Puumala Virus Dynamics in Bank Voles along Habitat and Community Gradients

The Ecology and Risk of an Emerging Infectious Disease

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Abstract

The majority of recent infectious disease outbreaks are zoonotic, i.e. caused by pathogens shared between humans and other vertebrates, and many of those originate in wildlife. The life cycle of zoonotic diseases is complex, and involves at least one non-human host. To adequately assess human risk, we need to understand relevant ecological interactions driving host and pathogen populations.

Puumala hantavirus (PUUV) is a directly transmitted pathogen, carried by the bank vole (*Myodes glareolus*), and causes a mild form of haemorrhagic fever in humans. Using long-term data from a 100 × 100 km study area, my project aimed to improve spatial and temporal predictions of PUUV risk in northern Sweden. I was interested in how community interactions influence bank vole abundance and infection rates, in an ecosystem where several species recently declined.

We found that either overall density or density of infected voles can be used to predict incidence in humans, and the predictor of choice depends on the seasonal relationship between bank vole density and PUUV prevalence. Also, bank vole density and distribution in the landscape at the beginning of a vole population cycle can predict peak human risk during that cycle, approximately 18 months later.

To identify plots with infected bank voles, we developed and successfully validated a model based on microhabitat variables. Amongst others, important variables were related to cover, e.g. large holes, and resource availability, e.g. bilberry shrubs.

Community interactions contributed to both host and pathogen dynamics, and we found evidence for the dilution effect, by which non-host species may reduce PUUV prevalence or host density. The decline in Tengmalm's owl (*Aegolius funereus*), an important predator of voles, coincided with a long-term increase in the density of infected bank voles, and owls were more likely to prey on infected bank voles in less isolated forest patches. PUUV prevalence declined with common shrew (*Sorex araneus*) density, while bank vole density decreased as field vole (*Microtus agrestis*) density increased in clear-cuts.

The present work enables public health professionals to forecast PUUV outbreaks and predict the spatial distribution of infected voles. Further, authorities and other stakeholders ought to conserve and promote functional diversity in the ecosystem, given the potential of competitors and predators to reduce human risk.

Keywords: bank vole; common shrew; dilution effect; disease ecology; field vole; grey-sided vole; *nephropathia epidemica*; Puumala virus; Tengmalm's owl; zoonosis

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Dedication

To my loving family

“Wisdom ceases to be wisdom when it becomes too proud to weep, too grave to laugh, and too selfish to seek other than itself.”

Khalil Gibran; A Lebanese writer, poet, and painter

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Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Khalil H, Ecke F, Evander M, Bucht G, Hörnfeldt B. Ecology meets epidemiology: Early warning of zoonotic risk. (Submitted manuscript).

II Khalil H, Olsson GE, Magnusson M, Evander, M, Hörnfeldt B, Ecke F. Spatial prediction and validation of zoonotic hazard through micro-habitat properties: where does Puumala hantavirus hole – up? (Submitted manuscript.)

III Khalil H, Ecke F, Evander M, Hörnfeldt B (2016). Selective predation on hantavirus-infected voles by owls and confounding effects from landscape properties. *Oecologia* 181(2), 597 – 606.

IV Khalil H, Ecke F, Evander M, Magnusson M, Hörnfeldt B (2016). Declining ecosystem health and the dilution effect. *Scientific Reports* 6, (31314).

Papers III and IV are reproduced with the permission of the publishers.

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The contribution of HK to the papers included in this thesis was as follows:

I Participated in laboratory analyses, analysed the data, and wrote most of the manuscript.

II Developed the idea with the co-authors, the models, and wrote most of the manuscript.

III Collected parts of the data, participated in lab work, analysed the data, and wrote most of the manuscript.

IV Took part in developing the idea, analysed the data, and wrote most of the manuscript.

Abbreviations

AUC	Area under curve
BRT	Boosted regression trees
GAM	Generalized additive model
GLM	Generalized linear model
GLS	Generalized least square regression
NE	Nephropathia epidemica
PUUV	Puumala virus

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1 Introduction

Infectious diseases have plagued humanity for hundreds of thousands of years, especially as agriculture and livestock keeping allowed larger human populations and settlements. Diseases have greatly influenced the course of our history, e.g. European conquest of the Americas, and left their mark on the human genome (Wolfe et al., 2007). Unsurprisingly, the origin of infectious diseases has been of interest since antiquity, and Hippocrates is credited with ascribing natural rather than super-natural causes to disease in his essay "On Airs, Waters, and Places", ca 2500 years ago.

Epidemiology – the study of patterns and causes of disease in a population – is the standard method to track and deal with infectious disease outbreaks in human populations. A classic example is the Cholera outbreak of London in the 1800s. Anaesthesiologist John Snow mapped where people infected with Cholera lived and worked, and suspected that the source of infection was a particular water pump. Further investigation revealed that using that pump was a common factor shared by Cholera-infected individuals. He convinced municipal officials to remove the handle of the water pump, which was sufficient to end the outbreak (Snow, 1936). After tracing and deciphering the origin(s) of an infectious disease, epidemiology aims to mitigate and eradicate it. This is often done by isolating infected individuals, quarantining suspected cases, and vaccinating susceptible individuals. Ever since the advent of germ theory of diseases, epidemiology has played a major role in reducing and eradicating infectious diseases such as smallpox.

Epidemiological studies often focus on the minimum number of species involved in the transmission chain of an infectious agent, and it is precisely this approach that made epidemiology effective in the fight against many pathogens (Ostfeld, 2012). However, when disease systems become more complex and

involve multiple interacting species, a reductionist approach can do little to predict outbreaks or emergence of pathogens.

The majority of recent outbreaks of infectious diseases are zoonotic, i.e. diseases shared between humans and other vertebrates (Taylor et al., 2001). Hosts and vectors of zoonoses, especially those originating in wildlife, are hard to isolate or vaccinate (but see example of bovine tuberculosis, where isolation of infected buffaloes succeeded in reducing infection prevalence (Michel et al., 2006)). Such difficulties necessitate a different and holistic approach to mitigate human risk. Indeed, a growing *corpus* of ecological literature deals with how changes in resource availability, weather conditions, or community composition are ultimately coupled to zoonotic risk, usually by driving host (and/or vector) density, its pathogen infection rates, and behaviour (Ostfeld et al., 2008).

Disease ecology seeks to understand the important links that govern the relationship between a pathogen and its host (and/or vector) on one hand, and the environment they interact within on the other. Once factors that drive disease dynamics in hosts and vectors are understood, it becomes possible to forecast outbreaks in time and space. For example, West Nile Virus causes encephalitis in humans and is mainly carried by migrating birds. Knowledge of bird migration patterns enables predicting the probability of West Nile Virus reaching previously unaffected locations such as Great Britain (Bessell et al., 2014). In the eastern United States, high production of acorns by Oak (*Quercus* spp.) leads to an increase in density of white-footed mice (*Peromyscus leucopus*) – hosts of the pathogenic spirochete *Borrelia burgdorferi*. This culminates in higher density of infected ticks that can bite and infect humans. By keeping track of acorn production, it is possible to predict human risk two years earlier (Ostfeld et al., 2006).

Intermittent increases in zoonotic risk may result from natural processes. For example, in Kazakhstan, cyclic host – pathogen interactions drive outbreaks of *Yersinia pestis* (plague). Gerbils, which carry the bacteria, must reach threshold densities for an epidemic to occur (Davis et al., 2004). The epidemic leads to the extinction of many gerbil colonies and a temporary reduction of zoonotic risk. The few surviving gerbil colonies then expand and proliferate, resulting in another epidemic and increasing human risk, and so on. However, over the past few decades anthropogenic activities may well have caused or exacerbated zoonotic outbreaks. Through climate and land-use change, loss of biodiversity,

and introduction of exotic species, humans have increased the global burden of zoonotic pathogens (Gortazar et al., 2014)

Consequently, we are in a race against time to establish generalities regarding disease ecology that we may use to avert future outbreaks (Ostfeld et al., 2008), and answer four essential questions concerning the emergence or outbreak of a given zoonosis:

1- Why now?
2- Why here?
3- Where and when next? and
4- What can we do about it?

Disease ecology has huge potential, yet, it must make further progress if it is to answer the above questions. To achieve sufficient understanding of a disease system, long-term studies are needed to capture regular and rarer variations in that system. These variations may be caused by changes in weather and resources, or ecological processes such as competition or predation. Similarly, the spatial scale needs to be large enough to account for differences among landscapes and communities (Ostfeld et al., 2005). Finally, to elucidate both patterns and processes governing the system under study, diverse approaches including descriptive and experimental studies, ought to be used.

Scientists generally, and ecologists especially, are familiar with the difficulty of procuring long-term research funding. It is next to impossible to keep a study going for many years at a large spatial scale. Fortunately for me, I was part of a project that has been running since 1971, in a 10 000 km^2 area near the city of Umeå in northern Sweden. Through this project, I had detailed information on the zoonosis I worked with, including extensive data on host density and infection rates, density of its competitors and one of its predators, and human incidence. Thanks to the work of my supervisors and other researchers, I had access to background data pertaining to the study area; from how many lingonberry bushes were in a plot to the total area of clear-cuts in the landscape.

I will first describe the disease system I studied, and then present the aims of my project and the hypotheses investigated herein.

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1.1 Puumala hantavirus – bank vole system

During the Korean War (1950 – 1953), a mysterious illness was making UN soldiers ill with fever and nephritis, and came to the fore of international attention. When it became clear that the illness was caused by an airborne agent, the Soviet Union, China, and USA grew interested in identifying it, given its potential to be weaponized (Lee et al., 2014). The etymological agent was finally isolated by Ho Wang Lee and his team in the late 1970's from the striped field mouse *Apodemus agrarius*. The virus was named after the Hantaan river, which runs through both North and South Korea (Lee et al., 1978). The genus was called *Hantavirus* and belongs to the family *Bunyaviridae*. In the decades that followed, dozens of other hantavirus species were isolated from old and new world rodents, insectivores, and bats in all continents but Oceania (Kruger et al., 2015).

Before its appearance in Korea, the disease was known in different places under different names from northern Scandinavia (Myrhman, 1934) to Japan. In Europe, two pathogenic hantaviruses are responsible for thousands of annual cases of hemorrhagic fever with renal syndrome, which leads in some cases to renal impairment, and in rare occasions to death (Vapalahti et al., 2003). Puumala virus (PUUV) was isolated from the bank vole (*Myodes glareolus*) in 1980 and named after the area where the infected voles were trapped (Brummer-Korvenkontio et al., 1980). PUUV causes a mild form of hemorrhagic fever known as *nephropathia epidemica* (NE) in Russia, Central and Western Europe, and northern Fennoscandia (Olsson et al., 2010). Whereas Dobrava hantavirus is responsible for most cases of hemorrhagic fever in Central and Eastern Europe, and is carried by yellow-necked mice and wood mice (*Apodemus* spp.) (Nemirov et al., 1999).

