The effect of small mammal population fluctuations on tick load and pathogen prevalence in two contrasting ecosystems

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II

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IV

Ackowlegdements

This master thesis was done at CEES (Centre for Ecological and Evolutionary Synthesis) at the Department of Biosciences, University of Oslo. The thesis was carried out under the supervision of main supervisor Atle Mysterud and co-supervisor Hildegunn Viljugrein. I would like to thank Atle Mysterud for the opportunity to take part in this project. Thank you, for excellent guidance, and for your good and constructive feedback as well as helping me stay on the right path when writing my thesis. I would also like to thank Hildegunn Viljugrein for helping me with the analysis and statistics work in this thesis.

Also, many thanks to Anders Herland, Vetle Malmer Stigum and Fredrik Munkelien for participating in fieldwork and lab work with me.

I would also like to thank my family for supporting and encouraging me in my education and in other aspects of life.

At last, a thank goes to my fellow students through the last five years here at the university.

VI

Abstract

Ticks and other arthropod vectors are responsible for the transmission of many human and animal infectious diseases. These diseases often have complicated transmission cycles that are affected by both biotic and abiotic factors. The generalist tick *Ixodes ricinus* is the principal vector of tick-borne pathogens in Europe. Small mammals such as rodents and shrews are widely distributed and are regarded as important hosts for early life-stages of ticks. They are also known to be competent reservoirs for tick-borne pathogens such as *Borrelia burgdorferi* sensu lato (s.l.) and *Anaplasma phagocytophilum.* Small mammal populations are known to be highly dynamic in space and time, but little is known about how this affects the tick population and infection prevalence of tick-borne pathogens. In this study, I investigated how annual fluctuations in small mammal populations affect tick abundances and infection prevalence of *B. burgdorferi* s.l. and *A. phagocytophilum* from two contrasting ecosystems in Førde, Sogn og Fjordane, Norway and Son, Akershus, Norway. I quantified and analyzed tick burdens of *I. ricinus* and *Ixodes trianguliceps* on small mammal hosts caught in Førde and Son in the years 2014 and 2015. The presence of tick-borne pathogens in hosts was also determined. Annual variation in the small mammal population was observed in both study areas. The most common tick species in both areas was *I. ricinus*. However, annual variation in infection prevalence of *I. ricinus* was only observed in Førde. Son had overall significantly higher rates of tick infestation and infection prevalence of *B. burgdorferi* s.l. and *A. phagocytophilum* and hence a higher disease hazard. In addition, the different species of small mammals showed different rates of tick infestation and infection prevalence of tick-borne pathogens. The two areas were clearly different in how tick abundances, infection prevalence and resulting disease hazard are affected by annual fluctuations in the host populations. This study highlights the importance of understanding the dynamics of small mammal populations and how they ultimately contribute to disease hazard. In a changing climate and with ticks expanding their distribution in the northern hemisphere more knowledge is needed for how host dynamics affect the risks associated with ticks and tick-borne diseases.

Table of contents

x

1   Introduction

Many human and animal infectious diseases are transmitted through arthropod vectors such as lice, fleas and ticks, and these diseases often have complicated transmission cycles that are largely affected by environmental conditions (Rogers & Randolph, 2006). In the last few decades, ticks and tick-borne diseases have become a growing concern in the northern hemisphere (Jore et al., 2014; Medlock et al., 2013). Ticks are notorious for being vectors of many pathogens causing diseases affecting both humans and livestock, including several types of bacteria (Nadelman & Wormser, 1998), protozoans (Zintl et al., 2003) and viruses (Rieille et al., 2014). Among these pathogens, spirochetes from the *Borrelia burgdorferi* sensu lato (s.l.) complex are the most common (Stanek & Strle, 2003). These bacteria are known for causing Lyme borreliosis in humans (Nadelman & Wormser, 1998), with 85 000 cases estimated annually in Europe and 300 000 estimated annually in the USA (Pritt et al., 2016). With ticks expanding their northern distribution range (Jore et al., 2014), there is an increasing risk of contracting Lyme borreliosis in Scandinavia and Europe in general (Jaenson et al., 2009). Another important pathogen is the bacteria *Anaplasma phagocytophilum*, of which one strain is known to cause tick-borne fever in several domestic animals (Woldehiwet, 2006). The disease is a major problem for the sheep (*Ovis aries*) industry in several European countries, including Norway (Stuen et al., 2006).

Hard ticks (Acari: *Ixodidae*) are ectoparasites that feed on a wide variety of terrestrial vertebrates (Gern et al., 1998). Common for all Ixodid ticks is the specific life-cycle consisting of three stages requiring a blood meal; larvae, nymph and adult. The larvae and nymphs are dependent on a blood meal to molt into the next stage and the adult female needs a large blood meal in order to produce a large number of eggs (2000-3000) thus completing its life-cycle (Milne, 1947). Ticks are intermittent parasites, which mean they spend most of their time off the host, either searching for hosts or in diapause (Anderson & Magnarelli, 1980). It is during the feeding the tick can contract the pathogen from a vertebrate host, or transmit it to the next host. In Europe, the sheep tick *Ixodes ricinus* is the main vector for most of the tick-borne diseases. Understanding their ecology is regarded particularly important, as they also attempt to feed on humans causing spillover of pathogens from wildlife and due to its wide distribution throughout Europe (Danielova et al., 2008; Jore et al., 2011). This makes the *I. ricinus* a common vector for human disease. The host range of different tick species is variable. *I. ricinus* is not very host specific and can feed on nearly any

terrestrial vertebrates sharing its habitat (Milne, 1947). Though this tick is a generalist, it is like most other parasites often aggregated on certain species and individuals (Brunner & Ostfeld, 2008). This is important when considering the ecology of these parasites and their role as vectors. Small mammals, such as rodents and shrews, generally have dense populations in certain areas and with large annual variations in population abundances (Gern et al., 1998). This annual variation in population abundances affects the host availability, especially for the immature ticks between years, but this has rarely been quantified. Thus, the variations in tick abundances result from a combination of biotic and abiotic factors (Boyard et al., 2008; Brunner & Ostfeld, 2008; Randolph & Storey, 1999).

Transovarial transmission of *B. burgdorferi* s.l. occurs rarely (Rollend et al., 2013). This means that larvae in general are uninfected. To act as a vector, the tick must acquire the pathogen at some point in its life-cycle and transmit it later. They may acquire infection along with their first blood meal and transmit it to a new host when obtaining their second blood meal as a nymph or their third as an adult, hence acting as vectors. The *B. burgdorferi* s.l. complex consists of many different genospecies (Franke et al., 2013). The relationships between the *Borrelia* genospecies, the vectors and their hosts form complex ecological systems (Estrada-Peña et al., 2015; Estrada-Pena et al., 2016). Though *I. ricinus* will feed on nearly any vertebrate in their habitat, not all hosts are created equal from the pathogens point of view (Mannelli et al., 2012). Nearly all human infections are caused by one of three *B. burgdorferi* s.l. genospecies: *B. burgdorferi* sensu stricto (s.s.), which is found both in North America and Europe, and *B. garinii* and *B. afzelii* which both are found in Europe (Pritt et al., 2016) and have the *I. ricinus* as its primary vector in Europe. The different *B. burgdorferi* s.l. genospecies have different reservoirs according to the reservoir competence of the different hosts (Mannelli et al., 2012). *B. burgdorferi* s.s. is a generalist and *B. afzelii* has a small mammal reservoir (Hanincova, Schafer, et al., 2003), while *B. garinii* has an avian reservoir (Hanincova, Taragelova, et al., 2003). To qualify as a reservoir or transmission host for the pathogen, the host must participate significantly in circulation of the pathogens, and the pathogens should be able to persist in the hosts system for a certain period (Mather et al., 1989). In addition, the hosts contribution also depends on the amount of ticks it feeds (LoGiudice et al., 2003; Schauber & Ostfeld, 2002). This also means that disease hazard, not only depends on the population density of the vector, but also on the infection prevalence within the vector population. Though the *I. ricinus* is known to be the primary vector for human disease in Europe, there are more specialized ticks feeding on certain species that may

act as reservoirs. These more specialized tick species include the *I. trianguliceps* (Kovalevskii et al., 2013; Mannelli et al., 2012). The *I. trianguliceps* is a small mammal specialist, mostly questing in burrows (Nilsson, 1974; Randolph, 1975). Though this tick species does not act as a vector for human or livestock disease, it may contribute to the circulation of pathogens in reservoir populations (Mannelli et al., 2012; Randolph, 1975). *A. phagocytophilum* is known to exist as at least four different ecotypes or strains with different enzootic cycles in Europe. Studies suggest that *I. trianguliceps* is also associated with one of these strains of *A. phagocytophilum* (Jahfari et al., 2014).

The qualitative aspects of the tick's life-cycle, how pathogens are transmitted and how reservoirs are maintained are quite well known. Despite this, the quantitative aspects are much less known at the ticks' northern distribution range. Small mammals are particularly important because they are assumed to feed the majority of tick larvae and due to their competence as reservoir hosts for *B. afzelii,* the bacteria most frequently causing Lyme borreliosis in Europe (Gern et al., 1998; Mather et al., 1989). Small mammal populations are highly dynamic both in space and time, and it is reasonable to assume that host availability and infection prevalence are influenced by these fluctuations (Mannelli et al., 2012). However, our current knowledge of the role of small mammals feeding ticks are from a single year in a single location (Mysterud et al., 2015; Radzijevskaja et al., 2013). The aim of this study is therefore to increase the understanding of how rodents and shrews are as hosts to different species and life stages of ticks, and as a reservoir of the tick-borne pathogens *B. burgdorferi* s.l. and *A. phagocytophilum,* in two contrasting ecosystems over two years (2014 and 2015). To achieve this, I will quantify the *I. ricinus* tick load on different rodent and shrew species from two contrasting ecosystems and relate it to traits like body mass, and other variables like season, year and elevation. I will also quantify the tick load of *I. trianguliceps* and compare it to the attachment patterns of *I. ricinus.* The infection prevalence of *B. burgdorferi* s.l. and *A. phagocytophilum* in the small mammal hosts will also be analyzed in attempt to identify factors that influence the pathogen reservoir and ultimately the disease hazard of tick-borne pathogens.

