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EXAMENSARBETE



Does type of habitat affect tick-burden in roe deer (*Capreolus capreolus*) neonates?

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Abstract

This study was investigating the relationships between: 1) habitat type and tick abundance, 2) habitat type and tick load on fawns, and 3) tick load and fawn survival. During two years and in two study areas, 105 fawns (57 fawns in Bogesund and 48 fawns in Grimsö) have been captured by hand and equipped with a radio-transmitter. The fawns' positions have then been triangulated almost every day until they died or had at least 30 positions. The surviving fawns were recaptured when they were estimated to have a weight of 3.6 kg. Ticks were collected from the fawns during both the capture and the recapture. By using the flagging-method, in which a white sheet is dragged along the ground, ticks were also collected from the vegetation. A vegetation map was used to determine the habitat on transects and the home range of the fawns. The study areas showed different results regarding in which habitat the ticks were found. At Grimsö ticks seems to favor deciduous forest and mixed forest not on mires. At Bogesund the favored tick habitat was instead coniferous forest with trees between five to fifteen meters. In Bogesund there was a positive correlation between tick-burden and percent of coniferous forest on lichen-dominated areas that covered fawn home ranges. No relationship could be found between ticks and the survival of the fawns. A positive correlation between surviving days and tick load during first capture could instead be found on fawns that died within 30 days.

Sammanfattning

Det här examensarbetet har undersökt sambandet mellan: 1) habitattyp och fästingförekomst, 2) habitattyp och fästingbördan på rådjurskid (*Capreolus capreolus*), och 3) fästingbörda och kidöverlevnad. Under två år och på två lokaler har sammanlagt 105 rådjurskid (57 stycken på Bogesund och 48 stycken på Grimsö) fångats för hand och försetts med sändarhalsband. Därefter triangulerades kidens position nästan varje dag fram tills de återfanns döda eller fram till att de hade minst 30 positioner. De kid som överlevde till en beräknad vikt på ca 3,6 kg, återfångades. Fästingar från kiden samlades in under både första fångsten och återfångsten. Fästingförekomst i olika habitat uppskattades genom flaggnings-metoden, där ett vitt lakan dras längs marken. För att bestämma habitattyp på flaggningssträckorna och kidens hemområde användes en vegetationskarta. De två studieområdena visade olika resultat, både på antalet fästingar som hittades och vart dessa fanns. Resultatet visade att fästingar från flaggningen på Grimsö föredrog lövskog och blandskog som inte var belägna på myrar. På Bogesund var det istället barrskog med träd mellan fem och femton meter som föredrogs. Kiden på Bogesund visade att det fanns ett positivt samband mellan fästingmängden och andelen lavdominerad barrskog som fanns i deras hemområden. Inget samband hittades mellan antalet fästingar och kidens mortalitet. Däremot hittades en positiv korrelation mellan antalet överlevande dagar och fästingbördan under första fångsten för de kid som dog inom 30 dagar.

Introduction

Ixodes sp. ticks are on a global level, after mosquitos, “the most important arthropod disease vectors of pathogens from wild animals to humans” (Lindström & Jaenson 2003). The most important vector in Europe is the castor bean tick (*Ixodes ricinus* Linnaeus, 1758) that can spread diseases such as ehrlichiosis, babesiosis, tularemia, tick-borne encephalitis and Lyme borreliosis (Lindström & Jaenson 2003). *I. ricinus* stands for 95% of the tick bites on humans (Vanhoenacker 2013) and is in Sweden the only vector for TBE. During 2011, there were 284 cases of TBE in Sweden (Jaenson et al. 2012b). Roe deer (*Capreolus capreolus* Linnaeus, 1758) are important hosts for ticks and the large roe deer population has been suggested to have benefitted the increase of ticks in Sweden (Jaenson et al. 2012b). Between 1955 and 2005, the population of roe deer increased from 100,000 to 375,000 (Bergström & Danell 2009) and during the latest 30 years, *I. ricinus* has expanded northwards and increased its range with 9.9 % between early 1990 and 2008 (Jaenson et al. 2012a).

I. ricinus nymphs have been shown to favor forest habitats before open habitats, where dry meadow seems to be the least favorable for the nymphs (Lindström & Jaenson 2003). The roe deer similarly favor forest before open habitat for the bed-sites of the neonates (Linnell et al. 1999). Micro-climatic condition is the main factor for the lower tick density in open habitats; where ticks will be more exposed for wind and sun resulting in desiccation. But it has also been suggested that roe deer indirectly can affect the different densities in habitats (Lindström & Jaenson 2003).

