OF TICKS, MICE AND MEN

shaping the ecology of tick-borne pathogens in Baden-Württemberg

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„Nature itself is just one unreplicated realization of a large stochastical process.“

Stephen Ellner

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Zusammenfassung

Zecken verursachen mehr Krankheitsfälle in der Nordhemisphäre und übertragen weltweit mehr verschiedene Krankheitserreger auf Mensch und Tier als jeder andere blutsaugende Arthropode. Während der letzten Jahrzehnte haben sich die Hinweise auf die Zunahme der Verbreitung und Häufigkeit human- und veterinärmedizinisch bedeutsamer Zeckenarten in Europa immer weiter verdichtet. Nach wie vor besteht jedoch ein beträchtlicher Mangel an detailliertem Verständnis der Faktoren, welche die Dichte und Dynamik von Zecken beeinflussen. Umfangreiche europäische Langzeitstudien über die komplexen Zusammenhänge zwischen Zecken, ihrer Umwelt und den von ihnen übertragenen Pathogenen sind kaum vorhanden.

Einflussreiche Studien aus den USA haben gezeigt, dass dort Kleinsäuger eine Schlüsselfunktion in der Regulation von Zecken und den von ihnen übertragenen Pathogenen besitzen: Sie dienen als Blutwirt für das erste (Larve) und zweite (Nymphe) Entwicklungsstadium von Zecken und sind empfänglich für die Infektion mit zahlreichen zeckenübertragenen Krankheitserregern; ohne jedoch selbst zu erkranken. Die Bedeutung dieser Studien in Bezug auf europäische Verhältnisse ist jedoch unbekannt. Daher war es das Ziel meiner Arbeit, den relativen Einfluss von Kleinsäugerpopulationen und Umweltfaktoren auf die Dichte und Dynamik von Zecken und zeckenübertragenen Pathogenen in Baden-Württemberg (BW) zu bestimmen.

An vier Waldstandorten wurden dazu monatlich von Frühling bis Herbst 2012 bis 2014 Kleinsäuger mit Lebendfallen gefangen, individuell markiert und wieder freigelassen. Charakteristika der gefangenen Tiere wurden erhoben und vorhandene Zecken abgesammelt. Zusätzlich wurden an den Standorten kontinuierlich Mikroklimadaten aufgezeichnet und Zecken von der Vegetation gesammelt um methodenbasierte Einflüsse vergleichen zu können. Alle gesammelten Zecken wurden im Anschluss auf Infektion mit den zeckenübertragenen Pathogenen *Babesia* spp., *Borrelia burgdorferi* s.l., *Rickettsia* spp., Frühsommermeningoenzephalitis-Virus und *Candidatus* Neoehrlichia mikurensis untersucht.

Ich konnte dadurch die strukturelle Stabilität der Kleinsäugergemeinschaften trotz ausgeprägter jährlicher und saisonaler Schwankungen der Populationsdichte zeigen. Der negative Zusammenhang zwischen Wirtspopulationsdichte und individueller

Zeckenbelastung offenbarte, dass Kleinsäugerpopulationen keinen Einfluss auf die Zeckendichte im Untersuchungsgebiet ausüben. Zudem zeigte sich ein Einfluss der jeweiligen Pathogenökologie auf ihre Prävalenz in Zecken.

Durch die Etablierung statistischer Modelle in Verbindung mit meinem umfangreichen Datensatz konnte ich zeigen, dass Temperatur und Sättigungsdefizit, sowie Wirtsfaktoren den Zeckenbefall von Kleinsäugern maßgeblich beeinflussen, wobei dieser jedoch keinen bedeutenden Einfluss auf die Gesamtdichte von Zecken oder Pathogenen hat.

Meine Arbeit (i) stellt damit das Paradigma von Kleinsäugern als Schlüsselwirte für zeckenübertragene Pathogene in Frage, (ii) identifiziert das Zecke-Wirt-Pathogen-System in BW als deutlich komplexer als in den USA und (iii) verdeutlicht damit die Notwendigkeit weiterer Langzeitfeldstudien um die Bedeutung der untersuchten Pathogene auf die öffentliche Gesundheit bestimmen zu können.

Abstract

Ticks cause more vector-borne diseases in humans and animals of the Northern Hemisphere and transmit more different pathogens than any other blood-feeding arthropod worldwide. Over the past decades, evidence for an increase in the distribution and abundance of ticks of medical and veterinary importance in Europe has accumulated. To date, however, there is still a remarkable lack in our understanding of the detailed understanding of the factors that influence tick distribution and dynamics. Comprehensive studies on the complex relationships between ticks, their environment and the pathogens they transmit are scarce. Influential studies in the US show that small mammals are keystone species regulating the abundance and dynamics of ticks and tick-borne pathogens (TBP): they serve as blood meal hosts for the first (larval) and second (nymphal) life history stages of ticks and are susceptible to a variety of TBPs without suffering from disease symptoms themselves. The relevance of these American studies to Europe is, however, unknown.

Therefore, the aim of my PhD thesis was to determine the relative influence of small mammal host populations and environmental factors on the distribution and dynamics of ticks and TBPs in Baden-Württemberg (BW).

In four forest sites, small mammals were trapped monthly from spring to autumn 2012 to 2014 using live traps. Individual features of the captured individuals were determined, they were individually marked upon first encounter, examined for ticks and released. In addition, weather data was recorded continuously using microclimate data loggers, and ticks were collected from vegetation to compare for potential methodological bias.

Ticks from hosts and vegetation were subsequently analyzed for the presence of the TBPs *Babesia* spp., *Borrelia burgdorferi* s.l., *Rickettsia* spp., tick-borne encephalitis virus and *Candidatus* Neoehrlichia mikurensis.

I could show the structural stability of small mammal hosts communities despite the occurrence of substantial inter- and intra-annual variability in population densities. The negative relationship between host population density and tick burdens on individual hosts demonstrated the absence of any influence of small mammal populations on tick abundance in the investigated area. In addition, the ecology of the respective pathogens was most likely to influence their prevalence in ticks.

Combining the detailed data from three years of sampling with the use of advanced statistical methods, I could show that temperature and saturation deficit as well as host factors influence tick burdens on rodent hosts, but that these do not contribute significantly to the overall abundance of ticks or pathogens. My study: (i) challenges the paradigm of rodents as central drivers of TBPs in the wild, (ii) identifies the tick-host-pathogen-systems in BW as being far more complex than those in the US, and (iii) emphasizes the urgent need for further, long-term field studies to determine the public health impact status of these disease agents in BW.

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Abbreviations

a	Adult
Af	*Apodemus flavicollis*
As	*Apodemus sylvaticus*
AIC	Akaike information criterion
ANOVA	Analysis of variance
AW	Auwald
Bbsl	*Borrelia burdorferi* sensu lato
BW	Baden-Württemberg
CNM	*Candidatus* Neoehrlichia mikurensis
D.	*Dermacentor*
HSD	Honestly significant difference
HW	Hardtwald
GAM	Generalized additive model
GLM	Generalized linear model
I.	*Ixodes*
j	Juvenile
JS	Jolly Seber
MB	Michaelsberg
Mg	*Myodes glareolus*
p	Probability of statistical significance
$\overline{R^2}$	Adjusted coefficient of determination
Rspp	*Rickettsia spp.*
s^2	Variance
Sa	*Sorex araneus*
sa	Subadult
Sm	*Sorex minutus*
SD	Standard deviation
SW	Schwarzwald
TBP(s)	Tick-borne pathogen(s)
TBD(s)	Tick-borne disease(s)
TBE	Tick-borne encephalitis
TBE-V	Tick-borne encaphalitis virus
VIF	Variance inflating factor

1 INTRODUCTION

1.1 What is ecology?

Ernst Haeckel, the founding father of ecology, defined it as follows:

"By ecology, we mean the whole science of the relations of the organism to the environment including, in the broad sense, all the "conditions of existence." These are partly organic, partly inorganic in nature; both, as we have shown, are of the greatest significance for the form of organisms, for they force them to become adapted. Among the inorganic conditions of existence to which every organism must adapt itself belong, first of all, the physical and chemical properties of its habitat, the climate (light, warmth, atmospheric conditions of humidity and electricity), the inorganic nutrients, nature of the water and of the soil, etc.

Haeckel (1866) Oecologie und Chorologie.

Translated by Stauffer (1957).

1.2 Small mammals

The term “small mammal” does not refer to a taxonomic group, but is used to comprise animals that are both mammals and small, with up to 31cm in size and a maximum weight of 5 kg (Merritt, 2010). However the definition of *small* is somewhat arbitrary and its interpretation varies largely among authors, suggesting weight limits between 120 g (Delany, 1974) and a maximum of 20 kg (Heusner, 1991).

Most small mammals are rodents (Rodentia), bats (Chiroptera), shrews (Soricomorpha) and hedgehogs (Erinaceomorpha) with some further contribution coming from primates (Primates), carnivores (Carnivora) as well as monotremes (Monotremata: echidna and platypus). As the focus of the present study lays predominantly on rodents and to a certain extent on shrews, these two orders will be discussed thoroughly and the term “small mammals” will be applied in reference to them.

In Central Europe, terrestrial small mammals comprise a total of 38 species from eight different taxonomic families or subfamilies, respectively, with mice (Murinae), voles (Arvicolinae) and shrews (Soricidae) being the most abundant and species-rich ones (Blatt and Resch, 2016a). They have short generation times and are widespread as well as abundant throughout Germany and BW (Braun and Dieterlen, 2005; Grimmberger, 2014). Besides their small body dimension and the fact that they are not under protection status in Germany, this makes small mammals ideal study organisms for ecological studies.

1.2.1 Biology and ecology of wild small mammal species in Germany

***Apodemus flavicollis* Melchior, 1834 –yellow-necked mouse (*Af*) (Rodentia: Muridae)**

The 26-32 g heavy animals have a snout to vent length of about 65-120 mm. Additionally, the tail, with an average of 65-130 mm can be the same length or longer than the body itself (Jenrich *et al.*, 2010). The fur shows a lot of contrast being blonde-reddish on the back and the sides while it is white on the belly, which gives a clear separation between the upper and the lower body side. A very distinct feature of the species is the “yellow” collar to the neck, in most cases represented by a continuous band and never prolonged to the belly (Jenrich *et al.*, 2010), which is the eponym of the common name of the species.

Occurrence, distribution and habitat preferences

Af is distributed throughout the western Palearctic, including Europe and the middle East (Mitchell-Jones *et al.*, 1999). It can be found throughout Germany, except for the extreme North-West and the Frisian islands (Grimmberger, 2014) and up to 2.300 m above sea level (Flowerdew, 1984; Hille and Mortelliti, 2010). The yellow-necked mouse is a typical forest species with a certain degree of flexibility both at habitat and microhabitat level (Hille and Mortelliti, 2010). It prefers mature deciduous forests such as oak and beech forests as well as mixed forests (Hille and Mortelliti, 2010; Quéré and Le Louarn, 2011). Additionally, it seems to be largely restricted to forest areas (Kraft, 2008). Important for the habitat suitability is a tree layer with high coverage, a high amount of fruitful trees, little leaf litter and an only slightly developed herbal layer (Jenrich *et al.*, 2010). Since *Af* is not closely bound to the coverage of shrubs, it can also be found in spruce stands (Kraft, 2008). Within the forest, it prefers structured areas with e.g. dead wood (Marsh and Montgomery, 2008). In more heterogenic landscapes, where areas of forest, hedges, succession and meadows occur, the density of *Af* is much higher than in homogenuous areas (Jenrich *et al.*, 2010).

Their burrows and nests are mainly build under tree trunks, stones or tree roots, which helps to protect them against predators such as foxes (*Vulpes vulpes*) or wild boar (*Sus scrofa*) (Marsh and Montgomery, 2008). The corridors lay in average around 50 cm below the ground but can be as deep as 150 cm. Every individual has one or two nests (Blatt and Resch, 2016b). Their territory varies and its size is highly dependent on the reproductive phase where it is about 1.6 ha for reproductive males and 0.7 ha for reproductive females (Jenrich *et al.*, 2010). Outside of the reproductive phase, the territory of a female is, with 0.3-0.4ha very small (Jenrich *et al.*, 2010). In autumn, due to increasing population density (see ecology), the territories become smaller and can be given up entirely. During that time of the year, *Af* individuals focus mainly on increasing their food resources (Jenrich *et al.*, 2010) and can cover distances up to 1 km (Marsh and Montgomery, 2008).

Ecology

Af is a crepuscular to nocturnal animal, which is also featured through their large eyes and prominent ears (Jenrich *et al.*, 2010a). Individuals are highly active and are able to not only run, but jump for long distances (Turni, 2005a) and to climb trees (Schröpfer, 1984;

Müller, 2011). During winter, *Af* lapses into a torpor and reduces its metabolic rate to about 156 kJ/ day. The average longevity of *Af* is about one year (Radda et al., 1969; Vogel, 1995) which is mostly a result of the high winter mortality rate of 80% (Jenrich *et al.*, 2010). The population density of this rodent lies between two Individuals/ha in spring and 15 Individuals/ha in fall (Vogel, 1995). After preceding tree mast events the population density can increase up to 55 Individuals/ha (Vogel, 1995). *Af* mainly feeds on pollen and nectar from flowers, seeds, mushrooms and berries as well as, to a lesser extent, on insects, larvae, earthworms, spiders, snails and small vertebrates such as bats or young birds (Hille and Mortelliti, 2010; Blatt and Resch, 2016b). Hazelnuts, acorns and beechnuts are stored in storerooms within the burrows to provide food supply during the winter period. Reproduction occurs between February and September (Niethammer, 1990a). Individuals born in spring become reproductive after two to three months.

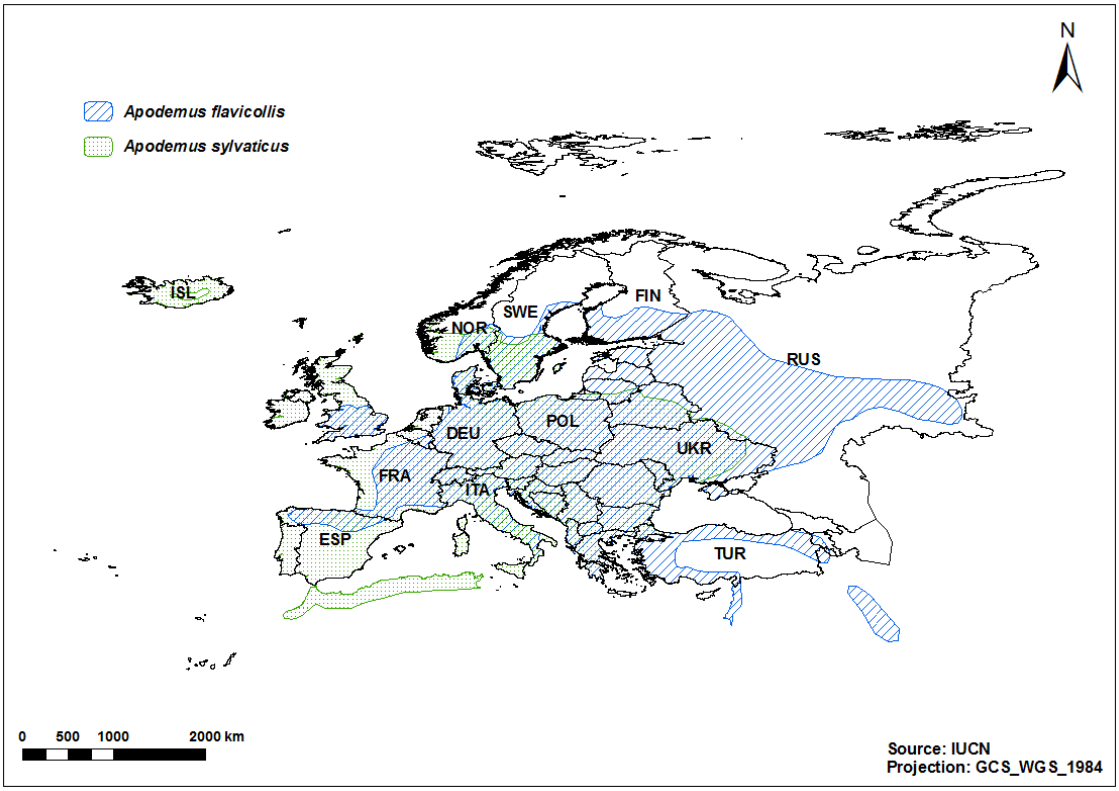


Figure 1-1: Distribution of the mouse species *A. flavicollis* and *A. sylvaticus* in the Palearctic (provided by Dr. Miriam Pfäffle)

Myodes glareolus Schreber, 1780 (*Mg*) – bank vole (Rodentia: Cricetidae)

Adult *Mg* weigh between 15-49 g (Jenrich *et al.*, 2010). The snout to vent length is about 70 - 130 mm, while the tail can reach lengths between35 - 70 mm (Jenrich *et al.*, 2010).

The color of the fur depends on their geographical distribution and the season (Jenrich *et al.*, 2010). In general it has a reddish color with greyish sides and sometimes a yellow appearing belly (Quéré and Le Louarn, 2011).

Occurrence, distribution and habitat preferences

Mg is distributed in the wooded belt of the western Palearctic, from the British Isles to the Lake Baikal (Mitchell-Jones *et al.*, 1999). In Germany it occurs in all potentially suitable habitats. It is sympatric with *Af* and represents one of the most abundant species in Central European forests (Hille and Mortelliti, 2010). Even though voles are considered to be habitat generalists and are known for their high trophic plasticity with no deep specialization on any particular plant species (Gebczynska, 1983), they prefer habitats with a lot of ground structure such as dead wood and shrubs for protection (Demuth-Birkert, 2004; Buesching *et al.*, 2008). The species flourishes in mixed and deciduous forests with a well-developed herb and shrub layer (Blatt and Resch, 2016c) and reaches high population densities in habitats with moist or wet soil (Kraft, 2008) or in the tall herbaceous vegetation of wetlands (Blatt and Resch, 2016c). In openly structured habitats, *Mg* can be found in hedges, which act as a substitute (Viro and Niethammer, 1982). *Mg* occurs in heights up to 2400 m above sea level, where it can survive in small blueberry patches (Hille and Mortelliti, 2010). This was also shown by Suchomel et al. (2014) in a study in the mountain forests in Western Carpathians where *Mg* was highly abundant in plantations with dicotyledonous plants, predominantly in the herb layer and patches of European blueberry. The burrows of voles consist of underground corridors that run only centimeters under the surface and often rise into the leaf litter (Stehr, 1982; Burkhardt and Schlund, 2005). Voles spend their resting time in the nest, use it as a storage and feeding spot and for raising their offspring (Viro and Niethammer, 1982). The territorial behavior of *Mg* depends on season, gender and age and territory size depends additionally on population density and resource availability. During the reproduction phase, sexually mature females are territorial (261-1292 m^2) (Shore and Hare, 2008). Typically, the territories of young animals overlap with those of males (380-2208 m^2), that also include the territories of several females (Shore and Hare, 2008). Except for the reproductive phase, there is no social binding between the individual animals (Jenrich *et al.*, 2010).

Ecology

The bank vole is both diurnal to nocturnal, though day activity occurs mostly during winter (Stehr, 1982) or at high population densities. In this way, *Mg* adapts to avoid *Af*, because the latter, being physically superior and aggressive, can attack voles and expel them from resources (Hille and Mortelliti, 2010). Though, with increasing population density, the usually peaceful individuals can become more aggressive for reasons of competition (Burkhardt and Schlund, 2005). The reproductive period of *Mg* varies according to food availability and environmental factors. In France, reproduction can therefore take place from February to October, while in the Swiss Alps it only lasts for 3.5 months (Burkhardt and Schlund, 2005). Females are sexually mature after 1-1.5 months, males after 2 months, though their sexual maturation can be delayed depending on the relative amount of adults at high population densities (Blatt and Resch, 2016c). On average, bank voles reproduce 3-4 times a year. Autumn offspring reproduces in the following spring. After a mast year, winter reproduction can occur, leading to high population densities in spring (Blatt and Resch, 2016c). Population density ranges between 9-34 individuals/ ha, favorable conditions can lead to over 50 Individuals/ ha (Quéré and Le Louarn, 2011). *Mg* can as well show arboreal behavior and was found up to five meters above ground (Claude, 1995; Buesching *et al.*, 2008). In Europe, there are two different types of *Mg* population fluctuations: In northern European countries, perennial cycles with population crashes every 3-5 years occur, while apart from that annual cycles occur, with a more or less steady population increase beginning in early summer, reaching high densities towards autumn and decline from winter until early spring, with the respective extent and timing highly depending on tree mast (Shore and Hare, 2008). Reasons for perennial cycles are explained by extreme climatic seasonality, nomadic predators (Blatt and Resch, 2016c). and the overall age of the population: During years of peak abundance, less selection occurs towards the fitness of individuals, the average population age increases and the population finally crashes in times of strong seasonality and short vegetation and reproductive periods (Burkhardt and Schlund, 2005). *Mg* reaches, on average, ages from 18-21 months, with the mortality in the first six weeks being up to 60%. Winter mortality ranges, in contrast to *Af*, about only 15% (Shore and Hare, 2008). *Mg* has a very diverse diet, depending on the available resources (Stehr, 1982): In spring, it mainly feeds on seedlings, grass and herbs, while moss, mushrooms, bark, fruit and seeds are added in

summer (Stehr, 1982; Hille and Mortelliti, 2010). In spring and summer about 40-60% of the diet consists of green plants, in autumn and winter 20-50% of seeds. Insects, worms, snails and sometimes bird eggs act as protein sources (Viro and Niethammer, 1982).

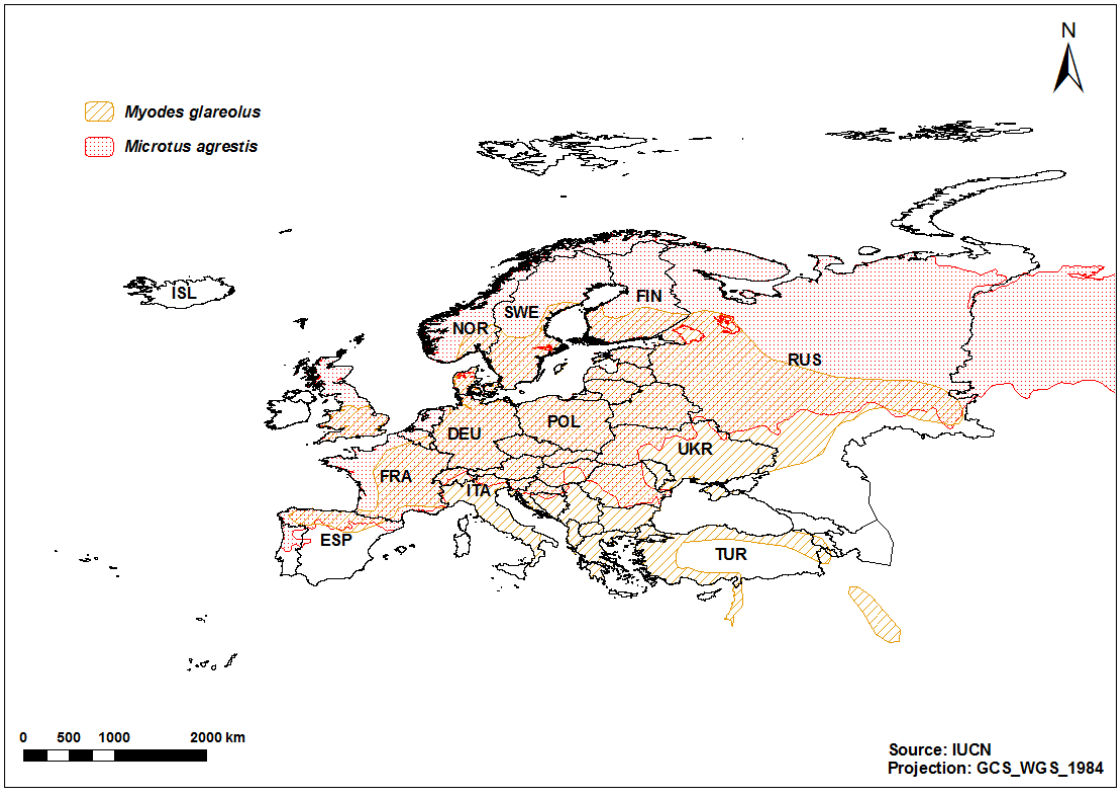


Figure 1-2: Distribution of the vole species *M. glareolus* and *M. agrestis* in the Palearctic (provided by Dr. Miriam Pfäffle)

Other less abundant species

Apodemus sylvaticus Linnaeus, 1758 (*As*) – wood mouse (Rodentia: Muridae)

The average weight of *As* lies between 13 and 44 g, with a snout to vent length of 62-110 mm and a tail length 53-110 mm (Jenrich *et al.*, 2010). With 19-24 mm the length of the hind feet is slightly shorter than that of *Af* (21-26 mm) and can act as a distinguishing feature between those very similar species. As *Af, As* shows an ochre yellow fur pattern on the chest, which is mainly expressed as a spot that can be prolonged in a thin line towards the belly. The spot, however, is never a full band, as seen in *Af* (Kraft, 2008).

Occurrence, distribution and habitat preferences

As is widely distributed in Northern Africa and Europe (Vogel, 1995b) and can be found up to 1900 m above sea level (Spitzenberger, 2001; Grimmberger, 2014). The wood mouse is a pioneer species, able to live in a variety of different habitat types (Quéré and Le Louarn, 2011) and prefers slightly different habitats than *Af*: While *Af* is mainly found in forest habitats, *As* can be found both in forests and open grassland, depending on the region (Hoffmeyer, 1973). The presence of *Af* might inhibit the activity of *As* in forest habitats and increases its occurrence in the grassland (Hoffmeyer, 1973). The size of its territory varies depending on the availability of resources and the individuals' gender. For adult males it is about 0.2-0.3 ha, for females 0.01-0.2 ha (Flowerdew, 1984).

Ecology

As is a crepuscular / nocturnal animal with with a highly similar ecology to *Af*.

***Microtus agrestis* Linnaeus, 1761 (*Mg*) – field vole (Rodentia: Cricetidae)**

Ma has a weight range between 20-55 g with a snout to vent length of 97-111 mm and a tail length of 30-42 mm (Jenrich *et al.*, 2010). Males tend to be heavier than females (Krapp and Niethammer, 2005). The fur is brown, being light grey, seldom yellow, on the belly (Jenrich *et al.*, 2010; Grimmberger, 2014).

Occurrence, distribution and habitat preferences

The distribution of the field vole ranges from Western Europe to the Lake Baikal, however the species is absent in Ireland and southern Europe (Mitchell-Jones *et al.*, 1999). It can be found all over Germany and up to heights of 2100 m above sea level (Spitzenberger, 2001; Grimmberger, 2014). It prefers marsh habitats with high vegetation (Kraft, 2008) and annual precipitation of at least 600 mm as well as average annuals temperatures not higher than 8°C (Blatt and Resch, 2016d). Typical habitats are uncut and damp meadows, moor, alluvial forests, forest clearings and clear cuttings (Kraft, 2008). Territory size depends on gender, reproductive activity and population density and territories almost never cross (Blatt and Resch, 2016d). The territory of a male is around 600 m^2 (reproductive season 1434 m^2) and that of a female is around 480 m^2 (reproductive season 773 m^2) (Lambin, 2008).

Ecology

The field vole has activity phases both during the day and the night (Blatt and Resch, 2016d). *Ma* is a herbivore and its diet consists mostly of green plant parts, seedlings and seeds (Jenrich *et al.*, 2010). In times of lack of food it can also feed on roots and bark of young trees (Blatt and Resch, 2016d). *Ma* is known for its high population densities which can be as high as 300 individuals/ha. Those phases are only short-termed because of factors such as lack of food, stress, parasites and predators and population densities decrease naturally on their own (Jenrich *et al.*, 2010). The life expectancy under natural conditions is normally 15 months and can be 39 months under laboratory conditions (Krapp and Niethammer, 2005).

***Sorex araneus* Linnaeus, 1758 (*Sa*) – common shrew (Eulipotyphla: Soricidae)**

The common shrew weighs between 6-15 g and a snout to vent length of 65-85 mm. The tail has a length of 32-47 mm (Jenrich *et al.*, 2010) . The fur is trichromic and features a brown-blackish back, lighter sides and a grey-white belly (Lugon-Moulin, 2003). However the fur is not clearly divided into the different colors and it exists a highly individual variation in fur color (Lugon-Moulin, 2003; Grimmberger, 2014). The upper side of the tail is darker and hairy, but hairless in older animals (Jenrich *et al.*, 2010).

Occurrence, distribution and habitat preferences

Sa is distributed in the Palearctic up to the arctic coast and the Lake Baikal (Mitchell-Jones *et al.*, 1999). In Germany, it is very abundant in all suitable habitats (Grimmberger, 2014) and can be found in heights up to 2480 m above sea level (Mitchell-Jones *et al.*, 1999). *Sa* prefers cool-humid habitats with high vegetation cover; it is however very adaptable to less favorable conditions and can also be found in dry habitats (Kraft, 2008). The territory size of the common shrew depends on the season and the habitat and can range between 90 and 2800 m², with 360-630 m² on average (Churchfield and Searle, 2008a). As nests, *Sa* often expand and shape the old nests of other small mammals species or builds nests over ground at secure spots (Lugon-Moulin, 2003; Jenrich *et al.*, 2010).

Ecology

Sa is both diurnal and nocturnal and lives solitary in defined territories. Here, the core areas are aggressively defended (Hausser *et al.*, 1990). Shrews have a very high and fast metabolism and have to cover daily nutritional requirements consisting of 80-90% of their own body weight (Hausser, 1995). Therefore, it constantly forages for food, which mainly consists of earthworms, insects, spiders, snails and other prey (Churchfield and Searle, 2008a). The reproductive season starts in March/April and lasts until September (Turni, 2005b; Jenrich *et al.*, 2010). In northern Europe, populations of *Sorex araneus* vary between 7 and 96 individuals/ha (Churchfield and Searle, 2008a) and underlie population crashes every 3 to 4 years (Jenrich *et al.*, 2010). The life expectancy of *Sa* is about 15-18 months, with a mortality rate in the first two months of 50% and a winter mortality rate of over 80% (Churchfield and Searle, 2008a).

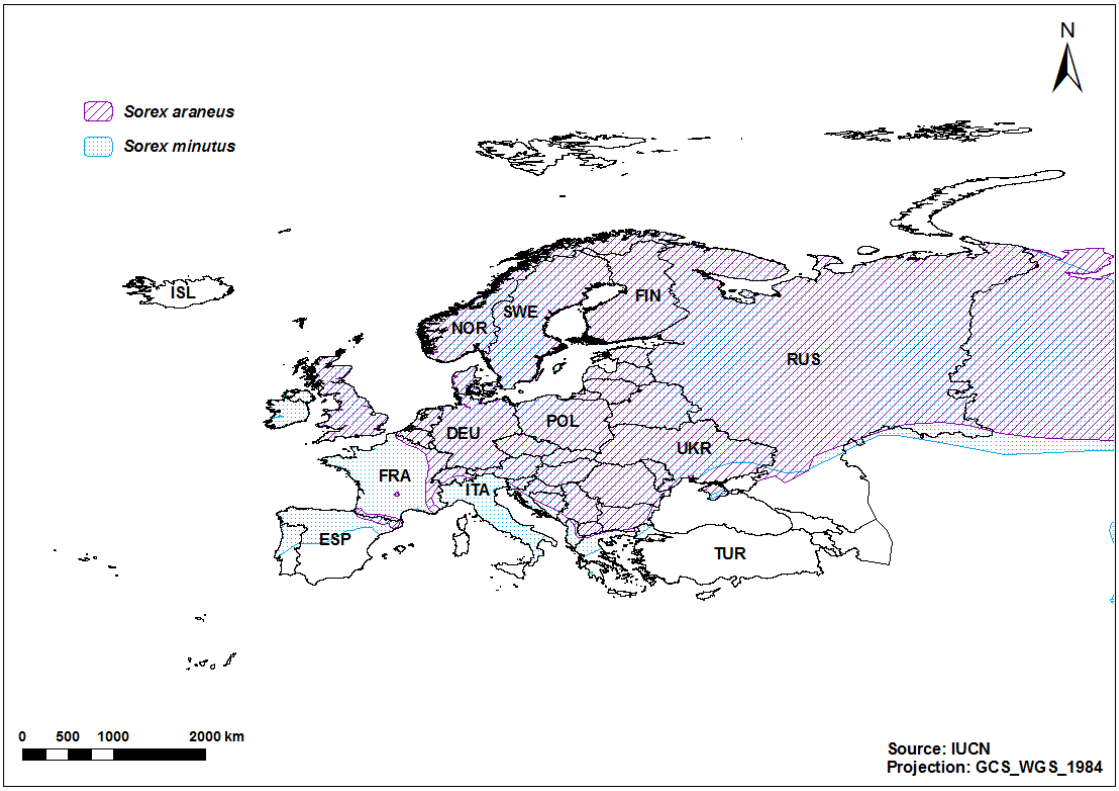


Figure 1-3: Distribution of the shrew species *S. araneus* and *S. minutus* in the Palearctic (provided by Dr. Miriam Pfäffle)

Sorex minutus Linnaeus, 1766 (*Sm*) – Pygmy shrew (Eulipotyphla: Soricidae)

Sm weighs between 2 and 5g. Its snout to vent length is 36-64 mm and its tail has a length of 32-46 mm (Jenrich *et al.*, 2010). The back fur is brown and becomes lighter at the sides

turning into a grey-white belly. However, there is a high age and habitat depend variability in fur color (Lugon-Moulin, 2003). The tail is thick and evenly covered with hair, although older animals tend to lose the tail hair (Jenrich *et al.*, 2010).

Occurrence, distribution and habitat preferences

Sm occurs from Portugal to the Lake Baikal (Mitchell-Jones *et al.*, 1999). In Germany, where it is also the smallest shrew, it is very abundant in all suitable habitats (Grimmberger, 2014) and can be found in heights up to 2260 m above sea level (Spitzenberger, 2001). Just like *Sa*, *Sm* prefers cool and humid habitats with intense ground vegetation (Kraft, 2008) and precipitation of at least 600 mm per year (López-Fuster, 2007). Its territory varies according to season and environmental factors ranges over 150-1000 m². In contrast to *Sa*, *Sm* is only found above ground , as its ability to dig is only little developed (Churchfield and Searle, 2008b; Jenrich *et al.*, 2010) but it also uses the nests of other small mammals (Adler, 2009; Jenrich *et al.*, 2010).

Ecology

The pygmy shrew is diurnal and nocturnal (Churchfield and Searle, 2008b). The life expectancy of *Sm* is about 13-16 month, with a mortality rate of 50% during the first seven months (López-Fuster, 2007). Because of its high metabolism, *Sm* needs to consume 1.25 times its body weight daily, which represents around 250 prey animals (Churchfield and Searle, 2008b). Its diet consists of insects, mites and snails, however, in contrast to *Sa*, earthworms and spiders are only a rare part of its diet(Hutterer, 1990; Jenrich *et al.*, 2010). The reproductive season starts in April and lasts until September. During this time a maximum of three litters with 4-6 pups can be produced (Jenrich *et al.*, 2010). Its population density can range between 4 to 42 individuals/ha (Churchfield and Searle, 2008b) and is potentially higher in absence of *Sa* (Churchfield and Searle, 2008b).

1.2.2 The role of small mammals as blood-feeding hosts for ticks

Af and *Mg* play an important role as blood-feeding hosts for *Ixodes ricinus* larvae and to a lesser amount for nymphs (Matuschka *et al.*, 1991; Dizij and Kurtenbach, 1995). *Mg* is able to acquire resistance to *I. ricinus*, resulting in reductions of fully engorged ticks, the

duration of attachment of partially engorged ticks and the adjacent moulting process, while *Af* shows no sign of acquired resistance to *I. ricinus* (Dizij and Kurtenbach, 1995). This results in *Af* being significantly more infested with *I. ricinus* than *Mg* in areas where both species co-exist (Matuschka *et al.*, 1990; Kurtenbach *et al.*, 1995; Siński *et al.*, 2006a). The infestation patterns on rodents are influenced by extrinsic factors such as season, relative humidity or vegetation cover (Kiffner *et al.*, 2011). In addition, tick burden increases with body mass (age) of the individual host and decrease with the increasing rodent population density (Kiffner *et al.*, 2011). *I. ricinus* infestation can vary between years and host individuals as well: Tälleklint and Jaenson (1997) conducted a study in south-central Sweden in showed that the infestation pattern of *I. ricinus* larvae on rodent host can either be unimodal or bimodal. Male *Mg* showed to carry significantly more ticks than females and older individuals more than younger ones (Tälleklint and Jaenson, 1997), even though *Mg* are supposed to acquire resistance to *I. ricinus*. However, *Af* carried significantly more ticks than *Mg*, as it also shown in other studies (Humair *et al.*, 1999; Randolph *et al.*, 1999; Sínski *et al.*, 2006; Paulauskas *et al.*, 2009; Burri *et al.*, 2011a; Silaghi *et al.*, 2012a; Pérez *et al.*, 2012).

Tälleklint and Jaenson (1994) further showed that Insectivores and rodent species are the most important hosts for larval *I. ricinus*, while nymphs preferred to feed on cervids and hare. In urban areas rats (*Rattus norvegicus*) might play a more important role than smaller woodland rodents such as *Af* and *Mg*, by hosting higher numbers of larvae and nymphs of *I. ricinus* but also by serving as capable reservoir hosts for *Borrelia burgdorferi* sensu lato *(Bbsl)* (Matuschka *et al.*, 1996).

1.2.3 Small mammals and the transmission of tick-borne pathogens

"Hosts that attract many vectors will tend to be the focus of transmission" (Perkins *et al.*, 2003): Tälleklint and Jaenson (1994) examined the role of different mammal species as reservoir hosts for *Bbsl*. The most important mammal reservoirs happened to be rodents and the common shrew (*Sa*), in total accounting for 91% of larval *I. ricinus* infection. It also seems that the infectivity of a rodent species is related to the number of infesting, potentially infected *I. ricinus* nymphs. The most significant reservoirs for *Bbsl* are small

mammals, especially ubiquitous rodents represented in Europe by *Af*, As, and *Mg* (Michalik *et al.*, 2003).

If infected with *Bbsl spirochetes*, *Af* and *As* remain infective for the rest of their lives, although the transmission rate (from reservoir host to tick) can differ between individuals and lays between 26.5 - 81.4% (Gern *et al.*, 1994). Continuous exposition to tick infestation enhances the infectivity for larval *I. ricinus* ticks (Gern *et al.*, 1994). Humair et al. (1993) states that *Af* and *As* present a higher potential infectivity for *Bbsl* than *Mg*. A study from Poland indicates that both the density of hosts, as well as the density of ticks are the most important risk factors for *Bbsl* (Siński *et al.*, 2006a). *Af* carries more infected ticks than *Mg*, which implies that factors influencing the density of *Af* in a habitat also influence the risk of humans being infected with *Bbsl* (Siński *et al.*, 2006a). However, Radzijevskaja et al. (2013) imply that according to calculated indices of specific infectivity, *Mg* but also the field vole *Microtus arvalis* are more efficient to transmit *Bbsl* to ticks than mice from the genus *Apodemus*. This could be related to the fact, that *Af* and *As* produce more specific antibodies against *Bbsl* than *Mg* when exposed to the same concentration of spirochetes and at the same time transmit lower numbers of spirochetes to ticks (Kurtenbach *et al.*, 1994). At the same time, simply regarding the transmission potential of a species neglects the fact that less larvae feeding on *Mg* will moult successfully to infected nymphs compared to those who have fed on *Apodemus* spp. (Humair *et al.*, 1999).

Af and *Mg* both seem to play a role in the transmission of *Candidatus* Neoehrlichia mikurensis (CNM) (Silaghi *et al.*, 2012a). Transmission of tick-borne encephalitis virus (TBE-V) occurs when ticks feed in co-feeding aggregation (Randolph, 2001). Hence the number and frequency of co-feeding groups provides an estimate of the potential rate of virus transmission. For *Af* sexual mature males with high body mass tend to be the most important individuals for transmission, since the aggregation of ticks are highest on them (Perkins *et al.*, 2003). A study from Slovakia further emphasizes the importance of *Af*, *Mg* but also hedgehogs (*Erinaceus roumanicus*) in the maintenance of TBE-V in the Tribec region (Kozuch *et al.*, 1967).

1.3 Ticks (Ixodida)

Ticks are obligate blood-feeding ectoparasites of the class Arachnida (phylum: Arthropoda) (Arthur, 1963; Ginsberg and Faulde, 2008; Guglielmone *et al.*, 2014) that infest every terrestric vertebrate class worldwide (Parola and Raoult, 2001b). The nearly 900 tick species known to date (Guglielmone *et al.*, 2014; Estrada-Peña *et al.*, 2014b) have conquered almost every terrestrial ecosystem and can be found from tropical islands and rainforests over deserts to the arctic circle (Barré *et al.*, 1995; Guglielmone *et al.*, 2010; Dantas-Torres, 2010; Hvidsten *et al.*, 2014).

The eleven tick species whose presence has been confirmed for Baden-Württemberg (BW) so far (Petney *et al.*, 2012, 2015) can be divided into the families of Argasidae (soft ticks) and Ixodidae (hard ticks). The argasids are represented by only two species (Petney *et al.*, 2012); *Argas vespertilionis* that predominantly feeds on bats (Chiroptera) and *A. reflexus* that usually feeds on domestic pigeons (*Columba livia* f. *domestica*). Both are rather host specific ticks (Dautel and Kahl, 1999) that inhabit the nests of their principal hosts and their close surroundings. However, both species have also been shown to feed on humans that invade tick-infested caves and burrows or live close to abandoned pigeon roosts (Estrada-Peña and Jongejan, 1999), causing skin irritations up to anaphylactic shocks in humans (Petney *et al.*, 2012).

The tick species that are known to infest small mammal hosts in the woodlands of Germany are *D. marginatus* (Sulzer, 1776), *D.reticulatus* (Fabricius, 1794), *Ixodes acuminatus* (Neumann, 1901)*, I. ricinus* (Linnaeus, 1758) and *I. trianguliceps* (Birula, 1895), and all belong to the the Ixodidae (Dautel and Kahl, 1999; Hartelt *et al.*, 2008; Kiffner *et al.*, 2011; Obiegala *et al.*, 2014; Rubel *et al.*, 2014). The most peculiar distinguishing feature of this family is the scutum, a shield-like cuticula formation on their back, which covers the entire back of male individuals and only parts of it in females, nymphs and larvae of this family (Figure 1-4)(Jongejan and Uilenberg, 2004).

The most prominent species among the small mammal-infesting tick species in BW is *I. ricinus*, the castor-bean tick (Matuschka *et al.*, 1990; Franke *et al.*, 2010a; Kiffner *et al.*, 2011; Silaghi *et al.*, 2012a; b) which represents the most abundant and medically important tick species in Germany and Central Europe (Dautel and Kahl, 1999; Oehme *et al.*, 2002; Süss *et al.*, 2010).

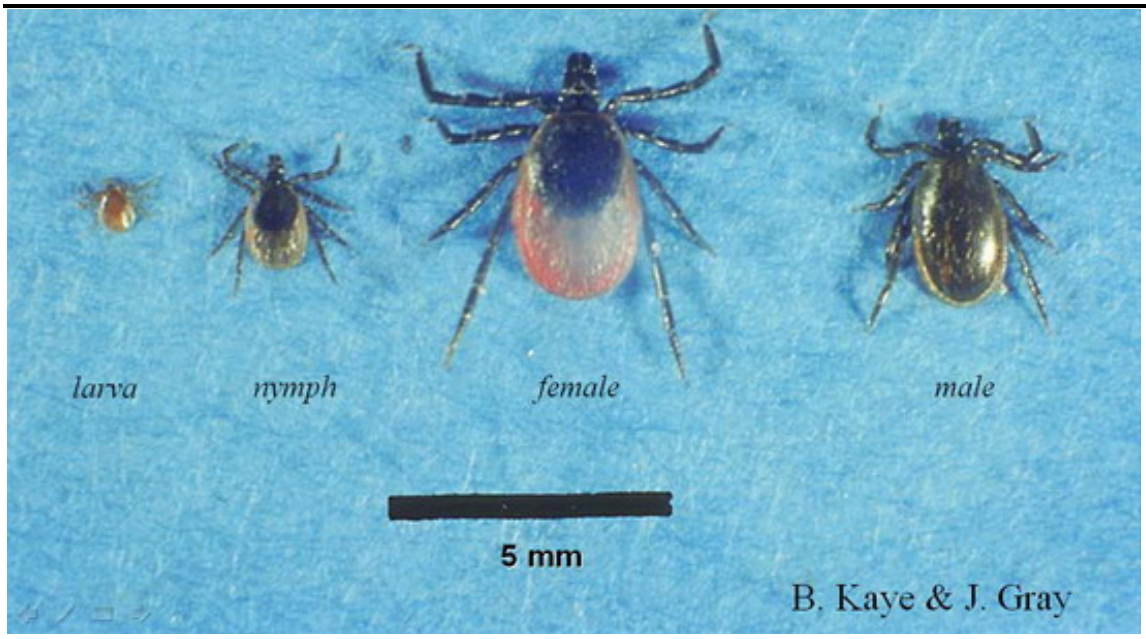


Figure 1-4: Overview of the morphology and body size of the active developmental stages of *I. ricinus* from larva to nymph to adult tick (female and male)

The tick species belongs to and is the eponym of *the I. ricinus* complex (Figure 1-5) (Keirans *et al.*, 1999; Petney *et al.*, 2012), a group of closely related species, that comprises 19 tick species of worldwide distribution (Keirans *et al.*, 1999; Xu *et al.*, 2003; Petney *et al.*, 2012). Among the species of this complex are *I. ricinus*, *I. persulcatus*, *I. pacificus* and *I. scapularis.* These species are the major vectors of tick-borne pathogens (TBPs) of medical and veterinary importance in temperate areas of the Nearctic and Palearctic and the predominant global vectors of Lyme disease (Piesman, 1989; Filippova, 1990; Diuk-Wasser *et al.*, 2016).

There is accumulating evidence for an increase in the geographical distribution and abundance of *I. ricinus* (Danielová *et al.*, 2006; Gray *et al.*, 2009; Jaenson *et al.*, 2012). This data suggests the expansion of *I. ricinus* ranges in latitude as well as altitude over the past decades. Populations of the vector tick have conquered higher-lying mountainous areas (Gray *et al.*, 2009), and have expanded their range up to the north of Sweden (Lindgren *et al.*, 2000; Jaenson *et al.*, 2012), this being attributed to climatic changes in the recent past (Lindgren *et al.*, 2000; Estrada-Peña *et al.*, 2012; Medlock *et al.*, 2013).

Due to its predominant role as an ectoparasite of small mammals and against the background of its importance in pathogen transmission, *I. ricinus* was the main focus of the present study. Further sections about the biology and ecology of ticks in BW will be

based on the characteristics of *I. ricinus* with reference to differences exhibited by other tick species infesting small mammal hosts.

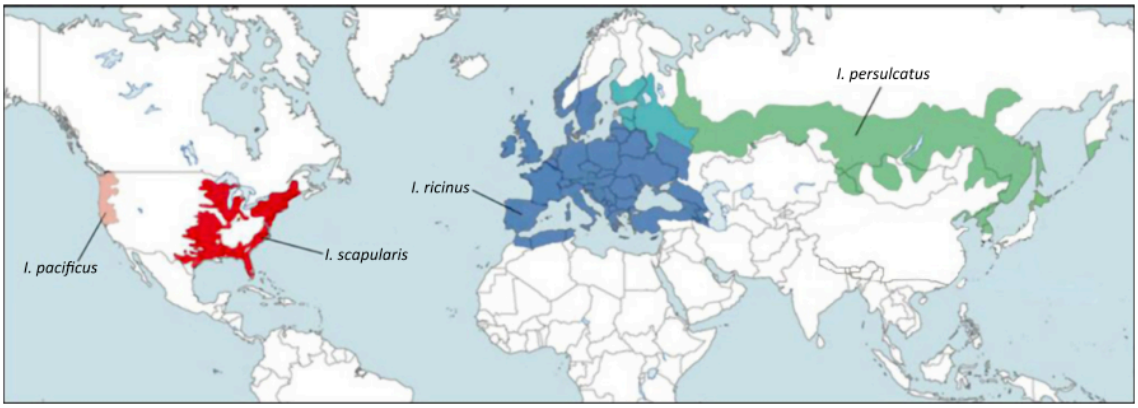


Figure 1-5: Geographic distribution of those *Ixodes spp.* that are the major vectors of human pathogens. *I. pacificus* (orange) is found along the Pacific west coast of the US, *I. scapularis* (red) occurs from the upper Midwest of the USA to the eastern Atlantic coast and the Gulf of Mexico in the South, *I. ricinus* (blue) is present throughout Europe, in parts of Northern Africa, Turkey and the Caucasus. Around the Baikals, *I. ricinus* is sympatric with *I. persulcatus* (turquoise), which is predominant throughout the South of Russia to the Far East (green) (Diuk-Wasser *et al.*, 2016)

1.3.1 Life cycle

I. ricinus and all other Ixodidae have four developmental stages: the immobile egg and the mobile larva, nymph and adults comprising female and male (Figure 1-4). The larve, nymphs and females feed only once, taking a large blood meal of several days from a vertebrate host in order to develop into the next stage or to produce eggs (Estrada-Peña *et al.*, 2004; Lucius and Loos-Frank, 2008a). An exception to this rule are male *Ixodes* spp. that do not require a blood meal prior to mating (Allan, 2013).

The life cycle starts with a fully engorged, gravid female that deposits its egg clutch in the leaf litter layer (Figure 1-6). While an *I. ricinus* female typically lays between 1000 and 2500 eggs (Balashov, 1972), egg clutch size for *Dermacentor* spp. can reach over 5000 eggs (Zahler and Gothe, 1997). After the larva hatches it seeks its first host, usually a small mammal or a ground foraging bird (Tälleklint and Jaenson, 1997). The larva feeds on its host for three to seven days (Allan, 2013) and then drops off to the ground where it hides in the leaf litter and molts into a nymph. The nymph follows the same pattern: it seeks a host, feeds on it for five to seven days and then drops off to molt in the leaf litter to become a female or male (Balashov, 1972). The adults in turn mate either off-host before the female seeks its third blood meal source (*Ixodes* spp.) or mate on-host (Gray, 1991; Jongejan and Uilenberg, 2004). After its final full engorgement, which can take up to 14

days (Allan, 2013), the female drops off the host and shelters in the leaf litter where it remains until egg development has finished and the egg clutch can be deposited (Petney *et al.*, 2013).

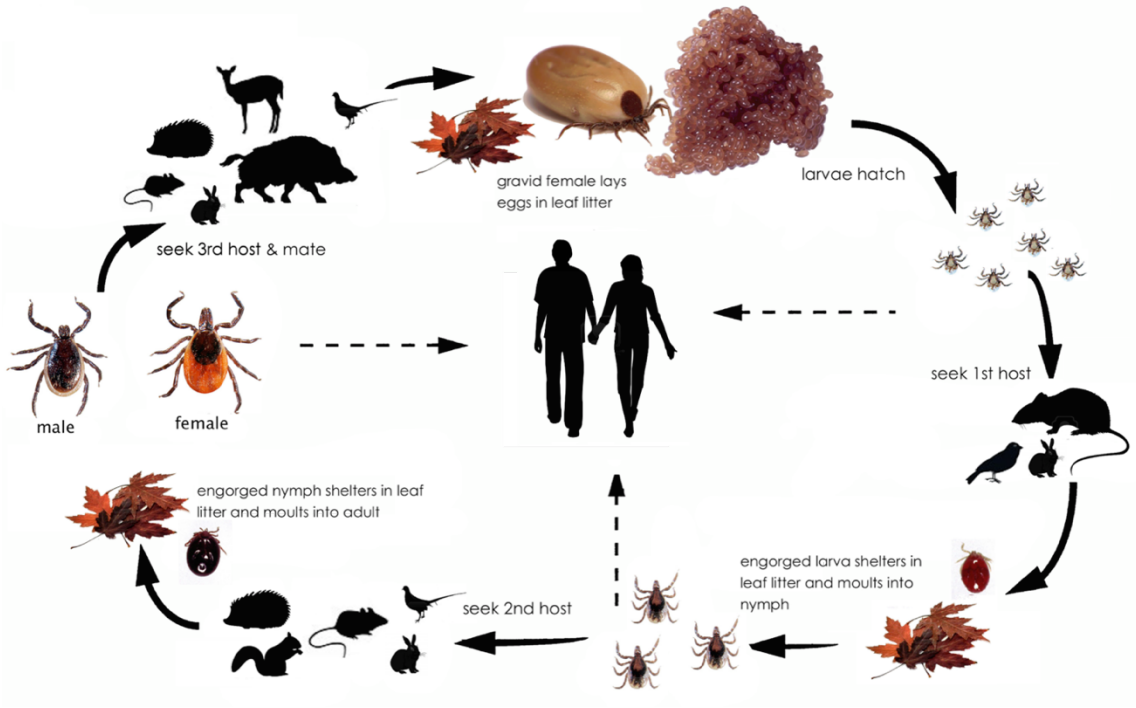


Figure 1-6: Typical three-host life cycle of Ixodidae, under reference to *I. ricinus*. Humans can serve as potential host of every instar.

Humans can serve as a blood-meal source of every developmental stage of *I. ricinus* (Figure 1-6) and have also been found to be infested by *Dermacentor* spp. (Estrada-Peña and Jongejan, 1999). Such data is, however, largely absent for *I. acuminatus* and *I. trianguliceps* (Bown *et al.*, 2008). The infestation of humans, however, is regarded as "ecological trap" for ticks, as it is usually accompanied by the death of the respective tick, and thus, can no longer contribute to the maintenance of the tick population (Taraschewski, 2006).

1.3.2 Host preferences

I. ricinus has a very wide host choice and infests over 300 different terrestrial to semi-aquatic vertebrate species, including reptiles, birds and mammals (Randolph, 2013). Its life cycle is heteroxenous, which means that the different life stages feed on different species or groups of hosts: larvae typically infest small mammal hosts (Tälleklint and

Jaenson, 1997), while nymphs prefer medium sized mammals like hedgehogs (Pfäffle *et al.*, 2011) and adults are mainly found on large mammals such as deer (Kiffner *et al.*, 2010). While the adult life stages of *D. marginatus* and *D. reticulatus* infest a range of different large mammals (Karbowiak, 2014), larvae and nymphs seem predominantly to be associated with small mammals like rodents and insectivores and are probably endophilic, living closely associated with small mammals in their burrows (Liebisch and Rahman, 1976; Hillyard, 1996; Pfäffle *et al.*, 2015a). The remaining two tick species known to infest small mammals in BW, *I. acuminatus* and *I. trianguliceps*, are endophilic, living in the burrows of their principal small mammal host (Boyard *et al.*, 2008). Due to this close association the life cycle of these species is likely to be homoxenous, involving only one species of host as blood source for all life stages (Petney *et al.*, 2012, 2015).

The more specialized a tick species is, and the closer the association with its principal host, the smaller is the resulting potential for humans to be infested.

1.3.3 Host seeking behavior

Several different ways are deployed by the small mammal-infesting Ixodidae to find their host: endophilic tick species do not show active host-seeking behavior like *I. ricinus* and adult *Dermacentor* spp., but rather infest their hosts directly inside their burrows (Pfäffle *et al.*, 2011; Petney *et al.*, 2012). The host-seeking strategy of *I. ricinus* and adult *Dermacentor* spp. can be described as “ambushing”: the tick climbs the vegetation until it reaches the tip (Lees and Milne, 1951) or a certain height (Balashov, 1972; Maier *et al.*, 2003) and waits for a host to cross its path, spreading its first pair of legs to expose Haller’s organ. This structure consists of a groove that is connected to a capsule filled with sensory hairs and is used by the tick to detect a host via the recognition of host-derived stimuli of chemical, mechanical or thermal origin (Lucius and Loos-Frank, 2008b). Once a host passes by the active questing tick, the tick lets itself be wiped off the vegetation onto the host’s body surface by way of its tarsal claws.

Larvae usually remain close to the ground, whereas nymphs and adults can climb higher, due to their comparatively larger tolerance for water loss, but also depending on the structure of the vegetation at the questing site (Kahl, 1989; Mejlon and Jaenson, 1997).This will be examined in the following section in more detail. The stage-dependent

height of host-seeking is likely to influence the "host preference" based on availability, rather than actual preference for a certain-sized vertebrate host (Mejlon and Jaenson, 1993; Randolph *et al.*, 2000).

1.3.4 Feeding behavior

Once the tick has found a suitable site for feeding, it uses its knife-like mouthparts, the chelicerae, to cut through the host's skin and subsequently inserts its furrow-shaped hypostome to start the feeding process (Allan, 2013). From attachment to engorgement, the tick not only ingests host-derived body fluids, but also injects its saliva into the host. This contains different components of vasodilatory, anticoagulant or immunomodulatory function (Dizij and Kurtenbach, 1995; Wikel, 1996; Wikel and Bergman, 1997). The entire process of feeding therefore represents the crucial step in TBP transmission that can lead either to the infection of the susceptible host by a previously infected tick, or to the infection of a naïve tick by an infected host individual (Kurtenbach *et al.*, 1998a; Randolph *et al.*, 1999; Liu and Bonnet, 2014).

1.3.5 What makes ticks tick? Factors influencing tick abundance and survival

Ticks are long-lived ectoparasites. In order to pass through all phases of development per stage, the individual developmental stage of *I. ricinus* requires a fairly long period of about one year (Gray, 1991). The completion of the entire lifecycle of *I. ricinus* in the field therefore takes about three years from a theoretical point of view, but can take up to six years, strongly depending on the suitability of environmental conditions (Gray, 1991). The developmental cycle of *D. reticulatus, I. acumintus* and *I. trianguliceps* can be completed within one to two years as they are less exposed to the environment outside of their host's nests (Immler, 1973; Liebisch and Rahman, 1976; Gingrich *et al.*, 2001). In comparison to these (semi-) endophilic species, *I. ricinus* spends about 99% of its entire life off-host (Randolph, 2013). Being exposed to the environment, the development and the phenology of *I. ricinus* depends largely on environmental conditions (Estrada-Peña *et al.*, 2013).

Therefore, exophilic ticks like *I. ricinus* require, besides a sufficient number of hosts as a blood meal source, a habitat that provides suitable environmental conditions (Pérez *et al.*, 2012).

Every step of the development of *I. ricinus* depends on environmental conditions: from the period of egg maturation to the development and activity of larvae, nymphs and adults, all processes are mainly driven by the influences of the abiotic factors temperature and humidity (Perret *et al.*, 2000, 2004; Gray, 2009; Estrada-Peña *et al.*, 2013).

The preferred habitats of *I. ricinus* in Germany are found in deciduous or mixed forests with an extensive humus and leaf litter layer and thick understory vegetation that provide stable and humid microclimatic conditions (Kahl *et al.*, 2002; Stanek, 2009; Schwarz *et al.*, 2009; Williams and Ward, 2010).

I. ricinus needs a threshold temperature above 5-7°C to become active in spring and requires a high humidity above 85% that prevents water loss (Dautel and Kahl, 1999; Süss *et al.*, 2008; Estrada-Peña *et al.*, 2013). It spends the active part of its off-host-life continuously balancing between the demands to find a host and to remain hydrated (Lees, 1946; Randolph, 2013; Herrmann and Gern, 2015).

During questing above the leaf litter layer, the ticks are likely to dehydrate, especially if the atmosphere is highly unsaturated (Randolph and Storey, 1999; Williams and Ward, 2010). To avoid desiccation, the tick needs to move back into the leaf litter to actively rehydrate (Herrmann and Gern, 2015) before it can climb back up to start over looking for a host to pass by. However, the tick only has the energy resources available from its last blood meal, and the life span of the developmental stages is limited to the capability of the tick to maintain these reserves until it finds a host. If dry conditions over a longer time coincided with the seasonal activity of ticks, this would substantially increase water stress in the tick population, leading to a shortened duration of the questing period and potential increase of tick mortality (Gern, 2009).

Steep increases in saturation deficit (calculated from temperature and humidity to describe the water deficit in the atmosphere) have been shown to lead to a decline in questing activity (Randolph and Storey, 1999; Perret *et al.*, 2000), and tick phenology patterns outside the sheltering leaf litter layer might have evolved based on the seasonally changing patterns of weather conditions.

1.3.6 Tick phenology

The phenology of ticks follows a seasonal pattern of activity and fluctuations are largely attributed to changing weather conditions influencing temperature and humidity (Perret *et al.*, 2000; Estrada-Peña *et al.*, 2004; Schwarz *et al.*, 2009). The schematic illustration of the generalized phenology of *I. ricinus* larvae, nymphs and adults (Figure 1-7) illustrates the main period of activity, at the same time showing the potential extent of differences between the physiological tolerance spectra of the instars (Gray, 1985a; Herrmann and Gern, 2015). Larvae are regarded to be the least tolerant instar towards water stress (Lees, 1946; Kahl, 1989). Their seasonal activity is assumed to be largely restricted to the summer months, exhibiting an either unimodal or bimodal peak of abundance (Kurtenbach *et al.*, 2006; Dobson *et al.*, 2011; Egyed *et al.*, 2012; Randolph, 2013). The nymphs and adults of *I. ricinus* typically exhibit a bimodal pattern, with abundance peaking in spring and autumn (Kurtenbach *et al.*, 2006; Schwarz *et al.*, 2009).

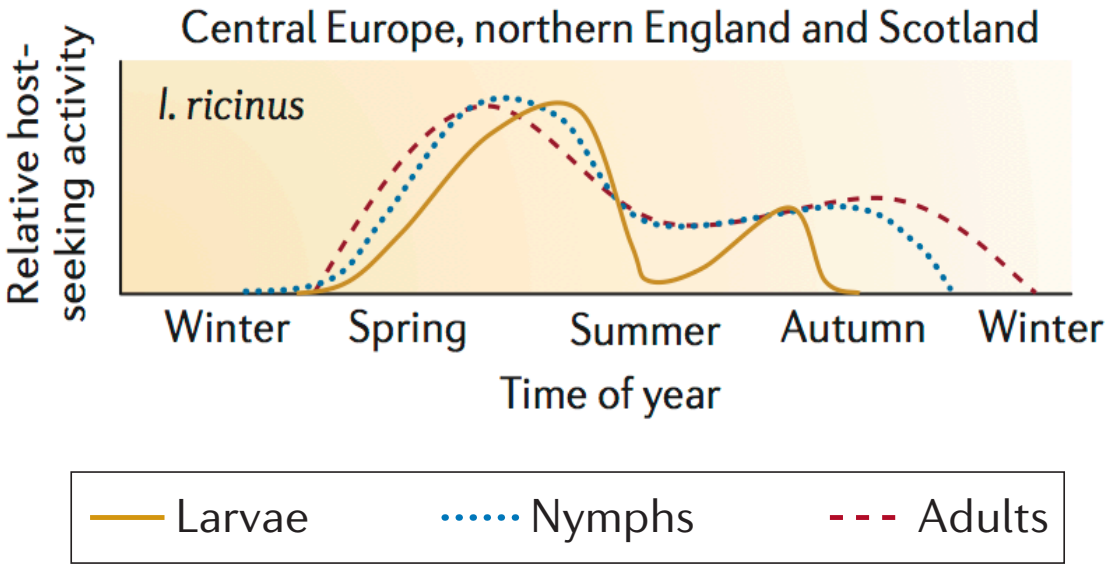


Figure 1-7: Schematic of the seasonal phenology of the instars (larvae, nymphs, adults) of *I. ricinus* shown for Central Europe, northern England and Scotland. (Kurtenbach et al. 2006)

1.3.7 The role of rodent-infesting tick species in disease transmission

The mere presence of ticks in Germany and BW is more of a nuisance than an actual health threat. The threat to human or animal health, however, arises from the competence of ticks in transmitting a variety of pathogens causing tick-borne diseases (TBDs).

The tick of highest medical importance is *I. ricinus* due to its widespread and frequent abundance throughout Central Europe, its importance as the confirmed vector of a variety of TBPs and its capability to infest humans (Parola and Raoult, 2001a; Estrada-Peña *et al.*, 2005; Schwarz *et al.*, 2009; Reye *et al.*, 2010; Schorn *et al.*, 2011; Randolph, 2013). *Dermacentor* spp. are likely to play a role for human as well as animal health, being known vectors of the human pathogenic *Rickettsia slovaca* and *R. raoultii*, which can cause a syndrome (SENLAT) characterized by scalp eschar and neck lymph adenopathy following tick bites (Angelakis *et al.*, 2010; Rieg *et al.*, 2011; Parola *et al.*, 2013), and which has not been shown to be transmitted by *I. ricinus* so far. Against the background of the current expansion of both *Dermacentor* species in Germany and the occasional infestation of humans, there is the potential for the emergence of new disease foci in BW. Both species are also competent sources of infection with *Babesia canis canis*, the causative agent of canine babesiosis with malaria-like symptoms in dogs (Parola and Raoult, 2001a; Földvári *et al.*, 2007; Silaghi *et al.*, 2012b; Špitalská *et al.*, 2012).

I. trianguliceps can transmit *Anaplasma phagocytophilum,* the most widespread pathogen in Europe affecting humans, companion animals and livestock, as well as *B. microti* (Randolph, 1991, 1995; Bown *et al.*, 2008; Turner *et al.*, 2014). Data confirming the vector role of *I. acuminatus* in the transmission of TBPs is not available. This species has only recently been described for Germany (Petney *et al.*, 2015), typically occurring at low densities and is therefore unlikely to be the focus of studies on pathogen ecology (Boyard *et al.*, 2008; Földvári *et al.*, 2011; Petney *et al.*, 2015). However, it has been associated with several TBPs, including *Coxiella burnettii*, the causative agent of Q-fever, and *Bbsl* (Obsomer *et al.*, 2013; Petney *et al.*, 2015). Thus, all species potentially infesting small mammal hosts in BW have been previously shown to be either competent reservoirs of TBP or are suspected to be involved in the transmission of TBPs.

Due to their differences with respect to their frequency of occurrence, distribution, host preferences and host association, the individual species are likely to contribute in a different way and to a different extent to the epidemiological cycles of TBPs in BW. Even though *Dermacentor* spp., *I. acuminatus* and *I. trianguliceps* might occasionally infest humans (Hillyard, 1996; Estrada-Peña and Jongejan, 1999) this is a comparatively rare event and is unlikely to play a major role in the direct transmission of TBD agents. Nevertheless, these tick species are competent vectors of a variety of TBPs that also

include *I. ricinus* as vector and might act as promoters in the maintenance of local enzootic TBP cycles (the circulation of the pathogen between tick vector and animal reservoir) in the field (Oliver *et al.*, 2003; Rizzoli *et al.*, 2014) or might be of direct veterinary concern (*Dermacentor* spp.). Those cycles are, however, not well investigated and an estimate of their epidemiological relevance is not possible to date. The sympatric occurrence of the generalist tick *I. ricinus*, and other specialist rodent-infesting tick species, might therefore act synergistically. The specialist tick species could maintain high levels of TBP prevalence (proportion of infected individuals with a respective pathogen) in ticks and reservoir hosts in the field and *I. ricinus* could serve as bridge vector in the transmission of a variety of different zoonotic pathogens between wildlife and humans (de la Fuente *et al.*, 2008; Pfäffle *et al.*, 2015b) leading to a potential increase in the transmission potential of TBPs to humans in such areas.

1.4 Tick-borne pathogens

TBDs have been accompanying humandkind for a very long time: recent findings indicate that the 5,300 year old Tyrolean Iceman "Ötzi" suffered from borreliosis (Keller *et al.*, 2012). The recognition of the widespread TBPs as the origin of human disease, however, only occurred much later in the 20th century, for example with the discovery of the TBE-V and *Borrelia burgdorferi* (Zilber, 1939; Burgdorfer *et al.*, 1982). Ever since, interest in research of TBD and the pathogens that cause them increased rapidly and deepened our understanding of the epidemiology of TBDs to a great extent.

A central characteristic of TBPs is their highly complex eco-epidemiology, which is influenced by a variety of different vector- and host-dependend factors in a spatially and temporally variable environment (Talleklint-Eisen and Lane, 2000; Brown *et al.*, 2001; Rosà *et al.*, 2003; Hartemink *et al.*, 2008; Perez *et al.*, 2016). These include, amongst others, the overall population density of ticks and the infection rate of the different developmental stages, the population density of hosts und their infection prevalence, the quality and susceptibility of available host species and the immune status of individual hosts (Sonenshine and Mather, 1994; Rosà *et al.*, 2003; Petney *et al.*, 2013). Due to the large number of components that are potentially involved in shaping the tick-host-

pathogen system, this complexity allows for large scale variations in the relative impact and relevance of individual factors within a changing environment and makes their examination a potentially challenging task.

The wide spectrum of pathogens transmitted by ticks of the family Ixodidae includes viruses, bacteria and protozoans (Durden, 2006). In Germany, the most important tick-borne virus is the tick-borne encephalitis virus (TBE-V) (Faulde and Hoffmann, 2001; Süss, 2008, 2011). In addition, a wide range of bacterial TBPs of human medical and veterinary relevance is present. Among these are at least five pathogenic genospecies of the *Bbsl* group*, Coxiella burnetii, Francisella tularensis, Candidaus* Neoehrlichia mikurensis*, Rickettsia raoultii* and *R. slovaca* (Faulde and Hoffmann, 2001; Süss *et al.*, 2004a). TBE-V and the agents of the *Bbsl* complex are, by far, the most important and abundant viral and bacterial vector-borne pathogens of humans in Germany, respectively (Humair and Gern, 2000; Süss *et al.*, 2004a; Lindgren and Jaenson, 2006). They are also the most extensively studied tick-borne pathogenic organisms not only in Germany, but throughout their range of occurrence in the Palearctic and Nearctic (only *Bbsl*) regions. In contrast to these well-studied pathogens, little is known about the epidemiology, pathogenicity, reservoir status of hosts and vector capacities of potential tick vectors of several emerging pathogens. These are mainly present among the family of Rickettsiaceae, comprising the rickettsial species *R. raoultii* and the recently discovered CNM. Protozoans, such as *Babesia divergens* are not as significant for human health, since most infections occur in patients with a decreased immune defense (Häselbarth *et al.*, 2007).

A comprehensive list of the TBPs that have been dicovered in BW so far, is given in Table 1.

Table 1: Overview of the tick-borne pathogens (in alphabetical order) that have been detected in Baden-Württemberg and are transmitted by the Ixodidae. Viruses are listed at first, then bacteria and finally protozoan parasites. CNS: Central nervous system, meningitis: inflammation of the brain envelope, encephalitis: inflammation of the brain, myelitis: inflammation of the spinal chord, leukopenia: leukocyte deficiency, erythema migrans: migrating rash. (Modified after Petney et al. 2013)

Pathogen	Vector	Reservoir host	Pathogenicity	Source
Eyach virus (Coltiviridae)	*Ixodes ricinus*	Rodents (?); lagomorphs	Fever	(Faulde and Hoffmann, 2001; Maier *et al.*, 2003; Hassler *et al.*, 2003)
TBE virus (Flaviviridae)	*Dermacentor* spp.; *Ixodes* spp.	Rodents; insectivores	Febrile illness; meningitis; encephalitis; myelitis	(Schrader and Süss, 1999; Süss *et al.*, 1999; Oehme *et al.*, 2002; Maier *et al.*, 2003; Alpers *et al.*, 2004; Süss and Schrader, 2004)
Tettnang virus (Iridoviridae)	*I. ricinus*	Rodents; insectivores	Similar to TBE infection	(Faulde and Hoffmann, 2001; Maier *et al.*, 2003)
Anaplasma phagocytophylum	*I. ricinus*	Deer; rodents; horses; dogs; cattle; sheep	Leukopenia, febrile illness	(Baumgarten *et al.*, 1999; Oehme *et al.*, 2002; Leonhard, 2005; Hartelt *et al.*, 2008; Skuballa *et al.*, 2010)
***Borrelia burgdorferi* sensu lato**	*Ixodes* spp.	Dogs; cats; horses; rodents; birds	Multisystemic illness; unspecific symptoms; CNS, joints or myocard frequently affected, Erythema migrans;	(Baumgarten *et al.*, 1999; Faulde and Hoffmann, 2001; Oehme *et al.*, 2002; Leonhard, 2005; Fingerle *et al.*, 2008)
***B. burgdorferi* sensu stricto**	*Ixodes* spp.	see above	See above	(Oehme *et al.*, 2002; Alpers *et al.*, 2004; Leonhard, 2005)
B. afzelii	*Ixodes* spp.	Rodents, European hedgehogs	See above; arthritids	(Wilske *et al.*, 1996; Humair *et al.*, 1999; Oehme *et al.*, 2002; Alpers *et al.*, 2004; Leonhard, 2005; Skuballa *et al.*, 2007)
B. garinii	*Ixodes* spp.	Fallow deer; birds (OspA3;OspA5-A8)	See above; neuroborreliosis	(Wilske *et al.*, 1996; Huegli *et al.*, 2002; Oehme *et al.*, 2002; Alpers *et al.*, 2004; Leonhard, 2005; Skuballa *et al.*, 2007; Fingerle *et al.*, 2008)
B. spielmanii	*Ixodes* spp.	Dormice; European hedgehogs	see above	(Leonhard, 2005; Richter *et al.*, 2006; Skuballa *et al.*, 2007; Fingerle *et al.*, 2008)
B. valaisiana	*Ixodes* spp.	Birds	see above	(Oehme *et al.*, 2002; Leonhard, 2005)
"*Candidatus*" Neoehrlichia mikurensis	*Ixodes spp.*	Rodents	Febrile illness	(Obiegala *et al.*, 2014; Andréasson *et al.*, 2015; Silaghi *et al.*, 2016)

Coxiella burnetii	*Dermacentor marginatus; D. reticulatus; Haemaphysalis punctata; I. ricinus; Rhipicephalus sanguineus*	Ruminants; mammals; birds	Q-fever: fever; myalgia; atypic pneumonia; hepatitis; fertility disorder; abort (ruminants)	(Maier *et al.*, 2003; Alpers *et al.*, 2004; Krauss, H Weber *et al.*, 2004; Sting *et al.*, 2004; Hartelt *et al.*, 2008)
Francisella tularensis	*D. marginatus; D. reticulatus; I. ricinus*	Rodents; lagomorphs	Tularemia (rabbit fever); fever; myalgia	(Faulde and Hoffmann, 2001; Hirsch *et al.*, 2001; Maier *et al.*, 2003; Krauss, H Weber *et al.*, 2004)
Rickettsia helvetica	*I. ricinus*	not confirmed	not confirmed	(Oehme *et al.*, 2002; Maier *et al.*, 2003)
R. raoultii	*D. marginatus; D. reticulatus*	domestic ruminants (?)	SENLAT (Scalp Eschar and Neck lymphadenopathy after tick bite)	(Hartelt *et al.*, 2008; Ortuño *et al.*, 2012; Switaj *et al.*, 2012; Parola *et al.*, 2013)
R. slovaca	*D. marginatus; D. reticulatus; I. ricinus*	Rodents; dogs (?)	SENLAT	(Maier *et al.*, 2003; Hartelt *et al.*, 2008; Parola *et al.*, 2013)
B. canis	*D. marginatus; D. reticulatus; H. punctata; I. ricinus*	Dogs	Babesiosis (malaria-like hemolytic anemia)	(Maier *et al.*, 2003; Barutzki *et al.*, 2007; Beelitz *et al.*, 2008)
B. divergens	*D. marginatus; H. punctata; I. ricinus*	Cattle	Babesiosis (immuno-comprimized persons)	(Maier *et al.*, 2003; Hartelt *et al.*, 2004; Leonhard, 2005)
B. microti	*D. marginatus; H. punctata; I. ricinus*	Rodents	Babesiosis (immuno-comprimized persons)	(Maier *et al.*, 2003; Hartelt *et al.*, 2004; Leonhard, 2005)

1.4.1 Transmission dynamics

In addition to the high diversity of etiological agents, the different modes of transmission by which these pathogens circulate between ticks and hosts further adds to the complexity and variability of tick-bone disease systems (Sonenshine and Mather, 1994):

- Horizontal transmission describes the direct or indirect infection of a tick by another tick. This can be facilitated via the infection of a naïve reservoir host by an infected tick on which a tick feeding subsequently can acquire infection (Figure 1-8) (Randolph *et al.*, 1999; Nuttall *et al.*, 2000). Another way is the passage of infection via co-feeding. It facilitates transmission between two ticks feeding in close proximity on the same host via the passage of pathogen-infected tick saliva, without the host necessarily developing a systemic viraemia (Randolph *et al.*, 1996; Harrison and Bennett, 2012).

