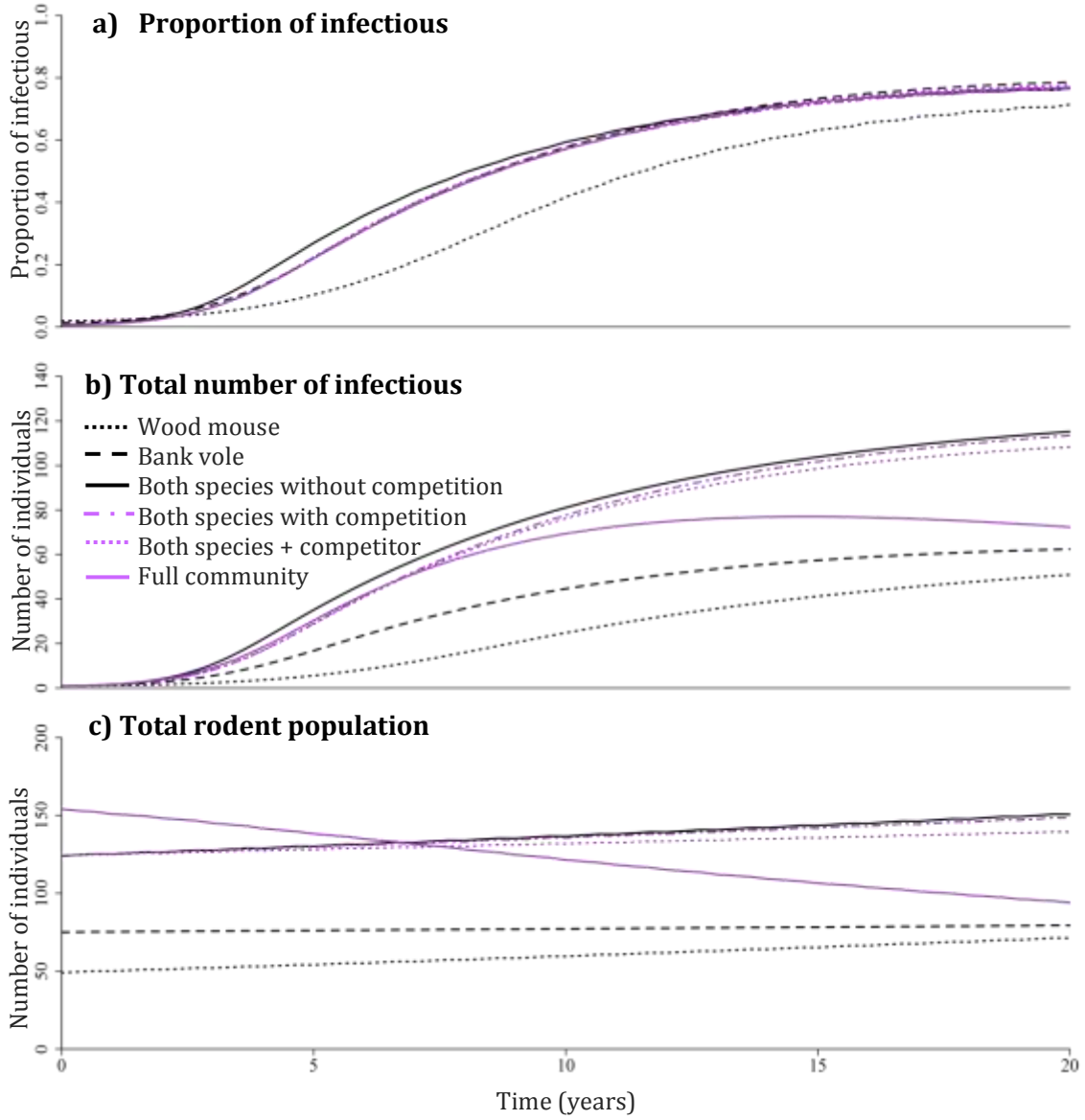


Fig. 5.9. Comparison between different community assemblages. Equilibrium values for proportion of infectious (right) and total number of infectious (left). Parameters value as in Fig. 5.7.

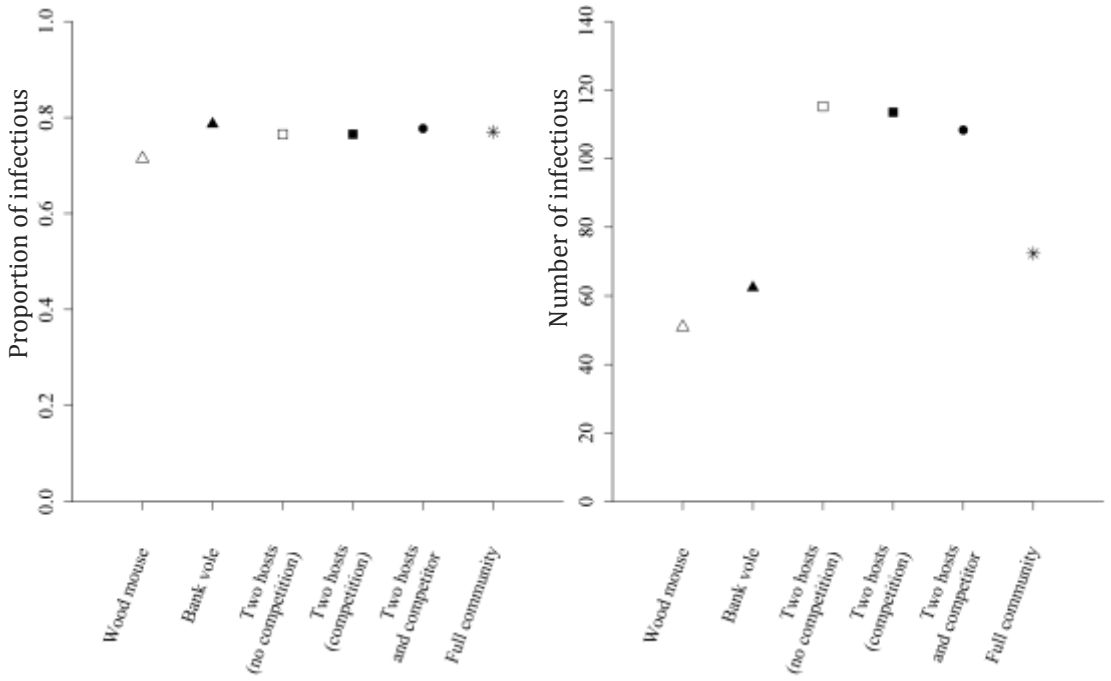


Fig. 5.10. Effect of community composition on pathogen transmission: total number of infectious. Communities: a) wood mouse; b) two-host species; c) two host species and non-host competitor; d) full community. Cowpox virus (light green solid line), *Leptospira* spp. (violet dashed line), Mouse hepatitis virus (Co-V) (dark green solid line), Murine Herpesvirus (MuHV-4) (violet solid line), Murine Norovirus (MNV) (dark violet solid line), *Mycobacterium microti* (light green dashed line), PUUV (black solid line). Pathogen related parameters are reported in Table 5.3, other parameters as in Fig. 5.7.

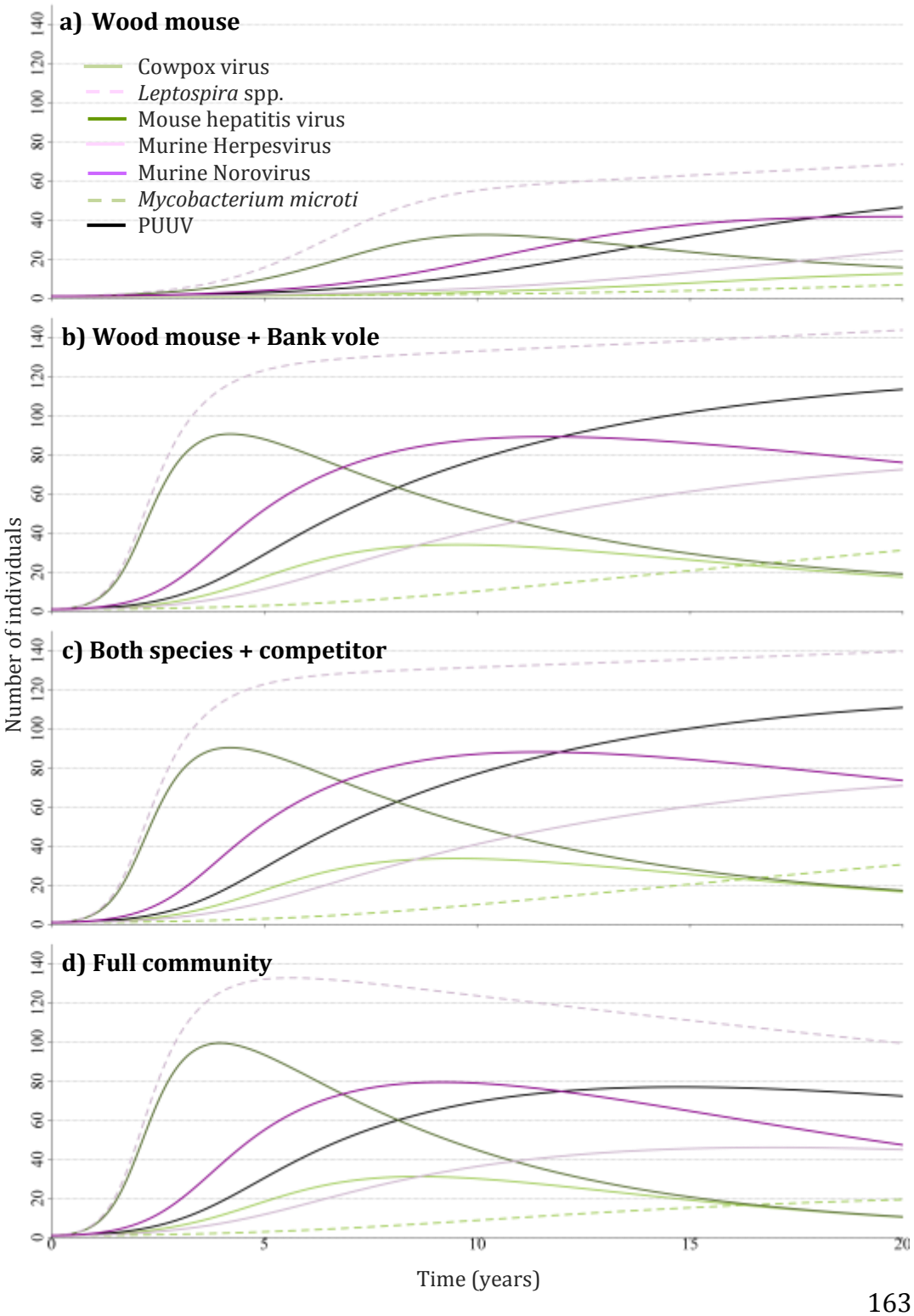
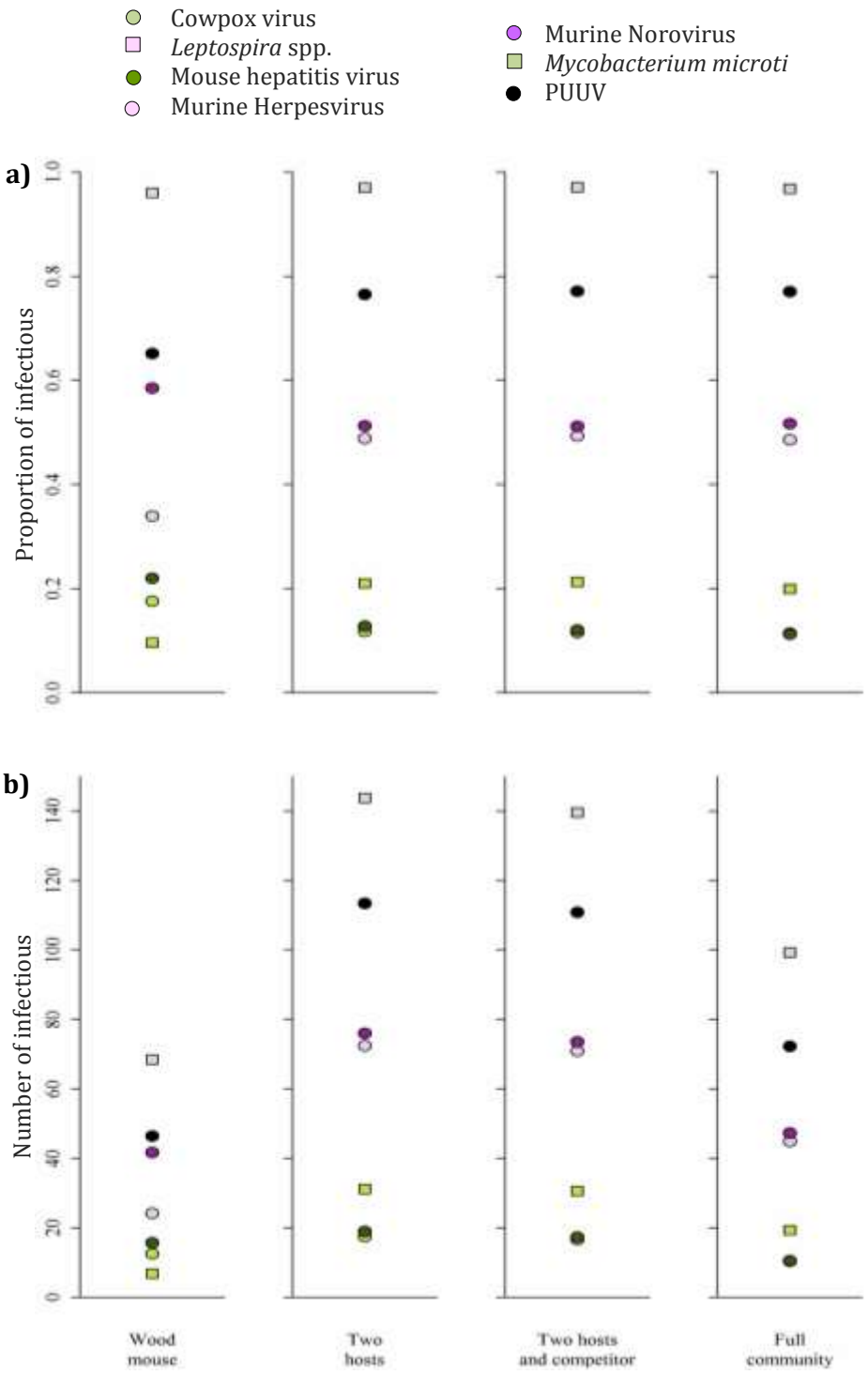


Fig. 5.11. Effect of community composition on pathogen transmission: equilibrium values for proportion of infectious a) and total number of infectious b). Cowpox virus (light green circle), *Leptospira* spp. (violet square), Mouse hepatitis virus (Co-V) (dark green circle), Murine Herpesvirus (MuHV-4) (violet circle), Murine Norovirus (MNV) (dark violet circle), *Mycobacterium microti* (light green square), PUUV (black circle). Communities: wood mouse; two host species; two host species and non-host competitor; full community. Pathogen-related parameters are reported in Table 5.3, other parameters as in Fig. 5.7.



5.2.4 Discussion

Before examining the results, some methodological choices with regards to model structure and parameter estimation will be discussed. Firstly, rodent populations were modelled including intra-annual variations, as supported by field data (Chapter 2), but no cyclic dynamics were included since these have been rarely observed in Britain (Petty, 1999; Sundell *et al.*, 2012; but see Lambin *et al.,* 2000); also, empirical data could not confirm multi-annual cycles in sampled populations. Secondly, more complex methodologies for growth rate estimation could not be employed due to the field data collection design (Chapter 2). Although the growth rates of rodent populations were found to have density and non-density dependent drivers (Aars and Ims, 2002), and density-dependent lag varied in different studies (e.g. Lambin *et al.*, 2000), evidence supports the idea that the non-breeding season density is a determinant in regulating rodents' growth rate (Ergon *et al.*, 2011; Smith *et al.*, 2006). Therefore, the methodology used, which estimated the rate of change between the pre-breeding recruitment population density (representing the non-breeding season) and the post-breeding peak population density, was considered appropriate to describe seasonal fluctuations of rodent populations. Thirdly, in Chapter 2, the estimation of daily individual intra and inter-specific contacts was performed via a bottom-up approach, namely contacts were estimated directly considering individuals' shared space (Buhnerkempe *et al.*, 2015). However, defining an epidemiologically relevant contact remains difficult, and estimating the probability of transmission per contact is problematic (Buhnerkempe *et al.*, 2015). Thus, in order to overcome inference limitations, contact rates employed were not fixed (i.e. absolute value estimated from empirical data), but were included as a function of individual density (see Parameter estimation Section 5.2.1), and the transmission term was deconstructed (McCallum *et al.*, 2017). In fact, the pathogen transmission term was split into contact rates (between susceptible and infectious individuals), and reservoir competence coefficient, in order to distinguish between key

components of transmission and increase model flexibility (McCallum *et al.*, 2017).

The results showed that, in the absence of inter-specific competition, the addition of host species led to an increase in the number of infectious hosts compared to the single-host scenario (Fig. 5.2). In addition, reservoir competence variation between the two hosts was not sufficient to reduce the additive effect of a second host species (Fig. 5.3). The competence sensitivity analysis revealed that, as expected, prevalence (i.e. proportion of infectious) and total infectious rose as the competence values increased, but this rise was slightly different among the two hosts due to demographic differences (i.e. different recruitment rates) (Fig. 5.4). The bank vole population had slightly steeper growth, and a greater carrying capacity than wood mouse, so the transmission was quicker than in wood mouse. Also, reservoir competence affected pathogen transmission only until a threshold value (~0.05 for both species) was reached; beyond that value, maximum transmission occurred and prevalence and number of infectious reached a plateau. For this reason, Fig. 5.4 shows reservoir competence ranging from 0 to 0.1 and not to the maximum algebraic value found. Therefore, if only variability in host reservoir competence is considered, the variation of community composition did not produce an actual dilution of pathogen transmission. In agreement with Roche *et al.* (2012), a simply additive increase in host species richness yielded a greater number of infectious individuals, simultaneously reducing pathogen prevalence (Fig. 5.2a).

The introduction of inter-specific competition negatively affected the susceptible host population growth rate, and altered the relative densities of host and non-host species in the community: the higher the competition, the lower the number of susceptible hosts, and so the lower the number of infectious individuals (Fig. 5.5 and Fig. 5.6). By contrast, prevalence showed the opposite trend, and this reinforces the importance of metric selection in interpreting epidemiological results (Roche *et al.*, 2012). These results supported the hypothesis that, in the context of rodent-borne pathogens, the observations of reduced (or diluted) infection prevalence might not represent a

true dilution effect, but a misinterpretation of empirical data (Dobson and Auld, 2016; Roche *et al.*, 2012). Consequently, both pathogen prevalence and the number of infectious individuals were always considered and compared in this study (also in the following sections). Although competition was not estimated empirically, this type of mutual density-dependent effect between sympatric species has been often observed in the field (Bryce *et al.*, 2001; Huitu *et al.*, 2004; Merritt *et al.*, 2001; Sundell *et al.*, 2012). In agreement with the results, inter-specific competition has been found to be an essential parameter in affecting intensity and trend of pathogen-diversity relationships for directly transmitted pathogens (O'Regan *et al.*, 2015; Strauss *et al.*, 2015).

Similarly, predation affected susceptible host population through top-down regulation (Ostfeld and Holt, 2004), reducing the number of infectious hosts (Fig. 5.7). Predator removal has been found to increase pathogen transmission in preys across different contexts (Holt and Roy, 2007; Packer *et al.*, 2003); conversely, predator species have been reported to decrease transmission, especially when they consume selectively infected preys (Hoverman and Searle, 2016). Although this was not modelled, it is likely that the effect of susceptible host regulation is amplified by the consumption of infected preys, which simultaneously limits the number of infectious, removes parasites from the system, and increases abundance of healthy individuals (assuming no predator-prey transmission) (Hoverman and Searle, 2016; Hudson *et al.*, 1992; Murray *et al.*, 1997). Thus, predators are essential to keep under control rodent abundance, which is correlated with zoonoses emergence (Bordes *et al.*, 2015). In the specific context of Hantavirus transmission, predator species presence has been associated with a decrease of Hantavirus prevalence and zoonotic risk (Dearing and Dizney, 2010; Orrock *et al.*, 2011). Further, generalist and specialist predation had different effects on prevalence and number of infectious (Fig. 5.7). The intensification of specialist predation slightly increased prevalence, while marginally decreasing the number of infectious individuals. By contrast, generalist predation had a much more marked effect on both outputs: the higher the generalist predation, the lower both the proportion and total number of infectious. This was to some extent expected considering how

predation was modelled (functional responses); generalist predation is considered to chronically suppress rodent populations (Ostfeld and Holt, 2004), and an important cause of population cycle dampening (Hanski *et al.*, 1991; Hanski and Henttonen, 1996; Hanski *et al.*, 2001; Korpela *et al.*, 2014). However, Lambin *et al.* (2000) have criticised the approach used to model generalist predation developed by Turchin and Hanski (1997), which was used in this study, since in their study they did not find evidence of field vole population cycles dampened by generalist predators. It is indeed very challenging to estimate predation parameters exactly from empirical data, and it may be that predation terms need to be modelled according to local dynamics (Lambin *et al.*, 2000), but it is undeniable that predators keep rodent populations at lower densities, decreasing pathogen transmission (Ostfeld and Holt, 2004).

Understanding the magnitude of the susceptible host regulation driven by competition and predation is paramount, since host densities and abundance are key parameters in pathogen transmission, with higher abundance and densities correlated to higher prevalence and pathogen diversity (Anderson and May, 1979; Begon *et al.*, 2002; Bordes *et al.*, 2015). Hence, a community perspective, which includes realistic ecological relationships and reliable parameter estimation from empirical data, may give critical insights into wildlife epidemiological patterns and may help to understand and predict their dynamics (Belden and Harris, 2007; Johnson *et al.*, 2015a; Koprivnikar and Johnson, 2016). This was confirmed by the fact that not only the number of species in the community, but also the degree of community complexity (in terms of interaction among species) affected pathogen transmission (Fig. 5.8 and Fig. 5.9). A true dilution effect, i.e. a decrease of the number of infectious hosts, occurred when species were added to the community together with their ecological relationships; no dilution occurred in a simple additive model.

Considering PUUV and other Hantaviruses, dilution has been often detected in field studies (Clay *et al.*, 2009a; Dearing and Dizney, 2010; Ruedas *et al.*, 2004; Tersago *et al.*, 2008). In light of the modelling results it may be that the observed phenomenon was not due, as hypothesised, to the variability of host

species reservoir competence, namely transmission reduction (Keesing *et al.*, 2006). In fact, it seems more likely that dilution, in directly transmitted pathogens, is caused by the mechanism of susceptible host regulation due to the interactions between host and non-host sympatric species (Faust *et al.*, 2017). Moreover, the mechanism of encounter reduction (between most susceptible hosts) due to the presence of non-host (or less susceptible) species was not formally tested in this study, but it seems a possible mechanism of dilution since rodent intra-specific contacts were found to be negatively correlated with shrew density (Khalil *et al.*, 2016). In addition, in this study a positive relationship was found between rodent density and inter-specific contacts (Chapter 2).

The results observed modelling PUUV were consistent when modelling other rodent-borne pathogens (Fig. 5.10 and Fig. 5.11), despite very different courses of epidemics. For example, mouse hepatitis virus (Co-V) displayed an early infectious peak due to the extremely short latency period and the short infectious period (Navas and Weiss, 2003; Siddell *et al.*, 1983; Weiss and Navas, 2005), whereas, *Mycobacterium microti* showed a slow and steady increase of infectious due to the extended latent and infectious periods (Cavanagh *et al.*, 2004; van Soolingen *et al.*, 1998; Wells, 1946). All these pathogens exhibited a decrease of total infectious when competition and predation reduced the number of susceptible individuals in the community, while prevalence was subject to minor alterations (Fig. 5.11). Considering infectious equilibrium values (Fig. 5.11b), the same hump shaped pattern could be identified, for all the pathogens (including PUUV), across the progressive increase of community complexity. In order to check the reliability of these results, pathogen prevalence was compared with relevant published prevalence records (epidemiological studies of wild rodent population in Britain). The model predicted prevalence values in general agreement with existing data, although pathogen prevalence data were not always available. For example, Cowpox virus prevalence was in the range found by Hazel *et al.* (2000), and in line with values predicted by Cavanagh *et al.* (2004) for the relative rodent densities. Murine Herpesvirus (MuHV-4) predicted prevalence was higher compared to

the values described by Telfer *et al.* (2007b), while *M. microti* values were in the range of values reported for British rodent populations (Cavanagh *et al.*, 2002; Wells, 1946). Regarding PUUV, this pathogen is not currently circulating in UK, but prevalence data from Fennoscandia revealed great variation across time and population phases (Razzauti *et al.*, 2009; Razzauti *et al.*, 2013). In the long term, the model predicted a high prevalence value (~80%) for all the community assemblages (Fig. 5.8a), and this result was comparable with the prevalence observed in the long-term longitudinal study by Voutilainen *et al.* (2016). This underlines the importance of long-term longitudinal studies to fully appreciate pathogen epidemiological differences, and gather high quality epidemiological data to compare pathogen transmission, and back up modelling work.

In conclusion, the model was effective in recognising susceptible host regulation via competition and predation as the most important dilution mechanism with regards to directly transmitted pathogens. The modelling results highlighted that estimation of the magnitude of competition and predation was essential to understand the strength of dilution. Additionally, this type of modelling approach might be suitable to identify parameters most affecting specific pathogen transmission in certain community assemblages, and to design efficient eco-epidemiological studies, maximising fieldwork efforts and providing essential information for wildlife disease management. Lastly, wild rodents are a significant reservoir of zoonotic pathogens, so the model might also find applications in the context of public health.

5.3 Tick-borne pathogen model: testing dilution the effect in different host-vector pathogen systems

The aim of the study was to investigate potential dilution effects in two host-tick-pathogen systems, analysing the effects of parameter variation and community composition, in order to highlight the effects on vector numbers and pathogen prevalence. The research focused on identifying key parameters affecting disease transmission in each system, and investigating the role of non-competent host species (not able to maintain pathogen transmission) in determining dilution (or amplification).

The study employed a deterministic multi-host single-vector compartmental model constituted by a set of differential equations (Eq. 1-18). All the results were produced using the function rk4 in the R package deSolve (R Core Team, 2016) which is based on the classical Runge-Kutta 4th order integration. The model was not explicitly spatial; the area, 1 ha, was considered constant since it was constrained by the sampling unit (Begon *et al.*, 2002), so the populations were expressed in individuals/ha and the parameters were scaled accordingly. Dilution was tested by modelling a progressively more complex community: species were added in turn following realistic assembly rules. As in section 5.2, the reservoir community was represented by ground-dwelling small rodents, and the model was parameterised (when possible) with data on such ground-dwelling small rodents collected during live trapping (see Chapter 2). Wood mouse and bank vole were the host species for the pathogen and at the same time to be host for the ticks. *Sorex* ssp. shrews were added to the community as sympatric competitor non-host species, since evidence of their presence were found in the sites sampled (Eq. 3), and they were also hosts for ticks. Finally, predation was added: specialist predators (mustelids; Eq. 4) were considered suitable hosts for ticks in this system, but the generalist predation term (e.g. avian predation) representing only generalist predation intensity did not affect tick population. Community assembly was performed starting with (1) one host

species and then (2) adding a second host species, (3) the competitor species, (4) the predation terms, and, finally, (5) a large grazing ungulate (i.e. sheep or deer [in this context]). The latter was included as a closed, constant population not affecting, or affected by any other population in the system, and unable to transmit the pathogen, except in that it is a suitable host for the vector. Rodent and shrew populations followed a logistic growth tending to species-specific carrying capacity and limited by intra-specific density-dependent reduction and inter-specific competition (Lotka, 1925; Hanski *et al.*, 1993; Volterra, 1926). Inter-specific competition among rodents, and between rodents and shrews was represented by a density dependent competition term (Huitu *et al.*, 2004; O'Regan *et al.*, 2015; Turchin and Hanski, 1997). Rodent species were considered more competitive than shrew species since shrew growth rate has been found to be reduced by rodent populations (Henttonen *et al.*, 1989; Huitu *et al.*, 2004). These species were all predated upon, but rodent species were considered preferential prey (Korpimaki and Norrdahl, 1989; Korpimaki, 1992). Generalist predation was modelled according to the alternative prey hypothesis (Holling type III functional response), while specialist predator population was modelled according to the Holling type II functional response based on Rosenzweig-MacArthur model (1963) with no preference among rodents (Elton, 1942; Erlinge, 1975; Hanski and Henttonen, 1996; Holling, 1959; Krebs and Myers, 1974; Turchin and Hanski 1997). Specialist predation parameters were based on *Mustela nivalis* (least weasel), which was the most common and most widespread mustelid across sampled sites. Tick population equations were based on the work by Norman *et al.* (1999) with the addition of density-dependent reduction in fecundity (Ogden *et al.*, 2007). Tick distribution on hosts was modelled in order to take into account aggregation, i.e. non-homogeneous distribution of the vectors on the host population (Rosà *et al.*, 2003). For parameter estimation details regarding host population dynamics, competition and predation see section 5.2.1 and 5.3.1, while for details about parameters regarding vector population and vector-borne pathogens see section 5.3.1.

The two systems chosen for the analysis were *Ixodes ricinus* – *Borrelia burgdorferi* s.l. and *I. trianguliceps* – *Babesia microti*. These ixodid ticks have a

three-host life cycle (larva, nymph, and adult), for which rodents act as hosts for all or just some life stages, depending on the tick species (Paziewska *et al.*, 2010). *Ixodes ricinus* (sheep tick) is a generalist tick and the key vector for *Borrelia burgdorferi* s.l. (causative agent of Lyme disease in humans) (Norman *et al.*, 2017). Larvae and nymphs feed on small mammals, while adults mostly prefer larger animals (Mysterud *et al.*, 2015). *I. trianguliceps* is a specialist tick for small mammals and all life stages feed on them (Bown *et al.*, 2006; Cotton and Watts, 1967; Mysterud *et al.*, 2015). In the UK, it has been found to be the key vector for *B. microti*, a potentially zoonotic protozoan, of which voles seem to be the main reservoir (Bown *et al.*, 2008; Hussein, 1980; Siński *et al.*, 2006). *I. ricinus* is sympatric with *I. trianguliceps* in many areas, but the role of the first in *Babesia* transmission, and the zoonotic potential of the second are still unclear (Bown *et al.*, 2006; Bown *et al.*, 2011; Kovalevskii *et al.*, 2013).

The time scale of the simulations was 20 years to allow the eco-epidemic dynamics to reach the equilibrium, and one day was the basic time step. The model included susceptible, infectious, and recovered compartments (SIR) for the host; and susceptible and infectious (SI) for the vector (Porco, 1999); non viraemic transmission through tick co-feeding was not considered because it was found to be a very minor, or inefficient route of transmission for the chosen pathogen (Jacquet *et al.*, 2016). Moreover, both pathogens were considered not to be vertically transmitted; consequently larvae could not be infectious, but could be infected and moult into infectious nymphs (Gray, 2006; Randolph, 1995; Wood and Lafferty, 2013). The reservoir competence value summarised susceptibility, ability of the pathogen to magnify and persist in the host/vector, and efficiency of transmission; all the individuals of same host species were equally competent, but different species might display different levels of competence. The initial density for each rodent species was set at the average value found from field data (Chapter 2) (pooled sites excluding Skomer). The inoculum was represented by a single infectious individual.

