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Nicolas De Pelsmaeker

# Altitudinal distribution and host-parasite relations of ticks in Norway

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**Ecology**

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Studying ecology is having the profound realization that all forms of life are connected and exercise an influence on one another, and on the world around them.

“We are all connected. To each other, biologically. To the earth, chemically. To the rest of the universe, atomically.”

– Neil deGrasse Tyson. Astrophysicist

# Preface

Ticks are paradoxical organisms. For most plants or animals, the more optimal their living conditions (i.e. abundance of food, niche parameters etc.), the longer their life span as they have the best conditions to thrive, and fewer threats that compromise their survival. The harsher the conditions, the more living conditions will chip away at an organism's fitness, and risk shortening or ending its life before reaching its maximum potential length.

With ticks, the opposite is true. The more optimal the conditions are (high humidity, suitable temperature and an abundance of available hosts) the quicker they will cycle through their life stages, mate, reproduce and die. The more suboptimal the conditions are (within a certain tolerance range), the longer their lifespan. Ticks are capable of many things, but there is one thing they truly excel at, and that is their capacity to wait. If conditions are not met (e.g. no host is available, temperature is too cold, or the air too dry), they will simply wait it out. For a very long time if need be. The sheep tick *Ixodes ricinus* can live up to seven years, and certain argasids (soft ticks) have been reported to live up to 20 years (Sonenshine and Roe 2013).

Ticks are also incredibly tenacious, and able to survive extreme environments for prolonged periods of time. Experiments on tick survival have shown that when fully submerged under water, *I. ricinus* females can live without gaseous oxygen for up to 13 days without showing any sign of weakness. Despite being considered air "breathing" terrestrial organisms, submerged eggs will gestate under water, and newly hatched larvae have been found to survive for 1.5 months, or even up to 80 days when submerged after hatching (MacLeod 1935, and others cited herein).

People sometimes ask me: "what is the use of ticks?" as in: "what is their role?" As an ecologist, I am always a bit bemused by such an anthropomorphized question. An organism does not need to have a "role" to exist, if it can occupy a certain niche in the world, it will do so simply because it can. From the ticks' point of view, its role is to feed on hosts, mate, reproduce and disperse. From a pathogens perspective, the ticks' role is to enable its epidemiology among reservoir hosts, and facilitate the spread to new geographical areas. To humans, its role is to be repulsive parasites that will sneakily crawl into areas of our body where we'd rather not have them, stealthily cement themselves into our skin and suck our blood unnoticed until repletion, providing pathogens the opportunity to hitch-hike from a previous host.

However, perhaps the anthropocentrism of that question is not so futile. Since the dawn of man, humans have been deriving benefits from nature to improve our lives. For example, the burr seeds from burdock plants (*Arctium spp.*) were the inspiration for the invention of Velcro. The kingfisher's bill (family: *Alcedinidae*) inspired the aerodynamic design of the Japanese bullet train. Understanding the flight of a bird, eventually gave rise to the airplane, and building upon that, the observation of how birds of prey curl up the tips of the flight feathers while soaring, was the basis for the fairly recent installation of now ubiquitous winglets on airplanes. Mother Nature is an endless classroom for those who are curious to observe and discover.

Recent discoveries have shown that tick saliva is a vast mixture of complex organic molecules (Hovius et al. 2008, Nuttall 2019), some of which prevent the blood of the host from coagulating (Maritz-Olivier et al. 2007). Certain compounds allow ticks to cement themselves onto the host, and others serve to dissolve the cement in order to detach when replete (Ramamoorthi et al. 2005). As ticks feed for several days, they must remain unnoticed by the host during that period, and specific immunosuppressive molecules in tick saliva prevent the host from developing an irritation where the tick is feeding, and subsequently noticing the infestation (Valenzuela 2004). There are some promising developments for these compounds to be adapted in the treatment and prevention of myocarditis, a condition mostly affecting young adults under the age of 30, which can cause sudden heart failure (Singh et al. 2017). The molecules that make up the cement of tick saliva are also being investigated for their potential to act as a "bioadhesive", which could perhaps one day reduce or even replace stitching, and be applied quickly during emergencies for severe lacerations to prevent critical blood loss

(Suppan et al. 2018). Certain types of cancer could be reduced or even cured by molecules derived from tick saliva (Sousa et al. 2015).

There are many reasons to study ticks in regard to their biology, physiology and ecology. The answer to the question of their role is definitely not singular, and the more we learn about them, the more we can learn from them. Once more illustrating their paradox, instead of merely being disease-transmitting parasites, ticks may actually come to improve our lives.

# Acknowledgements

A view from the top of the Erdal valley (1000 m.a.s.l.), looking down on the altitudinal transect of Lærdal. When first gazing down this valley, I remember thinking: "There are worse places in the world to do fieldwork."

First of all I would like to express my gratitude to my two PhD supervisors: Dr. Øyvind Steifetten (main supervisor) and Dr. Lars Korslund (co-supervisor). Thank you both for your support in regards to writing, analysis, and your patience during this profound learning experience (of which the curve was nearly vertical at times). Migrating back from a totally unrelated field in the private sector, you have placed your trust in me, even in times when I did not. Secondly, my deepest gratitude goes out to the PhD committee at USN: Prof. Dr. Andreas Zedrosser with the patience of a sage, and Dr. Veronica Tinnesand with the valiance of a champion, tirelessly fighting for the rights of us PhD students, both made an invaluable contribution in my experience as a PhD candidate. I would like to thank Prof. Dr. Andrew Jenkins, for being a confidant with whom I could share my concerns without fear of judgement, and for being the only person with whom I could have casual yet intellectually engaging conversations about ticks.

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# Abstract

With a warming climate and changes in land use during the last decades, ticks have been observed to occur at increasingly higher latitudes and altitudes in the northern hemisphere. With the range expansion of ticks comes the potential for new diseases to emerge in previously uninfected areas, as well the number of cases of existing ones to increase. The effects of climate change are expected to be disproportionally pronounced a high latitudes, and are expected to continue for the foreseeable future. However, the dynamics of tick range expansion and their altitudinal occurrence near their northern distribution limit remain poorly understood. This thesis examines the altitudinal occurrence and host-parasite relations of two tick species, the exophilic generalist *Ixodes ricinus* and the endophilic specialist *I. trianguliceps*, by investigating the burdens found on small mammals along two altitudinal gradients in two locations in southern Norway. Based on previous studies in Norway, the highest altitude at which *I. ricinus* was found was 583 m.a.s.l., but in this thesis we found that *I. ricinus* occur considerably higher than previously thought, up to an altitude of at least 1000 m.a.s.l. The effects of altitude were less pronounced on the occurrence of *I. trianguliceps*, indicating that the endophilic ecology of this species may enable it to survive at higher altitudes compared to the exophilic *I. ricinus*. A follow-up study expanding the range of the altitudinal gradient may reveal the actual distribution limit of ticks in these areas.

Furthermore, this study also investigated how ticks may utilize other hosts in areas characterized by multi-annual, high-amplitude rodent cycles, and how such cycles may inhibit the further progression of ticks. We found that non-cyclical shrew populations may have the potential to maintain tick populations in periods of low rodent availability, therefore enabling ticks to sustain a further upward progression, despite the periodic unavailability of some host species. A study encompassing one or more complete rodent cycles may shed more light on the roles of small mammals other than rodents in their capacity to act as a stable reserve of tick hosts, as well as on the specifics of the rodent cycles in these areas, and the influence of warming temperatures.

Lastly, we tested whether the choice of capture method (live or lethal trapping) may result in different perceived tick burdens, in order to determine whether the use of live trapping was unavoidable to accurately assess tick burdens. We found no significant differences in larval *I. ricinus* burdens on hosts captured between the two trap types, and we therefore propose that in light of animal welfare, lethal trapping of small mammals in studies assessing tick burdens is favored, as animals are not subjected to capture stress, while accuracy is maintained. The combined results in this thesis may serve as a starting point for further studies investigating the range expansion of ticks and tick-borne diseases in northern regions.

**Keywords:** Climate, range shift, expansion, ticks, hosts, *Ixodes ricinus*, *Ixodes trianguliceps*, rodents, shrews, altitude.

# List of papers

## Paper I

De Pelsmaeker, N, Korslund, L, Steifetten, Ø. Do bank voles (*Myodes glareolus*) trapped in live and lethal traps show differences in tick burden? *PLoS ONE 15(9):* e0239029.
doi: https://doi.org/10.1371/journal.pone.0239029

## Paper II

De Pelsmaeker, N, Korslund, L, Steifetten, Ø. High-elevational occurrence of two tick species, *Ixodes ricinus* and *I. trianguliceps,* at their northern distribution range., in review for *Parasites & Vectors*.

## Paper III

De Pelsmaeker, N, Korslund, L, Steifetten, Ø. Host in reserve: the role of common shrews (*Sorex araneus*) as a stable supplementary source of hosts in small mammal communities influenced by rodent population cycles. Manuscript.

# Abbreviations

| | |
|---|---|
| m.a.s.l. | meters above sea level |
| glm | Generalized Linear Model |

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# 1 Introduction

Parasites and vector-borne diseases have been a well-known cause of morbidity and mortality in both humans and animals throughout recorded history. For example, the bubonic plague epidemics were caused by the bacterium *Yersina pestis* which was transmitted by fleas (*Siphonaptera*), and caused millions of human deaths (McEvedy 1988). In recent decades, the importance of the role of ticks in the transmission of existing and emerging diseases to humans and both wild and domestic animals has become increasingly recognized (Heyman et al. 2010), and to date more than 16 tick-borne (or tick-induced) diseases in humans and 19 tick-borne diseases of livestock have been identified (Nicholson et al. 2019). In fact, ticks are believed to have the widest range of diseases of all vector parasites (Sonenshine and Roe 2013), and several European health ministries have declared tick-borne diseases as the biggest threat caused by environmental change (Randolph 2009).

Ticks are arthropod ectoparasites, consisting of approximately 896 currently recognized species worldwide, divided three families: the Ixodidae (702 species), the Argasidae (193 species) and the monotypic Nuttalliellidae (Guglielmone et al. 2010). They are obligate hematophagous parasites (Hovius et al. 2008), feeding solely on the blood of live vertebrate hosts such as mammals, birds and reptiles. During their life cycle ixodid ticks undergo several life stages, hatching from an egg as a larva, molting to a nymph, and finally to an adult. During every active life stage, ticks acquire a blood meal from a host (Sonenshine and Roe 2013). Ticks employ different ecological strategies to find a host on which to feed. Most species are exophilic, ambushing hosts by climbing up vegetation and latching onto hosts as they pass by and can be host specific or generalists. Other ixodid ticks are endophilic, spending the off-host part of their life cycle within the burrows or harborages of animals. For highly host-specific specialists, this strategy enables them to spend their time off-host within a sheltered environment, and to remain in close proximity of their host when ready to feed (Sonenshine and Roe 2013).

A warming climate has consequences on the distribution of species across several taxa (Walther et al. 2002, Parmesan and Yohe 2003, Root et al. 2003, Lenoir and Svenning

2015, Platts et al. 2019, Garcia-Vozmediano et al. 2020), and depending on their capacity for adaptation to rising temperatures, the distribution of a species can be affected in different ways. For some, the areas with hospitable conditions may increase in size, and hence those species may expand their distributional range (Ogden et al. 2008, Titelboim et al. 2019, Tombre et al. 2019, Osland and Feher 2020). For species adapted to colder habitats, their range limits may contract as fewer areas are suitable (Cole et al. 2011, Rubidge et al. 2012, Smale and Wernberg 2013). Lastly, a range shift may occur, with species moving away from inhospitable areas and towards more suitable areas (Parmesan and Yohe 2003, Berriozabal-Islas et al. 2018, Magalhaes et al. 2019).

The majority of species that have shifted their distribution range in the northern hemisphere have been observed to have done so northwards (Clements and DiTommaso 2012, Thompson and Finck 2013, Dawe and Boutin 2016, Pakanen et al. 2018, Virkkala et al. 2018). In most of Europe, ticks have had a historical presence, and people have recognized the dangers of tick-borne diseases for a long time (Parola and Raoult 2001a, Parola and Raoult 2001b). However, in recent decades a warming climate in combination with land-use changes, has caused ticks already present in those areas to increase in density, and coupled with that, an increase in cases of tick-borne infections (Kurtenbach et al. 2006). Aside from a merely numerical increase in tick abundance and disease incidence, the relative prevalence of several pathogens within ticks has also been observed to increase (Lindgren and Gustafson 2001). Although ticks have had a known presence in northern Europe for several decades (Arthur 1955, Nuorteva and Hoogstraal 1963, Oker-Blom et al. 1964, Brinck et al. 1967, Mehl 1983), they are increasingly being found in areas in which they were not present before (Jore et al. 2011, De Pelsmaeker et al. *in review*). Ticks have always been brought into areas outside their suitable niche by migrating hosts (Nuorteva and Hoogstraal 1963, Saikku et al. 1971, Hasle et al. 2009), but these ticks have not been able to survive in these new areas and establish permanent populations (Daniel 1993, Materna et al. 2008). One explanation is that the majority of a tick's life is spent detached from a host. Aside from temperature alone, ticks are also limited in their survival and distribution by the relative air humidity of the area. When ticks are not actively feeding, they must maintain their water balance against the drying power of the atmosphere (Knülle and Rudolph 1982). As both temperature and humidity in northern Europe are expected to increase in the future (Bindi and Olesen 2011, Barreca 2012), the most important climatic factors determining the occurrence of ticks are improving. Areas previously unsuitable for tick survival may become more favorable, and ticks could be able to establish new populations in new areas, with a potentially increased risk of tick-borne infections as a result.

In northern Europe, ticks have been observed to occur at increasingly higher latitudes since the 1990's as a result of climate change, and tick-borne diseases have been following the same pattern (Lindgren et al. 2000, Mejlon 2000). Moving along a latitudinal gradient northwards, the same environmental changes can be observed as along an altitudinal gradient, albeit on a larger scale (Jump et al. 2009). In central Europe, ticks have also been observed to move upwards in several countries (Daniel 1993, Danielova et al. 2002, Daniel et al. 2003, Daniel et al. 2009, Jouda et al. 2004, Daniel et al. 2005, Danielova et al. 2006, Danielová et al. 2008, Materna et al. 2008, Hornok and Farkas 2009). In these regions, tick-borne diseases have spread to higher altitudes as well, also believed to be a result of a changing climate (Daniel et al. 2004, Danielová et al. 2008). However, most of the countries where this altitudinal range shift has been studied are located at the geographical center of their distribution range. In these areas, the main impact of climate change may be on the seasonal dynamics and phenology of the ticks already present (Randolph 2001, Randolph et al. 2002). The conditions in regards to temperature and humidity are different at higher altitudes (Duane et al. 2008), hence the additive effect of latitude on climatic conditions may affect the occurrence of ticks along altitudinal gradients in northern regions. Given that the effects of climate change are expected to be stronger in northern Europe (Houghton 1996), warming temperatures, coupled with higher humidity and an increase in precipitation, may exacerbate the progression of ticks at higher altitudes, compared to lower latitude regions. To this day, little is known about the altitudinal distribution of ticks at their northern distributional range.

The potential distribution ranges of ticks are typically estimated by examining abiotic factors of a geographical region (i.e. climatic variables), or in some occasions topological characteristics or indices of landscape connectivity or fragmentation (Estrada-Peña and de la Fuente 2017). However, the availability (in density and abundance) of suitable hosts plays an important role in the persistence of tick populations and infection cycles. Yet, such biotic factors are often overlooked when evaluating habitat suitability of ticks (Estrada-Peña and de la Fuente 2017). Not all hosts are equally suitable for ticks, and different species have different carrying capacities. Studies on the deer tick *I. scapularis* in the USA showed that some host species can feed large amounts of ticks, while others only allow a small numbers of ticks to parasitize them (LoGiudice et al. 2003). In Europe, small mammals have long been recognized as important hosts for the immature stages of the common and well-studied sheep tick *I. ricinus* (Hillyard 1996), with rodents playing an important role in maintaining tick populations (Estrada-Pena et al. 2005, Bown et al. 2006, Boyard et al. 2008, Brunner and Ostfeld 2008, Ambrasiene et al. 2009). Rodents have also been shown to act as competent reservoirs for several tick-borne pathogens, and contribute to maintaining the infection cycles between host reservoirs and vector ticks (Bown et al. 2003, Bown et al. 2006, Bown et al. 2008, Bown et al. 2009, Vayssier-Taussat et al. 2012, Andersson et al. 2014). Rodents have therefore received ample attention in the study of tick ecology and tick-borne diseases (Brisson et al. 2008, Bown et al. 2011). However, the availability of rodent hosts in areas at the northern and altitudinal limit of the tick’s distribution range is not a temporal constant. At higher latitudes, rodent populations undergo multi-annual cycles with peaks occurring three to four years apart (Kaikusalo 1972, Hörnfeldt 1978), and the amplitude of these cycles increases with altitude (Taylor et al. 2013, Andreassen et al. 2020). For some rodent species, the population size during low points can reach near-zero (Boonstra et al. 1998, Kausrud et al. 2008). Periodically unavailable hosts may constitute a limiting factor for the further dispersal of ticks, despite climatic conditions being favorable. However, most tick species are not solely dependent on small rodents as hosts, and the presence of such other hosts could potentially offset any negative effects of low rodent numbers. Another common tick host for the immature stages of *I. ricinus* are shrews (*Soricidae*).

Shrew populations undergo intra-annual and inter-annual fluctuations, but these are typically non-cyclical and erratic (Buckner 1969, Henttonen et al. 1989, Churchfield et al. 1995). These insectivorous small mammals often occur in the same habitats as rodents (Nosek et al. 1972, Bown et al. 2011, Mysterud et al. 2015) and have been found to act as tick hosts as well (Gregson 1942, Telford III et al. 1990, Bakhvalova et al. 2001, Paramasvaran et al. 2009), indicating that shrews could serve as replacement hosts for ticks in times where rodent populations are at a low point. Furthermore, evidence demonstrates that shrews possess similar reservoir potential for tick-borne pathogens (Kozuch et al. 1967, Jaenson et al. 1994, Gern et al. 1998, Liz 2002, Bakhvalova et al. 2006, Bown et al. 2011), capable of maintaining the infection cycle of pathogens.

The abovementioned climatic and host related factors demonstrate that intricate biotic and abiotic relationships exist between ticks and their environment. Therefore, in order to gain a more complete understanding of the upward progression of ticks in northern Europe, both biotic and abiotic factors should be considered. In this thesis, I investigate the occurrence of a generalist and a specialist tick species along two altitudinal gradients in southern Norway. I tested the effects of altitude on the infestation rates of small mammals in order to determine whether the altitudinal limit of ticks could be found in these areas, and how altitude and other factors affected their occurrence. As rodents are important tick hosts, I aimed to elucidate whether shrews had the potential to serve as replacement hosts for ticks in times of low rodent availability, and if future range expansion of ticks could be limited by rodent population cycles. In order to answer these questions, my objective was to determine whether ticks are opportunistic in host selection, or whether a certain propensity exists towards a certain host type, and if this would differ between tick species or life stage. I also assessed the difference in tick burdens on a rodent host captured by two different trap types, to determine whether animal suffering could be mitigated in the study of tick densities on hosts.

# 2 Objectives

A large amount of research has been performed on ticks in Norway, but most often it is with a focus on the occurrence of tick-borne diseases. While pathogens are certainly worthy of study, the ecological aspects of tick dispersal as well as host-parasite relations and how these can affect range expansion remain somewhat neglected. The overall aim of this thesis was to gain a deeper ecological understanding of the altitudinal distribution of ticks, and of the host-parasite relations in a northern ecosystem as can be found in Norway.

## Capture methods for tick burden assessment (paper I)

I investigated the difference in tick burdens between two capture methods of hosts commonly used in tick research, to determine if there was a difference in observed burden size on bank voles captured alive and then euthanized, and lethally trapped bank voles. The goal was to assess whether the time between capture and collection influenced observed tick burdens, due to tick drop-off from a dead host. I aimed to test whether subjecting captive animals to capture stress and later euthanasia was indispensable in order to accurately determine burden size, or whether lethal trapping would be sufficient, therefore alleviating some ethical concerns in regards to animal suffering. Furthermore, I investigated whether host sex or body mass would influence trap selection, and if this could influence which capture method would be less biased.

## Altitudinal distribution of two tick species in Norway (paper II)

I used larval burden data of two common tick species (*I. ricinus* and *I. trianguliceps*) collected from bank voles trapped at ten different altitudes along two altitudinal gradients in Norway. The aim was to determine whether altitude had an effect on the occurrence of the two selected species, and if their altitudinal distribution limit could be found. I predicted that *I. ricinus,* an exophilic species, would be more strongly affected by the changes in environmental conditions related to altitude, and therefore would have a lower maximum altitude limit compared to *I. trianguliceps,* which is characterized as an endophilic species.

## Host type and tick burden (paper III)

I used data from two life stages (larvae and nymphs) of two common tick species (*I. ricinus* and *I.trianguliceps*) to investigate whether there would be a difference in tick burdens between sympatric rodents (bank voles) and shrews (common shrews) as the host population size and composition changed. I related this to geographical area, altitude and collection year, as well as to host type and air humidity. The aim was to investigate whether shrews have the same potential to act as hosts for ticks as voles, and if shrews could therefore be sufficient small mammal hosts in times of low rodent population density to maintain tick populations.

# 3 Materials and methods

## 3.1 Study area

The data for all three papers was collected from two study areas located along two mountain slopes in the east and in the west of southern Norway. The eastern study area was a southern facing mountain slope which is part of the Lifjell mountain massif, located north of Bø i Telemark (N59°26.495’ E9°0.603’) (Fig 1), approximately 66 km from the eastern coastline at the Skagerrak Sea. The area is situated within the boreonemoral to southern boreal zone, characterized by a continental climate. Below the tree line, which is situated between 800 and 900 m.a.s.l., the vegetation is a blend of deciduous and coniferous forests with birch (*Betula pubescens*) and spruce (*Picea abies*) as the dominant tree species, and blueberry (*Vaccinium myrtillus*) as the dominant species at ground layer. Above the tree line the vegetation is primarily dominated by common heather (*Calluna vulgaris*) and blueberry. Boulder fields occur frequently throughout the gradient, and the highest peak found on the plateau is 1288 m.a.s.l. Most of the data collection points were located on a topographically open hillside.