Ixodes larvae have shown to prefer younger roe deer. The reason for this could be longer resting phases and thinner skin (Vor et al. 2010). A study by Hair et al. (1992) showed that the tick-burden on fawns could affect the weight gain negatively and also result in weight loss. In the same study it was also shown that adult lone star ticks (*Amblyomma americanum*) can increase the mortality in fawns of white-tailed deer in America if the tick-burden is high enough for a period of time (Hair et al. 1992). It is possible that roe deer density affects tick-burden on roe deer, but it is also suggested that habitat structures could be more important for the tick-burden (Vor et al. 2010).

SLU-Grimsö runs a project where the aim is to improve the knowledge about how to prevent and control both the spreading and transmission of tick-borne diseases to humans. Grimsö are especially focusing on the relation between wildlife and ticks, and has provided the data for this thesis. The aim for this thesis is to investigate the relationships between 1) habitat type and tick abundance, 2) habitat type and tick load on roe deer fawns, and 3) tick load and fawn survival.

Material and methods

Study species

Roe deer is a small cervid with a weight of 20-30 kg (Andersen et al. 1998) and is the most widespread cervid in Europe. With a population of more than 9.5 million, it is also the most common one (Burbaitè and Csányi 2009). They are found in most habitats, both natural and man-made, but generally prefer habitats of early succession before climax habitats (Linnell, Duncan and Andersen. 1998). The does selection of habitat is related to the available resources in their home range. The habitat can therefore change between years as a response to changing availability of resources (Pellerin et al. 2010). The selection of habitat also varies

depending on point of time, open habitats is more used during nighttime while habitats with cover are more used during the day (Bonnot et al. 2013). The need of cover during daytime is for protection against predators (Tufto, Andersen and Linnell 1996). The size of a roe deer's home range may be determined by several factors, such as landscape structure (Saïd and Servanty 2005), population density (Kjellander et al. 2004), visibility and food supply (Tufto, Andersen and Linnell 1996). In a study by Cederlund (1983) the size of the daily home range varied from 1 ha and up to 120 ha.

Roe deer are browsing generalists that feed on a wide variety of different plant species (Duncan et al. 1998). But as concentrate selectors, they have a digestive system not optimized for plant fiber and therefore feed on high quality food that is easily digested (Hofmann 1989). Roe deer are solitary animals that often live alone or in small groups of two or three, which is often an adult doe with her fawns, but can also consist of an adult buck. Bigger groups can sometimes form during winter (Hewison, Vincent and Reby 1998). Between early spring to the end of the rut in August, adult bucks will defend their territory that can contain several does home range (Liberg et al. 1998). In May to June, does will give birth to one to three fawns (Gaillard et al. 1998). Roe deer have a synchronized birth period and 80 % are born within 30 days (Gaillard et al. 1993). As a hider species, the roe deer fawns will be hidden and separated from the doe for the first six to eight weeks and will rely on being still and odorless to avoid predation (Panzacchi et al. 2009). The doe will visit the neonates two to seven times per day to nurse and move them to a new bed-site (Jarnemo 2004).

I. Ricinus is a widespread tick in Europe that occurs from Sweden in north to Egypt in south and from the Ural Mountains (Russia) in east to Ireland in west (Randolph et al. 2002). Ticks go through four stages (egg, larva, nymph and adult) in their life. As a larva and nymph, the tick needs to feed to be able to develop to the next stage. An adult female also need to feed to be able to lay eggs (Gray 1998). The adult male can only be found on hosts in search for a female for reproduction (Kiffner et al. 2011). Adult females need a host larger than a hare, a deer for an example, while nymphs and larvae also can feed on smaller animals (Gray 1998). For the larva, the feeding will last for three to four days, for the nymph three to five days and for the adult female seven to ten days (Materna et al. 2008). *I. ricinus* sits in vegetation waiting for a host using an ambush strategy. Ticks are sensitive to desiccation and require a humidity of at least 80 % (Gray 1998).

Study areas

Data was collected in two different study areas (Figure 1). The first study area is Bogesund, which is located ten kilometers north-east of Stockholm (59°24'N, 18°12'E). The area covers 2,600 ha with 65 % forest, 25 % grazing and arable land, and 10 % bogs and bedrocks. The forest is dominated by Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*). Common deciduous species are willow (*Salix* spp.), birch (*Betula* spp.) and common oak (*Quercus robur*) (Jarnemo 2004; Kjellander, Gaillard and Hewison 2006).

The second study area is located in south-central Sweden at Grimsö (59°40'N, 15°25'E) and covers 13,000 ha, where 78 % consist of mixed conifer forest. 18 % is covered by mires, bogs and fens. Rivers and lakes covers 5 % and the last 3 % consist of farmlands (Kjellander and Nordström 2003).