Equations 1 to 7 represent the model of host-vector dynamics in the absence of pathogen, while equations 8 to 18 represent the SIR-SI model. Non-host

competitor and specialist predator equations (Eq. 3 and 4) are reported only once because the equations are identical with or without pathogen transmission. Model variables and parameters are listed in Table 5.4, while Fig. 5.12 graphically represents the transmission dynamics expressed by the equations 8 to 18.

$$\frac{dN_w}{dt} = r_w N_w \left(1 - \frac{(N_w - c_{wb}N_b - c_{wj}N_j)}{K_w}\right) - \frac{(gN_w^2)}{(N_w^2+h^2)} - (\alpha_w N_p N_w)/\left(\Delta_w + N_w + \frac{\Delta_w}{\Delta_b}N_b + \frac{\Delta_w}{\Delta_j}N_j\right) \quad \text{Eq. 1}$$

$$\frac{dN_b}{dt} = r_b N_b \left(1 - \frac{(N_b - c_{bw}N_w - c_{bj}N_j)}{K_b}\right) - \frac{(gN_b^2)}{(N_b^2+h^2)} - (\alpha_b N_p N_b)/\left(\Delta_b + N_b + \frac{\Delta_b}{\Delta_w}N_w + \frac{\Delta_b}{\Delta_j}N_j\right) \quad \text{Eq. 2}$$

$$\frac{dN_j}{dt} = (v_j - \rho_j)N_j \left(1 - \frac{(N_j - c_{jw}N_w - c_{jb}N_b)}{K_j}\right) - \frac{\left(gN_j^2\right)}{\left(N_j^2+h^2\right)} - (\alpha_j N_p N_j)/\left(\Delta_j + N_j + \frac{\Delta_j}{\Delta_w}N_w + \frac{\Delta_j}{\Delta_b}N_b\right) \text{Eq. 3}$$

$$\frac{dN_p}{dt} = (v_p - \rho_p)N_p \left(1 - qN_p/\left(\Delta_w + N_w + \frac{\Delta_w}{\Delta_b}N_b + \frac{\Delta_w}{\Delta_j}N_j\right)\right) \quad \text{Eq. 4}$$

$$\frac{dl}{dt} = \left(\beta_7 d_1 N_w a + \beta_7 d_1 N_b a + \beta_8 d_2 N_j a + \beta_9 d_3 N_p a + \beta_9 d_4 N_d a\right)\left(num_{egg} - s_v N_v\right) - \rho_v l$$
$$-\left(\beta_1 N_w l + \beta_1 N_b l + \beta_2 N_j l + \beta_3 N_p l + \beta_3 N_d l\right)(1 + 1/k) \quad \text{Eq. 5}$$

$$\frac{dn}{dt} = \left(\beta_1 d_1 N_w l + \beta_1 d_1 N_b l + \beta_2 d_2 N_j l + \beta_3 d_3 N_p l + \beta_3 d_4 N_d l\right) - \rho_v n$$
$$-\left(\beta_4 N_w n + \beta_4 N_b n + \beta_5 N_j n + \beta_6 N_p n + \beta_6 N_d n\right)(1 + 1/k) \quad \text{Eq. 6}$$

$$\frac{da}{dt} = \left(\beta_4 d_1 N_w n + \beta_4 d_1 N_b n + \beta_5 d_2 N_j n + \beta_6 d_3 N_p n + \beta_6 d_4 N_d n\right) - \rho_v a$$
$$-\left(\beta_7 N_w a + \beta_7 N_b a + \beta_8 N_j a + \beta_9 N_p a + \beta_9 N_d a\right)(1 + 1/k) \quad \text{Eq. 7}$$

$$\frac{dS_w}{dt} = r_w N_w \left(1 - \frac{\left(N_w - c_{wb}N_b - c_{wj}N_j\right)}{K_w}\right) - \beta_4 \tau_v S_w I_n - \beta_7 \tau_v S_w I_a - \frac{(gS_w^2)}{(S_w^2 + h^2)}$$

$$-(\alpha_w N_p S_w)/\left(\Delta_w + N_w + \frac{\Delta_w}{\Delta_b}N_b + \frac{\Delta_w}{\Delta_j}N_j\right) \quad \text{Eq. 8}$$

$$\frac{dI_w}{dt} = \beta_4\tau_v S_w I_n - \beta_7\tau_v S_w I_a - \sigma_w I_w - \frac{(gI_w^2)}{(I_w^2+h^2)}$$

$$-(\alpha_w N_p I_w)/\left(\Delta_w + N_w + \frac{\Delta_w}{\Delta_b}N_b + \frac{\Delta_w}{\Delta_j}N_j\right) \quad \text{Eq. 9}$$

$$\frac{dR_w}{dt} = \sigma_w I_w - \frac{(gR_w^2)}{(R_w^2+h^2)} - (\alpha_w N_p R_w)/\left(\Delta_w + N_w + \frac{\Delta_w}{\Delta_b}N_b + \frac{\Delta_w}{\Delta_j}N_j\right) \quad \text{Eq. 10}$$

$$\frac{dS_b}{dt} = r_b N_b\left(1 - \frac{(N_b - c_{bw}N_w - c_{bj}N_j)}{K_b}\right) - \beta_4\tau_v S_b I_n - \beta_7\tau_v S_b I_a - \frac{(gS_b^2)}{(S_b^2+h^2)}$$

$$-(\alpha_b N_p S_b)/\left(\Delta_b + N_b + \frac{\Delta_b}{\Delta_w}N_w + \frac{\Delta_b}{\Delta_j}N_j\right) \quad \text{Eq. 11}$$

$$\frac{dI_b}{dt} = \beta_4\tau_v S_b I_n - \beta_7\tau_v S_b I_a - \sigma_b I_b - \frac{(gI_b^2)}{(I_b^2+h^2)} - (\alpha_b N_p I_b)/\left(\Delta_b + N_b + \frac{\Delta_b}{\Delta_w}N_w + \frac{\Delta_b}{\Delta_j}N_j\right) \quad \text{Eq. 12}$$

$$\frac{dR_b}{dt} = \sigma_b I_b - \frac{(gR_b^2)}{(R_b^2+h^2)} - (\alpha_b N_p R_b)/\left(\Delta_b + N_b + \frac{\Delta_b}{\Delta_w}N_w + \frac{\Delta_b}{\Delta_j}N_j\right) \quad \text{Eq. 13}$$

$$\frac{dl}{dt} = (\beta_7 d_1 N_w + \beta_7 d_1 N_b + \beta_8 d_2 N_j + \beta_9 d_3 N_p + \beta_9 d_4 N_d)(S_a + I_a)(num_{egg} - s_v N_v) - \rho_v l -$$

$$(\beta_1 N_w l + \beta_1 N_b l + \beta_2 N_j l + \beta_3 N_p l + \beta_3 N_d l)(1 + 1/k) \quad \text{Eq. 14}$$

$$\frac{dI_n}{dt} = (\beta_1 d_1 I_w \tau_w l + \beta_1 d_1 I_b \tau_b l)(1 + 1/k) - \rho_v I_n - (\beta_4 N_w I_n + \beta_4 N_b I_n + \beta_5 N_j I_n + \beta_6 N_p I_n +$$

$$\beta 6 N d I n 1 + 1/k \quad \text{Eq. 15}$$

$$\frac{dS_n}{dt} = (\beta_1 d_1 (S_w + R_w) l + \beta_1 d_1 (S_b + R_b) l + \beta_2 d_2 N_j l + \beta_3 d_3 N_p l + \beta_3 d_4 N_d l) - \rho_v S_n$$

$$-(\beta_4 N_w S_n + \beta_4 N_b S_n + \beta_5 N_j S_n + \beta_6 N_p S_n + \beta_6 N_d S_n) \quad \text{Eq. 16}$$

$$\frac{dI_a}{dt} = \left(\beta_4 d_1 N_w I_n + \beta_4 d_1 N_b I_n + \beta_5 d_2 N_j I_n + \beta_6 d_3 N_p I_n + \beta_6 d_4 N_d I_n\right) + (\beta_4 d_1 I_w \tau_w S_n + \beta_4 d_1 I_b \tau_b S_n)(1 + 1/k)$$

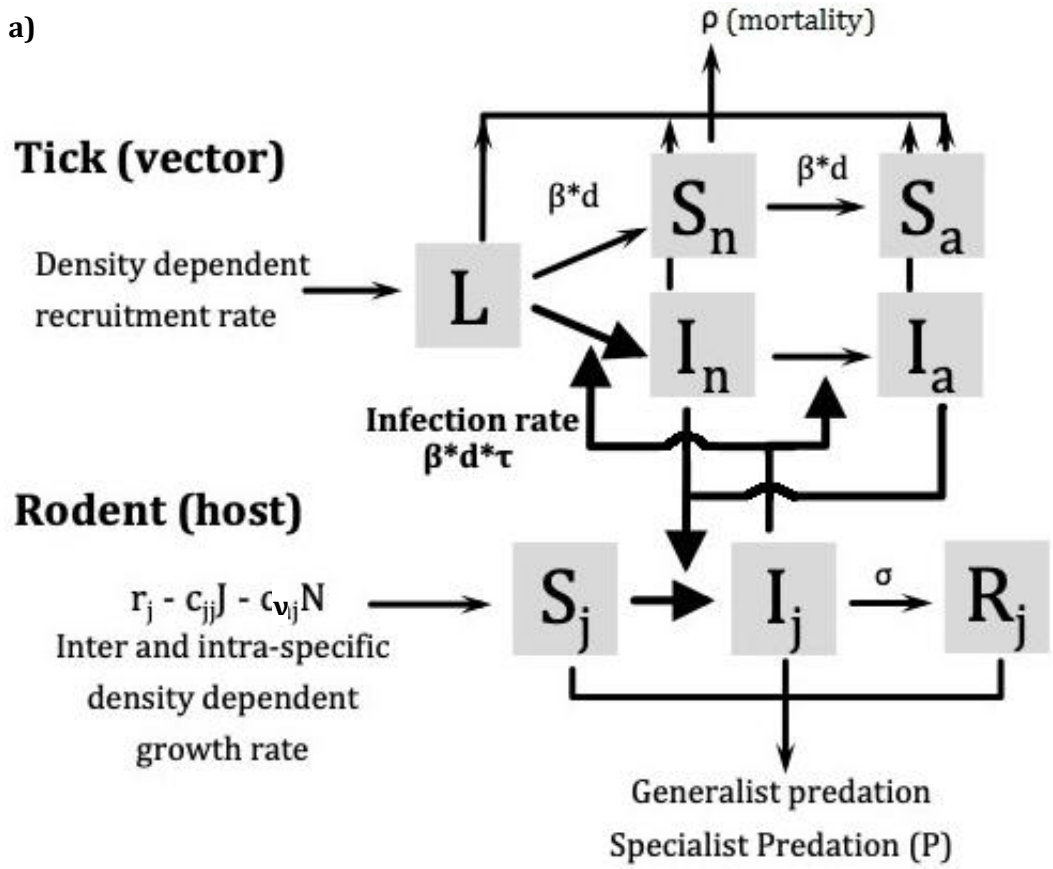
$$-\rho_v I_a - \left(\beta_7 N_w I_a + \beta_7 N_b I_a + \beta_8 N_j I_a + \beta_9 N_p I_a + \beta_9 N_d I_a\right)(1 + 1/k) \quad \text{Eq. 17}$$

$$\frac{dS_a}{dt} = \left(\beta_4 d_1 (S_w + R_w) S_n + \beta_4 d_1 (S_b + R_b) S_n + \beta_5 d_2 N_j S_n + \beta_6 d_3 N_p S_n + \beta_6 d_4 N_d S_n\right) - \rho_{v4} S_a -$$

$$\left(\beta_7 N_w S_a + \beta_7 N_b S_a + \beta_8 N_j S_a + \beta_9 N_p S_a + \beta_9 N_d S_a\right) \quad \text{Eq. 18}$$

Fig. 5.12. Tick-borne disease compartmental model a) and tick life cycle b). Boxes represent epidemiological compartments in which each population is subdivided: L = tick larval stage, S = susceptible, I = infectious, R = recovered. J (host population density) = S+I+R. Subscripts: n = tick nymphal stage, a = tick adult stage; j = rodent species (host), ν = non-host competitor (shrew species). r = growth rate (determined by competition and density of rodent and shrew species), c = competition coefficient, N = non-host competitor population density, P = specialist predator population density, β = host-vector encounter rate, d = tick moulting success, ρ = tick death rate, σ = recovery rate, τ = reservoir competence. Arrows indicate the direction of movement of individuals between classes. Arrows pointing outside the boxes represent mortality. Vectors can feed on J, N, and P.

a)



b)

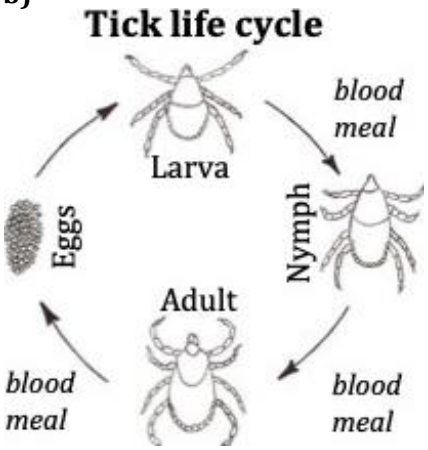


Table 5.4. List of model variables and parameters. For details on parameter estimation see section 5.2.1 and section 5.3.1.

Symbol	Description (source where relevant)	
S	Number of susceptible individuals	
I	Number of infectious individuals	
R	Number of recovered individuals	
N	Total number of individuals (population size)	
w, b	Host-species for the pathogen: wood mouse (w); bank vole (b)	
j	Non-host (for the pathogen) competitor species	
p	Specialist predator	
v	Vector (ixodid tick)	
d	Grazing species (ungulate)	
l	Number of tick larvae	
n	Number of tick nymphs	
a	Number of tick adults	
S_n	Number of susceptible nymphs	
S_a	Number of susceptible adults	
I_n	Number of infectious nymphs	
I_a	Number of infectious adults	
r	Rodent growth rate	(this study)
c	Competition factor	(O'Regan *et al.*, 2015; this study)
K	Carrying capacity	(Bolzoni *et al.*, 2008; De Leo and Dobson, 1996)
τ	Reservoir competence of transmission	(Giardina *et al.*, 2000; LoGiudice *et al.*, 2003; Harrison *et al.*, 2011; Hartemink *et al.*, 2008)
β	Encounter rate vector-host	(Dobson *et al.*, 2011; Hancock *et al.*, 2011)
d	Moulting/feeding success	(LoGiudice *et al.*, 2003)
num_{egg}	Maximum number of per capita adult female tick eggs production	(*Ixodes ricinus*: Norman *et al.*, 1999; *I. trianguliceps*: Krasnov *et al.*, 2007)
s_v	Density-dependent reduction of tick growth rate	(Ogden *et al.*, 2007)
σ	Recovery rate	(Harrison *et al.*, 2011; Hartemink *et al.*, 2008; Randolph, 1995; Randolph *et al.*, 1996)
ν	Birth rate	(Bolzoni *et al.*, 2008; De Leo and Dobson, 1996)

Table 5.4 (continued). List of model variables and parameters. For details on parameter estimation see section 5.2.1 and section 5.3.1.

Symbol	Description (source where relevant)	
$\boldsymbol{\rho}$	Death rate	(Bolzoni *et al.*, 2008; De Leo and Dobson, 1996)
$\boldsymbol{\rho_v}$	Tick death rate	(Dobson *et al.*, 2011)
$\boldsymbol{g}$	Saturation rate of generalist predation	(Turchin and Hanski, 1997)
$\boldsymbol{h}$	Prey density at which generalist predation rate is half of the maximum	(Turchin and Hanski, 1997)
$\boldsymbol{\alpha}$	Maximum consumption rate of specialist predator	(Turchin and Hanski, 1997)
$\boldsymbol{\Delta}$	Half-saturation constant (specialist predator)	(Turchin and Hanski, 1997)
$\boldsymbol{q}$	Specialist predator-prey ratio constant	(Turchin and Hanski, 1997)
$\boldsymbol{k}$	Tick aggregation parameter	(this study; Rosà *et al.*, 2003)

5.3.1 Parameter estimation

All the parameters relating to host species, inter-specific competition and predation were estimated as in section 5.2. Wood mouse and bank vole growth rates (r) were calculated from field data according to Lambin *et al.* (2000) (section 2.2.3.3), but using log_e instead of log_{10} for the purpose of the inclusion in the model. The values obtained for breeding and non-breeding season were scaled by day, as were all other parameters in the model, and seasonality was included in the model considering breeding season from March to October, and non-breeding season November to February (Hörnfeldt, 1994; Stenseth *et al.*, 2002) (Table 5.5). Carrying capacity for all the species, birth rate, and death rate of shrew and weasel were estimated allometrically with the following formulae: $K = 16.2w^{-0.70}$, $\nu = w^{-0.25}$, $\rho = 0.4w^{-0.25}$ (where w is the mean body mass in g) (Bolzoni *et al.*, 2008; De Leo and Dobson, 1996). The body mass values used were: average adults and sub-adults (this study) for rodent species (Skomer voles were excluded due to their larger size), while adult average values from PanTHERIA database (Jones *et al.*, 2009) were used for common shrew and least

weasel. The competition factor (c) values were computed algebraically, ranging from 0 (competition absent) to the maximum value for the competing species to co-exist (for example, in the case of competition of species *i* over species *j*, the maximum value is K_j/K_i), but here the values were fixed as illustrated in Table 5.5. The parameters relative to predation were estimated with the formulae proposed by Turchin and Hanski (1997), but using empirical data from this study or data relative to UK, as in section 5.2.1. In this case no sensitivity was performed for predation intensity and the parameter values used are reported in Table 5.5. Maximum number of per capita adult female tick egg production in *I. ricinus* and *I. trianguliceps* was set as suggested by Norman *et al.* (1999) and Krasnov *et al.* (2007) respectively (Table 5.4 and Table 5.5). Tick death rate, specific for each life stage, was estimated from Dobson *et al.* (2011) empirical study (Table 5.4 and Table 5.5). According to Ogden *et al.* (2007) the tick approach to equilibrium was modelled with a density-dependent reduction in fecundity: $s_v = 0.5 + (0.049 \log(1.01 + \frac{a}{2}/N))$, where *a* was the number of adult ticks, and *N* the total density of tick hosts. In addition, tick distribution on hosts was modelled in order to represent aggregation, i.e. non-homogeneous distribution of the vectors on the host population (Rosà *et al.*, 2003). The aggregation parameter, *k*, was calculated from empirical data as the dispersion parameter of the negative binomial distribution fitting the data collected in the field about the distribution of ticks on rodent hosts (all rodent data pooled together: k = 0.18, μ = 0.23). Values for reservoir competence of hosts and vector, host-vector encounter rates, and moulting/feeding success were taken from relevant literature and sensitivity analysis was carried out as described in section 5.3.2 (Table 5.4 and Table 5.5). Recovery rate (σ) values used in the model were pathogen specific and given by 1/infectious period (see Table 5.4 and Table 5.5 for sources and numeric values).

Table 5.5. List of starting conditions and parameter values used for the simulations. When a range of values is provided, sensitivity analysis has been performed (see section 5.3.2).

Symbol	Description	Value
N_w	Wood mouse population	49 (ind/ha)
N_b	Bank vole population	75 (ind/ha)
N_j	Shrew population	20 (ind/ha)
N_p	Weasel population	3 (ind/ha)
N_d	Ungulate population (constant)	5 (ind/ha)
N_v	Tick population	100 (ind/ha)
cbw, cjw	Competition of wood mouse over bank vole, and shrew respectively	0.20 1.04
cwb, cjb	Competition of bank vole over wood mouse, and shrew respectively	0.20 1.03
cwj, cbj	Competition of shrew over wood mouse, and bank vole respectively	0.11 0.12
d_r d_s d_l	Moulting/feeding success on rodents, shrews, and large hosts respectively (range)	0 – 0.593 0 – 0.496 0 – 0.639
g	Saturation rate of generalist predation	0.49
h	Prey density at which generalist predation rate is half of the maximum	9.9
k	Tick aggregation parameter	0.18
num_{egg}	Maximum number of per capita adult female tick eggs production: *Ixodes ricinus, I. trianguliceps*	1500 1000
q	Specialist predator-prey ratio constant	56
r_{b+}, r_{b-}	Bank vole growth rate breeding season (+), and non-breeding season (-)	0.007; -0.002
r_{w+}, r_{w-}	Wood mouse growth rate breeding season (+); non-breeding season (-)	0.04; -0.006
α	Maximum rodent consumption rate of specialist predator	1
α_s	Maximum shrew consumption rate of specialist predator	7.67
β_{sl}, β_{ll}	Encounter rate small host, large host-larva (range)	0.0043 – 0.073
β_{sn}, β_{ln}	Encounter rate small host, large host-nymph (range)	0.04 – 0.0975
β_{sa}, β_{la}	Encounter rate small host, large host-adult (range)	0.043 – 0.105
Δ	Half-saturation constant (rodent)	11.31
Δ_s	Half-saturation constant (shrew)	22.62
ρ_v	Tick death rate: larvae, nymphs, adults	0.0014 0.0005 0.0004
σ_{bb}	Recovery rate *Borrelia burgdorferi* s.l.	0.0083
σ_{bm}	Recovery rate *Babesia microti* s.l.	0.4
τ	Reservoir competence (range)	0 – 0.90

5.3.2 Sensitivity analyses

Sensitivity analysis was performed on parameters that were hypothesised to affect model outputs but could not be directly estimated, namely reservoir competence, host-vector encounter rate, moulting/feeding success. These parameters were chosen because they were considered among the most important affecting transmission in similar tick-borne pathogen systems (Dunn *et al.*, 2013; Roche *et al.*, 2013). The range of values chosen for the sensitivity analyses were selected from empirical papers that investigated the same or similar host-vector-pathogen systems; when available, data from the same host and vector species were preferred (Table 5.4 and Table 5.5). Firstly, sensitivity analysis was carried out on encounter rates via systematic sampling; 10 intervals were input into the sensitivity between the minimum and the maximum value found in literature. In this way the impact of different encounter rates (host-larva, host-nymph, or host-adult) on pathogen transmission across different community assemblages was examined. Next, sensitivity analysis through Latin hypercube sampling (Iman *et al.*, 1981a,b) was executed for the moulting/feeding success parameter assembling a progressively more complex community in order to identify the relative importance of different hosts in sustaining tick population and pathogen transmission. Finally, reservoir competence was split into five parameters for each rodent species: host to larva, host to nymph, host to adult, nymph to host, and adult to host. Sensitivity analysis was performed through Latin hypercube sampling (Iman *et al.*, 1981a,b) on each of those to investigate the effect of variation of every competence parameter on pathogen transmission among rodents and ticks. For each of these parameters (moulting success and reservoir competence) values were randomly chosen between the minimum and the maximum. Sensitivity analyses were performed separately for the two systems under consideration, keeping constant pathogen-specific parameters such as recovery rate, and tick-specific parameters such as the maximum number of per capita adult female tick eggs production.

5.3.3 Results

Fig. 5.13 shows the two vector populations across different host species assemblages in the absence of pathogen transmission. *Ixodes ricinus*, compared to *I. trianguliceps*, was more prolific and displayed an overall higher number of individuals, but due to adult lower preference of feeding on small sized hosts, the proportion of nymphs and adults was lower, especially in the assemblages without larger hosts (Fig. 5.13a, b, c). Thus, assembling the community, *I. ricinus* population increased, especially the proportion of adults. Conversely, *I. trianguliceps* did only feed on small hosts (rodents and shrews) therefore there was no substantial change in tick numbers across different communities. However, a slight increase in individuals is noticeable when the shrew population was added, due to the overall host increase (Fig. 5.13g), and also in the full community because predation, lessening small host populations, determined a relaxation of the host-density dependent fecundity reduction.

Considering the system *I. ricinus*-*Borrelia burgdorferi*, sensitivity analysis on host-vector encounter rates revealed that the parameter most affecting the outputs of interest, proportion and number of infectious nymphs and hosts, was the encounter rate between small host and larva (Fig. 5.14 and Fig. 5.15). This meant that for each value of this parameter the alteration of other parameters representing encounter rates did not determine much variation in the outputs. In the single-host and two host scenario (Fig. 5.14a,b and Fig. 5.15a,b), increasing the small host-larva encounters caused a decrease of proportion and number of infectious hosts and a decrease of number of infectious nymphs, while an increase in nymph prevalence (proportion of infectious individuals). This pattern was produced because tick fecundity is regulated by tick density and host density. The addition of the shrew to the community (Fig. 5.14c and Fig. 5.15c) increased the total number of hosts available for the ticks, and, consequently, the encounter rate value was irrelevant in determining host and nymphal prevalence, and number of infectious hosts. The minimum value of the parameter was enough to saturate transmission. However, compared to the less

complex communities, host prevalence was higher and nymph prevalence was lower, due to the ticks feeding also on non-competent hosts for the pathogen. In the full community (Fig. 5.14d and Fig. 5.15d), the presence of many alternative tick hosts determined an increase in nymph prevalence, without an increase of the number of infectious nymphs, compared with the previous scenario. Similarly, the presence of predators affected prevalence and number of infectious hosts decreasing actual number of infectious hosts and increasing host prevalence at the same time.

The second host-vector-pathogen system presented substantially different results (Fig. 5.16 and Fig. 5.17), especially with regards to the values of infectious hosts, which were considerably lower than the previous case, due to the shorter infectious period of *Babesia microti*. The outputs were not remarkably affected by different community assemblages, but, in general, the progressive addition of competitor and predator species progressively reduced the number of infectious hosts (due to the susceptible host regulation). However, the pattern of the results along the range was different because of the lower fecundity of *I. trianguliceps* and the lower amount of hosts suitable for this tick species (only rodent species and shrew). The increase of encounter rates determined a subsequent increase in infectious nymphs (with no change in prevalence as the total nymphal population increased as well), while prevalence and total number of infectious hosts reached a peak, then a decline followed by a slow rise (most likely caused by the interaction between host and tick population size and patterns of ticks aggregation).

Sensitivity analysis on moulting success parameters demonstrated that, in both systems, rodents and shrews were the hosts more affecting pathogen transmission (Fig. 5.18 to Fig. 5.21). Taking into account the *I. ricinus*-*B. burgdorferi* system, in the community constituted only by rodents and shrew, the number of infectious nymphs increased with the increase of the moulting success on rodents (the opposite trend could be observed for prevalence because of the rise of total tick population), while the moulting success on shrews did not affect the results (Fig. 5.18a, c). Likewise, higher moulting success on rodents determined a rise of infectious hosts (but, unlike nymphal

prevalence, host prevalence increased too) (Fig. 5.19a, c). Moulting success on shrews mostly affected proportion and number of infectious nymphs in the full community because of the higher predation on rodents than shrews (Fig. 5.18b, d). The rise in shrew relative density compared to the former scenario boosted their role in supporting tick population. By contrast, in terms of patterns of results, no difference could be noticed with regards to proportion and number of infectious hosts between the two communities; moulting success on rodents was the most important parameter affecting the outputs (Fig. 5.19b, d). However, in the full community, an overall higher number of infectious nymphs and a lower number of infectious hosts could be observed compared to the less complex assemblage. A threshold value could be observed above which the maximum transmission occurred and the proportion and number of infectious hosts reached a plateau, likely due to the inclusion in the model of tick aggregation on hosts (Fig. 5.19).

Considering sensitivity analysis on the same parameters, the *I. trianguliceps*-*B. microti* results did not significantly differ from the previous case in terms of trends and patterns (Fig 5.20 and Fig. 5.21). Moulting success on rodents was the main parameter affecting numbers of infectious nymphs and hosts, but in the full community, the relative rise of shrew population determined the same phenomenon as described earlier (Fig. 5.20d). Comparing the two host assemblages, there was no change in number of infectious nymphs, while a decrease in the number of infectious hosts could be observed in the more complex assemblage (Fig. 5.21b, d). However, there were two important differences between the systems. Firstly, the higher amount of wasted bites (in terms of pathogen transmission) of *I. ricinus* and the higher encounter rate of *I. trianguliceps* with small sized hosts (including rodents, competent in transmitting the pathogen) yielded an overall lower amount of nymphs were infected by *B. burgdorferi*. Secondly, the higher fecundity of *I. ricinus* and the higher number of alternative hosts (in the full community) determined that a lower value of moulting success on rodents was sufficient to reach the maximum number of infectious hosts in this system.

Results regarding the sensitivity analysis on competence of transmission were difficult to interpret due to the relative contribution to transmission of all the five different parameters (rodent to larva, nymph, adult, nymph or adult to rodent). In general, as expected, the proportion and the number of infectious nymphs and hosts increased together with the increase in competence of transmission. However, Fig. 5.22 to Fig. 5.25 display the pair of parameters for which the model was more sensitive in each case. In both systems, the proportion and number of infectious nymphs were influenced by multiple parameters, and the graphical visualisation of the results did not reveal any specific pattern, with the exception that it seemed that an increase in nymphal competence of transmission to rodents decreased the number of infectious of *I. ricinus* nymphs, while the opposite was observed for *I. trianguliceps* (in this case the same occurred for nymph prevalence) (Fig. 5.22 and Fig. 5.24). In the second system a small value of competence was enough to reach the maximum level of transmission, as the vast majority of the available hosts were competent in transmitting the pathogen. In this system, increasing community complexity, led to a marked rise of infectious nymphs due to the addition of shrews, which increased host availability and boosted tick population (Fig. 5.24f). Conversely, the higher number of alternative hosts for *I. ricinus* produced an overall lower number of infectious nymphs in the full community (Fig. 5.22f). With regards to the effect of competence on hosts, the competence of transmitting the pathogen from nymph to rodent was the most important parameter in the *I. ricinus*-*B. burgdorferi* system, with a low value sufficient to reach maximum transmission (Fig. 5.23). Also, in assembling the community, we observed an increase of host prevalence, but a lower number of infectious hosts because of the overall reduction of rodent populations due to competition and predation (Fig. 5.23c, f). In the alternative system, the effect of competence was less clear because a low level of competence for any of the parameters was enough to sustain transmission. However, the parameters the increase of which produced a more marked amplification of proportion and number of infectious hosts were primarily competence of transmission from rodent to larva (the effect of bank vole or wood mouse was similar, although for the plotting purpose only the

parameter relative to bank vole was shown), and nymph to rodent. Finally, also in this case, competition and predation reduced the number of infectious hosts compared to the communities consisting of only rodent species because reduced the availability of competent hosts (Fig. 5.25d, e, f).

Fig. 5.13. Host-vector dynamics: comparison between *Ixodes ricinus* (a, b, c, d) and *I. trianguliceps* (e, f, g, h) systems. Log transformed number of individual larvae (dashed line), nymphs (solid line), and adults (dotted line) across different community assemblages (values were log transformed). Bank vole (a, e); wood mouse and bank vole (b, f); two rodent species and shrew (c, g); full community (two rodent species, shrew, generalist and specialist predation, ungulate) (d, h).

Parameter name	Parameter symbol	Value (or range)
Fig. 5.13 a), b), c)		
Contact rate rodent/larva	β_{sl}	0.025
Contact rate rodent/nymph	β_{sn}	0.040
Contact rate rodent/adult	β_{sa}	0.010
Fig. 5.13 d)		
Contact rate rodent/larva	β_{sl}	0.025
Contact rate rodent/nymph	β_{sn}	0.040
Contact rate rodent/adult	β_{sa}	0.010
Contact rate large animal/larva	β_{ll}	0.025
Contact rate large animal/nymph	β_{ln}	0.040
Contact rate large animal/adult	β_{la}	0.060

Parameter name	Parameter symbol	Value (or range)
Fig. 5.13 e), f), g)		
Contact rate rodent/larva	β_{sl}	0.040
Contact rate rodent/nymph	β_{sn}	0.040
Contact rate rodent/adult	β_{sa}	0.040
Fig. 5.13 h)		
Contact rate rodent/larva	β_{sl}	0.040
Contact rate rodent/nymph	β_{sn}	0.040
Contact rate rodent/adult	β_{sa}	0.040
Contact rate large animal/larva	β_{ll}	0.000
Contact rate large animal/nymph	β_{ln}	0.000
Contact rate large animal/adult	β_{la}	0.000

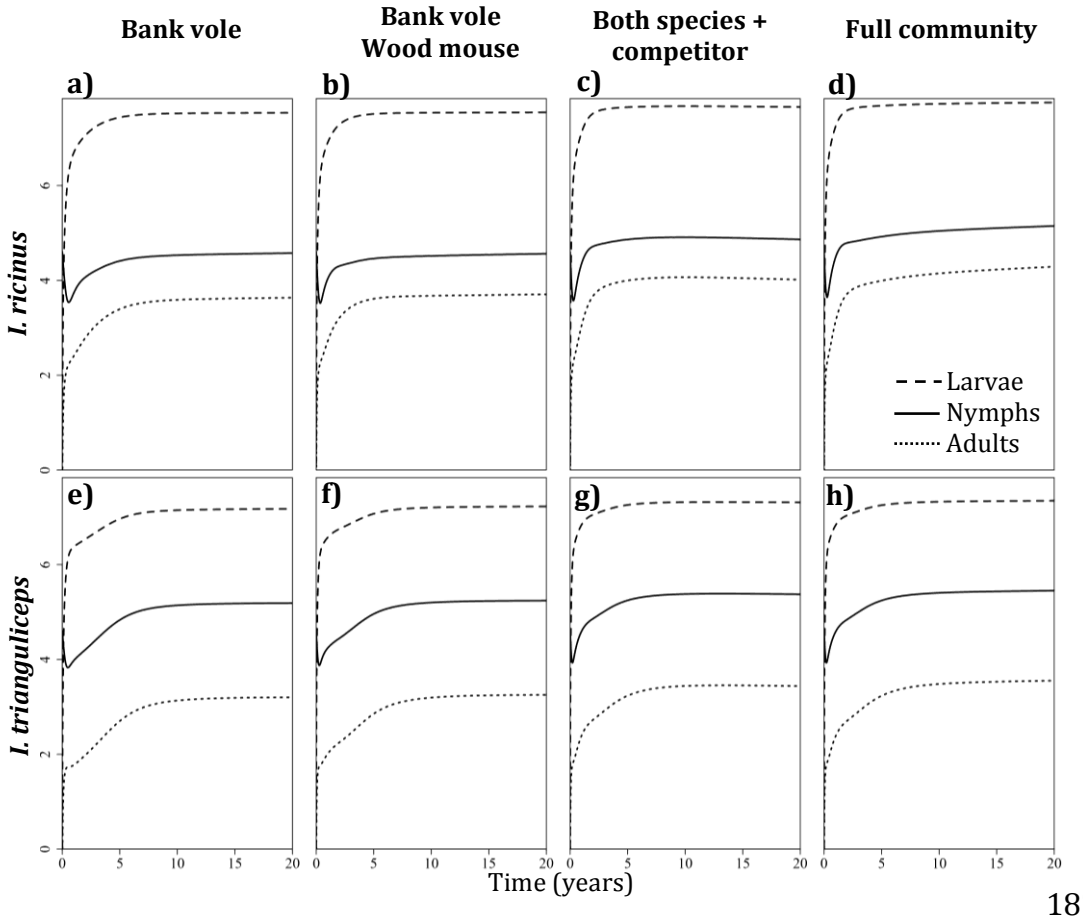


Fig. 5.14. ***Ixodes ricinus-Borrelia burgdorferi*****: sensitivity analysis on encounter rates. Proportion of infectious hosts (purple) and infectious nymphs (green) across different community assemblages: a) bank vole; b) wood mouse and bank vole; c) two rodent species and shrew; d) full community (two rodent species, shrew, generalist and specialist predation, ungulate). Box and whiskers plots of the results (1000 combinations) sorted according to the encounter rate small host (rodents and shrews)-larva.**

Parameter name	Parameter symbol	Value (or range)
Moulting success on rodents	d_r	0.198
Moulting success on shrews	d_s	0.165
Moulting success on large host	d_l	0.213
Reservoir competence host to tick	τ_{ht}	0.500
Reservoir competence tick to host	τ_{th}	0.800
Contact rate rodent/larva	β_{sl}	0.0043-0.073
Contact rate rodent/nymph	β_{sn}	0.04-0.0975
Contact rate rodent/adult	β_{sa}	0.043-0.105
Contact rate large animal/larva	β_{ll}	0.0043-0.73
Contact rate large animal/nymph	β_{ln}	0.04-0.0975
Contact rate large animal/adult	β_{la}	0.043-0.105

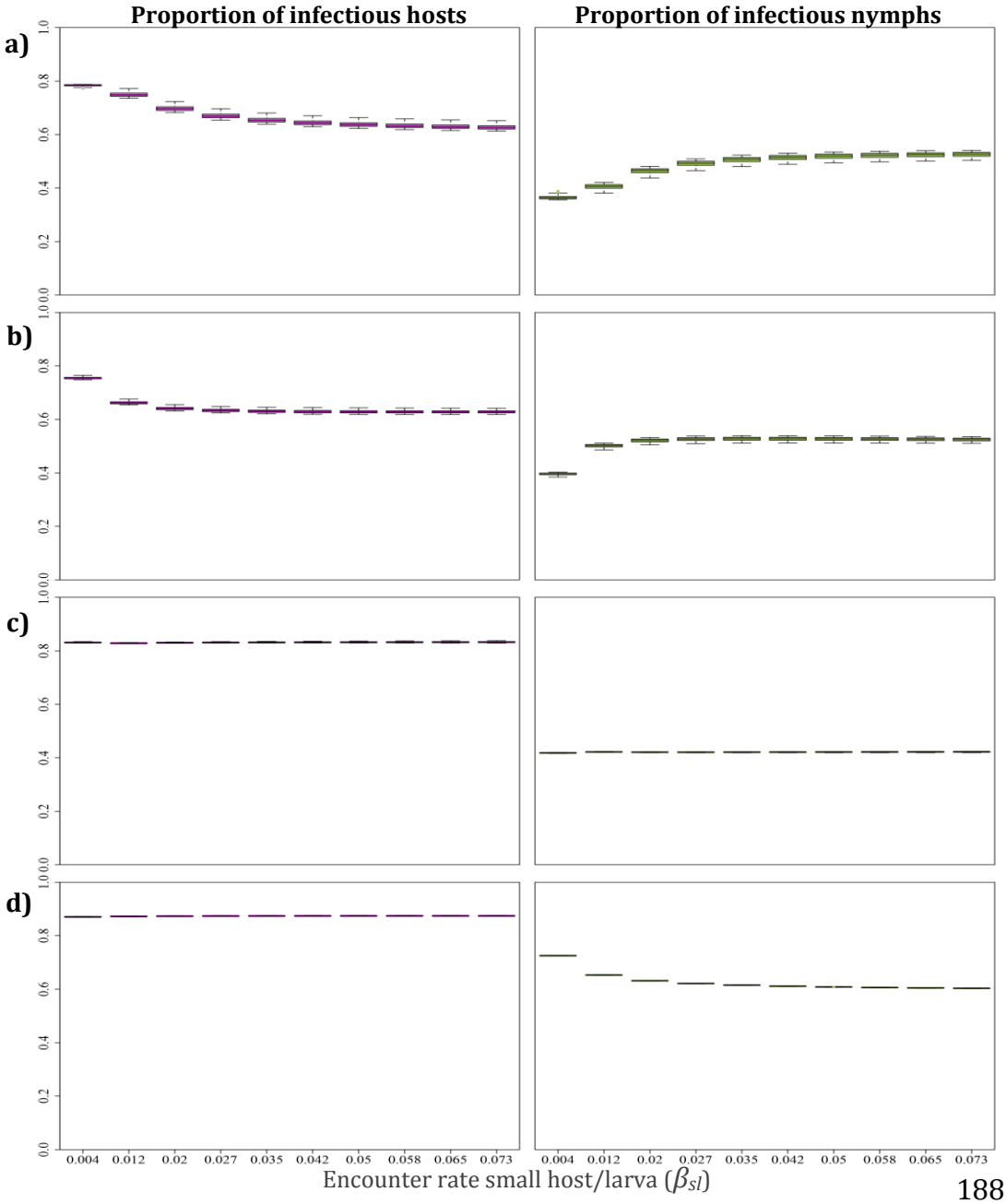


Fig. 5.15. ***Ixodes ricinus-Borrelia burgdorferi*****: sensitivity analysis on encounter rates. Number of total infectious hosts (purple) and infectious nymphs (green) across different community assemblages: a) bank vole; b) wood mouse and bank vole; c) two rodent species and shrew; d) full community (two rodent species, shrew, generalist and specialist predation, ungulate). Box and whiskers plots of the results (1000 combinations) sorted according to the encounter rate small host (rodents and shrews)-larva.**