2   Materials and methods
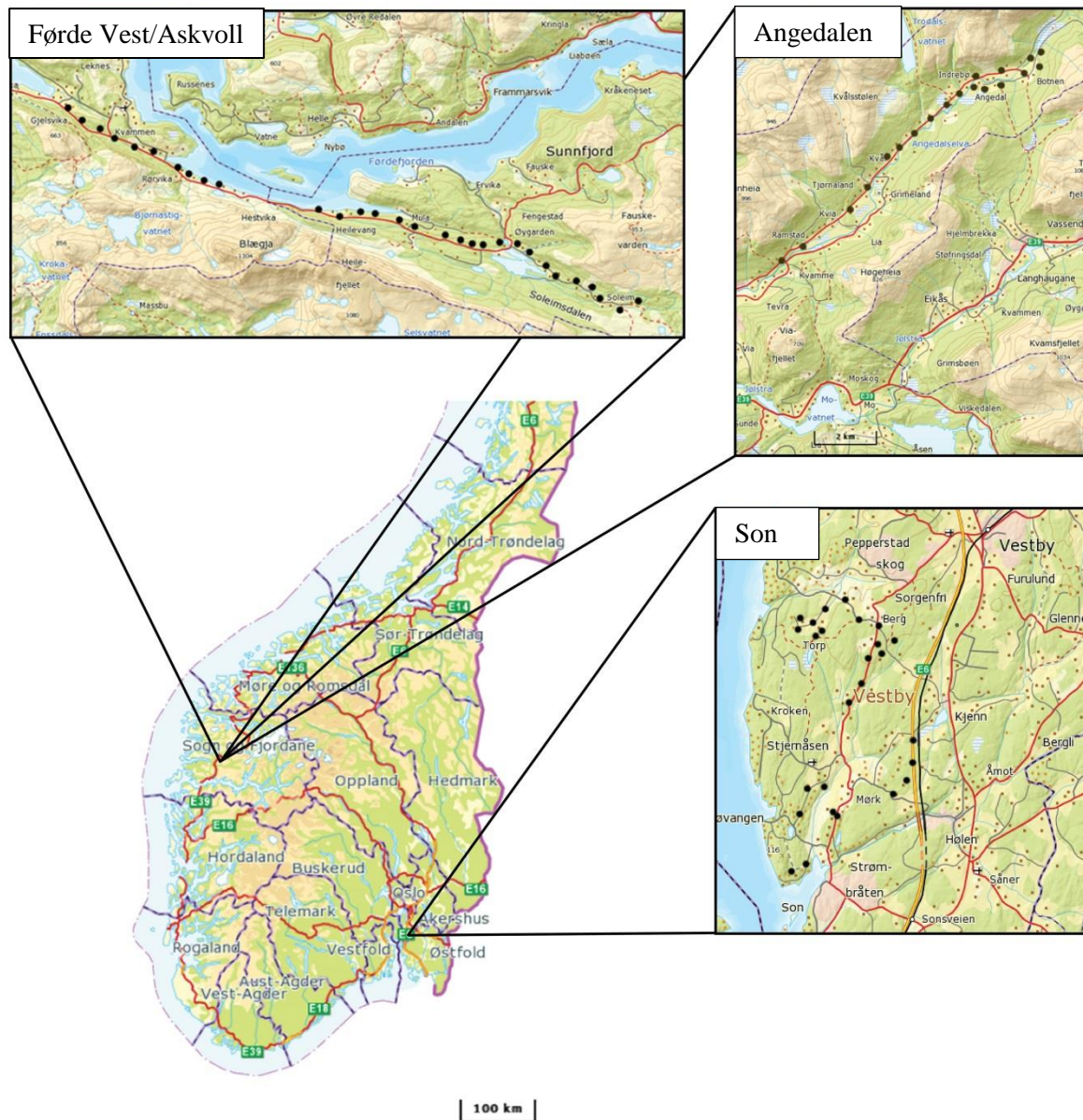
2.1   Study areas

Figure 1. Map over the three different transects Førde Vest/Askvoll (Sogn og Fjordane, Norway), Angedalen (Sogn og Fjordane, Norway) and Son (Akershus, Norway). Trapping stations are represented by black dots. Maps were retrieved from Kartverket, www.norgeskart.no

The study areas consist of two contrasting ecosystems. One is located in Førde and Askvoll municipalities in the county of Sogn og Fjordane along the western coast of Norway (called Førde hereafter). The other study area is located close to, and in the areas around the town of Son, in Vestby municipality, Akershus county in the south-eastern part of Norway (called Son hereafter).

Førde lies mostly within the boreonemoral vegetation zone. The bedrock is mostly dominated by gneiss, granite and other plutonic rock types. The vegetation mostly consists of mixed forests with deciduous woodland dominated by birch (*Betula* spp.) and alder (*Alnus incana*). The field layer is dominated by grasses with a mix of herbaceous plants. Other parts consist of coniferous forests, mainly dominated by Scots pine (*Pinus sylvestris*) and areas of planted with Norway spruce (*Picea abies*) (Mysterud et al., 2002), with the field layer consisting mainly of mosses, lichens and heath species. In addition, some areas consist of marshes or pastures and grass fields for grass production. The topography is characterized by steep mountain hills and valleys, with rivers and streams (Abrahamsen et al., 1977). Elevation among the trapping stations ranges between 11 and 355 m a.s.l. Among the large mammals in the area, the red deer (*Cervus elaphus*) is the most common, and the most important host for the adult *I. ricinus*. The coastal areas generally have a milder climate with higher humidity compared to the colder and dryer inland climate. Temperature and humidity also decrease with increased elevation (Langvatn et al., 1996). The study area typically has cold summers and mild winters. The average temperature in the years 2014 and 2015 was 5.3° and 4.2° respectively (Norwegian Meteorological Institute 2015).

The vegetation in Son is also mostly within the boreonemoral zone, situated in the geological area named the Oslo field ("Oslofeltet"). The bedrock here mostly consists of gneisses. The vegetation is characterized by mixed forests, with both deciduous woodlands and coniferous forests with relatively large areas of agricultural landscape through the whole study area. The deciduous forests are dominated by birch or oak (*Quercus robur*) with elements of alder, goat willow (*Salix caprea*), hazel (*Corylus avellana*) and aspen (*Populus tremula*), with a mix of grasses, shrubs and herbaceous plants covering the field layer. Coniferous forests are mostly made up of Norway spruce or Scots pine, with the field layer mostly consisting of mosses, mainly *Sphagnum* spp. and heath species. The terrain is typically flat and less elevated than in Førde, with the stations ranging from 58 to 137 m a.s.l. The area differs from Førde when it comes to the community of larger mammals. While Førde is dominated by red deer, the area around Son is home to large populations of roe deer

(*Capreolus capreolus*), which serve as the main host for the mature *I. ricinus.* Moose (*Alces alces*) is also found in the area. The average temperature in the years 2014 and 2015 was 3.4° and 2.7° respectively (Norwegian Meteorological Institute 2015).

2.2   Study design

Capturing was done in spring and fall in both 2014 and 2015 for both Førde and Son, of which 2015 was done as part of this thesis and 2014 available from earlier work (Mysterud et al., 2015). In Førde, trapping was done in two different transects. One transect is located in Angedalen near the town of Førde, in Førde municipality, Sogn og Fjordane county. This transect consists of 20 trapping stations, each with 4 traps giving a total of 80 traps. The second transect is in the western part of Førde municipality (called Førde West) extending into the adjacent municipality of Askvoll. This transect consists of 30 trapping stations and 120 traps in total. In the other main study area Son, there are 25 trapping stations with a total of 100 traps. Common for all trapping stations was the approximate minimum distance of minimum 500 m between each station. This was done to avoid local depletion of populations. The four traps were placed in the corners of a 15x15 m square according to the small quadrate method (Myllymäki et al., 1971). The traps are positioned in the terrain to maximize the probability of capture within 2 m of each corner. All trapping stations were also situated along, or in proximity of main roads with a minimum distance of 50 m. Trap coordinates and elevation had been collected using a handheld GPS.

2.3   Data collection

2.3.1 Capturing of small mammals

The traps used were "Ugglan"-traps. These are live traps, in which the animals are caught within a small cage. The trap is covered with a metal sheet to protect the animals from the weather and to keep predators away. They are baited with oats for food and a piece of carrot for water to sustain the animals before the traps are emptied. The traps were set and baited the first day, and checked daily for three consecutive days. Small mammals that were caught, were culled by cervical dislocation, and then stored in individual closed zip-lock bags to prevent the ticks from escaping. The bags were marked with an individual number, trap number and date of capture before they were stored in a freezer for later examination in the laboratory.