- Vertical transmission involves the persistence of pathogens between different life history stages of ticks (Figure 1-8): the pathogen can either be propagated transstadially, from one molting stadium to the next, ensuring the preservation of infection throughout the tick's life span (Bonnet *et al.*, 2007; Jahfari *et al.*, 2012). Alternatively, the pathogen can be passed on transovarially from the infected mother tick to her offspring, leading to larvae that are infected. The transmission success varies strongly among different groups of pathogens and tick partners (Parola *et al.*, 2005; Socolovschi *et al.*, 2012; Rollend *et al.*, 2013).

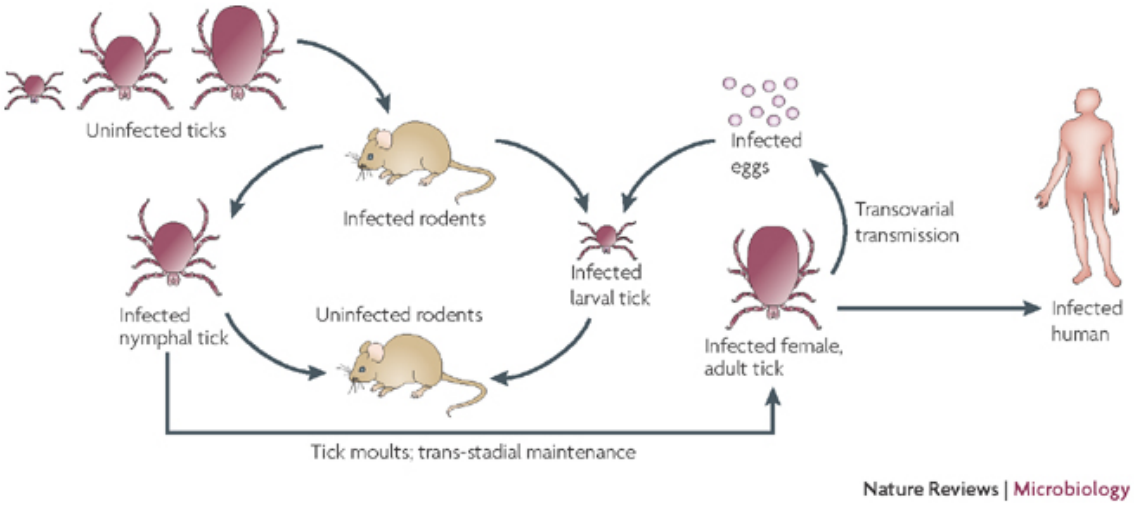


Figure 1-8: Routes of TBP transmission between ticks and hosts including the potential involvement of humans as ecological sink in the transmission cycle, leading to the death of the tick and the end of pathogen propagation (Walker and Ismail, 2008)

Babesia spp., *Bbsl*, CNM as well as *Rickettsia* spp.*(Rspp)* and TBE-V can be transmitted horizontally, via the saliva of a feeding tick, which is injected into the host. If the pathogen encounters a host that is suceptible, it is able to reproduce within the host and can be subsequently ingested by a tick during its blood meal (Labuda *et al.*, 1997; Nuttall *et al.*, 2000). However, *Bbsl* and TBE-V -infected ticks rarely transmit these pathogens transovarially to their offspring (Kurtenbach *et al.*, 1995; Lindquist and Vapalahti, 2008). This is more likely to occur for a variety of *Rspp* which occur intracellularly (Parola and Raoult, 2001a; Katargina *et al.*, 2015).

Interestingly, all TBDs are zoonotic, which means that they occur endemically and cycle between animals and their respective tick vectors (Sonenshine and Mather, 1994; Gage *et al.*, 1995; Comer, 2001; Vorou *et al.*, 2007). In this scenario, humans represent dead-end hosts (Figure 1-8), as their infestation and infection usually leads to the death of the

tick and the end of pathogen propagation (Estrada-Peña and Jongejan, 1999; Walker and Ismail, 2008; Pugliese and Rosà, 2008).

1.4.2 Important tick-borne pathogens in Germany

In the following paragraph, the biology of the major TBPs of relevance to the present study will be examined with respect to the involvement of small mammal hosts and ticks as reservoirs and vectors.

Tick-borne encephalitis virus

TBE-V is an RNA-virus belonging to the family Flaviviridae and related to the etiologic agents of other vector-borne viral disease of global impact, namely yellow-fever, dengue fever and West-Nile, amongst others (Gresikova and Kaluzova, 1997; Süss, 2008). It occurs across Central and Eastern Europe as well as in parts of Russia, China and Japan and can be divided into three subtypes: The European subtype, the Siberian subtype and the Far Eastern subtype (Dobler, 1998; Süss, 2003). Tick-borne encephalitis (TBE), the disease caused by an infection with TBE-V, is the most abundant and significant athropod-borne disease of viral origin in Europe (Oehme *et al.*, 2002; Süss *et al.*, 2004b).

Infection is often inapparent, however, if the disease breaks out, the clinical manifestations of infection include inflammation of the central nervous system including the meninges, the brain or the spinal marrow (Bröker and Gniel, 2003). The epidemiology of TBE is closely related to the ecology and biology of its ixodid tick vectors and its endemiological cycle is maintained by infected ticks and their wild vertebrate hosts within narrowly defined, forested foci (Donoso-Mantke *et al.*, 2011). A natural focus describes the occurrence of a pathogen during a period of time within a certain location (Süss, 2003). TBE strains of the European subtype are predominantly transmitted by *I. ricinus* (Gresíková and Nosek, 1966; Gresikova and Kaluzova, 1997), while the Siberian and Far Eastern subtype are mostly transmitted by *I. persulcatus,* a tick species closely related to *I. ricinus* (Gresikova and Kaluzova, 1997; Rumer *et al.*, 2011). TBE-V infected ticks remain infected throughout their life from one developmental stage to the other via transstadial transmission (Donoso-Mantke *et al.*, 2011). In addition, TBE-V can be transmitted transovarially and by co-feeding. Even though transovarial transmission is regarded as a

rare event for the European subtype and *I. ricinus*, it occurs more frequently for *I. persulcatus* and the other subtypes (Süss, 2011). Furthermore, this mode of transmission can suffice to maintain the virus population under certain conditions (Donoso-Mantke *et al.*, 2011).

Natural reservoir hosts for TBE-V include rodents, insectivores and carnivores (Kozuch *et al.*, 1967; Süss, 2003), with rodents as well as insectivores being able to maintain the pathogen over winter (Donoso-Mantke *et al.*, 2011). The fluctuation in transmission is therefore closely related to the dynamics of the reservoir host populations and - while it is not clear which role birds play in the epidemiology of TBE - they are, together with larger vertebrates, of importance for the introduction of the virus to new areas by carrying infected ticks (Süss, 2003). The prevalence of infected *I. ricinus* in an area was shown to be of high importance for the overall "risk" of human infection, as it is for any other TBPs (Dobler, 1998). However, large scale investigations on the abundance of TBE-V in ticks and wildlife are scarce and our knowledge about the detailed interactions between ticks, hosts and TBE-V is not sufficient to explain or predict the establishment and persistence of TBE foci (Oehme et al., 2002; Süss et al., 2004b). German studies imply that in endemic regions about 0.1% of ticks are infected with TBE-V, however, in known TBE foci the infection rate can be much higher, ranging around 0.5% to 5% (Maier *et al.*, 2003; Süss *et al.*, 2004b, 2006; Lucius and Loos-Frank, 2008b).

***Borrelia burgdorferi* s.l**

Bbsl describes a group of extracellular, spirochaetal bacteria that are the causative agents of Lyme disease (Spielman *et al.*, 1985; Lindgren and Jaenson, 2006), the most common TBD in the Northern Hemisphere (Alpers *et al.*, 2004; Stanek, 2005; Poggensee *et al.*, 2008). Lyme disease, or Lyme borreliosis, is a multi-systemic disease which can affect the skin, heart, nervous system, muscles and the skeleton, and can present a variety of different symptoms and disease patterns (Stanek, 2005; Strle and Stanek, 2009; Krause and Fingerle, 2009). The clinical symptoms of patients with *Bbsl* infection are mainly restricted to a certain organ system (Huppertz *et al.*, 1999). These different manifestations are hypothesized to be species-specific and therefore attributed to the heterogenity of the *Bbsl* complex (Lipsker and Jaulhac, 2009; Krause and Fingerle, 2009).

From the 19 known genospecies worldwide belonging to the *Bbsl* complex to date, seven have been identified in Germany (Figure 1-9). At least five of them are known to cause Lyme disease in humans in Germany (Stanek and Reiter, 2011; Franke *et al.*, 2013), including *B. afzelii, B. burgdorferi s.s., B. garinii, B. spielmanii* and *B. bavariensis*, with the three former being the most important both in Europa and Asia (Hovius *et al.*, 2007; Stanek and Reiter, 2011). It is unclear whether other genospecies (Figure 1-9) are human pathogenic as well (Margos *et al.*, 2011; Franke *et al.*, 2013).

The major vectors of *Bbsl* are ticks of the genus *Ixodes* belonging to the *I. ricinus* complex (Anderson, 1991; Franke *et al.*, 2013). However, the different genospecies are associated with different reservoir hosts and different vector species (Lindgren and Jaenson, 2006; Kurtenbach *et al.*, 2006). In this context, *Bbsl* possesses bridge vectors that can transmit *Bbsl* to humans (Diuk-Wasser *et al.*, 2016), as well as other vectors that typically do not come into contact with humans and act to maintain the pathogen in the enzootic cycles (Rudenko *et al.*, 2011).

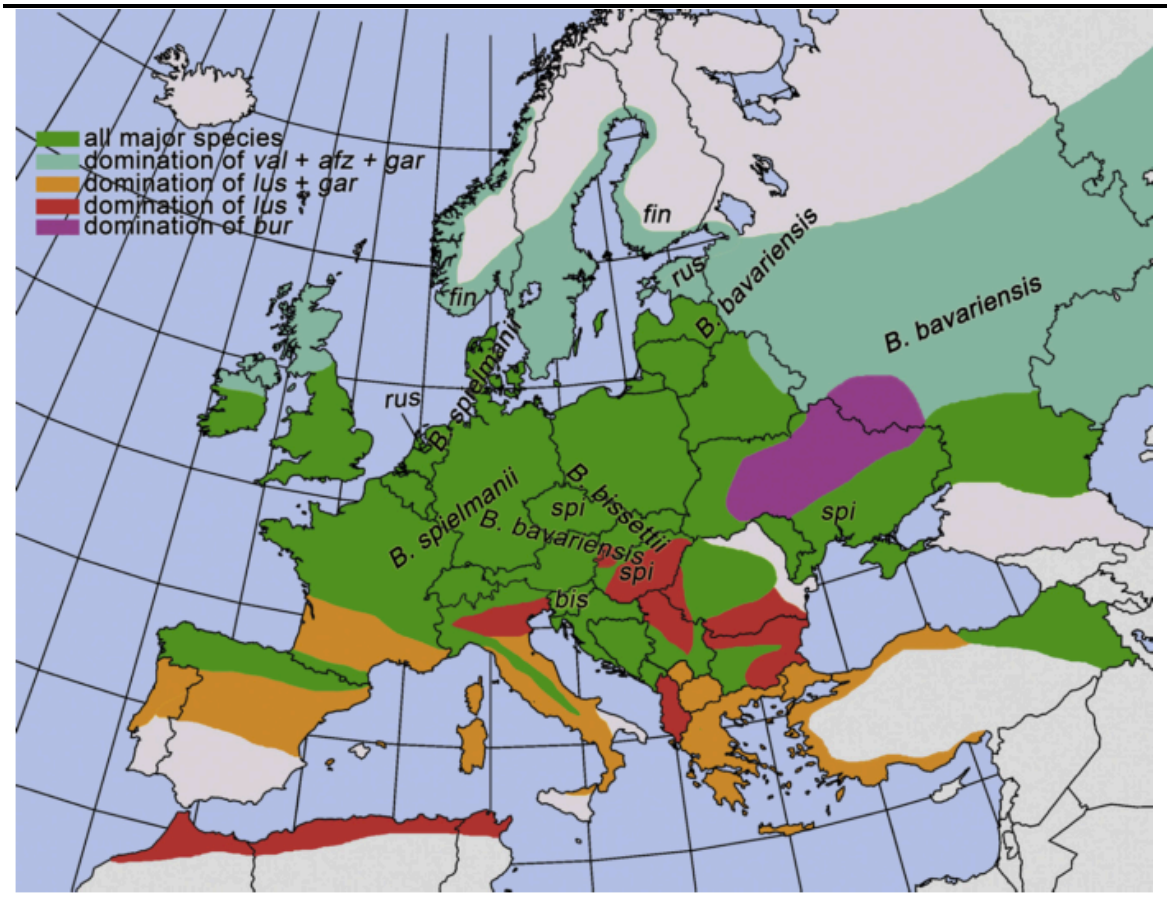


Figure 1-9: Schematic overview of the distribution of *Borrelia* spp. in Europe, North Africa and Western Asia based on a Meta-analysis by Franke et al., 2013). Major species are *B. afzelii, B. burgdorferi s.s., B. garinii, B. lusitaniae* and *B. valaisiana.* Other species include *B. bissettii* (bis), *B. finlandensis* (fin), Candidatus *B. ruski* (rus) and *B. spielmanii* (spi) (Franke *et al.*, 2013)

However, due to the lack of xenodiagnostic studies, knowledge on the natural transmission cycles, particularly concerning relevant reservoir hosts for most *Bbsl*, is still poor (Franke *et al.*, 2013). *B. afzelii* causes chronic skin lesions or acrodermatitis chronica atrophicans and uses rodents as reservoir hosts (Humair *et al.*, 1999; Kurtenbach *et al.*, 2006; Strle and Stanek, 2009). It has been detected in several small mammal species including *Apodemus* spp. (Humair *et al.*, 1999; Pérez *et al.*, 2012). *B. garinii*, on the other hand, is associated with neuroborreliosis and uses different bird species as reservoir hosts (Olsén *et al.*, 1993; Hanincova *et al.*, 2003; Kurtenbach *et al.*, 2006; Gern, 2008; Strle and Stanek, 2009). *B. burgdorferi* s.s. is often found in arthritic manifestations of the disease and seems not to be host specific as it is found in both bird and rodent-enzootic cycle (Kurtenbach *et al.*, 2006; Gern, 2008; Strle and Stanek, 2009). This shows that it is most likely that two different enzootic transmission cycles play an important role in the

distribution and dispersion of *Bbsl*., comprising a rodent and a bird cycle (Kurtenbach *et al.*, 1998a; b, 2001, 2002).

Candidatus Neoehrlichia mikurensis

The obligate intracellular, gram-negative bacterium CNM is an emerging TBP that parasitizes the cytoplasm of rodent endothelial cells (Kawahara *et al.*, 2004). It was first described in the Netherlands in 1999 (Schouls *et al.*, 1999). Until now, the isolation and cultivation of this bacterium has not been possible under laboratory conditions (Kawahara *et al.*, 2004; Grankvist *et al.*, 2015). The first clinical case of CNM infection in Germany was reported in 2010 (Von Loewenich *et al.*, 2010). Its clinical manifestation includes systemic inflammatory syndromes like relapsing fever and its pathogenicity is predominantly expressed in immunocomprimized patients (Jahfari *et al.*, 2012; Andréasson *et al.*, 2015; Silaghi *et al.*, 2016). However, unspecific symptoms involved in the clinical picture of infection (fever, cough, anaemia, headache), particularly in the absence of any serological tests, make the diagnosis of CNM-associated disease difficult (Vayssier-Taussat *et al.*, 2012). To our current knowledge, CNM occurs from Europe to Asia and circulates endemically among tick vectors of the genus *Ixodes* and several rodent reservoir hosts including the genera *Apodemus*, *Microtus*, *Myodes* and *Rattus* (Jahfari *et al.*, 2012; Li *et al.*, 2013; Grankvist *et al.*, 2014). It has been shown to be transmitted transstadially, but transovarial transmission has not been demonstrated (Vayssier-Taussat *et al.*, 2012; Jahfari *et al.*, 2012; Burri *et al.*, 2014; Silaghi *et al.*, 2016)

Rickettsia spp.

Rspp are gram-negative, obligate intracellular bacteria that parasitize endothelial cells and macrophages (Walker and Ismail, 2008). They are associated with blood-feeding arthropods and can be transmitted to vertebrates via saliva, feces, blood and aerosols (Hartelt *et al.*, 2004; Parola *et al.*, 2013). The genus *Rickettsia* is classically subdivided into three groups: the spotted fever group (SFG) with more than 20 different species, the typhus group (TG, *R. prowazekii*, *R. typhi*) and the scrub typhus group (STG, *R. canadensis*, *R. bellii*) (Fournier and Raoult, 2009). The presence of six different *Rickettsia* species with human pathogenic potential in Germany has been confirmed so far (*R. helvetica, R. monacensis, R. massiliae, R. slovaca, R. raoultii, R. felis*) (Richter *et al.*, 2002; Simser *et al.*,

2002; Dobler and Wölfel, 2009; Dobler *et al.*, 2009; Silaghi *et al.*, 2011). Except for *R. felis*, which is mainly associated with flea-vectors, all *Rspp* belong to the SFG *Rickettsiae.* These circulate exclusively between vertebrate hosts and ixodid ticks (Parola and Raoult, 2001a; Silaghi *et al.*, 2011) and are the causative agents of human rickettioses worldwide (Parola *et al.*, 2013). *I. ricinus* is regarded as principal vector for *R. helvetica* and *R. monacensis*, whereas *Dermacentor* spp. are predominantly associated with *R. slovaca* and *R. raoultii* (Bouyer *et al.*, 2001; Dobler and Wölfel, 2009). The role of mammals as reservoirs for different *Rspp* has not been clearly defined (Parola and Raoult, 2001a), as is the influence of birds and reptiles on the developmental cycle. However, it has been suggested that birds play, as for *A. phagocytophilum* and *Babesia* spp., an important role in the the distribution of these bacteria (Tijsse-Klasen *et al.*, 2010; Franke *et al.*, 2010b; Movila *et al.*, 2011; Václav *et al.*, 2011; Hildebrandt *et al.*, 2011). Ticks can act as vectors as well as reservoirs of *Rickettsia* and the pathogen can be transmitted transovarially, transstadially, via co-feeding and transplacently in mammals (Socolovschi *et al.*, 2009; Biernat *et al.*, 2016). To date, data on rickettsial occurrence and prevalence in Germany are rare (Dobler and Wölfel, 2009; Schex et al., 2011). In a study conducted in BW, the prevalence of *Rickettsia helvetica* was shown to be 8.9% in *I. ricinus* (Hartelt *et al.*, 2004).

Babesia spp.

The protozoans of the genus *Babesia* are haemoparasites that invade vertebrate eukaryotes (Krause, 2002). Clinical manifestations in human *Babesia* infections (Table 1) are typically related to *Babesia divergens* or *Babesia microti* (Herwaldt *et al.*, 2003) infections in immunocomprimizd patients. Canine babesiosis, caused by *Babesia canis* (Table 1), is a widely distributed disease of dogs that causes malaria-like symptoms including anemia and jaundice, and has a high rate of lethality if untreated (Barutzki *et al.*, 2007).

To fulfill their life cycle, *Babesia* need both a vertebrate and an invertebrate host. The invertebrate host is always an ixodid tick (Homer *et al.*, 2000). All medically relevant *Babesia* species in Germany are transmitted by ticks of the genera *Ixodes* or *Dermacentor* (Uilenberg, 2006; Hunfeld *et al.*, 2008). Besides rodents, birds can act as vertebrate hosts for *Babesia* spp. (Süss *et al.*, 2004a). As the human pathogenic *Babesia divergens* and

Babesia sp. EU1 both can be transmitted transovarially, ticks not only only serve as vectors in the transmission of the pathogens, but also as reservoirs for the amplification and maintenance of their life cycles (Bonnet *et al.*, 2007, 2009). In contrast, *Babesia microti* is only transmitted transstadially and has been detected in various small mammal hosts including *Apodemus flavicollis*, *Microtus agrestis, Myodes glareolus* and *Sorex araneus*, all acting as potential reservoirs in Europe (Karbowiak, 2004; Siński *et al.*, 2006b; Bown *et al.*, 2011; Tadin *et al.*, 2012).

1.5 Overview

Rodents are the most abundant and diverse order of living mammals in the world. They have been known as source of human disease since their involvement in the plague epidemic in medieval times. To date, the threat they pose to public health, with respect to vector-borne diseases, emerges from their putative role as keystone hosts in the transmission of a variety of TBPs like Lyme disease, based on long-term studies from the Northeastern US.

However, comprehensive long-term data on factors influencing tick burdens on small mammal hosts and implications for pathogen transmission are still scarce for Central Europe. The vast majority of available studies consider only parts of the system, focusing on the impact of abiotic influences on tick phenology on vegetation, mainly against the background of climate change (Perret *et al.*, 2000; Estrada-Peña *et al.*, 2004, 2016; Gray *et al.*, 2009; Schwarz *et al.*, 2009; Tack *et al.*, 2012b; Lauterbach *et al.*, 2013; Schulz *et al.*, 2014), or they monitor pathogen prevalence in ticks or hosts with reference to human risk of acquiring TBDs (Bown and Begon, 2003; Michalik *et al.*, 2003; Franke *et al.*, 2010a; Burri *et al.*, 2011b; Egyed *et al.*, 2012; Silaghi *et al.*, 2012a; Jahfari *et al.*, 2012; Gassner *et al.*, 2013; Biernat *et al.*, 2016). Those studies taking into account the differential reasons for variable tick burdens on rodent hosts in Central Europe (Humair *et al.*, 1993; Kurtenbach *et al.*, 1995; Tälleklint and Jaenson, 1997; Randolph *et al.*, 1999; Gray *et al.*, 1999; Korenberg *et al.*, 2003; Siński *et al.*, 2006a; Rosà *et al.*, 2007; Krasnov *et al.*, 2007; Paulauskas *et al.*, 2009; Paziewska *et al.*, 2010; Kiffner *et al.*, 2011; Burri *et al.*, 2011a; Mihalca *et al.*, 2012), are mainly based on short-term data of one year or less (Paziewska *et al.*, 2010; Mihalca *et al.*, 2012; Mysterud *et al.*, 2015), data with large intervals between sampling events (Krasnov *et al.*, 2007; Paulauskas *et al.*, 2009; Kiffner *et al.*, 2011; Mysterud *et al.*, 2015), or data based on single sampling sites (Gray *et al.*, 1999; Korenberg *et al.*, 2003; Rosà *et al.*, 2007; Paziewska *et al.*, 2010). Due to these impairments, such studies are unlikely to grasp the variability present in tick-host-pathogen-systems.

Extensive long-term studies have been carried out for over a decade in the largely oak-dominated woodlands of New York State (Ostfeld *et al.*, 1996, 1998a, 2001, 2006; Jones, 1998; Schmidt *et al.*, 1999; Goodwin *et al.*, 2001; Brunner and Ostfeld, 2008; Jones *et al.*, 2015). The studied system is based on *I. scapularis*, one of the most abundant tick species

in the Eastern US and closely related to *I. ricinus,* and the rodent *Peromyscus leucopus,* the preferred host for immatures of *I. scapularis* (Davidar *et al.*, 1989; Ostfeld *et al.*, 1998b; Schmidt *et al.*, 1999) and major reservoir for *Bbsl* in the eastern US (Anderson and Magnarelli, 1984; Mather *et al.*, 1989). Using the acquired long-term data, Ostfeld and colleagues developed a conceptual framework model, stating mast as the main driver of *Borrelia* prevalence in the northeast of North America. They postulate that acorn production strongly influences rodent abundance in the following season, which increases the total number of larval *I. scapularis* finding a blood-meal host and coincidentally feeding on a competent *Bbsl* reservoir. This in turn is assumed to result in an increased abundance of nymphs in the following season as well as a higher *Bbsl* infection prevalence in nymphs (Ostfeld *et al.*, 1996, 2001, 2006; Jones, 1998; Goodwin *et al.*, 2001). Climatic conditions were repeatedly shown to be of minor influence on the tick-host-pathogen-system (Ostfeld *et al.*, 2001, 2006), but the universal applicability of the "acorn-host-tick-*Borrelia*-framework" has already been critizised in the past, suggesting the increasing importance of (micro-) climatic influences if narrowly defined ranges of these conditions are overstepped (Ostfeld *et al.*, 1998b; Schmidt *et al.*, 1999; Jones and Kitron, 2000; Schauber *et al.*, 2005). Given the seven *Borrelia* genospecies and multiple species strains (Fingerle *et al.*, 2008; Margos *et al.*, 2011; Stanek and Reiter, 2011; Skuballa *et al.*, 2012) together with the large amount of available host and tick species in the Palearctic (Humair and Gern, 2000; Pichon *et al.*, 2003; Tilly *et al.*, 2008), it can be hypothesized that the the European tick-host-pathogen-system is substantially more complex than the system postulated by Ostfeld *et al.* (1996, 2001, 2006; Jones, 1998; Goodwin *et al.*, 2001).

1.6 Objectives

As described in the previous sections, the tick-host-pathogen-system is based on a complex network of interactions and, to date, no clear pattern has emerged on the factors controlling the system. For a deeper understanding of the relative importance and contribution of abiotic and biotic factors on the tick-host-pathogen system it is essential to regard the system holistically, not piecemeal as most previous studies have done so far.

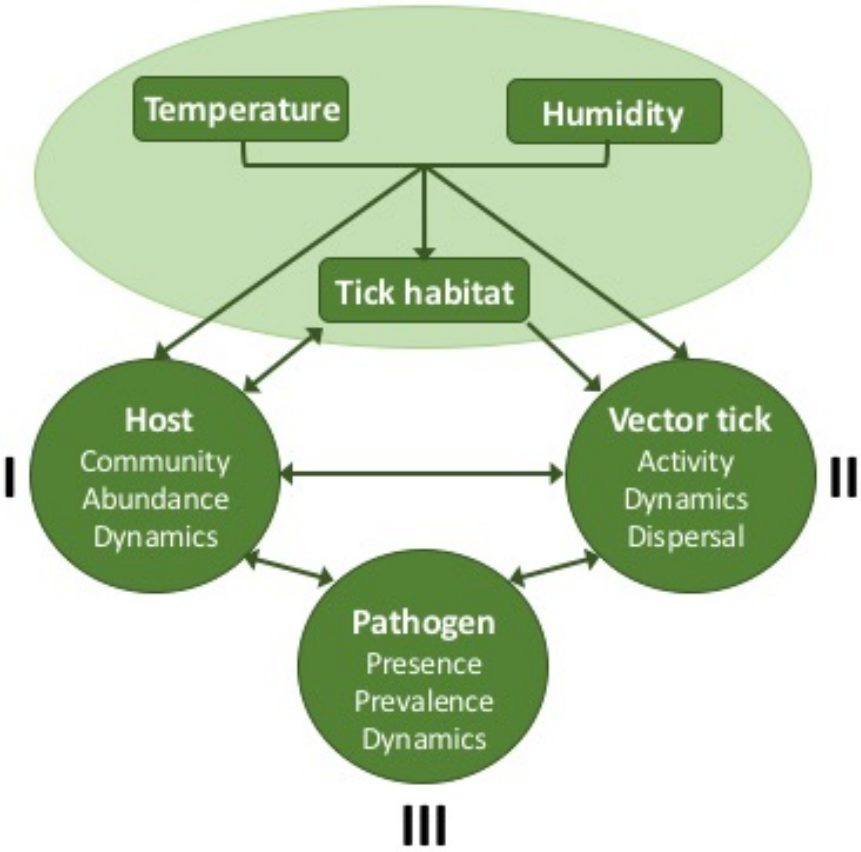


Figure 1-10: Overview of the objectives, serving as the structure of the thesis. The dark green circles "Host" (I), "Vector tick" (II) and "Pathogen" (III) indicate the three main components and their order of examination in the thesis, including individual points of focus in each circle. The light-green ellipse comprises the abiotic and biotic environmental factors of interest in the study. Modified after Lindgren (1998), Ecological Modelling 110:55-63.

Therefore, my overall objective is to elucidate if and how small mammal host populations, habitat and microclimatic conditions influence tick-host-pathogen-interactions in BW. To achieve this aim, a comprehensive and detailed study of the above factors was carried out at four different forest sites in BW over a study period of three years, focusing on the most

widespread and medically important tick species, *I. ricinus,* in its preferred habitat, the forest.

My first aim (**I** in Figure 1-10) was the characterization of the community structure, abundance and dynamics of small mammal host populations. In order to achieve this, I used population models to estimate the abundance of small mammal host populations and examined differential distributions of intrinsic host characteristics among host species of potential influence on tick infestation levels and dynamics.

My second objective was to examine the influence of small mammal hosts and environmental factors on the abundance (activity), dynamics and dispersal of *I. ricinus* populations in the investigated areas (**II** in Figure 1-10). To achieve this, I assessed a variety of measures to quantify tick burden on hosts, examined the spatio-temporal dynamics of the ticks on hosts with respect to identifying patterns of tick-host-interaction, compared the patterns of tick abundance on hosts and on vegetation and, finally, used advanced statistical modelling approaches to examine the relative influence of abiotic and biotic influences on tick abundance on hosts and on vegetation.

My third objective was to identify how tick-host interactions influence the presence, prevalence and dynamics of tick borne pathogens (**III** in Figure 1-10). The focus of this chapter lies on the more commonly detected pathogens, *Rspp* and *Bbsl* and tends to analyze the dynamics of prevalence and potential causes. Again, I used advanced statistical modelling approaches to analyze the factors influencing pathogen dynamics in ticks on hosts and on vegetation.

Finally, my fourth objective was to provide a synthesis of the first three objectives, stating in which way the complex networks between hosts, ticks and pathogens in a spatially and temporally variable environment interact and which implications can be drawn from this.

2 MATERIAL & METHODS

2.1 Study area

Four woodland areas in the northwest of BW (in the districts of Karlsruhe and Calw) have been chosen as model sites for small mammal sampling and tick collection in this study (Figure 2-1). They differ in forest type, soil composition and elevation above sea level (Table 2-1) and represent typical forests for BW.

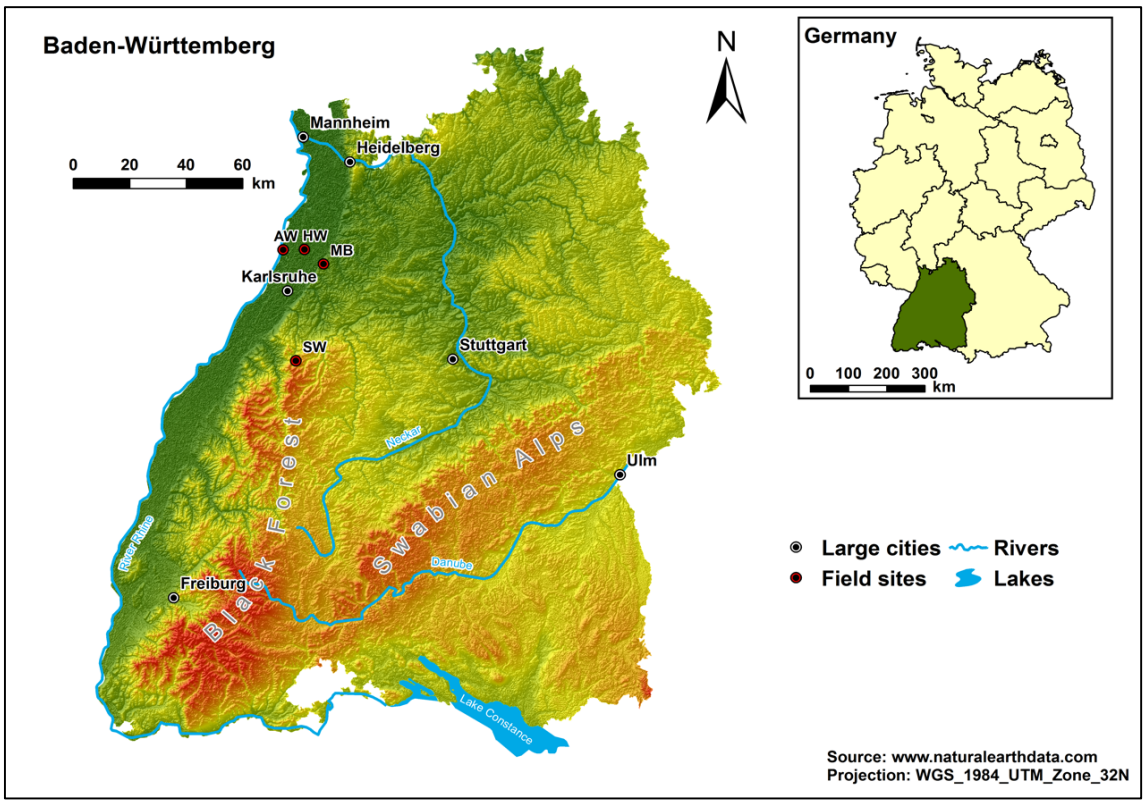


Figure 2-1: Locations of the four study sites Auwald (AW), Hardtwald (HW), Michaelsberg (MB) and Schwarzwald (SW) in BW (red circles) and Germany

Auwald

This forest area is located at 111 m.a.s.l. to the east of the city of Linkenheim within the borders of the Rheinniederungskanal (east) and the river Rhine (west) (coordinates, Table 2-1). It represents a typical hardwood alluvial forest with oaks (*Quercus robur*) and ashes (*Fraxinus excelsior*) (see Table 2-1) and a series of other tree species. This forest is further characterized by medium humidity, a high proportion of ground cover by plants or dead wood and an outstanding floral and faunal species richness (Lechner and Zimmermann, 2007). Due to its unique ecological value, the Auwald is an area under protection status according to the *Natura 2000* protocol, a European Union (EU) wide network of nature reserves for the conservation of endangered or regionally typical and therefore worth protecting biotopes and species (http://ec.europa.eu/environment/nature/natura2000/index_en.htm).

Figure 2-2: Auwald alluvial forest in August 2013. Dense ground cover, deciduous trees (Nina Littwin)

Hardtwald

The Hardtwald study site is located between the towns of Friedrichstal and Graben-Neudorf (coordinates, see Table 2-1), delimited in the west by an agricultural area and by a railway line in the east (Figure 2-3). This forest is an especially dry mixed forest with a low overall plant cover, built on inland dunes, acidic soil and drift sand (Zimmermann and Brinkmann, 2006). It is dominated by the Common Beech (*Fagus sylvatica*) and Pine (*Pinus sylvestris*). Due to its unique habitat properties, this forest is of high relevance for conservation, just as the previously described Auwald and represents a “Flora, Fauna, Habitat” (FFH) - nature reserve (Zimmermann and Brinkmann, 2006).

Figure 2-3: Hardtwald in August 2013. Low plant cover, thick layer of leaf litter, beeches (*Fagus sylvatica*) (N. Littwin)

Michaelsberg

Figure 2-4: Michaelsberg in August 2013. Thick leaf litter layer and dead wood on the ground, Common Beech (*Fagus sylvatica*) trees and saplings (Nina Littwin)

The Michaelsberg forest site is situated above the town of Untergrombach, between the cities of Weingarten and Bruchsal (coordinates Table 2-1). Just like the previously described forest sites Auwald and Hardtwald, the Michaelsberg sampling site is located within a protected nature reserve area (http://themenpark-umwelt.baden-wuerttemberg.de/servlet/is/10768/?path=7160;7111;). The soil at Michaelsberg is medium humid and consists of loess, the forest is a mixed forest dominated by the Common Beech (*F. sylvatica*).

Table 2-1: Study sites: Description of habitat location and properties (m.a.s.l.: meters above sea level, reference system: North Sea)

	Auwald (AW)	Hardtwald (HW)	Michaelsberg (MB)	Schwarzwald (SW)
Coordinates	49° 8'1.76" N 08°22'35.50" E	49° 8'7.34" N 08°28'47.79" E	49° 5'17.44" N 08°34'21.39" E	48°46'26.58" N 08°26'33.12" E
Elevation (m.a.s.l.)	111 m	117 m	253 m	610 m
Forest type	Alluvial forest	Mixed forest	Beech forest	Mixed forest
Deciduous: Coniferous	100:0	50:50	100:0	75:25
Major tree species	*Fraxinus excelsior* *Quercus robur* *Populus spec* *Carpinus betulus*	*Fagus sylvatica* *Pinus sylvestris*	*F. sylvatica*	*F. sylvatica* *Abies alba*
Minor tree species	none	*Picea abies* *C. betulus* *Q. robur*	*Acer spec.* *C. betulus*	*P. abies* *Larix decidua*
Soil humidity	Medium humid	Dry	Medium dry	Medium dry
Soil properties/type	clay	sand (acidic)	loess	rankers

Schwarzwald

This forest is located in the Gaistal (Table 2-1), a part of the northern Black Forest, southwards of Bad Herrenalb and, with an elevation of 610 m.a.s.l. it represents the most high-lying sampling site of this study. The soil type here is rankers, a shallow soil built on solid, lime-deficient rock and typical for the area (Scheffer and Schachtschnabel, 1984).

Figure 2-5: Representative picture of the study site Schwarzwald. The entire area is located along a slope, trees shown here are White Fir and Beech. Thin leaf litter due to the high proportion of conifers, medium ground cover with saplings and dead wood (Nina Littwin)

Due to its limited suitability for a variety of tree species, the forest here is dominated by the Common Beech (*F. sylvatica*) and the White Fir (*Abies alba*).

2.2 Mast

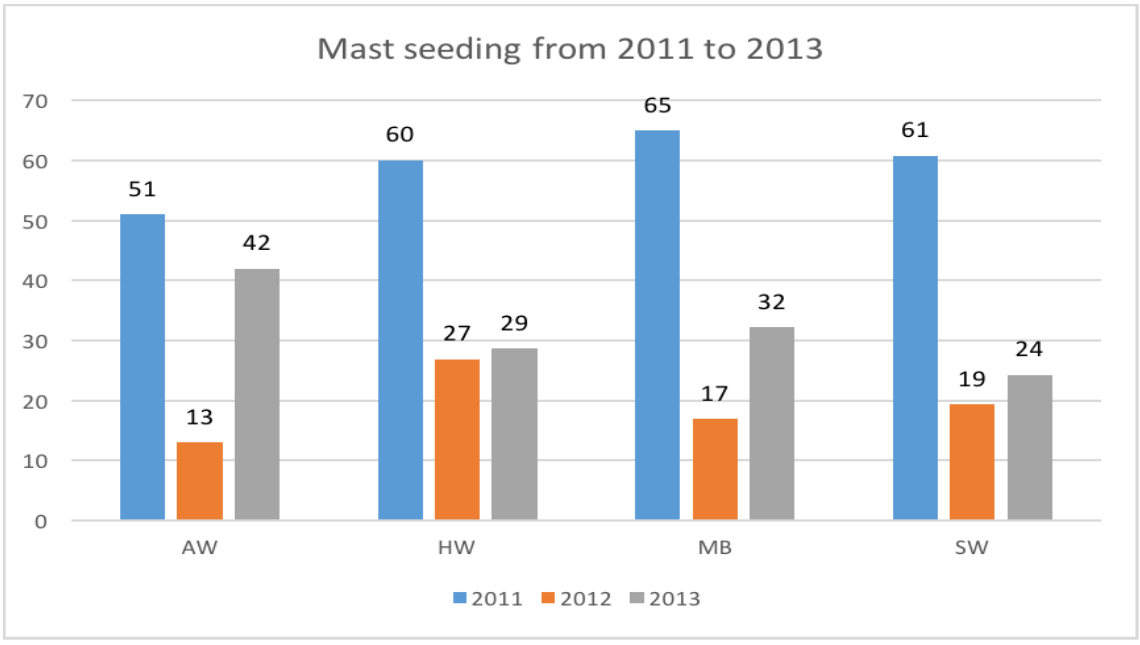


Figure 2-6: Differences in mast seeding of trees (in %) at the sampling sites during the study period. Major species account for 2/3 of total mast and minor species for the remaining 1/3. Values of 0-9% indicate the absence of mast, 10-39% indicates partial mast, 40- 69% indicates half-mast, and 70-100% reflects full mast

Mast is a measure (%) of tree fructification (e.g. beech, oak) per season(Schütt *et al.*, 2007). The corresponding data was provided by the forestry management Freiburg for BW and the Upper Rhine rift.

2.3 Field methods

2.3.1 Monitoring of microclimate

In collaboration with the Institute for Geology and Geoecology (IfGG) of the KIT, climate loggers (SDI-Log Data Logger, UP GmbH with HygroClip 2 Sensors, Rotronic) have been installed for the constant monitoring of weather conditions at the four study sites. Every 10 minutes, temperature and relative humidity of the air was measured at two different heights (50 cm and 200 cm above the ground) and temperature and humidity of the soil. Measurements were taken every ten minutes. Saturation deficit has been calculated after Randolph and Storey (1999):

$$SD = \left(1 - \frac{RH}{100}\right) 4.9463 e^{0.0621\mathrm{T}}.$$

2.3.2 Small mammals

At each of the four sampling sites, three grids of 40 x 40 m (1.600 m^2) have been established for small mammal trapping (see Figure 2-8). In each grid patch, 25 consecutively numbered bamboo sticks were placed at 10 m intervals (see Figure 2-7). The locations for the three grids at each site have been chosen according to the variability of the habitat to cover all variations of present vegetation structure with an equal amount of traps. Therefore, an area of 4800 m^2 per site have been used for sampling in total.

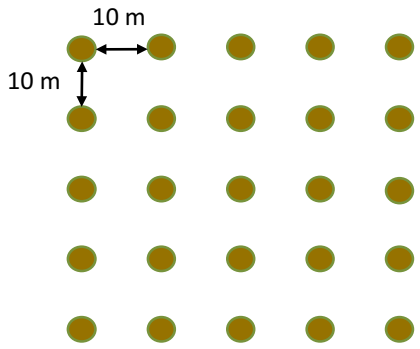


Figure 2-7: Schematic of the grid patches. Dots indicate bamboo sticks with numbers from 1-25 (Patch 1), 26-50 (Patch 2) and 51-75 (Patch 3) respectively

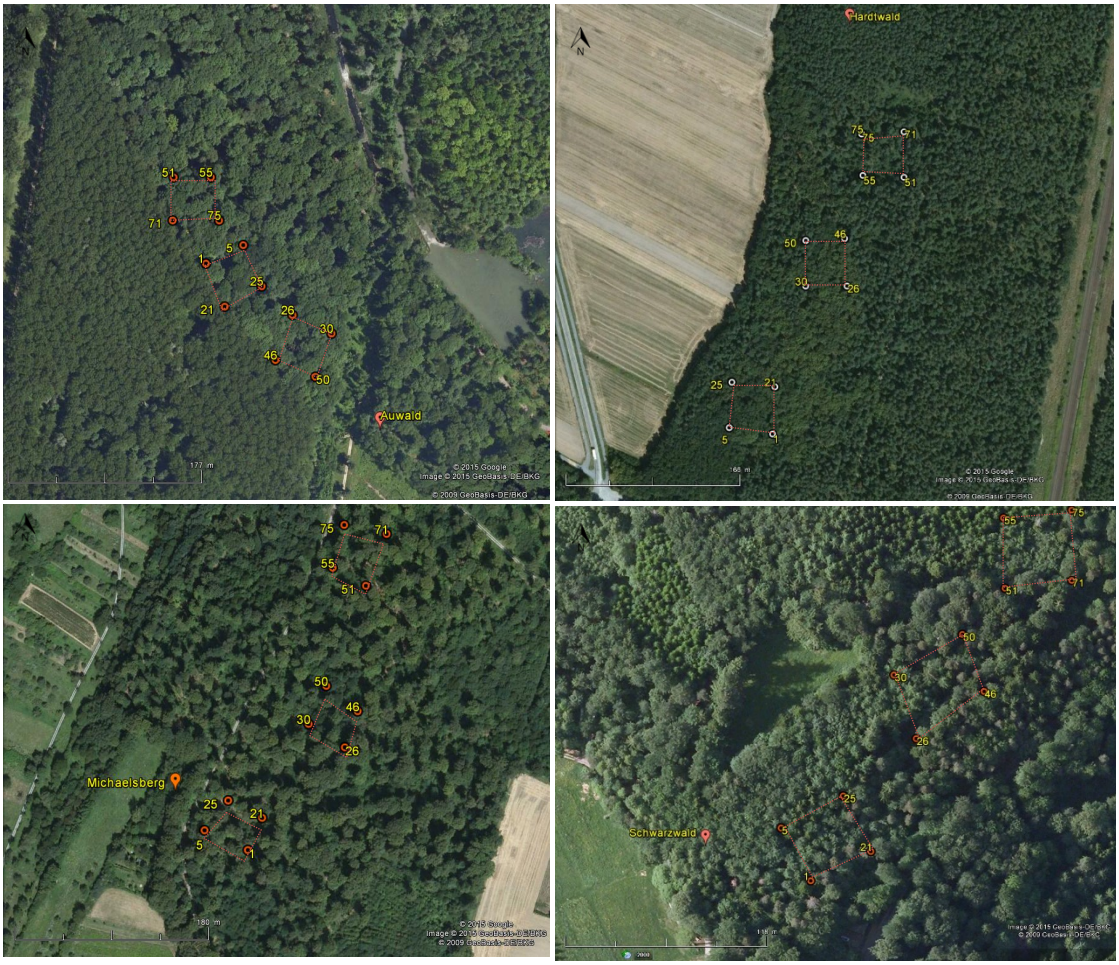


Figure 2-8: Overview of the site-specific locations of the three trapping grids at the sampling sites: Upper left: AW, upper right: HW, lower left: MB and lower right: SW. Numbering and dashed lines indicates the arrangement of the patches (1-25: patch 1, 26-50: patch 2, 51-75: patch 3). Source: GoogleEarth, © 2015 Google Image Landsat, SW, MB and HW pictures taken 01/01/2009, AW picture taken 01/01/2010, height: ca. 1km

Trapping

The methods used for small mammal trapping have been modified after Goodwin *et al.* (2001). Trapping sessions took place from May to October 2012 and March to October 2013 and 2014.

Live trapping of the small mammals is essential for the assessment of population dynamics via Capture-Mark-Recapture (CMR) as well as for the reproducibility of the collection of ticks from host individuals (see section “Tick collection – small mammal hosts”). During winter, however, cold and humid weather conditions are likely to increase trap-related mortality of the small mammals. Therefore, no trapping sessions took place from November until February. During the sampling seasons, Longworth small mammal live

traps (see Figure 2-9) were set within a radius of 2 meter in maximum around each marking stick (see Figure 2-7), one per position. So, a total of 25 Longworth live traps per grid patch, and a total of 75 traps per site were set on the evening of the first day of the trapping session and set open. To reduce trap-related stress, traps were provided with hay for shelter and with mealworms and sunflower seeds as food supply for the captured individuals. The traps were set out in the evening of day 1 of each trapping session before sunset. They were then controlled the next morning (day 2) after sunrise and in the evening of day 2 and again a third time after sunset of the following day (day 3) when the traps were removed from the site again.

Figure 2-9: Longworth live trap with nesting box in the back, attached to a tunnel with a lid in front. The lid closes after mechanical stimulation of a trip wire at the end of the tunnel

Examination and Marking

Captured small mammals were determined to species (Braun and Dieterlen, 2005; Kraft, 2008), gender (Kunz *et al.*, 1996), age (Sínski *et al.*, 2006) and reproductivity (McCravy and Rose, 1992; Kunz *et al.*, 1996). They were then examined for ticks (see section "**Small mammal hosts**") and it was checked whether or not they had been captured before. Newly captured rodents were individually marked by tattooing a unique, continuous number (ID) on their toe pads. This was realized using the Micro Tattoo System (Fine Science Tools, Heidelberg) together with Ketchum Green Tattoo Paste (Ketchum, Manufacturing Inc., Brockville, Ontario) and hypodermic

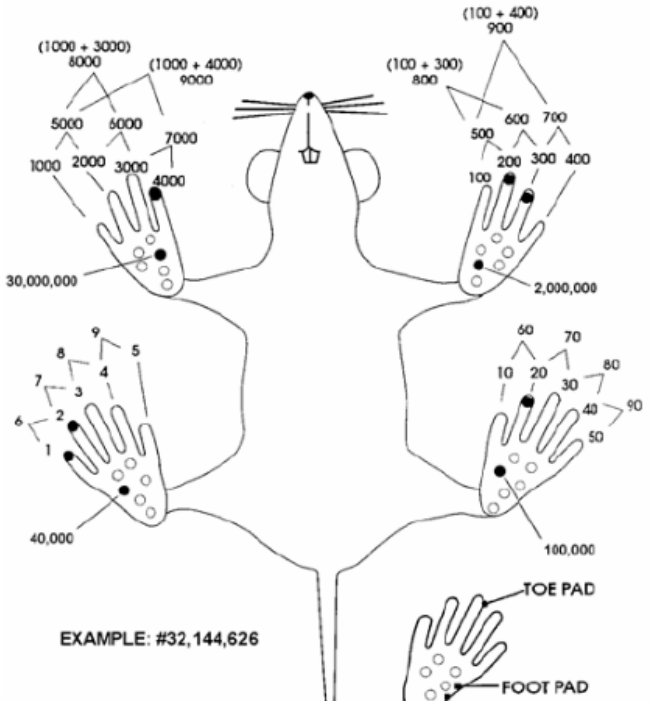


Figure 2-10: Schematic of the tattoo code for toe pads used to mark the small mammals individually. Obtained from the Manual for Micro Tattoo System by Fine Science Tools

needles size 27G x ½“, 0.4 x 12 mm (Sterican, B.Braun Melsungen AG, Melsungen) for adult and subadult individuals and size 30G x ½“, 0.3 x 13 mm (BD MicrolanceTM 3, BD, Drogheda, Ireland) for juvenile individuals and for shrews, respectively. For reasons of applicability due to their comparably small size, shrews were marked on their tail instead of their toes using a combination of one to two colors and one to five dots to give them their individual numbers. If animals had been previously caught (recaptured), their individual number was recorded.

Finally, animals were weighed using spring scales (for animals up to 50g: Micro-Line Spring Scale, metric, 60 g, Pesola AG, Baar, Schweiz, for animals over 50g: LightLine Spring Scale, metric, 100 g, Pesola AG, Baar, Schweiz) and then released at the site of capture.

Small mammal age class determination

The classification of age classes for each species was carried out after Sínski *et al.* (2006) for *A. flavicollis* and *M. glareolus*. To address the comparably more difficult classification of age in shrews (*Sorex araneus* and *S. minutus*), the information given in Braun & Dieterlen (2005) has been used as reference to determine individuals as adult or not.

Table 2-2: Overview of determination characteristics for the age classification of small mammals of different species. For thedetermination of the age class of *Apodemus sylvaticus* individuals, the specifications of *A. flavicollis* were used, for individuals of *Microtus agrestis*, the weight ranges of *M. glareolus* were used

	A. flavicollis	*M. glareolus*	*S. araneus*	*S. minutus*
juvenile	< 20 g	< 15 g	---	---
subadult	20 - 30 g	15 – 19.5 g	---	---
adult	> 30 g	> 19.5 g	≥ 6.5 g	≥ 2.6 g

2.3.3 Ticks

Small mammal hosts

This method represents an indirect manual method of tick collection. Small mammal individuals captured during a trapping session (as described in the sections above) have been inspected thoroughly for ticks by eye, a method that has been shown to give reliable results on actual tick burdens (Schmidt *et al.*, 1999; Goodwin *et al.*, 2001). The areas for preferred attachment of ticks on the small mammal hosts and therefore the most extensively screened parts were the head, the feet, the axles, and the tail of the hosts. Ticks were removed using forceps (Dumont, Biologie No. 5, Bioform, Nürnberg) from each

individual and transferred to a reaction tube (Reagiergefäß 1.5 ml, Sarsted, Nürnbrecht). The tick samples were then stored for a minimum of three days at -20°C until further examination. Small mammal trapping was approved by the local ethics committee (Regierungspräsidium Karlsruhe, Az 35-9185.82/A-11/12).

Flagging

Flagging is a well-established and widely used method for the collection of ticks from vegetation (Ginsberg and Ewing, 1989; Hillyard, 1996; Estrada-Peña *et al.*, 2013) In each of the four habitats, ticks were collected monthly, along with each small mammal trapping session, by dragging a white cotton cloth of 1m in length and width ($1m^2$) over the vegetation surface.

Figure 2-11: Flagging. A white cotton cloth is attached to a wooden stick and dragged over the vegetation surface to collect ticks (Lena Kratzer)

After a distance of 10 meters, the cotton cloth was examined for the presence of ticks on the cloth, attached ticks were then removed with fine forceps (Dumont, Biologie No. 5, Bioform, Nürnberg) and transferred to a reaction tube (Reagiergefäß 1.5 ml, Sarsted, Nürnbrecht). This procedure was repeated ten times in total, covering different heights of vegetation. If possible, two samples were taken across high vegetation (>30 cm above the ground), four samples across medium-high vegetation (10-30 cm) and another four samples were taken from leaf litter or low vegetation (0-10 cm). The tick samples were then stored at -20°C for a minimum of three days until further examination.

Taxonomic classification and engorgement status

The tick samples obtained by flagging and by collection from host animals were determined to life stage and species according to Arthur (1963), Hillyard (1996) and Pérez-Eid (2007) using the SMZ 1000 Stereomicroscope (Nikon, Düsseldorf) in combination with the ED Plan 2x Objective. Flagged ticks were pooled per species and developmental stage per session at each site in 2ml reaction tubes (Rotilabo®-safety microcentrifuge tubes, PP

2.0ml, Roth, Karlsruhe). Ticks collected from small mammals were determined separately per host individual per site and session and subdivided by developmental stage and species. Samples were then further subdivided according to the tick's level of engorgement, which served as proxy for the duration that a tick has been sucking blood on the host individual before collection and can allow for deeper insights into transmission patterns of TBPs. Ticks were classified from 1= (almost) unfed, 2= medium engorged to 3= (almost) fully engorged. This sub-classification was done visually and carried out only for the samples collected in 2012 and 2013. Due to financial restraints, this was not done with the samples collected in 2014.

2.4 Molecular methods

2.4.1 Homogenization of ticks

The ticks collected by flagging and from small mammal hosts were transferred according to the protocol shown in the table below (Table 2-3) to 2ml safe lock tubes (Eppendorf, Hamburg) containing aliquots of 400 µl MEM Earle (1x) cell culture medium w 2.2g/l $NaHCO_3$, w stable glutamine (Biochrom AG, Berlin) and three steel beads of 3mm in diameter (VWR, Bruchsal) each. The tubes were then placed in a vibration mill (MM400, Retsch, Haan) and homogenized at 30 Hz for 10 minutes. All homogenates were subsequently centrifuged at 3000 rfc at 4°C (5804R cooling centrifuge, Eppendorf, Hamburg) and stored at -20°C until further use.

Table 2-3: Overview of the separation of tick specimen for homogenization

Origin of sample	Larvae	Nymphs	Adults
Flagging	Maximum of 50 ticks pooled per session and site	single	single
Host	Maximum of 50 ticks pooled per host individual (In 2012 and 2013 subdivided by level of engorgement)	single	single

2.4.2 Nucleic acid purification

The extraction of nucleic acids from the homogenized tick samples was done using the automated NucliSense® easyMag™ (BioMérieux Deutschland GmbH, Nürtingen) system,

providing the extraction device and all neccessary expendable materials. This method uses the BOOM® technology, based on the principle of silica extraction:

- Lysis: Samples are first being lysed by guanidinthiocynanate (GIT), which denatures proteins and inactivates potentially present nucleases in the sample.
- Incubation: After the lysis step, magnetic silica particles are added and bind the nucleic acids reversibly.
- Washing: A magnet inside the NucliSense® easyMag™ attracts the magnetic beads and thereby enables the purification of the bound nucleic acids by washing.
- Elution: After washing, the nucleic acids are released from the beads by a heating step, followed by a final separation of the silica particles from the eluate by the integrated magnet inside the device.

Protocol:

- Homogenized samples were centrifuged at 6000 g for 5 minutes (Mini centrifuge 5415D, Eppendorf, Hamburg) and pooled according to the protocol in Table 2-4
- Transfer of the respective volume (see Table 2-4) to the NucliSense® easyMag™ cartridge slots (NucliSense® easyMag™ Disposables) and insertion into device. Automated lysis with NucliSense® easyMag™ lysis buffer for 10 minutes
- One part silica particles and one part nuclease free water were mixed in a 2 ml safe lock tube (Eppendorf, Hamburg), pre-aliquoted in a 96-well (Greiner Bio-one Gmbh, Frickenhausen) and then 100µl were applied per catridge slot using a multi-channel pipette (Biohit, Rosbach)
- Automatic nucleic acid purification (with NucliSense® easyMag™ Extraction buffers 1,2 and 3)
- Purified nucleic acids were eluated in 55µl Elution buffer
- Transfer of eluate in 0.5 ml reaction tubes (Eppendorf, Hamburg)

Table 2-4: Overview of the protocol for the pooling of samples before nucleic acid purification including the volumes applied to the cartridge slots for purification with the NucliSense® easyMag™

	Flagging	Max. volume	Host	Max. volume
Larvae	Max. 10 homogenates à 95µl	950µl	Max. 10 homogenates à 95µl	950µl
Nymphs	Max. 10 homogenates à 95µl	950µl	Max. 10 homogenates à 95µl	950µl
Adults	Max. 5 homogenates à 95µl	475µl	Single	95µl

2.4.3 Quantitative Real-Time PCR (qrt-PCR)

Light cyclers (LC) 1.0 and 1.5 (Roche, Mannheim) and the GeneAmp PCR System 9700 (Applied Biosystems, Darmstadt) have been used to analyze the purified DNA samples obtained from ticks collected either by flagging or from host animals regarding the presence of different TBPs. They were screened for *Babesia* spp., *Bbsl*, CNM, *Rspp* and TBE-V. The target sequences and protocols used here are listed below:

Pathogen	Target	Protocol used & modified after
Bbsl	Flagellin gene (fla)	(Schwaiger *et al.*, 2001)
Rspp	Citrate synthase (glt A)	(Wölfel *et al.*, 2008)
TBE V	Non-coding 3´- region	(Schwaiger and Cassinotti, 2003)
***Babesia* spp.**	18S rRNA	(Radzijevskaja *et al.*, 2008)
CNM	Heat Shock Protein (groEL)	(Silaghi *et al.*, 2012a)

***B. burgdorferi* s.l. (LC)**

Primer	Sequence (5’ - 3’)
fla-for	AGC AAA TTT AGG TGC TTT CCA A
fla-rev	GCA ATC ATT GCC ATT GCA GA
fla-probe	FAM-TGC TAC AAC CTC ATC TGT CAT TGT AGC ATC TTT TAT TTG-TAMRA

Master mix

water		6.4µl
MgCl2	25 mM	1.6µl
Primer fla-for (for)	5 pmol	2.0µl
Primer fla-rev (rev)	5 pmol	2.0µl
Probe fla	4 pmol	2.0µl
LightCycler DNA master HybProbe		2.0µl
+ DNA template (eluate)		5.0µl

Temperature profile

Initialisation	60 sec	94 °C	
Denaturation	4 sec	94 °C	50 cycles
Annealing	45 sec	60 °C	
Cooling	30 sec	40 °C	

TBE-V (LC)

Primer	Sequence (5’ - 3’)
TBE1 for	GGG CGG TTC TTG TTC TCC
TBE1 rev	ACA CAT CAC CTC CTT GTC AGA CT
TBE - WT - probe	FAM-TGA GCC ACC ATC ACC CAG ACA CA-TAMRA

Master mix

water		3.2µl
Mn		1.3µl
Primer TBE1 for	5 pmol	1.0µl
Primer TBE1 rev	5 pmol	1.0µl

Probe TBE - wt	4 pmol	1.0µl
LightCycler RNA Master hybrid		7.5µl
+ DNA template (eluate)		5.0µl

Temperature profile

Reverse transcription	20 min	60 °C	
Initialisation	2 min	95 °C	
Denaturation	5 sec	95 °C	} 50 cycles
Annealing	15 sec	60 °C	
Cooling	30 sec	40 °C	

***Rickettsia* spp.**

Primer	Sequence (5’ – 3’)
PanRick2 - for	ATA GGA CAA CCG TTT ATT T
PanRick2 - rev	CAA ACA TCA TAT GCA GAA A
PanRick3 Taq - probe	FAM-CCT GAT AAT TCG TTA GAT TTT ACC G-TAMRA

Master mix

water		6.4µl
MgCl2	25 mM	1.6µl
Primer PanRick2 - for	5 pmol	2.0µl
Primer PanRick2 - rev	5 pmol	2.0µl
Probe PanRick3 Taq	4 pmol	1.0µl
LightCycler DNA Master HybProbe		2.0µl
+ DNA template (eluate)		5.0µl

Temperature profile

Initialisation	60 sec	94 °C	
Denaturation	4 sec	94 °C	} 50 cycles
Annealing	45 sec	55 °C	
Cooling	30 sec	40 °C	

Candidatus Neoehrlichia mikurensis (LC)

Primer	Sequence (5’ - 3’)
NMikGroEL-for2	CCT TGA AAA TAT AGC AAG ATC AGG TAG
NMikGroEl-rev1	CCA CCA CGT AAC TTA TTT AGC ACT AAA G
NMikGroEl-rev2	CCA CCA GTA ACT TAT TTA GTA CTA AAG
NMikGroEl-P2a	FAM-CCT CTA CTA ATT ATT GCT GAA GAT GTA GAA GGT GAA GC-BHQ

Master mix

water		6.4µl
MgCl2	25 mM	1.6µl
Primer NMikGroEL-for2	10 pmol	2.0µl
Primer NMikGroEl-rev1	10 pmol	1.0 µl
Primer NMikGroEl-rev2	10 pmol	1.0 µl
Probe NMikGroEl-P2a	4 pmol	1.0µl
LightCycler DNA master HybProbe		2.0µl
+DNA template (eluate)		5.0µl

Temperature profile

Initialisation	60 sec	94 °C	
Denaturation	4 sec	94 °C	} 50 cycles
Annealing	45 sec	60 °C	
Cooling	30 sec	40 °C	

Babesia spp. (LC)

Primer	Sequence (5’ - 3’)
BdiF	CAG CTT GAC GGT AGG GTA TTG G
BdiR	TCG AAC CCT AAT TCC CCG TTA
BdiT	FAM-CGAGGCAGCAACGG-TAMRA
BdiTMic	FAM-CGGGGCGACGACGG-TAMRA

Master mix

water		6.4µl
MgCl2	25 mM	1.6µl

Primer BdiF	5 pmol	2.0µl
Primer BdiR	5 pmol	2.0µl
Probe BdiT	4 pmol	0.5 µl
Probe BdiTMic	4 pmol	0.5 µl
LightCycler DNA Master HybProbe		2.0µl
+DNA template (eluate)		5.0µl

Temperature profile

Initialisation	5 min	95 °C	
Denaturation	15 sec	95 °C	} 40 cycles
Annealing	60 sec	60 °C	
Cooling	30 sec	40 °C	

In all runs, a negative control (aqua bidest and master mix) and a positive control (pre-amplified DNA of the respective pathogen and master mix) were included.

2.4.4 Identification of *Rickettsia* spp. genospecies

PCR

After determination of the presence of *Rspp* DNA by qrt-PCR (see previous section "Quantitative Real-Time PCR (qrt-PCR)"), a representative amount of positively tested samples from hosts and from flagging has been deployed to identify the genospecies of *Rspp*: About 10 larval samples per year and site were chosen randomly, consisting of a minimum of five larvae per individual host due to sensitivity restrictions. Additionally, all available samples of *I. ricinus* and *D. reticulatus* nymphs from small mammal hosts, as well as a subset of samples from flagging were used to identify the genospecies of *Rickettsia*. This was done by amplification of the 341bp target fragment of the citrate synthase (gltA) by conventional PCR with the primer pair RH314-rev and Rh654-for by Nilsson et al. (1999) using the PCR-Cycler C 1000TM Thermal Cycler (Bio-Rad, München).

Primer	Sequence (5’ – 3’)
Rh314 - for	AAACAGGTTGCTCATCATTC
Rh654 - rev	AGAGCATTTTTTATTATTGG

Master mix

Primer Rh314 - for	2 µM	4.0 µl
Primer Rh654 - rev	2 µM	4.0 µl
iProof reaction buffer	5x	3.25 µl
DMSO		0.75 µl
dNTPs	10mM	0.33 µl
iProof High Fidelity DNA Polymerase	2U/µl	0.18 µl
+ DNA template (eluate)		5.0 µl

Temperature profile

Initialisation	180 sec	95 °C	
Denaturation	10 sec	98 °C	
Annealing	30 sec	54 °C	50 cycles
Elongation	15 sec	72 °C	

The final elongation step was performed for 5’ at 72°C. The obtained amplicons were checked by a 2% Agarose gel including Midori Green (Nippon Genetics) in 1x Tris-borate-EDTA (TBE) buffer and visualized using the ChemiDoc XRS gel documentation system (BioRad, München).

DNA sequencing

The amplified fragments were sequenced by Eurofins Genomics (Germany).

CLC Genomics Workbench (Version 8.5.1 for Mac OS X) was used for visualization and editing of the obtained sequences. Sequences were controlled and edited visually and trimmed automatically using the in-built function in the CLC application (quality scores with a limit of 0.05 and trimming using ambiguous nucleotides of 2 residues). Sequences were then aligned and compared for sequence identity and similarity with entries in the GenBank database using NCBI BLAST (U. S. National Institutes of Health, Bethesda, Maryland) via the in-built function “*BLAST at NCBI”* in the CLC Genomics Workbench.

2.5 Statistical analysis

Descriptive statistical analysis was carried out using Microsoft Excel 2011 for Mac (Version 14.3.9). Inductive statistical analysis was carried out using JMP® 12 for Mac OS X (SAS Institute Inc., Cary, NC, 2015), IBM SPSS 22 (Windows) and R for Mac OS X Version 3.2.0 (R Development Core Team, 2015). All significances were calculated based on a 5% (0.05) level of significance.

Data on ticks, hosts and pathogens were always pre-checked for individual years and sites and only in the case of absence of significant deviations within, data was pooled for further analysis.

2.5.1 Testing differences between categorical variables

Pearson χ^2 testing was used to compare expected versus observed frequency distributions of categorical variables based on contingency tables. The test was applied to assess if the distribution between small mammals of different species, age or gender at the four sampling sites and between the three years of sampling was balanced and therefore stochastically independent (H_0) or if there was evidence to reject the null hypothesis and instead accept the alternative hypothesis (H_A) that there are some significant differences within the range of a certain level of probability (here: 95%). It was further used to examine differences in tick loads on hosts according to individual host characteristics in space and time as well as to investigate differences in pathogen prevalence distribution based on different host species, age, gender, tick load, co-infestation of larvae and nymphs on hosts, the overall abundance of larvae on hosts, the mean abundance of hosts per month and the number of questing nymphs. If sample sizes were low (rule of thumb: less than 100 observations) or one of the expected values in any of the contingency table cells was below 5 (below 10 if there was only 1 degree of freedom), Fisher's Exact Test was used instead. All tests were carried out in JMP 12 for Mac.

2.5.2 Testing for differences between continuous variables

To investigate differences in tick loads and related measures either per host individuals or in time and space, non-parametric tests were applied to account for the non-normal

distribution of the respective dependent variable (Sahu *et al.*, 2015). Here, the Wilcoxon-Mann-Whitney-Tests (two-level comparison) and the Kruskal-Wallis-Tests (more than two-level comparison) were used.

2.5.3 Parasite dispersion

Aggregation of ticks on hosts was analysed by calculating the ratio of variance to mean ($s^2/\bar{x}$) according to Anderson and Gordon (1982). A variance/mean ratio >1 indicates aggregation of ticks on hosts.

2.5.4 Dominance structure of small mammal communities

The dominance index reflects the relative abundance of a species within a species community. The dominance structure of a species community is the arrangement of all present species according to their dominance index in descending order.

The dominance index (D_i) of a species *i* is calculated according to Untersteiner (2007) as

$$D_i = (N_i * 100)/N$$

with

N_i = number of individuals of species $_i$

N = total number of individuals in the species community

The resulting dominance classes are defined after Engelmann (1978) as

d_i [%]	name	group name
32 – 100	eudominant	Primary species
10 – 31.99	dominant	
3.2 – 9.9	subdominant	
1 – 3.19	recedent	Secondary species
0.32 – 0.99	subrecedent	
< 0.32	sporadic	

2.5.5 Capture-Mark-recapture (CMR) models of small mammal abundance

In addition to the number of captured individuals per hectare and session, a capture-mark-recapture analysis has been carried out to estimate small mammal abundance in the four forest habitats during the study. CMR models offer the opportunity to estimate population demographic parameters from individual encounter histories with imperfect detection rate (Lebreton *et al.*, 1992). The analysis of CMR data are applied to estimate a variety of population parameters like population size, survival, recruitment, emigration and immigration and has undergone enormous advancements during the past decades (Seber and Schwarz, 1986; Pollock, 2000).

Following Nichols and Conroy (1996) and Pollock and Alpizar-Jara (2005), we analyzed the small mammal data using the Jolly-Seber (JS) model (Jolly, 1965; Seber, 1965), an open population capture-recapture model. Such models estimate population size (*N*), survival rates (Φ), capture probabilities (*p*) and birth rates (*b*). They are suitable tools to identify trends in populations (Lavadinovic, 2012) and are usually applied to long-term data, when gains and losses are likely to occur between sampling occasions (e.g. Seber and Schwarz, 1999). Here, we chose the POPAN formulation (Schwarz and Arnason, 1996) of the JS model since it allows both the estimation of population sizes ($\widehat{N}$) and to incorporate losses-on-capture.

In the present study, POPAN Jolly-Seber models were used to estimate the population sizes ($\widehat{N}$) of the subadult and adult individuals of the two dominant small mammal species *A. flavicollis* and *M. glareolus*. The species were analyzed separately to minimize potential heterogeneity of capture probabilities. Further, juveniles were removed from the dataset due to low sample size.

I conducted goodness of fit tests using the complete dataset (all species and sites pooled together) instead of subdivided datasets. It increased our ability to detect structural failure in the dataset. If no violation was observed in the whole dataset using the fully time dependent model, I could assume that there was no violation when using the same model for each subsets of the data.

Then, I specified the time intervals between sampling occasions to account for the longer time-window between sampling occasions during winter (five-month-interval) than between other sampling occasions (one-month-interval). Such tests were performed using RELEASE from within MARK.

Table 2-5: Overview of the results of the of the Goodness of Fit testing carried out using RELEASE in MARK. Results are listed by Group. All groups represent a combination of small mammal species and site (e.g. group 1 = *A. flavicollis* in AW)

Group	Chi-square	df	p-level
1	16.9880	39	0.9992
2	41.1215	55	0.9177
3	6.0436	27	1.0000
4	9.0812	18	0.9578
5	13.8409	43	1.0000
6	25.8638	34	0.8404
7	9.4957	21	0.9847
8	4.6373	8	0.7955

Given the results of the goodness-of-fit test for each group (combination of species*site), no lack of fit was detected in the dataset (Table 2-5).

For each separate dataset, I then examined the sensibility of the respective, biological hypotheses underlying all possible models and tested the following models: (i) fully time-dependent model {p(t),phi(t),pent(t)}, (ii) model assuming constant probability of survival of the individuals between sampling occasions over time {p(t),phi(.),pent(t)}, (iii) model assuming constant probability of encounter over time {p(.),phi(t),pent(t)} and (iv) model assuming constant probability of encounter and constant survival over time{p(.),phi(.),pent(t)}. For each dataset, I selected the model with the lowest AICc (Akaike Information Criterion adjusted for sample size, Burnham and Anderson 2002) (Table 2-5).

Once the best model was selected, I derived the estimated population size $\widehat{N}$ as following:

$$\widehat{N}_1 = \widehat{B}_0$$

Table 2-6: Overview of all created and compared models for the two small mammal species. The chosen models for every group are indicated in yellow. p= capture probability, phi= survival rate, pent= birth rates. AICc= Corrected AIC that accounts for sample size, Delta AICc= difference of AICc between the best model and the next-best model, the AICc weights (after Burnham and Anderson 1998) represents a quality criterion used in model averaging. It is based on the likelihood of the respective model divided by the sum of likelihoods across all models

site	species	Model	AICc	Delta AICc	AICc Weights	Model Likelihood
AW	*AF*	{p(t),phi(.),pent(t)}	968	0	0.99	1
AW	*AF*	{p(.),phi(t),pent(t)}	984	16	0.0004	0.0004
AW	*AF*	{p(.),phi(.),pent(t)}	989	22	0.00002	0
AW	*AF*	{p(t),phi(t),pent(t)}	993	25	0	0
AW	*MG*	{p(t),phi(.), pent(t)}	1,409	0	0.96	1
AW	*MG*	{p(.),phi(.), pent(t)}	1,416	7	0.03	0.04
AW	*MG*	{p(t),phi(t), pent(t)}	1,418	9	0.01	0.01
AW	*MG*	{p(.),phi(t), pent(t)}	1,423	14	0.001	0.001
HW	*AF*	{p(t),phi(t),pent(t)}	553.3	0	0.96	1
HW	*AF*	{p(.),phi(t),pent(t)}	561.3	7.97	0.02	0.02
HW	*AF*	{p(t),phi(.),pent(t)}	561.68	8.38	0.02	0.02
HW	*AF*	{p(.),phi(.),pent(t)}	562.19	8.89	0.01	0.01
HW	*MG*	{p(t),phi(t),pent(t)}	335.12	0	0.99	1
HW	*MG*	{p(.),phi(.),pent(t)}	344.26	9.15	0.01	0.01
HW	*MG*	{p(.),phi(t),pent(t)}	346.19	11.07	0.004	0.004
HW	*MG*	{p(t),phi(.),pent(t)}	348.17	13.06	0.001	0.002
MB	*AF*	{p(.),phi(t),pent(t)}	839.72	0	0.97	1
MB	*AF*	{p(t),phi(t),pent(t)}	846.82	7.1	0.03	0.03
MB	*AF*	{p(t),phi(.),pent(t)}	855.58	15.86	0.0004	0.0004
MB	*AF*	{p(.),phi(.),pent(t)}	883.33	43.61	0	0
MB	*MG*	{p(.),phi(.),pent(t)}	578.86	0	0.64	1
MB	*MG*	{p(t),phi(.),pent(t)}	580.03	1.17	0.36	0.56
MB	*MG*	{p(.),phi(t),pent(t)}	591.23	12.36	0.001	0.002
MB	*MG*	{p(t),phi(t),pent(t)}	604.65	25.79	0	0
SW	*AF*	{p(t),phi(t),pent(t)}	453.73	0	0.99	1
SW	*AF*	{p(t),phi(.),pent(t)}	474.03	20.31	0.00004	0
SW	*AF*	{p(.),phi(t),pent(t)}	483.47	29.74	0	0
SW	*AF*	{p(.),phi(.),pent(t)}	509.5	55.77	0	0
SW	*MG*	{p(t), phi(t), pent(t)}	392.63	0	0.98	1
SW	*MG*	{p(.), phi(t), pent(t)}	400.74	8.11	0.02	0.02
SW	*MG*	{p(.), phi(.), pent(t)}	421.41	28.79	0	0
SW	*MG*	{p(t), phi(.), pent(t)}	429.42	36.79	0	0

$$\widehat{N_{t+1}} = \hat{N}_t \varphi_t + \hat{B}_t$$

Here, it has to be considered that using the POPAN formulation, the first and the last estimate of abundance $\hat{N}$ can be biased (Schwarz and Arnason, 1996).

2.5.6 Statistical modeling

Stochastic models have been developed to analyze the relative influence of small mammal populations (*A. flavicollis* and *M. glareolus*) and their intrinsic factors together with the relative influence of environmental influences (habitat and microclimate) on the abundance/activity of (a) the number of larvae on small mammal hosts and (b) the number of questing nymphs at the four investigated forest sites. In a second approach, the relative influence of small mammal host characteristics and habitat on *Bbsl* and *Rspp* prevalence in *I. ricinus* larvae and nymphs on hosts and nymphs on vegetation were analyzed.

Ticks on hosts and on vegetation

In collaboration with Roberto Rosà (San Michele all'Adige, Italy) and Agustìn Estrada-Peña (Zaragoza, Spain), generalized linear models (GLM) and further generalized additive models (GAM) have been developed to describe the relationships between ticks and their abiotic and biotic environment. GLM extend the linear modelling framework to response variables with non-normal error distribution and are commonly used to model binary or count data (McCullagh and Nelder, 1989).

GAM in turn represent an advancement of GLM; they are GLM where the linear predictor depends on smooth functions of covariates (Wood, 2006). Using a GAM instead of a GLM, it is possible to account e.g. for the non-linear nature of the relationship between the response variable and a covariate by the introduction of a smoother function for that covariate. This can be advantageous regarding the influence of microclimatic factors on the dynamics of *I. ricinus* on hosts and on vegetation, as the effect here is potentially non-linear but rather follows a (unknown) function.

The analysis of the data started with a preselection process:

Here, the data collected by the microclimate loggers at each site were checked for their influence on the phenology of ticks: Daily means of temperature (°C) and relative humidity in the soil, the leaf litter layer and at 50 cm height have been obtained by Denise Boehnke (KIT, IfGG). Additionally, the saturation deficit (SD) has been calculated after Perret *et al.* (2000).