1.2 Disease incidence in humans and bank vole dynamics

The number of annual cases of NE is strongly linked to bank vole density in Finland (Kallio et al., 2009), Sweden (Nyström, 1977; Niklasson et al., 1995; Olsson et al., 2009), and central-western Europe (Tersago et al., 2010; Reil et al., 2015). In Fennoscandia most NE cases occur in winter, when infected bank voles infest human dwellings (Olsson et al., 2003). Humans become sick after inhaling aerosolized viral particles, secreted or excreted by infected bank voles through saliva, urine, and feces. Among bank voles, PUUV transmission is horizontal via direct contact, e.g. grooming or biting, or through viral particles secreted or excreted in the environment (Hardestam et al., 2008). PUUV

infection and viral shedding in bank voles are chronic (Voutilainen et al., 2015) and the infection is considered to be asymptomatic (but see Kallio et al., 2007).

The bank vole is the only natural host of PUUV, and is a common and widely distributed species (Baillie et al., 2004). In boreal Fennoscandia, vole populations undergo 3 to 4 year cycles, with hundred-folds differences in density between high and low density phases (Hörnfeldt, 1978, 1994; Hansson et al., 1985). Bank vole distribution in the landscape also expands and contracts depending on the cycle phase (Hörnfeldt, 2004). In Central and Western Europe, bank vole populations generally exhibit seasonal variation, yet intermittently reach extreme densities following high production of oak (*Quercus* spp.) and beech (*Fagus* spp.) seeds, which provide energy for reproduction and survival through winter (Tersago et al., 2008).Bank voles are generally considered a forest-dwelling species, but achieve considerable densities in other habitat types during high-density years (Ecke et al., 2002). Also, it often persists at low species diversity and tolerates habitat disturbance caused by forestry (Ecke et al. *submitted manuscript*).

Between 2006 and 2008 in Sweden, more than 2000 people were diagnosed with NE. During winter 2006/2007, Umeå hospital struggled to cope with the influx of cases. This occurred a few weeks after a sudden increase in temperature in northern Sweden, causing out-of-season rains, snow-melt, and ground freezing. The abnormal weather conditions preceding the outbreak revealed the role of winter weather and snow conditions in exacerbating NE risk during winter (Pettersson et al., 2008; Khalil, Olsson, et al., 2014). A year later there was an even larger outbreak in 2007/2008, driven by very high bank vole densities (Olsson et al., 2009).

1.3 PUUV risk in time and space

While NE incidence is strongly related to bank vole density, many questions related to temporal and spatial aspects of NE risk in boreal Fennoscandia are yet to be answered. For example, given what we know now through my PhD project, the outbreaks of 2006/2007 and especially 2007/2008 could have been predicted even earlier, allowing hospitals and public health officials to raise awareness and preparedness.

In temperate Europe, NE risk can be predicted two years in advance based on weather conditions that promote high seed production from broad-leaf trees such as oak and beech. At northern latitudes, despite extensive knowledge on

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the population cycles of small mammals (Hörnfeldt, 1978, 1994, 2004; Hansson et al., 1985; Huitu et al., 2004), existing studies predict NE incidence only a few months before an outbreak (Kallio et al. 2009; Olsson et al. 2009; Khalil, Olsson, et al. 2014).

The lack of a very early warning system for NE in Fennoscandia could perhaps be attributed to two reasons. First, to predict NE risk based on cyclic fluctuations in bank vole populations, density and infection data for at least a few population cycles are required. This corresponds to at least a decade of seasonal field and lab work. The second reason is the assumption that PUUV dynamics follow similar patterns from one cycle to the next, or at least that the differences among cycles are negligible. This is clearly not the case, and a quick look at Figure 1 demonstrates that not all cycles are equal in terms of their PUUV dynamics. Indeed, it is precisely the difference among cycles that I focused on to improve the prediction of NE.

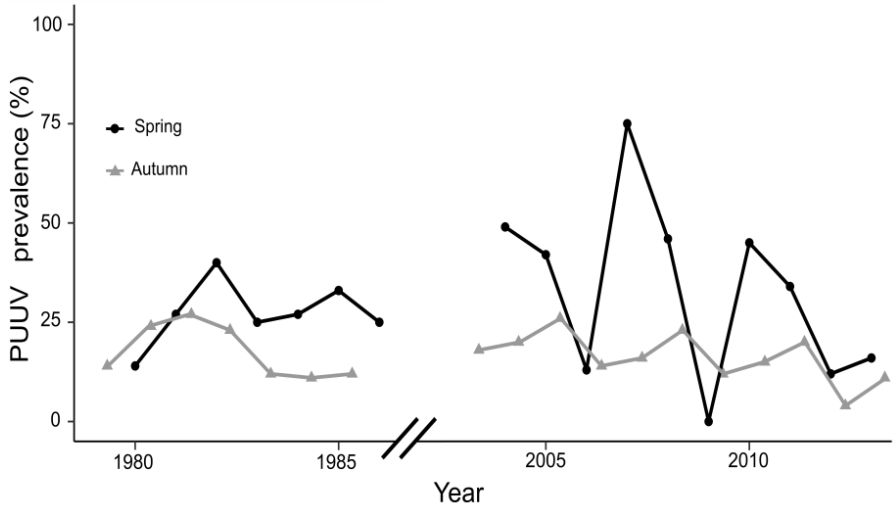

Figure 1. Puumala virus (PUUV) prevalence in bank voles from autumn 1979 to spring 1986 and autumn 2003 to autumn 2013. No infection data was available in 1971-1978 and 1987-2002. Paper I

Our understanding of what determines the spatial patterns of PUUV infection in bank voles remains deficient. We know that high-quality habitats can sustain more bank voles (Ecke et al., 2001, 2002; Olsson et al., 2005), leading to increased infection prevalence through direct or delayed density-dependent mechanisms (Davis et al., 2005). Host survival is also higher in good quality

habitats, allowing older individuals to pass on the virus to the next cohort (Calisher et al., 2001). Yet, microhabitat properties may influence host movement patterns, contact rates, and host **and** viral survival, yet have rarely been used to account for PUUV presence. Also, studies commonly consider habitat quality for the host but not for the virus. Indirect transmission through the environment is necessary for the long-term persistence of PUUV (Sauvage et al., 2003), and extended survival outside the host is proposed to be related to moisture and temperature (Kallio, Klingström, et al., 2006). For instance, forests with dense canopy have lower forest floor temperature, higher humidity, and get less ultraviolet light penetration (Goodin et al., 2009). Hence, microhabitat data can shed light on how host and PUUV environmental requirements come together to determine spatial risk of PUUV.

Additionally, little is currently known about the properties of infection "refugia" where hantaviruses persists when bank vole density is low (but see Glass et al., 2007). For PUUV, this is partly due to rarity of long-term studies, which are needed to describe the ebb and flow of infection on a landscape scale. Characterizing habitats where infected animals are frequently found and from which infection subsequently spreads reduces the scale needed for surveillance.

1.4 A deteriorating ecosystem and the dilution effect

Long before the 2006 – 2008 NE outbreaks, several species in our study area had already declined. This was most likely due to milder winters and forestry (Magnusson et al., 2015). Today, approximately 40% of the landscape consists of forest that has been clear-cut at some point (Ecke et al., 2013). Young even-aged forests lack the extensive three-dimensional structures and ground cover found in older forests (Stenbacka et al., 2010), which provide shelter and food for forest-dwelling voles (Ecke et al., 2002).

Tengmalm's owl (*Aegolius funereus*) is a main predator of small mammals. It has declined over the past three decades, most likely due to the decline of vole species, which constitute > 90 % of the owl's diet (Hörnfeldt et al., 1990, 2005). The grey-sided vole (*Myodes rufocanus*) (Figure 2b) is the main competitor of bank voles in forests, and has drastically declined to become regionally extinct (Hörnfeldt et al., 2006; Ecke et al., 2010; Magnusson et al., 2015). The field vole (*Microtus agrestis*) has also declined since the 1970's (Figure 2c), yet persists in the landscape mainly in open areas dominated by

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grasses in the field layer, such as meadows and clear-cuts (Hansson, 1971; Magnusson et al., 2015).

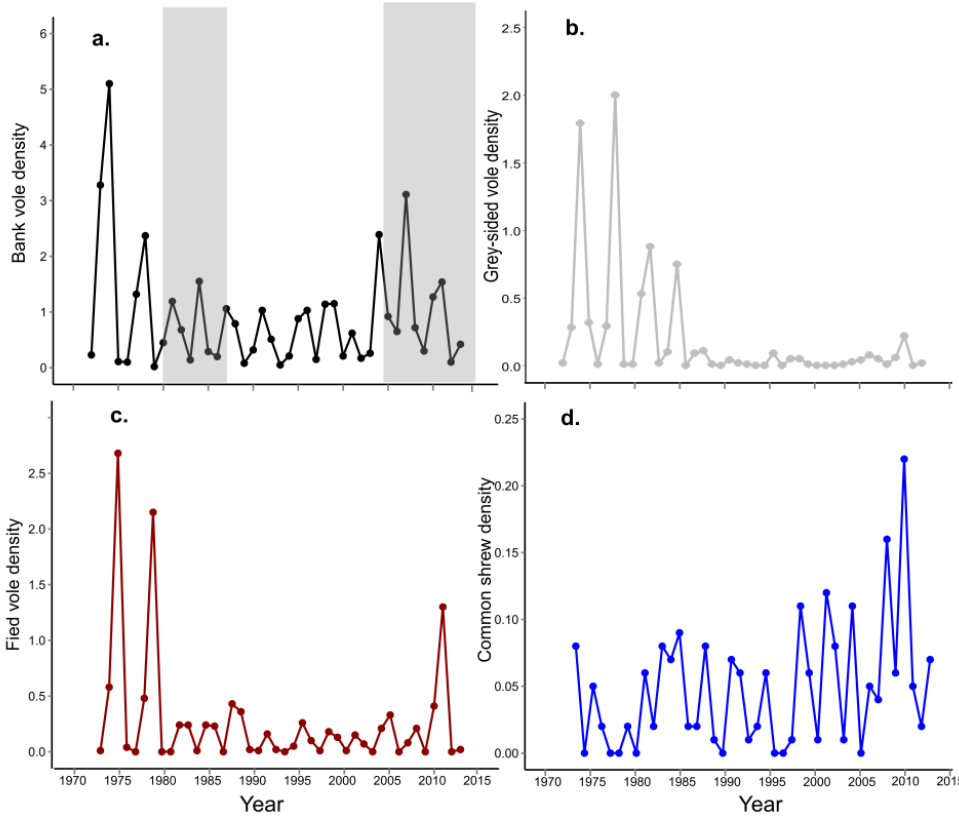

Figure 2. Small mammal density in spring (trapped individuals per 100 trap nights) in 1971 – 2013. **a.** Bank vole, **b.** Grey-sided vole, **c.** Field vole, **d.** Common shrew. The shaded area represents time periods where Puumala hantavirus infection data was available. Adapted from Paper IV

