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The effect of ticks (*Ixodes ricinus*) on early survival of roe deer (*Capreolus capreolus*) fawns

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Preface

This thesis is written at the Faculty of Environmental Sciences and Natural Resource Management (MINA), and constitutes the final part of my master's degree in Ecology at the Norwegian University of Life Sciences (NMBU).

This study was made in cooperation with the Swedish Infrastructure for Ecosystem Science (SITES), in this case at Grimsö Wildlife Research Station. I would like to thank Petter Kjellander and Madeleine Christensson at Grimsö Wildlife Research Station for letting me be a part of the research team collecting data for the project “The interplay between ticks, tick-borne diseases and wildlife in Sweden”, and for giving me the opportunity to do this topic for my master thesis, and for helping me with data and information. It was really a unique experience and I learned so much about ticks, roe deer and wildlife in Sweden during fieldwork and while writing my thesis.

I would especially like to thank Leif Egil Loe for being my supervisor, and for guiding me through hours of challenging analyses and writing, always staying positive and enthusiastic about the thesis and throughout the working process. Your thoughts and feedback have been very much appreciated. I would also like to thank Atle Mysterud for providing much needed feedback and additional supervising regarding my writing.

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Of course it would not be possible to do this paper without the effort from all the fieldworkers at Bogesund and Grimsö throughout the years for gathering information and data used in this project. Thanks to all of the volunteers and other people associated with the project whom I learned a lot from during fieldwork and who helped me with my data, especially Ulrika Alm Bergvall, Giulio Grandi, Anders Jarnemo, Linda Höglund and Charlotte Öhman.

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Abstract

Parasites can be a burden for many animals and may in some cases affect the survival and reproduction of their hosts. The effect ticks may have on the early survival of roe deer (*Capreolus capreolus*) fawns is less known. Knowing what affects mortality of neonatal roe deer is important to estimate reproduction and growth potential in populations. A total of 213 roe deer fawns were captured in Sweden at two different study sites (Bogesund and Grimsö). Fawns were sampled for ticks (*Ixodes ricinus*) at initial capture and at recaptures during the first 30 days after birth. I first investigated variation in tick burden between individuals, and if ticks induced a physiological response in roe deer fawns (i.e., a body temperature increase). Thereafter I tested if ticks affected early survival using Cox Proportional Hazard models.

The number of ticks on fawns below the age of 30 days was generally low, where 45% of total observations ($n = 552$) had 10 ticks or less, and 9% of observations had no ticks attached at all. Number of ticks increased with increasing age and body mass. Body temperature increased significantly with increasing number of ticks attached to the fawn, demonstrating a physiological response to tick attachments.

A total of 68 out of the 213 fawns died within their first 30 days (32%). Of total mortality, 6% died from unknown causes, 16% died from natural causes and 78% died from predation. In the univariate Cox Proportional Hazard models, number of ticks and body temperature had no effect on survival. Further, the univariate models showed that fawn mortality increased over time, where risk of dying increased with age in the first 30 days, indicating that mortality is high in the first month after birth. In addition, habitat had an effect on survival, where the risk of dying increased in open field habitat compared to forest. When separating study sites, fawns with a higher body mass than expected for their age were found to have a decreased risk of dying at Bogesund, whereas neither individual traits nor habitat characteristics at capture sites were found to affect survival in Grimsö. Fawns that died from predation also had a higher risk of dying in open field habitat, while fawns that died from natural causes had a decreased risk of dying with higher body mass.

Low amount of ticks on young fawns may relate to the hiding behaviour in the first period after birth, and higher activity level and more exposure to questing ticks is considered to be the main reason for the tick increase as the fawn gets older and heavier. The increase in body temperature caused by ticks may indicate a fever response to pathogens associated with ticks. However, neither amount of ticks nor body temperature had any effect on survival of the fawn. The effect of ticks might be disguised in populations with high mortality rates. It is recommended to further investigate the effect of ticks on the survival of roe deer fawns along a predation pressure and tick abundance gradient, in addition to the effect of tick-borne pathogens that were not addressed in this study.

Sammendrag

Store mengder parasitter kan være en krevende byrde for mange dyr, og i mange tilfeller kan de påvirke både overlevelse og reproduksjon hos vertsdyret. Effekten av flått på tidlig overlevelse hos rådyrkalv (*Capreolus capreolus*) er lite kjent. Det er viktig å vite hva som påvirker dødeligheten til svært unge kalver for å kunne estimere reproduksjon og vekstpotensial i populasjoner.

Til sammen ble 213 rådyrkalver merket på to forskjellige studieområder i Sverige (Bogesund og Grimsö). Kalvene ble undersøkt for flått (*Ixodes ricinus*) ved merking (første fangst) og ved gjenfangst i løpet av de 30 første dagene etter fødsel. Jeg testet først om det fantes variasjon i flåttmengde mellom individer, deretter om flått induiserte en fysiologisk respons hos kalvene (dvs. en økning i kroppstemperatur). Deretter testet jeg om flått påvirket tidlig overlevelse ved hjelp av Cox Proportional Hazard-modeller.

Kalver i sin første levemåned hadde et generelt lavt flåttantall, hvor 45% av de samlede observasjonene ($n = 552$) hadde 10 flått eller mindre, og 9% av observasjonene hadde ingen flått. Antall flått økte med økende alder og kroppsmasse. kroppstemperatur økte signifikant med økende antall flått, og demonstrerte en fysiologisk respons på flåttmengde hos kalven.

Totalt 68 av de 213 kalvene døde innen de første 30 dagene (32%). Av total dødelighet døde 6% av ukjente årsaker, 16% døde av naturlige årsaker og 78% døde som følge av predasjon. I de univariate Cox Proportional Hazard-modellene hadde antall flått og kroppstemperatur ingen effekt på overlevelse. Videre viste modellene at dødelighet økte over tid i den første levemåned, ved at risiko for å dø økte med økende alder. I tillegg var det en økt risiko for å dø i åpne habitater (open field) i forhold til skogshabitater. Ved å teste studieområdene hver for seg ble det funnet at kalver med høyere kroppsmasse enn forventet for deres alder, hadde en redusert risiko for å dø ved Bogesund, mens det ikke fantes noen individuelle egenskaper eller habitategenskaper ved fangststedene som påvirket overlevelse ved Grimsö. Kalver som døde av predasjon, hadde også en høyere risiko for å dø i åpne habitater, mens kalver som døde av naturlige årsaker, hadde en redusert risiko for å dø med høyere kroppsmasse.

Lite bevegelse i trykkeperioden den første tiden etter fødsel kan være grunnen til et lavt antall flått på kalvene, og høyere aktivitetsnivå og mer eksponering for vertssøkende flått anses å være den viktigste årsaken til at antall flått øker med alder og kroppsmasse. Økningen i kroppstemperatur forårsaket av flått kan indikere en respons som følge av feber på grunn av patogener forbundet med flått. Imidlertid hadde verken flåttmengde eller kroppstemperatur noen effekt på kalvenes overlevelse. Det er mulig at effekten av flått ikke kommer frem i populasjoner med høy dødelighet. Det anbefales videre å undersøke effekten av flått på overlevelse til rådyrkalv i populasjoner med lite predasjon og mye flått, i tillegg til effekten av flått-bårne patogener, ettersom det ikke ble undersøkt i denne studien.

Table of contents

| | |
|---|----|
| 1 Introduction | 1 |
| 2 Material and methods | 4 |
| 2.1 Study sites | 4 |
| 2.2 Study species | 6 |
| 2.2.1 Roe deer: fawn behaviour and mortality causes | 6 |
| 2.2.2 Ixodes ricinus: ecology and effects on mammals | 8 |
| 2.3 Field methods and sampling | 9 |
| 2.3.1 Locating roe deer fawns | 9 |
| 2.3.2 Marking and recaptures of fawns | 10 |
| 2.4 Data organisation and definition of variables | 11 |
| 2.5 Statistical analyses | 13 |
| 3 Results | 16 |
| 3.1 Number of ticks increase with age and weight | 16 |
| 3.2 Body temperature increase with number of ticks and age | 18 |
| 3.3 Survival analyses | 18 |
| 3.3.1 Proportion of dead and death causes | 18 |
| 3.3.2 Difference in weight between study sites | 19 |
| 3.3.3 Univariate models | 19 |
| 3.3.4 Multivariate models | 23 |
| 4 Discussion | 25 |
| 4.1 Number of attached ticks increase with age and body mass | 25 |
| 4.2 Higher tick numbers increase fawn body temperature | 26 |
| 4.3 No effect of ticks and body temperature increase on survival | 26 |
| 4.4 Factors affecting risk of dying from predation and natural causes | 28 |
| 5 Conclusion | 30 |
| References | 31 |

1 Introduction

Parasites can be a burden for many animals and may in some cases affect the survival and reproduction of their hosts (Albon et al. 2002; Lehmann 1993; Tompkins & Begon 1999). Hair and weight loss (Glines & Samuel 1989), reduced feeding or behavioural change (Hagemoen & Reimers 2002) and paralysis (Hanson et al. 2007) are just some of the detrimental effects parasites can cause on their hosts, which may affect young hosts more (Hawlana et al. 2006; Lehmann 1993). Such adverse effects of parasites may not only be related to them being high in numbers, but also by transferring pathogens causing disease or infections (Aguirre et al. 1999; Stuen 2007). The emergence of ticks and tick-borne diseases is of growing concern. The common tick *Ixodes ricinus* is a vector of many pathogens such as *Anaplasma* spp., *Babesia* spp., *Borrelia* spp. and Tick-borne encephalitis virus (family Flaviviridae) (Jongejan & Uilenberg 2004; Lindquist & Vapalahti 2008; Zintl et al. 2003). These pathogens do not only affect the health of livestock and wildlife (Bram 1983; Stuen 2007), but are also a threat to human health (Gritsun et al. 2003; Rudenko et al. 2011).