2.3.2 Identification of small mammals and ticks

In the lab, the animals were weighed, determined to species and examined for ticks. Each animal was defrosted before they were examined under a magnifying glass using a tweezer to locate and remove on-host ticks. A total of 998 rodents and shrews captured in 2014 and 2015 were examined. The set standardized time for examining each host-animal was 20 minutes. This time was set to ensure that each animal was given the same level of examination. This was based on experience from an earlier, similar study (Mysterud et al., 2015). Species determination of small mammals was done either by external morphological characters (shrews and *Apodemus* sp.), or by examination of teeth (*Microtus agrestis* and *Myodes glareolus*). To ensure the correct determination of species, a large subsample of both rodents and shrews were controlled by an expert (Jeroen van der Kooij). During examination, the ticks were removed from the animal and placed aside for identification. After removal, the ticks were counted and identified to species and developmental stage (larvae, nymph or adult) under a stereomicroscope using morphological characters. The ticks were then placed in tubes together with silica-gel and stored in the freezer for later analysis. Other ectoparasites like mites and fleas were also collected, but were not used in further analyses in this study. Also, a tissue sample from each animal's ear was collected to be used in molecular pathogen detection of *B. burgdorferi* s.l and *A. phagocytophilum*.

2.4 Pathogen detection

The presence of the tick-borne pathogens *B. burgdorferi* s.l. and *A. phagocytophilum* in the small mammals were determined by using an established PCR-protocol (Mysterud et al., 2013). This protocol is based on Allender et al. (2004). Biological material from 991 small mammals was sent to the lab at CEES for molecular pathogen detection (performed by Vetle M. Stigum under supervision of W.R. Easterday). The procedure analyses the samples for *A. phagocytophilum* and *B. burgdorferi* s.l., using multiplex real-time PCR (Courtney et al., 2004) on a Roche Lightcycler® 480 Real-Time PCR instrument.

2.5 Statistical analysis

For analysis, host animals caught in 2014 and 2015 from both Førde and Son was included. One species of shrew, the *Neomys fodiens* was excluded due to small sample size (n=4). One individual of the common shrew (*Sorex araneus*) was also excluded due to missing value for body weight. This gave a total sample size of 991 small mammals. All statistical analyses were performed in R software version 3.1. (R Development Core Team, 2016). Tick abundance data and other types of parasite data are often subject to overdispersal relative to the Poisson distribution often used for count data (D. J. Shaw & Dobson, 1995). This is mainly due to parasites often being highly aggregated on certain individuals rather than evenly distributed among the host animals. In statistical terms, this means that the variance is larger than the mean. Negative binomial distribution allows for this, so that the estimated negative binomial frequency distribution does not significantly differ from the observed parasite frequency distribution (D. J. Shaw & Dobson, 1995). Secondly, there is another challenge, related to the study design. Since each trapping station consists of 4 individual traps, the variance within stations is smaller than the variance between them (Mysterud et al., 2015). This must be handled statistically because it violates the assumption of independent observations. This was done by including the covariate "station" as a random effect. The data for tick burdens on small mammals were analyzed with generalized mixed effect models in the library glmmADMB in R, since this package allow inclusion of both fixed and random effects with a negative binomial distribution (Littell et al., 2006). The prevalence of the pathogens *B. burgdorferi* s.l. and *A. phagocytophilum* was analyzed with generalized linear models with a binomial distribution.

For the analysis, models were built separately for each tick species in each main study area (Førde and Son) due to differences in host community and to avoid multivariate models. This gave a total of four different models for *I. ricinus* and *I. trianguliceps* larvae. Nymphs were quantified, but not included in the analysis. Partly due to low sample size, but also since the objective was limited to investigate differences in larval tick burdens in two different ecosystems over two years. However, nymph numbers were used as a covariate in pathogens prevalence analyses. For Førde, the two transects Førde Vest/Askvoll and Angedalen were included in the same analysis and entered as a factor variable. The common response variable in all the models was the number of ticks per individual host. In the full model the covariates included; log (body weight), elevation, transect (Førde only), year (2014/2015), host species and season. The model also considered the following interactions; year*transect, year*season, year* elevation, year*log(bodyweight), season*elevation and transect*season. For the pathogens, the models were built separately for each pathogen in each study area. This gave a total of four models. The full model included the same covariates as for the tick analyses, but in addition the three variables total ticks on a given host (all life stages of both species of tick), number of *I. ricinus* nymphs and number of *I. trianguliceps* nymphs were also tried added to the models. The common response variable was infection prevalence.

I used the Akaike Information Criterion (AIC) for the model selection, i.e. to compare performance of different models. The AIC uses a parsimonious principle aiming to find the best balance between the deviance as a measure of fit, and it adds a term to penalize more complex models, i.e. models with more parameters. It aims to find the model that would best explain the observed data with the fewest number of parameters (Bolker et al., 2009). The term ΔAIC is used for the difference in AIC between a given candidate model relative to the best current model, and a model is considered better if the AIC-value is lower than the models it is compared to. A rule of thumb is to define the model with the fewest parameters differing with ΔAIC<2 from the lowest AIC-value (Burnham and Anderson 2004). I used this as a criterion in the model selection. For the model selection, I chose a backwards selection approach in the case of tick loads. This means, starting with the full model and removing the covariates one by one, and determine if the removal of a certain covariate lowers the AIC-value. For the pathogens, I used a combination of backwards and forwards selection.

3 Results

A total of 998 small mammals were captured in the two areas. The species composition of small mammals differed between the two areas. In Son, five species of small mammals were caught, this included *Apodemus sylvaticus* (n=45)*, Myodes glareolus* (n=163)*, Sorex araneus* (n=200), *S. minutus* (n=34) and *Neomys fodiens* (n=1). In Førde, the following eight species were found: *A. sylvaticus* (n=35)*, M. glareolus* (n=38)*, S. araneus* (n=291), *S. minutus* (n=39), *S. isodon* (n=101), *A. flavicollis* (n=13)*, Microtus agrestis* (n=32) and *N. fodiens* (n=3) (Table 1)*.*

In Førde, a total of 2258 ticks were collected of which 56.6% (n=1279) were *I. ricinus* larvae, and 34.7% (n=784) were *I. trianguliceps* larvae. Only 1.2% (n=28) of the total ticks were *I. ricinus* nymphs and 6.8% *I. trianguliceps* nymphs (n=153). Among all the ticks collected in Førde, only 0.6% (n=14) were adult *I. trianguliceps*, while no adult males were found. From the 4379 collected in Son, 92% (n=4029) were *I. ricinus* larvae and 4% (n=176) were *I. trianguliceps* larvae. There were 2.7% (n=117) *I. ricinus* nymphs and 1.1 % (n=51) *I. trianguliceps* nymphs. Of all the ticks from Son only 0.02 % (n=9) were adult female *I. trianguliceps*. No male *I. trianguliceps* were found. As expected, no adult *I. ricinus* were found on small mammals in either area.

Of the 549 small mammals caught in Førde, the prevalence of *I. ricinus* larvae was 51.3% with a range of 1-104 in 2014, and 33% with a range of 1-14 ticks in 2015 (Table 1). For *I. trianguliceps* larvae, the prevalence was 50% with a range of 1-11 in 2014, and 20 % with a range of 1-9 in 2015 (Table 2). Among the 442 small mammals that were caught in Son, the prevalence of *I. ricinus* larvae was 84.4 % with a range of 1-117 in 2014, and 83.8 % with a range of 1-112 in 2015 (Table 1). For *I. trianguliceps* larvae*,* the prevalence was 26.6 % with a range of 1-25 in 2014, and 12.8 % with a range 1-9 in 2015 (Table 2).
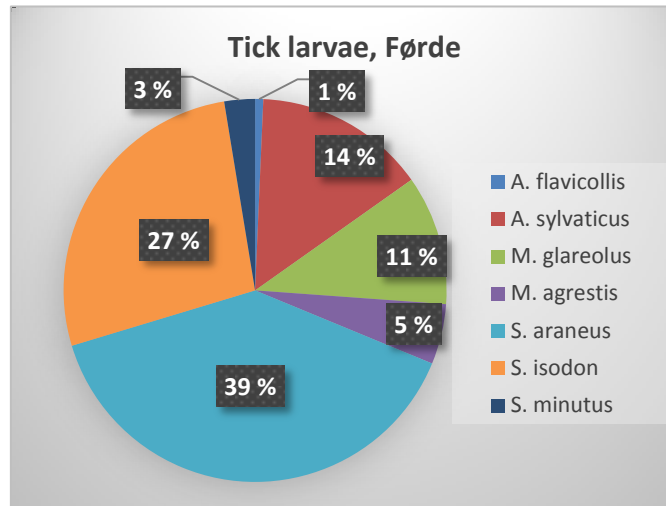
Figure 2. The proportion of tick larvae fed by each host species in Førde, Sogn og Fjordane, Norway, in 2014 and 2015 combined.
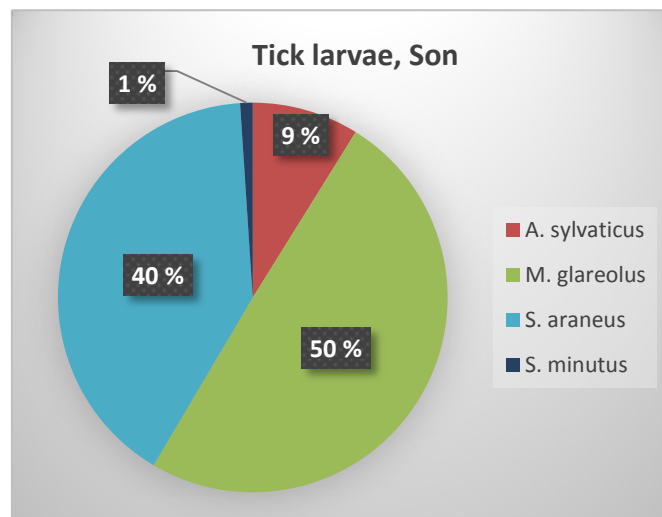

Figure 3. The proportion of tick larvae fed by each host species in Son, Akershus, Norway, in 2014 and 2015 combined.