Although the bank vole declined in the 1980's and 1990's, unlike its competitors, its populations recovered in the 2000's (Figure 2a). The decline in competitors and a main predator of bank voles has presumably contributed to lower functional diversity in the ecosystem, which may ultimately be detrimental to human health through the loss of a valuable ecosystem service: the "dilution effect". The link between diversity and disease risk is interesting from public health and scientific perspectives. Briefly stated, in disease systems where species vary in their susceptibility to infection by a pathogen, higher diversity often results in lower disease risk (reviewed in Civitello et al., 2015). The dilution effect (Ostfeld et al., 2000) and its framework for zoonotic

systems was developed for the tick-borne Lyme disease system (Schmidt et al., 2001; Ostfeld, 2012). It has also been shown for the mosquito-borne West Nile Virus. Passerine birds are the most competent hosts for the virus, and Ezenwa et al. (2006) showed that mosquito infections rates are lower where non-passerine species richness was high. By diverting mosquito bites away from the most competent hosts, non-passerine birds dilute infection, resulting in lower human incidence.

A key component of the dilution effect is that species-assemblages are nested, where host species tend to persist at low diversity (Mills, 2006; Johnson et al., 2013; Huang et al., 2013). The bank tends to be a habitat generalist and most importantly tolerates human disturbance, mainly in the form of clear-cutting in our study area. This stands in contrast to its competitor, the forest specialist grey-sided vole (Figure 2a, b).

For directly transmitted pathogens such as hantaviruses, the dilution effect occurs when inter-specific processes such as predation or competition reduce host density or alter its behavior such that transmission is reduced. For example, in Central and South America, agricultural activities resulted in changes in the composition of rodent assemblages, which became species-poor. The species that persisted were hantavirus hosts, and their release from competition in agricultural and peri-domestic areas increased human risk (Mills, 2006).

The lack of interspecific competition may allow bank voles to reach high densities in habitats that otherwise would be occupied by competitively superior species, e.g. the field vole in open habitats (Henttonen et al., 1977) and the grey-sided vole in forests (Löfgren, 1995). The field vole could cause a "dilution effect" due to its ability to affect both bank vole behavior and survival (Eccard et al., 2007). The common shrew (*Sorex araneus*) is a competitor and nest predator of bank voles (Liesenjohann et al., 2011). This solitary small-sized insectivore can be found in most habitat types (Hanski et al., 1989), and has not declined in our study area (Figure 2d). Recent studies have shown that the presence of common shrews influences the behavior and home range of lactating female bank voles (Liesenjohann et al., 2011, Liesenjohann et al. 2015). Thus, the common shrew may dilute PUUV infection in bank voles through influencing its behavior.

Predators also have the potential to reduce the risk of zoonotic diseases spread by small mammals (Ostfeld et al., 2004). In an experimental setting, the

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perceived absence of barn owls resulted in greater foraging activity and different habitat use of rodent prey compared when owls where present (Brown et al., 1988). Increased activity of hosts may lead to higher transmission rates and prevalence, i.e. proportion of infected individuals. By limiting host densities or even selectively taking infected animals, predators may reduce the number of infected prey or prevalence in host populations. No study according to our knowledge has explored the role of predation in controlling zoonotic diseases beyond theoretical models (e.g. Ostfeld and Holt 2004; Levi et al. 2012). Tengmalm's owls breed in tree holes and readily use nest boxes to cache their prey as a buffer against food shortage. Caching of bank voles provides the opportunity to compare PUUV prevalence in prey to that in the general population, and may reveal a role for owls in reducing the number of infected bank voles.

1.5 Aims

My project investigated the PUUV – bank vole system in a well-studied area affected by forestry, milder winters, and a decline in diversity. My thesis had dual but not mutually exclusive concerns. First, from a public health perspective, I was interested in assessing spatiotemporal risk of NE, to enable early prediction of risk and characterize key habitats of PUUV-infected voles. With data available at multiple trophic levels, the thesis also tackled questions pertaining to disease ecology in a community context, for which this one–host, one–pathogen system is an ideal and simple model. Broadly, I was interested in how inter-specific interactions and habitat factors influence bank vole density, PUUV prevalence, or both.

Factors determining bank vole density or PUUV infection prevalence are either intrinsic to host populations, i.e. related to reproduction and demography, or extrinsic, e.g. habitat properties, resource availability, and predation and competition. The intrinsic factors influencing PUUV dynamics in bank voles have been thoroughly studied and I believe that they are well understood (reviewed in Khalil, Hörnfeldt, et al., 2014). The present work focused more on the extrinsic factors influencing the PUUV – bank vole system. Here, I present the aims of this thesis (Figure 3) and then develop the hypotheses further at the beginning of the methods section.

1- How can we use long-term data on bank vole density and PUUV prevalence for very early prediction of NE risk? (Paper I)
2- What characterizes habitats where infected voles are frequently found, and can we predict their presence in an independent area? (Paper II)
3- Are infected voles more likely to be preyed upon by Tengmalm's owls? (Paper III)
4- Do Tengmalm's owls, common shrews, and field voles influence bank vole density or PUUV prevalence? (Paper IV)

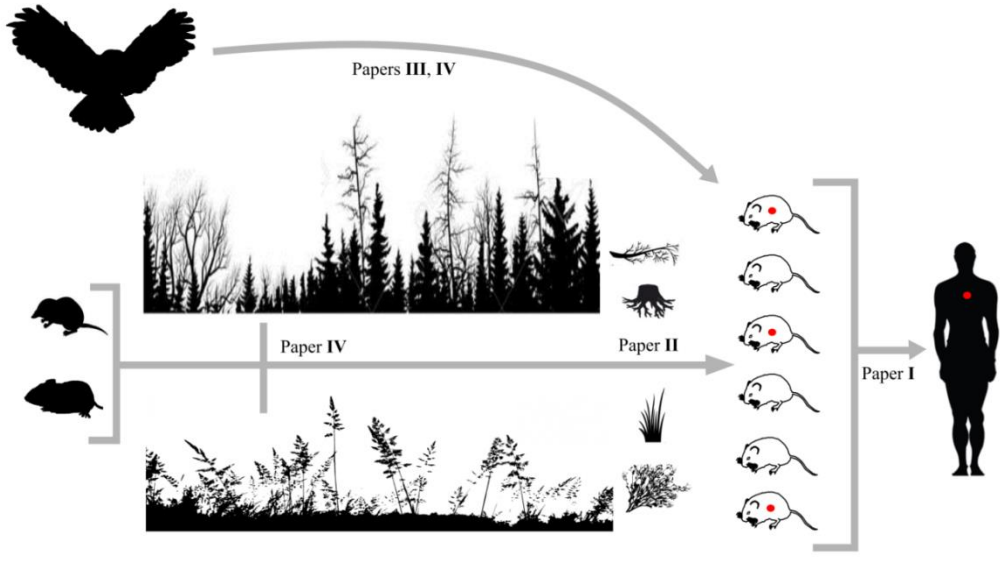

Figure 3. Graphical illustration of the aspects covered in the thesis based on Papers I – IV. Paper I focused on the prediction of disease incidence in humans. In Paper II, we used microhabitat properties to predict the presence of bank voles infected with Puumala virus. Papers III and IV investigated the influence of Tengmalm's owl and two bank vole competitors on bank vole density and PUUV prevalence, accounting for landscape and habitat properties.

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2 Methods

The time series on bank vole density and PUUV infection (42 and 17 years, respectively), coupled with extensive microhabitat data from several habitat types provided a rare opportunity for improving temporal predictions of NE incidence and environmental hazard posed by infected bank voles (Papers I and II).

By exploring the relationship between bank vole density and PUUV prevalence at the time of sampling, we attempted to improve the prediction of seasonal NE incidence (Paper I). We then used available infection data and bank vole landscape distribution to develop a simple model that can predict peak NE risk as early as possible, i.e. in the first spring of the vole population cycle (increase phase) when densities are normally at a 3 – 4 year minimum (Hörnfeldt, 1994).

After exploring the temporal aspects of the PUUV – bank vole – NE system, we developed a Boosted Regression Trees (BRT) model to identify microhabitat characteristics important for the presence of infected bank voles in spring and autumn. We used PUUV infection data in autumn 2003 – autumn 2013, to match with microhabitat data collected in 2012 – 2013. We validated the models in an independent area by predicting seasonal presence of infected voles in a total period of five years.

The latter part of the thesis was concerned with community interactions and the dilution effect. The response variables I was interested in were density of bank voles and infection prevalence; the latter linking host population density to the zoonotic hazard they pose. I investigated the relationship between the aforementioned variables to two competitors and a main predator. After accounting for landscape properties, I asked whether owls were more likely to

prey upon and cache infected bank voles compared to what is found in the population (Paper III). In Paper IV, I tested whether the field vole and common shrew diluted PUUV infection by reducing bank vole density or its PUUV prevalence, and if the dilution effect was habitat dependent.

2.1 Study area and species data

2.1.1 Monitoring area (Papers I, II, IV)

My study area was near the city of Umeå in northern Sweden (64 ºN, 20 ºE), and belongs to the middle boreal zone (Ahti et al., 1968). It is part of the ongoing Swedish National Environmental Monitoring Program for small rodents, to which I will subsequently refer as the monitoring area. Within a 100 ×100 km area, trapping of small mammals takes place in 58 systematically placed 1-ha plots of at least 2.5 km inter-distance (Hörnfeldt, 1978, 1994, 2004). Habitat types within the monitoring area include old forest dominated by Norway spruce (*Picea abies*) or Scots pine (*Pinus sylvestris*) (> 80 years-old), intermediate-aged forest (20 – 80 years), clear-cuts (0 – 20 years), and mires and meadows. The majority of the plots are located within forests and all major forest vegetation types are represented, i.e. lichen, mesic, moist, and wet (see further Ecke et al., 2013). Two sampling plots are on meadows dominated by grasses in the field layer; for small mammals a habitat type often functionally similar to clear-cuts (Hansson, 1971).