Roe deer (*Capreolus capreolus*) are known to be important hosts for adult reproducing individuals of *I. ricinus* in Sweden (Jaenson et al. 1994) and elsewhere in Europe (Kiffner et al. 2010), but the extent to which ticks affect roe deer is less well known. In Scandinavia, ticks are expected to increase in abundance and distribution as a result of climate change, creating more favourable conditions for ticks (Jaenson et al. 2012; Jore et al. 2014; Lindgren et al. 2000). Growing populations of hosts, such as the roe deer, contribute to this increase (Jaenson et al. 2012; Mysterud et al. 2016). Adult roe deer can have large amounts of ticks attached to their body, head and neck (Carpi et al. 2008; Talleklint & Jaenson 1997; Vor et al. 2010), and disease or parasite infestation may affect the risk of dying (Aguirre et al. 1999; Stuen et al. 2001). Tick transmitted pathogens such as *Anaplasma phagocytophilum* may cause fever and loss of appetite in mammals (Engvall & Egenvall 2002; Stuen et al. 2013), whereas *Babesia divergens* can cause anaemia but also fever in cattle (Zintl et al. 2003). Importantly, *Babesia capreoli* may potentially be fatal to roe deer (Hinaidy 1987). Wild roe deer get infected by the pathogens carried by ticks naturally (de la Fuente et al. 2008b; Overzier et al. 2013), which has been suggested to influence survival of fawns (Stuen et al. 2001). Although a high parasite load on roe deer would increase the probability of disease and infection by these pathogens, negative effects of the tick load itself is rarely investigated and mainly mentioned in other studies focusing on pathogens. Little negative effects on adult roe deer has been found (Talleklint &

Jaenson 1997; Vor et al. 2010), but few studies has so far investigated the effect of tick load on young roe deer fawns.

Several factors are expected to contribute to the expected effect of ticks on fawn survival. Firstly, roe deer fawns adopt a hiding strategy to avoid predation the first period after birth (Lent 1974), which involves low activity and less movement in their home range (Andersen et al. 2004; Espmark 1969). Fawns will hide at random places in the vegetation, while their mother is feeding elsewhere, only to interact with the fawn for nursing few times a day in the early period (Espmark 1969). Ticks have low mobility and do questing to find their host. The questing behaviour involves crawling onto ends of leaves and grasses to grab potential hosts that pass by. Being hiders, very young roe deer fawns may seldom be exposed to questing ticks as they move less through the vegetation, thereby largely avoiding the negative direct effects associated with the tick such as blood loss (Pfäffle et al. 2009), and potentially disease. Secondly, natural predation rates of fawns are often high in roe deer populations. On average, 67% of total mortality for ungulates is caused by predation, and in some cases the predation can account for up to 100% of the mortality in areas with predators (review in (Linnell et al. 1995)). Reports have been made of red fox (*Vulpes vulpes*) predation on roe deer fawns accounting for up to 88% of known mortality (Jarnemo & Liberg 2005). Even if ticks have the capacity to affect natural mortality rate it could still remain undetected in populations with high neonatal loss to predators. Finally, if some fawns despite the hiding strategy pick up a substantial number of ticks, it is possible that a high parasite burden might impact survival at the neonatal stage as suggested by Stuen et al. (2001).

Knowing what affects mortality of neonatal roe deer is important to estimate recruitment and growth potential in populations (Albon et al. 2002; Gaillard et al. 1998b), and it is therefore important to investigate if ticks may affect roe deer fawn survival in addition to predator loss. A total of 213 VHF-collared roe deer fawns were captured in Sweden at two separate study sites, Bogesund and Grimsö. Age was determined using a scoring system based on behavioural assessment and umbilical cord appearance. Fawns were recaptured and sampled for ticks (all *I. ricinus*) in their first 30 days after birth in four consecutive years to investigate the effect of ticks on early fawn survival. In addition, body mass, body temperature and habitat at capture site was recorded. When receiving a VHF mortality signal, death causes were determined by field examinations in combination with necropsy protocols to be able to investigate the effect of ticks for predation or natural deaths.

I predict that:

- 1) Young roe deer fawns will have few ticks because they are hidiers and move less through vegetation. Number of ticks will increase with age and body mass of the fawn as they become more mobile and hence more exposed to questing ticks in the vegetation.
- 2) Body temperature will increase with number of ticks as fawns with high amount of ticks may display a temperature response from having large amounts of parasites, either because of stress-related effects or from potential fever.
- 3) Tick burden may therefore have a negative effect on the survival of roe deer fawns as it could indicate some disease, stress or blood loss, and by this increase the risk of dying.

2 Material and methods

2.1 Study sites

Data collection were conducted at two separate study sites in south-central and south-eastern Sweden, at Grimsö and Bogesund respectively (Figure 1). The Grimsö study site is based around the Grimsö Wildlife Research Station (centred at, 59°43'43"N, 15°28'19"E) which is located in a mostly coniferous forest area with dominating species being Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*). Mixed and deciduous forest make up a small proportion of the forest in the area. Annual precipitation is on average 758 mm (years 1961-2014; SMHI 2017) and temperatures ranging from -13.9 to 24 °C (year 2014; SMHI 2017). Forest constitutes the largest portion of the area (74%) with approximately 23% wetland (bogs, mires and fens) and lakes (Kjellander & Nordström 2003). Agricultural land only make up less than 5% of the land use within the study site. Predators on roe deer in the area include red fox (*Vulpes vulpes*) which is considered the main predator of fawns (Jarnemo et al. 2004). Known populations of wolf (*Canis lupus*) and eurasian lynx (*Lynx lynx*) exist in the area and may also be potential predators of roe deer fawns (Linnell et al. 1995). Lynx predation on roe deer is common in the area (Davis et al. 2016). Other important prey species for predators are moose (*Alces alces*), wild boar (*Sus scrofa*) and badger (*Meles meles*). Roe deer density in the area is 0.9 ind/km² (mean), and fox density is 0.08 ind/day observed (mean) (Öhman 2017). The amount of ticks in the area is generally low (average 0.1 ±0.8 larvae and 0.3 ±0.7 nymphs per 100 m) compared to more southern and coastal parts of Sweden, as the study site represents the edge of tick distribution (Gandy 2015; Jaenson et al. 1994).

Bogesund (centred at, 59°24'28"N, 18°11'18"E) is located in the archipelago east of Stockholm, where the western part of the peninsula constitutes the study site (12.5 km²). Dominating forest type is coniferous and mixed forest with larger proportion of deciduous species than in Grimsö. Dominating forest species are Norway spruce, Scots pine and birch (*Betula* spp.) with more common occurrences of willow (*Salix* spp.) and oak (*Quercus robur*) (Kjellander et al. 2006). Bogesund has a coastal climate with average annual precipitation of 594.2 mm (years 1961-2014; SMHI 2017) and temperatures ranging from -8.7 to 24.8 (year 2014; SMHI 2017). Although primarily forest (approx. 65%), Bogesund has a larger agricultural area (approx. 25%) than Grimsö (Kjellander et al. 2006), with fields for grazing cattle and horses, and otherwise livestock fodder production. Bogesund has high numbers of red fox (mean 0.2 ind/day observed) (Öhman 2017) which is considered as the main predator

of fawns. Other predators such as lynx and wolf are unknown and unlikely but cannot be excluded as possible predators in the area. Other prey species notable in the area is a high number of hares (*Lepus* spp.), wild boar, and some observations of moose. Bogesund has a clearly higher roe deer density than Grimsö (mean 5.18 ind/km²) (Öhman 2017). The coastal areas in Sweden generally have high amounts of ticks, and Bogesund has very high tick numbers compared to Grimsö (average 15.4 ±27.8 larvae and 7.8 ±8.1 nymphs per 100 m; Gandy 2015).

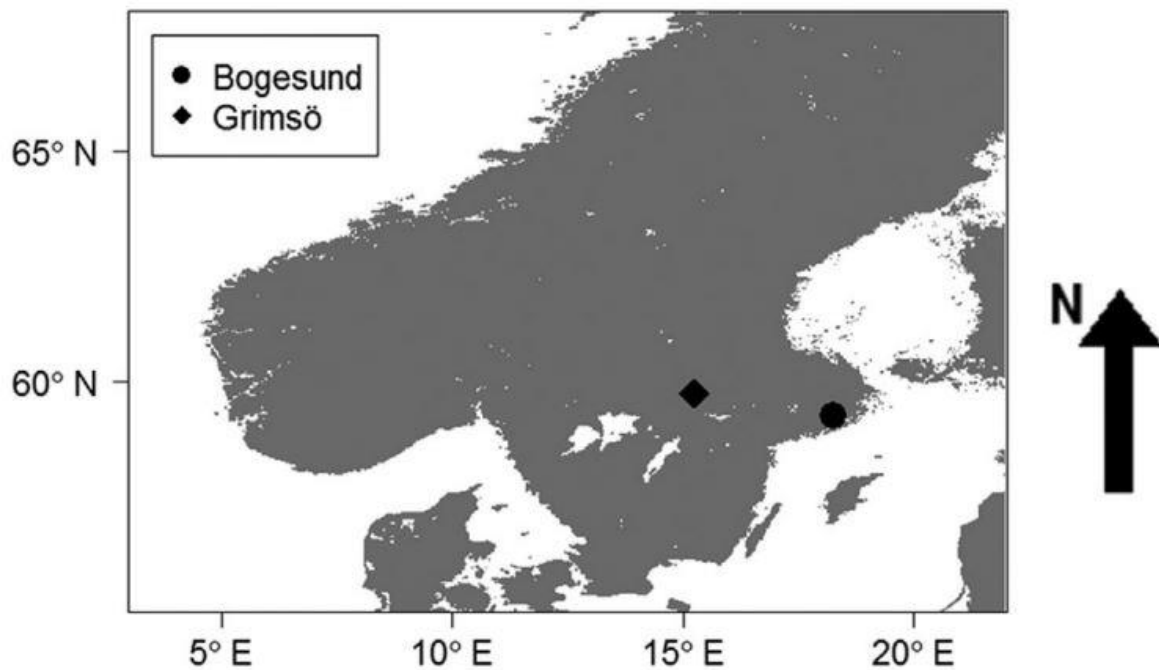


Figure 1. Map of southern part of Scandinavia showing the location of the study sites Bogesund and Grimsö. Map retrieved from Davis et al. (2016).