The western study area was a northern facing mountain slope in the Erdal valley near Lærdalsøyri (N61°05.817’ E7°24.688’) (Fig 1), hereafter referred to as Lærdal. It is located close to the innermost part of the Sognefjorden fjord, approximately 150 km from the western coastline. Due to its close proximity to the fjord, it has a more coastal climate, characterized by milder winters and cooler summers when compared to Lifjell. The tree line is here situated between 900 and 1000 m.a.s.l., and below the tree line the vegetation consist primarily of homogeneous deciduous forests with birch and alder (*Alnus glutinosa*) as the dominant tree species. At ground layer the vegetation is dominated by blueberry, different species of ferns (Polypodiales) and tall perennial herbs. Above the tree line common heather, dwarf birch (*Betula nana*), common juniper (*Juniperus communis*) and crowberry (*Empetrum nigrum*) are the dominant species. Surrounding the study area are several mountain peaks exceeding 1500 m.a.s.l.

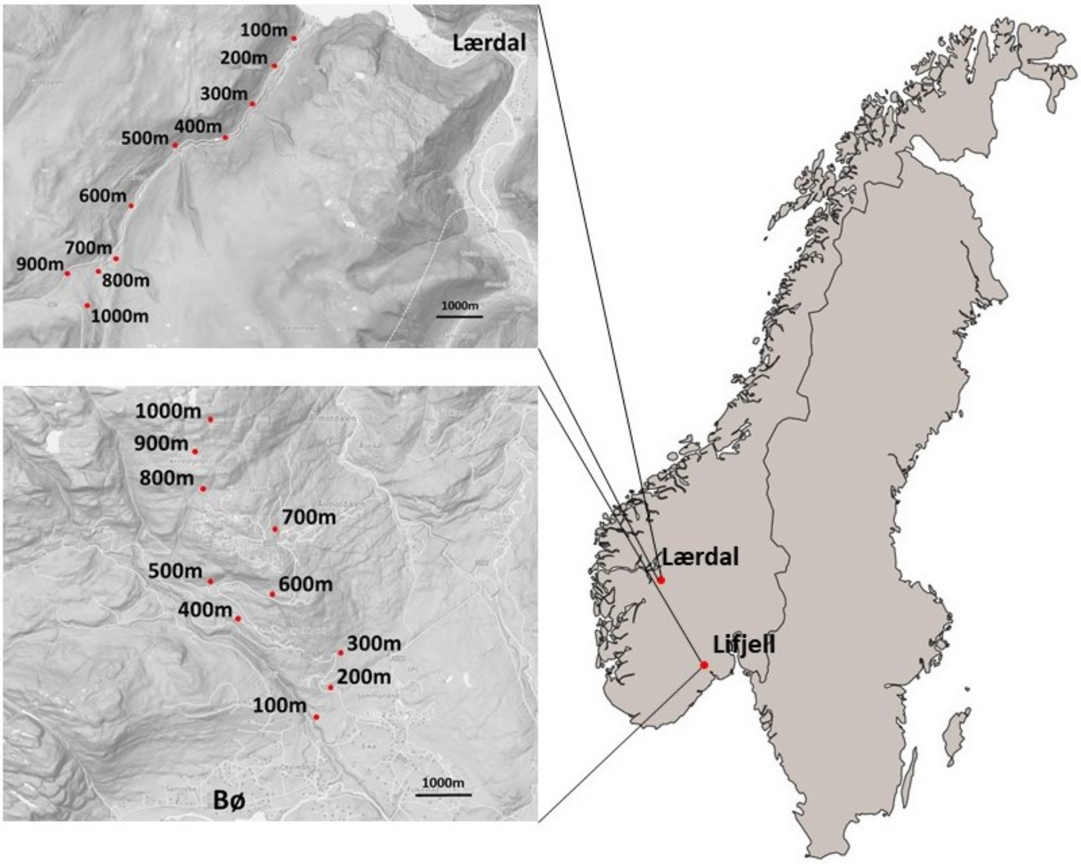

Fig 1. Map of Norway. Inset maps are the study areas indicating the location of every trapping station.

## 3.2 Study species

### 3.2.1 *Ixodes ricinus*

The sheep tick *I. ricinus* is the most widespread and common tick species in Europe (Estrada-Peña et al. 2018), as well as the most important vector of tick-borne diseases (Petney et al. 2012). Its distribution ranges latitudinally from North Africa to Scandinavia, and longitudinally from Ireland to Russia (Gem and Humair 2002, Randolph et al. 2002). *I. ricinus* is a generalist three-host tick, feeding on different hosts depending on its life stage (Nicholson et al. 2019). Larvae and nymphs will feed on a range of different hosts, ranging from small mammals and birds to large ungulates, whereas adults tend to be found more exclusively on larger host such as cattle; or cervids such as roe deer (*Capreolus capreolus*) (Talleklint and Jaenson 1997, Medlock et al. 2013). Considered an exophilic species, it spends the majority of its life in soil litter or vegetation. It ambushes its host by climbing up vegetation and spreading the front pair of legs (a behavior called “questing”), latching on to a host when it passes by (Medlock et al. 2013). While feeding, *I. ricinus* will maintain several consecutive days of attachment on the host (Anderson and Magnarelli 2008). It is during this parasitic phase that pathogens can be transmitted from a reservoir host into naïve ticks, and subsequently from infected ticks to other naïve competent hosts. Sensitive to desiccation, *I. ricinus* is limited by environmental factors such as temperature and humidity, which determines its climatic distribution (Bowman and Nuttall 2008). In central Europe, *I. ricinus* has been observed to have increased its altitudinal distribution limit to 1100 m.a.s.l. in the Czech Republic (Daniel et al. 2003), and up to 1650 m.a.s.l. in northern Italy (Martello et al. 2014). In Norway *I. ricinus* has so far been found up to altitudes of 583 m.a.s.l. (Jore et al. 2011).

### 3.2.2 *Ixodes trianguliceps*

The vole tick *I. trianguliceps* is a specialist tick, feeding on small mammals during all its life stages (Aeschlimann et al. 1970, Ulmanen 1972, O’Donnell 1973, Nilsson 1974, Randolph 1975, Kolonin 2007), and rarely parasitizes humans (Hillyard 1996). It does not quest for hosts in open vegetation, but rather drops off inside the host’s burrow when fully engorged (Bown et al. 2006). After molting to the next stage, it will search for a new host within the burrow. Due to this behavior, *I. trianguliceps* may be more sheltered from the external climatic factors in its surroundings that would otherwise limit its distribution. It ranges from Italy to well above the arctic circle (Korenberg and Lebedeva 1969, Balashov 1997, Estrada-Peña et al. 2018), and has been found in Europe up to altitudes of 1500 m.a.s.l. in Switzerland (Aeschlimann et al. 1970). *I. trianguliceps* is generally considered one of the most cold-resistant tick species of the genus *Ixodes*. The highest altitude at which *I. trianguliceps* was recorded was 2300 m.a.s.l. in the Caucasus mountains of Georgia (Korenberg and Lebedeva 1969).

### 3.2.3 Bank vole (*Myodes glareolus*)

Bank voles are commonly found rodents throughout the European continent (Stenseth 1985). It occupies a wide variety of habitats (Haapakoski and Ylönen 2010) and occurs in all types of forests (Hansson 1971, Banach 1988, Mazurkiewicz 1994). Of all forest-dwelling rodents, it is often the most numerous (Mazurkiewicz and Rajska-Jurgiel 1987), and has a wide altitudinal distribution, found from coastal forests and up to 1400 m.a.s.l. in Norway (Stenseth 1985). It is predominantly diurnal, with activity throughout the whole day, but with highest activity around dusk and dawn (Greenwood 1978). At high latitudes, bank vole populations undergo multi-annual cycles, peaking every three to four years (Kaikusalo 1972, Hörnfeldt 1978), and the amplitude of the population cycles increases with altitude and the length of the winter season (Andreassen et al. 2020). Bank voles are common hosts for the immature stages of *I. ricinus,* and an important host for all stages of *I. trianguliceps* (Hillyard 1996). It is a reservoir for several tick-borne pathogens such as *Borrelia burgdorferi* sensu lato (Jaenson 1991, Matuschka et al. 1992, Humair et al. 1999, Cayol et al. 2018), *Candidatus* Neoehrlichia mikurensis (Vayssier-Taussat et al. 2012, Andersson et al. 2014), tick-borne encephalitis virus (Tonteri et al. 2013) and *Anaplasma phagocytophylum* (Bown et al. 2003, Bown et al. 2008, Perez et al. 2020).

### 3.2.4 Common shrew (*Sorex araneus*)

Common shrews are small, mostly insectivorous mammals found throughout Eurasia, ranging longitudinally from England to Russia (Churchfield 1982a, Bakhvalova et al. 2001) and latitudinally from southern Europe to Fennoscandia (Canova 1992, Henttonen et al. 1989). They are often found in the same habitats as bank voles (Churchfield 1990), and are parasitized by similar tick fauna (Arthur 1963, Randolph 1975). Common shrews are active throughout a 24h day period, aside from some intermittent periods of reduced activity (Churchfield 1982b). Shrew populations do not display regular cycles, but rather intra-annual and inter-annual fluctuations that are typically erratic and non-cyclical (Henttonen et al. 1989, Churchfield et al. 1995). Like rodents, common shrews have been found to act as reservoirs for tick-borne pathogens such as tick-borne encephalitis virus (Kozuch et al. 1967, Bakhvalova et al. 2001), *Anaplasma phagocytophylum* (Bown et al. 2011), *B. burgdorferi* s.l. (Gern et al. 1998) and granulocytic *Ehrlichia* (Liz 2002).

## 3.3 Host capturing

We trapped small mammals during spring (May 20th-30th), summer (July 20th-30th) and autumn (September 20th-30th) of 2017 and 2018. During the spring season of 2017, trapping exceptionally occurred from June 1st until June 7th and only up to 700 m.a.s.l., as too much snow was present in both study areas to allow capturing earlier and above this altitude. In both study locations, ten trapping stations were deployed along a vertical gradient ranging from 100 to 1000 m.a.s.l. (Fig 1). At every altitude, two plots consisting of 20 traps each were deployed, arranged in a 4 by 5 grid, with 10 m spacing between each trap (Fig 2). Because assessing tick burdens on live animals under field conditions can yield inaccurate results (Lydecker et al. 2019), can be stressful and lead to injury or death of the examined animal (Lindsjö et al. 2016), we opted for a post-mortem full body examination, and live captured animals were sacrificed by means of cervical dislocation of the head. Full body examination of both live and lethal captures offered a higher degree of sensitivity (Mooring and McKenzie 1995). One plot was set with live traps (Ugglan Special Nr. 2, Grahnab AB, Sweden, www.grahnab.se), and the other with lethal traps (Rapp2 Mousetrap, www.rapfellene.no) (Fig 3). We chose the new Rapp2 mousetraps over classic snap traps as these reduce the risk of non-lethal capture (e.g. leg or tail), which can cause considerable suffering. Lethal traps killed animals instantly through cervical fracturing. Both plots were spaced a minimum of 100 m apart to avoid host home range overlap (Korn 1986, Bond and Wolff 1999), but in similar habitats. Live traps were baited with apple slices for hydration and whole oats for sustenance, and a bedding of sawdust was provided on the trap floor. Lethal traps were baited with peanut butter for practical reasons as it is easily applied to the inside of the trap body and remains in place. Traps were checked every 24h, starting at 8h30 at the top of the gradient (1000 m.a.s.l.). We employed a bag limit of 50 animals per altitude and season, to prevent unnecessary depletion of local host populations. This means that if during any capturing season a total of 50 animals was reached (live and lethal traps combined), the traps in the two plots at that altitude were deactivated and capturing ceased. All captured animals were sealed individually in coded plastic bags, and frozen at -20 °C at the end of every day of trapping. Activated traps were rebaited and reset.

Tick activity, phenological patterns, host-seeking behavior and survival are all influenced by environmental factors such as temperature and humidity (Perret et al. 2000). As a measure of environmental conditions, a temperature and humidity logger (TinyTag Plus 2 - TGP 4017) was housed in a DataMate instrument cover (ACS - 5050), and mounted on a pole 50 cm above ground level, in between live and lethal plots at every altitude station. These loggers recorded temperature and relative humidity (%) at a 1-hour interval for the entire duration of the study period (June 2017 - September 2018).

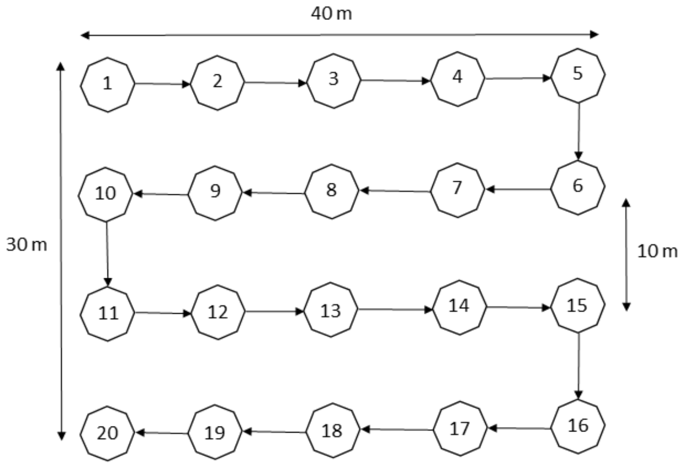

Fig 2. Schematic representation of a capture plot. The traps were checked from 1 - 20 in a zig-zag fashion for efficiency.

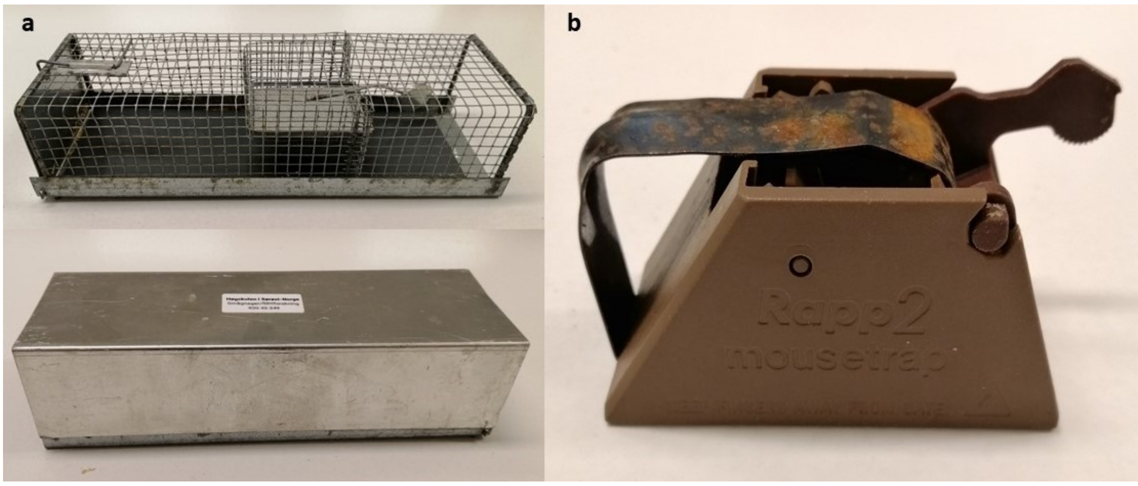

Fig 3. The two trap types used in the study: (a) Ugglan Special Nr 2 Live trap (top), covered with an Ugglan Special Long Roof metal cover (bottom); and (b) Rapp2 lethal mousetrap.

## 3.4 Laboratory processing

After each trapping season, all captured animals were examined for ticks in the laboratory. The day before the examination, the animals were removed from the freezer and thawed overnight in a cold room at 10 °C. The animals were taken out of the plastic bags and underwent an individual full body examination. The empty bags were checked for ticks that might have dropped off. It was our observation that a fraction of the tick burden dropped off the host when placed in the freezer, possibly in an attempt to escape the extreme temperatures. Animals that were wet after thawing were first dried using a hairdryer. The hosts were examined above a white plastic tray and checked for ticks starting with the ears, snout, neck and throat, followed by the back and abdomen, legs, feet and tail. Ticks attached or found present on the host were removed with tweezers and placed in a 1.5 mL Eppendorf tube containing a 70 % ethanol solution (1 vial per host). Other ectoparasites such as fleas, lice and mites found on the host were also collected. After the examination, a lice comb was brushed through the fur of the animal from tail to head (against the hair orientation), and the animal was shaken by the tail above the tray for 5 seconds, to collect any ticks that might have been missed during the examination. The hosts were then weighed to the nearest 10th of a gram, and determined to species based on morphological characteristics and dental features (Van

Der Kooij 1999). In the case of rodents, the sex was also determined. After the examination, each animal was resealed in newly coded plastic bags and refrozen for long term storage at -20 °C.

The ticks were individually determined for species and life stage under a Zeiss Discovery V20 stereomicroscope (Fig 4), using established publication keys as reference (Arthur 1963, Hillyard 1996). Ticks that were too mutilated for determination were discarded from the study.

Fig 4. Parasite burden of a bank vole examined under a Zeiss Discovery V.20 stereomicroscope. The burden consisted of several engorged adult *I. trianguliceps* females, as well as larvae of both *I. ricinus* and *I. trianguliceps* at different stages of engorgement. One flea was also collected.

## 3.5 Statistical analysis

All data analyses were performed using R version 3.5.3 (paper I and II) and version 4.0.2 (paper III) (R Development Core Team 2019). Visual representations of the results were either produced in base R, or using the *ggplot2* package (Wickham 2016). A p-value of <0.05 was considered significant in all model selection.

### 3.5.1 Paper I: Differences in tick burdens on bank voles captured in live lethal traps

For the assessment of the differences in tick burden size between trap types, only *I. ricinus* was used as this species represented the majority of the burden (88.1 %) on bank voles. Of the collected ticks, 94.0 % were larvae, and therefore only this life stage was retained for the study. Because bank voles constituted 86.2 % of all captured rodents, and because it is a common *I. ricinus* host in most of Europe (Humair et al. 1999, Ambrasiene et al. 2009), only this host species was used in the analysis.

In order to assess differences in tick burdens between the two capture methods, we used general linear regression models, using *I. ricinus* larval burden (i.e. the number of tick larvae on one individual host) as the response variable. Trap type (live or lethal) was used as a categorical predictor variable to compare burdens between trap types. Study area (Lifjell and Lærdal), sampling year (2017 and 2018), season (spring, summer and autumn), and daily average temperature (°C) were used as extrinsic covariates, and the host characteristics body mass (in grams) and vole sex (male or female) were used as intrinsic covariates. To account for the possible effect of temperature on tick drop-off from hosts, temperature was averaged for a 24-hour period spanning from 12:00 AM on the day the trap was set until 12:00 AM on the day of capture. Furthermore, two-way interactions between trap type and all other covariates were included. We started with a full model containing all predictor variables and interactions, and used a stepwise backward selection approach (type II ANOVA test) to progressively remove non-significant variables, until a nested model yielded only significant predictor variables. The response variable, larval tick burden, was skewed towards zero, hence a Poisson distribution was used to model the data in the glm-function. Because overdispersion was detected, we corrected standard errors accordingly using a quasi-glm approach, following Zuur et al. (2009). To investigate whether hosts selected a particular trap type, host trappability was regressed using trap type as a binomial response variable and the host-specific variables body mass and sex as predictors, adding an overdispersion parameter ϕ (Zuur et al. 2009). A Wilcoxon rank sum test was used to assess whether body mass of captured voles differed between the sexes.

### 3.5.2 Paper II: Infestation prevalence along altitude

As for paper I, we used bank voles as the only host species in the analysis of this paper. From the collected *I. ricinus* and *I. trianguliceps*, 94.4 % and 75.4 % were larvae, respectively, and thus only larvae were used in the study. The analysis of the larval burdens of each tick species was performed separately. Tick burdens are often aggregated and not evenly distributed among hosts (Harrison and Bennett 2012). This was the case for both tick species with 46.8 % and 18.1 % of the bank voles feeding all of the larvae of *I. ricinus* and *I. trianguliceps*, respectively. We therefore chose to apply generalized linear modelling with a binomial distribution (i.e. logistic regression), using larval presence/absence as the response variable. Here, the term “prevalence” is defined as the probability of encountering a larva on a host. As predictor variables we considered altitude (ranging from 100 to 1000 m.a.s.l.) as a continuous variable, study area (Lifjell and Lærdal), collection year (2017 and 2018), season (spring, summer and autumn) and relative humidity (%). Temperature was negatively correlated with altitude (Pearson correlation test, $t= -8.1578$, $df= 1323$, $p< 0.001$, $r= -0.219$). Hence, temperature was not included to avoid introducing collinearity into the model. We considered two-way interactions between altitude and season, study area and year, as well as between year and study area and season. Host body mass and sex were also included in the starting model. Using a backward stepwise model selection process, we progressively removed non-significant predictors from the model (by comparing the difference in the residual deviance of two competing models to a Chi-square distribution), until a final model, containing only significant variables, was reached.