2.1.2 Small mammal data (Papers I, II, IV)

Small mammal data from the monitoring area were available in spring and autumn 1971 – 2013. Trapping takes place twice a year: spring (May) and autumn (September). Each sampling plot contains 10 trapping stations placed 10 m apart; unless any of the trapping stations are within non-trappable locations such as lakes. Each plot was trapped with five snap-traps per station for three consecutive nights and the total effort is 150 trap nights. We calculated an index of density for each species as the number of individuals trapped per 100 trap nights, and I subsequently refer to this index as density.

Given the cyclic fluctuations of small mammal populations in this area, we classified years based on the phase of the population cycle as follows: ‘increase’, ‘peak’, and ‘decline’ years. In total, the bank vole time series spanned 12 complete population cycles (numbered I – XII) and exhibited marked differences in amplitude and peak densities during the 43-year period.

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Trapping of animals was approved by the Swedish Environmental Protection Agency (latest permission: NV-01124-15) and the Animal Ethics Committee in Umeå (latest permissions: Dnr A 61-11 and A121-11). Applicable institutional and national guidelines for the use of animals were followed.

2.1.3 Microhabitat data in the monitoring and validation areas (Paper II)

The field surveys of microhabitat properties were done in autumn 2012 and 2013 in all 58 plots. Vegetation and structural habitat variables were recorded within a quadratic plot with 2.5 m sides centered on each trapping station (see Paper II and Ecke et al., 2002 for details on similar sampling of habitat variables).

To validate the microhabitat models predicting the occurrence of infected bank voles, we used unpublished trapping data from a project focusing on the response of small mammals to a forest fire. The study area for that project was 200 km north of the monitoring area (approx. 66 º N, 20 º E). Small mammal sampling and recording of microhabitat data followed a similar protocol as that for the monitoring area. Sampling occurred from spring 2007 to autumn 2010 as well as spring and autumn 2015 in 17 1-ha plots (n = 7 old forests, n = 7 burnt forest, n = 3 were clear-cuts) (Nematollahi Mahani, 2016).

2.1.4 Tengmalm's owl data and nest box study (Papers III and IV)

Data on Tengmalm's owl breeding was available for this study between 1980 and 2013 from nest boxes placed in trees, at approximately 1 km interval, in an area partially overlapping with the monitoring area (Hörnfeldt et al., 1990). The number of nest boxes checked per year varied and ranged between 275 and 500 (Löfgren et al., 1986; Hipkiss et al., 2013), and breeding attempts were confirmed through systematic visits in spring. Tengmalm's owl reproduction is largely dependent on vole density (Hörnfeldt et al., 1990) and is reflected in inter-annual variation in box occupancy (%) by breeding owls.

In Paper III, nest boxes with breeding owls were identified in late February 2014 and were visited every third day from March until mid-May 2014. We collected bank voles cached in nest boxes and replaced them with marked frozen voles or mice. Once ≥10 bank voles were collected, we trapped small mammals within a 500 m radius around the nest box. We only trapped in closed-canopy forest to control for habitat differences (i.e. we avoided trapping

in young-forest, clear-cuts, and meadows). Trapping effort around different nest boxes varied and ranged between one and four occasions. However, trapping effort around each nest box was always more than 100 trap nights (mean = 188, range: 120 – 270).

In total, we collected and trapped voles from 15 pairs of nest box-surrounding patches over a period of seven weeks; amounting to 497 bank voles. We calculated the number of trapped bank voles per 100 trap nights (density), and the number of collected bank voles from a nest box per visit.

The nest box study in Paper III was approved by the Animal Ethics Committee in Umeå (Dnr A 11-14, A 12-14 and A 13-14), and we followed the national and institutional and national guidelines for the use of animals.

2.2 Bank vole infection data (Papers I – IV)

PUUV infection data from the monitoring area was already available between autumn 1979 and autumn 1986 (Paper I and IV). Within my PhD project, we accessed bio-banked bank voles to obtain infection data in autumn 2003 – autumn 2013 (Papers I, II, IV); and analyzed the samples from the nest box study, collected in spring 2014 as detailed above (Paper III).

We analyzed lung samples by enzyme-linked immunosorbent assay (ELISA) to detect anti-PUUV IgG antibodies and identify sero-positive individuals (Lindkvist et al., 2008). Sero-positive bank voles weighing <14.4g may carry maternal antibodies (Kallio, Poikonen, et al., 2006) and were excluded from further analyses as their status may not reflect genuine infection (n = 348 in 1979 – 1986; n = 902 in 2003 – 2013). I refer to sero-positive voles as infected, since PUUV infection is chronic (Voutilainen et al., 2015). Also, using a subsample of bank vole lung biopsies, we found that the results from serology closely matched those from PCR.

The analyses of available PUUV infection data from the monitoring area were based on 2064 and 4294 bank voles in 1979 – 1986 and 2003 – 2013, respectively (Papers I, II, IV), and 497 from the nest box study (Paper III).

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2.3 NE incidence data (Paper I)

NE has been a notifiable disease in Sweden since 1989, and incidence data was available from the website of the Public Health Agency of Sweden (https://www.folkhalsomyndigheten.se/ [*In Swedish]*). NE incidence (no. cases per 100,000 inhabitants) was divided into two six month periods: spring – summer period (April – September, hereon referred to as **summer**) and autumn – winter period (October – March, hereon referred to as **winter**), which approximately match spring and autumn trapping, respectively. In subsequent analyses, we used NE incidence data in 1990 – 2013, as preliminary analysis suggested that NE incidence was under-diagnosed in the first year of reporting, 1989, which was a negative outlier (Khalil, Olsson, et al., 2014).

2.4 Brief summary of analyses

I performed the majority of hypothesis testing using generalized linear models (GLMs, Papers I, III, IV), generalized additive models (GAMs, Paper I), and generalized least square models (GLS, Paper IV). When running statistical tests (Papers I, III, IV), I tried to keep the number of included independent variables and their interactions to a minimum to avoid type I errors and spurious relationships. If there was no *a priori* reason to believe that a variable influenced the response, I resisted the urge to include it. In Paper II, I used BRT, which does not provide *p*-values. Instead, it focuses on predictive power and the performance of a fitted model is evaluated through cross-validation or preferably using independent data. Below is a short account of the analyses run in each paper.

2.4.1 PUUV risk in time and space (Papers I and II)

We used GAMs to look for long-term changes in NE incidence in summer and winter between 1990 and 2013. We then tested for direct and delayed density dependence of PUUV prevalence on bank vole density. To predict NE incidence in summer and winter 2003 – 2013, we used either overall bank vole density or density of infected voles in spring and autumn, respectively. Finally, we used bank vole density in spring of the increase phase (*sensu* Hörnfeldt 1994) to predict the maximum density of infected voles reached later in the cycle. We correlated the proportion of the 58 1-ha plots occupied in spring of the increase phase as defined above with density of infected bank voles in spring of the following year, a peak year.

To predict the presence of bank voles in general and the presence of infected bank voles using microhabitat properties, we fitted BRT models to spring and autumn data using the variables listed in table 1 in Paper II. This resulted in two models per season: one to predict general bank vole presence and the other for predicting the presence of infected voles. We validated the models by predicting the seasonal presence of infected bank voles in an independent area over a total period of five years. See the methods section in Paper II for more details on BRT and the measures used to evaluate the performance of the models.

2.4.2 A deteriorating ecosystem and the dilution effect

To determine if Tengmalm's owl declined, we first fitted a GLS model to the time-series of owl nest box occupancy (%). We also fitted a GLS model to the % bank voles relative to other small mammals in 1971 – 2013 to test if the contribution of bank voles to the small mammal community changed over time.

In the nest box study (Paper III), we tested if PUUV prevalence in voles collected from nest boxes was higher than prevalence in trapped voles. We predicted the infection probability of a bank vole by fitting a GLM model using the following predictors: source of bank vole (collected or trapped), weight (g), patch isolation, and the statistical interaction between patch isolation (%) and source of the bank vole.

In Paper IV, we tested whether competitors diluted PUUV infection in bank voles by reducing prevalence (encounter reduction) or suppressing bank vole density (susceptible host regulation) (Keesing et al., 2006). We fitted GLM models to predict seasonal bank vole density and infection probability at a plot level in 2003 – 2013. As predictors, we used field vole and common shrew densities and habitat (forest succession stage). Additionally, for the models predicting infection probability, we also used bank vole density and weight (g) as predictors.

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3 Results

I present the most important results of this work, and begin by those pertaining to temporal and spatial investigations of PUUV risk and hazard. Then, I list the main findings in relation to how community interactions influence bank vole and PUUV dynamics. For more detailed results, see the respective papers.

3.1 PUUV risk in time and space (Papers I and II)

Between 1990 and 2013, NE incidence generally increased in the summer period, but not in the winter period (GAM: $F_{20.4} = 4.48$; $p < 0.05$ for summer, $F_{21.5} = 0.37$; $p = 0.48$ for winter).

PUUV prevalence in spring depended on bank vole density during the current spring and previous autumn. Autumn prevalence, however, was not significantly related to current or previous bank vole density.

Both bank vole density and density of infected voles in spring predicted NE incidence well in summer (Figure 4a, b), explaining 76 % and 87 % of the variation, respectively. For NE incidence in winter, bank vole density in autumn was significant and explained 75 %. However, the density of infected bank voles was borderline non-significant and only explained 47 % of the variation in winter incidence (Figure 4c, d). NE incidence appeared to increase linearly with bank vole density in summer but exponentially in winter.