2.2 Study species

2.2.1 Roe deer: fawn behaviour and mortality causes

Roe deer display a synchronized birth strategy with a birth peak between mid-May to early June in Europe (Gaillard et al. 1993; Jarnemo 2004a; Plard et al. 2013). The first 30 days after birth represents the main part of the fawn hiding behaviour, where escape from their bed site is less likely (Andersen et al. 2004; Fitzgibbon 1990). In the first period after birth, fawns display a freezing response (bradycardia) when startled, which causes the fawn to lay completely still (Espmark & Langvatn 1985; Lent 1974). Eventually, as the fawn gets older, bradycardia ceases, and escape response is initiated more often (Espmark 1969; Espmark & Langvatn 1985). In the first 10 days after birth, all fawns seem to stay in their bed site when approached by humans (Andersen et al. 2004). At three weeks, 25% escape, and further 50% escape when they reach 25 days old. At one month's age, most of the fawns escape when approached by humans, and at two months all escape from their bed sites with few exceptions (Andersen et al. 2004). As they get older, fawns become more active, and there is a relationship between an increase in fawn activity with age and weight as the hiding behaviour ceases gradually (Espmark 1969; Espmark & Langvatn 1985; Fitzgibbon 1990). In the first days, fawns rely highly on their mother for feeding, however grazing of plant material starts early, when the fawn is between six to eight days old (Espmark 1969).

The main death cause for neonatal roe deer is predation (Linnell et al. 1995) and especially by the red fox. Predation pressure from red fox on fawns varies greatly, with total mortality ranging from 74% to up to more than 90% in some cases (Aanes & Andersen 1996; Jarnemo et al. 2004; Jarnemo & Liberg 2005; Linnell et al. 1995). The risk of predation is determined by many factors. Fawns born either very early or very late related to the birth peak have a higher predation risk (Jarnemo 2004a). Fawn body mass and early body growth have been shown to be positively related to early survival (Gaillard et al. 1997). Body mass determines the ability to initiate the escape response (for example if encountered by fox), where a higher body mass will start this response earlier as the fawn is more developed (Espmark & Langvatn 1985). Different types of habitat are also linked to differences in predation risk. The fawn chooses bed site after cover and concealment (Linnell et al. 1999; Van Moorter et al. 2009). When hiding, the fawn uses all types of areas and habitats within the maternal home range, which is suggested to make it harder for predators to predict the position of the hiding fawn (Linnell et al. 1999). Survival seems to be determined by the availability of good bed sites within the maternal home

range (Van Moorter et al. 2009), and there seems to be a higher risk of red fox predation for fawns residing in open grassland than for those who use more forest type of habitat (Aanes & Andersen 1996). In a forest-farm mosaic landscape, there is often a trade-off between good feeding habitat for the mother and survival of the fawn (Jarnemo 2004a). Compared to a denser habitat such as the forest, open grasslands would increase detectability of the hiding fawns for the fox as it can easier observe mother-fawn interactions (Jarnemo 2004b; Panzacchi et al. 2009; Panzacchi et al. 2010). In addition, high productive areas may result in shorter prey movements and thereby increasing predictability of fawn position (Panzacchi et al. 2009). For sex, there is contradicting evidence of predation risk. In most studies reporting a difference, males seem to have the higher risk of predation, arguably because of behavioural differences (Aanes & Andersen 1996). Other studies seem to find no difference in red fox predation between sexes (Jarnemo et al. 2004). In addition, other important factors such as fox prey switching in years when rodent population numbers are low, may increase predation rates on fawns (Kjellander & Nordström 2003). So called “family effects” can in turn impact predation risk of fawns, where studies show that predation risk increases after the fawns siblings are found by fox (Panzacchi et al. 2009). Survival related to family effects is also found in populations without predators (Gaillard et al. 1998a)

Natural deaths have been reported to make up about 5 - 7% of known fawn mortality (Aanes & Andersen 1996; Jarnemo 2004a), depending on predation pressure. In areas without predators, natural mortality is higher (Andersen & Linnell 1998). High roe deer densities may ultimately lead to fawn starvation as less food resources are available for the fawn in a growing population (Vincent et al. 1995), or by poor maternal care (abandonment and malnutrition) (Monestier et al. 2015). Hypothermia is also a common death cause for fawns (Andersen & Linnell 1998; Jarnemo 2004a), and a small proportion may die from organ abnormalities (found in this study) or disease. Adult roe deer have been reported to die from systemic diseases, and bacterial or parasitic infections in Sweden (Aguirre et al. 1999) and it is reasonable to believe that the same diseases can affect mortality of young roe deer fawns. Stuen et al. (2001) reported the death of a roe deer fawn in Norway, where the fawn had died from a bacterial infection (*Escherichia coli* septicaemia) following heavy infestation of ticks and development of anaplasmosis, a disease caused by the bacterium *Anaplasma phagocytophilum* carried by ticks. This seems to be the first case of reported possible anaplasmosis contributing to the death of a roe deer fawn.

2.2.2 *Ixodes ricinus*: ecology and effects on mammals

The most common tick species in Sweden is the hard tick *Ixodes ricinus*, also known as sheep tick or castor bean tick. In Sweden, *I. ricinus* are found mostly in the southern part (all of Götaland county and large parts of Svealand county), but also extending far north in Norrland county along the eastern coast and inland lakes (Figure 2; Jaenson et al. 1994).

I. ricinus life cycle is complex and have four development stages (egg, larvae, nymph and adult) (Oliver 1989). To be able to moult into the next life stage, ticks require a blood meal from a host. *I. ricinus* are not host-specific but commonly use rodents, smaller carnivores and cervids to feed (Mejlon & Jaenson 1997). Larvae and nymphs use mainly smaller to medium sized hosts, whereas adult ticks use larger animals (Oliver 1989). As adults, the female tick needs to have a blood meal to be able to reproduce, whereas the male tick only has smaller blood meals and remains on the host to search for females to mate with. Ticks find their host by performing behaviour called questing (Lees & Milne 1951). It involves the tick crawling upon the tips of vegetation to attach to potential hosts that are walking by. Questing is considered the main method for ticks to find their hosts, where they stay at vegetation tips for many days (Lees & Milne 1951). The most important factors determining presence and abundance of *I. ricinus* is moisture and temperature in addition to adequate vegetation cover (Milne 1950; Tack et al. 2012). Questing behaviour is dependent on temperature, air humidity and soil moisture (Medlock et al. 2008; Schulz et al. 2014). The ticks need high moisture to avoid water loss and desiccation, and show increased mortality at high temperatures when moisture becomes less available (Bertrand & Wilson 1996). For this reason, ticks thrive in habitats with stable microclimates and well developed shrub layers, thereby favouring closed forest habitats over open habitats (Bertrand & Wilson 1996; Estrada-Peña 2001).

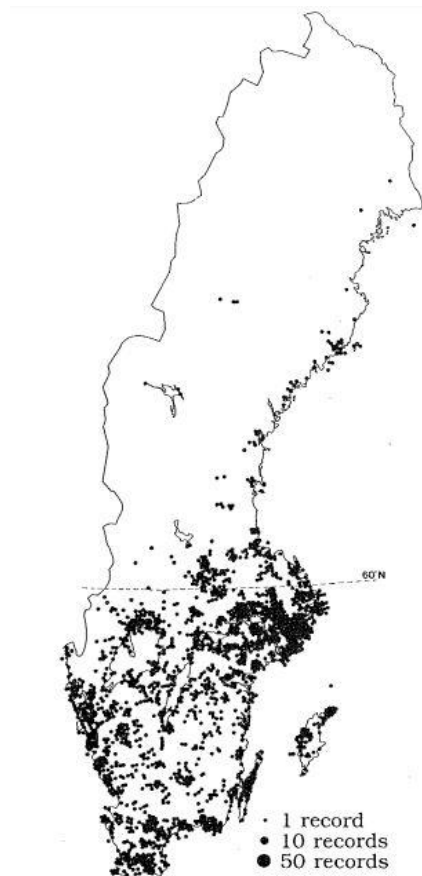


Figure 2. Map over Sweden showing records of *Ixodes ricinus* distribution. Each record represent one or more ticks recorded at one locality or host, by one collector. Map and text retrieved from Jaenson et al. (1994).

As for many other tick species, *I. ricinus* is associated with, and a carrier of many vector borne diseases (de la Fuente et al. 2008a), facilitated by its hosts such as roe deer and red deer (*Cervus elaphus*) (Mysterud et al. 2016). One of the diseases known to affect domestic ruminants is Tick-borne fever (TBF) which is caused by the bacterium *Anaplasma phagocytophilum* transferred by *I. ricinus* (Stuen et al. 2013). Cattle and sheep are main hosts for *A. phagocytophilum* where the bacteria cause high fever (Stuen et al. 2011), and have further been found to affect the growth of lambs (Grøva et al. 2011). Species of *Anaplasma* infect roe deer naturally and prevalence in fawn blood samples at the study sites is 20% (based on 77 fawns, Kjellander unpublished data). *Babesia divergens* is a protozoan blood parasite known to be the causing agent of bovine babesiosis or “redwater fever” and is also zoonotic to humans (Zintl et al. 2003). Of the *Babesia* spp. prevalent in roe deer, *B. venatorum* and *B. capreoli* are the most relevant for roe deer health (Andersson et al. 2016), which have been observed both fatal but also asymptomatic in roe deer (Hinaidy 1987). *Babesia* spp. prevalence in blood sampled from fawns are estimated to be 38% at the study sites (based on 77 fawns, Kjellander unpublished data). Previous studies have found that there is a higher prevalence of *Babesia* spp. in roe deer blood samples (fawns, juveniles and adults in total) at Grimsö than in Bogesund (Andersson et al. 2016). Here, *B. divergens* was not found in the blood sampled at the study sites.

