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Examensarbete i ämnet biologi
Department of Wildlife, Fish, and Environmental studies
Umeå
2014

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Credits: 30 HEC
Level: A2E
Course title: Master degree thesis in Biology at the Department of Wildlife, Fish, and Environmental Studies
Course code: EX0633
Programme/education: Management of Fish and Wildlife Populations

Place of publication: Umeå
Year of publication: 2014
Cover picture: Emma Tjärnström
Title of series, no: Examensarbete i ämnet biologi
Number of part of series: 2014:4
Online publication: http://stud.epsilon.slu.se

Keywords: *Capreolus capreolus, Ixodes ricinus,* roe deer neonatal, tick burden, bed site

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Abstract

This thesis deal with tick burden on roe deer (*Capreolus capreolus*) fawns, in relation to age, weight, hind foot length, and as well as to vegetation and habitat on bed sites. Roe deer fawns (N = 25) were captured from May 27 to June 27. Samples of ticks were collected and tick burden were estimated. Of all sampled ticks the nymphs and adults were *Ixodes ricinus*, while the larvae were not identified. 4.4 % of the sampled ticks were larvae, 55.0 % nymphs, 26.8 % adult females, and 13.8 % adult males. The average tick burden were rather small (14 ticks per fawn), with individual variation from 0 to 80 ticks per fawn. Tick burden were positively correlated with age, weight and hind foot length. However, tick burden did not have any effect on weight gain or growth rate. There was a significant difference in tick burden on roe deer neonatal in the heavier weight group between vegetation types, where the fawns that had been captured in short grass, herbs, blueberry shrubs, and moss in closed habitats had a higher tick burden than fawns that was captured in high grass and herbs in open habitats. This was consistent with the result from sampling ticks on cloth drags, where the highest amount of ticks was found among short grass, herbs and moss with a mean of 69.1 ticks/100m², followed by blueberry shrubs and moss with 44.9 ticks/100m² and high grass and herbs with a mean of 6.6 ticks/100m². The highest prevalence of *I. ricinus* was found on roe deer fawns that were more frequently located in coniferous forest, while the lowest prevalence was found on the fawns that were more frequently located in farmlands. The analysis of the collected ticks' shows that the presence of nymph, adult female, and adult male ticks on roe deer fawns is dependent of the habitats the fawn has been in, but that presence of larvae is independent of habitat. Adult tick burden was higher on fawns captured in closed habitats then on fawns in open habitats. Overall, the tick burden was relatively low and did not have any visible effect on the roe deer fawns during this study period. Since age and tick burden was positively correlated, we cannot reject the risk that tick burden will reach a level that might have a negative effect on weight gain and/or growth rate as the fawns' ages. To examine this further, the fawns should therefore be followed for a longer period of time.

Keywords: *Capreolus capreolus*, *Ixodes ricinus*, roe deer neonatal, tick burden, bed site

Sammanfattning

Detta examensarbete tar upp fästingbördan hos rådjurskid (*Capreolus capreolus*), i förhållande till ålder, vikt, haslängd samt vegetation och habitat på legorna. Tjugofem rådjurskid fångades mellan den 27 maj och 27 juni. Ett urval av fästingar samlades in och antal fästingar uppskattades. Av alla insamlade fästingar var nymferna och adulterna av arten *Ixodes ricinus* medan larverna inte var artbestämda. 4.4 % av de insamlade fästingarna var larver, 55.0 % nymfer, 26.8 % adulta honor och 13.8 % adulta hanar. Det genomsnittliga antalet fästingar per individ var relativt få (14 fästingar per kid), med en individuell variation på 0 till 80 fästingar. Det visade sig att antalet fästingar var positivt korrelerade med ålder, vikt och haslängd. Däremot hade antalet fästingar inte någon inverkan på viktökning eller tillväxt. Det fanns en signifikant skillnad i antalet fästingar hos rådjurskid i den tyngre viktgruppen mellan vegetationstyper, där kid som hade fångats i kort gräs, örter, blåbärsris och mossa i slutet habitat hade ett högre antal fästingar än kid som fångats i högt gräs och örter i öppet habitat. Detta stämde överens med resultatet från fästingflaggningen, där det högsta antalet fästingarna hittades bland kort gräs, örter och mossa med ett medelvärde på 69,1 fästingar/100m², följt av blåbärsris och mossa med 44,9 fästingar/100m² och högt gräs och örter med 6.6 fästingar/100m². Den högsta förekomsten av *I. ricinus* hittades på kid som i större utsträckning vistats i barrskog, medan den lägsta förekomsten hittades på kid som oftare befann sig i jordbruksmarker. Analysen av de insamlade fästingarna visar att förekomsten av nymfer, adulta honor och adulta hanar bland fästingarna på kiden är beroende av de habitat kiden har befunnit sig i, men att förekomsten av larver är oberoende av habitat. Antalet adulta fästingar var högre på kid som fångats i slutet habitat än på kid i öppna habitat. Överlag var fästingbördan relativt låg och har inte haft någon synlig effekt på rådjurskiden under denna studieperiod. Eftersom antal fästingar var positivt korrelerade med kidens ålder, kan vi inte bortse från risken att antalet fästingar kan nå en nivå som skulle kunna ha en negativ effekt på viktökning och/eller tillväxt. För att undersöka detta vidare bör kiden följas under en längre tidsperiod.