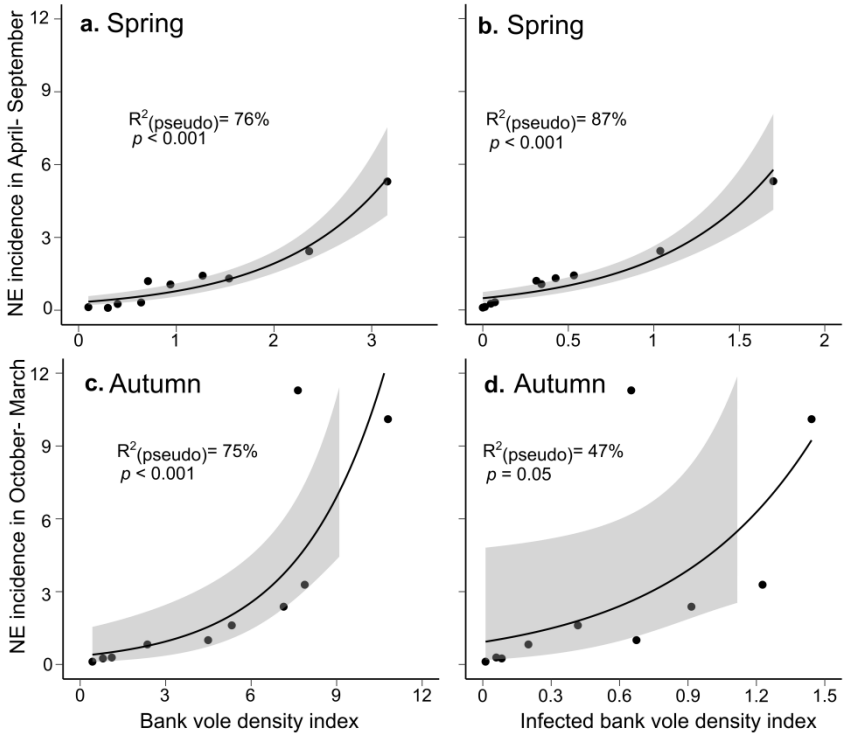

Figure 4. Explaining nephropathia epidemica incidence in Sweden (no. cases / 100 000 inhabitants), in (**a.**,**b.**) "summer" (April – September) and (**c.**,**d.**) "winter" (October – March) using overall bank vole density or infected bank vole density in spring (**a.**,**b.**) and autumn (**c.**,**d.**). The black lines correspond to fitted generalized linear models with Poisson errors and the gray shaded area represents standard error around fitted line. Paper I

Bank vole density in the spring of the increase phase predicted the maximum attained density of infected voles – typically in autumn, 18 months later – during the same cycle (*Pearson r* = 0.96, *t* = 4.3, *df* = 3, $p < 0.01$). Likewise, the proportion of sampling plots occupied by bank voles in spring of the increase phase was strongly correlated with density of infected bank voles the following spring peak (Figure 5).

The presence and frequency of occurrence of PUUV-infected voles showed considerable spatial and seasonal variation. In spring, bank voles and infected bank voles were present in 7 – 81 % and 2 – 70 % of the 58 1-ha plots, respectively. In autumn, bank voles were present in 30 to 98 % of the plots,

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whereas infected bank voles were present in 2 – 74 %. There were plots where PUUV-infected voles were frequently found in spring, when bank vole densities are at an annual low (Figure 6), including four plots where infected animals were trapped on six or more occasions out of ten.

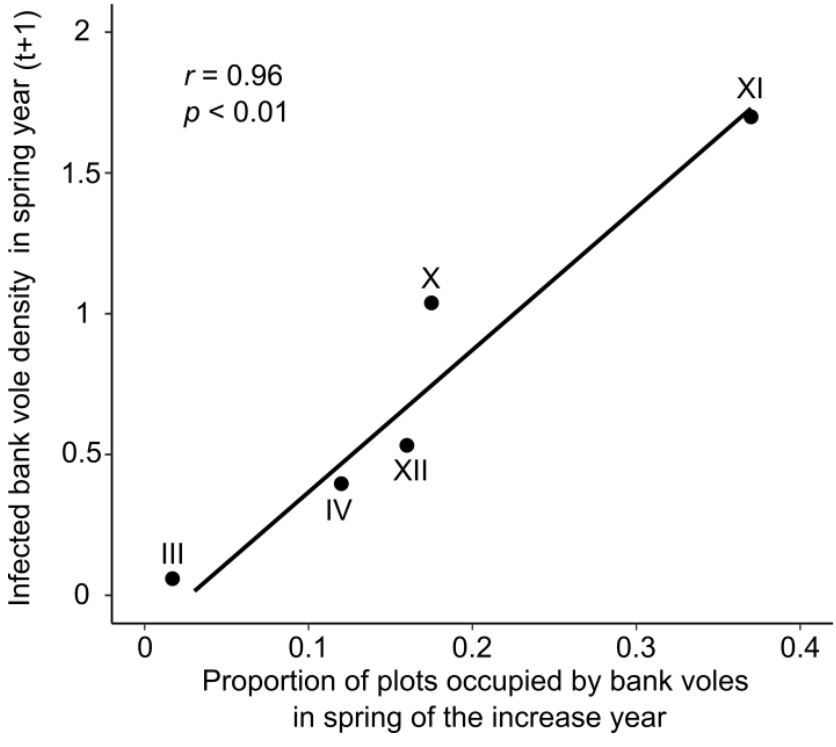

Figure 5. Dependence of infected bank vole density in spring year $_{t+1}$, in the five cycles with infection data (III, IV, X, XI, XII), on bank vole occupancy of the landscape (proportion) in spring of the increase phase, when average autumn density had reached >1 individual per 100 trap nights. r is Pearson correlation coefficient. Paper I

There was large inter-annual variation in landscape occupancy of infected bank voles, depending on the phase of the population cycle (Figure 7). For example, in the spring of 2007 – a peak year – infected bank voles were present in 38 out of 58 1-ha trapping plots, whereas by the end of the cycle in spring 2009 there was only one plot with infected animals.

In the four models predicting overall bank vole and infected bank vole presence in spring and autumn, microhabitat variables related to availability of cover and food were, as expected, important. All models performed well; Area under curve (AUC) was ≥ 84 and 25 % to 40 % of the deviance was explained. 'Bilberry' and 'Large holes' were present in all four models, and the relative importance (%) of 'Large holes' was above 10 % in three models out of four (see the tables in Paper II for detailed results on important variables and model performance).

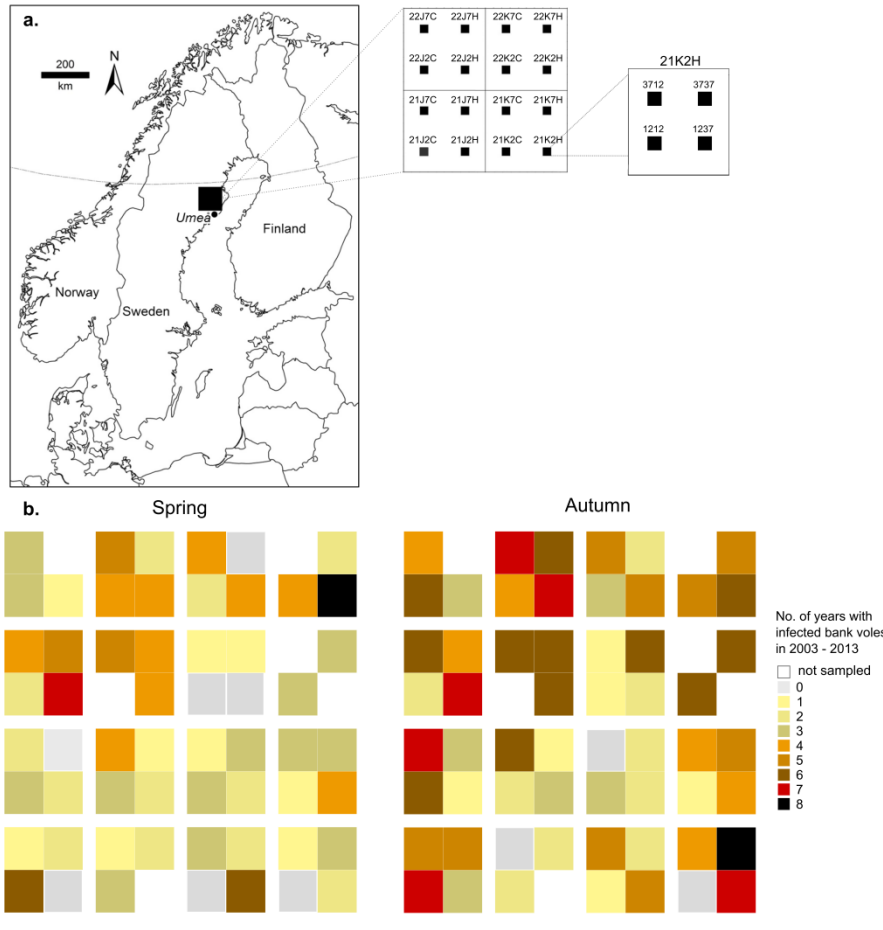

Figure 6. The study area in northern Sweden (black square) near the city of Umeå (a); the curved line indicates 65ºN. The blow-up shows the 16 landscapes, containing four 1-ha trapping plots each, totaling 58 trappable out of 64 plots (six plots encompassed for example water bodies and were not sampled). b) Each tile in the spring and autumn panels represents a 1-ha plot; the color coding reflects the number of years when infected bank voles were trapped (autumn 2003 – autumn 2013). Paper II

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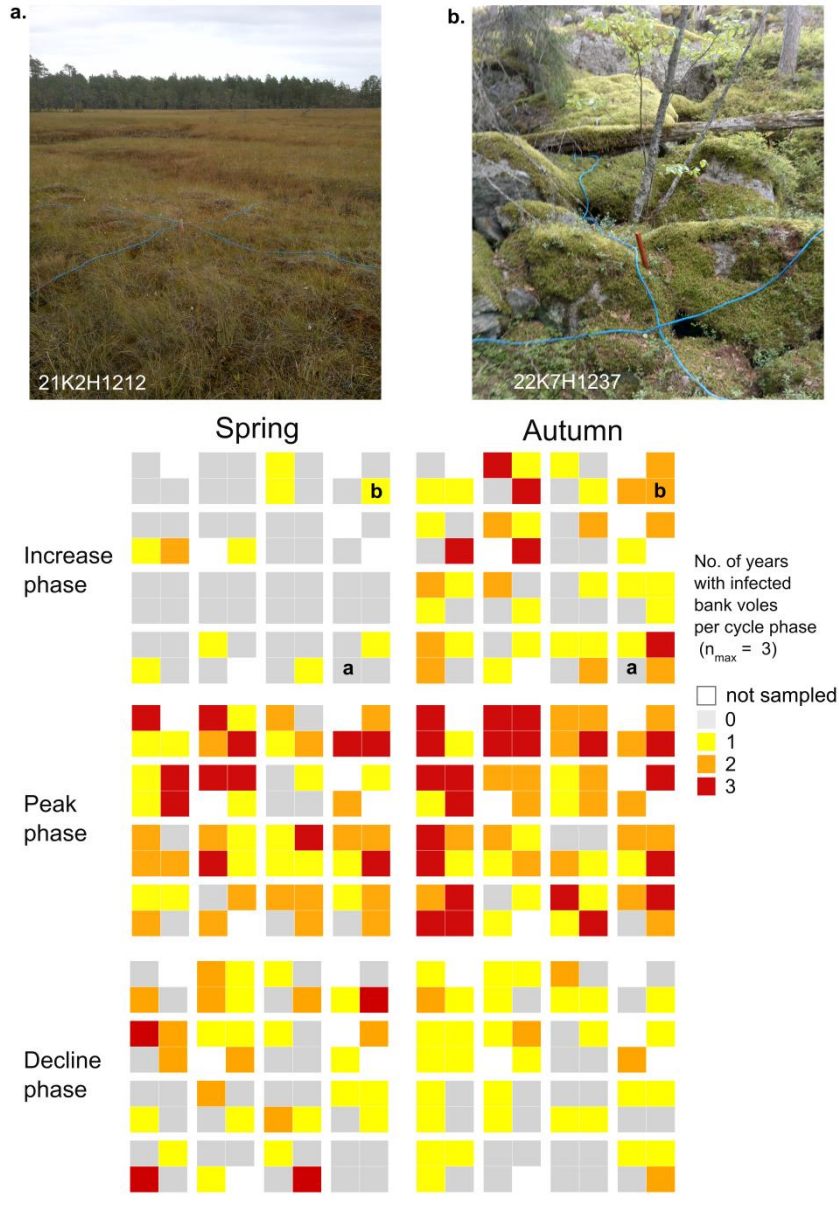