3.1   Tick load of *Ixodes ricinus*

3.1.1 Førde

The most parsimonious model for explaining the variation in tick load of *I. ricinus* larvae among small mammal hosts in Førde included the variables host species (with 7 levels) elevation, body weight, and year (as a category), and the interactions year*elevation and season*elevation (Table 1). The covariate transect was not included in the most parsimonious model, neither were the interactions year*transect, year*season, year* body weight or transect*season. The model was also run without the variable body weight to see if the effect of host species was different when body weight was accounted for.

The tick load of *I. ricinus* in Førde was positively correlated with body mass of the host, but the relationship was not linear. Between 3 and 11 g of body mass, tick load increased significantly, while body mass had little effect above 11-12 g (Figure 4). Host species was also significant. There was difference in tick load between the different species of small mammals, with the *A. sylvaticus* having the highest tick load (Table 1). Part of the host species effect on tick load result from the different body weight ranges of the species. This is especially evident for the small *S. minutus* with a range of 3-5 g in body weight, which is well below the other host species. *S. minutus* had lower tick load compared to the other hosts, but not after the effect of body mass was accounted for. Tick load was significantly higher in 2014 than in 2015. Elevation and season also influenced tick load, but their effect interacted. Elevation had greater effect in the spring season than in the fall, with the tick load decreasing more with increasing elevation in the spring, than it did in the fall. Tick load decreased significantly with increasing elevation in the spring both in 2014 and 2015, but the effect was stronger in 2014 vs. 2015 which shows the interaction between year and elevation (Figure 5). Lastly, elevation was also negatively correlated with tick load in the fall season of 2014, but not in the fall of 2015 (Figure 5).

Table 1. Parameter estimates from the most parsimonious model explaining variations in tick load of *I. ricinus* larvae among small mammal hosts in Førde, Sogn og Fjordane, Norway. Baseline for species is *S. araneus*, for season is fall and for year 2014. SE = standard error.

Parameter	Estimate	SE	z	p
Intercept	-0.25437	0.98091	-0.26	0.795
Apodemus flavicollis	-1.34625	0.86068	-1.56	0.118
Apodemus sylvaticus	0.53478	0.47550	1.12	0.261
Microtus agrestis	-0.87014	0.57628	-1.51	0.131
Myodes glareolus	0.03285	0.51116	0.06	0.949
Sorex isodon	-0.16021	0.25609	-0.63	0.532
Sorex minutus	-1.83459	0.58524	-3.13	0.002
Year 2015	-2.07215	0.38880	-5.33	<0.001
Season spring	0.79735	0.53841	1.48	0.139
Elevation	-0.00509	0.00250	-2.04	0.041
Log (body weight)	0.8213	0.40729	2.02	0.044
Year 2015*Elevation	0.00491	0.00221	2.22	0.026
Season spring*Elevation	-0.00686	0.00298	-2.30	0.021

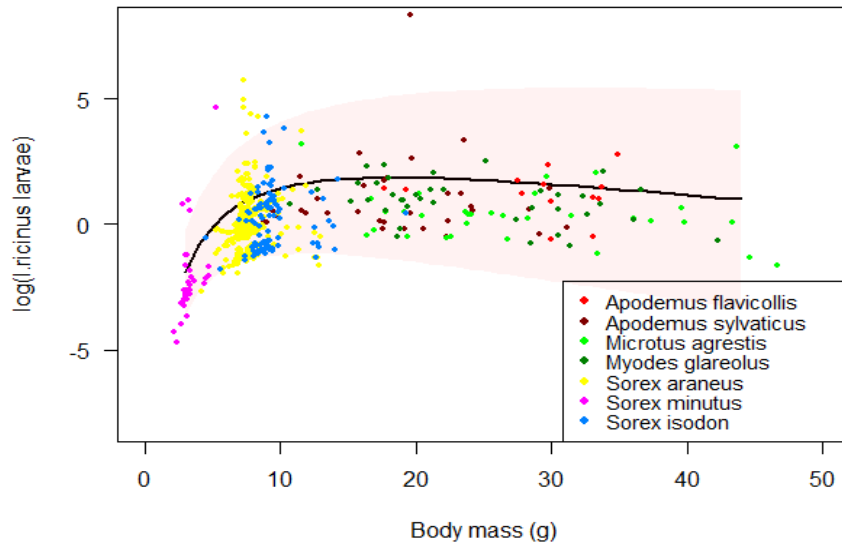**Figure 4.** The number of *I. ricinus* larvae per individual host as a function of body mass in a community of small mammal hosts in Førde, Norway. Residuals are shown in different colors for the different host species.
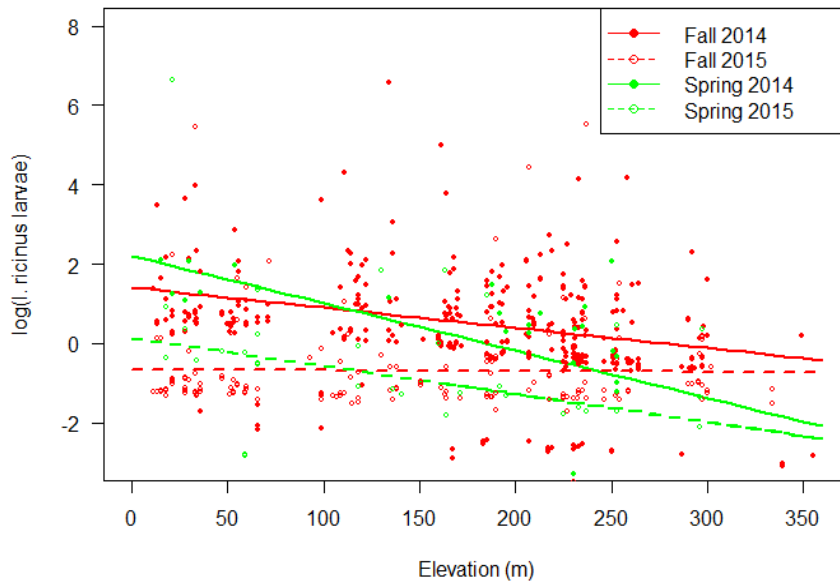

Figure 5. The number of I. ricinus larvae per individual as a function of elevation in spring and fall season in 2014 and 2015 in Førde, Norway. Residuals are shown in different colours for spring and fall.

3.1.2 Son

The most parsimonious model for explaining the variation in tick load of *I. ricinus* larvae among small mammal hosts in Son included the variables host species (with 4 levels), body weight, season, year, and the interactions year*season and year*body weight. Elevation was not included in the most parsimonious model, neither were the interactions year*elevation and season*elevation.

Tick load of *I. ricinus* in Son was positively correlated with body weight of the host animal. Like in Førde, the effect of body weight was non-linear. Host species were also significant, with the *S. araneus* having the highest tick load, and the *S. minutus* having the lowest (Table 2). However, in this case the *S. minutus* had significantly lower tick load compared to the baseline species *S. araneus* also when body mass was accounted for. There was interaction between season and year (Table 2). Tick load was similar in spring and fall 2014, higher in spring 2015 than in the spring of 2014 and higher in fall 2015 compared to spring 2015. Body weight of host animal also interacted with year. In both years there was positive correlation between body weight of host animal and tick load, but the effect of body weight in 2015 was smaller than in 2014 (Table 2).

Table 2. Parameter estimates from the most parsimonious model for explaining variations in tick load of *I. ricinus* larvae among small mammal hosts in Son, Akershus, Norway. Baseline for species is *S. araneus*, for season is fall and for year 2014. SE = standard error.

Parameter	Estimate	SE	z	p
Intercept	-0.00065	0.39906	0	0.999
Apodemus sylvaticus	-0.37699	0.26019	-1.45	0.147
Myodes glareolus	-0.42596	0.20418	-2.09	0.037
Sorex minutus	-1.48895	0.30446	-4.89	<0.001
Year 2015	0.91368	0.42039	2.17	0.029
Season spring	-0.00907	0.23521	-0.04	0.969
Log (body weight)	0.89598	0.18949	4.73	<0.001
Year 2015*Season spring	1.01665	0.33972	2.99	0.003
Year 2015*Log (body weight)	-0.44972	0.18198	-2.47	0.014

Table 3. Prevalence and intensities of *I. ricinus* larvae infestation among small mammal hosts in Førde, Sogn og Fjordane, Norway in 2014 and 2015.

Host	n	Infected (n)	Prevalence (%)	Mean	Median	Range	n	Infected (n)	Prevalence (%)	Mean	Median	Range
			2014		Intensity				**2015**		Intensity	
A. flavicollis	11	3	27,3 %	4,0	5	1-6	2	0	0,0 %	0	0	0
A. sylvaticus	27	14	51,9 %	15,9	4	1-104	8	3	37,5 %	2,3	2	1-4
M. glareolus	29	25	86,2 %	4,7	4	1-23	9	5	55,6 %	2,4	2	1-4
M. agrestis	21	11	52,3 %	4,3	3	1-20	11	3	27,2 %	4,3	2	1-10
S. araneus	178	90	50,6 %	4,1	3	1-24	113	42	37,1 %	3,3	2	1-14
S. isodon	49	30	61,2 %	9,7	3	1-63	52	12	23,0 %	2,5	1	1-13
S. minutus	34	6	17,6 %	1,7	1	1-5	5	1	20,0 %	1	1	1
Total	**349**	**179**	**51,3 %**	**6,0**	**3**	**1-104**	**200**	**66**	**33,0 %**	**3,1**	**2**	**1-14**

Table 4. Prevalence and intensity of *I. ricinus* larvae infestation among small mammal hosts in Son, Akershus, Norway in 2014 and 2015.