Parameter name	Parameter symbol	Value (or range)
Moulting success on rodents	d_r	0.198
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Moulting success on large host	d_l	0.213
Reservoir competence host to tick	τ_{ht}	0.500
Reservoir competence tick to host	τ_{th}	0.800
Contact rate rodent/larva	β_{sl}	0.0043-0.073
Contact rate rodent/nymph	β_{sn}	0.04-0.0975
Contact rate rodent/adult	β_{sa}	0.043-0.105
Contact rate large animal/larva	β_{ll}	0.0043-0.73
Contact rate large animal/nymph	β_{ln}	0.04-0.0975
Contact rate large animal/adult	β_{la}	0.043-0.105

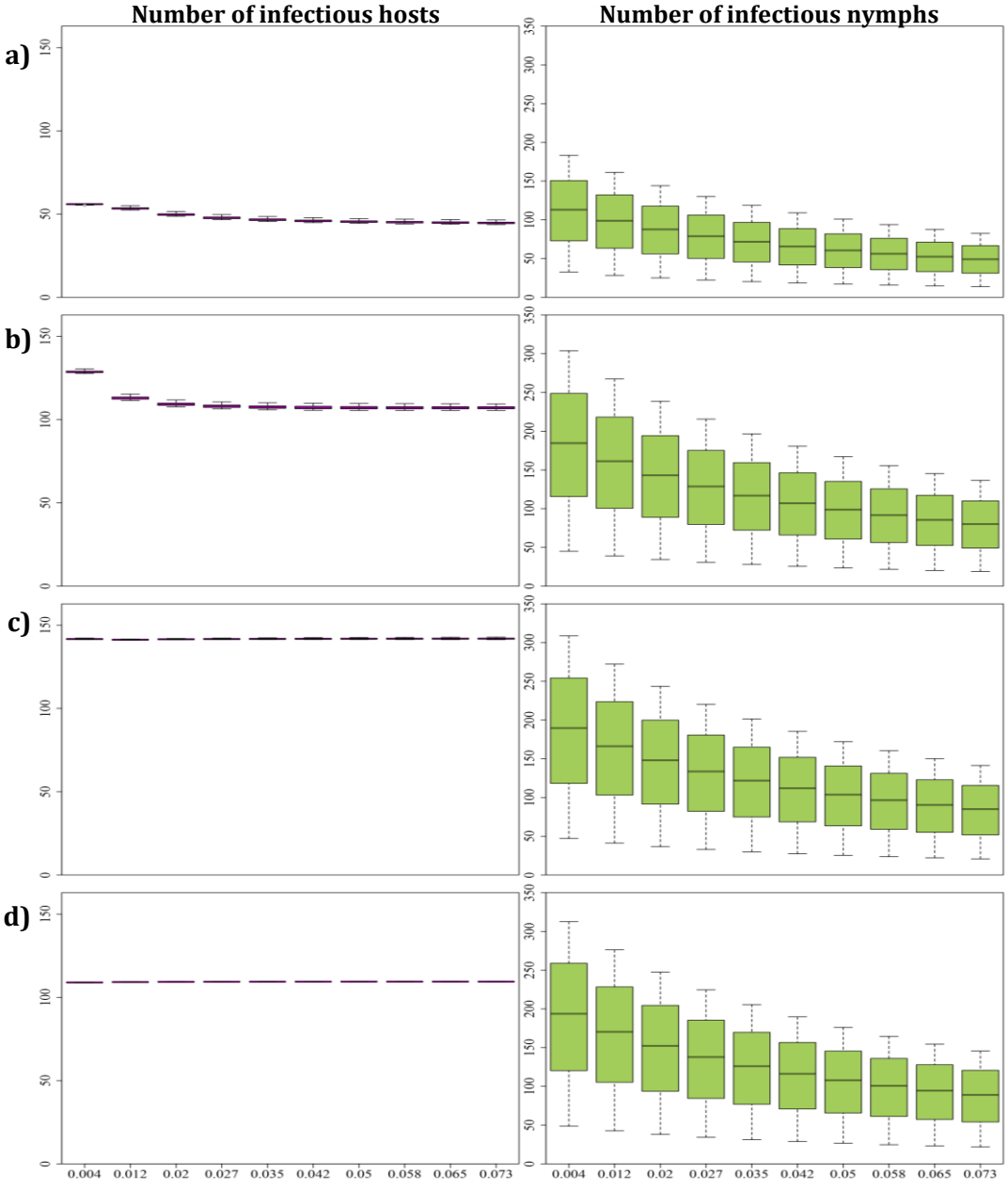


Fig. 5.16. ***Ixodes trianguliceps-Babesia microti*****: sensitivity analysis on encounter rates. Proportion of infectious hosts (purple) and infectious nymphs (green) across different community assemblages: a) bank vole; b) wood mouse and bank vole; c) two rodent species and shrew; d) full community (two rodent species, shrew, generalist and specialist predation, ungulate). Box and whiskers plots of the results (1000 combinations) sorted according to the encounter rate small host (rodents and shrews)-larva.**

Parameter name	Parameter symbol	Value (or range)
Moulting success on rodents	d_r	0.198
Moulting success on shrews	d_s	0.165
Moulting success on large host	d_l	0.213
Reservoir competence host to tick	τ_{ht}	0.500
Reservoir competence tick to host	τ_{th}	0.800
Contact rate rodent/larva	β_{sl}	0.0043-0.073
Contact rate rodent/nymph	β_{sn}	0.04-0.0975
Contact rate rodent/adult	β_{sa}	0.043-0.105
Contact rate large animal/larva	β_{ll}	0.0043-0.73
Contact rate large animal/nymph	β_{ln}	0.04-0.0975
Contact rate large animal/adult	β_{la}	0.043-0.105

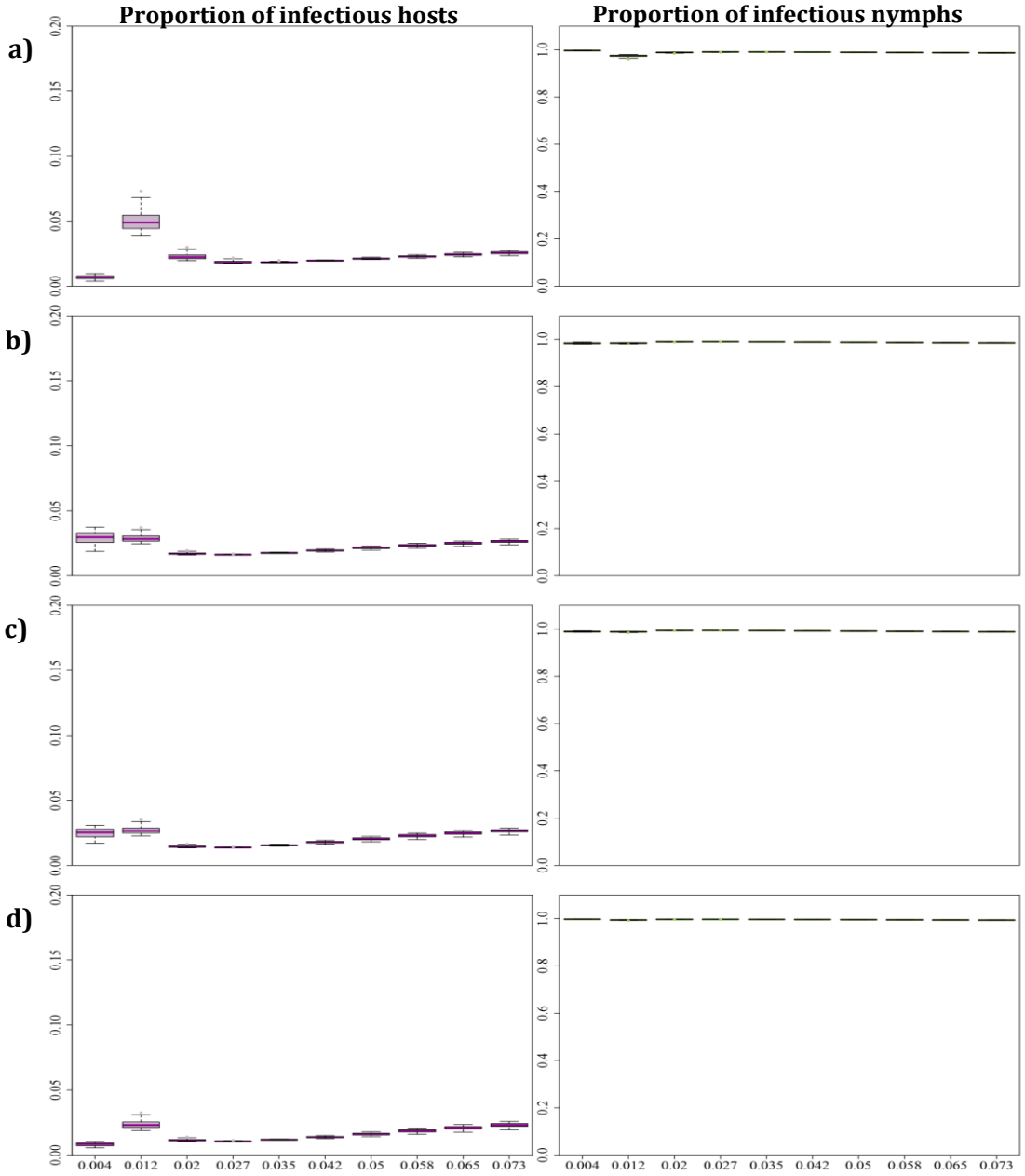


Fig. 5.17. ***Ixodes trianguliceps-Babesia microti*****: sensitivity analysis on encounter rates. Number of total infectious hosts (purple) and infectious nymphs (green) across different community assemblages: a) bank vole; b) wood mouse and bank vole; c) two rodent species and shrew; d) full community (two rodent species, shrew, generalist and specialist predation, ungulate). Box and whiskers plots of the results (1000 combinations) sorted according to the encounter rate small host (rodents and shrews)-larva.**

Parameter name	Parameter symbol	Value (or range)
Moulting success on rodents	d_r	0.198
Moulting success on shrews	d_s	0.165
Moulting success on large host	d_l	0.213
Reservoir competence host to tick	τ_{ht}	0.500
Reservoir competence tick to host	τ_{th}	0.800
Contact rate rodent/larva	β_{sl}	0.0043-0.073
Contact rate rodent/nymph	β_{sn}	0.04-0.0975
Contact rate rodent/adult	β_{sa}	0.043-0.105
Contact rate large animal/larva	β_{ll}	0.0043-0.73
Contact rate large animal/nymph	β_{ln}	0.04-0.0975
Contact rate large animal/adult	β_{la}	0.043-0.105

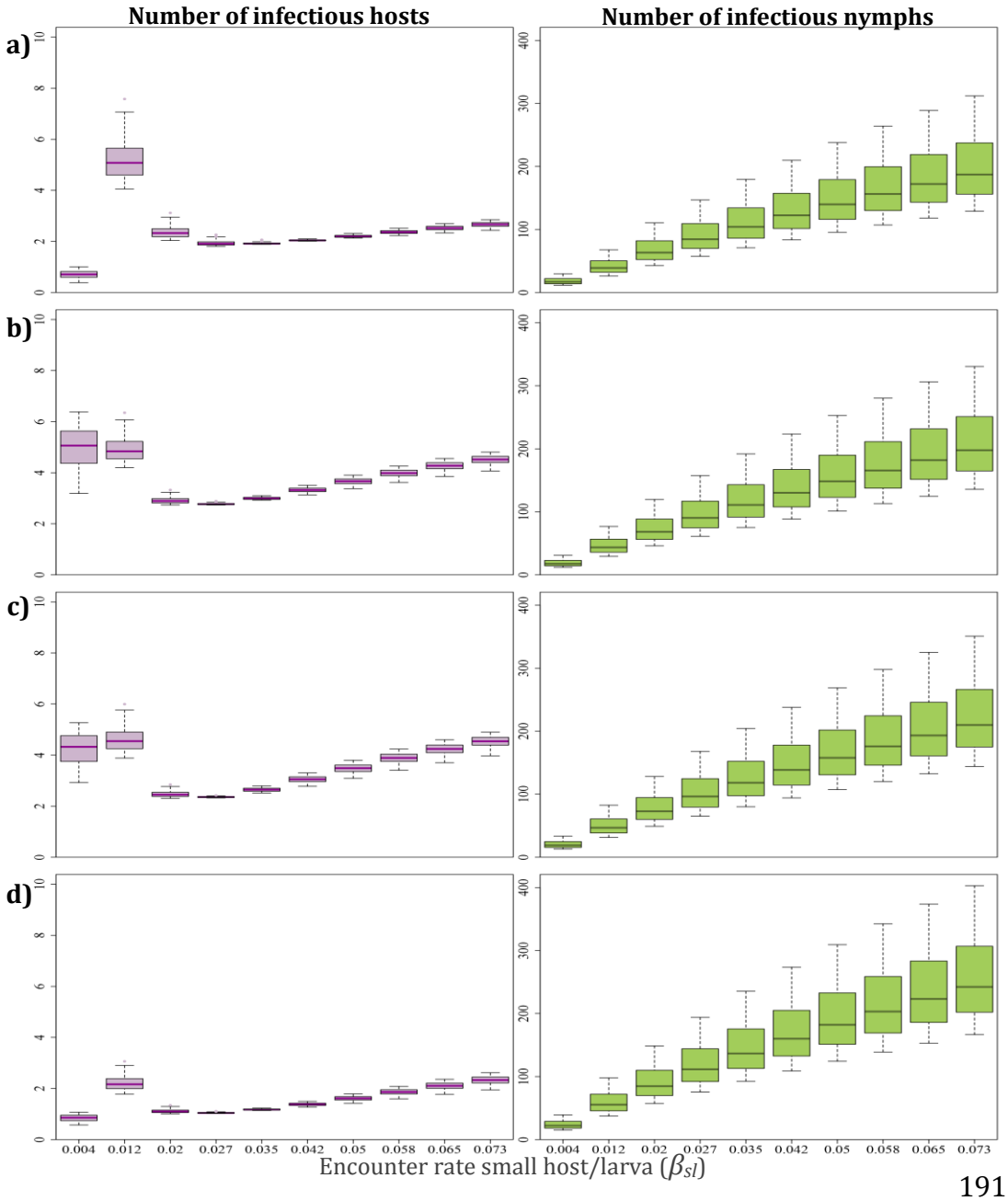


Fig. 5.18. ***Ixodes ricinus-Borrelia burgdorferi*****: sensitivity analysis on moulting-feeding success. On the z-axis equilibrium values for proportion (a, b) and number of infectious nymphs (c, d) across different community assemblages: a) and c) two rodent species plus shrew; b) and d) full community. Latin hypercube sampling 1000 replicates.**

Parameter name	Parameter symbol	Value (or range)
Moulting success on rodents	d_r	0-0.593
Moulting success on shrews	d_s	0-0.496
Moulting success on large host	d_l	0-0639
Reservoir competence host to tick	τ_{ht}	0.500
Reservoir competence tick to host	τ_{th}	0.800
Contact rate rodent/larva	β_{sl}	0.025
Contact rate rodent/nymph	β_{sn}	0.040
Contact rate rodent/adult	β_{sa}	0.010
Contact rate large animal/larva	β_{ll}	0.025
Contact rate large animal/nymph	β_{ln}	0.040
Contact rate large animal/adult	β_{la}	0.060

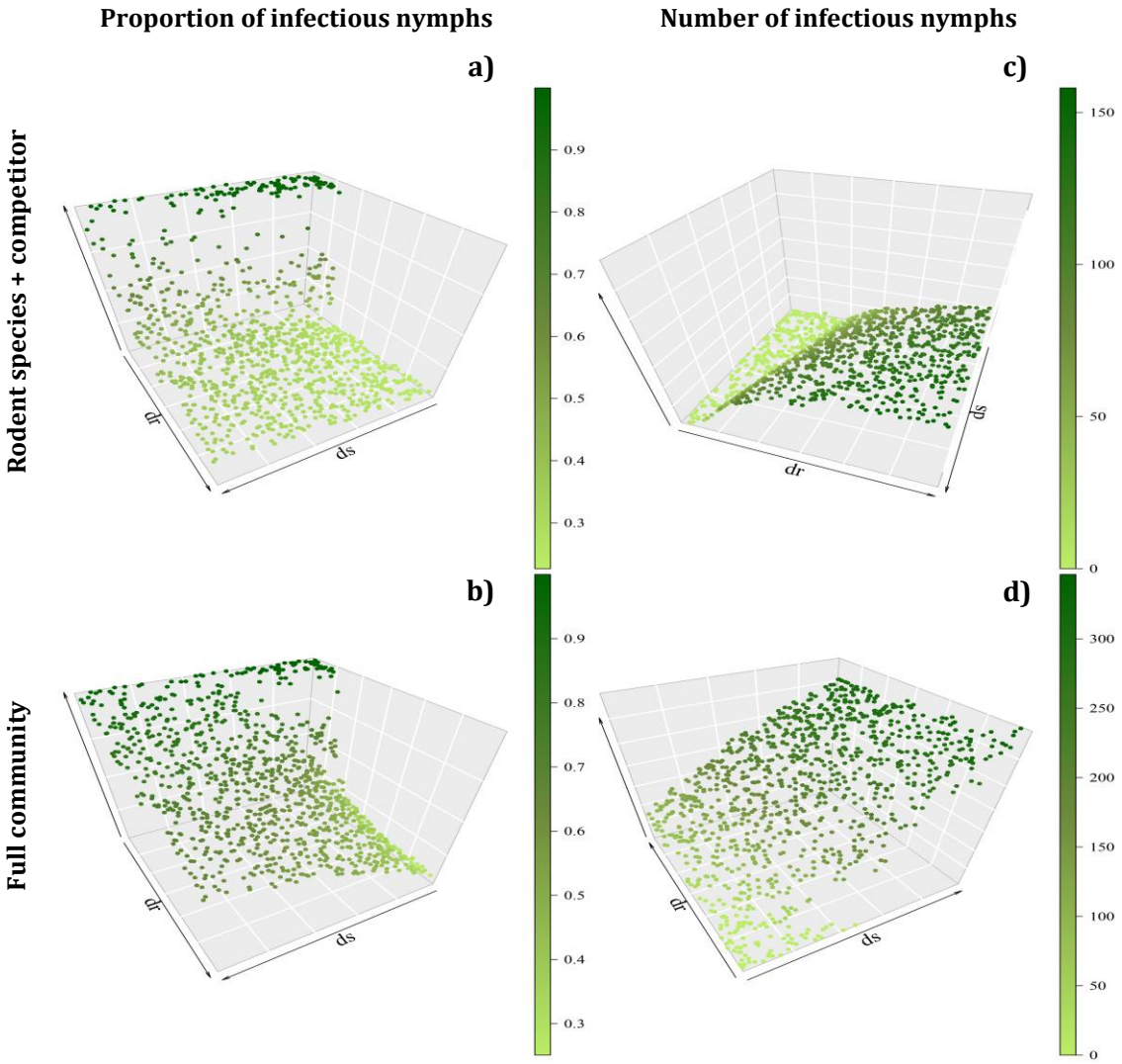


Fig. 5.19. ***Ixodes ricinus-Borrelia burgdorferi*****: sensitivity analysis on moulting-feeding success. On the z-axis equilibrium values for proportion (a, b) and number of infectious hosts (c, d) across different community assemblages: a) and c) two rodent species plus shrew; b) and d) full community. Latin hypercube sampling 1000 replicates.**

Parameter name	Parameter symbol	Value (or range)
Moulting success on rodents	d_r	0-0.593
Moulting success on shrews	d_s	0-0.496
Moulting success on large host	d_l	0-0639
Reservoir competence host to tick	τ_{ht}	0.500
Reservoir competence tick to host	τ_{th}	0.800
Contact rate rodent/larva	β_{sl}	0.025
Contact rate rodent/nymph	β_{sn}	0.040
Contact rate rodent/adult	β_{sa}	0.010
Contact rate large animal/larva	β_{ll}	0.025
Contact rate large animal/nymph	β_{ln}	0.040
Contact rate large animal/adult	β_{la}	0.060

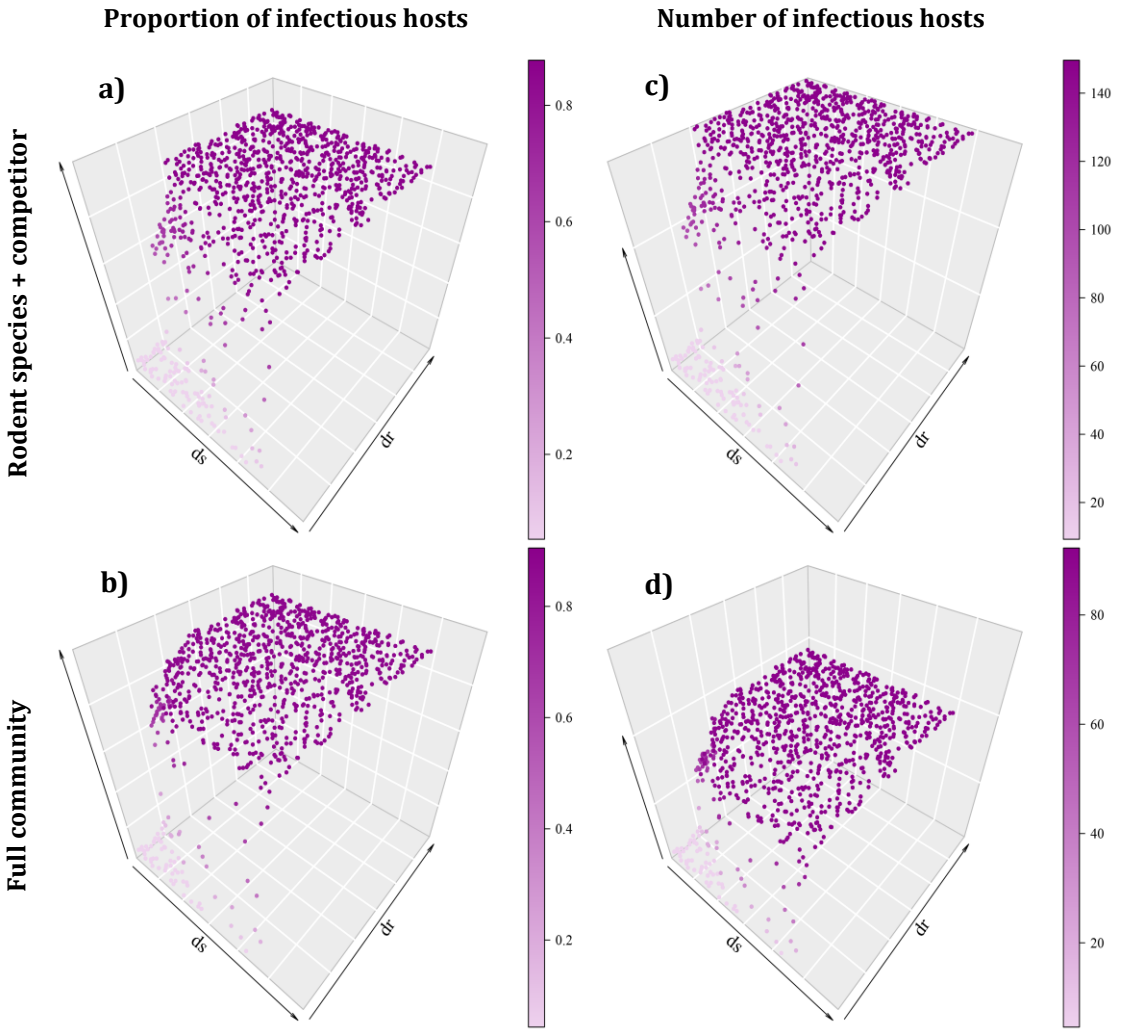


Fig. 5.20. ***Ixodes trianguliceps-Babesia microti*****: sensitivity analysis on moulting-feeding success. On the z-axis equilibrium values for proportion (a, b) and number of infectious nymphs (c, d) across different community assemblages: a) and c) two rodent species plus shrew; b) and d) full community. Latin hypercube sampling 1000 replicates.**

Parameter name	Parameter symbol	Value (or range)
Moulting success on rodents	d_r	0-0.593
Moulting success on shrews	d_s	0-0.496
Moulting success on large host	d_l	0-0639
Reservoir competence host to tick	τ_{ht}	0.500
Reservoir competence tick to host	τ_{th}	0.800
Contact rate rodent/larva	β_{sl}	0.040
Contact rate rodent/nymph	β_{sn}	0.040
Contact rate rodent/adult	β_{sa}	0.040
Contact rate large animal/larva	β_{ll}	0.000
Contact rate large animal/nymph	β_{ln}	0.000
Contact rate large animal/adult	β_{la}	0.000

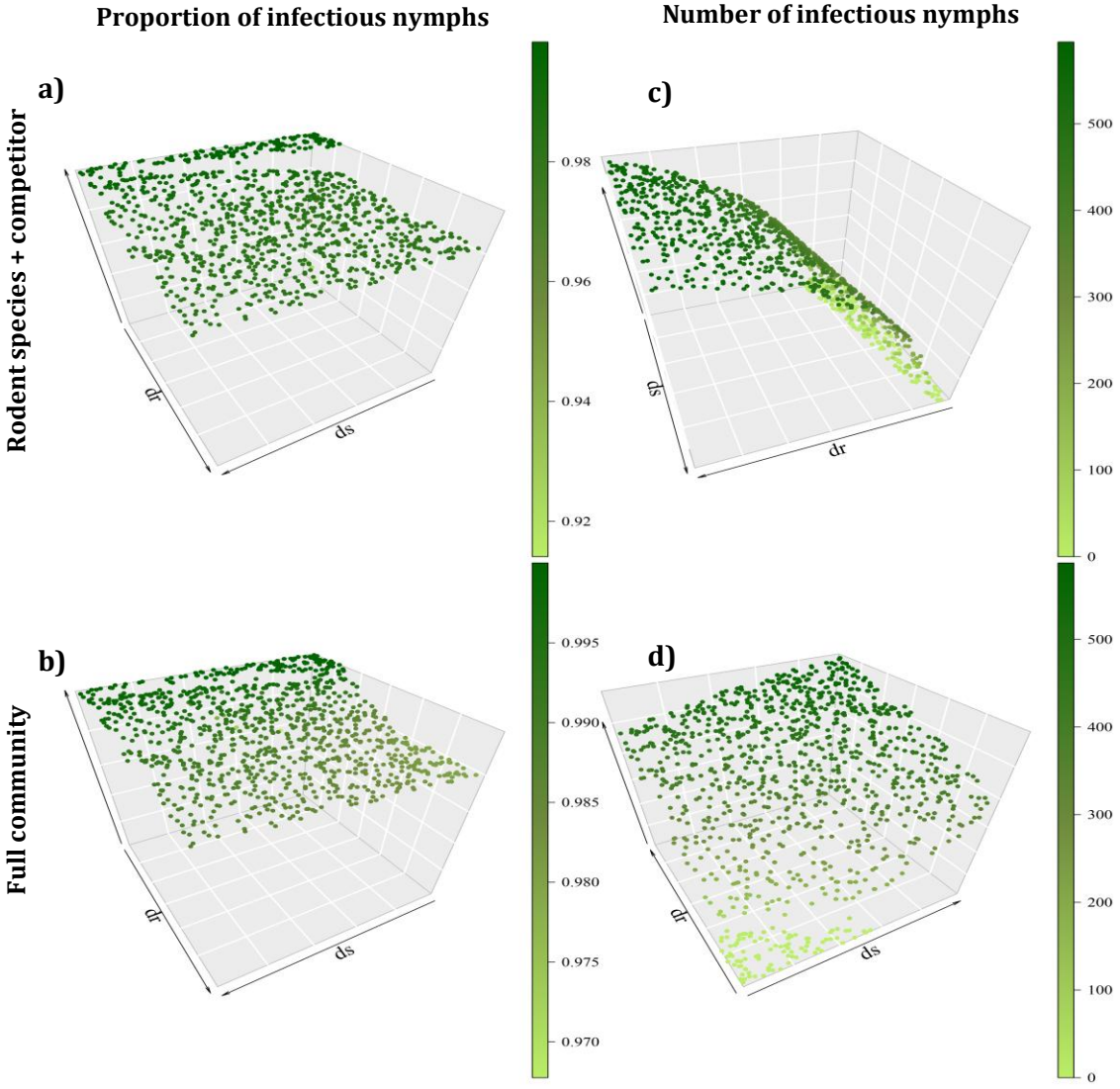


Fig. 5.21. ***Ixodes trianguliceps-Babesia microti*****: sensitivity analysis on moulting-feeding success. On the z-axis equilibrium values for proportion (a, b) and number of infectious hosts (c, d) across different community assemblages: a) and c) two rodent species plus shrew; b) and d) full community. Latin hypercube sampling 1000 replicates.**

Parameter name	Parameter symbol	Value (or range)
Moulting success on rodents	d_r	0-0.593
Moulting success on shrews	d_s	0-0.496
Moulting success on large host	d_l	0-0639
Reservoir competence host to tick	τ_{ht}	0.500
Reservoir competence tick to host	τ_{th}	0.800
Contact rate rodent/larva	β_{sl}	0.040
Contact rate rodent/nymph	β_{sn}	0.040
Contact rate rodent/adult	β_{sa}	0.040
Contact rate large animal/larva	β_{ll}	0.000
Contact rate large animal/nymph	β_{ln}	0.000
Contact rate large animal/adult	β_{la}	0.000

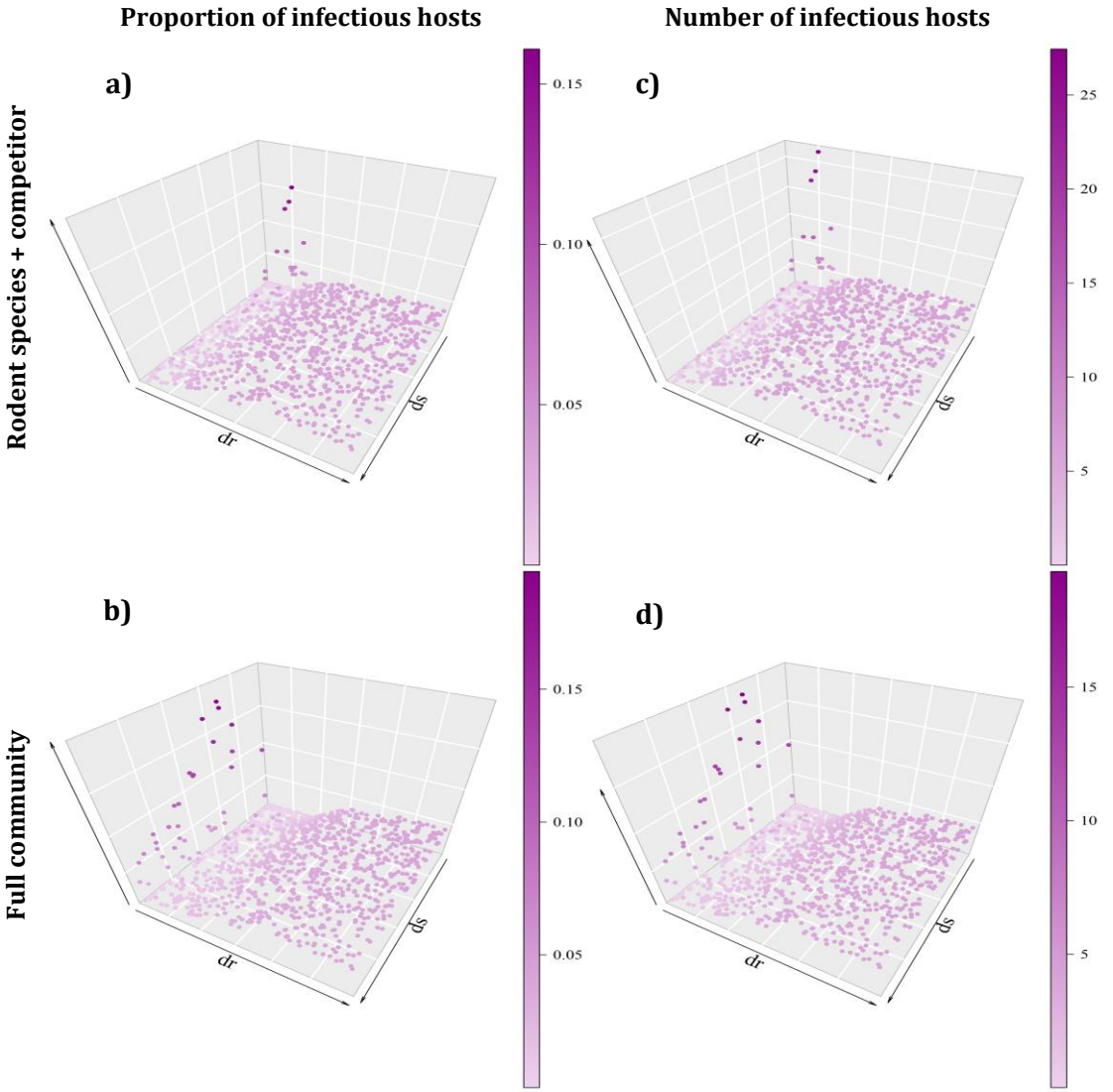


Fig. 5.22. ***Ixodes ricinus-Borrelia burgdorferi*****: sensitivity analysis on reservoir competence. On the z-axis equilibrium values for proportion (a, b, c) and number of infectious nymphs (d, e, f) across different community assemblages: a) and d) bank vole; b) and e) wood mouse and bank vole; c) and f) full community. Latin hypercube sampling 1000 replicates. BV: bank vole.**