Figure 7. Number of years when infected bank voles were trapped per season and phase of the population cycle. Data was available for three cycles, two 3-year cycles (autumn 2003 – autumn 2008; and autumn2013) and one 4-year cycle (2009 – 2012). We excluded year 2012, which was the only "low" phase year and thus values ranged between zero and three. The photos (**a.** and **b.**) show examples of plots where **a.** infected bank voles were not trapped in either season over the study period, and in **b.** infected bank voles were frequently trapped. The plot in **a.** is an open mire and generally lacks a heterogeneous structure and cover, whereas the plot in **b.** is rich in large holes. Photo Copyright: Magnus Magnusson. Paper II

In spring, the microhabitat variables important for predicting overall bank vole presence and infected bank vole presence were similar, with the two models sharing seven out of the eight important variables. The presence of bank voles in overall and of infected bank voles were predicted by microhabitat variables typical of spruce forests, and variables related to cover and food availability, such as 'Coarse woody debris', 'Bilberry', and 'Lingonberry'. However, autumn models diverged and only shared four variables out of ten. For example, 'Spruce' was not an important predictor of overall bank vole presence in autumn, but was important for the presence of infected voles. Infected voles were likely to occur in plots rich in cover such as 'Large holes', 'Fine woody debris', and 'Umbrella vegetation'.

In the independent area (17 1-ha plots × 5 years = 85 instances per season), both spring and autumn models predicted the presence of infected bank voles well. Model performance was fair in spring (AUC = 74) and good in autumn (AUC = 83) (Swets, 1988). The models predicted absences less successfully than presences, especially in spring.

3.2 A deteriorating ecosystem and the dilution effect (Papers III and IV)

Between 1971 and 2013 in the monitoring area, the percentage of bank voles relative to total number of small mammals increased. Owl nest box occupancy (%) decreased in 1980 – 2013 (Figure 8), while the number of infected voles per cycle was higher in the 2003 – 2013 time-frame compared to that in 1979 – 1986, and the difference was most evident in spring.

Out of a total of 497 bank voles collected from and trapped around each of the 15 nest boxes, 133 were infected (prevalence = 27 %). Bank vole density was higher in isolated patches (*Pearson* $r = 0.53$, $t = 2.3$, $p = 0.04$) and we collected more voles per visit from nest boxes around which bank vole density was high (*Pearson* $r = 0.54$, $t = 2.3$, $p = 0.04$).

PUUV prevalence in voles cached by owls was higher at low patch isolation but not at high patch isolation (approx. > 15%), where infection probability in trapped voles was highest (Figure 9). There was an interaction between the source of a bank vole, i.e. collected from the nest box or trapped around it, and

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patch isolation. See table 2 and the results section in Paper III for detailed information.

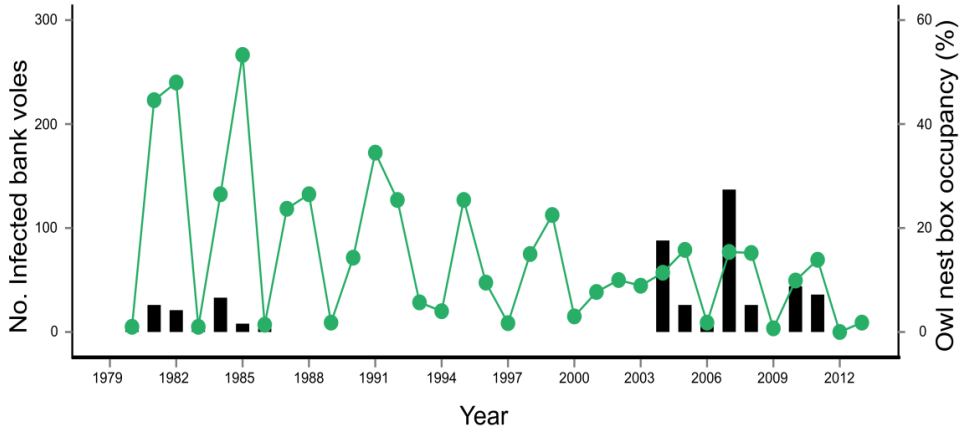

Figure 8. The number of infected bank voles (bars and left-hand axis) in spring in two time periods: 1979-1986 and 2003-2013 and Tengmalm's owl nest box occupancy (%) (line and right-hand axis) in springs 1980-2013. Adapted from Paper IV

In the monitoring area, infection probability increased with bank vole density and decreased as common shrew density increased (Figure 10). In autumn, the best model suggested that infection probability also increased with bank vole weight. Neither habitat nor field vole density index influenced infection probability in either season.

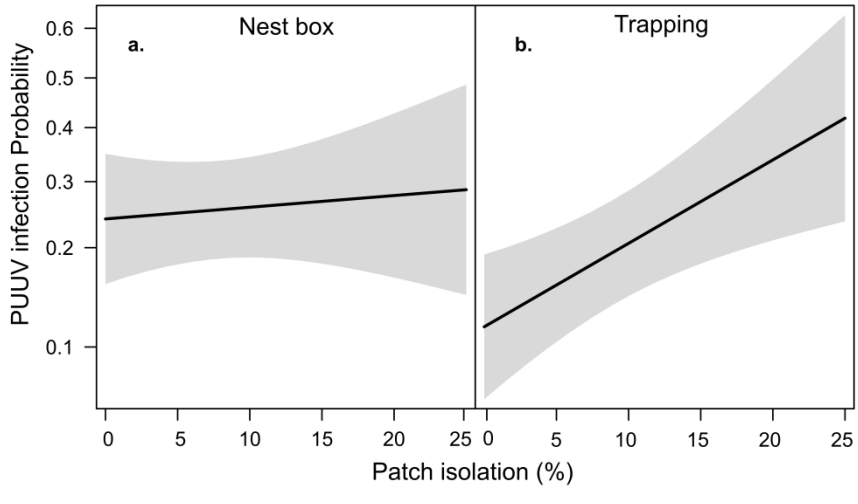

Figure 9. Model predictions of bank vole infection probability in **a.** nest boxes and **b.** traps as function of patch isolation. The grey shading encompasses the 95 % confidence interval of the model prediction. Paper III

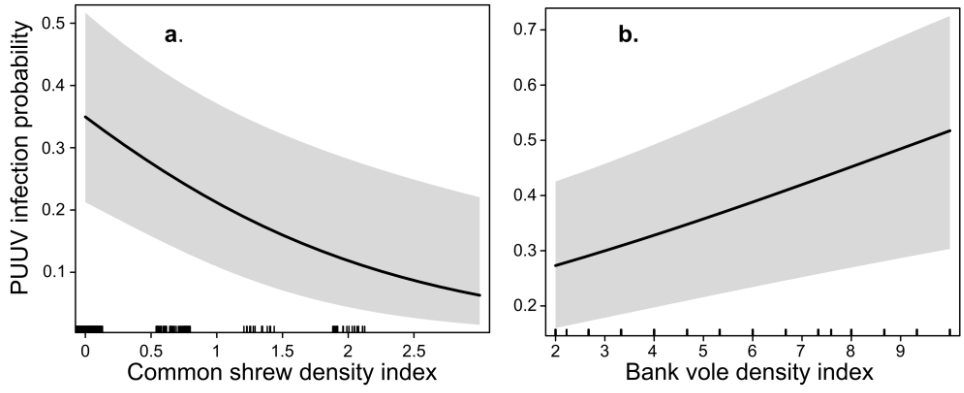

Figure 10. The model-predicted probability of a bank vole being infected in autumn relative to **a.** common shrew density index, **b.** bank vole density index. The grey-shaded area represents the 95% confidence interval of coefficient estimates. Vertical black marks on the x-axis show how predictor values are distributed across predictor range, denser marks indicate a concentration of predictor values. Paper IV

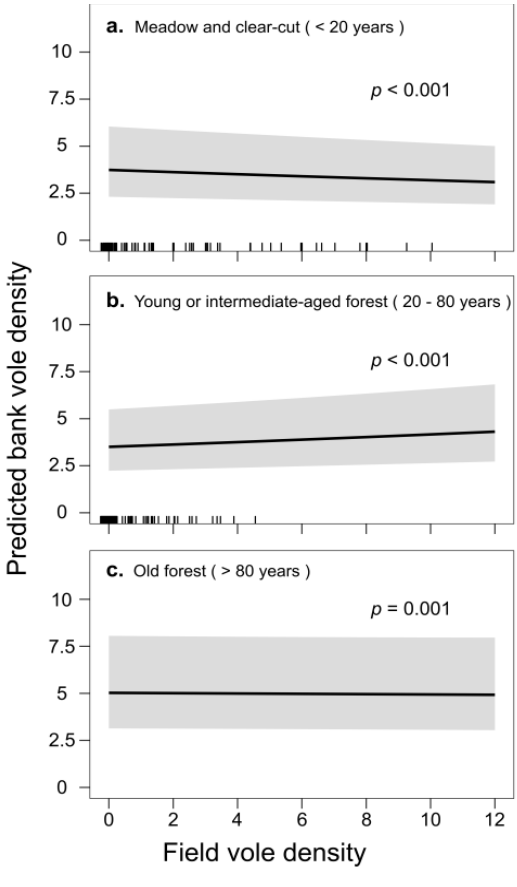

Figure 11. Model-predicted bank vole density index in autumn relative to field vole density index in different (**a. - c.**) habitat succession stages. The grey-shaded area represents the 95% confidence interval of coefficient estimates. Vertical black marks on the x-axis (rug plots) show how predictor values are distributed across predictor range, denser marks indicate a concentration of predictor values. Paper IV

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In the model with bank vole density as a response variable, there was an interaction between field vole density and habitat. In spring, bank vole density generally increased with field vole density. But in autumn we found the opposite scenario, and bank vole density decreased when field vole density increased, but only in core field vole habitat, i.e. meadows and clear-cuts (Figure 11). See table 1 in Paper IV for more detailed information on the results and model coefficients.