These microclimatic factors are regarded as important influencers of tick phenology and quantitatively measurable (Kahl, 1989; Estrada-Peña *et al.*, 2004; Ogden *et al.*, 2005; Pfäffle *et al.*, 2013). They act as short-term indicators for tick-activity and long-term influential factors of mortality and survival of exophilic ticks like *I. ricinus* (Perret *et al.*, 2000; Gray *et al.*, 2009; Estrada-Peña *et al.*, 2013; Pfäffle *et al.*, 2013).

From the daily means of these variables, the means for time intervals from 3 days up to 15 days prior to sampling for every month of the investigation period have been calculated. This time frame has been chosen due to its biological importance, regarding the influence of microclimatic conditions on tick activity and taking into account the larval and nymphal feeding time (several days) on hosts.

In order to find out which time interval at which detection height had the strongest influence on the number of questing nymphs and larvae on hosts, a preliminary analysis was carried out.

This was realized by building simple GAM using the number of ticks (*I. ricinus* larvae on host and *I. ricinus* nymphs on vegetation) as response variable and the respective microclimatic variable (°C, RH, SD) for the three heights of measurement (soil, leaf litter and 50cm) and time intervals (3,6,9,12 and 15 days before sampling) as predictors in separate models.

Significant values of each height were compared using the Akaike information criterion (AIC) (Aho *et al.*, 2014) and the intervals with the lowest relative AIC were chosen to be used in the basic full model. To also address long-term influences of microclimate on the number of *I. ricinus* larvae on hosts and nymphs on vegetation, the daily mean temperature, saturation deficit and absolute humidity (AH) have been accumulated from the first sampling session of each year up to the sampling session within this year. The selection procedure was the same as for the short-term microclimatic variables, but only one accumulated variable was chosen to be used in the basic full model due to collinearity resulting from the nature of accumulation of the variables.

Subsequent to the pre-selection process of the microclimatic variables of greatest importance towards tick load, a set of variables associated with availability and structure of host populations were further incorporated as predictors. Here, the species, age, gender, weight and relative abundance of small mammal hosts at the four investigation areas were included. The abundance of small mammals was included as relative abundance estimates of *A. flavicollis* and *M. glareolus* previously calculated using a Jolly-Seber model in MARK (Capture-Mark-recapture (CMR) models of small mammal abundance). Furthermore, the amount of culled deer and wild boar in the previous hunting seasons was incorporated as predictors to account for the availability of hosts for adult ticks. As the number of deer and wild boar is of potential influence on the host finding and feeding success of tick females, it is likely to have an effect on the clutch size and therefore also on the number of larvae in the following season, when the small mammals have been trapped and ticks have been collected from them. In addition to that, habitat related factors were also added to account for the potential influence of the different forest sites on tick dynamics. Therefore, the percentage of coniferous trees, which do not contribute to the extent and maintenance of leaf litter, an important factor for tick survival providing a relatively stable microclimatic environment and preventing ticks from desiccation, was included.

For the model on questing nymphs on vegetation another factor was introduced to the model setup: The total larval density in the year previous to the actual sampling season (t-1). Here, the average rodent density per session was multiplied by the average larval load per rodent at each session to provide a better measure of the actual total amount of larvae present in a given area.

All these predictor variables have then been used to set up a second, comprehensive collinearity analysis, including all variables of interest. This was done by collating only those variables with a correlation coefficient of $r>0.6$ and a variance inflating factor (VIF) <4 (using the *corvif* function in R, provided in the HighstatLibV6.R library by Alain Zuur) in the same model. The correlation coefficient R thereby displays the strength of the relationship between two variables, whereas the VIF indicates the extent to which the variance is increased due to collinearity among variables. With the set of remaining variables, a GLM was set up as first approach to a full model with tick numbers (number of *I. ricinus* larvae on hosts and number of questing *I. ricinus* nymphs, respectively) as

response variables. This was followed by a stepwise reduction of non-significant variables ($p>0.05$) until all remaining variables were significant at the 5% level. In parallel, an automated model averaging process was carried out and the results of both approaches were then compared afterwards and were congruent without exception. The minimum models then passed through a multi-step validation procedure by screening them for the presence of outliers and influential observations, as well as for homogeneity, normality and independence of the model's residuals. This first approach revealed a strong temporal pattern and potentially other confounding influences in the residuals and therefore, generalized linear mixed models (GLMM) were deployed as second approach to the data. These models are used if there is a random variable that describes your data sample as a subset of the data you could have collected. Applying this to the data of interest, the site of sampling and sampling session were used for the model on the questing nymphs as response variable, whereas the individual host ID was used as random factor for larvae on small mammal hosts. The introduction of random terms did not significantly reduce the pattern in the model residuals, so another approach was necessary. GAM were then chosen as third approach. In a GAM, a variable that is potentially causing a pattern in the residuals e.g. by not expressing a linear relationship towards the response variable can be introduced in the model using a smoother function to account for this pattern. Here, the pattern in the model residuals were assumed to be of temporal origin. So, a smoother function was introduced for the day of the year in which the respective sample was taken, also accounting for the different years of sampling. This approach lead to a considerably reduced pattern in the residuals and was therefore used as final approach to model the data.

B. burgdorferi s.l. and *Rickettsia* spp. prevalence in ticks on hosts and on vegetation

In a second modeling approach, the prevalence of *Bbsl* and *Rspp* in larval and nymphal *I. ricinus* as well as the prevalence of the respective pathogens in questing nymphs was modeled as binomial response variable (infected versus uninfected). The predictors applied to assess their influence on larval and nymphal prevalence was the same used in the respective approach to model ticks on hosts and on vegetation but without microclimatic factors, as they were assumed to directly influence tick and hosts activity and abundance, but to rather be of indirect or cryptic influence on pathogen prevalence

(Gray, 2008). The selection process of predictor variables to avoid collinearity in the respective models was done analogous to the first approach to model ticks on hosts and vegetation. The comparison and choice of the best model was as well carried out in the same way, using the AIC.

Here, the resulting model type of choice was a GLM, because neither the introduction of random variables (GLMM) under the assumption of underlying substructures in the dataset, nor the use of smoother terms (GAM) to account for temporal patterns in the data reached the desired effect of model improvement. Therefore, the simpler approach using a GLM of the binomial family with logit link function was chosen and applied.

3 RESULTS

In this chapter I will take you on a guided tour from small mammal hosts to pathogen prevalence in BW forests. I will show you how the dynamic abundance of small mammals (part 1) can modify tick dispersal (part 2) and how this can pave the way for changes in the occurrence of TBPs (part 3). There will also be a short sidestep, to introduce the use of differential methods of tick sampling. At every step of the way, I will reveal the tremendous variability and complexity of the tick-host-pathogen system in time and space. I will present a comprehensive approach that models tick loads based on the network of influences by hosts, habitat and microclimate before I'll finally turn to the culmination of all preceding events and show the effect of all these interactions on the abundance of TBPs in BW forests.

3.1 Small mammals

3.1.1 Small mammal species community

A total of 2,909 small mammal individuals were captured at the four forest sites Auwald (AW), Hardtwald (HW), Michaelsberg (MB) and Schwarzwald (SW) during the study (see Table 3-1). The trapped individuals belonged to the orders Rodentia (rodents) and Eulipotyphla (shrews). The most prevalently captured species at all four sites and in every year of the study from 2012 to 2014 were the yellow-necked mouse *Apodemus flavicollis* (*Af*, 27.6% – 70.9%) and the bank vole *Myodes glareolus* (*Mg*, 11.4% – 72.1%).

According to the dominance index by Engelmann (1978), these two rodent species represent the primary species at all sites, ranging between the two categories of highest abundance, namely dominant (10% - 31.99% of all individuals in the community) and eudominant (32% - 100% of all individuals) (see Table 3-1). Besides these two highly abundant species, individuals of the common shrew *Sorex araneus* and the pygmy shrew *Sorex minutus* were captured to a lesser extent (*S. araneus*: 0.3 - 10.5%, *S. minutus*: 0% - 6%) at all four sites. In addition, four individuals at HW were identified as wood mice (*Apodemus sylvaticus,* 0.6% - 1.2%) and two individuals of the field vole *M. agrestis* (0.6%) were trapped in AW in 2012 (see Table 3-1). The latter four species are considered as secondary or minor species according to the index of dominance (Engelmann 1978),

except for the common shrew, which was relatively more abundant at SW in 2013 and 2014 (with a maximum of 20 individuals in total per year).

Table 3-1: Total number of small mammal individuals captured in 2012 (May-October), and 2013 and 2014 (March-October). Numbers in brackets indicate the percentage of the respective species in relation to the annual number of all small mammal individuals. Sampling at SW only started in June 2012 and in April 2013 due to unfavorable weather conditions. Small mammal pictures (by courtesy of www.kleinsaeuger.at) indiciate species according to the order of names in the table






	A. flavicollis		*A. sylvaticus*		*M. agrestis*		*M. glareolus*		*S. araneus*		*S. minutus*		Σ
2012	626	(53.3%)	3	(0.3%)	2	(0.2%)	499	(42.5%)	24	(2.0%)	20	(1.7%)	1174
AW	150	(47.3%)	0	(0.0%)	2	(0.6%)	155	(48.9%)	8	(2.5%)	2	(0.6%)	317
HW	164	(63.8%)	3	(1.2%)	0	(0.0%)	77	(30.0%)	4	(1.6%)	9	(3.5%)	257
MB	185	(63.1%)	0	(0.0%)	0	(0.0%)	95	(32.4%)	6	(2.0%)	7	(2.4%)	293
SW	127	(41.1%)	0	(0.0%)	0	(0.0%)	172	(56.0%)	6	(2.0%)	2	(0.7%)	307
2013	249	(42.3%)	0	(0.0%)	0	(0.0%)	285	(48.5%)	42	(7.1%)	12	(2.0%)	588
AW	82	(27.6%)	0	(0.0%)	0	(0.0%)	214	(72.1%)	1	(0.3%)	0	(0.0%)	297
HW	61	(70.9%)	0	(0.0%)	0	(0.0%)	18	(20.9%)	7	(8.1%)	0	(0.0%)	86
MB	69	(51.9%)	0	(0.0%)	0	(0.0%)	42	(31.6%)	14	(10.5%)	8	(6.0%)	133
SW	37	(51.4%)	0	(0.0%)	0	(0.0%)	11	(15.3%)	20	(27.8%)	4	(5.6%)	72
2014	623	(54.3%)	1	(0.1%)	0	(0.0%)	460	(40.1%)	42	(3.7%)	21	(1.8%)	1147
AW	284	(51.3%)	0	(0.0%)	0	(0.0%)	266	(48.0%)	3	(0.5%)	1	(0.2%)	554
HW	107	(59.8%)	1	(0.6%)	0	(0.0%)	64	(35.8%)	3	(1.7%)	4	(2.2%)	179
MB	152	(50.7%)	0	(0.0%)	0	(0.0%)	117	(39.0%)	21	(7.0%)	10	(3.3%)	300
SW	80	(70.2%)	0	(0.0%)	0	(0.0%)	13	(11.4%)	15	(13.2%)	6	(5.3%)	114
Σ	1498	(51.5%)	4	(0.1%)	2	(0.1%)	1244	(42.8%)	108	(3.7%)	53	(1.8%)	2909

3.1.2 Community dynamics

In 2012, a total of 1,174 individuals belonging to six different small mammal species were captured at the four sites (see Table 3-1). The overall abundance of small mammals (AW: n= 317, HW: n= 257, MB: n= 293, SW: n= 307), the species community composition, as well as the relative abundance of the respective species, was comparable between the four sites in 2012, with *Af* and *Mg* consistently being the most prevalent species (Table 3-1). At AW, the number of *Af* and *Mg* was almost equal, with 150 individuals of the yellow-necked mouse and 155 individuals of the bank vole being captured during the sampling season. Shrews were captured to a lesser extent (n= 8 *S. araneus* and n= 2 *S. minutus*) and both individuals of *M. agrestis* found during the entire study were captured

at AW during this season. At HW, over twice as many individuals of *Af* (n= 164) than *Mg* (n= 77) were trapped. Compared to the two dominant species, again, a considerably lower number of shrews were found here (n= 4 *S. araneus* and n= 9 *S. minutus*). Three out of the four individual captures of *Ap. sylvaticus* occurred in HW in 2012. At MB, the ratio of captured individuals of *Af* (63.1%, n= 185) to *Mg* (32.4%, n= 95) was comparable to the findings of HW. This held true for the relative abundance of shrews, with a total of six individuals of *S. araneus* and seven individuals of *S. minutus* being trapped at MB in 2012. In contrast to the three other sites, the number of bank voles (n=172) exceeded the number of yellow-necked mice (n= 127) at SW. The number and relative abundance of shrews (n= 6 *S. araneus* and n= 2 *S. minutus*) in turn, was comparable to the three other sites.

In 2013, the overall number of small mammals trapped decreased by 49.1% to only 588 individuals captured during the entire season at all four sites (Table 3-1). These belonged to only four of the six species that had been detected in the previous year 2012. The four sampling sites contributed differently to this pattern: while the total number of individuals at AW remained at a very similar level compared to the previous year (n_{2012}= 317, n_{2013}= 297), the numbers of small mammals trapped decreased dramatically at HW, MB and SW by 54.6% (MB), 76.5% (SW) and 66.5% (HW). *Af* and *Mg* remained the most prevalent species, accounting for 42.3% and 48.5% of total captures at all sites, but with a considerable amount of variation between the sites (Table 3-1Table 3-1). At AW, the number of voles captured in 2013 even increased significantly from 155 to 214 individuals (Table 3-2, χ^2= 9.1, p= 0.003), whereas the number of *Af* decreased significantly from 150 individuals in 2012 to 82 individuals in 2013 (χ^2= 19.9, p < 0.0001). Only one *S. araneus* was trapped throughout the season at AW. At HW, the decrease in small mammal abundance affected both dominant species significantly (Table 3-2), with the relative proportion of *Mg* to *Af* shifting slightly towards the yellow-necked mouse (Table 3-1Table 3-1). The relative abundance of *Af* increased from 63.8% of all captures in 2012 to 70.9% of all captured individuals at HW in 2013, though. The relative proportion of bank voles, in contrast, decreased from 30.0% in 2012 to 20.9% in 2013. The number of shrews captured at HW remained at a similar level with n= 7 *S. araneus* captured in 2013, but due to the reduced number of both *Af* and *Mg* in this year, these seven individuals accounted for 8.1% of total captures at HW in 2013 (Table 3-1).

Table 3-2: Comparison of the changes in frequency of abundance of the dominant host species *A. flavicollis* and *M. glareolus* between 2012 and 2014. Differences between years were analyzed in pairs (overall difference between years at individual sites: p< 0.0001): 2012 / 2013, 2013 / 2014 and 2012 / 2014 at AW, HW, MB and SW. χ^2-values and corresponding p-values are shown to indicate significant changes (p< 0.05) between the respective years. Analysis was only carried out for *A. flavicollis* and *M. glareolus* because sample size was large enough to obtain reliable results

	2012/2013		2013/2014		2012/2014	
Af	χ^2	p	χ^2	p	χ^2	p
AW	19.9	< 0.0001	111.5	< 0.0001	41.4	< 0.0001
HW	46.4	< 0.0001	12.6	0.0004	11.6	0.0007
MB	52.3	< 0.0001	30.6	< 0.0001	3.3	0.07
SW	49.4	< 0.0001	15.7	< 0.0001	10.7	0.001
Mg						
AW	9.1	0.003	5.9	0.02	29.3	< 0.0001
HW	25.3	< 0.0001	25.8	< 0.0001	1.2	0.3
MB	19.3	< 0.0001	35.4	< 0.0001	2.7	0.09
SW	141.6	< 0.0001	0.2	0.7	136.7	< 0.0001

At MB, the total number of captures of both dominant species, the yellow-necked mouse and the bank vole, was significantly affected by the population decline in 2013 (*Af* 12/13: χ^2= 52.3, p < 0.0001, *Mg* 12/13: χ^2= 19.3, p < 0.0001). The total numbers of *Af* went from 185 in 2012 down to only 69 individuals (Table 3-1Table 3-1) in 2013. *Mg* captures decreased to a slightly lesser extent than *Af*, from 95 in 2012 to 42 in 2013. Here, the number of both shrew species increased in 2013: 14 individuals of *S. araneus* (10.5%) have been captured together with a total of eight individuals of the pygmy shrew *S. minutus (*6%), leading to a considerable increase in relative abundance of shrews in the small mammal species community at MB in 2013. As stated above, the strongest decrease in population numbers among the sampling sites was observed at SW. The total number of *Af* dropped by 70.9% to 37 individuals (χ^2= 49.4, p< 0.0001), the number of bank voles decreased even more dramatically by 93.6% from 172 individuals in 2012 to 11 individuals in 2013 (χ^2= 141.6, p< 0.0001). Here, the the yellow-necked mouse individuals accounted for 51.4% of all captures, whereas bank voles accounted for only 15.3%. The number of shrews at SW in 2013 was elevated in comparison to 2012, in numbers as well as in percentage of total captures. In 2013, the 20 individuals of *S. araneus* accounted for 27.8% of all captured individuals at SW and the four individuals of *S. minutus* accounted for a further 5.6%.

In the following year 2014, 1,147 small mammals of five different species were captured – almost exactly the same total number as in 2012 - and a substantial increase compared to the 588 captures in 2013. All four sites contributed to this pattern, as the overall number of trapped small mammals increased at all sites AW, HW, MB and SW compared to 2013 (Table 3-1), but to a different extent. At AW, the total number of trapped individuals exceeded both previous seasons (Table 3-1). The number of both dominant species rose to a similar final extent, with both species accounting for about 50% of all captures at AW (Table 3-1). While population numbers *Af* increased substantially from 2013 to 2014 (χ^2= 111.5, p< 0.0001) the number of *Mg* increasing as well (χ^2= 5.9, p= 0.02), but less dramatically. The number of Soricidae at AW in 2014 (n= 3 *S. araneus* and n= 1 *S. minutus*) increased slightly, but not in comparison to 2013 (n= 1 *S. araneus*), but remained marginally lower than in 2014 (n= 8 *S. araneus* and n= 2 *S. minutus*). These slight fluctuations did not change the overall pattern of low abundance of shrews in the traps throughout the three years of sampling at AW. At HW, there were substantially more individuals captured in 2014 (n= 179) than in 2013 (n= 86), but total numbers recovered by only 69.6% compared the extent of total captures in 2012 (n= 257). The ratio of *Af* to *Mg* found at HW in 2014 was comparable to the findings in 2012, and even similar to the findings in 2013. In 2014, *Af* accounted for 59.8% (n=107) and *Mg* for 35.8% (n= 64) of all individuals. In 2013, the relative proportion of yellow-necked mice was somewhat higher (70.9%), accompanied by a reduced relative proportion of *Mg* (20.9%), whereas the ratio between the two major species in HW in 2012 was very similar to 2014 with 63.8% being *Af* and 30% *Mg*. Moreover, the comparison of total numbers showed that the population of *Af* did not fully recover after the breakdown in 2013 (12/14: χ^2= 11.6, p= 0.0007) whereas the population of *Mg* at HW did (χ^2= 1.2, p= 0.3). As in 2012 and 2013, the total number and relative abundance of shrews at HW remained at a low level in 2014, with the two species accounting for 1.7% (*S. araneus*) and 2.2% (*S. minutus*) of all captures. After the substantial decrease in small mammal numbers in 2013, the number of captured individuals at MB fully recovered in 2014 to almost exactly the same number of individuals as in 2012 (Table 3-2, *Af* 12/14: χ^2= 3.3, p< 0.07, *Mg* 12/14: χ^2= 2.7, p= 0.09). The relative proportion of *Af* (50.7%) exceeded that of *Mg* (39.0%) in 2014, just as during the preceding sampling seasons in 2012 and 2013, but again with some fluctuations in the ratio of captures per species. *Mg* was most prevalent at MB in 2014, with the highest relative

abundance (2014: 39%, 2013: 31.6%, 2012: 32.4%), as well as the highest total number of captures (2014: n= 117, 2013: n= 42, 2012: n= 95). In contrast, the relative abundance of *Af* tended to be highest in 2012 (63.1%), compared to 2013 (51.8%) and 2014 (50.7%). The total number of shrews captured at MB and the total number of individuals per *Sorex* species increased in 2014 (*S. araneus* n= 21, *S. minutus* n= 10) compared to both preceding seasons, but still the relative proportion of shrews at MB in 2014 remained somewhat lower than in 2013 (10.5% *S. araneus* and 6% *S. minutus*). At SW, the total number of captures in 2014 was slightly elevated compared to 2013, but remained at a low overall level compared to 2012 (Table 3-1). Of the 114 small mammals captured, only 13 were bank voles but 80 individuals were yellow-necked mice. This comparably low number of *Mg* was similar to 2013 (13/14: χ^2= 0.2, p= 0.7) and indicated that the *Mg* population had not recovered from the decrease in 2013 at all (12/14 χ^2= 136.7, p< 0.0001). In contrast, the number of yellow-necked mice increased again significantly from 2013 to 2014 (13/14: χ^2= 15.7, p< 0.0001) but still remained at significantly lower levels compared to 2012 (12/14 χ^2= 10.7, p= 0.001). Just as in 2013, the small mammal community at SW in 2014 consisted of a noticeable percentage of shrews, with 15 individuals of *S. araneus* (13.2%) and six individuals of *S. minutus* (5.3%). This does not hold true for 2012 when there were substantially more trapped individuals in total at SW. Here, the total and the relative amount of *Sorex* spp. was considerably lower (n= 8, 2.7%) than in 2013 and 2014.

3.1.3 Small mammal population dynamics

Not only the overall abundance but also the dynamics of the small mammal populations showed strong fluctuations and different patterns between years and sites during the investigation period from 2012 to 2014.

It is important to state that the beginning of the sampling season in 2012 was delayed in comparison to 2013 and 2014. In 2012, sampling took place from May until October (SW: June - October) but from March until October in the following two years (SW 2013: April - October). For this reason, there are no trapping data available for March and April of 2012 to compare with those of the two following years of sampling.

In 2012, the small mammal populations at the four sites AW, HW, MB and SW showed a similar pattern (Figure 3-1Figure 3-1). The four sites were similar not only in terms of total

abundance of small mammals (Table 3-1), but also in the pattern of dynamic changes taking place during the season of sampling in 2012. There were differences between the sites in the total abundance of small mammals at the beginning of the sampling season in 2012 (Figure 3-1, e.g. in May 2012). This ranged between about 88 individuals/ha (ind/ha) in HW and 144 ind/ha in AW and was followed by a decrease in trapped ind/ha in June 2012 at AW, HW and MB (not observable in SW as sampling started only in June). At SW, the sampling season started in June 2012 with about 164 ind/ha, the highest abundance observed among all sites, during the entire season of 2012 and is followed by a "summer decline" of captured individuals in July 2012, one month later than at the three other sampling sites. In July 2012, the numbers at AW, HW and MB increased again. Such an increase could not be observed at SW in the following month (August 2012), but rather the population density remained stable at about the same level as at the AW and MB (~ 125 ind/ha). At HW, though, there was another decline in August, followed by another increase in small mammal density in the following month. In September, all four sites showed a similar density of small mammals per hectare ranging between 104 ind/ha at HW to 128 ind/ha at SW. At the last session of the first sampling season in 2012, the numbers of small mammals uniformly decreased at all four sites.

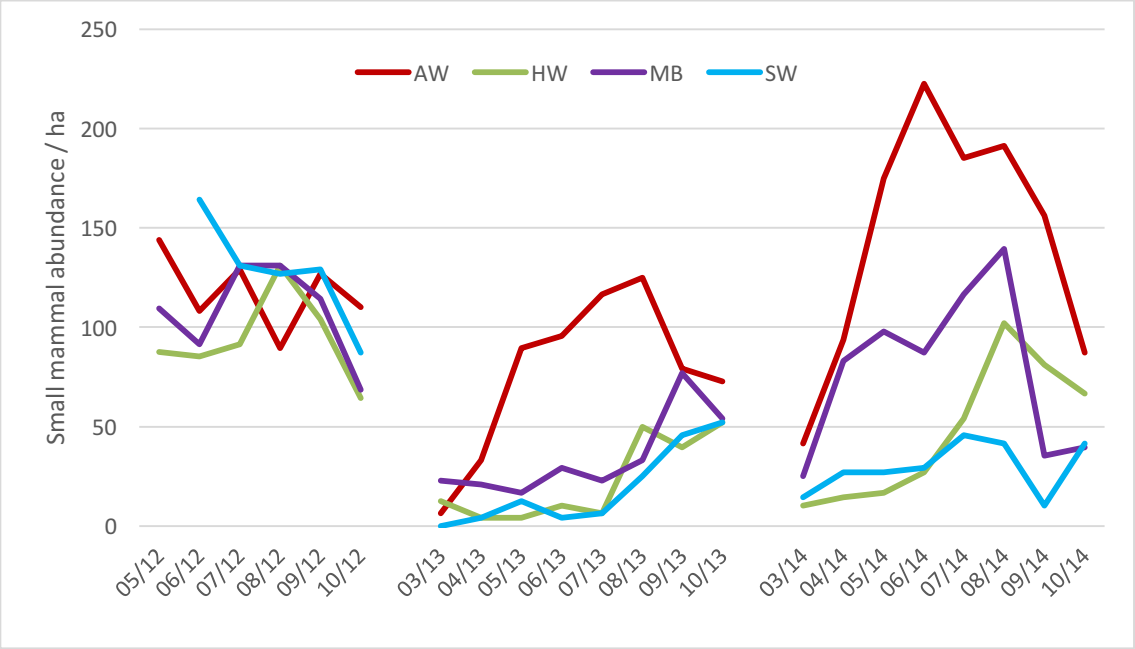


Figure 3-1: The abundance of individuals of all captured small mammal species per hectare during the sampling seasons from May 2012 until October 2014 (June 2012 and April 2013 in SW). The number of captured individuals per month on the trapping grids were converted to ind/ha

In 2013, a strong population decrease occurred at three out of the four investigated sites, namely at HW, MB and SW. Only the small mammal population at AW did not experience such a substantial breakdown in numbers (Figure 3-1). At HW, MB and SW, the small mammal abundance stayed at a very low level until August 2013, ranging between 6.2 ind/ha at HW and SW and 22.9 ind/ha at MB, and increased only towards the end of the sampling season. The overall abundance of ind/ha at HW, MB and SW did not reach the level of the previous sampling season (Figure 3-1). In contrast to these three sites, the abundance of small mammals was indeed low at the beginning of the sampling season in March 2013 (12.5 ind/ha), but rocketed to a first plateau level of 89.4 individuals per hectare in March 2013. After this steep increase in captured individuals, a further increase of small mammal density took place and reached 124.8 ind/ha in August 2013. After this peak, small mammal numbers decreased again towards the end of the sampling season (as in 2012), but remained at a slightly higher level compared to the three other sites even in October 2013.

In 2014, the dynamics of small mammal abundance at the four sites differed again from the preceding two years of sampling. Nevertheless, the pattern of small mammal population dynamics showed some temporal similarities between the sites: the number of individuals per hectare was low at the beginning of the third season of sampling at AW, HW, MB and SW (see Figure 3-1). At AW and MB, the low number of captures in March 2014 was followed by a steep increase in small mammal numbers during spring, leading to a first peak of 97.8 ind/ha in MB (in May 2014) and 222.6 ind/ha in AW (in June 2014). At HW and SW, in contrast, the number of small mammals/ha remained at a remarkably low level after the onset of the sampling season in 2014. At SW and HW, capture numbers increased rather slowly during the first half of the year, from 6.2 ind/ha at SW and 8.3 ind/ha at HW in March 2014 to less than 30 individuals per hectare in June (HW: 25.0 ind/ha, SW: 29.1 ind/ha). At AW, the first peak of the season in June 2014 was followed by a moderate decline to 185.1 ind/ha, followed by a second, but comparably smaller peak in August (191.4 ind/ha) and a steep decline in abundance towards the end of the season in September (156.0 ind/ha) and October 2014 (85.3 ind/ha). A similar pattern was observable at MB for the second half of the sampling season in 2014: after the first peak in a small mammal abundance, captures decreased slightly during summer to 85.3 ind/ha in June 2014. Subsequently, small mammal abundance reached a second peak at MB as

well, reaching 124.8 ind/ha in August of 2014. At MB, this second peak turned out larger than the first in May 2014, but both peaks, as well as the overall abundance, remained at a substantially lower level than at AW. A considerable drop in small mammal numbers from August to September followed the second peak at MB with only 25.0 ind/ha detectable in September. The number of captures remained low until the end of sampling in October 2014 (27.0 ind/ha). At HW, small mammal abundance reached its first and only peak with 99.8 ind/ha in August 2014. This increase followed a decline of captured individuals towards the last two months of sampling with 77.0 individuals per hectare in September and 62.4 individuals per hectare in October 2014. The pattern of small mammal abundance at HW in 2014 resembled that of the previous season in 2013, with a slight, but continuous increase in abundance throughout the season. The small mammal abundance at SW kept on increasing slowly but steadily from March until August 2014 and finally reached 39.5 ind/ha. The number of captured individuals then dropped to only 6.2 individuals per hectare in September, followed by a re-increase up to 37.4 ind/ha in October 2014, at the end of the season. The overall pattern of the small mammal dynamics at the four sites showed that in 2013 the number of small mammal captures was very low at the beginning and remained at a low overall level at HW, MB and SW, three out of the four sites of the study. This clearly contrasts with the findings of the first year at all four sites. In the third year, numbers at AW recovered and even increased compared to both previous years of sampling, numbers at MB recovered as well after the breakdown in 2013. At HW there was some increase in small mammal abundance after the crash in 2013, but numbers remained lower than in 2014. SW, when small mammal abundance was highest in 2012 capture numbers did not recover in 2014 after the population breakdown in 2013.

The total number of captures of both *Sorex* species and especially the isolated captures of *M. agrestis* and *Ap. sylvaticus* occurred considerably more rarely compared to the number of captured individuals of the two dominant species *Af* and *Mg* (Table 3-1). With a maximum of 21 *S. araneus* at MB throughout 2014, capture numbers were too low to analyze their dynamics and their influence on ticks or TBPs in the present study in detail. Therefore, the further sections will predominantly focus on *Af* and *Mg,* the two major species, their dynamics, population structure and their influence on ticks and TBPs.

3.1.4 Estimated abundance of the dominant small mammal species

The abundance of small mammal populations of *Af* and *Mg* was estimated by Capture-Mark-Recapture analysis using a POPAN Jolly-Seber model in MARK. The method was chosen because it has the advantage (compared to e.g. the count of individuals/ha) that the information of the capture-recapture pattern of every small mammal individual could be used to estimate the overall population abundance at a given site and at a given time despite incomplete recapture of individuals.

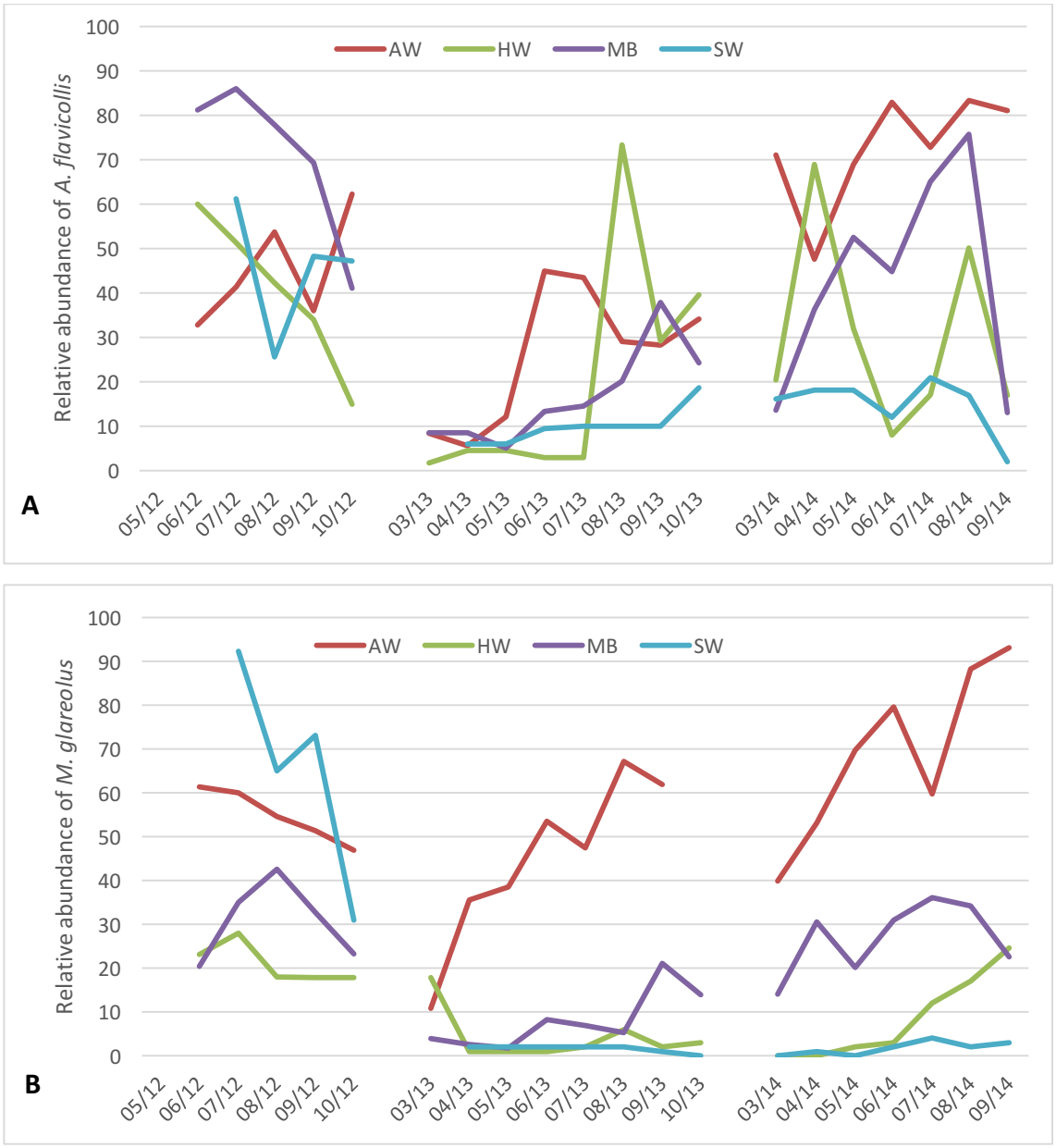


Figure 3-2: Relative abundance of the two dominant small mammal species *A. flavicollis* and *M. glareolus* at the four investigation sites from 2012 to 2014. For the estimation of the relative abundance, the Jolly Seber method (POPAN formulation) was used within the software application of MARK (White & Burnham 1999). The abundance estimates for the first and last session are not shown because they are likely to be confounded

In 2012, the overall abundance of *Af* at AW, HW and SW was at a similar level, with different patterns between the sites. The overall abundance of *Af* at MB exceeded the abundance at the three other sites (Figure 3-3Figure 3-2). The pattern of relative abundance of *Mg* differed for all the four sites in 2012 by level of overall abundance as well as the dynamics in the course of the first sampling season. Thus, for both species, there was no common peak in abundance between the sites. If you compared the dynamics of both species at the same site, there were some similarities detectable though: At AW, the overall estimated abundance of *Af* and *Mg* was similar, whereas the pattern of abundance differed for both species. The abundance of *Mg* started at its highest in June 2012 with 61.4 individuals and slowly decreased without any peak to 46.9 in October 2012. *Af*, however, started with a comparably low abundance in June 2012 (32.8) and showed two peaks of abundance, first in August 2012 (53.7) and again in October 2012 (62.2). At HW, the pattern of abundance of both species was similar (and similar to the pattern of *Mg* abundance at AW in 2012), but the overall abundance of *Af* was higher at HW. The abundance of *Af* started with 60.1 individuals in June 2012 and then decreased continuously, reaching only 15.0 individuals at the end of the season in October 2012. *Mg* started at a comparably low abundance with 23.1 individuals, followed by a slight increase in June (27.9 individuals) and remained at an overall lower level than *Af* until the end of sampling in October (17.9 individuals). At MB there were again substantially more *Af* than *Mg* present throughout the season of 2012 (Figure 3-2Figure 3-2). Here, just as at HW, the abundance patterns of both species were very similar to each other. After an increase in abundance at the beginning of the sampling season peaking in July (*Af*: 86.0 individuals) and August (*Mg*: 42.62 individuals), respectively, estimated small mammal abundances kept decreasing until the end of sampling at HW in 2012 (Figure 3-2Figure 3-2). Finally, at SW there was again a similar pattern observable in the dynamics of the estimated small mammal abundance during the sampling season in 2012. After the initial estimate of abundance in July 2012, a steep decrease in small mammal abundance followed, and then numbers recovered again in September 2012 (Figure 3-3). The abundance of *Mg* decreased again considerably towards October of 2012 (31.0 individuals) whereas the estimated population size of *Af* remained rather stable (Figure 3-3). At SW, as previously observed at HW and MB, the overall abundance of both species differed substantially, but this time the estimated abundance of the bank vole

exceeded that of the yellow-necked mouse with an estimated population size of 92.3 compared to 61.2 in July 2012.

In 2013, the overall estimated abundance of *Af* showed a somewhat similar level at all four sites. These rough similarities could also be observed for the overall abundance of *Mg* at HW, MB and SW, but not for AW. Here, the number of bank voles exceeded the three other sites by far. The overall pattern of *Af* and *Mg* abundances was similar at HW, MB and SW, but not at AW, with small mammal abundance increasing not before the second half of the sampling season in 2013 at these three sites.

At AW, the dynamics of both species showed a similar pattern, with the estimated abundance of *Af* (45.0 individuals) and *Mg* (53.6 individuals) peaking in June 2013. Estimated abundance of the bank vole increased even further and reached a second peak with 67.2 individuals in August 2013, followed by a decline. The estimated abundance of *Af* did not show a second peak in abundance for the species at AW in 2013, but only a slight increase in October 2013 from an estimated number of 28.3 individuals in September to 34.2 in October. At HW, abundance estimated at the beginning of the sampling season remained rather low for *Af* but peaked in August of 2013, reaching 73.3 individuals, followed again by a decrease of about 50% in September and October 2013 (Figure 3-3). The numbers of *Mg* were rather high at the beginning of the season (17.8 individuals), but then dropped and remained very low until the end of the 2013 sampling season. Abundance estimates for the bank vole population at HW in 2013 did not exceed six individuals after March. At MB, both, abundance patterns and the level of abundance of both species were very similar in 2013 (Figure 3-3). Both species did not show a substantial increase in abundance during the first half of the year. Only after July/August did population abundance estimates start to increase and reached their peak in September of 2013 with 37.9 individuals of *Af* and 21.0 individuals of *Mg*. At SW, the dynamics and overall abundance of both species were similar. Here, both species remained at a low level throughout the year 2013. Estimates of *Af* abundance did not exceed 10 individuals until October 2013, when there was a slight increase up to 18.7 individuals. The population of *Mg* at SW remained at an even lower level in 2013 and did not exceed more than two individuals.

In 2014, the patterns of abundance at the four sites differed from each other in terms of overall levels of abundance between sites and in terms of the pattern of small mammal

dynamics for both *Af* and *Mg*. The abundance pattern of *Af* showed some similarities between the sites as they all had roughly two peaks of abundance, but they differed greatly in the overall extent of abundance and with regard to the time of occurrence of the respective peaks. The dynamics of *Mg* showed some similarities between MB and AW at both sites; there were two peaks in 2013, but again, these differed greatly in terms of overall abundance between the sites. Abundance at AW exceeded the abundance of the bank voles at all other sites in 2013.

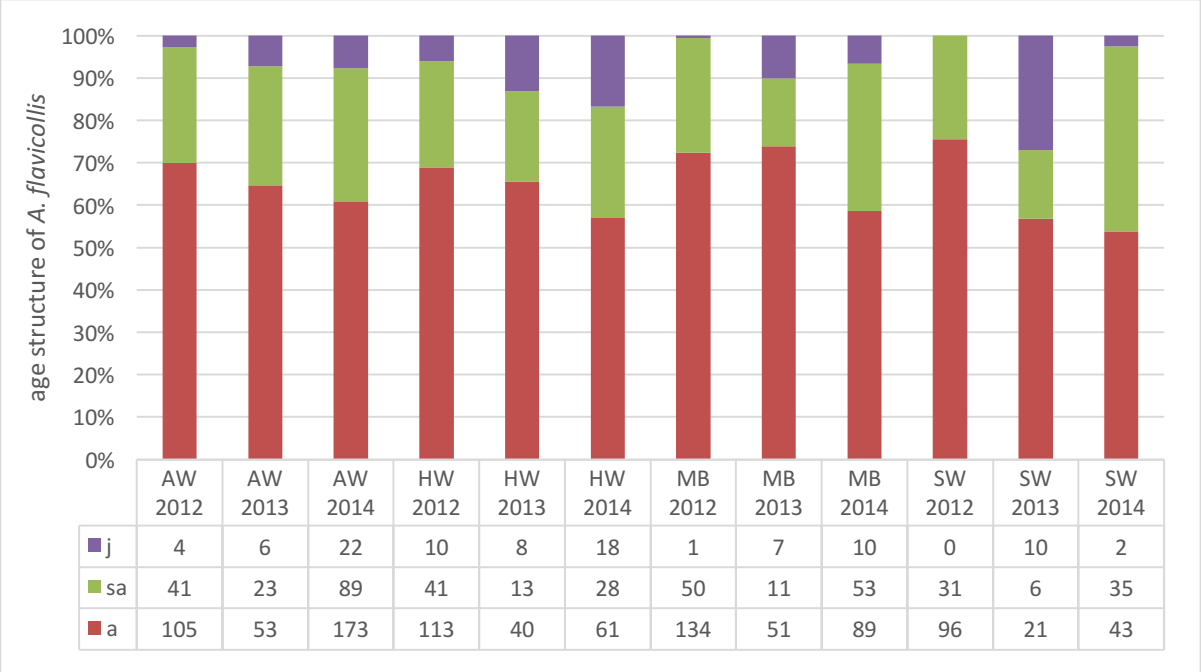
At AW, small mammal abundance estimates of the bank vole and the yellow-necked mouse were remarkably similar with respect to overall abundance and the dynamics of abundance. For both species, there was a first peak observable in June of 2014, with about 80 estimated individuals each. Then, the estimated abundance decreased slightly, before reaching a second peak in September (*Af*: 81.0 individuals)/ October and *Mg*: 93.1 individuals). At HW, overall numbers and dynamics of the abundance of the two species differed again greatly. Estimates of the overall abundance of *Af* exceeded that of *Mg* at HW in 2014 and showed two peaks during the season in this year. The first peak occurred in April 2014 with 69.0 estimated individuals of the yellow-necked mouse at HW followed by 50.2 individuals in August 2014. The abundance of *Mg* did not show a peak in abundance for the first half of 2014, but then increased up to 25.6 individuals in September 2014. At MB, the pattern of abundance of both species was very similar throughout the sampling season of 2014, but not the level of abundance. The overall estimates of abundance of *Af* clearly exceeded those of *Mg* (Figure 3-2). Both species showed similar abundance dynamics, though. After a first increase in abundance, the abundance estimates of both species reached a first peak with 30.5 individuals of the bank vole in April of 2014 and 52.5 individuals of the yellow-necked mouse in May of 2014. The estimates of both species then showed a decrease in abundance and a second peak in abundance in late summer (*Af*: 75.8 individuals in August, *Mg*: 36.1 individuals in July 2014). At SW, the abundance level and pattern of both species were similar to each other, with a slightly larger estimated population size for *Af* compared to *Mg* in 2014. The abundance of the bank vole remained at a constantly low level throughout 2014. It never exceeded an estimate of more than four individuals. There were some fluctuations observable for *Af* at SW in 2014 (Figure 3-3), but no distinct peak in abundance estimates.

The overall patterns of abundance of both species at SW in 2014 therefore also resembled those observed in 2013.

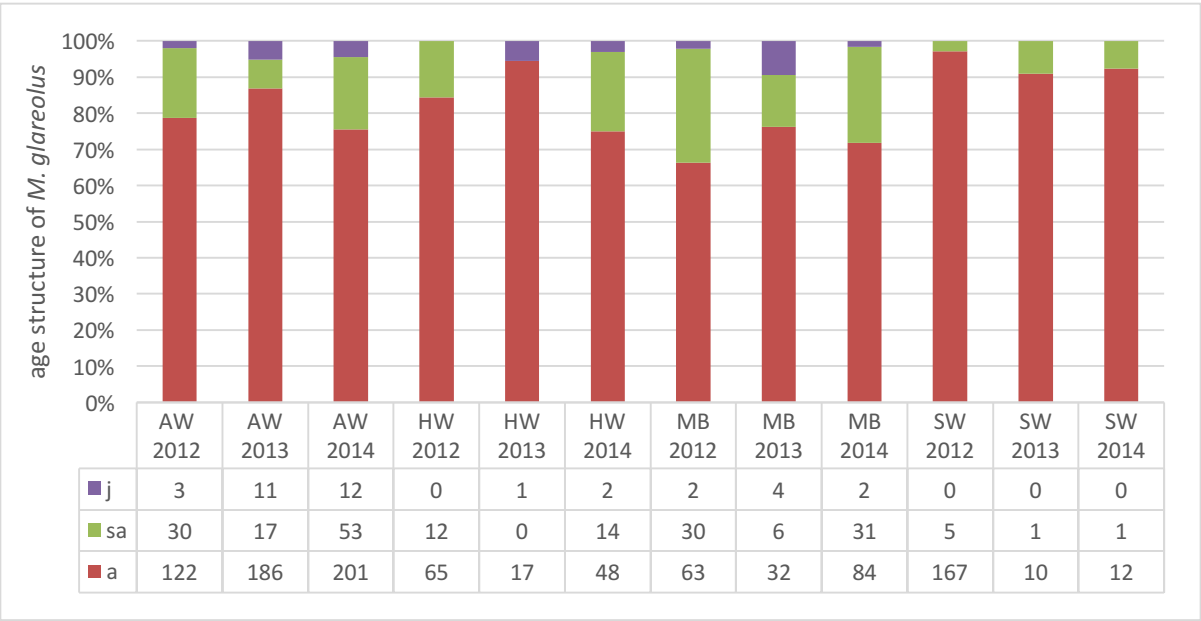
3.1.5 Age structure of *Af* and *Mg* populations

Throughout the study, adult individuals comprised the largest part of the respective populations of both species at every site (AW, HW, MB and SW) and in every year from 2012 to 2014. A total of 64.8% (n=979) of all yellow-necked mice and 82.5% (n=1,007) of all bank voles were adults, followed by an overall proportion of 26.8% subadult *Af* (n=421) and 14.7% subadult *Mg* (n=200). Juveniles made up the smallest proportion of both species, with 8.4% (n=98) of all *Af* and 2.8% (n=37) *Mg* individuals being juveniles. Subadult and juvenile *Af* occurred more frequently than for *Mg* (). A χ^2-test supported this assumption of differences in age class distribution for the two species as it revealed the strong influence of species on the relative abundance of different age classes (χ^2= 83.797, p<0.0001). The overall relative number of subadult *Af* at the sampling sites ranged between 24.7% (n= 82) at HW and 29.7% (n= 153) at AW, and between 4.4% (MB: n=18) and 10.8% (HW: n=36) for juvenile individuals. The percentage of subadult *Mg* ranged between 3.6% (SW: n=7) and 26.4% (MB: n=67); juveniles did not occur at SW (n=0) and accounted for only up to 4.1% (n=26) at AW. *Af* showed similar patterns with respect to the proportion of adult individuals for the individual years at the different sites.

In 2012, the percentage of adult individuals at all four sites was highest (AW: 70.0%, HW: 68.9%, MB: 72.4%, SW: 75.6%), followed by the proportion of adult individuals in 2013 (AW: 64.6%, HW: 65.6%, MB: 73.9%, SW: 56.8%), and in turn was followed by the relative number of adults in 2014 (AW: 60.9, HW: 57.0, MB: 58.6, SW: 53.8). The percentage of adults at MB in 2013 is slightly higher than in 2012, but here the substantially lower number of individuals during the entire season of 2013 must be taken into account. The relative proportion of subadults and juveniles of *Af* was not distributed as evenly as the adult individuals of the yellow-necked mouse. At AW and HW, the proportions of subadult individuals were relatively evenly distributed throughout the three years, with 27.33% to 31.34% subadult mice at AW and 21.31% to 26.17% subadults at HW. At MB and SW though, the relative



	AW 2012	AW 2013	AW 2014	HW 2012	HW 2013	HW 2014	MB 2012	MB 2013	MB 2014	SW 2012	SW 2013	SW 2014
j	4	6	22	10	8	18	1	7	10	0	10	2
sa	41	23	89	41	13	28	50	11	53	31	6	35
a	105	53	173	113	40	61	134	51	89	96	21	43



	AW 2012	AW 2013	AW 2014	HW 2012	HW 2013	HW 2014	MB 2012	MB 2013	MB 2014	SW 2012	SW 2013	SW 2014
j	3	11	12	0	1	2	2	4	2	0	0	0
sa	30	17	53	12	0	14	30	6	31	5	1	1
a	122	186	201	65	17	48	63	32	84	167	10	12

Figure 3-3: Age structure of the major small mammal species *A. flavicollis* and *M. glareolus* at the four investigation sites Auwald (AW), Hardtwald (HW), Michaelsberg (MB) and Schwarzwald (SW) from 2012 to 2014

amount of subadult *Af* strongly decreased in 2013 (see Figure 3-3), when the overall small mammal population breakdown occurred at HW, MB and SW (see Figure 3-1). At MB, the mean abundance of subadult yellow-necked mice in 2012 and 2014 was 30.95% compared to 15.94% in 2013. At SW, the mean abundance of 2012 and 2014 was 34.08% and decreased to 16.22% in 2013. The pattern of the relative abundance of juvenile yellow-necked mice can again be compared between (i) AW and HW and (ii) MB and SW. At AW

and HW, juvenile abundance was low in 2012 and comparably higher in 2013 and 2014 with 2.7% at AW in 2012 (n=4) and 7.3% (n=6) to 7.8%(n=22) in 2013 and 2014. At HW, there were 6.1% juvenile *Af* present in 2012 (n=10), and between 13.1% (2013, n=8) and 16.8% (2014, n= 18) in the following years of sampling. At MB there were only 0.54% (n=1) juvenile *Af* captured throughout 2012 and no juvenile individuals of this species at all at SW in 2012. In the year of the population decline, the relative proportion of juvenile individuals of *Af* increased at both sites to 10.1% (n=7) at MB and 27.0% at SW (n=10), followed by a steep decrease in the following year 2014 with 6.6% (n= 10) *Af* juveniles at MB and 2.5% (n=2) at SW. As indicated above, the overall proportion of adult individuals of *Mg* was even higher than the percentage of adult *Af* (Figure 3-3). An overall percentage, between 70.5% (MB) and 96.4% (SW) of all captured bank voles from 2012 to 2014 were adult. Here, it should to be taken into account that capture numbers at SW were extremely low in 2013 and 2014 (Figure 3-3) and cannot be regarded as representative in these two years. The proportion of adult individuals reached its peak levels in 2013, when from 76.2% (MB) up to 94.4% (HW) of the individuals were adult at the time of capture (SW excluded). A further pattern that could be observed was that the relative number of subadult individuals of *Mg* in 2013 was lowest among the three years at all of the sites (exluding SW), ranging from 0% at HW (n= 0) to 14.3% at MB (n=6). In comparison, the proportion of subadult bank voles in 2012 ranged between 2.9% (SW: n=5) and 31.6 (MB: n=30) and between 19.9% (AW: n=53) and 26.5% (MB: n=31) in 2014 (excluding SW). Interestingly, the proportion of juvenile bank voles was slightly higher at all sites in 2013. In 2012, the relative number of juvenile *Mg* ranged between 0% (HW: n=0) and 2.1% (MB: n=2). During the sampling season of 2014, there were between 1.7% (MB: n=2) and 4.5% (AW: n=12) bank voles captured as juveniles. In contrast, the number of juvenile *Mg* increased to a total of 5.1% (AW: n=11) up to 9.5% (MB: n=4). Therefore, the overall proportions of the age structures at the four investigated sites were similar between the sites and between the years but with some differences between the small mammal species.

3.1.6 Gender distribution of small mammal populations

In general, the overall proportion of females and males for both *Af* and *Mg* seemed rather evenly distributed (Figure 3-4). The relative proportion of *Af* females per site ranged between 41.0% (HW) and 49.5% (MB) and between 50.5% (MB) to 59.0% (HW) male individuals of the yellow-necked mouse (Table 3-3). In comparison, the proportion of

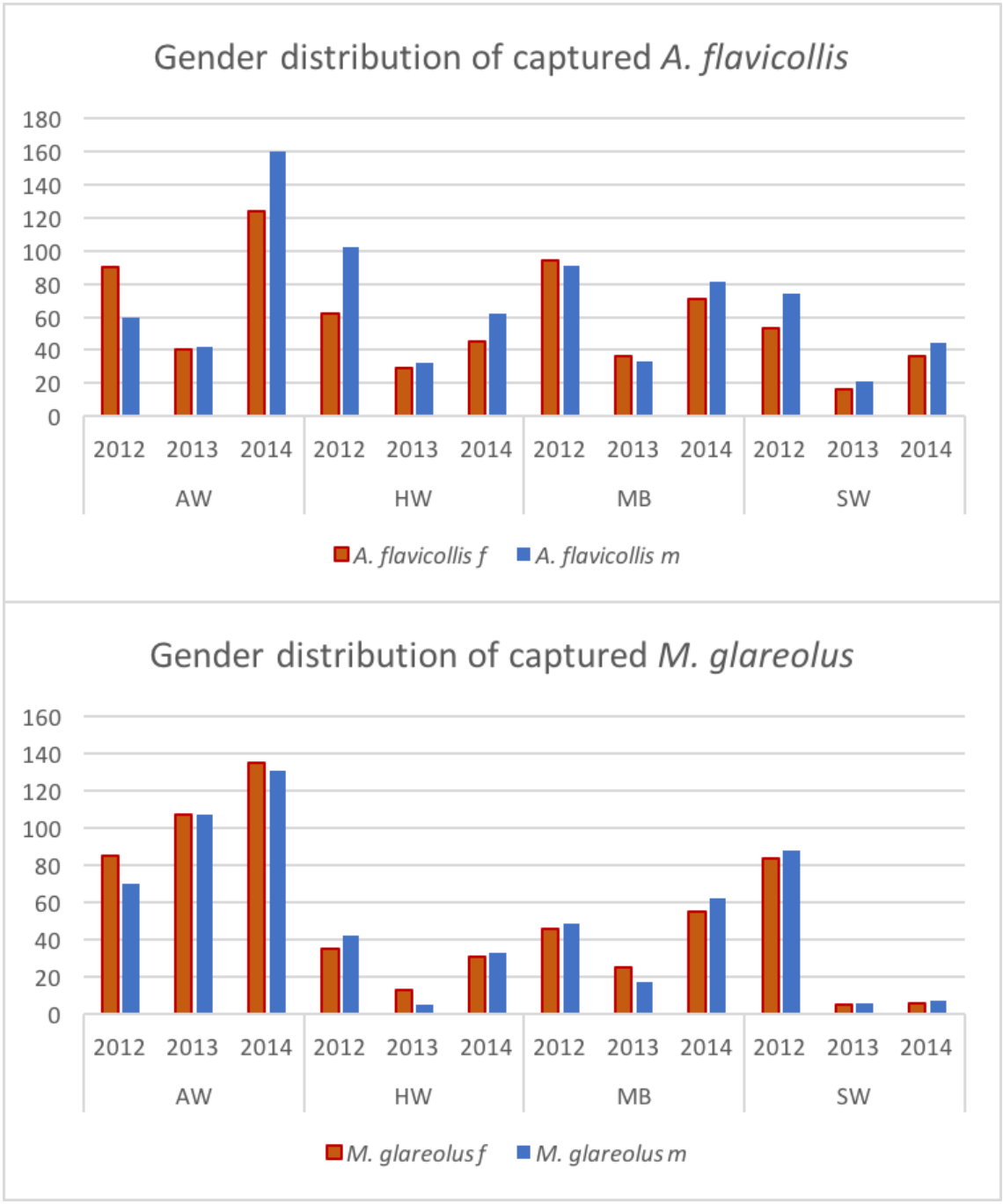


Figure 3-4: Comparison of the frequency of female and male individuals of the major small mammal species *A. flavicollis* and *M. glareolus* at the four sites AW, HW, MB and SW from 2012 to 2014. Individuals of all age classes were included

female *Mg* ranged between 48.5% (SW) and 51.5% (AW) and an overall percentage

between 48.5% (AW) to 51.5% (SW) male bank voles in total (overall mean from 2012 to 2014, Table 3-3). But there were as well some fluctuations observable between the ratio of males to females at the four sites through the three years of sampling: It seemed that the relation between both sexes was more balanced for *Mg* than it was for *Af*.

Af males tended to be more prevalently captured than females: At SW, elevated numbers of males compared to female individuals were observed throughout the three years of sampling (see Table 3-3). Here, the relative proportion of males lay between 55.0% (n=44) and 58.3% (n= 71), whereas the proportion of female *Af* was always below 50%, ranging between 41.7% (n= 53) and 45.0% (n= 36). The strongest differences in female to male ratios of *Af* could be observed at HW, where in 2012 the relative proportion of female *Af* was as low as 37.8% (n= 62) with 62.2% (n= 102) males. This pattern held true for 2013 and 2014. At AW, *Af* males were more commonly captured in 2013 (51.2%, n= 42) and 2014 (56.3%, n= 160), but not in 2012 (40.0% males, n=60). At MB, the proportion of both genders was evenly distributed, with slightly more female individuals in 2012 (50.8%, n= 91) and 2013 (52.2, n= 36). *Af* males, in turn, were slightly more abundant only in 2014 (53.3%, n= 81). The numerical imbalance between the genders in captured individuals of *Af* at the four sites and during the three years of sampling was further confirmed by a χ^2-test. It revealed that there were no significant differences in male over female abundance between the three years of sampling (χ^2= 3.56, p= 0.31), but that at the different sites there were significant differences between the relative number of males and females (χ^2= 8.29, p= 0.04).

The presence of a larger numbers of captured males than females at specific times also held true for *Mg* at SW, but here, capture numbers were comparably closer to 50% and very low in total in 2013 and 2014 (Figure 3-4). The strongest deviation from equivalence was observable at HW in 2013 (Table 3-3), where the proportion of *Mg* females compared males was elevated with a total of 72.2% (n= 13) versus 27.8% (n= 5). However, here the overall capture numbers were substantially lower than in the other two years of sampling (Figure 3-4). In 2012 and 2014, males were captured slightly more often than females, but the ratio was very close to 1:1 (2012: 50.3% males, 2014: 51.6% males). At AW, there were equally to slightly more female *Mg* captured during the three years of sampling, ranging between 50.0% (n= 107) and 54.8 (n= 85). At MB, *Mg* males were captured more often in 2012 and 2014, but only to a minor degree. In 2012 males accounted for 51.6% (n= 49)

and for 53.0% (n= 61) in 2014. The opposite pattern was observed in 2013, when female bank voles accounted for 59.5% of captures, but with a total of only 25 captured female individuals and 17 males at MB in 2013.

These patterns were further confirmed by a χ^2-test. The proportion of male to female individuals of the bank vole was not significantly different either between the sites (χ^2= 1.04, p= 0.60), or between the individual years of sampling (χ^2= 0.69, p= 0.88).

Table 3-3: Gender of *A. flavicollis* and *M. glareolus* individuals captured from May 2012 until October 2014 at AW, HW, MB and SW. f= female, m= male. All age classes are included

	A. flavicollis				*M. glareolus*			
	f (n)	**f (%)**	**m (n)**	**m (%)**	**f (n)**	**f (%)**	**m (n)**	**m (%)**
AW	**254**	49.22	**262**	50.78	**327**	51.50	**308**	48.50
2012	90	60.00	60	40.00	85	54.84	70	45.16
2013	40	48.78	42	51.22	107	50.00	107	50.00
2014	124	43.66	160	56.34	135	50.75	131	49.25
HW	**136**	40.96	**196**	59.04	**79**	49.69	**80**	50.31
2012	62	37.80	102	62.20	35	45.45	42	50.31
2013	29	47.54	32	52.46	13	72.22	5	27.78
2014	45	42.06	62	57.94	31	48.44	33	51.56
MB	**201**	49.51	**205**	50.49	**126**	49.61	**128**	50.39
2012	94	50.81	91	49.19	46	48.42	49	51.58
2013	36	52.17	33	47.83	25	59.52	17	40.48
2014	71	46.71	81	53.29	55	47.01	62	52.99
SW	**105**	43.03	**139**	56.97	**95**	48.47	**101**	51.53
2012	53	41.73	74	58.27	84	48.84	88	51.16
2013	16	43.24	21	56.76	5	45.45	6	54.55
2014	36	45.00	44	55.00	6	46.15	7	53.85

But there were as well some fluctuations observable between the ratio of males to females at the four sites through the three years of sampling:

Af males tended to be more prevalently captured than females: At SW, elevated numbers of males compared to female individuals have been observed throughout the three years of sampling (Table 3-3). Here, the relative amount of males lied between 55.0% (n= 44) and 58.3% (n= 71) whereas the proportion of female *Af* in turn was always below 50.0%, ranging between 41.7% (n= 53) and 45.0% (n=36). The strongest differences in female to male ratios of *Af* could be observed at HW, where in 2012 the relative abundance of female *Af* was as low as 37.8% (n= 62) and the respective amount of male individuals of this species was 62.2% (n= 102), but this pattern held also true for 2013 and 2014. At AW,

Af males were predominantly captured in comparison to females in 2013 (51.2%, n= 42) and 2014 (56.3%, n= 160), but nor in 2012 (40.0% males, n= 60). At MB, the proportion of both genders was evenly distributed, with slightly more female individuals in 2012 (50.8%, n= 91) and 2013 (52.2%, n= 36). *Af* males, in turn, were slightly more abundant only in 2014 (53.3%, n= 81).

The presence of a larger amount of captured males than females held also true for *Mg* at SW, but here, capture numbers were comparably closer to 50% and very low in total in 2013 and 2014. The strongest deviation from equivalence was observable at HW in 2013, where the proportion of *Mg* females compared to males was elevated with a total of 72.2% (n= 13) versus 27.8% (n= 5). However, the overall capture numbers were substantially lower than in the other two years of sampling (Figure 3-4). In 2012 and 2014, males were captured slightly more often than females, but the ratio was very close to 1:1 (2012: 50.3% males, 2014: 51.6% males). At AW, there were equally to slightly more female *Mg* captured during the three years of sampling, ranging between 50.0% (n= 107) and 54.8 (n=85). At MB, *Mg* males were captured more often in 2012 and 2014, but only to a minor degree. In 2012 males accounted for 51.6% (n= 49) and for 53.0% (n=61) in 2014. The opposite pattern was observed in 2013, when female bank voles accounted for 59.5% of captures, but with a total of only 25 captured female individuals and 17 males at MB in 2013. Overall, it seemed that the relation between both genders was more balanced for *Mg* than it was for *Af*.

These patterns were further confirmed by a χ^2-test. The proportion of male to female individuals of the bank vole seemed to be rather similar among years and among sites. Neither between the four sites (χ^2= 1.04, p= 0.60), nor between the individual years of sampling (χ^2= 0.69, p= 0.88) was there any significant deviation of the bank vole populations from the balanced pattern of occurrence of male and female individuals. Analogous to that, the visually noticed patterns of disequilibrium between the sexes in captured individuals of *Af* between the sites and during the three years of sampling were also tested for significance by χ^2-test testing. It revealed that there were significant overall deviations from stochastic independence occurring for male and female individuals of *Af* during the three years of sampling (2012: χ^2= 18.06, p= 0.0004) and that there were significant differences between the relative amount of males and females observable at the different sites (χ^2 =10.49, p=0.0053) as well.

3.2 Ticks

3.2.1 Ticks on small mammal hosts

To observe and analyse the basic host-parasite-system, the overall abundance of ticks on small mammals and later the individual tick burden of host individuals will be examined without accounting for other conditions such as microclimate. Small mammals that were dead on capture (n= 10) were not examined. They have been included as captured individuals in the previous sections on small mammal dynamics (Small mammals), but were removed for further calculations regarding tick dynamics on hosts as well as the dynamics of TBPs.

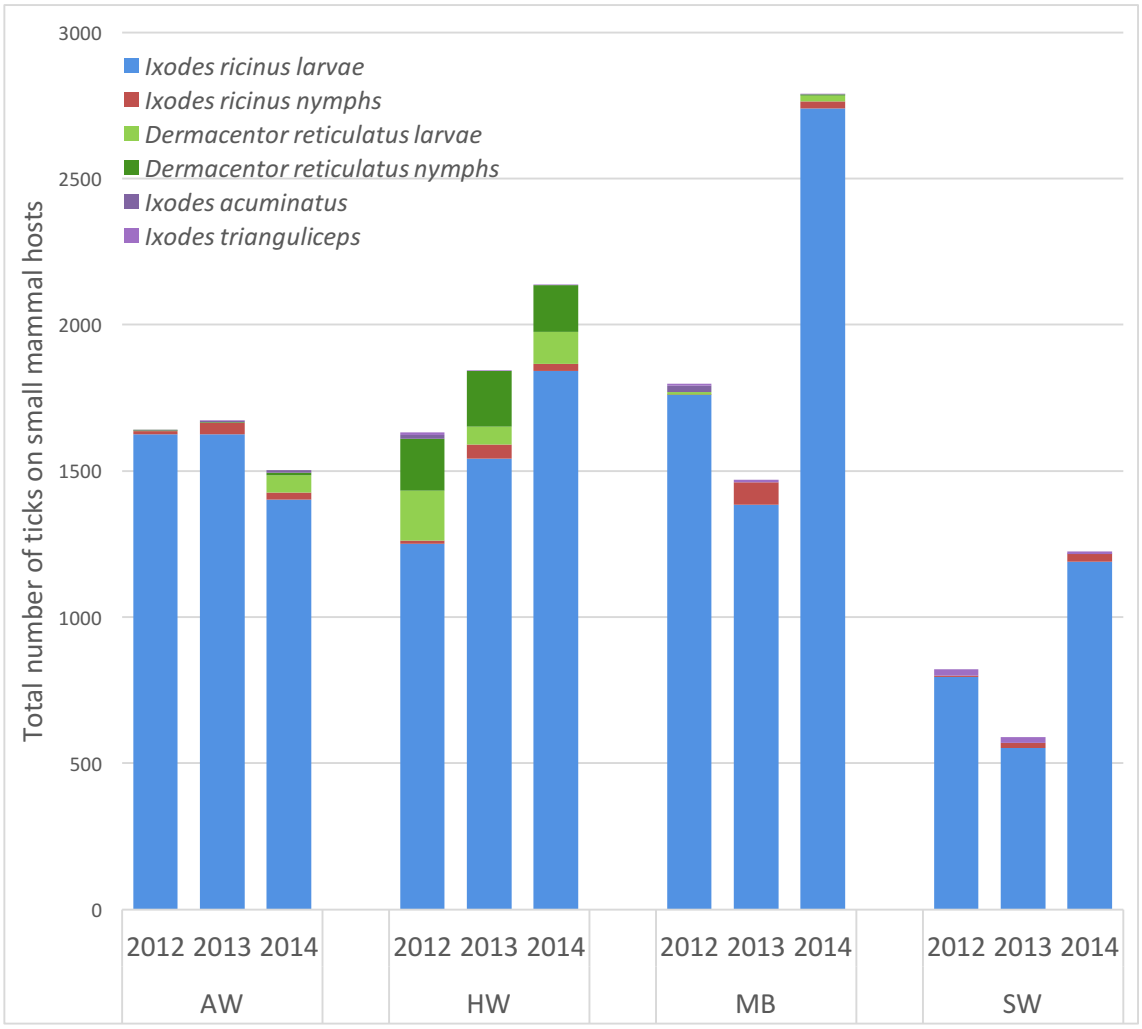


Figure 3-5: Total number of ticks collected from all small mammal hosts between 2012 to 2014 at the four sites AW, HW, MB and SW. *I. ricinus* and *D. reticulatus* were separated by instar (larvae and nymphs)

During the sampling periods from 2012 to 2014, 19,125 ticks were collected from small mammals at the four sites AW, HW, MB and SW (5,892 ticks in 2012, 5,577 ticks in 2013, 7,656 ticks in 2014) (Table 3-4). These belonged to four different species: *D. reticulatus*, *I.*

acuminatus, *I. ricinus* and *I. trianguliceps* (Figure 3-5). Among them the castor bean tick *I. ricinus* was most prevalent, being found at all sites and in all years. It comprised 92.6% - 95.0% of all ticks (n_{total}= 17,712). The second most abundant species was *D. reticulatus*, the ornate dog tick (4.6 % – 6.1 %). It was found at all sites except SW and occurred predominantly at HW (Figure 3-5). In fact, 869 out of the 974 *D. reticulatus* on small mammal hosts (89.2 %) were found at HW (Table 3-4). Additionally, rare tick species collected from hosts at all four sites during the study were the southern rodent tick, *I. acuminatus* (n_{total}= 51, 0.1% – 0.6%), representing the first record of this species in Germany (Petney *et al.*, 2015) and the vole tick, *I. trianguliceps* (n_{total}= 82, 0.2 – 0.6%). These two species are, as their common names imply, species that are known to typically infest small mammals (Arthur, 1963; Hillyard, 1996).

The most abundant instar in all three years was the larval stage (2012: 96.3%, 2013: 92.9%, 2014: 96.3%), followed by nymphs (2012: 3.7%, 2013: 7.0%, 2014: 3.7%) and sporadically adults (one *I. ricinus* female at HW in 2013, one *I. trianguliceps* female at SW in 2012, six *I. trianguliceps* females at SW in 2013).

In 2012, the overall numbers of ticks at AW (n= 1,640), HW (n= 1,632) and MB (n= 1,798) were comparable, whereas at SW there were only about half as many ticks collected in total (n= 822). The highest relative abundance at all sites in 2012 showed the larval stage of *I. ricinus* accounting for 99.1% (n= 1,626) of all ticks at AW, 97.9% (n= 1,761) at MB and 96.8% (n=796) at SW. At HW the relative abundance of *I. ricinus* larvae was lower compared to the other three sites, but still as high as 76.6% of all ticks collected in 2012 (Table 3-4). The second most abundant species and instar at HW in 2012 were *D. reticulatus* nymphs (10.8%, n= 177) and larvae (10.5%, n= 171), which only accounted for a maximum of 0.1% (AW: n= 1) to 0.4% (MB: n= 8) at the other sites in 2012 (Figure 3-5). The nymphs of *I. ricinus* were captured less frequently compared to the larval stage of the species at all sites and accounted for 0.1% (MB: n= 1) to 0.7% (HW: n= 11) of all ticks collected on small mammal hosts. *I. acuminatus* was found sporadically at AW (0.1%, n= 1), HW (0.9%, n=15) and MB (1.2% n= 21), but not as SW in 2012, while *I. trianguliceps* was found at HW (0.5%, n= 8), MB (0.4%, n= 7) and SW (2.7%, n=22) (Table 3-4).

Table 3-4: Overview of tick species and instars collected from small mammal host at AW, HW, MB and SW between 2012 and 2014

Tick species and instar	2012				2013				2014				
	AW	HW	MB	SW	AW	HW	MB	SW	AW	HW	MB	SW	Σ
D. reticulatus larvae	2	171	8	0	0	61	0	0	60	109	20	0	431
D. reticulatus nymphs	1	177	0	0	2	191	0	0	8	160	4	0	543
I. acuminatus larvae	1	12	21	0	1	0	0	0	2	0	0	1	38
I. acuminatus nymphs	0	3	0	0	3	0	0	0	7	0	0	0	13
I. ricinus larvae	1626	1250	1761	796	1626	1541	1385	551	1402	1843	2741	1190	17712
I. ricinus nymphs	10	11	1	4	39	50	76	19	24	23	23	25	305
I. ricinus females	0	0	0	0	0	1	0	0	0	0	0	0	1
I. trianguliceps larvae	0	3	6	18	2	1	4	8	0	1	1	6	50
I. trianguliceps nymphs	0	5	1	3	0	0	4	6	0	2	1	3	25
I. trianguliceps females	0	0	0	1	0	0	0	6	0	0	0	0	7
Σ	1640	1632	1798	822	1673	1845	1469	590	1503	2138	2790	1225	19125

In 2013, there were some fluctuations but these were not as substantial in the total number of ticks compared to 2012, even though small mammal numbers had declined dramatically at HW, MB and SW (Figure 3-1). At AW, the number of ticks collected in 2013 remained very similar to the previous year (n= 1,673), while there was a slight increase in total tick numbers at HW (n= 1,845) and a certain decrease at MB (n= 1,469) and SW (n= 590) compared to the previous year of sampling (Table 3-4). At all sites, *I. ricinus* larvae were again the most prevalently collected species and instar on host individuals, accounting for 83.5% (n= 1,541) at HW and up to 97.2% (n= 1,626) at AW (Table 3-4). The second most abundant species and instars at HW in 2013 were again *D. reticulatus* nymphs and larvae, with about the same number of nymphs (10.4%, n= 191) as in the previous season but with a comparatively lower abundance of *D. reticulatus* larvae (3.3%, n= 61). In contrast to the first season of sampling, an elevated level of *I. ricinus* nymphs

could be observed at all sites in 2013: while the overall abundance of *I. ricinus* nymphs in 2012 was 0.4% (n= 26), it increased to 3.3% (n= 184) of all collected ticks in 2013.

I. ricinus nymphs accounted for 2.3% of all ticks (n= 39) at AW, 2.7% (n= 50) of all ticks collected on-host at HW, while a total of 76 nymphs of *I. ricinus* (5.2%) were collected at MB, and 19 nymphs of *I. ricinus* (3.2%) at SW. *I. acuminatus* was only found at AW in 2013 (0.2%, n= 4), whereas *I. trianguliceps* was collected sporadically from hosts at all four sites and ranged between 0.1% (n= 1) at HW) and 3.4% (n=20) at SW.

In 2014, the overall number of ticks on hosts at AW remained at a similar level. At HW, an ongoing increase in tick numbers compared to both previous years was observed (Table 3-4). The pattern of increasing overall numbers of ticks in 2014 becomes even clearer and more dramatic at MB and SW in 2014. At MB, the total number of ticks collected from host individuals at the site increased by 89.9% from 1,469 ticks in 2013 to 2,790 ticks in 2014. This was even exceeded at SW where although the overall amount of ticks still remained lower than at the three other sites, it rocketed up from 590 ticks in 2013 to 1,225 ticks in 2014 (Table 3-4), which represents an increase of 107.6%. The most prevalent species and instar at all sites in 2014 remained the larval stage of *I. ricinus*, accounting for 86.2% (n= 1,843) at HW up to 98.2% of all ticks at MB (n= 2,741). The previously described overall increase of tick numbers at HW was mainly facilitated by an increase of *I. ricinus* larvae (n_{2013}= 1,541 n_{2014}= 1,843). This was observable for the substantial increases in total tick numbers at MB and SW as well. Here, the abundance of larval *I. ricinus* increased from 1,385 in 2013 to 2,741 in 2014 at MB and from 551 in 2013 to 1,190 in 2014 at SW. This represents an increase of 97.9% at MB and of 116.0% at SW, respectively, and does - in both cases - account for the entire increase in tick numbers. As in 2012 and 2013 the second most abundant species and instars in 2014 were those of *D. reticulatus*. The abundance of *D. reticulatus* larvae at HW (n= 109, 4.0%) was slightly higher than in the previous year of sampling, but still lower than in 2012. The abundance of *D. reticulatus* nymphs on hosts at HW (n= 172, 7.5%) was comparable to the overall number collected in 2012 and 2013, but the relative abundance was slightly lower due to the overall increase in tick numbers at HW in 2014. Besides, there was a considerable increase in *D. reticulatus* abundance at AW and MB in 2014. Sixty larvae (4.0%) and 8 nymphs (0.5%) of *D. reticulatus* were collected at AW and 20 larvae (0.7%) with 4 nymphs

(0.1%) were found at MB in 2014 (Table 3-4). *I. acuminatus* and *I. trianguliceps* were again only sporadically found (Table 3-4).

Hence, the overall pattern of *I. ricinus* as the dominant tick species, followed by *D. reticulatus* and the isolated occurrence of *I. acuminatus* and *I. trianguliceps* was consistently observable throughout the study. However, there was also a considerable amount of variation between sites and between years in terms of the overall level of abundance as well as the dynamic pattern of tick abundance in space and time.