Introduction

Inter- and intraspecific differences in habitat selection is the mainly source of variation in life-history traits among cervid (*Cervidae*) species, where the habitat quality creates trade-offs between predation pressure, forage quality and availability, and possibilities to find shelter and hide (Bowyer *et al*. 1998; Linnell *et al*. 2004; Nilsen *et al*. 2004). Closed habitats, as an example, offer suitable shelter for cervids against predators and harsh weather (Krebs and Kacelnik 1991), while open habitat offer the best forage (Godvik *et al*. 2009). This trade-off is shown in roe deer (*Caprelous caprelous*) neonatal where fawns in open areas have a lower survival rate than fawns in forest habitats during years of high red fox (*Vulpes vulpes*) abundance, but a higher survival rate in years of low fox abundance (Jarnemo *et al*. 2004a). Lower roe deer fawn survival rate in open habitats, as agricultural fields, may also be due to mowing machines (Jarnemo 2002). Therefore, the resulting choice is the outcome of trade-offs between the cost and benefits sensed by the cervid (Lima and Dill 1990). Several studies have been made on the relationship between adult roe deer and ticks (*e.g*. Kiffner *et al*. 2010; Vor *et al*. 2010; Kiffner *et al*. 2011), still far too little attention has been paid to the relationship between roe deer neonatal and ticks. Since it is claimed that the roe deer is the main reason to the increased tick abundance and range expansion of the tick population in Sweden (Jaenson *et al*. 2012), it is of interest to get a better understanding of the fawn-tick relationship. In this study, the focus will be on tick burden in roe deer neonatal, and then especially regarding the relationship between vegetation- and habitat selection and tick burden. The roe deer is an important host for a numerous of tick species, where *Ixodes spp.* (Latreille 1795) and *Dermacentor spp*. (Koch 1844) is the primarily species in Europe (Walker *et al*. 2001; Rizzoli *et al*. 2007). On the Swedish mainland, almost all (>95 %) ticks that is found in larger mammals as human, cattle, horses, moose, roe deer, mountain hare, and European hare, belongs to *Ixodes ricinus* (Jaenson *et al*. 1994). Tick burden does not differ significantly between male and female roe deer (Vor *et al*. 2010) where an adult roe deer can be infested with more than 2000 ticks (Tälleklint and Jaensson 1997). Ticks may serve as vectors of tick borne diseases such as tick-borne encephalitis (TBE), Lyme disease, tick-borne fever and Babeosis (Aguire *et al*. 1999; Randolph *et al*. 1996; Bruno *et al*. 2000). Some scientist claim that the roe deer itself is not susceptible to tick-borne diseases such as TBE (Labuda *et al*. 2002; Hartemink *et al.* 2008; Rizzoli *et al*. 2007) and Lyme disease (Hartemink *et al*. 2008; Pugliese and Rosà 2008), but that they are competent reservoirs for Babeosis (Aguire *et al*. 1999; Malandrin *et al*. 2010) and for tick-borne fever (Alberdi *et al*. 2000; Silaghi *et al*. 2008). Ticks also have a direct effect on the host by reduced fitness caused by significant blood loss, which in worst case can lead to mortality in deer (Bolte *et al*. 1970; Barker *et al*. 1973; Hair *et al*. 1992). In a study by Hair *et al*. (1992) regarding white-tailed deer fawns infested with lone star tick (*Amblyomma americanum*), they detected a correlation between tick infestation level and fawn mortality, where fawns with 150 to 540 adult lone star ticks per week for approximately 30 days died. The primary cause of fawn mortality in their study was due to hematological reductions attributed to blood loss and tick-induced toxins. Alberdi *et al*. (2000) suggest that another outcome can be that the immunosuppressive effects of infection with the bacterium *Anaplasma phagocytophilum* (that causes tick-borne fever) may aggravate already present infections, as for example diseases from flukes (*Fasciola hepatica*) and lungworm (*Dictyocaulus spp*.).

The roe deer is a solitary (Hewison *et al*. 1998), small sized ungulate with an adult body mass of 20 to 30 kg (Andersen *et al*. 1998). Since the roe deer is a generalist herbivore it can forage from a broad range of different plant species (Vor *et al.* 2010) but as a concentrate selector (*i.e*. forage high quality -but low fibre- food; Hofmann 1985) it prefer herbs and deciduous browse (Duncan *et al.* 1998). The roe deer is consider to be adapted to forest habitat with earlier succession (Liberg and Wahlström 1995), but since the roe deer can feed on a lot of different plant species it is found in a wide variety of habitats (Vor *et al.* 2010). The habitat preference ranges from coniferous and deciduous habitat to agricultural land, moorlands, shrublands and marshes (Linnell *et al.* 1998a; Alberdi *et al.* 2000) and because of this, the roe deer is the most widespread and common ungulate in Europe (Liberg and Wahlström 1995). The roe deer has a synchronized birth period where 80 % of the fawns are born within a period of 20 to 30 days during late May and early June (Gaillard *et al*. 1993b; Aanes and Andersen 1996). The litter size normally varies between one and three fawns (Andersen *et al*. 1998). There is no difference in body mass and growth rate between male and female roe deer neonates (Gaillard *et al.* 1993a), where the fawns have a birth weight of approximately 1500 g and a growth rate of 150 g per day (Linnell 1994; Linnell and Andersen 1998). Body mass is a good predictor for individual performance in ungulates, where heavier individuals have a higher survival rate -regardless of age (Clutton-Brock 1991; Gaillard *et al.* 1997). This is consistent with Andersen and Linnell's (1998) study where roe deer fawns that had a lower survival rate also suffered from a reduced growth rate. There has also been shown among terrestrial vertebrates that early growth affects adult body size positively (Shine 1990). Regarding ticks, Hair *et al*. (1992) detected a correlation between weight loss, reduced weight gain, and lone star tick burden in white-tailed deer fawns, which has also been seen in cattle infested with the cattle tick (*Boophilus microplus*; Little 1963). Hair *et al*. (1992) believed that the reduced weight gain was a result of the fawns' suppressed metabolic function, due to tick toxins together with reduced food intake. Therefore it is of interest to distinguish if ticks have a negatively effect on weight gain or growth rate on the roe deer neonatal. However, there are a lot of other factors that may have an impact on birth weight, growth rate and body mass. For instance, fawn body mass is sensitive to changes in habitat quality (Pettorelli *et al*. 2003), climate (Toïgo *et al*. 2006), and population density both in their own (Kjellander *et al*. 2006) and in competing populations (Richard *et al*. 2010). In cervids, birth weight and growth rate are related to the mothers' condition, where mothers in better condition than average have offspring with higher birth weight and growth rate (Moore *et al*. 1988). There is also a trade-off between growth rate and the number of siblings due to the fawns' dependence on the lactational capabilities of the mother (Lavigueur and Barrette 1992) which results in a negative correlation between litter size and growth rate (Andersen and Linnell 1997).

In the role of mother-neonate relationship the roe deer is a typical hider species as the fawns lie hidden in vegetation –separated from the mother, motionless, odorless and with a reduced metabolic rate- for the first eight weeks. During the hiding phase, the doe visits the neonate approximately two to seven times a day for nursing and for change of bed site (Werner and Anholt 1993; Linnell 1994; Lima 1998), but overall the fawn spends up to 80 % lying in their bed site for the first four weeks (Linnell 1994; Linnell *et al*. 1998b). The fawn stays in their mother's home range and doesn't leave it until its first winter appears (Hewison *et al.* 1998). Hiding behavior is an anti predator strategy adapted for living in forest or in other dense habitats (Lent 1974) where the red fox is a major predator on roe deer fawns (Aanens and Andersen 1996; Jarnemo *et al*. 2004b). At approximately two weeks of age hider ungulates adopt flight behavior as an alternative to hiding from predators (Nelson and Woolf 1987). Hiding also provides shelter during harsh weather

(Bowyer *et al*. 1998). To find a proper bed site, the fawn is dependent of high vegetation and canopy cover, *e.g*. shrubs and herbaceous layers. The vegetation and habitat preference for fawns' bed sites then change with the succession of vegetation development during spring and summer. From early May, the bed site is often located in coniferous forest and moorland among heather, shrubs and other evergreen or woody plants, and in late May and early June, the bed sites is in open pastures and fields among herbaceous layers. In late June, when the harvest of the fields has occurred, the use of old fields and deciduous forests among herbaceous layers increase (Linnell *et al*. 1999; Linnell *et al*. 2004). But overall, forest is preferred over open habitats (Linnell *et al*. 1999; Bongi *et al*. 2008). The roe deer fawns preference of closed habitats with dense vegetation, together with their daily rhythm of feeding and hiding phases, makes them an easy target for questing ticks (Walker *et al*. 2001; Vor *et al*. 2010).