4 Discussion

The PUUV – bank vole system is a simple one compared to vector-borne zoonoses such as the Lyme disease (Ostfeld et al., 2006). This made it feasible to explain a substantial proportion of risk and incidence using host population fluctuations and associated infection prevalence. The results from the first part of this thesis, i.e. improving the prediction of NE incidence and presence of PUUV infected voles (Papers I and II), can be easily employed by public health officials to anticipate outbreaks and assess ecological risk at a local scale. Further, we found that non-host species that compete with or prey upon the host may mitigate human risk. Given the worrying decline of several species in our study area, it is pertinent that authorities and other stakeholders try to conserve the remaining functional diversity.

In northern Sweden, forestry is one of the most important economic sectors. Forests are also valuable culturally, and the majority of northerners have a strong attachment to nature. People experience forests through hunting, collecting berries and mushrooms, running, or walking their dogs. From a public health point of view and given the results presented here, we ought to reflect on the kind of forests we want close to our homes, and the considerations that must be made by the forestry industry when planning timber harvest.

4.1 PUUV risk in time and space (Papers I and II)

NE incidence has been increasing in summer since the 1990's, coinciding with a spring increase in bank vole density and PUUV infection prevalence. We have explored and evaluated the seasonal and multi-annual relationship

between host abundance and human incidence. This association can be used for risk prediction and to guide data collection for its future assessment. For example, sampling of bank voles during the increase phase of the population cycle can be used to predict a possible outbreak 18 months later.

We found that spring PUUV prevalence was dependent on bank vole density in autumn and over-winter survival, reflected by density in spring. In Finland, Voutilainen et al. (2016) reported that most PUUV transmission among bank voles occurred during winter, which highlights the importance of vole density during that period. A positive correlation between prevalence and host density was reported in other rodent-borne zoonoses, e.g. in plague (Davis et al., 2004), and suggests an increase in contact rates with host density (Davis et al., 2005).

In autumn, we found no evidence for a relationship between bank vole density and PUUV prevalence at the scale of the whole study area. The influx of new-born voles during the reproductive season probably masked any increase in PUUV transmission with density (Niklasson et al., 1995; Roche et al., 2012; Lehmer et al., 2012). As is typical for horizontally transmitted pathogens, infection probability increased with host age (Kuenzi et al., 2001; Olsson et al., 2002). In autumn, 41 % of trapped bank voles weighed <17 g and were likely born in the same season, compared to 1 % in spring. This demographic bias towards younger individuals in autumn could be responsible for the idiosyncratic relationship between density and prevalence.

The observed exponential increase in winter NE incidence with autumn bank vole density (Figure 4c) is compatible with high winter transmission of PUUV among bank voles, leading to an exponential increase in the density of infected animals. The acceleration in transmission may occur when bank voles share nests (Glorvigen et al., 2012) and previously immune voles lose maternal antibodies (Kallio, Poikonen, et al., 2006). In spring however, bank vole populations consisted of overwintered individuals, and we suspect that most transmission events had already occurred. Unsurprisingly, the relationship between spring bank vole density and summer NE incidence appeared linear (Figure 4a), and was improved by using infected bank vole density as a predictor.

The density and landscape distribution of bank voles at population minima in a population cycle accurately predicted NE risk at its highest level, represented by maximum density of infected bank voles in that cycle. When host density

drops below a certain threshold, the pathogen may go locally extinct and infection rates take longer to build from that low level (Luis et al., 2015). In cyclic host populations with predictable changes in growth rates, density minima determine the starting point of host and pathogen proliferation. Relatively higher host densities during those minima act as a springboard for pathogen transmission and culminate in higher risk in the near future, when peak densities of infected voles are reached. Due to the comparatively small number of bank voles that need to be collected and analyzed in the first spring of a vole cycle, surveillance during that period provides early and cost-effective assessment of NE risk and possibly for other zoonotic diseases such as Lyme disease, where bank voles are part of a more complex host community (Hofmeester et al., 2016).

Now that the temporal risk of NE can be reasonably predicted, spatial investigation of PUUV hazard can help identify habitats that function as sources of infected bank voles in human dwellings. We are unaware of previous studies on PUUV that validated habitat models and predicted infected host presence using independent data. Here, we found that the presence of PUUV-infected voles could be predicted using microhabitat properties and extrapolated to an independent area.

In general, moist and mesic spruce forests with abundance of structures that provide cover such as large holes, and dwarf shrubs that provide both cover and food, were most likely to harbor infected bank voles. Interestingly, bank voles in autumn were present in a wide range of habitats and had a broad landscape distribution, yet the presence of PUUV-infected voles remained restricted to habitats where especially cover was abundant.

When host landscape distribution declined in winter and during low-density years, PUUV-infected voles were frequently found in a few focal patches (Figure 7). These habitats functioned as infection "refugia" from which we suspect future colonization of the landscape occurs (Glass et al., 2007; Magnusson et al., 2015). However, no plot harbored infected bank voles throughout the 10-year study period, and PUUV-infected voles were trapped in different plots during low density phases of different cycles. This suggests that although some plots promoted the persistence of infected voles during adverse periods, there remains an element of stochasticity in the occurrence of infected voles at plot level.

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Bank voles in spring were frequently trapped in old spruce forests, characterized by availability of microhabitat structures that provide cover (e.g. fine and coarse woody debris, large holes, and shrubs) and food (e.g. lingonberry and blueberry). Similarly, Ecke et al. (2002) found that although bank vole densities were high in clear-cuts and young forests, their over-winter survival was lower there than in old forests. In Belgium, bank voles were found in preferred habitats with dense cover during low density years (Escutenaire et al., 2002). In the U.S., hantavirus hosts survived in habitats with more cover where predation risk was hypothesized to be lower (Root et al., 1999).

In autumn, the contrast between predictors of overall occurrence of bank voles and the occurrence of infected animals is nontrivial. It provides an opportunity to explore potential divergence between host and virus ecology, compared to an earlier study in the same region that was limited to one autumn (Olsson et al., 2005). We suspect that habitats with abundant cover can enhance virus survival outside the host by maintaining moisture and reducing penetration of ultraviolet radiation (Kallio, Klingström, et al., 2006; Voutilainen et al., 2012). Bank voles may also survive longer in plots where cover is abundant if predation rates are lower. Large holes found under cobbles, logs, and stumps were the most important predictor of the presence of PUUV-infected voles in autumn. We hypothesize that such holes are akin to "infection hubs", and voles may use these holes as nesting sites or corridors. Consequently, moist and mesic spruce forests may better maintain PUUV compared to habitats with less undergrowth, such as dry pine forests.

High quality bank vole habitat in northern Sweden is in theory ideal for PUUV survival in the environment, namely mesic and moist forests of the dwarf-shrub type rich in cover. However, this does not have to be the case for other hantavirus – host systems. In Paraguay, animals infected with Jaborá virus were more likely to be found where forest cover was thicker and the soil likely retained more moisture. However, such habitat was less preferred by the host, the montane grass mouse (*Akodon montensis*) (Goodin et al., 2009), which was more abundant in human-disturbed habitats. The difference in habitat association between infected and non-infected *Akodon montensis* points to the importance of microhabitat structure for viral survival, and supports differences between host and pathogen ecology.

The microhabitat models developed for the 58 1-ha plots near Umeå were especially good at identifying plots with PUUV-infected bank voles in an area approx. 200 km north. Nevertheless, spring models overestimated presence of

PUUV-infected voles. This may be due to lack of sufficient positive plots in the training data to produce a model better able to discriminate negatives from false positives.

We attempted to maintain a balance between the feasibility of data collection to fit the models and the generality of their predictions on one hand, and explanatory power and interpretation on the other. For example, given the variables included here, practitioners do not need to trap bank voles to assess the likelihood of presence of infected bank voles. Nevertheless, bank vole density would have greatly improved predictions, as human risk is expected to be more closely linked to the number of infected bank voles (Paper I) rather than their presence.

4.2 A deteriorating ecosystem and the dilution effect (Papers III and IV)

Community interactions were consequential for both host density and infection prevalence, indicating that functional diversity is important in controlling PUUV. Specifically, bank vole competitors either reduced prevalence in bank voles or their density. The role of predation by Tengmalm's owl merits further investigation, but our results and long-term trends in the data imply that owl decline allowed infected bank vole to reach higher densities.

Previous studies from the monitoring area documented dramatic declines of the field vole and the grey-sided vole during the past three decades (Hörnfeldt et al., 2005, 2006; Ecke et al., 2010; Magnusson et al., 2015). Bank vole populations also declined, but partly recovered during the 2000's, leading to an increase in bank vole dominance within the small mammal community. Concurrent with Tengmalm's owls decline, PUUV prevalence and infected bank vole density in spring were higher in 2003 – 2013 compared to 1979 – 1986 (Figure 8).

In our nest box experiment in Paper III, we found that PUUV prevalence in voles collected from nest boxes and in trapped voles did not show a straightforward difference. Instead, our results suggest a complex relationship involving predation, landscape structure, and infection prevalence in bank voles.

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Only in connected forest patches, PUUV prevalence in voles collected from nest boxes was higher than that in voles trapped in surrounding forest, which suggests that owls in those patches were more likely to pick out infected animals (Figure 9). In connected patches, trapped and cached bank voles may belong to the same "population" of prevalence. We could not confirm that we trapped in the exact same localities where owls hunted. Yet, we retrieved more bank voles per visit from nest boxes in patches with higher bank vole density. This suggests that owls could have been hunting in proximity to where we trapped.

The interaction between prevalence and patch isolation is intriguing, and we propose the following explanation. The home range size of a male owl varies with habitat composition (Santangeli et al., 2012). Hence, owls breeding in nest boxes positioned in isolated forests patches may hunt in areas defined by a radius larger than the 500 meters limit within which we trapped. They may prey on bank voles from habitats characterized by disparate PUUV prevalence and vole densities. Since the diet of owls is dominated by bank voles and field voles (Hörnfeldt et al., 1990), it is indeed likely that owls prefer hunting along forest edges bordering field vole habitats such as fields, meadows, and clear cuts. To further explore whether owls selectively pick infected voles, future studies ought to be conducted in e.g. continuous landscapes, where landscape properties such as isolation are not expected to play a significant role in PUUV prevalence variation.