Host	n	Infected (n)	Prevalence (%)	Mean	Median	Range	n	Infected (n)	Prevalence (%)	Mean	Median	Range
			2014		Intensity				**2015**		Intensity	
A. sylvaticus	28	25	89,3 %	9,2	5	1-59	17	16	94,1 %	17,4	7	1-102
M. glareolus	103	98	95,1 %	13,8	8	1-117	60	55	91,7 %	9,3	10	1-31
S. araneus	112	87	77,7 %	8,3	4	1-49	88	73	83,0 %	11,8	6	1-112
S. minutus	20	12	60,0 %	1,9	1	1-4	14	6	42,9 %	2,2	1.5	1-5
Total	**263**	**222**	**84,4 %**	**10,5**	**4**	**1-117**	**179**	**150**	**83,8 %**	**11,4**	**6**	**1-112**

16

3.2 Tick load of *Ixodes trianguliceps*

3.2.1 Førde

The most parsimonious model for explaining the variation in tick load of *I. trianguliceps* among small mammal hosts in Førde included the variables host species (with 7 levels), year, season, elevation. The interactions year*season and year*elevation were also included. The variables transect, log (weight) and the interaction season*elevation were not included in the most parsimonious model (Table 5).

The tick load of *I. trianguliceps* larvae in Førde differed between the host species with *M. glareolus* having the highest tick load of all the host species. The effect of season and year interacted, so that 2014 had higher tick load in the spring than in the fall, but in 2015, there were no differences between spring and fall in terms of tick load. Tick loads of 2014 were very different compared to 2015. In 2015, the tick load was below 1 for both spring and fall. There was also interaction between elevation and year, with the effect of elevation being stronger in 2015 compared to 2014 (Table 5).

Table 5. Parameter estimates from the most parsimonious model for explaining variations in tick load of *I. trianguliceps* among small mammal hosts in Førde Sogn og Fjordane, Norway. Baseline for species is *S. araneus*, for season is fall and for year 2014. SE = standard error.

Parameter	Estimate	SE	z	p
Intercept	0.95845	0.31647	3.03	0.002
Apodemus flavicollis	-0.57903	0.60184	-0.96	0.336
Apodemus sylvaticus	0.25736	0.36653	0.70	0.483
Microtus agrestis	-0.08992	0.39104	-0.23	0.818
Myodes glareolus	0.83237	0.34180	2.44	0.015
Sorex isodon	0.61392	0.25346	2.42	0.015
Sorex minutus	-0.25692	0.35474	-0.72	0.469
Year 2015	-0.71044	0.37850	-1.88	0.061
Season spring	1.26179	0.38277	3.30	<0.001
Elevation	-0.00416	0.00157	-2.66	0.008
Year 2015*Season spring	-1.43429	0.58833	-2.44	0.015
Year 2015*Elevation	-0.00587	0.00245	-2.40	0.016

3.2.2 Son

The most parsimonious model for explaining the variation in tick load of *I. trianguliceps* among small mammal hosts in Son included the variables host species (with 4 levels), year, season and the interaction year*season. The variables body weight of host animal, elevation or any other interactions were not included in the most parsimonious model (Table 6).

The tick load of *I. trianguliceps* larvae differed also between the different host species, with *M. glareolus* having the highest tick load among the host species. Like in Førde, there were differences between seasons, and the season and year variables interacted, so the season effect differed between the two years. In 2014, the fall season had significantly higher tick loads than the spring season. In 2015, the opposite was the case, with the spring season having higher tick loads than the fall season. The variation within the year of 2015 was significantly greater than that of 2014, with the spring of 2015 having the highest overall tick load and the fall of 2015 having the lowest overall tick load of all the seasons.

Table 6. Parameter estimates from the most parsimonious model for explaining variations in tick load of *I. trianguliceps* among small mammal hosts in Son, Akershus, Norway. Baseline for species is *S. araneus*, for season is fall and for year 2014. SE = standard error.

Parameter	Estimate	SE	z	p
Intercept	-1.178	0.258	-4.57	<0.001
Apodemus sylvaticus	-0.137	0.453	-0.30	0.763
Myodes glareolus	0.939	0.285	3.29	0.001
Sorex minutus	-0.378	0.583	-0.65	0.517
Year 2015	-2.340	0.404	-5.79	<0.001
Season spring	-1.141	0.528	-2.16	0.031
Year 2015*Season spring	3.878	0.755	5.14	<0.001

Table 7. Prevalence and intensities of *I. trianguliceps* larvae infestation among small mammal hosts in Førde, Sogn og Fjordane, Norway in 2014 and 2015.

				2014						2015		
					Intensity						Intensity	
Host	n	Infected (n)	Prevalence (%)	Mean	Median	Range	n	Infected (n)	Prevalence (%)	Mean	Median	Range
A. flavicollis	11	8	72,7 %	2,38	1,5	1-6	2	0	0,0 %	0,00	0,0	0
A. sylvaticus	27	16	59,3 %	4,20	2,5	1-15	8	1	12,5 %	1,00	1,0	1
M. glareolus	29	18	62,0 %	3,55	2,5	1-14	9	6	66,7 %	5,00	3,0	1-14
M. agrestis	21	12	57,1 %	3,60	1,5	1-17	11	1	9,0 %	1,00	1,0	1
S. araneus	178	81	45,5 %	3,07	2,0	1-15	113	17	15,0 %	2,05	1,0	1-7
S. isodon	49	32	65,3 %	5,60	3,5	1-25	52	12	23,0 %	4,50	1,0	1-20
S. minutus	34	7	20,5 %	5,14	2,0	1-17	5	2	40,0 %	3,00	3,0	1-5
Total	**349**	**174**	**49,9 %**	**3,80**	**2,0**	**1-25**	**200**	**39**	**19,5 %**	**3,26**	**2,0**	**1-20**

Table 8. Prevalence and intensity of *I. trianguliceps* larvae infestation among small mammal hosts in Son, Akershus, Norway in 2014 and 2015.

				2014						2015		
					Intensity						Intensity	
Host	n	Infected (n)	Prevalence (%)	Mean	Median	Range	n	Infected (n)	Prevalence (%)	Mean	Median	Range
A. sylvaticus	28	5	17,8 %	1,60	1,0	1-3	17	4	23,5 %	2,00	1,5	1-4
M. glareolus	103	38	36,9 %	2,03	1,0	1-10	60	14	23,3 %	1,64	1,0	1-9
S. araneus	112	22	19,6 %	2,19	2,0	1-11	88	4	4,5 %	2,00	2,0	1-3
S. minutus	20	5	25,0 %	1,20	1,0	1-2	14	0	0,0 %	0,00	0,0	0
Total	**263**	**69**	**26,2 %**	**1,98**	**1,0**	**1-11**	**179**	**22**	**12,2 %**	**1,78**	**1,0**	**1-9**

3.3   Prevalence of *B. burgdorferi s. l.* and *A. phagocytophilum*

3.3.1   *B. burgdorferi s. l.*

Førde

The most parsimonious model for explaining infection prevalence of *B. burgdorferi* s.l. in Førde included the variables year, body weight of host animal (with 7 levels) and total ticks. The variables elevation, transect, season and host species were not included. Neither were the variables number of *I. ricinus* nymphs or *I. trianguliceps* nymphs, or any interactions between the covariates (Table 9).

The infection prevalence differed between years, with the prevalence being 4.5 % in 2014 and 8.5 % in 2015. Infection prevalence was also positively correlated with both body weight of host animal and with total ticks. When the variable host species was included, the model failed to converge. This is mainly due to lack of data (few positives). Host species were only significant when the variable body weight was not included. The variable was also tried with 3 levels by including the *Sorex* sp. into one group, the *Apodemus* sp. into another, and *M. agrestis* and *M. glareolus* into a third group. In this case the model did converge, but only when the variable body weight was not included. This is mainly because the model was not able to separate the effects of body weight and host species, so host species was left out. There was also little data for the nymphs, both *I. ricinus* and *I. trianguliceps,* i.e. the

Table 9. Parameter estimates from the most parsimonious model explaining infection prevalence of *B. burgdorferi* s. l. in a community of small mammal hosts in Førde, Sogn og Fjordane, Norway. Baseline for year is 2014. SE= standard error.

Parameter	Estimate	SE	z-value	p-value
Intercept	-5.85621	0.90268	-6.488	<0.001
Year 2015	0.89128	0.40557	2.198	0.028
Log (body weight)	1.00441	0.31938	3.145	0.002
Total ticks	0.03662	0.01496	2.448	0.014

Son

The most parsimonious model for explaining infection prevalence of *B. burgdorferi* s.l. among small mammal hosts in Son included the variables year, host species and body weight of host animal. The variables elevation and season were not included, neither were the variables total ticks, *I. ricinus* nymphs or *I. trianguliceps* nymphs or any interactions between the covariates (Table 10).

The overall infection prevalence of *B. burgdorferi* s.l. in Son varied between years, with the prevalence being 18.6 % in 2014 and 32 % in 2015 (Table 10). Body weight was also positively correlated with infection prevalence. The infection prevalence also varied between the host species. Unlike the model for Førde, the Son model converged when host species and body weight were included. In this case, the number of infected animals was much higher, and the variable host species had 4 levels, instead of 7 like in Førde.