The tick life cycle consist of three development stages; larvae, nymph and adult (male and female). All life stages parasitize on roe deer (Gray 1991; Kiffner *et al*. 2010), where only one feed occur per life stage before transition to the next stage (Gray 1991), except for the adult male who only search for a female for reproduction and do not feed (Kiffner *et al*. 2010). The feeding last for approximately 3-4 days in larvae, 3-5 days in nymphs and 7-10 days in females (Materna *et al*. 2008). All tick life stages often feed simultaneously on the roe deer where close aggregation of the ticks allow transmission of virus and bacteria between tick life stages (Randolph *et al*. 1996; Alberdi *et al*. 2000). There is a possibility that it is a difference between tick life stages in pathogen transmission where less than 1 % of host seeking larvae are infected by the Lyme disease spirochetes whereas 10 to 30 % of the nymphs and 15 to 40 % of adult ticks are infected (Mejlon and Jaenson 1993; Gray *et al*. 1998). Ticks are sensitive to desiccation and therefore the climate, as temperature, humidity and rainfall, has a major influence on tick development and survival. Ticks are thereby dependent of habitats that offer optimal vegetation cover (Estrada-Peña 2001; Lindström and Jaenson 2003). In Scotland, Walker *et al*. (2001) recorded the highest abundance of *I. ricinus* nymphs in coniferous forest, and slightly lower abundance in deciduous forest followed by open pastures with the least abundance. In contrast to this, in Spain (Estrada-Peña 2001) oak (*Quercus*) and mixed forest had the highest *I. ricinus* abundance, although the open habitat was consistent with Walker *et al*. (2001) findings where ticks were absent. Findings in studies performed in Sweden (Mejlon and Jaenson 1993; Lindström and Jaenson 2003) is similar to Estrada-Peña (2001), with the highest nymphal abundance in mixed spruce (*Picea*) and mixed deciduous forest, followed by spruce and Scots pine (*Pinus sylvestris*) forest and open pastures and grassy areas. But overall, ticks survival and abundance is greater in closed habitats than in open habitats (Majlon and Jaenson 1993; Estrada-Peña 2001; Lindström and Jaenson 2003). Walker *et al*. (2001) investigated tick preference of vegetation types, where they found that blueberry shrubs (*Vaccinium myrtillus*) and heather (*Calluna vulgaris*) had the highest tick abundance of the main vegetation types they were investigating. Bentgrass (*Agrostis*), fescue (*Festuca*) and soft-grass (*Holcus*) had a lower abundance followed by bracken (*Pteridium*) vegetation. Adult life stages may be found on vegetation 1.5 meter above ground, whereas the larvae are more sensitive to ambient humidity and thereby stay closer to the ground (Mejlon and Jaenson 1997).

This thesis is a part of a long term research project of the relationship between roe deer, ticks and tick-borne diseases. This research project is conducted by Grimsö Wildlife Research Station, SLU, together with Linköping University, the National Veterinary

Institute, and the Public Health Agency of Sweden. The aim of this thesis is to examine tick burden on roe deer fawns in relation to their age, weight and hind foot length. Further, the relationship between tick burden and bed site is investigated, and then at two levels, *i.e*. the vegetation on the bed site and the habitat where the bed site is located. In order to that, the following issues were examined: (1) Is there any correlation between tick burden on the fawn and the fawns' age, weight and hind foot length? (2) Is there a negative correlation between tick burden, weight gain and growth rate of the fawn? (3) Is there a correlation between tick burden on the fawns and the vegetation on their bed site? (4) Can we see any difference in tick burden (both number and life stage/sex) in these vegetation types? (5) Is there a correlation between tick burden on the fawns and the habitat? (6) Can we see any difference in tick burden (life stage/sex) in these kinds of habitats?

Materials and Methods

Study area

The study was implemented at a research area located on Bogesund (59°24'N, 18°12'E), a peninsula situated in the hemiboreal zone north of Stockholm (Figure 1). The study area (1300 ha) consists of approximately 65% forest which is mainly dominated by the coniferous species Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*). Common oak (*Quercus robur*), willow (*Salix* spp.) and birch (*Betula* spp.) is also widespread species, but then in deciduous and mixed forest in the area. Agricultural land represents 25% of the area and consists of oats, wheat, rape and hay, where a minor part function as pasture for instance horses and cattle. Further, 10% consist of bogs and stony areas. Herb species are highly represented in most of the habitat types (Jarnemo 2004; Kjellander *et al*. 2006). The winter population density of roe deer in this area was 12.08 roe deer per km^2 in the year 2013 (Kjellander *et al*. unpubl.).

Figure 1. The study area (1300 ha) was located on Bogesund (59°24'N, 18°12'E), a peninsula situated in the hemiboreal zone north of Stockholm.

Capturing roe deer fawns

During May 27 to June 27 searches for neonate roe deer fawns were conducted, where fawns (N=25) were captured and recaptured by hand. Fawns were found either by observing marked and unmarked does that displayed signs of having fawns until they revealed their fawn. Other fawns were seen by chance as the fawn was spotted accompanying the doe. Some fawns were found by searches of siblings in surrounding areas of previously marked fawns, since the distance between siblings is usually less than 50 m during their first week of life (Panzacchi 2007). For each fawn, the weight and hind foot length (hereafter mention as *HFL*) were recorded. The recording of HFL was chosen as a complement to the weight since the skeleton is less affected by nutritional inadequacy in the diet than weight (Klein 1964). The fact that the birth weight of roe deer neonatal normally is 1500 g with a weight gain of 150 g per day (Linnell 1994; Linnell and Andersen 1998) were used as guidelines in determining the age. Other parameters that was included in the age determination was the appearance of umbilical cord and behavioral observation before, during, and after the capture (Jullien *et al*. 1992 in Andersen and Linnell 1998). The behavioral observations included head position, ear movement, and if the eyes were open or closed while approaching the fawn before capture. Further, behavior during the capture, as screaming and struggling, was noticed, as well if the fawn stayed at the bed site after the capture. The fawns' body was examined for ticks when captured, where the body was divided into two distinct sections: head and abdomen (where leg creases was included). This is because of the *Ixodes ricinus* preferred feeding site on the head and neck of roe deer (Kiffner *et al*. 2011) and the good overview over the abdomen due to sparse coat. The sections were systematically inspected to identify the number of ticks on the fawn. If the number of ticks in one section was below ten ($N < 10$) the precise number of ticks was recorded, although if it exceeded ten ($N > 10$), the total number of ticks was estimated. Ten randomly chosen ticks from each section were removed using a tweezers and then placed in test tubes for further examination. All collected ticks were frozen in order to simplify the determination of species, life stage, and sex of the ticks. Furthermore the fawns were ear-tagged and equipped with VHF transmitters (Televilt International AB, Lindesberg Sweden) with expandable collar with a drop-off function. If the fawn were captured when it was hidden, its GPS-position, the vegetation on the bed site and the habitat which the bed site was located in, was recorded. If the fawn wasn't hidden only the habitat and GPS-position was recorded. The marked fawns were recaptured one or two times, dependent on age. The fawns were monitored almost daily using radio tracking equipment (beacon receiver and antenna) from 27 May until 27 June. The positions were located either through visits, where the GPS-position were recorded, or by triangulation from fixed sites along roads, where the middle points were counted as the position of the fawn.