### 3.5.3 Paper III: Propensity of parasitism with a changing host population size and composition

For this study, we used bank voles and common shrews as model species to investigate whether the burdens of the generalist *I. ricinus* and the specialist *I. trianguliceps* were influenced by rodent availability, and if shrews have the potential to act as a stable and adequate source of hosts for juvenile ticks in periods of low rodent abundance. We hypothesized that opportunistic ticks would display an equal likelihood of parasitizing a host, regardless of the host species, whereas a propensity towards either host type would result in different burden ratios. In order to assess this, we used the propensity of ticks to parasitize either host type as the response variable in a multiple linear regression model with a normal distribution. We defined propensity (*P*) as:

$$P = \log \frac{\left(\overline{burden_{shrew}} + 0.05\right)}{\left(\overline{burden_{vole}} + 0.05\right)}$$

where $\overline{burden_{shrew}}$ is the mean burden of ticks parasitizing common shrews, and $\overline{burden_{vole}}$ is the mean burden of ticks parasitizing bank voles. Propensity (*P*) would then be positive if the mean burdens of shrews were higher, negative if the mean burdens of voles were higher, and zero if both hosts have an equal mean burden. Thus, a propensity (*P*) close to zero indicates that the mean tick burdens of bank voles and common shrews are similar, and that ticks parasitize hosts opportunistically, whereas a slope deviating from zero indicates a disproportional level of parasitism towards a certain host type, and that mean burdens are higher on a particular host. Because the function is undefined if the mean burden of one or both hosts is zero, a small constant (0.05) was added to every estimated mean tick burden. The main independent variables of interest were the ratio of voles in the entire host population, and the total population size (the sum of all captured voles and shrews at that station per study area per year). We tested this for four separate datasets, one for each tick species and life stage (*I. ricinus* and *I. trianguliceps* larvae and nymphs). In order to maximize the number of observations, we pooled all captures form every station (live and lethal traps) in each study area (Lifjell and Lærdal), from all seasons in each year (2017 and 2018), treating every station as a separate population. This resulted in a maximum of 40 replicates per station (2 years, 2 sites, 10 stations), but no captures or no ticks at a certain station or year resulted in fewer replicates in the datasets (*I. ricinus* larvae: n= 35; *I. ricinus* nymphs: n= 23, *I. trianguliceps* larvae: n= 33, *I. trianguliceps* nymphs: n= 33). Additional covariates used in the starting model were *year* (2017 and 2018), *study area* (Lifjell and

Lærdal), *altitude* (ranging from 100 to 1000 m.a.s.l.), and *humidity* (%). Similar to paper II, temperature was not included in the starting model to avoid collinearity. Starting with a full model containing all predictor variables, we used a backward step selection approach, progressively removing non-significant covariates using a type II ANOVA test until an optimal model was retained, containing only significant predictors.

## 3.6 Ethics statement

This study was carried out in strict accordance with regulations issued by the Norwegian Environment Agency, and a permit was provided prior to the start of the sampling (Miljødirektoratet, reference number: 2017/4651) for the duration of the trapping period. The trapping protocol for animal capture was approved by the Animal Ethics Committee of the Department of Natural Sciences and Environmental Health (University of South-Eastern Norway). All efforts were made to minimize animal suffering. Lethal traps killed animal instantly through cervical fracturing. Cervical dislocation of the head of animals captured in live traps was also instantly fatal.

# 4 Results and discussion

During the collection years of 2017 and 2018, a total of 43920 trap nights were performed, collecting 3703 animals. The majority of the captures were bank voles (45.4 %) and common shrews (46.8 %). Other captured rodents were grey red-backed voles (*Myodes rufocanus*, n= 88 (2.4 %)) field voles (*Microtus agrestis*, n= 130 (3.5 %)), tundra voles (*M. oeconomus*, n= 26 (0.7 %)), wood mice (*Apodemus sylvaticus*, n= 23 (0.6 %)), yellow-necked mice (*A. flavicollis*, n= 1 (0.03 %)) and house mice (*Mus musculus*, n= 1 (0.03 %)). Other shrews captured were pigmy shrews (*S. minutus*, n= 16 (0.4 %)) and water shrews (*Neomys fodiens*, n= 2 (0.05 %)). About twice as many captures were made during 2017 compared to 2018 (2480 and 1223 captures, respectively), and most animals were captured at low to intermediate altitudes (up to 800 m.a.s.l.). The majority of captures were made in Lærdal, compared to Lifjell (59.3 % and 40.7 %, respectively).

From all the captured hosts, 15819 ticks were collected. From the bank voles and common shrews (n= 1328 and n= 1143, respectively), 13404 ticks were collected. *I. ricinus* accounted for 80.5 % (of which 94.4 % were larvae and 5.6 % were nymphs) and *I. trianguliceps* accounted for 19.5 % (of which 75.4 %, 21.1 % and 3.5 % were larvae, nymphs and adult females, respectively) of all the collected ticks. No *I. ricinus* adults were collected.

## 4.1 Paper I: An opportunity for reduced animal suffering while maintaining accuracy

A total of 1318 bank voles were used for this study. More bank voles were captured in both study areas in 2017 compared to 2018 (413 and 31 in Lifjell, 556 and 318 in Lærdal, respectively). A total of 4735 *I. ricinus* larvae were collected and retained for the statistical analysis. Of the captured voles, 47.0 % were infested with at least one tick larva, and voles captured in live and lethal traps had similar infestation rates (44.4 % and 49.3 % for euthanized and lethally trapped voles, respectively). *I. ricinus* burden sizes ranged from 1 - 100, and the average infestation was 3.6 larvae per bank vole.

The primary goal of this study was to determine whether capture method had an influence on larval burden size, in order to assess if trapping voles alive and euthanizing them was unavoidable to accurately determine tick burdens on voles, or whether the use of lethal trapping was sufficient. Lethally trapped voles had 5.7 % larger burdens compared to euthanized voles. However, the effect of trap type alone was not significant (p= 0.420), and trap type was retained in the model due to a significant interaction between trap type and season (F= 4.13, df= 2, p= 0.02). A post-hoc Wilcoxon rank sum test showed that only during autumn did lethal captures have higher burdens compared to euthanized captures (W= 26371, p< 0.001, n= 520) (Fig 5). Thus, our results indicate that both trapping methods had similar burdens, suggesting that tick drop-off rates were not significantly higher in lethal captures compared to euthanized captures.

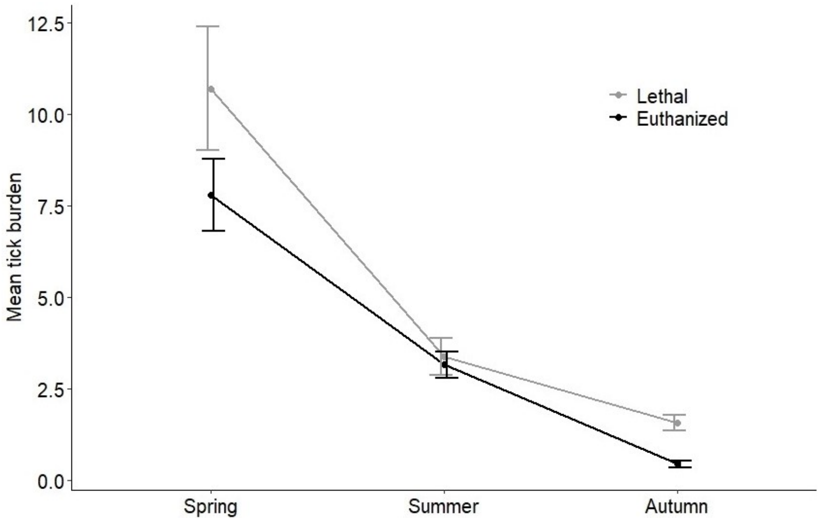

Fig 5. Mean tick burden of *I.ricinus* larvae on lethally captured and euthanized bank voles per season. Mean tick burdens are for both collection years, study areas and all altitudes combined. Error bars represent standard errors.

Wild animals that are captured alive an later euthanized may experience capture stress from the time spent in captivity (Letty et al. 2000). The stress responses of captive animals occur almost immediately after capture (Delehanty and Boonstra 2009) and increase with time (White et al. 1991, Schütz et al. 2006). Captivity can lead to the development of capture myopathy (Dickens et al. 2010, Blumstein et al. 2015, Breed et al. 2019), which may be fatal in itself (Herraez et al. 2007). When selecting which capture method to use for assessing tick burdens on free-ranging small mammals, the potential drop-off rate of ticks between the time of capture and the moment of collection can be a deciding factor. Although several studies have investigated detachment rates of fully engorged ticks from live animals (Piesman et al. 1987, Yeh et al. 1995, Meiners et al. 2006), research on the detachment process from a dead host is very limited. Nakao and Sato (1996) found that taiga ticks (*I. persulcatus*) started to abandon euthanized laboratory mice three hours after death, and Piesman (1991) found that deer ticks (*I. dammini*) detached from dead hamsters over the course of two days after host death. However, both these studies occurred under laboratory conditions, and we can reasonably assume that field conditions (e.g. diurnal and nocturnal temperature fluctuations, variations in humidity, differences in light regime, etc.) can affect drop-off rates of attached ticks and abandonment behavior. The rate at which a dead host cools down will depend on host morphology and environmental conditions, and ticks might continue to either feed for a while after death, or detach from the host, but remain in the fur before abandoning the host. The results from this study show that if any effect of trap type is present (e.g. in autumn), lethally trapped voles had higher burdens compared to euthanized voles (Fig 5). Possibly the time voles spent in captivity inside the live traps provided a window of opportunity for feeding ticks to reach full engorgement and drop off the host, as neither trap types would not have prevented replete ticks from leaving.

Although male voles were captured more frequently than females, the capture ratio between the sexes was similar in both trap types (Fig 6). Males have larger home ranges and larger mobility compared to females (Nilsson and Lundqvist 1978, Haapakoski and Ylönen 2010), which may explain their higher frequency of capture. Males also had larger larval burdens compared to females, but also here the differences in tick burden between the sexes was similar in both trap types, indicating that trap type does not influence either trappability between the sexes, nor burden size. Male hosts have been found to carry higher tick loads than females (Laurance and Coan 1987, Craine et al. 1995, Harrison et al. 2010, Kiffner et al. 2011, Dallas et al. 2012, Pollock et al. 2012), which may also be explained by their higher mobility. Ticks (particularly larvae) do not disperse very far from the place where they hatched from the egg batch or dropped off a host, and hence their distribution is highly aggregated (Harrison and Bennett 2012). Due to the higher levels of movement of male voles, the probability of encountering and acquiring *I. ricinus* larvae is greater than for females.

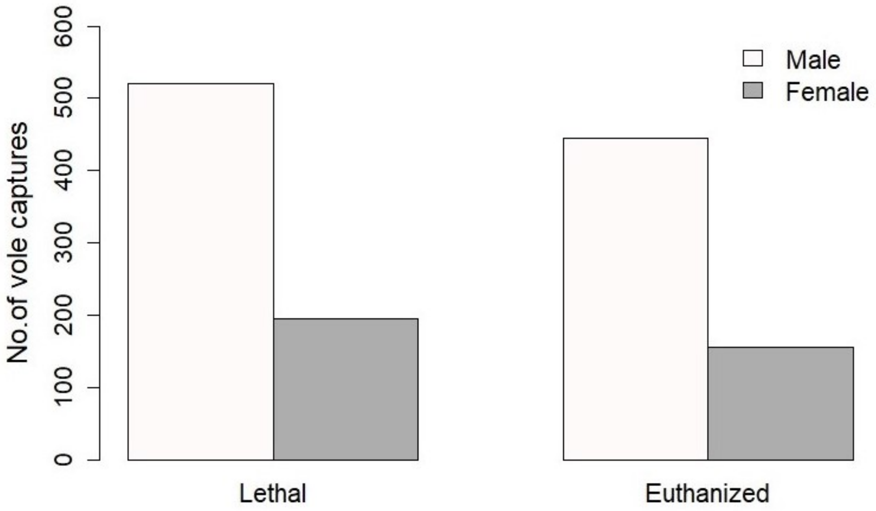

Fig 6. Total number of male and female voles captured per trap type. Total captures are for both collection years, study areas and all altitudes combined.

Location was the most influential factor driving larval burdens, indicating that site specific factors such as temperature and humidity, and/or overall host availability are of greater influence on tick burden compared to trap type. Temperature and humidity are determining factors for tick survival, and due to the relative surface to volume ratio, larvae are particularly sensitive to desiccation (Knülle 1966). Both study areas differ in climate, and possibly the local climatic conditions are contributing to the overall abundance of ticks. The results presented in this paper show that drop-off rates are not significantly different between lethal and euthanized captures. This can make the use of lethal traps in tick burden studies a more favorable option, as animals are not exposed to capture stress, while offering the same degree of accuracy as live capturing and euthanasia. In addition, as there does not seem to be a sex bias between both capture methods, and lethal trapping is often more practical and economical, we propose that the use of lethal traps is preferable.

## 4.2 Paper II: Two tick species found at high altitudes in Norway

For this paper, a total of 5372 tick larvae were collected from 1325 trapped bank voles. *I. ricinus* and *I. trianguliceps* larvae accounted for 88.1 % and 11.9 % of all larvae, respectively. *I. ricinus* burdens ranged from 1 - 100 and *I. trianguliceps* burdens from 1-29. A majority of voles (57.4 %) were infested with at least one larva of either tick species, and 7.3 % of voles carried at least one larva of both species simultaneously.

The main goal of this study was to assess the occurrence of both tick species along an altitudinal gradient ranging from 100 to 1000 m.a.s.l. Under the influence of climate change, ticks have increased their distribution range northwards in Scandinavia (Lindgren et al. 2000, Jaenson et al. 2012). In Norway, *I. ricinus* was believed to be limited to coastal areas, but it has recently been observed further inland and shifted its distribution range approximately 400 km northwards since the early 1980s (Jore et al. 2011). Until now, the highest recorded observation for *I. ricinus* was 583 m.a.s.l. (Jore et al. 2011). The results of this study show that both *I. ricinus* and *I. trianguliceps* are occurring at an altitude of at least 1000 m.a.s.l. (Fig 7). As we found larvae actively feeding on rodents, we can be reasonably confident that in these two study areas in the eastern and western part of southern Norway, both species can be assumed to be established up to at least 1000 m.a.s.l.

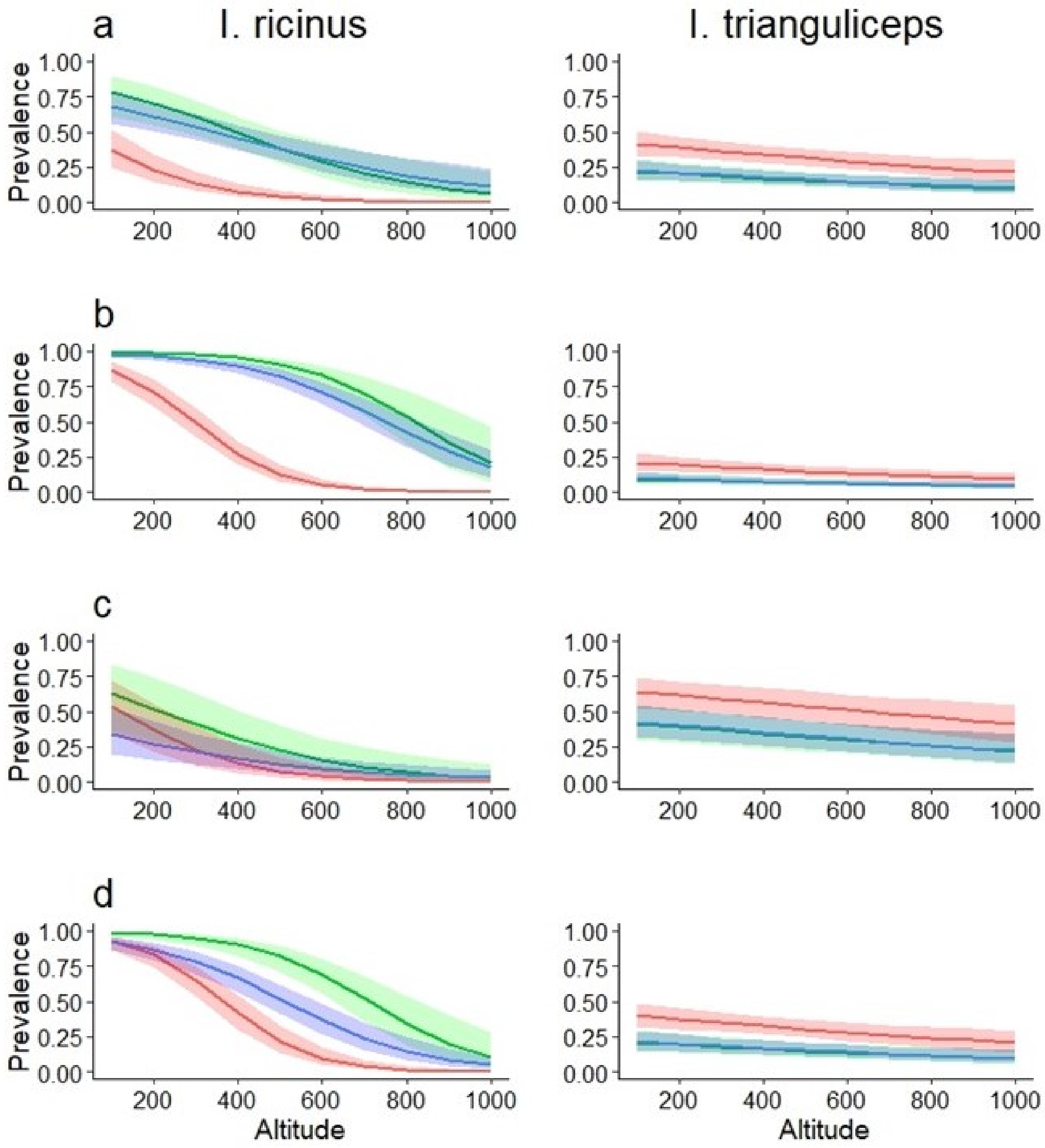

Fig 7. Predicted tick prevalence on bank voles along altitude in (a) Lifjell 2017, (b) Lærdal 2017, (c) Lifjell 2018, (d) Lærdal 2018. A prevalence of 1.00 represents a certainty of encountering at least one tick. Lines: spring (green), summer (blue), autumn (red). Ribbons represent standard error 95 % confidence intervals. For *I. trianguliceps,* spring and summer curves are closely overlapping.

We expected that the altitude effect would be stronger for *I. ricinus* than for *I. trianguliceps* (Fig 7), mostly due to its exophilic behavior making *I. ricinus* more exposed to climatic conditions as opposed to *I. trianguliceps*. Our results seem to confirm this expectation. The higher abundances of *I. ricinus* at lower altitudes might be a consequence of the generalist behavior of *I. ricinus*, able to parasitize multiple types of hosts such as cervids or other hosts (Talleklint and Jaenson 1997, Medlock et al. 2013). Field experiments have demonstrated that roe deer exclusions reduce the larval burdens of *I. ricinus* on small mammals, but that such exclusions have no effect on *I. trianguliceps* burdens (Bown et al. 2008).

The autumn season showed the strongest effect on *I. ricinus* decline (Fig 7), during which the prevalence above 900 m.a.s.l. was zero, while still infesting a substantial part of the hosts at lower altitudes. At higher altitudes, *I. trianguliceps* may be less affected by environmental conditions due to its endophilic nature, whereas *I. ricinus* might be more limited by lower temperatures and/or a longer snow period. Tick hosts are generally present at altitudes above that at which ticks occur, hence ticks are likely to be limited by other factors, such as microclimatic conditions (Daniel 1993). Materna et al. (2008) found that ticks (especially engorged females) can survive above their natural altitudinal range limit for quite a long time, and that their range limit is not limited by survival per se (Daniel 1993). Neither is the molting success or oviposition by females limited by altitude. Rather, it appears that it is a reduced hatching success of the egg batch that is hindering the developmental cycle of ticks, preventing them from establishing permanent populations (Materna et al. 2008). Ticks only disperse very short distances by themselves and are reliant on hosts such as birds (Hasle et al. 2009, Hasle 2011, 2013) or large mammals for long-distance dispersal (Medlock et al. 2013). The occurrence of feeding larvae at altitudes up to 1000 m.a.s.l. in both study areas presents further evidence that both *I.ricinus* and *I. trianguliceps* are capable of fully completing their life cycle at these altitudes. Because this study only investigated infestation rates up to 1000 m.a.s.l., the actual altitudinal range limit of these two tick species in Norway remains unknown, but we found that *I.ricinus* is present well above the previously found altitude of 583 m.a.s.l. (Jore et al. 2011).

During the spring, the prevalence of *I.ricinus* was generally higher at all altitudes compared to other seasons. Only during the summer of 2017, the prevalence was approximately equally high in Lifjell (Fig 7). *I. ricinus* larvae tend to show a bimodal activity pattern, peaking during the spring and autumn, or showing a unimodal summer peak (Cotton and Watts 1967, Jouda et al. 2004, Petney et al. 2012, Dantas-Torres and Otranto 2013). In 2017, spring and summer showed similarly high prevalences, but the spring burdens of *I. ricinus* seemed to drop before the summer in 2018. Although humidity was not a reliable predictor for infestation of both tick species, the exceptionally dry spring and summer of 2018 may help explain the lower prevalences during the summer. *I. ricinus* depend on a humid environment to quest and survive (Piesman and Gern 2004), and it appears that larvae are generally not limited by summer drought in these regions of Norway, and the conditions are generally cool and humid enough to enable them to quest throughout the summer until the autumn. Despite Lærdal having a lower relative humidity than Lifjell, infestation prevalence was higher in Lærdal. As humidity was not correlated with altitude in neither study areas, it was unlikely to have limited the presence of *I. ricinus* at any altitude. Although *I. trianguliceps* infested voles in lower numbers in both areas compared to *I. ricinus*, it was found during all active seasons at every altitude. We had no data on humidity inside the burrows, but as *I. trianguliceps* is dependent on a very humid environment (Estrada-Peña et al. 1992), it is conceivable that humidity was higher inside the burrows than above the surface (Bulova 2002, Shenbrot et al. 2002). As *I. trianguliceps* was most abundant during the autumn, it is possible that generally higher precipitation and cooler temperatures during autumn lead to more favorable conditions during this part of the year.

Larval prevalence of *I. ricinus* was higher on male voles compared to females. The larger home ranges and levels of mobility of males (Crawley 1969, Haapakoski and Ylönen 2010) may explain the higher presence of *I. ricinus* larvae questing in open vegetation (Perret et al. 2000, Randolph et al. 2002) and dependent on the passing by of a host (Estrada-Peña et al. 2018). No host sex difference was found for *I. trianguliceps*, which may be due to it being nidiculous (Bowman and Nuttall 2008). As burrows are visited by both male and female voles, both sexes may be equally exposed to parasitism by *I. trianguliceps*. This is supported by the findings of (Cotton and Watts 1967), who found no host sex difference in *I. trianguliceps* prevalence on voles in Britain. The results of this paper indicate that ticks may be able to survive and establish at altitudes higher than previously thought, and their range limit may be even higher than the occurrences found in this study.