2.3 Field methods and sampling

Collection of data happened as a part of the RåFäst project (The interplay between ticks, tick-borne diseases and wildlife in Sweden) conducted by the Swedish University of Agricultural Sciences (SLU) at Grimsö Wildlife Research Station. All fawns were captured, sampled and marked in the study period from mid-May to end of June, with some recaptures in July, over the years 2013 to 2016.

2.3.1 Locating roe deer fawns

Finding the fawns were based on locating and observing marked and unmarked roe deer females. Marked females had either Global Positioning System (GPS)-collars or Very High Frequency (VHF)-collars so they could be located and assessed for pregnancy or recent birth based on scoring belly and udder size. Unmarked females were mainly located while driving around or walking in the study area at dusk or dawn, checking fields and observing or following females with suspected birth. Fawns were then located by either seeing the fawn itself, or

searching the area where the female had been for a long time expressing watchful/suspicious behaviour. If the fawn was not found within 30 minutes of searching, the area was left and searched another day to avoid too much disturbance of the females.

2.3.2 Marking and recaptures of fawns

Field workers consisted of employees from Grimsö and otherwise volunteers, mostly students or internship-workers from SLU or other universities. All field workers had passed the web-based ethical course on research animals “Swedish Legislation, Ethics and Animal Use” and completed practical training to get marking permission issued by Naturvårdsverket and Uppsala Djurforsöksetiska Nämnd. After capturing a fawn, the time spent for marking and sampling was not to exceed 30 minutes and preferably done in as short time as possible.

The roe deer fawns were fitted with VHF-collars weighing 60 grams (about 4% of body mass at birth (estimated birth weight of 1500 grams (Jarnemo 2004a); Followit Sweden AB) with a mortality signal function to allow for recaptures and survival checks. The collar was designed to expand as the fawn gets older, and fall off after weathering at constructed weak parts of the collar. Fawns were recaptured for measurements between one to four times (including first marking) resulting in different observation number for each fawn. Sometimes it was not possible to do recapture if the fawn was escaping and impossible to catch, or had died. Fawns were checked for survival signal and position every day, also after recapturing was ended (when fawns became too mobile for recaptures of humans by foot). Young fawns are easily picked up by the observer as they have not yet started their escape behaviour when encountered in their first early weeks. For older fawns, it was sometimes necessary to use a capture net to avoid escape and to be able to do the sampling. Fawns less than a day old (determined by white or red/bloody umbilical cord) were only fitted with VHF-collar and not handled, measured or sampled before second recapture to avoid abandonment from the mother. Body temperature was measured by rectal temperature in °C (hereafter referred to as body temperature) using a standard temperature measuring device inserted in the fawns rectum with some Vaseline to ease insertion. Temperature was measured at the start of capture before other measurements were taken to get a good estimate of the fawn’s temperature before handling. Temperature was also taken after marking, before weighing and leaving the fawn back in its bed site. Only first measurement of temperature was used for analyses. Ticks were searched for by looking, and feeling the ears, nose, head and belly. Found ticks that were attached were then counted accurately if the total tick number was 10 or below, or noted in tick number categories of 11-

20, 21-30, 31-50 or 51-100. Ticks found were separated into belly/body and head categories. All life stages of ticks were noted (larvae, nymph and adult male or female). The fawn was weighed at the end of marking in a canvas bag of known weight with a standard hanging hook weighing scale. After weighing, the weight of the bag and collar (60 g) was subtracted to get fawn weight.

Date of death was determined after finding the fawns body or collar after receiving a mortality signal from the VHF-collars. Fawns may have disappeared and died before the collar was found and mortality signal received, but it is impossible to know this date of death for sure. Therefore, the mortality signal in combination with finding the body or collar had to be fulfilled in order to set the date of death as some fawns could not be accounted for (lost signal, collar out of reach) but then reappeared days or months later. Cause of death was determined by examination in field together with necropsy protocols done by veterinaries at Grimsö Research Station or at the faculty of veterinary medicine and animal science at SLU.

2.4 Data organisation and definition of variables

Variables used in analyses were age (in days), ticks (count number of ticks), ticks.res (higher or lower amount of ticks than expected for age), mass (body mass in grams), mass.res (higher or lower weight than expected for age), habitat type (open field/forest), cause of death (predation, natural, other and human), body temperature in °C (temp), sex (M/F), study site (Bogesund/Grimsö), and survival status (0/1).

Age: Age (in days) of the fawn was calculated based on a scoring system after Öhman (2017), based on state of appearance of the umbilical cord combined with behaviour assessment. Siblings that were captured and marked at the same date but were scored with different age after the calculations, were adjusted to the lowest age calculated for either of the siblings. The scoring system to determine age did not involve fawn weight, as it would create a confounding effect between age and weight in the analyses.

Ticks: Number of ticks found on head and on body/belly were combined as a single tick variable (ticks), assuming tick load were similarly affecting the fawn regardless of position on the individual. Tick life stages (larvae, nymph and adult) were also combined for analyses. Ticks found only crawling on the fawn (not attached) were not included in the analyses. In order to get a count variable for ticks to use in the analyses, the sampling categories above 10 ticks was

noted as an average number for each tick category (e.g. 15 for tick category 11-20 etc.). In addition, another variable for ticks were added in order to test the effect of having higher or lower tick numbers than expected for their age, or age-expected tick number (ticks.res). This was done by using a linear regression to fit the relationship between ticks and age at first capture, and extracting the residuals. I used a combination of Generalized Additive Models (GAM) and Linear Models (LM). GAM was first used to check for non-linearity in the relationship between response variables and predictors (library “mgcv” in R (Wood 2006)). If relationships were linear or close to linear, linear models with a gaussian link (assuming normally distributed residuals) were used in further analyses. Positive values are higher amount of ticks than expected (residuals above regression line) and negative values are lower than expected (residuals below regression line).

Age-expected body mass (mass.res): In order to test individuals that had a heavier or lighter weight than expected for their age, age-expected body mass was calculated with the same procedure as age-expected tick number (ticks.res), from linear models including mass and age at first capture. The residuals in the model would represent fawns with a higher weight than expected (residuals above regression line) or with a lower weight than expected (residuals below regression line) for their age.

Cause of death: Cause of death was separated into four categories; “predation”, “natural”, “human” and “other”. A fawn was considered dead by predation if necropsy forms suggested this, or only collar was found in a condition that indicated death by predator, such as bite marks, blood on collar or if the break point were not on a natural weak part of the collar. A fawn was considered dead by natural causes if the necropsy form and observer comments suggested no signs of predation, or if the fawn was in an abnormal condition, such as for organ abnormality or if it had starvation or hypothermia symptoms. Fawns killed by traffic or agricultural mowing were put in the category for human caused deaths. Fawns that had an unknown or indecisive death cause (either unsure predation, natural or human caused) based on necropsy and observer comments, or that could not be termed as predation, natural or human caused death were categorised as other. Individuals that ended in the human caused deaths category (4 deaths) were removed from the analyses, as they were considered not relevant for the purpose of this study. Survival status of the fawns were either set as 0 (survived) or 1 (dead) for all observations.

Habitat: Habitat category was determined based on fawn bed site descriptions collected during markings, and would fit into two categories either favouring high or low numbers of ticks; respectively “forest” or “open field”. Forest would be descriptions involving the actual word forest, it being deciduous, coniferous or mixed. Bed site descriptions containing forest species such as blueberries or other *Vaccinium* spp., mosses (Bryophyta) and ferns (Polypodiopsida) were also categorized as forest, together with bog areas which are assumed to be more associated with forest than agricultural fields. Open field would be descriptions involving the actual word open field, in addition to pastures, areas used for agriculture and livestock or horses, or natural meadows. Sometimes the bed site descriptions would be unclear, and set as NA in the dataset.

Study site: Bogesund or Grimsö. The study sites have marked differences in roe deer density, predator species and density, tick amount, land use and climate (see above).

2.5 Statistical analyses

Analyses were performed in R version 3.3.1 through the interface R studio version 0.99.491 (RStudio Team 2015). To investigate factors affecting number of ticks on fawns, prediction (1), I used a negative binomial General Linear Model (function “glm.nb” in library “MASS” in R; Venables & Ripley 2002), because of a highly skewed distribution of tick numbers (Figure 3). I created two models to predict the number of ticks on fawns, one where mass was the predictor variable and another where age was the predictor variable. To test the effect of number of ticks on body temperature (°C), prediction (2), I fitted a Linear Model (LM) with body temperature as the response variable and number of ticks (log+1 transformed) as predictor variable.

Survival analysis for prediction (3) was performed using the Cox Proportional Hazard (CPH) model (Cox 1972), to investigate if any of the candidate variables (ticks, temp, age, habitat, mass, sex, mass.res and ticks.res) affected survival of the roe deer fawns (function “coxph” in the “survival” library in R; Therneau 2015). The CPH is a multiple linear regression model which describes the relation between the event (death) expressed by the hazard function and the covariates tested in the model (Bradburn et al. 2003a; Muenchow 1986). The hazard is the probability that the individuals (the fawns) at a time t experiences the event (death) at that time (Clark et al. 2003a). The hazard ratio (exponentiated coefficients) produced in the test, give the effect size of the covariates (Bradburn et al. 2003a). To be able to test the observations in CPH

models, the data was organized as survival intervals ($n = 339$). The data is of the type staggered-entry and right-censored (animals survive past the end of the study, potential radio-collar failure etc.), starting for each fawn ID from first marking until recapture events or death (time-to-event) with each interval ending in either survived (0) or dead (1). The CPH model assumes proportional hazards, meaning that the covariates act multiplicatively on the baseline hazard (Bradburn et al. 2003a). Assumption of proportional hazards was tested on all variables using model diagnostics, which tests proportionality of the predictor by creating time interactions (function “cox.zph” in the “survival” library in R). A p-value of below 0.05 would indicate that the hazard would be non-proportional and significantly increase with time (called “time-dependent” or “updated” variables) (Borucka 2014; Clark et al. 2003b; Therneau 1996).