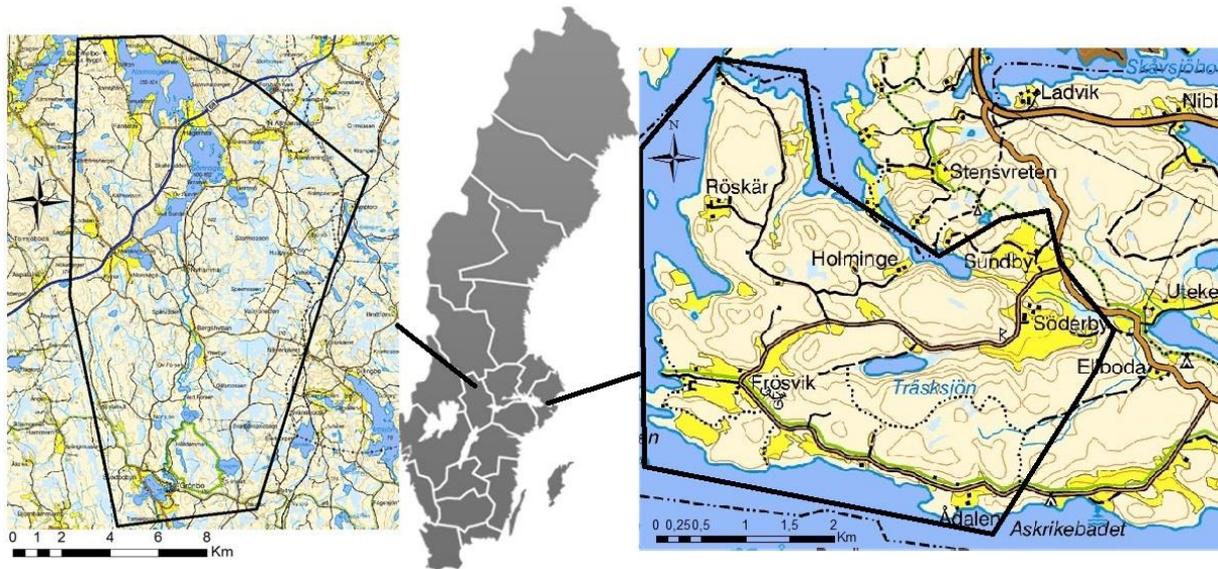


Figure 1. The left map shows an overview of Grimsö and the right map shows Bogesund.

Capturing roe deer

Newborn roe deer fawns were captured during May 27 to July 1, 2013 and May 21 to June 26, 2014. Fawns were found by observing does with a slim belly but with a big udder (suggesting that they had given birth) and waiting for them to visit bedded fawns. Sibling fawns were found by searching the close surroundings of already marked fawns. A third method was to search core areas of GPS-collared females.

After being captured, the fawns were sexed and weighed, the hind foot length was measured and the rectal temperature was taken. The fawns behavior before, during and after were noted and also the presence and appearance of the umbilical cord. They were also ear-tagged and equipped with a VHF radio-transmitter with a mortality function (Followit, Lindesberg, Sweden) on an expandable collar with a drop-off function. The transmitter has a weight of 70 g and a battery life of nine months. In open terrain the transmitter can send a signal to a receiver from a distance at maximum 1.5 km. With the receiver it is possible to determine in which direction the fawn is positioned by listening in which direction the signal is strongest. By doing it from three different locations, a position of the fawn can be found. The number of ticks was counted on the fawns' abdomen and head. When the number of ticks was below ten, the ticks were counted precisely and if the number was over ten, the number was estimated on a scale (11-20, 21-30, 31-50, 51-100, >100). The chosen body sections had been determined because of *I. ricinus* preferred feeding sites (Kiffner et al. 2011). Blood was also sampled during capture. Fawn survival and bed-site habitats were monitored daily through telemetry until late August. The fawns were recaptured and measured again when they were calculated to weigh approximately 3.6 kg, which was done by using an average weight gain of 150 g per day. To estimate the age of the fawns at their first capture, they were estimated to have a birth weight of 1,500 g and have a daily weight gain as previously mentioned (Jarnemo 2004).

Collecting ticks

Tick abundance in different habitat types was measured by flagging (explained below), which is the method that is most commonly used (Gherman et al. 2012). Every second week from

the end of April to the beginning of August, thirteen transects at Bogesund and twenty places at Grimsö were flagged by dragging a white sheet along the ground for 2 x 100 m. After 100 m, the ticks were collected from the sheet. The same flagging places were used every time. An exception was sites at Grimsö, where some new ones were taken because of different reasons, e.g. unmarked transects. The transects at Grimsö was the first time placed systematical as it was expected to cover all the different habitats. In Bogesund most of the transects had been randomly placed. As some habitats were missed in this randomness, it was decided to place extra transects in those habitats. The ground had to be dry when flagging was performed; if it was wet the flagging was scheduled to the following day. Back at the laboratory, the ticks were counted and determined according to species and life stage.