Parameter name	Parameter symbol	Value (or range)
Moulting success on rodents	d_r	0.198
Moulting success on shrews	d_s	0.165
Moulting success on large host	d_l	0.213
Reservoir competence host to tick	τ_{ht}	0-0.90
Reservoir competence tick to host	τ_{th}	0-0.90
Contact rate rodent/larva	β_{sl}	0.025
Contact rate rodent/nymph	β_{sn}	0.040
Contact rate rodent/adult	β_{sa}	0.010
Contact rate large animal/larva	β_{ll}	0.025
Contact rate large animal/nymph	β_{ln}	0.040
Contact rate large animal/adult	β_{la}	0.060

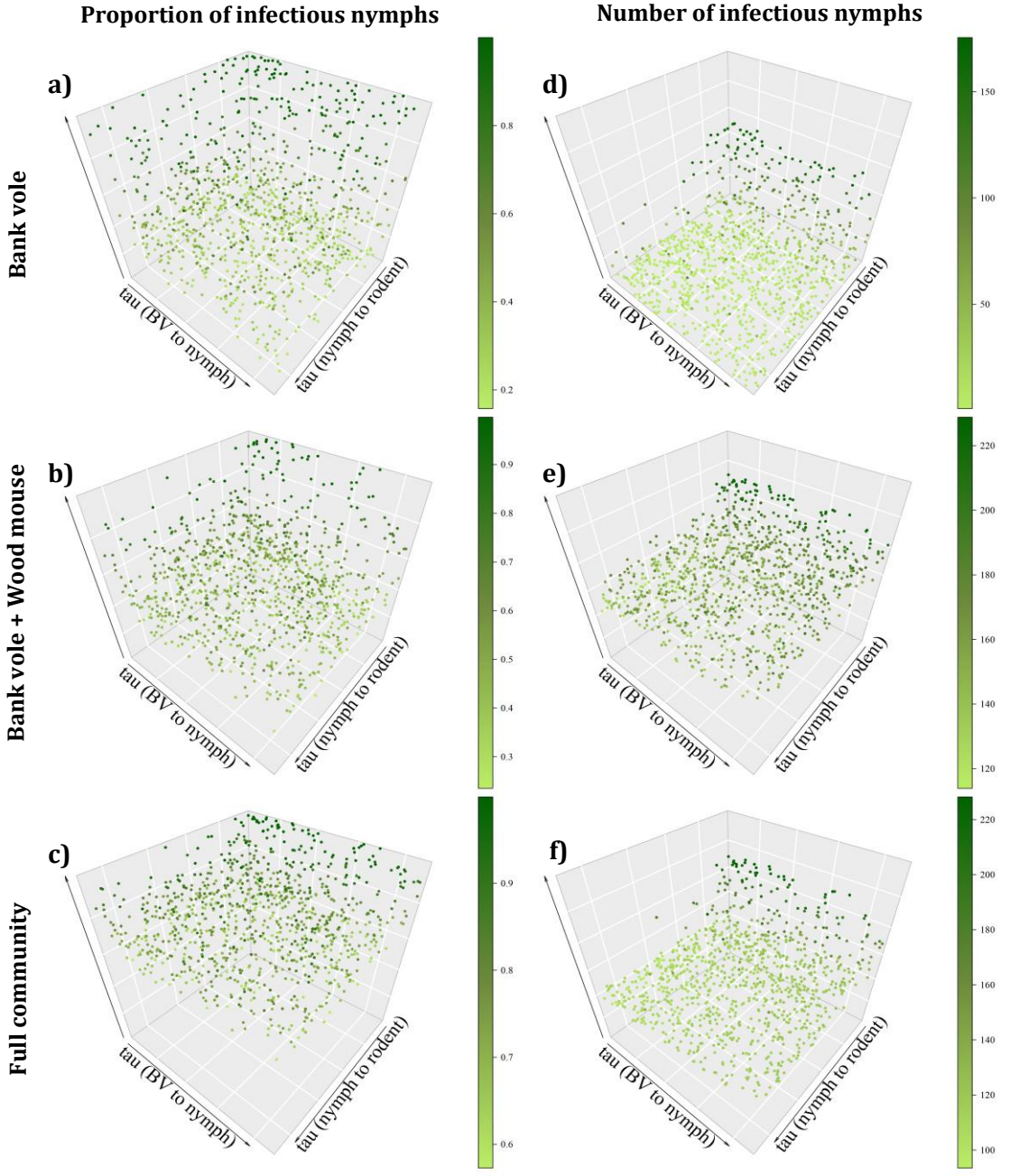


Fig. 5.23. ***Ixodes ricinus-Borrelia burgdorferi*****: sensitivity analysis on reservoir competence. On the z-axis equilibrium values for proportion (a, b, c) and number of infectious nymphs (d, e, f) across different community assemblages: a) and d) bank vole; b) and e) wood mouse and bank vole; c) and f) full community. Latin hypercube sampling 1000 replicates. BV: bank vole.**

Parameter name	Parameter symbol	Value (or range)
Moulting success on rodents	d_r	0.198
Moulting success on shrews	d_s	0.165
Moulting success on large host	d_l	0.213
Reservoir competence host to tick	τ_{ht}	0-0.90
Reservoir competence tick to host	τ_{th}	0-0.90
Contact rate rodent/larva	β_{sl}	0.025
Contact rate rodent/nymph	β_{sn}	0.040
Contact rate rodent/adult	β_{sa}	0.010
Contact rate large animal/larva	β_{ll}	0.025
Contact rate large animal/nymph	β_{ln}	0.040
Contact rate large animal/adult	β_{la}	0.060

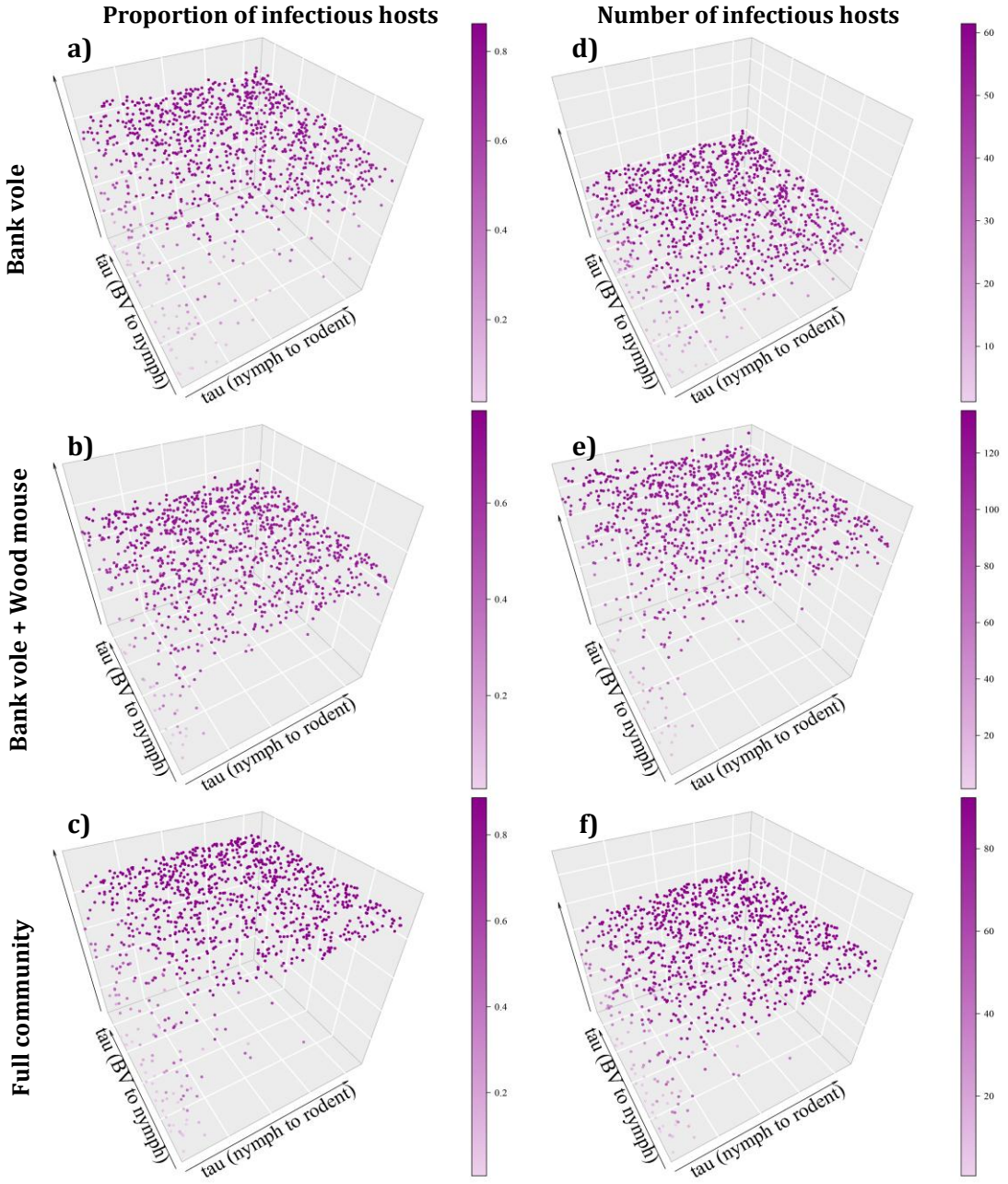


Fig. 5.24. ***Ixodes trianguliceps-Babesia microti*****: sensitivity analysis on reservoir competence. On the z-axis equilibrium values for proportion (a, b, c) and number of infectious nymphs (d, e, f) across different community assemblages: a) and d) bank vole; b) and e) wood mouse and bank vole; c) and f) full community. Latin hypercube sampling 1000 replicates. BV: bank vole.**

Parameter name	Parameter symbol	Value (or range)
Moulting success on rodents	d_r	0.198
Moulting success on shrews	d_s	0.165
Moulting success on large host	d_l	0.213
Reservoir competence host to tick	τ_{ht}	0-0.90
Reservoir competence tick to host	τ_{th}	0-0.90
Contact rate rodent/larva	β_{sl}	0.040
Contact rate rodent/nymph	β_{sn}	0.040
Contact rate rodent/adult	β_{sa}	0.040
Contact rate large animal/larva	β_{ll}	0.000
Contact rate large animal/nymph	β_{ln}	0.000
Contact rate large animal/adult	β_{la}	0.000

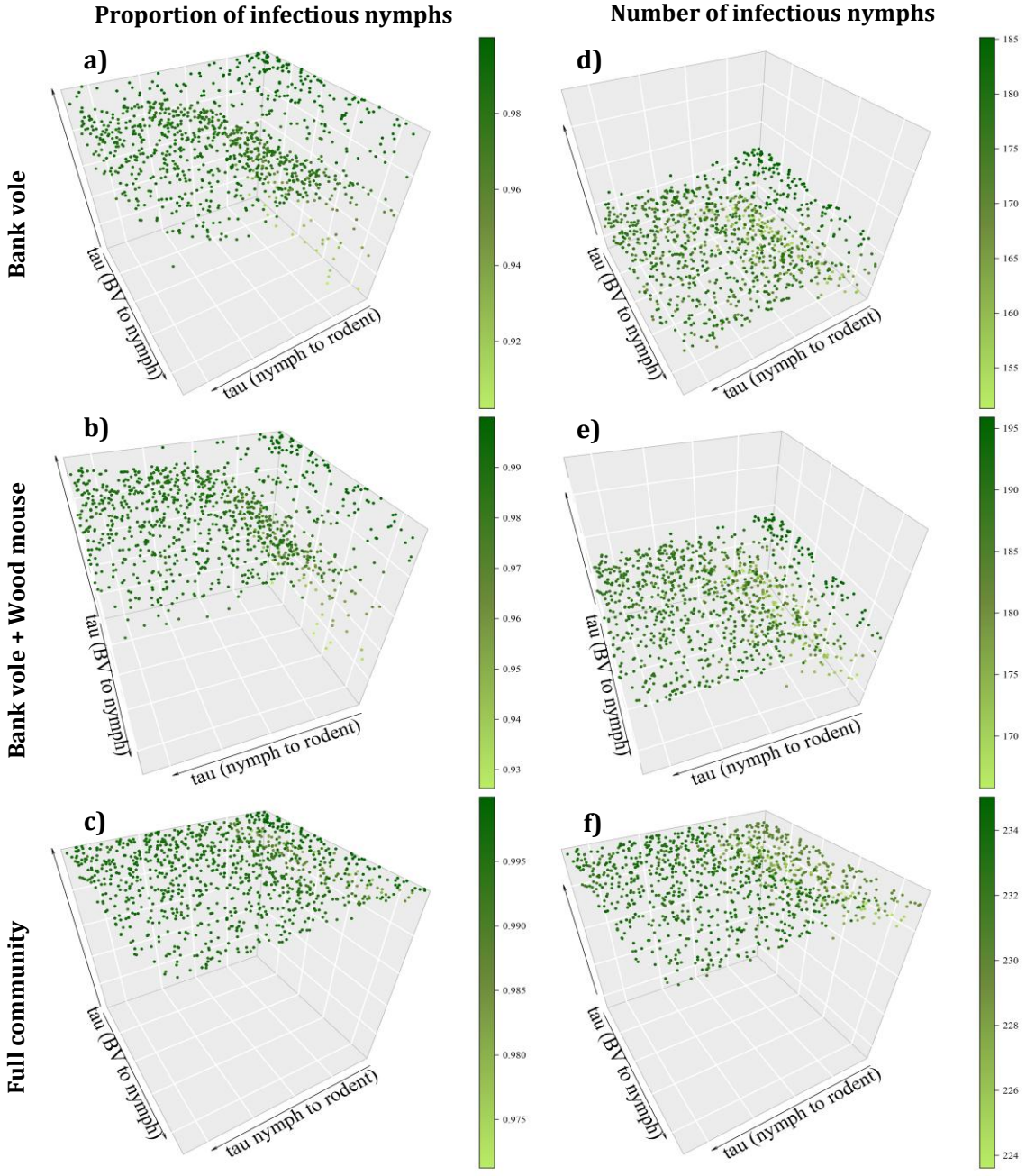
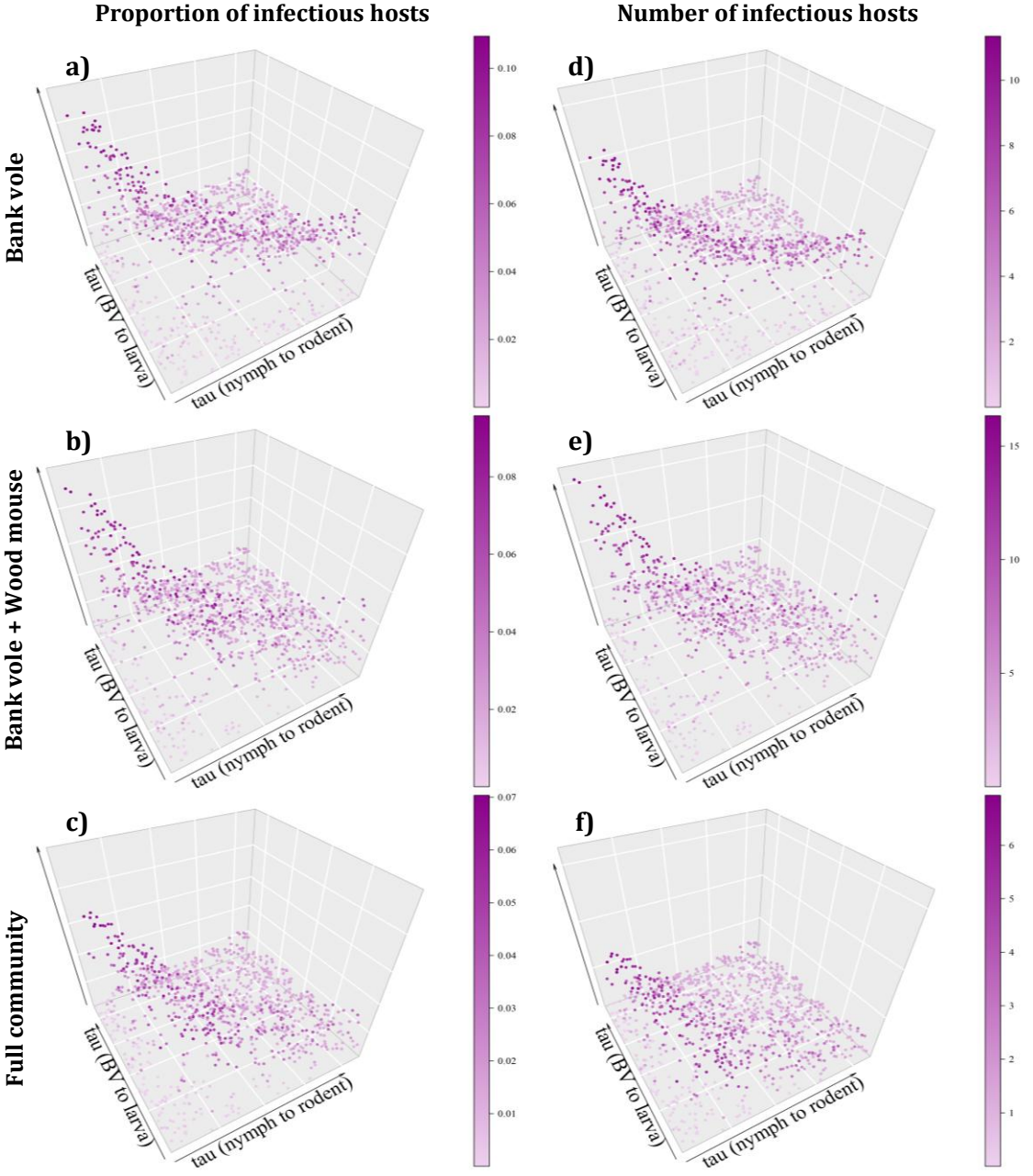


Fig. 5.25. ***Ixodes trianguliceps-Babesia microti*****: sensitivity analysis on reservoir competence. On the z-axis equilibrium values for proportion (a, b, c) and number of infectious nymphs (d, e, f) across different community assemblages: a) and d) bank vole; b) and e) wood mouse and bank vole; c) and f) full community. Latin hypercube sampling 1000 replicates. BV: bank vole.**

Parameter name	Parameter symbol	Value (or range)
Moulting success on rodents	d_r	0.198
Moulting success on shrews	d_s	0.165
Moulting success on large host	d_l	0.213
Reservoir competence host to tick	τ_{ht}	0-0.90
Reservoir competence tick to host	τ_{th}	0-0.90
Contact rate rodent/larva	β_{sl}	0.040
Contact rate rodent/nymph	β_{sn}	0.040
Contact rate rodent/adult	β_{sa}	0.040
Contact rate large animal/larva	β_{ll}	0.000
Contact rate large animal/nymph	β_{ln}	0.000
Contact rate large animal/adult	β_{la}	0.000



5.3.4 Discussion

The structure of the model seemed suitable to highlight differences in the host-vector-pathogen dynamics. The empirical data collected in the field were useful to develop a model suitable to describe the systems under investigation. First, aggregation of the vector on the host was included (non-uniform distribution on the host population), as found in the field, in order to have a more realistic pathogen transmission patterns, as the increase of vectors does not linearly lead to an increase in infections because of the "20/80 rule" (Perkins *et al.*, 2003; Woolhouse *et al.*, 1997). The aggregation parameter was estimated from both species and for all tick life stages due to the uneven amount of data for each of those variables; although, this may be host and life stage specific (Krasnov *et al.*, 2007). Intensity of infestation has been reported to predict pathogen prevalence (Van Buskirk and Ostfeld, 1995; Foley and Piovia-Scott, 2014; Hofmeester *et al.*, 2017), therefore the aggregation term parameterised according to the tick distribution found in the field seemed an optimal way to incorporate this relationship in the model (Calabrese *et al.*, 2011). Then, the often counterintuitive relationship between host density and ecto-parasites abundance/prevalence (e.g. Fichet-Calvert *et al.*, 2003; McCauley *et al.*, 2008; Stanko *et al.*, 2006) was the reason to include the tick and host density dependent fecundity reduction function to the tick recruitment equation (Ogden *et al.*, 2007). In fact, ecto-parasites do not necessarily linearly increase following host abundance increase. Further, no transmission through tick co-feeding (non-viraemic) was introduced, as, in the animals sampled, co-feeding was an extremely rare event. Also, the pathogens considered were assumed not to have non-viraemic transmission (Jacquet *et al.*, 2016), although different pathogens and strains were found to have different efficacy of infection through co-feeding (Levin and Fish, 2000; Richter *et al.*, 2002; Tonetti *et al.*, 2015; Voordouw, 2015). Ecological differences between the two tick species have been observed, e.g. different host preferences and different seasonal peaks representing different environmental preferences (e.g. temperature, humidity) (Mysterud *et*

al., 2015), and the two species have often not been recorded together (e.g. this study), therefore it was reasonable to model the two systems independently. Further, *B. burgdorferi* has never been isolated in *I. trianguliceps* (Mysterud *et al.*, 2015).

The vector populations modelled were comparable with other empirical and modelling studies with regards to tick density (taking into account the spatial scale and the host densities), and the proportion between larvae, nymphs and adults (~1000:100:2) (Dobson *et al.*, 2011; Harrison *et al.*, 2011). It might seem that a very small number of ticks were collected in this study (see Chapter 3) compared to the size of the population modelled, but ticks were not sampled from the environment, where the vast majority of the tick real population is found (Dobson, 2014). Moreover, other very important tick vertebrate hosts were not sampled (i.e. shrews: Bray *et al.*, 2007; Mysterud *et al.*, 2015).

According to LaDeau *et al.* (2011), multiple data sources were integrated in developing the model in order to maximise existing ecological and epidemiological knowledge. As advised by Roche *et al.* (2013), no fixed relationship was introduced between host abundance and reservoir competence (competence of transmission was parameterised according to previous empirical studies), and seasonality was included in the host population dynamics. Despite seasonality not explicitly being included in the tick population dynamics, the period of the year in which they were expected to peak was included in the rodents' breeding season, so, this caused an indirect seasonal effect. Lastly, the model might have been improved by including the links between tick population dynamics and habitat, which is essential to understand local dynamics (and avoid ecological fallacy), as it has been observed that tick prevalence may be more dependent upon site rather than host species (Maaz *et al.*, 2018), but this was beyond the scope of this study.

The prevalence values predicted by the model were plausible for the pathogen considered. *B. burgdorferi* s.l. prevalence in *I. ricinus* in Europe differs regionally, ranging from 3.3 to 36.2% (reviewed in Obiegala *et al.*, 2017). In Germany, Obiegala *et al.* (2017) found that the majority of species of the order Rodentia

were highly prevalent (25.4–62.5%), while insectivores and carnivores were all negative, and Khanakah *et al.* (2006) found all rodents species positive with prevalence ranging from 13.3 to 77.0%. By contrast, *B. microti* has been found at low prevalence across rodents (Hussein, 1980; Welc-Falęciak *et al.*, 2008), although it seems to widely vary according to rodent species and methodology used (blood smear or PCR) (Siński *et al.*, 2006).

The model outputs of interest were proportion and number of infectious hosts, and proportion (NIP) and number of infectious nymphs (DIN), since the latter are common metrics used to estimate human disease risk, as nymphs are the most numerous infectious tick life stage (no transovarial transmission), and the most likely to bite humans (Barbour and Fish, 1993; LoGiudice *et al.*, 2003; Piesman, 1989; Wood and Lafferty, 2013). Both the proportion and number of infectious were included as they represent different metrics, and might respond in divergent ways to parameter and community composition variations, according to the finding in section 5.2.

The encounter rate parameter that most influenced the pathogen transmission was, in both systems, the one between small hosts and larvae, most likely because this was also the parameter most impacting the recruitment of tick populations. However, the increase in encounters had a negative effect on infectious nymphs in *I. ricinus*, and the opposite in *I. trianguliceps* because the population of the first tick species, being more prolific, was subjected to a progressive population reduction due to the greater fecundity reduction, which was density-dependent. The addition of the shrew population, which was not competent in transmitting the disease proved evidence of dilution through transmission reduction (wasted tick bites) in the *I. ricinus*-*B. burgdorferi* system; in fact, despite the rise of overall hosts available for ticks, there was no increase of infectious nymphs, and nymph prevalence dropped (because of the overall increase in nymph population). This effect was not observed in the alternative system, as prevalence and number of infectious nymphs did not vary across different community assemblages. The reason for this phenomenon might be found in the different disease-related parameters, and the aggregation

term that determined that maximum transmission was reached already in the community with only two rodents, and the addition of more ticks into the system was irrelevant. Comparing the third scenario with the richest community assemblage, it was possible to find evidence of dilution (with regards to the host) through susceptible host regulation in both systems, as the number of infectious hosts decreased due to predation. Also, this effect acted in synergy with transmission reduction in the first host-vector-pathogen system, as *I. ricinus* fed also on the non-competent species added to the last assemblage (predator, ungulate). In general, it was observed that there was usually, for each case, a threshold value of the encounter rate higher than which the output under consideration reached a plateau. The explanation might be found in the constraints of transmission that were represented by the values of competence lower than 100%, and the presence of the aggregation term.

Moulting success on rodents was the parameter that most affected pathogen transmission in both systems, especially with regards to host infection. Moulting success on shrews did have an impact on prevalence and number of infectious nymphs only in the community where predation was modelled, since predators preyed preferentially on rodents and the shrew population became relatively more important in sustaining the tick population, and subsequently pathogen circulation. However, even in this case the numerical differences between the two systems were maintained; compared to *I. trianguliceps*, the *I. ricinus* population was larger, yet overall fewer infectious nymphs were present in both community assemblages, because of the presence of alternative hosts, and the different host preferences. By contrast, a lower value of moulting success on rodents was sufficient to reach the maximum number of infectious hosts because of the greater number of ticks. Hence, the higher number of wasted bites (in terms of pathogen transmission) of *I. ricinus* and the higher encounter rate of *I. trianguliceps* with small sized hosts (including rodents, competent in transmitting the pathogen) yielded an overall lower amount of nymphs infected by *B. burgdorferi*. Nonetheless, the lower population size of *I. trianguliceps* determined that a higher value of moulting success was necessary to reach the plateau with regard to infectious hosts, of which the number varied more along

the range of values of moulting success than of encounter rates. Therefore, moulting success on rodents rather than encounter rate, in the second system, was the parameter that regulated pathogen transmission. Comparing the two community assemblages, there was evidence, as described earlier, of dilution through susceptible host regulation by predation in both systems.

Considering competence of transmission, in the *B. microti* system, this parameter did not massively influence transmission among ticks, as *I. trianguliceps* fed almost exclusively on competent hosts, so a small value of competence was enough to reach the maximum level of transmission among nymphs. With regards to infectious hosts, the two competence parameters that seemed to be more decisive were rodent to larva and nymph to rodent, probably due to the fact that a higher number of infectious larvae can moult in infectious nymphs, while adults are a minor part of the population, confirming the crucial role of nymphs in transmission. In the full community there was evidence of susceptible host regulation (the number of infectious hosts decreased compared to the two-host scenario), but the presence of shrews did cause an increase in infectious nymphs. By contrast, in the alternative system, competence was essential in determining the transmission among ticks, with all the different competence parameters contributing to the infection of nymphs; therefore, it was visually impossible to display any pattern. However, with regards to transmission among hosts, the parameter most influencing the number of infectious was competence of transmission from nymph to rodent. A low value of this parameter was sufficient to reach the maximum number of infectious hosts, as the nymphal life stage is the most numerous among infectious ticks. Also in this case there was evidence of dilution through susceptible host regulation by predation in both systems. It seemed that, due to the higher proportion of wasted bites, competence of transmission was crucial in regulating the pathogen circulation in this system. Finally, the increase in nymphal competence of transmission to rodents decreased the number of infectious *I. ricinus* nymphs because this increased the number of infectious adults, without altering the total vector prevalence.

So, it seemed that the parameters most affecting the juvenile stages of the ticks were the ones most affecting transmission, and this has been previously observed by Van Buskirk and Ostfeld (1995). They demonstrated that the density of hosts for adult ticks impacted transmission only at very low densities of hosts for juvenile ticks, and provided evidence that Lyme disease risk was more efficiently decreased controlling small mammal populations not ungulates.

The results provide evidence that in the system with the more generalist vector, *I. ricinus*, the dilution effect was more significant and more mechanisms were represented, and this is in agreement with the classic assumptions about dilution formulated by Schmidt and Ostfeld (2001). The modelling study was also useful to identify the key parameters regulating transmission in the two systems. Taking into account the more complex community, moulting success seemed to be crucial in the case where the tick species had a smaller population and fewer alternative hosts, because transmission was limited by the number of ticks. Whereas, for *I. ricinus*, the regulating factor was competence of transmission, since this species had to maximise the efficiency of transmission of the lower proportion of feeding events on competent hosts. Consequently, in the two systems the key dilution mechanisms were also different: transmission reduction for *I. ricinus*-*B. burgdorferi*, and susceptible host regulation for *I. trianguliceps*-*B. microti*. Furthermore, the parameters most affecting the systems were among the most uncertain, demonstrating that more empirical studies are needed to estimate the most relevant parameters for these systems and so improve model prediction power. This modelling approach may be useful to better direct and design field studies, according to the system under consideration.

Transmission reduction due to the presence of fewer, or non-competent, hosts was the basic mechanisms of dilution described by the majority of the studies about vector-borne pathogens (e.g. Levi *et al.*, 2016; LoGiudice *et al.*, 2008; Ostfeld and LoGiudice, 2003). This was observed in this study, but was only relevant to the number of infectious hosts because the increase in host (for the vector) abundance also enhanced vector abundance, and subsequently

infectious vector abundance, as shown by other modelling works (Roche *et al.*, 2013; Ruyts *et al.*, 2018). Nevertheless, this phenomenon was overlooked by many studies, as vector prevalence, in the presence of more alternative hosts, decreased producing spurious dilution (Dobson and Auld, 2016). Considering dilution in the host, shrew population dilution power has been observed empirically, as shrews often possess higher tick burdens than rodents, representing an important source of wasted bites for the tick population, although in North America shrew species have been found to be fairly competent to transmit *B. burgdorferi* (Halsey *et al.*, 2018). Nevertheless, shrew species are typically less abundant than rodents, and therefore their abundance, degree of competition with rodents, and degree of competence will strongly determine their role in pathogen transmission, so a better understanding, at a local scale, is needed to draw definitive conclusions.

The second mechanism of dilution, observed in both systems, was susceptible host regulation, mainly due to predation (a minor reduction of rodent populations came from the competition with the shrew population, but that parameter was chosen conservatively since it was very uncertain). In agreement with this finding, predation has been previously observed to lower the density of reservoir-competent hosts (Levi *et al.*, 2012; Ostfeld and Holt, 2004). In addition, it has also been found to have other indirect effects. Predators might reduce the density of infectious nymphs (DIN) via non-lethal effects on prey as many prey species show decreased movement and increased refuging behaviour; predator activity has been found to be negatively correlated with rodent larval burden, and therefore with DIN, and, finally, animals that move more, acquiring more ticks, are more easily predated upon (selective predation on highly infested animals) (Hofmeester *et al.*, 2017). Moreover, predators, such as mustelids and foxes, are not heavily parasitised by ticks (Lorusso *et al.*, 2011; Meyer-Kayser *et al.*, 2012) so they do not contribute markedly to the increase in tick population.

In summary, dilution or amplification effects might not be mutually exclusive and depend on the metric under consideration, which has to be selected

according to the aim of the study (e.g. public health or wildlife management/conservation) (Dobson and Auld, 2016; Lou *et al.*, 2014). In the presented cases more complex communities led to fewer infectious hosts providing evidence for dilution, but also led to an amplification of human disease risk due to the increase of density of infectious nymphs. Still, with regards to the *I. trianguliceps-B. microti* system the quantification of human disease risk is challenging because *I. trianguliceps* is present at lower densities than *I. ricinus*, and has not been found involved in zoonotic transmission yet (Kovalevskii *et al.*, 2013). Nonetheless, *B. microti* has been isolated in *I. ricinus* (e.g. Abdullah *et al.*, 2018), and the two tick species may be sympatric, so where they occur together, *I. ricinus* might amplify human disease risk as it happens for the system *B. microti-I. spinipalpis-I. scapularis* in North America (Castro *et al.*, 2001).

Finally, the model did not take into consideration the presence of multiple species of vectors, and pathogen co-infection, which could be included in a further development of the model. Although in a study investigating *Anaplasma phagocytophilum* (another rodent-borne pathogen), infection probability in ticks was not related to tick species richness, diversity, or evenness (Foley and Piovia-Scott, 2014), tick species have been often reported to have differential transmission competence (e.g. as illustrated for *B. burgdorferi* and *B. microti*). In addition, pathogens can facilitate or inhibit different pathogen infection; for example, the tick *I. scapularis* has been infected with *B. microti* and *B. burgdorferi* more often than expected by chance, whereas the opposite has been observed with *A. phagocytophilum* and *B. microti* (Diuk-Wasser *et al.*, 2016; Hersh *et al.*, 2014b).

5.4 Flea-borne pathogen model: is the dilution effect detectable in presence of reinfection and vertical transmission?

As previously, a deterministic multi-host compartmental model constituted by a set of differential equations (Eq. 1-9) was employed to investigate the dynamics of a flea-borne pathogen. All the results were produced using the function rk4 in the R package deSolve (R Core Team, 2016) which is based on the classical Runge-Kutta 4^{th} order integration. Also this model was not explicitly spatial; the area, 1 ha, was considered constant since it was constrained by the sampling unit (Begon *et al.*, 2002), so the populations were expressed in individuals/ha and the parameters were scaled accordingly. The dilution effect was tested by assembling a progressively more complex community: host-species, competitor and predator species were added in turn following realistic assembly rules.

In this case the pathogen chosen for the simulation was *Bartonella* sp., which was isolated in the fleas collected in this study (see Chapter 4). *Bartonella* is a gram-negative bacterium that is transmitted via fleas (and probably other vectors), of which several species have been commonly found to infect rodent species (Birtles *et al.*, 2000; Telfer *et al.*, 2007a,c; Paziewska *et al.*, 2012). These species do not seem to be associated with a particular flea assemblage, but it has been discovered that a great variety of strains have different infectivity among rodents, as they do not develop full cross-immunity, so they can get re-infected by a different strain (Gutiérrez *et al.*, 2015; Kosoy, 2010). Further, *Bartonella* strains displayed some degree of species-specificity among rodents (Withenshaw *et al.*, 2016), and vertical transmission has also been observed (Brook *et al.*, 2017; Kosoy *et al.*, 1998; Morick *et al.*, 2013). This host-vector-pathogen system has been chosen with the aim of testing whether the dilution mechanisms observed in the previous sections were still detectable in a vector other than tick, a specialist of rodents, and in the case of a pathogen that does not cause life-long immunity, and also presents vertical transmission.

The host community for both pathogen and vector was represented by ground-dwelling small rodents, and, in particular, the model was parameterised where possible with data collected during live trapping (see Chapter 2). The rodent species considered were wood mouse and bank vole. *Sorex* ssp. shrews were added to the community as a sympatric competitor non-host species, since evidence of their presence was found in the sites sampled (Eq. 8). *Bartonella* species have been isolated in shrews, but they have been hypothesised to be specific to shrew species (Bray *et al.*, 2007), therefore in the model it was assumed that they were not competent in transmitting in rodent species. Also, shrews were not considered hosts for rodent fleas, as very little information is available about flea-shrew dynamics, and it was assumed that they did not share the same flea assemblages. Finally, predation was introduced with two terms representing generalist (e.g. avian predation) and specialist (mustelids) predation (Eq. 9). Community assembly was performed starting from one host species, then adding a second host species, and finally including the competitor species (shrew) and the predation terms.