Our study to investigate PUUV dilution by non-host competitors through species-specific hypotheses was the first of its kind according to our knowledge (Paper IV). We considered the densities of the two species which we had *a priori* identified as potentially important in influencing bank vole behavior or density, instead of using an index of diversity. We found that the probability of bank vole being infected decreased with increasing common shrew density. Field voles affected PUUV prevalence indirectly by suppressing bank vole density in autumn in meadows and clear-cuts. Our study thus found evidence for the dilution effect by two non-host species, and through different pathways that lead to fewer infected animals. Through the "encounter reduction" pathway, host density may remain the same, yet intraspecific encounters and pathogen transmission decline, leading to lower infection prevalence. Whereas "susceptible host regulation" occurs when interspecific interactions lead to lower host density, which likely leads to fewer infected animals (Keesing et al., 2006).

Paper IV adds to the growing *corpus* of evidence for the dilution of infection in a range of new and old world hantavirus – host systems. For example, in an experimental study in Panama, both infection prevalence and host density increased when small mammal diversity was reduced (Suzán et al., 2009). In the United States, Dizney and Dearing (2016) found that hosts of Sin Nombre hantavirus in more diverse sites spent less time engaged in behaviors related to transmission and were less likely to be infected. An observational study in Argentina found that animals infected with Andes hantavirus were more likely to be found near human dwellings where small mammal diversity was low (Piudo et al., 2011). In Europe, Voutilainen et al. (2012) found evidence for the dilution of PUUV infection in bank voles through pooling densities of non-host small mammals. Here, by studying the potential of common shrews and field voles to influence PUUV infection in bank voles independently, we were able to infer mechanisms and conditions that promote dilution of PUUV.

The common shrew is found in a wide range of habitats (Hanski et al., 1989). It is smaller and competitively inferior to the bank vole (Henttonen et al., 1989; Huitu et al., 2004), and is unlikely to regulate bank vole densities. Nevertheless, dilution through "encounter reduction" limits infection in host populations irrespective of host density (Figure 10) (Clay et al., 2009). In an experimental study, the presence of common shrews changed bank vole behavior, resulting in lactating females visiting fewer supplementary feeding stations (Liesenjohann et al., 2015). Common shrews are opportunistic predators and may prey on vole nestlings, and the two species share above ground runways and tunnels (Liesenjohann et al., 2011). In North America, the short-tailed shrew (*Blarina brevicauda*) restricts spatial use of the meadow vole (*Microtus pennsylvanicus*) (Fulk, 1972) and may prey on it (Martinsen, 1969). As a response to risk, bank voles may avoid common shrews and increase time spent protecting nestlings. Ultimately, we expect that a reduction in movement of infected voles limited the spatial scale of PUUV shedding and number of encounters with susceptible voles.

Competition can also control disease by reducing host density. Field voles suppressed bank vole density in meadows and clear-cuts in autumn (Figure 11), when bank vole density is often highest. In the reproductive season, field vole populations reach peak densities after bank voles (Huitu et al., 2004), and competition between the two species is most likely space-driven after reproduction (Myllymäki, 1977). This may explain why we found evidence for interference competition only in autumn. Because field voles also alter bank vole behavior (Eccard et al., 2007), we expected field voles to directly reduce

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PUUV infection in bank voles in meadows and clear-cuts. But we found no evidence for dilution through "encounter reduction". We speculate that space-driven interference competition occurred for a limited time-period after reproduction, outside of which bank vole behavior, encounter rates, and PUUV transmission were not sufficiently altered to be reflected in PUUV infection rates.

The study area is heavily managed by forestry (Ecke et al., 2013; Magnusson et al., 2015) with a species-poor small mammal community (Hörnfeldt, 1994). The drastic decline of the grey-sided vole (Hörnfeldt et al., 2006), driven by habitat loss (Magnusson et al., 2015), probably released the bank vole from competition in forest habitats and allowed the latter to expand its niche (*sensu* Löfgren, 1995). The grey-sided vole prefers forests with boulder fields and large holes (Magnusson et al., 2013), and its decline possibly enabled the bank vole to prevail in such forests, which likely contributed to increased PUUV hazard (Paper II).

Our results are based on long-term time series collected systematically, over a large area with plots 2.5 km apart. This enabled us to test the dilution effect at the mechanistically important local scale, while accounting for habitat differences. It is at the plot level where changes in bank vole density and behavior are expected to affect PUUV infection within populations. The simple system with directly transmitted pathogen and few non-host small mammal species enabled us to include density of non-host species rather than species richness or other measurements of diversity. Nevertheless, our inferences of dilution mechanisms were based on observational data. Experimental testing in large enclosures is needed to establish a direct link between behavioral and density changes in bank voles (e.g. Ylönen et al., 1990; Sundell et al., 2003; Eccard et al., 2007) to changes in transmission rates. For example, experimental work on the dilution effect is ongoing in the United States on Sin Nombre virus system (reviewed by Dearing et al., 2015).

4.3 Implications and future perspectives

After discussing spatial and temporal patterns in PUUV dynamics, and how community interactions can modify host density or infection prevalence, I will briefly address the implications of our results for forestry and urban or semi-urban planning. I also touch on future directions and outlook.

Near our study area, isolated patches of old forests are valued and maintained around human dwellings, and will continue to be promoted as part of the 'green infrastructure' in Europe. However, patches of old forest, especially spruce-dominated ones can act as infection 'refugia' when regional bank vole density declines (Paper II). Also, infection prevalence increases with patch isolation (Paper III), and the latter is typical of forests in urban areas in Sweden. These patches are probably less likely to sustain predators or high densities of non-hosts species, undermining the valuable ecosystem service provided by the dilution effect (Papers III and IV). I suspect that such isolated patches close to houses and frequented by people, may pose the greatest threat to humans, especially in winter when bank voles infest houses, cabins, and woodsheds.

To mitigate the potential risk from isolated forest patches in urban areas, I suggest the following. First, maintain enough connectivity and edge habitat between forest patches so that local bank vole populations are not forced into a confined area where they may reach high densities and infection prevalence (Paper III). Where suitable, having pine forests instead of spruce near houses may result in lower probability of infected-vole presence, due to the relative lack of undergrowth and lower moisture in pine forests compared to spruce (Paper II). From our experience, Tengmalm's owls breed readily in nest boxes close to houses. Through a citizen-science driven project, more nest boxes can be positioned at forest edges near human houses. Breeding owls would provide excitement and engagement, education, and possibly protection from voles attempting to infest houses.

Similarly, timber harvesting ought to be done in a manner ensuring enough connectivity between patches to sustain higher diversity of small mammals, while heterogeneity of habitats in each given landscape may provide enough prey to sustain predators (Papers III, IV). Some might wonder whether forestry reduces risk by destroying core bank vole habitat. I believe it may do so, but only temporarily. Forest plantations need to reach 80 or more years of age to provide good quality timber. Thus after clear-cutting, and a brief reduction in bank vole density (and infected bank vole density), bank voles will re-establish as the plantations grow and develop in structure, providing cover and resources. On the other hand, specialist species such as the grey-sided vole and predators might not survive the initial extensive clear-cutting and fail to recover, ultimately resulting in a further depleted ecosystem and exacerbated risk for humans.

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In this work, I did not establish a direct link between ecological risk of PUUV in different habitats and direct risk in human buildings. Despite higher bank vole density and frequency of occurrence of infected bank voles in old forests, perhaps the lack of structure in younger forests make them better sources of infected voles in human dwellings, especially when weather or snow conditions deteriorate. Thus, it is not enough to study ecological risk in a forest patch; we ought to ascertain which patches supply infected animals to human buildings. Future studies should investigate the propensity of infected bank voles to enter dwellings from different habitat types, and link bank vole movement to weather and the microhabitat characteristics of patches near houses and cabins.

Despite the wealth of data I had at my disposal, I would have liked to diversify the methods used to collect it. The nest box work was exciting, and in the future, live trapping and behavioral studies would be necessary to move on from correlational studies – albeit data rich and extensive – to mechanistic ones. The technology to tag owls with light weight Global Positioning System (GPS) and track their hunting behavior is now available. Passive integrated transponders (PIT tags) can shed light on bank vole movement in relation to microhabitat properties, among patches, and in response to risk of predation or competition. Also, PIT tagged bank voles found in human dwellings can possibly be traced back to the patch where they came from, which would reveal the source of infestation.

4.4 Conclusion

The take home message from this work is that we can learn a good deal about a disease system by focusing on the dynamics of host and pathogen only, but this knowledge will very likely lack precision. When the complex web of interactions in which both host and pathogen are involved changes, so too will host and pathogen dynamics, rendering our earlier assumptions and assessments obsolete. A holistic approach indeed complicates things, but enables putting together a more reliable framework for risk assessment and mitigation. Thanks to ecological studies, we now know that neighbors and enemies of hosts of dangerous pathogens may indeed protect our health, and in this particular case it was the owls, field voles, and shrews.

Popular Science Summary

Several recent outbreaks of infectious diseases have been caused by pathogens originating in wildlife. In Sweden, Puumala virus (PUUV) causes a form of hemorrhagic fever, and is carried and transmitted to humans by the bank vole, a very common small mammal. Humans risk is highest during winter months, when infected animals infest houses, cabins, and woodsheds. Our ability to predict and lower the risk of PUUV outbreaks depends on understanding the processes that lead to high abundance of infected bank voles.

My thesis was based on a long-term study of PUUV in bank voles in a large study area in northern Sweden. We developed models that allowed us to predict human risk early, and estimate the probability of finding infected animals based on habitat properties. Also, we investigated how interactions between the bank vole and its small mammal competitors and an owl predator affected the abundance of bank voles and their infection with PUUV.

Bank vole populations undergo predictable and regular changes in abundance, which results in 3 to 4 year population cycles, and characterized by high and low density years. Based on this knowledge and on how infection probability in bank voles depends on their abundance, we were able to predict the highest human risk during a bank vole population cycle, almost a year and a half beforehand. Using fine-scale habitat data, we found that moist spruce forests with plenty of cover and food were the habitats where infected bank voles are most likely to be found, even when bank vole general abundance is low.

Tengmalm's owls are an important predator of bank voles, and were more likely to hunt infected individuals, but only under specific conditions. Two small mammal competitors of bank voles: field voles and common shrews, were able to either limit the abundance of bank voles or their PUUV infection.

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Hence, owls, field voles, and shrews have the potential to perform a valuable service and protect our health.

The findings in this thesis can be used by public health officials, to raise awareness and better prepare for periods with high human risk. Also, our results highlight the importance of biodiversity. In northern Sweden, land use and climate change have probably caused the decline of several species, and conserving the remaining diversity should be of highest priority.

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