Statistical analysis

The statistical program R (R Core Team 2014) was used to calculate the fawns' home ranges. For all fawns that had twenty or more positions, not counting the first capture position, a home range was established by calculating Mcp95. Mcp95 calculates the home range by creating the smallest possible convex polygon using 95 % of the GPS points in the data (Calenge 2011). By using the average size of these home ranges, separated into the different areas and different years, home ranges were also created for the fawns below twenty positions. A circle was created from the average size and given to all the fawns; the midpoint of this circle was placed over the midpoint for the positions that had been taken of the fawn. The home ranges that were created by R were then used to extract habitat data from a raster map from Swedish database on land and vegetation cover, where every square has a different value depending on the habitat type (Table 1). Fawns that had less than five positions were not analyzed in statistics for home range, but were used when looking for significance in the number of ticks and in those cases where the fawns had died. R was also used to get the habitat from transects. A line was created between the start and end of transects. The habitat was then extracted from the habitat map from where the line was placed.

In the program IBM SPSS statistics, the data from the roe deer and transect was analyzed separately, the data was also divided into the different locations as the number of ticks differed significantly (Figure 2 and 3). Linear regression was used to analyze both the average and total number of ticks on roe deer in each habitat. The habitat was inserted as the independent and the ticks were inserted as the dependent. Besides using the habitat in Table 1, the habitats were also combined into groups with open habitats, coniferous, deciduous and mixed forest which was also analyzed. Lastly a group combined of all the different forest habitats was also used. Linear regression was also used for the analysis of transects, using both ticks/km and the total amount of ticks that was found. Habitat was inserted as independent while ticks were inserted as dependent. As with the roe deer, the same habitats and combined group of habitats were used.

Pearson correlation test was used to analyze if there occurred a relation between ticks and fawn mortality, both the total number of ticks found on the fawns and the average were analyzed. A separated analysis with linear regression was also done between the number of ticks from the first capture and on the fawns that died during the first month (30 days) after the capture, using the number of surviving days as the dependent factor. Linear regression was also used to analyze the tick load on the fawns during their first capture and the age of the fawns. Both a Pearson correlation test and one-way ANOVA was used to analyze the fawns

that died during the first month after capture and the ratio of forest habitat in their home range. Finally, fawns that were estimated to be around the same age when caught for the first time, were compared to see if a pattern could be found between the ones that survived and the ones that died within 30 days.

Table 1. The different habitat classes used for both the fawns and transects. The definition of a forest on mires is a wooded area in a wetland with a total canopy cover of at least 30%. To determine if it is a deciduous or coniferous forest, at least 75 % of the canopy need to be either one (Swedish environmental protection agency 2014)

Habitat
Arable land
Clear-felled areas
Coniferous forest >15 m
Coniferous forest 5-15 m
Coniferous forest on lichen-dominated areas
Coniferous forest on mires
Deciduous forest not on mires
Deciduous forest on mires
Mixed forest not on mires
Mixed forest on mires
Other mires
Pastures
Sand and gravel pits
Solitary houses with property
Thickets
Wet mires
Younger forest

Results

A total of 57 fawns were captured in Bogesund; 24 during 2013 and 33 during 2014. In Grimsö a total of 48 fawns were captured; 23 in 2013 and 25 in 2014. 83 fawns were used in the home range statistics. The fawns in Grimsö had a larger average home area (120 ha) than fawns in Bogesund (23 ha). Both transects and fawns showed a significant higher amount of ticks in Bogesund as opposite to Grimsö (Figure 2 and 3).

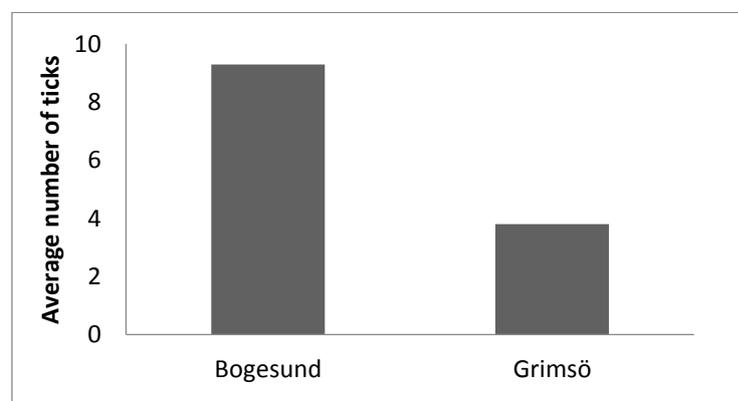


Figure 2. The average number of ticks found on fawns during capture on the different areas were significantly different with one way ANOVA ($f=8.192$, $p=0.005$).