Analyses with Cox Proportional Hazards were started as univariate models to find significant variables associated with fawn survival. The use of CPH is common to determine survival in ecological and medical studies, and this can be done using multiple approaches (see Bradburn et al. 2003a; Forrest et al. 2003; Muenchow 1986; Rivrud et al. 2016). It seemed appropriate to test the variables individually to determine each variable’s effect on survival following the method by STHDA (2017). Significant variables would then be fitted as multivariate models to see how the factors impact survival together (accounting for the effect of other variables). Variables in the univariate models were first tested as overall models of the entire dataset, and then tested as a subset separating the study sites Bogesund and Grimsö, and death cause predation from natural deaths. This was done to reveal any effects on survival specific to each study site or cause of death. Death cause “other” was not tested separately for survival effects in the death cause subset. The number of events (deaths) were too few ($n = 4$) to reveal any meaningful results in a separate analysis, and the death type did not seem appropriate to combine with predation or natural deaths in the test.

Kaplan-Meier survivorship curves were used to visualize the results showing the survival probability for different categories of a variable (function “survfit” in the “survival” library in R). This is a stepped survivorship curve, which adds information to the plot as deaths occur over time (Crawley 2007). To make the Kaplan-Meier survivorship curves, the last observation for each fawn was extracted to a new dataset containing only one observation per fawn ID ($n = 213$). Depending on whether the fawn had died or were still alive after 30 days, survival status were set to either (0) or died (1). Two extra variables was created for the purpose of the survivorship curves. Age-expected tick number at first capture markings was used to create two

tick categories “many” and “few”, representing fawns with high or low number of ticks at first capture. The same was done for weight, using age-expected body mass at first capture to separate fawns into weight categories “heavy” and “light”, based on their initial body mass. For habitat categories used in the survivorship curves, only first capture habitat observation could be used because there was little information about habitat and where the fawn was found at the last observation or death. Using only last observations of habitat would cut the dataset dramatically (only 9 deaths in total had information about habitat at death). Using habitat at first capture for survivorship curves would also make it more coherent with the other variables used in the survivorship curves that were also based on first capture information and sampling (tick category and weight category).

3 Results

3.1 Number of ticks increase with age and weight

Number of ticks on fawns below the age of 30 days were generally low, where 45% of fawn observations had 10 ticks or less (Figure 3), and 9% of observations had no ticks at all. The highest number registered on a fawn was 80 ticks.

Linear models showed that number of ticks increase with increasing age (estimate =0.071, SE = 0.010, $p < 0.001$, Figure 4) and increasing body mass (estimate = 0.0005, SE = 0.00006, $p < 0.001$, Figure 5).

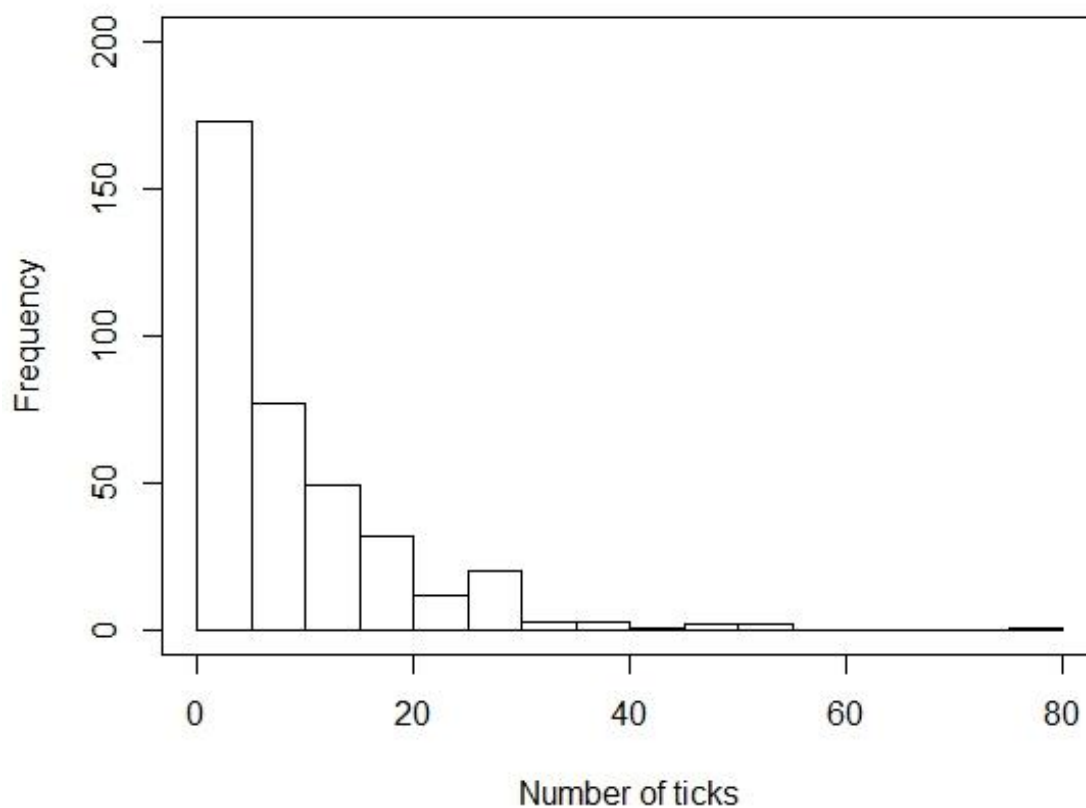


Figure 3. The distribution of number of ticks attached on roe deer fawns below the age of 30 days for all recaptures at both study areas (Bogesund and Grimsö) and years (2013-2016) combined.

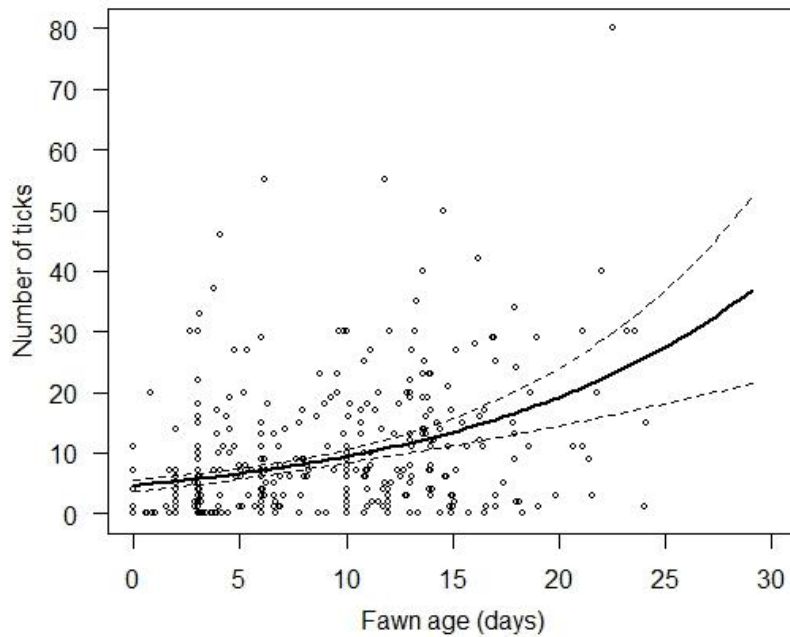


Figure 4. Relationship between fawn age (days) and number of ticks attached on fawns below the age of 30 days at Bogesund and Grimsö 2013-2016. Solid line is the back-transformed slope estimate from a negative binomial regression with number of ticks as response and mass as predictor variable, and stippled lines are ± 1 SE. Points are raw data.

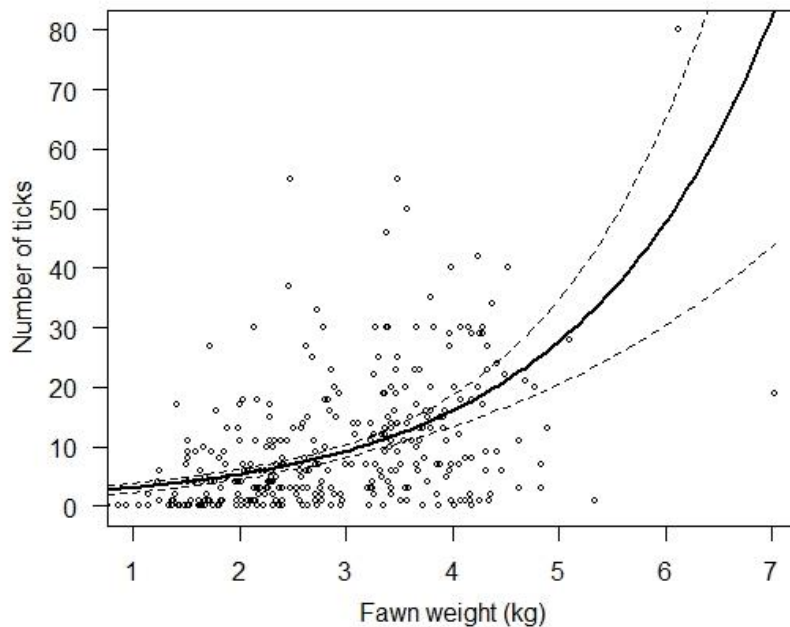


Figure 5. Relationship between fawn body mass (kg) and number of ticks attached on fawns below the age of 30 days at Bogesund and Grimsö 2013-2016. Solid line is the back-transformed slope estimate from a negative binomial regression with number of ticks as response and mass as predictor variable, and stippled lines are ± 1 SE. Points are raw data.