The abundance of the less frequently occurring tick species in the present study (*I. acuminatus, I. trianguliceps, D. reticulatus* except for HW) was insufficient to draw reliable conclusions on the following topics or to statistically analyse their dynamics. Moreover, the larval instar of *I. ricinus* alone accounted for the majority of all ticks collected during the study period (92.6%, n= 17,712). Hence, the further results and conclusions will mainly focus on *I. ricinus* as it is also known to be of major human and veterinary medical importance (Jaenson *et al.*, 1994). Even though the nymphal stage of *I. ricinus* was not as abundant as the larval stage (Figure 3-5) during the study, it is of potentially great importance in the epidemiology of TBPs (Humair and Gern, 2000; Randolph, 2004; James *et al.*, 2012) and will be considered in this context. The infestation patterns of *D. reticulatus* larvae and nymphs on small mammal hosts have been described in Pfäffle *et al*. (2015) and therefore will not be examined explicitly here.

3.2.2 Measures of parasite burden - Infestation of small mammal species

In the previous section, the total numbers, species and instars of ticks present at the four sites of sampling during the sampling period from 2012 to 2014 were shown. There was considerable variation between the tick communities at the different sites and years of sampling. The next question to be addressed was whether these ticks were evenly distributed among host species.

Of the 2,899 small mammal individuals that were examined, 2,295 (79.2%) were infested, harboring all 19,125 ticks collected (Table 3-4). A total of 18,182 of these ticks (95.1%) were collected from *Af* and *Mg* hosts of which 94.4% were *I. ricinus*.

In the following section, the infestation patterns of *I. ricinus* larvae and nymphs on *Af* and *Mg* will be examined for the study period from 2012 to 2014 at AW, HW, MB and SW. The occurrence and distribution of the other tick species will be addressed briefly.

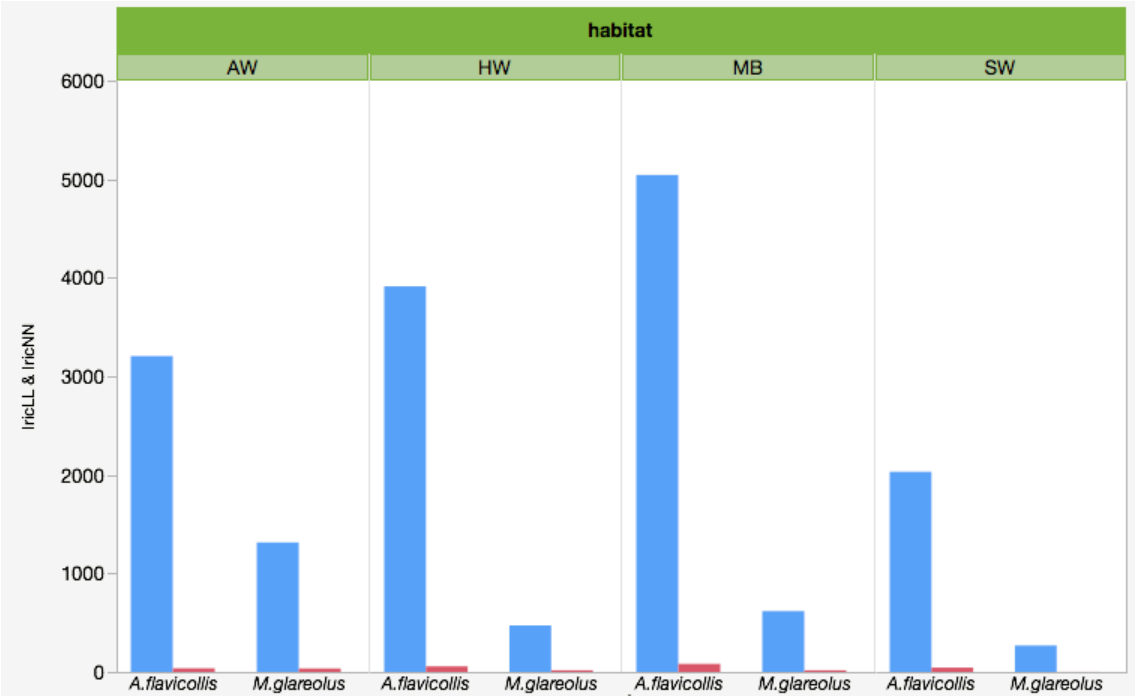


Figure 3-6: Overview of total numbers of *I. ricinus* larvae (blue bars) and nymphs (red bars) collected on *A. flavicollis* and *M. glareolus* at AW, HW, MB and SW from 2012 to 2014 (united).IricLL=*I. ricinus* larvae, IricNN= *I. ricinus* nymphs

3.2.3 Infestation patterns of *I. ricinus* on *A. flavicollis* and *M. glareolus*

Considerably more *I. ricinus* larvae and nymphs were collected from *Af* (n= 14,192 larvae, n= 220 nymphs) compared to *Mg* (n= 2,673 larvae, n= 70 nymphs), shown in Figure 3-6), with larvae being substantially more abundant than nymphs on both species.

Both small mammal species showed a high prevalence (the proportion of infested to uninfested individuals among the entire sample) of *I. ricinus* larval infestation (Table 3-5, 79.2–96.7% *Af* and 47.7–90.9% *Mg* depending on the collection site), with comparably lower prevalence of *I. ricinus* nymphs (0.5–35.1% infested *Af* and 0–16.7% infested *Mg*) and with *Af* showing consistently higher prevalence of larvae and nymphs of *I. ricinus* at all sites and in all years of the study (Table 3-5). The significance of the elevated infestation prevalence of *Af* compared to *Mg* was confirmed by a χ^2 test (χ^2_{Larvae}= 215.2, $p < 0.0001$, χ^2_{Nymphs}= 19.271, $p < 0.0001$).

This pattern was perpetuated by a persistently higher mean abundance ($\bar{x}_a$, indicates the mean number of ticks collected on all examined small mammals) of *I. ricinus* larvae on *Af* compared to *Mg* (Mann-Whitney U-test: $p < 0.0001$), and also held true for the overall pattern of mean abundance of the nymphal stage of *I. ricinus* on yellow-necked mice (Mann-Whitney U-test: $p < 0.0001$). Only at AW and HW in 2014 were there slight deviations from the otherwise very clear pattern: at AW, the mean abundance of *I. ricinus* nymphs on *Af* (0 ± 0.3) was the same as for *Mg* in 2014. At HW, the average infestation of *Mg* with *I. ricinus* nymphs (0.2 ± 0.5) was slightly higher than that of *Af* (0.1 ± 0.5), but if these minor differences are further compared to the mean intensity of larvae and nymphs

Table 3-5: Number of *I. ricinus* larvae and nymphs on *A. flavicollis* (A) and *M. glareolus* (B). The prevalence gives the percentage of infested host individuals among all examined hosts. The mean abundance gives the average number of ticks ± standard deviation (SD), including all examined individuals. The mean intensity is the average tick burden of infested host individuals ± SD. The range gives the intensity of individual tick burdens on hosts.

A		2012				2013				2014			
Af		AW	HW	MB	SW	AW	HW	MB	SW	AW	HW	MB	SW
hosts	n	150	163	184	127	82	61	69	37	284	107	151	80
larvae	n	1212	977	1518	636	935	1404	1196	285	1058	1532	2329	1110
prevalence	%	94.0	85.3	94.0	81.9	92.7	96.7	94.1	94.6	79.2	95.3	95.4	88.8
abundance	$\bar{x}$ ± SD	8.1 ± 8.2	6.0 ± 7.6	8.3 ± 8.2	5.0 ± 7.5	11.4 ± 11.1	23.0 ± 22.4	17.3 ± 20.5	7.7 ± 8.1	3.7 ± 4.1	14.5 ± 15.8	15.4 ± 19	13.9 ± 20.7
intensity	$\bar{x}$ ± SD	8.6 ± 8.2	7 ± 7.8	8.8 ± 8.2	6.1 ± 7.9	12.3 ± 11.1	23.8 ± 22.4	18.6 ± 20.8	8.1 ± 8.1	4.7 ± 4.1	15.2 ± 15.9	16.2 ±19.1	15.6 ± 21.3
range	Min-Max	0 – 50	0 - 66	0 - 59	0 - 60	0 - 46	0 - 105	0 - 78	0 - 30	0 - 27	0 - 86	0 - 130	0 - 110
nymphs	n	6	6	1	4	17	44	62	18	14	7	19	22
prevalence	%	4.0	3.7	0.5	3.1	14.6	29.5	15.9	35.1	3.9	5.6	7.9	16.3
abundance	$\bar{x}$ ± SD	0 ± 0.2	0 ± 1.8	0 ± 0.1	0 ± 0.2	0.2 ± 0.6	0.7 ± 1.8	0.9 ± 3.9	0.5 ± 0.9	0 ± 0.3	0.1 ± 0.3	0.1 ± 0.5	0.3 ± 0.9
intensity	$\bar{x}$ ± SD	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1.4 ± 0.7	2.4 ± 2.7	5.6 ± 8.7	1.4 ± 1	1.3 ± 0.6	1.4 ± 0.5	1.6 ± 0.8	1.7 ± 1.5
range	Min-Max	0 - 1	0 - 1	0 - 1	0 - 1	0 - 3	0 - 12	0 - 3	0 - 4	0 - 3	0 - 2	0 - 3	0 - 6

B		2012				2013				2014			
Mg		AW	HW	MB	SW	AW	HW	MB	SW	AW	HW	MB	SW
hosts	n	155	77	93	172	213	18	42	11	266	64	117	13
larvae	n	290	125	150	153	687	118	158	70	337	229	310	46
prevalence	%	69.0	68.8	73.1	47.7	76.5	88.9	81.0	90.9	51.1	76.6	73.5	76.9
abundance	$\bar{x}$ ± SD	1.9±2.3	1.6±1.8	1.6±1.7	0.9±1.2	3.2±3.9	6.6±9.3	3.8±3.9	6.4±7.2	1.3±2.2	3.6±4.9	2.6±3.3	3.5±4.8
intensity	$\bar{x}$ ± SD	2.7 ± 2.3	2.4 ± 1.7	2.2 ± 1.6	1.9 ± 1.2	4.2 ± 3.9	7.4 ± 9.6	4.6 ± 3.9	7.0 ± 7.3	2.5 ± 2.6	4.7 ± 5.1	3.6 ± 3.4	4.6 ± 45.0
range	Min-Max	0 - 12	0 - 7	0 - 7	0 - 6	0 - 25	0 - 40	0 - 18	0 - 18	0 - 22	0 - 25	0 - 18	0 - 18
nymphs	n	3	2	0	0	22	4	13	1	10	11	4	0
prevalence	%	1.3	2.6	0.0	0.0	6.6	16.7	11.9	9.1	2.6	12.5	2.6	0.0
abundance	$\bar{x}$ ± SD	0 ± 0.2	0 ± 0.2	0 ± 0	0 ± 0	0.1 ± 0.5	0.2 ± 0.5	0.3 ± 1.4	0.1 ± 0.3	0 ± 0.3	0.2 ± 0.5	0 ± 0.2	0 ± 0
intensity	$\bar{x}$ ± SD	1.5 ± 0	1 ± 0	0 ± 0	0 ± 0	1.6 ± 0.9	1.3 ± 0.6	2.6 ± 3.6	1 ± 0	1.4 ± 1.1	1.4 ± 0.7	1.3 ± 0.6	0 ± 0
range	Min-Max	0 - 2	0 - 1	0	0	0 - 4	0 - 2	0 - 9	0 - 1	0 - 4	0 - 3	0 - 2	0

($\bar{x}_I$, a more precise measure of tick load on hosts, which represents the actual mean number of ticks on infested hosts only) it was clearly observable that yellow-necked mice always carried larger tick loads than voles. In all three years and at all sites, the mean intensity of *I. ricinus* larvae was significantly higher on *Af* hosts (Table 3-5, Mann-Whitney U-Test: $p_{Larvae} < 0.0001$). The pattern was not as obvious for the mean intensity of the less frequently occurring nymphs, but seemed rather balanced between both host species (Mann-Whitney U-test: $p_{Nymphs} = 0.57$). The range of larvae and nymphs in turn showed that *Af* infestation could reach higher peak levels compared to *Mg*, ranging between 0 to 130 larvae and 0 to 12 nymphs on individuals of the yellow-necked mouse, compared to 0 to 40 larva and 0 to 9 nymphs on individual bank voles.

3.2.4 Spatio-temporal infestation dynamics

In 2012, the overall infestation patterns of *I. ricinus* larvae on small mammal hosts were similar. The infestation of *Af*, in terms of prevalence, mean abundance and intensity were highly similar among AW and MB, somewhat lower at HW and substantially lower at SW. For *Mg*, the prevalence, mean abundance as well as the mean intensity were overall comparable at AW, HW and MB, while they were somewhat lower at SW (Table 3-5).

The prevalence of *Af* was exactly the same at AW and MB (94%, Fisher's Exact-Test $p>0.05$), while it was comparatively lower at HW (85.3%, $p_{AW>HW} = 0.009$, $p_{MB>HW} = 0.006$) and even lower at SW (81.9%, $p_{AW>SW} = 0.002$, $p_{HW>SW} > 0.05$, $p_{MB>SW} = 0.0008$). The mean abundance of larvae on yellow-necked mice was again highly similar between AW and MB and lower at but similar between HW and SW (Table 3-5). A comparison of the mean abundances of larvae on *Af* between all sites in 2012 (by Tukey-Kramer Honestly significant difference (HSD) Test) supported this, by indicating clearly significant differences between MB and SW ($p = 0.002$), between AW and SW ($p = 0.008$), as well as lower significant differences between MB and HW ($p = 0.04$).

The prevalence of larval infestation on bank voles was very similar at AW (69%), HW (68.8%) and MB (73.1%), but again substantially lower at SW (47.7%, $p_{AW>SW} < 0.0001$, $p_{HW>SW=} 0.001$, $p_{MB>SW} < 0.0001$). The mean abundance of *I. ricinus* larvae on bank voles showed the same pattern with AW, HW and MB being comparable (Tukey-Kramer HSD $p>0.05$), whereas these three sites showed significant differences compared to SW

(Tukey-Kramer HSD $p_{AW-SW}<0.0001$, $p_{HW-SW}=0.016$, $p_{MB-SW}=0.01$). The analysis of the mean intensity of *I. ricinus* larvae on both host species between the sites in 2012 (Tukey-Kramer HSD) showed the same pattern as for the prevalence and the mean abundance. Again, SW was seperated from the other three sites; exhibiting the strongest differences between the mean abundances of MB and SW (p= 0.04) for *Af* and between AW and SW for *Mg* (p= 0.01). The range of total abundance of *I. ricinus* larvae on hosts was somewhat similar between all sites, with slightly elevated total numbers of larvae on individual *Mg* at AW and a slightly smaller range on *Af* at AW in 2012 (Nymph abundance was low at all sites on both host species in 2012, ranging between a maximum of 0 to 2 nymphs per host and a total of 0 to 6 nymphs collected per site during the entire sampling season in 2012.

In 2013, when small mammals suffered population crashed at HW, MB and SW, the larval and nymphal infestation of hosts increased at all sites, but to a different extent. The distinct overall increase of tick infestation at HW, MB and SW was accompanied by a rather moderate increase at AW. The prevalence of *Af* carrying *I. ricinus* larvae reached over 90% at all four sites. Prevalence at AW and MB remained at equivalent levels to 2012 (Fishers-Exact-Test p> 0.05) whereas the proportion of infested yellow-necked mice increased to 96.7% at HW (Fisher's Exact Test $p_{2013>2012}$= 0.01) and to 94.6% at SW ($p_{2013>2012}$= 0.04) As prevalence were close to 100%, the mean abundance and mean intensity of larvae on yellow-necked mice became almost equal. Both parameters increased as well at all sites in 2013. The strongest increase was observable at HW ($\bar{x}_{a(HW)}$= 23.0 ± 22.4, $\bar{x}_{i\ (HW)}$= 23.8 ± 22.4) and MB ($\bar{x}_{a(MB)}$= 17.3 ± 20.5, $\bar{x}_{i\ (MB)}$= 18.6 ± 20.8), followed by AW ($\bar{x}_{a(\ AW)}$= 11.4 ± 11.1, $\bar{x}_{i\ (AW)}$= 12.3 ± 11.1) and survivalSW ($\bar{x}_{a(SW)}$= 7.7 ± 8.1, $\bar{x}_{i\ (SW)}$= 8.1 ± 8.1), which remained the site with lowest mean abundance and intensity in 2013 as well (Table 3-5). The similarity of the levels of mean abundance and intensity between HW and MB and the differences compared to AW and SW were confirmed by a Tukey-Kramer HSD test, showing the strongest differences in terms of mean abundance and mean intensity between HW and SW ($p\bar{x}_a$ < 0.0001, $p\bar{x}_i$= 0.0002), MB and SW ($p\bar{x}_a$ = 0.03,$p\bar{x}_i$ = 0.004) and between HW and AW ($p\bar{x}_a$ = 0.004 , $p\bar{x}_i$ = 0.0008). Furthermore, the larval range of *Af* was also only elevated at HW and MB (Table 3-5).

The infestation of *I. ricinus* larvae on *Mg* increased at all sites in 2013. While there was a moderate increase in infestation prevalence at AW from 69.0% to 76.5% (Fisher's Exact Test p> 0.05), resulting in the lowest prevalence at the four sites in this year, the

prevalence at HW, MB and SW rocketed up to 81.0% (MB), 88.9% (HW) and 90.9% (SW), respectively. The resulting mean abundance and intensity was comparable between AW and MB and between HW and SW, but only (roughly) significantly different between the most distinct sites AW and HW ($p\bar{x}_a$= 0.02, $p\bar{x}_i$= 0.05). The total range of *I. ricinus* larvae on *Mg* was only elevated at HW.

The prevalence of nymphal *I. ricinus* on both host species showed the same overall pattern as the larvae, with a strong overall increase at most sites in 2013 (Fisher's Exact test p> 0.05), and a less drastic increase at AW. The prevalence of *I. ricinus* nymphs on *Af* increased by 10.1% at AW (from 4% in 2012 to 14.6% in 2013), while the increase at the three other sites ranged between 15.4% (MB) and 32% (SW). The prevalence of nymphal infestation on *Mg* increased by 5.3% to 6.6% in 2013 at AW, while the increase in prevalence on bank voles at HW, MB and SW ranged between 9.1% (SW) up to 14.1% (HW) (Fisher's Exact Test p> 0.05). Mean abundances and intensities were lowest on *Af* at AW (Tukey-Kramer HSD p>0.05) whereas they were rather balanced on *Mg* at AW, HW and SW and slightly higher at MB (Tukey-Kramer HSD p>0.05) (Table 3-5).

In 2014, the larval and nymphal infestation of hosts at AW was the lowest for the entire period of sampling from 2012 to 2014. At HW and MB, the overall levels of infestation were intermediate, falling between those of 2012 and 2013. At SW, however, tick burdens, predominantly those on *Af,* reached even higher levels than before. At AW, the prevalence of *I. ricinus* larvae on both host species (*Af*: 79.2%, *Mg*:51.1%) and of nymphs on *Af* (3.9%) was lowest among the four sites. This was accompanied by the lowest abundance and mean intensity of larval infestation on both host species at AW for the entire study period (Table 3-5). The nymphal abundance and intensity on hosts at AW in 2014 was lower than 2013, but comparable to the numbers in 2012 (Table 3-5). The range of larval *I. ricinus* was again lowest on *Af* in 2014 (0-27), but comparable to the previous sampling season for *Mg* (0-22). At HW and MB, the prevalence of larvae on *Af* was only slightly lower than in 2013. Nymphal prevalence at both sites and on both host species fell between those from 2012 and 2013, as did the larval and nymphal ranges except for larvae on *Af* at MB, which increased to a maximum of 130 (Table 3-5). At SW, the prevalence of larvae on hosts and of nymphs on *Af* in 2014 fell between the prevalence of 2012 and 2013 (Fisher's Exact Test p> 0.05), while nymphs on *Mg* were absent. The abundance and intensity of larvae on *Af*, as well as the larval range at SW, was by far

highest in 2014 and comparable to HW and SW (Table 3-5), whereas nymphal abundance and intensity were comparable to those in 2013. The mean larval burden (abundance and intensity) on bank voles fell between 2012 and 2013. The overall similarities of larval mean abundance and intensity on hosts between HW, MW and SW, in contrast to AW, were supported by a Tukey-Kramer HSD test. Here, significant differences were shown for both host species and all sites compared to AW in 2014. For *Af*, all pairs were significantly different from AW with $p\bar{x}_a < 0.0001$, $p\bar{x}_i < 0.0001$). For *Mg*, the abundance and intensity of *I. ricinus* larvae at HW, MB and SW were significantly different from AW as well (MB-AW $p\bar{x}_a < 0.0001$, $p\bar{x}_i = 0.0004$; HW-AW $p\bar{x}_a < 0.0001$, $p\bar{x}_i < 0.0001$; SW-AW $p\bar{x}_a < 0.0001$, $p\bar{x}_i = 0.05$, roughly significant).

These results show that tick distribution on hosts is non-uniform in space, in time and on different host species. The mean abundance and intensity were substantially higher (up to 4-fold) in 2013 at all three sites when the population crash occurred, but it is remained at a lower level at AW where the population remained stable compared to the previous year. Thus it seems in large parts like a similar number of ticks was distributed on a smaller number of hosts when less host individuals were available. This pattern raises the question of if and how small mammal populations have an influence on the abundance of ticks in a given area.

3.2.5 Seasonal interaction between hosts and ticks

The mean abundance of ticks on hosts is the result of the abundance and activity of the overall available (active) ticks and hosts in a given area at any time. *Af* and *Mg* exhibit different host capacities; however, individual tick burdens are the result of interspecific interactions and overall host density and should be considered as a unified measure for the examination of seasonal dynamics. Therefore, the seasonal abundance of *Af* and *Mg* were pooled to represent the majority of hosts available for tick infestation at a given time and at a given site. In the following section, I will show how the mean abundance of *I. ricinus* larvae interacts with the abundance of small mammal hosts at the four sampling sites during the three seasons of sampling from 2012 to 2014 (Figure 3-7).

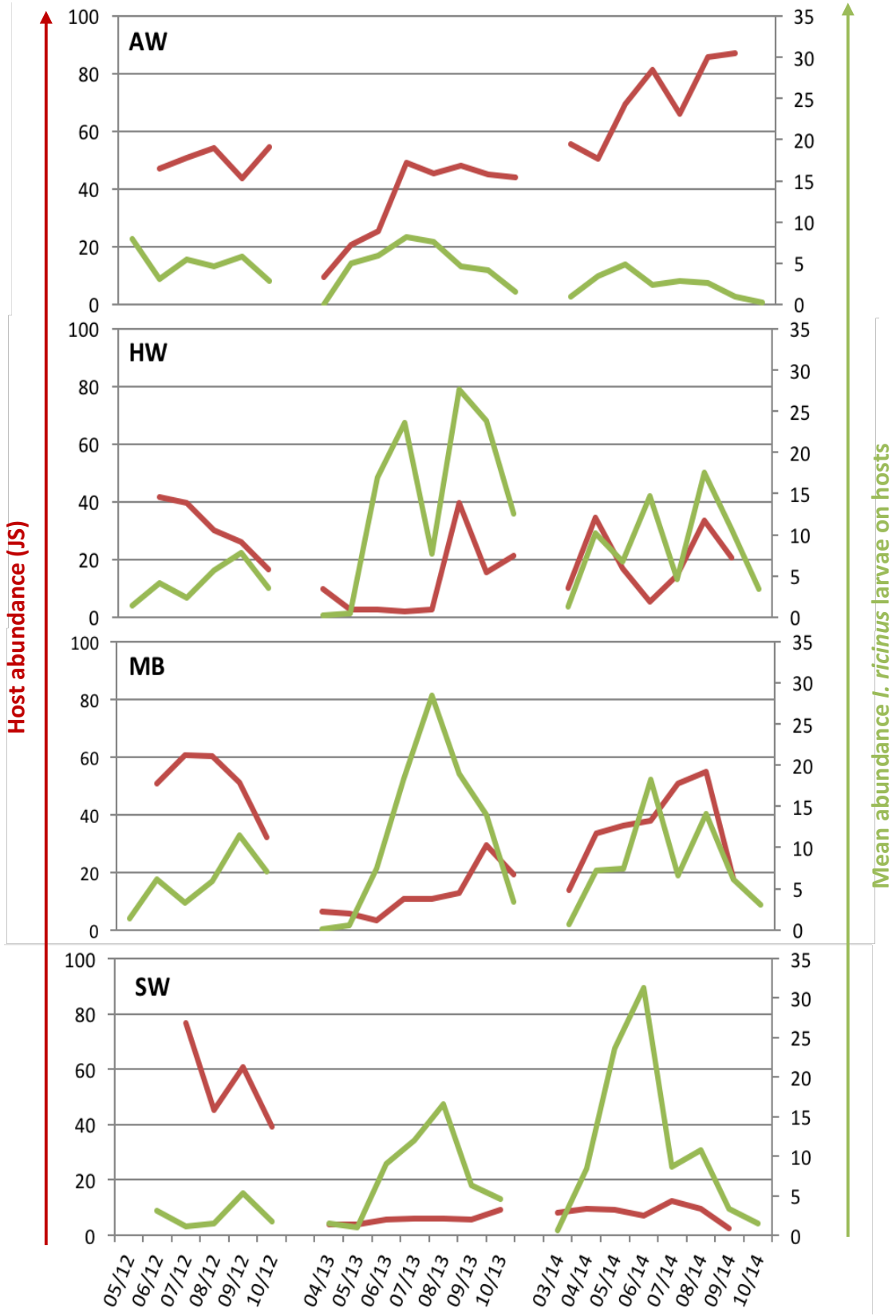


Figure 3-7: Seasonal pattern of the estimated abundance of small mammal hosts (*A. flavicollis* and *M. glareolus*) compared to the mean abundance of *I. ricinus* larvae from May 2012 to October 2014 (June 2012 at SW). The abundance is shown for June 2012 to September 2014 (July 2012 at SW), to avoid potentially confounded estimates of the first and last month of sampling

Just as the populations of their small mammal hosts undergo seasonal fluctuations, so does the abundance of ticks on hosts (Figure 3-7).

The activity patterns differed substantially between the individual sites, even though they were located relatively close to one another (10 - 58km), as well as between the three years of sampling. Depending on site and sampling season, there were differences between peak tick activity and the total number of peaks per season. Altogether, there were from one to three peaks of larval activity on hosts observable at all sites and in all years. The most frequently found pattern was two peaks of *I. ricinus* mean abundance on hosts per season the first in late spring/early summer (around June) and the second in autumn (Figure 3-7). At AW, a hybrid form between uni- and bimodal activity was found in all three years. After a first peak in June of each year, there was either a slow decrease in mean abundance until the end of the season (2013) or a slight, second peak (2012, 2014). At HW, larval abundance on hosts peaked twice in 2012 and 2013 and three times in 2014, whereas at MB, the activity pattern of *I. ricinus* larvae was bimodal in 2012 and 2014, but unimodal in 2013. At SW, the peaks of tick activity seemed slightly delayed in comparison to the other three sites. Two peaks were observable in 2012 and to a certain extent also in 2014, with the second peak in late summer being less pronounced. In 2013, larvae exhibited a unimodal pattern of activity with a distinct late-summer peak. These patterns suggest that habitat-related differences between the four sites, with resulting differences in microclimatic conditions, can have a strong influence on the pattern of host-seeking activity of ticks.

Furthermore, it was striking that the mean abundance of ticks on hosts in the course of a season behaved in any way opposite to small mammal abundance during this year.

If small mammal abundance was high, the mean abundance of ticks on hosts was relatively low, whereas during times of low small mammal abundance it was substantially elevated. This relationship could be detected by observing the seasonal dynamics of ticks on hosts (Figure 3-7), by examining the mean abundance of ticks on hosts per year (Table 3-5) as well as by looking at the total numbers of ticks found on small mammal hosts. This finding has important implications for the transmission dynamics of TBDs (discussed below).

Another clearly distinguishable pattern at all sites and in all years was that the main activity of *I. ricinus* larvae on hosts was not - as usually reported in the literature

(Kurtenbach *et al.*, 2006) - restricted to the summer months. Moreover, it could be shown that from the very beginning of small mammal sampling each spring, ticks were found infesting their hosts (Figure 3-7).

However, after three years of observation, there was no distinct, continuous pattern detectable at any of the four sites.

Table 3-6: Results of ANOVA analysis (F-test) of all predictor variables in the best resulting model (GLM)

Variable	F	p
Species	1506.9	< 0.0001
Site	58.2	< 0.0001
Session	38.2	< 0.0001
Species*Site	8.2	< 0.0001
Species*Session	9.8	< 0.0001
Site*Session	11.6	< 0.0001

In addition to this visual representation of the enormous amount of spatiotemporal variation, a generalized linear model (GLM) was carried out to determine the influence that time (session), space (site) and hosts (species) can have on the abundance of *I. ricinus* larvae on hosts (Table 3-6). Moreover, interactions between all explanatory variables were included to assess their relative importance. The resulting best fit model (GLM of the negative binomial family with log link function) still included all initially present predictor variables including all interactions, which gives a first impression of their importance as well as of their interconnectedness (Table 3-6). The variables themselves (site, session, species) as well as all two-way interactions were assigned as highly significant factors influencing tick burden on hosts. This shows again that these factors are not only of high relative importance regarding tick load on hosts but that there are multiple interactions in time and space leading to the enormous amount of variation in the data and the non-uniform patterns between the years and the sites.

3.2.6 Dispersion patterns of ticks on hosts

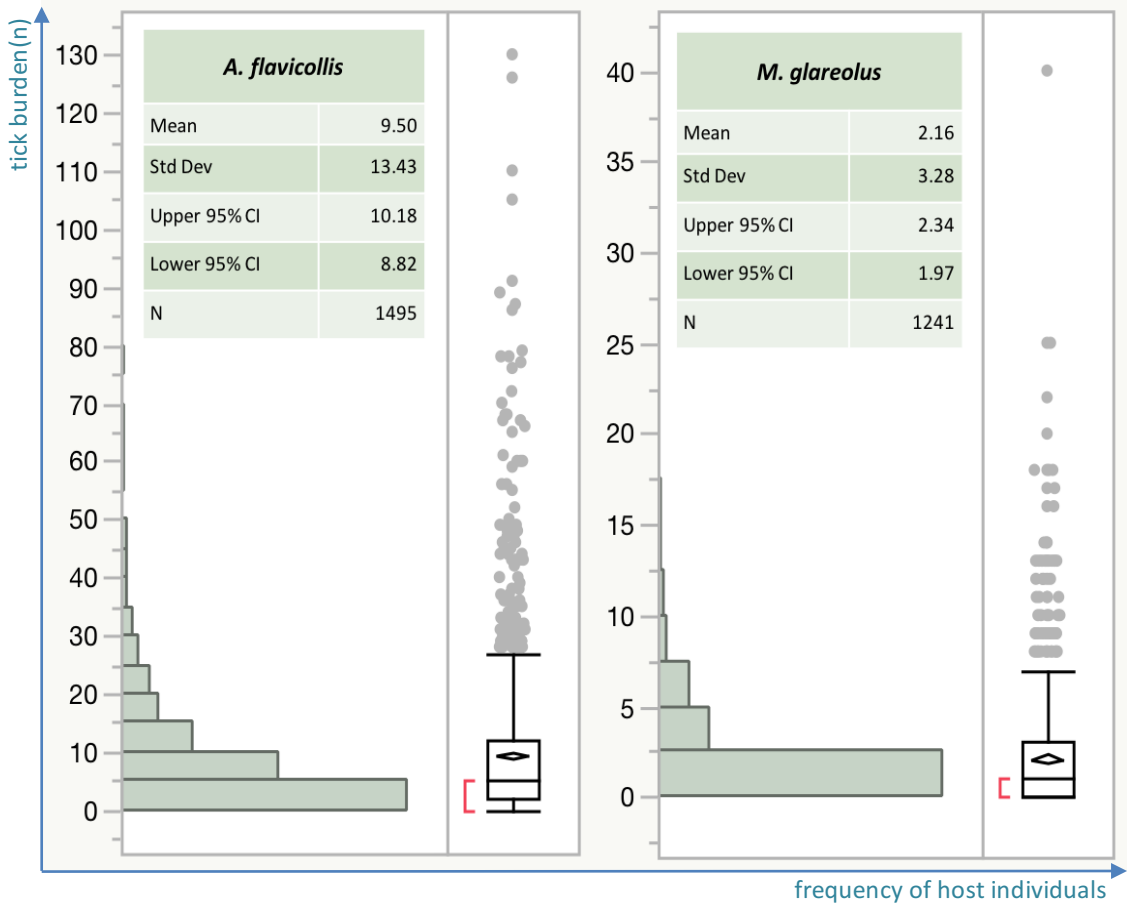


Figure 3-8: Frequency distribution of *I. ricinus* larval burden on *A. flavicollis* (left) and *M. glareolus* (right). The data of all sites and years were pooled. The X-axis of the histogram indicates the frequency of host individuals; the Y-axis shows the individual tick burden. The boxplot on the right of both histograms encompasses the lower quantile (25%) as lower border of the box, the median as vertical line in center of the box and the 75% quantile as upper border of the box. The range spanned between the mean and the confidence intervals of the distribution is shown as rhomb within the box. The red bracket indicates the densest area of the distribution, holding 50% of all data. Potential outliers are shown as dots

The overall distribution of *I. ricinus* larvae among individuals of both dominant species, *Af* (n= 1,495) and *Mg* (n= 1,241), is shown in Figure 3-8. It is obvious at first sight that the larvae of *I. ricinus* were not evenly distributed among hosts, either for *Af* (left) or for *Mg* (right). The majority of hosts seemed to harbour none to only a few ticks, with a mean abundance of 9.5 (± 13.4) on *Af* and a mean abundance of 2.2 (± 3.3) for *Mg.* Whereas only a few hosts (shown as outliers) harboured substantially more than the mean number of ticks, with up to 130 larvae on individuals of the yellow-necked mouse and up to 40 larvae on bank voles. Therefore, in both cases, the dispersion of *I. ricinus* larvae on hosts

does not follow a normal (gaussian) distribution, but is strongly right-skewed and overdispersed. This pattern is typical for parasitological data and holds true for a variety of macroparasite-host systems (Crofton, 1971; Shaw and Dobson, 1995). It is usually described best by a negative binomial distribution (Shaw *et al.*, 1998).

3.2.7 The influence of host characteristics on tick abundance

So far, host species accounted for substantial differences in tick loads with *Af* exhibiting much higher prevalence, mean abundances, mean intensities, total ranges of tick burden compared to *Mg*. However, within these species, some individuals tend to have more ticks than others (Figure 3-8, Figure 3-9, Figure 3-10). The next question to be addressed is: What intrinsic factors could have caused these differences in tick dispersion on hosts? Based on the literature, gender and age (Shaw *et al.*, 1998; Kiffner *et al.*, 2011) were investigated for their influence on the abundance of *I. ricinus* larvae and nymphs on hosts.

Gender

As the patterns of tick infestation per gender were consistent in space and time for *Af* and *Mg*, the respective data was pooled and revealed the overall influence of gender on individual *I. ricinus* larvae burden with a significantly higher abundance of larvae on male individuals of both host species (Mann-Whitney U-test $p<0.0001$ for both *Af* and *Mg*).

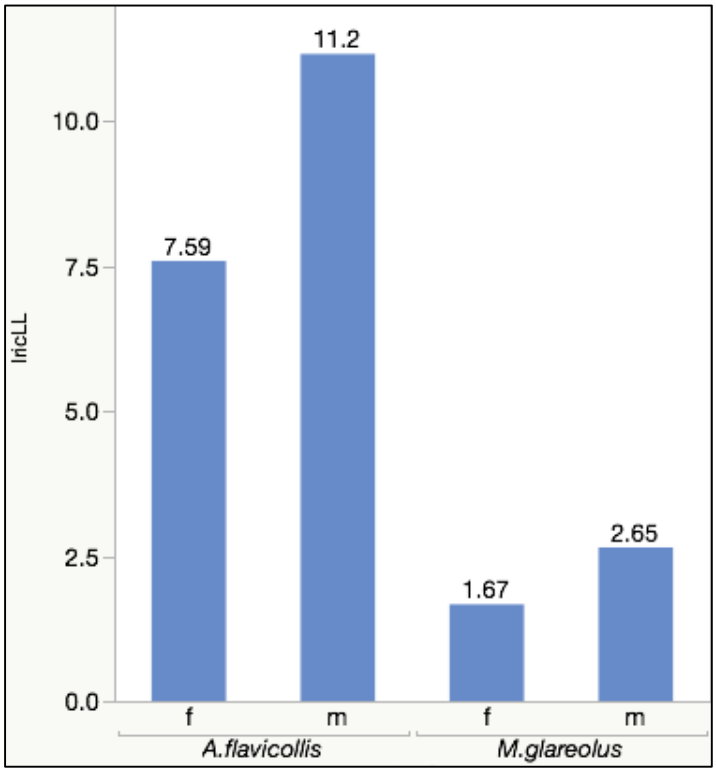


Figure 3-9: Infestation (mean) of *A. flavicollis* and *Mg* individuals with *I. ricinus* larvae according to host gender. Data was pooled after confirmation of rectified results for all sites and years

The numbers of *I. ricinus* nymphs on hosts were substantially lower than those of larvae, but at all sites and in all years of sampling the abundance of nymphs on hosts, as well as all

significant results obtained by comparing the nymphal burden between genders at the individual sites and years, pointed in the same direction, indicating a significantly higher number of nymphs on male individuals of both host species (*Af* at HW 2013: p= 0.0003, *Mg* at AW 2013: p= 0.0008). When the abundance of nymphs on host species was pooled among sites, the influence of gender on tick abundance was even clearer for *Af* but also observable for *Mg* (Mann-Whitney U-test; *Af* p<0.0001, *Mg* p= 0.009).

Age class

The influence of host affiliation to a certain age class (juvenile, subadult, adult; after Sínski *et al.*, 2006) was the second host characteristic that has been investigated for its potential influence on tick burden.

The overall influence of individual age classes was first investigated for differences in tick burden between all three groups (df=2, Kruskal-Wallis-test) and then for significant differences between pairs (df= 1, Mann-Whitney U-test). If the patterns were consistent, data was pooled to give an overall measure of significance. The comparison of *I. ricinus* larval burden on the three age classes for individual sites and years showed significantly higher tick burdens on adult *Af* (Kruskal-Wallis-test $p_{AW\ 2013}$= 0.04, p_{SW2012}= 0.03) and *Mg* (Kruskal-Wallis-test p_{MB2012}= 0.0002, p_{AW2014}= 0.02). The subsequent comparison of age classes in pairs confirmed the higher burden of *I. ricinus* larvae on adult versus subadult *Af* (Figure 3-10, Mann-Whitney-test p_{AW2013}= 0.02, p_{HW2012}= 0.05, p_{MB2014}= 0.0003, p_{SW2012}= 0.03) and juveniles (p_{HW2013}= 0.03, p_{HW2014}=0.04, p_{MB2014}= 0.008) and showed significantly higher larval tick burdens on adult *Mg* compared to subadult individuals
(Figure 3-10, p_{AW2012}= 0.02, p_{AW2013}= 0.04, p_{AW2014}= 0.008).

Furthermore, there were individual, statistically significant differences showing higher burdens on subadult versus juvenile *Af* (p_{MB2013}= 0.04 (n=7)) and juvenile versus subadult *Mg* (p_{AW2012}= 0.02 (n=3), p_{AW2013}= 0.003 (n=11)). However, the significant results involving juvenile individuals for single sites and years were based on extremely low sample sizes, especially for *Mg* and were therefore not likely to be representative of the actual relationship between age class and tick burden (and therefore not shown in Figure 3-10).

To verify the universality and reliability of the individually obtained results, an overall comparison of age classes was made. This revealed significantly higher *I. ricinus* burdens on adult versus subadult individuals of both host species (*Af* p< 0.0001, *Mg* p= 0.0002),

and significantly higher larval burdens on juvenile (n= 98) versus subadult (n= 419) *Mg* (p=0.002).

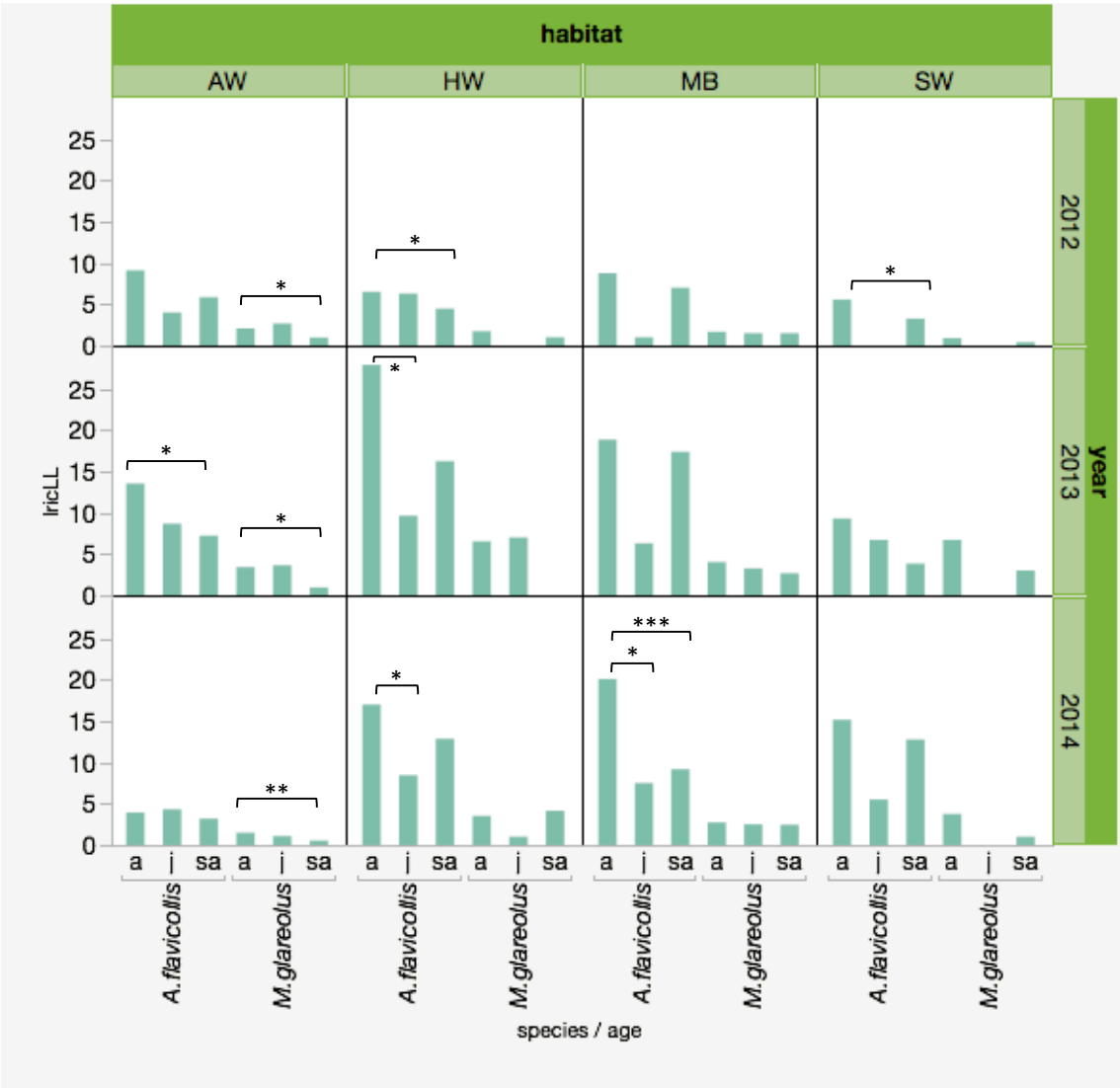


Figure 3-10: *I. ricinus* larvae (mean) on *A. flavicollis* and *M. glareolus* at AW, HW, MB and SW from 2012 to 2014 (united), according to host age class associaton. a= adult, sa= subadult, j= juvenile. Asterisks indicate significant differences between age class affiliations (Mann-Whitney-U-Test *: p<0.05, **: p<0.01: ***: p< 0.001)

A significant influence of age class on nymphal burden could be shown for *Af*, but not *Mg*. The overall comparison of all age groups revealed a significant difference in nymphal burden at HW in the year of highest aggregation of ticks, 2013 (Kruskal-Wallis test p= 0.008). Further comparison of age classes in pairs confirmed these findings for adult *Af* versus subadults at HW in 2013 (p= 0.02) and at SW in 2014 (p= 0.03). Data on nymphal burden on *Af* were then pooled to check the universality of this result. The Mann-Whitney

U-test supported the previous findings that adult individuals of *A. flavicolllis* harboured significantly more nymphs than subadults overall ($p < 0.0001$).

Thus, both of the intrinsic host characteristics investigated, gender and age class, had a significant influence on tick load among sites and years between the two dominant small mammal host species and with respect to both *I. ricinus* larvae and nymphs.

3.2.8 Measuring and comparing aggregation

The aggregation of ticks on individual hosts within a group is an important parameter for the characterization of tick dispersion and can be of great importance for the transmission potential of TBPs. The patterns of tick dispersal on hosts can be analyzed by the variance to mean ratio ($s^2/\bar{x}$) (Anderson and Gordon 1982, Parasitology 85: 373-398).

If the distribution of parasites on hosts was random (i.e. followed a Poisson distribution), then $s^2/\bar{x} = 1$, meaning that there were no effects of host characteristics (or other factors) on parasite burden. An overdispersed distribution, with most hosts harboring very few ticks and a few hosts having very high tick burdens, can be characterised by a variance that exceeds the mean ($s^2/\bar{x} > 1$).

In the present study, the overall pattern of *I. ricinus* dispersion on hosts showed a moderate to highly aggregated distribution ($s^2/\bar{x} > 1$) at all sites and in every year of the study from 2012 to 2014 (Table 3-7). Values were continuously higher for *Af* than *Mg* (Table 3-7). A comparison of the individual estimates of aggregation for both species for all sites and all sampling sessions (Figure 3-11) confirmed the

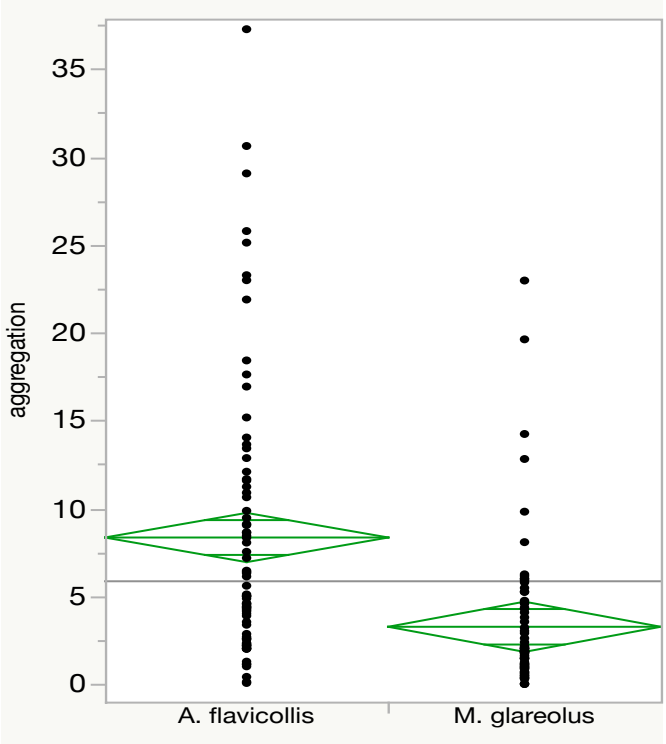


Figure 3-11: Comparison of tick aggregation on host species. The black line indicates the overall sample mean. Black dots indicate individual values of aggregation per session and site. The green boxes comprise the group mean (center line), 95% confidence intervals (CI, outer line) and standard errors (between mean and CI)

lower overall level of aggregation on *Mg* versus *Af* at a higher resolution and an ANOVA analysis revealed the highly significant nature of this difference ($p < 0.0001$). The extent of differences in aggregation between the two species varied between sites and over time from slight differences to almost five fold larger values for *Af* versus *Mg* (Table 3-7). The level of aggregation for the two species were similar among sites in 2012; values at the sites reflected the overall mean. A strong overall increase in aggregation was observable from 2012 to 2013, but the individual sites contributed differently to the overallpattern. At AW, aggregation levels remained rather stable, whereas the increase at HW and MB was dramatic. The low measures of aggregation for SW in 2013 might not be representative as it is based on very few individuals. In 2014, the differences between the aggregation levels at the four sites were even more distinct: While aggregation levels at AW and HW (slightly) decreased compared to 2013, they remained at very high levels at MB and further increased at SW:

Table 3-7: Degree of aggregation ($s2/\overline{x}$) of I. ricinus larvae on host species (A. flavicollis and M. glareolus) and the ratio of aggregation between the two species at the sampling sites from 2012 to 2014

	Af	*Mg*	*Af : Mg*
2012	9.3	2.3	4.0
AW	8.3	2.9	2.9
HW	9.7	2	4.9
MB	8.2	1.7	4.8
SW	11.2	1.7	6.6
2013	20.7	5.9	3.5
AW	10.9	4.6	2.4
HW	21.9	13.2	1.7
MB	24.4	4.1	6.0
SW	8.5	8.2	1.0
2014	22.9	5.2	4.4
AW	4.6	4	1.2
HW	17.3	6.7	2.6
MB	23.4	4.1	5.7
SW	30.8	6.4	4.8

The overall ratio of aggregation between *Af* and *Mg* was comparable between years. The comparison of aggregation levels among the individual sites showed that the relative aggregation on *Af* was more pronounced for AW at MB and SW than at AW and HW (excluding SW 2013).

3.2.9 The phenology of questing ticks

In addition to the monthly collection of ticks from small mammal hosts at the four sites, ticks were collected from vegetation by flagging (e.g. Ginsberg and Ewing 1989). As shown above, the most abundant instar on small mammal hosts was *I. ricinus* larvae, with only

few nymphs were found (Figure 3-5). The engorged larvae from small mammal hosts, in turn, will develop to nymphs, which will be active on vegetation in the following seasons. As the nymphs of *I. ricinus* are the most important instar for human infection with TBPs (Vassallo *et al.*, 2000; Wirtz, 2001), flagging allows the monitoring and analysis of the contribution of small mammal hosts to the prevalence and dynamics of TBPs.

The peak height, as well as the number and timing of peak activity of flagged *I. ricinus* larvae, differed clearly in time and space (Figure 3-12). There were usually one to two peaks per season at all four sites, however, no larval activity was detectable at SW in 2012 and three peaks were observed at MB in 2013. In 2012, the larvae collected by flagging showed substantial differences in total abundance, with the largest numbers at HW (n_{2012}= 200), whereas there were substantially less larvae at AW (n_{2012}=25) and MB (n_{2012}=93) and none at SW. There was a spring peak observable at HW and MB, a distinct autumn peak observable at HW and a slight increase in larvae collected from the vegetation at MB and AW in autumn 2012. In 2013, the overall number of larvae was similar at HW (n= 136), MB (n= 190) and SW (n= 165), but much lower at AW (n= 54), and there was no synchrony of larval peak activity observable between the sites. In 2014, the overall pattern was again completely different from the two previous years, showing low overall larval abundance at AW (n= 29), MB (n= 45) and SW (n= 70), and a high abundance at HW (n= 274). There was a slight spring peak at AW, HW and MB between April and June, and a second increase between August and October at all four sites, although substantially different numbers of larvae were collected (Figure 3-12). Therefore, neither the overall period of activity, nor the interannual dynamics of larval activity with respect to the overall abundance or the time of larval peak activity on vegetation showed a consistent pattern between years or among sites. Furthermore, the results do not correspond with the main time of larval activity being summer, as postulated in literature (Kurtenbach *et al.*, 2006).

Figure 3-12: Seasonal abundance patterns of larvae (top), nymphs (center) and females (bottom) of *I. ricinus* collected per 100m^2 by monthly flagging at the four sampling sites AW, HW, MB and SW from 2012 to 2014

In contrast, nymphal abundance on vegetation showed some temporal fluctuations between the sites and years but, overall, showed a very similar pattern. The seasonal activity lasted throughout the entire sampling season, from March to October, with major nymphal activity observable between spring and early summer. In 2012, the overall numbers of nymphs and their seasonal dynamics were highly similar between AW (n= 221), HW (n= 225) and MB (n= 237). At these three sites there was a first, large peak in nymphal activity between June (AW and MB) and July (HW), followed by a decline and a

second, slight, increase towards the end of the sampling season at all four sites. The overall numbers of nymphs were substantially lower at SW (n= 36) and there was no peak observable during 2012. A similar pattern occurred in 2013, with comparable numbers at AW (n= 226), HW (n= 297) and MB (n= 259) but lower numbers at SW (n= 70). A first activity peak could be observed between April (HW) and May (AW, MB and comparatively low at SW) and a second, lower peak, occurred during June (HW), July (MB and SW) or August (AW), depending on the respective site (Figure 3-12). In 2014, the overall number of nymphs was again similar at HW (n= 230), slightly lower at AW (n= 163) and MB (n= 149) and higher to previous years at SW (n= 166), leading to a comparable number of nymphs at AW, MB and SW in 2014. The seasonal dynamics again showed a similar pattern, with a large, first peak and a lower (to no) second peak. At AW, the first peak was interrupted by a decline in nymph nymbers in April but then recovered in May. At HW and MB, seasonal dynamic patterns of nymphs were again highly similar, with the first peak of the season taking place between April and May 2014. This was followed by a continuous decrease of nymphal activity towards the end of the sampling season. The dynamics of nymphal abundance at SW in the last season of sampling corresponded for the first season: a first, large peak was observed in May, followed by a second, somewhat smaller peak in in August (Figure 3-12).

I. ricinus females were less abundant than larvae and nymphs, and their activity could last throughout the entire season from March until October, but depending on site and year. The only common peak in female activity at all four sites was observable in May 2014, when there was also a peak in nymphal abundance at all four sampling sites. Apart from this, collection of females by flagging showed a high level of spatio-temporal variation and a low level of overall abundance.

3.2.10 Vegetation versus vertebrates – A comparison of tick activity patterns on host and by flagging

Tick phenology is the result of complex interactions between abiotic and biotic variables (Pérez et al. 2012, Léger et al. 2013). It is not only the evaluation of the relative contributions of influencing variables to the resulting tick population and their dynamics, but also the simple analysis of tick activity patterns in the field which poses problems for

scientists. Here, I compare the seasonal dynamics of juvenile *I. ricinus* questing on vegetation with the infestation patterns of *I. ricinus* on small mammals at the four sampling sites, highlighting the differences and reliability between these two sampling methods for the different developmental stages of *I. ricinus*.

Larvae

The estimates of larval activity obtained by the two methods clearly differed from each other (Figure 3-13). As stated in the previous section, the overall larval abundance on vegetation showed an extensive level of asynchronous and spatially non-comparable variation, and therefore revealed no consistent pattern. There was a certain amount of overall variation observable for the mean abundance of larvae on hosts between the sites and years (Figure 3-13), but there was a longer overall duration of larval activity on hosts. Whenever hosts were captured, larvae were also found. These findings suggest that data on *I. ricinus* larval activity obtained by flagging are of limited reliability for determining the actual, temporal spectrum of activity of this developmental stage. For studies of transmission dynamics, the seasonal activity of *I. ricinus* larvae collected from hosts is more reliable than from flagging. Furthermore, the main larval activity on host individuals was not generally observable during summer months (June, July) and not generally unimodal distributed, but varied between sites and years (Figure 3-13). In fact, there were several kinds of activity patterns present at the four investigated sites, varying between major larval activity in spring (AW 2012, AW 2014), in summer (AW 2013, MB 2013, MB 2014, SW 2014) or in autumn (HW 2013, HW 2014, MB 2014, SW 2013), but most often showing a combination of major larval activity at different times of the tick activity season with up to three activity peaks during one season (HW 2014).

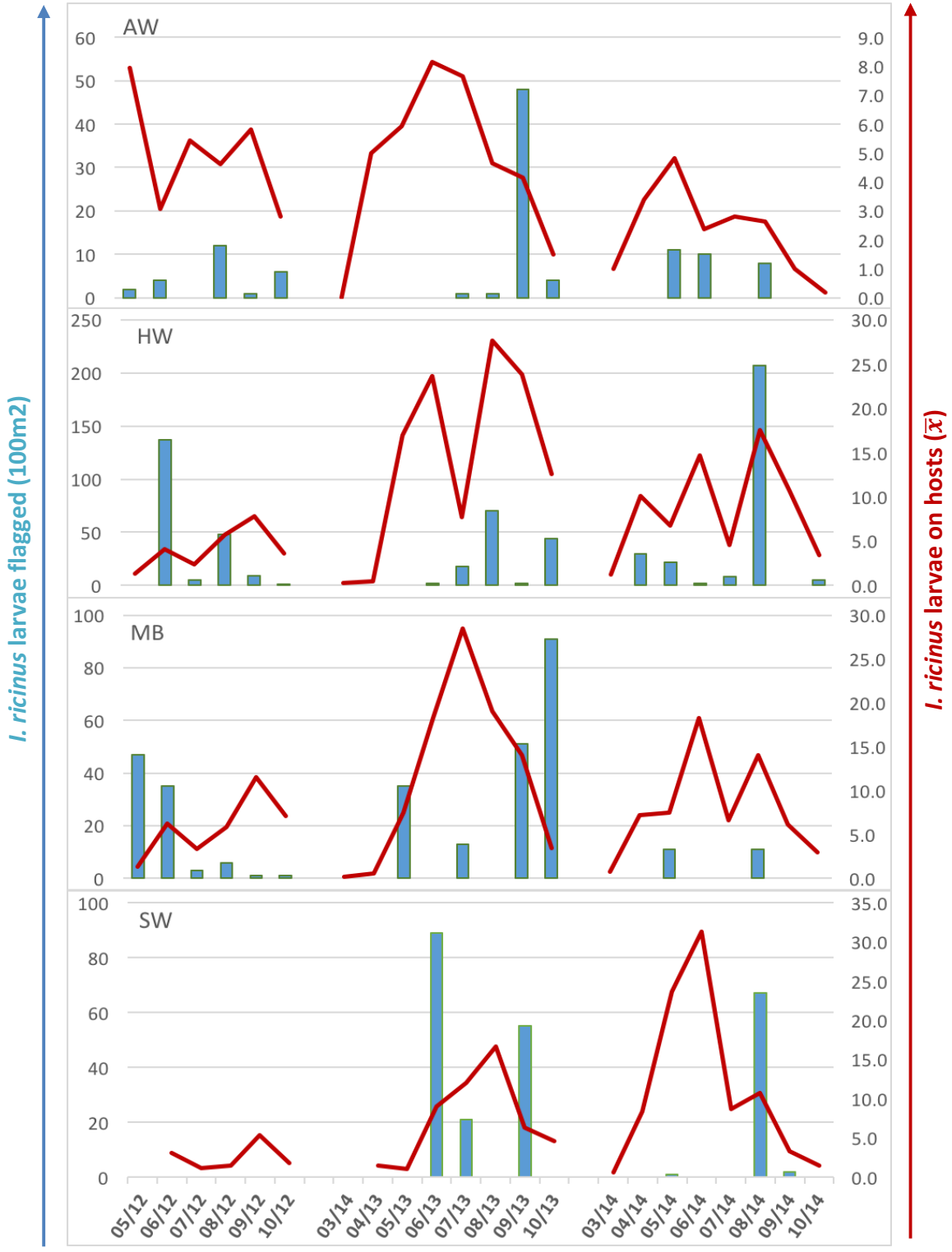


Figure 3-13: Comparison of the seasonal abundance dynamics *of I. ricinus* larvae collected by flagging (blue bars) on 100m^2 and the mean abundance of *I. ricinus* larvae (red line) on the major host species (*A. flavicollis* and *M. glareolus* at AW, HW, MB and SW from 2012 to 2014. For reasons of better visualization of the relationships between the two methods, the axes were chosen according to the respective values and can differ between the sites

Nymphs

The comparison of nymphs by flagging and by their mean abundance on hosts showed a different pattern to that found for the larvae (Figure 3-13). As previously indicated, a clear pattern of seasonal nymphal activity could be observed by flagging at each site (Figure 3-12), with the period of nymphal activity lasting throughout the entire sampling period of every season from 2012 to 2014 (except MB in October 2014). Major activity occurred in the spring with comparable total numbers of nymphs per season (except SW). However, the mean abundance of nymphs on hosts showed no clear pattern. This was extremely low (Table 3-5) and spatially as well as temporally inconsistent. In 2012 and 2014, there were hardly any *I. ricinus* nymphs at all on small mammal hosts. But even in 2013, when the aggregation of ticks on hosts was elevated due to the decline in host populations at HW, MB and SW, mean numbers of nymphs on hosts were still low and did not show a recognizable pattern among sites. Therefore, the flagging method seemed to represent the more accurate and adequate way of tick collection for determining seasonal dynamics.

Larvae on hosts versus nymphs on vegetation

The previous two sections on determining the comparative seasonal abundance of *I. ricinus* larvae and nymphs by flagging and on host individuals has shown that the accessibility of the instars differs greatly between the different sampling methods. The sampling of *I. ricinus* larvae showed a more reliable pattern by collection from small mammal hosts (Figure 3-13), whereas nymphal patterns of activity exhibited much more distinct seasonal peaks and distributions by flagging (Figure 3-14). Both sampling methods, considered to be more adequate to grasp the seasonal activity dynamics of the respective instar, showed a comparatively more continuous and transferable pattern of activity between sites and years. But still there was a lot of spatio-temporal variation.

A larger proportion of nymphs than larvae became active earlier in the year, with the peak of nymphal activity usually being located between spring and early summer (Figure 3-15). In contrast, even though a few larvae of *I. ricinus* were already found on hosts early in the year, they generally exhibited their major seasonal activity after the onset of nymphal activity (Figure 3-15), between late spring and autumn. By comparing the abundance of *I. ricinus* larvae on small mammals in one year with the number of flagged nymphs in the next year it is possible to determine the influence of small mammal populations (feeding

larvae) to the abundance of nymphs in the following season. This showed was no consistent pattern, either between years at one site, or between the sites. Only the site

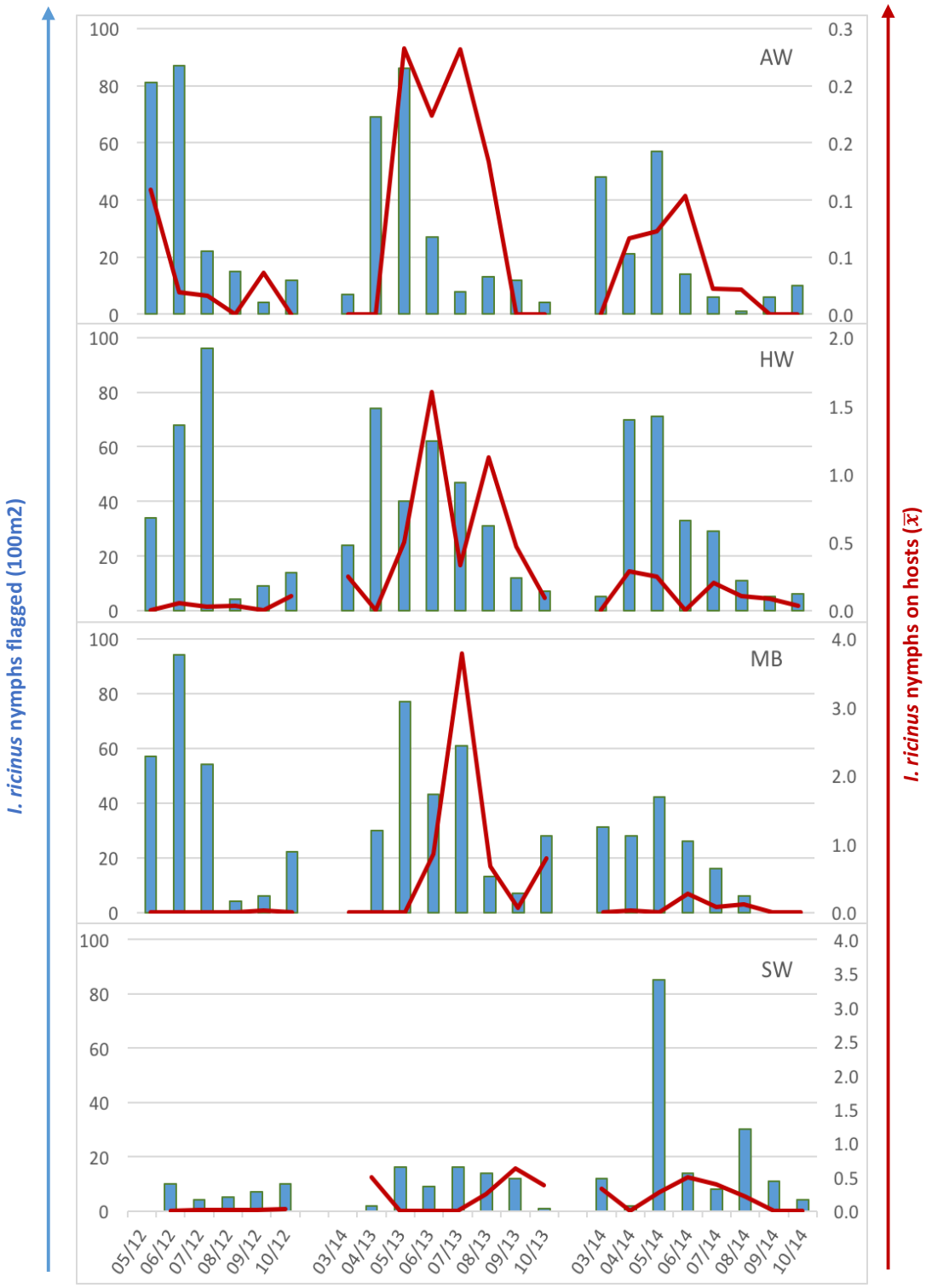


Figure 3-14: Comparison of the seasonal abundance dynamics of *I. ricinus* nymphs collected by flagging (blue bars) on 100m^2 and the mean abundance of *I. ricinus* nymphs (red line) on the major host species *A. flavicollis* and *M. glareolus* at AW, HW, MB and SW from 2012 to 2014. For reasons of better visualization of the relationships between the two methods, the axes were chosen according to the respective values and can differ between the sites

SW showed a pattern which would be expected if the small mammal population influenced the number of nymphs in the following year. First, in 2012, a low mean number of larvae was found feeding on hosts ($\bar{x}_{2012}$= 2.6 ± 5.3), followed by low numbers of nymphs in the next season ($\bar{x}_{2013}$= 10 ± 1.1). Second, higher numbers of larvae per host in 2013 ($\bar{x}_{2013}$= 7.7 ± 12.2) were followed by a higher number of nymphs on the vegetation in the next season ($\bar{x}_{2014}$= 20.8 ± 2.2). However, a pattern like this was not observable at any of the other three sites, and the opposite pattern could even be observed, e.g. at AW, where a higher larval abundance on hosts in 2013 ($\bar{x}_{2012}$= 5.1 ± 7.1; $\bar{x}_{2013}$= 5.5 ± 7.6) was followed by a decrease in nymphal abundance on vegetation in 2014 ($\bar{x}_{2013}$= 28.3 ± 2.7; $\bar{x}_{2014}$= 20.4 ± 2.2).

This raises the question of whether or not rodent populations are important for determining the abundance of nymphs in a given area.

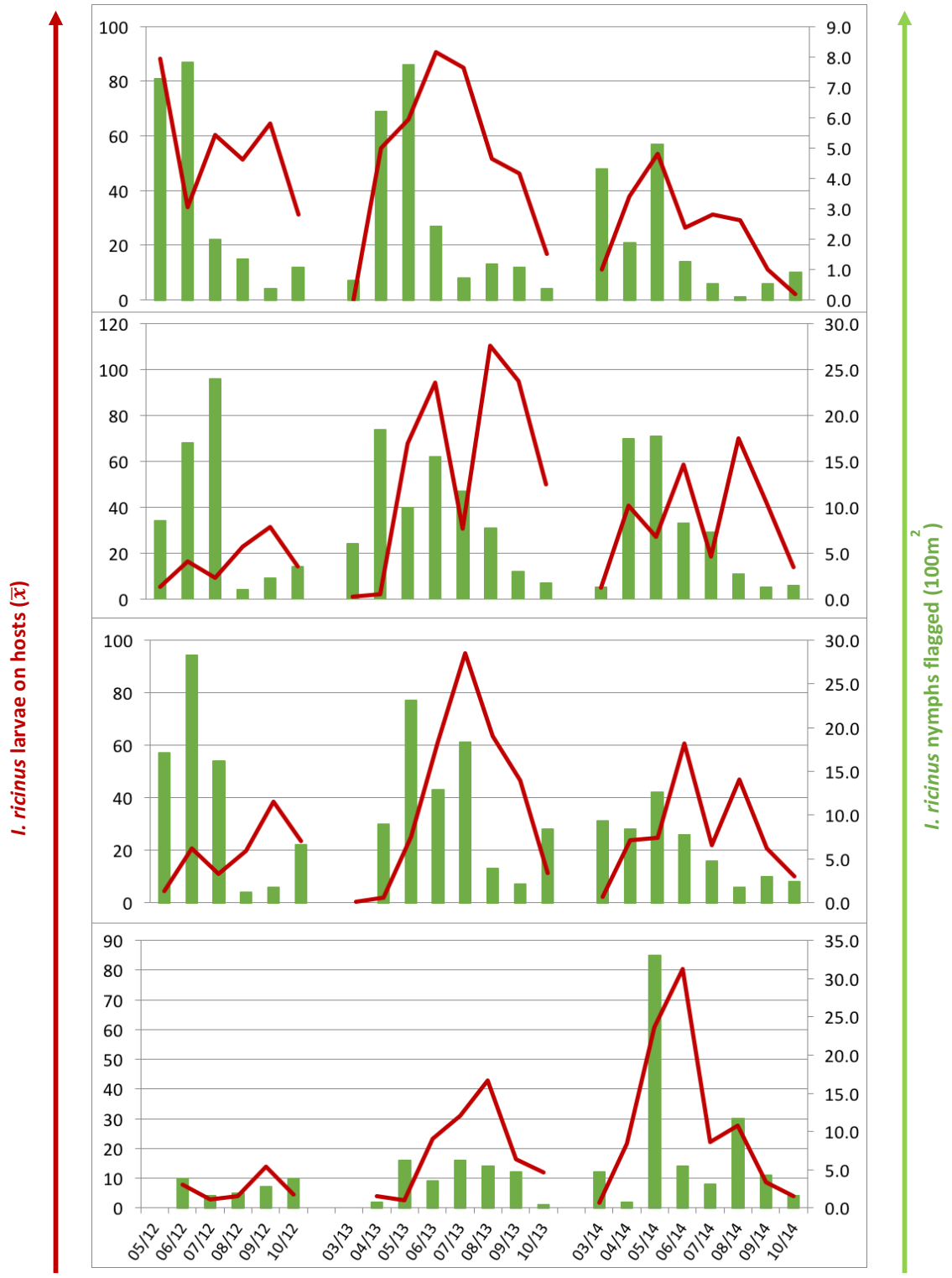


Figure 3-15: Comparison of the relationship between the mean abundance of *I. ricinus* larvae (red line) on the major host species (*A. flavicollis* and *M. glareolus*) and the seasonal abundance of *I. ricinus* nymphs per 100m2 (green bars) collected by flagging at AW, HW, MB and SW from 2012 to 2014. For reasons of better visualization of the relationships between the two methods, the axes were chosen according to the respective values and can differ between the sites

3.2.11 How do biotic and abiotic factors shape the abundance of ticks? A statistical approach

This statistical modelling approach using GAM aimed to assess the most important factors that shape tick abundance and activity at the four sampling sites. Models were established to examine (i) the larval *I. ricinus* burden of small mammal hosts (*Af* and *Mg*, taken together and separately) and (ii) the questing activity of *I. ricinus* nymphs at the four sites.

Larvae on small mammal hosts

Three different models were used to investigate the larval burden on small mammal hosts, one for both dominant host species (*Af* and *Mg*) together and one for each species separately. This was done to examine the influence of the overall number of available hosts on the tick-host-system among the sites, as well as to elucidate potential host species-specific influences on tick burdens. The deviance explained by the best estimate models was 51.1% ($\bar{R}^2$= 0.4) for the complete approach (*Af* and *Mg*, n= 1,518), 49.8% for *Af* ($\bar{R}^2$= 0.373, n= 819) and 30.9% for *Mg* ($\bar{R}^2$= 0.31, n= 699).

Altogether, the patterns of larval infestation on small mammal hosts were based on a complex network of interactions (Table 3-8), with the abundance of *I. ricinus* larvae on hosts being related to intrinsic characteristics of the host population and microclimatic factors (long-term and short-term). The leaf litter layer was shown to have the greatest influence on small mammal infestation with *I. ricinus* larvae. All three models included significant microclimatic variables measured in the leaf litter layer (short-term, 9 days mean °C, long-term accumulated saturation deficit), but neither 50cm nor 200cm height should significant effects. There was a significant, positive, short-term effect of the mean temperature in the leaf litter layer for the interval of 9 days prior to small mammal sampling. Thus, a higher temperature during this time interval in this layer led to an increase in larval abundance on hosts among the four sites. This effect was observable in the combined host approach (A) and the *Af* (B) approach. The second microclimatic variable present in all three models (A, B and C) was the long-term accumulated saturation deficit measured in the leaf litter layer. This was chosen as proxy for humidity limiting tick activity, and was shown to have a significant, negative effect on the abundance of *I. ricinus* larvae on small mammals overall (Table 3-8, model A), as well as on *Af* and *Mg* individually. A significant positive effect of male gender on *I. ricinus* burden was also identified (Table

3-8, models A, B and C), as was host weight (used as numerical proxy for age class affiliation), which was positively correlated with tick load, indicating that the heavier an individual was, the higher the larval *I. ricinus* burden became, regardless from which species the respective host originated.

With an increasing density of small mammal hosts, the individual tick burden decreased significantly (Table 3-7, model A). *Mg* was shown to harbour significantly less *I. ricinus* larvae than *Af* (Table 3-8, model A). In addition, a higher proportion of *Mg* led to a significant decrease in *I. ricinus* larval burdens (Table 3-7, models B and C).

These results showed the direct relationship between the dynamics of the tick-host-system and its environment and also depicted the complexity of this dynamic system.

Nymphs on vegetation

The final GAM describing *I. ricinus* nymphal activity on vegetation at the four sites was much simpler than the previously examined models describing *I. ricinus* larval burden on small mammals (Table 3-8, Table 3-9). The explained deviance of the best estimate model here was 51% ($\bar{R}^2$= 0.45, n= 56). Table 3-9 shows that the remaining significant effects were both due to microclimatic variables. There was a positive short-term influence shown for the mean soil moisture for the interval of 9 days prior to flagging on the abundance of *I. ricinus* nymphs on vegetation. In addition, there was a significantly negative influence of the long-term accumulated mean temperature measured at 50cm (Table 3-9). Thus, the abundance of *I. ricinus* nymphs on vegetation at the four sampling sites was related to long-term and short-term microclimatic factors, but there were no direct, significant effects of small mammal populations. This result was consistent with the comparison of flagged nymphs and larvae on host (Figure 3-15) where host abundance does not have an effect on the number of questing nymphs in the next year.