Vegetation and habitat

The vegetation on the bed sites was recorded and divided into three different vegetation types: "high grass and herbs", "short grass, herbs and moss", and "blueberry shrubs and moss". The computer software ArcMap 10 was used for habitat determination in the area the fawn was located and cross-checked by visits. The habitats was divided into seven categories: (1) Farmland, open land used for cultivation of grass and herbs for silage and hay production (2) Coniferous forest, closed habitat with gramineous- and herbaceous layers, blueberry shrubs and moss (3) Deciduous forest, closed habitat with gramineous- and herbaceous layers (4) Pasture, open habitat except for some few trees and bushes, with mainly gramineous- and herbaceous layers grazed by cattle and horses (5) Old field or Forest glade, small open areas surrounded by closed habitat, with gramineous- and herbaceous layers (6) Clear-cut, open habitat with gramineous- and herbaceous layers, ferns (*Pteridophyta*) and mosses (7) Forest edge, with gramineous- and herbaceous layers. The categories were decided in relation to the prevalent three species, where the threshold for standtype was that ≥65 percent of the trees are of the same species, and mixed forest contains of 35 to 65 percent deciduous species (Swedish National Forest Inventory 2012). Even a rough classification of the vegetation and habitat was made to get fewer variables in the analysis: "high grass and herbs in open habitat", "high grass and herbs in closed habitat", and "short grass, herbs, blueberry shrubs, and moss in closed habitat" (Table 1).

Table 1. Categories of vegetation types on the bed site and habitat in the area. Threshold for categorizing a particular standtype is when a species group share ≥ 65 percent.

Vegetation	Habitat	Vegetation and Habitat
1.High grass and herbs	1.Farmland	1.High grass and herbs
2.Short grass, herbs and moss	2.Coniferous forest	in open habitat
3.Blueberry shrubs and moss	3.Deciduous forest	2.High grass and herbs
	4.Pasture	in closed habitat
	5.Old field or Forest glade	3.Short grass, herbs, blueberry
	6.Clear-cut	shrubs, and moss in closed habitat
	7.Forest edge	

Ticks in vegetation

Ticks were sampled using "the woolen flannel cloth dragging method" (Mejlon and Jaenson 1993) for evaluation of the vegetation. The size of the woolen flannel cloth was one times one meter. Both ends of the cloth were hemmed and one of the ends was attached with a wooden stick and a string. The dragging method was implemented by dragging the cloth slowly in two transects á 140 m and one transect á 100 m through the vegetation. At every 30 m the cloth was inspected and the attached ticks were removed and placed in test tubes. With this method eleven randomly chosen study locations was investigated one or two times each. Three vegetations were represented: "high grass and herbs", "short grass, herbs and moss", and "blueberry shrubs and moss". The sampling was excluded when the vegetation was wet from rain and before 11 a.m. due to dew. Even here, all collected ticks were frozen in order to simplify the counting and determination of species, life stage and sex of the ticks.

Statistical analysis

The relationship between the fawns' age, weight and HFL, and tick burden, were tested using the Spearman Rank Order Correlations. The age, weight and HFL functions as a data point and therefore it does not matter that a fawn appears more than once. The Spearman Rank Order Correlations was also used in order to investigate if there were a negative correlation between weight gain, growth rate and the number of ticks on the fawn.

Potential differences in the number of ticks on the fawns between different vegetation types on the bed site were tested using Kruskal-Wallis ANOVA by Ranks, where the vegetation was the independent grouping variable and only the vegetation types that was recorded during captures was used. This test was chosen because the data were not normally distributed. The analysis tested all individual fawns, but also the fawns divided into two age groups (younger fawns ≤ 7 days < older fawns) and two weight groups (less heavier fawns < 2.33kg ≤ heavier fawns). Owing to the small sample size, even a non significant but close to significant difference, may indicate that there might be a difference. Therefore, the

Mann-Whitney U Test was performed for each weight class between the vegetation and habitats "high grass and herbs in open habitat" and "short grass, herbs, blueberry shrubs, and moss in closed habitat". Correlations between the total number of ticks on the fawn (*i.e.* the sum of all estimated ticks) and the vegetation on their bed site were tested with Spearman Rank Order Correlations. Correlations between the total number of ticks and the habitat the fawn have been located in were tested with the parametric test Pearson correlation coefficient since the data was normally distributed. Here all recorded data regarding vegetation and habitat between the first and the last catch of each fawn, *i.e.* locations from captures, visits and triangulations, was used.

Kruskal-Wallis ANOVA by Ranks was used to test if the life stage/sex of the ticks on the fawns differed between vegetation types, the rough classification on vegetation and habitat, habitat types, and in open or closed habitats. The tick life stages were tested separately and with the different vegetation and habitat types as the independent grouping variable.

The data from "the woolen flannel cloth dragging method" was estimated to ticks per 100 m². The data was not normally distributed and therefore Kruskal-Wallis ANOVA by Ranks was implemented to investigate if the number of sampled ticks differed between different vegetation types and between the rough classifications on the vegetation and habitat types. The vegetation and habitat was the independent grouping variable in both tests.

Results

In total, 25 individual roe deer fawns were examined one, two or three times where a total of 52 captures took place. Of all randomly removed ticks from the fawns were the nymphs and adults *Ixodes ricinus* while the larvae were not identified. The mean tick burden per fawn was 14 ticks, but the tick burden could range from 0 to a maximum of 80 ticks. The number of days between captures had a mean of 6 days and at the first capture the fawns had a mean tick burden of 8.6 ticks, followed by 17.9 ticks at their second capture, and 20.3 ticks at their third capture. The number of ticks per capture is represented in figure 2.

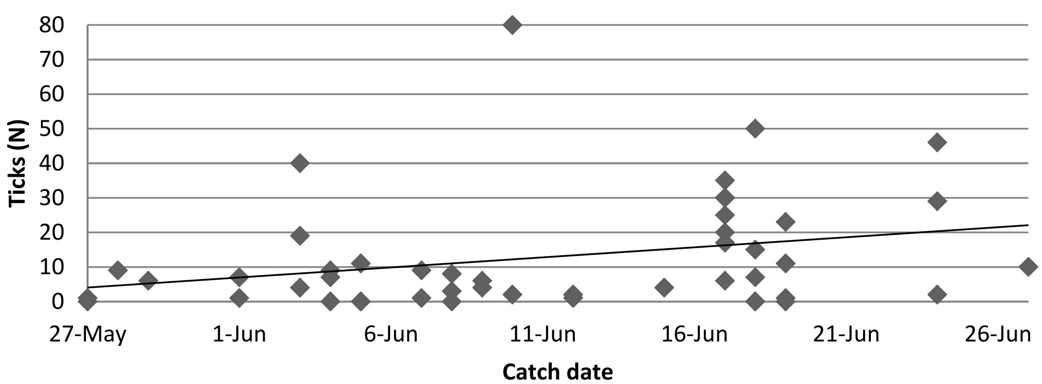

Figure 2. Roe deer fawns were captured and recaptured during May 27 to June 27, and tick burden was estimated. During the first capture the fawn had an average tick burden of 8.6 ticks, followed by 17.9 ticks at their second capture, and 20.3 ticks at their third capture.