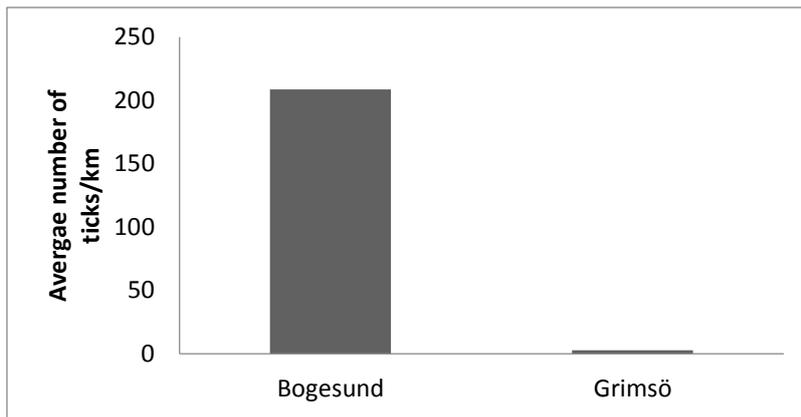


Figure 3. The average number of ticks/km found on transects showed a significant difference between the two study areas when using one way ANOVA ($f=154.997$, $p=0.000$).

In Bogesund, the average number of ticks on the fawns showed a significant (Figure 4) increase with increasing percent of coniferous forest on lichen-dominated areas. This pattern could not be found on fawns in Grimsö, nor on ticks/km on transects in both study areas. With the total number of ticks found on transects, a significant relationship could be found in Bogesund with increasing ratio coniferous forest on lichen dominated areas ($r = 0.42$, $f = 4.708$, $df = 23$, $p = 0.041$). The transects in Bogesund also showed a significant relationship between ticks/km and increasing ratio coniferous forest between five and fifteen meters (Figure 5). In Grimsö the transects showed a significant relationship between ticks/km and increasing ratio deciduous forest not on mires (Figure 6), and ticks/km and mixed forest not on mires (Figure 7). At Grimsö there was also a significant relationship between average number of ticks/km and increasing ratio deciduous forest ($r = 0.321$, $f = 10.706$, $df = 94$, $p = 0.001$) and increasing ratio of all type of forest ($r=0.234$, $f=5.398$, $df = 94$, $p=0.022$).

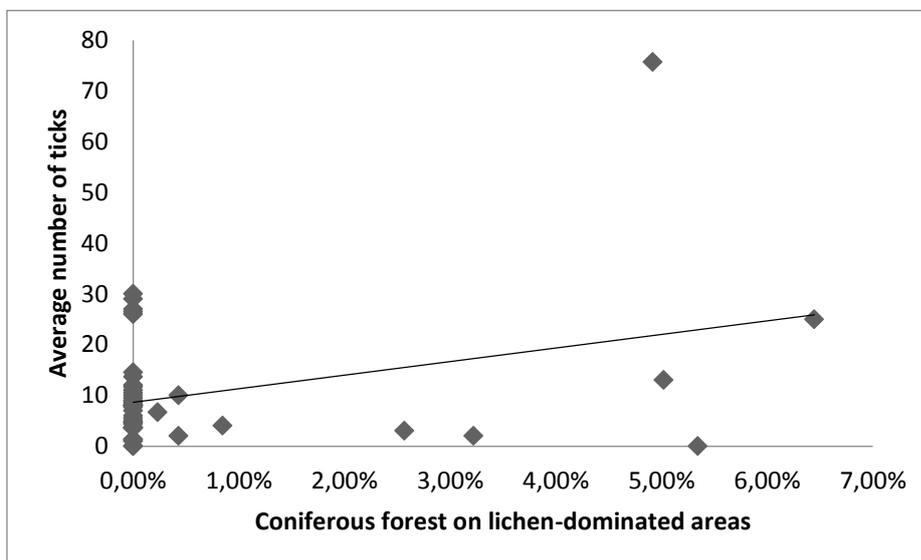


Figure 4. Linear regression between the average number of ticks found on fawns in Bogesund and the percent of the home range containing coniferous forest on lichen-dominated areas ($r=0.341$, $f=6.036$, $df =47$, $p=0.018$).

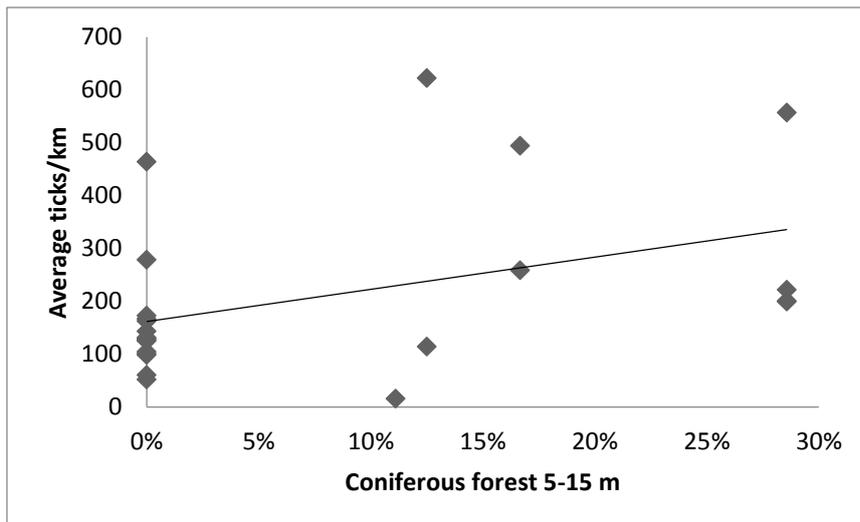


Figure 5. With linear regression a significant relationship could be found in Bogesund between the average ticks/km on transects and the percent of coniferous forest five to fifteen meter ($r=0.416$, $f=4.595$, $df=23$, $p=0.043$).