Similarly to the previous models, rodent and shrew populations were modelled according to the Lotka-Volterra system, namely they followed a logistic growth tending to species-specific carrying capacity and limited by intra-specific density dependent reduction and inter-specific competition (Lotka, 1925; Hanski *et al.*, 1993; Volterra, 1926). Inter-specific competition among rodents, and among rodents and shrews was represented by a density dependent competition term (Huitu *et al.*, 2004; O'Regan *et al.*, 2015; Turchin and Hanski, 1997). Rodent species were considered better competitors than shrew species since shrew density has been observed to be negatively correlated with rodent density (Henttonen *et al.*, 1989; Huitu *et al.*, 2004). These species were all predated upon, but rodent species were considered preferential prey (Korpimaki and Norrdahl, 1989; Korpimaki, 1992). Generalist predation was modelled according to the alternative prey hypothesis (Holling type III functional response), while specialist predator population was modelled according to the Holling type II functional response based on the Rosenzweig-MacArthur model (1963) with no preference among rodents (Elton, 1942;

Erlinge, 1975; Hanski and Henttonen, 1996; Holling, 1959; Krebs and Myers, 1974; Turchin and Hanski 1997). Parameters associated with specialist predation were referred to *Mustela nivalis* (least weasel), which was the most common and widespread mustelid across sampled sites. For details about parameters estimation see next section (5.4.1). The time scale of the simulations was 20 years, and one day was the basic time step.

The epidemiological structure of the model followed the one proposed by Anguelov and colleagues (Anguelov *et al.*, 2010; Brettschneider *et al.*, 2012); the vector was split into three compartments, susceptible, exposed, infectious (SEI), while to the host population was also added the recovered compartment (SEIR). It was assumed that flea recruitment was determined by host abundance, and limited by a seasonal carrying capacity (Eq. 12) that regulated flea birth and death rate over time (Eq. 10-11) (Anguelov *et al.*, 2010; Brettschneider *et al.*, 2012). The transition between maximum abundance (spring/summer as found in field), and low abundance (autumn/winter) was set at ~20% and modelled using the periodic environmental carrying capacity, which also comprised the average flea infestation for each species. The epidemiologically relevant population consisted of adult fleas, since the larvae are rarely parasitic and feed on debris in the host burrows (Krasnov *et al.*, 2004a). Fleas were assumed to remain infectious for their entire life span (~1 year) (Brettschneider *et al.*, 2012), but no vertical transmission in fleas was included (Morick *et al.*, 2010). Further, transmission only occurred through the vector, i.e. no horizontal transmission occurred among rodents (Bown *et al.*, 2004; Colton *et al.*, 2011), and vertical transmission was assumed to be possible (Kosoy *et al.*, 1998; Morick *et al.*, 2013). *Bartonella* infection has not been reported to cause increased mortality or any other effect on vector and host population dynamics (Birtles *et al.*, 2001; Jones *et al.*, 2008b). The force of infection parameter combined the reservoir competence and infectivity, while the contact rate was determined by host-vector dynamics.

Initial density for each host-species was set at the average value found from field data (Chapter 2) (pooled sites excluding Skomer). The inoculum was

represented by a single infectious individual. Model equations are reported below, and variables and parameters are listed in Table 5.6.

$$\frac{dS_f}{dt} = \nu_f(t) - \lambda_f\left(\frac{(mf_iI_i+mf_jI_j)}{(mf_iN_i+mf_jN_j)}\right)S_f - \rho_f(t)S_f \quad \text{Eq. 1}$$

$$\frac{dE_f}{dt} = \lambda_f\left(\frac{(mf_iI_i+mf_jI_j)}{(mf_iN_i+mf_jN_j)}\right)S_f - \left(\kappa_f + \rho_f(t)\right)E_f \quad \text{Eq. 2}$$

$$\frac{dI_f}{dt} = \kappa_f E_f - \rho_f(t)I_f \quad \text{Eq. 3}$$

$$\frac{dS_i}{dt} = r_iN_w\left(1 - \frac{(N_i - c_{ji}N_j - c_{nj}N_n)}{K_i}\right) + \gamma_i\sigma_iI_i - \lambda_i\left(\frac{mf_iI_f}{(mf_iN_i+mf_jN_j)}\right)S_i$$
$$-\varepsilon_ir_iN_w\left(1 - \frac{(N_i - c_{ji}N_j - c_{nj}N_n)}{K_i}\right)I_i - \frac{(gS_i^2)}{(S_i^2+h^2)} - \frac{(\alpha N_pS_i)}{\left(\Delta_i+N_i+\frac{\Delta_i}{\Delta_j}N_j+\cdots\frac{\Delta_i}{\Delta_n}N_n\right)} \quad \text{Eq. 4}$$

$$\frac{dE_i}{dt} = \lambda_i\left(\frac{mf_iI_f}{(mf_iN_i+mf_jN_j)}\right)S_i - \kappa_iE_i - \frac{(gE_i^2)}{(E_i^2+h^2)} - \frac{(\alpha N_pE_i)}{\left(\Delta_i+N_i+\frac{\Delta_i}{\Delta_j}N_j+\cdots\frac{\Delta_i}{\Delta_n}N_n\right)} \quad \text{Eq. 5}$$

$$\frac{dI_i}{dt} = \kappa_iE_i + \varepsilon_ir_iN_w\left(1 - \frac{\left(N_i - c_{ji}N_j - c_{nj}N_n\right)}{K_i}\right)I_i - \sigma_iI_i - \frac{\left(gI_i^2\right)}{\left(I_i^2+h^2\right)}$$
$$-\frac{(\alpha N_pI_i)}{\left(\Delta_i+N_i+\frac{\Delta_i}{\Delta_j}N_j+\cdots\frac{\Delta_i}{\Delta_n}N_n\right)} \quad \text{Eq. 6}$$

$$\frac{dR_i}{dt} = \sigma_iI_i - \frac{(gR_i^2)}{(R_i^2+h^2)} - \frac{(\alpha N_pR_i)}{\left(\Delta_i+N_i+\frac{\Delta_i}{\Delta_j}N_j+\cdots\frac{\Delta_i}{\Delta_n}N_n\right)} \quad \text{Eq. 7}$$

$$\frac{dN_n}{dt} = (\nu_n - \rho_n)_i N_n \left(1 - \frac{\left(N_n - c_{in}N_i - \dots c_{jn}N_j\right)}{K_c}\right) - \frac{(gN_n^2)}{(N_n^2 + h^2)}$$

$$- \frac{(\alpha_s N_p N_n)}{\left(\Delta_n + N_n + \frac{\Delta_n}{\Delta_i}N_i + \cdots \frac{\Delta_n}{\Delta_j}N_j\right)} \qquad \text{Eq. 8}$$

$$\frac{dN_p}{dt} = \left(\nu_p - \rho_p\right)N_p \left(1 - \frac{qN_p}{\left(N_i + \frac{\Delta_i}{\Delta_j}N_j + \cdots \frac{\Delta_i}{\Delta_n}N_n\right)}\right) \qquad \text{Eq. 9}$$

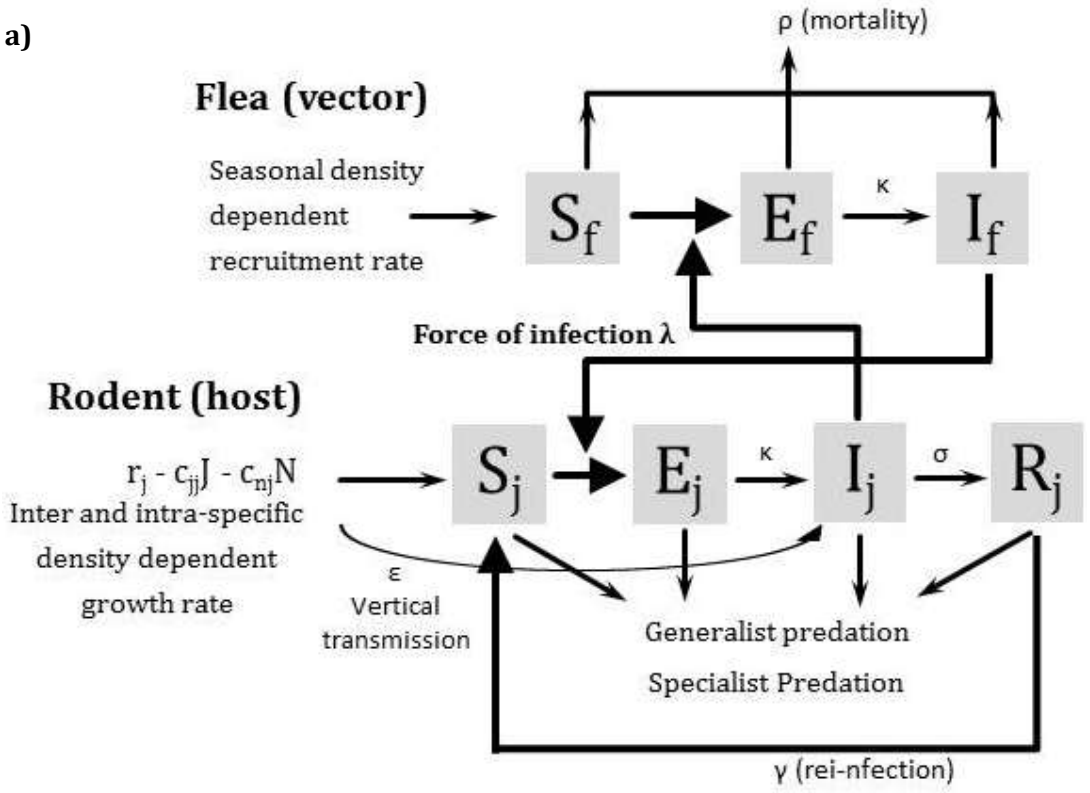
$$\nu_f(t) = \rho_f K_f(t) + \nu_f max\left(K_f(t) - N_f, 0\right) \qquad \text{Eq. 10}$$

$$\rho_f(t) = \rho_f + \nu_f max\left(1 - \frac{K_f(t)}{N_f}, 0\right) \qquad \text{Eq. 11}$$

$$K_f(t) = \left(mf_i N_i + mf_j N_j\right) min\left(1.1, max(0.65 + \cos 2\pi t, 0.2)\right) \qquad \text{Eq. 12}$$

Fig. 5.26. Flea-borne disease compartmental model a) and female flea life cycle b). Boxes represent epidemiological compartments in which each population is subdivided: S = susceptible, E = exposed (not infectious), I = infectious, R = recovered. F (vector population density) = S+E+I, J (host population density) = S+E+I+R. Subscripts: f = flea, j = rodent species (host), n = non-host competitor (shrew species). r = growth rate (determined by competition and density of rodent and shrew species), c = competition coefficient, N = non-host competitor population density, γ = reinfection rate, ε = rate of vertical transmission, κ = rate at which exposed became infectious (latent period), λ = force of infection, ρ = flea death rate, σ = recovery rate. Arrows indicate the direction of movement of individuals between classes and are labelled by the transition rates. Arrows pointing outside the boxes represent mortality. Vectors can feed only on J.

a)



b)

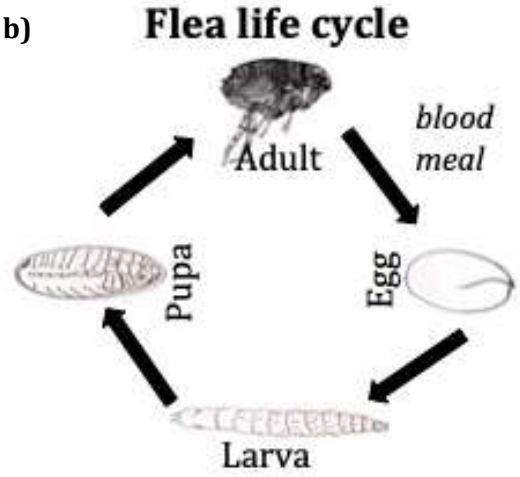


Table 5.6. List of model variables and parameters. For details about parameters estimation see section 5.2.1 and section 5.4.1.

Symbol	Description (source where relevant)	
S	Number of susceptible individuals	
E	Number of exposed individuals	
I	Number of infectious individuals	
R	Number of recovered individuals	
N	Total number of individuals (population size)	
i, j	Host-species for the pathogen: wood mouse; bank vole	
n	Non-host competitor species	
p	Specialist predator	
f	Vector (flea)	
r	Rodent growth rate	(this study)
c	Competition factor	(O'Regan *et al.*, 2015; this study)
g	Saturation rate of generalist predation	(Turchin and Hanski, 1997)
h	Prey density at which generalist predation rate is half of the maximum	(Turchin and Hanski, 1997)
q	Specialist predator-prey ratio constant	(Turchin and Hanski, 1997)
α	Maximum consumption rate of specialist predator	(Turchin and Hanski, 1997)
γ	Rate of reinfection	(Anguelov *et al.*, 2010; Brettschneider *et al.*, 2012)
Δ	Half-saturation constant (specialist predator)	(Turchin and Hanski, 1997)
ε	Rate of vertical transmission	(Anguelov *et al.*, 2010; Brettschneider *et al.*, 2012; Kosoy *et al.*, 1999)
K	Carrying capacity	(Bolzoni *et al.*, 2008; De Leo and Dobson, 1996)
κ	Rate at which exposed became infectious	(Anguelov *et al.*, 2010; Brettschneider *et al.*, 2012; Kosoy *et al.*, 1999; Morick *et al.*, 2013)
κ_f	Rate at which exposed became infectious fleas	(Anguelov *et al.*, 2010; Brettschneider *et al.*, 2012; Kosoy *et al.*, 1999; Morick *et al.*, 2013)
λ	Force of infection to rodent	(Anguelov *et al.*, 2010; Brettschneider *et al.*, 2012; Brook *et al.*, 2017)
λ_f	Force of infection to flea	(Anguelov *et al.*, 2010; Brettschneider *et al.*, 2012; Brook *et al.*, 2017)
mf	Maximum average flea burden	(this study)

Table 5.6 (continued). List of model variables and parameters. For details about parameters estimation see section 5.2.1 and section 5.4.1.

Symbol	Description (source where relevant)	
N	Birth rate	(Bolzoni *et al.*, 2008; De Leo and Dobson, 1996)
ν_f	Flea birth rate	(Anguelov *et al.*, 2010; Brettschneider *et al.*, 2012)
ρ	Death rate	(Bolzoni *et al.*, 2008; De Leo and Dobson, 1996)
ρ_f	Flea death rate	(Shrewsbury, 2005)
σ	Recovery rate	(Koesling *et al.*, 2001; Kosoy *et al.*, 1999)

5.4.1 Parameter estimation

All the parameters relating to host species, inter-specific competition and predation were estimated as in section 5.2 and section 5.3, and the values used for the simulations in this section are reported in Table 5.7, as no sensitivity analysis was performed on any of these parameters.

Flea birth and death rates were estimated according to Brettschneider *et al.* (2012) and Shrewsbury (2005), and used to parametrise time-dependent birth and death functions (Eq. 10-11). The average flea burden of infested individuals was estimated according to the empirical data in this study (see Chapter 3).

Pathogen-related parameters such as rate at which exposed became infected (κ) and recovery rate (σ) were taken from relevant literature (Table 5.6 and Table 5.7), while sensitivity analysis was performed on force of infection (both to flea and to host), rate of reinfection, and vertical transmission. The ranges of values used for sensitivity analysis are shown in Table 5.7, while the methodology of sensitivity analysis is described in section 5.4.2.

Table 5.7. List of starting conditions and parameter values used for the simulations. When a range of values is provided, sensitivity analysis has been performed (see section 5.4.2).

Symbol	Description	Value
N_w	Wood mouse population	49 (ind/ha)
N_b	Bank vole population	75 (ind/ha)
N_j	Shrew population	20 (ind/ha)
N_p	Weasel population	3 (ind/ha)
N_v	Flea population	100 (ind/ha)
cbw	Competition of wood mouse over bank vole, and shrew respectively	0.20
cjw		1.04
cwb	Competition of bank vole over wood mouse, and shrew respectively	0.20
cjb		1.03
cwj	Competition of shrew over wood mouse, and bank vole respectively	0.11
cbj		0.12
g	Saturation rate of generalist predation	0.49
h	Prey density at which generalist predation rate is half of the maximum	9.9
mf	Maximum average flea burden of infested individuals: wood mouse; bank vole	1 1.53
q	Specialist predator-prey ratio constant	56
r_{b+}, r_{b-}	Bank vole growth rate breeding season (+), and non-breeding season (-)	0.007 -0.002
r_{w+}, r_{w-}	Wood mouse growth rate breeding season (+); non-breeding season (-)	0.04-0.006
α	Maximum rodent consumption rate of specialist predator	1
α_s	Maximum shrew consumption rate of specialist predator	7.67
γ	Rate of reinfection (range)	0-1
Δ	Half-saturation constant (rodent)	11.31
Δ_s	Half-saturation constant (shrew)	22.62
ε	Rate of vertical transmission (range)	0-0.5
κ	Rate at which exposed became infectious	0.24
κ_f	Rate at which exposed became infectious fleas	0.26
λ	Force of infection to rodent (range)	0-3
λ_f	Force of infection to flea (range)	0-10
ν_f	Flea birth rate	0.03727
ρ_f	Flea death rate	0.00273
σ	Recovery rate	0.021

5.4.2 Sensitivity analysis

As mentioned in the previous section, sensitivity analysis has been performed on both vector and host force of infection. Force of infection to flea ranged from 0 to 10 (range found in the selected sources) and 21 intervals were input into the sensitivity between the minimum and the maximum. For each of these values, 100 force of infection to host values were randomly chosen between the minimum and the maximum (0 to 3) via Latin square sampling (Iman *et al.* 1981a,b). In this analysis, it was assumed there were no reinfection and no vertical transmission among rodents.

Systematic sampling was used for the sensitivity analysis of reinfection and vertical transmission rates; 33 intervals were input into the sensitivity between the minimum (0 = no effect) and the maximum values, which were 1 (all the infectious did get reinfected) and 0.5 (all the infectious individuals reproducing gave birth to infectious offspring) respectively.

5.4.3 Results

The sensitivity on the force of infection revealed, as expected, that the increase of this parameter (both flea and host force of infection) increased pathogen transmission, in this case represented by cumulative incidence and total infectious host over total time due to the periodic nature of this epidemic (absence of an endemic equilibrium) (Fig. 5.27 to Fig. 5.35). In general, the increase of the force of infection to flea induced a more marked effect on flea incidence, while the increase of the force of infection to rodents had a more obvious effect on host incidence. Starting from the single-host scenario (Fig. 5.27 to Fig. 5.29), transmission seemed to reach a maximum above specific values of force of infection, especially noticeable in the plot showing the total infectious hosts over time (Fig. 5.29). In this case, the increase of the force of infection to flea determined the magnitude of maximum transmission and the higher this parameter the lower force of infection to host required to reach this maximum (steeper curve with longer plateau).

The two-host community displayed the same patterns (Fig. 5.30 to Fig. 5.32), but incidence was overall slightly lower (for any λ value) compared to the previous scenario because transmission slowed down due to the host-vector dynamics (host-vector ratio) (Fig. 5.30 and Fig. 5.31). The increase of force of infection to bank vole had a moderately bigger impact on transmission, since bank vole had a higher flea load; in fact, this effect was more evident looking at the steeper increase of flea incidence on the force of infection to bank vole axis in Fig. 5.30. Pathogen transmission in this community, for any equivalent value of force of infection, led to a higher number of total infectious hosts (Fig. 5.32).

In the full community flea cumulative incidence showed the same patterns and values (Fig. 5.33) as the two-host scenario, but host incidence was lower than both previous assemblages (Fig. 5.34). Similarly, total infectious hosts over time, in this scenario, decreased compared to the two-host community, but were higher than in the single-host case (Fig. 5.35).

Generally, at force of infection values greater than two, the increase of incidence and total infectious hosts was progressively slower, especially with regards to host-related outputs, and in the more complex community. Therefore, for the following sensitivity analysis the force of infection (to flea and to host) value was set at one in order to model a moderate force of infection.

Considering the sensitivity analysis on the reinfection rate, in the single-host scenario, the greater this parameter the greater host incidence and total infectious hosts (Fig. 5.36b, c), but flea incidence reached a peak at $\gamma = 0.72$ and above that value gradually decreased (Fig. 5.36a). As expected from the previous results, in the two-host community, for any value of γ, flea and host incidence were lower than the previous scenario (Fig. 5.36d, e), while total infectious hosts showed the opposite trend (Fig. 5.36f). It seemed that the increase of wood mouse reinfection rate determined a slightly steeper increase of all the outputs taken into account. Assembling the full community, incidence and total infectious hosts went down compared to the two-host scenario (Fig. 5.36g, h, i), as it happened in the previous sensitivity analysis; and this effect was extremely evident in host related outputs (Fig. 5.36h, i). Host incidence values were lower than both previous assemblages, and total infectious hosts were not so remarkably higher than the single-host community. Further, with regards to these results, the difference between no reinfection and maximum reinfection rate was much lower than in the other two cases.

The vertical transmission rate sensitivity analysis was then performed assuming moderate force of infection ($\lambda = 1$) and moderate reinfection rate ($\gamma = 0.5$). There was a sigmoid relationship between ε and the model outputs displayed in Fig. 5.37, especially clear in the total infectious hosts (Fig. 5.37b, d, f). Analogously to the previous analyses, flea incidence, for any given value of vertical transmission rate, decreased along the gradient of assemblage complexity (Fig. 5.37a, c, e). Comparing the single-host to the two-host scenario, total infectious hosts values did not substantially differ, contrasting the previous analyses results, where a marked increase of infectious was observed with the addition of the second host. Also in this scenario it was clear that there was a maximum transmission level, and high values of vertical transmission

determined the plateauing of the total infectious hosts. Wood mouse population had a major effect in this case, and this can be observed by the steepness of the curve on the wood mouse axis in Fig 5.37c to f. In the full community, the number of total infectious hosts was lower than the previous scenarios only at values of vertical transmission below 0.4, while, above this threshold, the number showed a slight increase compared to the maximum transmission in the previous community assemblages. However, it was striking that the number of total infectious hosts for just moderate values of vertical transmission differed from previous analyses by almost one order of magnitude.

Fig. 5.27. Single-host scenario: sensitivity analysis on force of infection (λ). Cumulative incidence of fleas. a) to i) λ_f = 0.5, 1, 1.5, 2, 2.5, 3, 5, 7.5, 10. On the x-axis, rodent force of infection, λ, ranging from 0 to 3. Reinfection rate (γ) = 0; vertical transmission (ε) = 0.

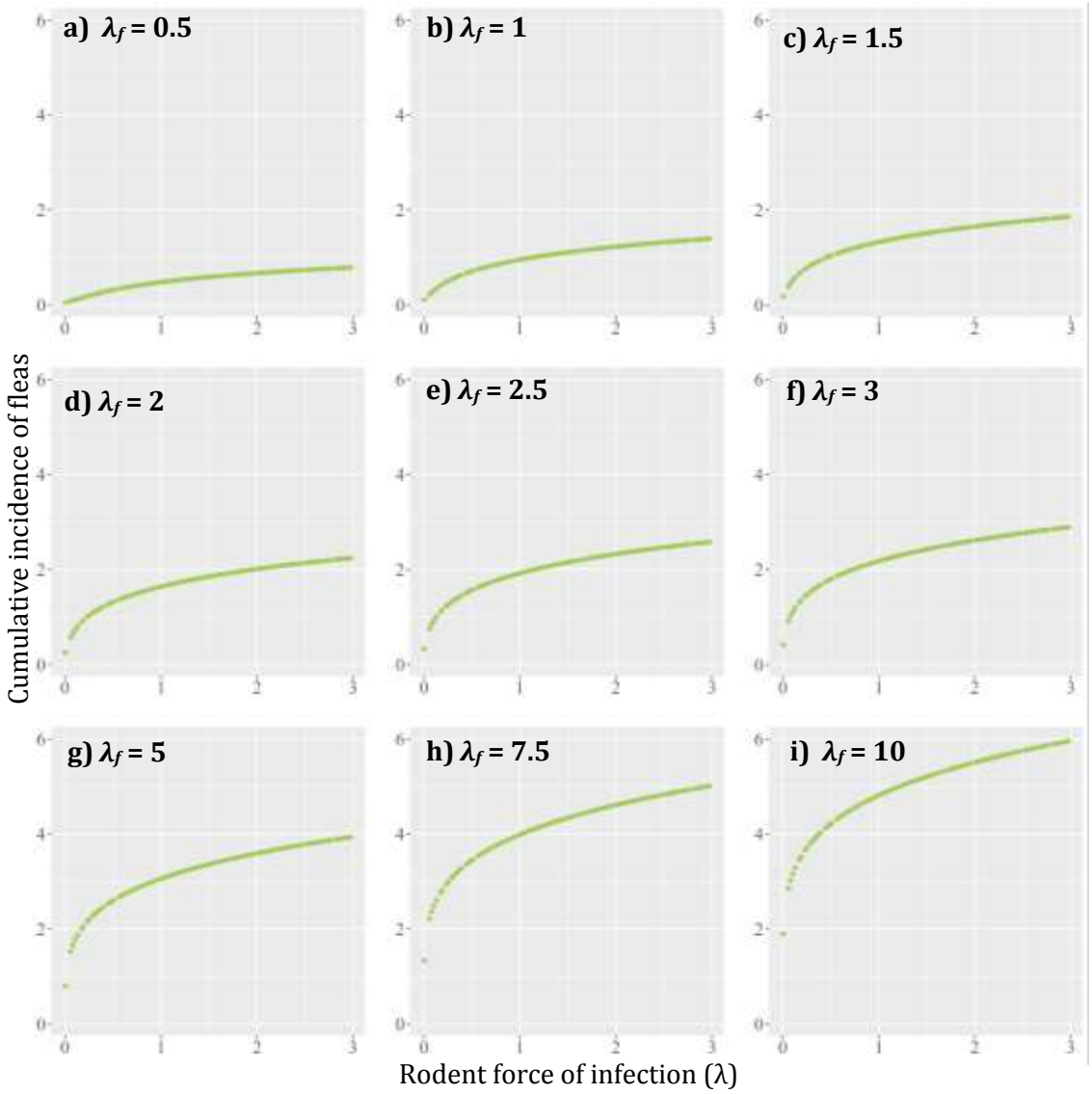


Fig. 5.28. Single-host scenario: sensitivity analysis on force of infection (λ). Cumulative incidence of hosts. a) to i) λ_f = 0.5, 1, 1.5, 2, 2.5, 3, 5, 7.5, 10. On the x-axis, rodent force of infection, λ, ranging from 0 to 3. Reinfection rate (γ) = 0; vertical transmission (ε) = 0.

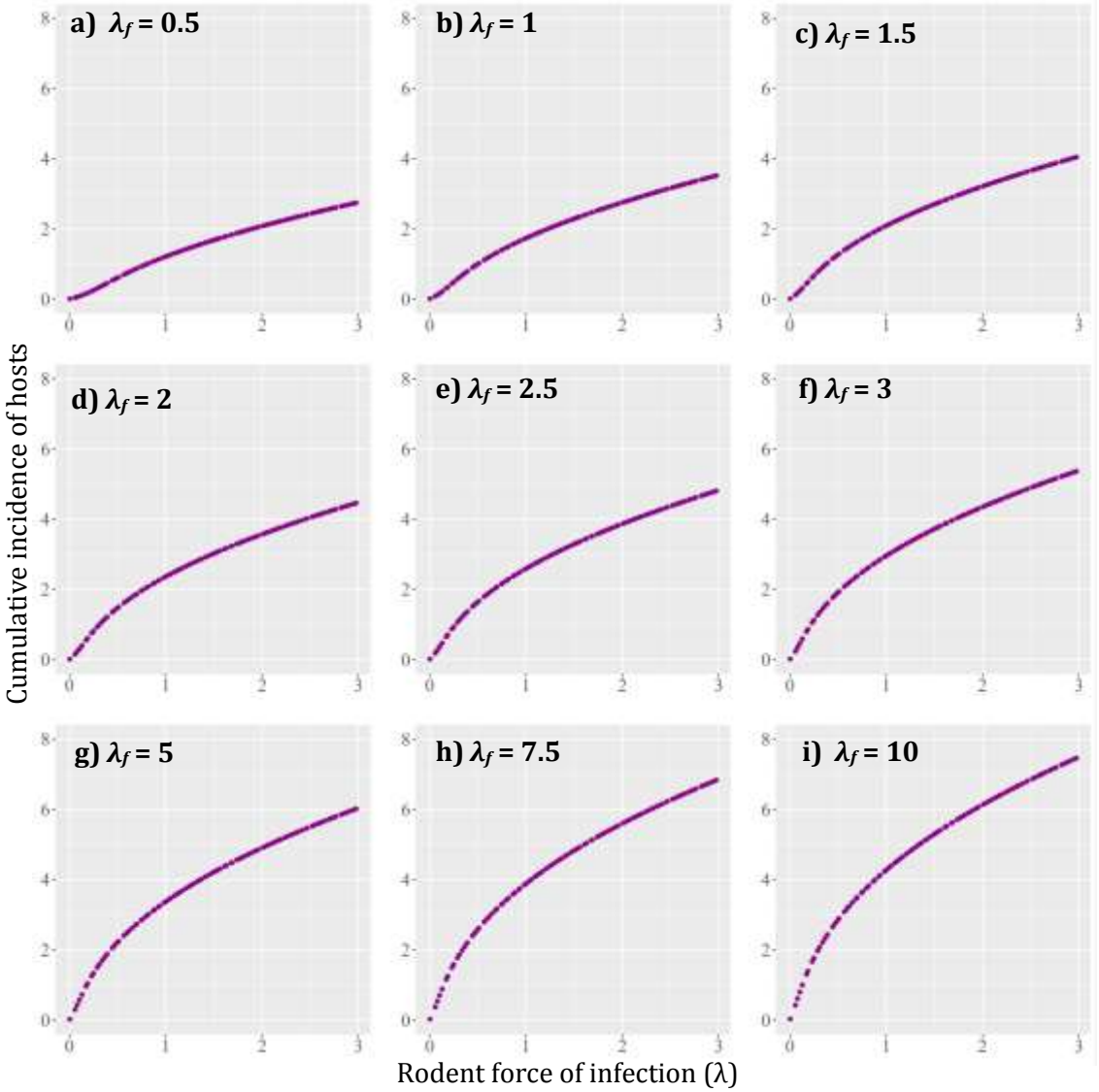


Fig. 5.29. Single-host scenario: sensitivity analysis on force of infection (λ). Total infectious hosts over total time (divided by 100000). a) to i) λ_f = 0.5, 1, 1.5, 2, 2.5, 3, 5, 7.5, 10. On the x-axis, rodent force of infection, λ, ranging from 0 to 3. Reinfection rate (γ) = 0; vertical transmission (ε) = 0.

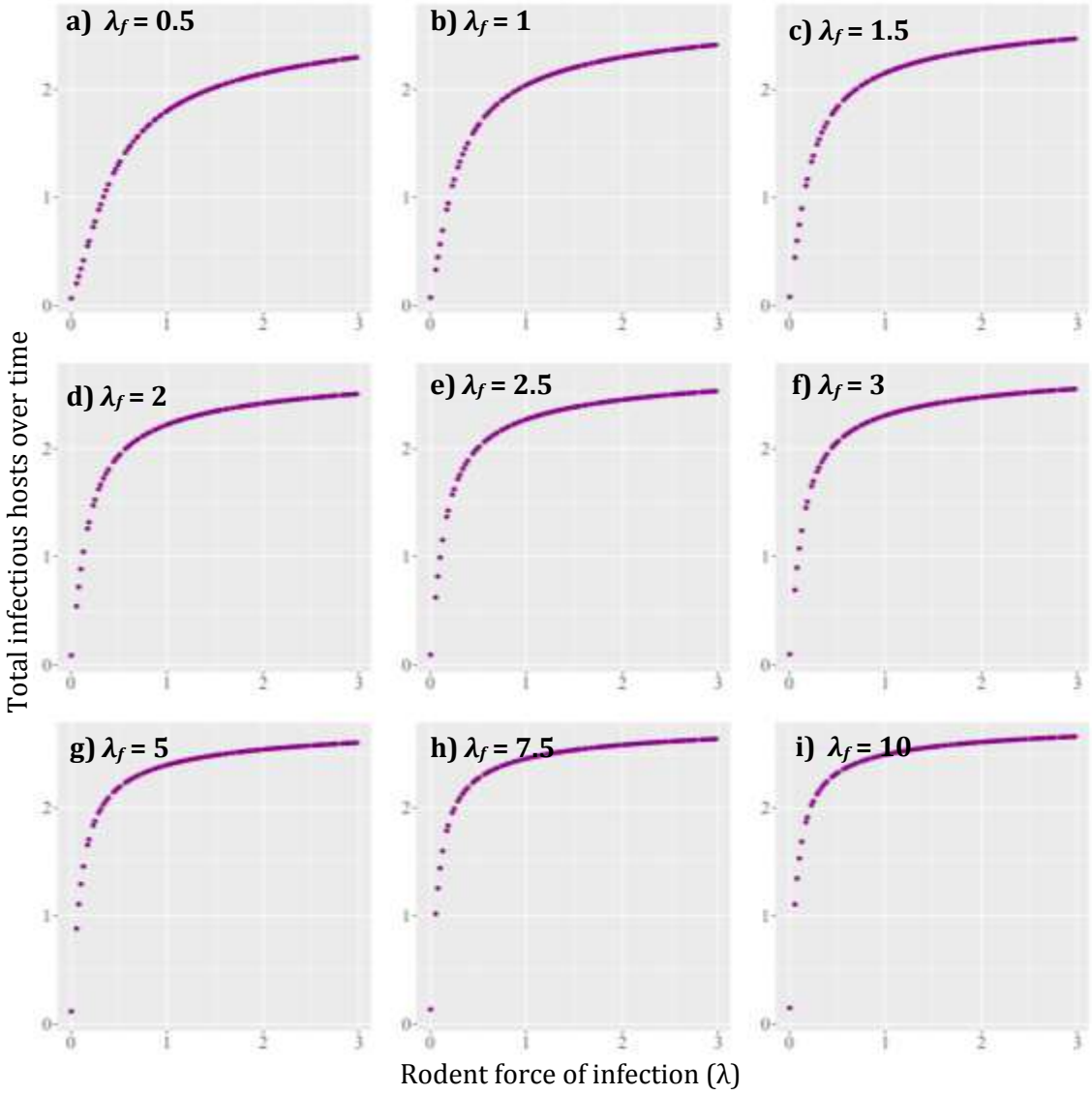


Fig. 5.30. Two-host scenario: sensitivity analysis on force of infection (λ). Cumulative incidence of fleas. a) to i) λ_f = 0.5, 1, 1.5, 2, 2.5, 3, 5, 7.5, 10. On the x-axis, rodent force of infection, λ, ranging from 0 to 3. Reinfection rate (γ) = 0; vertical transmission (ε) = 0. BV: bank vole; WM: wood mouse.