Age and Body mass
During the captures the fawns' ages ranged between 1 and 20 days, the weight between 1230 and 4290 g, and the HFL between 17.5 and 24.5 cm. The mean weight gain was approximately 151 g per day, followed by a mean growth rate of 0.26 cm per day. Fawns' age was positively correlated with the number of ticks, and the same applied for the fawns weight and HFL (Table 2; Figure 3). However, the number of ticks did not affect the weight gain or growth rate during this study period (Table 2).

Table 2. Spearman Rank Order Correlations tested if roe deer fawns age, weight and hind foot length (HFL) correlate with tick burden. The same test also investigated if the total number of ticks on the fawns had a significant influence on weight gain and growth rate of the fawn. Correlations are significant at $p < 0.05$.

Spearman Rank Order Correlations	Valid N	Spearman R	t(N-2)	p-level
Age (days) & Ticks (N)	45	0.67	5.86	<0.0001
Weight (kg) & Ticks (N)	49	0.70	6.70	<0.0001
HFL (cm) & Ticks (N)	45	0.65	5.65	<0.0001
Weight Gain (g/day) & Ticks Total (N)	21	0.24	1.06	0.30
Growth rate (cm/day) & Ticks Total (N)	19	-0.11	0.47	0.64

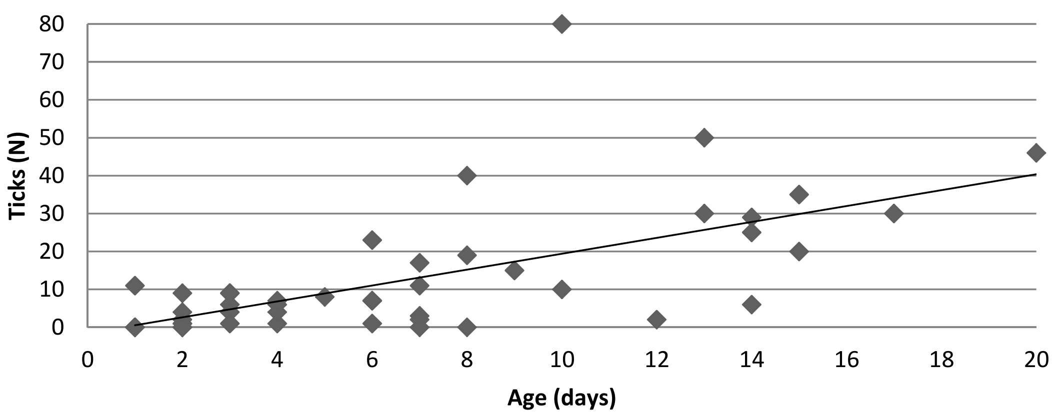

Figure 3. According to Spearman Rank Order Correlations the tick burden on roe deer fawns was significantly correlated to their age ($p < 0.0001$).

Vegetation on bed site
Vegetation types on bed sites was determined in 41 occasions during captures, where high grass and herbs was the most represented vegetation type (85.4%) followed by short grass, herbs and moss (9.8%), and blueberry shrubs and moss (4.9%). In relation to open or closed habitats (the rough classification of vegetation and habitats) 48.8 % of the bed sites was located among high grass and herbs in open habitats, followed by high grass and herbs in closed habitats (41.5%), and short grass, herbs, blueberry shrubs, and moss in closed habitat (9.8%).

No significant differences in the number of ticks on all individual fawns were observed between different vegetation types on bed site (Kruskal-Wallis test: H (2, N=40) = 0.75 p = 0.69). Further, this was also the case for the two age classes (Table 3). Anyhow, the ANOVA of the two weight classes showed almost a significant difference in the heavier weight class between the rough classification of vegetation and habitat ($p = 0.07$), and since the sample was rather small it may indicate that there might be a difference (Figure 4). Therefore, Mann-Whitney U Test was performed for the heavier weight class between the vegetation/habitats “high grass and herbs in open habitat” and “short grass, herbs, blueberry shrubs, and moss in closed habitat”. This nonparametric test detected a significant difference between the two vegetation types ($U = 1.00$ $Z = -2.01$ $p = 0.04$) where the amount of ticks was higher in the fawns that had been captured in short grass, herbs, blueberry shrubs, and moss in closed habitat. By investigating all bed site vegetation types between the first and the last capture (*i.e.* all bed sites located through captures or visits at 43 occasions) the Spearman Rank Order Correlations did not show a significant correlation between the total number of ticks and vegetation type.

Table 3. It was examined if the tick burden in roe deer fawns, where the fawns were divided in two different age groups, differed between different vegetation types on the bed site or between vegetations in relation to open and closed habitat: “high grass and herbs in open habitat” and “short grass, herbs, blueberry shrubs, and moss in closed habitat”. The table presents the results of the Kruskal-Wallis ANOVA by Ranks where the vegetation and vegetation/habitat was the independent grouping variable. Tests are significant at $p < 0.05$.

Kruskal-Wallis ANOVA by Ranks	**Vegetation**		**Vegetation/Habitat**	
	Fawns ≤ 7 days	Fawns > 7 days	Fawns ≤ 7 days	Fawns > 7 days
H	0.98	1.75	0.15	3.71
p	0.61	0.42	0.93	0.16

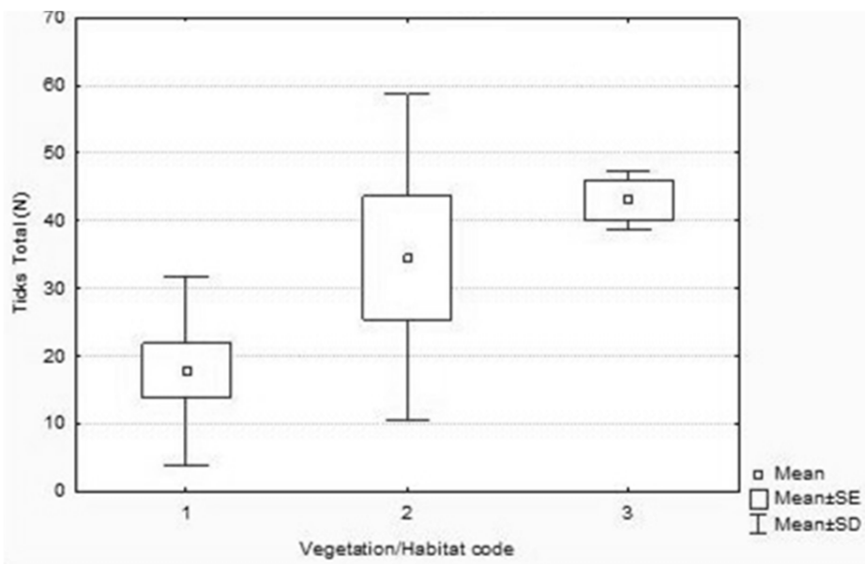

Figure 4. Tick burden on the fawns with a weight over 2.33 kg in different vegetation and habitat types, where number 1 = High grass and herbs in open habitat, 2 = High grass and herbs in closed habitat, and 3 = Short grass, herbs, blueberry shrubs, and moss in closed habitat.