## 4.3 Paper III: Rodent cycles may not inhibit tick range expansion

In this study, 2380 captures were made (54.5 % bank voles and 45.5 % common shrews) in both study areas. From 2017 to 2018, bank vole captures dropped by 90.8 %, and shrew captures by 59.5 % in Lifjell, and in Lærdal bank vole captures dropped by 38.5 %, whereas shrew captures increased by 23.9 %. The substantial drop in the number of bank voles captured indicate that in both study areas the bank vole populations are in a declining phase, and more so in Lifjell compared to Lærdal. A total number of 13326 ticks was collected (76.2 % *I. ricinus* larvae, 4.1 % *I. ricinus* nymphs, 15.8 % *I. trianguliceps* larvae and 4.0 % *I. trianguliceps* nymphs), and ticks were 3.7 times more numerous in Lærdal compared to Lifjell (10478 and 2848, respectively).

The primary goal of this study was to determine if and how the propensity (i.e. the mean tick burden ratio between bank voles and common shrews) would change if the size and composition of the host population changed. As ticks have been observed to progress upwards under the influence of climate change (Mejlon 2000, Daniel et al. 2003, Materna et al. 2008, Jore et al. 2011, Martello et al. 2014), we aimed to determine whether rodent population cycles could potentially inhibit the range expansion in altitude, or if ticks would have the potential to utilize alternate hosts as a stable reserve for survival. Furthermore, we assessed if there would be a difference in propensity towards a specific host type dependent on tick species or life stage. We found that *I. ricinus* larvae parasitized voles and shrews equally (Fig 8), regardless of the composition of the host population, but that the overall population size favored the parasitism of voles (Fig 9). We found similar results for nymphs, although voles were parasitized more regardless of the size and composition of the host population (Fig 8 and Fig 9). As changes in the population size were mainly influenced by changes in rodent availability, we can conclude that although nymphs had overall higher levels of parasitism on voles, both life stages may have the potential to utilize shrews as reserve hosts in time of low rodent availability. This is in agreement with other studies demonstrating that although each life stage of *I. ricinus* shows a preference for certain hosts (Talleklint and Jaenson 1997, Medlock et al. 2013), all stages can occur on different hosts. The results seem to confirm the generalist ecology of *I. ricinus*, and that a periodic unavailability of rodent hosts would probably not inhibit any future range expansion, given favorable climatic conditions. As this study focused solely on the tick burdens found on small mammals, we did not have information on the parasitism by *I. ricinus* on other (larger) host species, and it is possible that both larvae and nymphs may utilize hosts other than small mammals if rodents are at a low-point. This generalist ability may have contributed to *I. ricinus* becoming the most widespread tick species in Europe.

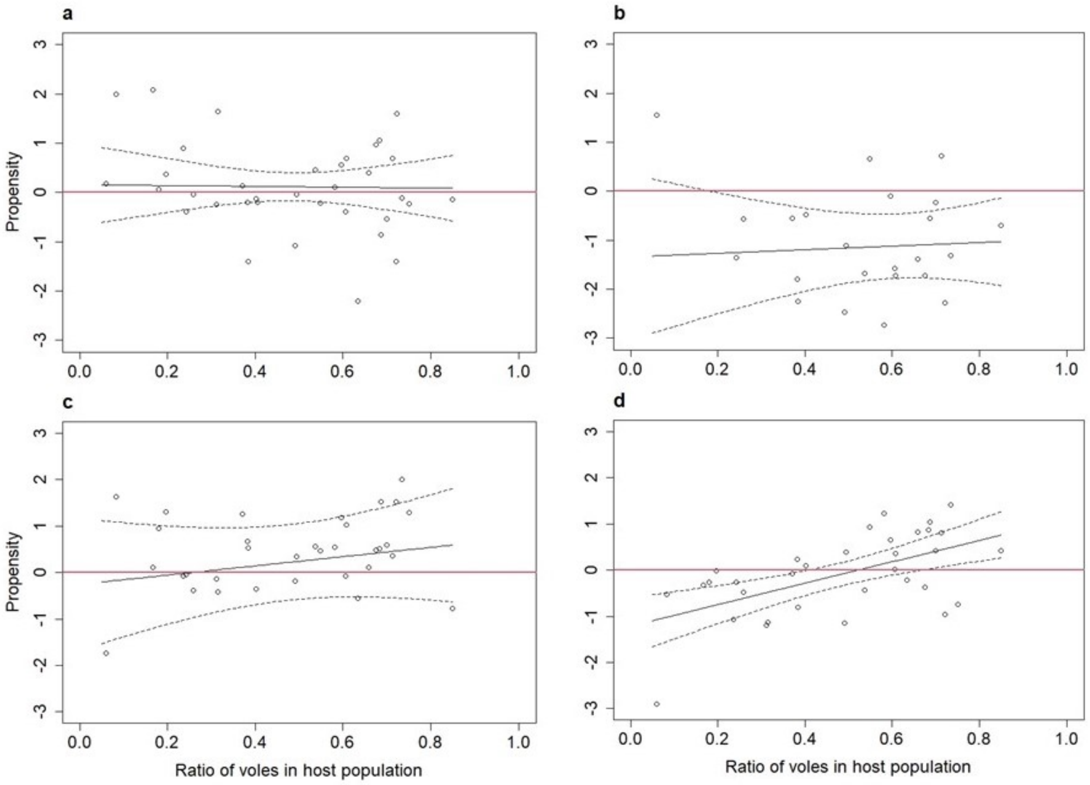

Fig 8. The effect of vole ratio in the host population on the propensity of (a) *I. ricinus* larvae (p= 0.920), (b) *I. ricinus* nymphs (p= 0.782), (c) *I. trianguliceps* larvae (p= 0.431), and (d) *I. trianguliceps* nymphs (p= 0.001) to parasitize either voles or shrews, as predicted by the final models. Dashed lines represent 95 % confidence intervals. The red line represents an equal propensity towards both host types.

In the case of *I. trianguliceps*, neither population size nor composition affected the propensity of larvae to parasitize either host (Fig 8 and Fig 9). Being a nidiculous (endophilic) tick species, spending its time off host within the burrows of the host, this species may be particularly dependent on other small mammal hosts when rodents are less numerous or not available. Larvae tended overall to parasitize shrews more compared to voles, regardless of the number of rodents or available hosts in general. This demonstrates that *I. trianguliceps* larvae may have a similar potential to *I. ricinus* to utilize other small mammals. Although generally considered an endophilic species (Bown et al. 2006), the larvae of *I. trianguliceps* have been reported to quest for hosts in small mammal harborages and animal trails (Hillyard 1996), although this is yet to be confirmed by other studies. This somewhat exophilic behavior may facilitate encounters between larvae and other potential small mammal hosts. The parasitism of *I. trianguliceps* nymphs on voles and shrews remained equal at all population sizes (Fig 9), and the propensity was close to zero when the number of shrews in the population was similar to the number of voles (Fig 8). However, as the ratio of voles in the population increased, nymphs paradoxically tended to favor the relatively less abundant shrews. A decline in rodent populations may leave some burrows vacant for prolonged periods, and this may leave nymphs without a host for several years. Depending on climatic conditions, the life cycle of *I. trianguliceps* typically lasts two to five years (Balashov 1997). A single life stage would likely exhaust its energy reserves in less time than that, hence subpopulations of *I. trianguliceps* at the burrow level, may become extinct inside a vacant burrow. As ticks are dependent on the movement of hosts for dispersal (Medlock et al. 2013), it may take some time for voles to recruit *I. trianguliceps* and allow them to colonize new or existing burrows during periods of rodent recovery.

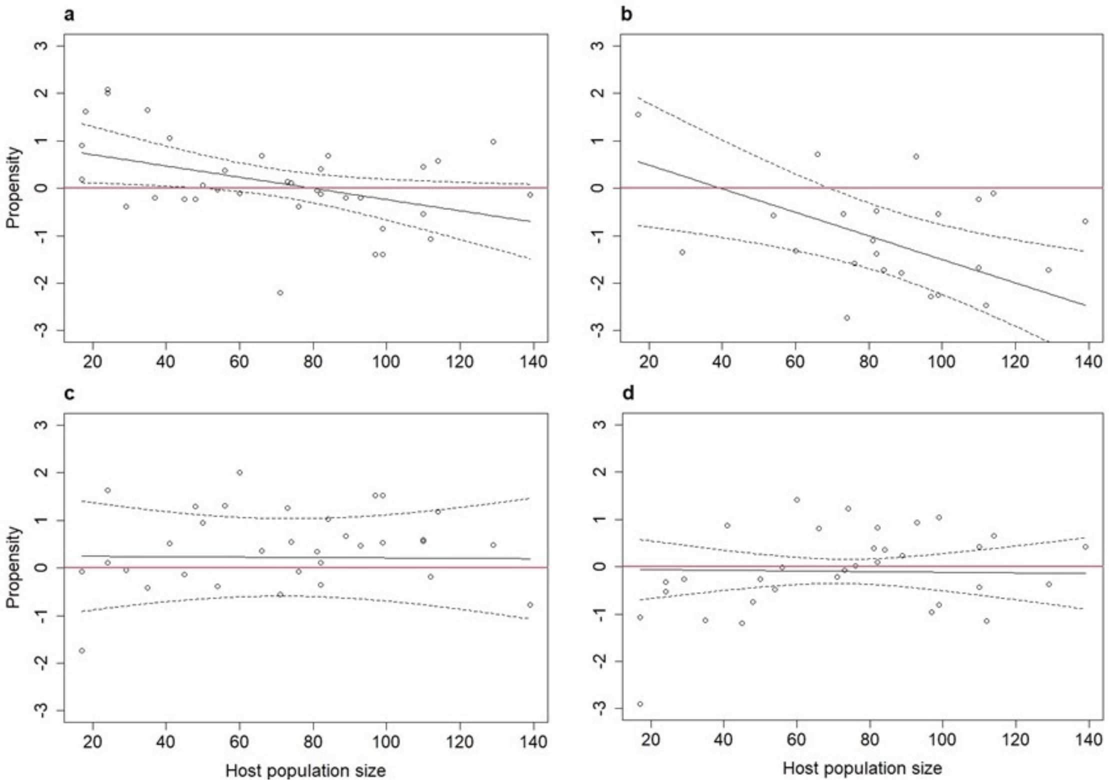

Fig 9. The effect of host population size on the propensity of (a) *I. ricinus* larvae (p= 0.035), (b) *I. ricinus* nymphs (p= 0.010), (c) *I. trianguliceps* larvae (p= 0.956), and (d) *I. trianguliceps* nymphs (p= 0.900) parasitizing either voles or shrews, as predicted by the final model. Dashed lines represent 95 % confidence intervals. The red line represents an equal propensity towards both host types.

# 5 Conclusions and perspectives

In recent years, increased attention has been paid to the range expansion of parasites under the influence of climate change and land use changes (Heyman et al. 2010). Invasive parasites may become pests to humans and livestock, and the ability to transmit pathogens may cause existing diseases to intensify, as well as introduce new emerging pathogens in areas previously free of infection. With continuing warming temperatures and changes in land use, the range expansion of parasites northwards and upwards is expected to continue in the future. Because the effects of climate change are supposed to be disproportionally stronger in northern regions (Smol 2012), the range expansion of parasites and the intensification of existing diseases, as well as the emergence of new pathogens, could be particularly exacerbated in these areas.

Several arthropod parasites have recently been documented to have shifted their range northwards. The deer ked (*Lipotena cervi*) has shifted its range limit northwards by almost 1000 km in the last 50 years (Kaitala et al. 2009). The Asian tiger mosquito (*Aedes albopictus*) is an aggressive invasive mosquito with a high vector potential, which has expanded its range in North America, and the expansion is predicted to continue during this century (Rochlin et al. 2013, Ogden et al. 2014). Other tick species such as the deer tick (*I. scapularis*) and the western black-legged tick (*I. pacificus*) have also expanded their northern range (Hahn et al. 2016, Clow et al. 2017).

In Norway, *I. ricinus* has been documented to have expanded its latitudinal range approximately 400 km in recent decades (Jore et al. 2011). Historically believed to be limited to coastal areas, it has now moved further inland and upwards. The results presented in this thesis seem to confirm the pattern of a continuing altitudinal progression. While *I. ricinus* has previously been found at altitudes up to 583 m.a.s.l., the results of this study show that this species is now able to parasitize small mammals at altitudes of 1000 m.a.s.l. This is a considerable increase in its altitudinal distribution, which had not been previously observed. Most altitudinal studies on the occurrence of *I. ricinus* in Europe have been done at lower latitudes, and the results of this study may provide valuable insights to the altitudinal distribution patterns of ticks at their northern range. Thus, the findings of this study may require a re-evaluation of disease risk in these areas. A substantial part of Norwegian society spends leisure time in mountainous areas. Many citizens visit cabins during weekends and holidays, and an increase in the presence of ticks at higher altitudes may lead to an increase in bite risk, and a subsequent transmission of tick-borne pathogens. *B. burgdorferi* s.l. (the causal agent of Lyme disease) is a commonly transmitted tick-borne pathogen to humans worldwide, and in Norway between 400 and 500 cases are diagnosed annually (Norwegian Surveillance System for Communicable Diseases - http://www.msis.no). An increased bite risk at both low and high elevations could therefore cause the number of human cases to rise in the future. Also, Norwegian sheep farmers tend to let their sheep range freely in the mountains outside of the winter season, and sheep have been found to suffer from morbidity and mortality caused by tick-borne anaplasmosis, damaging the sheep husbandry (Grøva et al. 2011). The social and economic consequences of tick-borne diseases have so far been limited to low altitude areas, but a higher occurrence combined with a longer active tick period may lead to increased damages to the livestock industry. Aside from the infection of domestic animals, the role of livestock as tick hosts may further increase the number of ticks at high altitude, increasing the potential for range expansion.

The potential shift in altitude may nevertheless be limited by other factors than warming temperatures alone. Aside from a mere favorable climatic environment, ticks are dependent on the availability of hosts to acquire a blood meal. Immature stages of ticks are often dependent on small mammals such as rodents for sustenance, and although rodents are common in most terrestrial ecosystems, their distinct population dynamics may constitute a limiting factor in the range expansion of ticks. Rodents usually undergo multi-annual population cycles 3-4 years long (Kaikusalo 1972, Hörnfeldt 1978), and the amplitude of the fluctuations increases with altitude (particularly in northern regions) (Taylor et al. 2013). During the low-phase of the population cycle densities can reach near-zero levels (Kausrud et al. 2008). This periodic unavailability of important hosts for juvenile ticks may inhibit any further expansion of ticks, as a potential expansion that occur during rodent peaks years may be set back by a strong rodent population decline.

However, during the low phase in rodent cycles, generalist ticks may utilize other small mammal hosts in larger numbers to survive. Shrews have been found to act as hosts of immature ticks (Gregson 1942, Telford III et al. 1990, Bakhvalova et al. 2001, Paramasvaran et al. 2009), with similar reservoir potential for pathogens as that of rodents (Kozuch et al. 1967, Jaenson et al. 1994, Gern et al. 1998, Liz 2002, Bakhvalova et al. 2006, Bown et al. 2011). Despite being somewhat neglected as potential hosts (Bown et al. 2011), several studies comparing tick burdens between rodents and shrews, have found concurrently higher infestation levels on shrews compared to rodents (Nilsson 1974, Randolph 1975, Nakao and Miyamoto 1993, Talleklint and Jaenson 1994, Bown et al. 2011, Mysterud et al. 2015). As shrews generally do not undergo periodic population cycles, and their numbers fluctuate less than those of rodents, they may have the potential to serve as replacement hosts during periods of low rodent abundance. The utilization of other hosts could compensate for the lack of available rodents, and therefore maintain the potential for range expansion, as well as disease progression. Alternate host utilization by ticks and the implications in range expansion is to date poorly understood, and the results presented here show that such a potential may exist for *I. ricinus*. This species did not show a significant preference towards either host type, and seems therefore to be sufficiently opportunistic to parasitize shrews in such numbers so that any expansion is not limited by periodic low bank vole densities. Furthermore, it may be that lower altitudes will become too hot or too dry for ticks during certain seasons of the year (Gern et al. 2008), inhibiting the questing activity of ticks (Perret et al. 2000, Jouda et al. 2004). Hence, a range shift towards higher elevation (where moisture stress is less probable), rather than an overall expansion, may occur. If so, risk areas may not increase in size, but rather shift upwards. But as climatic changes in latitudinal and altitudinal gradients are similar (albeit at different scales), the most likely scenario is that ticks will continue to expand their distribution limit both northwards and upwards.

As the range expansion of ticks is likely to continue in the future (Ogden et al. 2006), there is a need for adequate monitoring to identify possible high-risk areas. Several ways exist to track the progression of ticks through the landscape. Tick collection methods such as flag dragging or collecting ticks from animals and humans can provide information on the local occurrences of ticks, and microbiological studies can determine the accompanying disease prevalence. Veterinary and medical data collection of diagnosed cases of tick-borne infections can offer a more widespread indirect estimation of infection risk. However, although these methods have proven valuable and will continue to be of importance in the future, such studies generally only provide localized data and need to be widespread and combined to provide a regional or nationwide overview of tick occurrence (Jore et al. 2011). Another possibility is to call upon citizen science to crowdsource information gathering (Shimada et al. 2003, Eichenberger et al. 2015). Such research projects can be valuable as they are relatively low cost and passive in effort, but are limited in coverage and accuracy due to incomplete responses or erroneous reporting (e.g. wrongful identification) (Schmidt 1997). The online survey CATS (Cats As Tick Sentinels) is an ongoing project started in 2018 (and originally part of this PhD project) aimed at tracking the latitudinal (and altitudinal) distribution of ticks in mainland Norway, using ticks on domestic cats as a proxy. Cat owners throughout the country are invited to report the incidences of ticks found on their cats, and will allow for an estimation of the distribution of *I. ricinus* ticks, as well as an estimation of range expansion and density of tick infestation on cats. Citizens and veterinarians have been asked to participate by using social and online media outlets as tools for promotion and communication to the public. As cats are usually more homebound than e.g. dogs, and people tend to travel less with them, ticks found on cats are likely to be more representative of the area where it lives.

Altitudinal range expansion has been observed in several central European countries (Daniel et al. 2003, Jouda et al. 2004, Danielová et al. 2008, Gern et al. 2008, Materna et al. 2008, Daniel et al. 2009), but the information on the elevational progression of ticks in northern regions (where overall temperature is lower) remains scarce. Although the results presented in this thesis have shown ticks to be parasitizing small mammals at altitudes of at least 1000 m.a.s.l., sampling was only performed during the active seasons of ticks, and doesn't provide any answers on whether ticks can survive the harsh conditions during winter at such altitudes. In order to answer this, a tick survival experiment was performed on one of the gradients during the winter of 2019 - 2020. Ticks were placed in small enclosed tubes, made of fabric to allow sufficient air flow, at all altitude stations in autumn and left over the winter, and survival was recorded after the snowmelt. Preliminary results show a notable degree of survival at all altitudes, and further data analysis may reveal how environmental factors (e.g. length of the snow covered season) will influence tick winter survival, and whether such conditions may inhibit the further expansion of ticks to higher altitudes in Norway.

Combining the results of this thesis with future studies may provide a more comprehensive image of the current status of tick distribution along an altitudinal gradient in a northern region, and of the factors influencing tick survival. Prolonging a study similar to paper III and encompassing two or more complete rodent population cycles, may provide further insights into the role of small mammal hosts, how they can facilitate or inhibit tick expansion and the impact of a changing climate on the amplitude and frequency of these cycles.

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## Paper I

De Pelsmaeker, N, Korslund, L, Steifetten, Ø. Do bank voles (*Myodes glareolus*) trapped in live and lethal traps show differences in tick burden? *PLoS ONE 15(9):* e0239029. doi: https://doi.org/10.1371/journal.pone.0239029

PLOS ONE

RESEARCH ARTICLE

# Do bank voles (*Myodes glareolus*) trapped in live and lethal traps show differences in tick burden?

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## Abstract

In studies assessing tick abundance, the use of live traps to capture and euthanize rodent hosts is a commonly used method to determine their burden. However, captive animals can experience debilitating or fatal capture stress as a result prior to collection. An alternative method is the use of lethal traps, but this can potentially lead to tick drop-off between the time of capture and collection. In this study, in order to determine whether subjecting animals to capture stress is inevitable, we tested the difference in sheep tick (*Ixodes ricinus*) larval burdens between bank voles (*Myodes glareolus*) captured alive and euthanized, and lethally trapped bank voles. During 2017 and 2018, 1318 bank voles were captured using live (Ugglan Special no. 2) and lethal (Rapp2 Mousetrap) traps during two consecutive years over three seasons in two locations in Norway. Voles captured alive would remain captive until euthanized, while lethally trapped voles were killed instantly upon capture. Log-linear models, accounting for overdispersion, were used to determine whether trap type was influencing observed tick burden. Bank voles captured in lethal traps carried 5.7% more larvae compared to euthanized voles captured in live traps, but this difference was not significant (p = 0.420). Males were overall captured 2.7 times more frequently than females, and the sex ratio was equal in both trap types. This study shows that the use of lethal traps to determine tick burden of rodents is sufficiently reliable, without having to subject animals to potentially lethal stress, hereby reducing some ethical concerns of animal suffering and the results thereof, without compromising accuracy. Lethal trapping is also often more economical and practical, further favoring this collection method.