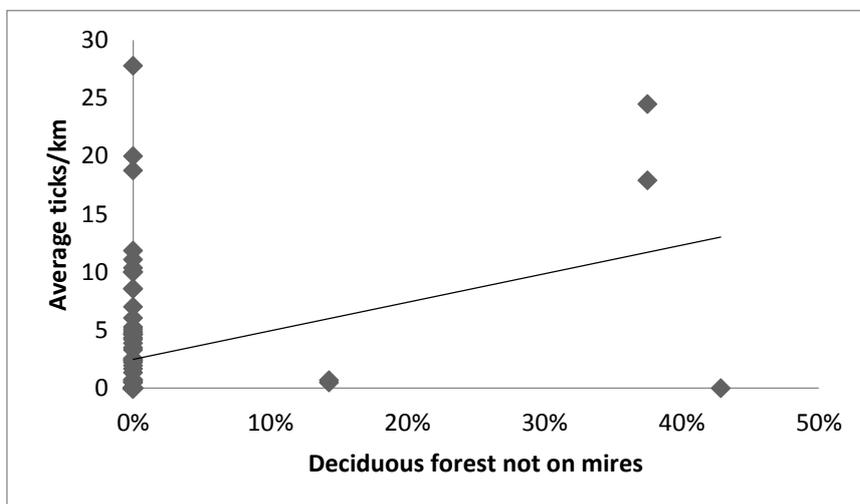


Figure 6. Using linear regression average ticks/km on transects in Grimsö showed a significant relationship with the percent of deciduous forest not on mires ($r=0.321$, $f=10.706$, $df=94$, $p=0.001$).

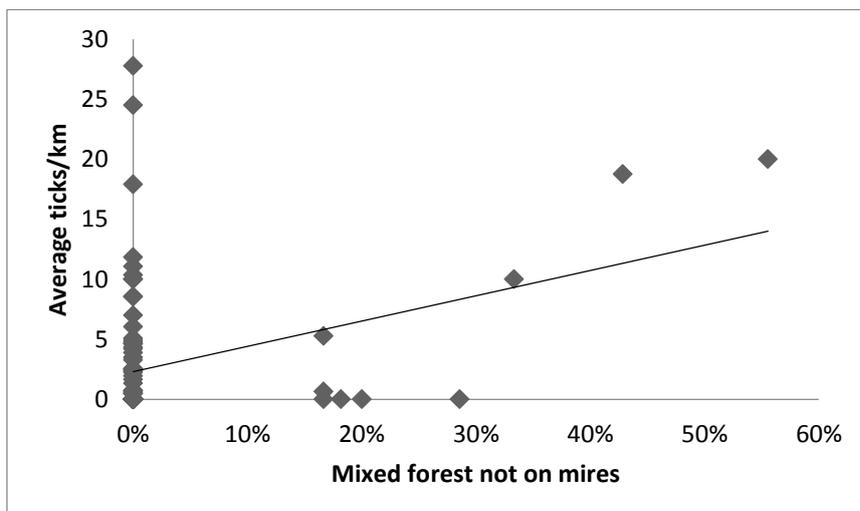


Figure 7. Transects in Grimsö showed a significant relationship when using linear regression between the average ticks/km on transects and the percent of mixed forest not on mires ($r=0.349$, $f=12.911$, $df=94$, $p=0.001$).

Of the 105 captured roe deer, a total of 44 fawns (41.2 %) died during the summer (Bogesund = 25, Grimsö = 19). Predation accounted for 54.2 % of the deaths in Bogesund, while in Grimsö it accounted for 33 % of the deaths but 100 % of the known causes (Table 2). 75 % of the predation in Bogesund happened during the last week of June or later, where the average estimated age of the fawns were twenty days. The average age of captured fawns in Bogesund was 4 days (range 0-18), while it was 6 days in Grimsö (range 0-26). The tick-burden on fawns during first capture in Bogesund showed a positive correlation with age on the fawns ($r = 0.589$, $f = 27.575$, $df = 53$, $p = 0.000$), which could not be seen on fawns in Grimsö.

Table 2. The fawns cause of death.