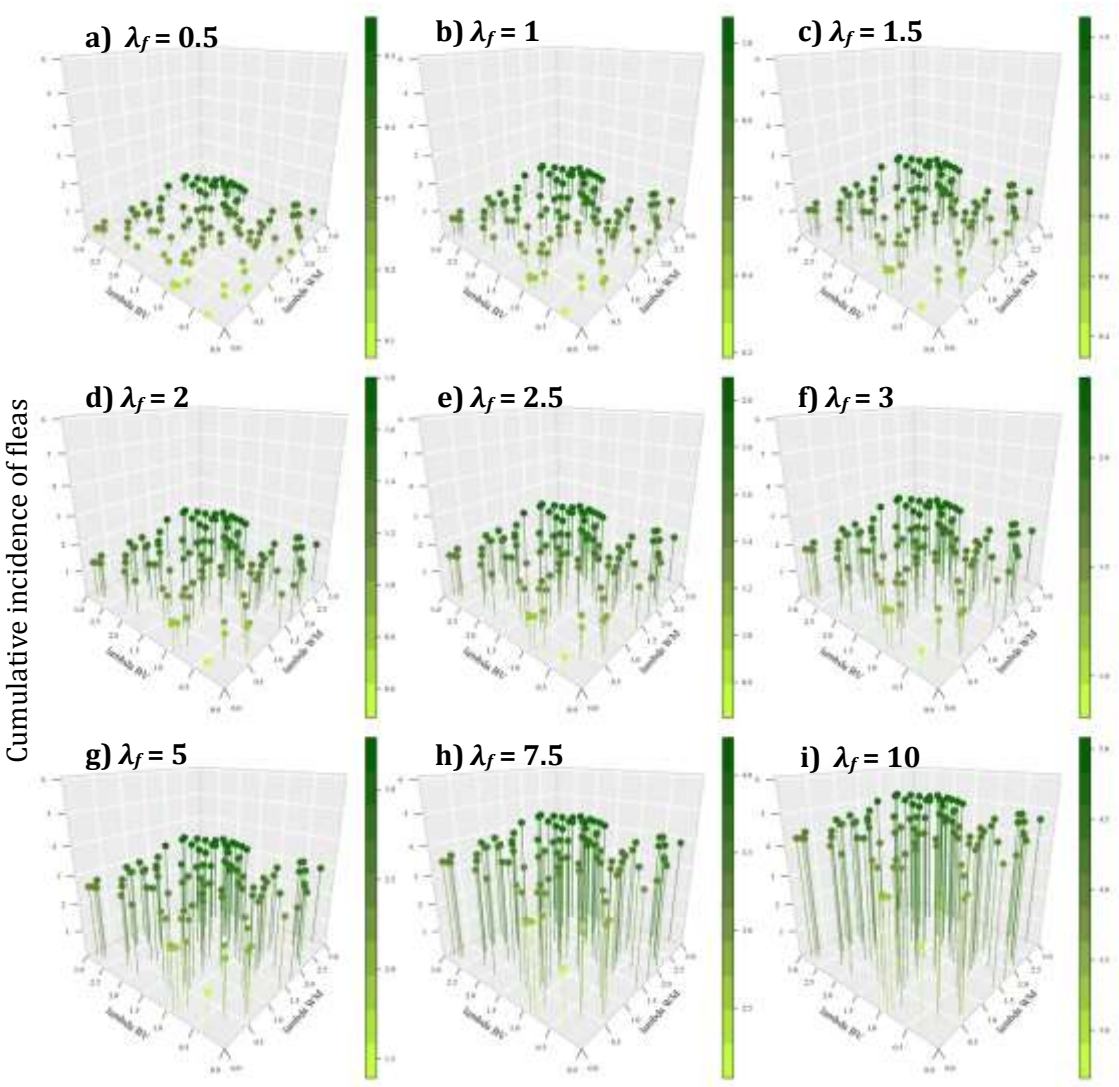


Fig. 5.31. Two-host scenario: sensitivity analysis on force of infection (λ). Cumulative incidence of hosts. a) to i) λ_f = 0.5, 1, 1.5, 2, 2.5, 3, 5, 7.5, 10. On the x-axis, rodent force of infection, λ, ranging from 0 to 3. Reinfection rate (γ) = 0; vertical transmission (ε) = 0. BV: bank vole; WM: wood mouse.

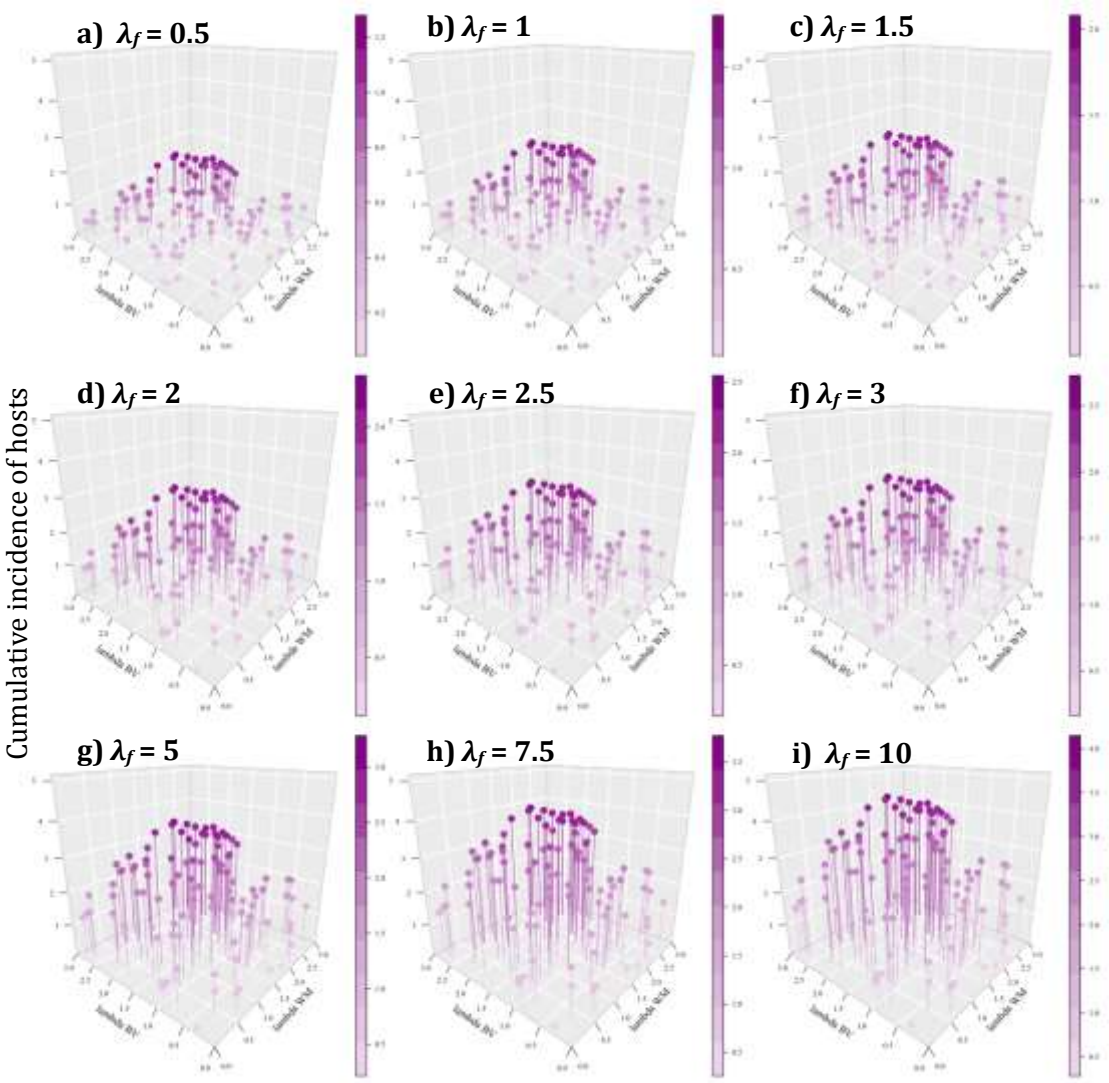


Fig. 5.32. Two-host scenario: sensitivity analysis on force of infection. Total infectious hosts over total time (divided by 100000). a) to i) λ_f = 0.5, 1, 1.5, 2, 2.5, 3, 5, 7.5, 10. On the x-axis, rodent force of infection, λ, ranging from 0 to 3. Reinfection rate (γ) = 0; vertical transmission (ε) = 0. BV: bank vole; WM: wood mouse.

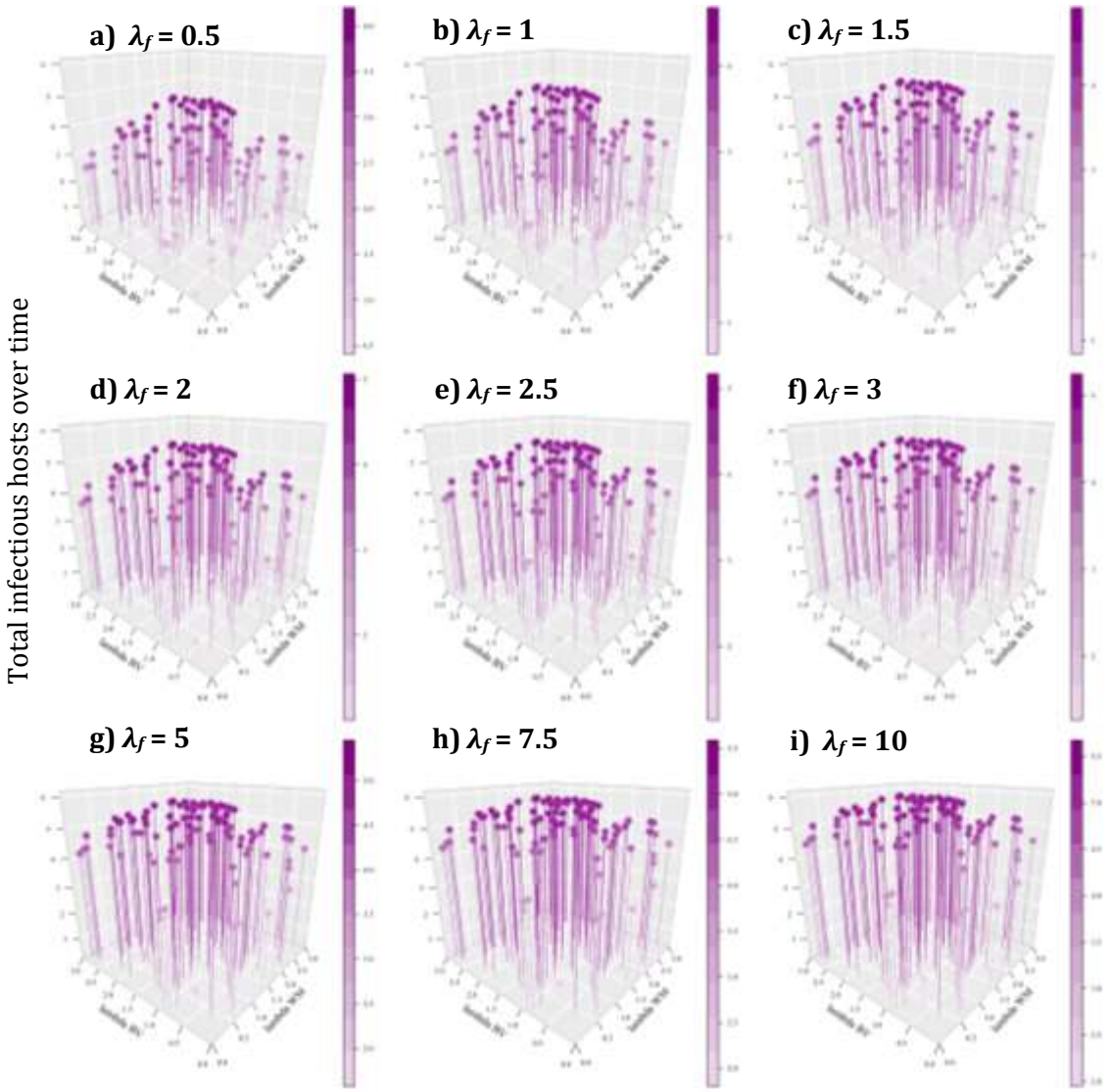


Fig. 5.33. Full community scenario: sensitivity analysis on force of infection. Cumulative incidence of fleas. a) to i) λ_f = 0.5, 1, 1.5, 2, 2.5, 3, 5, 7.5, 10. On the x-axis, rodent force of infection, λ, ranging from 0 to 3. Reinfection rate (γ) = 0; vertical transmission (ε) = 0. BV: bank vole; WM: wood mouse.

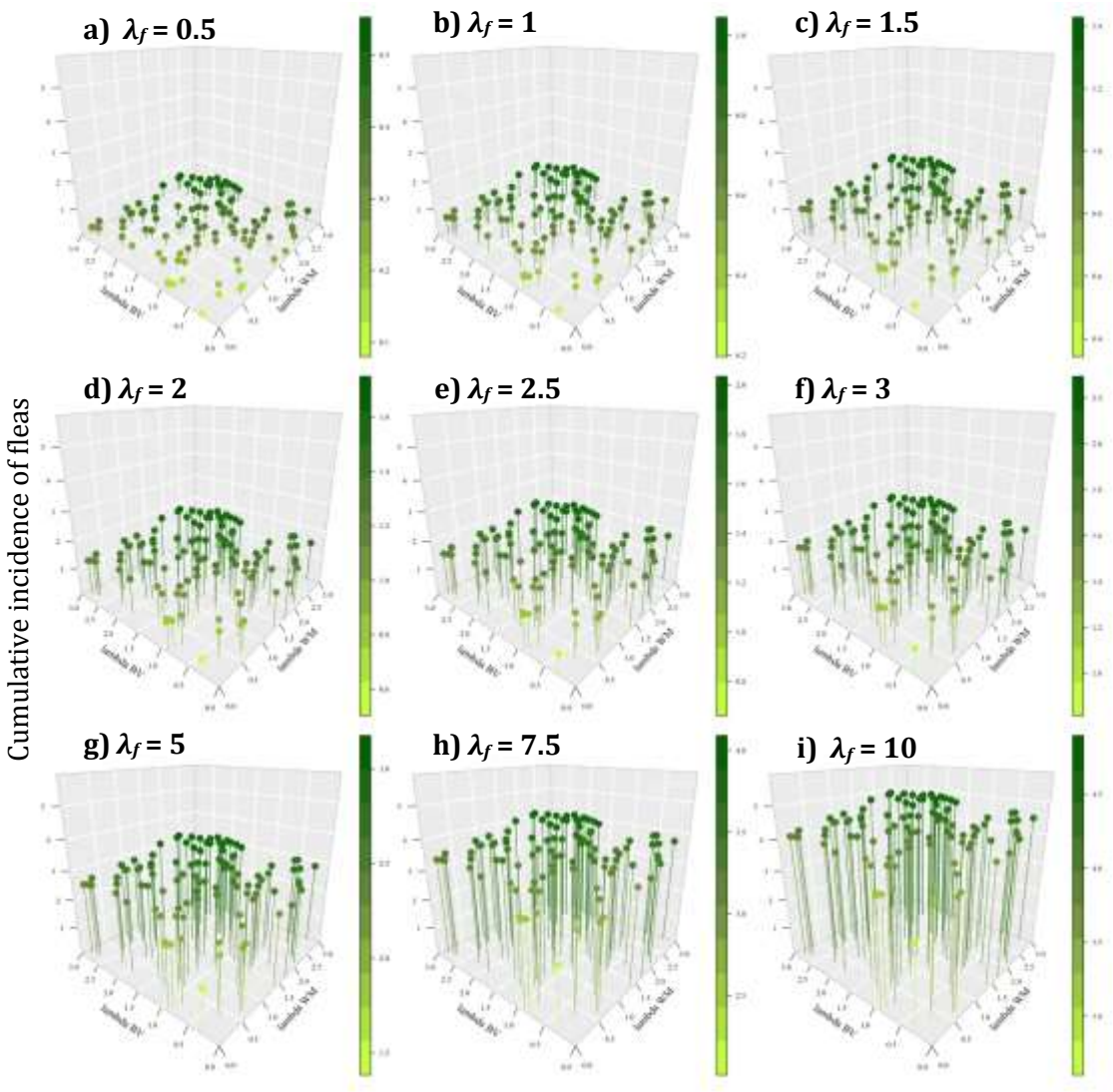


Fig. 5.34. Full community scenario: sensitivity analysis on force of infection. Cumulative incidence of hosts. a) to i) λ_f = 0.5, 1, 1.5, 2, 2.5, 3, 5, 7.5, 10. On the x-axis, rodent force of infection, λ, ranging from 0 to 3. Reinfection rate (γ) = 0; vertical transmission (ε) = 0. BV: bank vole; WM: wood mouse.

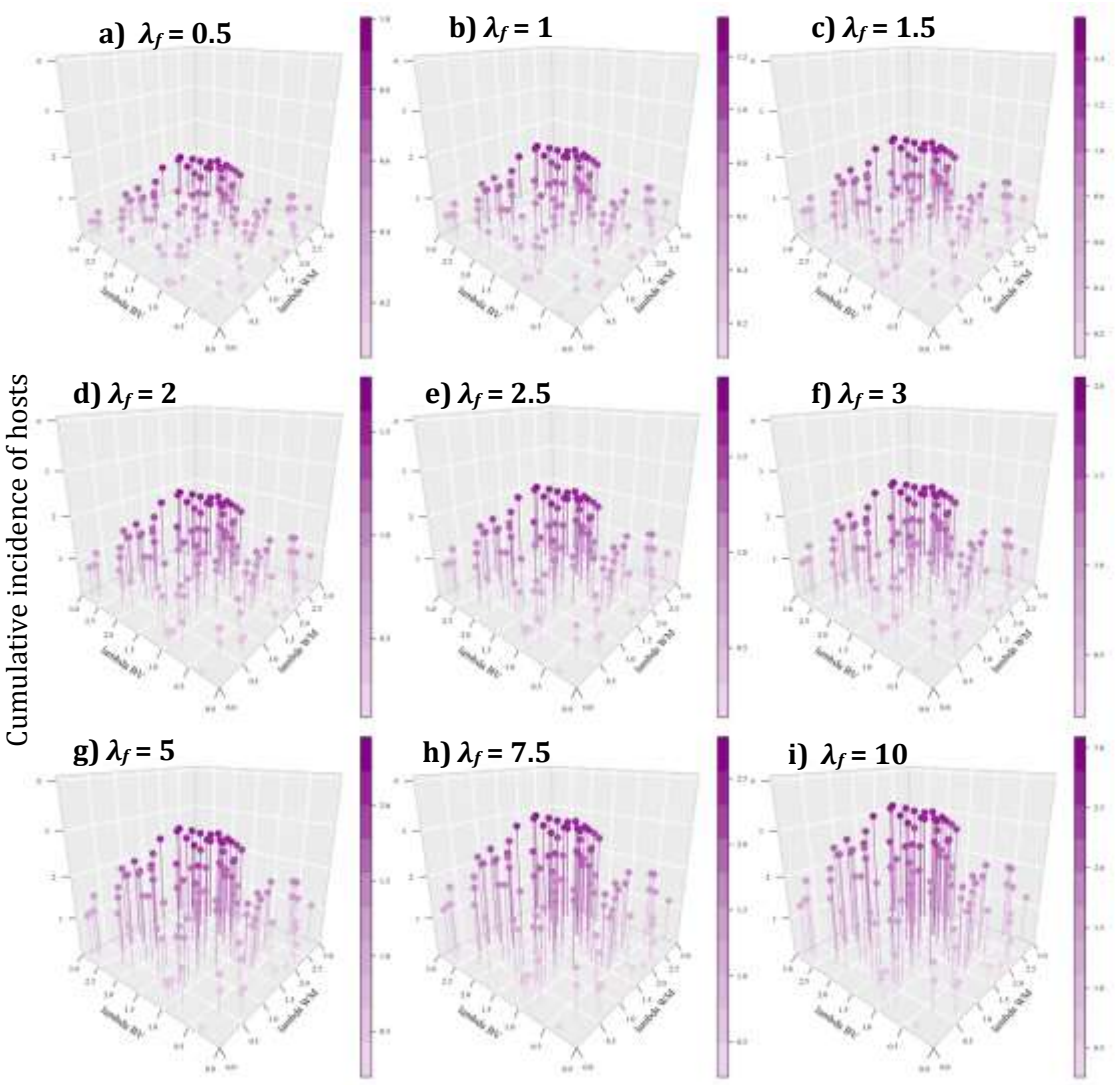


Fig. 5.35. Full community scenario: sensitivity analysis on force of infection. Total infectious hosts over total time (divided by 100000). a) to i) λ_f = 0.5, 1, 1.5, 2, 2.5, 3, 5, 7.5, 10. On the x-axis, rodent force of infection, λ, ranging from 0 to 3. Reinfection rate (γ) = 0; vertical transmission (ε) = 0. BV: bank vole; WM: wood mouse.

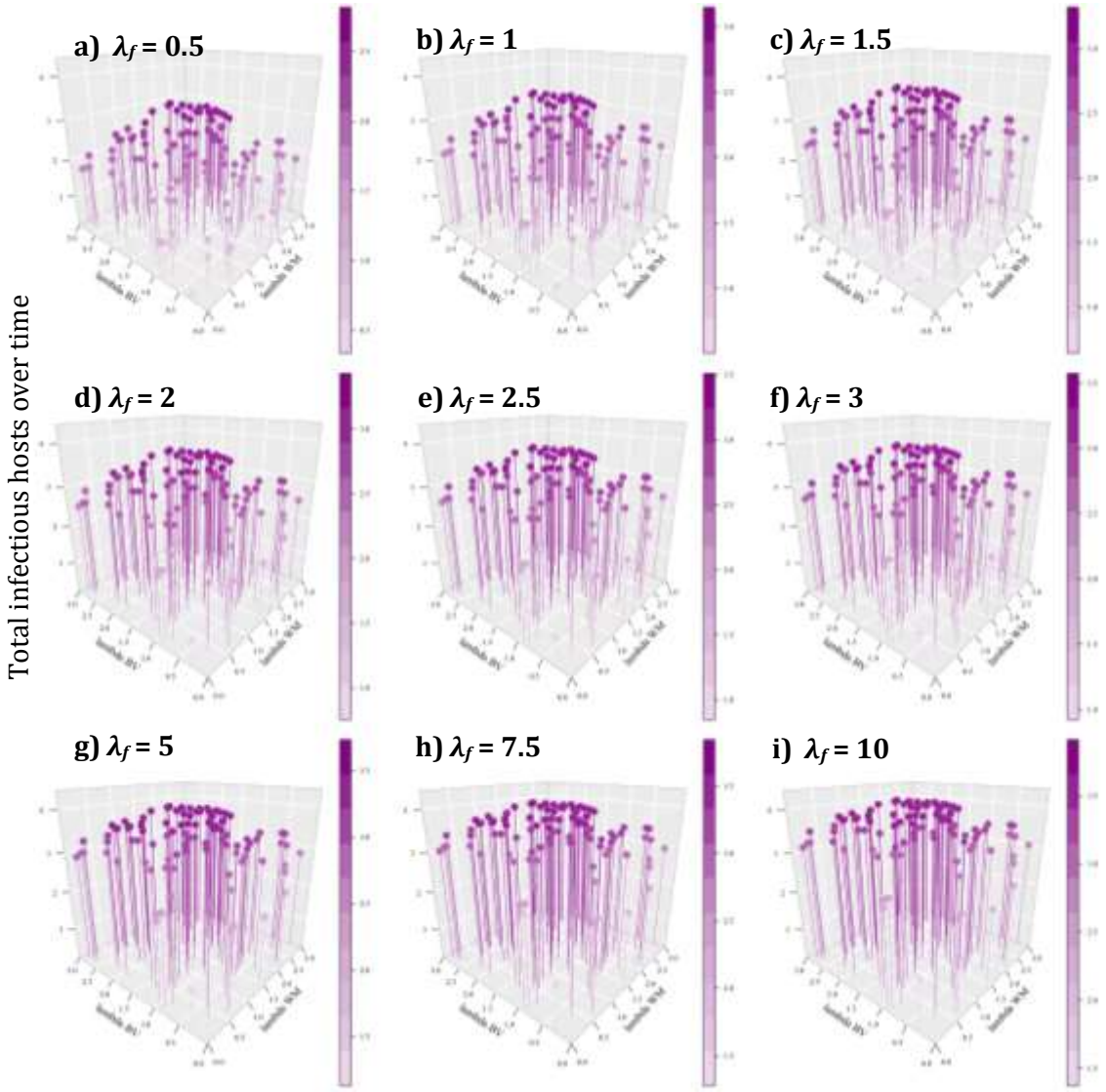


Fig. 5.36. Sensitivity analysis on reinfection rate (γ). Cumulative incidence of fleas (a, d, g), cumulative incidence of hosts (b, e, h), and total infectious hosts over total time (divided by 100000) (c, f, i) across different community assemblages: single-host scenario (a, b, c); two-host scenario (d, e, f); full community (g, h, i). Flea force of infection (λ_f) = 1; host force of infection (λ) = 1; vertical transmission (ε)= 0. BV: bank vole; WM: wood mouse.

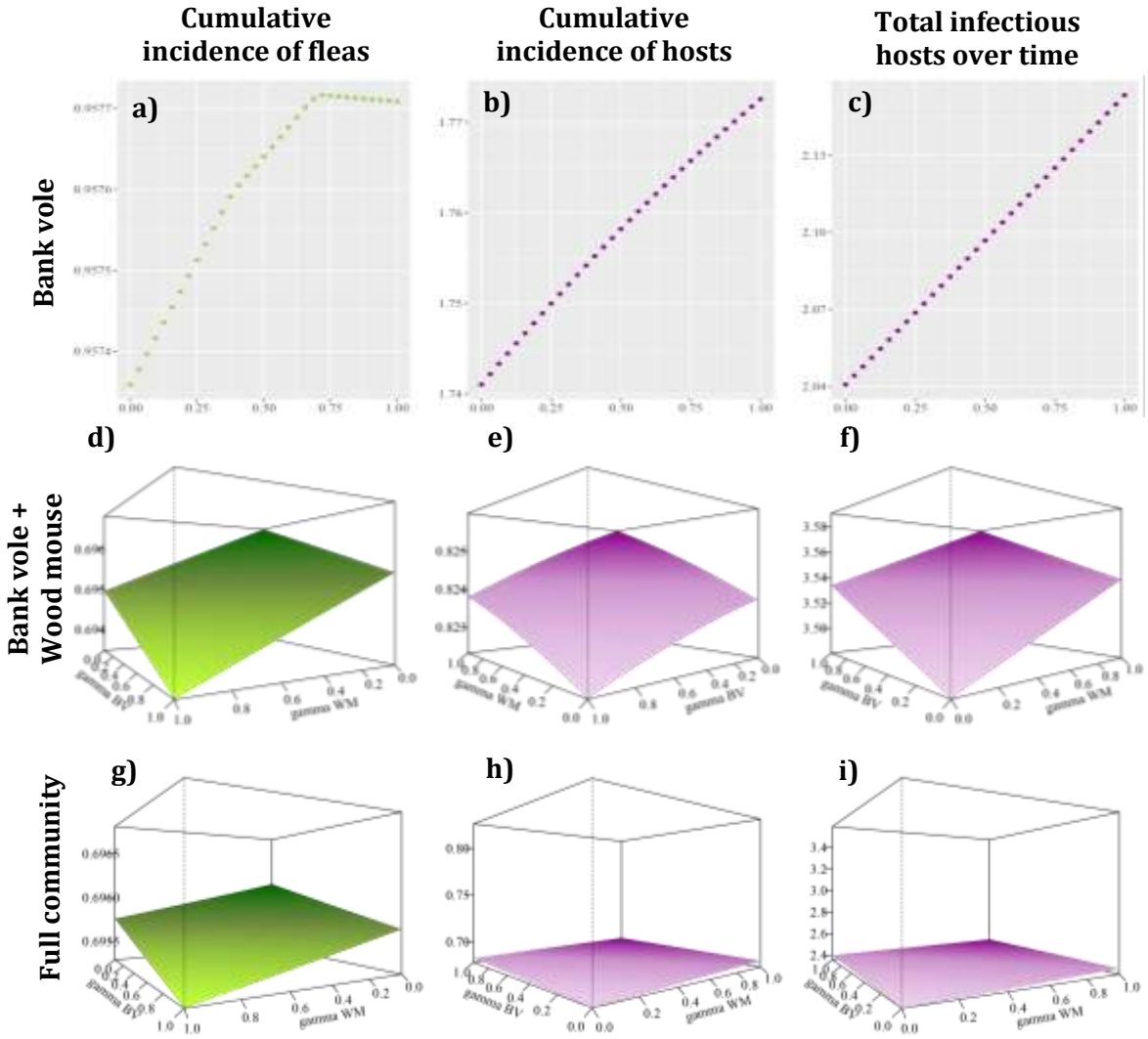
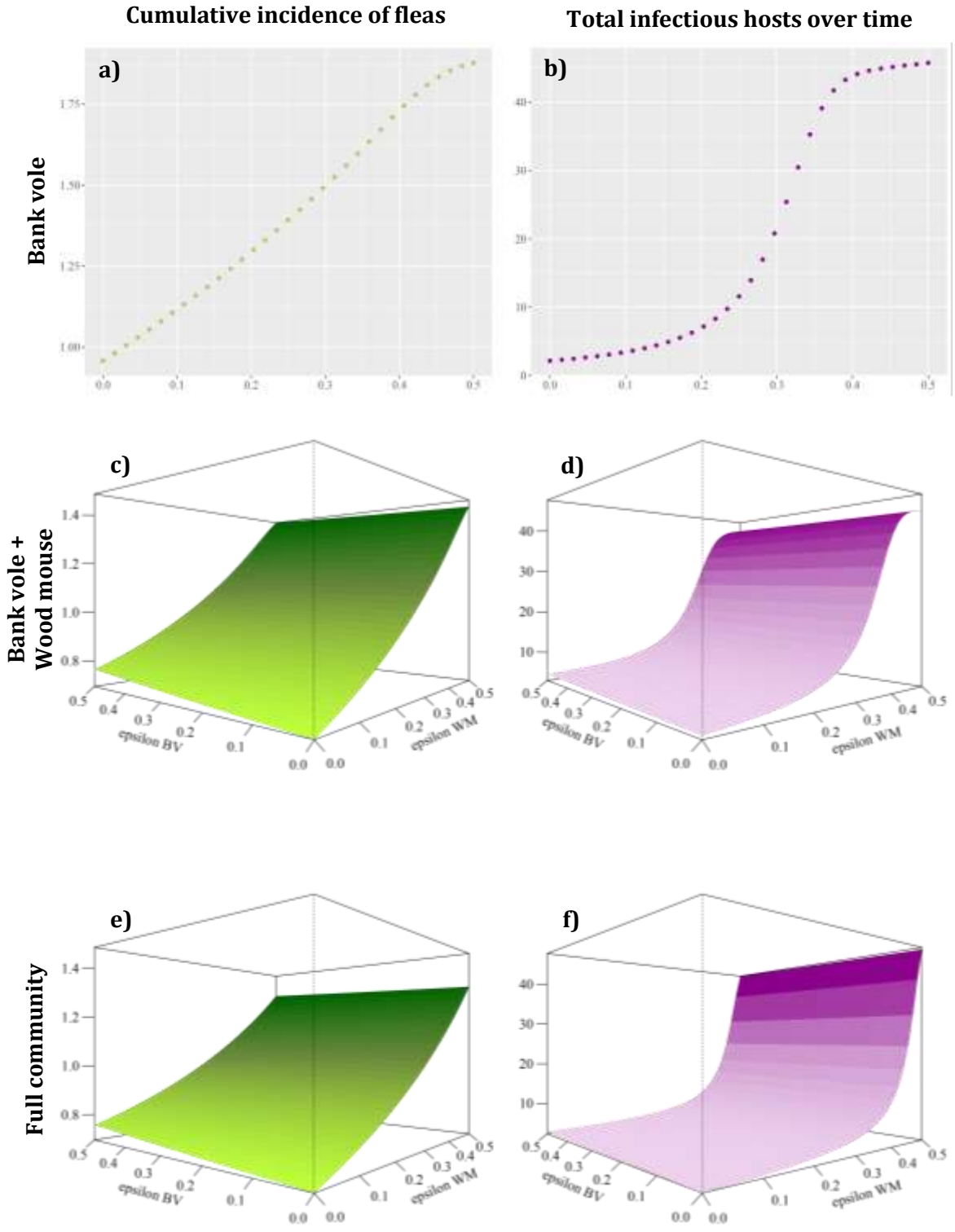


Fig. 5.37. Sensitivity analysis on vertical transmission rate (ε). Cumulative incidence of fleas (a, c, e), and total infectious hosts over total time (divided by 100000) (b, d, f) across different community assemblages: single-host scenario (a, b); two-host scenario (c, d); full community (e, f). Flea force of infection (λ_f) = 1; host force of infection (λ) = 1; reinfection rate (γ) = 0.5. BV: bank vole; WM: wood mouse.



5.4.4 Discussion

Several researchers have reported seasonal patterns of infection with *Bartonella* among wild rodents; for example, the seasonal prevalence peak in Ireland changed according to pathogen species (Telfer *et al.*, 2007c), and prevalence was influenced by rodent demographic processes in USA (Kosoy *et al.*, 2004a). In Poland, peak prevalence was reported in summer months, most likely because it coincided with the peak of flea population (Pawelczyk *et al.*, 2004; Paziewska *et al.*, 2012), and the prevalence variation between years and seasons was mostly explained by year, season, and their interactions as extrinsic factors (Pawelczyk *et al.*, 2004). Thus, the structure of the model, capturing the seasonality of rodent and flea populations proved to be ideal to describe realistic *Bartonella* transmission dynamics. Further, since the disease displayed seasonal oscillations, the outputs chosen to describe the effect of parameter variation and community composition were incidence and total infectious hosts over time in order to capture more information about the course of the epidemic.

Bartonella has been often reported, in field and laboratory studies, to have a long infectious period, presenting long bacteraemia rather than high incident rates (Birtles *et al.*, 2001; Koesling *et al.*, 2001; Kosoy *et al.*, 1997; Kosoy *et al.*, 1999). This finding is in agreement with model results, which showed rather high total infectious hosts over time with moderate values of incidence. However, the infectious period selected, ~47-48 days, may be an underestimation, since Kosoy *et al.* (2004a) found that infection in cotton rats can be as long as 11 months. This variable could also be species or strain specific to *Bartonella* genus, which displays a very high diversity among mammalian hosts (Kosoy, 2010).

Considering the sensitivity analysis on the force of infection, in general, the increase of the force of infection to flea induced a more marked effect on flea incidence, while the increase of the force of infection to rodents had a more obvious effect on host incidence. In all community assemblages, there seemed to

be evidence of values of force of infection above which there was no change in the outputs, representing maximum transmission. The reason for this phenomenon was most likely the way in which host-vector dynamic was modelled, with the carrying capacity and average flea burden that limited flea population growth and host-vector contacts. Comparing the single- and two-host communities, a decrease of incidence was observed because of the slower input of susceptibles in the host population (reduction of rodent growth rates due to inter-specific competition), and of the host-vector ratio. In fact, the way fleas were modelled assumed no linear increase of fleas with increasing host density according to Krasnov *et al.* (2002) and Smith *et al.* (2005). Fleas live mostly in host burrows and, when host density increase, their density increases at first, but, above certain values of host density, transient hosts (i.e. not living in burrows) rise so there is no further flea increase.