Table 3-8: Final model formulations of the GAM of *I. ricinus* larval burdens on the small mammal hosts *A. flavicollis* and *M. glareolus* (A), *A. flavicollis* (B), *M. glareolus* (C). Parameter estimates of all significant variables are shown. Short-term means were calculated as mean value of the interval before sampling. Long-term accumulated variables were accumulated from the first sampling session of the year on

(A) *I. ricinus* larvae on small mammals total - generalized additive model (GAM)

I. ricinus larvae ~ host species + gender + weight + abundance *A.flavicollis* and *Mg* + °C leaf litter (9 days mean) + long-term accumulated saturation deficit leaf litter + smoother(day/year), family= negative binomial, link= log

Parameter	Estimate	z	p
Mg	-1.1	-13.51	< 0.0001
gender (male)	0.46	8.6	< 0.0001
weight	0.03	4.4	< 0.0001
small mammal host abundance total	-0.01	-17.78	< 0.0001
short-term (9 days) mean °C leaf litter	0.14	6.29	< 0.0001
long-term accumulated saturation deficit leaf litter	-0.003	-3.97	< 0.0001

(B) *I. ricinus* larvae on *Af* - generalized additive model (GAM)

I. ricinus larvae ~ gender + weight + abundance *Mg* + °C leaf litter (9 days mean) + long-term accumulated saturation deficit leaf litter + smoother(day/year), family= negative binomial, link= log

Parameter	Estimate	z	p
gender (male)	0.3	4.68	< 0.0001
weight	0.02	6.22	< 0.0001
abundance of *Mg*	-0.03	-15.61	< 0.0001
short-term (9 days) mean °C leaf litter	0.13	5.24	< 0.0001
long-term accumulated saturation deficit leaf litter	-0.01	-4.65	< 0.0001

(C) *I. ricinus* larvae on *Mg* - generalized additive model (GAM)

I. ricinus larvae ~ gender + weight + gender:weight + culled deer/ha (t-1) + abundance *Mg* + abundance *Af* + long-term accumulated saturation deficit leaf litter + smoother(day/year), family= negative binomial, link= log

Parameter	Estimate	z	p
weight	0.04	3.08	0.002
abundance of *Mg*	-0.02	-6.9	< 0.0001
long-term accumulated saturation deficit leaf litter	-0.01	-4.38	< 0.0001

Table 3-9: Final formulation of the best estimate GAM of *I. ricinus* nymphal abundance on vegetation (D). Parameter estimates of all significant variables are shown. Short-term means were calculated as mean value of the interval (days) before sampling. Long-term accumulated variables were accumulated from the first sampling session of the year on

(D) *I.ricinus* nymphs on vegetation - generalized additive model (GAM)

I. ricinus nymphs ~ culled deer/ha (t-1) + mean soil moisture (6 days) + s(Long-term accumulated °C at 50 cm), famly= negative binomial, link= log			
Parameter	**Estimate**	**z**	**p**
short-term (6 days) mean soil moisture	0.02	2.02	0.04
long-term accumulated °C at 50cm	-0.001	-5.77	< 0.0001

3.3 Tick-borne pathogens

3.3.1 Pathogen presence, absence and prevalence

The ticks collected from hosts and from vegetation during the sampling seasons from 2012 to 2014 at the four sampling sites AW, HW, MB and SW were analyzed by qrtPCR for the presence of infection with five different TBPs of human and veterinary medical importance: *Babesia* spp., *Bbsl*, CNM, *Rspp* and TBE-V.

At first, I will give an impression of the overall infection prevalence in the tick species and hosts found at the different sites and then focus on the prevalence and patterns in *I. ricinus* larvae and nymphs as the major species and instars found on the dominant host species and on vegetation, and as the most important vectors of TBPs. Subsequently, I will examine the contribution of host characteristics and individual tick loads on the prevalence in rodent hosts and finally compare the prevalence in ticks on hosts with those from ticks collected by flagging on vegetation.

The presence of infection varied greatly. *Bbsl* and *Rspp* were the most abundant pathogens found in ticks on hosts and on vegetation, whereas *Babesia* spp. could not be detected at all. Tick infection with TBE-V was only detected in two *I. ricinus* larval samples from the same *S. araneus* individual (HW in 2012). A subset of the samples in 2013 was further examined for the presence of the emerging pathogen CNM, and will be addressed separately.

In the upcoming sections, I will therefore survey the prevalence of tick infection focusing on *Bbsl* and *Rspp*.

3.3.2 Host-tick-pathogen relationships- pathogens in ticks on small mammal hosts

In Figure 3-16, the overall prevalence for *Bbsl* and *Rspp* found at the four sites of investigation is shown together with the relative contribution of the four tick species present. The relative contribution was based on the number of positive tick samples per tick species on all hosts, to give a first, broad overview of the occurrence of *Bbsl* and *Rspp* in tick species. The largest proportion of ticks with *Bbsl* and *Rspp* infections could be allotted to *I. ricinus* larvae and nymphs, followed by ticks of the species *D. reticulatus*.

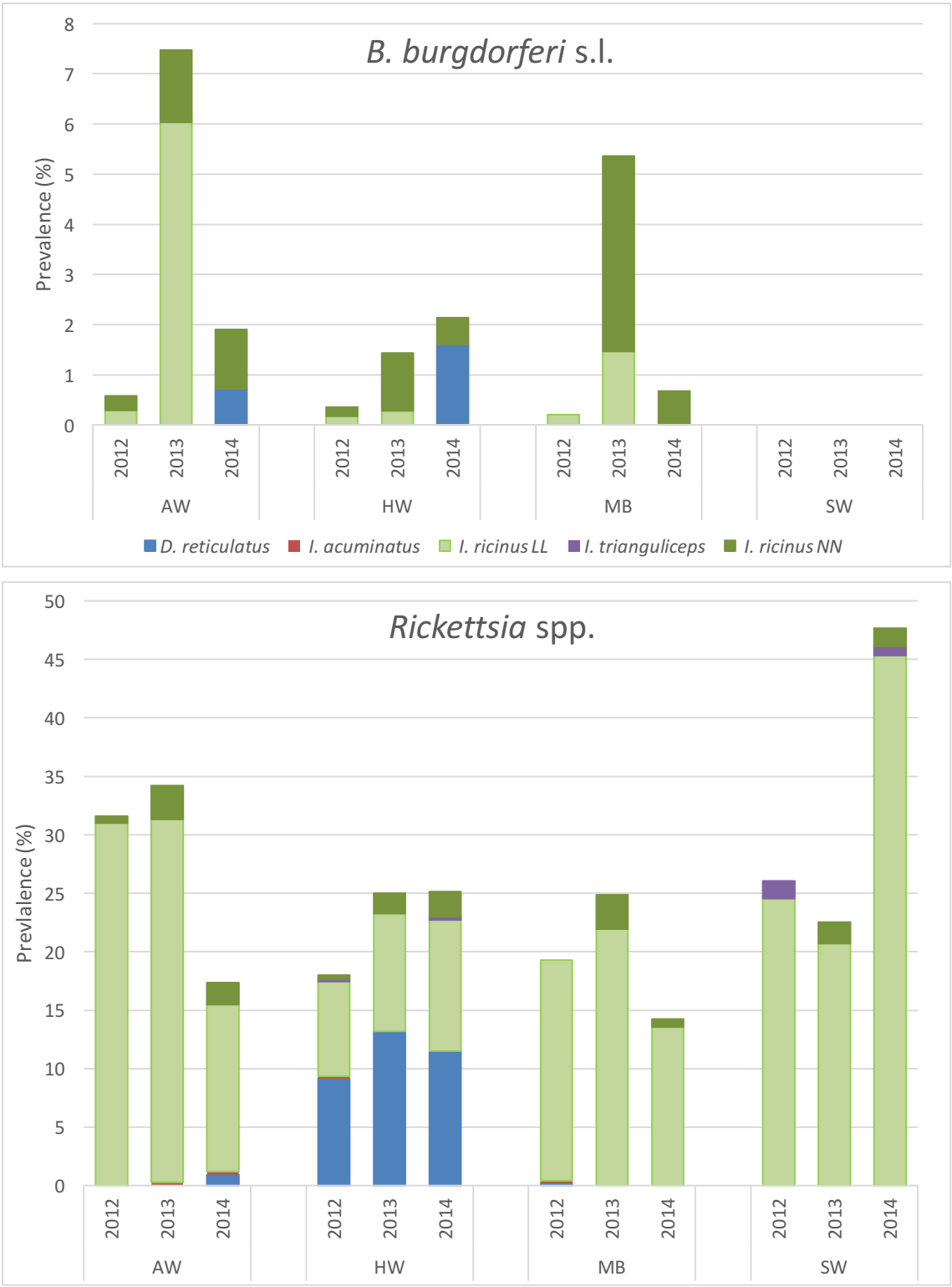


Figure 3-16: Overview of the overall prevalence (in %) for the most frequently occurring pathogens *B. burgdorferi* s.l. and *Rickettsia* spp. at the four field sites (AW, HW, MB and SW) from 2012 to 2014. Values include all host species and show the relative contribution (proportion of positive samples) of all present tick species and instar in the case of *I. ricinus*. LL= larvae, NN= nymphs

In the case of *Bbsl,* it seemed that both instars of *I. ricinus* contributed to the overall prevalence, with emphasis on the nymphal contribution (except for AW in 2013), whereas

for *Rspp*, *I. ricinus* larvae seemed to account for most of the overall prevalence (Figure 3-16). The contribution of *D. reticulatus* to the occurrence of *Bbsl* and *Rspp* was rather focal due to its restricted distribution (Figure 3-5). The infection of *D. reticulatus* with *Bbsl* was only detectable in 2014 at the sites AW and HW, while *Rspp* could be detected in this tick species in all three years of sampling at HW, and also at AW in 2014. *I. acuminatus* and *I. trianguliceps* did not contribute to the overall prevalence for *Bbsl*, and only to a limited extent for rickettsial prevalence, with the main presence of *I. trianguliceps* prevalence at SW (Figure 3-16).

The overall prevalence of *Bbsl* was highly variable between sites, with a total absence in ticks on hosts at SW. At the other three sites, prevalence was also highly variable between years within each site. But there was also a significant increase in *Bbsl* prevalence in *I. ricinus* larvae and nymphs from 2012 to 2013 at all three sites (Fisher's Exact Test: p_{AW}< 0.001, p_{HW}= 0.03, p_{MB}<0.001). The following decrease in *Bbsl* prevalence in 2014 was not significant (Fisher's Exact Test: p> 0.05).

The overall prevalence of *Rspp* was higher and more evenly distributed compared to *Bbsl*. The pathogen could be detected at all four sites and in all four years of sampling, with some variation between sites and between years, but not as extensive as for *Bbsl*. Larvae of *I. ricinus* accounted for the largest proportion of *Rspp* prevalence in total. At HW, a remarkable proportion of *D. reticulatus* was infected in all years of sampling, which contrasts with the remaining three sites.

3.3.3 Pathogen prevalence in *I. ricinus* on *A. flavicollis* and *M. glareolus*

We now know that *Af* harbors significantly more ticks than *Mg*. The next question to be addressed was whether the two species carry a different proportion of infected ticks. To examine this, the prevalence of *Bbsl* and *Rspp* in *I. ricinus* larvae and nymphs were analyzed.

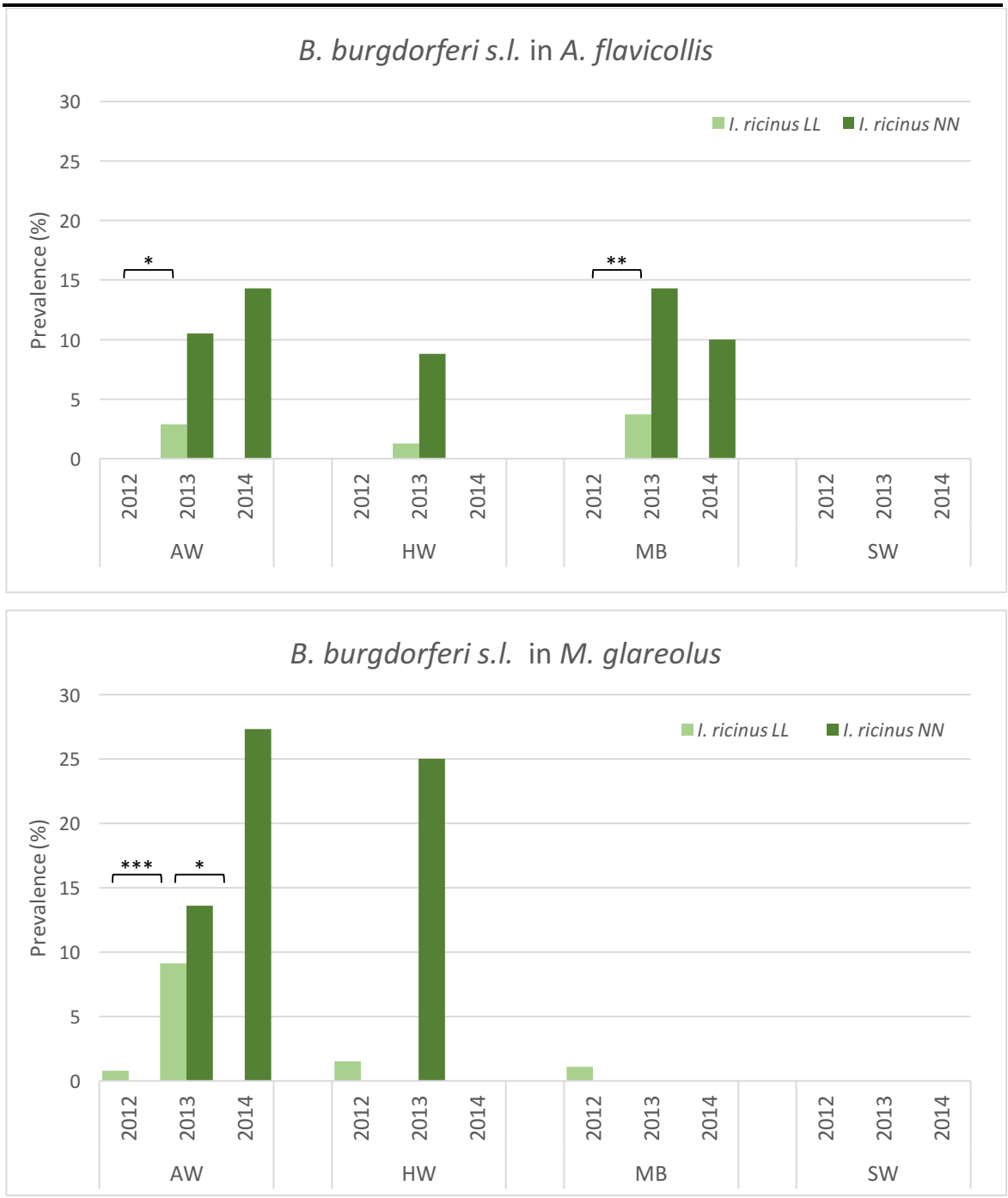


Figure 3-17: B. *burgdorferi s.l.*infection prevalence (%) in *I. ricinus* nymphs and larvae on *A. flavicollis* (top) and *M. glareolus* (bottom) at AW, HW, MB and SW from 2012 to 2014. Prevalence was calculated as percentage of positive / total samples. Asterisks indicate significant differences (p< 0.05) in larval prevalence according to Fisher's Exact Test (*: p< 0.05, **: p< 0.01, ***: p< 0.001). LL= larvae, NN= nymphs

B. burgdorferi s.l.

The prevalence rates of *Bbsl* (Figure 3-17) were different between the two host species with a high variability between years, sites and instars of ticks for both host species (Figure 3-17, Table 3-10). In *Af*, larval infection with *Bbsl* could only be detected in 2013 (2.9% at

AW, 1.3% at HW, 3.7% at MB). In *Mg*, infection of *I. ricinus* larvae with *Bbsl* could be found at all sites with *Bbsl* occurrence (0.8% at AW, 1.5% at HW and 1.1% at MB) in 2012, only at the site AW in 2013 (9.1%), but not in 2014 (Table 3-10). Nymphs of *I. ricinus* were not found to be infected with *Bbsl* in any of the dominant host species in 2012. In 2013, all sites with *Bbsl* occurrence revealed nymphal infection in ticks from *Af* (10.5% at AW, 8.8 % at HW, 14.3% at MB), as well as in ticks from *Mg* at AW (13.6%) and HW (25%). In 2014, nymphal prevalence of *Bbsl* in ticks from *Af* was only found at AW (14.3%) and MB (10%), but not at HW, and only at AW for *Mg* derived nymphs (27.3%).

At all sites and in all years, nymphs were more likely to be infected with *Bbsl* than larvae (Table 3-10). This pattern was consistent for *Mg* (Fisher's Exact Test: p=0.005) and *Af* (Fisher's Exact Test: p< 0.0001). It could further be shown that larval *Bbsl* prevalence were highest in 2013 (Figure 3-17, Table 3-10). For *Af* this pattern was detectable at all individual sites with *Bbsl* (AW, HW and MB), showing significantly increased prevalence rates at AW and MB for *Af* and at AW for *Mg* (Figure 3-17). There was an overall significant increase for *Af* (Fisher's Exact Test 2012/2013: p< 0.001, 2013/2014: p= 0.02). The same trend could be confirmed for *Mg* at AW (Fisher's Exact Test 2012/2013: p= 0.001, 2013/2014 p= 0.01). Moreover, larvae feeding on *Mg* were more likely to be positive for *Bbsl* than larvae feeding on *Af* (Fisher's Exact test, p_{Larvae}< 0.001).

***Rickettsia* spp.**

The pattern of rickettsial prevalence in *I. ricinus* larvae and nymphs from *Af* and *Mg* was different to that of *Bbsl* (Figure 3-17, Figure 3-18). Prevalence of *Rspp* was at an overall higher level than *Bbsl* (Table 3-10). *Rspp* was found at all four sites and in all years of sampling with some variation in space and time. *Rspp* could be detected in larvae throughout the sampling sites and years, while the occurrence of the pathogen in nymphs showed stronger fluctuations in space and time (Figure 3-18). The prevalence of *Rspp* in *I. ricinus* larvae was consistently higher than in nymphs (except for SW in 2013) and was significantly higher in *Af* (Fisher's Exact Test p< 0.0001), but not in *Mg* (p> 0.05). Furthermore, *Af* harbored a significantly higher proportion of *Rspp* positive larvae than *Mg* (χ^2= 6.58, p= 0.006). The most obvious trend observable in *I. ricinus* larvae was the

overall increase in prevalence rates in both host species from 2012 over 2013 to 2014 (Figure 3-18, Table 3-10).

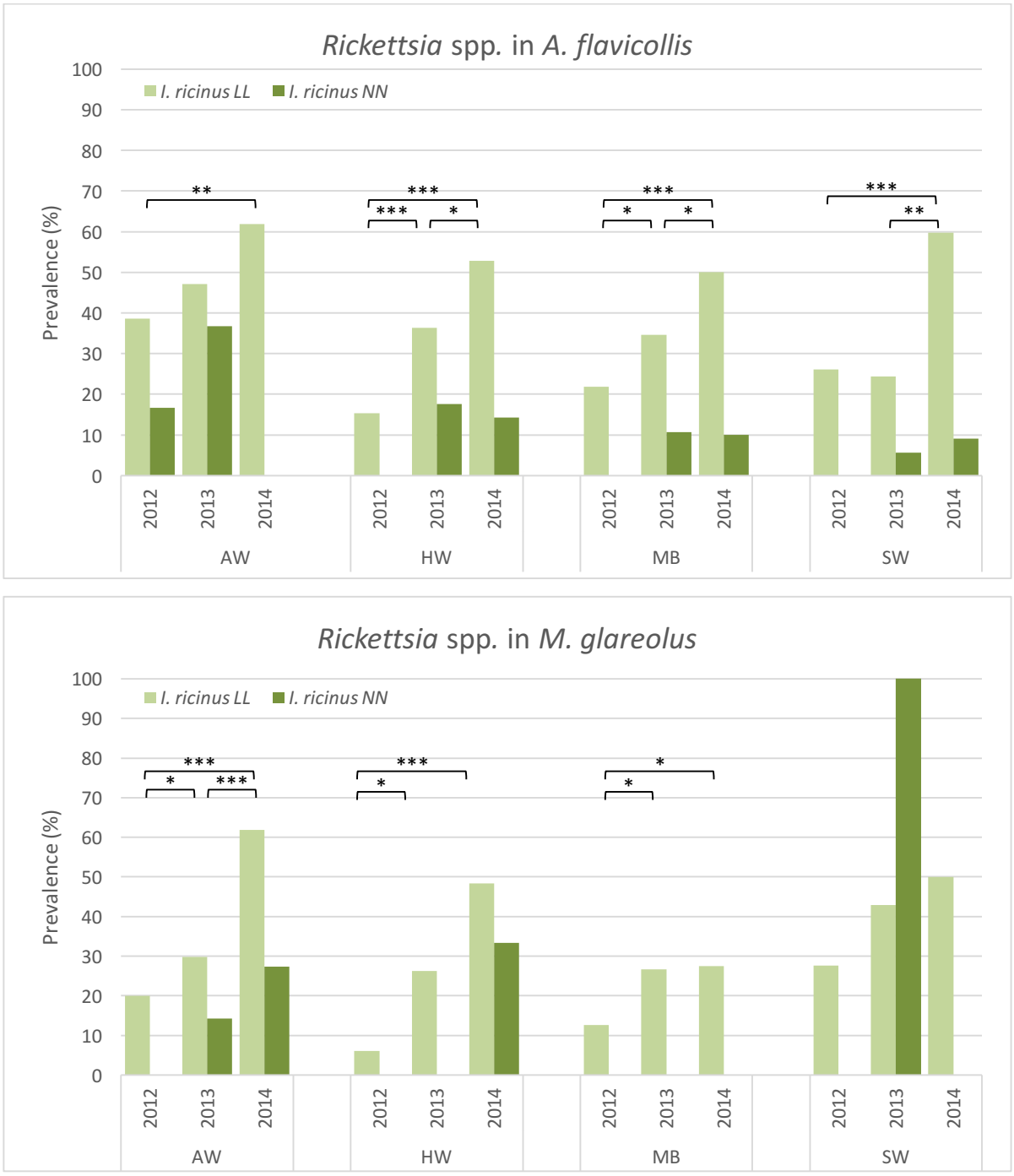


Figure 3-18: *Rspp* infection prevalence (%) in *I. ricinus* nymphs and larvae on *A. flavicollis* (top) and *M. glareolus* (bottom) at AW, HW, MB and SW from 2012 to 2014. Prevalence were calculated as percentage of positive / total samples. Asterisks indicate significant differences (p< 0.05) in larval prevalence according to Fisher's Exact Test (*: p< 0.05, **: p< 0.01, ***: p< 0.001). LL = larvae, NN = nymphs

Table 3-10: Prevalence of *Bbsl* and *Rspp* in *I. ricinus* larvae and nymphs at the sampling sites from 2012 to 2014. The number of larvae per pool is given as the mean (x̄) ± standard deviation (SD). Nymphs were analyzed individually. The prevalence is calculated as the ratio of infected/total samples, and the percentage of positive samples (%). In 2014, 120 randomly selected samples per site have been investigated, due to financial restrictions

A. flavicollis

		I. ricinus larvae		*I. ricinus* nymphs		*I. ricinus* total	
	larvae (x̄±SD)	n infected / analyzed (%)		n infected / analyzed (%)		n infected / analyzed (%)	
		Bbsl	*Rspp*	*Bbsl*	*Rspp*	*Bbsl*	*Rspp*
2012	5 ± 5.8	0 / 919 (0)	222 / 919 (24.2)	0 / 15 (0)	3 / 15 (20)	0 / 934 (0)	225 / 934 (24.1)
AW	6.9 ± 7.5	0 / 184 (0)	71 / 184 (38.6)	0 / 6 (0)	1 / 6 (16.7)	0 / 190 (0)	72 / 190 (37.9)
HW	4.2 ± 5	0 / 246 (0)	38 / 246 (15.4)	0 / 5 (0)	2 / 5 (40)	0 / 251 (0)	40 / 251 (15.9)
MB	4.7 ± 4.8	0 / 347 (0)	76 / 347 (21.9)	0 / 2 (0)	0 / 2 (0)	0 / 349 (0)	76 / 349 (21.8)
SW	4.5 ± 6.4	0 / 142 (0)	37 / 142 (26.1)	0 / 2 (0)	0 / 2 (0)	0 / 144 (0)	37 / 144 (25.7)
2013	12.8 ± 13.5	7 / 306 (2.3)	116 / 306 (37.9)	13 / 127 (10.2)	20 / 127 (15.7)	20 / 433 (4.6)	136 / 433 (31.4)
AW	9.1 ± 10	3 / 104 (2.9)	49 / 104 (47.1)	2 / 19 (10.5)	7 / 19 (36.8)	5 / 123 (4.1)	56 / 123 (45.5)
HW	18.3 ± 15.6	1 / 80 (1.3)	29 / 80 (36.3)	3 / 34 (8.8)	6 / 34 (17.6)	4 / 114 (3.5)	35 / 114 (30.7)
MB	15 ± 15	3 / 81 (3.7)	28 / 81 (34.6)	8 / 56 (14.3)	6 / 56 (10.7)	11 / 137 (8)	34 / 137 (24.8)
SW	7.2 ± 7.7	0 / 41 (0)	10 / 41 (24.4)	0 / 18 (0)	1 / 18 (5.6)	0 / 59 (0)	11 / 59 (18.6)
2014	10.9 ± 13.5	0 / 230 (0)	129 / 230 (56.1)	4 / 63 (6.3)	10 / 63 (15.9)	4 / 293 (1.4)	139 / 293 (47.4)
AW	4.7 ± 4.1	0 / 42 (0)	26 / 42 (61.9)	2 / 14 (14.3)	5 / 14 (35.7)	2 / 56 (3.6)	31 / 56 (55.4)
HW	15.2 ± 16.4	0 / 53 (0)	28 / 53 (52.8)	0 / 7 (0)	1 / 7 (14.3)	0 / 60 (0)	29 / 60 (48.3)
MB	15.9 ± 16.2	0 / 58 (0)	29 / 58 (50)	2 / 20 (10)	2 / 20 (10)	2 / 78 (2.6)	31 / 78 (39.7)
SW	14.4 ± 15	0 / 77 (0)	46 / 77 (59.7)	0 / 22 (0)	2 / 22 (9.1)	0 / 99 (0)	48 / 99 (48.5)

M. glareolus

		I. ricinus larvae		*I. ricinus* nymphs		*I. ricinus* total	
	larvae (x̄±SD)	n infected / analyzed (%)		n infected / analyzed (%)		n infected / analyzed (%)	
		Bbsl	*Rspp*	*Bbsl*	*Rspp*	*Bbsl*	*Rspp*
2012	2 ± 1.6	3 / 389 (0.8)	69 / 389 (17.7)	0 / 5 (0)	0 / 5 (0)	3 / 394 (0.8)	69 / 394 (17.5)
AW	2.3 ± 2.0	1 / 130 (0.8)	26 / 130 (20)	0 / 3 (0)	0 / 3 (0)	1 / 133 (0.8)	26 / 133 (19.5)
HW	2.2 ± 1.9	1 / 66 (1.5)	4 / 66 (6.1)	0 / 2 (0)	0 / 2 (0)	1 / 68 (1.5)	4 / 68 (5.9)
MB	1.7 ± 1.1	1 / 95 (1.1)	12 / 95 (12.6)	0 / 0 (0)	0 / 0 (0)	1 / 95 (1.1)	12 / 95 (12.6)
SW	1.8 ± 1.2	0 / 98 (0)	27 / 98 (27.6)	0 / 0 (0)	0 / 0 (0)	0 / 98 (0)	27 / 98 (27.6)
2013	4 ± 4.4	18 / 276 (6.5)	82 / 276 (29.7)	4 / 30 (13.3)	4 / 29 (13.8)	22 / 306 (7.2)	86 / 305 (28.2)
AW	3.7 ± 3.6	18 / 198 (9.1)	59 / 198 (29.8)	3 / 22 (13.6)	3 / 21 (14.3)	21 / 220 (9.5)	62 / 219 (28.3)
HW	6.4 ± 9	0 / 19 (0)	5 / 19 (26.3)	1 / 4 (25)	0 / 4 (0)	1 / 23 (4.3)	5 / 23 (21.7)
MB	3.8 ± 3.4	0 / 45 (0)	12 / 45 (26.7)	0 / 3 (0)	0 / 3 (0)	0 / 48 (0)	12 / 48 (25)
SW	5.9 ± 6.1	0 / 14 (0)	6 / 14 (42.9)	0 / 1 (0)	1 / 1 (100)	0 / 15 (0)	7 / 15 (46.7)
2014	3.3 ± 3.6	0 / 134 (0)	64 / 134 (47.8)	3 / 36 (8.3)	10 / 36 (27.8)	3 / 170 (1.8)	74 / 170 (43.5)
AW	2.4 ± 2.6	0 / 55 (0)	34 / 55 (61.8)	3 / 11 (27.3)	3 / 11 (27.3)	3 / 66 (4.5)	37 / 66 (56.1)
HW	5.2 ± 6	0 / 29 (0)	14 / 29 (48.3)	0 / 21 (0)	7 / 21 (33.3)	0 / 50 (0)	21 / 50 (42)
MB	3.4 ± 3.3	0 / 40 (0)	11 / 40 (27.5)	0 / 4 (0)	0 / 4 (0)	0 / 44 (0)	11 / 44 (25)
SW	4.6 ± 5.0	0 / 10 (0)	5 / 10 (50)	0 / 0 (0)	0 / 0 (0)	0 / 10 (0)	5 / 10 (50)

As indicated by the asterisks in Figure 3-18, a significant increase of *Rspp* prevalence between individual years of sampling (2012/2013 and 2013/2014) could be observed for *Af* at HW, MB and SW (SW: only 13/14), and for *Mg* at AW, HW and MB (HW and MB: only 12/13). The overall increase of *Rspp* prevalence in larvae from 2012 to 2014 was shown to be significant at all sites and for both host species, except for *Rspp* in ticks on *Mg* at SW.

Tick engorgement status and pathogen prevalence

The engorgement status of larvae on hosts was assessed as a reference to the time a tick had been attached to its host at the time of collection. The relationship between the level of engorgement and the infection of *I. ricinus* larvae with *Bbsl* and *Rspp*. is shown in Table 3-11.

Regarding *Bbsl* infection according to the level of engorgement it could be shown that in ticks from *Af*, there was no positive sample of *Bbsl* for engorgement class 1. The predominant source of *Bbsl* infection could be detected in larvae of the most common engorgement class 2. Furthermore, a single sample of class 3 larvae was found positive for *Bbsl* on *Af*. The pattern of *Bbsl* infection in ticks on *Mg* was similar to that of *Af*: only one sample of class 1 was positive for *Bbsl* on *Mg*. The highest proportion of positive samples was again found for larvae of engorgement class 2, and only a single sample of class 3 was positive.

For *Rspp*, the pattern of prevalence among the three classes of engorgement differed substantially from that of *Bbsl* infection*:* here, the pathogen was more widely and evenly distributed among larvae of different engorgement classes (Table 3-11). The proportion of positive larval samples was comparable between engorgement classes and for both host species (Fisher's Exact Test $p > 0.05$).

Table 3-11: *Bbsl* and *Rspp* prevalence in *I. ricinus* larvae on *A. flavicollis* and *M. glareolus* at the four sampling sites in 2012 and 2013 according to the level of engorgement. Engorgement classes refer to 1= (almost) unfed, 2= medium engorged, 3= (almost) fully engorged. Prevalence are calculated as proportion of infected/ analyzed samples and (%)

***B. burgdorferi* s.l. in *I. ricinus* larvae**

	n infected / analyzed (%)					
	A. flavicollis			***M. glareolus***		
class	**1**	**2**	**3**	**1**	**2**	**3**
2012	0/185 (0)	0/581 (0)	0/153 (0)	0/45 (0)	3/290 (1)	0/54 (0)
AW	0/29 (0)	0/147 (0)	0/8 (0)	0/6 (0)	1/113 (0.9)	0/11 (0)
HW	0/53 (0)	0/147 (0)	0/46 (0)	0/11 (0)	1/42 (2.4)	0/13 (0)
MB	0/81 (0)	0/185 (0)	0/81 (0)	0/16 (0)	1/65 (1.5)	0/14 (0)
SW	0/22 (0)	0/102 (0)	0/18 (0)	0/12 (0)	0/70 (0)	0/16 (0)
2013	0/47 (0)	6/243 (2.5)	1/16 (6.3)	1/16 (6.3)	16/230 (7)	1/30 (3.3)
AW	0/20 (0)	2/75 (2.7)	1/9 (11.1)	1/11 (9.1)	16/167 (9.6)	1/20 (5)
HW	0/9 (0)	1/67 (1.5)	0/4 (0)	0/1 (0)	0/17 (0)	0/1 (0)
MB	0/16 (0)	3/64 (4.7)	0/1 (0)	0/2 (0)	0/34 (0)	0/9 (0)
SW	0/2 (0)	0/37 (0)	0/2 (0)	0/2 (0)	0/12 (0)	0/0 (0)
Total	0/232 (0)	6/824 (0.7)	1/169 (0.6)	1/61 (1.6)	19/520 (3.7)	1/84 (1.2)

***Rickettsia* spp. in *I. ricinus* larvae**

	n infected / analyzed (%)					
	A. flavicollis			***M. glareolus***		
class	**1**	**2**	**3**	**1**	**2**	**3**
2012	26/185 (14.1)	162/581 (27.9)	34/153 (22.2)	8/45 (17.8)	54/290 (18.6)	7/54 (13)
AW	2/29 (6.9)	67/147 (45.6)	2/8 (25)	1/6 (16.7)	22/113 (19.5)	3/11 (27.3)
HW	6/53 (11.3)	22/147 (15)	10/46 (21.7)	2/11 (18.2)	2/42 (4.8)	0/13 (0)
MB	17/81 (21)	41/185 (22.2)	18/81 (22.2)	3/16 (18.8)	9/65 (13.8)	0/14 (0)
SW	1/22 (4.5)	32/102 (31.4)	4/18 (22.2)	2/12 (16.7)	21/70 (30)	4/16 (25)
2013	10/47 (21.3)	103/243 (42.4)	3/16 (18.8)	6/16 (37.5)	72/230 (31.3)	4/30 (13.3)
AW	8/20 (40)	39/75 (52)	2/9 (22.2)	4/11 (36.4)	52/167 (31.1)	3/20 (15)
HW	0/9 (0)	29/67 (43.3)	0/4 (0)	0/1 (0)	5/17 (29.4)	0/1 (0)
MB	2/16 (12.5)	25/64 (39.1)	1/1 (100)	1/2 (50)	10/34 (29.4)	1/9 (11.1)
SW	0/2 (0)	10/37 (27)	0/2 (0)	1/2 (50)	5/12 (41.7)	0/0 (0)
Total	36/232 (15.5)	265/824 (32.2)	37/169 (21.9)	14/61 (23)	126/520 (24.2)	11/84 (13.1)

3.3.4 Pathogen prevalence in ticks on hosts versus ticks on vegetation

In the following section, the prevalence of *Bbsl, Rspp* and CNM in *I. ricinus* larvae and nymphs on hosts (*Af* and *Mg*) were compared to the prevalence obtained from *I. ricinus* larvae and nymphs from flagging. Furthermore, the results of the analysis of *Rspp* genospecies in ticks from hosts and from vegetation is shown.

***B. burgdorferi* s.l.**

Bbsl was hardly found in flagged larvae at the four sampling sites during the study, and could only be detected at SW in 2013. Here, 3 out of 5 larval pools (60%) tested positive for *Bbsl* (Table 3-14). This clearly contrasted to the prevalence found in larvae from small mammal hosts, which was detected at all sites except SW (Figure 3-19) at a low overall level and being highest in 2013 (max. of 7% at AW). The prevalence of *Bbsl* in nymphs on vegetation showed a more continuous pattern than larvae. *Bbsl* could be detected at all sites and most years (not at HW in 2014, not at SW in 2012), but with a remarkable amount of variation between years (Figure 3-19). At AW, MB and SW, nymphal *Bbsl* prevalence on vegetation was highest in 2013 among sampling years. Only at HW did it decrease over time (Figure 3-19). Nymphal prevalence on vegetation tended to be higher compared to the *Bbsl* prevalence in host-derived ticks in 2012 and 2013.

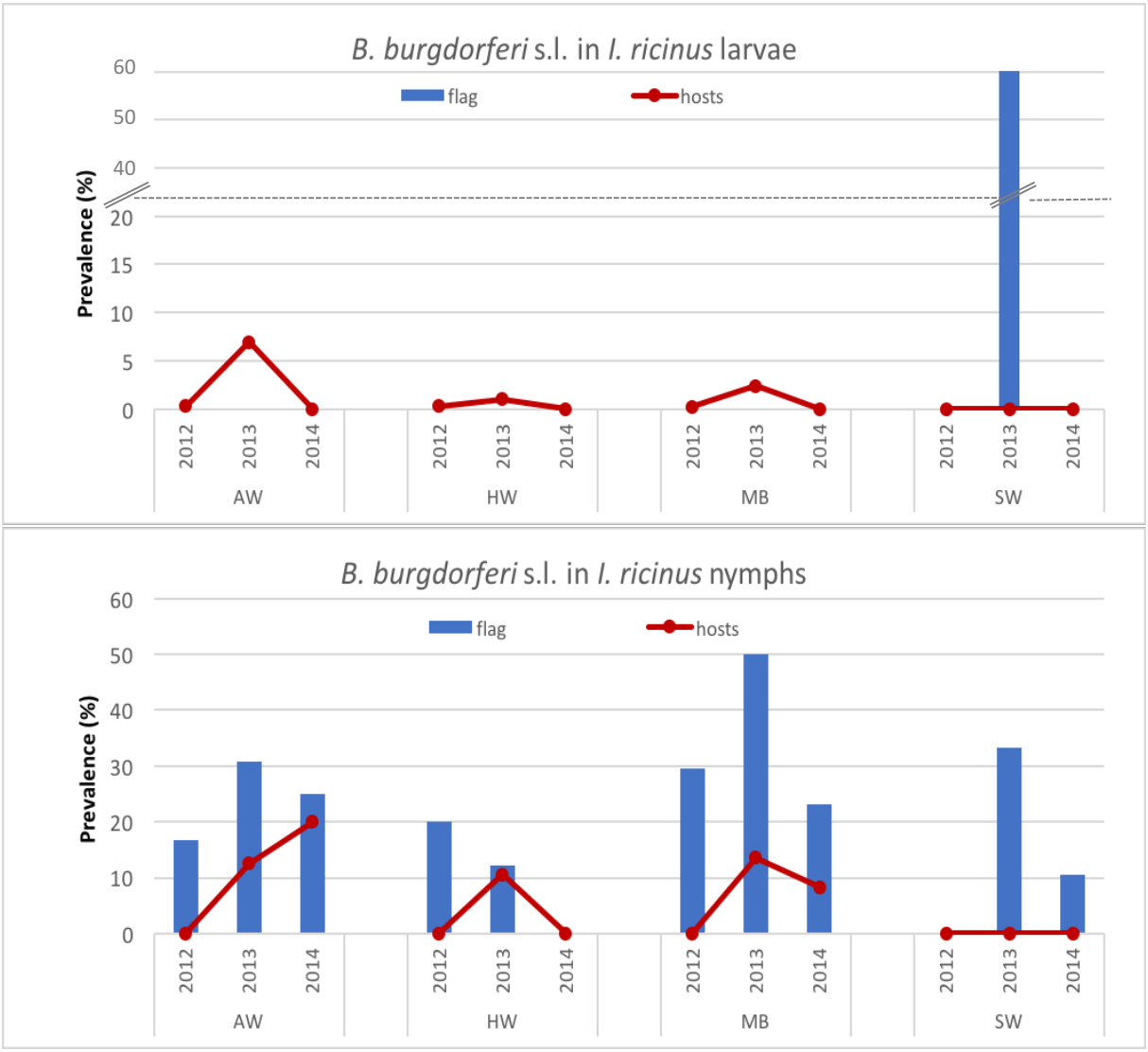


Figure 3-19: Comparison of *B. burgdorferi* s.l. prevalence in *I. ricinus* larvae (top) and nymphs (bottom) collected on host individuals (red line) and on vegetation (blue bars) at AW, HW, MB and SW from 2012 to 2014. Prevalence are based on positive/examined samples (%).The vertical axis in the above part of the diagram was interrupted for better visualization

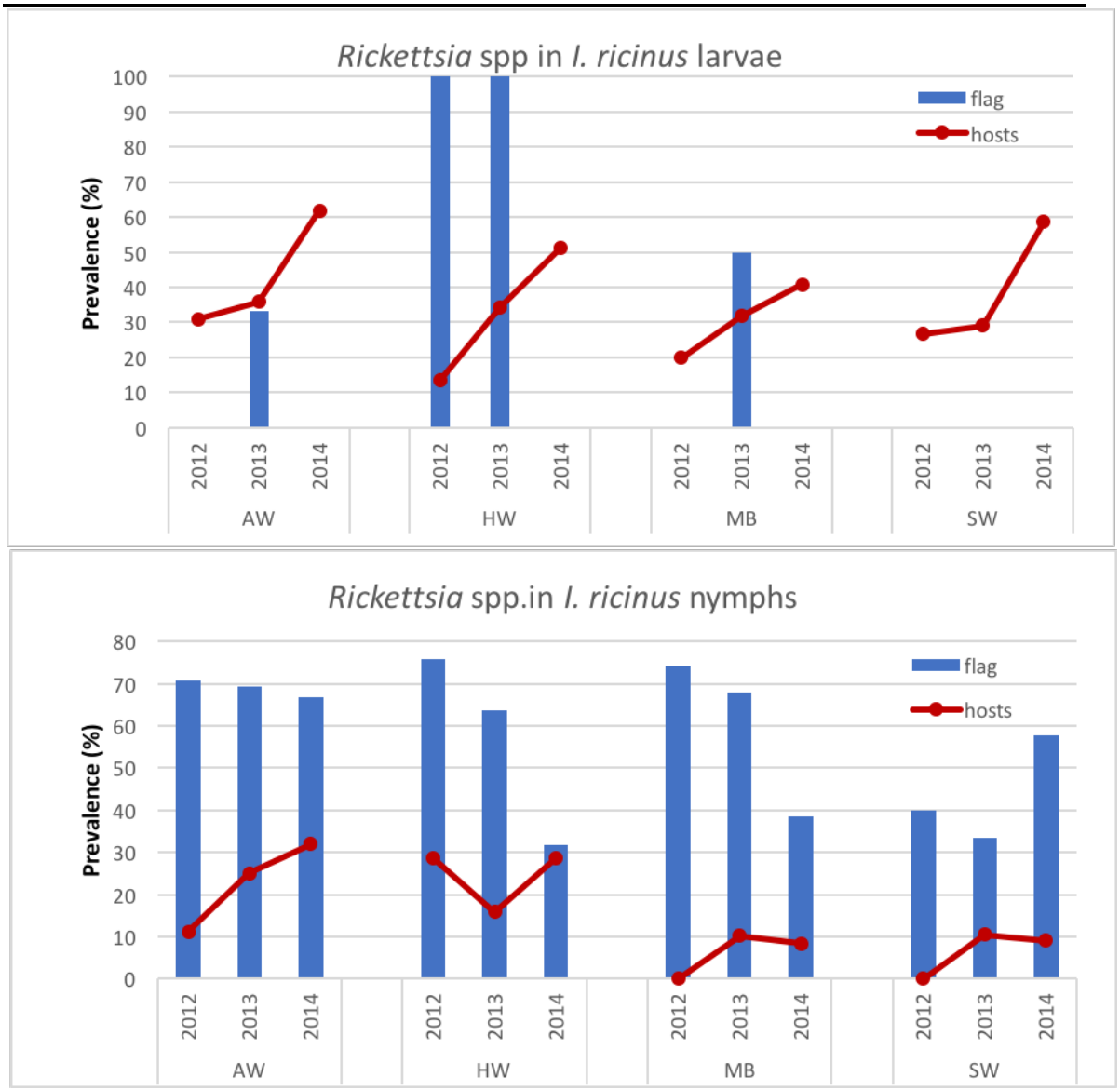


Figure 3-20: Comparison of *Rickettsia* spp. prevalence in *I. ricinus* larvae (top) and nymphs (bottom) collected on host individuals (red line) and on vegetation (blue bars) at AW, HW, MB and SW from 2012 to 2014. Prevalence are based on positive/examined samples (%)

Rickettsia spp.

A patchy occurrence with high amounts of variation in the *Rspp* prevalence of flagged larvae could be observed between the sites and years (Figure 3-20). The highest overall prevalence rates per site were detected in the 2013. This pattern held true for AW, HW and MB (100% at HW in 2012 and 2013), while there were no *Rspp* found in larvae on vegetation at SW in any year (Table 3-14). The prevalence of *Rspp* in flagged larvae was more variable than larval prevalence of *Rspp* in ticks on hosts (Figure 3-20). Distinct

patterns or tendencies were therefore hard to detect. The overall increase of *Rspp* prevalence as observed in larvae on hosts could not be confirmed for larvae on vegetation.

Table 3-12: *Bbsl* and *Rspp* prevalence in *I. ricinus* larvae and nymphs collected by flagging at the sampling sites AW, HW, MB and SW from 2012 to 2014 (Larvae only 2012 and 2013). Larval prevalence is given as positive/analyzed pools (%), as samples were analyzed in pools per month without examining the number per sample. Nymphal prevalence is shown as infected samples/ examined samples (%) with the number of nymphs per pool ($\bar{x}$ ± SD)

***B. burgdorferi* s.l.**	**Larvae infected / analyzed (%)**	**Nymphs infected / analyzed (%)**	**Nymphs per pool ($\bar{x}$ ± SD)**
2012	0 / 8 (0)	17 / 81 (21)	8.9 ± 2.2
AW	0 / 2 (0)	4 / 24 (16.7)	9.1 ± 2.3
HW	0 / 3 (0)	5 / 25 (20)	9 ± 2
MB	0 / 3 (0)	8 / 27 (29.6)	8.9 ± 2.3
SW	0 / 0 (0)	0 / 5 (0)	7.2 ± 2.8
2013	3 / 20 (15)	29 / 96 (30.2)	8.8 ± 2.3
AW	0 / 3 (0)	8 / 26 (30.8)	8.7 ± 2.4
HW	0 / 6 (0)	4 / 33 (12.1)	9 ± 2.4
MB	0 / 6 (0)	14 / 28 (50)	9.3 ± 1.9
SW	3 / 5 (60)	3 / 9 (33.3)	7.4 ± 3
2014	NA	8 / 63 (12.7)	8.3 ± 2.8
AW	NA	3 / 12 (25)	7.8 ± 3
HW	NA	0 / 19 (0)	8.3 ± 3
MB	NA	3 / 13 (23.1)	8.1 ± 2.8
SW	NA	2 / 19 (10.5)	8.6 ± 2.7
Total	13 / 28 (46.6)	54 / 240 (22.5)	8.7 ± 2.4
***Rickettsia* spp.**	**Larvae infected / analyzed (%)**	**Nymphs infected / analyzed (%)**	**Nymphs per pool ($\bar{x}$ ± SD)**
2012	3 / 8 (37.5)	58 / 81 (71.6)	8.9 ± 2.2
AW	0 / 2 (0)	17 / 24 (70.8)	9.1 ± 2.3
HW	3 / 3 (100)	19 / 25 (76)	9 ± 2
MB	0 / 3 (0)	20 / 27 (74.1)	8.9 ± 2.3
SW	0 / 0 (0)	2 / 5 (40)	7.2 ± 2.8
2013	10 / 20 (50)	61 / 96 (63.5)	8.8 ± 2.3
AW	1 / 3 (33.3)	18 / 26 (69.2)	8.7 ± 2.4
HW	6 / 6 (100)	21 / 33 (63.6)	9 ± 2.4
MB	3 / 6 (50)	19 / 28 (67.9)	9.3 ± 1.9
SW	0 / 5 (0)	3 / 9 (33.3)	7.4 ± 3
2014	NA	30 / 63 (47.7)	8.3 ± 2.8
AW	NA	8 / 12 (66.7)	7.8 ± 3
HW	NA	6 / 19 (31.6)	8.3 ± 3
MB	NA	5 / 13 (38.5)	8.1 ± 2.8
SW	NA	11 / 19 (57.9)	8.6 ± 2.7
Total	13 / 28 (46.6)	149 / 240 (62.1)	8.7 ± 2.4

Rspp prevalence in nymphs obtained by flagging showed, in contrast to larval prevalence of *Rspp*, a continuous presence of the pathogen at all sites and in all years of sampling. Prevalence rates of *Rspp* in flagged nymphs decreased over time at HW and MB, while they remained stable at AW and even increased at SW. The overall *Rspp* prevalence in flagged nymphs was higher than in larvae, and substantially elevated compared to nymphal prevalence in ticks collected from hosts (Figure 3-20). Besides the substantial differences in overall prevalence levels between nymphs on vegetation and on hosts, the pattern of *Rspp* dynamics in nymphs on vegetation did not resemble that of the nymphs on hosts.

Rickettsia genospecies determination

A total of 157 *I. ricinus* samples (larvae and nymphs) from host individuals (n=93) and from flagging (n=32) that have previously been analysed as *Rspp*-positive by gltA-qrtPCR were randomly chosen among the sampling sites and years to determine the rickettsial genospecies present in ticks in the study area. The sequencing of the gltA fragment (obtained by conventional PCR) revealed the exclusive presence of *R. helvetica* in *I. ricinus* ticks of both instars from hosts and from flagging.

Table 3-13: Results of *Rickettsia* genospecies identification shown separately for the investigated *I. ricinus* instars (larvae and nymphs) and whether the sample originated from host collection (Host) or flagging (Flag). Total (n) indicates the number of *Rickettsia*-positive samples by qPCR, NA indicates the number of samples for which no distinct sequence profile could be obtained (by PCR or sequence analysis*). R. helvetica* indicates the number and % of samples shown to belong to the genospecies

	I. ricinus larvae			*I. ricinus* nymphs			
origin	Total (n)	*R. helvetica* n (%)	NA	Total (n)	*R. helvetica* n (%)	*R. spec.* n (%)	NA
Host	93	61 (100)	32	6	3 (100)	0 (0)	3
Flag	-	-	-	58	43 (91.5)	4 (8.5)	11

Candidatus Neoehrlichia mikurensis

The presence of the emerging pathogen CNM was examined for ticks on small mammal hosts at the four sites AW, HW, MB, SW in 2013 and for ticks on vegetation in 2012 and 2013. The results shown in this paragraph give an overview of the spatial occurrence of this human pathogen at the four study sites and the relationship between the prevalence rate of CNM detected in the different instars and by different sampling methods. As tick samples were analyzed in pools of several host individuals (across species), no inferences

about the role of host species could be made. Therefore, exclusively the comparison of the results on CNM prevalence in ticks on hosts and in ticks on vegetation are given.

Table 3-14: CNM in *I. ricinus* larvae and nymphs on hosts (*A. flavicollis* and *M. glareolus*) at the four sampling sites in 2013. The mean number of larvae and nymphs per sample ($\bar{x}$) ± the standard deviation (SD) are shown, as well as the proportion of positive and analyzed samples (n) and the resulting prevalence (%)

	larvae ($\bar{x}$ ± SD)	n infected / analyzed (%)	nymphs ($\bar{x}$ ± SD)	n infected / analyzed (%)
2013	**49.1 ± 57.9**	**38 / 103 (36.9)**	**4.8 ± 3.7**	**12 / 33 (36.4)**
AW	42.5 ± 34.1	18 / 37 (48.6)	5.9 ± 3	4 / 7 (57.1)
HW	64.2 ± 77.6	7 / 25 (28)	3.6 ± 3.2	4 / 11 (36.4)
MB	58.4 ± 71.7	7 / 22 (31.8)	5.4 ± 4.5	4 / 11 (36.4)
SW	31.4 ± 42.4	6 / 19 (31.6)	4.8 ± 4.1	0 / 4 (0)

Table 3-15: CNM in *I. ricinus* nymphs on vegetation at the four sampling sites in 2012 and 2013. Larval prevalence is not shown because no CNM infection could be detected. The mean number of nymphs per sample ($\bar{x}$) ± the standard deviation (SD) is shown as well as the proportion of positive and analyzed samples (n) and the resulting prevalence (%)

	nymphs ($\bar{x}$ ± SD)	n infected / analyzed (%)
2012	**8.6 ± 2.3**	**19 / 80 (23.8)**
AW	9.1 ± 2.3	13 / 24 (54.2)
HW	9.3 ± 1.7	2 / 24 (8.3)
MB	8.9 ± 2.3	3 / 27 (11.1)
SW	7.2 ± 2.8	1 / 5 (20)
2013	**8.6 ± 2.4**	**29 / 96 (30.2)**
AW	8.7 ± 2.4	9 / 26 (34.6)
HW	9 ± 2.4	14 / 33 (42.4)
MB	9.3 ± 1.9	6 / 28 (21.4)
SW	7.4 ± 3	0 / 9 (0)
Total	**8.9 ± 2.3**	**48 / 176 (27.3)**

A total of 5,216 *I. ricinus* collected from hosts (5,057 larvae and 159 nymphs) and 1,564 nymphs (plus 20 larval samples) from vegetation were analyzed for the presence of infection with CNM. This pathogen was present at all four sites and in ticks collected from small mammal hosts (Table 3-14), as well as from vegetation (

Table 3-15), and reached remarkably high overall prevalence (up to 36.9% in larvae and 36.4% in nymphs). Ticks on hosts showed comparable CNM prevalence between sites (χ^2_{Larvae}= 3.52, p= 0.32, χ^2_{Nymphs}= 3.59, p= 0.31), and for both instars (χ^2= 0.003, p= 0.96). Flagged *I. ricinus* showed no infection with CNM for larvae in 2012 and 2013. Nymphal prevalence was highly variable between sites in 2012, ranging between 8.3% and 54.2% (HW and AW) and resulting in an overall prevalence of 23.8%. In 2013, the infection of flagged nymphal *I. ricinus* was more evenly distributed among sites, but with no detection at SW. The overall prevalence rate increased up to 30.2% in 2013, but this was significant only at HW (Fisher's Exact test: p= 0.004). A comparison between nymphal prevalence from hosts and on vegetation (2013) showed slightly elevated overall prevalence in nymphs on hosts (36.4%) over flagged nymphs (30.2%), but this was not significant ($\chi^2_{Nymphs2013}$= 2.01, p= 0.16).

3.3.5 The relative influence of hosts and ticks in shaping pathogen prevalence

Pathogen prevalence in ticks on hosts are highly dynamic (Figure 3-17, Figure 3-18s) over time and differ in various aspects between small mammal hosts (*Af* and *Mg*). In order to gain a more detailed insight regarding the factors that influence pathogen abundance and dynamics, host characteristics (species, gender, age/weight), as well as individual tick load and the presence of nymphs (co-infestation) were examined for their importance for *Bbsl* and *Rspp* prevalence.

In contrast to tick loads on host individuals, simple, individual relationships between hosts, ticks and pathogen prevalence (*Bbsl* and *Rspp*) could hardly be identified. Except for male individuals of *Mg* being more likely to be infested with *Rspp* infected larvae (χ^2= 4.69, p= 0.03), neither individuals of different age classes, nor of a particular gender were more or less likely to harbor infected ticks.

For this reason, the prevalence of *Bbsl* and *Rspp* in larvae and nymphs on hosts and in nymphs on vegetation was analyzed using a more comprehensive and advanced statistical framework: GLM. The procedure followed a two-step process, including an overall model for all four sampling sites at first and then for the four sites individually, to assess the

transferability of the overall approach in this context and to examine processes and patterns between the individual sites.

Table 3-16: Overall formulation of the GLM of *B. burgdorferi* s.l. (*Bbsl*, A) and *Rickettsia* spp (*Rspp*, B) prevalence in *I. ricinus* larvae and nymphs on hosts (*A. flavicollis* and *M. glareolus*). Models were established for all sites (AW, HW, MB, SW) together and separate. Parameter estimates (Estimate) including standard error (SE), χ2 value and corresponding p-value (p) of all variables in the final formulation of the models for all sites (together) and all significant variables for the models of the individual sites are shown. Significances are indicated by asterisks following the p-value. No models containing significant variables could be obtained for *Bbsl* in larvae at SW (no *Bbsl* in hosts) and nymphal *Bbsl* and *Rspp* prevalence at individual sites (except for Rspp at HW). JS= Jolly-Seber, (t-1) = previous season

(A) *B. burgdorferi* s.l. prevalence in *I. ricinus* on hosts - GLM

Bbsl (0,1) ~ host species + gender + weight + host abundance (JS) + ticks + average larvae/host + coinfestation + nymphs questing (100m2) + deer/ha (t-1), family= binomial, link= logit				
Parameter	**Estimate**	**SE**	**χ**	**p**
Larvae				
All sites (n= 2248)				
ticks	-0.05	0.02	6.82	0.009*
host species (*Af*)	1.68	0.32	37.43	<.0001*
weight	-0.12	0.03	11.90	0.0006*
nymphs questing	-0.01	0.01	2.75	0.1
AW (n= 712)				
host species (*Af*)	1.54	0.42	21.41	<.0001*
weight	-0.15	0.04	12.75	0.0004*
gender (female)	0.50	0.24	5.00	0.03*
HW (n= 493)				
deer/ha (t-1)	2.06	1.07	4.93	0.03*
MB (n= 664)				
nymphs questing	-0.06	0.03	6.80	0.009*
mean larvae/host	-0.20	0.05	15.49	<.0001*
Nymphs				
All sites (n= 276)				
nymphs questing	-0.02	0.01	9.30	0.002*

(B) *Rickettsia* spp. prevalence in *I. ricinus* on hosts - GLM

Rspp (0,1) ~ host species + gender + weight + host abundance (JS) + ticks + mean larvae/host + coinfestation + nymphs questing (100m2) + deer/ha (t-1), family= binomial, link= logit				
Parameter	**Estimate**	**SE**	χ^2	**p**
Larvae				
All sites (n= 2248)				
ticks	-0.06	0.01	118.51	<.0001*
nymphs questing	-0.01	0.002	7.38	0.007*
weight	-0.01	0.01	2.68	0.1
host abundance JS	-0.01	0.003	5.91	0.02*
deer/ha (t-1)	0.04	0.01	10.51	0.001*
AW				
ticks	-0.11	0.02	62.74	<.0001*
nymphs questing	-0.01	0.003	3.90	0.049*
host abundance	-0.04	0.01	39.39	<.0001*
HW				
ticks	-0.04	0.01	11.44	0.0007*
deer(t-1)	-0.77	0.21	15.19	<.0001*
weight	-0.03	0.01	4.86	0.03*
mean larvae/host	-0.08	0.02	19.33	<.0001*
MB				
Ticks	-0.04	0.01	20.02	<.0001*
Nymphs questing	-0.02	0.003	33.64	<.0001*
SW				
ticks	-0.07	0.01	27.79	<.0001*
deer/ha (t-1)	0.16	0.07	6.14	0.013*
Nymphs				
All sites (n= 276)				
gender (female)	0.46	0.23	5.37	0.02*
weight	0.04	0.02	3.93	0.048*
HW				
gender (female)	1.28	0.76	7.86	0.005*

B. burgdorferi s.l. *and Rickettia* spp. in larvae and nymphs on hosts

The analysis of *Bbsl* prevalence in larvae and nymphs on hosts revealed the significance of several host-derived characteristics, as well as tick-derived variables and revealed certain differences between the overall model and individual sites (Table 3-16A). The variables remaining in the overall final model for all sites could mainly be retrieved in the models fitted for the individual sites with the same set of initial predictor variables, showing the significant influences of host species, weight and the number of questing nymphs.

In addition to that, a negative association between total tick loads on small mammal hosts with *Bbsl* prevalence in larvae (χ^2=6.82, p= 0.009) was detected for the overall model of infection only.

The number of questing nymphs remained in the final overall model, which suggested that it was of some importance towards *Bbsl* prevalence, but without being overall significant (χ^2= 2.75, p= 0.1). For MB, this variable could be shown to be of significant influence on *Bbsl* prevalence in larvae (χ^2= 6.8, p= 0.009), and was further shown to be of significance overall on *Bbsl* prevalence in nymphs (χ^2= 9.3, p= 0.002).

The importance of the remaining two significant predictors of *Bbsl* prevalence in the overall model could be confirmed at individual sites: *Af* individuals were shown to be associated with a significantly higher likelihood of harboring *Bbsl* infected larvae (χ^2= 37.43, p< 0.0001). This result could be confirmed at AW (χ^2= 21.41, p< 0.0001). Secondly, the weight of host individuals was negatively associated with *Bbsl* prevalence at all sites (χ^2= 11.9, p= 0.0006) as well at AW for individual sites (χ^2= 12.75, p= 0.0004).

The only variables shown to be of significant influence on *Bbsl* prevalence in larvae at individual sites were the average number of larvae per host (χ^2= 15.49, p< 0.0001) at MB and the number of deer culled in the previous season, the only significant predictor at HW (χ^2= 4.93, p= 0.03).

Rickettsia spp. in larvae and nymphs on host

The prevalence of *Rspp* in ticks on hosts at all sites appeared to predominantly be the result of interactions between tick-related factors (Table 3-16B). All variables of overall importance towards *Rspp* prevalence in larvae could be rediscovered in the final models at the individual sites and one out of two remaining significant variables of importance on

overall nymphal prevalence could be confirmed at the individual site-level as well, showing a higher level of conformity compared to *Bbsl* prevalence on hosts. The significant, negative association between tick load and *Rspp* prevalence found for the combined sites (χ^2= 118.51, p< 0.0001) was confirmed for all individual sites (Table 3-16). The negative overall relationship between the number of questing nymphs per 100m^2 and *Rspp* prevalence in larvae (χ^2= 7.38, p= 0.007) could also be shown for AW and MB. The remaining, but not significant negative influence of weight on *Rspp* in larvae at all sites (χ^2= 2.68, p= 0.1) was significant at HW, and further with respect to the overall *Rspp* prevalence in nymphs. A decreasing overall effect on *Rspp* prevalence associated with increasing host abundance (χ^2= 5.91, p= 0.02) was found at AW as well, just as the positive overall association of the number of culled deer/ha in the previous season (χ^2= 10.51, p= 0.001) could be also identified at HW and SW. In addition, the average number of larvae per host was shown to be negatively associated with *Rspp* prevalence in larvae only and individually at MB. Female host individuals could be shown to infer with increasing *Rspp* prevalence in nymphs at all sites (χ^2= 5.37, p= 0.02) and could be confirmed for HW as the only remaining, significant model parameter for an individual site.

B. burgdorferi s.l. and *Rickettsia* spp.in nymphs on vegetation

The final models for pathogen prevalence in nymphs on the vegetation (Table 3-17) appeared simpler and more uniform compared to the models describing *Bbsl* and *Rspp* prevalence in larvae and nymphs on hosts (Table 3-16). Both remaining significant variables in the final model of *Bbsl* prevalences at all sites could be rediscovered in the models established for the individual sites. The number of questing nymphs was shown to have a significant, positive overall effect on *Bbsl* prevalence in nymphs on vegetation (χ^2= 7.2, p= 0.007). The effect could be confirmed at SW. The second remaining overall significant parameter, the mean abundance of larvae on hosts in the previous sampling season, showed a strong positive association with *Bbsl* prevalence in questing nymphs at all sites (χ^2=43.59, p< 0.0001) and was consistent for HW, MB and SW (Table 3-17A).

Rspp prevalence in questing nymphs (Table 3-17B) showed slightly less conformity between the overall and individual-site models compared to *Bbsl* (Table 3-17A): the significant, negative influence of host abundance in the previous season (Host abundance JS [t-1]) on *Rspp* prevalence (χ^2= 38.35, p< 0.0001) shown for the site-spanning model,

could not be discovered in any of the site-specific models. Furthermore, there was a significantly negative association between the number of questing nymphs and *Rspp* prevalence at AW, but a contrasting positive association at HW (Table 3-17).

Besides that, there was consistency between the overall model and the site-specific models: the second remaining variable of significance in the overall model of *Rspp* prevalence in nymphs on vegetation, the mean number of larvae per host in the previous sampling season, was shown to be highly significant and positively associated with *Rspp* prevalence at the site-spanning model (χ^2= 172.71, p< 0.0001), as well as in every single-site model (AW: p< 0.0001, HW: p< 0.0001, MB: p< 0.0001, SW: p= 0.03).

Table 3-17: Overall formulation of the GLM of *B. burgdorferi* s.l. (*Bbsl*, A) and *Rickettsia* spp (*Rspp*, B) prevalence in *I. ricinus* nymphs on vegetation. Models were established for all sites (AW, HW, MB, SW) together and separate. Parameter estimates (Estimate) including standard error (SE), χ2 value and corresponding p-value (p) of all variables in the final model for all sites and all significant variables for the models of the individual sites are shown. Significances are indicated by asterisks following the p-value. No models containing significant variables could be obtained for Bbsl at AW. JS= Jolly-Seber, (t-1)= previous season

(A) ***Bbsl* prevalence in *I. ricinus* nymphs on vegetation - GLM**

Bbsl (0,1) ~ host abundance JS (t-1) + mean larvae/ host (t-1) + nymphs questing (100m^2) + deer/ha (t-1), family= binomial, link= logit				
Parameter	**Estimate**	**SE**	χ^2	**p**
All sites				
nymphs questing	0.01	0.005	7.20	0.007*
mean larvae/host (t-1)	0.28	0.04	43.59	<.0001*
HW				
mean larvae/host (t-1)	0.40	0.11	22.13	<.0001*
MB				
mean larvae/host (t-1)	0.20	0.06	10.42	0.001*
SW				
nymphs questing	0.05	0.02	11.54	0.0007*
mean larvae/host (t-1)	0.18	0.24	0.18	0.7*

(B) ***Rspp* prevalence in *I. ricinus* nymphs on vegetation - GLM**

Rspp (0,1) ~ host abundance JS (t-1) + mean larvae/ host (t-1) + nymphs questing (100m^2) + deer/ha (t-1), family= binomial, link= logit				
Parameter	**Estimate**	**SE**	χ	**p**
All sites				
mean larvae/host (t-1)	0.42	0.04	172.71	<.0001*

host abundance JS (t-1)	-0.03	0.01	38.35	<.0001*
AW				
mean larvae/host (t-1)	0.31	0.08	15.46	<.0001*
nymphs questing	-0.03	0.01	15.83	<.0001*
HW				
nymphs questing	0.02	0.01	7.44	0.006*
mean larvae/host (t-1)	0.47	0.06	110.68	<.0001*
MB				
mean larvae/host (t-1)	0.34	0.05	55.03	<.0001*
SW				
mean larvae/host (t-1)	0.45	0.20	4.54	0.03*

4 DISCUSSION

A central outcome of my thesis shows the pervasive presence of substantial spatio-temporal variability of all three cornerstones of my investigation (Figure 4-1): small mammal hosts, ticks and pathogens underwent seasonal fluctuations of various shape and extent during the three years, which differed between the four forest sites in BW. This variability makes the identification of the major factors influencing the tick-host-pathogen-system a challenging task.

In the following sections, I will analyze the relative influence of the variety of factors that are likely to determine the abundance and dynamics of small mammal hosts*, I. ricinus* ticks and pathogens in BW.

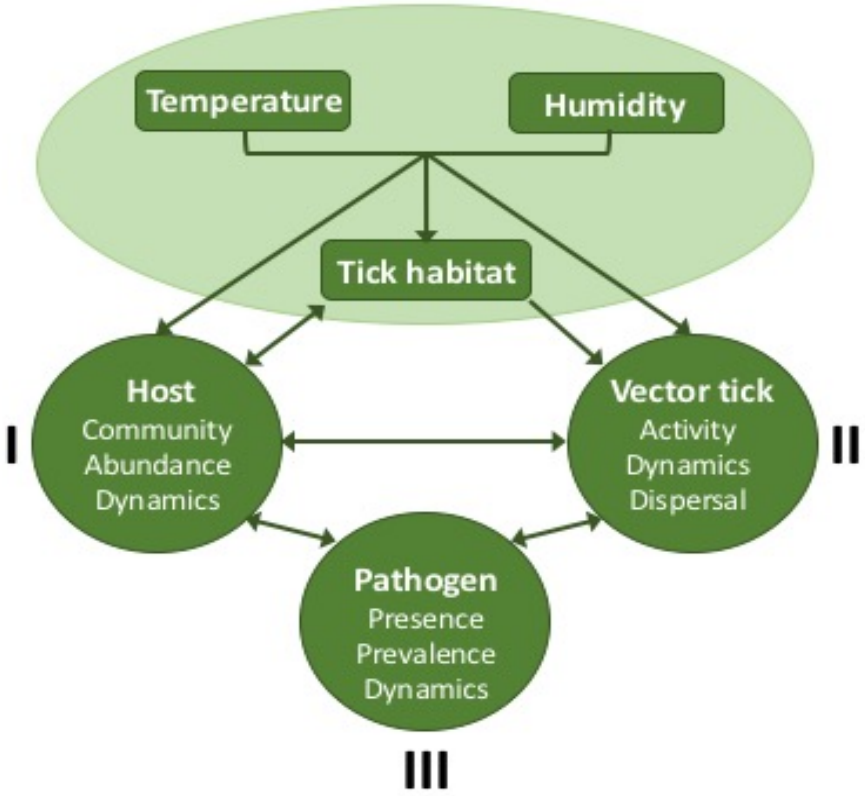


Figure 4-1: Overview of the thesis' structure. Here, the dark green circles "Host" (I), "Vector tick" (II) and "Pathogen" (III) indicate the three main components of the discussion, including respective points of focus. The light-green ellipse comprises the abiotic and biotic environment as influencing factors of interest in the study

The discussion is divided into three parts, analogous to the introduction and the results chapter:

The first part of the discussion will be dedicated to the analysis of the small mammal communities at the field sites. At first, I will examine the overall community structure, abundance and dynamics of the small mammals captured at the four sites during the three seasons of investigation and will then analyze the population dynamics in more detail for the two major species *Af* and *Mg*.

The second part of the discussion will be dedicated to the exploration of the abundance (activity), dispersal and dynamics of ticks on these hosts, the relationships between hosts and ticks and the comparison of tick dynamics on hosts and ticks collected from vegetation. The section will then be concluded with a comprehensive examination of the relative influence of abiotic and biotic factors on tick abundance using advanced statistical modeling.

In the third section, I will examine the general occurrence and prevalence of the five TBPs of human and veterinary concern that were investigated in the present study. I will explore the influence of host and tick dynamics on the infection prevalence with these pathogens and complete this section with a comprehensive, statistical examination of the relative influence of hosts and ticks on shaping pathogen prevalence in the field.

The final section will then provide a synthesis of the previous three parts of the discussion, integrating the individual results to a final outcome.

4.1 Small mammal hosts

Small mammals are important elements in ecosystems (Mihalca and Sándor, 2013). They perform key ecological functions such as habitat formation or ecological engineering (Jones *et al.*, 1996), are involved in the dispersal of seeds and spores (Morand *et al.*, 2006), and serve as prey for predators (Maternowski, 2007). Their omnipresence throughout Central Europe (Meerburg *et al.*, 2009), together with their limited activity range, good accessibility and detailed knowledge about their ecology (Flowerdew *et al.*, 1985) makes small mammals an ideal model group of organisms with which to address a variety of ecological questions (Barrett and Peles, 1999).

For ticks and TBPs, small mammals are of special importance as they act as the main blood-meal hosts for immature ticks (Mihalca and Sándor, 2013), as well as being competent reservoir hosts for a multitude of TBPs (Tälleklint and Jaenson, 1994; Jongejan and Uilenberg, 2004; Ostfeld *et al.*, 2006; Bown *et al.*, 2011; Jahfari *et al.*, 2012; Mihalca and Sándor, 2013).

The four forest sites in this study represent typical forest types of BW. They are suitable as habitats for ticks, small mammals and larger hosts, providing for the potential presence of ticks as well as for the biotic aspect of tick survival. However, they differed regarding forest type and structure, soil or microclimatic conditions. The sampling on trapping grids was chosen (over transects) to cover structural different core areas of each habitat and at the same time to reduce edge effects, leading to more centralized catchment areas (Barnett and Dutton, 1995; Efford, 2004; Tioli *et al.*, 2009). The sampling period from March to October (May to October in the first year) was chosen to cover the main seasonal activity of both, ticks and small mammals (Smith *et al.*, 1975; Barnett and Dutton, 1995; Estrada-Peña *et al.*, 2006) and minimizing the risk of death of captured individuals because of hypothermia or context-related stress (Barnett and Dutton, 1995). The sampling interval of monthly captures for three consecutive days and nights was chosen as trade-off between a large enough, small enough and long enough timeframe. Large enough to minimize disturbances of the small mammals, which could introduce bias by causing behavioral changes like trap-happiness or trap-shyness (Seber and Schwarz, 1986; Grimm *et al.*, 2014) and to (re-) acquire tick burdens (Jones *et al.*, 2015) that can be regarded as independent from the previous sampling. Short enough to ensure that changes regarding the small mammal populations and the ticks parasitizing them can be observed between sampling events. Long enough sampling per session was intended to e.g. minimize the impact of behaviorally altered individuals on the representativeness of captures to provide the base for a reliable capture-mark-recapture study (Lebreton *et al.*, 1992; Schwarz and Arnason, 1996; Seber and Schwarz, 1999). The overall sampling over three years was intended to set up a reliably long dataset to grasp possible differences between sampling years and to provide a sound scaffold for further analysis.

4.1.1 Community structure and dynamics

Small mammal populations in Central European forests usually consist of three to fourteen species (Gurnell, 1985; Suchomel *et al.*, 2014). Thus, the six different small mammal species (rodents and insectivores) found at my study sites were within the range of expectations.

During the three years of study, the overall abundance of captured small mammals varied greatly among the four forest sites. Despite these fluctuations, *Af* and *Mg* remained the dominant species at all sites and in all years. Both species are highly abundant rodent species in Central European forests (Stenseth *et al.*, 2002; Suchomel *et al.*, 2014). They have been found to be the most abundant small mammal species in a variety of other German studies (Klenke *et al.*, 2004; Maternowski, 2007; Essbauer *et al.*, 2009; Schex, 2011). If both species occur within the same area, they tend to minimize niche overlap: bank voles change their diurnal rhythm to daylight activity (Shore and Hare, 2008). In this way, they can coexist in suitable habitats without considerable competition with the night active yellow necked mouse (Gurnell, 1985). In addition, both species are known as hosts for immature tick stages and are competent reservoirs for a variety of TBPs (Mihalca and Sándor, 2013). Taken together, this indicates their importance as fundamental ecological factors at all four sites and their potential influence as the main hosts for ticks and reservoirs for TBPs in the present study.

Shrews of the species *S. araneus* and *S. minutus* were permanently present in low numbers throughout the study. Both species are widely distributed throughout BW (Nagel and Nagel, 2005; Turni, 2005b). They are solitarily living (Nagel and Nagel, 2005; Jenrich *et al.*, 2010) and usually co-occur in multi-species communities. As both Sorex species are insectivorous, occur in low numbers and show avoidance behavior towards rodents they are unlikely to be in direct competition with rodents (Rautenbach *et al.*, 2014). Nevertheless, competition can still arise when total densities rise and space becomes a limiting factor (Gurnell, 1985). This effect is more likely to occur if vole densities increase, as they share similar habitat preferences, including areas with high ground cover (Gurnell, 1985). This pattern could consistently be observed at the site AW, where the relative and overall abundance of *Mg* was continuously high, and the relative abundance of shrews was comparatively lower than at the other sites. A long-term study from Finland (Tast *et al.*, 2005) observed some connectedness between shrews and rodent populations by

increasing shrew numbers one year after rodent numbers had increased, suggesting that high rodent numbers prevented the increase of shrews, but the underlying mechanisms remain unknown. In the present study, this could be confirmed, to some extent, by the increased overall relative abundance of *S. araneus* in 2013 at the three sites where the rodent populations decreased significantly. However, it is not possible to determine whether the increased relative abundance of *Sorex* individuals in the traps could be attributed to a real increase in abundance, as suggested by Task *et al.*, or an increased trappability (Southwood and Henderson, 1989) because of reduced competition with the dominant rodents.

The occurrence of single individuals of *A. sylvaticus* and *M. agrestis*, the former being closely related to *A. flavicollis* (Turni, 2005b) and the latter sharing major ecological traits with *M. glareolus* (Blatt and Resch, 2016d) also fits the pattern commonly seen in German forests. *A. sylvaticus* and *M. agrestis*, make use of wider habitat ranges with comparably lower preference for forests than *Af* and *Mg* (Quéré and Le Louarn, 2011; Bugarski-Stanojević et al., 2013).

Consequently, the overall community composition of the small mammals found at the four sites of investigation in the present study seemed to be representative, stable and to embody a typical community structures for forest sites in BW.

4.1.2 Small mammal population dynamics

Not only the overall abundance of hosts, but also their inter- and intra-annual dynamic abundance patterns can influence tick infestation patterns (Goodwin *et al.*, 2001; Krasnov *et al.*, 2007) and feeding success on hosts (Tälleklint and Jaenson, 1994; Kurtenbach *et al.*, 1995). This in turn is likely to affect the transmission dynamics of TBPs and will be discussed in the next sections.

The community structure of small mammal populations within a certain habitat, e.g. forest, depends on the structure and diversity of the habitat and the adaptability of individual species (Flowerdew *et al.*, 1985; Suchomel *et al.*, 2014). Population dynamics in temperate parts of Europe are usually driven by a set of key influencing variables that can be divided into extrinsic (density-independent) and intrinsic (density-dependent) factors (Krasnov *et al.*, 2007). Extrinsic influences comprise food supply, microclimate, weather,

habitat structure, predation, parasites and disease, while the intrinsic factors of potential importance are even more complex and have either a social, physiological or genetic aspect (Krebs, 2013). Nevertheless, after decades of extensive studies, the search for causes of small mammal population fluctuations is not over and population ecologists still discuss the topic as passionate as controversially (Hudson and Bjørnstad, 2003). As the aim of the present study was the ecological characterization of small mammal communities including their fluctuations in space and time as foundation with which to understand tick and pathogen dynamics, it is not of interest to provide a comprehensive analysis of all factors that can potentially influence small mammal populations. Instead, I will analyze their structure and dynamics with respect to the influence of the density-independent components habitat (with respect to landscape and microclimate) and food supply (mast seeding of trees) and the density-dependent overall abundance of small mammals.