Cause of death	Bogesund	Grimsö
Predation	54.2%	33.3%
Mower	8.3%	0%
Sick	25%	0%
Unkown	12.5%	66.7%

There was no relationship between tick load and fawn mortality. Fawns that died within 30 days from capture in Bogesund had a positive correlation between surviving days and tick load during first capture (Figure 8). This could not be found in the fawns in Grimsö. No relationship could be found in neither of the two study areas between fawns that died within 30 days and the ratio of forest habitat in their home range. The comparison between fawns that were estimated to be the same age during first capture shows that surviving fawns in Bogesund often had a slightly higher tick load compared to the ones that died within 30 days (Figure 9).

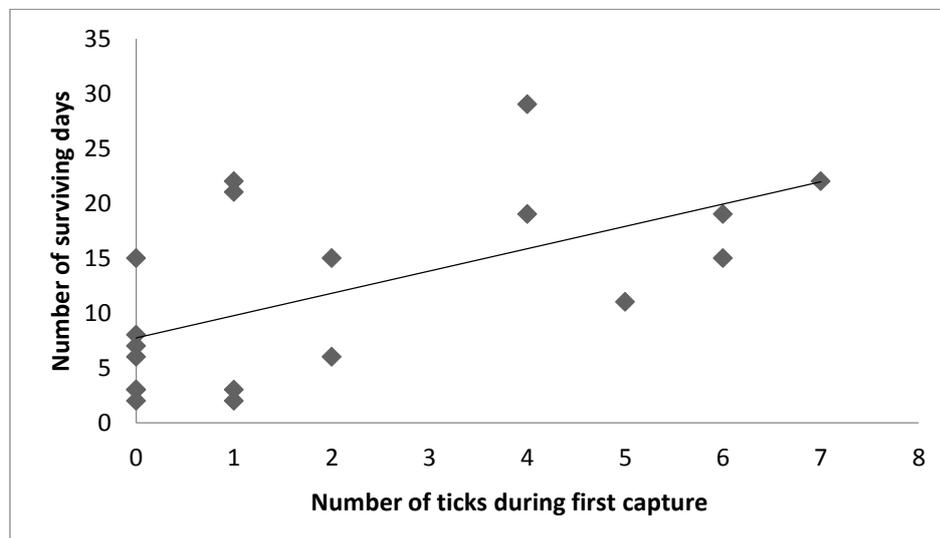


Figure 8. Fawns in Bogesund that died within 30 days after the capture showed a significant relationship with the number of ticks they had during their first capture ($r=0.591$, $f=9.114$, $df=18$, $p=0.008$).

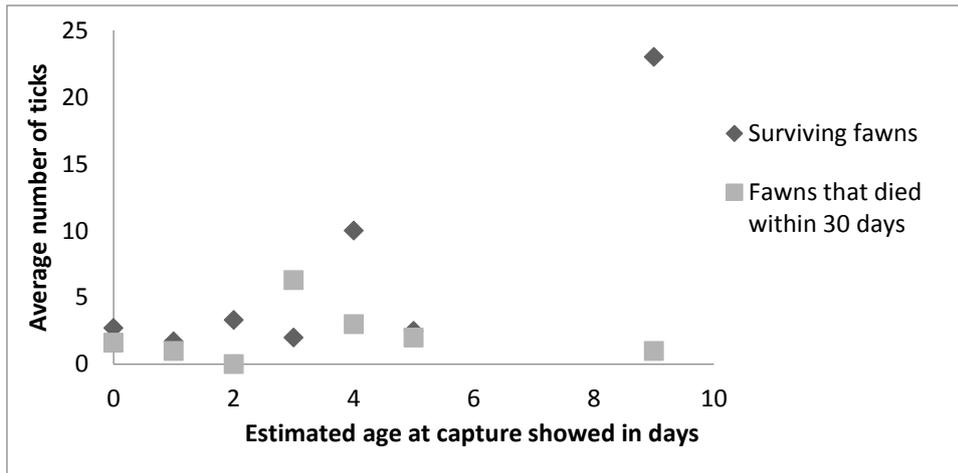


Figure 9. Comparison of the average tick load during the first capture between fawns that died during the first month and those who survived. Only days/ages which have both surviving fawns and fawns that died are used.

Discussion

In this study I investigated the possible relationships between habitat type and tick abundance, between habitat type and tick load on roe deer fawns, and between tick load and fawn mortality. The results showed that tick abundance varied between habitat types. There was also a difference between in which habitat we found ticks on transects and the habitat that resulted in more ticks on the fawns. The results showed no overall relationship between tick load and fawn mortality. However, and contradictory to the predictions, I found that within the group of fawns that died within 30 days of capture, a higher tick-burden on the first capture resulted in more surviving days.