3.2 Body temperature increase with number of ticks and age

Mean body temperature was 38.9 °C (min. 34.3 °C and max. 41.5 °C). Body temperature increased significantly with increasing number of ticks attached to the fawn ($p < 0.001$, estimate = 0.187 (log), SE = 0.034, $R^2 = 0.079$). Body temperature also increased with increasing age (days) ($p < 0.001$, estimate = 0.053, SE = 0.006, $R^2 = 0.144$), which could confound the effect of ticks. As the correlation between age and number of ticks was only 37%, they could be added as predictor variables in the same model. Body temperature still increased significantly with increasing number of ticks after accounting for the age effect (Table 1).

Table 1. The relationship between body temperature °C of roe deer fawns and number of ticks (log scale) and age (days) in the first 30 days after birth at Bogesund and Grimsö 2013-2016.

| Variable | Estimate | SE | t-value | <i>p</i> |
|-----------------|----------|-------|---------|----------|
| (Intercept) | 38.318 | 0.078 | 494.115 | < 0.001 |
| log (ticks + 1) | 0.102 | 0.035 | 2.901 | < 0.001 |
| age | 0.044 | 0.007 | 6.569 | < 0.001 |

3.3 Survival analyses

3.3.1 Proportion of dead and death causes

A total of 217 roe deer fawns (100 females and 117 males) were measured and marked for the purpose of this study with a VHF collar in the study period from May until end of June 2013-2016 at Bogesund and Grimsö. Of the marked fawns, 97 died (45%). The majority (70%) of total mortality occurred within the first 30 days and all analyses focused on this period. After removing observations of fawns older than 30 days and those with deaths caused by human activity, the final dataset for analyses consisted of 213 marked fawns, 97 females (46%) and 116 males (54%) of which 68 died (32%) within the first 30 days after birth. Of total dead fawns in the analyses ($n = 68$, Table 2), 78% died from predation, 16% died naturally and 6% died from unknown or undetermined causes. Dead fawns consisted of 27 females (40%) and 41 males (60%). Mortality was higher at Bogesund compared to Grimsö (Table 3).

Table 2. Distribution of deaths of fawns below the age of 30 days used in analyses (n = 68) in the categories natural, predation and other separated between years, in total (TOT) and by males (M) and females (F) at Bogesund and Grimsö combined.

| | NATURAL | | | PREDATION | | | OTHER | | | TOTAL | |
|--------------|---------|---|---|-----------|----|----|-------|---|---|------------|-----|
| | TOT | F | M | TOT | F | M | TOT | F | M | Dead/Alive | |
| 2013 | 1 | 0 | 1 | 11 | 5 | 6 | 1 | 1 | 0 | 13/47 | 28% |
| 2014 | 4 | 1 | 3 | 16 | 6 | 10 | 0 | 0 | 0 | 20/55 | 36% |
| 2015 | 3 | 2 | 1 | 6 | 3 | 3 | 2 | 0 | 2 | 11/52 | 21% |
| 2016 | 3 | 2 | 1 | 20 | 7 | 13 | 1 | 0 | 1 | 24/59 | 41% |
| TOTAL | 11 | 5 | 6 | 53 | 21 | 32 | 4 | 1 | 3 | 68/213 | 32% |

Table 3. Distribution of deaths of fawns below the age of 30 days used in analyses (n = 68) between study sites Bogesund and Grimsö, years 2013-2016.

| | NATURAL | | | PREDATION | | | OTHER | | | TOTAL | |
|-----------------|---------|---|---|-----------|---|---|-------|---|---|------------|-----|
| | TOT | F | M | TOT | F | M | TOT | F | M | Dead/Alive | |
| BOGESUND | 7 | | | 34 | | | 3 | | | 44/114 | 39% |
| GRIMSÖ | 4 | | | 19 | | | 1 | | | 24/99 | 24% |

3.3.2 Difference in weight between study sites

Linear models showed that there was a significant difference in body mass between study sites, where fawns at Grimsö had higher observed body mass than fawns at Bogesund (estimate = 414.25, SE = 94.76, $p < 0.001$). In addition to combined analyses I therefore also ran separate survival analyses for each area.

3.3.3 Univariate models

Overall univariate CPH models revealed that fawns risk of dying significantly increased with age (Table 4) and with being in habitat open field (Table 4, Figure 6). Fawn body temperature and number of ticks had no effect on fawn survival, neither had body mass or sex (Table 4, Figure 6). The habitat variable did not meet the assumptions of constant hazards, meaning that hazard changes significantly with time, and must therefore be interpreted with caution (assumption test $p = 0.026$).

Univariate models separated by study site showed that fawns risk of dying significantly increased with age (Table 5) and decreased with increasing age-expected body mass (Table 5) at Bogesund, whereas no variables were significantly affecting survival at Grimsö (Table 5). The age variable for the Grimsö subset did not meet the assumptions of constant hazards (assumptions test $p = 0.012$).

Univariate models separated by cause of death showed that fawns risk of dying from predation significantly increased with age (Table 6) and with being in habitat open field (Table 6; but note a violation of the assumption of constant hazards $p = 0.025$). The risk of dying from natural causes decreased with increasing body mass (Table 6) and increasing age-expected mass (Table 6). In addition there was an unexpected tendency that a high body temperature decreased the risk of dying from natural causes (Table 6).

Table 4. Cox proportional hazard models for both study areas combined, showing univariate model variables effect on fawns survival 30 days after birth (variable explanation: temp = body temperature (rectal temperature in °C), habitat = habitat open field compared to habitat forest, mass = fawn body weight (g), sex = sex male compared to female, mass.res = age-expected body mass, tick.res =age-expected tick number). Significant p-values in bold.

| Variables | N/ev | Coefficient | Hazard ratio | 95% CI | z-value | p |
|-----------|--------|-------------|--------------|---------------|---------|-------------------|
| age | 339/68 | 0.114 | 1.121 | (1.058-1.187) | 3.904 | < 0.001 |
| ticks | 239/52 | -0.007 | 0.993 | (0.963-1.024) | -0.439 | 0.661 |
| temp | 287/63 | -0.082 | 0.922 | (0.764-1.111) | -0.855 | 0.393 |
| habitat | 291/63 | 0.617 | 1.853 | (1.040-3.301) | 2.092 | 0.036 |
| mass | 269/60 | 0.0002 | 1.000 | (0.999-1.001) | 1.228 | 0.219 |
| sex | 339/68 | 0.062 | 1.064 | (0.626-1.808) | 0.229 | 0.819 |
| mass.res | 269/60 | -0.201 | 0.818 | (0.612-1.093) | -1.358 | 0.174 |
| ticks.res | 239/52 | -0.022 | 0.978 | (0.944-1.014) | -1.192 | 0.233 |

N/ev = Number of observations tested in model/Number of events (survival status 1). CI = Confidence intervals.

Table 5. Cox proportional hazard models showing univariate model variables effect on fawns survival 30 days after birth for Bogesund and Grimsö (variable explanation: temp = body temperature (rectal temperature in °C), habitat = habitat open field compared to habitat forest, mass = fawn body weight, sex = sex male compared to female, mass.res = age-expected body mass, tick.res = age-expected tick number). Significant p-values in bold.

| STUDY SITE | | | | | | |
|-------------------|-------------|--------------------|---------------------|---------------|----------------|--------------|
| Variables | N/ev | Coefficient | Hazard ratio | 95% CI | z-value | p |
| Bogesund | | | | | | |
| age | 182/44 | 0.108 | 1.115 | (1.037-1.198) | 2.952 | 0.003 |
| ticks | 132/34 | -0.018 | 0.982 | (0.944-1.023) | -0.868 | 0.386 |
| temp | 157/42 | 0.329 | 1.389 | (0.798-2.420) | 1.161 | 0.246 |
| habitat | 152/40 | 0.404 | 1.497 | (0.723-3.100) | 1.087 | 0.277 |
| mass | 161/41 | 0.000052 | 1.000 | (1.000-1.001) | 0.214 | 0.83 |
| sex | 182/44 | 0.061 | 1.063 | (0.511-2.209) | 0.163 | 0.871 |
| mass.res | 161/41 | -0.393 | 0.675 | (0.458-0.995) | -1.986 | 0.047 |
| ticks.res | 132/34 | -0.037 | 0.964 | (0.917-1.012) | -1.483 | 0.138 |
| Grimsö | | | | | | |
| age | 157/24 | 0.029 | 1.030 | (0.925-1.146) | 0.531 | 0.596 |
| ticks | 107/18 | -0.099 | 0.906 | (0.786-1.044) | -1.365 | 0.172 |
| temp | 130/21 | -0.148 | 0.863 | (0.630-1.181) | -0.923 | 0.356 |
| habitat | 139/23 | 0.603 | 1.827 | (0.611-5.465) | 1.078 | 0.281 |
| mass | 108/19 | -0.0002 | 1.000 | (0.999-1.001) | -0.633 | 0.527 |
| sex | 157/24 | 0.205 | 0.814 | (0.280-2.371) | -0.377 | 0.706 |
| mass.res | 108/19 | -0.403 | 0.669 | (0.318-1.408) | -1.060 | 0.289 |
| ticks.res | 107/18 | -0.089 | 0.915 | (0.814-1.029) | -1.490 | 0.136 |

N/ev = Number of observations tested in model/Number of events (survival status 1). CI = Confidence intervals.