Table 10. Parameter estimates from the most parsimonious model explaining infection prevalence of *B. burgdorferi* s. l. in a community of small mammal hosts in Son, Akershus, Norway. Baseline for species is *S. araneus* and baseline for year is 2014. SE = standard error.

Parameter	Estimate	SE	z-value	p-value
Intercept	-7.2256	1.0905	-6.626	<0.003
Year 2015	0.4975	0.2531	1.966	0.049
Apodemus sylvaticus	-3.5228	0.7949	-4.432	<0.001
Myodes glareolus	-3.3496	0.6978	-4.800	<0.001
Sorex minutus	0.8283	0.8902	0.930	0.352
Log (body weight)	3.1109	0.5640	5.516	<0.001

Table 11. Infection prevalence of *B. burgdorferi* s. l. among species in a community of small mammals in the years 2014 and 2015 in Førde, Sogn og Fjordane, Norway.

Species	2014				2015			
	n	Positive	Negative	Prevalence	n	Positive	Negative	Prevalence
A. flavicollis	11	0	11	0.0 %	2	0	2	0.0 %
A. sylvaticus	27	2	25	7.0 %	8	0	8	0.0 %
M. agrestis	21	2	19	9.0 %	11	5	6	45.0 %
M. glareolus	29	1	28	3.4 %	9	2	7	22.0 %
S. araneus	178	9	169	5.0 %	112	8	104	7.1 %
S. isodon	49	1	48	2.0 %	52	2	50	3.8 %
S. minutus	34	1	33	2.9 %	5	0	5	0.0 %
Total	**349**	**16**	**333**	**4.5 %**	**199**	**17**	**182**	**8.5 %**

Table 12. Infection prevalence of *B. burgdorferi* s. l. among species in a community of small mammals in the years 2014 and 2015 in Son, Akershus, Norway.

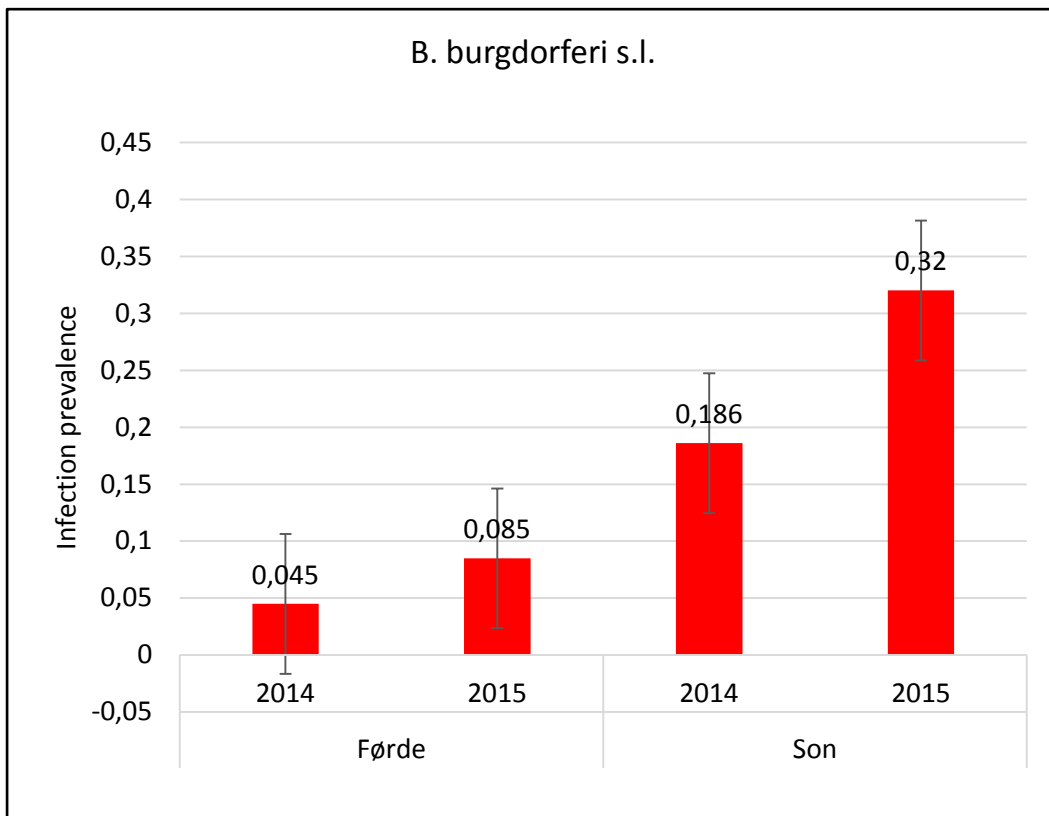| Species	2014				2015			
	n	Positive	Negative	Prevalence	n	Positive	Negative	Prevalence
A. sylvaticus	28	5	23	17.9 %	20	5	15	25.0 %
M. glareolus	103	19	84	18.4 %	60	25	35	41.7 %
S. araneus	112	23	89	20.5 %	87	28	59	32.1 %
S. minutus	20	2	18	10.0 %	14	0	14	0.0 %
Total	**263**	**49**	**214**	**18.6 %**	**181**	**58**	**123**	**32.0 %**

Figure 6. Infection prevalence of *B. burgdorferi s.l.* in small mammal hosts in Førde, Sogn og Fjordane, Norway and Son, Vestby, Akershus in the years 2014 and 2015.

3.3.2 *Anaplasma phagocytophilum*

Førde

The most parsimonious model for explaining infection prevalence of *A. phagocytophilum* among small mammal hosts in Førde, included the variables elevation, host species (3 levels) and body weight of host animal. The variables year, season and transect were not included. Neither were the variables *I. ricinus* nymphs, *I. trianguliceps* nymphs, total ticks or any interactions between the covariates (Table 13).

The prevalence of *A. phagocytophilum* in Førde was 13.5 % and 12.5 % for 2014 and 2015 respectively. Infection prevalence was negatively correlated with elevation. In this case, the model did not converge unless the variable elevation was rescaled by subtracting the mean from the actual values. When this was done, the model was able to handle the variable. The infection prevalence also varied between host species. For the model to converge when including this variable, it had to be reduced from 7 to 3 levels. This is mainly due to data deficiency, with some of the species having few positives. This was done by grouping several host species together, with all three *Sorex* spp. into one group, *Apodemus* spp. into another, and *M. glareolus* and *M. agrestis* into a third group. It was seen as the most biologically relevant to group the more closely related species together. Infection prevalence was also positively correlated with body weight of the host animal.

Table 13. Parameter estimates from the most parsimonious model explaining infection prevalence of *A. phagocytophilum* in a community of small mammal hosts in Førde, Sogn og Fjordane, Norway. Baseline for species is Species group 1 (*Sorex* sp.). Species group 2 contains *Apodemus* sp. and Species group 3 contains *M. glareolus* and *M. agrestis*. SE = standard error.

Parameter	Estimate	SE	z-value	p-value
Intercept	-4.25268	1.09040	-3.900	<0.001
Elevation	-0.00709	0.00200	-3.532	<0.001
Species group 2	-1.63795	0.76074	-2.153	0.031
Species group 3	-2.89840	0.98431	-2.945	0.003
Log (body weight)	1.09614	0.52620	2.083	0.037

Son

In the analysis of infection prevalence of *A. phagocytophilum* among small mammal hosts in Son, none of the covariates showed any measurable effects. The model only including the intercept without covariates, for explaining infection prevalence of *A. phagocytophilum* in the current area was the most parsimonious. Overall prevalence was 7.9 % and 9.8 % for 2014 and 2015 respectively. 95 % of the infected individuals were *M. glareolus* or *S. araneus.*

4   Discussion

With the tick expanding its distribution ranges over the last decades, and the subsequent increase in disease risk from tick-borne pathogens, increased knowledge of the ticks' ecology is more important than ever (Jore et al., 2011; Medlock et al., 2013). The primary aim of this study was to increase the understanding of the pattern of tick attachment, and the circulation of tick-borne pathogens among small mammal hosts at the ticks' northernmost distribution limits. The knowledge on this subject today is limited, and previous studies from northern areas stem from single year and location type of studies (Mysterud et al., 2015; Radzijevskaja et al., 2013). In this thesis, I have investigated factors that influence tick load of *I. ricinus* and *I. trianguliceps* larvae, and prevalence of the pathogens *B. burgdorferi* s.l. and *A. phagocytophilum* among small mammal hosts in two contrasting ecosystems over two years. A common feature in both areas was variation in tick load between host species. Tick load of both *I. ricinus* and *I. trianguliceps* in both study areas was affected by host species to a certain degree. For *I. ricinus,* tick load correlated positively with the body weight of the host animal, different from the case of *I. trianguliceps.* All analyses also showed annual and seasonal differences in tick load, however, with different directions across years and seasons. The two areas also differed from one another, both in terms of tick load and infection prevalence of tick-borne pathogens. Infection prevalence of *B burgdorferi* s.1. differed between 2014 and 2015 in both Førde and Son. This was not the case for *A. phagocytophilum*. Such variation in larval tick load and infection prevalence is likely to yield variation in disease hazard the year after.