In this first section, I will examine the inter-annual fluctuation between the overall small mammal population including all captured individuals and species between the four sites. In this way, I will analyze the dynamic abundance of all available hosts for ticks in the respective areas.

Very substantial seasonal variation in density is a known characteristic of small mammal populations. These usually exhibit population cycles of expansion and contraction (Gurnell, 1985). Typically, after a period of overwintering, the overall numbers of small mammals are low in early spring, before reproduction sets in. After the onset of reproduction in March or April (Boonstra *et al.*, 1998; Stenseth *et al.*, 2002), population density increases and often culminates in a population peak towards autumn (Kirkland, 1985; Pucek *et al.*, 1993; Vogel, 1995a). This autumn peak is usually correlated with the emergence of ripe fruit on fruiting trees (e.g. beech, oak) and is therefore stronger in species that are predominantly herbivorous (Pucek *et al.*, 1993; Marsh and Montgomery, 2008). Furthermore, it has been shown that shrews generally fluctuate to a lesser extent than any rodent species (Tast *et al.*, 2005). Changes in overall abundance are therefore likely to be attributed to rodents rather than shrews.

In 2012, small mammal populations at all four sites exhibited a comparatively high and very similar overall abundance and dynamics. 2011 was a year of full mast with extensive

fructification of over 2/3 (68%) of all major tree species in BW (Meining *et al.*, 2011). This was followed by the mild spring in 2012 (average air temperature in March 2012: 8.6°C, German Weather Service (DWD), station: Rheinstetten). Taken together, this combination of preceding events is likely to have led to winter reproduction among small mammals (Jensen, 1982; Pucek *et al.*, 1993). The early onset of reproductive activity thus potentially gave rise to a dramatic population increase in rodent abundance (Marsh and Montgomery, 2008; Shore and Hare, 2008). This pattern of the extensive population growth of seed-eating rodents after mast events has been documented for several European and North American forest rodents (Bergstedt, 1965; Pucek *et al.*, 1993; Ostfeld *et al.*, 2001; Stenseth *et al.*, 2002; Turni, 2005a; Shore and Hare, 2008; Sundell *et al.*, 2012). As spring abundance mainly represents the consequence of winter mortality which is higher in colder winters (Stenseth *et al.*, 2002) and the abundance of small mammals at all sites was comparatively high in May 2012, when sampling started, this conclusion seems justified for the overall population dynamics of 2012.

In the following sampling seasons, the number of small mammals captured in spring of each trapping season was lower than in May of 2012 (exception AW in 2014). This pattern corresponds with the typically low population density of small mammals at the beginning of years without winter reproduction (Gurnell, 1985). In years after population increase due to a previous heavy mast, population crashes are likely to occur (Radda *et al.*, 1969a; Burkhardt and Schlund, 2005). The factors causing this phenomenon are diverse and can differ spatially as well as in their applicability to different small mammal species (Flowerdew *et al.*, 1985; Gurnell, 1985). Population decline during winter is a consequence of density-dependent factors (more individuals competing for less resources), as well as density-independent factors (microclimate) (Solonen 2006). Such a decline is commonly favored by the duration and harshness of a winter period, as food becomes scarce and predation pressure increases (Wenk 2007). The winter of 2012/2013 was comparatively cold and long (average air temperature in March 2013: 3.4 °C, DWD, station: Rheinstetten). In addition, the year 2012 was not a mast year. Mast levels of the major fruiting trees were low (25% overall mast), reaching only the level of partial mast (e.g. *F. sylvatica* 20%, *P. sylvestris* 45%, *Q. petraea* 5%, Forestry Office Karlsruhe). Food competition by other animals can further influence the population density of small mammals. Forcadi et al. (2000) showed that wild boar actively plunder food reservoirs of

mice, which can lead to starvation among small mammal populations during times of limited food supply. Deer, in turn, can alter habitats and lead to a decrease in vole abundance (Flowerdew and Ellwood, 2001). This could have led to substantial food shortages, indicating that that there was not enough food available to provide for the increased number of small mammals during the following winter of 2012/2013, leading to higher winter mortality.

Taken together, this is a likely cause of the extensive decrease in population numbers at three out of the four sites of investigation in 2013. The exception was AW, which in 2013 had a population dynamic and abundance comparable to 2012. Compared to the other sites, AW seems to provide a more stable microstructural framework, thus a better long-term living environment for small mammal populations than the other sites. This hardwood alluvial forest has a high plant biodiversity, including an intense herbal layer, while at the same time providing high stand density and habitat structure. This entails a richer and more evenly spread food supply as well as suitable microclimatic conditions close to the ground compared to the other sites (Ellenberg 1963). All of these factors combined might have prevented a population decline at this site during 2013. Still, a common pattern observable at all four sites in 2013 was the autumn peak of populations, which is a typical feature of rodent populations and has been described previously (Tkadlec and Zejda, 1998; Krasnov *et al.*, 2007).

In 2014, small mammal populations recovered, for the most part, and in certain parts even reached a higher density than in 2012. Overall levels of tree fructification in autumn 2013 (34%) were higher compared to the previous season (*F. sylvatica* 20%, *P. sylvestris* 30%, *Q. petraea* 60%, *Acer pseudoplatanus* 40%, Forestry Office Karlsruhe) leading to a food increase, which, together with the comparatively short winter period (average air temperature for March 2014: 8.7 °C, DWD, station: Rheinstetten) could have led to low winter mortality of small mammals. In addition, most individuals in the present population were probably born in the previous autumn, which means that the population was young and already reproductive, in order to produce large litters and viable offspring (Boonstra *et al.*, 1998; Tkadlec and Zejda, 1998), which in turn would have favored the overall recovery of small mammal populations in 2014. The only exception to the pattern constituted the site SW, which only showed a slight increase in overall numbers from 2013 to 2014. The reasons for the missing recovery at this site can be manifold. A potential

reason can be found in the putatively less favorable climatic conditions in the Black Forest together with a habitat that does not provide as much shelter for small mammals as the other sites due to low leaf litter coverage and shallow soils. Another possible explanation might be the higher presence of predators such as mustelids (Gaistal ranger, oral communication) or food competition by wild boar, as previously discussed.

It seems that food and weather are of potentially great importance towards shaping small mammal dynamics, but that they are not sufficient in explaining the entire pattern of these dynamics.

This already shows how complex the interactions between small mammals and their environment can be.

4.1.3 Population dynamics of *A. flavicollis* and *M. glareolus*

In this section, I will focus on the interspecific population dynamics of the major host species *Af* and *Mg* at the individual sites with respect to their species ecology.

As *Af* and *Mg* represent the majority of all captured individuals in this study and are known to be important hosts for ticks and TBPs they can be regarded as representative for the overall small mammal population. As stated before, it is of particular interest to examine the dynamic abundance of both species at the four sampling sites over time individually. This is because their roles as hosts for ticks and as reservoirs for TBPs differ significantly (Kurtenbach *et al.*, 1998a; Radzijevskaja *et al.*, 2013; Burri *et al.*, 2014). Furthermore, *Af* and *Mg* differ to some extent in their species-specific ecology, and thus are likely to respond differently to extrinsic or intrinsic influences (Flowerdew *et al.*, 1985; Gurnell, 1985).

It might be that the number of captured individuals per unit area cannot always grasp population developments in detail, as the information it provides is limited to the counting of individuals of a population with incomplete capture. Capture-mark-recapture-based population models can give a deeper insight in fluctuation events and be more precise in estimating individual numbers per unit time, because they consider the capture histories of individuals and, based on that, generate species-specific estimates of survival, encounter and birth / death to estimate population abundance for individual species. Therefore, this approach is more precise in observing population processes in detail. The

specific method of choice in the present study was POPAN Jolly-Seber, which provides the combined opportunity to model populations dynamics in which births and deaths occur and to derive estimates for population abundance (Schwarz and Arnason, 1996; Cooch and White, 2012). Both were vital requirements, because population changes are highly likely to happen in small mammal populations over three years, as they are short-lived and have one to two reproduction periods per year (Jensen, 1985; Niethammer, 1990b; Burkhardt and Schlund, 2005) and because the focus of model usage here was the estimation of abundance for *Af* and *Mg* to analyze their dynamic changes.

While the annual overall abundance of *Af* was rather similar among sites, the abundance of *Mg* differed to a greater extent. Furthermore, the abundance of *Af* was comparatively higher at all sites and in all years, except for AW. It seems like, beside the variation between years, the suitability of the forest sites as habitats differed between both species, with all four sites providing comparatively suitable habitats for *Af*, but not for *Mg*. This pattern is unlikely to be attributed to the adaptability of *Af* because the species was shown to have more narrowly defined habitat requirements compared to the bank vole, with definite restriction to mature forests (Watts, 1968; Gurnell, 1985; Turni, 2005a). The bank vole, despite its general ability to adapt, requires a substantially higher amount of ground cover and humidity than *Af* (Gurnell, 1985). *Af,* in contrast, prefers open woodlands (Jenrich *et al.*, 2010) with high canopy cover but less ground cover (Quéré and Le Louarn, 2011). The forest with the highest equivalence to the suitability requirements for *Af* and the lowest suitability for *Mg*, was the site HW: This mature, mixed forest was dominated by fructifying trees (*F. sylvatica* and *P. sylvestris*), had an intense canopy cover, the least ground cover with an almost not existing herbal layer among sites and very little dead wood. Besides, it was the driest forest among all four sampling sites (Zimmermann and Brinkmann, 2006). These assumptions were confirmed by the substantially higher abundance of *Af* to *Mg* at that site in all three years of sampling.

The forest site MB largely provided a more suitable habitat for *Af* as well, being dominated by the *F. sylvatica*, with densely closed treetops and little ground cover over most of the site. But at the same time, the area was comparably more humid than HW, with larger substructures within the forest site, including patchy areas of potential suitability for *Mg* like shrubs and large amounts of dead wood (Hassler, 1998). This relative habitat suitability was reflected by a pattern of relative abundance between the two species that

was by trend similar to that at HW, with *Af* being more abundant than *Mg* throughout the study, but with a higher relative amount of *Mg* compared to HW. Furthermore, the total productivity or capacity at MB seems to be higher than at HW, allowing for a comparatively higher total abundance of small mammals at the site, as observable in 2012.

SW, the site located within the northern Black Forest (610m a.s.l.), is assumed to be comparatively more suitable for *Mg* than *Af. Mg* has been shown to be highly abundant in mountain forests of Germany, Austria and Italy, reaching high population densities (Ladurner and Cazzolli, 2000; Jerabek and Reiter, 2003a; Burkhardt and Schlund, 2005). In addition to that, the site is located along a slope, dominated by fructifying trees (*F. sylvatica* and White Fir *A. alba*) with medium canopy cover, and medium ground cover but with large amounts of dead wood and rocks and a dense occurrence of saplings and herbs. The comparatively high abundance of *Mg* (mainly in 2012) is consistent with the assumption of habitat suitability for the species. Together with the comparatively lower but overall high abundance of *Af* at SW in 2012, it seems that the site can be highly productive as well. However, there must be some factors influencing their abundance, apart from extrinsic factors of habitat structure or food, preventing the recovery of both species' populations after the dramatically high abundance in 2012. A possible explanation is the presumably higher predation pressure compared to the other three sites by an increased presence of foxes and mustelids (oral communication Gaistal Ranger G. Eberhardt). It has been shown that predator populations increase after an increase in the populations of their prey organisms (Hudson and Bjørnstad, 2003; Sundell *et al.*, 2012), which is likely to have happened after 2012, but exact numbers are missing for the area. In addition to that, the colder weather conditions might be less favorable compared to the other three sites and might further impair the recovery process of small mammal populations, e.g. by high winter mortality (Flowerdew *et al.*, 1985; Barrett and Peles, 1999).

The site AW, an alluvial forest with most intense ground cover and herbal layer among the four sites, coincided with the highest overall abundance of *Mg*. This matches the previously stated habitat preference of *Mg* for humid areas (like alluvial forests) with thick herbal layers to feed on and to avoid predation (Viro and Niethammer, 1982) and an overall denser vegetation than *Af* (Stenseth *et al.*, 2002; Jenrich *et al.*, 2010). The higher

proportion of *Mg* compared to *Af* at this site might therefore be attributed to the comparatively higher habitat suitability for *Mg*, allowing the *Mg* population to thrive (Jerabek and Reiter, 2003b; Kraft, 2008). But the site harbors an overall high number of *Af* as well. Furthermore, the site AW possessed the most stable overall small mammal population among all four sites over the entire study period. This might indicate its extraordinarily high and stable productivity, providing the necessary capacity for persistent coexistence of both species due to the high degree of habitat substructures, differential neighboring succession stages, a uniquely large variety of tree species and other vegetation, shrubs, deadwood etc. (Lechner and Zimmermann, 2007), as stated in the previous section.

Moreover, it has been shown that in areas where both species, *Af* and *Mg*, co-occur, this can lead to a reduced long-term abundance of bank voles (Burkhardt and Schlund, 2005) without direct competition. Instead, this is attributed to a natural interspecific dominance hierarchy, with *Af* being superior to *Mg* (Andrzejewski and Olszewski, 1963). Due to its higher habitat adaptability, *Mg* can avoid competition by evading the area of concern (Flowerdew *et al.*, 1985). In fact, this could add to explain the proportional differences between the two species at the sampling sites HW, MB and SW, being the result of the realized niche of *Mg* in contrast to its much larger fundamental niche due to the presence of *Af*.

The dynamic changes of both species at the four sites will now be regarded with respect to these presuppositions.

Examining the abundance of *Af* and *Mg* in 2012, it is obvious that the similar overall pattern of small mammal populations at all sites in 2012 does not simply correspond to the abundance of the individual species. At none of the sites there is an equivalent abundance of *Af* and *Mg* observable. Instead, both species contribute differently to the observed overall pattern of similarity. This stresses again the importance of species-specific examination of population dynamics. A reason for the overall similarity at the four sampling sites in 2012 might be the synchronization of overall populations due to the simultaneously occurring, extensive mast in the previous autumn which has potentially lead to maximum-capacity winter reproduction under the respective extrinsic conditions (Gurnell, 1985; Barrett and Peles, 1999) and to an earlier end of the reproduction phase (Jerabek and Reiter, 2003b). But, taking into account the harsh period of extremely cold

weather at the beginning of 2012, there might as well have been some weather-related limitations in small mammal reproduction acting across sites (Deutscher Wetterdienst, 2012), which should be taken into account when comparing the conditions of 2012 to other years. The pattern of abundance in 2012 suggests that the (first) peak of abundance has been missed by delayed onset of sampling in the year (compared to 2013 and 2014), in which winter reproduction after extensive mast seeding in the autumn of 2011 is likely to have occurred.

The peak of rodent density might then have resulted in a density-dependent population development which was highly similar in shape among sites (Stenseth *et al.*, 2002; Liebhold *et al.*, 2004; Krasnov *et al.*, 2007).

At first sight, it can be seen that both species are affected by the population decline at HW, MB and SW in 2013. The fact that mast seeding was substantially reduced in 2012 can be potentially best observed with respect to *Af* as they are predominantly granivorous and more likely to be affected by the absence of mast (Pucek *et al.*, 1993; Turni, 2005a), whereas *Mg* has a larger trophic diversity and is less dependent on seed abundance (Don, 1979; Gurnell, 1985). It is therefore imaginable that, under such harsh conditions, *Af* might exert some pressure on *Mg* by its interspecific dominance, leading to a decline in bank vole numbers (Andrzejewski and Olszewski, 1963; Boonstra *et al.*, 1998). This seems reasonable for HW, MB and SW observing the interaction of abundance between the species at HW, MB and SW against this background. The larger dependence of *Af* on wood crops compared to *Mg* can be even observed by the slight decrease in *Af* abundance even at AW in 2013, the site which presumably provides the most buffering attributes due to its extraordinarily high vegetation diversity and habitat structure, whereas there was no effect on *Mg* abundance observable. Population abundance at HW, MB and SW did not increase until the second half of the sampling season in 2013 and was more pronounced for *Af* than *Mg* (except at AW). While *Af* reached overall estimated abundances in 2014 that were comparable to 2012 (except for SW) and even exceeded the population density of 2013 at AW by far, *Mg* populations, mainly at HW and MB, did not seem to have fully recovered until the end of the last sampling season in 2014 (no recovery at SW). This leads to the assumption that populations of *Af* might recover sooner or that *Mg* populations take longer to recover (Jensen, 1982; Boonstra *et al.*, 1998; Taylor *et al.*, 2013). Reasons for that might be found in the slower population turnover and the longevity of *Mg*

compared to *Af* (Radda *et al.*, 1969a; Niethammer, 1990b; Burkhardt and Schlund, 2005) and again to the dominance of *Af* over *Mg* under harsh conditions (Andrzejewski and Olszewski, 1963).

Furthermore, the extremely mild winter of 2013/2014 (Deutscher Wetterdienst, 2014) might have favored small mammal survival in general and in particular the increase of the potentially more quickly regenerating species, *Af* ,compared to *Mg* at all sites in 2014.

The examination of the dynamics of the two major small mammal species in the present study showed the importance of species-specific examination of population processes to understand the underlying processes as well as it showed the intense variability that occurred between the two species during three years of investigation at four different forest sites.

Of course a variety of further aspects might have been acting on the small mammals in the present study, shaping individual species' abundance peaks etc. Here, it was of main interest to monitor species abundance and dynamics and to give an overview of the factors that might influence these dynamic patterns.

4.1.4 Distribution and dynamics of age and gender among *A. flavicollis* and *M. glareolus*

Another aspect of small mammal populations with potential effect on tick and TBP abundance and dynamics are the distribution of individuals of different age classes (juvenile, subadult and adult) and different genders (female and male). Individuals can differ in their susceptibility to tick infestation and infection with TBP according to their immune status, which is related to the age of an individual (Tälleklint *et al.*, 1993; Shaw and Dobson, 1995). The same relationship applies to individuals of different gender (Zuk and McKean, 1996; Hughes and Randolph, 2001; Dallas *et al.*, 2012) and will be discussed with reference to ticks and TBPs in the respective sections.

It cannot be completely excluded that there might be an effect of age and gender towards trap-response in small mammals of both major species, as these two factors are likely to influence behavior (Turni, 2005a; Shore and Hare, 2008). Adult individuals usually have larger home ranges than juveniles (Viro and Niethammer, 1982; Turni, 2005a) and depending on a variety of extrinsic and intrinsic influences males and females can show

differential activity patterns (Gurnell, 1985). Nevertheless, the main focus of this study was not to assess the exact proportion of age classes of individuals at the sites during the sampling period but to examine the influence of the respective individual attributes on the ecology of tick infestation and infection with TBPs. Capture-mark-recapture analysis and minimum-invasive life-trapping of small mammals, as applied in the present study, ensures for a minimized potential bias and representative surveillance of hosts (Goodwin *et al.*, 2001; Brunner and Ostfeld, 2008) and their associated macro- and micro-parasites.

Adult individuals represent the most abundantly captured age class of hosts with respect to both species, all sites ad all years of investigation and therefore provided the most reliable source of information regarding further examinations. This result corresponds with the typical overall age structure composition found in wild rodent populations (Flowerdew *et al.*, 1985). The proportion of adults among populations is even higher in *Mg* compared to *Af*, which is likely due to the comparative longevity of bank voles, leading to an increase of the relative abundnace of adult individuals in populations (Marsh and Montgomery, 2008; Shore and Hare, 2008). The second most abundant age class comprised subadult individuals and was consistent for both species and throughout the study. The predominance of subadults over juvenile individuals is a typical pattern as well, as the juvenescence represents the shortest part of the lifespan in both species (Jenrich *et al.*, 2010). Besides that, juvenile individuals are less likely to capture as they are substantially less active in terms of foraging behavior and home range than larger and reproductively active subadult or adult individuals (Schlund and Scharfe, 1993; Turni, 2005a).

Besides these overall proportions between the age classes, there were also some observable differences during the sampling seasons as well as between the two major rodent species:

In addition to the previously discussed influences, temporal fluctuations in age structure and recovery time of *Mg* populations might be as well attributed to “senescence-maternal effects”: The underlying theory proposes that the maturation of juveniles born within a population peak is delayed as a density-dependent intrinsic effect. Together with the usually earlier ending reproductive phase during seasons of high individual abundance, the resulting adult population in the following spring is much older than in years without

substantial population increase. Furthermore, the relative amount of individuals of low fitness is expected to be largest after peak years, due to comparatively eased survival. This is assumed to confer deleterious maternal effects, leading to smaller litters of potentially weaker constitution and a slower population expansion in the following year after a peak (Boonstra *et al.*, 1998). The low number of subadult individuals and comparatively higher abundance of juvenile individuals of *Mg* in 2013 found at the sampling sites coincides with this hypothesis and has been found in other European studies on *Mg* as well (Tkadlec and Zejda, 1998; Jerabek and Reiter, 2003b). The individuals that were born in the previous season have already reached adulthood and as reproduction sets in with delay, there are only few subadult individuals to expect in the population during the sampling season. The effect has been stated and examined by several studies on voles (Boonstra *et al.*, 1998; Tkadlec and Zejda, 1998), but was until now attributed a negligible role in mice (Havelka and Millar, 2004).

The earlier onset of population recovery in *Af*, as examined in the previous section, might have led to an increase in the proportion of juvenile individuals in 2013, as the remaining individuals after the decline are entirely involved in reproduction and therefore in the expansion of the population. After the following winter 2013/2014 with potentially low winter mortality and in concurrence with a higher abundance of individuals, this could have fed on to the even larger proportion of juvenile *Af* in 2014. The comparatively higher proportion of juvenile *Af* trapped during the study might be attributed to behavioral differences between the two rodent species: Juvenile *Mg* tend to stay in their nests longer and express less foraging behavior compared to the juveniles of *Af* (Burkhardt and Schlund, 2005; Turni, 2005a).

The proportion of male to female *Af* and *Mg* was mainly balanced at the overall level between sites and years, with some slight fluctuations and differences over time and between species.

While the amount of captured individuals of both genders was equivalent for *Mg*, the numbers of captures *Af* slightly exceeded those of females in this study. The balanced occurrence of both genders is a usual finding for *Mg* (Jerabek and Reiter, 2003b), and even though females have been shown to live slightly longer than males, the proportion of both genders tends to be 1:1 in field observations (Schlund and Scharfe, 1993; Burkhardt and Schlund, 2005).The excess of male individuals of *Af* (Bergstedt, 1965) is a commonly

observed phenomenon as well, attributed to the higher activity of male *Af* compared to female individuals of the species (Hammond and Anthony; Turni, 2005a).

If female individuals are captured more frequently than males, this is likely to be related to an increased energy requirement, for example during breeding season, leading to increased foraging behavior (Jerabek and Reiter, 2003b; Turni, 2005a)

It can therefore be assumed that the proportion of male and female individuals was generally balanced among sites and years.

The results presented here show an enormous amount of variation in small mammal abundance and dynamics between sites and between years of sampling. Therefore, it seems highly unlikely to assume that any generalizable or valid information can be derived from data from only one site and/or covering only one year of observation.

4.2 Ticks

Ticks are the major vectors of zoonotic pathogens in Central Europe, being able to transmit a large variety of viral, bacterial and protozoan pathogens to humans and animals (Estrada-Peña *et al.*, 2013; Estrada-Peña and de la Fuente, 2014). The distribution and density of tick populations is the result of a complex network of interactions (Estrada-Peña *et al.*, 2004; Ogden *et al.*, 2008; Gray, 2008), based on two cornerstones: firstly, suitable microclimatic and habitat conditions are needed to provide the framework for tick survival and activity in a given area (Randolph and Storey, 1999; Estrada-Peña *et al.*, 2004), and secondly, the presence of suitable hosts is necessary to provide for the maintenance of tick populations (Goodwin *et al.*, 2001; Mihalca *et al.*, 2012). Small mammals are of special importance as hosts for tick immatures (Tälleklint and Jaenson, 1997), but beyond their role as blood-meal source for ticks, host populations are far more important (Randolph and Storey, 1999). Their community structure, abundance and dynamics are assumed to strongly impact the abundance and aggregation of ticks on host individuals as well as populations and hence can be of great importance in the transmission dynamics of TBPs.

4.2.1 Ticks on small mammal hosts

I. ricinus was the dominant species throughout the study (with 17,712 out of 19,125 ticks collected in total). This coincides with the situation found in many other Central and Northern European studies (Jaenson *et al.*, 1994; Dorn *et al.*, 1999; Estrada-Peña *et al.*, 2005; Paziewska *et al.*, 2010; Pérez *et al.*, 2012; Mihalca and Sándor, 2013), and confirms *I. ricinus* as being the most prevalent tick species throughout Central Europe (Estrada-Peña *et al.*, 2006), Germany (Rubel *et al.*, 2014; Schulz *et al.*, 2014) and BW (Petney *et al.*, 2013). *I. ricinus* is known to infest a wide range of hosts, including small mammals (Estrada-Peña *et al.*, 2015) and it is the main vector species of tick-borne infections in Europe (Bown *et al.*, 2006; Medlock *et al.*, 2013). For these reasons, *I. ricinus* is of particular interest in this study.

The second most prevalent species found was *D. reticulatus*. This species is widespread throughout the temperate regions of Europe, with an increasing range in Germany (Dautel *et al.*, 2006). The recent spread of the species has been attributed to the effects of climatic changes, increasing the localities providing suitable habitats for the species (Gray *et al.*, 2009; Karbowiak, 2014; Mierzejewska *et al.*, 2016). Compared to *I. ricinus*, it has a highly focal distribution (Obsomer *et al.*, 2013; Karbowiak, 2014; Pfäffle *et al.*, 2015a). The immature stages of *D. reticulatus* are nest dwellers known to infest small mammals (Hillyard, 1996; Dautel *et al.*, 2006). Furthermore, the species is also a competent vector of certain TBPs, transmitting, e.g., *Babesia canis*, *Rickettsia slovaca* and *R. raoultii* (Silaghi *et al.*, 2012b; Špitalská *et al.*, 2012). The patchy occurrence of *D. reticulatus* was confirmed by the data collected in the present study, as the majority of individuals were collected at the site HW only. The collection of larvae and nymphs throughout the three years of sampling at HW indicates a stable population of the tick species at the site (Pfäffle *et al.*, 2015a). Besides woodlands, *D. reticulatus* has been found to inhabit grasslands and pastures. Despite the fact that it has long been considered to prefer habitats with a high moisture content like alluvial forests (Liebisch and Rahman, 1976) it seems that it can as tolerate areas of comparatively low humidity as well (Dautel and Kahl, 2009). This finding coincides with the presence of the species in stream valleys and fallow land in Eastern European countries, representing habitats that provide medium vegetation cover with high ground water level and drying soils (Karbowiak, 2014; Mierzejewska *et al.*, 2015, 2016).

The remaining two tick species were only collected to a minor extent. *I. acuminatus* and *I. trianguliceps* are both closely associated with certain species of small mammals (Bown *et al.*, 2006; Boyard *et al.*, 2008; Kovalevskii *et al.*, 2013). They are suggested to be capable vectors of a variety of TBPs (Bown and Begon, 2003; Földvári *et al.*, 2007; Kovalevskii *et al.*, 2013). Hence, all four tick species represent typical tick species found on small mammal hosts.

The dominance of larvae, accompanied by the presence of a few nymphs on small mammal hosts was an expected result confirming that the study can be generalized to other areas in Europe (Gray, 1985a; Goodwin *et al.*, 2001; Sínski *et al.*, 2006; Brunner and Ostfeld, 2008; Paziewska *et al.*, 2010; Kiffner *et al.*, 2010; Mihalca *et al.*, 2012). The ratio of larvae:nymphs:adults is assumed to be the result of the vertically higher questing activity of nymphs and adults, resulting in lower probabilities of contact between nymphs or adults and rodents (Randolph and Storey, 1999)..

Thus my results regarding tick species composition and relative abundance on rodent hosts at the four field sites during the study, as well as the ratio of occurrence of different instars mirror the results of other, shorter term studies that have been conducted in Germany and other parts of Europe. The present analysis is therefore based on the common situation and can be considered to be generally applicable.

The overall number of ticks collected at the sites during the three years of sampling showed a certain level of variability in space and time. However, the fluctuations in total tick abundance do not reflect the substantial differences in host abundance, especially with respect to the population collapse at HW, MB and SW in 2013. This contrasts with studies from the US, stating that an increase or decrease of the rodent host population is accompanied by a proportional increase or decrease of the total number of ticks they harbor (Goodwin *et al.*, 2001). During the first two years of study, 2012 and 2013, the total abundance of ticks at AW, HW and MB was comparable, despite the substantial differences in host abundance. This might be related to widespread, potentially weather-related events in preceding years (Ruiz-Fons *et al.*, 2012) that had an influence on the general survival of the eggs deposited in the leaf litter at all sites, leading to a similar number of larvae in the following season of activity. Host-related effects that act over a large area, such as that preceding years of mast (Ostfeld *et al.*, 2001; Rizzoli *et al.*, 2009), might as well have led to the flourishing of suitable hosts for female ticks, resulting in large

and comparable quantities of eggs laid and larvae hatching among the sites AW, HW and MB. However, as the year of extensive mast took place in 2011, but not in 2012, this is unlikely to have caused the similarity in total tick abundance among sites in 2012 and 2013. Furthermore, the total number of ticks at MB increased drastically in 2014, but not at any of the other sites; not even at AW, the site with the most stable habitat structure and food availability, was there a comparable increase in tick numbers observable in this year, despite a comparable increase in host abundance.

Despite the overall comparable host population structure, no generalizable pattern could be identified between the total abundance of hosts and the total number of ticks per sampling season, which indicates that the situation in BW is more complex than in the northeast of the USA. This will be examined in more detail in the following sections.

A clearly observable pattern, though, is the lower overall number of ticks collected on hosts at SW throughout the sampling period. Potential reasons for this can be either host-related or weather-related. As the species composition and abundance of small mammal hosts in 2012 was comparable to the other sites, this is unlikely to be the reason for the lower tick abundance at SW. The lack of suitable hosts for adult ticks is also unlikely as the area in which the sampling took place is known to harbor red deer and roe deer (personal communication Gaistal ranger G. Eberhardt).

Therefore, the lower abundance of ticks at SW might be related to microclimatic or, more generally, abiotic influences. The reduced availability of shelter for ticks from extreme weather events due to the thin leaf litter at SW might reduce tick survival in general, leading to a lowered number of ticks available per season (Estrada-Peña, 2008). As the site also exhibits the strongest drop in relative humidity and among the four sites, this is likely to be associated with increased tick mortality due to water loss of either the eggs or the active instars (Kahl, 1989; Estrada-Peña *et al.*, 2013)

The three minor tick species found on hosts, *D. reticulatus*, *I. acuminatus* and *I. trianguliceps,* are endophilic tick species (*D. reticulatus*: only immatures), living in close association with their small mammal hosts and being only detectable by collection from host individuals (Randolph, 2013). Therefore, they rarely infest humans (Randolph and Storey, 1999; Bown *et al.*, 2008). However, as they are capable of transmitting TBPs, they are likely to be involved in maintaining the enzootic cycle of these pathogens in the field (Oliver *et al.*, 2003; Rizzoli *et al.*, 2014). *I. ricinus,* in contrast*,* is the only exophilic species

among the four tick species found during the study. It spends most of its life apart from hosts in or on vegetation (Schulz *et al.*, 2014). Furthermore, it is the only generalist tick species among the four species collected, with over 300 known host species (Stanek, 2009; Silaghi *et al.*, 2012a), including humans. *I. ricinus* is therefore likely to serve as the only potential bridge vector in the transmission of a variety of different zoonotic pathogens between wildlife and humans (de la Fuente *et al.*, 2008; Pfäffle *et al.*, 2015b).

4.2.2 Measures of parasite burden – relative infestation of *A. flavicollis* and *M. glareolus*

Tick infestation patterns on hosts are based on a complex network of interactions between intrinsic and extrinsic factors shaping their extent and dynamics (Krasnov *et al.*, 2007; Brunner and Ostfeld, 2008; Calabrese *et al.*, 2011; Kiffner *et al.*, 2011) and hosts typically differ distinctively in matters of tick infestation between species, populations and at the individual level (Durden and Beati, 2014).

The potentially differential tick burden between the two major small mammal host species in this study will be examined in this section. The differences between tick burdens of *Af* and *Mg* at the individual sampling sites and their dynamics over time, as well as the influence of intrinsic host attributes on tick burden and aggregation will be addressed in the following sections.

Even though it is known that both, *Af* and *Mg*, are competent reservoirs for several TBP of veterinary and human medical concern (Mather *et al.*, 1989; Tälleklint and Jaenson, 1994; Gassner *et al.*, 2013), it still remains unknown which rodent species exhibits a higher effective transmission potential, leading to most infected *I. ricinus* ticks. A study conducted in Poland by Sínski *et al.* (2006) found that individuals of *Af* were not only more likely to be infested with *I. ricinus* larvae than *Mg*, but that they also harbored substantially larger numbers of ticks compared to the bank vole.

The comparison of tick burdens between the two major small mammal hosts in the present study clearly confirmed these strong differences between the two rodent species: *Af* was found to have a consistently higher level of tick infestation compared to *Mg*. This pattern was consistent at all sites in all years of sampling, comprising larval as well as

nymphal burdens of *I. ricinus* infestation and covering all measures of parasite burden used to characterize the distribution of ticks between the two species (prevalence, mean abundance, mean intensity, range of infestation).

The lowered tick burden on *Mg* might be attributed to the species' ability to acquire immunological resistance against *I. ricinus* infestation over time (Wikel, 1996). This can consequently lead to reduced feeding success, lowered engorgement weight and impaired survival rate of ticks (Dizij and Kurtenbach, 1995). *Af,* on the other hand, is not able to build up such a resistance against *I. ricinus* (Dizij and Kurtenbach, 1995).

Besides the immunological differences of potential influence on tick burdens, the two rodent species differ in their ecology: individuals of *Af* have much larger home ranges and more extensive activity periods than *Mg*, which increases the potential contact rate between host individuals and ticks and might be involved in comparatively increased tick burdens of the species as well. In addition, yellow-necked mice tend to show comparatively less pronounced self and foreign grooming than voles (Gurnell, 1985), which is likely to contribute to the higher tick load on *Af* as well.

Patterns of parasite abundance and dispersion have previously been shown to not only be related to the quality of hosts (Vázquez *et al.*, 2005) but also to depend on their frequency of occurrence (Arneberg *et al.*, 1998). This does not seem to be generally applicable to the two major species in the present study, though, as *Af* was shown to harbor higher tick loads even when or where *Mg* was the overall most abundant species (e.g. SW 2012, AW 2013).

Similar proportions of the relative infestation patterns of *I. ricinus* between *Af* and *Mg,* with higher larval tick burdens on *Af,* have not only been stated for Poland (Sínski *et al.*, 2006; Paziewska *et al.*, 2010), but in fact seem to represent the general pattern observable in Central Europe (Humair *et al.*, 1993; Gray *et al.*, 1999; Hanincová *et al.*, 2003; Boyard *et al.*, 2008; Mihalca *et al.*, 2012; Gassner *et al.*, 2013; van Duijvendijk *et al.*, 2015) and Germany (Kiffner *et al.*, 2011; Silaghi *et al.*, 2012b).

The consistency of this trend suggests that the higher tick load on *Af* compared to *Mg* is attributable to intrinsic, species-specific attributes that lead to a generally better suitability of *Af* as host for *I. ricinus*.

4.2.3 Seasonal dynamics of infestation

Tick burdens are often subject to substantial variation over time (Kurtenbach *et al.*, 1995; Tälleklint and Jaenson, 1997; Randolph *et al.*, 1999; Rosà *et al.*, 2007) which is a key element in the quantification of the transmission of TBP (Kiffner *et al.*, 2011). Therefore, I examined how the inter-annual dynamics of hosts (*Af* and *Mg)* between the three sampling seasons influenced tick abundance parameters at the four sites with respect to their potential influence on pathogen transmission. As the dynamic patterns of tick burdens on *Af* and *Mg* within sites were comparable, but differed in the extent of the burden on individuals, they will mainly be addressed as "rodents".

Though it is generally known that tick burdens show strong seasonal variation (Kurtenbach *et al.*, 1995; Tälleklint and Jaenson, 1997; Randolph *et al.*, 1999; Rosà *et al.*, 2007), no European study exists to date in which the influence of rodent host dynamics on tick infestation parameters over several years and at several study sites has been examined in detail.

My data clearly show, for the first time, the extremely variable and highly complex nature of the *I. ricinus*-rodent system in Central Europe in both space and time. This emphasizes the absolute importance of long-term data in order to identify the factors affecting tick burdens on hosts and to distinguish between the exception and the rule.

As small mammal numbers and host species composition at the sites, were highly similar in 2012, the significant differences in tick burdens on hosts among sites are likely to represent differences in general tick abundance or activity and therefore be attributable to influences that are extrinsic to the rodent host population (Krasnov *et al.*, 2007). It might be that the slightly lower tick presence on both rodent species at HW and the substantially lower tick burdens at SW simply reflect the comparatively higher environmental suitability of AW and MB for ticks (Kurtenbach *et al.*, 1995; Estrada-Peña, 2001; Tack *et al.*, 2012a). Both of the latter sites represent humid forest habitats at younger successional forest stages (Hassler, 1998; Lechner and Zimmermann, 2007) including large amounts of thicket and overall extensive ground cover, which potentially provides more stable microclimatic conditions at AW and MB which has previously been found to favor tick abundance (Lauterbach *et al.*, 2013). In contrast, HW and SW are potentially unfavorable tick habitats compared to AW and MB as they are both drier in terms of soil moisture (HW) and relative humidity (SW), respectively (Jensen *et al.*, 2000).

In addition, these sites have more mature forests with substantially less overall ground cover (Zimmermann and Brinkmann, 2006). The relatively drier conditions at HW and SW is likely to impair tick survival (Kahl, 1989; Estrada-Peña *et al.*, 2013); the shallow soil and lower abundance of leaf litter at SW might lead to increased mortality of ticks due to reduced shelter against extreme temperatures or very low humidity, especially affecting larval ticks (Schwarz *et al.*, 2009; Estrada-Peña *et al.*, 2012). Together with the potentially prolonged developmental period of ticks at SW, due to the lower mean temperatures (highest-lying site at 610m a.s.l.), this might again increase mortality (Gray, 2009; Dallas *et al.*, 2012). A shorter period of tick activity at SW is rather unlikely to explain the lower abundance of ticks on hosts, as tick activity could be observed throughout the sampling season as soon as hosts were active.

The overall low abundance of nymphs on both host species at all sites in 2012 might have an impact on *Bbsl* transmission, as nymphs are generally assumed to infect small mammal hosts with the aetiological agent (Kurtenbach *et al.*, 2006); these in turn infect larvae feeding on these hosts later in the season. As nymphs were largely absent from rodents in 2012, it is questionable whether this epidemiological cycle has been realized during this season.

However, as other blood-feeding as well as reservoir hosts might be involved, more specific deductions comparing the four sampling sites can only be made examining the comparative dynamics of tick infestation patterns on different hosts species.

When small mammal populations decreased dramatically at three of the four sites in 2013, it became obvious that the relationship between host abundance and tick infestation in the present study was not as expected under the "mast-host-tick-framework" established for the northeastern US (Goodwin *et al.*, 2001; Ostfeld *et al.*, 2001, 2006). Opposite to their findings, there was a substantial amount of spatial variability in tick infestation present between the closely located woodland sites (10-58km apart from each other) (Goodwin *et al.*, 2001). Additionally, the positive relationship found between host species abundance and tick density for tick burdens of *I. scapularis* on *P. leucopus* does not fit to the data on tick abundance on rodents in the present study. Rather, the opposite pattern could be observed at the sites with reduced small mammal host abundance in 2013: the reduced number of rodents harbored a substantially increased number of larvae per individual (Krasnov *et al.*, 2007). Infestation levels at AW,

the only site where fluctuations were of minor extent, were remarkably constant. This leads to the hypothesis that an equivalently large stock of ticks was present in both years and dispersed on the reduced number of available hosts. The question in this context is whether the abundance of ticks really depends on small mammal hosts (Ostfeld *et al.*, 1996, 1998b; Goodwin *et al.*, 2001). The same pattern as reported here has been found by Krasnov *et al.* (2007), investigating *I. ricinus* abundance on rodent hosts in Slovakia. They attempted to explain this phenomenon by the "dilution hypothesis" after (Hamilton, 1971), which was originally based on predator-prey interactions, describing the lower probability of a prey individual eaten if the herd size increases, and transferred it to tick-host-systems. The differential relationship between hosts and tick along with the increased tick burdens on hosts in 2013 is of special interest as it might have a further impact on the transmission of TBP between ticks and hosts (Brunner and Ostfeld, 2008; Harrison and Bennett, 2012; Jones *et al.*, 2015).

At AW, where the population decrease was restricted to *Af* only and did not lead to a lower overall number of small mammal hosts at that site (compared to 2012), this coincided with a slight increase in parasite burdens on *Af* as well as *Mg*. This pattern might be due to the dramatic reduction of the comparatively more suitable host species, *Af*, which consequently might have caused a shift of parasite pressure from *Af* to *Mg*. Furthermore, the larval tick burden on individual hosts at AW did not increase for *Af* but did for *Mg*, pointing in the same direction regarding higher infestation pressure acting on the "replacement host" *Mg* and indicating a temporal host switch of *I. ricinus* larvae at that site in 2013. This might be of further interest regarding the differential reservoir competence of *Af* and *Mg* for TBP (Kurtenbach *et al.*, 1994; Dizij and Kurtenbach, 1995).

Tick burdens on hosts in 2013 increased at SW as well, but the site still showed the lowest tick burdens on hosts among the four sites. This confirmed the previous indications of its generally lower suitability as tick habitat, which led to a certain increase in individual burdens as host numbers crashed, but much lower than at the other sites, potentially due to the comparatively lower habitat and weather related long-term availability of tick larvae (Randolph, 2004; Lindgren and Jaenson, 2006).

The pattern for nymphs on rodent hosts in 2013 was different from 2012: here, the numbers of nymphs infesting both rodent species increased at all sites in comparison to the preceding season. As the rate of contact between nymphs and rodents is rather small

because of the high questing activity of nymphs in areas above the activity range of rodents (Randolph and Storey, 1999), it might be that unfavorable, probably weather-related conditions reduced the energy reserves of nymphs early in 2013 and led to spatially lower questing activity of nymphs, therefore increasing the probability of contact between rodents and nymphs (Kurtenbach *et al.*, 2006). This hypothesis finds support in the fact that the effect was observed at all sites, irrespective of host population density. This suggests that extrinsic, weather-related explanations are more likely than intrinsic ones (Schauber *et al.*, 2005). The winter preceding the sampling season of 2012 was mainly characterized by an extraordinarily high variability in temperature and precipitation, followed by an unusually prolonged cold weather period until the end of March, which might have reduced the nymphs' energy reserves at a very early stage in the questing season (Herrmann and Gern, 2010, 2013). This would lead to lower vertical questing activity to maximize the period of questing activity (Randolph and Storey, 1999). Furthermore, studies on rodents in fields, where the microclimatic conditions are less favorable for ticks than in woodlands, and might therefore lead to accelerated tick energy reserve reduction, showed comparatively higher nymph burdens on rodent hosts (Boyard *et al.*, 2008; Paziewska *et al.*, 2010). As nymphs play a key role in the epidemiology of pathogens, their considerably increased presence on hosts in 2013 is potentially of great importance for transmission dynamics.

In 2014, tick infestation patterns on rodents differed again from the previous years and among sites. This confirms the larger variability between woodland habitats compared to the US (Goodwin *et al.*, 2001) and, taking together the three years of sampling, stresses the necessity of multiple sampling sites to analyze the complex and dynamic relationships between ticks and their hosts over time.

The tick-host-infestation parameters obtained for MB and SW in 2014 make it obvious that there is more potential complexity to the system than previously identified (Brunner and Ostfeld, 2008; Meentemeyer *et al.*, 2012); that some additional factors of unknown nature are involved in shaping the abundance of ticks or the attachment of ticks to hosts at these sites under certain circumstances (Schauber *et al.*, 2005; Krasnov *et al.*, 2007). The overall increased number of larvae on hosts at both sites resulted in a comparatively high mean abundance and intensity at SW and intermediate values for the infestation parameters at MB in relation to the abundance of rodents at these sites in 2014. It might

be that some extrinsic, habitat related characteristics have actually increased the available number of tick larvae at MB and SW in the last year of sampling. This could be related to an increased feeding success of female *I. ricinus* at the two sites in the previous season, but this cannot be verified. Interpretations of this pattern are therefore speculative due to the limited availability of data, but its presence gives further insight in the complexity of the system, demonstrating that different numbers of tick larvae might be available between woodland sites over time and that the stock of ticks present at the sites is not static.

This again increases the possible set of factors influencing the dynamics in the tick-host-pathogen system and further complicates the fine-scale generalization of effects in the present system.

The overall decrease of nymph abundance on hosts at all sites in 2014 in comparison to 2013 seems to affirm the assumption that increased nymph abundance on hosts in 2013 was probably caused by unfavorable environmental conditions preceding the sampling season (Randolph and Storey, 1999). However, as the nymph numbers dropped below those of 2013 (except for a minor increase at SW), but remained above those of 2012, this raises the question of whether nymph abundance in 2012 might have been extraordinarily low. It might well be that the values and patterns of 2012 have been "confounded" by the relatively harsh winter period preceding the first sampling seasons (oral communication, Olaf Kahl, Berlin), and that the situation observed in 2014 is more likely to represent the "normal" tick population dynamics in Germany. This relationship will be further addressed when tick burdens are compared with ticks collected from vegetation.

So far, the variability observed for the relationship between *I. ricinus* and its rodent hosts at the four sites leaves some room for speculation, and the causes are possibly only identifiable with a longer series of data. What can be concluded from the spatio-temporal dynamics of ticks on rodent hosts is that there is no overall generalizable pattern, that the present system seems to be highly dynamic in space and time and more complex than the system in the northeastern US. Thus, my data suggests that the eco-epidemiology of the *I. ricinus* system in Europe differs substantially from the *I. scapularis* system in the USA, with potentially different regulatory mechanisms shaping tick-host-associations depending on the geographic range (Stanko *et al.*, 2006).

4.2.4 Seasonal interactions of larval infestation and host abundance

The previous section examined the patterns and dynamics of tick burden on hosts. In this section, I will analyze the seasonal dynamics of tick infestation in comparison to host abundance and illustrate how they relate to each other.

The fluctuations in tick abundance over time are assumed to be the result of the natural developmental cycle of *I. ricinus* (Tälleklint and Jaenson, 1997; Estrada-Peña *et al.*, 2004), influenced by many intrinsic and extrinsic factors. In Central Europe, larvae usually have a unimodal to bimodal pattern of activity during one season (Humair *et al.*, 1993; Tälleklint and Jaenson, 1997; Kurtenbach *et al.*, 2006; Paziewska *et al.*, 2010). These basic patterns could be detected at the four sampling sites. However, the activity patterns differed to some extent between the sites, despite their geographical proximity, as well as between the three years of sampling, exhibiting between one and three peaks of larval activity on hosts per season. The differential distribution of larval peak activity on hosts among sites, as well as between years, again suggests that multiple factors, potentially interacting in time and space, are involved in generating these patterns (Stanko *et al.*, 2006; Krasnov *et al.*, 2007). However, it was not possible to discern a regular, consistent pattern for any of the sites.

A GLM showed the complexity and dynamic nature of tick burdens on hosts, indicating that a variety of different factors acting in space and time, including multiple interactions, contribute to the diversified patterns in the seasonal relationship between tick infestation and host abundance in the present system. This underlines again the importance of long-term studies to identify, explain and predict ecological processes and mechanisms in this field (Brown *et al.*, 2001)

Moreover, it is evident from my data that the predominant activity of *I. ricinus* larvae is not restricted to summer as previously stated based on data acquired by flagging ticks from vegetation (Gray, 1985a, 2008; Matuschka *et al.*, 1992; Estrada-Peña *et al.*, 2004). Instead, whenever small mammal hosts were collected between March and October, they were already infested with *I. ricinus* ticks. This coincides with the results of a broad range of studies from Central Europe collecting larvae from rodent hosts (Tälleklint and Jaenson, 1997; Rosà *et al.*, 2007; Paziewska *et al.*, 2010; Burri *et al.*, 2011a; Pérez *et al.*, 2012), and suggests that the examination of larval activity of *I. ricinus* derived by collecting individuals

from rodent hosts can provide a higher resolution of larval activity in the field than collecting larvae from vegetation.

In addition, the seasonal interaction pattern between the abundance of larvae on hosts and host density confirms the previous hypothesis regarding the presence of the host dilution effect (Hamilton, 1971; Krasnov *et al.*, 2007) at the seasonal level: Tick abundance on small mammal hosts was, in turn, to be inversely correlated to small mammal abundance in any given year. Thus, high small mammal abundance was associated with a comparatively lower larval abundance per host, whereas lower small mammal abundance coincided with an up to five-fold increased level of abundance.

Considering the "dilution-effect-based" relationship between small mammal population density and mean abundance of *I. ricinus* larvae on hosts, the question remains as to how this affects the overall density of nymphs emerging from these larvae in the following season, due to potentially different reactivity of the host immune system and in particular the density of infected nymphs.

In summary, it seems that if there is a comparable number of active larvae, more larvae infest individual hosts if host abundance is low and *vice versa*. This strongly contradicts the results on tick-host-relationships in the northeastern US by Ostfeld *et al.* (2001, 2006), indicating a stable mean abundance of ticks on hosts, but an increase in total tick numbers proportional to the increase of host abundance. In contrast, my data shows the consistent involvement of the dilution hypothesis in shaping tick-host-relationships in BW. As the lower abundance of small mammal hosts coincides with a proportionally increased mean abundance of ticks on these hosts, this leads to the hypothesis that small mammal abundance probably does not have a significant influence on how many larvae find a host. This, in turn, calls into question whether small mammal density together with the factors influencing this abundance (e.g. mast) in a given season, exert any influence on the abundance of nymphal *I. ricinus* in the following year. Further data will be needed to support or dismiss this hypothesis.

4.2.5 Gender, age and tick abundance on hosts

Besides the species-related differences of tick burdens on *Af* and *Mg*, it is known that additional host-related factors lead to higher or lower tick burdens on certain hosts (Randolph *et al.*, 1999). According to my data, both host gender and age are of importance in shaping larval as well as nymphal burdens between the respective groups of hosts.

Different mean tick burdens on hosts of different genders have been shown to occur in various studies and across different groups of hosts for both the ecto- and endoparasite fauna (Poulin, 1996; Zuk and McKean, 1996; Wilson *et al.*, 2001; Moore and Wilson, 2002). Increased parasitism on males is most pronounced for parasites of birds and mammals (Poulin, 1996). Higher parasite burdens on males have been confirmed by a number of studies on tick burdens on rodent hosts (Davidar *et al.*, 1989; Zuk and McKean, 1996; Schmidt *et al.*, 1999; Hughes and Randolph, 2001; Morand *et al.*, 2004; Obiegala *et al.*, 2014). The same pattern was found in the present study: both major host species, *Af* and *Mg*, exhibited significantly higher numbers of *I. ricinus* larvae and nymphs on male individuals compared to females of the same species. This pattern was consistent in space and time and reveals the importance of host gender as a driver of parasite burden. Furthermore, this can potentially influence TBP transmission. The causes for the higher infestation of male individuals has been mainly attributed to two aspects: on the one hand, behavioral differences between individuals of the two genders can lead to higher exposure of males to ticks (Wilson *et al.*, 2001). The generally higher activity of males with their comparatively larger home ranges and territories (Moore and Wilson, 2002), as well as their aggressive behavior against opponents or reduced grooming behavior compared to females could lead to higher tick burdens on male individuals (Tälleklint and Jaenson, 1997; Morand *et al.*, 2004; Krasnov *et al.*, 2012). This effect is assumed to be even larger when food is scarce or population densities are low (Wilson *et al.*, 2001) and will be discussed in the following section. On the other hand, physiological differences, such as the immunosuppressing effects of androgenes, like testosterone, can make males more susceptible to tick infestations (Hughes and Randolph, 2001). An experimental increase of testosterone in male rodents (*Apodemus* and *Myodes*) led to reduced innate and acquired immunity to ticks and was hypothesized to make those individuals more susceptible to TBP (Hughes *et al.* 2001).

Tick burdens on small mammal hosts differ substantially between individuals of different age groups (or body mass of non-pregnant individuals as a proxy for host age). Increasing age or body weight, was shown to be positively correlated with tick burden. Thus, adult individuals are most likely to carry the largest number of ticks (Schmidt *et al.*, 1999; Sínski *et al.*, 2006; Brunner and Ostfeld, 2008; Harrison *et al.*, 2010; Kiffner *et al.*, 2011; Mysterud *et al.*, 2015). The synonymy of age class (dividing individuals in groups based on pelage, sexual maturity and weight) and body mass is still discussed controversially, although, as larger individuals might be larger tick-targets, and the individuals might trade off weight gain at the cost of reduced immunity (Harrison *et al.*, 2010). Even though, none of the factors influencing tick abundance on hosts are mutually exclusive (Brunner and Ostfeld, 2008; Calabrese *et al.*, 2011; Dallas *et al.*, 2012), both approaches are legitimate in the context of roughly estimating the influence of increasing age on tick burdens.

High levels of infestation on adult animals have predominantly been attributed to the increased spatial activity related to reproductive activity and the increased hormone levels of sexually active individuals (Randolph *et al.*, 1999). Conversely, the fewer ticks on juvenile individuals has been related to their comparatively shorter time of exposure to ticks in the field (Schmidt *et al.*, 1999), an argument which is difficult to follow as tick turnover on a host is usually less than a week - the time taken to engorge and detach (Piesman *et al.*, 1987; Meiners *et al.*, 2006).

My data revealed that adult individuals of both major host species showed a significantly high infestation with larvae of *I. ricinus* than juveniles. This held further true for the abundance of nymphs on *Af*, whereas no association of nymphal burdens was found for any of the age classes of *Mg*. This lack of significance might be ascribed to the low nymphal infestation on *Mg* compared to *Af*.

The influence of these intrinsic host characteristics on tick burdens showed that there are generalizable patterns detectable within the vast levels of variability in space and time. The relative proportion of sexually mature males of *Af* within a community, representing the species, the gender and the age group of highest tick burdens, as well as being competent reservoir hosts for a variety of TBPs, is therefore highly likely to impact on TBP transmission.

4.2.6 Aggregation

The aggregation of macroparasites on individual hosts, in terms of the disproportionate accumulation of larvae on certain host individuals, is a widespread phenomenon, assumed to be based on differences in individual exposure of hosts to ticks and physiological differences amongst the host individuals. This leads to a situation in which few hosts within a population feed the majority of parasites (Shaw and Dobson, 1995). A concept that is broadly accepted in this context is the "Pareto principle" or the"80-20-rule" of parasitism, which states that 20% of the host population usually feeds 80% of the parasite population in a given area and is therefore responsible for the transmission of TBP (Woolhouse *et al.*, 1997; Brunner and Ostfeld, 2008). Those hosts harboring and successfully feeding the majority of the tick (vector) population are also responsible for the main part of pathogen transmission (Brunner and Ostfeld, 2008; Harrison and Bennett, 2012). Moreover, the level of aggregation is of crucial importance with respect to the epidemiology of TBP, as it has the potential to influence the establishment and persistence of TBPs (Woolhouse *et al.*, 1997; Rosà and Pugliese, 2007; Kiffner *et al.*, 2011; Harrison and Bennett, 2012). The higher the level of aggregation, the higher the probability that a host has previously fed a nymph and has acquired infection with a TBP, for example, *Bbsl*. This host will subsequently feed a large number of larvae that will in turn acquire infection from the infected host (Kiffner *et al.*, 2011).

In the present study, *I. ricinus* larvae on both host species were demonstrated not to be equally dispersed among host individuals, as demonstrated by a variance to mean ratio (VMR, $s^2/\bar{x}$) that was consistently larger than 1 (Anderson and Gordon, 1982), pointing to a situation in which ticks are aggregated on individual hosts within the host population with some variation according to the site of collection and the respective year. This represents the typical, heterogeneous distribution of parasites within host populations (Shaw *et al.*, 1998). Levels of aggregation were found to be consistently higher for *Af* compared to *Mg*. This indicates that *Af* not only harbored more ticks on average, but that these ticks were also more highly aggregated on fewer individuals than was the case for *Mg.* Furthermore, the extent of aggregation of tick larvae on hosts appeared to be related to the population density of the hosts: in 2012, the aggregation of *I. ricinus* larvae on *Af* and *Mg* was comparable among sites for each species, whereas in 2013, the VMR reached up to three times higher at HW, MB and SW, but remained lower at AW. The low VMR at

AW even decreased towards 2014, which coincides with the further increase of small mammals in this year and further confirms the hypothesis of relatedness between host abundance and aggregation. The positive correlation between the level of aggregation and mean burdens on hosts has previously been shown for the rodent-tick-system in the northeastern US (Shaw and Dobson, 1995; Shaw *et al.*, 1998), and seems to occur in Central Europe as well (Stanko *et al.*, 2006; Krasnov *et al.*, 2007; Ferreri *et al.*, 2014).

Thus, my results suggest that the mean tick burdens on hosts in 2013 were not only higher at the population level and not only clumped on fewer individuals, but that, those few highly infested hosts even harbored an increased number of ticks (Krasnov *et al.*, 2007). This increases the prominence of those individuals that harbor a disproportionately high number of ticks in such years of low host abundance with regard to their role in the epidemiology of TBP, making them potential superspreaders (= individuals that make a disproportionate contributions to community-wide disease transmission relative to their abundance, [Paull *et al.*, 2012]) of pathogens if they indeed are infected and competent reservoirs (Ferreri *et al.*, 2014; Johnson *et al.*, 2015).

Even such high larval tick loads as those observed in the present study (max. 130 larvae on *Af*, 40 larvae on *Mg*) were shown not to affect the physical condition of small mammal hosts (Tälleklint and Jaenson, 1997; Hersh *et al.*, 2014), in contrast to other tick hosts like hedgehogs (Pfäffle *et al.*, 2009; Hersh *et al.*, 2014). This might additionally increase the effectiveness of small mammals as key hosts in the epizootiology of TBP. The factors that are responsible for such extreme individual intra-species differences in tick infestation are not well understood (Krasnov *et al.*, 2007; Brunner and Ostfeld, 2008; Calabrese *et al.*, 2011).

The question remains: if larvae occur largely clumped in the leaf litter or on vegetation, why do many small mammals have only few larvae while a few harbor many? To date, this question cannot be answered properly (Shaw *et al.*, 1998; Rosà, 2003; Calabrese *et al.*, 2011; Jones *et al.*, 2015). It is known that, besides general host attributes, individual host behavior can further influence tick burden, with host grooming and general avoidance behavior representing the first defense line of hosts to actively and individually reduce parasite infestation (Morand et al., 2006). The extent and influence of this impact factor is hard to estimate in detail, being roughly applicable at the species level and with respect to gender, but hardly determinable for individuals.

The second line of defense is represented by the innate and acquired immune responses of the host (Davidar *et al.*, 1989; Kurtenbach *et al.*, 1994; Hughes and Randolph, 2001; Morand *et al.*, 2006). The assessment of this parameter is cost and labor intensive and is further complicated by the fact that the individual host's immune status can change over time, making it a “moving target” (Wikel, 1996; Wikel and Bergman, 1997).

Many attempts have been made to explain aggregation on hosts, but even advanced statistical approaches using long-term data have largely failed (Brunner and Ostfeld, 2008; Calabrese *et al.*, 2011; Ferreri *et al.*, 2014). It is also necessary to determine to which extent host characteristics, associated with age, gender and species, as well as extrinsic factors, or a combination of both, account to the final tick burden on an individual host within a population (Shaw *et al.*, 2003; Brunner and Ostfeld, 2008; Calabrese *et al.*, 2011; Mysterud *et al.*, 2015). Further research, including long-term studies of defined extent, including host individuals in more detail (including immunological competence, life history), will be needed to address this topic in detail.

4.2.7 Vegetation versus vertebrates – a comparison of tick activity patterns on hosts and by flagging

The accessibility of instars or even tick species can be limited by the application of certain methods of tick collection (Estrada-Peña *et al.*, 2013). To date, there is no method available to cover tick abundance in its entirety. However, the examination of several tick instars and their dynamic, co-related activity is essential to understand tick dynamics in order to analyze the occurrence and prevalence patterns of TBP (Perret *et al.*, 2004; Gray, 2008; Pérez *et al.*, 2012). Therefore, the use of differential methods of tick sampling is essential not only to monitor ticks and TBP, but also to provide the opportunity to understand their abundance and dynamics in the field.

The tick collection from vegetation by flagging was carried out complementarily to the tick collection from small mammal hosts. This allows the direct assessment of tick activity in the field without dependence on the abundance of host individuals (Burri *et al.*, 2011a; Pérez *et al.*, 2012) and is a suitable method for exophilic tick species like *I. ricinus,* which exhibit host-seeking behavior by questing on vegetation (Tack *et al.*, 2012a). Even though all developmental stages of *I. ricinus* can be collected by flagging, my main focus was to

examine the questing activity of nymphs, the instar that represents not only the outcome of successful larval engorgement in the preceding seasons, but also the major source of TBD-infection for humans (Vassallo *et al.*, 2000; Wirtz, 2001). Analyzing the abundance and dynamics of nymphs on vegetation by flagging therefore allows the indirect analysis of the contribution of small mammal hosts and their tick burdens to the number of questing nymphs and, finally, their influence on the prevalence of TBPs.

Larvae

The mean abundance of larvae on hosts showed the continuous and extensive presence of larvae from spring to autumn, including the previously discussed variability over time and between the sites. Whereas the dynamics of larval infestation on rodents was generally in correspondence with the expected pattern of larval activity according to recent studies (Burri *et al.*, 2011a; Randolph, 2013), no distinct pattern could be observed for the questing activity of larvae, either for the sites or for year of sampling. The larval period of activity observed by flagging was neither as consistent, nor as long as shown by host sampling, indicating that the beginning and end of larval questing does not coincide with the activity of larvae in general (Pérez *et al.*, 2012). The strong variation in the data indicated erratic changes in larval activity, with a substantial proportion of sampling events without the collection of any larvae, but sudden increases to up to more than 200 larvae. As all individuals of an activity “peak” were usually collected within the same flag drag, they probably belonged to a single nest of larvae, which accordingly reduces the explanatory power of this result substantially and suggests the potentially biased nature of these results (Gray, 1985b). Due to the nature of tick oviposition in egg clutches, and the low ability of larvae to disperse (Gray, 1985a), this life history stage typically occurs in a highly aggregated pattern (Balashov, 1972). The density of larvae obtained by flagging is therefore unlikely to represent the actual density of larvae in a given area (Lauterbach *et al.*, 2013) unless extremely large areas could be covered per flagging event. This would be time and labor consuming on the one hand, and the high numbers of ticks removed would be likely to introduce some bias to following flagging events, especially over a long period of time such as in the present study.

According to the hypothesis by Brunner *et al.* (2008), larval abundance on vegetation is positively correlated with larval abundance on hosts. This is not the case for my data

regarding the tick burden on the investigated rodent hosts, but as rodents are not the exclusive hosts for *I. ricinus* larvae this does not provide sufficient scaffolding for the potential rejection of this hypothesis

Despite the fact that flagging has been used in a multitude of studies to estimate the abundance or activity of larvae in the field (Ginsberg and Ewing, 1989; Randolph and Storey, 1999; Ostfeld *et al.*, 2001; Brunner and Ostfeld, 2008; Dallas *et al.*, 2012; Egyed *et al.*, 2012; Schulz *et al.*, 2014), and has been shown to be useful to assess larval activity in evenly structured habitats like meadows (Gray, 1985a, 2008; Talleklint-Eisen and Lane, 2000), this method does not appear to produce reliable results in forest habitats of Central Europe. This was indicated not only by the present data but also several other studies (Burri *et al.*, 2011a; Pérez *et al.*, 2012; Lauterbach *et al.*, 2013), and the results obtained for larvae by flagging should be regarded with caution (Burri *et al.*, 2011a; Pérez *et al.*, 2012; Lauterbach *et al.*, 2013). Deductions from the abundance of flagged larvae in Central European forests should therefore be used more restrictively, as qualitative (present/ absent) rather than quantitative measures (larvae per unit area) of larval activity.

In conclusion, my data suggest that *I. ricinus* larval activity obtained by flagging is of limited reliability for determining the actual, temporal spectrum of activity of this developmental stage and should only be used, if at all, as a qualitative indicator only. For studies of tick phenology and pathogen transmission dynamics, the seasonal activity of *I. ricinus* larvae collected from hosts is more reliable than from flagging.

Nymphs

In comparison with larvae, nymphal abundance on hosts was extremely low (Perret *et al.*, 2000, 2004), and their general occurrence did not reveal any clear pattern. The only inference that could be made from the dynamics of nymphs on hosts was their substantial increase in 2013 (not at SW). This might be explained by nymphal exhaustion due to unfavorable weather constellation in the preceding spring that lead to a lower vertical questing activity, hence increasing the contact rate with small mammals, as previously discussed (Randolph and Storey, 1999; Kurtenbach *et al.*, 2006).

Contrary to the low amount and large variability in questing larval activity, nymphs showed a consistent level of abundance as well as a traceable pattern of activity on

vegetation between the sites and between the years of sampling. They exhibited a strong peak of activity in spring and a more or less pronounced peak towards autumn, which is in accordance with the accepted phenology of *I. ricinus* nymphs in Central Europe and Germany (Perret *et al.*, 2000, 2004; Vassallo *et al.*, 2000; Kurtenbach *et al.*, 2006; Egyed *et al.*, 2012; Schulz *et al.*, 2014).

Nymph abundance on vegetation was not reduced in 2013. This might be due to the fact that the sampling protocol for flagging included the coverage of all present heights of vegetation, including leaf litter. Therefore, even if nymphs quested more closely to the ground, this would most likely not have been reflected in a reduced number of nymphs collected by flagging as was found in other studies (Randolph and Storey, 1999).

The comparatively lower abundance of nymphs at SW (in 2012 and 2013) might relate to the lower overall temperature and relative humidity, which could lead to a smaller stock of nymphs at SW as well as to a shorter seasonal activity (Randolph, 2004, 2013). The similar abundance and dynamics of questing nymphs at the four sites during the study (despite strong fluctuations in host abundance) again raises the question of whether small mammal hosts and their feeding capacity of larvae in fact influence the abundance of nymphs. Furthermore, coinciding peaks of nymphal questing activity at all sites, as observed most clearly in May 2014, indicate the strong influence of weather-related, abiotic influences on tick activity (Jones and Kitron, 2000; Estrada-Peña *et al.*, 2004; Burri *et al.*, 2011a) at the investigated sites.

In conclusion, small mammal sampling was not able to detect specific pattern in nymph phenology except for the coinciding occurrence of nymphs on hosts and on vegetation, whereas flagging revealed nymphal activity patterns nicely at the four sites. This verified the use of flagging as a complementary sampling method to tick collection from hosts and emphasizes the importance of combining differential sampling methods to allow a more comprehensive view on tick phenology (Gray, 1985a; Estrada-Peña *et al.*, 2013). This is essential to understand tick dynamics and the factors influencing them, and can provide the basis for the analysis of TBP abundance and dynamics (Randolph, 2004; Brunner and Ostfeld, 2008; Estrada-Peña *et al.*, 2013).

Larvae on hosts versus nymphs on vegetation

As a final step, the two most reliable methods of larval and nymphal activity assessment were used to review the impact of larval activity (on hosts) on nymph abundance (on vegetation).

As shown previously, the total number of larvae feeding on rodent hosts remained remarkably stable over time, despite the strong fluctuations in host density throughout the three seasons of sampling, whereas the mean abundance of larvae per host did vary substantially over time and between sampling sites.

The comparison between the mean abundance of *I. ricinus* larvae on hosts and the abundance of questing nymphs in the following seasons did not reveal a clear relationship. At the site SW, a pattern as expected under the assumption of a true relationship between larval abundance on hosts and nymph abundance on vegetation was observed, but as this did not hold true for any of the other sites, the nature and quality of this observation is doubtful.

According to the hypothesis by Ostfeld and colleagues, the total number of larvae that feed on small mammal hosts is proportional to host density and directly influences the abundance of host-seeking nymphs in the following season (Ostfeld *et al.*, 1998b, 2001, 2006; Goodwin *et al.*, 2001). Substantial differences in the overall number of nymphs on vegetation would therefore be expected, especially between 2012 and 2013. After the mast year of 2011, rodent abundance should increase (which occurred) and the increased number of rodents should feed a larger total number of larvae in 2012, leading to increased nymphal abundance on vegetation in 2013 (which did not occur). According to this hypothesis, the opposite pattern would have been expected after the population collapse in 2013 (except for AW), leading to strongly decreased abundance of nymphs on vegetation. This did not apply either. Furthermore, the comparable number of questing nymphs at the sites throughout the study, including the, by trend, lower abundance of nymphs on vegetation at SW, conforms with the total number of larvae collected from hosts per season and suggests that the abundance of hosts is in fact of minor influence on the future number of host-seeking nymphs.

As Ostfeld's hypothesis did not apply at the four investigated sites, this confirms once more the strong differences and potentially higher complexity of the European tick-host-system compared to that in the northeastern USA, and the inappropriateness of the

application of the ”mast-mouse-tick-pathogen-hypothesis” (Ostfeld *et al.*, 1998a; b, 2001, 2006; Goodwin *et al.*, 2001) in Central Europe.

However, as the larval infestation level on individual hosts was considerably higher and more aggregated in 2013, this might impact on the number of infected nymphs on vegetation emanating from successfully fed larvae in the following season. The increased presence of host individuals that can act as super spreading organisms within the rodent host population under such circumstances might lead to transmission hotspots for TBP, providing for increased pathogen transmission in the case of, e.g. *Bbsl*, due to the highly aggregated feeding patterns of larvae, as suggested by my data.

4.2.8 How do biotic and abiotic factors shape the abundance of ticks? A statistical approach.

Tick abundance and dynamics on hosts can be influenced by a variety of factors, and the relative importance of these factors can fluctuate over time, even at a local scale. In order to identify the underlying mechanisms of a generalizable nature within the enormous amount of variation in the present data, an advanced, comprehensive statistical approach using GAM was employed. Hereby, the comprehensive analysis of the potential factors influencing tick activity allows the identification of the relative importance of these factors instead of their isolated potential relationship.

Larvae on small mammal hosts

The abundance of *I. ricinus* larvae on small mammal hosts was shown to be based on a complex network of interactions. The results obtained by applying GAM to the data revealed the direct relationship between the dynamics of the tick-host-system and its abiotic environment and further depicts the intricacy of this dynamic system itself as shown for Germany (Kiffner *et al.*, 2011) and the US (Brunner and Ostfeld, 2008). The congruence between the three final models for larvae on hosts, and independently on *Af* and *Mg*, confirmed the reliability of the modeling approach.

The complex interactions between intrinsic host attributes and environmental traits, which are in most cases likely attributed to microclimatic factors, has previously been

shown to act synergistically in shaping tick burden on small mammal hosts (Goodwin *et al.*, 2001; Shaw *et al.*, 2003; Mihalca *et al.*, 2012; Byrkjeland, 2015).

Microclimatic influences

The leaf litter layer was shown to be the most influential microclimatic factor influencing larval infestation on hosts. This is reasonable as larvae do not quest up to a height of 50 cm (= location of the next highest microclimate logger sensor), are known to be the most sensitive developmental stage with respect to microclimatic conditions and to shelter in the leaf litter even for long periods (Mejlon and Jaenson, 1997). All three final models included significant microclimatic factors measured in the leaf litter layer as predictors of tick activity on hosts.

The positive correlation between the short-term mean temperature in the leaf litter (within nine days prior to sampling) and larval burden can be attributed to the activity-inducing effect of temperature on ticks (Perret *et al.*, 2000; Jaenson and Lindgren, 2011), as well as to the increased proportion of larval activity towards the summer months in general (Kurtenbach *et al.*, 2006; Pérez *et al.*, 2012). The absence of this effect for larval burdens on *Mg* might be related to the ecology of this species: bank voles prefer microhabitats with high ground cover and extensive herb layers (Flowerdew *et al.*, 1985) where microclimatic conditions are more stable than in the surrounding areas (Ellenberg, 1996). The lesser extent of microclimatic fluctuations could affect larval activity in these narrowly defined areas, and hence could mask the influence of temperature on *Mg* infestation compared to the overall approach and the final model for *Af*. Alternatively, the lower abundance of bank voles and the lower abundance of ticks on bank voles might have added to the non-significance of short-term temperature on tick burdens on voles.

In contrast to short-term microclimate variables, the significant, negative association between long-term saturation deficit and the activity of tick larvae was present in all three host models, indicating the generality of this relationship across species. Saturation deficit was chosen as a proxy for the main drivers of tick survival and mortality, temperature and humidity (Kahl, 1989; Estrada-Peña *et al.*, 2013; del Fabbro *et al.*, 2015). Both are united in saturation deficit, which expresses the water deficit in the atmosphere (Kahl, 1989). A high saturation deficit leads to tick water and energy loss (Randolph, 2013; Estrada-Peña *et al.*, 2013). In consequence, high saturation deficit restrains the host-seeking activity of

ticks, as well as leading to increased tick mortality if it is large enough over a certain period of time (Perret *et al.*, 2000, 2003; Tagliapietra *et al.*, 2011). The consistent and uniform importance of saturation deficit for all three host models indicates the significance of this variable for tick activity, rather than small mammal species attributes.

Host attributes

The host characteristics identified as significant predictors of larval burden on rodents within my applied statistical framework (species, gender and weight as proxy for age class affiliation) are in accordance with the factors that potentially influence tick abundance according to the literature (Zuk and McKean, 1996; Tälleklint and Jaenson, 1997; Schmidt *et al.*, 1999; Wilson *et al.*, 2001; Sínski *et al.*, 2006; Kiffner *et al.*, 2011; Krasnov *et al.*, 2012). They also correspond with the nature of the relationships that have been identified when individually examining the influence of host species, age classes and genders on differential tick burdens between the respective groups of hosts. This confirms the validity of the present comprehensive statistical approach to the data. The theoretical background associated with the significantly lower larval abundance on *Mg* compared to *Af*, as well as the significant positive association of larval abundance on male versus female hosts and on adult versus subadult and juvenile individuals has been analyzed individually in previous sections and will therefore not be repeated to avoid redundancy. The negative association between abundance of small mammal hosts and individual larval *I. ricinus* burden, as shown in the general host model (species combined) and for both species-specific models, further substantiates the previously made hypothesis of the dilution effect (Krasnov *et al.*, 2007) as driver of the tick-host relationship in the present study. This confirms the observation that, according to my data, host abundance significantly influences individual tick burdens on hosts, potentially diluting pathogen prevalence in ticks as the abundance of hosts increase, but that it does not influence the total number of ticks. The fact that the increasing abundance of *Mg* rather than the abundance of *Af* was shown to be of significant negative influence on larval burdens is likely to represent a statistical artefact. Both species encountered similar overall changes in population densities during the study. Due to the resulting collinear nature of the densities of *Af* and *Mg*, it was not possible to include both predictors in the same model and the density of *Af* was removed from the initial model due to a comparatively higher

correlation factor. The resulting relationship between host density and larval abundance on hosts, as observed for *Mg* in the species-specific final model for *Af*, is therefore likely to be the masked effect for the same relationship of the species *Af.*

As previously discussed, *Mg* harbored significantly lower tick burdens than *Af* (Kurtenbach *et al.*, 1995) and can acquire resistance to *I. ricinus* by repeated infestation over time (Dizij and Kurtenbach, 1995). Such an acquired resistance is associated with a prolonged feeding duration, reduced engorgement weight and decreased molting success of ticks feeding on resistant *Mg* (Wikel, 1996; Humair *et al.*, 1999; Pérez *et al.*, 2012). A larger relative proportion of *Mg* within a small mammal community may therefore reduce larval survival and could eventually trigger a long-term decrease in tick abundance (Kurtenbach *et al.*, 1995). Such effects are most likely to have an impact in years of low host abundance (Krasnov *et al.*, 2007), as the reduced number of hosts will be exposed to a larger numbers of ticks per individual host over a comparatively shorter time span. This can lead to the accelerated acquisition of tick resistance in *Mg* (Jones *et al.*, 2015). In contrast, in areas with a relatively larger abundance of *Af*, tick populations and pathogen prevalence will thrive, even in years of low host abundance (Sínski *et al.*, 2006), as *Af* is a high capacity blood-meal host compared to *Mg*, as well as being a competent reservoir of TBP. In addition, *Af* is more effective in the transmission of TBP at higher infestation frequencies (Gern and Siegenthaler, 1994; Jones *et al.*, 2015), and an individual can remain infectious throughout its lifetime (Gern and Siegenthaler, 1994; Randolph *et al.*, 2002a).