Bank voles had a moderately bigger impact on transmission than wood mice, as they had a slightly higher average flea load, and this was mostly evident with regards to flea incidence. Although, transmission was somewhat slower, the addition of the second host species led to, for any equivalent value of force of infection, a higher number of total infectious hosts. In the full community, due to a decrease of the rodent population determined by inter-specific competition with shrew and predation, host incidence decreased further, but flea incidence remained essentially unchanged, because the host decrease was probably not sufficient to cause major changes in flea population. In this case, the total infectious hosts over time were lower than the two-host community, but higher than the single-host case, so with regard to host population there was evidence of dilution through susceptible host regulation (Keesing *et al.*, 2006). Susceptible host regulation was also observed by Young *et al.* (2014) who, in their field experiment, recorded fewer infectious rodents in the plots where they did not remove large fauna that played a major role in controlling rodent populations. Differently from the tick system there was no chance of dilution through transmission reduction, as there were no wasted bites; nonetheless, increasing host richness, and so overall host abundance, a mechanism similar to encounter reduction (between flea and host) might occur considering the

constraint of the maximum average flea burden and the host-vector relationship (Kosoy *et al.*, 2004b; Krasnov *et al.*, 2002). Telfer *et al.* (2007a) found that at high host densities the flea population is divided between more potential hosts and consequently individual hosts are less likely to be infested, which might be an encounter reduction type of dilution effect. In Ireland, the addition of the non-native and non-competent bank vole to the rodent community determined the reduction of *Bartonella* prevalence among the competent wood mice, demonstrating that the increase in vector population did not outweigh encounter reduction (Telfer *et al.*, 2005). Also, in agreement with these results, Telfer *et al.* (2007a) observed that *Bartonella* infection dynamics appeared to be more strongly influenced by competent host density than flea abundance. However, increased flea abundance due to the addition of non-competent host species in the community might increase the number of infectious fleas in the environment. Unlike ticks though, most rodent fleas are considered negligible in terms of human disease risk (but see Zeppelini *et al.*, (2016) for a review on plague human risk), and their potential in transmitting the recognised zoonotic *Bartonella* species (e.g. *B. henslae*) is still undetermined (Eisen and Gage, 2012). Maaz *et al.* (2018) have reported that, among all the flea species collected on mice and voles in Germany, only *Monopsyllus sciurorum* and *Nosopsyllus fasciatus* have been found to attack humans (Brinck-Lindroth and Smit, 2007). Both species had a wide host array, and *N. fasciatus* was highly prevalent; in this study, *Nosopsyllus fasciatus* and other species from the same family (Ceratophyllidae) were collected from sampled rodents (see Chapter 3).

Examining reinfection rate sensitivity analysis, it was observed that, in the single-host scenario, 0.72 was the threshold at which maximum flea incidence occurred, while both host incidence and total infectious hosts increased linearly with the increase of reinfection values. So, above a reinfection rate of 0.72, the infection of fleas was slowed down most likely because of the constraint of the maximum average flea burden (i.e. when most of the fleas were already infected and there was a maximum number of fleas allowed on each host, it was more difficult to find a susceptible flea to be infected).

As before, incidence decreased in the more complex community assemblages, while total infectious hosts showed the opposite trend. In this case, wood mouse reinfection rate seemed to have a greater influence on pathogen transmission because of the faster recruitment rate that, over time, increased their dominance in the host community. Nonetheless, susceptible host regulation was obvious in this case too, as total infectious hosts decreased in the full community compared to the two-host assemblage. In addition, in the full community, the lower amount of susceptible hosts and the slower recruitment rate determined that reinfection rate values had a much lower impact on number of infectious hosts compared to the alternative scenarios.

Reinfection is a very likely event in *Bartonella* transmission dynamics (Paziewska *et al.*, 2012), as the multiple strains and species, circulating in the same location (this study Chapter 4; Streicker *et al.*, 2010; Telfer *et al.*, 2007a,c; Withenshaw *et al.*, 2016), do not provide full cross-immunity (Birtles *et al.*, 2001). In fact, it has been suggested that high prevalence found in rodent populations may be due to the high diversity of bartonellae, which determine reinfection and coinfection (Gutiérrez *et al.*, 2015; Holmberg *et al.*, 2003; Kosoy, 2010). Although traditionally it was thought that there was no host species-specificity among *Bartonella* species in rodent communities (Kosoy *et al.*, 2004a), in depth molecular studies have revealed that different strains were infecting preferentially some rodents (or fleas), or occupying different ecological and epidemiological niches (Buffet *et al.*, 2013; Gutiérrez *et al.*, 2015; Withenshaw *et al.*, 2016; Ying *et al.*, 2002). Telfer *et al.* (2007c) found that *B. taylorii* and *B. doshiae* were more prevalent in wood mice than bank voles, whereas *B. birtlesii* was more prevalent in bank voles, and this was probably due to different infection and recovery rates in different hosts and different *Bartonella* species. A different recovery rate was also observed between yellow-necked wood mice (*Apodemus flavicollis*) and bank voles in Poland, where the first displayed a much longer infection (Paziewska *et al.*, 2012). Kosoy *et al.* (2000) hypothesised that this species-specificity was associated with the flea vector, but Withenshaw *et al.* (2016) provided evidence that this was associated

with the host, being the fleas more likely to be infected with the strain specific to the species on which they were feeding.

Hence, the degree and the origin of species-specificity are still unclear and may vary depending on the flea-rodent assemblages, and it was not included in the model. Nonetheless, it is definitely a matter of interest and warrants further investigation, as it could potentially change the outcome of the simulations, since one of the basic assumptions for dilution is the generalism of the pathogen (Ostfeld and Keesing, 2000; Ostfeld and Keesing, 2012; Schmidt and Ostfeld, 2001; Wood *et al.*, 2014).

According to the above, vertical transmission rate sensitivity analysis was performed assuming moderate force of infection and some degree of reinfection. As in the previous cases, and for analogous reasons, flea incidence decreased along the gradient of assemblage complexity. In this case, the number of total infectious hosts was much higher than in previous analyses, with maximum values greater by almost one order of magnitude. However, the total number of infectious hosts did not substantially differ between the single-host and two-host community, most likely because the recruitment of one host population, tending freely to its carrying capacity, was similar to the recruitment of the two populations exerting inter-specific competition upon each other. The sigmoid relationship exhibited by vertical transmission rate and model outputs, especially clear in the single-host case, represented the existence of a maximum transmission due to host-vector dynamics (constraints), force of infection, and reinfection rate, therefore beyond a certain value of vertical transmission no more infections could occur. The wood mouse population was mostly responsible for the increase of flea incidence and total infectious hosts due to vertical transmission, because of its greater growth rate compared to bank vole (this study). Consequently, the wood mouse input of new infectious individuals to the community was much greater.

Upon assembly of the full community, susceptible host regulation was present only at values of vertical transmission lower than 0.4, since, above this threshold, the value increased compared to the previous assemblages, and

vertical transmission outweighed dilution. Nonetheless, because of the inter-specific competition with shrews and the predation that reduced rodent population, a rather high value of vertical transmission was necessary to counterbalance dilution. Interestingly, in the full community, with values of vertical transmission greater than 0.4, the number of total infectious hosts was higher than in the previous scenarios. This was probably due to the shrews exerting slightly higher competition on voles, resulting in wood mice being more dominant compared to the two-host scenario. As they reproduce faster than voles, this community could support more infectious individuals.

Vertical transmission has been documented by Kosoy *et al.* (1998) and Kosoy *et al.* (2004a), who hypothesised this to be the reason for the high prevalence frequently recorded in wild rodents. In particular, it may be plausible that the prevalence peaks observed in cotton rats by Kosoy *et al.* (2004a) in the late reproductive season were due to the introduction into the population of vertically infected individuals. In general, higher prevalence was reported often in the most dominant species in the community (Kosoy *et al.*, 1997; Kosoy *et al.*, 2000; Kosoy *et al.*, 2004a), and model results were in agreement, with the more abundant and fastest growing species contributing more to the pathogen transmission. Nevertheless, there have been cases in which *Bartonella* was not infecting the numerically dominant species in the community (e.g. Holmberg *et al.*, 2003). Assuming some level of species-specificity of *Bartonella*, as mentioned before, it may be possible that high prevalence in numerically dominant species occurs because a higher proportion (higher probability of encounters) of fleas feed on those species and get infected with species-specific strains being less able to infect alternative host species. In this study most of the fleas positive for *Bartonella* were collected from bank voles (Chapter 4), which were dominant, while wood mice density was slightly negatively associated with flea infestation (Chapter 3), so it might be possible that across the sampled sites wood mice diluted flea infestation with regards to the dominant host species.

In the future, aspects that could be investigated with regards to *Bartonella* infection include alternative transmission routes, for example alternative

vectors (Morick *et al.*, 2013; Telfer *et al.*, 2007c). Ticks have been found to be competent in transmitting *Bartonella* experimentally, and *B. henslae* have been isolated in ticks from cats and dogs (Cotté *et al.*, 2008). In the wild, *Bartonella* have been recorded in ticks and other several ecto-parasites infesting mammals in Poland, Netherlands, but these may not be efficient vectors (Jardine *et al.*, 2006; Tsai *et al.*, 2011). The presence of alternative routes of transmission could strongly affect the dynamics illustrated by this model, and nullify dilution mechanisms. In addition, co-infections may affect dilution, as *Bartonella* has been often found in association with other pathogens (Pawelczyk *et al.*, 2004; Vaumourin *et al.*, 2013), but no interaction has been observed between *Borrelia* and *Bartonella* in bank voles (Vaumourin *et al.*, 2013).

In summary, the parameters under investigation that most affected pathogen transmission and the degree of dilution were force of infection and vertical transmission, which were also among the most uncertain in the system, and require further investigation. With regards to force of infection, it seemed that highest variability in the results occurred setting values between 0 (no transmission) and ~2-3, probably because the constraints to transmission mentioned above made highest values unable to remarkably increase transmission. A crucial value to determine these transmission constraints was the maximum average flea burden that, in this study, was estimated from empirical data, representing realistic local dynamics, and that should be adapted according to the host-flea community under consideration. Vertical transmission also needs further research, as above a certain threshold it reversed the effect of susceptible host regulation, and even moderate values increased the number of total infectious hosts by an order of magnitude compared the results of other analyses. By contrast, reinfection rate did not affect dilution mechanisms, and in the full community did not considerably impact the number of total infectious hosts.

From the epidemiological point of view, the metrics chosen to examine modelling results were more informative than prevalence, since this seemed inappropriate due to the cyclic nature of the pathogen chosen and the misleading interpretation that it can produce (as illustrated in the previous

sections of this chapter). Whereas incidence and abundance of infectious hosts were recommended as among the best metric for *Bartonella* disease risk (Salkeld and Lane, 2010; Young *et al.*, 2014). In particular, the total number of infectious hosts over time was the most informative output to understand the effects of parameter variations and community composition on this pathogen transmission.

Finally, the results of this modelling study were supported by several studies that found *Bartonella* infection to vary among communities differing in diversity and compositions (e.g. Bai *et al.*, 2009; Kosoy *et al.*, 2004b). Bai *et al.* (2009) showed a negative correlation between *Bartonella* prevalence and community diversity, suggesting that transmission was more common within species than between species. So, increasing community diversity and host diversity may decrease *Bartonella* transmission reducing the probability of encounters between hosts and fleas (and also between host and flea infected with matching the species-specific pathogen species/strain), and limiting or regulating host numbers through competition and predation.

Chapter 6

Final discussion: summary of research, innovations, limitations, and future perspectives

6.1 Summary of experimental chapters

Chapter 2. Small rodent species captured during the live trapping were wood mouse, bank vole, and rarely field vole. Bank vole was dominant when present, likely because it exhibits less dispersal abilities and smaller home-ranges than mice species (Kozakiewicz *et al.*, 1999). The differences found in demography of the two most represented species seemed to reflect the asynchronous breeding peaks (Mallorie and Flowerdew, 1994; Huitu *et al.*, 2004).

Body mass and population density analysis confirmed that the sampling seasons chosen were effective in capturing the seasonal differences of the population. Adults and sub-adults individuals were lighter in the post-breeding peak phase (autumn), reflecting the introduction in the population of the individuals born previously in the breeding season, and in this season higher densities were recorded. Population densities were also higher in woodland habitats compared to grasslands, and Skomer voles' density was much higher than mainland bank voles. Further, the estimated seasonal growth rate clearly showed the difference between non-breeding and breeding season, validating the intra-annual population fluctuations remarked by density estimates.

In order to include relevant epidemiological information about contacts between individuals, daily individual contact rates were estimated. The analysis revealed that the distribution of both intra and inter-specific contacts was highly aggregated, meaning that a small number of individuals were responsible

for a large proportion of interactions. However, bank voles performed more intra-specific contacts than wood mice, while wood mice tent to interact more with other species. In addition, it was found that older, heavier wood mice might be more mobile and have more inter-specific transmission potential, while heavier bank voles might have more intra-specific transmission potential. In both species, heaviest individuals could not be distinguished in terms of sex or reproductive status.

Chapter 3. The collection of ticks and fleas recovered from sampled rodents showed that the proportion of the population parasitised was very small supporting the "20/80 Rule" (see Perkins *et al.*, 2003; Woolhouse *et al.*, 1997). Bank voles were more prevalent than wood mice with regards to both ticks and fleas, and also exhibited higher intensity of infestation. According to the molecular identification, *I. trianguliceps* accounted for 87% of the ticks collected, with only three cases of two different species of ticks co-feeding on the same individual. Fleas collected displayed higher species diversity, with *Ctenophtalmus nobilis*, *Hystrichopsylla talpae*, and *Megabothris turbidus* dominating the flea community.

In this study, data showed that gender and season were the factors influencing ecto-parasite burden. Both ticks and fleas were more prevalent and abundant in spring, and among males. However, when flea data were analysed by genus, *Hystrichopsylla*, *Ctenophthalmus*, and *Megabothris* were more prevalent in autumn, suggesting that different flea genera might adopt different reproductive strategies, which may be dependent by climatic variables and taxonomic characteristics.

Intensity and prevalence of infestation were analysed in relation to host density, but no clear relationship was found between ecto-parasites and host density. The only finding was a not well-supported negative relationship between wood mice density and ecto-parasite prevalence, likely due to the fact that wood mice were significantly less parasitised. Alternatively, it may indicate that the species dilutes ecto-parasite prevalence, although, this does not necessarily mean a decrease in total parasite abundance. The finding may also reflect an actual absence of relationship between host density and ecto-parasites, as supported

by other studies that have explored the mechanisms of host density and parasite intensity/prevalence decoupling (e.g. McCauley *et al.*, 2008).

These findings are particularly interesting in the context of pathogen transmission because they shed light on host preferences, vector assemblages, vector seasonality, and host-vector dynamics.

Ecto-parasite molecular analysis confirmed that 16S and 18S genes gave less phylogenetic resolution than COI, due to slower mutation rates, but can be used as complementary to COI, when this fails to produce reliable results. COI, which is the most used fragment of mtDNA for barcoding, might still represent the best choice, but more research is needed especially for obscure taxa where the combination of morphological and molecular approaches is still crucial. Especially in the case of fleas, the combination of phenotypic and genetic approaches was essential to determine species identification, and allow the characterisation of flea community. Also, the BLAST search alone was mostly insufficient to provide definitive information on species identification, so more work is necessary to increase the number of sequences and their quality on GenBank.

Chapter 4. The pathogen investigation in rodent faeces revealed very low prevalence of Herpesvirus and *Escherichia coli*, and absence of *Mycobacterium microti* among the individuals sampled. The positive samples of Herpesvirus displayed high similarity to human HVs, and no Murid Herpesviruses were isolated. *E. coli* positive sample were collected in autumn almost exclusively from bank voles, and this may be explained by the relationship between gut microbiota and diet. In fact, it is likely that different food items determine presence and abundance of *E. coli*. No individuals were found infected by *M. microti*, and this might represent true absence, or may be due to the absence of gastro-intestinal lesions and consequent absence of mycobacteria in the faeces. However, confidence of freedom analysis revealed that the prevalence data were likely to be reliable.

Regarding macroparasites, the result of host heterogeneity analysis revealed that bank voles were key hosts for nematodes, while wood mice for cestodes. Bank voles contributed to transmission pool mainly in terms of host abundance

and prevalence (i.e. super-abundant and super-infected host), while wood mice represented exclusively super-shedder hosts. Clearly, the methodology might have influenced the results, for example, unequal detection and sampling of host species, or of parasite eggs. However, this demonstrates that even in an apparently simple and common multi-host-parasite system, over a small spatial and temporal scale, host heterogeneities are detectable. Identification of the type of contribution of different hosts is essential in designing control strategies, understanding effects of host community change on pathogen transmission, and eco-epidemiological modelling.

Tick-borne pathogens detected in the sampled ticks were *Anaplasma phagocytophilum* and *Babesia microti*. *A. phagocytophilum* was detected only in one site, in ticks recovered on bank voles. The very low presence of *Ixodes ricinus* (amplification vector) at the sampling sites, and the short infectious period reported in rodents (short amount of time for the ticks to be infected) (Bown *et al.*, 2003) may explain the low recovery of this pathogen. Only one tick sample was positive for the protozoan *B. microti*. The strain recovered from the positive sample displayed high relatedness to a European strain (Munich) isolated from ticks and rodents in several European countries, and involved in the first human case of *B. microti*-caused babesiosis in Spain (Arsuaga *et al.*, 2016). It seems likely that, in UK, different strains of *B. microti* are circulating, and these might not only differ with regards to host and vector preferences, but also in terms of zoonotic risk (Gray, 2006). *Borrelia burgdorferi* s.l. did not occur in the ticks collected, probably because *I. trianguliceps*, which accounted for the vast majority of ticks sampled, is not considered of major importance in transmitting the spirochete (Kilpatrick *et al.*, 2017a; Stanek *et al.*, 2012). The results found were likely due to the combination of factors such as true absence/low prevalence of the pathogens, low competence of rodents and/or vectors, negative impact on the pathogens tested of other undetected infections.

Bartonella was detected in the most represented flea species in the sampled pool (*Amalaraeus penicilliger*, *Megabothris turbidus*, *Hystrichopsylla talpae*, and *Ctenophthalmus nobilis*). Statistical analyses did not reveal any pattern of association between *Bartonella* infection and factors related to fleas or hosts

(e.g. flea species, host from which the flea was collected). As expected, *Bartonella* infections were recorded in fleas collected from bank voles, as this represented the dominant species in the rodent community (Bai *et al.*, 2009; Gutiérrez *et al.*, 2015; Kosoy *et al.*, 1997). The sequences chosen to represent the results of molecular analysis were appropriate to reveal that at least three species (or three different groups with high similarity to *B. taylorii*, *B. grahamii*, and *B. rochalimae*) were circulating at the sampling sites. While *B. taylorii* and *B. grahamii* have been widely recorded in rodent fleas (in UK and other countries) (Birtles *et al.*, 2000; Špitalská *et al.*, 2017; Telfer *et al.*, 2007a,c; Withenshaw *et al.*, 2016), it was the first time that *B. rochalimae* (or a highly similar species) has been isolated in UK. This species, which has a zoonotic potential (Eremeeva *et al.*, 2007), has rarely been isolated from fleas, so the role of fleas in transmission has not been clarified yet. This result may provide additional evidence that *B. rochalimae* circulates among small rodents, and it is very likely that fleas act as a vector for transmission.

Chapter 5. Modelling results of a directly transmitted pathogen (PUUV) confirmed that reduced (or diluted) infection prevalence might not represent a true dilution effect, since prevalence could decrease simultaneously to the increase of infectious individuals. Consequently, both pathogen prevalence and number of infectious individuals were always considered and compared in this study. The model was effective in recognising susceptible host regulation via inter-specific competition and predation as the most important dilution mechanism with regards to directly transmitted pathogens, and the results highlighted that estimation of the magnitude of competition and predation was essential to understand the strength of dilution. Hence, a community perspective, which includes realistic ecological relationships and reliable parameter estimation from empirical data, may give critical insights into wildlife epidemiological patterns and may help to understand and predict their dynamics (Belden and Harris, 2007; Johnson *et al.*, 2015a; Koprivnikar and Johnson, 2016). This was confirmed by the fact that not the number of species in the community, but the degree of community complexity (in terms of interaction among species) affected pathogen transmission. The results

observed modelling PUUV were consistent when modelling other rodent-borne pathogens, regardless of very different course of epidemics.

Modelling two similar but different host-tick-pathogen systems showed that, in general, the parameters most affecting the juvenile stages of the ticks were the ones most affecting transmission. The results provided evidence that in the system with the more generalist vector, *Ixodes ricinus*, the dilution effect was more significant and more mechanisms were represented, in agreement with the classic assumptions about dilution formulated by Schmidt and Ostfeld (2001). The modelling study was also useful to identify the key parameters regulating transmission in the two systems. Moulting success seemed to be crucial in the case of *I. trianguliceps*, which had smaller population and less alternative hosts than *I. ricinus*, because transmission was limited by the number of ticks in the system. Whereas, for *I. ricinus*, the regulating factor was competence of transmission, since this species had to maximise the efficiency of transmission of the lower proportion of feeding events on competent hosts. Consequently, in the two systems the key dilution mechanisms were also different: transmission reduction for *I. ricinus*-*Borrelia burgdorferi*, and susceptible host regulation for *I. trianguliceps*-*Babesia microti*. However, here, more complex communities led only to fewer infectious hosts (evidence for dilution effect), because, at the same time, there was also an increase (or a stability) of infectious nymphs, representing amplification of human disease risk.

In the context of the flea-borne *Bartonella*, the modelling study, focusing on testing dilution in a pathogen determining reinfection and vertical transmission, identified force of infection and vertical transmission as the parameters most affecting transmission and degree of dilution (through susceptible host regulation adding inter-specific competition and predation). With regards to force of infection, it seemed that highest variability in the results occurred setting values between 0 (no transmission) and ~2-3, probably because the constraints to transmission due to host-vector dynamics made highest values unable to remarkably increase transmission. A crucial value to determine

transmission constraints was the maximum average flea burden that, in this study, was estimated from empirical data. Vertical transmission rate, above a certain threshold, reversed the effect of susceptible host regulation, and even moderate values increased the number of total infectious hosts by an order of magnitude compared to the other analysis results. By contrast, reinfection rate did not affect dilution mechanisms, and in the full community did not considerably impact the number of total infectious hosts. The results of modelling were supported by several studies that found *Bartonella* infection to vary among communities differing in diversity and compositions. In particular, since transmission seems more common within species than between species (Bai *et al.*, 2009; Withenshaw *et al.*, 2016), increasing community and host diversity may decrease transmission in two ways: reducing encounters between hosts and fleas with the correct species-specific *Bartonella* species/strain; and limiting or regulating susceptible hosts through competition and predation.

6.2 Limitations, innovations, and future perspectives

This study integrated empirical data about local host communities with mathematical modelling techniques to develop a realistic eco-epidemiological approach for the investigation of rodent-borne pathogens with different transmission modes. First, it was chosen to explore rodent communities through live trapping in selected sites across Wales in order to gather information about local population dynamics, and perform multi-host parasite and pathogen screening focusing on all host-species sampled. Then, mathematical models were developed to describe pathogen transmission in rodent populations.

The study presented some limitations mostly due to the fieldwork design and the lack of explicit spatial scale in the model. Specifically, the epidemiological study did not include a longitudinal pathogen screening due to the impossibility of identifying individuals between different trapping seasons (i.e. fur clipping did not allow identification between seasons), the lack of authorisation for sample collection apart from faeces and ecto-parasites, and the impossibility of more intensive trapping (i.e. lack of resources to increase trapping frequency). The model did not include an explicitly spatial scale, which is essential to analyse the effect of patches connectivity, individual dispersion, and meta-populations on pathogen transmission (Cohen *et al.*, 2016), but this was beyond the scope of this study. In addition, host populations in the model might have split according to age and/or sex groups to better define the different contribution of individuals to pathogen transmission, for example sub-adult individuals seemed to be crucial in bartonellae transmission (Kosoy *et al.*, 2004a; Jardine *et al.*, 2006; Telfer *et al.*, 2007a).

Despite the limitations, this innovative modelling approach included rodent population dynamics parameterised with data collected through the live trapping, and detailed ecological and epidemiological dynamics, representing a realistic Welsh community assemblage of host and non-host species interacting

with each other. This approach was inspired by the recommendations for future work on diversity–disease relationships by Johnson *et al.* (2015b). In their seminal review, the distinction between response and predictor variable was clearly delineated. In particular, the effect of host species richness and non-host species functional diversity on different epidemiological metrics representing wildlife and human disease risk separately was taken into account. In addition, more empirical and laboratory data were collected, and the modelling methodology expanded from purely additive versus purely substitutive community structures, considering more realistic patterns, according to Mihaljevic *et al.* (2014). The influence of multiple diversity components on pathogen transmission was investigated, allowing the identification of distinct dilution mechanisms and the species responsible for each mechanism to occur; so, this approach was different from the purely theoretical (e.g. Roche *et al.*, 2012, 2013), or purely observational ones (e.g. Clay *et al.*, 2009; Kosoy *et al.*, 2004b). Moreover, it was explored how the relationships between host diversity, community structure and disease risk varied among different host-pathogen systems, assembling the community according to realistic ecological criteria and identifying how community complexity played a different role in each system differing for pathogen transmission mode, in contrast with previous studies that focused on a single type of transmission (e.g. Clay *et al.*, 2009b; Clay *et al.*, 2014; Keesing *et al.*, 2006; Ostfeld and Keesing, 2000; Ostfeld and LoGiudice, 2003; Roche *et al.*, 2012; Schmidt and Ostfeld, 2001).

Commonly, modelling studies in disease ecology have focused on a single host species or on a single aspect such as the transmission term (e.g. Begon *et al.*, 2002), the variance of reservoir competence (e.g. Roche *et al.*, 2012), the inter-specific competition (e.g. O'Regan *et al.*, 2015), or the predator-prey-pathogen dynamics (e.g. Roberts and Heesterbeek, 2013), and often employing theoretical frameworks (e.g. Dobson and Auld, 2016). By contrast, in this study, these were all combined in a unique framework to describe pathogen transmission dynamics in a realistic community, to identify potential dilution mechanisms and the key parameters in each system.

Especially novel was the application of this modelling technique to the flea-borne pathogen *Bartonella*, as, to the best of this author's knowledge, it was the first time that such a comprehensive approach was used to describe flea-host-pathogen dynamics. In fact, other studies described host-flea dynamics (e.g. Krasnov *et al.*, 2002), or the difference in flea and pathogen prevalence among sympatric rodent species (e.g. Brettschneider *et al.*, 2012; Kedem *et al.*, 2014), but this study put all these aspects together in a unified eco-epidemiological framework. In addition, the work on fleas was particularly valuable, since little information is available on the taxon (but see Krasnov *et al.*, 2002, 2015, 2016), and this study contributed to knowledge about small rodents' flea communities, flea-host associations, and flea-borne bartonellae circulating in Wales, isolating for the first time in UK a *Bartonella rochalimae*-like species.

One of the most interesting outcomes of the modelling work was that the parameters most affecting pathogen transmission, in each system, were also the most uncertain, suggesting that more ad hoc empirical studies are needed to improve model reliability (Johnson *et al.*, 2015a). The modelling results also pointed out, in each system, which were the parameters that were mostly affecting the transmission, representing a useful tool for designing future empirical data collection. Moreover, the search of these parameters in literature suggested that, when available, these values should be provided with clearer information about their units (or methods of estimation), so they can be meaningfully used in modelling studies.

In this study more than one epidemiological metric was reported to illustrate modelling results (in contrast with the widespread tendency of reporting exclusively pathogen prevalence), in order to distinguish between true and spurious dilution effect (Dobson and Auld, 2016), and identify which metric was the most appropriate to evaluate the effect of community composition and parameter variation on wildlife and human disease risk independently.

Lastly, this investigation provided not only qualitative, but also quantitative information about rodent populations and their parasites and pathogens. This knowledge may be useful to implement and improve management practices in

place at the sampling sites, since all the pathogens found have a human zoonotic potential (Bitam *et al.*, 2010; Eremeeva *et al.*, 2007; Gray, 2006; Homer *et al.*, 2000).

In the future, it would be ideal, for each pathogen system considered, to have precise estimation of the parameters on which assumptions were made or sensitivity analysis was performed. Additionally, another field to explore via modelling would be the systems in which multiple vectors or multiple transmission modes are involved (Webster *et al.*, 2017), and an example may be *Bartonella*, which has been isolated also in ticks (e.g. Chang *et al.*, 2001; Cotté *et al.*, 2008; Tsai *et al.*, 2011). The parallel implementation of the theoretical and empirical approach would facilitate the identification of key hosts and key transmission pathways, as well as the identification of effective disease control strategies (Webster *et al.*, 2017). Likewise, this methodology might be employed to understand the effects of human disturbance on pathogen transmission, since it has been shown that several common human environmental impacts can contribute to disease emergence, and emerging infectious diseases (EIDs) are a substantial threat to biodiversity, human health and economic well-being (Rogalski *et al.*, 2017). Indeed, it could be very interesting for future research to monitor epidemiological variations where conservation measures are put in place compared to control sites (with no conservation efforts) (Keesing *et al.*, 2010). Finally, since the debate about existence, generality, and definition of the dilution effect is still open, it will be worth to find new ways of approaching dilution estimation. For example, Ruyts *et al.* (2018), in the context of tick-borne pathogens, introduced a new indicator referred to as "potential dilution", which represents the proportion of larvae feeding on dilution hosts in the host community, and can be derived from the proportion of dilution hosts in the total host community weighted by the species-specific average larval burden.

6.3 Conclusions

This study supported the idea that the dilution effect is not a universal principle, but it can be observed in some systems considering the appropriate epidemiological metrics and/or outputs (Johnson and Thieltges, 2010). Moreover, it was taken into account the response of community composition of just one pathogen at a time, but it is still very much unclear what is the overall relationship between diversity and the overall pathogen and parasite community, if there is one (Johnson *et al.*, 2015b). Nonetheless, in the context of pathogen (and disease) emergence some risk factors were identified, especially human disturbance and consequent changes in biological communities (i.e. species introductions and extirpations, higher human-wildlife-livestock interface; Johnson and Thieltges, 2010). Hence, it may be more effective to focus on pathogen control investigating specific cases and gathering data to develop specific control measures instead of trying to find a one-fits-all disease-diversity relationship.

Considering public health, the emphasis on the potential spillover risk in areas with high parasite diversity has been criticised by Plowright *et al.* (2017), who demonstrated that spillover requires a complex series of processes. Also, pathogen/parasite richness is the result of interplay between parasite lifestyle, host ecology, host defences (Krasnov *et al.*, 2016), and so the effort should be put in the eco-epidemiological investigation of the systems with distinct risk factors. Infectious disease distribution has been found to be uneven, with human zoonoses being particularly concentrated in some geographical areas, but the drivers of this phenomenon are not clarified yet, and it is also difficult to predict how this distribution will change in the future due to the aforementioned environmental changes (Morand and Krasnov, 2010). Therefore, eco-epidemiological studies to explore this might involve the investigation of patterns of reservoir competence, of the relationship between resilience and competence, and between life traits and immune response.

In conclusion, the health ecology (i.e. one health) approach has been revealed to be crucial in this era, in which infectious diseases are tightly linked to human driven environmental changes (Morand and Krasnov, 2010). In order to understand the connections between biodiversity, wildlife disease ecology and zoonotic risk it is essential to overcome boundaries between disciplines such as veterinary science, epidemiology, microbiology, parasitology, evolutionary biology, wildlife and landscape ecology and integrate ecological niche modelling, macroecology, biogeography with the final goal of developing effective strategies in managing wildlife disease to conserve biodiversity and reduce human disease risk (Morand and Krasnov, 2010; Vander Wal *et al.*, 2014).

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Appendix I

Statistical analyses R code

I. 1 Chapter 2

Normality test:

```
require("nortest")
ad.test(data)
```

Linear regression between log-transformed rodent population density and the log-transformed rodent population density in the following season:

```
density_next_density.glm<-
lm(log_density~next_seas_density,data,na.omit)
```

Linear regression between rodent growth rate and log-transformed rodent population density:

```
growth_density.glm<-lm(growth_rate~log_density,data,na.omit)
```

Permutation test to investigate differences in intra and interspecific contact rates between seasons, sites, and rodent species, sex, age class, and breeding condition:

```
require("coin")
oneway_test(contacts~variable,na.omit(data),distribution=appro
ximate(B=9999))
```

Negative binomial regression between intra and interspecific contacts and rodent weight:

```
require("MASS")
contact_weight.glm.nb<-glm.nb(contacts~weigth,data,link="log")
```

I. 2 Chapter 3

Ecto-parasite prevalence analyses:

```
prevalence.glm<-glm(prevalence~variable,data,family=poisson)
```

Regression between ecto-parasite prevalence and rodent population density:

```
prevalence.density.lm<-lm(density~prevalence,data)
```

Chi-square test for independence for differences among ecto-parasite species distribution on host species:

```
Chisq.test(table(data$var1,data$var2)
```

Non-parametric statistics to analyse patters of intensity of infestation:

```
Infestation.test<-kruskal.test(parasite.burden~variable,data)
Infestation.test<-wilcox.test(parasite.burden~variable,data)
```

I. 3 Chapter 4

Pathogen prevalence analysis:

```
prevalence.glm<-
glm(prevalence~variable,data,family=binomial,na.omit)

prevalence<-kruskal.test(prevalence~variable,data)

prevalence<-wilcox.test(prevalence~variable,data)
```

Helminth burden analysis:

```
Helminth.burden.glm<-
glm(burden~variable,data,family="binomial",na.omit)
```

Appendix II

Confidence of Freedom of prevalence found for Herpesvirus, *Escherichia coli* and *Mycobacterium microti*

Confidence of Freedom of prevalence found for Herpesvirus, *E. coli* and *M. microti* was estimated (Christensen and Gardner, 2000; Humphry *et al.*, 2004; Romeo and Ferrari, 2017) (Fig. II.1, Fig. II.2, and Fig. II.3 respectively).

The method considers that confidence of freedom equals the Herd-level Negative Predictive Value (HNPV) (i.e. Negative Predictive Value = probability that a test-negative individual is truly negative), which represents the probability that a test-negative herd (or a wild population) is truly negative and depends on prevalence, specificity, sensitivity and sample size.

$$HNPV = \frac{(1-eP)HSp}{(1-eP)HSp + eP(1-HSe)}$$

where *eP* is the prevalence, *HSp* is the herd specificity: $HSp = Sp^N$ (i.e. the probability that an uninfected herd will give a negative result to a defined testing protocol, it depends only on the sample size *N*), and HSe is the herd sensitivity: $HSe = 1-[(1-(ePSe)+(1-eP)(1-Sp))]^N$ (i.e. the probability that an infected herd will give a positive result to a defined testing protocol, it depends on sample size and prevalence). Since specificity (Sp) and sensitivity (Se) values were not available for the detection tests employed in the study, sensitivity analysis on those variables was performed.

Fig. II.1. Herpesvirus prevalence Confidence of Freedom estimated according to Christensen and Gardner (2000), Humphry *et al.* (2004), and Romeo and Ferrari (2017). Herd sensitivity (HSe) values, from 0 to 1, are represented by the lines from lighter to darker shade.