## Introduction

In the pursuit of reliable data in animal studies, killing animals is sometimes necessary and unavoidable, and without such practices many aspects of animal ecology, demography, physiology and biology would remain unstudied [1]. It allows for a detailed study of the captured animal and can reveal much more about the biology of a specimen than from a live examination, and it also permits the collection of animals for later dissection and preservation, and

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offers possibilities that are not available if an animal is to remain intact or alive. However, wild animals that are first captured and later euthanized may still experience some degree of stress from the time spent in captivity and from handling by the researcher [2]. Trap induced stress responses usually occur within minutes of capture [3], and longer periods of captivity lead to increasingly more stress [4, 5]. Animals have shown to develop capture myopathy [6–8] which often has fatal consequences [9], and animals that are kept in captivity after capture can die from secondary stress responses [10]. Consequently, there are ethical considerations in the practice of capture, and efforts should be made to reduce the discomfort of captive animals.

In parasitological studies, the parasite burden of small mammals can be used as a proxy for parasite distribution and infestation rates, as small mammals act as hosts for a number of parasitic species, including ticks [11–13]. One of the main hosts of larval ticks are small rodents, which are considered good indicators of tick abundance as they occupy the same physical space [14]. Small rodents can act as reservoirs for zoonotic pathogens [15–18], and generalist ectoparasites such as ticks can further transmit these pathogens to humans [19–21]. Some of these pathogens can be dangerous to humans [22, 23]. Ticks can themselves be hosts to a number of pathogens and readily transmit these pathogens to new hosts [24–26], and thus rodent parasites are an important element in the study of epidemiology [27]. One method often used when studying the distribution and prevalence of ticks is to measure the burden on live hosts in the field before returning them [11–13], but this is very stressful for the animal being handled, and it can be difficult to accurately determine the precise numbers in this way due to sub-optimal conditions, leading to imprecise measurements. Most such studies involve visual inspection and mechanical removal of the parasites from the animal using a comb or a tweezer, while physically restraining it or using anesthetics [21, 28–31]. Estimating and removing ticks from a live host is particularly complicated as ticks are cemented into the skin surface while feeding [32]. The unavoidable and intensive handling of an animal during examination can also be a major cause of stress in itself [33, 34], and may potentially lead to the death of the animal [9]. A more reliable alternative to live examination is to euthanize the captured animals when collected, in order to examine them thoroughly in a controlled environment (hereafter referred to as euthanized captures). Although this method allows for a more reliable assessment of ticks on hosts [35], this does not prevent any stress related discomfort experienced by the captive animal between time of capture and collection. An option to reduce capture-induced stress in small mammals such as rodents is to employ lethal traps, where the animal is killed instantly upon capture (hereafter referred to as lethal capture). When studying ticks however, this might constitute a problem, as ticks require a live host in order to complete a blood meal. It is possible that upon the death of a host, ticks will start to progressively abandon the dead host (drop-off) in order to molt or to quest for a new host in an attempt to complete the blood meal (refeeding) [36, 37]. During field studies, there can be a considerable amount of time (several hours) between a lethal capture and collection of the animal. Therefore, it is possible that during that time period at least some of the ticks will drop off after the host dies, and the number of ticks found on the animal may not represent the total burden at the time of death. It is unknown which cues (or lack thereof) might cause ticks to detach from a dead host, but in laboratory conditions ticks have been observed to start detaching from mice three hours after death [36].

In this study, we investigated if larval tick burden size on small rodents differed between euthanized captures and lethal captures to determine whether live trapping followed by euthanasia is necessary to reliably assess the total tick burden on small rodents, or whether the use of lethal traps is sufficient for this purpose. The aim was to determine if lethal trapping could be a viable alternative to euthanized captures in order to reduce the stress animals are likely to experience in live traps. Because little is known on how ticks behave after the host dies, we had no clear expectation on the outcome of the study, and we could not predict which trapping method would yield the largest tick burdens.

## Materials and methods

### Study area

The study was part of a larger research project investigating the distribution of ticks along an altitudinal gradient, conducted in two separate areas in Norway during 2017 and 2018. The first study area was a southern facing mountain slope on the Lifjell massif (N59˚ 26.495' E9˚ 02.603') near Bø i Telemark. Lifjell is located within the boreonemoral to southern boreal zone, characterized by mixed coniferous and deciduous forest, and is characterized by a continental climate. The second study area was the northern facing Erdal valley (N61˚ 05.817' E7˚ 24.688') near Lærdalsøyri (hereafter referred to as Lærdal) close to the innermost part of the Sognefjorden fjord. It is located in the middle to northern boreal zone, and lies approximately 150 km east of the western coastline. The vegetation is dominated by deciduous forest and it has a coastal climate.

### Host trapping

This study was carried out in strict accordance with the regulations issued by the Norwegian Environment Agency, and a permit was provided prior to the start of the sampling (Miljødirektoratet, reference number: 2017/4651) for the duration of the trapping period. The protocol for capturing animals was approved by the Animal Ethics Committee of the Department of Nature, Health and Environment (University of South-Eastern Norway). All efforts were made to minimize animal suffering.

At each study area, 10 trapping stations were established along an altitudinal gradient from 100 up to 1000 m. a. s. l., at every 100 m altitude interval. At every altitude station, two capture plots were constructed. One with 20 live traps for euthanized captures (Ugglan Special Nr. 2, Grahnab AB, Sweden; www.grahnab.se) (Fig 1A) and the other with 20 lethal traps (Rapp2

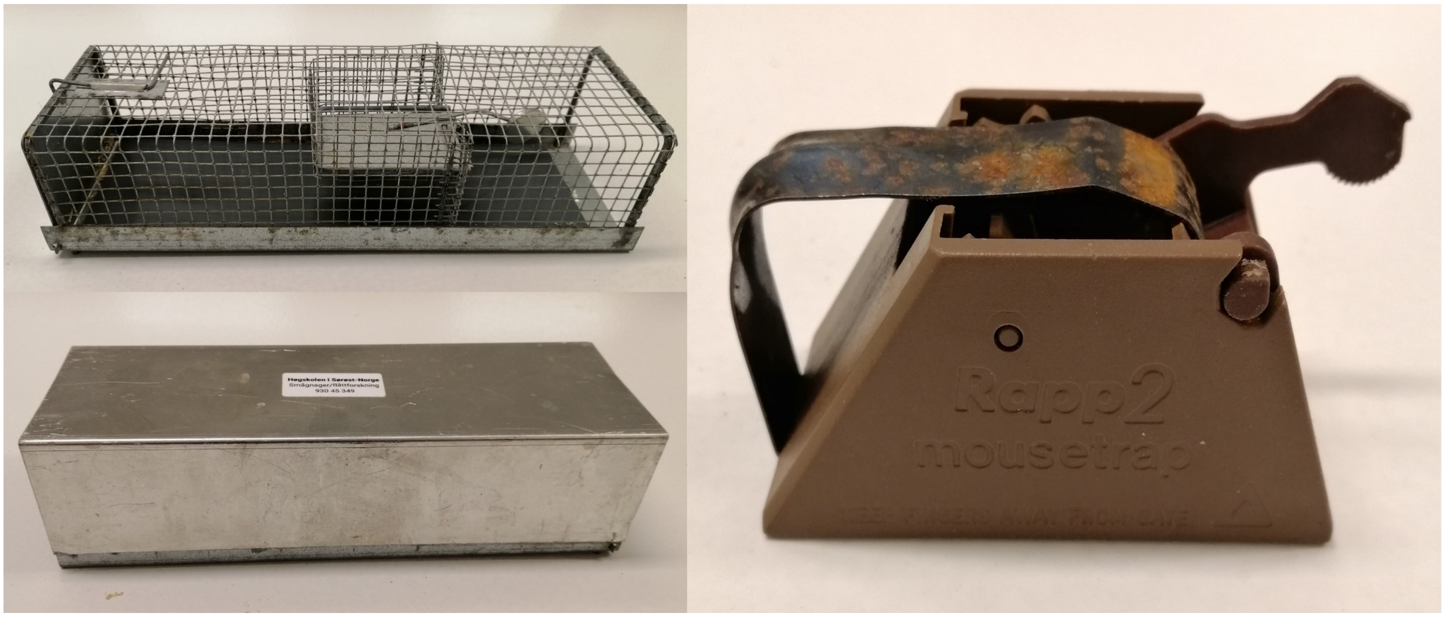

**Fig 1. The two trap types used in the study.** (A) Ugglan special Nr 2 live trap (top), covered with an Ugglan special long roof metal cover (bottom). (B) Rapp2 lethal mousetrap.

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Mousetrap, www.rappfellene.no) (Fig 1B). Rapp2 mousetraps were chosen over classic snap traps as they reduce the risk of non-lethal capture (e.g. leg or tail), which can cause considerable suffering. Lethal trapping killed the animals instantly by cervical fracturing. Both trap types were arranged in a 4 by 5 grid, with 10 meters spacing between each trap. Live and lethal plots were spaced a minimum of 100 m apart to avoid home range overlap of hosts [38, 39]. Live traps were baited with a slice of apple for hydration and whole oats for caloric value, and a lining of sawdust was provided as insulation on the trap floor. Lethal traps were baited with peanut butter for practical reasons as it is easily applied to the inside of the trap body. The trapping of small mammals took place three times per year, during spring (May 20th– 30th), summer (July 20th– 30th), and autumn (September 20th– 30th). The only exception to this schedule was during the spring season of 2017, when capturing took place from June 1st until June 7th in both study areas, and only up to 700 m. a. s. l., as there was too much snow in both areas to permit trapping earlier and above this altitude. Traps were checked for captures once every 24 hours during the trapping period, and collection of the captures started at 8h30 every day. Triggered traps were rebaited and reset. Handling rodents and examining them for ticks in the field can be stressful and can cause harm or injury to the animals [40], and it is difficult to accurately determine tick burden on live small mammals [41]. Therefore, we opted for a post-mortem full body examination, and voles captured in live traps were euthanized and collected. Full body examinations of both euthanized and lethal captures provided a higher degree of sensitivity [42]. Individuals captured in live traps were euthanized in the field by cervical dislocation of the head, and sealed in separately coded plastic bags. Cervical dislocation of the head was also instantly fatal. After every collection day, all animals were placed in a freezer at –20˚C. Because bank voles (*Myodes glareolus*) constituted more than 86% of all rodents captured, we only used bank voles in the analysis.

Humidity and temperature have a direct influence on tick activity, and are important drivers of phenological patterns and host-seeking behavior [43]. For this reason, a temperature logger (TinyTag Plus 2 –TGP 4017) was placed inside a DataMate instrument cover (ACS-5050), and mounted on a pole 50 cm above ground level in between live and lethal plots at every altitude station. These loggers recorded temperature at a 1-hour interval for the duration of the study period (June 2017 –September 2018), as a measure of environmental conditions throughout the transect.

## Laboratory processing

After every field season the captured bank voles were checked for ticks. The voles were taken out of the freezer the evening before examination, and left to thaw in a cold room at 10˚C overnight. The animals were then removed from the plastic bags and the empty bags were checked for ticks that might have dropped off. Wet animals were first dried using a hairdryer to more easily detect the ticks. The voles were then processed one by one, and ticks were removed, counted and placed in a 1.5mL plastic vial containing a 70% ethanol solution (1 vial per host). Ticks found attached or just present on the host were removed using a tweezer. The hosts were then checked starting with the head, ears and snout, followed by neck and throat, back and abdomen, legs, feet and tail. Finally a lice comb was used over the whole body of the animal from tail to head (against the hair orientation), and the animal was subsequently shaken by the tail during 5 seconds above a white plastic tray to collect any ticks that were potentially missed during the examination. The minimum time needed to process one vole was 20 minutes. The hosts were also weighed to the nearest 10th of a gram and their sex was determined. Once the examination was completed the processed animals were bagged in new plastic bags and refrozen at -20˚C.

Individual ticks were determined for life stage and species using the determination keys published by Arthur [44] and Hillyard [45] under a Zeiss Discovery V20 stereomicroscope. Because more than 80% of all ticks collected were sheep ticks (*Ixodes ricinus)*, and of these more than 94% were larvae, only *I. ricinus* larvae were used for this study. A minority of vole ticks (*I. trianguliceps*) was also encountered, but was not used in this analysis, as it is a rodent specialist solely living in burrows of the host [46], and is not encountered in open vegetation. Ticks that were too damaged to allow for precise identification were discarded from the study altogether. After determination, the ticks were replaced in vials for long-term storage.

## Data analysis

All statistical analyses were performed using R version 3.5.3 [47]. We used general linear regression models, using larval burden (i.e. the number of tick larvae on an individual) as the response variable. To compare the larval burden between live and lethal traps, trap type was used as a categorical predictor. Additionally, study area (Lærdal and Lifjell), collection year (2017 and 2018), season (spring, summer and autumn) and daily average temperature (˚C) were used as extrinsic covariates, and the individual characteristics host weight (in grams) and sex (male or female) were used as intrinsic covariates. To investigate if ambient temperature could affect any drop off of ticks from the captured host, temperature was averaged for a 24-hour period spanning from 12:00 AM the day before capture until 12:00 AM on the day of capture. Two-way interactions between all covariates and trap type were considered, and we used a stepwise backwards model selection approach. We started with a full model containing all predictors and interactions, and progressively removed interactions or predictor variables that were not significant in a type II ANOVA test, until a nested model only yielded significant predictor variables. Larval burden on bank voles was a count, and we therefore used a Poisson distribution in the glm-function to model the data. Overdispersion was detected, hence we corrected the standard errors using a quasi–GLM model according to Zuur et al. [48], where the variance is given by $\varphi \times \mu$, where $\mu$ is the mean and $\varphi$ the dispersion parameter [48]. To visually represent the relationships between larval burden and the predictor variables, we used effect plots from the *ggplot2* package [49]. To test whether there was a trap selection for a particular trap type, we regressed host trappability by using trap type as a binomial response variable and the host specific variable sex and weight as predictors, adding an overdispersion parameter φ [48]. A Wilcoxon rank sum test was performed to test if the body mass of the captured voles differed between sexes. A p- value $< 0.05$ was considered significant.

## Results

For both years and study areas a total of 43920 trap nights were performed, capturing 1318 bank voles (Table 1). Other captured rodents were field voles (*Microtus agrestis*), tundra voles (*M. oeconomus*), grey red-backed voles (*Myodes rufocanus*), wood mice (*Apodemus sylvaticus*), yellow-necked mice (*A. flavicollis*) and house mice (*Mus musculus*). Three species of shrews

**Table 1. Number of bank voles captured per trap type in each study area during 2017 and 2018.**

| | 2017 | | | | | | 2018 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Site** | **Spring** | | **Summer** | | **Autumn** | | **Spring** | | **Summer** | | **Autumn** | | |
| | **Live** | **Lethal** | **Live** | **Lethal** | **Live** | **Lethal** | **Live** | **Lethal** | **Live** | **Lethal** | **Live** | **Lethal** | **Total** |
| **Lifjell** | 61 | 22 | 61 | 108 | 107 | 54 | 11 | 3 | 6 | 2 | 3 | 6 | 444 |
| **Lærdal** | 55 | 48 | 117 | 109 | 64 | 163 | 31 | 37 | 57 | 70 | 29 | 94 | 874 |
| **Total** | 116 | 70 | 178 | 217 | 171 | 217 | 42 | 40 | 63 | 72 | 32 | 100 | 1318 |

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**Table 2. Number of larval *Ixodes ricinus* ticks collected from bank voles per trap type in each study area during 2017 and 2018.** Below are the mean (SD) burdens per vole.

| Site | 2017 | | | | | | 2018 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Spring | | Summer | | Autumn | | Spring | | Summer | | Autumn | |
| | Live | Lethal | Live | Lethal | Live | Lethal | Live | Lethal | Live | Lethal | Live | Lethal |
| **Lifjell** | 144 | 63 | 36 | 182 | 18 | 20 | 21 | 3 | 0 | 0 | 21 | 3 |
| | 2.4 ± 6.5 | 2.9 ± 6.0 | 0.6 ± 1.6 | 1.7 ± 3.8 | 0.2 ± 0.7 | 0.4 ± 1.1 | 1.9 ± 5.7 | 1.0 ± 1.7 | 0.0 ± 0.0 | 0.0 ± 0.0 | 7.0 ± 6.1 | 0.5 ± 0.8 |
| **Lærdal** | 678 | 772 | 527 | 527 | 32 | 328 | 389 | 340 | 197 | 270 | 18 | 146 |
| | 12.3 ± 11.5 | 16.1 ± 21.3 | 4.5 ± 6.5 | 4.8 ± 11.0 | 0.5 ± 1.4 | 2.0 ± 5.1 | 12.5 ± 18.7 | 9.2 ± 15.6 | 3.4 ± 8.2 | 3.9 ± 9.3 | 0.6 ± 1.3 | 1.6 ± 2.2 |

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were also captured: common shrews (*Sorex araneus*), pigmy shrews (*S. minutus*) and water shrews (*Neomys fodiens*). In both study areas fewer captures were made in 2018 compared to 2017 (413 and 31 in Lifjell, 556 and 318 in Lærdal for 2017 and 2018, respectively). In total 4735 *I. ricinus* larvae were collected, and 47.0% of the captured voles were infested with at least one larvae. Infestation rates between capture methods were similar (44.4% and 49.3% of euthanized and lethally trapped voles, respectively). Larval burden ranged from 1 to 100, and voles were infested with on average 3.6 larvae. Mean burdens per study area and year are summarized in Table 2.

Voles that were lethally trapped had 5.7% larger tick burdens in comparison to euthanized captures, but the effect of trap type alone was not significant (Table 3), and trap type was only retained in the model due to the significant interaction between trap type and season (F = 4.13, df = 2, p = 0.02). Larval burdens on bank voles trapped in lethal traps were somewhat higher in spring and autumn, and burdens were nearly equal between traps in summer (Fig 2). Burdens differed however substantially and significantly between seasons overall (F = 186.89, df = 2, p< 0.001), showing the largest burdens in spring, with a nearly linear decline throughout the trapping period for both euthanized and lethal captures (Fig 2). A post-hoc Wilcoxon rank sum test showed no significant differences in tick burdens between capture types in each season, with the exception of autumn (W = 26371, p< 0.001, n = 520). The final model, best describing the variation in larval burden between individual bank voles, included an additive effect of trap type, study area, year, season, host sex and temperature, as well as an interaction between trap type and season (Table 3). Study area was the most influential predictor of larval burden, as it differed significantly between study areas (F = 158.10, df = 1, p< .001), and was

**Table 3. Estimated regression parameters, standard errors, t-values and p-values for the final model, describing the factors influencing larval burdens on bank voles.**

| | Estimate | Std. Error | *t*-value | p-value |
|---|---|---|---|---|
| Intercept | 0.234 | 0.304 | 0.772 | 0.440 |
| Site Lærdal | 1.395 | 0.176 | 7.915 | < 0.001 |
| Year 2018 | -0.585 | 0.143 | -4.081 | < 0.001 |
| Season Summer | -1.477 | 0.190 | -7.780 | < 0.001 |
| Season Autumn | -1.567 | 0.214 | -7.329 | < 0.001 |
| Temperature | 0.101 | 0.023 | 4.436 | < 0.001 |
| Sex Female | -0.281 | 0.125 | -2.243 | 0.025 |
| Trap type Live: Season Autumn | -0.864 | 0.444 | -1.944 | 0.052 |
| Trap type Live | -0.121 | 0.150 | -0.806 | 0.420 |
| Trap type Live: Season Summer | -0.092 | 0.232 | -0.396 | 0.692 |

Non-significant predictors are retained in the model because of their involvement in an interaction or other predictor variable which is significant.

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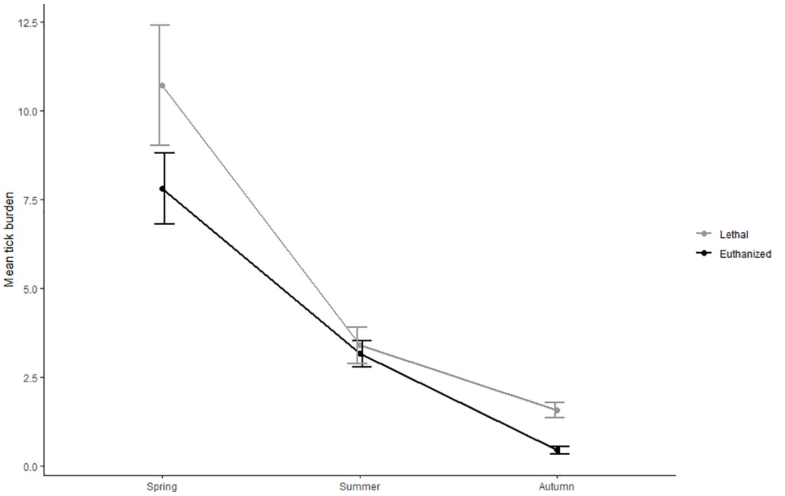

**Fig 2. Mean tick burden of *I.ricinus* on lethally captured and euthanized bank voles per season.** Mean tick burdens are for both collection years, study areas and all altitudes combined. Error bars represent standard errors.

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overall more than four times higher in Lærdal compared to Lifjell (4.8 and 1.2 ticks per vole, respectively). The only exception was in autumn 2018, where mean burden in Lifjell was twice that of Lærdal [2.6 ± 4.1(sd) and 1.3 ± 2.1(sd), respectively]. Overall, burdens were significantly lower in 2018 compared (F = 33.51, df = 1, p< 0.001).

Temperature had a significant positive effect on burden (F = 36.82, df = 1, p< 0.001), and this effect was equally strong irrespective of trap type, as shown by the non-significant interaction between temperature and trap type (F = 0.06, df = 1, p = 0.802). Males carried overall more ticks than females (F = 9.93, df = 1, p = 0.002). The Wilcoxon rank sum test showed that the difference in body mass between the trap types was statistically significant (W = 109980, p < 0.001, n = 1318). Voles captured in lethal traps were significantly heavier than voles captured in live traps (F = 7.86, df = 1, p = 0.005), but the mean difference was only 0.9 g. Male voles were captured 2.7 times more frequently than females (Fig 4), and the body mass of captured females (Mdn = 25.9) was higher than that of males (Mdn = 20.3). The sex of the captured vole was not significantly different in either trap type (F = 0.14, df = 1, p = 0.707).