Habitat on bed site

Habitat types used by fawns was determined in 93 occasions between the first and the last capture, where 60.2 % belonged to coniferous forest followed by farmland (16.1 %), pasture (7.5 %), deciduous forest (6.5 %), old field or forest glade (4.3 %), clear-cut (3.2 %), and forest edge (2.2 %). Some fawn locations were recorded after the last capture of the fawn, but were not included in the statistical tests. These locations are nonetheless interesting to get a better understanding in which habitats the fawns has been in. Looking at the habitats pre and post the field harvest, which occurred mainly during June 22 to June 23 and where a possible change in habitat use could occur (Linnell *et al*. 2004), a total of 111 locations were determined before the harvest and 15 locations after. Before the harvest, 54.1 % of the fawn locations were positioned in coniferous forest, followed by farmland (24.3 %), deciduous forest (7.2 %), pasture (5.4 %), clear-cut (4.5 %), old field or forest glade (3.6 %), and forest edge (0.9 %). After the harvest of the fields, the use of pastures increased up to 33.3 % but still with the highest habitat use of coniferous forest (53.3 %), followed by deciduous forest (6.7 %), and forest edge (6.7 %). Farmland, old field or forest glades, and clear-cuts were not used during this period. Therefore, the use of closed habitats was higher than the use of open habitats during the whole study period.

Coniferous forest and old field or forest glade was accordingly to the test positively correlated with the total number of ticks, and farmland was negatively correlated with the total number of ticks (Table 4). *I.e.* the highest prevalence of *I. ricinus* was found on roe deer fawns that more frequently was positioned in coniferous forest and old field or forest glades, while the lowest prevalence was found on the fawns that mostly were located in farmland. No correlation was found in the remaining habitats.

Table 4. Pearson Correlation coefficient tested whether the number of ticks on the fawn correlate with the habitats they have been in. Correlations are significant at $p < 0.05$.

Pearson Correlation coefficient	**Mean**	**Std.Dv.**	**r(X,Y)**	**r²**	**t**	**p**	**N**
Ticks Total (N) & Farmland	0.32	0.40	-0.53	0.28	-2.91	0.01	25
Ticks Total (N) & Coniferous forest	0.48	0.34	0.61	0.37	3.59	0.002	25
Ticks Total (N) & Deciduous forest	0.06	0.14	-0.10	0.01	-0.46	0.65	25
Ticks Total (N) & Pasture	0.07	0.19	-0.09	0.01	-0.40	0.69	25
Ticks Total (N) & Old field or Forest glade	0.02	0.07	0.54	0.30	3.05	0.01	25
Ticks Total (N) & Clear-cut	0.02	0.05	0.07	0.01	0.35	0.73	25
Ticks Total (N) & Forest edge	0.03	0.09	-0.13	0.02	-0.61	0.55	25

Tick life stages

A total of 362 ticks were collected, where all tick life stages and sexes was represented. Nymphs belonged to the most numerous life stages (55.0 %), followed by adult females (26.8 %), adult males (13.8 %), and larvae (4.4 %). No significant difference was found in tick life stages/sexes between different vegetation types. The same was regarded for the life stages/sexes between the rough classifications of vegetation and habitats. However, the ANOVA detected significant differences in nymphs, adult females, and adult males between different habitat types (Table 5). There were more nymphs on the fawns captured in deciduous forest, old field or forest glade, pasture, and clear-cut, than on the fawns in farmlands and coniferous forest. Adult females were more abundant on fawns in coniferous forest, pastures, and clear-cuts, than in farmlands and deciduous forest. Adult males had a higher abundance on fawns captured in coniferous- and deciduous forest than in farmlands and pastures (Figure 5). The presence of males is consistent with the fact that the number of adult female and male ticks on fawns captured in closed habitat was significant higher than the amount on fawns in open habitats. Nonetheless the amount of larvae and nymphs was independent of open or closed habitats (Table 5).

Table 5. It was examined if the life stage and sex of the present ticks on the fawns differed between habitat types and between open and closed habitats. The table presents the results of the Kruskal-Wallis ANOVA by

Ranks where the habitat types (farmland, coniferous forest, deciduous forest, pasture, old field or forest glade, clear-cut, and forest edge), and open and closed habitats was the independent grouping variable. Tests are significant at $p < 0.05$.

Kruskal-Wallis ANOVA by Ranks					
Habitat		Larvae	Nymph	Female	Male
	H	5.57	17.15	15.54	13.76
	p	0.35	0.004	0.01	0.02
Open and Closed Habitat					
		Larvae	Nymph	Female	Male
	H	0.29	0.002	5.08	5.97
	p	0.59	0.96	0.02	0.02

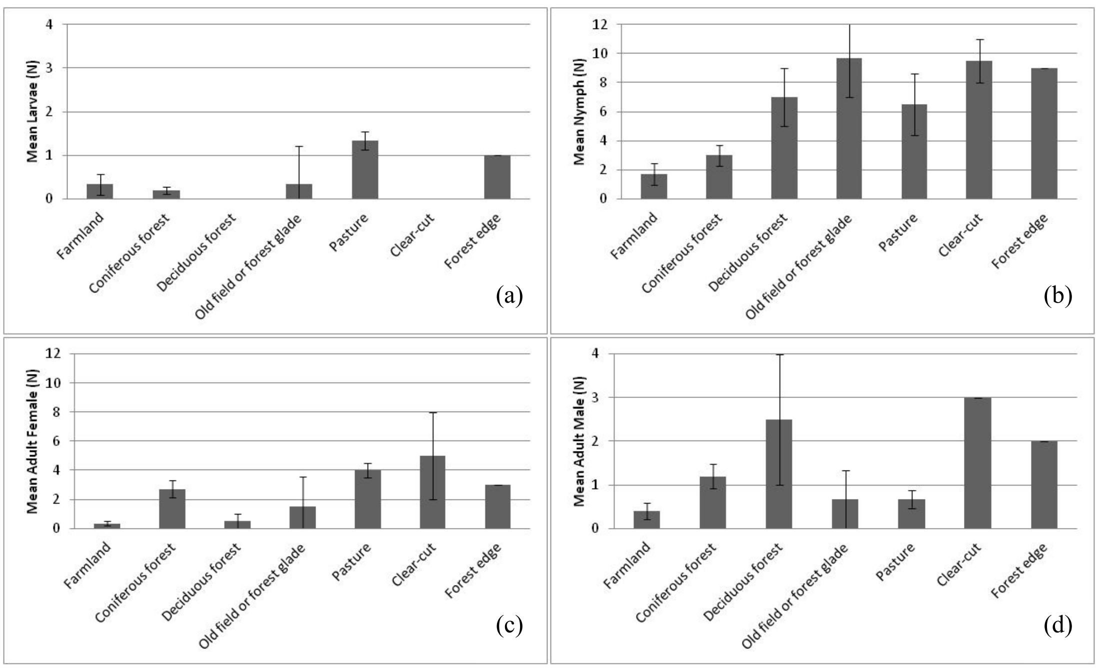

Figure 5 a-d. These figures shows how the tick life stages/sexes differed between roe deer fawns captured in different habitat types. Each bar represents the mean number of ticks with standard error. The bars that have no standard error had only one fawn caught in that habitat.