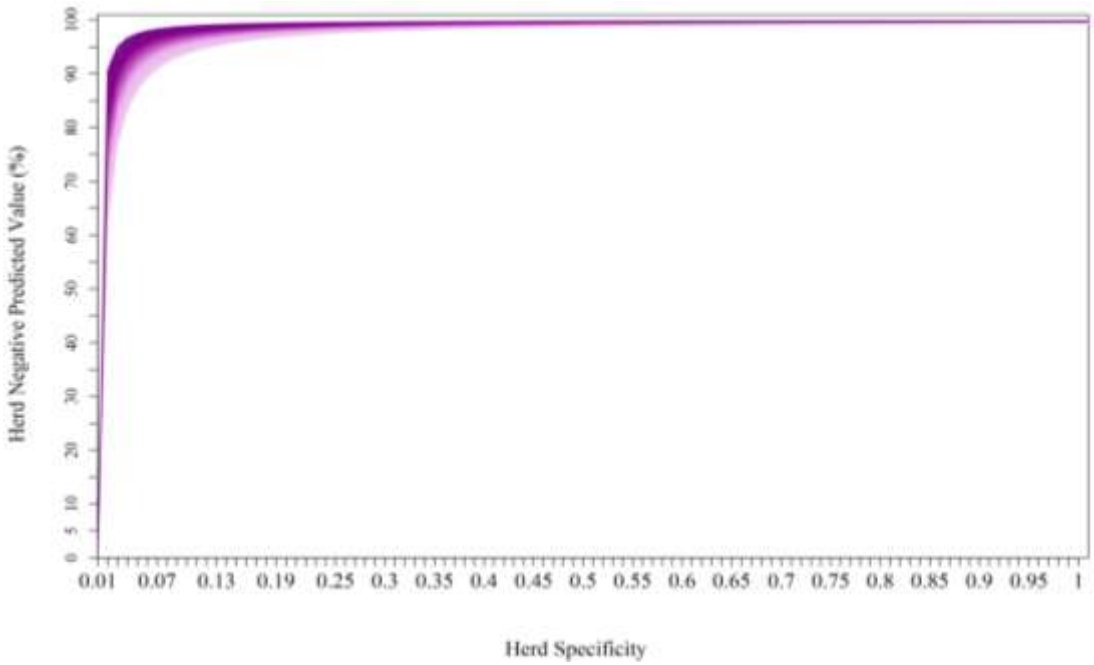


Fig. II.2. *Escherichia coli* prevalence Confidence of Freedom estimated according to Christensen and Gardner (2000), Humphry *et al.* (2004), and Romeo and Ferrari (2017). Herd sensitivity (HSe) values, from 0 to 1, are represented by the lines from lighter to darker shade.

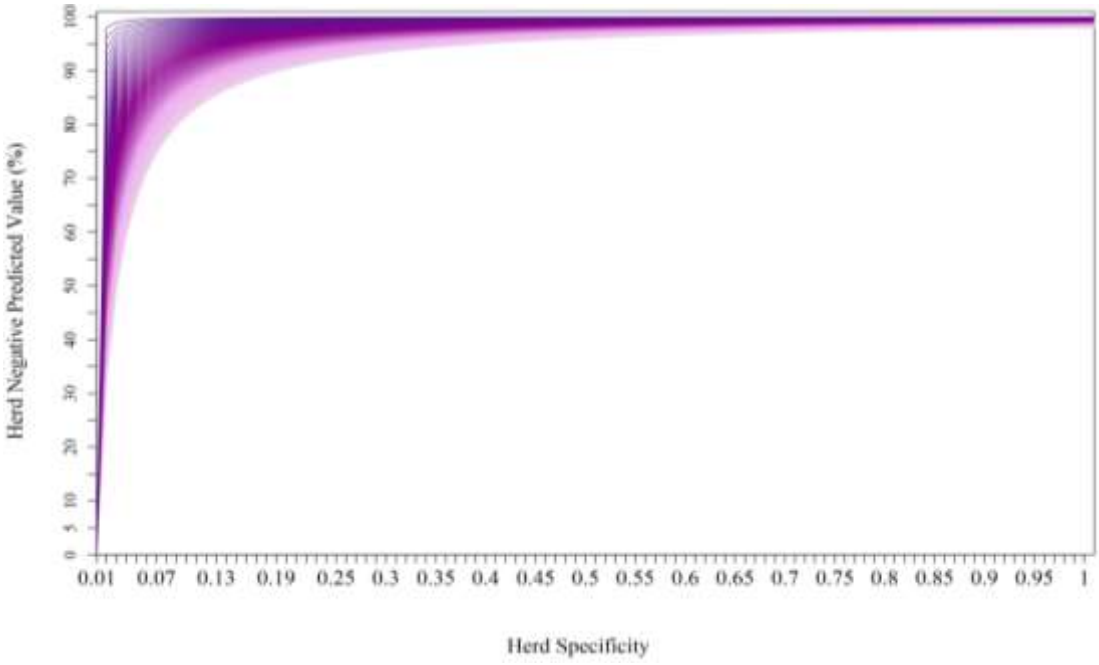
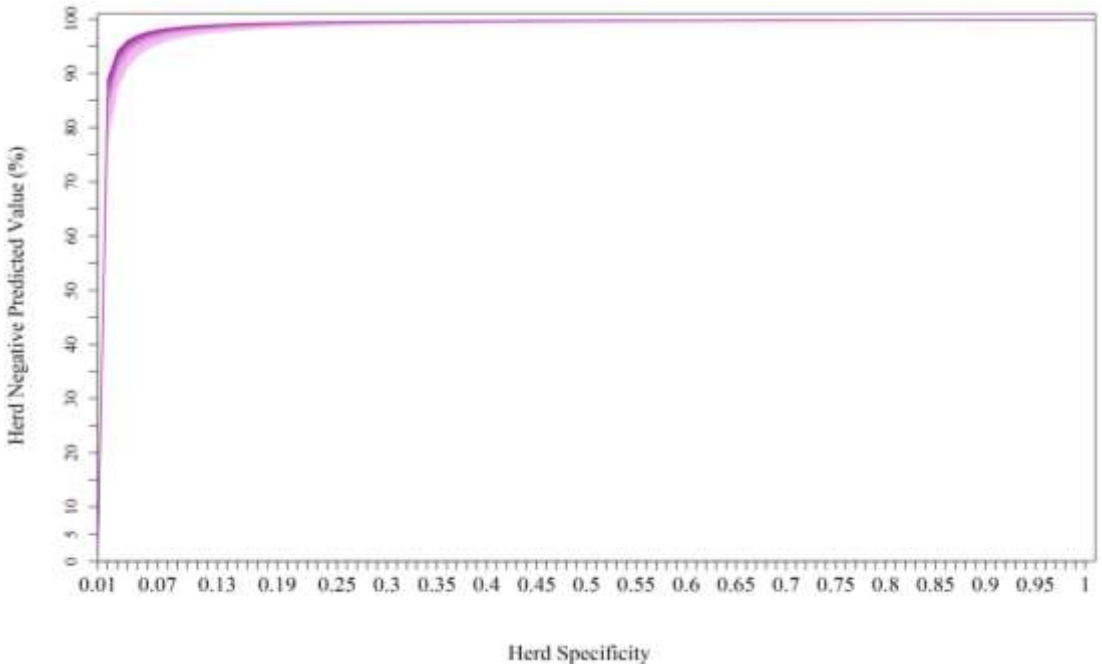


Fig. II.3. *Mycobacterium microti* prevalence Confidence of Freedom estimated according to Christensen and Gardner (2000), Humphry *et al.* (2004), and Romeo and Ferrari (2017). Herd sensitivity (HSe) values, from 0 to 1, are represented by the lines from lighter to darker shade.



Appendix III

R code of eco-epidemiological models of infection

III.1 Directly-transmitted pathogen model

```
#requires the package deSolve
require("deSolve")

# define the function
multihostSEIR.model <- function( t, x, parameters )

{ sw <- x[1]
ew <- x[2]
iw <- x[3]
rw <- x[4]
sb <- x[5]
eb <- x[6]
ib <- x[7]
rb <- x[8]
sf <- x[9]
ef <- x[10]
iF <- x[11]
rf <- x[12]
nj  <- x[13]
np <- x[14]

with(as.list(parameters),
     { nw <- sw + ew + iw + rw
     nb <- sb+eb+ib+rb
     nf <- sf+ef+iF+rf
     nj  <- nj
     np <- np
     r.temp.w<-ifelse(t %% 1 < 2/3, gw[2],gw[1])
     r.temp.b<-ifelse(t %% 1 < 2/3, gb[2],gb[1])
     r.temp.f<-ifelse(t %% 1 < 2/3, gb[2],gb[1])
     lambdaw <-
(tauw*(0.23+(0.01*nw))*iw)+(tauw*(0.71+(0.02*nb))*ib)+(tauw*(0.71+(0
.02*nf))*iF)
     lambdab <-
(taub*(0.53+(0.01*nb))*ib)+(taub*(0.39+(0.02*nw))*iw)+(taub*(0.23+(0
.01*nf))*iF)
     lambdaf <-
(tauf*(0.53+(0.01*nf))*iF)+(tauf*(0.39+(0.02*nw))*iw)+(tauf*(0.23+(0
.01*nb))*ib)
     mw <- M[1]
     mb <- M[2]
```

```
      mf <- M[3]
      mj  <- M[4]
      mp <- M[5]
      vj  <- (mj**-0.25)
      vp <- (mp**-0.25)
      rhoj  <- (0.4*(mj**-0.25))
      rhop <- (0.4*(mp**-0.25))
      kw <- k[1]
      kb <- k[2]
      kf <- k[3]
      sigmaw <-  sigma[1]
      sigmab <-  sigma[2]
      sigmaf <- sigma[3]
      Kw <- (16.2*(mw**-0.70))
      Kb <- (16.2*(mb**-0.70))
      Kf <- (16.2*(mf**-0.70))
      Kj <- (16.2*(mj**-0.70))
      Kp <- (16.2*(mp**-0.70))
      cwb<-c.mat[1]
      cwj<-c.mat[7]
      cbw<-c.mat[2]
      cbj<-c.mat[8]
      cjw<-c.mat[10]
      cjb<-c.mat[11]
      cwf <- c.mat[5]
      cbf <- c.mat[6]
      cfw <- c.mat[3]
      cfb <- c.mat[4]
      cfj <- c.mat[9]
      cjf <- c.mat[12]
      deltaw <- delta[1]
      deltab <- delta[2]
      deltaf <- delta[3]
      deltaj <- delta[4]

      dsw <- r.temp.w*nw*((Kw-nw-(cwb)*nb-(cwf)*nf-(cwj)*nj)/Kw) -
lambdaw*sw - ((g*sw^2)/(sw^2+h^2)) -
((alfa*np*sw)/(deltaw+nw+((deltaw/deltab)*nb+(deltaw/deltaf)*nf+(del
taw/deltaj)*nj)))
      dew <- lambdaw*sw -kw*ew- ((g*ew^2)/(ew^2+h^2)) -
((alfa*np*ew)/(deltaw+nw+((deltaw/deltab)*nb+(deltaw/deltaf)*nf+(del
taw/deltaj)*nj)))
      diw <- kw*ew - sigmaw*iw - ((g*iw^2)/(iw^2+h^2))-
((alfa*np*iw)/(deltaw+nw+((deltaw/deltab)*nb+(deltaw/deltaf)*nf+(del
taw/deltaj)*nj)))
      drw <- sigmaw*iw - ((g*rw^2)/(rw^2+h^2)) -
((alfa*np*rw)/(deltaw+nw+((deltaw/deltab)*nb+(deltaw/deltaf)*nf+(del
taw/deltaj)*nj)))
      dsb <- r.temp.b*nb*((Kb-nb-(cbw)*nw-(cbf)*nf-(cbj)*nj)/Kb) -
lambdab*sb - ((g*sb^2)/(sb^2+h^2)) -
((alfa*np*sb)/(deltab+nb+((deltab/deltaw)*nw+(deltab/deltaf)*nf+(del
tab/deltaj)*nj)))
      deb <- lambdab*sb -kb*eb - ((g*eb^2)/(eb^2+h^2))-
((alfa*np*eb)/(deltab+nb+((deltab/deltaw)*nw+(deltab/deltaf)*nf+(del
tab/deltaj)*nj)))
      dib <- kb*eb - sigmab*ib - ((g*ib^2)/(ib^2+h^2))-
((alfa*np*ib)/(deltab+nb+((deltab/deltaw)*nw+(deltab/deltaf)*nf+(del
tab/deltaj)*nj)))
```

```
     drb <- sigmab*ib - ((g*rb^2)/(rb^2+h^2)) -
((alfa*np*rb)/(deltab+nb+((deltab/deltaw)*nw+(deltab/deltaf)*nf+(del
tab/deltaj)*nj)))
     dsf <- r.temp.f*nf*((Kf-nf-(cfw)*nw-(cfb)*nb-(cfj)*nj)/Kf) -
lambdaf*sf - ((g*sf^2)/(sf^2+h^2)) -
((alfa*np*sf)/(deltaf+nf+((deltaf/deltaw)*nw+(deltaf/deltab)*nb+(del
taf/deltaj)*nj)))
     def <- lambdaf*sf -kf*ef- ((g*ef^2)/(ef^2+h^2)) -
((alfa*np*ef)/(deltaf+nf+((deltaf/deltaw)*nw+(deltaf/deltab)*nb+(del
taf/deltaj)*nj)))
     diF <- kf*ef - sigmaf*iF - ((g*iF^2)/(iF^2+h^2))-
((alfa*np*iF)/(deltaf+nf+((deltaf/deltaw)*nw+(deltaf/deltab)*nb+(del
taf/deltaj)*nj)))
     drf <- sigmaf*iF - ((g*rf^2)/(rf^2+h^2)) -
((alfa*np*rf)/(deltaf+nf+((deltaf/deltaw)*nw+(deltaf/deltab)*nb+(del
taf/deltaj)*nj)))
     dnj  <- (vj-rhoj)*nj*((Kj-nj-(cjw)*nw-(cjb)*nb-(cjf)*nf)/Kj) -
((g*nj^2)/(nj^2+h^2))-
((alfas*np*nj)/(deltaj+nj+((deltaj/deltaw)*nw+(deltaj/deltab)*nb+(de
ltaj/deltaf)*nf)))
      dnp <- (vp-rhop)*np*(1-
((q*np)/(nw+(deltaw/deltab)*nb+(deltaw/deltaj)*nj)))
     res <-
c(dsw,dew,diw,drw,dsb,deb,dib,drb,dsf,def,diF,drf,dnj,dnp)
     list(res)
     }
)}

# parameters
times <- seq(0,20,length=7300)
gw<-c(-0.006,0.04)
gb<-c(-0.002,0.007)
M<-c(0.02025503,0.01906557,0.02175, 0.0092,0.07845)
k<- c(0.14,0.14,0.14)
alfa <- 1
alfas<-7.674456522
delta <- c(11.31050155,11.31050155,11.31050155,22.621)
cwb_sens.an <- seq(0, 0.9851319, length.out = 6)
cbw_sens.an <- seq(0, 1.015092, length.out = 6)
cfw_sens.an <- seq(0, 0.9513746,length.out = 6)
cfb_sens.an <- seq(0,0.9119129,length.out = 6)
cwf_sens.an <- seq(0,1.051111,length.out = 6)
cbf_sens.an <- seq(0,1.096596,length.out = 6)
cwj_sens.an <- seq(0, 0.5744988, length.out = 6)
cbj_sens.an <- seq(0, 0.5831694, length.out = 6)
cfj_sens.an <- seq(0,0.5475578,length.out = 6)
cjw_sens.an <- seq(0, 1.740648, length.out = 6)
cjb_sens.an <- seq(0, 1.714768, length.out = 6)
cjf_sens.an <- seq(0, 1.826291,length.out = 6)
c.mat<-
c(cwb_sens.an[2],cbw_sens.an[2],cfw_sens.an[2],cfb_sens.an[2],cwf_se
ns.an[2],cbf_sens.an[2],cwj_sens.an[1],cbj_sens.an[1],cfj_sens.an[1]
,cjw_sens.an[6],cjb_sens.an[6],cjf_sens.an[6])
sigma<-c(0.011,0.011,0.011)
g= 0.49312
h = 9.9
q= 56
taub<-0.05
tauf<-0.03
```

```
# perform the realisation
parameters <-
c(M=M,alfa=alfa,alfas=alfas,delta=delta,c.mat=c.mat,g=g,h=h,q=q,
sigma=sigma,tauw=tauw,taub=taub,tauf=tauf,gw=gw,gb=gb)
xstart<-
c(sw=48,ew=0,iw=1,rw=0,sb=75,eb=0,ib=0,rb=0,sf=30,ef=0,iF=0,rf=0,
nj=20, np=3)
output<-
as.data.frame(rk4(xstart,times,multihostSEIR.model,parameters))
output$Prev.w<-output$iw/apply(output[,2:5],1,sum)
output$Prev.b<-output$ib/apply(output[,6:9],1,sum)
output$Prev.f<-output$iF/apply(output[,10:13],1,sum)
```

III.2 Tick-borne pathogen model

```
#requires the package deSolve
require("deSolve")

# define the function
multihostTICK.model <- function( t, x, parameters )

{ l <- x[1]
sn <- x[2]
In <- x[3]
sa <- x[4]
ia <- x[5]
sw <- x[6]
iw <- x[7]
rw <- x[8]
sb <- x[9]
ib <- x[10]
rb <- x[11]
nj  <- x[12]
np <- x[13]

with(as.list(parameters),
     { nv <- l+sn+In+sa+ia
     nw <- sw+iw+rw
     nb <- sb+ib+rb
     nj  <- nj
     np <- np
     nd <- 0
     prop_l <- l/(l+In+sn+ia+sa)
     prop_In <- In/(l+In+sn+ia+sa)
     prop_sn <- sn/(l+In+sn+ia+sa)
     prop_sa <- sa/(l+In+sn+ia+sa)
     prop_ia <- ia/(l+In+sn+ia+sa)
     mw <- M[1]
     mb <- M[2]
     mj  <- M[3]
     mp <- M[4]
     r.temp.w<-ifelse(t %% 1 < 2/3, gw[2],gw[1])
     r.temp.b<-ifelse(t %% 1 < 2/3, gb[2],gb[1])
     vj  <- (mj**-0.25)
     vp <- (mp**-0.25)
     rhoj  <- (0.4*(mj**-0.25))
     rhop <- (0.4*(mp**-0.25))
     Kw <- (16.2*(mw**-0.70))
     Kb <- (16.2*(mb**-0.70))
     Kj <- (16.2*(mj**-0.70))
     Kp <- (16.2*(mp**-0.70))
     sigmaw <-  sigma[1]
     sigmab <-  sigma[2]
     cwb<-c.mat[1]
     cwj<-c.mat[7]
     cbw<-c.mat[2]
     cbj<-c.mat[8]
     cjw<-c.mat[10]
```

```
     cjb<-c.mat[11]
     deltaw <- delta[1]
     deltab <- delta[2]
     deltaj <- delta[4]
     sv <- (0.5+(0.049*log((1.01+((sa+ia)/2))/(nw+nb+nj+np+nd))))

     dl <-
(beta[7]*d[1]*nw+beta[7]*d[1]*nb+beta[8]*d[2]*nj+beta[9]*d[3]*np+bet
a[9]*d[4]*nd)*(sa+ia)*(num_egg-sv*nv)-rhov[2]*l-
(beta[1]*nw*l+beta[1]*nb*l+beta[2]*nj*l+beta[3]*np*l+beta[3]*nd*l)*(
1+1/k)
     # nymphal stage
     dIn <- (beta[1]*d[1]*iw*tauw[1]*l+beta[1]*d[1]*taub[1]*ib*l)*
(1+1/k)-rhov[3]*In-
(beta[4]*nw*In+beta[4]*nb*In+beta[5]*nj*In+beta[6]*np*In+beta[6]*nd*
In)*(1+1/k)
     dsn <-
(beta[1]*d[1]*(rw+sw)*l+beta[1]*d[1]*(rb+sb)*l)+beta[2]*d[2]*nj*l+be
ta[3]*d[3]*np*l+beta[3]*d[4]*nd*l-rhov[3]*sn-beta[4]*nw*sn-
beta[4]*nb*sn-beta[5]*nj*sn-beta[6]*np*sn-beta[6]*nd*sn
     # adult stage
     dia <-
beta[4]*d[1]*nw*In+beta[4]*d[1]*nb*In+beta[5]*d[2]*nj*In+beta[6]*d[3
]*np*In+beta[6]*d[4]*nd*In+(beta[4]*d[1]*ib*taub[2]*sn+
beta[4]*d[1]*iw*tauw[2]*sn)*(1+1/k)-rhov[4]*ia-
(beta[7]*nw*ia+beta[7]*nb*ia+beta[8]*nj*ia+beta[9]*np*ia+beta[9]*nd*
ia)*(1+1/k)
     dsa <-
(beta[4]*d[1]*(rw+sw)*sn+beta[4]*d[1]*(rb+sb)*sn)+beta[5]*d[2]*nj*sn
+beta[6]*d[3]*np*sn+beta[6]*d[4]*nd*sn-rhov[4]*sa-beta[7]*nw*sa-
beta[7]*nb*sa-beta[8]*nj*sa-beta[9]*np*sa-beta[9]*nd*sa

     # hosts
     dsw <- r.temp.w*nw*((Kw-nw-cwb*nb-cwj*nj)/Kw) -
tauv[1]*beta[4]*sw*In-tauv[2]*beta[7]*sw*ia - ((g*sw^2)/(sw^2+h^2))
- ((alfa*np*sw)/(deltaw+nw+((deltaw/deltab)*nb+(deltaw/deltaj)*nj)))
     diw <- tauv[1]*beta[4]*sw*In + tauv[2]*beta[7]*sw*ia -sigmaw*iw
-((g*iw^2)/(iw^2+h^2))-
((alfa*np*iw)/(deltaw+nw+((deltaw/deltab)*nb+(deltaw/deltaj)*nj)))
     drw <- sigmaw*iw - ((g*rw^2)/(rw^2+h^2)) -
((alfa*np*rw)/(deltaw+nw+((deltaw/deltab)*nb+(deltaw/deltaj)*nj)))
     dsb <- r.temp.w*nb*((Kb-nb-cbw*nw-cbj*nj)/Kb) -
tauv[1]*beta[4]*sb*In-tauv[2]*beta[7]*sb*ia- ((g*sb^2)/(sb^2+h^2)) -
((alfa*np*sb)/(deltab+nb+((deltab/deltaw)*nw+(deltab/deltaj)*nj)))
     dib <- tauv[1]*beta[4]*sb*In + tauv[2]*beta[7]*sb*ia -
sigmab*ib - ((g*ib^2)/(ib^2+h^2))-
((alfa*np*ib)/(deltab+nb+((deltab/deltaw)*nw+(deltab/deltaj)*nj)))
     drb <- sigmab*ib - ((g*rb^2)/(rb^2+h^2)) -
((alfa*np*rb)/(deltab+nb+((deltab/deltaw)*nw+(deltab/deltaj)*nj)))
     dnj  <- (vj-rhoj)*nj*((Kj-nj-cjw*nw-cjb*nb)/Kj) -
((g*nj^2)/(nj^2+h^2))-
((alfas*np*nj)/(deltaj+nj+((deltaj/deltaw)*nw+(deltaj/deltab)*nb)))
     dnp <- (vp-rhop)*np*(1-
((q*np)/(nw+(deltaw/deltab)*nb+(deltaw/deltaj)*nj)))
     res <- c(dl,dsn,dIn,dsa,dia,dsw,diw,drw,dsb,dib,drb,dnj,dnp)
     list(res)

     }
)}
```

```
# parameters
times <- seq(0,20,length=7300)
gw<-c(-0.006,0.04)
gb<-c(-0.002,0.007)
M<-c(0.02025503,0.01906557,0.02175, 0.0092,0.07845)
alfa <- 1
alfas<-7.674456522
delta <- c(11.31050155,11.31050155,11.31050155,22.621)
cwb_sens.an <- seq(0, 0.9851319, length.out = 6)
cbw_sens.an <- seq(0, 1.015092, length.out = 6)
cfw_sens.an <- seq(0, 0.9513746,length.out = 6)
cfb_sens.an <- seq(0,0.9119129,length.out = 6)
cwf_sens.an <- seq(0,1.051111,length.out = 6)
cbf_sens.an <- seq(0,1.096596,length.out = 6)
cwj_sens.an <- seq(0, 0.5744988, length.out = 6)
cbj_sens.an <- seq(0, 0.5831694, length.out = 6)
cfj_sens.an <- seq(0,0.5475578,length.out = 6)
cjw_sens.an <- seq(0, 1.740648, length.out = 6)
cjb_sens.an <- seq(0, 1.714768, length.out = 6)
cjf_sens.an <- seq(0, 1.826291,length.out = 6)
c.mat<-
c(cwb_sens.an[2],cbw_sens.an[2],cfw_sens.an[2],cfb_sens.an[2],cwf_se
ns.an[2],cbf_sens.an[2],cwj_sens.an[1],cbj_sens.an[1],cfj_sens.an[1]
,cjw_sens.an[6],cjb_sens.an[6],cjf_sens.an[6])
beta <- c(0.025,0.025,0.025,0.04,0.04,0.04,0.01,0.01,0.06)
d <- c(0.415,0.496,0.639,0.563)/3
num_egg = 1500
rhov <- c(0.002,0.001428,0.000476,0.000408)
g= 0.49312
h = 9.9
q= 56
sigma <- c(0.0083,0.0083)
tauw <- c(0.5,0.5,0.4)
taub <- c(0.5,0.5,0.4)
tauv <- c(0.8,0.8)
k = 0.18

# perform a realisation
parameters <-
c(M=M,alfa=alfa,alfas=alfas,delta=delta,c.mat=c.mat,g=g,h=h,q=q,
sigma=sigma,tauw=tauw,taub=taub,tauv=tauv,k=k,gw=gw,gb=gb)
xstart<-c(l=0,In=0,sn=100,ia=0,sa=0,sw=48,iw=1,rw=0,sb=75,ib=0,rb=0,
nj=20, np=3)
output<-
as.data.frame(rk4(xstart,times,multihostTICK.model,parameters))
```

III.3 Flea-borne pathogen model

```
#requires the package deSolve
require("deSolve")

# define the function
multihostFLEA.model <- function( t, x, parameters )

{sF <-x[1]
 eF <-x[2]
 iF <-x[3]
 sw <-x[4]
 ew <-x[5]
 iw <-x[6]
 rw <-x[7]
 sb <-x[8]
 eb <-x[9]
 ib <-x[10]
 rb <-x[11]
 nj <-x[12]
 np <-x[13]

with(as.list(parameters),
     { nF <- sF+eF+iF
     nw <- sw+ew+iw+rw
     nb <- sb+ew+ib+rb
     nj  <- nj
     np <- np
     mw <- M[1]
     mb <- M[2]
     mj  <- M[3]
     mp <- M[4]
     r.temp.w<-ifelse(t %% 1 < 2/3, gw[2],gw[1])
     r.temp.b<-ifelse(t %% 1 < 2/3, gb[2],gb[1])
     rhoj  <- (0.4*(mj**-0.25))
     rhop <- (0.4*(mp**-0.25))
     rhoF<- 0.00273
     aF<-0.04-rhoF
     vj  <- (mj**-0.25)
     vp <- (mp**-0.25)
     Kw <- (16.2*(mw**-0.70))
     Kb <- (16.2*(mb**-0.70))
     Kj <- (16.2*(mj**-0.70))
     Kp <- (16.2*(mp**-0.70))
     kappaw <-  kappa[1]
     kappab <-  kappa[2]
     cwb<-c.mat[1]
     cbw<-c.mat[2]
     cwj<-c.mat[3]
     cbj<-c.mat[4]
     cjw<-c.mat[5]
     cjb<-c.mat[6]
     deltaw <- delta[1]
     deltab <- delta[2]
     deltaj <- delta[3]
```

```
      MAXFw<- MAXF[1]
      MAXFb<- MAXF[2]
      epsilonw<-epsilon[1]
      epsilonb<-epsilon[2]
      sigmaw<-sigma[1]
      sigmab<-sigma[2]
      lambdaw<-lambda[1]
      lambdab<- lambda[2]

      # flea
      Cf.t<-min(1.1,max(0.65+cos(2*pi*t),0.2))*(nw*MAXFw+nb*MAXFb)
      alphaF_t<-rhoF*Cf.t+aF*max(Cf.t-nF,0)
      rhoF_t<-rhoF+aF*max(1-Cf.t/nF,0)
      dsF <-alphaF_t-
kappaF*((MAXFw*iw+MAXFb*ib)/(MAXFw*nw+MAXFb*nb))*sF-rhoF_t*sF
      deF <- kappaF*((MAXFw*iw+MAXFb*ib)/(MAXFw*nw+MAXFb*nb))*sF-
(lambdaF+rhoF_t)*eF
      diF <- lambdaF*eF-rhoF_t*iF

      # hosts
      dsw <- r.temp.w*nw*((Kw-nw-cwb*nb-cwj*nj)/Kw) - epsilonw*iw +
1/2*kappaw*iw - sigmaw*((MAXFw*iw)/(MAXFw*nw+MAXFb*nb))*sw -
((g*sw^2)/(sw^2+h^2)) -
((alfa*np*sw)/(deltaw+nw+((deltaw/deltab)*nb+(deltaw/deltaj)*nj)))
      dew <- sigmaw*((MAXFw*iw)/(MAXFw*nw+MAXFb*nb))*sw - lambdaw*ew+
epsilonw*iw - ((g*ew^2)/(ew^2+h^2)) -
((alfa*np*ew)/(deltaw+nw+((deltaw/deltab)*nb+(deltaw/deltaj)*nj)))
      diw <- lambdaw*ew - kappaw*iw -((g*iw^2)/(iw^2+h^2))-
((alfa*np*iw)/(deltaw+nw+((deltaw/deltab)*nb+(deltaw/deltaj)*nj)))
      drw <- kappaw*iw - ((g*rw^2)/(rw^2+h^2)) -
((alfa*np*rw)/(deltaw+nw+((deltaw/deltab)*nb+(deltaw/deltaj)*nj)))
      dsb <- r.temp.w*nb*((Kb-nb-cbw*nw-cbj*nj)/Kb) - epsilonb*ib +
1/2*kappab*ib - sigmab*((MAXFb*ib)/(MAXFw*nw+MAXFb*nb))*sb -
((g*sb^2)/(sb^2+h^2)) -
((alfa*np*sb)/(deltab+nb+((deltab/deltaw)*nw+(deltab/deltaj)*nj)))
      deb <- sigmab*((MAXFb*ib)/(MAXFw*nw+MAXFb*nb))*sb - lambdab*eb
+ epsilonb*ib - ((g*eb^2)/(eb^2+h^2)) -
((alfa*np*eb)/(deltab+nb+((deltab/deltaw)*nw+(deltab/deltaj)*nj)))
      dib <- lambdab*eb - kappab*ib  - ((g*ib^2)/(ib^2+h^2))-
((alfa*np*ib)/(deltab+nb+((deltab/deltaw)*nw+(deltab/deltaj)*nj)))
      drb <- kappab*ib - ((g*rb^2)/(rb^2+h^2)) -
((alfa*np*rb)/(deltab+nb+((deltab/deltaw)*nw+(deltab/deltaj)*nj)))
      dnj  <- (vj-rhoj)*nj*((Kj-nj-cjw*nw-cjb*nb)/Kj) -
((g*nj^2)/(nj^2+h^2))-
((alfas*np*nj)/(deltaj+nj+((deltaj/deltaw)*nw+(deltaj/deltab)*nb)))
dnp <- (vp-rhop)*np*(1-
((q*np)/(nw+(deltaw/deltab)*nb+(deltaw/deltaj)*nj)))
      res <- c(dsF,deF,diF,dsw,dew,diw,drw,dsb,eb,dib,drb,dnj,dnp)
      list(res)

      }
)}

# parameters
times <- seq(0,20,length=7300)
gw<-c(-0.006,0.04)
gb<-c(-0.002,0.007)
M<-c(0.02025503,0.01906557,0.02175, 0.0092,0.07845)
alfa <- 1
```

```
alfas<-7.674456522
delta <- c(11.31050155,11.31050155,11.31050155,22.621)
cwb_sens.an <- seq(0, 0.9851319, length.out = 6)
cbw sens.an <- seq(0, 1.015092, length.out = 6)
cfw_sens.an <- seq(0, 0.9513746,length.out = 6)
cfb_sens.an <- seq(0,0.9119129,length.out = 6)
cwf_sens.an <- seq(0,1.051111,length.out = 6)
cbf_sens.an <- seq(0,1.096596,length.out = 6)
cwj_sens.an <- seq(0, 0.5744988, length.out = 6)
cbj_sens.an <- seq(0, 0.5831694, length.out = 6)
cfj_sens.an <- seq(0,0.5475578,length.out = 6)
cjw_sens.an <- seq(0, 1.740648, length.out = 6)
cjb_sens.an <- seq(0, 1.714768, length.out = 6)
cjf_sens.an <- seq(0, 1.826291,length.out = 6)
c.mat<-
c(cwb_sens.an[2],cbw_sens.an[2],cfw_sens.an[2],cfb_sens.an[2],cwf_se
ns.an[2],cbf_sens.an[2],cwj_sens.an[1],cbj_sens.an[1],cfj_sens.an[1]
,cjw_sens.an[6],cjb_sens.an[6],cjf_sens.an[6])
Cf.vals.partial<-sapply(times,Cf.func.partial)
MAXF<- c(1,1.5)
g= 0.49312
h = 9.9
q= 56
sigma <- c(0.021,0.021)
epsilon <- c(0.5,0.5)
lambdaF <- 1 # rate from exposed to infective
lambda <- 1
kappa <- c(0.024,0.024)
kappaF <- 0.26

# perform a realisation
parameters <-
c(M=M,alfa=alfa,alfas=alfas,delta=delta,c.mat=c.mat,g=g,h=h,q=q,
sigma=sigma,MAXF=MAXF,kappaF=kappaF,epsilon=epsilon,lambdaF=lambda,l
ambda=lambda,eta=eta,gw=gw,gb=gb)
xstart<-
c(sF=100,eF=0,iF=1,sw=48,ew=0,iw=1,rw=0,sb=75,eb=0,ib=0,rb=0, nj=20,
np=3)
output<-
as.data.frame(rk4(xstart,times,multihostFLEA.model,parameters))
```

Appendix IV

Rodent species sampled during the study

Rodent species captured during the live-trapping (Chapter 2) were *Apodemus sylvaticus* (wood mouse), *Myodes glareolus* (bank vole), *Myodes glareolus skomerensis* on Skomer Island (Skomer vole), and *Microtus agrestis* (field vole) (Fig. IV.1).

Rodents (Order Rodentia) are a various taxonomic group, of which the main common feature is the number and structure of incisors, a rootless pair, on either the upper and the lower jaw, that have a continuous growing (Single *et al.*, 2001). The aforementioned species are included in the subfamilies Arvicolinae (voles) and Murinae (mice). Voles usually feed on grass and other types of vegetation, while mice are more generalists, consuming seeds, buds, fruits, nuts, but also animal matter (Single *et al.*, 2001). The first are usually active during day and night; they use to live in burrows in woodlands, but also scrubs, grasslands and hedgehogs (Single *et al.*, 2001). Mice are nocturnal and excavate burrows in various habitats, from woodlands to arable lands. Wood mice are more adaptable than voles, and usually are the first recolonizing areas after disturbance (Single *et al.*, 2001). These small rodents are heavily predated by owls and carnivores (Single *et al.*, 2001).

Fig. IV.1. Rodent species sampled during the study. The photographs were taken during this study.