According to the model results on tick infestation levels and dynamics on hosts, as well as the results obtained from the individual analysis of species infestation patterns, one can hypothesize that a high proportion of yellow-necked mice in the small mammal community will lead to increased survival of the overall tick population, as well as to an increased net infection rate of tick larvae (Humair *et al.*, 1999; Sínski *et al.*, 2006). Considering the dynamic influences of microclimatic parameters on tick abundance and activity, this constellation could, in fact, lead to substantial changes in TBP prevalence in nymphs on vegetation in the following season and increase the human risk of TBP-infection.

Nymphs questing on vegetation

The fundamental question behind this modeling approach was to identify whether the small mammal population and/or the number of larvae feeding on small mammals is/are of significance for the number of nymphs questing on vegetation in the following season. The analysis of the questing activity of nymphs on vegetation revealed a much simpler final model than the GAM analyzing the factors influencing larval burdens on hosts. Interestingly, the only remaining significant predictors of *I. ricinus* nymph abundance were of microclimatic origin. The final model showed that a combination of short-term temperature of the soil (6-day-interval before sampling) together with the long-term accumulated temperature (at 50cm height) could explain 51% of the variability in questing nymph density. The positive association between short-term soil moisture and nymphal abundance on vegetation is related to the accelerated rehydration rate of ticks under more humid conditions (Schwarz *et al.*, 2009; Schulz *et al.*, 2014), potentially allowing for a comparatively more extensive activity of nymphs on vegetation. The leaf litter layer represents the stratum where ticks find shelter when they suffer from water loss in the presence of a large saturation deficit over time or low relative humidity further up the vertical level (Perret *et al.*, 2000; Schwarz *et al.*, 2009; Tagliapietra *et al.*, 2011). The negative influence of the long-term accumulated temperature on the abundance of nymphs questing on vegetation can be ascribed either to the earlier onset of nymphal host-seeking activity and the associated potentially earlier host encounter leading to a more rapid loss of nymphs from the vegetation if temperatures increase (Süss *et al.*, 2008; Estrada-Peña *et al.*, 2013). Or, if the abundance of suitable hosts for nymphs is low, the earlier “activation” of nymph questing activity is likely to lead to the more rapid exhaustion of the ticks’ fat reserves (Randolph *et al.*, 2002b).

The results obtained within the modeling framework on the factors influencing nymphal activity on vegetation are therefore consistent with the comparison of ticks (larvae) on hosts and ticks (nymphs) collected by flagging, indicating that larval abundance on hosts had no effect on the number of questing nymphs in the next year, which has also been shown by Rosà *et al.* (2007).

The potential relationship between the total number of larvae feeding on small mammal hosts per session and the number of questing nymphs in the following season might be of minimal influence on the number of host-seeking nymphs, either due to the presence of

other host species, or the relatively greater importance of microclimatic influences on the activity of nymphs at a given site. It should be taken into account that the number questing nymphs does not reflect the total stock of nymphs, but rather the proportion of nymphs that are active at a given time due to their developmental history and the current microclimatic conditions (Dobson, 2014). However, as only the active part of the nymphal stock poses a potential infection risk to humans, this was of minor concern in the present study.

As the abundance of neither rodent hosts, nor the abundance of larvae collected from these hosts was shown to influence the presence of nymphal abundance on vegetation, the question remains whether there might be other hosts that have an impact on nymph abundance in the investigated areas or, if the influence that rodent hosts and the larvae they carry might influence the prevalence of TBP in these nymphs and therefore alter the infection potential for humans and companion animals.

To conclude, the fewer remaining predictors of significance and their microclimatic background showed the predominant importance of abiotic influences on the questing activity of nymphs. The positive effect of humidity together with the negative influence of long-term temperature were shown to define the abundance of questing nymphs, as together they reflect how well ticks can balance water loss and avoid desiccation and how the early onset of questing activity of nymphs can lead to the sooner finding of a host or the sooner exhaustion and potential death of the nymph.

4.3 Tick-borne pathogens

A variety of different protozoan, bacterial and viral pathogens are known to be transmitted by ticks in Germany (Petney *et al.*, 2013). The ecology of TBPs is highly complex (Silaghi *et al.*, 2012b) and based on the abundance and dynamics of their vectors, as well as populations of vertebrate that behave as reservoirs (Sonenshine and Mather, 1994; Kahl *et al.*, 2002).

In the following section, I will address my third objective, exploring the ecology of TBPs by the examination of interactions among pathogens, vector ticks and rodent hosts in a

spatially and temporally variable environment with focus on the ensuing pathogen prevalence in ticks.

The occurrence of pathogens was determined by examining their prevalence in the ticks collected on hosts and on vegetation. This provided several practical as well as analytical advantages compared to the analysis of host's blood or tissues: the practical advantage lies in the continuous monitoring of small mammal without the necessity to harm or euthanize individuals, providing a representative estimation of the circulation of the pathogen (Schmidt et al., 1999; Goodwin et al., 2001). The analytical advantage is that prevalence of the pathogens in ticks is a better estimation, since it considers the circulation potential. The infected larvae will develop into nymphs, the most aggressive stage for humans and therefore of importance to measure the hazard of infection (Wirtz, 2001).

4.3.1 Pathogen occurrence and prevalence

All five pathogens examined in the present study, *Babesia* spp., *Bbsl*, CNM, *Rspp* and TBE-V are causative agents of zoonotic diseases of humans or are of veterinary concern (Süss *et al.*, 2006; Burri *et al.*, 2011b; Silaghi *et al.*, 2012b; Parola *et al.*, 2013; Obiegala *et al.*, 2014). In either case, small mammals have a keystone function in the transmission cycle of a multitude of species comprised within the respective pathogen genera, and the predominant tick vector of the investigated pathogens is likely to be *I. ricinus* (Humair *et al.*, 1993; Ulrich *et al.*, 2009; Andreassen *et al.*, 2012; Silaghi *et al.*, 2012b; Mihalca and Sándor, 2013; Katargina *et al.*, 2015; Kalmár *et al.*, 2016).

Some of the etiologic agents of tbd, like the spirochete bacteria of the *Bbsl*-complex and TBE-V, the flavivirus causing tick-borne encephalitis, are usually referred to as established TBPs (Franke *et al.*, 2010a): they are the most abundant TBD agents of substantial medical relevance in Germany (Süss *et al.*, 2008; Hartelt *et al.*, 2008; Schwarz *et al.*, 2009) and have been extensively studied. A great deal of knowledge has been accumulated on their epidemiology with respect to disease ecology, transmission patterns, reservoirs, vectors, etc. (Barbour and Fish, 1993; Tälleklint and Jaenson, 1994; Eisen *et al.*, 2003; Süss *et al.*, 2006, 2010; Hubálek and Rudolf, 2012; Radzijevskaja *et al.*, 2013). In conjunction with

these two established pathogens, my aim was to determine whether the occurrence (TBE-V) and prevalence (*Bbsl*) conformed with previous findings.

Other pathogens, like the protozoan *Babesia* spp. and the bacterial *Rspp* and CNM belong to the causative agents of (re-) emerging infectious diseases (EID) (Silaghi *et al.*, 2012b; Parola *et al.*, 2013; Obiegala *et al.*, 2014). While it is known that they are present in Germany with potentially increasing geographical occurrence and the ability to reach remarkably high prevalence (Franke *et al.*, 2010a; Silaghi *et al.*, 2012a), there are still large knowledge gaps regarding their distribution. In addition, our understanding of their endemic transmission cycles and general epidemiology remain poor (Radzijevskaja *et al.*, 2008; Franke *et al.*, 2010a; Nicholson *et al.*, 2010; Andersson *et al.*, 2014). The examination of tick samples for the occurrence of these three EID-associated pathogens therefore aimed at identifying their general presence and/or prevalence in BW forests.

The human medical relevance of *Babesia* spp. infections is rather low (Siński *et al.*, 2006b; Häselbarth *et al.*, 2007) compared to the threat that these protozoans represent to animal health (Földvári *et al.*, 2007; Radzijevskaja *et al.*, 2008). The focus in the present study was to identify whether *Babesia* spp. were present in ticks feeding on small mammals and on vegetation focusing on the potential detection of *B. canis,* the causative agent of canine babesiosis that is mainly associated with *Dermacentor* ticks (Földvári *et al.*, 2007; Radzijevskaja *et al.*, 2008). The complete absence of *Babesia* spp. from ticks in the present study was interesting, as rodent *Babesia* Infections (e.g. *B. divergens* and *B. microti*) in ticks have been shown to be widespread throughout Germany and Europe (Pauliks, 2005; Siński *et al.*, 2006b; Földvári *et al.*, 2007; Hildebrandt *et al.*, 2008; Radzijevskaja *et al.*, 2008; Silaghi *et al.*, 2012b). This result confirmed again the complex nature of tick-host-pathogen systems with the potential absence of a pathogen despite the fulfillment of the theoretical requirements for its presence. The absence of *B. canis* in *D. reticulatus* ticks at the study sites coincides with the recent increases in both distribution and abundance of this species in Germany (Dautel *et al.*, 2006; Karbowiak, 2014) and the lag phase of pathogens that usually accompanies the invasion of new territories (Phillips *et al.*, 2016).

The nature of occurrence of TBE-V is highly focal (Süss *et al.*, 2004b) and mainly characterized by a low overall prevalence (Süss *et al.*, 2006). The pathogen thereby

establishes and persists in local foci where it is assumed to cycle between wild rodent hosts and ticks (Ginsberg and Faulde, 2008). The examination of ticks for TBE-V infection in the present study therefore aimed at the qualitative examination of the TBE-V distribution (presence/ absence). The isolated detection of TBE-V in two *I. ricinus* larvae collected from the same *S. araneus* host at HW confirms its expected low frequency, and raises the question of the role shrews might play in its epidemiological cycle of TBE-V (Tonteri and Jääskeläinen, 2011; Bown *et al.*, 2011). Insectivores are solitary living individuals, are usually trapped in substantially lower numbers compared to rodents and are more prone to stress-induced mortality in the course of trapping (Barnett and Dutton, 1995; Turni, 2005b; Nilsson and Lundqvist, 2009). Due to the higher difficulty of capture and lower overall abundance, they are often neglected in studies of TBPs, but recent studies indicate their potential importance as maintenance hosts of TBP (Bown *et al.*, 2011; Mysterud *et al.*, 2015). In the present study, *Sorex* spp. were excluded from statistical analysis due to their low abundance, but will be respected in the implications regarding TBP occurrence and persistence in the study area.

The remaining three pathogens of interest in the present study, *Bbsl*, CNM and *Rspp*, were found to be more abundant. This allowed for the examination of their prevalence in space and time and will be discussed for each pathogen individually.

While a representative subset of ticks collected in 2012 and 2013 was examined for the presence of infection with CNM as an emerging pathogen of potential impact in BW, the prevalence of *Bbsl* and *Rspp* was analyzed for ticks on hosts and ticks on vegetation throughout the three years of study.

The central focus of the discussion will therefore be the analysis of prevalence patterns of *Bbsl* and *Rspp* in BW forests along with an individual section about CNM infection prevalence.

4.3.2 *B. burgdorferi* s.l. and *Rickettsia* spp. in ticks from small mammal hosts

Rspp and *Bbsl* were expected to occur frequently and and widely distributed *in I. ricinus* (Korenberg *et al.*, 2003; Burri *et al.*, 2011b). The contribution of tick species and instars to the overall prevalence of *Bbsl* and *Rspp* observed allowed me to demonstrate that the

majority of ticks infected with *Bbsl* and *Rspp* in this study belonged to the species *I. ricinus,* followed by *D. reticulatus.*

The overall higher prevalence of *Rspp* compared to *Bbsl* might be attributed to the highly complex nature of infection acquisition by *Bbsl* compared to *Rspp*. Transovarial transmission is likely to occur and is highly effective for *Rspp* (Socolovschi *et al*., 2012), but highly unlikely for *Bbsl* (Korenberg *et al.*, 2003; Špitalská *et al.*, 2012). This potentially leads to higher *Rspp* prevalence in larvae, which can persist transstadially throughout the lifetime of the tick (Brouqui *et al.*, 2007). Furthermore, the infection of larvae with *Bbsl* can, depending on the respective genospecies involved, be facilitated by feeding on infected rodent hosts. Transmission efficiency of *Bbsl* to the feeding larvae can hence be impaired by several factors, for example the prevalence in hosts or the host immune status (Kurtenbach *et al.*, 1995; Bouchard *et al.*, 2011; Radzijevskaja *et al.*, 2013).

The differences in the ratio to which larvae and nymphs contributed to the overall pattern for both major pathogens are likely to be related to the contrasting transmission cycles of *Bbsl* and *Rspp*. The infection prevalence of *Bbsl* is expected to increase from larvae to nymphs, as *Bbsl* typically cycles between ticks and their hosts, and larvae are infected mainly by feeding on hosts that acquired *Bbsl* infection by having fed an infected nymph earlier in the season (Tälleklint and Jaenson, 1994; Gern *et al.*, 1997; Humair and Gern, 2000). The infection probability of larvae with *Bbsl* is proportional to the amount of host blood intake (Tälleklint and Jaenson, 1994). Therefore, the overall pathogen prevalence in larvae will be expected to be lower for feeding larvae compared to the prevalence observed in nymphs on rodent hosts, as they comprise large numbers of partly to mostly unengorged individuals. Furthermore, variations can arise when other host species are involved in feeding and potentially infecting *I. ricinus* larvae, which can also contribute to differences in nymphal infection prevalence (Gern *et al.*, 1997; Korenberg *et al.*, 2003).

The absence of *Bbsl* at SW might be attributed to the low overall amount of ticks at SW, which might not be sufficient to facilitate the stable circulation of *Bbsl*. This can be further impaired if a certain amount of the present larvae feeds on incompetent hosts for *Bbsl*, which would eventually lead to further attenuation of the pathogen prevalence (Schmidt *et al.*, 1999; LoGiudice *et al.*, 2003; Pfäffle *et al.*, 2015b).

In the case of *Rspp*, ticks, including *I. ricinus,* are regarded as the principal reservoir host of the pathogen and larvae are likely to be infected via transovarial transmission (Halos *et*

al., 2010; Špitalská *et al.*, 2012), but the role of rodents as reservoir host of *Rspp* is still controversially discussed (Špitalská *et al.*, 2012). Therefore, the prevalence in larvae and nymphs should be similar to each other.

The role of *I. trianguliceps* as potential reservoir or vector of *Rspp*, which is comparatively prominent at SW only, remains unclear as well. The tick species has been shown to be a competent vector of *A. phagocytophylum* (Bown *et al.*, 2008, 2011), a bacterium of the same order as *Rspp*, but the confirmation of the actual reservoir capacity of *I. trianguliceps* for *Rspp* warrants further investigation.

Contrary to its dubious role in the transmission of *Bbsl*, the involvement of *D. reticulatus* as a reservoir and vector for *Rspp* has previously been shown (Parola and Raoult, 2001b; Hartelt *et al.*, 2008; Paddock and Telford, 2010; Špitalská *et al.*, 2012). *D. reticulatus* is regarded as a minor concern for human health, but could be involved in the maintenance of pathogens like *R. raoultii* and *R. slovaca* which are responsible for causing SENLAT (scalp eschar and neck lymphadenopathy after tick bite) in humans (Dobler and Wölfel, 2009; Angelakis *et al.*, 2010; Switaj *et al.*, 2012; Parola *et al.*, 2013).

4.3.3 Host-tick-pathogen interactions – *B. burgdorferi* s.l. and *Rickettsia* spp. prevalence in *I. ricinus* on *A. flavicollis* and *M. glareolus*

After the identification of small mammal host population fluctuations and the examination of tick burden dynamics with respect to intrinsic and extrinsic conditions, the next step will be to identify whether host species, host abundance and/or tick burdens in space and time can impact the tick-borne prevalence of *Bbsl* and *Rspp* in BW.

B. burgdorferi s.l.

The overall *Bbsl* prevalence in *I. ricinus* larvae and nymphs on *Af* and *Mg* ranged at a comparable level to other Central Europe n studies (Hanincová *et al.*, 2003; Michalik *et al.*, 2003; Sínski *et al.*, 2006; Radzijevskaja *et al.*, 2013), indicating the potential importance of both species in the *Bbsl* transmission cycle at the sampling sites. The prevalence patterns observed for *Bbsl* in the two rodent species revealed the importance of host species as well as the influence of host and tick dynamics.

The pattern of *Bbsl* occurrence in ticks collected from *Af* and *Mg* indicates that these hosts contribute to the overall transmission dynamics and ecology of *Bbsl* in species-specific ways. This is presumably related to the intrinsic differences between mice and voles, such as the potential of *Mg* to acquire resistance against *I. ricinus* infestation and the antibody-mediated impaired transmission of *Bbsl* by *Af* (Kurtenbach *et al.*, 1994; Dizij and Kurtenbach, 1995). The higher total prevalence in ticks collected on *Mg* compared to *Af* matches these hypotheses and, according to Humair and Gern (1999), suggests a higher infectivity of bank voles compared to yellow-necked mice. This higher infectivity or transmission efficiency of the bank vole has been confirmed by other studies (Radzijevskaja *et al.*, 2013), and is probably most clearly observed at AW: this site showed the largest abundance of *Mg* and pathogen prevalence in both larvae and nymphs were higher and most consistently traceable among the sampling sites. Furthermore, in 2012, when the lowest number of nymphs was found on hosts, larval prevalence of *Bbsl* was only detected on *Mg*, despite the comparatively low overall infestation level of bank voles. This suggests a higher reservoir potential of *Mg* (Humair *et al.*, 1999; Radzijevskaja *et al.*, 2013), but ignores several other factors that should be taken into account to define transmission host quality. Besides the fact that *Mg* feeds substantially fewer larvae than *Af*, those that fed on *Mg* have been shown to have a significantly lower molting success (Dizij and Kurtenbach, 1995; Humair *et al.*, 1999; Pérez *et al.*, 2012). Thus, it seems that larvae feeding on bank voles are more easily infected with *Bbsl*, but *Af* feeds substantially more larvae in total and prospectively generates more *Bbsl* infected nymphs (Humair *et al.*, 1999). In addition, *Bbsl* infected individuals of *Af* have been shown to remain infectious throughout their entire life (up to 40 months) and a repeated infestation with non-infected *I. ricinus* larvae can even increase the transmission of *Bbsl* from host to tick (Gern and Siegenthaler, 1994; Jones *et al.*, 2015). All this makes *Af* the species with the higher reservoir potential for *Bbsl* (Humair *et al.*, 1999; Sínski *et al.*, 2006). This indicates potentially wide-ranging effects on *Bbsl* prevalence, depending on species community composition and relative abundance of *Af* and *Mg* on the resulting cumulative reservoir competence of the rodent community at a given locality and impact on pathogen prevalence in a pronounced way.

Besides the overall patterns of *Bbsl* prevalence in *I. ricinus* between *Af* and *Mg*, the overall infection prevalence of larvae and nymphs feeding on rodent hosts confirmed the

complex and highly dynamic nature of *Bbsl* infection in wild rodents as well as the ticks they carry.

The most prominent pattern observable for both host species (more clearly for *Af*, only at AW for *Mg*) at the sampling sites was the high larval *Bbsl* prevalence in 2013, the year in which the host population crash and the increased larval and nymphal abundance on hosts occurred.

In 2012 and 2014, the potential partitioning of larvae and nymphs on different hosts might nearly have effaced the overall prevalence of *Bbsl* (Randolph and Storey, 1999; Bouchard *et al.*, 2011). This is supported by a study by Rosà and Pugliese (2007) who state that a pathogen attenuation effect with competent hosts is possible. This means that a higher density of competent hosts, including *Af* and *Mg*, and a coinciding low tick infestation rate of hosts will lead to a lower density of infected ticks. Contrary to this, the strong increase of nymphal abundance on hosts in 2013, together with the strongly increased larval burdens on both *Af* and *Mg*, are likely to have increased the probability of *Bbsl* transmission by boosting the probability of an infected nymphs feeding on a reservoir-competent rodent. There would be an increase in the number of larvae that can acquire infection by feeding on infected reservoir hosts (Tälleklint and Jaenson, 1994; Kurtenbach *et al.*, 1995; Gern *et al.*, 1997). Furthermore, the increase in aggregation was more pronounced in *Af* than *Mg*, especially in 2013, which might have further enhanced the probability of *Bbsl* transmission and therefore led to an increase in the prevalence of *Bbsl* in larvae (Brunner and Ostfeld, 2008; Harrison and Bennett, 2012). My results therefore showed that a higher proportion of nymphs, as well as a higher tick burden on small mammal hosts, can lead to a higher prevalence of *Bbsl* in the larvae of the same year.

The increase in nymphal prevalence in 2014, after the host population breakdown and the condensed occurrence of ticks on hosts, might be related to the more extensive *Bbsl* transmission efficiency in 2013, but the actual involvement of rodents still needs to be confirmed by identification of the genospecies of *Bbsl* in nymphs: the different species of the *Bbsl* complex have been found to be highly species-specific in their host association (Hanincová *et al.*, 2003), which is assumed to be related to differential *Borrelia*-specific host complement reactivity (Kurtenbach *et al.*, 2002). The identification of *B. afzelii*, the *Bbsl* species that is cycling almost exclusively among rodent species (Humair *et al.*, 1999;

Hanincová *et al.*, 2003; van Overbeek *et al.*, 2008) in nymphs would therefore support the hypothesis of larval feeding on rodent hosts as the origin of *Bbsl* infection.

So far, it can be hypothesized that a lower small mammal density with an associated higher aggregation of larvae on hosts in one year can increase the abundance of infected nymphs and the risk for humans in the next year.

Nevertheless, there is a large variability between *Bbsl* prevalence in larvae and nymphs of *Ir* in space and time, potentially arising from intrinsic mechanisms of competition among the reservoirs or their species-specific contact rates to ticks as well as to a variety of environmental factors that potentially influence pathogen transmission (Kurtenbach *et al.*, 2006; Ogden and Tsao, 2009).

Rickettsia spp.

The overall prevalence of *Rspp* was substantially higher compared to previous findings in ticks examined in BW and Bavaria (Hartelt *et al.*, 2008; Dobler and Wölfel, 2009), as well as substantially higher compared to *Bbsl* prevalence in *I. ricinus*. These differences were particularly distinct for *I. ricinus* larvae. This indicates the need to acquire more information on the ecology of this genus comprising several emerging pathogens in Germany.

The occurrence of *Rspp* in both major host species at the four sampling sites over time revealed a prevalence pattern quite distinct from *Bbsl*. The species of host, the population crash in 2013 or the higher individual tick burdens did not have any significant effect on the prevalence patterns of *Rspp* in ticks derived from hosts. The *Rspp* prevalence patterns in larvae of *I. ricinus* were identical for both *Af* and *Mg*, with respect to either the overall prevalence and the patterns in space and time. Furthermore, *Rspp* prevalence did not show a strong increase or decrease at any of the sampling sites, as was observed in the case of *Bbsl*. This indicates the negligibility of any environmental trait or intrinsic effect, be it host population fluctuations or the increased tick burden on individual hosts, on *Rspp* prevalence.

The patterns found for *Rspp* probably arose from the special tick-pathogen interaction of many *Rspp*, as the bacteria can be vertically transmitted from mother tick to offspring (Socolovschi *et al.*, 2009). Such vertical transmission is facilitated by the transstadial maintenance of *Rspp* infection from one developmental stage to the next after which the

infection moves from one generation to the next by the gravid female passing on its infection to the egg clutch (Dobler and Wölfel, 2009; Socolovschi *et al.*, 2009). This will result in freshly hatched larvae being infected. This route of transmission has been shown to be highly effective in case of the most prevalent rickettsial species found in *I. ricinus* ticks in Germany, *R. helvetica* (Parola *et al.*, 2005). In contrast, the reservoir status of small mammals is still discussed controversially (Brouqui *et al.*, 2007; Parola *et al.*, 2013; Biernat *et al.*, 2016). Thus, the pathogen can exist virtually independently of vertebrates as reservoir hosts, and can flourish within its designated reservoir, the vector tick (Socolovschi *et al.*, 2009). Therefore, rodent host or tick abundance or dynamics are unlikely to affect *Rspp* prevalence, and my results correspond with this hypothesis. The larger degree of variability in nymphal prevalence between years might arise, among other potential factors, from the comparatively lower number of nymph samples on rodents in total, especially for *Mg*. The comparatively lower prevalence in nymphs than larvae might also indicate deleterious effects of *Rspp* infection in ticks over time. *Rickettsia conorii* infection in *Rhipicephalus sanguineus* ticks increases the mortality of ticks undergoing diapause (Socolovschi *et al.*, 2012).

The most prominent pattern of prevalence dynamics of *Rspp* in ticks from rodent hosts was the continuously increasing prevalence in larvae on both major species of hosts. The reasons for this steady rise in the proportion of infected larvae can only be speculated upon. It might be that *Rspp* has been introduced only recently to the area and will, due to its independence of competent vertebrate reservoirs as well as the widespread occurrence of *I. ricinus* in BW (Boehnke *et al.*, 2015) continuously increase until it reaches a certain threshold of prevalence. Due to the differences between the study sites with respect to habitat structure, tick and host dynamics and simply location, the synchronous introduction of *Rspp* at all sites to a similar extent seems rather unlikely. It might be that some event in the past, acting across the sampling sites, synchronously led to a substantially reduced prevalence of *Rspp* in ticks. A possible event of this character could be weather-related, acting in a similar way as the relationship between *R. conorii* infection and increased tick mortality in nymphs that undergo diapause (Socolovschi *et al.*, 2009, 2012). If this putative period of higher sensitivity coincided with detrimental weather conditions, this might have “reset” the *Rspp* prevalence.

Consequently, not only the overall *Rspp* prevalence in *I. ricinus* ticks was shown to be higher than in any other study in Germany so far, but the pattern of consistent increase in *Rspp* prevalence is alarming as well. Even though the only rickettsial species found in *I. ricinus* was *R. helvetica*, which is of comparatively low pathogenicity in humans, there are still large gaps in our knowledge of the ecology of the pathogen, its endemic transmission cycle or the potential pathogenicity in certain cohorts like immunocompromised people (Häselbarth *et al.*, 2007).

The univariate approach towards the analysis of *Bbsl* and *Rspp* prevalence in *Af* and *Mg* has already provided a first insight into the factors influencing tick-host-pathogen dynamics in BW forests, but there still remains a lot of variability that cannot be explained.

4.3.4 Tick engorgement status and pathogen prevalence

The influence of the engorgement status of *I. ricinus* larvae as proxy for the duration of host attachment revealed an increase of *Bbsl* prevalence with ongoing engorgement. The comparatively strong increase of *Bbsl* prevalence between class 1 and class 2 of engorgement correlates with the proportional uptake of spirochetes during a blood-meal, which is regarded as the usual source of infection in larvae (Gern and Siegenthaler, 1994; Radzijevskaja *et al.*, 2013) and provides the basis for *Bbsl* detection in larvae. The further increase in *Bbsl* prevalence in *I. ricinus* larvae between class 2 and class 3 might not have been fully accessible due to the predominance of larvae belonging to class 2. Nevertheless, the increase of *Bbsl* prevalence in larvae on both hosts along with increased feeding duration suggests an influence of the larval level of engorgement on pathogen prevalence and further confirms the status of rodent hosts as source of *Bbsl* infection in *I. ricinus* larvae.

In contrast, *Rspp* prevalence was already remarkably high in virtually to almost all unfed larvae of engorgement class 1 and remained constantly high for classes 2 and 3, with some variation. Anyhow, the overall pattern of *Rspp* prevalence in *I. ricinus* larvae did not reveal a significant influence of engorgement level on pathogen prevalence. The absence of any detectable relationship between different levels of engorgement and *Rspp* prevalence fits

the fact that the ticks are likely to acquire infection in a different way or by a different host. As the larvae's blood meal on small mammal hosts constitutes the first time they feed on blood after they have hatched from the egg, the potential source of infection must have happened prior to this event. This indicates infection by transovarial transmission as previously discussed (Parola *et al.*, 2005, 2013).

The findings obtained by the examination of the relationship between pathogen prevalence and engorgement status gave further insight into the system of tick-pathogen-interaction and confirmed the previous findings regarding the involvement or minor influence of hosts on tick-derived pathogen prevalence, but at a higher resolution.

4.3.5 Pathogen prevalence in ticks on hosts versus ticks on vegetation

The larval abundance on vegetation as well as the nymphal abundance on rodent hosts were shown to be of rather low and of patchy occurrence. These two measures of tick activity are therefore considered to be of limited reliability, not only towards the observation of tick dynamics, but also as reflecting the prevalence patterns of *Bbsl* and *Rspp*. Therefore, they will be used as qualitative indicators of pathogen presence, but not as a source for the elucidation of pathogen prevalence and dynamics.

***B. burgdorferi* s.l.**

The examination of the relationship between larval and nymphal *Bbsl* prevalence at the four study sites confirmed the previous finding that the abundance of infected *I. ricinus* nymphs on rodent hosts drives the prevalence of infection in larvae on these hosts. Moreover, rodent-borne-effects on *Bbsl* prevalence do not determine the abundance of *Bbsl* infected nymphs on vegetation.

The absence of *Bbsl* in larvae collected by flagging was expected due to the scarce occurrence of transovarial transmission for the causative agent of Lyme disease and the first blood-meal as source of *Bbsl* infection (Kurtenbach *et al.*, 2006; Tilly *et al.*, 2008). Interestingly, the only site with *Borrelia* infection in larvae on vegetation was SW, the site where *Bbsl* infection in ticks derived from rodents was completely absent throughout the study. Even though transovarial transmission of *Bbsl* has been sporadically reported (Tilly *et al.*, 2008; van Duijvendijk *et al.*, 2016), it is regarded as an unlikely event in the

endemiological cycle of the pathogen (Kurtenbach *et al.*, 2006). A possible explanation for the presence of unfed but infected larvae at SW is provided by a newly emerging pathogen, *Borrelia miyamotoi*, which can cause relapsing fever and Lyme disease-like manifestations in humans (Platonov *et al.*, 2011). This species and has previously been detected in *I. ricinus* ticks in Germany (Richter and Matuschka, 2010). The transmission of this *Borrelia* species involves transovarial transmission (van Duijvendijk *et al.*, 2016). In fact, Rollend *et al.* (2013) showed that infections in unfed larvae of *I. scapularis* are the results of transovarial transmission of *B. miyamotoi* and not *Bbsl*. Rodents have been confirmed as zoonotic reservoirs (Burri *et al.*, 2014; van Duijvendijk *et al.*, 2016), but birds and deer, as well as other large grazers have also been associated with *B. miyamotoi* infection (Pichon and Rogers, 2005; Richter and Matuschka, 2010; Burri *et al.*, 2014). This might explain the absence of infection in ticks on rodents, indicating a transmission cycle without rodent involvement at SW. The impending *Borrelia* genospecies analysis of these samples will clarify the potential presence of *B. miyamotoi* in larvae and nymphs at SW.

The increase in nymphal *Bbsl* prevalence on vegetation after a year with a peak of small mammal population density, as observed at AW, MB and SW in 2013, was also shown in a long-term study conducted in Russia (Korenberg *et al.*, 2003). They attributed the elevated level of *Bbsl* infection in nymphs to the higher transmission efficiency in years of high rodent abundance. However, this is not supported by my data, as *Bbsl* infection was almost absent in larvae during 2012 and could therefore not provide the rodent-borne basis of the rise of nymphal *Bbsl* prevalence in 2013. It rather seems that the larger abundance of nymphs on hosts led to an increase in larval prevalence of infection, as previously discussed for the individual host species, but that the *Bbsl* prevalence in nymphs on vegetation was not associated with any rodent-based effects. This again contradicts the hypothesis by Ostfeld *et al.* (1998, 2001, 2006) in the US, stating that rodent dynamics drive the occurrence of *Bbsl*.

The increase of tick burden on hosts together with the increased nymphal abundance on rodents in 2013 was accompanied by an overall increase in larval *Bbsl* prevalence (Krasnov *et al.*, 2007; Kiffner *et al.*, 2011) on rodent hosts. The hypothesized more effective and extensive transmission of *Bbsl* (Pérez *et al.*, 2012) did not lead to an increase of nymphal prevalence in the following season though. Together with the absence of any observable rodent-borne impact on *Bbsl* occurrence in nymphs on vegetation in 2013, my data

suggest that the endemic cycle of *Bbsl* transmission at the sampling sites is not only based on rodent-facilitated transmission of the pathogen, but also that it is likely to be influenced by other factors, and potentially other host species, which further increase the complexity of tick-host-pathogen interactions. This can mask the patterns of rodent-borne *Bbsl* transmission at the individual sites.

***Rickettsia* spp.**

The examination of *Rspp* prevalence in larvae on hosts and nymphs on vegetation might indicate a certain level of influence of rodent population dynamics on nymphal *Rspp* prevalence. Due to the extraordinary effectivity of transovarial transmission for *Rspp*, e.g. *R. helvetica* in *I. ricinus* (Parola *et al.*, 2005), leading to up to 100% infected larvae hatching from an egg clutch, the continuous increase of *Rspp* prevalence in larvae on rodent hosts might reflect the steady expansion of the pathogen as long as the infected larvae are able to find a blood-meal host to ensure their survival (Socolovschi *et al.*, 2009). From a theoretical point of view, this seems to be more likely for individual larvae in years of high rodent abundance, even though a similar total number of larvae were fed on rodent hosts despite strong fluctuations in hosts abundance (Goodwin *et al.*, 2001; Stanko *et al.*, 2006; Brunner and Ostfeld, 2008). This might be, in part, reflected by the strong decrease in nymphal *Rspp* prevalence on vegetation from 2013 to 2014. Furthermore, the prevalence of *Rspp* infection in nymphs collected on vegetation at all sites in 2012 and 2013 was higher than the prevalence in larvae feeding on hosts. This might indicate the involvement of vertebrate hosts in the transmission of *Rspp* at the four sites of investigation, leading to the potential infection of nymphs during a blood-meal (Socolovschi *et al.*, 2009; Parola *et al.*, 2013). Rodents, including *Myodes* spp. and *Apodemus* spp. as *Rspp*-susceptible vertebrate hosts of immature *I. ricinus,* might be involved in *Rspp* transmission as potential amplifying hosts (Schex *et al.*, 2011). The same is true for other small mammals like squirrels and birds (Parola *et al.*, 2005; Hornok *et al.*, 2014). Due to the relatively short rickettsiaemia of vertebrate hosts (Parola *et al.*, 2005; Hornok *et al.*, 2014), the influence of abiotic or biotic factors on tick or host abundance and dynamics might influence the extent of the impact of this putative additional source of infection. However, this remains speculative. After all, there is still an enormous amount of variation and complexity in the tick-host-*Rspp* system that cannot be explained by my data.

The ubiquitous abundance of *Rspp* at high prevalence in both larvae and nymphs accentuates the necessity for further research on the ecology and the transmission cycle of *Rspp* in BW.

4.3.6 The relative influence of hosts and ticks in shaping pathogen prevalence

B. burgdorferi s.l. *and Rickettia* spp. prevalence in ticks on hosts

Due to the expectable – and verified – absence of any simple, individually assessable relationships between pathogen prevalence and host- or tick-derived characteristics, a comprehensive statistical approach was used to analyze the relative influence of different predictors of *Bbsl* and *Rspp* prevalence in larvae and nymphs on hosts as well as in nymphs on vegetation. GLM were chosen for parsimony reasons (Blumer *et al.*, 1987), as the introduction of any explanatory terms to adjust for hierarchical (GLMM) or temporal (GAM) effects in the dataset failed to account for the extensive substructures of unknown origin that were encountered in the data and therefore did not improve the models. However, even the use of this advanced statistical approach, attempting to analyze the tick-host-pathogen system as a functional unit (Rosà *et al.*, 2007) demonstrated that the identification of the factors influencing pathogen prevalence in the field is much more challenging than for ticks.

The overall model for pathogen prevalence in larvae on hosts revealed the overall importance of host derived characteristics, like species and weight, for the presence of *Bbsl* infection, whereas the species of rodent host did not seem to affect the prevalence of *Rspp* in larvae on hosts. This coincides with my previous findings regarding the ecology of both pathogens, indicating the overall involvement of rodents and the differential competence of *Af* and *Mg* in the transmission of *Bbsl* (Tälleklint and Jaenson, 1994; Humair *et al.*, 1999; Gassner *et al.*, 2013), and the predominant role of rodents as blood-meal hosts for *Rspp* (Wodecka *et al.*, 2014). If the overall final models for *Bbsl* and *Rspp* prevalence were coherent between the individual sites, this would suggest that tick-host-pathogen-dynamics could be grasped by the available variables acting in a comparable way among sites. However, this was not the case.

The overall models were largely incompatible with the site-specific models and not applicable to the individual sites, leading to the conclusion that the applied modeling framework could simply not encompass the processes and relationships shaping the prevalence at the individual sites. It appears that different variables drive the larval prevalence of both pathogens at the individual sites and that this variability seems to be larger than the presence of any uniform site-spanning relationship. This lack of consistency between the overall and the site-specific models for both pathogens and instars made it hard to infer general principles or relationships and to distinguish between the exception and the rule. However, it serves as proof that the tick-host-pathogen system in space and time is in fact more complex than expected. As it is questionable whether the real character or meaning of a certain predictor can be assessed properly if another synergistic or antagonistic variables is present among the set of available predictors, any further attempt to relate the resulting predictors at the individual sites to actually conceivable relationships between pathogen prevalence and tick or host factors would be of dubious value.

B. burgdorferi s.l. and *Rickettsia* spp. in nymphs on vegetation

As the infection rate of nymphs on vegetation is believed to mirror the risk to humans of acquiring an infection with TBP (Wirtz, 2001; Rizzoli *et al.*, 2014), the second part of the pathogen modelling approach was dedicated to identifying factors influencing the prevalence of *Bbsl* and *Rspp* in questing nymphs. The overall models for *Bbsl* and *Rspp* prevalence in nymphs on vegetation revealed significant predictors of reasonable explanatory value. The significant positive relationship between nymphal *Bbsl* prevalence and the overall number of questing nymphs is consistent with the current epidemiological concept, as the number of questing nymphs is likely to indicate increased feeding success of larvae in the previous season (Randolph *et al.*, 1999). In addition, the probability of detection of infected ticks due to the low prevalence of *Bbsl* in the present study is increased (Schwaiger and Cassinotti, 2003; Hildebrandt *et al.*, 2008; Schorn *et al.*, 2011). This positive relationship between *Rspp* prevalence and the mean abundance of larvae per host might result from the increased number of larvae present at a given site rather than the individual burden on hosts. This is because *Rspp* is more likely to be transmitted by transovarially than to cycle between rodents and ticks. Therefore, this would

contribute positively to the increased feeding success of the larval population and feed on to an increased number of nymphs in the following season (Nilsson *et al.*, 1999; Parola *et al.*, 2013). The negative connection between host abundance and *Rspp* prevalence in questing nymphs in the following season can be explained by the dynamic nature host abundance. After a period of high abundance, host populations commonly crash (Flowerdew *et al.*, 1985; Pucek *et al.*, 1993; Rosà *et al.*, 2007). This would lead to a decreased number of hosts for ticks, including those infected with *Rspp*, and thus decrease the abundance of infected nymphs on vegetation in the following year. However, again, the final models were of rather limited reliability, with low overall variability of *Bbsl* (R^2= 0.113) being explained and the considerable lack of fit for the *Rspp* model. The application of site-specific models for nymphal prevalence on vegetation did not lead to reliable results with respect to the remaining predictor variables and therefore will, not be discussed for their relevance.

Directly traceable reasons for the limited adequacy of the modeling approach might be the low overall *Bbsl* prevalence, which impedes the detection of patterns and relationships, or the potentially minor influence of vertebrate hosts like rodents on the general transmission patterns of *Rspp*. As rodents do not represent the only hosts involved in the transmission and maintenance of *Bbsl* and *Rspp*, further investigations should include other potential hosts like birds, carnivores or other small mammals (Tälleklint and Jaenson, 1994; Oehme *et al.*, 2002; Bouchard *et al.*, 2011; Hornok *et al.*, 2014; Wodecka *et al.*, 2014). Additionally, microclimatic influences might have an indirect influence on the pathogens themselves, but directly influence the transmission patterns by affecting vertebrate hosts and tick vectors (Estrada-Peña *et al.*, 2004, 2014a; Burri *et al.*, 2011a). The large amount of variability observed in the system can only be addressed by a longer-term investigation than the three years presented here. In this way, the validity of the overall models could be refined, verified or rejected and site-specific models of increased reliability could be established.

There are a few important, additional points with respect to the general applicability and relevancy of tick-borne-pathogen models and their placement in the continuum of a universal modeling approach to pathogen transmission that should be considered (Gunawardena, 2014).

Even though I used a uniquely comprehensive and continuous dataset, consisting of three years of comprehensive data collected monthly on rodent hosts, their ticks and the pathogens they carried, it was not possible to derive any general patterns of pathogen transmission at the four investigated sites. All sites exhibited their own individual processes and patterns acting to shape pathogen prevalence in ticks on rodent hosts and on vegetation. The vast amount of variability that I encountered examining *Bbsl* and *Rspp* prevalence across four similarly shaped and closely located forest sites, challenges the universal feasibility of large-scale approaches aiming at modeling pathogen prevalence or/and disease "risk" in general. We might be able to grasp the behavior of a certain tick-borne-pathogen-system at a specific site using a large set of carefully chosen variables (Randolph, 2002; Lindgren and Jaenson, 2006; Ogden *et al.*, 2007, 2013; Gray *et al.*, 2009), but it remains questionable whether this approach can be transferable to any other site, much less be generally applicable. Even though health authorities crave for such models to explain pathogen dynamics and predict the risk of infection with TBD agents, such time and effort-consuming approaches might end up explaining the mechanisms driving pathogen prevalence at only one or a few geographical locations (Humair and Gern, 2000). Besides these analytical impediments, the situation is further complicated by the subjective perception and elusive nature of "risk". It is hard to deduce a general risk for a human population as individual exposure and other anthropogenic factors like habitat fragmentation or the urbanization of human settlements have to be taken into account (Stoddard *et al.*, 2009; Pfäffle *et al.*, 2013).

The establishment of theoretical models in order to explain the importance of eco-epidemiological aspects of TBD is of use in examining relationships and mechanisms in theory, but may only be of limited value under field conditions. Due to the highly specific and complex nature of TBD systems, it is hard to derive exact measures to use as a foundation for these models. This, together with the highly complex nature of theoretical models, as they attempt to grasp the behavior of the system, provides plenty of room for variability and interpretation.

A considerable number of studies make use of statistical modeling approaches to analyze pathogen prevalence in a specific locality based on a restricted dataset and/or a narrowly defined set of variables. Deductions made from confined datasets are likely to have limited explanatory power or to be based on spurious relationships if the biological

significance of the relationship is doubtful (Jensen *et al.*, 2000; Halos *et al.*, 2010; Barrios *et al.*, 2012).

The complexity, together with the variability of tick-borne-pathogen or -disease systems, might not allow the establishment of a single, universal model to explain pathogen dynamics in space and time, regardless of how many variables are acquired. As stated by Humair and Gern (2000), it is rather likely that every single focus of Lyme borreliosis exhibits its own transmission patterns because every single focus has its own ecological history. Instead, a sensible approach to deepen our understanding of the patterns and processes underlying the dynamics of tick-host-pathogen systems might be to initiate small-scale local studies, acquiring comprehensive long-term data of several sampling sites against the background of our current, interdisciplinary knowledge on the ecology of TBP (Eisen, 2008; Reye *et al.*, 2010; Dobson *et al.*, 2011; Johnson *et al.*, 2015). However, one should always keep in mind that models are only a tool representing a simplified version of nature that allows us to identify patterns within the range of variables we allow the model to “see”. In other words: the outcome of a model is limited to the quality and extent of variables we insert and to the ability of the modeler to choose an appropriate approach of the respective dataset.

5 SYNOPSIS AND OUTLOOK

In which way does the complex network between hosts, ticks and pathogens in a spatially and temporally variable environment interact and what implications can be drawn from this?

My study at four study sites in BW over a study period of three years provides a much more comprehensive and detailed framework than any other study in Germany so far on the epidemiological cycle of TBDs. It shows that numerous abiotic and biotic factors have a significant impact on the abundance, activity and development of *I. ricinus* and the pathogens that it transmits. I have shown that the tick-host-pathogen system present in BW is highly complex and dynamic and that even the large number of potential influential factors that have been taken into account do not suffice to completely explain the dynamic interactions between hosts, ticks and pathogens.

5.1 Small mammals

In BW, all of the areas examined showed a very similar community structure with respect to the potential small mammal hosts of ticks. Within this community, *Af* and *Mg* were the most conspicuous species, dominating the trapping data. This consistency indicated that ticks (particularly larvae) have a high probability of encountering one of these species.

5.2 Ticks

I have shown that *Af* and *Mg* differ in their roles as hosts for ticks, as well as for TBP. Both species were substantially more often infested with larvae than nymphs of *I. ricinus*. *Af* was more frequently and more heavily infested with both immature stages of *I. ricinus*, demonstrating its better suitability for ticks compared to bank voles, which can acquire resistance to *I. ricinus* infestation. The number of larvae per host was dependent on host abundance (i.e. the fewer hosts the more larvae per host), but the overall number of nymphs found within the host population was not related to rodent abundance in the previous year.

In general, there was a great deal of variation in the dynamics of tick populations, for all life history stages, both spatially and temporally. This lack of consistency was unexpected and is contrary to the current paradigm that larvae show a summer peak and that adults and nymphs show spring and autumn peaks. The reasons for this variability could be assigned to various abiotic factors but were not obvious from direct examination of the data. Therefore, a more advanced modelling approach was used (see below).

5.3 Pathogens

While *Mg* was more infective for larvae in relation to the acquisition of *Bbsl*, *Af* was shown to carry infected *Bbsl* larvae more often. In contrast, both species carried the same proportion of *Rspp*-infected ticks, indicating that the mere presence of host rather than any species-related attributes influences *Rspp* prevalence. Thus, both host species were shown to contribute differently to the cumulative reservoir competence.

Despite the influence of the overall abundance of nymphs on hosts on *Bbsl* prevalence in host-derived larvae, this does not seem to influence the abundance of infected nymphs on vegetation, whereas *Rspp* prevalence in nymphs seems to be influenced by host abundance.

5.4 Modelling

Advanced statistical analysis (GLM, GAM) was used to identify the most important factors influencing tick abundance and pathogen prevalence in BW and provide a deeper insight in the complex system of interactions between ticks, hosts and pathogens in a spatially and temporally variable environment. This allowed me to draw first conclusions regarding the mechanisms and relationships acting to shape the system:

While the abundance of ticks on hosts was based on the interaction between microclimatic factors and host population characteristics and showed the importance of male adult *Af* as predominant host for tick larvae, the abundance of nymphs on vegetation did only rely on microclimatic short-term and long-term conditions, not showing any influence of rodent host populations.

The major result of the pathogen models was that the behavior of the tick-host-pathogen system is difficult to grasp within a short time frame and probably between different sites.

It could be shown that rodents are of potential relative importance to the prevalence of *Bbsl* and *Rspp* with respect to their species community composition (*Bbsl*) or their overall abundance (*Rspp*), but that they are unlikely to be the exclusive hosts involved in *Bbsl* transmission.

One of the central outcomes of my study is therefore to show clearly that the paradigm of rodents as central drivers of TBPs in the wild, i.e. the hypothesis put forward by Ostfeld *et al.* based on data from the northeastern USA, does not apply in Central Europe. This hypothesis, which fits the North American data, has frequently been assumed to apply to Europe as well. This is not the case. Rather, my data shows that the epidemiological patterns vary between habitats and years making any form of generalization difficult. Each habitat must be considered as a separate entity in which the pattern of tick-host-pathogen interactions must be studied over a long time span in order to define the fine scale interconnections. In fact, the fundamental aspect of every ecological study is the need to deal with enormous amounts of variability in the system, mainly due to complexity and the limited options to control for general parameters under field conditions. If a study limits its scope to only one site of investigation or by an observation period of only one year or less, it is unlikely to provide an accurate picture of the system under consideration.

Although the general “risk” posed by the presence of ticks in an area can be, to some extent, determined and predicted, health practitioners and decision makers demanding risk maps for TBPs should take into account the enormous dynamic capacities inherent to this complex system and realize that “risk” is a factor that cannot be easily modelled, or maybe not all.

5.5 Outlook

Tick-host-pathogen systems in BW and Germany are far more complex than in those in the eastern US. In the US, the main agent of Lyme disease is *B. burgdorferi* s.s., the main reservoir is *P. leucopus* and the main vector *I. scapularis*. Pathogen diversity is much larger in Germany, a variety of different host species can serve as tick hosts and these have different capacities to act as disease reservoirs. In order to consolidate our knowledge on the interactions within the tick-host-pathogen-environment network and to be able to differentiate between the exception and rule, further years of continuous field studies, taking more host species into consideration (for example deer which act as the major hosts for female *I. ricinus*) are needed. This should be combined with the analysis of the transmission characters of the TBPs under controlled laboratory conditions and the molecular determination of the species and strains/ ecotypes of the pathogens involved.

6 LITERATURE

Adler, S. (2009). Zwergspitzmaus *Sorex minutus*. In *Atlas der Säugetiere Thüringens* (ed. Görner, M.), pp. 90–91. Arbeitsgruppe Artenschutz Thüringen e. V. und Landesjagdverband Thüringen e. V., Jena.

Aho, K., Derryberry, D. and Peterson, T. (2014). Model selection for ecologists: the worldviews of AIC and BIC. *Ecology* 95 (3) pp. 631–636.

Allan, S. A. (2013). Tick rearing and in vitro feeding. In *Biology of ticks, Vol.2*, pp. 445–473.

Alpers, K., Stark, K., Hellenbrand, W. and Ammon, A. (2004). Zoonotische Infektionen beim Menschen. *Bundesgesundheitsblatt - Gesundheitsforschung - Gesundheitsschutz* 47 (7) pp. 622–632.

Anderson, J. F. (1991). Epizootiology of Lyme borreliosis. *Scandinavian Journal of Infectious Diseases.* 77 (Suppl 1) pp. 23–34.

Anderson, R. M. and Gordon, D. M. (1982). Process influencing the distribution of parasite numbers wihin host populations. *Parasitology* 85 pp. 373–398.

Anderson, J. F. and Magnarelli, L. A. (1984). Avian and mammalian hosts for spirochete-infected ticks and insects in a Lyme disease focus in Connecticut. *The Yale Journal of Biology and Medicine* 57 (4) pp. 627–41.

Andersson, M., Scherman, K. and Raberg, L. (2014). Infection dynamics of the tick-borne pathogen "*Candidatus* Neoehrlichia mikurensis" and coinfections with *Borrelia afzelii* in bank voles in southern Sweden. *Applied and Environmental Microbiology* 80 (5) pp. 1645–1649.

Andreassen, A., Jore, S., Cuber, P., Dudman, S., Tengs, T., Isaksen, K., Hygen, H. O., Viljugrein, H., Anestad, G., Ottesen, P. and Vainio, K. (2012). Prevalence of tick borne encephalitis virus in tick nymphs in relation to climatic factors on the southern coast of Norway. *Parasites & Vectors* 5 (177) pp. 1–12.

Andréasson, K., Jönsson, G., Lindell, P., Gülfe, A., Ingvarsson, R., Lindqvist, E., Saxne, T., Grankvist, A., Wenneras, C. and Marsal, J. (2015). Recurrent fever caused by *Candidatus* Neoehrlichia mikurensis in a rheumatoid arthritis patient treated with rituximab. *Rheumatology (United Kingdom)* 54 (2) pp. 369–371.

Andrzejewski, R. and Olszewski, J. (1963). Social behaviour and interspecific relations in *Apodemus flavicollis* (Melchior, 1834) and *Clethrionomys glareolus* (Schreber, 1780). *Acta Theriologica* 7 (10) pp. 155–168.

Angelakis, E., Waton, J., Imbert, P., Socolovschi, C., Edouard, S. and Dellamonica, P. (2010). Scalp eschar and neck lymphadenopathy caused by *Bartonella henselae* after tick bite. *Clinical Infectious Diseases* 50 pp. 549–551.

Arneberg, P., Skorping, A., Grenfell, B. and Read, A. F. (1998). Host densities as determinants of abundance in parasite communities. *Proceedings of the Royal Society B: Biological Sciences* 265 (1403) pp. 1283–1289.

Arthur, D. R. (1963). *British ticks*. London: Butterworths, London.

Balashov, Y. S. (1972). Bloodsucking ticks (Ixodoidea) - vectors of diseases of man and animals. In *Miscellaneous Publications of the Entomological Society of America*, pp. 163–376.

Barbour, A. G. and Fish, D. (1993). The biological and social phenomenon of Lyme disease. *Science* 260 (5114) pp. 1610–1616.

Barnett, A. and Dutton, J. (1995). Expedition Field Techniques: Small mammals (excluding bats). In *Geography outdoors*, p. 140. London.

Barré, N., Garris, G. and Camus, E. (1995). Propagation of the tick *Amblyomma variegatum* in the Caribbean. *Rev Sci Tech* 14 (3) pp. 841–855.

Barrett, G. W. and Peles, J. D. (1999). Small Mammal Ecology: A Landscape perspective. In *Landscape Ecology of Small Mammals*, pp. 1–10. Springer Science & Business Media, New York.

Barrios, J. M., Verstraeten, W. W., Maes, P., Aerts, J.-M., Farifteh, J. and Coppin, P. (2012). Using the gravity model to estimate the spatial spread of vector-borne diseases. *InternationalJjournal of Environmental Research and Public Health* 9 (12) pp. 4346–4364.

Barutzki, D., Reule, M., Scheunemann, R. and Heile, C. (2007). Die Babesiose des Hundes. Eine autochthone Erkrankung in Deutschland. *Deutsches Tierärzteblatt* 3 pp. 284–292.

Baumgarten, B. U., Röllinghoff, M. and Bogdan, C. (1999). Prevalence of *Borrelia burgdorferi* and granulocytic and monocytic ehrlichiae in *Ixodes ricinus* ticks from southern Germany. *Journal of Clinical Microbiology* 37 (11) pp. 3448–3451.

Beelitz, P., Wild, D., Wild, S. and Pfister, K. (2008). Update on *Babesia canis* infections in dogs in Bavaria and Baden-Wuerttemberg, Germany. *Kleintierpraxis* 53 (2) pp. 88–94.

Bergstedt, B. (1965). Distribution, reproduction, growth and dynamics of the rodent species *Clethrionomys glareolus* (Schreber), *Apodemus flavicollis* (Melchior) and *Apodemus sylvaticus* (Linne) in Southern Sweden. *Oikos* 16 (1/2) pp. 132–160.

Biernat, B., Stańczak, J., Michalik, J., Sikora, B. and Wierzbicka, A. (2016). Prevalence of infection with *Rickettsia helvetica* in *Ixodes ricinus* ticks feeding on non-rickettsiemic rodent hosts in sylvatic habitats of west-central Poland. *Ticks and Tick-borne Diseases* 7 (1) pp. 135–141.

Blatt, C. and Resch, S. (2016a). www.kleinsaeuger.at.

Blatt, C. and Resch, S. (2016b). Gelbhalsmaus - *Apodemus flavicollis*. In *Internethandbuch über Kleinsäugerarten im mitteleuropäischen Raum: Körpermerkmale, Ökologie und Verbreitung*, kleinsaeuger.at, Salzburg.

Blatt, C. and Resch, S. (2016c). Rötelmaus - *Myodes glareolus*. In *Internethandbuch über Kleinsäugerarten im mitteleuropäischen Raum: Körpermerkmale, Ökologie und Verbreitung*, kleinsaeuger.at, Salzburg.

Blatt, C. and Resch, S. (2016d). Erdmaus - *Microtus agrestis*. In *Internethandbuch über Kleinsäugerarten im mitteleuropäischen Raum: Körpermerkmale, Ökologie und Verbreitung*, www.kleinsaeuger.at, Salzburg.

Blumer, A., Ehrenfeucht, A., Haussler, D. and Warmuth, M. K. (1987). Occam's Razor. *Information Processing Letters* 24 (6) pp. 377–380.

Boehnke, D., Brugger, K., Pfäffle, M., Sebastian, P., Norra, S., Petney, T., Oehme, R., Littwin, N., Lebl, K., Raith, J., Walter, M., Gebhardt, R. and Rubel, F. (2015). Estimating *Ixodes ricinus* densities on the landscape scale. *International Journal of Health Geographics* 14 (1) pp. 23–34.

Bonnet, S., Jouglin, M., Malandrin, L., Becker, C. A. M., Agoulon, A., L'Hostis, M. and Chauvin, A. (2007). Transstadial and transovarial persistence of *Babesia divergens* DNA in *Ixodes ricinus* ticks fed on infected blood in a new skin-feeding technique. *Parasitology* 134 (Pt 2) pp. 197–207.

Bonnet, S., Brisseau, N., Hermouet, A., Jouglin, M. and Chauvin, A. (2009). Experimental in vitro transmission of *Babesia* sp. (EU1) by Ixodes ricinus. *Veterinary Research* 40

(3) pp. 21–27.

Boonstra, R., Krebs, C. and Stenseth, N. (1998). Population cycles in small mammals: the problem of explaining the low phase. *Ecology* 79 (5) pp. 1479–1488.

Bouchard, C., Beauchamp, G., Nguon, S., Trudel, L., Milord, F., Lindsay, L. R., Bélanger, D. and Ogden, N. H. (2011). Associations between *Ixodes scapularis* ticks and small mammal hosts in a newly endemic zone in southeastern Canada: implications for *Borrelia burgdorferi* transmission. *Ticks and Tick-borne Diseases* 2 (4) pp. 183–190.

Bouyer, D. H., Stenos, J., Crocquet-Valdes, P., Moron, C. G., Popov, V. L., Zavala-Velazquez, J. E., Foil, L. D., Stothard, D. R., Azad, A. F. and Walker, D. H. (2001). *Rickettsia felis*: Molecular characterization of a new member of the spotted fever group. *International Journal of Systematic and Evolutionary Microbiology* 51 (2) pp. 339–347.

Bown, K. J. and Begon, M. (2003). Seasonal dynamics of *Anaplasma phagocytophila* in a rodent-tick (*Ixodes trianguliceps*) system, United Kingdom. *Emerging Infectious Diseases* 9 (1) pp. 63–70.

Bown, K. J., Begon, M., Bennett, M., Birtles, R. J., Urthie, S., Lambin, X., Telfer, S., Woldehiwet, Z. and Ogden, N. H. (2006). Sympatric *Ixodes trianguliceps* and *Ixodes ricinus* ticks feeding on field voles (*Microtus agrestis*): Potential for increased risk of Anaplasma phagocytophilum in the United Kingdom? *Vector Borne and Zoonotic Diseases* 6 (4) pp. 404–410.

Bown, K. J., Lambin, X., Telford, G. R., Ogden, N. H., Telfer, S., Woldehiwet, Z. and Birtles, R. J. (2008). Relative importance of *Ixodes ricinus* and *Ixodes trianguliceps* as vectors for *Anaplasma phagocytophilum* and *Babesia microti* in field vole (*Microtus agrestis*) populations. *Applied and Environmental Microbiology* 74 (23) pp. 7118–7125.

Bown, K. J., Lambin, X., Telford, G., Heyder-Bruckner, D., Ogden, N. H. and Birtles, R. J. (2011). The common shrew (*Sorex araneus*): a neglected host of tick-borne infections? *Vector Borne and Zoonotic Diseases* 11 (7) pp. 947–953.

Boyard, C., Vourc'h, G. and Barnouin, J. (2008). The relationships between *Ixodes ricinus* and small mammal species at the woodland-pasture interface. *Experimental & Applied Acarology* 44 (1) pp. 61–76.

Braun, M. and Dieterlen, F. (2005). *Die Säugetiere Baden-Württembergs. Band 2: Insektenfresser (Insectivora), Hasentiere (Lagomorpha), Nagetiere (Rodentia), Raubtiere (Carnivora), Paarhufer (Artiodactyla)*. Stuttgart (Hohenheim).

Bröker, M. and Gniel, D. (2003). New foci of tick-borne encephalitis virus in Europe: consequences for travellers from abroad. *Travel medicine and infectious disease* 1 (3) pp. 181–184.

Brouqui, P., Parola, P., Fournier, P. E. and Raoult, D. (2007). Spotted fever rickettsioses in southern and eastern Europe. *Federation of European Microbiological Societies* 49 (1) pp. 2–12.

Brown, J. H., Whitham, T. G., Morgan Ernest, S. K. and Gehring, C. A. (2001). *Complex species interactions and the dynamics of ecological systems: long-term experiments*.

Brunner, J. L. and Ostfeld, R. S. (2008). Multiple causes of variable tick burdens on small-mammal hosts. *Ecology* 89 (8) pp. 2259–72.

Buesching, C. D., Newman, C., Twell, R. and Macdonald, D. W. (2008). Reasons for arboreality in wood mice *Apodemus sylvaticus* and bank voles *Myodes glareolus*. *Mammalian Biology* 73 (4) pp. 318–324.

Bugarski-Stanojević, V., Blagojević, J., Adnađević, T., Jovanović, V. and Vujošević, M.

(2013). Identification of the sibling species *Apodemus sylvaticus* and *Apodemus flavicollis* (Rodentia, Muridae)—Comparison of molecular methods. *Zoologischer Anzeiger* 252 (4) pp. 579–587.

Burgdorfer, W., Barbour, A., Hayes, S., Benach, J., Grunwaldt, E. and Davis, J. (1982). Lyme disease-a tick-borne spirochetosis? *Science* 216 (4552) pp. 1317–1319.

Burkhardt, J. F. and Schlund, W. (2005). Rötelmaus *Clethrionomys glareolus* (Schreber, 1780). In *Die Säugetiere Baden-Württembergs. Band 2*, pp. 289–296. Ulmer Verlag, Stuttgart (Hohenheim).

Burri, C., Bastic, V., Maeder, G., Patalas, E. and Gern, L. (2011a). Microclimate and the zoonotic cycle of tick-borne encephalitis virus in Switzerland. *Journal of Medical Entomology* 48 (3) pp. 615–627.

Burri, C., Dupasquier, C., Bastic, V. and Gern, L. (2011b). Pathogens of emerging tick-borne diseases, *Anaplasma phagocytophilum, Rickettsia* spp., and *Babesia* spp., in *Ixodes* ticks collected from rodents at four sites in Switzerland (Canton of Bern). *Vector Borne and Zoonotic Diseases* 11 (7) pp. 939–944.

Burri, C., Schumann, C., Schumann, O. and Gern, L. (2014). Are *Apodemus* spp. mice and *Myodes glareolus* reservoirs for *Borrelia miyamotoi, Candidatus* Neoehrlichia mikurensis, *Rickettsia helvetica, R. monacensis* and *Anaplasma phagocytophilum*? *Ticks and Tick-borne Diseases* 5 pp. 245–251.

Byrkjeland, R. (2015). The role of small rodents and shrews as hosts for ticks and reservoirs of tick-borne pathogens in a northern coastal forest ecosystem. Master Thesis.

Calabrese, J. M., Brunner, J. L. and Ostfeld, R. S. (2011). Partitioning the aggregation of parasites on hosts into intrinsic and extrinsic components via an extended poisson-gamma mixture model. *PLoS ONE* 6 (12) pp. e29215.

Churchfield, S. and Searle, J. B. (2008a). Common shrew *Sorex araneus*. In *Mammals of the British Isles* (eds. Harris, S. and Yalden, D. W.), pp. 257–265. The Mammal Society, Southhampton.

Churchfield, S. and Searle, J. B. (2008b). Pygmy shrew *Sorex minutus*. In *Mammals of the British Isles* (eds. Harris, S. and Yalden, D. W.), pp. 267–271. The Mammal Society, Southampton.

Claude, C. (1995). *Clethrionomys glareolus* (Schreber, 1780). In *Die Säugetiere der Schweiz: Verbreitung, Biologie und Ökologie* (ed. Hausser, J.), Birkhäuser Verlag, Basel.

Comer, J. (2001). Urban zoonoses caused by *Bartonella, Coxiella, Ehrlichia*, and *Rickettsia* species. *Vector Borne Zoonotic Diseases* 1 (2) pp. 91–118.

Cooch, E. and White, G. C. (2012). *Program MARK "A gentle introduction."* 11th ed. (eds. Cooch, Evan and White, G.).

Crofton, H. D. (1971). A quantitative approach to parasitism. *Parasitology* 62 (02) pp. 179–193.

Dallas, T. A., Foré, S. A. and Kim, H.-J. (2012). Modeling the influence of *Peromyscus leucopus* body mass, sex, and habitat on immature *Dermacentor variabilis* burden. *Journal of Vector Ecology* 37 (2) pp. 338–341.

Danielová, V., Rudenko, N., Daniel, M., Holubová, J., Materna, J., Golovchenko, M. and Schwarzová, L. (2006). Extension of *Ixodes ricinus* ticks and agents of tick-borne diseases to mountain areas in the Czech Republic. *International Journal of Medical Microbiology* 296 (Suppl 1) pp. 48–53.

Dantas-Torres, F. (2010). Biology and ecology of the brown dog tick, *Rhipicephalus sanguineus*. *Parasites & Vectors* 3 (26) pp. 1–11.

Dautel, H. and Kahl, O. (1999). Ticks (Acari: Ixodoidea) and their medical importance in the urban environment. *Proceedings of the Third International Conference on Urban Pests: 19–22 July 1999.* pp. 73–82.

Dautel, H. and Kahl, O. (2009). Die Auwaldzecke breitet sich aus. *LWF aktuell* 73 pp. 28–29.

Dautel, H., Dippel, C., Oehme, R., Hartelt, K. and Schettler, E. (2006). Evidence for an increased geographical distribution of *Dermacentor reticulatus* in Germany and detection of *Rickettsia* sp. RpA4. *International Journal of Medical Microbiology* 296 (Suppl 1) pp. 149–156.

Davidar, P., Wilson, M. and Ribeiro, J. M. C. (1989). Differential distribution of immature *Ixodes dammini* (Acari: Ixodidae) on rodent hosts. *Journal of Parasitology* 75 (6) pp. 898–904.

de la Fuente, J., Estrada-Peña, A., Venzal, J. M., Kocan, K. M. and Sonenshine, D. E. (2008). Ticks as vectors of pathogens that cause disease in humans and animals. *Frontiers in Bioscience* 13 pp. 6938–6946.

del Fabbro, S., Gollino, S., Zuliani, M. and Nazzi, F. (2015). Investigating the relationship betwen environmental factors and tick abundance in a small, highly heterogeneous region. *Journal of Vector Ecology* 40 (1) pp. 107–116.

Delany, M. J. (1974). The ecology of small mammals. *Studies in biology **51**,* pp. 1–60.

Demuth-Birkert, M. (2004). Mammalia: Insectivora , Rodentia (Kleinsäuger). *Naturwaldreservate in Hessen 6/2 Schönbuche. Zoologische Untersuchungen 1990-1992. Kurzfassung*.

Deutscher Wetterdienst (2012). *Kältewelle in Europa und Asien im Spätwinter 2011 / 2012*.

Deutscher Wetterdienst (2014). *Pressemitteilung: Deutschlandwetter im Winter 2013/ 2014*. Offenbach.

Diuk-Wasser, M. A., Vannier, E. and Krause, P. J. (2016). Coinfection by *Ixodes* tick-borne pathogens: ecological, epidemiological, and clinical consequences. *Trends in Parasitology* 32 (1) pp. 30–42.

Dizij, A. and Kurtenbach, K. (1995). *Clethrionomys glareolus*, but not *Apodemus flavicollis*, acquires resistance to *Ixodes ricinus* L., the main European vector of *Borrelia burgdorferi*. *Parasite immunology* 17 (4) pp. 177–83.

Dobler, G. (1998). Durchseuchung von Zecken in Bayern und Baden-Württemberg mit FSME. *Der Internist* 39 (2) pp. 231–232.

Dobler, G. and Wölfel, R. (2009). Fleckfieber und andere Rickettsiosen: Alte und neu auftretende Infektionen in Deutschland. *Deutsches Ärzteblatt* 106 (20) pp. 348–354.

Dobler, G., Essbauer, S. and Wölfel, R. (2009). Isolation and preliminary characterisation of "*Rickettsia monacensis*" in south-eastern Germany. *Clinical Microbiology and Infection* 15 (Suppl 2) pp. 263–264.

Dobson, A. D. M. (2014). History and complexity in tick-host dynamics: discrepancies between "real" and "visible" tick populations. *Parasites & Vectors* 7 (1) pp. 231.

Dobson, A. D. M., Taylor, J. L. and Randolph, S. E. (2011). Tick (*Ixodes ricinus*) abundance and seasonality at recreational sites in the UK: hazards in relation to fine-scale habitat types revealed by complementary sampling methods. *Ticks and Tick-borne Diseases* 2 (2) pp. 67–74.

Don, B. A. C. (1979). Gut analysis of small mammals during a sawfly (*Cephalcia lariciphila*) outbreak. *Journal of Zoology* 188 (2) pp. 290–294.

Donoso-Mantke, O., Karan, L. S. and Růžek, D. (2011). Tick-Borne Encephalitis Virus: A General Overview. *Flavivirus Encephalitis* pp. 133–156.

Dorn, W., Messutat, S., Kipp, S., Sünder, U., Feldman, A. and Flügel, C. (1999). Seasonal variation in the infestation of rodents with *Ixodes ricinus* (Acari: *Ixodidae*) and prevalence of infection with *Borrelia burgdorferi* in a recreation area. *Proceedings of the 3rd International conference on urban pests* pp. 463–469.

Durden, L. A. (2006). Taxonomy, host associations, life cycles and vectorial importance of ticks parasitizing small mammals. In *Micromammals and Macroparasites* (eds. Morand, S., Krasnov, B., and Poulin, R.), pp. 91–102. Springer Japan, Tokyo.

Durden, L. A. and Beati, L. (2014). Modern tick systematics. In *Biology of ticks* (eds. Sonenshine, D. E. and Roe, R. M.), pp. 17–58. Oxford University Press, New York City, New York.

Efford, M. (2004). Density estimation in live-trapping studies. *Oikos* 106 pp. 598–610.

Egyed, L., Elő, P., Sréter-Lancz, Z., Széll, Z., Balogh, Z. and Sréter, T. (2012). Seasonal activity and tick-borne pathogen infection rates of *Ixodes ricinus* ticks in Hungary. *Ticks and Tick-borne Diseases* 3 (2) pp. 90–4.

Eisen, L. (2008). Climate change and tick-borne diseases: A research field in need of long-term empirical field studies. *International Journal of Medical Microbiology* 298 (Suppl 1) pp. 12–18.

Eisen, R. J. R., Eisen, L., Castro, M. M. B. and Lane, R. R. S. (2003). Environmentally related variability in risk of exposure to Lyme disease spirochetes in Northern California: effect of climatic conditions and habitat type. *Environmental Entomology* 32 (5) pp. 1010–1018.

Ellenberg, H. (1996). Lebensbereich der wichtigen Baumarten. In *Vegetation Mitteleuropas mit den Alpen*, pp. 116–122.

Engelmann, H.-D. (1978). Zur Dominanzklassifizierung von Bodenarthropoden. *Pedobiologia* 18 pp. 378–380.

Essbauer, S., Schex, S., Splettstoesser, W., Pfeffer, M., Ulrich, R. G., Seibold, E., Dobler, G., Wölfel, R. and Bäumler, W. (2009). Nagetier-übertragene Zoonosen : Beispiele aus Untersuchungen in Süd- und Westdeutschland. *Wirbeltierforschung in der Kulturlandschaft* 421 pp. 37–48.

Estrada-Peña, A. (2001). Forecasting habitat suitability for ticks and prevention of tick-borne diseases. *Veterinary Parasitology* 98 pp. 111–132.

Estrada-Peña, A. (2008). Climate, niche, ticks, and models: what they are and how we should interpret them. *Parasitology Research* 103 (Suppl 1) pp. 87–95.

Estrada-Peña, A. and de la Fuente, J. (2014). The ecology of ticks and epidemiology of tick-borne viral diseases. *Antiviral Research* 108 pp. 104–128.

Estrada-Peña, A. and Jongejan, F. (1999). Ticks feeding on humans: a review of records on human-biting Ixodoidea with special reference to pathogen transmission. *Experimental & Applied Acarology* 23 (9) pp. 685–715.

Estrada-Peña, A., Martinez, J. M., Sanchez Acedo, C., Quilez, J. and Del Cacho, E. (2004). Phenology of the tick, *Ixodes ricinus*, in its southern distribution range (central Spain). *Medical and Veterinary Entomology* 18 (4) pp. 387–97.

Estrada-Peña, A., Osácar, J., Pichon, B. and Gray, J. S. (2005). Hosts and pathogen detection for immature stages of *Ixodes ricinus* (Acari: Ixodidae) in North-Central

Spain. *Experimental & Applied Acarology* 37 pp. 257–268.

Estrada-Peña, A., Venzal, J. M. and Sánchez Acedo, C. (2006). The tick *Ixodes ricinus*: distribution and climate preferences in the western Palaearctic. *Medical and Veterinary Entomology* 20 (2) pp. 189–97.

Estrada-Peña, A., Ayllón, N. and de la Fuente, J. (2012). Impact of climate trends on tick-borne pathogen transmission. *Frontiers in Physiology* 3 (March) pp. 64.

Estrada-Peña, A., Gray, J. S., Kahl, O., Lane, R. S. and Nijhof, A. M. (2013). Research on the ecology of ticks and tick-borne pathogens-methodological principles and caveats. *Frontiers in Cellular and Infection Microbiology* 3 (29) pp. 1–12.

Estrada-Peña, A., Ostfeld, R. S., Peterson, A. T., Poulin, R. and de la Fuente, J. (2014a). Effects of environmental change on zoonotic disease risk: an ecological primer. *Trends in Parasitology* pp. 1–10.

Estrada-Peña, A., Nava, S. and Petney, T. N. (2014b). Description of all the stages of *Ixodes inopinatus* n. sp. (Acari: Ixodidae). *Ticks and Tick-borne Diseases* 5 (6) pp. 734–743.

Estrada-Peña, A., de la Fuente, J., Ostfeld, R. S. and Cabezas-Cruz, A. (2015). Interactions between tick and transmitted pathogens evolved to minimise competition through nested and coherent networks. *Scientific Reports* 5 pp. e10361.

Estrada-Peña, A., Alexander, N. and Wint, G. R. W. (2016). Perspectives on modelling the distribution of ticks for large areas: so far so good? *Parasites & Vectors* 9 (1) pp. 179.

Faulde, M. and Hoffmann, G. (2001). Vorkommen und Verhütung vektorassoziierter Erkrankungen des Menschen in Deutschland unter Berücksichtigung zoonotischer Aspekte. *Bundesgesundheitsblatt - Gesundheitsforschung - Gesundheitsschutz* 44 (2) pp. 116–136.

Ferreri, L., Giacobini, M., Bajardi, P., Bertolotti, L., Bolzoni, L., Tagliapietra, V., Rizzoli, A. and Rosà, R. (2014). Pattern of tick aggregation on mice: larger than expected distribution tail enhances the spread of tick-borne pathogens. *PLoS Computational Biology* 10 (11) pp. e1003931.

Filippova, N. A. (1990). The taxonomic aspects of the transmission of the causative agent of Lyme disease. *Parazitologiia* 24 (4) pp. 257–267.

Fingerle, V., Schulte-Spechtel, U. C., Ruzic-Sabljic, E., Leonhard, S., Hofmann, H., Weber, K., Pfister, K., Strle, F. and Wilske, B. (2008). Epidemiological aspects and molecular characterization of *Borrelia burgdorferi* s.l. from southern Germany with special respect to the new species *Borrelia spielmanii* sp. nov. *Int J Med Microbiol* 298 (3-4) pp. 279–90.

Flowerdew, J. R. (1984). *Woodmice and yellow-necked mice. Mammal Society Series*. (ed. Burton, R.) The Mammal Society, Shrewsbury.

Flowerdew, J. R. and Ellwood, S. A. (2001). Impacts of woodland deer on small mammal ecology. *Forestry* 74 (3) pp. 277–287.

Flowerdew, J. R., Gurnell, J. and Gipps, J. H. W. (1985). The ecology of woodland rodents : bank voles and wood mice. In *Symposia of the Zoological Society of London No. 55* (eds. Flowerdew, JR, Gurnell, J and Gipps, J.), p. 418. Clarendon Press, Oxford.

Földvári, G., Márialigeti, M., Solymosi, N., Lukács, Z., Majoros, G., Kósa, J. P. and Farkas, R. (2007). Hard Ticks Infesting Dogs in Hungary and their Infection with *Babesia* and *Borrelia* Species. *Parasitology Research* 101 (Suppl 1) pp. 25–34.

Földvári, G., Rigó, K., Jablonszky, M., Biró, N., Majoros, G., Molnár, V. and Tóth, M. (2011). Ticks and the city: ectoparasites of the Northern white-breasted hedgehog (*Erinaceus roumanicus*) in an urban park. *Ticks and Tick-Borne Diseases* 2 (4) pp. 231–

4.

Fournier, P.-E. and Raoult, D. (2009). Current knowledge on phylogeny and taxonomy of *Rickettsia* spp. *Annals of the New York Academy of Sciences* 1166 pp. 1–11.