The woolen flannel cloth dragging method

A total of 1262 ticks, all nymphs and adult *I. ricinus* and larvae unidentified, were sampled in eleven transects at 18 occasions. The highest tick abundance was recorded among short grass, herbs and moss with a mean of 69.1 ticks/100m². This was followed by blueberry shrubs and moss (44.9 ticks/100m²), and the lowest tick abundance was recorded in high grass and herbs (6.6 ticks/100m²; Figure 6). There were no significant difference in tick abundance among different vegetation types according to the ANOVA ($H (2, N=18) = 5.44$ $p = 0.07$), nor in the rough classification of vegetation and habitats ($H (2, N=18) = 1.28$ $p = 0.83$). This was also the case in the analysis of the tick life stages/sexes where there were no significant differences in the different life stages/sexes between vegetations (Larvae: $H (2, N=18) = 5.82$ $p = 0.05$, Nymphs: $H (2, N=18) = 1.01$ $p = 0.61$, Adult female: $H (2, N=18) = 1.22$ $p = 0.54$, Adult male: $H (2, N=18) = 5.51$ $p = 0.06$).

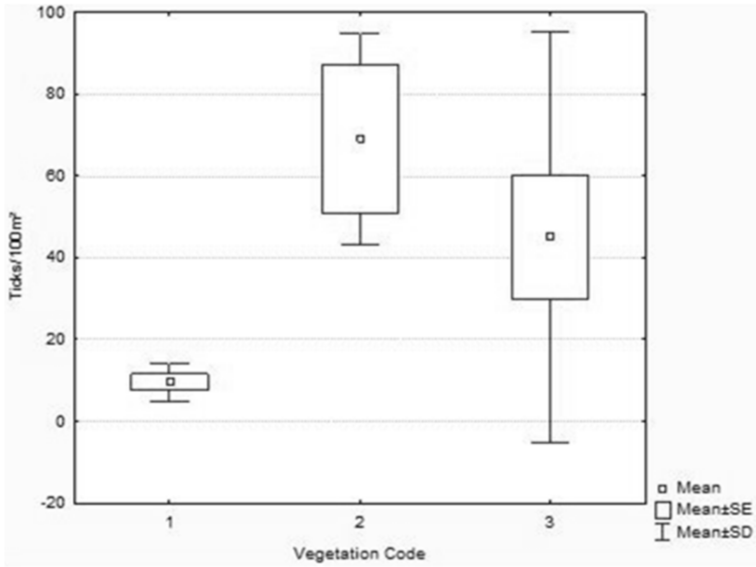

Figure 6. Tick sampling by the woolen flannel cloth dragging method during May 27 to June 27, where three vegetation types were represented: (1) High grass and herbs, (2) Short grass, herbs and moss, and (3) Blueberry shrubs and moss.

Discussion

One of the most interesting finding was that tick burden was positively correlated with roe deer fawn age, weight and HFL, with a higher tick burden on older, heavier and larger fawns. Vor *et al*. (2010) found no significant correlation in tick abundance with age, weight or HFL in adult roe deer. However, they found a positively correlation between adult tick burden and age, weight and HFL, and a negatively correlation between larvae tick burden and age. Vor *et al*. (2010) considered the latter correlation to indicate a larvae preference for younger host, due to the fawns longer resting phases and thinner skin, but in this study the larvae burden was only 4.4 % of the collected ticks. Although, even if the tick sampling was carried out with extraordinary accuracy, I cannot exclude that larvae ticks were underrepresented in the sample due to their small size. A possible explanation for the positive correlation between tick burden and age in this study may be a combination of several components. Since the seasonal activity patterns of questing nymphs at Bogesund differs among years but with no peak during summer (Tälleklint and Jaenson 1996), a higher activity pattern in ticks in late June may be neglected as an explanation. This is supported by Walker *et al*. (2001) study in the U.K. where the highest *I. ricinus* nymph abundance was in April, and then rapidly decreased in May, June, and July. Fawns tend to select bed site locations pursuant to the succession of vegetation development. In late May and early June the bed site is mostly located in open pastures and fields, while in late June the use of old fields and deciduous forests among herbaceous layers increases, due to the harvest of fields (Linnell *et al*. 1999; Linnell *et al*. 2004). The increased use of closed habitat may result in a higher tick burden, however in this study the fawns tended to use a greater amount of closed habitats, as coniferous forest, both before and after the harvest. Although, there were a decrease in the use of farmlands and old field or forest glades, and a increase in the use of pastures, after the harvest. Overall, the fawn preference of closed habitats was higher than open habitats during the whole study period. Roe deer fawn spends up to 80 % lying in their bed site for the first four weeks, and during these weeks the doe visits the neonate approximately two to seven times a day for nursing and for change of bed site (Linnell 1994; Linnell *et al*. 1998b). No fawn was older than 20 days when the last capture took place, and thereby it is assumed that these fawns spent a large part of the day in their bed sites. Since they are inactive large parts of the day, it takes a larger amount of time to be infested by a greater number of ticks from the bed site, as well as on the way to a new bed site. However, it is possible that fawns become more active with age during their first weeks in life (Fitzgibbon 1990) where hider ungulates change anti predator strategy from hiding to fleeing when they are approximately two weeks old (Nelson and Woolf 1987). This may result in higher activity and the fawns may thereby become more infested with questing ticks in a predator environment. The outcome of both of these latter alternatives, together with the use of closed habitats, may explain why roe deer fawns become more infested with ticks as older they get. Further studies, where roe deer fawn movement is examined, is required to establish if it is an increased movement that may have an influence on tick burden early in life.