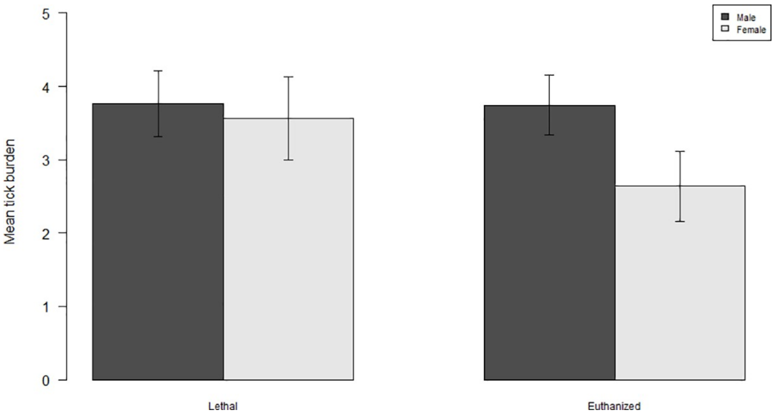

**Fig 3. Mean tick burden of *I. ricinus* on bank voles per trap type and vole sex for lethal and euthanized captures.** Mean tick burdens are for both collection years, study areas and all altitudes combined. Error bars represent standard errors.

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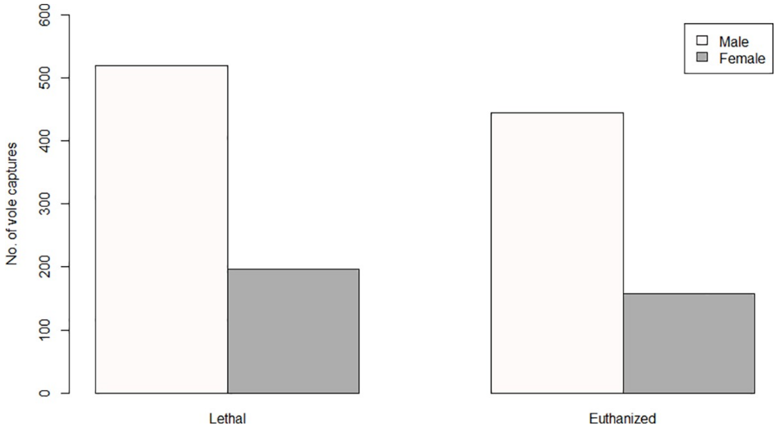

**Fig 4. Total number of male and female voles captured per trap type.** Total captures are for both collection years, study areas and all altitudes combined.

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## Discussion

This study aimed to determine if larval tick burdens differed between euthanized voles captured in live traps and voles killed in lethal traps. Our data showed that there was no significant difference in the number of ticks between bank voles that were lethally trapped, and those that remained alive until collection, suggesting that the use of lethal traps does not result in a different drop-off rate compared to live traps. In fact, our data showed that, if any, lethally trapped voles had borderline significantly higher burdens during autumn in comparison to euthanized voles.

Potential drop-off rates from dead hosts can be a deciding factor in selecting which trapping method to employ, and research on this process is limited. Nakao and Sato [36] found that Taiga ticks (*I. persulcatus*) began to abandon dead laboratory mice three hours after time of death, and Piesman [37] demonstrated that deer tick larvae (*I. dammini*) abandoned hamsters during two days following the death of the host. However, these studies represented laboratory conditions and it is reasonable to assume that the field conditions in our study (i.e. diurnal and nocturnal temperature fluctuations, as well as differences in humidity and light regime) could have affected the behavior of larvae and the duration of attachment after the death of a host. It may have taken a certain amount of time for larvae to decide to leave a dead host as the body of hosts probably cooled down at different rates based on environmental conditions. Attached larvae might have continued to feed for a little while even after death. Larvae might also have detached, but remained in the fur of a dead host for a time before dropping off completely. Investigating detachment rates under field conditions could prove valuable in understanding the cues ticks use to determine host death and their decision to abandon and/or attempt refeeding. Several studies have documented tick drop-off rates of live hosts when ticks reach full engorgement under laboratory conditions [31, 50, 51], and it is possible that under field conditions the time voles spent captive in live traps provided a window of opportunity for a fraction of the attached larvae to have reached full engorgement and dropped off the host before it was collected, and hence would not have been counted during the laboratory assessment. Both live and lethal traps would have allowed for engorged larvae to leave. Dizij and Kurtenbach [52] found that the longest attachment duration for *I. ricinus* larvae on bank voles was 79 hours. Once an animal entered a live trap, it was isolated from the environment and probably did not acquire new ticks. With a maximum of 24 hours between live capture and euthanasia, this means that potentially over a third of the burden could potentially have reached full engorgement and have dropped off within that period (24 hours = 30,4% of 79 hours). More realistically, bank voles are most active right before dusk, and right after dawn [53], so the time spent in captivity was more likely to be either 9–12 hours, meaning a potential drop off of 11–15% in the time captive before euthanasia, or about 2–5 hours, resulting in a drop off of 2–6% of the burden at the time of collection. The mean burden in live traps was 7% lower to lethal traps and 12% fewer larvae were collected in total. However, voles captive in live traps may have had time to remove some of the ticks through grooming, potentially explaining the lower larval burdens found in live traps.

Females carried on average fewer larvae than males (Fig 3), hence larvae dropping off females during captivity could have made a larger proportional difference compared to males. Pollock [55)] found that western black-legged ticks (*I*. *pacificus*) fed faster and dropped off quicker on female lizards (*Sceloporus occidentalis*) during the mid-summer season [54]. During spring, male voles carried on average twice as many larvae compared to females (10.5 and 4.9 respectively). This could indicate that not only would the proportional difference in drop-off be larger in females compared to males, but that the difference drop-off in females could be exacerbated by faster feeding and less time required for ticks to reach full engorgement. Multiple studies have found that tick infestation rates are skewed towards male rodent hosts [14, 55–59]. Bank vole males have larger home ranges than females [60, 61], and therefore roam around more than females. Hence, the likelihood of males to encounter questing ticks is higher than for females. In particular, *I*. *ricinus* larvae do not disperse far from the place where the adult female tick oviposited the egg batch, and hence their distribution is spatially clumped. Because of differences in roaming behavior, males have a higher probability of encountering aggregated tick larvae, resulting in larger burdens. In fact, the five highest burdens encountered in this study (n = 81, 81, 84, 97, 100) all occurred on males. In general, the sex ratio of bank voles is close to 1:1 [62], or fluctuating between sexes with generational turnover [63, 64]. The difference in activity between the sexes can help explain why males were more trappable than females, and the ratio between male and female captures was nearly identical in both trap types, indicating that trap type itself did not have an effect on the likelihood of a vole of either sex to enter the trap. Although captured females were heavier than males, and voles in lethal traps were heavier than voles in live traps, we do not consider that trap selection was biased between the sexes, given the fact that the weight difference between trap types was small. Live traps baited with apple slices and oats seems to perform equally well compared to lethal traps baited with peanut butter.

The main factor driving larval burden on bank voles in this study was location, indicating that environmental factors such as temperature and humidity, and overall host community, are more influential on burden than trap type. Ticks are sensitive to environmental factors such as temperature and humidity, and due to the relative surface to volume ratio, larvae are expected to be particularly sensitive to desiccation [65]. Both study areas differ in climate, and possibly the local climatic conditions are contributing to the overall abundance of ticks. Adults of *I*. *ricinus* quest for hosts higher in the vegetation, compared to subadult life stages [66], and tend to prefer larger mammalian hosts such as roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) [25, 67]. Performing a survey of cervids was outside the scope of this study, and it is possible that differences in local abundances of larger hosts may contribute to a higher abundance of larvae. Roe deer is more abundant in eastern Norway (Lifjell), whereas red deer is more abundant in western Norway (Lærdal) (Reimers et al., 1990 and Solberg et al., 2009, as cited in Handeland et al. [68]). A local survey of cervids or other large mammalian hosts may help elucidate whether adult tick host availability is influential on the larval infestation rates of voles.

Burden increased with temperature, and this is in accordance with other studies, in which above a certain tolerance limit in humidity, temperature can increase the activity of questing ticks [68–70], and can subsequently result in an increase in larval burden. The effect of temperature on tick burden was however, almost identical in both trap types, showing little influence of the trap type itself in relation to temperature. Although temperature had a positive effect on tick burdens, 2018 was not only warmer, but also drier than 2017 in both study areas (84 and 37 days of precipitation less in Lifjell and Lærdal, respetively; data: Norwegian Meteorological Institute), and this decrease in humidity may explain why overall burdens were lower in 2018 compared to 2017. The seasonal effects of tick abundance and rates of tick parasitism are well documented, and are dependent on life stage and locality. Questing nymphs and adults generally show a bimodal activity pattern during spring and autumn, and less activity during the summer [21, 71], whereas larvae generally show a single activity peak in summer to autumn [71–77]. Burdens on hosts tend to follow the same pattern for each life stage in rodents [55, 78]. Larval burdens in this study indeed showed a single spring peak in May, and a decline through summer (Fig 4). Norway being relatively cool and humid during the summer, ticks may not have been limited by dry conditions and high temperatures. The seasonal patterns found in this study might be due to ticks that have not found a host in spring, continuing questing during the summer until low autumn temperatures limited their activity.

## Conclusions

In the past decades, animal welfare has become increasingly important in animal studies [1, 2, 79–81], and efforts are continuously being made to reduce the discomfort or suffering animals might experience during research activities. Although killing the animals is sometimes necessary to achieve research goals, efforts should nonetheless be made to prevent or minimize avoidable suffering. Here we show that lethal traps are just as reliable as live traps in assessing tick burdens on rodents, and by avoiding the stress experienced by animals in live traps before euthanasia, lethal traps are the preferred choice. Given the fact that in this study, live captures were euthanized when collected, and both capture methods resulted in the death of the animal, we argue that in the scope of animal welfare, the use of lethal traps is the preferred option. An argument could be made that lethal traps might behave differently than live traps regarding trappability and random captures, thereby making them less suitable for ecological studies. However, our results show that both trap types are equally effective at capturing bank voles, and that intrinsic factors such as the weight or sex of the animal does not influence the likelihood of a vole to enter either trap type. Lethal traps are generally also cheaper than live traps, and usually smaller, lighter, less bulky, and quicker to operate, which further adds to the benefits of using lethal traps. In conclusion, we propose lethal traps should be considered as an alternative to live capture and euthanasia to prevent unnecessary suffering of the animals, without sacrificing reliability in tick burden assessment on rodents.

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## Author Contributions

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## Paper II

De Pelsmaeker, N, Korslund, L, Steifetten, Ø. High-elevational occurrence of two tick species, *Ixodes ricinus* and *I. trianguliceps,* at their northern distribution range., in review for *Parasites & Vectors*.

# High-elevational occurrence of two tick species, *Ixodes ricinus* and *I. trianguliceps,* at their northern distribution range

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## Abstract

**Background:** During the last decades a northward and upward range shift has been observed among many organisms across different taxa. In the northern hemisphere, ticks have been observed to have increased their latitudinal and altitudinal range limit. However, the elevational expansion at its northern distribution limit remains largely unstudied. In this study we investigated the altitudinal distribution of the exophilic *Ixodes ricinus* and endophilic *I. trianguliceps* on two mountain slopes in Norway by assessing larval infestation rates on bank voles (*Myodes glareolus*).

**Methods:** During 2017 and 2018, 1325 bank voles were captured during spring, summer and autumn at 10 trapping stations ranging from 100 m to 1000 m.a.s.l. in two study areas in southern Norway. We used generalized logistic regression models to estimate the prevalence of infestation of both tick species along altitude, considering study area, collection year and season, temperature, humidity and altitude interactions as extrinsic variables; and host body mass and sex as intrinsic predictor variables.

**Results:** We found that both *I. ricinus* and *I. trianguliceps* infested bank voles at altitudes up to 1000 m.a.s.l., which is a substantial increase in altitude compared to previous findings for *I. ricinus* in this region. The infestation rates declined more rapidly for *I. ricinus* compared to *I. trianguliceps*, indicating that the endophilic ecology of *I. trianguliceps* may provide shelter from limiting factors tied to altitude. Seasonal effects limited the occurrence of *I. ricinus* during autumn, but *I. trianguliceps* was found to infest rodents at all altitudes during all seasons of both years.

**Conclusions:** This study provides new insights into the altitudinal distribution of two tick species at their northern distributional limit, one with the potential to transmit zoonotic pathogens to both humans and livestock. With warming temperatures predicted to increase, and especially so in the northern regions, the risk of tick-borne infections is likely to become a concern at increasingly higher altitudes in the future.

**Keywords: Ticks. Altitude. *Ixodes ricinus*. *Ixodes trianguliceps*. Bank vole. *Myodes glareolus*. Distribution. Range shift.**

## Background

During the last decades increasing temperatures have been shown to have an impact on the distribution of species across a wide range of taxonomic groups [1, 2]. Depending on the species' adaptability to a warmer climate and the ability to disperse, species may either 1) increase their distribution range due to conditions becoming more favourable in areas that were previously inhospitable [3, 4], 2) contract in range as their habitat becomes increasingly unsuitable [5, 6], or 3) move away from areas that have become unsuitable towards habitats that have become more favourable [7, 8]. To date most species appear to expand their natural distribution range, and for the majority of species both a northward [9-12] and an upward [13-15] range expansion seems to be the most common movement pattern.

Along with other organisms, certain ectoparasites such as ticks have undergone similar range expansions [16-18]. Ticks are generally limited in their distribution by environmental factors such as temperature and humidity [19, 20], but as hematophagous parasites they also depend on the presence of adequate hosts [21]. With a changing climate, ticks have been observed to have increased their distribution range northwards in the northern hemisphere [22-24], and several studies have also demonstrated the occurrence of ticks to increasingly higher altitudes in central Europe [16, 25, 26]. For example, in Switzerland, *Ixodes ricinus* has been recorded at altitudes up to 1070 m.a.s.l. [27, 28], and in the Czech Republic it has shifted its altitudinal range limit up to 700 m.a.s.l. since the 1990s [29], and in one instance up to 1100 m.a.s.l. [30]. According to Danielova et al. (2006) it may survive up to 1200 m.a.s.l. or higher if the habitat is favourable [31]. Another tick species, *I. trianguliceps,* has been recorded at altitudes as high as 2300 m.a.s.l. in Switzerland [32], but because it does not quest in open vegetation like *I. ricinus*, it is likely to be less exposed to limiting environmental conditions, especially temperature, and hence able to survive in more extreme environments [21]. With any upward shift in range limit, the risk of exposure to tick-borne infections is likely to increase for both humans and livestock [26, 30], and is predicted to further increase in the future [33-35].

Most studies investigating the altitudinal dynamics of *I. ricinus* have occurred at its geographical center in Europe [16, 28, 31, 36], and very little is known about its altitudinal distribution at the northern range limit. Because the effects of climate change are expected to be stronger at higher latitudes [37], the increase in altitude expansion is likely to be more pronounced in Scandinavia. In Norway, *I. ricinus* has undergone a northward range shift similar to other Scandinavian countries [38-40], and it has been found as far north as 69°N [17] and is considered to be permanently established at 66°N [41]. It has also expanded upwards in altitude and based on direct and indirect multi-source analysis reported by citizens, hunters, health professionals and veterinarians, has been observed up to 583 m.a.s.l. [17]. Even so, data are scarce, and the occurrence of ticks in relation to altitude and its range boundary remain largely unknown. To the best of our knowledge no recent field studies have investigated the altitudinal distribution of ticks at their northern distributional limit in Europe. This study aims to determine the altitudinal distribution patterns of the generalist tick *I. ricinus* and the specialist tick *I. trianguliceps* in Norway, by studying the infestation rates of both species on a commonly found rodent, the bank vole (*Myodes glareolus*). Because of the nest-dwelling behavior of *I. trianguliceps*, we expect it to be less limited by altitude compared to *I. ricinus*.

## Methods

### *Study areas*

This study took place along two mountain slopes in Norway during 2017 and 2018 (Fig 1). The first study area was a southern facing mountain slope on the Lifjell massif (N59°26.495’ E9°0.603’), north of Bø i Telemark. It is characterized by a continental climate, located within the boreonemoral to southern boreal zone. Below the tree line, which is situated between 800 and 900 m.a.s.l., the vegetation is a blend of deciduous and coniferous forests with birch (*Betula pubescens*) and spruce (*Picea abies*) as the dominant tree species, and blueberries (*Vaccinium myrtillus*) as the dominant species at ground layer. Below the tree line the vegetation is mostly homogeneous. Above the tree line the vegetation is primarily dominated by common heather (*Calluna vulgaris*) and blueberries. Boulder fields occur frequently throughout the gradient, and the highest peak found on the plateau is 1288 m.a.s.l. Most of the data collection points were located on topographically open hillside. Temperature and precipitation normals for the study areas can be found in Appendix 3. The second study area was located in the Erdal valley (N61°05.817’ E7°24.688’) near Lærdalsøyri (hereafter referred to as Lærdal). It is a northern facing mountain slope close to the innermost part of the Sognefjorden fjord, approximately 150 km east of the western coastline. Due to its proximity to the fjord, the climate is more maritime than Lifjell, characterized by cooler summers and milder winters (Appendix 3). Sampling points were mostly located within the valley formed by the Erdal river. The tree line is here situated between 900 and 1000 ma.s.l., and below the tree line the vegetation consist primarily of homogeneous deciduous forests with birch and alder (*Alnus glutinosa*) as the dominant tree species. At ground layer the vegetation is dominated by blueberries, and different species of ferns and tall perennial herbs. Above the tree line common heather, dwarf birch (*Betula nana*), common juniper (*Juniperus communis*) and crowberry (*Empetrum nigrum*) are the dominant species. Surrounding the study area are several mountain peaks exceeding 1500 m.a.s.l.

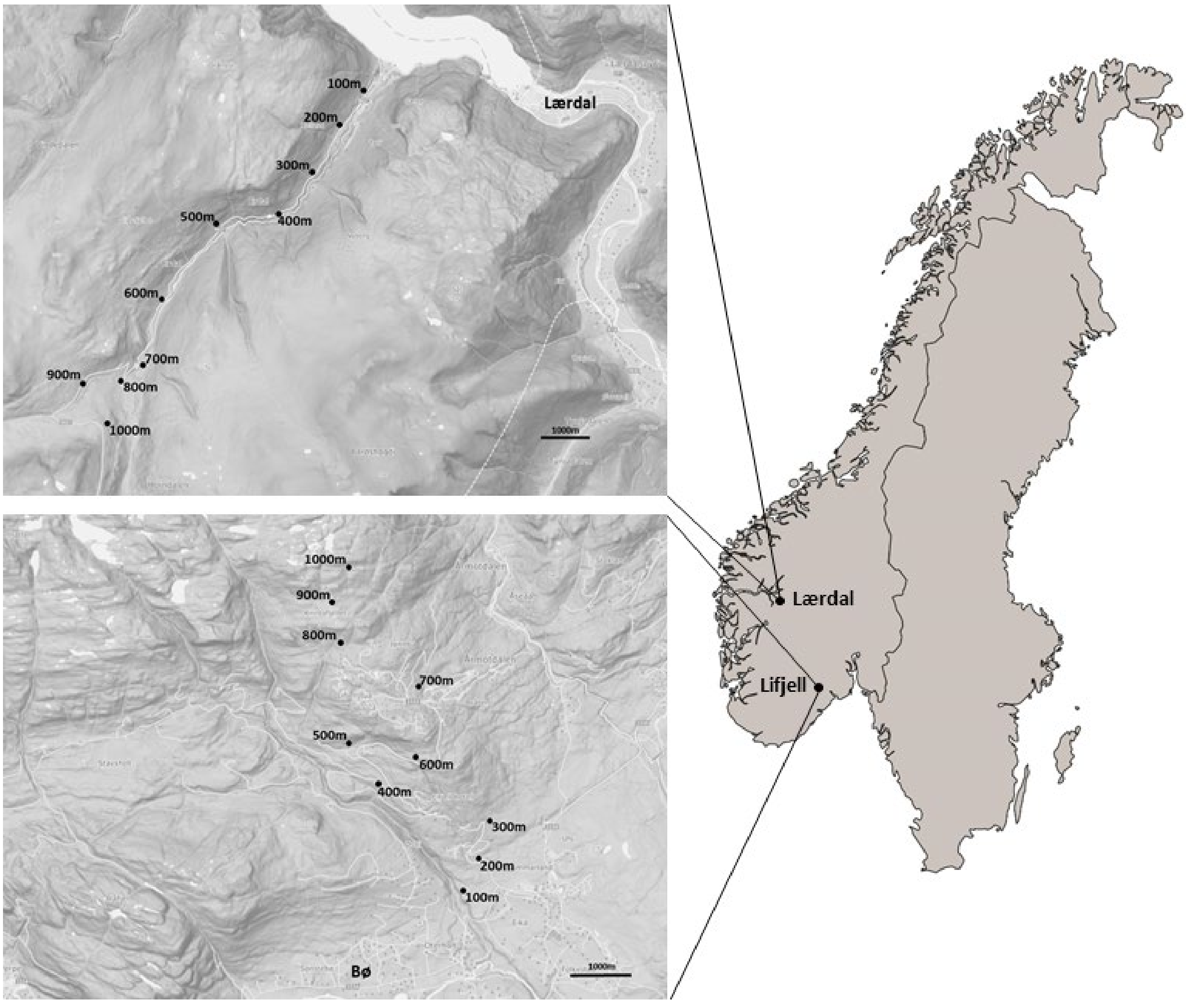

Fig 1. Location of the two study areas in Norway. Inset maps show the exact location of each trapping station along the altitudinal gradient in Lifjell and Lærdal. Each trapping station was placed at 100 m altitude intervals ranging from 100 m to 1000 m above sea level.

*Study species*

*I. ricinus* is the most common and the most studied tick species in Europe [20]. It ranges latitudinally from North Africa to Scandinavia, and longitudinally from Ireland to Russia [42]. It is a three-host tick feeding on a wide range of mammals, birds and reptiles [43, and sources cited herein]. Immature life stages (larvae and nymphs) parasitize small mammals in larger proportions [38, 44], whereas adults tend to feed on larger mammals [43]. It is common to deciduous, and to some extent, coniferous forests, and is dependent on sufficient temperature and humidity to be able to quest (actively seeking a host). It is sensitive to desiccation and temperature extremes [45].