Table 6. Cox proportional hazard models showing univariate model variables effect on fawns survival 30 days after birth for fawns dead by predation or natural deaths (variable explanation: temp = body temperature (rectal temperature in °C), habitat = habitat open field compared to habitat forest, mass = fawn body weight, sex = sex male compared to female, mass.res = age-expected body mass, tick.res = age-expected tick number). Significant p-values in bold and strong trends in cursive.

| DEATH CAUSE | | | | | | |
|--------------------|-------------|--------------------|---------------------|---------------|----------------|-------------------|
| Variables | N/ev | Coefficient | Hazard ratio | 95% CI | z-value | p |
| Predation | | | | | | |
| age | 308/53 | 0.111 | 1.118 | (1.048-1.193) | 3.364 | < 0.001 |
| ticks | 217/41 | -0.010 | 0.990 | (0.953-1.027) | -0.550 | 0.582 |
| temp | 262/49 | 0.091 | 0.913 | (0.751-1.110) | -0.914 | 0.360 |
| habitat | 263/48 | 0.891 | 2.438 | (1.225-4.852) | 2.539 | 0.011 |
| mass | 244/46 | 0.0003 | 1.000 | (1.000-1.001) | 1.635 | 0.102 |
| sex | 308/53 | -0.065 | 0.937 | (0.505-1.739) | -0.206 | 0.837 |
| mass.res | 244/46 | -0.107 | 0.898 | (0.627-1.286) | -0.586 | 0.558 |
| ticks.res | 217/41 | -0.031 | 0.969 | (0.926-1.015) | -1.344 | 0.179 |
| Natural | | | | | | |
| age | 224/11 | 0.071 | 1.073 | (0.906-1.271) | 0.819 | 0.413 |
| ticks | 158/8 | -0.045 | 0.956 | (0.851-1.073) | -0.769 | 0.442 |
| temp | 189/10 | -0.760 | 0.468 | (0.217-1.009) | -1.936 | <i>0.053</i> |
| habitat | 190/11 | 0.232 | 1.261 | (0.257-6.189) | 0.285 | 0.775 |
| mass | 180/10 | -0.003 | 0.997 | (0.994-0.995) | -2.291 | 0.022 |
| sex | 224/11 | 0.123 | 1.131 | (0.304-4.207) | 0.184 | 0.854 |
| mass.res | 180/10 | -1.537 | 0.215 | (0.072-0.644) | -2.746 | 0.006 |
| ticks.res | 158/8 | -0.045 | 0.956 | (0.854-1.071) | -0.768 | 0.442 |

N/ev = Number of observations tested in model/Number of events (survival status 1). CI = Confidence intervals.

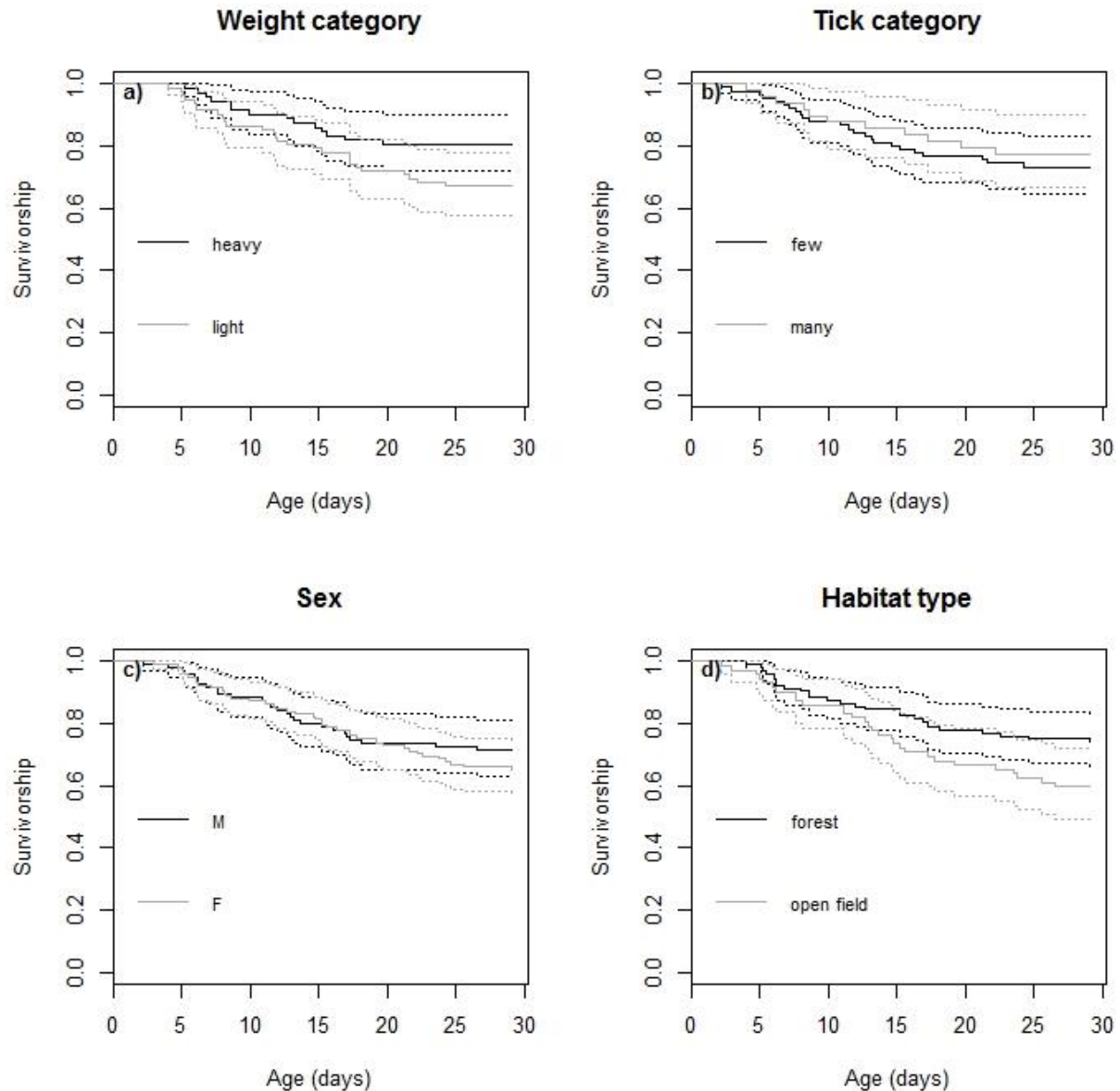


Figure 6. Kaplan Meier survivorship curves displaying survival probability within the fawns first 30 days of life in relation to a) weight category at first capture (heavy $n = 72$, light $n = 82$), b) tick category at first capture (many $n = 49$, few $n = 90$), c) sex (male (M) $n = 118$, female (F) $n = 95$) and d) first capture habitat (forest $n = 104$, open field $n = 72$) at Bogesund and Grimsö 2013-2016. Dotted lines show 95% confidence intervals.

3.3.4 Multivariate models

Significant variables predicting survival in the univariate models were tested together in multivariate models to see if their effects changed when accounting for other variables. Only the overall combined dataset, the Bogesund subset and the Predation subset had more than one significant variable needed to develop multivariate models. As it is not meaningful to combine both body mass and age-expected body mass in the same model, a multivariate model could not be developed for natural deaths (as these two variables were the only significant).

The positive effect of age on survival remained significant in all of the multivariate models (Table 7). However, the effect of habitat disappeared when accounting for the effect of age (Table 7A). In the multivariate model for Bogesund, the positive effect of residual body mass on survival dropped marginally out of significance (Table 7B). Similarly, the higher risk of dying from predation in open habitat dropped marginally out of significance when accounting for age (Table 7C).

Table 7. Cox proportional hazard models showing multivariate model variables effect on fawns survival 30 days after birth for overall combined both study sites, study site Bogesund and death cause predation (variable explanation: habitat = habitat open field compared to habitat forest, mass.res = age-expected body mass). Significant p-values in bold and strong trends in cursive.

| Variables | N/ev | Coefficient | Hazard ratio | 95% CI | z-value | p |
|---------------------|--------|-------------|--------------|---------------|---------|--------------|
| A) Overall | | | | | | |
| age | 291/63 | 0.110 | 1.116 | (1.044-1.193) | 3.233 | 0.001 |
| habitat | | 0.367 | 1.444 | (0.786-2.650) | 1.184 | 0.236 |
| B) Bogesund | | | | | | |
| age | 161/41 | 0.106 | 1.112 | (1.030-1.201) | 2.709 | 0.007 |
| mass.res | | -0.345 | 0.708 | (0.492-1.020) | -1.851 | <i>0.064</i> |
| C) Predation | | | | | | |
| age | 263/48 | 0.095 | 1.100 | (1.021-1.185) | 2.490 | 0.013 |
| habitat | | 0.670 | 1.953 | (0.954-3.999) | 1.832 | <i>0.067</i> |

N/ev = Number of observations tested in model/Number of events (survival status 1). CI = Confidence intervals.

4 Discussion

Detrimental effects of parasites are in general well documented, but studies examining the effect of ticks have mainly focused on livestock. How the tick *I. ricinus* affects populations of wild roe deer is poorly known. Here I found that roe deer fawns in general hosted few ticks as expected from their hider strategy with low mobility and contact with questing ticks. However, increasing tick burden correlated with an increase in body temperature, possibly reflecting fever-causing pathogens carried by ticks. Despite this physiological effect, neither tick burden nor body temperature affected survival. This either implies that ticks have no effect on fawn survival (at the current tick and roe deer densities) or that the effect of ticks is masked by a high predation level (fawns are killed by foxes before the cumulative burden of parasites affects survival). I detected that risk of dying from natural causes decreased with increasing body mass. Any parasite or pathogen significantly affecting early body mass of roe deer therefore has a potential to affect survival. The physiological response of ticks reported in my study calls for a continued study of the effect of ticks and their pathogens on roe deer fawns, especially as the abundance and distribution of ticks are expected to increase in the future.

4.1 Number of attached ticks increase with age and body mass

This study gives insight to what affects the number of attached ticks on very young fawns before their first month of age. Majority of fawns were found to have few ticks attached, supporting prediction 1) where low tick numbers were expected as fawns move less through vegetation and have low exposure to questing ticks. However, large individual variation occurred across both age and weight of the fawn (Figure 4 and 5). Supporting prediction 1) further, number of ticks attached on fawns increased with increasing age and body mass, probably representing an increase in activity pattern and a greater exposure probability to questing ticks in the vegetation. After the first week, the fawn is actively searching for food. Activity periods are gradually increasing in duration starting the first two weeks after birth, and now involves walking and eating (Espmark 1969). This type of behaviour would increase the possibility of being exposed to questing ticks in the vegetation. Fawn age and body mass is therefore correlated with the increase in mobility and the expected exposure to questing ticks. The strategy of spending their first weeks of living with low mobility may therefore be effective towards avoiding both predation and parasites.