In contrast to earlier findings, tick burden did not have any negative effect on weight gain or growth rate in this study. Hair *et al*. (1992) found that lone star tick burden in neonatal white-tailed deer was negative correlated with weight gain, and the same applies in Little (1963), where the same effect was seen in cattle infested with the cattle tick. The lack of correlation in this study may be due to a combination of the short amount of time the fawn was examined and the young age of the fawns and thereby a rather low tick burden. Since age and tick burden was positively correlated, we cannot reject the risk that tick burden will reach a level that might have a negative effect on weight gain and/or growth rate as the fawn ages. To investigate this further, the fawns should be followed for a longer time period. One also has to remember that in this study the tick burden was only estimated on head and abdomen. Since the number of *I. ricinus* on the head represent a large proportion of the total tick burden on the whole body, the head have been found effective for estimate total tick burden on roe deer (Kiffner *et al*. 2010). Even if the total tick burden was not estimated in this case, I corroborate the tick estimation on the head and abdomen as a convenient method since I wanted an accurate estimated tick burden, and that the handling time of the fawn would be as short as possible. This was possible as a result of good overview due to rather sparse coat on the abdomen and the tick preferred attachment site on the fawns' ears. However, this method may underestimate the tick burden which supports the non effect of tick burden on neonatal weight gain and growth rate during the first 20 days of life.

There was a significant difference in tick burden between vegetation types on roe deer neonatal in the heavier weight group, where the fawns that had been captured in short grass, herbs, blueberry shrubs, and moss in closed habitats had a higher tick burden than fawns that was caught in high grass and herbs in open habitats. This was consistent with the result from sampling ticks on cloth drags, where the highest amount of ticks was found among short grass, herbs and moss with a mean of 69.1 ticks/100m², followed by blueberry shrubs and moss with 44.9 ticks/100m², and high grass and herbs with 6.6 ticks/100m². According to Walker *et al*. (2001), the highest tick abundance is found in blueberry shrubs and heather, followed by bent-grass, fescue, and soft-grass. The lack of significant difference in tick burden, and in tick life stages/sexes, between vegetation types alone maybe due to the ticks' preference of closed habitat (Mejlon and Jaenson 1993; Estrada-Peña 2001; Lindström and Jaenson 2003). The vegetation type high grass and herbs was present in both open habitats, as well as in closed forests, and therefore the tick burden on fawns with the bed site among high grass and herbs had a wide variety. Possibly, the significant difference in tick burden between short grass, herbs, blueberry shrubs, and moss in closed habitats and high grass and herbs in open habitats depends to a larger extent on the habitat than the vegetation. One contributing factor may also be the fawns' use of multiple vegetation and habitat types, which implies that they can be infested by questing ticks from other vegetations and habitats than the ones they been located in. The reason why no significant difference was found in the less heavy weight group may be due to inactive behavior and thereby a lower tick burden, independent of vegetation and habitat.

Another important finding was that the highest prevalence of ticks was found on roe deer fawns that were positioned more frequently in coniferous forest or, surprisingly, in old field and forest glades, while the lowest prevalence was found on the fawns that were more frequently located in farmlands. It is known that ticks have a lower survival and abundance in open habitats (Mejlon and Jaenson 1993; Estrada-Peña 2001; Lindström and Jaenson 2003), therefore the findings that old field and forest glade was positively correlated with tick burden were unexpected. Since old fields and forest glades was rather small areas surrounded by more dense forest habitats, together with the possibility that the fawns have been in other habitats than the ones I have recorded, there is a high possibility that their other bed sites has been located in nearby closed habitats and been infested by questing ticks there. Looking at the fawns that was located in old fields or forest glades, they had also been located in coniferous forest, and then to a higher amount. As a result of this, the correlation between tick burden and the habitats old fields and forest glades might be biased. The highest prevalence of ticks in coniferous forest was on the other hand expected.

The analysis of the collected ticks shows that the presence of larvae on roe deer fawns is independent of the habitats the fawns has been in, but that the presence of the other life stages/sexes is dependent of habitat. Nymphs were more abundant on fawns captured in deciduous forest, old field or forest glade, pasture, and clear-cut, than on fawns in farmlands and coniferous forest. Adult females were found to a larger extent on fawns in coniferous forest, pastures, and clear-cuts, than in farmlands and deciduous forest. Adult males had a higher abundance on fawns captured in coniferous- and deciduous forest than in farmlands and pastures. The higher nymph abundance in deciduous forest is consistent with findings of other studies in Sweden (Mejlon and Jaenson 1993; Lindström and Jaenson 2003). Though, in these studies they had also high nymph abundance in coniferous forest, followed by a low abundance in open pastures and grassy areas, which is not consistent with the findings of the present study. Walker *et al*. (2001) had similar result with the highest abundance of *I. ricinus* nymphs in coniferous forest and the lowest abundance in open pastures. The higher adult tick burden in fawns captured in closed habitats than on fawns in open habitats supports previous findings that ticks is more abundant in closed habitat (Mejlon and Jaenson 1993; Estrada-Peña 2001; Lindström and Jaenson 2003). Nonetheless the amount of larvae and nymphs was independent of open or closed habitats. Mejlon (1997) had the opposite findings in his study, where the abundance of *I. ricinus* larvae and nymphs was significantly higher in forest compared to open meadows, and where the abundance of adults was low at both sites. More already mentioned, it is possible that the fawns have been in other habitat types than the ones I have recorded, and thereby been infested by ticks there. This may result why there is a high tick burden on fawns that only have been located in hostile habitat types for ticks.

Concluding remarks and future effort
Habitat selection can have large effect in life-history traits among roe deer fawns, where the habitat quality creates trade-offs between costs and benefits (Lima and Dill 1990; Bowyer *et al*. 1998; Linnell *et al*. 2004; Nilsen *et al*. 2004). Roe deer fawns in open habitats are more vulnerable to fox predation (Aanes and Andersen 1996; Jarnemo *et al*. 2004a), as well as to mowing machines in farmlands (Jarnemo 2002), than fawns in closed habitats. However, observations in the present study show that fawns who more frequently uses open habitats experience a lower tick burden than fawns that uses closed habitats. Yet, it is still rather unknown what negative impact tick burden may have on roe deer neonatal, and how it affects them in the long term. Since there is a positive correlation between the fawns age and tick burden, together with earlier findings of negative correlation between tick burden and weight gain (Little 1963; Hair *et al*. 1992), it is possible that tick burden will reach a level that have a negative effect on weight gain and/or growth rate of the roe deer fawn. Therefore, further research of the relationship between roe deer fawns and tick burden is required during a longer time period.

Acknowledgement

First of all I would like to thank my assistant supervisor Ulrika Alm Bergvall for the great advice, support and feedback throughout the field work, statistical analysis, and writing.

This thesis is a part of a long term research project of the relationship between roe deer, ticks and tick-borne diseases, and therefore I would like to thank the leader and supervisor of the project, Petter Kjellander, SLU, Grimsö, Sweden, for the opportunity to be a part of the project. Further, the long term project is financed by the Swedish Environmental Protection Agency's wildlife management fund. I will also thank the National Property Board of Sweden who is the landowner of Bogesundsland, and the Swedish Association for Hunting and Wildlife Management in Stockholm for lending the study area.

I would like to thank Torsten Berg for great advice and help during the field work, Emma Tjärnström for the companionship during the field work and discussions during the writing of this work and my supervisor Göran Ericsson. Finally, I'm grateful for the support from my boyfriend Fredrik Hansson.

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