*I. trianguliceps* occurs throughout Europe, ranging latitudinally from Italy up to well above the Arctic circle [46]. Contrary to *I. ricinus*, *I. trianguliceps* specializes primarily on rodents and small mammals during all life stages [47, 48]. It is endophilic (nest-dwelling), spending its off-host time within the burrows of its host to molt and quest [49]. It occurs in widely different habitats, ranging from meadows, peat bogs to dark-coniferous forests, mixed and deciduous forests, as well as high altitude treeless zones [44, 47]. It is generally considered to be one of the most cold resistant ticks of the genus *Ixodes* in the Palearctic region [47]. Since it rarely infests humans or livestock due to its host seeking behaviour [50], cases of tick-borne infection are considered exceptional [17], but it does contribute to maintaining the infection cycle of several pathogens between *I. ricinus* and their hosts such as *Borrelia burgdorferi* [51] and *Anaplasma phagocytophilum* [52].

The bank vole is a commonly found rodent throughout Europe and occurs virtually everywhere in Fennoscandia [53]. It is the most common rodent species in both study areas. Other small mammal species present are the field vole (*Microtus agrestis*), tundra vole (*M. oeconomus*), grey red-backed vole (*Myodes rufocanus*), wood mouse (*Apodemus sylvaticus*), yellow-necked mouse (*A. flavicollis*) and house mouse (*Mus musculus*). The common shrew (*Sorex araneus*), pigmy shrew (*S. minutus*) and water shrew (*Neomys fodiens*) are also found in these areas. The bank vole is a reservoir host for several tick-borne pathogens such as *B. burgdorferi* [54], *Babesia microti* [55], *Candidatus* Neoehrlichia mikurensis [56], and *A. phagocytophylum* [57]. It is possibly the most important host for all life stages of *I. trianguliceps* [44], and is heavily infested by the immature stages of *I. ricinus* [58, 59]. Because it was common at both study areas and at all altitudes, we chose to base our analysis on this species.

### *Host trapping*

Ticks are commonly collected by two methods: flag dragging for questing (unfed) ticks and host examination for feeding ticks. The flag dragging method is only applicable for the collection of exophilic species, and only when the vegetation is dry. During most days of data collection the vegetation was either partly wet or weather conditions did not allow for flag dragging. Additionally, as we aimed to include the infestations of *I. trianguliceps* in this study, we focused solely on the capturing of hosts. However, examining tick burdens accurately from live small mammals can be difficult [60], therefore we opted for a combination of lethal traps and euthanized live captures. In both study areas, 10 trapping stations were set up along a vertical gradient ranging from 100 to 1000 m.a.s.l. at every 100 m altitude interval. Bank voles have relatively small home ranges [61], and altitude stations were located several hundred meters from eachother, hence we can be reasonably confident that ticks collected from host at a certain altitude were also acquired in the immediate vicininy. At each trapping station, two plots of 20 traps each were deployed, one with live traps (Ugglan Special Nr. 2, Grahnab AB, Sweden, www.grahnab.se), the other with lethal traps (Rapp2 Mousetrap, www.rappfellene.no). The traps in both plots were arranged in a 4 by 5 grid, with 10 meters spacing between each trap. Live traps were baited with a slice of apple for hydration and whole oats for caloric value, and a lining of sawdust was provided on the trap floor as insulation. Lethal traps were baited with peanut butter for practical reasons as it is easily applied to the inside of the trap body. Trap type does not influence tick burden size on the captured animals [62]. At each altitude, the live and lethal plots were placed at approximately 100 m distance from each other, but in locations with similar vegetation structure and habitat characteristics. As humidity and temperature have a direct influence on tick activity, they are important drivers of phenological patterns and host-seeking behavior [63]. For this reason, a datalogger (TinyTag Plus 2 – TGP 4017, housed in a DataMate instrument cover ACS-5050) was placed in between the two plots at each trapping station, approximately 50 cm above ground level measuring air temperature and relative air humidity at a 1-hour interval for the duration of the trapping period. The height of 50 cm was chosen to capture the general variation in environmental conditions at each altitude. Trapping took place during the spring (May 20th – 30th), summer (July 20th-30th) and autumn (September 20th-30th) seasons of the years 2017 and 2018. As an exception, during the spring season of 2017, capturing took place from June 1st until June 7th, and only up to 700 m.a.s.l. in both areas, as there was too much snow to allow for the operation of traps earlier and above this altitude. During each trapping period, traps were checked every 24 hours, and the collection of trapped animals started at 8h30. When checking the trapping grids, triggered lethal traps were rebaited and reset. As examining live small mammals for ticks can be stressful and cause injury or death [64], all animals captured in live traps were euthanized by cervical dislocation of the head upon collection, and each individual was kept separately in a sealed and coded plastic bags. Activated live traps were emptied of the remaining contents, and new insulation and food was provided before resetting the traps. At the end of every collection day, all animals were place in a freezer at -20°C.

### *Laboratory processing*

At the end of every trapping season, captured bank voles were examined for ticks in the laboratory, as a full body post-mortem examination provides the highest degree of sensitivity [65] The day before the examination, the voles were removed from the freezer and left to thaw overnight at 10°C. The voles were examined one by one and taken out of the plastic bags individually. The empty bags were checked for ticks that might have dropped off. It was our observation that a number of ticks would drop off the host when the animals were placed in the freezer, possibly in an attempt to escape the extreme temperatures. Animals that were wet were dried with a hairdryer before examination. The hosts were checked for ticks starting with the head, ears and snout, followed by the neck and throat, back and abdomen, legs, feet and tail. Attached or detached ticks were removed from the host using tweezers. Collected ticks were removed and placed in a 1.5 mL plastic Eppendorf tube containing a 70 % ethanol solution (1 vial per host). Finally, a lice comb was brushed through the fur of the animal from tail to head (against the hair orientation), and the vole was shaken by the tail above a white plastic tray to collect any ticks that might have been missed during the examination. The hosts were then weighed to the nearest 10th of a gram, and the sex was determined. The minimum amount of time needed to process one animal was 20 minutes. After the examination the animals were bagged in new plastic bags and refrozen at -20°C.

Ticks were determined for life stage and species under a Zeiss Discovery V20 stereomicroscope, using an established publication key as reference [66]. Because more than 94 % and 75 % of all *I. ricinus* and *I. trianguliceps* collected were larvae, only the larval stage was included in the analysis.

*Data analysis*

The statistical analyses were performed using the software package R version 3.5.3 [67]. The analysis of *I. ricinus* larvae and *I. trianguliceps* larvae was performed separately. As is usually the case with tick presence on small mammals, neither species was evenly distributed on the hosts [68], and 13.8 and 82.0 % of the hosts had no *I. ricinus* and *I. trianguliceps* larvae present, respectively. We therefore chose to use the presence or absence of larvae as the response variable and applied generalized linear modeling with a binomial distribution, i.e. logistic regression. The probability of encountering a tick is defined as prevalence. As predictor variables we considered altitude (ranging from 100 to 1000 ma.s.l. – as a continuous variable), study area (Lifjell and Lærdal), collection year (2017 and 2018), season (spring, summer and autumn) and humidity (%). Because altitude and temperature were negatively correlated (Pearson correlation test, $t=-8.1578$, $df=1323$, $p<0.001$, $r=-0.219$), temperature per se was not used as a predictor variable to avoid introducing collinearity into the model. Two-way interactions between season and altitude, study area and altitude, year and altitude, study area and year, as well as year and season were included in the starting model. We also considered individual bank vole body mass (in grams) and sex (male or female) as intrinsic co-variates. Starting with a full starting model containing all variables and the two-way interactions listed above, we used a backward step model selection process to progressively remove non-significant predictors - by comparing the residual deviance and degrees of freedom of nested models using a Chi-square test - until an optimal model, containing only significant predictor variables (α=0.05), was found. To visually represent the infestation probabilities of both species across study areas, collection years and seasons, we created multiple regression line plots using the *ggplot2* package in R [69].

### *Ethics statement*

This study was carried out in strict accordance with regulations issued by the Norwegian Environment Agency, and a permit was provided prior to the start of the sampling (Miljødirektoratet, reference number: 2017/4651) for the duration of the trapping period. The trapping protocol for animal capture was approved by the Animal Ethics Committee of the Department of Nature, Health and Environment (University of South-Eastern Norway). All efforts were made to minimize animal suffering.

## Results

During 2017 and 2018, a total of 43920 trap nights was performed, capturing 1325 bank voles (976 and 349 captures in 2017 and 2018, respectively). Altogether 5372 tick larvae were collected from the trapped voles at all altitudes of the gradient. Larvae of *I. ricinus* were 7.4 times more numerous compared to *I. trianguliceps* (88.1 and 11.9 % of ticks, respectively). Larval burdens ranged from 1-100 ticks for *I. ricinus* (mean = 3.6 ± 0.5 (95% CI)), and from 1-29 for *I. trianguliceps* (mean = 0.5 ± 0.1 (95% CI)), and more than half of the bank voles (57.4 %) were infested with at least one larva of either tick species. For *I. ricinus* and *I. trianguliceps,* respectively, 46.8 % and 18.0 %, of voles carried at least one larva. Of all the voles, 7.3 % were infested with at least one larva of both tick species. For a total number of ticks collected from voles at every altitude see Fig 2. Mean burdens can be found in Appendix 1. An overview of all captured host species and infestation rates is listed in Appendix 2.

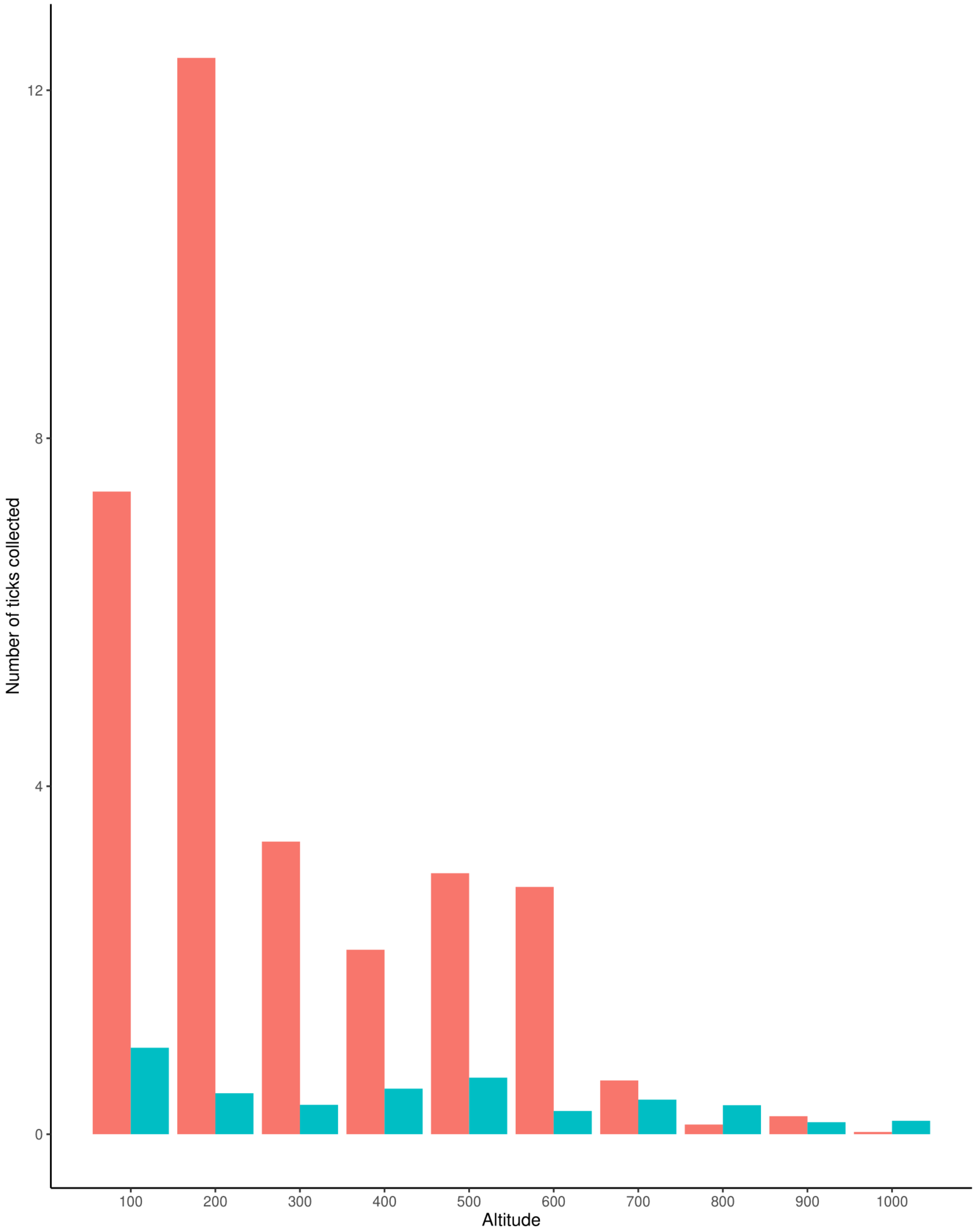

Fig 2. Total number of tick larvae collected from bank voles per altitude, for both study areas, years and seasons combined (red bars, *I. ricinus*; blue bars, *I. trianguliceps*).

### *I. ricinus*

The best model shows that there was a clear negative overall effect of altitude on the prevalence of *I. ricinus* presence (z=-3.954, p<0.001), and a significant interaction between altitude and season ($c^2$=12.3, df=2, p=0.002) illustrate that this effect was stronger in autumn than in spring and summer (Table 1, Fig 3). In addition, a significant interaction between altitude and site ($c^2$=11.1, df=1, p<0.001) shows that the negative altitude effect was stronger in Lærdal than in Lifjell (Table 1). This is mainly caused by the fact that, while the prevalence is low at 1000 m at both sites, the prevalence at the lower altitudes was higher in Lærdal (close to 1) compared to Lifjell (around 0.75 or less) (Fig 3). The model further shows that the overall prevalence of *I. ricinus* was highest among voles captured in spring, and lower, but not significantly so, among voles captured in summer (z=-1.14, p=0.25) and significantly lower among voles captured in autumn (z=-2.65, p=0.08) (Table 1, Fig 3). The prevalence of *I. ricinus* was overall lower in 2018 (z=-2.06, p=0.04), but the season effect, with lower prevalence in autumn, was much stronger in 2017 than in 2018, as indicated by a significant interaction between year and season (($c^2$=26.7, df=2, p<0.001) (Table 1, Fig3). Finally, the significant interaction between site and season shows that the season effect was stronger in Lærdal than in Lifjell ($c^2$=12.3, df=2, p=0.002) with especially the autumn prevalence being lower in Lærdal (z=-2.73, p=0.006) (Table 1, Fig 3).

While host weight did not affect the prevalence of *I. ricinus* ($c^2$=0.106, df=1, p=0.75), it was significantly more often found on male than on female host, resulting in a significant sex effect in the best model ($c^2$=15.0, df=1, p<0.001).

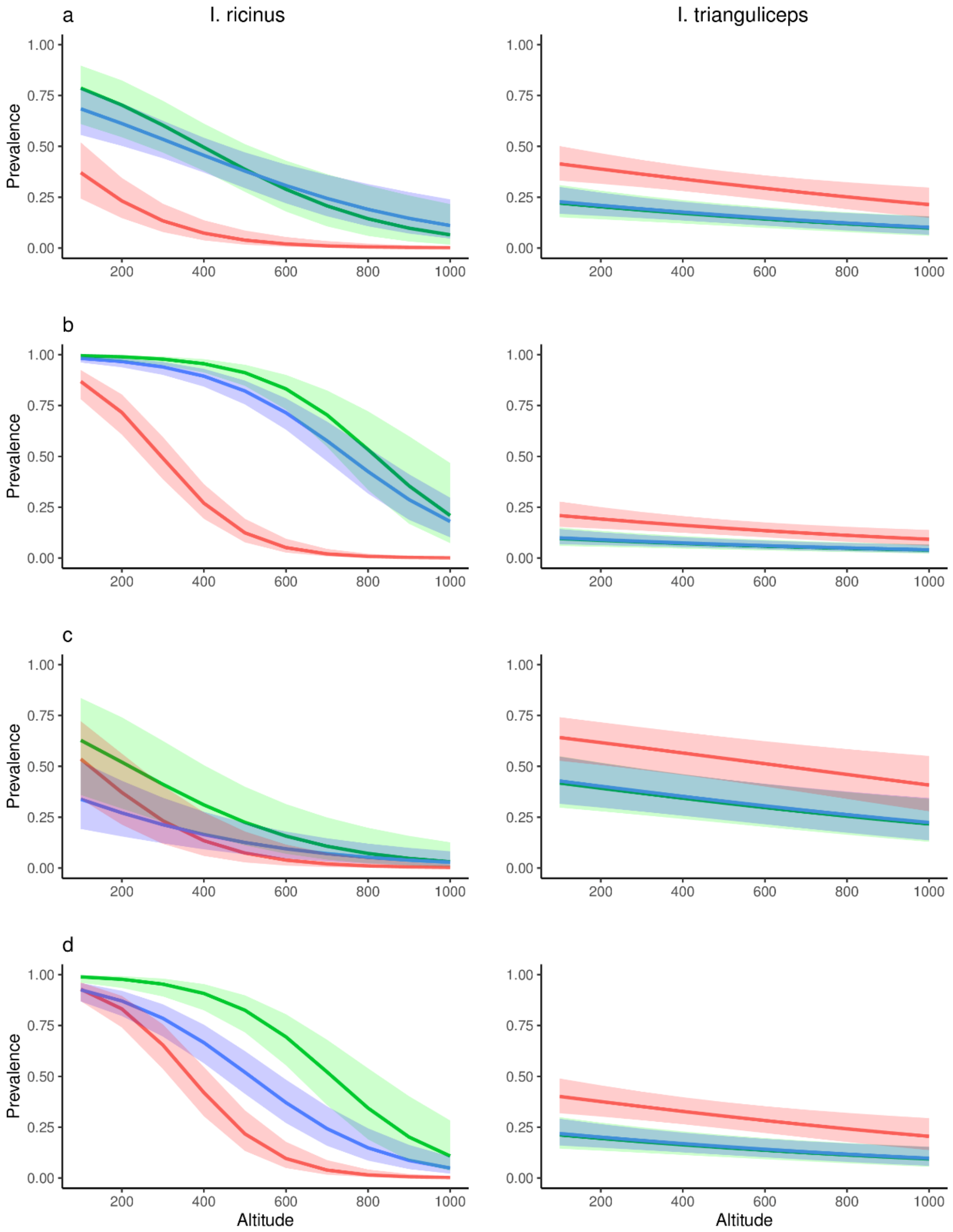

Fig 3. Tick prevalence on bank voles along altitude in a) Lifjell 2017, b) Lærdal 2017, c) Lifjell 2018, d) Lærdal 2018. A prevalence of 1.0 represents a certainty of encountering at least one tick. Lines: spring (green), summer (blue), autumn (red). Ribbons represent standard error 95 % confidence intervals. For *I. trianguliceps,* spring and summer curves are closely overlapping. (green), summer (blue), autumn (red). Ribbons represent standard error 95 % confidence intervals. For *I. trianguliceps,* spring and summer curves are closely overlapping.

### *I. trianguliceps*

The best model, explaining the prevalence of *I. trianguliceps* on bank voles, ended up being much simpler than that for *I. ricinus* (Table 1). Also for this species there was a clear negative overall effect of altitude on the prevalence ($c^2=11.7$, df=1, $p<0.001$) (Table 1). However, the overall prevalence was much lower than what was found for *I. ricinus* (Fig 3). The overall prevalence of *I. trianguliceps* was significantly lower in Lærdal than in Lifjell ($c^2=34.7$, df=1, $p<0.001$) and significantly higher in 2018, compared to 2017 ($c^2=27.0$, df=1, $p<0.001$) (Table 1, Fig 3). The model further shows a clear effect of season ($c^2=35.7$, df=2, $p<0.001$) with the overall prevalence of *I. trianguliceps* being higher among voles captured in autumn, and with an equal prevalence in spring and summer (Table 1, Fig 3).

Neither host weight ($c^2=2.66$, df=1, $p=0.10$) nor host sex ($c^2=1.11$, df=1, $p=0.29$) did affect the prevalence of *I. trianguliceps* presence on bank voles.