Tick load on fawns was positively related to amount of lichen-dominated coniferous forest in the home range in Bogesund. This could suggest that ticks favor this kind of habitat. This relationship could not be found on transects in Bogesund. However, this could be due to that only one transect was located in lichen-dominated coniferous forest. On the other hand, this transect is on top half of transects with most ticks/km. It is possible that additional transects in this kind of habitat would have shown the same result as with fawns. As it was now, a relationship was instead found in Bogesund between the total number of ticks found on transects and increasing ratio of lichen-dominated coniferous forest. The flagging in Bogesund also showed that with increasing percent of coniferous forest with trees between five and fifteen meters, ticks/km also increased. The same relationship could not be found with the fawns. However, as host density can increase the density of ticks in different vegetation (Lindström & Jaenson 2003), it is possible that there are other important hosts in this vegetation.

The results from Grimsö were not in line with the results from Bogesund. No correlation could be found between tick load on fawns and the habitat. Transects showed that the ticks in Grimsö favored deciduous and mixed forest not on mires. A straight-forward explanation for this difference between the areas is that there were no transects in Bogesund in deciduous forest not on mires. Lack of fawns and possible roe deer in mixed forest not on mires in Grimsö could be the reason for the variation in the habitat between the study areas, as it has been shown that lack of roe deer can increase the number of questing ticks (Perkins 2006; Rand et al. 2004).

As expected, in both Bogesund and Grimsö a significant correlation between tick abundance on transects and tick load on fawns was found in forest habitats. Other than that, neither transects, fawns or the different locations showed any similarities.

Transects in Grimsö also showed that the ticks favor all kind of forest habitats before open habitats, which supports Lindström & Jaenson (2003). The same could not be seen in Bogesund and the reason might be because only three transects had less than 100 % forest habitat. Therefore it might be profitable in the future to place additional transects in habitats that at present are represented in one study site but not in the other. As it is now, four habitats are only represented in Grimsö and two are only represented in Bogesund. However, it is possible that some habitats cannot be found in both study areas. What can be done is placing a new transect in Bogesund that will be in lichen-dominated coniferous forest. The reason for this would be to see if the relationship that was found on fawns between tick load and lichen-dominated coniferous forest also can be found on transects.

When it comes to fawns, it could be possible to get a better result in the future with more fawns with a higher amount of triangulations. If only fawns with at least 30 locations were used in the statistic, it would produce a more accurate result. With the result so far, if only the fawns with more than twenty triangulations would be used, there would only be data from seventeen fawns in Bogesund. In other words, 40 fawns in Bogesund that was used in this statistic would not have been used. On the other hand, it is not always possible to get at least 30 locations on fawns as they have a mortality risk.

Even though no relationship could be found between tick load and overall fawn mortality, there was a significant relationship between the fawns in Bogesund that died within 30 days from the first capture and tick load during that capture. The higher amount of ticks they had, the longer they survived. It is possible that this has nothing to do with tick load and instead is due to other factors affecting predation risk. The fawns that survived longer could have a higher ratio of habitat with lower predation risk, such as forest (Panzacchi et al. 2009). This could, however, not be seen with the analysis on forest habitat as no relationship could be found between the ratio of forest in the fawns home range and if they died. Birth date can also affect the predation risk, where fawns that are born in the beginning or in the end of the birth period can suffer from a higher predation risk (Jarnemo 2004). However, the fawns that died in Bogesund were a mix of fawns born across the whole birth period with an average age of twenty days, suggesting that the early or later born fawns did not have a harder time. Nevertheless, the effect of predation on the result cannot be rejected.

Another explanation could be that if the fawns are older when caught for the first time, they will have a higher tick load, which was observed on fawns caught in Bogesund. However, by comparing the average tick load on fawns that were estimated to be about the same age when caught, there was still a pattern showing that fawns that survived longer than 30 days often had slightly more ticks than those that died within 30 days.

Could it be possible that the ticks can sense if a fawn is not healthy and might die before they are done feeding and therefore wait for a healthier host? A study on one ectoparasitic mite, *Spinturnix myoti*, showed that *S. myoti* favor bats that has a better nutritional status (Christe et al. 2003). It is possible that *I. ricinus* favor fawns that are healthier.

Conclusion

One habitat in Bogesund clearly showed that even a small percent of lichen-dominated coniferous forest in the home range, would increase the tick-burden on fawns. On the other hand did the transects show different results, implying that you cannot draw a definite conclusion between where we will find ticks when we use the flagging method and which habitat will result in a higher tick-burden on hosts. Higher tick-burden on transects can instead indicate absence of hosts. Since open habitats and habitats on mires are not suited for *I. ricinus*, there is no coincidence that these habitats did not result in higher tick-burden. The ticks need a humidity of at least 80 % around the whole year and will have a risk of desiccation in both open habitats and mires during dry summers. Finally, how strange it might seem, a higher tick-burden increased the number of surviving days for the fawns that died during their first month. Since predation and the age of the fawns did not seem to explain this, there could be two explanations; either it occurred by coincidence or ticks are able to sense if the fawns are less healthy – a hypothesis that is worth further studies.

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