4.2 Higher tick numbers increase fawn body temperature

In support of prediction 2), fawn body temperature increased with tick burden (with and without correcting for the effect of age). Changes in body temperature can represent a response to distress for animals (review in Mellor et al. 2000). A body temperature increase correlating with high tick numbers could be a direct stress response to large amounts of parasites and represent an added burden to the fawn. Having to cope with large amounts of parasites has been shown to be detrimental for other cervid species such as for reindeer (*Rangifer tarandus*) with oestrid flies (Oestridae) causing a decrease in feeding and lying behaviour (Hagemoen & Reimers 2002), and for moose (*Alces alces*) with the winter tick (*Dermacentor albipictus*) causing hair and weight loss (Glines & Samuel 1989). However, in neither of these cases, body temperature response was examined. In addition to a potential heavy load in terms of energy drain on their host, ticks also transfer pathogens. The development of fever is one of the most basic responses in the host when infected with a pathogen, raising body temperature above normal temperature (Elsasser et al. 2000). Both *Anaplasma* spp. and *Babesia* spp. are known to cause fever in mammals, and especially for livestock (Bram 1983; Engvall & Egenvall 2002; Zintl et al. 2003). A body temperature increase positively correlating with amount of ticks attached, may represent an increase of the fever-causing pathogens in the fawn. With higher amounts of ticks, probability of getting these pathogens will also increase. *Babesia* spp. prevalence is 38% in blood samples from roe deer fawns at the study sites, which means that fawns here with large amounts of ticks are likely exposed to the pathogens. Whether the temperature variable in this case is a good representation of actual disease within the animal remains to be tested. As blood samples are needed to reveal any pathogens, the true cause for an increase in body temperature showed in this study remains uncertain.

4.3 No effect of ticks and body temperature increase on survival

Whether parasites such as the tick *I. ricinus* has an impact on the recruitment of roe deer is important to establish as the distribution and population of ticks is expected to increase. Despite that ticks induced a body temperature response, there was no effect of neither ticks nor body temperature on fawn survival and by this not supporting prediction 3) where survival was expected to be negatively affected by tick burden. Previous studies on the direct effects of tick infestation have also shown no marked impact of blood loss on roe deer, supporting the results in this study (Talleklint & Jaenson 1997). Talleklint and Jaenson (1997) also concluded that

even if some hosts are more severely affected by tick infestation, it is unlikely that this will have a large impact on the population dynamics overall. For adult roe deer, studies reporting high tick numbers tell of little visible negative effects on the animals (Talleklint & Jaenson 1997; Vor et al. 2010). In addition, reports have been made that the pathogens seem to have low virulence in roe deer and show little clinical signs of disease (Hinaidy 1987; Scharf et al. 2011; Schmid et al. 2008; Zintl et al. 2003). It is well recognized that parasite load may impact survival, by inducing distress (Glines & Samuel 1989), transfer disease (Stuen 2007) or causing loss of blood, fat reserves and affect nutrient uptake (Pfäffle et al. 2009; Stien et al. 2002). For roe deer, the fairly low tick burden might be manageable for the fawn. Parasite amount might not be enough to cause a decrease in survival, and interactions with other factors such as pathogens, forage availability (Gulland 1992), or other environmental or demographic factors might be important (Hatcher et al. 2006; Lehmann 1993). Stuen et al. (2001) reported a fawn's death after heavy infestation with *I. ricinus*. They showed that the fawn did not die from the parasite load itself, but from *E. coli* infection, possibly in combination with the developed anaplasmosis detected in the fawn. Even though the ticks transferred the pathogen causing anaplasmosis, the combination of the two bacterial infections and the heavy tick infestation together was probably decisive for the lethal outcome, and not only the ticks or the disease as single factors. Presence of pathogens in the blood could affect the animal's survival more than the actual tick burden. Although a greater risk of getting the pathogens would increase with number of ticks, the results only show that the actual blood loss caused by the amount of ticks is not affecting survival. Amount of ticks may have a bigger impact on the fawns as the burden increase with age, but these effects may also be counteracted by being a stronger individual. The impact of increasing tick populations on roe deer population recruitment is therefore considered to be low. However, it does not rule out the effect of ticks in combination with other negative factors not examined here (such as pathogens or environmental and demographic stressors), which might be more prominent in other areas. The effects from parasite load may be too small to be revealed in this study using this type of analysis. Although testing only natural deaths for the purpose of revealing any combinations with fawns that are already weak, no effect of ticks was found. Datasets that are too small may not be able to detect the impact of a variable on survival (Bradburn et al. 2003b). The small sample size for natural deaths may not reveal the actual mechanism or interaction with tick numbers.

Predation was the dominant death cause for fawns in this study, and populations without the same predation pressure shown here have the potential to develop a high parasite burden over

time (Stuen et al. 2001). Natural mortality is also higher in areas without predators (Andersen & Linnell 1998; Linnell et al. 1995). This could suggest that predation might be compensatory and remove weak or diseased animals as mentioned by Collins and Kays (2011) and Jarnemo (2004a), masking the real effect of ticks. Bogesund represents an area with high tick abundance, high roe deer density and a high predation pressure by fox. In areas with high predation by fox, the effect of parasites may be disguised by the high mortality. Although mortality is lower at Grimsö, tick abundance is also lower here. Many roe deer populations reside on coastal islands without predation pressure from fox. Coastal habitats can generally harbour saturated tick populations as it favours the climatic conditions for ticks (Porretta et al. 2013). This study found no effect of ticks in areas with a moderate to high predation level, however the effect of ticks can not be determined for areas without predators and with high abundance of ticks.

4.4 Factors affecting risk of dying from predation and natural causes

Fawn mortality increased over time, where risk of dying increased with age in the first 30 days (reflecting a steady decrease in survivorship; Figure 6), indicating that mortality is high in the first month after birth. This is coherent with previous findings for natural mortality (Andersen & Linnell 1998), and for predation, where survival in the first week is high and the period after the first week is associated with an increased predation risk by red fox (Aanes & Andersen 1996), possibly because the fawn becomes more active at this time and hence more exposed, while still not able to escape when encountered by a predator. In addition, fawns residing in open field habitat had a higher risk of dying than fawns in forest, in line with previous studies on predation risk by fox (Aanes & Andersen 1996; Panzacchi et al. 2009), and also confirmed by the models only including predation deaths. The results using the habitat variable must be interpreted reminding that it did not meet the assumptions of proportional hazards, and therefore can not be concluded with certainty. When accounting for the effect of age, habitat became non-significant in the combined model, but remained a tendency to affect survival for fawns who died from predation.

Despite a low sample size, both increasing body mass and age-expected body mass (mass.res) were associated with a lower risk of dying from natural causes. Conditions that results in low body mass (organ abnormality, starvation, infections), are some of the main causes of natural mortality (Andersen & Linnell 1998), which could explain the findings of a higher mortality risk for lower weights in this category. Age-expected body mass also determined survival in

Bogesund, and remained a tendency to affect survival also after adjusting for the effect of age. Bogesund have a higher predator density of foxes than Grimsö, which means that a body mass-related effect on survival might relate to an increased predation risk for fawns that are underdeveloped compared to their peers (Espmark & Langvatn 1985). As the Bogesund study area also have a larger proportion of agricultural land use and hence more open habitat, the results might reflect the importance of being at higher weights to be able to escape from predator in a risky environment. However, age-expected body mass did not predict survival for predation deaths, which is contradicting when the variable affected survival in Bogesund, an area where predation pressure is high. This could be related to that Bogesund have the highest mortality of fawns overall, both from predation and natural mortality, compared to Grimsö. Alternatively, it might be related to other demographic differences between the sites. Bogesund had lower fawn weights than Grimsö. Body mass is known to be an important determinant of survival for cervids, and early body growth for roe deer fawns have been found positively related to survival (Gaillard et al. 1997). In addition, fawns born in years with high roe deer density have been proved to weigh less than those born in years with lower densities (Hewison et al. 2002). The effect of being at higher weights compared to other fawns in the area might impact survival more where roe deer population densities are higher, and where fawn weights are generally low, as it is in Bogesund compared to Grimsö.

Contrary to the expectations of a fever induced negative effect on survival, I found that the risk of dying a natural death was negatively associated with body temperature. Average body temperature for cervids range between 37-41 °C (Soppela et al. 1986). The lowest body temperature recorded in my study (34.3 °C) was below the normal temperature range and considered as hypothermia for deer (Munerato et al. 2008). Low outside temperatures may cause fawns to be exposed to hypothermia, causing a drop in body temperature, which is also a common death cause for fawns (Andersen & Linnell 1998; Jarnemo 2004a). This may cause the CPH model to indicate a lower risk of dying with increasing body temperatures.

5 Conclusion

This study shows that the number of ticks on roe deer fawns increase over time as a function of age and weight. This provides insight to the distribution of the roe deer parasite *I. ricinus* on fawns as hosts. I found that ticks have a potential to increase body temperature of fawns, possibly reflecting fever-causing pathogens carried by ticks. Despite that ticks induced a body temperature response, there was no effect of neither ticks nor body temperature on fawn survival. High mortality among fawns in roe deer populations may have masked an effect of ticks, and as such, my study reflects the effect of ticks in areas with moderate to high predation levels. An effect of ticks on survival can not be excluded in roe deer populations with low predation rates and high tick abundance (for example on coastal islands without foxes). I recommend to further investigate the effect of ticks on the recruitment in roe deer populations across a predation pressure and tick abundance gradient, also considering the pathogens carried by ticks. Little is known about the effects of tick-borne diseases on fawns, which may have a higher impact on mortality or reproduction in roe deer populations than tick burden and blood loss per se.

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