Table 1. Parameter estimates for the infestation prevalence on bank voles indicating the probability to be infested with at least one tick, using a binomial distribution model. Baseline for study area is Lifjell, year 2017, spring season and male voles. Variables in bold indicate significant values.

| | Estimate | Std. Error | *z* - value | *p* -value |
|---|---|---|---|---|
| *I. ricinus* larvae | | | | |
| Intercept | 1.7429 | 0.5243 | 3.32 | 0.001 |
| **Study area Lærdal** | **4.2441** | **0.5824** | **7.29** | **< 0.001** |
| **Altitude** | **-0.0044** | **0.0011** | **-3.95** | **< 0.001** |
| **Sex Female** | **-0.6724** | **0.1719** | **-3.91** | **< 0.001** |
| **Year 2018** | **-0.7789** | **0.3780** | **-2.06** | **0.039** |
| Season Summer | -0.6515 | 0.5701 | -1.14 | 0.253 |
| **Season Autumn** | **-1.6039** | **0.6048** | **-2.65** | **0.008** |
| Altitude : Season Summer | 0.0012 | 0.0012 | 1.03 | 0.305 |
| Altitude : Season Autumn | -0.0023 | 0.0014 | -1.59 | 0.112 |
| **SiteLærdal : Altitude** | **-0.0029** | **0.0009** | **-3.39** | **0.001** |
| Study area Lærdal : Season Summer | -0.7681 | 0.4656 | -1.65 | 0.099 |
| **Study area Lærdal : Season Autumn** | **-1.5402** | **0.5646** | **-2.73** | **0.006** |
| Year 2018 : Season Summer | -0.6665 | 0.4740 | -1.41 | 0.160 |
| **Year 2018 : Season Autumn** | **1.4512** | **0.4858** | **2.99** | **0.003** |
| *I. trianguliceps* larvae | | | | |
| Intercept | -1.1554 | 0.2461 | -4.70 | < 0.001 |
| **Study area Lærdal** | **-0.9831** | **0.1690** | **-5.82** | **< 0.001** |
| **Altitude** | **-0.0011** | **0.0003** | **-3.37** | **0.001** |
| **Year 2018** | **0.9297** | **0.1785** | **5.21** | **< 0.001** |
| Season Summer | 0.0412 | 0.2257 | 0.18 | 0.855 |
| **Season Autumn** | **0.9133** | **0.2114** | **4.32** | **< 0.001** |

## Discussion

Climate change is one of the most important drivers of the expansion of ticks in Europe [70], and poses a health risk to both humans and animals [30]. In fact, tick-borne diseases were identified by several European Ministries of Health to be the biggest health risk resulting from climate change [71]. Ticks have increased their distribution range northwards in Scandinavia [22, 72], and whereas *I. ricinus* was historically assumed to be limited to coastal areas in Norway, it is now increasingly occurring further inland, with both adult and subadult stages being transported by hosts such as birds [73] or large mammals [43], and its latitudinal distribution limit has shifted approximately 400 km northward since the early 1980s [17]. Based on multi-source analysis, the highest recorded observation for *I. ricinus* has up until now been 583 m.a.s.l. [17], but here we show that both *I. ricinus* and *I. trianguliceps* were present at an altitude of at least 1000 m.a.s.l. in two locations located in the eastern and western part of Southern Norway. We can therefore assume that in this region, both species are established at altitudes up to 1000 m.a.s.l.

### *The effect of altitude on prevalence*

Although both tick species decreased in abundance as altitude increased, both were found at all altitudes of the gradient. We expected altitude to have a stronger effect on *I. ricinus* compared to *I. trianguliceps*, and our results support this. Although in both study areas, at low altitudes, the prevalence with *I. ricinus* larvae was generally higher than that of *I. trianguliceps*, infestation of the former showed a stronger decline with increasing altitude during all three seasons. The prevalence of *I. trianguliceps* larvae also declined with altitude, but less so. The stronger decline for *I. ricinus* is mostly a result of a much higher prevalence of this species at lower altitudes. The strongest decline in *I. ricinus* occurred during the autumn season, where the prevalence at the top of the gradient (above 900 m.a.s.l.) was zero, while still a substantial part of hosts were infested at lower altitudes. The generally higher prevalence found for *I. ricinus* at lower altitudes compared to *I. trianguliceps* might be a direct consequence that *I. ricinus* is able to make use of more types of hosts, such as cervids. Indeed, field experiments have shown that excluding roe deer (*Capreolus capreolus*) as tick hosts, reduced the larval burden of *I. ricinus* on small rodents, but not that of *I. trianguliceps* [74]. At higher altitudes it is possible that both tick species are limited by the lower overall temperatures and/or the shorter snow free season, but that *I. trianguliceps* is less affected by such environmental conditions due to its endophilic nature. It is reasonable to expect that *I. ricinus*, which requires a minimum temperature to initiate questing [75], cannot be active for as many days at 1000 m compared to at lower altitudes. This would reduce the probability of finding a host, leading to a lower population density. *I. trianguliceps* however, seeking a small mammal host within its burrows, is not as limited by low ambient temperatures [76] and should thus be more capable of completing its life cycle at higher altitudes. This distinction might explain the difference in regression slope between the two tick species in our study.

Daniel (1993) found that suitable tick hosts are generally present at altitudes above the distribution limit of ticks, hence other factors, such as microclimatic conditions (e.g. temperature or humidity), are probably limiting the occurrence of ticks above a certain altitude [19]. Materna et al. (2008) found that this range limit is not necessarily restricted by survival per se [30]. In fact, ticks (especially engorged females, imported by the movement of large hosts) can survive above their natural distribution limit for quite a long time [19]. The molting success of immature stages or oviposition by females also doesn't seem to be limited by altitude. According to Materna et al. (2008), it appears that a reduced hatching success of the egg batch is what is hindering the developmental cycle of ticks, thus preventing them from establishing permanent populations [30]. The presence of feeding larvae on rodents at high altitudes in this study indicates that the two species are capable of completing their life cycle. Because we only investigated infestation rates up to 1000 m.a.s.l. the actual altitudinal range limit of *I. ricinus* and *I. trianguliceps* in these areas of Norway is still unknown, but we have demonstrated *I. ricinus* to be present at altitudes well exceeding that of 583 m.a.s.l. found by Jore et al. (2011) [17]. A follow-up study at altitudes above 1000 m.a.s.l. may reveal the actual distribution limit of ticks in these areas.

### *The effect of season on prevalence*

The prevalence of *I. ricinus* larvae was generally highest in spring, compared to the other seasons at all altitudes, with the exception of Lifjell in 2017, where summer and spring prevalence was more or less equally high. Other studies in Switzerland [25], Italy [77] and Great Britain [46] showed that *I. ricinus* larvae have a bimodal activity pattern, peaking in spring and autumn, or only peaking in early summer [78]. In our study, spring and summer of 2017 had almost equally high prevalences, but seemed to drop before the summer of 2018. In 2018 both spring and summer were somewhat drier, and although humidity was not a reliable predictor of infestation for the two species, this could explain the lower levels of *I. ricinus* in summer of 2018. Overall it seems that *I. ricinus* larvae are not considerably inhibited by summer drought in this region, and the relatively cool and humid conditions enable it to continue questing for hosts throughout the summer until low temperatures in autumn inhibit their activity. *I. ricinus* depends on a relatively humid environment for questing and survival [79]. Surprisingly, higher infestation rates were found in Lærdal despite having a lower relative humidity than Lifjell. As humidity was not correlated with altitude in neither study areas, it is unlikely to have had a limiting effect on the presence of *I. ricinus* throughout the seasons at any altitude.

Larvae of *I. trianguliceps* were overall less frequent in both locations compared to *I. ricinus*, but were infesting voles during all active seasons of the year and at all altitudes of the gradient. In contrast to *I. ricinus*, *I. trianguliceps* was most frequently found on bank voles in autumn. *I. trianguliceps* is dependent on a very humid environment [80]. We had no data on the relative humidity inside the burrows for *I. trianguliceps*, but it could potentially be higher than above the surface [81, 82], and it is possible that generally more rain and lower temperatures in autumn leads to more favorable conditions for *I. trianguliceps* at this part of the year.

### *The effect of host on prevalence*

Vole sex was a determining factor in prevalence of *I. ricinus* larvae, with males having higher infestations than females. Male voles have larger home ranges [83] and have higher mobility than females [84]. As *I. ricinus* quest for a host in open vegetation [63, 85], they are dependent on a passing host in order to attach [20], especially in the case of larvae as this life stage disperses the least, and their distribution is highly clumped. As males roam around more than females, the probability of encountering ticks is higher, and this could explain the sex difference in the infestation rates of *I. ricinus*. No significant difference was found in infestation with *I. trianguliceps* larvae between male and female voles. As *I. trianguliceps* is a nidiculous tick [45], and as both male and female voles visit these burrows, both sexes are potentially equally exposed to infestations of *I. trianguliceps* larvae. This is in concurrence with the findings of Cotton and Watts (1967) in Britain, where *I. trianguliceps* infested male and female voles equally [46].

## Conclusions

Historically, *I. ricinus* was believed to be limited to coastal regions in Norway [86], but has since been found to expand further inland [17]. With ticks expanding to higher altitudes as well, there is an increasing risk for tick bites in such areas, and therefore an increased risk of human infection with tick-borne diseases. *I. ricinus* is a well known vector of several European zoonotic pathogens in humans [87-89], and between 400 and 500 cases of tick-borne Lyme borreliosis are diagnosed annually in Norway (Norwegian Surveillance System for Communicable Diseases - http://www.msis.no). Also, Norwegian sheep farmers tend to let the sheep graze freely outside of the winter season, and mortality due to tick-borne anaplasmosis has been found to be damaging to the sheep husbandry [90]. Thus, the occurrence of tick-borne infections has both social and economical consequences, but so far these problems have been restricted to low altitude areas. Even though *I. ricinus* occurrence decreases with altitude, the prevalence of tick-borne pathogens that are potentially dangerous to humans and livestock, does not necessarily decrease with tick abundance. Danielova et al. (2006) found that although fewer ticks are present at higher altitudes, the prevalence of *B. burgdorferi* s.l. (the causal agent of Lyme disease) in those ticks did not decrease, and they retained the same infection rates at high altitudes [31].

Although human cases of tick-borne infections via *I. trianguliceps* are rare [17], this species helps to maintain the prevalence of pathogens in small mammal host populations by transferring tick-borne pathogens between hosts [52, 91]. Although several recent studies have investigated the occurrence of tick-borne pathogens in *I. ricinus* ticks in Norway [e.g. 92-94], no investigation has yet focused on disease dynamics in relation to altitude. In combination with the results of this study, an in-depth survey along altitudinal gradients investigating the prevalence of pathogens in ticks could provide valuable insights into the actual disease risk to humans and livestock in Norwegian mountains. As projections indicate a continuing trend in warming temperatures, particularly in the northern regions [95], the northward and upward progression of ticks under the influence of climate change is expected to continue in the future [70]. This will further expose humans and livestock to tick-borne infections in areas where no infection risk was present before.

Appendix 1

Table 2 Mean larval burden sizes ± SD of *I. ricinus* and *I. trianguliceps* found on bank voles at every altitude during 2017 and 2018 in Lifjell and Lærdal. NA indicates either that no voles were captured, or that not enough data was available to compute a standard deviation.

| | | **Lifjell** | | | | | | **Lærdal** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **2017** | | | **2018** | | | **2017** | | | **2018** | | |
| | | **Spring** | **Summer** | **Autumn** | **Spring** | **Summer** | **Autumn** | **Spring** | **Summer** | **Autumn** | **Spring** | **Summer** | **Autumn** |
| **100 m** | ***I. ric.*** | 0.3 ± 0.7 | 0.8 ± 1.0 | 0.2 ± 0.5 | 19.0 ± NA | 0.0 ± 0.0 | 4.8 ± 5.3 | 84.0 ± 25.2 | 19.7 ± 20.3 | 10.5 ± 9.8 | 43.3 ± 32.6 | 6.3 ± 4.6 | 2.1 ± 1.6 |
| | ***I. tri.*** | 1.3 ± 2.2 | 0.2 ± 0.7 | 1.6 ± 3.4 | 0.0 ± NA | 1.0 ± 1.4 | 4.6 ± 6.5 | 0.3 ± 0.6 | 0.1 ± 0.3 | 0.6 ± 15 | 0.0 ± 0.0 | 0.2 ± 0.4 | 1.6 ± 2.8 |
| **200 m** | ***I. ric.*** | 15.0 ± 10.9 | 8.1 ± 7.4 | 2.9 ± 1.6 | NA | NA | NA | 23.8 ± 9.8 | 13.2 ± 9.1 | 8.9 ± 4.9 | 31.6 ± 25.0 | 19.4 ± 19.1 | 4.3 ± 3.0 |
| | ***I. tri.*** | 0.4 ± 1.0 | 0.0 ± 0.0 | 0.9 ± 1.1 | NA | NA | NA | 0.0 ± 0.0 | 0.3 ± 0.7 | 0.4 ± 08 | 0.5 ± 0.9 | 0.1 ± 0.3 | 1.4 ± 3.5 |
| **300 m** | ***I. ric.*** | 1.1 ± 0.7 | 1.5 ± 2.4 | 0.6 ± 1.4 | NA | NA | NA | 19.5 ± 13.7 | 3.1 ± 3.3 | 0.7 ± 1.1 | 10.6 ± 6.9 | 2.8 ± 4.0 | 0.5 ± 0.7 |
| | ***I. tri.*** | 0.0 ± 0.0 | 0.0 ± 0.2 | 1.6 ± 2.8 | NA | NA | NA | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.7 ± 14 | 0.2 ± 0.4 | 0.2 ± 0.4 | 0.4 ± 1.0 |
| **400 m** | ***I. ric.*** | 0.9 ± 1.3 | 0.9 ± 1.9 | 0.0 ± 0.0 | 0.5 ± 1.0 | 0.0 ± 0.0 | 0.0 ± NA | 8.1 ± 7.3 | 1.6 ± 1.6 | 0.2 ± 0.6 | 9.5 ± 15.3 | 1.0 ± 1.4 | 0.3 ± 0.6 |
| | ***I. tri.*** | 0.3 ± 0.6 | 0.6 ± 1.1 | 2.3 ± 4.9 | 0.5 ± 0.6 | 0.0 ± 0.0 | 4.0 ± NA | 0.0 ± 0.0 | 0.0 ± 0.2 | 0.3 ± 05 | 0.3 ± 0.5 | 0.6 ± 1.2 | 1.2 ± 3.3 |
| **500 m** | ***I. ric.*** | 0.5 ± 0.8 | 0.1 ± 0.4 | 0.0 ± 0.2 | 0.5 ± 1.2 | NA | NA | 14.5 ± 8.0 | 4.1 ± 2.6 | 0.0 ± 0.2 | 12.7 ± 10.5 | 2.6 ± 3.2 | 1.0 ± 1.8 |
| | ***I. tri.*** | 0.3 ± 0.6 | 0.5 ± 0.9 | 1.5 ± 3.9 | 1.2 ± 1.3 | NA | NA | 0.1 ± 0.3 | 0.3 ± 1.4 | 0.5 ± 1.9 | 0.1 ± 0.3 | 1.4 ± 4.3 | 1.6 ± 2.3 |
| **600 m** | ***I. ric.*** | 0.2 ± 0.4 | 1.1 ± 1.2 | 0.1 ± 0.3 | 0.0 ± NA | 0.0 ± 0.0 | NA | 13.8 ± 12.9 | 1.6 ± 1.5 | 0.0 ± 0.0 | 2.1 ± 2.6 | 0.4 ± 0.9 | 0.2 ± 0.4 |
| | ***I. tri.*** | 0.4 ± 0.7 | 0.1 ± 0.9 | 0.5 ± 1.1 | 0.0 ± NA | 0.5 ± 0.7 | NA | 0.1 ± 0.4 | 0.0 ± 0.0 | 0.1 ± 0.3 | 0.3 ± 0.8 | 0.5 ± 0.9 | 0.7 ± 1.1 |
| **700 m** | ***I. ric.*** | 2.8 ± 7.9 | 0.0 ± 0.0 | 0.0 ± 0.0 | NA | 0.0 ± NA | NA | 2.0 ± 3.3 | 1.4 ± 1.9 | 0.0 ± 0.0 | 0.3 ± 0.6 | 0.4 ± 0.9 | 0.0 ± 0.0 |
| | ***I. tri.*** | 0.0 ± 0.0 | 1.3 ± 5.9 | 0.2 ± 0.5 | NA | 0.0 ± NA | NA | 0.3 ± 0.8 | 0.1 ± 0.5 | 0.1 ± 0.4 | 0.0 ± 0.0 | 0.2 ± 0.4 | 1.1 ± 3.0 |
| **800 m** | ***I. ric.*** | NA | NA | 0.0 ± 0.0 | 0.0 ± 0.0 | NA | 0.0 ± 0.0 | NA | 0.2 ± 0.4 | 0.0 ± 0.0 | 0.2 ± 0.4 | 0.4 ± 0.9 | 0.2 ± 0.4 |
| | ***I. tri.*** | NA | NA | 0.2 ± 0.7 | 0.0 ± 0.0 | NA | 2.0 ± 1.4 | NA | 0.1 ± 0.4 | 0.5 ± 1.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 1.0 ± 2.4 |
| **900 m** | ***I. ric.*** | NA | 0.1 ± 0.4 | 0.0 ± 0.0 | NA | NA | NA | NA | 0.9 ± 0.9 | 0.0 ± 0.0 | NA | 0.0 ± 0.0 | NA |
| | ***I. tri.*** | NA | 0.1 ± 0.4 | 0.3 ± 0.8 | NA | NA | NA | NA | 0.0 ± 0.0 | 0.1 ± 0.6 | NA | 0.2 ± 0.7 | NA |
| **1000 m** | ***I. ric.*** | NA | 0.2 ± 0.4 | 0.0 ± 0.0 | NA | 0.0 ± NA | 0.0 ± | NA | 0.0 ± 0.0 | 0.0 ± 0.0 | NA | 0.0 ± NA | 0.0 ± 0.0 |
| | ***I. tri.*** | NA | 0.0 ± 0.0 | 0.4 ± 0.5 | NA | 0.0 ± NA | 0.0 ± NA | NA | 0.0 ± 0.0 | 0.2 ± 0.4 | NA | 0.0 ± NA | 0.0 ± 0.0 |

Appendix 2

Table 3 Total number of hosts captured of each mammal species during 2017 and 2018, relative infestation rates (%) and mean burdens ± SD of *I. ricinus* and *I. trianguliceps*. NA indicates that not enough captures were made to compute a standard deviation.

| | | ***I. ricinus*** | | ***I. trianguliceps*** | |
|---|---|---|---|---|---|
| **Host species** | **No. of captures** | **prevalence** | **mean burden** | **prevalence** | **mean burden** |
| *Apodemus flavicollis* | 1 | 100.0 | 62.0 ± NA | 100.0 | 1.0 ± NA |
| *Apodemus sylvaticus* | 23 | 56.5 | 6.4 ± 13.5 | 34.8 | 0.9 ± 1.7 |
| *Microtus agrestis* | 130 | 18.5 | 1.7 ± 7.9 | 6.9 | 0.1 ± 0.3 |
| *Microtus oeconomus* | 26 | 50.0 | 1.5 ± 2.5 | 23.1 | 0.3 ± 0.7 |
| *Mus musculus* | 1 | 100.0 | 11.0 ± NA | 0.0 | 0.0 ± NA |
| *Myodes glareolus* | 1325 | 46.8 | 3.6 ± 9.0 | 18.0 | 0.5 ± 1.8 |
| *Myodes rufocanus* | 88 | 19.3 | 0.5 ± 1.4 | 19.3 | 0.6 ± 2.0 |
| *Neomys fodiens* | 2 | 100.0 | 1.0 ± 0.0 | 50.0 | 7.5 ± 10.6 |
| *Sorex araneus* | 1733 | 30.0 | 3.3 ± 12.5 | 20.8 | 0.8 ± 3.4 |
| *Sorex minutus* | 16 | 31.3 | 0.4 ± 0.7 | 56.3 | 3.0 ± 4.4 |

Appendix 3

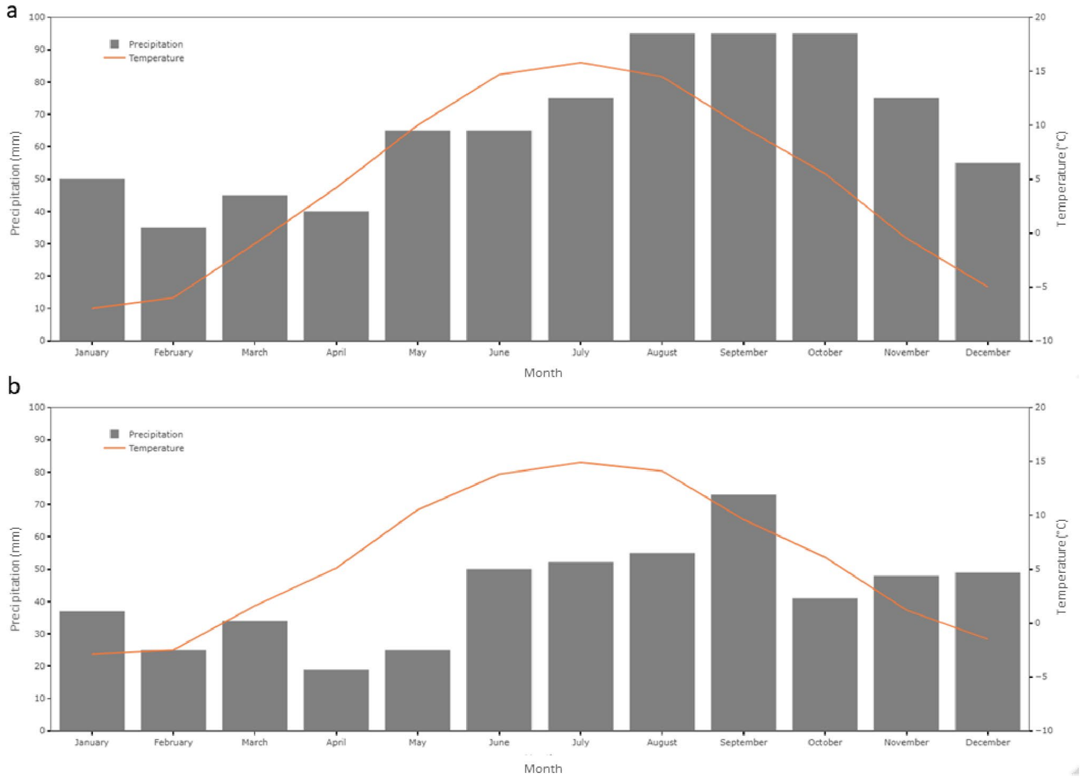

Fig 4. Temperature and precipitation normals in a) Lifjell, and b) Lærdal. Data: Norwegian Meteorological Institute.

**Declarations**

Ethics statement - Permission for capturing small mammals was issued by the Norwegian Environment Agency (Miljødirektoratet, reference number: 2017/4651).

Consent – All contributing authors are in agreement on the contents of the manuscript and to its submission.

Data Availability - Data set is deposited at usn.figshare.com. doi:10.23642/usn.12917960.

Competing interests – All contributing authors declare that there are no conflicts of interest involved in this study.

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Author's contributions – Nicolas De Pelsmaeker: data collection, formal analysis, manuscript writing; Lars Korslund: formal analysis, manuscript writing, supervision; Øyvind Steifetten: manuscript writing, supervision.

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## Paper III

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