4.1   Distribution of life stages of *I. ricinus* and *I. trianguliceps* on small mammals

The findings of this study agree with previous studies that suggest rodents and shrews are hosts for early life stages of *I. ricinus,* and all life stages of *I. trianguliceps* (Nilsson, 1974; Nilsson & Lundqvist, 1978). Larvae and nymphs of both species were found, as well as a few adult *I. trianguliceps*. This was expected due to the *I. trianguliceps* being a rodent specialist depending on small mammals for the entire life cycle (Randolph, 1975). The absence of adult *I. ricinus* was also expected, since adults are known to primarily feed on larger mammals, such as deer (Mysterud et al., 2014). Larvae of both tick species were found on all host species included in the analysis. However, their relative numbers differed between the two

study areas. *I. ricinus* was the dominant tick species in both Førde and Son. The reduction in number of ticks from larvae to nymph was much greater for *I. ricinus* than *I. trianguliceps*. Though much of the reduction can be contributed to naturally high mortality rates, *I. ricinus* nymphs are more likely to feed on different hosts in the habitat than the larvae, but this is not the case for *I. trianguliceps* nymphs (Matuschka et al., 1991). This is mostly a result of their different questing behaviors. The *I. ricinus* nymphs often quest higher in the vegetation where they are less likely to encounter smaller mammals such as rodents and shrews as opposed to the *I. trianguliceps* nymphs which mostly quest in burrows (Matuschka et al., 1991; Nilsson, 1974). Studies from other areas have shown that specialized ticks are important in maintaining infection levels in a small mammal reservoir (Brown & Lane, 1992), and therefore have an indirect effect on disease hazard. The role of *I. trianguliceps* in maintaining infection levels of *B. burgdorferi* s.l. in reservoir populations in a northern ecosystem have not been thoroughly studied.

4.1.1 Tick load on the different small mammal host species

The two areas in this study had different small mammal communities in terms of species composition. In Førde a total of eight species were found. These include *A. flavicollis, A. sylvaticus, M. agrestis, M. glareolus, S. isodon, S. araneus, S. minutus* and *N. fodiens*. The latter was not included in the analysis due to low sample size. In Son, only four species were found, *A. sylvaticus, M. glareolus, S. araneus* and *S. minutus*. This study showed that the proportions of tick larvae fed by the different host species were different in the two areas (Fig. 3). In Førde the most important species were two shrew species (*S. araneus* and *S. isodon*) feeding 66 % all tick larvae, while in Son *M. glareolus* and *S. araneus* were the most important species, feeding 90 % of all ticks in total. Tick prevalence also varied between the two areas, with the overall prevalence for tick larvae in Førde being 44.6% compared to 84.2% in Son, which means that almost twice as many individuals were infected with ticks in Son, compared to Førde.

In Førde, 11 % of all tick larvae were fed by *M. glareolus*, whereas the same species fed 50 % in Son, mostly due to the number of *M. glareolus* being higher in Son compared to Førde. The proportion of larvae fed by *A. sylvaticus* was 15 % in Førde and 9 % in Son. The proportion of tick larvae fed by *S. araneus* was similar in both areas (39% and 40%), but in Førde another shrew species, *S. isodon* fed 27 %. The additional species that were only present in Førde fed only 6 % of all ticks combined. This indicates that the relative

27

importance of different hosts varies between the two areas to a certain degree. One explanation for interspecific variation in tick load could be linked to different grooming rates between the species. All blood-sucking arthropods are at risk to some level of defensive behavior, and the animals could potentially reduce the number of ticks by grooming behavior (Anderson & Magnarelli, 2008; Murray, 1987). The time and energy spent grooming can also vary both between and within species, and be related to size and age (Harrison et al., 2010; M. T. Shaw et al., 2003). This could explain individual differences in tick load, since individuals who neglect grooming will suffer from higher infestation of ticks over time.

Based on counts of larva, *I. ricinus* was the most common tick species in both areas, but the prevalence of *I. ricinus* larvae on small hosts differed nevertheless between Førde and Son, with the overall prevalence for *I. ricinus* larvae in Førde being 44.6% compared to 84.2% in Son. The analyses for tick load of *I. ricinus* larvae showed that the variables host species, body weight of host species, seasonality and year were significant in explaining variation in both areas. Additionally, the landscape variable elevation was significant in Førde, but not in Son. *M. glareolus* had the highest prevalence in both areas. Mean intensity is the mean number of ticks among the infected hosts, and together with range (lowest and highest number of ticks found on one individual) says something about how the ticks are aggregated. In Førde in 2014, *A. sylvaticus* had the highest range of ticks between individuals (1-104) and the highest mean intensity (15.9) compared to *M. glareolus* (mean intensity = 4.7, range = 1-23), despite *A. sylvaticus* having much lower prevalence of tick larva than *M. glareolus* (Table 3). Hence, the ticks are more aggregated in the case of *A. sylvaticus* than *M. glareolus,* with fewer infected individuals, but the ones infected have higher burdens. The aggregation of ticks has implications on the transmission of pathogens from vector to host and host to vector, since hosts feeding a high number of ticks are more likely to be infected, and in turn feed a higher number of previously uninfected ticks (Randolph et al., 1996). *M. glareolus* had the highest prevalence of *I. ricinus* in both areas (Førde = 78.9 % and Son = 93.9%), with *A. sylvaticus* having the second highest (Førde = 48.6 % and Son = 91.1 %). *S. arane*us had the third highest prevalence of *I. ricinus* (Førde = 45.3 % and Son = 80.4 %). *S. minutus* had relatively low prevalence of *I. ricinus* (Førde = 17.2 % and Son = 52.9 %). Among the species only present in Førde, *M. agrestis* had 42.4 %, *S. isodon* had 41.6 % and *A. flavicollis* had 23 % (Table 3). The *I. ricinus* larvae seem to infect all the hosts present in its habitat in both areas, as is expected from a generalist species (Estrada-Peña et al., 2015). It is still debated whether the *I. ricinus*, though being globally considered a generalist, can be a local specialist,

showing different host ranges from area to area (McCoy et al., 2013). Though the findings in this study show that shrews feed a high proportion of tick larvae, the catch data might not represent the true relative abundance of all the host species. The abundance of certain host species may be over- or underestimated. Since the traps are baited with oats and carrots, insectivores like shrews are not attracted to traps because of the food, but are rather caught when randomly walking into traps placed where they would normally pass through. This would in fact give an underestimate of the abundance of shrews. Other species that prefer certain habitats, such as *M. agrestis*, could also be underestimated if a majority of the traps are placed in other habitats. However, this will not affect the estimated tick load or prevalence of the different species. Also, the fact that species composition in the two areas was very different indicates that the data reflects some of the actual variation in community composition. I will also, in all my inference, assume that this possible trapping bias is unchanged both between areas and between years, and will therefore not affect the main qualitative results of this study.

The prevalence of *I. trianguliceps* was different than for *I. ricinus*. Firstly, the overall prevalence of *I. trianguliceps* was significantly lower compared to *I. ricinus*, but when comparing the two areas, the prevalence in Førde was higher than in Son, unlike *I. ricinus*. Analyses for tick load of *I. trianguliceps* showed that host species, year and seasonality could explain some of the variation. Though there were differences, the prevalence of *I. trianguliceps* for the different hosts were more similar, compared to *I. ricinus.* Tick load of *I. trianguliceps* was not influenced by body weight (see also (Mysterud et al., 2015)), which was also expected due to their specialization to small mammal hosts.

Tick load and landscape

The two areas are different in terms of abiotic factors, which could help to explain the observed differences of both tick load and abundance of host animals. Firstly, the terrain in Førde is much more variable with higher differences in elevation compared to Son, in terms of elevation range and topography. Elevation was significant for explaining tick load in Førde, but not in Son. This is likely the result of Førde having wider range of elevation than Son. Tick load of both species in Førde was negatively correlated with elevation in the spring, but not in fall (Figure 5). Tick activity peaks earlier at lower elevations due to earlier spring warming and snow melting. Ticks are vulnerable to desiccation while questing, and studies have shown that ticks in dry habitats tend to quest lower in the vegetation, closer to the more

humid base of the vegetation compared to ticks in more humid habitats. Here, they more easily come in to contact with smaller hosts (Craine et al., 1995; Randolph & Storey, 1999). This could explain the difference in prevalence and tick abundance of *I. ricinus* larvae between Førde and Son, as it is possible that *I. ricinus* larvae in Førde, feed on larger hosts such as deer rather than rodents and shrews. However, this was not a part of this study, but further studies should focus on investigating the differences between Førde and Son.

Annual variation in tick load

The abundance of small mammals was highest for 2014 in both the Førde and Son area. The annual difference in abundances was bigger in Førde, but both areas had significantly lower numbers of small mammals in 2015. The variable year was significant in explaining variations in tick load of both tick species, but the effect of year was different. In Førde there was significantly lower tick load of *I. ricinus* larvae in 2015 compared to 2014, whereas in Son, tick load was highest in 2015 (Table 3 & 4). The overall prevalence of *I. ricinus* in Førde, was significantly lower in 2015 compared to 2014 (51.3 % vs. 33.0 %), while there was no annual difference in prevalence in Son being around 84 % in both years. The aggregation of *I. ricinus* larvae in Førde was much lower in 2015 (mean intensity = 3.1, range = 1-14) compared to 2014 (mean intensity = 6.0, range = 1-104). *S. araneus* and *S. isodon* had the smallest annual variations both in intensities and tick prevalence in Førde. Though these two shrew species had lower tick prevalence than the rodent species, the mean intensities and ranges was smaller in 2015 compared to 2014 even for shrews. This could indicate that shrews can sustain the tick larvae when densities of rodents are lower, due to their high numbers and smaller magnitudes in their population fluctuations. At least that was observed over the two years with contrasting small mammal abundances in this study. Studies have also shown that shrew populations have different degrees of cyclicity (Zakharov et al., 1991). The importance of shrews as hosts for ticks and reservoirs for pathogens is not as well-known as the role of rodents (Brisson et al., 2008).

In Son, there was no annual difference in the overall prevalence of *I. ricinus* larvae, and the overall prevalence was high. However, the mean intensity and ranges of ticks on the different hosts varied from 2014 to 2015 (Table 4). *M. glareolus* had lower mean intensity and range in 2014 compared to 2015. On the other hand, *A. sylvaticus* and *S. araneus* had higher mean intensities and ranges in 2015 than in 2014 (Table 4). This again highlights the

30

main difference between Førde and Son, with Førde showing large annual variation in tick abundance and Son not.

4.2 Infection prevalence of *B. burgdorferi* s.l. and *A. phagocytophilum*

All the small mammals (n=992) were analyzed for infection of the pathogens *B. burgdorferi* s.l. and *A. phagocytophilum*. The overall prevalence of *B. burgdorferi* s.l. differed between Førde and Son. In Førde the overall prevalence of *B. burgdorferi* s.l. for 2014 and 2015 were 4.5 % and 8.5 %, respectively, which is significantly lower than for Son, where the prevalence was 18.6 % and 32.0 %, respectively. For *A. phagocytophilum* the prevalence was relatively low in both areas, with minor variation between years, and consequently higher prevalence in Førde (14.0 % and 13.0 %, respectively, for 2014 and 2015) than in Son (7.9 % and 9.8 %, respectively). A problem for more detailed analysis of variation in pathogen prevalence was the lack of data due to relatively few positives, especially for *A. phagocytophilum*.

4.2.1 *B. burgdorferi* s.l.

The average overall prevalence for the two years was almost twice as high in Son (25.3 %) vs. Førde (13.0 %). Infection prevalence of *B. burgdorferi* s.l. was significantly different between years and positively correlated with body weight of host animals in both Førde and Son. In Son, host species were also significant for explaining variation in infection prevalence of *B. burgdorferi* s.l., though Førde also had interspecific differences in infection prevalence. Among the different species in Førde, *M. agrestis* had with 27.0 % the overall highest prevalence of *B. burgdorferi* s.l. in both years. The second highest overall prevalence of *B. burgdorferi* s.l. was found in *M. glareolus* with an average of 12.7 %. In Son, *M. glareolus* had the highest overall prevalence with 27.0 %. This indicates that voles like *M. glareolus* and *M. agrestis* are the most important small mammal reservoirs for *B. burgdorferi* s.l. in both Førde and Son. All the small mammals included in this study have earlier been established as competent reservoirs for *B. burgdorferi* s.l. (Gern et al., 1998). It is clear, that the effects of the annual variation in host populations on infection prevalence of *B. burgdorferi* s.l. are different between Førde and Son.

As discussed earlier, the host communities are different between the areas. Førde has a richer small mammal community, and with immature *I. ricinus* also possibly feeding on other hosts, that are not competent reservoirs, such as red deer. Such a system could lead to a dilution effect resulting in the low infection prevalence of *B. burgdorferi* s.l. seen in Førde (Mannelli et al., 2012). Son also has populations of larger mammals such as moose and roe deer, and larval *I. ricinus* are not found on roe deer in Son (Hügli & Mysterud, unpublished data). Reservoir competence for *B. burgdorferi* s.l. is also known to vary between small mammal species, and the presence of lesser competent hosts can result in dilution of the pathogens, since they are not able to infect new ticks (Barbour et al., 2015; LoGiudice et al., 2003). However, I was not able to find any significant differences between the different host species in Førde, due to the limited data, but this effect cannot be excluded. Very few nymphs were collected in this study, and they are the main vector in small mammal populations. They are also responsible for reinfection of the reservoir population in spring, since the pathogen survives in the tick during winter (Talleklint & Jaenson, 1995).

4.2.2 *A. phagocytophilum*

For *A. phagocytophilum*, the overall prevalence in hosts was relatively low in both areas with an average of 13.5 % in Førde, and 8.9 % in Son. None of the tested variables from the Son data had any significant effect. However, I found that host species, elevation and body weight were positively correlated with infection prevalence of *A. phagocytophilum* in Førde. When looking at the different host species, there are some interesting differences. In, Førde the highest prevalence of *A. phagocytophilum* was found in *S. isodon* (51.0 % and 34.6 %). With more than half of the individuals infected in 2014, this suggests that this species is a highly competent reservoir for *A. phagocytophilum*. Though these observations were not statistically significant with the current sample size, they may be biologically relevant, since *S. isodon* differs so much from the other hosts in terms of infection prevalence of *A. phagocytophilum*. In contrast, the closely related *S. araneus* and *S. minutus* both had low prevalence of *A. phagocytophilum*. There is little published research on the role of shrews and other insectivores in the life-cycle of *A. phagocytophilum* (Stuen et al., 2013). In Europe, one study found prevalence in *S. araneus* being up to 18.7 % (Bown et al., 2011), but the data from the present study points to *S. isodon* being more important with the prevalence being over 50 %. The other common group of small mammals, rodents, is known as competent reservoirs for *A. phagocytophilum* in Europe, with studies having found prevalence up to 19.2 %, with voles

on average having higher prevalence (Stuen et al., 2013). This agrees with the present study, with voles having higher prevalence than the mice species. As for *B. burgdorferi* s.l., there was no correlation between tick load of nymphs and infection of *A. phagocytophilum*. However, this may be due to the low sample sizes.

4.2.3 Variation in disease hazard

In a public health perspective, ticks are not particularly interesting by themselves. The interesting aspect is how they are associated with the disease hazard of the tick-borne pathogens. Disease hazard is defined as the number of infected nymphs (Ostfeld et al., 2006). In this case, it is mainly nymphal *I. ricinus* ticks that are relevant, since they are the main vector of human disease in Europe. Nymphal numbers are again determined by larval tick abundance and infection prevalence in the previous year. The number of tick larvae that contract pathogens during the summer, determine the number of infected nymphs the following year. This is because the surviving infected larvae will eventually moult into nymphs and then infect the reservoirs in spring and early summer the next year (Milne, 1947), or they may bite humans or livestock and cause disease if they are infected. Hence, some simple calculations of next year disease hazard (disregarding the contributions from birds) can be made with just quantifying the number of larvae that fed on an infected host the year before, assuming equal survivability. This means that I could calculate the disease hazard in Førde and Son of *B. burgdorferi* s.l. and *A. phagocytophilum* contributed by the small mammal populations in 2014 and 2015. The number of traps in Førde is 200 compared to 100 in Son. To have comparable estimates of disease hazard between the two areas, the number of larvae that fed on an infected host (estimated disease hazard) must be scaled. One way of doing this, is to divide the number of infected larvae from one by the number of traps in the same area.

In Førde, the estimated disease hazard for *B. burgdorferi* s.l. (number of infected nymphs per trap) was 1.10 and 0.11 in 2015 and 2016 respectively. It is worth pointing out, that 104 of the 217 ticks that fed on an infected host in 2014 were on one single individual host. This is a key characteristic of ectoparasites often being aggregated on few individuals (D. J. Shaw & Dobson, 1995). In Son, disease hazard for *B. burgdorferi* s.l. was 6.65 and 5.56 in 2014 and 2015 respectively. As seen from this, disease hazard was very different between the two areas. Disease hazard was much higher in Son in general, with Førde having large annual variation and Son not. There were annual variations in the small mammal populations

in both areas. One interesting difference is that this only seemed to affect abundance of tick larvae and disease hazard in Førde, but not in Son. On the other hand, Son had significantly higher densities of small mammals in both years, when adjusting for the difference in trap numbers. This is what seems to be the main reason for the differences in tick abundance and disease hazard of *B. burgdorferi* s.l. between the two areas.

For *A. phagocytophilum* the estimated disease hazard in Førde was 1.08 and 0.04 for 2015 and 2016 respectively. In Son, disease hazard was 1.07 and 2.84 for 2015 and 2016 respectively. Though the differences between areas were much smaller and not subject to formal statistical testing, Son had higher disease hazard in general, though having lower prevalence of *A. phagocytophilum.* This is mostly due to a higher number of *I. ricinus* larvae. This indicates that the contribution of small mammals to the disease hazard of *A. phagocytophilum* is relatively small. Many of the species have few positives and small sample sizes and may be vulnerable to random sampling error. Another potential source of error is the possibility that pathogen detection using tissue samples from the animal is not 100 % accurate. This is relevant for both pathogens where the number of positives is low. When the sample size and number of positives are as low as they were for several of the species, only a few errors will have large impact on the infection prevalence.

5   Conclusion

This study sheds light on the relationship between small mammal populations and tick abundance and their implications for disease hazard of tick-borne pathogens. This is the first study from northern areas that stems from more than one year and comparing two contrasting localities. From such a comparison, we can see considerable variation between areas and years in the production of infected larvae, which is linked to next year's disease hazard. Understanding the dynamics of the small mammal fluctuations, as well as the tick load and infection prevalence, will hence increase our understanding of how disease hazard and in turn disease incidence varies in space and time.

Given the clear predictions of a warming globe, ticks will likely continue their expansion in the northern hemisphere in the future. With them, tick-borne pathogens will become an increasing public health issue (Jaenson et al., 2009; Jore et al., 2014). In addition to the public health aspect, the veterinary aspect is also significant with ticks and *A. phagocytophilum* already being a major concern in the sheep industry (Stuen et al., 2006). Climate will also affect the host populations (Cornulier et al., 2013), which over time will have implications for tick abundance and prevalence of tick-borne pathogens. Further studies should be aimed at investigating annual variations in small mammal hosts, tick abundance and infection prevalence over the longer term. This is required to investigate how climate and other long-term processes contribute to disease hazard. This will give insight into the long-term effect of small mammal fluctuations for disease hazard.

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