Franke, J., Fritzsch, J., Tomaso, H., Straube, E., Dorn, W. and Hildebrandt, A. (2010a). Coexistence of pathogens in host-seeking and feeding ticks within a single natural habitat in central Germany. *Applied and Environmental Microbiology* 76 (20) pp. 6829–6836.

Franke, J., Meier, F., Moldenhauer, A., Straube, E., Dorn, W. and Hildebrandt, A. (2010b). Established and emerging pathogens in *Ixodes ricinus* ticks collected from birds on a conservation island in the Baltic Sea. *Medical and Veterinary Entomology* 24 (4) pp. 425–432.

Franke, J., Hildebrandt, A. and Dorn, W. (2013). Exploring gaps in our knowledge on Lyme borreliosis spirochaetes - Updates on complex heterogeneity, ecology, and pathogenicity. *Ticks and Tick-borne Diseases* 4 (1-2) pp. 11–25.

Gage, K., Ostfeld, R. S. and Olson, J. (1995). Nonviral vector-borne zoonoses associated with mammals in the United States. *Journal of Mammalogy* 76 (3) pp. 695–715.

Gassner, F., Takken, W., Plas, C. L. Der, Kastelein, P., Hoetmer, A. J., Holdinga, M. and van Overbeek, L. S. (2013). Rodent species as natural reservoirs of *Borrelia burgdorferi* sensu lato in different habitats of *Ixodes ricinus* in the Netherlands. *Ticks and Tick-borne Diseases* 4 (5) pp. 452–458.

Gebczynska, Z. (1983). Feeding habits. In *Ecology of the bank vole. Acta Theriologica 28* (ed. Petrusewicz, K.), pp. 40–48.

Gern, L. (2008). *Borrelia burgdorferi* sensu lato, the agent of Lyme borreliosis: life in the wilds. *Parasite* 15 (3) pp. 244–247.

Gern, L. (2009). Life cycle of *Borrelia burgdorferi* sensu lato and transmission to humans. In *Lyme Borreliosis. Current problems in dermatology* (eds. Lipsker, D. and Jaulhac, B.), pp. 18–30. Karger AG, Basel.

Gern, L., Siegenthaler, M., Hu, C. M., Leuba-Garcia, S., Humair, P. F. and Moret, J. (1994). *Borrelia burgdorferi* in rodents (*Apodemus flavicollis* and *A. sylvaticus*): Duration and enhancement of infectivity for *Ixodes ricinus* ticks. *European Journal of Epidemiology* 10 (1) pp. 75–80.

Gern, L., Rouvinez, E., Toutoungi, L. N. and Godfroid, E. (1997). Transmission cycles of *Borrelia burgdorferi* sensu lato involving *Ixodes ricinus* and/or *I. hexagonus* ticks and the European hedgehog, *Erinaceus europaeus*, in suburban and urban areas in Switzerland. *Folia Parasitologica* 44 (4) pp. 309–314.

Gingrich, J. B., Harlan, H. J., Perkins, P. V and Trosper, J. H. (2001). *Regional Disease Vector Ecology Profile Central Europe*.

Ginsberg, H. S. and Ewing, C. P. (1989). Comparison of flagging, walking, trapping, and collecting from hosts as sampling methods for northern deer ticks, *Ixodes dammini*, and lone-star ticks, *Amblyomma americanum* (Acari:Ixodidae). *Experimental & Applied Acarology* 7 (4) pp. 313–322.

Ginsberg, H. S. and Faulde, M. K. (2008). Ticks. In *Public health significance of urban pests* (eds. Bonnefoy, X., Kampen, H., and Sweeney, K.), pp. 303–345. World Health Organization (WHO) Regional Office for Europe, Copenhagen, Denmark.

Goodwin, B. J., Ostfeld, R. S. and Schauber, E. M. (2001). Spatiotemporal variation in a Lyme disease host and vector: Black-legged ticks on white-footed mice. *Vector Borne and Zoonotic Diseases* 1 (2) pp. 129–138.

Grankvist, A., Andersson, P.-O., Mattsson, M., Sender, M., Vaht, K., Höper, L., Sakiniene, E., Trysberg, E., Stenson, M., Fehr, J., Pekova, S., Bogdan, C., Bloemberg, G. V and Wennerås, C. (2014). Infections with the tick-borne bacterium "*Candidatus* Neoehrlichia mikurensis" mimic noninfectious conditions in patients with B cell malignancies or autoimmune diseases. *Clinical Infectious Diseases* 58 (12) pp. 1716–1722.

Grankvist, A., Moore, E. R. B., Svensson Stadler, L., Pekova, S., Bogdan, C., Geißdörfer, W., Grip-Lindén, J., Brandström, K., Marsal, J., Andréasson, K., Lewerin, C., Welinder-Olsson, C. and Wennerås, C. (2015). Multilocus Sequence Analysis of Clinical "*Candidatus* Neoehrlichia mikurensis" strains from Europe. *Journal of Clinical Microbiology* 53 (10) pp. 3126–3132.

Gray, J. S. (1985a). Studies on the larval activity of the tick *Ixodes ricinus* L. in Wicklow, Ireland. *Experimental & applied acarology* 1 pp. 307–316.

Gray, J. S. (1985b). A carbon dioxide trap for prolonged sampling of *I. ricinus* populations. *Experimental & Applied Acarology* 1 (1) pp. 35–44.

Gray, J. S. (1991). The development and seasonal activity of the tick *Ixodes ricinus*: a vector of Lyme borreliosis. *Review of Medical and Veterinary Entomology* 79 (6) pp. 323–333.

Gray, J. S. (2008). Ixodes ricinus seasonal activity: Implications of global warming indicated by revisiting tick and weather data. *International Journal of Medical Microbiology* 298 pp. 19–24.

Gray, J. S. (2009). The development and questing activity of *Ixodes ricinus* (L.) (Acari: Ixodidae) under field conditions in Ireland. *Bulletin of Entomological Research* 72 (2) pp. 263.

Gray, J. S., Kirstein, F., Robertson, J. N., Stein, J. and Kahl, O. (1999). *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks and rodents in a recreational park in south-western Ireland. *Experimental and Applied Acarology* 23 (9) pp. 717–729.

Gray, J. S., Dautel, H., Estrada-Peña, A., Kahl, O. and Lindgren, E. (2009). Effects of climate change on ticks and tick-borne diseases in Europe. *Interdisciplinary Perspectives on Infectious Diseases* pp. 1–12.

Gresikova, M. and Kaluzova, M. (1997). Biology of tick-borne encephalitis virus. *Acta Virologica* 41 (2) pp. 115–124.

Gresíková, M. and Nosek, J. (1966). Isolation of tick-borne encephalitis virus from *Haemaphysalis inermis* ticks. *Acta virologica* 10 (4) pp. 359–61.

Grimm, A., Gruber, B. and Henle, K. (2014). Reliability of different mark-recapture methods for population size estimation tested against reference population sizes constructed from field data. *PLoS ONE* 9 (6) pp. e98840.

Grimmberger, E. (2014). *Die Säugetiere Deutschlands*. Quelle & Meyer, Wiebelsheim.

Guglielmone, A. A., Robbins, R. G., Apanaskevich, D. A., Petney, T. N., Estrada-Peña, A., Horak, I. G., Shao, R. and Barker, S. C. (2010). The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida) of the world: a list of valid species names. *Zootaxa* pp. 1–28.

Guglielmone, A. A., Robbins, R. G., Apanaskevich, D. A., Petney, T. N., Estrada-Peña, A. and Horak, I. G. (2014). *The Hard Ticks of the World*. Springer Netherlands, Dordrecht.

Gunawardena, J. (2014). Models in biology: "accurate descriptions of our pathetic thinking." *BMC Biology* 12 (1) pp. 29.

Gurnell, J. (1985). Woodland rodent communities. In *The ecology of woodland rodents: bank voles and wood mice* (eds. Flowerdew, J., Gurnell, J., and Gipps, J. H. W.), pp. 377–411. Clarendon Press, Oxford.

Haeckel, E. (1866). Oecologie und Chorologie. In *Generelle Morphologie der Organismen*, pp. 286–289.

Halos, L., Bord, S., Cotté, V., Gasqui, P., Abrial, D., Barnouin, J., Boulouis, H.-J., Vayssier-Taussat, M. and Vourc'H, G. (2010). Ecological factors characterizing the prevalence of bacterial tick-borne pathogens in *Ixodes ricinus* ticks in pastures and woodlands. *Applied and Environmental Microbiology* 76 (13) pp. 4413–4420.

Hamilton, W. D. (1971). Geometry for the selfish herd. *Journal of Theoretical Biology* 31 (2) pp. 295–311.

Hammond, E. L. and Anthony, R. G. Mark-recapture estimates of population parameters for selected species of small mammals. *Journal of Mammalogy* 87 (3) pp. 618–627.

Hanincova, K., Taragelova, V., Koci, J., Schäfer, S. M., Hails, R., Ullmann, A. J., Piesman, J., Labuda, M. and Kurtenbach, K. (2003). Association of *Borrelia garinii* and *B. valaisiana* with songbirds in Slovakia. *Applied and Environmental Microbiology* 69 (5) pp. 2825–2830.

Hanincová, K., Schäfer, S. M., Etti, S., Sewell, H. S., Taragelová, V., Ziak, D., Labuda, M. and Kurtenbach, K. (2003). Association of *Borrelia afzelii* with rodents in Europe. *Parasitology* 126 pp. 11–20.

Harrison, A. and Bennett, N. C. (2012). The importance of the aggregation of ticks on small mammal hosts for the establishment and persistence of tick-borne pathogens: an investigation using the R(0) model. *Parasitology* 139 (12) pp. 1605–1613.

Harrison, A., Scantlebury, M. and Montgomery, W. I. (2010). Body mass and sex-biased parasitism in wood mice *Apodemus sylvaticus*. *Oikos* 119 (7) pp. 1099–1104.

Hartelt, K., Oehme, R., Frank, H., Brockmann, S. O., Hassler, D. and Kimmig, P. (2004). Pathogens and symbionts in ticks: prevalence of *Anaplasma phagocytophilum (Ehrlichia* sp.), *Wolbachia* sp., *Rickettsia* sp., and *Babesia* sp. in Southern Germany. *International Journal of Medical Microbiology* 293 (Suppl 1) pp. 86–92.

Hartelt, K., Pluta, S., Oehme, R. and Kimmig, P. (2008). Spread of ticks and tick-borne diseases in Germany due to global warming. *Parasitology Research* 103 (Suppl 1) pp. 109–116.

Hartemink, N. a, Randolph, S. E., Davis, S. a and Heesterbeek, J. a P. (2008). The basic reproduction number for complex disease systems: defining R(0) for tick-borne infections. *The American Naturalist* 171 (6) pp. 743–754.

Häselbarth, K., Tenter, A. M., Brade, V., Krieger, G. and Hunfeld, K. P. (2007). First case of human babesiosis in Germany - Clinical presentation and molecular characterisation of the pathogen. *International Journal of Medical Microbiology* 297 (3) pp. 197–204.

Hassler, M. (1998). *Der Michaelsberg. Naturkunde und Geschichte des Untergrombacher Hausbergs*. 90th ed. (eds. Hassler, M. für U. B.-W.) Landesanstalt für Umweltschutz Baden-Württemerg, Karlsruhe.

Hassler, D., Oehme, R., Kimmig, P. and Dobler, G. (2003). Eyach virus: relative of the Colorado tick fever virus rediscovered in Baden-Württemberg. *Deutsche Medizinische Wochenschrift* 128 (37) pp. 18–24.

Hausser, J. (1995). *Sorex araneus* (L. 1758). In *Die Säugetiere der Schweiz: Verbreitung, Biologie und Ökologie* (ed. Hausser, J.), pp. 23–27. Birkhäuser Verlag, Basel.

Hausser, J., Hutterer, R. and Vogel, P. (1990). *Sorex araneus* Linnaeus, 1758 - Waldspitzmaus. In *Handbuch der Säugetiere Europas: Insektenfresser, Herrentiere* (eds. Niethammer, J. and Krapp, F.), Aula Verlag, Wiesbaden.

Havelka, M. A. and Millar, J. S. (2004). Maternal age drives seasonal variation in litter size of *Peromyscus leucopus*. *Journal of Mammalogy* 85 (5) pp. 940–947.

Herrmann, C. and Gern, L. (2010). Survival of *Ixodes ricinus* (Acari: Ixodidae) under challenging conditions of temperature and humidity is influenced by Borrelia burgdorferi sensu lato infection. *Journal of Medical Entomology* 47 (6) pp. 1196–1204.

Herrmann, C. and Gern, L. (2013). Survival of *Ixodes ricinus* (Acari: Ixodidae) nymphs under cold conditions is negatively influenced by frequent temperature variations. *Ticks and Tick-borne Diseases* 4 (5) pp. 445–451.

Herrmann, C. and Gern, L. (2015). Search for blood or water is influenced by *Borrelia burgdorferi* in *Ixodes ricinus*. *Parasites & Vectors* 8 (1) pp. 4–11.

Hersh, M., LaDeau, S., Previtali, M. and Ostfeld, R. S. (2014). When is a parasite not a parasite? Effects of larval tick burdens on white-footed mouse survival. *Ecology* 95 (5) pp. 1360–1369.

Herwaldt, B. L., Cacciò, S., Gherlinzoni, F., Aspöck, H., Slemenda, S. B., Piccaluga, P. P., Martinelli, G., Edelhofer, R., Hollenstein, U., Poletti, G., Pampiglione, S., Löschenberger, K., Tura, S. and Pieniazek, N. J. (2003). Molecular characterization of a non-*Babesia divergens* organism causing zoonotic babesiosis in Europe. *Emerging Infectious Diseases* 9 (8) pp. 942–948.

Heusner, A. A. (1991). Size and power in mammals. *The Journal of Experimental Biology* 160 (1) pp. 25–54.

Hildebrandt, A., Tenter, A. M., Straube, E. and Hunfeld, K. P. (2008). Human babesiosis in Germany: Just overlooked or truly new? *International Journal of Medical Microbiology* 298 (Suppl 1) pp. 336–346.

Hildebrandt, A., Fritzsch, J., Franke, J., Sachse, S., Dorn, W. and Straube, E. (2011). Co-circulation of emerging tick-borne pathogens in Middle Germany. *Vector Borne Zoonotic Diseases* 11 (5) pp. 533–537.

Hille, S. M. and Mortelliti, A. (2010). Uso differenziale dei microambienti da parte di *Apodemus flavicollis* e *Myodes glareolus* in ambienti subalpini: Primi risultati. *Hystrix* 21 (2) pp. 157–163.

Hillyard, P. D. (1996). *Ticks of North-West Europe*. 52nd ed. (eds. Barnes, R. and Crothers, J.) Synopses of the British Fauna (New Series). Field Studies Counci, Shrewsbury.

Hirsch, F. W., Kleemann, D. and Grunow, R. (2001). Case reports of tularemia. *Deutsche Medizinische Wochenschrift* 126 (14) pp. 393–394.

Hoffmeyer, I. (1973). Interaction and habitat selection in the mice *Apodemus flavicollis* and *A. sylvaticus*. *Oikos* 24 (1) pp. 108–116.

Homer, M. J., Aguilar-Delfin, I., Telford, S. R., Krause, P. J. and Persing, D. H. (2000). Babesiosis. *Clinical Microbiology Reviews* 13 (3) pp. 451–469.

Hornok, S., Kováts, D., Csörgő, T., Meli, M. L., Gönczi, E., Hadnagy, Z., Takács, N., Farkas, R. and Hofmann-Lehmann, R. (2014). Birds as potential reservoirs of tick-borne pathogens: first evidence of bacteraemia with *Rickettsia helvetica*. *Parasites & Vectors* 7 pp. 128.

Hovius, J. W. R., van Dam, A. P. and Fikrig, E. (2007). Tick-host-pathogen interactions in Lyme borreliosis. *Trends in Parasitology* 23 (9) pp. 434–438.

Hubálek, Z. and Rudolf, I. (2012). Tick-borne viruses in Europe. *Parasitology Research*.

Hudson, P. J. and Bjørnstad, O. N. (2003). Vole stranglers and Lemming cycles. *Science* 302 pp. 797–798.

Huegli, D., Hu, C. M., Humair, P. F., Wilske, B. and Gern, L. (2002). *Apodemus* species mice are reservoir hosts of *Borrelia garinii* OspA serotype 4 in Switzerland. *Journal of Clinical Microbiology* 40 (12) pp. 4735–4737.

Hughes, V. L. and Randolph, S. E. (2001). Testosterone depresses innate and acquired resistance to ticks in natural rodent hosts: a force for aggregated distributions of parasites. *The Journal of Parasitology* 87 (1) pp. 49–54.

Humair, P. and Gern, L. (2000). The wild hidden face of Lyme borreliosis in Europe. *Microbes and Infection* 2 (8) pp. 915–22.

Humair, P. F., Turrian, N., Aeschlimann, A. and Gern, L. (1993). *Borrelia burgdorferi* in a focus of Lyme borreliosis: epizootiologic contribution of small mammals. *Folia Parasitologica* 40 (1) pp. 65–70.

Humair, P., Rais, O. and Gern, L. (1999). Transmission of *Borrelia afzelii* from *Apodemus* mice and *Clethrionomys* voles to *Ixodes ricinus* ticks: differential transmission pattern and overwintering maintenance. *Parasitology* pp. 33–42.

Hunfeld, K. P., Hildebrandt, A. and Gray, J. S. (2008). Babesiosis: Recent insights into an ancient disease. *International Journal for Parasitology* 38 (11) pp. 1219–1237.

Huppertz, H. I., Böhme, M., Standaert, S. M., Karch, H. and Plotkin, S. A. (1999). Incidence of Lyme borreliosis in the Würzburg region of Germany. *European Journal of Clinical Microbiology and Infectious Diseases* 18 (10) pp. 697–703.

Hutterer, R. (1990). *Sorex minutus* Linnaeus, 1766 - Zwergspitzmaus. In *Handbuch der Säugetiere Europas: Insektenfresser, Herrentiere* (eds. Braun, M. and Dieterlen, F.), pp. 42–47. Ulmer Verlag, Stuttgart.

Hvidsten, D., Stuen, S., Jenkins, A., Dienus, O., Olsen, R. S., Kristiansen, B.-E., Mehl, R. and Matussek, A. (2014). *Ixodes ricinus* and *Borrelia* prevalence at the Arctic Circle in Norway. *Ticks and Tick-borne Diseases* 5 (2) pp. 107–12.

Immler, R. (1973). Untersuchungen zur Biologie und Ökologie der Zecke *Dermacentor reticulatus* (Fabricius, 1794) (Ixodidae) in einem endemischen Vorkommensgebiet. *Mitteliungen der Schweizer Entomologischen Gesellschaft* 46 pp. 1–70.

Jaenson, T. G. T. and Lindgren, E. (2011). The range of *Ixodes ricinus* and the risk of contracting Lyme borreliosis will increase northwards when the vegetation period becomes longer. *Ticks and Tick-borne Diseases* 2 (1) pp. 44–49.

Jaenson, T. G. T., Tälleklint, L., Lundqvist, L., Olsen, B., Chirico, J. and Mejlon, H. (1994). Geographical distribution, host associations, and vector roles of ticks (Acari: Ixodidae, Argasidae) in Sweden. *Journal of Medical Entomology* 31 (2) pp. 240–256.

Jaenson, T. G. T., Jaenson, D. G. E., Eisen, L., Petersson, E. and Lindgren, E. (2012). Changes in the geographical distribution and abundance of the tick *Ixodes ricinus* during the past 30 years in Sweden. *Parasites & Vectors* 5 (1) pp. 8–22.

Jahfari, S., Fonville, M., Hengeveld, P., Reusken, C., Scholte, E.-J., Takken, W., Heyman, P., Medlock, J., Heylen, D. J. A., Kleve, J. and Sprong, H. (2012). Prevalence of *Neoehrlichia mikurensis* in ticks and rodents from North-west Europe. *Parasites & Vectors* 5 (1) pp. 74.

James, M. C., Bowman, A. S., Forbes, K. J., Lewis, F., McLeod, J. E. and Gilbert, L. (2012). Environmental determinants of Ixodes ricinus ticks and the incidence of *Borrelia burgdorferi* sensu lato, the agent of Lyme borreliosis, in Scotland. *Parasitology* pp. 1–

10.

Jenrich, J., Löhr, P. W., Müller, F. and Lang, J. (2010). *Kleinsäuger: Körper- und Schädelmerkmale, Ökologie. Beiträge zur Naturkunde in Osthessen*. Verein für Naturkunde in Osthessen e.V., Michael Imhof Verlag, Fulda.

Jensen, T. S. (1982). Seed production and outbreaks of non-cyclic rodent populations in deciduous forests. *Oecologia* 54 (2) pp. 184–192.

Jensen, T. (1985). Seed-seed predator interactions of European beech, *Fagus silvatica* and forest rodents, *Clethrionomys glareolus* and *Apodemus flavicollis*. *Oikos* 44 pp. 1-6.

Jensen, P. M., Hansen, H. and Frandsen, F. (2000). Spatial risk assessment for Lyme borreliosis in Denmark. *Scandinavian Journal of Infectious Diseases* 32 (5) pp. 545–550.

Jerabek, M. and Reiter, G. (2003a). Die Kleinsäugerfauna von Bergwäldern im Karwendel (Österreich): Verbreitung, Habitatwahl und Populationsentwicklung. *Berichte des Naturwissenschaftlich-Medizinischen Vereins Innsbruck* 90 pp. 231–259.

Jerabek, M. and Reiter, G. (2003b). Populationsbiologie der Rötelmaus *Clethrionomys glareolus* (Rodentia : Arvicolidae) im Karwendel (Tirol , Österreich). *Berichte des naturwissenschaftlich-medizinischen Vereins Innsbruck* 90 pp. 261–276.

Johnson, P. T. J., de Roode, J. C. and Fenton, A. (2015). Why infectious disease research needs community ecology. *Science* 349 (6252) pp. 1069–1080.

Jolly, G. M. (1965). Explicit estimates from data with both death and immigration-stochastic model. *Biometrika* 52 (1/2) pp. 225–247.

Jones, C. G. (1998). Chain Reactions Linking Acorns to Gypsy Moth Outbreaks and Lyme Disease Risk. *Science* 279 (5353) pp. 1023–1026.

Jones, C. J. and Kitron, U. D. (2000). Populations of Ixodes scapularis (Acari: Ixodidae) are modulated by drought at a Lyme disease focus in Illinois. *Journal of Medical Entomology* 37 (3) pp. 408–415.

Jones, C. G., Lawton, J. H. and Shachak, M. (1996). Organisms as ecosystem engineers. In *Ecosystem Management* (eds. Samson, F. B. and Knopf, F. L.), pp. 130–147. Springer New York, NY.

Jones, C. R., Brunner, J. L., Scoles, G. A. and Owen, J. P. (2015). Factors affecting larval tick feeding success: host, density and time. *Parasites & Vectors* 8 (1) pp. 340.

Jongejan, F. and Uilenberg, G. (2004). The global importance of ticks. *Parasitology* 129 (Suppl 7) pp. 3–14.

Kahl, O. (1989). Untersuchungen zumWasserhaushalt von Zecken (Acari:Ixodoidea) im Laufe ihrer postembryonalen Entwicklung unter besonderer Berücksichtigung der aktiven Wasserdampfsorption bei gesogenen Stadien. Thesis.

Kahl, O., Gern, L., Eisen, L. and Lane, R. S. (2002). Ecological research on *Borrelia burgdorferi* sensu lato: Terminology and some methodological pitfalls. In *Lyme Borreliosis: Biology, Epidemiology, and Control* (eds. Gray, J., Kahl, O., Lane, R. S., and Stanek, G.), p. 480. CABI Publishing, Oxon, New York.

Kalmár, Z., Sprong, H., Mihalca, A. D., Gherman, C. M., Dumitrache, M. O., Coipan, E. C., Fonville, M. and Cozma, V. (2016). *Borrelia miyamotoi* and *Candidatus* Neoehrlichia mikurensis in *Ixodes ricinus* Ticks, Romania. *Emerging Infectious Diseases* 22 (3) pp. 550–551.

Karbowiak, G. (2004). Zoonotic reservoir of *Babesia microti* in Poland. *Polish Journal of Microbiology* 53 pp. 61–65.

Karbowiak, G. (2014). The occurrence of the *Dermacentor reticulatus* tick-its expansion

to new areas and possible causes. *Annals of parasitology* 60 (1) pp. 37–47.

Katargina, O., Geller, J., Ivanova, A., Värv, K., Tefanova, V., Vene, S., Lundkvist, Å. and Golovljova, I. (2015). Detection and identification of *Rickettsia* species in *Ixodes* tick populations from Estonia. *Ticks and Tick-borne Diseases* 1999 pp. 1–6.

Kawahara, M., Rikihisa, Y., Isogai, E., Takahashi, M., Misumi, H., Suto, C., Shibata, S., Zhang, C. and Tsuji, M. (2004). Ultrastructure and phylogenetic analysis of "*Candidatus* Neoehrlichia mikurensis" in the family Anaplasmataceae, isolated from wild rats and found in *Ixodes ovatus* ticks. *International Journal of Systematic and Evolutionary Microbiology* 54 (5) pp. 1837–1843.

Keirans, J. E., Needham, G. R. and Oliver, J. H. J. (1999). The *Ixodes ricinus* complex worldwide: Diagnosis of the species in the complex, hosts and distribution. In *Acarology IX: Symposia* (eds. Needham, G. R., Michell, R., Horn, D. J., and Welbourn, W. C.), pp. 341–347. Ohio Biological Survey, Columbus.

Keller, A., Graefen, A., Ball, M., Matzas, M., Boisguerin, V., Maixner, F., Leidinger, P., Backes, C., Khairat, R., Forster, M., Stade, B., Franke, A., Mayer, J., Spangler, J., McLaughlin, S., Shah, M., Lee, C., Harkins, T. T., Sartori, A., Moreno-Estrada, A., Henn, B., Sikora, M., Semino, O., Chiaroni, J., Rootsi, S., Myres, N. M., Cabrera, V. M., Underhill, P. A., Bustamante, C. D., Vigl, E. E., Samadelli, M., Cipollini, G., Haas, J., Katus, H., O'Connor, B. D., Carlson, M. R. J., Meder, B., Blin, N., Meese, E., Pusch, C. M. and Zink, A. (2012). New insights into the Tyrolean Iceman's origin and phenotype as inferred by whole-genome sequencing. *Nature Communications* 3 (698) pp. 1–9.

Kiffner, C., Lödige, C., Alings, M., Vor, T. and Rühe, F. (2010). Abundance estimation of *Ixodes* ticks (Acari: Ixodidae) on roe deer (*Capreolus capreolus*). *Experimental & Applied Acarology* 52 (1) pp. 73–84.

Kiffner, C., Vor, T., Hagedorn, P., Niedrig, M. and Rühe, F. (2011). Factors affecting patterns of tick parasitism on forest rodents in tick-borne encephalitis risk areas, Germany. *Parasitology Research* 108 pp. 323–335.

Kirkland, G. L. J. (1985). Small mammal communities in temperate North American forests. *Australian Mammalogy* 8 pp. 137–144.

Klenke, R., Biedermann, M., Keller, M., Lämmel, D., Schorcht, W., Tschierschke, A. and Zillmann, F. (2004). Habitatansprüche, Strukturbindung und Raumnutzung von Vögeln und Säugetieren in forstwirtshaftlich genutzten und ungenutzten Kiefern- und Buchenwäldern. *Beiträge Forstwirtschaft und Landschaftsökologie* 38 (Fkz 0339757).

Korenberg, E. I., Kovalevskii, Y. V and Gorelova, N. B. (2003). Tick – host – Borrelia population interactions : long-term records in Eastern Europe. *Ticks and Tick-borne Diseases* 28 pp. 225–229.

Kovalevskii, Y. V., Korenberg, E. I., Gorelova, N. B. and Nefedova, V. V. (2013). Ecology of *Ixodes trianguliceps* and its significance in natural foci of ixodid tick-borne borrelioses in the Middle Urals. *Зоологический Журнал* 92 (5) pp. 505–516.

Kozuch, O., Gresíková, M., Nosek, J., Lichard, M. and Sekeyová, M. (1967). The role of small rodents and hedgehogs in a natural focus of tick-borne encephalitis. *Bulletin of the World Health Organization* 36 pp. 61–66.

Kraft, R. (2008). *Mäuse und Spitzmäuse in Bayern: Verbreitung, Lebensraum, Bestandssituation*. Eugen Ulmer, Stuttgart (Hohenheim).

Krapp, F. and Niethammer, J. (2005). *Microtus agrestis* (Linnaeus, 1761) - Erdmaus. In

Handbuch der Säugetiere Europas: Nagetiere II (ed. Niethammer, J. and Krapp, F.), pp. 312–319. Aula Verlag, Wiesbaden.

Krasnov, B. R., Stanko, M. and Morand, S. (2007). Host community structure and infestation by ixodid ticks: repeatability, dilution effect and ecological specialization. *Oecologia* 154 (1) pp. 185–194.

Krasnov, B. R., Bordes, F., Khokhlova, I. S. and Morand, S. (2012). Gender-biased parasitism in small mammals: patterns, mechanisms, consequences. *Mammalia* 76 (1) pp. 1–13.

Krause, P. J. (2002). Babesiosis. *Medical Clinics of North America* 86 (2) pp. 361–373.

Krause, A. and Fingerle, V. (2009). Lyme borreliosis. *Zeitschrift für Rheumatologie* 68 pp. 239–254.

Krauss, H Weber, A., Appel, B., Enders, M., von Graevenitz, A., Isenberg, H., Schiefer, H. G., Slenczka, W. and Zahner, H. (2004). *Zoonosen: Von Tier zu Mensch übertragbare Infektionskrankheiten*. 3rd ed. Deutscher Ärzte-Verlag, Köln.

Krebs, C. J. (2013). *Population fluctuations in rodents*. The University of Chicago Press, Chicago and London.

Kunz, T. H., Wemmer, C. and Hayssen, V. (1996). Sex, age and reproductive conditions of mammals. In *Measuring and Monitoring Biological Diversity: Standard Methods for Mammals* (eds. Wilson, D. E., Cole, F. R., Nichols, J. D., Rudran, R., and Foster, M. S.), pp. 279–290. Smithsonian Institution Press, Washington and London.

Kurtenbach, K., Dizij, A., Seitz, H. M., Margos, G., Moter, S. E., Kramer, M. D., Wallich, R., Schaible, U. E. and Simon, M. M. (1994). Differential immune responses to *Borrelia burgdorferi* in European wild rodent species influence spirochete transmission to *Ixodes ricinus* L. (Acari: Ixodidae). *Infection and Immunity* 62 (12) pp. 5344–5552.

Kurtenbach, K., Kampen, H., Dizij, A., Arndt, S., Seitz, H. M., Schaible, U. E. and Simon, M. M. (1995). Infestation of rodents with larval *Ixodes ricinus* (Acari; Ixodidae) is an important factor in the transmission cycle of *Borrelia burgdorferi* s.l. in German woodlands. *Journal of Medical Entomology* 32 (6) pp. 807–817.

Kurtenbach, K., Peacey, M., Rijpkema, S. G. T., Hoodless, A. N., Nuttall, P. A. and Randolph, S. E. (1998a). Differential transmission of the genospecies of *Borrelia burgdorferi* sensu lato by game birds and small rodents in England. *Applied and Environmental Microbiology* 64 (4) pp. 1169–1174.

Kurtenbach, K., Sewell, H.-S., Ogden, N. H., Randolph, S. E. and Nuttall, P. A. (1998b). Serum complement sensitivity as a key factor in Lyme disease ecology. *Infection and Immunity* 66 (3) pp. 1248–1251.

Kurtenbach, K., De Michelis, S., Sewell, H. S., Etti, S., Schäfer, S. M., Hails, R., Collares-Pereira, M., Santos-Reis, M., Hanincová, K., Labuda, M., Bormane, A. and Donaghy, M. (2001). Distinct combinations of *Borrelia burgdorferi* sensu lato genospecies found in individual questing ticks from Europe. *Applied and Environmental Microbiology* 67 (10) pp. 4926–4929.

Kurtenbach, K., De Michelis, S., Etti, S., Schäfer, S. M., Sewell, H.-S., Brade, V. and Kraiczy, P. (2002). Host association of *Borrelia burgdorferi* sensu lato-the key role of host complement. *Trends in Microbiology* 10 (2) pp. 74–9.

Kurtenbach, K., Hanincová, K., Tsao, J. I., Margos, G., Fish, D. and Ogden, N. H. (2006). Fundamental processes in the evolutionary ecology of Lyme borreliosis. *Nature Reviews MIcrobiology* 4 (9) pp. 660–9.

Labuda, M., Kozuch, O., Zuffová, E., Elecková, E., Hails, R. S. and Nuttall, P. A. (1997). Tick-borne encephalitis virus transmission between ticks cofeeding on specific immune natural rodent hosts. *Virology* 235 (1) pp. 138–143.

Ladurner, E. and Cazzolli, N. (2000). *Die Kleinsäugerfauna von Ritten und Montiggl Populationsökologie und Habitatnutzung*. Bozen.

Lambin, X. (2008). Field vole *Microtus agrestis*. In *Mammals of the British Isles* (ed. Harris, S. and Yalden, D. W.), pp. 100–107. The Mammal Society, Southampton.

Lauterbach, R., Wells, K., O'Hara, R. B., Kalko, E. K. V and Renner, S. C. (2013). Variable strength of forest stand attributes and weather conditions on the questing activity of *Ixodes ricinus* ticks over years in managed forests. *PloS one* 8 (1) pp. e55365.

Lavadinovic, V. (2012). The usage of capture-Mark-recapture/resight methods in estimating population abundance. In *First Serbian Forestry Congress - Future with Forest*, pp. 320–327. Belgrade.

Lebreton, J.-D., Burnham, K. P., Clobert, J. and Anderson, D. R. (1992). Modeling survival and testing biological hypotheses using marked animals: a unified approach with case studies. *Ecological Monographs* 62 (1) pp. 67–118.

Lechner, F. and Zimmermann, P. (2007). *Rheinaue bei Linkenheim-Hochstetten*. Staatliche Naturschutzverwaltung Baden-Württemberg, Regierungspräsidum Karlsruhe, Karlsruhe.

Lees, A. D. (1946). The water balance in *Ixodes ricinus* L. and certain other species of ticks. *Parasitology* 37 pp. 1–20.

Lees, A. D. and Milne, A. (1951). The seasonal and diurnal activities of individual sheep ticks (*Ixodes ricinus* L). *Parasitology* 41 (3-4) pp. 189–208.

Leonhard, S. (2005). Untersuchungen zur Häufigkeit von *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum* und *Babesia* spp. in *Ixodes ricinus* aus Bayern und Baden-Württemberg. PhD Thesis.

Li, H., Jiang, J., Tang, F., Sun, Y., Li, Z., Zhang, W., Gong, Z., Liu, K., Yang, H., Liu, W. and Cao, W. (2013). Wide distribution and genetic diversity of "*Candidatus* Neoehrlichia mikurensis" in rodents from China. *Applied and environmental microbiology* 79 (3) pp. 1024–7.

Liebhold, A., Koenig, W. D. and Bjørnstad, O. N. (2004). Spatial Synchrony in population dynamics. *Annual Review of Ecology, Evolution, and Systematics* 35 pp. 467–490.

Liebisch, A. and Rahman, M. S. (1976). Prevalence of the ticks *Dermacentor marginatus* (Sulzer, 1776) and *Dermacentor reticulatus* (Fabricius, 1794) and their importance as vectors of diseases in Germany. *Tropenmedizin und Parasitologie* 27 (4) pp. 393–404.

Lindgren, E. and Jaenson, T. G. T. (2006). *Lyme borreliosis in Europe: influences of climate and climate change, epidemiology, ecology and adaptation measures*. Copenhagen.

Lindgren, E., Tälleklint, L. and Polfeldt, T. (2000). Impact of climatic change on the northern latitude limit and population density of the disease-transmitting European tick *Ixodes ricinus*. *Environmental Health Perspectives* 108 (2) pp. 119–23.

Lindquist, L. and Vapalahti, O. (2008). Tick-borne encephalitis. *The Lancet* 371 (9627) pp. 1861–1871.

Lipsker, D. and Jaulhac, B. (2009). *Lyme Borreliosis - Biological and clinical aspects*. 37th ed. Karger AG, Basel.

Liu, X. Y. and Bonnet, S. (2014). Hard tick factors implicated in pathogen transmission. *PLoS Neglected Tropical Diseases* 8 (1) pp. e2566.

LoGiudice, K., Ostfeld, R. S., Schmidt, K. A. and Keesing, F. (2003). The ecology of

infectious disease: effects of host diversity and community composition on Lyme disease risk. *Proceedings of the National Academy of Sciences of the United States of America* 100 (2) pp. 567–71.

López-Fuster, J. (2007). *Sorex minutus* Linnaeus, 1766. In *Atlas y libro rojo de los Mamíferos terestres de España* (eds. Palomo, L. J., Gisbert, J., and Blanco, J. C.), pp. 99–101. Dirección General para la Biodiversidad - Secemu, Madrid.

Lucius, R. and Loos-Frank, B. (2008a). Allgemeine Aspekte der Biologie von Parasiten. In *Biologie von Parasiten*, pp. 3–102.

Lucius, R. and Loos-Frank, B. (2008b). Acari. In *Die Biologie von Parasiten*, pp. 423–451.

Lugon-Moulin, N. (2003). *Les musaraignes: Biologie, écologie, répartition en Suisse*. Porte-Plumes Verlag, Ayer.

Maier, W., Grunewald, J., Habedank, B., Hartelt, K., Kampen, H., Kimmig, P., Naucke, T., Oehme, R., Vollmer, A., Schöler, A. and Schmitt, C. (2003). Mögliche Auswirkungen von Klimaveränderungen auf die Ausbreitung von primär humanmedizinisch relevanten Krankheitserregern über tierische Vektoren sowie auf die wichtigen Humanparasiten in Deutschland. UBA *Forschungsbericht 200 61 218/11*.

Margos, G., Vollmer, S. A., Ogden, N. H. and Fish, D. (2011). Population genetics, taxonomy, phylogeny and evolution of *Borrelia burgdorferi* sensu lato. *Infection Genetics and Evolution* 11 (7) pp. 1545–1563.

Marsh, A. C. W. and Montgomery, W. I. (2008). Yellow-necked-mouse *Apodemus flavicollis*. In *Mammals of the British Isles* (ed. Harris, S. and Yalden, D. W.), pp. 137–141. The Mammal Society, London.

Maternowski, H.-W. (2007). Ergebnisse zweier Gewölleanalysen aus der Maiwaldsiedlung, einem Ortsteil von Renchen im Ortenaukreis. *Mitteilungen aus unserer Säugerwelt, Arbeitsgruppe Wildlebende Säugetiere (AGWS) Baden-Württemberg e. V.* 14 pp. 13–16.

Mather, T. N., Wilson, M. L., Moore, S. I., Ribeiro, J. M. C. and Spielman, A. (1989). Comparing the relative potential of rodents as reservoirs of the Lyme Disease spirochete (*Borrelia burgdorferi*). *American Journal of Epidemiology* 130 (1) pp. 143–150.

Matuschka, F.-R. R., Lange, R., Spielman, A., Richter, D. and Fischer, P. (1990). Subadult *Ixodes ricinus* (Acari, Ixodae) on Rodents In Berlin, West-Germany. *Journal Of Medical Entomology* 27 (3) pp. 385–390.

Matuschka, F. R., Fischer, P., Musgrave, K., Richter, D. and Spielman, A. (1991). Hosts on which nymphal *Ixodes ricinus* most abundantly feed. *American Journal of Tropical Medicine and Hygiene* 44 (1) pp. 100–107.

Matuschka, F.-R. R., Fischer, P., Heiler, M., Blümcke, S. and Spielman, A. (1992). Stage-associated risk of transmission of the Lyme disease spirochete by European *Ixodes ticks*. *Parasitology Research* 78 (8) pp. 695–698.

Matuschka, F. R., Endepols, S., Richter, D., Ohlenbusch, A., Eiffert, H. and Spielman, A. (1996). Risk of urban Lyme disease enhanced by the presence of rats. *The Journal of infectious diseases* 174 (5) pp. 1108–1111.

McCravy, K. W. and Rose, R. K. (1992). An analysis of external features as predictors of reproductive status in small mammals. *Journal of Mammalogy* 73 (1) pp. 151–159.

McCullagh, P. and Nelder, J. A. (1989). *Generalized Linear Models*. 2nd ed. Chapman and Hall, London.

Medlock, J. M., Hansford, K. M., Bormane, A., Derdakova, M., Estrada-Peña, A., George,

J.-C., Golovljova, I., Jaenson, T. G. T., Jensen, J.-K., Jensen, P. M., Kazimirova, M., Oteo, J. A., Papa, A., Pfister, K., Plantard, O., Randolph, S. E., Rizzoli, A. P., Santos-Silva, M. M., Sprong, H., Vial, L., Hendrickx, G., Zeller, H. and Van Bortel, W. (2013). Driving forces for changes in geographical distribution of *Ixodes ricinus* ticks in Europe. *Parasites & Vectors* 6 pp. 1–11.

Meentemeyer, R. K., Haas, S. E. and Václavík, T. (2012). Landscape epidemiology of emerging infectious diseases in natural and human-altered ecosystems. *Annual Review of Phytopathology* 50 pp. 379–402.

Meerburg, B. G., Singleton, G. R. and Kijlstra, A. (2009). Rodent-borne diseases and their risks for public health. *Critical Reviews in Microbiology* 35 (3) pp. 221–270.

Meiners, T., Hammer, B., Göbel, U. B. and Kahl, O. (2006). Determining the tick scutal index allows assessment of tick feeding duration and estimation of infection risk with *Borrelia burgdorferi* sensu lato in a person bitten by an *Ixodes ricinus* nymph. *International Journal of Medical Microbiology* 296 (S1) pp. 103–107.

Meining, S., Von Wilpert, K., Augustin, N. and Kramer, P. (2011). *Waldzustandsbericht 2011*.

Mejlon, H. and Jaenson, T. G. (1993). Seasonal prevalence of *Borrelia burgdorferi* in *Ixodes ricinus* in different vegetation types in Sweden. *Scandinavian Journal of Infectious Disease* 25 pp. 449–456.

Mejlon, H. A. and Jaenson, T. G. T. (1997). Questing behaviour of *Ixodes ricinus* ticks (Acari: Ixodidae). *Experimental & Applied Acarology* 21 (12) pp. 747–754.

Merritt, J. F. (2010). *The Biology of Small Mammals*. JHU Press.

Michalik, J., Hofman, T., Buczek, A., Skoracki, M. and Sikora, B. (2003). *Borrelia burgdorferi* s.l. in *Ixodes ricinus* (Acari: Ixodidae) ticks collected from vegetation and small rodents in recreational areas of the city of Poznań. *Journal of Medical Entomology* 40 pp. 690–697.

Mierzejewska, E. J., Welc-Faleciak, R., Karbowiak, G., Kowalec, M., Behnke, J. M. and Bajer, A. (2015). Dominance of *Dermacentor reticulatus* over *Ixodes ricinus* (Ixodidae) on livestock, companion animals and wild ruminants in eastern and central Poland. *Experimental and Applied Acarology* 66 (1) pp. 83–101.

Mierzejewska, E. J., Estrada-Peña, A., Alsarraf, M., Kowalec, M. and Bajer, A. (2016). Mapping of *Dermacentor reticulatus* expansion in Poland in 2012-2014. *Ticks and Tick-borne Diseases* 7 (1) pp. 94–106.

Mihalca, A. D. and Sándor, A. D. (2013). The role of rodents in the ecology of *Ixodes ricinus* and associated pathogens in Central and Eastern Europe. *Frontiers in Cellular and Infection Microbiology* 3 pp. 56.

Mihalca, A. D., Dumitrache, M. O., Sándor, A. D., Magdaş, C., Oltean, M., Györke, A., Matei, I. A., Ionică, A., D'Amico, G., Cozma, V. and Gherman, C. M. (2012). Tick parasites of rodents in Romania: host preferences, community structure and geographical distribution. *Parasites & Vectors* 5 pp. 266.

Mitchell-Jones, A. J., Amori, G., Bogdanowicz, W., Kryštufek, B., Reijnder, P. J. H., Spitzenberger, F., Stubbe, M., Thiessen, J. B. M., Vohralik, V. and Zima, J. (1999). *The Atlas of European Mammal*. Academic Press, London.

Moore, S. L. and Wilson, K. (2002). Parasites as a viability cost of sexual selection in natural populations of mammals. *Science* 297 (5589) pp. 2015–2018.

Morand, S., De Bellocq, J. G., Stanko, M. and Miklisová, D. (2004). Is sex-biased ectoparasitism related to sexual size dimorphism in small mammals of Central

Europe? *Parasitology* 129 (4) pp. 505–510.

Morand, S., Krasnov, B. and Poulin, R. (2006). Micromammals and macroparasites: Who is who and how they interact? In *Micromammals and Macroparasites* (eds. Morand, S., Krasnov, B., and Poulin, R.), pp. 3–13. Springer, Tokyo.

Movila, A., Reye, A. L., Dubinina, H. V, Tolstenkov, O. O., Toderas, I., Hübschen, J. M., Muller, C. P. and Alekseev, A. N. (2011). Detection of *Babesia* sp. EU1 and members of spotted fever group rickettsiae in ticks collected from migratory birds at Curonian Spit, North-Western Russia. *Vector Borne and Zoonotic Diseases* 11 (1) pp. 89–91.

Müller, P. (2011). *Gelbhalsmaus* (Apodemus flavicollis). In *Die Säugetiere des Fürstentums Lichtenstein. (Mammalia)*, pp. 112–113. Amtlicher Lehrmittelverlag, Vaduz.

Mysterud, A., Byrkjeland, R., Qviller, L. and Viljugrein, H. (2015). The generalist tick *Ixodes ricinus* and the specialist tick *Ixodes trianguliceps* on shrews and rodents in a northern forest ecosystem– a role of body size even among small hosts. *Parasites & Vectors* 8 (1) pp. 639.

Nagel, A. and Nagel, R. (2005). Zwergspitzmaus *Sorex minutus* Linneaus, 1766. In *Die Säugetiere Baden-Württembergs. Band 2* (eds. Braun, M. and Dieterlen, F.), pp. 42–47. Ulmer, Stuttgart (Hohenheim).

Nichols, J. D. and Conroy, M. J. (1996). Techniques for estimating abundance and species richness. In *Measuring and Monitoring Biological Diversity: Standard Methods for Mammals* (eds. Wilson, D. E., Cole, F. R., Nichols, J. D., Rudran, R., and Foster, M. S.), pp. 177–234. Smithsonian Institution Press, Washington and London.

Nicholson, W. L., Allen, K. E., McQuiston, J. H., Breitschwerdt, E. B. and Little, S. E. (2010). The increasing recognition of rickettsial pathogens in dogs and people. *Trends in Parasitology* 26 (4) pp. 205–212.

Niethammer, J. (1990a). *Apodemus sylvaticus* (Linnaeus, 1758) − Waldmaus. In *Handbuch der Säugetiere Europas. Band 1/I, Nagetiere I*, pp. 337–358. Akademische Verlagsgesellschaft: Wiesbaden, Wiesbaden.

Niethammer, J. (1990b). *Apodemus flavicollis* - Gelbhalsmaus. In *Handbuch der Säugetiere Europas. Band 1/I, Nagetiere I*, pp. 325–336. Akademische Verlagsgesellschaft, Wiesbaden.

Nilsson, A. and Lundqvist, L. (2009). Host selection and movements of *Ixodes ricinus* (Acari) larvae on small mammals. *Oikos* 31 (3) pp. 313–322.

Nilsson, K., Lindquist, O., Liu, A. J., Jaenson, T. G., Friman, G. and Påhlson, C. (1999). *Rickettsia helvetica* in *Ixodes ricinus* ticks in Sweden. *Journal of Clinical Microbiology* 37 (2) pp. 400–3.

Nuttall, P. A., Paesen, G. C., Lawrie, C. H. and Wang, H. (2000). Vector-host interactions in disease transmission. *Journal of Molecular Microbiology and Biotechnology* 2 (4) pp. 381–386.

Obiegala, A., Pfeffer, M., Pfister, K., Tiedemann, T., Thiel, C., Balling, A., Karnath, C., Woll, D. and Silaghi, C. (2014). *Candidatus* Neoehrlichia mikurensis and *Anaplasma phagocytophilum* : prevalences and investigations on a new transmission path in small mammals and ixodid ticks. *Parasites & Vectors* 7 (1) pp. 563.

Obsomer, V., Wirtgen, M., Linden, A., Claerebout, E., Heyman, P., Heylen, D. J. A., Madder, M., Maris, J., Lebrun, M., Tack, W., Lempereur, L., Hance, T. and Van Impe, G. (2013). Spatial disaggregation of tick occurrence and ecology at a local scale as a preliminary step for spatial surveillance of tick-borne diseases: general framework and health implications in Belgium. *Parasites & Vectors* 6 (1) pp. 190.

Oehme, R., Hartelt, K., Backe, H., Brockmann, S. O. and Kimmig, P. (2002). Foci of tick-borne diseases in southwest Germany. *International Journal of Medical Microbiology* 291 Suppl (July) pp. 22–29.

Ogden, N. H. and Tsao, J. I. (2009). Biodiversity and Lyme disease: Dilution or amplification? *Epidemics* 1 (3) pp. 196–206.

Ogden, N. H., Bigras-Poulin, M., O'Callaghan, C. J., Barker, I. K., Lindsay, L. R., Maarouf, A., Smoyer-Tomic, K. E., Waltner-Toews, D. and Charron, D. (2005). A dynamic population model to investigate effects of climate on geographic range and seasonality of the tick *Ixodes scapularis*. *International Journal for Parasitology* 35 (4) pp. 375–389.

Ogden, N. H., Bigras-Poulin, M., O'callaghan, C. J., Barker, I. K., Kurtenbach, K., Lindsay, L. R. and Charron, D. F. (2007). Vector seasonality, host infection dynamics and fitness of pathogens transmitted by the tick *Ixodes scapularis*. *Parasitology* 134 pp. 209–227.

Ogden, N. H., Bigras-Poulin, M., Hanincová, K., Maarouf, A., O'Callaghan, C. J. and Kurtenbach, K. (2008). Projected effects of climate change on tick phenology and fitness of pathogens transmitted by the North American tick *Ixodes scapularis*. *Journal of Theoretical Biology* 254 pp. 621–632.

Ogden, N. H., Lindsay, L. R. and Leighton, P. a. (2013). Predicting the rate of invasion of the agent of Lyme disease *Borrelia burgdorferi*. *Journal of Applied Ecology* 50 pp. 510–518.

Oliver, J. H., Lin, T., Gao, L., Clark, K. L., Banks, C. W., Durden, L. a, James, a M. and Chandler, F. W. (2003). An enzootic transmission cycle of Lyme borreliosis spirochetes in the southeastern United States. *Proceedings of the National Academy of Sciences of the United States of America* 100 (20) pp. 11642–5.

Olsén, B., Jaenson, T. G., Noppa, L., Bunikis, J. and Bergström, S. (1993). A Lyme borreliosis cycle in seabirds and *Ixodes uriae* ticks. *Nature* 362 (6418) pp. 340–342.

Ortuño, A., Pons, I., Quesada, M., Lario, S., Anton, E., Gil, An., Castellá, J. and Segura, F. (2012). Evaluation of the presence of *Rickettsia slovaca* infection in domestic ruminants in Catalonia, Northeastern Spain. *Vector Borne and Zoonotic Diseases* 12 (12) pp. 1019–1022.

Ostfeld, R. S., Jones, C. G. and Wolff, J. O. (1996). Of Mice and Mast. *BioScience* 46 (5) pp. 323–330.

Ostfeld, R. S., Keesing, F., Jones, C. G., Canham, C. D. and Lovett, G. M. (1998a). Integrative ecology and the dynamics of species in oak forests. *Integrative Biology: Issues, News, and Reviews* 1 (5) pp. 178–186.

Ostfeld, R. S., Jones, C. G., Richard, M. P., Schauber, E. M. and Wolff, J. O. (1998b). Tick population trends and forest type - Response. *Science* 281 (5375) pp. 349–351.

Ostfeld, R. S., Schauber, E., Canham, C., Keesing, F., Jones, C. G. and Wolff, J. (2001). Effects of acorn production and mouse abundance on abundance and *Borrelia burgdorferi* infection prevalence of nymphal *Ixodes scapularis* ticks. *Vector Borne and Zoonotic Diseases* 1 (1) pp. 55–63.

Ostfeld, R. S., Canham, C. D., Oggenfuss, K., Winchcombe, R. J. and Keesing, F. (2006). Climate, deer, rodents, and acorns as determinants of variation in Lyme-disease risk. *PLoS Biology* 4 (6) pp. 1058–1068.

Paddock, C. and Telford, S. I. (2010). Through a glass, darkly: The global incidence of tick-borne diseases. In *Critical needs and gaps in understanding prevention, amelioration*

and resolution of Lyme and other tick-borne diseases: the short-term and long-term outcomes., pp. 2–43. National Academic Press, Washington D.C.

Parola, P. and Raoult, D. (2001a). Tick-borne bacterial diseases emerging in Europe. *Clinical Microbiology and Infection* 7 pp. 80–83.

Parola, P. and Raoult, D. (2001b). Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. *Clinical Infectious Diseases* 32 (6) pp. 897–928.

Parola, P., Paddock, C. and Raoult, D. (2005). Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. *Clinical Microbiology Reviews* 18 (4) pp. 719–756.

Parola, P., Paddock, C. D., Socolovschi, C., Labruna, M. B., Mediannikov, O., Kernif, T., Abdad, M. Y., Stenos, J., Bitam, I., Fournier, P. E. and Raoult, D. (2013). Update on tick-borne rickettsioses around the world: A geographic approach. *Clinical Microbiology Reviews* 26 (4) pp. 657–702.

Paulauskas, A., Rosef, O., Radzijevskaja, J., Turcinaviciene, J. and Ambrasiene, D. (2009). Infestation of mice and voles with *Ixodes ricinus* ticks in Lithuania and Norway. *Estonian Journal of Ecology* 58 (2) pp. 112–125.

Pauliks, K. (2005). Prävalenz von *Borrelia burgdorferi* s.l. und *Babesia* spp. in *Ixodes ricinus*-Zecken im Naherholungsgebiet Zeitzgrund in Thüringen. PhD Thesis.

Paull, S. H., Song, S., Mc CLure, K. M., Sackett, L. C., Marm, K. A. and Johnson, P. T. J. (2012). From superspreaders to disease hotspots: linking transmisson across hosts and space. *Frontiers in Ecology and the Environment* 10 (2) pp. 75–82.

Paziewska, A., Zwolińska, L., Harris, P. D., Bajer, A. and Siński, E. (2010). Utilisation of rodent species by larvae and nymphs of hard ticks (Ixodidae) in two habitats in NE Poland. *Experimental & Applied Acarology* 50 (1) pp. 79–91.

Perez, G., Bastian, S., Agoulon, A., Bouju-Albert, A., Durand, A., Faille, F., Lebert, I., Rantier, Y., Plantard, O. and Butet, A. (2016). Effect of landscape features on the spatio-temporal relationship between *Ixodes ricinus* ticks and their small mammal hosts. *Parasites & Vectors*.

Pérez, D., Kneubühler, Y., Rais, O. and Gern, L. (2012). Seasonality of *Ixodes ricinus* ticks on vegetation and on rodents and *Borrelia burgdorferi* sensu lato genospecies diversity in two lyme borreliosis-endemic areas in Switzerland. *Vector Borne and Zoonotic Disease* 12 (8) pp. 633–44.

Perkins, S. E., Cattadori, I. M., Tagliapietra, V., Rizzoli, A. P. and Hudson, P. J. (2003). Empirical evidence for key hosts in persistence of a tick-borne disease. *International Journal for Parasitology* 33 (9) pp. 909–917.

Perret, J.-L., Guigoz, E., Rais, O. and Gern, L. (2000). Influence of saturation deficit and temperature on *Ixodes ricinus* tick questing activity in a Lyme borreliosis-endemic area (Switzerland). *Parasitology Research* 86 (7) pp. 554–557.

Perret, J.-L., Guerin, P. M., Diehl, P. A., Vlimant, M. and Gern, L. (2003). Darkness induces mobility, and saturation deficit limits questing duration, in the tick *Ixodes ricinus*. *The Journal of Experimental Biology* 206 (Pt 11) pp. 1809–1815.

Perret, J.-L., Rais, O. and Gern, L. (2004). Influence of climate on the proportion of *Ixodes ricinus* nymphs and adults questing in a tick population. *Journal of Medical Entomology* 41 (3) pp. 361–365.

Petney, T. N., Pfäffle, M. P. and Skuballa, J. D. (2012). An annotated checklist of the ticks (Acari : Ixodida) of Germany. *Systematic & Applied Acarology* 17 (69145) pp. 115–170.

Petney, T. N., Pfäffle, M., Skuballa, J. and Taraschewski, H. (2013). Zecken und zeckenübertragene Krankheiten in Baden-Württemberg – Ökologie und Epidemiologie. *Carolinea* 71 pp. 55–99.

Petney, T., Moser, E., Littwin, N.-V., Pfäffle, M., Muders, S. V. and Taraschewski, H. (2015). Additions to the "Annotated Checklist of the Ticks of Germany": *Ixodes acuminatus* and *Ixodes inopinatus* . *Systematic and Applied Acarology* 20 (2) pp. 221–224.

Pfäffle, M. P., Petney, T. N., Elgas, M., Skuballa, J. and Taraschewski, H. (2009). Tick-induced blood loss leads to regenerative anaemia in the European hedgehog (*Erinaceus europaeus*). *Parasitology* 136 (4) pp. 443–52.

Pfäffle, M., Petney, T. N., Skuballa, J. and Taraschewski, H. (2011). Comparative population dynamics of a generalist (*Ixodes ricinus*) and specialist tick (*I. hexagonus*) species from European hedgehogs. *Experimental and Applied Acarology* 54 (2) pp. 151–64.

Pfäffle, M., Littwin, N., Muders, S. V and Petney, T. N. (2013). The ecology of tick-borne diseases. *International Journal for Parasitology* 43 (12-13) pp. 1059–77.

Pfäffle, M., Littwin, N. and Petney, T. (2015a). Host preferences of immature *Dermacentor reticulatus* (Acari: Ixodidae) in a forest habitat in Germany. *Ticks and Tick-borne Diseases* 6 (4) pp. 508–515.

Pfäffle, M., Littwin, N. and Petney, T. N. (2015b). The relationship between biodiversity and disease transmission risk. *Research and Reports in Biodiversity Studies* 4 pp. 9–20.

Phillips, B. L., Kelehear, C., Pizzatto, L., Brown, G. P., Barton, D., Shine, R., Phillips, B. L., Kelehear, C., Pizzatto, L., Brown, G. P., Barton, D. and Shine, R. (2016). Parasites and pathogens lag behind their host during periods of host range advance. *Ecology* 91 (3) pp. 872–881.

Pichon, B. and Rogers, M. (2005). Blood-meal analysis for the identification of reservoir hosts of tick-borne pathogens in Ireland. *Vector Borne and Zoonotic Diseases* 5 (2) pp. 172–180.

Pichon, B., Egan, D., Rogers, M. and Gray, J. S. (2003). Detection and identification of pathogens and host DNA in unfed host-seeking Ixodes ricinus L. (Acari: Ixodidae). *Journal of Medical Entomology* 40 (5) pp. 723–31.

Piesman, J. (1989). Transmission of Lyme disease spirochetes (*Borrelia burgdorferi*). *Experimental & Applied Acarology* 7 (1) pp. 71–80.

Piesman, J., Mather, T. N., Sinsky, R. J. and Spielman, A. (1987). Duration of tick attachment and *Borrelia burgdorferi* transmission. *Journal of Clinical Microbiology* 25 (3) pp. 557–558.

Platonov, A. E., Karan, L. S., Kolyasnikova, N. M., Makhneva, N. A., Toporkova, M. G., Maleev, V. V., Fish, D. and Krause, P. J. (2011). Humans infected with relapsing fever spirochete *Borrelia miyamotoi*, Russia. *Emerging Infectious Diseases* 17 (10) pp. 1816–1823.

Poggensee, G., Fingerle, V., Hunfeld, K. P., Kraiczy, P., Krause, A., Matuschka, F. R., Richter, D., Simon, M. M., Wallich, R., Hofman, H., Kohn, B., Lierz, M., Linde, A., Schneider, T., Straubinger, R., Stark, K., Süss, J., Talaska, T. and Jansen, A. (2008). Lyme-Borreliose: Forschungsbedarf und Forschungsansätze. *Bundesgesundheitsblatt - Gesundheitsforschung - Gesundheitsschutz* 51 (11) pp. 1329–1339.

Pollock, K. H. (2000). Capture-recapture models. *Journal of the American Statistical*

Association 95 (449) pp. 293–296.

Pollock, K. H. and Alpizar-Jara, R. (2005). Classical Open-population Capture-Recapture Models. In *Handbook of Capture-Recapture Analysis* (ed. Amstrup, S. C., McDonald, T. L., and Manly, B. F. J.), pp. 36–55. Princeton University Press, Princeton & Oxford.

Poulin, R. (1996). Sexual inequalities in helminth infections: a cost of being a male? *American Naturalist* 147 (2) pp. 287–295.

Pucek, Z., Jedrzejewski, W., Jedrzejewska, B. and Pucek, M. (1993). Rodent population dynamics in a primeval deciduous forest (Bialowieza National Park) in relation to weather, seedcrop and predation. *Acta Theriolologica* 38 pp. 199–232.

Pugliese, A. and Rosà, R. (2008). Effect of host populations on the intensity of ticks and the prevalence of tick-borne pathogens: how to interpret the results of deer exclosure experiments. *Parasitology* 135 (13) pp. 1531–1544.

Quéré, J.-P. and Le Louarn, H. (2011). *Les rongeurs de France: Faunistique et biologie.* 3rd ed. Editions Quae, Versailles.

R Development Core Team (2015). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

Radda, A., Pretzmann, G. and M, S. H. (1969a). Bionomische und ökologische Studien an österreichischen Populationen der Gelbhalsmaus (*Apodemus flavicollis*, Melchior 1834) durch Markierungsfang. *Oecologia* 3 pp. 351–373.

Radda, A., Hofmann, H. and Pretzmann, G. (1969b). Threshold of viraemia in *Apodemus flavicollis* for infection of *Ixodes ricinus* with tick-borne encephalitis virus. *Acta Virologica* 13 (1) pp. 74–77.

Radzijevskaja, J., Paulauskas, A. and Rosef, O. (2008). Prevalence of *Anaplasma phagocytophilum* and *Babesia divergens* in *Ixodes ricinus* ticks from Lithuania and Norway. *International Journal of Medical Microbiology* 298 (S1) pp. 218–221.

Radzijevskaja, J., Paulauskas, A., Rosef, O., Petkevičius, S., Mažeika, V. and Rekašius, T. (2013). The propensity of voles and mice to transmit *Borrelia burgdorferi* sensu lato infection to feeding ticks. *Veterinary Parasitology* 197 (1-2) pp. 318–325.

Randolph, S. E. (1991). The effect of *Babesia microti* on feeding and survival in its tick vector, *Ixodes trianguliceps*. *Parasitology* 102 pp. 9–16.

Randolph, S. E. (1995). Quantifying parameters in the transmission of *Babesia microti* by the tick *Ixodes trianguliceps* amongst voles (*Clethrionomys glareolus*). *Parasitology* 110 (3) pp. 287–295.

Randolph, S. E. (2001). The shifting landscape of tick-borne zoonoses: tick-borne encephalitis and Lyme borreliosis in Europe. *Philosophical Transactions of the Royal Society of London* 356 (1411) pp. 1045–1056.

Randolph, S. E. (2002). Predicting the risk of tick-borne diseases. *International Journal of Medical Microbiology* 291 pp. 6–10.

Randolph, S. E. (2004). Tick ecology: processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. *Parasitology* 129 (7) pp. S37–S65.

Randolph, S. E. (2013). Ecology of non-nidicolous ticks. In *Biology of ticks* (ed. Sonenshine, D. E. and Roe, R. M.), p. 491. Oxford University Press, Oxford.

Randolph, S. E. and Storey, K. (1999). Impact of microclimate on immature tick-rodent host interactions (Acari: Ixodidae): implications for parasite transmission. *Journal of Medical Entomology* 36 (6) pp. 741–748.

Randolph, S. E., Gern, L. and Nuttall, P. (1996). Co-feeding ticks: epidemiological significance for tick-borne pathogen transmission. *Parasitology Today* 12 (12).

Randolph, S. E., Miklisová, D., Lysy, J., Rogers, D. J. and Labuda, M. (1999). Incidence from coincidence: patterns of tick infestations on rodents facilitate transmission of tick-borne encephalitis virus. *Parasitology* 118 pp. 177–186.

Randolph, S. E., Green, R. M., Peacey, M. F. and Rogers, D. J. (2000). Seasonal synchrony: the key to tick-borne encephalitis foci identified by satellite data. *Parasitology* 121 (1) pp. 15–23.

Randolph, S. E., Chemini, C., Furlanello, C., Genchi, C., Hails, R. S., Hudson, P. J., Jones, L. D., Medley, G., Norman, R. A., Rizzoli, A. P., Smith, G. and Woolhouse, M. E. J. (2002a). The ecology of tick-borne infections in wildlife reservoirs. In *The ecology of wildlife diseases* (eds. Hudson, P. J., Rizzoli, A. P., Grenfell, B. T., Heesterbeek, H., and Dobson, A. P.), pp. 240–267. Oxford University Press.

Randolph, S. E., Green, R. M., Hoodless, A N. and Peacey, M. F. (2002b). An empirical quantitative framework for the seasonal population dynamics of the tick Ixodes ricinus. *International Journal for Parasitology* 32 (8) pp. 979–89.

Rautenbach, A., Dickerson, T. and Schoeman, M. C. (2014). Diversity of rodent and shrew assemblages in different vegetation types of the savannah biome in South Africa: no evidence for nested subsets or competition. *African Journal of Ecology* 52 (1) pp. 30–40.

Reye, A. L., Hübschen, J. M., Sausy, A. and Muller, C. P. (2010). Prevalence and seasonality of tick-borne pathogens in questing *Ixodes ricinus* ticks from Luxembourg. *Applied and Environmental Microbiology* 76 (9) pp. 2923–2931.

Richter, D. and Matuschka, F.-R. (2010). Elimination of lyme disease spirochetes from ticks feeding on domestic ruminants. *Applied and Environmental Microbiology* 76 (22) pp. 7650–2.

Richter, J., Fournier, P. E., Petridou, J., Häussinger, D. and Raoult, D. (2002). *Rickettsia felis* infection acquired in Europe and documented by polymerase chain reaction. *Emerging Infectious Diseases* 8 (2) pp. 207–208.

Richter, D., Postic, D., Sertour, N., Livey, I., Matuschka, F. R. and Baranton, G. (2006). Delineation of *Borrelia burgdorferi* sensu lato species by multilocus sequence analysis and confirmation of the delineation of *Borrelia spielmanii* sp. nov. *International Journal of Systematic and Evolutionary Microbiology* 56 (4) pp. 873–881.

Rieg, S., Schmoldt, S., Theilacker, C., de With, K., Wölfel, S., Kern, W. V and Dobler, G. (2011). Tick-borne lymphadenopathy (TIBOLA) acquired in Southwestern Germany. *BMC infectious diseases* 11 (1) pp. 167–170.

Rizzoli, A. P., Hauffe, H. C., Tagliapietra, V., Neteler, M. and Rosà, R. (2009). Forest structure and roe deer abundance predict tick-borne encephalitis risk in Italy. *PLoS ONE* 4 (2) pp. e4336.

Rizzoli, A., Silaghi, C., Obiegala, A., Rudolf, I., Hubálek, Z., Földvári, G., Plantard, O., Vayssier-Taussat, M., Bonnet, S., Å Pitalskã¡, E. and KazimÃ-rovÃ¡, Mã¡. (2014). *Ixodes ricinus* and its transmitted pathogens in urban and peri-urban areas in Europe: new hazards and relevance for public health. *Frontiers in Public Health* 2 pp. 1–26.

Rollend, L., Fish, D. and Childs, J. E. (2013). Transovarial transmission of *Borrelia* spirochetes by *Ixodes scapularis*: A summary of the literature and recent observations. *Ticks and Tick-borne Diseases* 4 (1-2) pp. 46–51.

Rosà, R. (2003). The importance of aggregation in the dynamics of host-parasite interaction in wildlife: a mathematical approach. Thesis.

Rosà, R. and Pugliese, A. (2007). Effects of tick population dynamics and host densities on the persistence of tick-borne infections. *Mathematical Biosciences* 208 (1) pp. 216–240.

Rosà, R., Pugliese, A., Norman, R. and Hudson, P. J. (2003). Thresholds for disease persistence in models for tick-borne infections including non-viraemic transmission, extended feeding and tick aggregation. *Journal of Theoretical Biology* 224 (3) pp. 359–376.

Rosà, R., Pugliese, A., Ghosh, M., Perkins, S. E. and Rizzoli, A. (2007). Temporal variation of *Ixodes ricinus* intensity on the rodent host *Apodemus flavicollis* in relation to local climate and host dynamics. *Vector Borne and Zoonotic Diseases* 7 (3) pp. 285–295.

Rubel, F., Brugger, K., Monazahian, M., Habedank, B., Dautel, H., Leverenz, S. and Kahl, O. (2014). The first German map of georeferenced ixodid tick locations. *Parasites & Vectors* 7 (1) pp. 477–481.

Rudenko, N., Golovchenko, M., Grubhoffer, L. and Oliver, J. H. J. (2011). Updates on *Borrelia burgdorferi* sensu lato complex with respect to public health. *Ticks and Tick-borne Diseases* 2 (3) pp. 123–128.

Ruiz-Fons, F., Fernández-de-Mera, I. G., Acevedo, P., Gortázar, C. and de la Fuente, J. (2012). Factors driving the abundance of *Ixodes ricinus* ticks and the prevalence of zoonotic *I. ricinus*-borne pathogens in natural foci. *Applied and Environmental Microbiology* 78 (8) pp. 2669–2676.

Rumer, L., Sheshukova, O., Dautel, H., Mantke, O. D. and Niedrig, M. (2011). Differentiation of medically important euro-asian tick species *Ixodes ricinus , Ixodes persulcatus, Ixodes hexagonus*, and *Dermacentor reticulatus* by polymerase chain reaction. *Vector-Borne and Zoonotic Diseases* 11 (7) pp. 899–905.

Sahu, P. K., Pal, S. R. and Das, A. K. (2015). Non-parametric test. In *Estimation and Inferential Statistics*, pp. 145–179. Springer India, New Delhi.

Schauber, E. M., Ostfeld, R. S. and Evans, A. S. J. (2005). What is the best predictor of annual Lyme disease incidence: weather, mice, or acorns ? *Ecological Applications* 15 (2) pp. 575–586.

Scheffer, F. and Schachtschnabel, P. (1984). *Lehrbuch der Bodenkunde*. 11th ed. (eds. Schachtschnabel, P., Blume, H.-P., Hartge, K.-H., and Schwertmann, U.) Ferdinand Enke Verlag, Stuttgart.

Schex, S. Y. (2011). *Rickettsia* spp. in free-ranging small mammals in south-eatern Germany. PhD Thesis.

Schex, S., Dobler, G., Riehm, J., Müller, J. and Essbauer, S. (2011). *Rickettsia* spp. in wild small mammals in Lower Bavaria, South-Eastern Germany. *Vector Borne and Zoonotic Diseases* 11 (5) pp. 493–502.

Schlund, W. and Scharfe, F. (1993). Säugetiere der MIssen bei Oberreichenbach. *Beiheft zu Veröffentlichungen in Naturschutz und Landschaftspflege Baden-Würrtembergs* 73 pp. 493–506.

Schmidt, K. A., Ostfeld, R. S. and Schauber, E. M. (1999). Infestation of *Peromyscus leucopus* and *Tamias striatus* by *Ixodes scapularis* (Acari: Ixodidae) in relation to the abundance of hosts and parasites. *Journal of Medical Entomology* 36 (6) pp. 749–757.

Schorn, S., Pfister, K., Reulen, H., Mahling, M. and Silaghi, C. (2011). Occurrence of *Babesia* spp., *Rickettsia* spp. and *Bartonella* spp. in *Ixodes ricinus* in Bavarian public parks, Germany. *Parasites & Vectors* 4 (135) pp. 1–9.

Schouls, L. M., Van De Pol, I., Rijpkema, S. G. T. and Schot, C. S. (1999). Detection and identification of *Ehrlichia, Borrelia burgdorferi* sensu lato, and *Bartonella* species in Dutch Ixodes ricinus ticks. *Journal of Clinical Microbiology* 37 (7) pp. 2215–2222.

Schrader, C. and Süss, J. (1999). A nested RT-PCR for the detection of tick-borne encephalitis virus (TBEV) in ticks in natural foci. *Zentralblatt für Bakteriologie* 289 (3) pp. 319–28.

Schröpfer, R. (1984). Gelbhalsmaus – *Apodemus flavicollis* (Melchior, 1834). In *Die Säugetiere Westfalens* (eds. Schröpfer, R., Feldmann, R., and Vierhaus, H.), pp. 196–204. Abhandlungen des Westfälischen Museums für Naturkunde.

Schulz, M., Mahling, M. and Pfister, K. (2014). Abundance and seasonal activity of questing *Ixodes ricinus* ticks in their natural habitats in southern Germany in 2011. *Journal of Vector Ecology* 39 (1) pp. 56–65.

Schütt, P., Schuck, H. J. and Stimm, B. (2007). *Lexikon der Baum- und Straucharten*. Nikol Verlag, Hamburg.

Schwaiger, M. and Cassinotti, P. (2003). Development of a quantitative real-time RT-PCR assay with internal control for the laboratory detection of tick borne encephalitis virus (TBEV) RNA. *Journal of Clinical Virology* 27 (2) pp. 136–145.

Schwaiger, M., Péter, O. and Cassinotti, P. (2001). Routine diagnosis of *Borrelia burgdorferi* (sensu lato) infections using a real-time PCR assay. *Clinical Microbiology and Infection* 7 pp. 461–469.

Schwarz, C. J. and Arnason, A. N. (1996). A general methodology for the analysis of capture-recapture experiments in open populations. *Biometrics* 52 (3) pp. 860–873.

Schwarz, A., Maier, W. A., Kistemann, T. and Kampen, H. (2009). Analysis of the distribution of the tick *Ixodes ricinus* L. (Acari: Ixodidae) in a nature reserve of western Germany using Geographic Information Systems. *International Journal of Hygiene and Environmental Health* 212 (1) pp. 87–96.

Seber, G. A. F. (1965). A note on the multiple-recapture census. *Biometrika* 52 (1/2) pp. 249–259.

Seber, G. A. F. and Schwarz, C. J. (1986). A review of estimating animal abundance. *Biometrics* 42 (2) pp. 1–126.

Seber, G. A. F. and Schwarz, C. J. (1999). Estimating animal abundance: Review III. *Statistical Science* 14 (4) pp. 427–456.

Shaw, D. J. and Dobson, A. P. (1995). Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. *Parasitology* 111 (Suppl) pp. 111–133.

Shaw, D. J., Grenfell, B. T. and Dobson, A. P. (1998). Patterns of macroparasite aggregation in wildlife host populations. *Parasitology* 117 (6) pp. 597–610.

Shaw, M., Keesing, F., McGrail, R. and Ostfeld, R. S. (2003). Factors influencing the distribution of larval blacklegged ticks on rodent hosts. *The American Journal of Tropical Medicine and Hygiene* 68 (4) pp. 447–452.

Shore, R. F. and Hare, E. J. (2008). Bank vole *Myodes glareolus*. In *Mammals of the British Isles* (eds. Harris, S. and Walden, D. W.), pp. 88–99. The Mammal Society, London.

Silaghi, C., Hamel, D., Thiel, C., Pfister, K. and Pfeffer, M. (2011). Spotted fever group rickettsiae in ticks, Germany. *Emerging Infectious Diseases* 17 (5) pp. 890–892.

Silaghi, C., Woll, D., Mahling, M., Pfister, K. and Pfeffer, M. (2012a). *Candidatus* Neoehrlichia mikurensis in rodents in an area with sympatric existence of the hard ticks *Ixodes ricinus* and *Dermacentor reticulatus*, Germany. *Parasites & Vectors* 5 (1)

pp. 285–292.

Silaghi, C., Woll, D., Hamel, D., Pfister, K., Mahling, M. and Pfeffer, M. (2012b). *Babesia* spp. and *Anaplasma phagocytophilum* in questing ticks, ticks parasitizing rodents and the parasitized rodents--analyzing the host-pathogen-vector interface in a metropolitan area. *Parasites & Vectors* 5 (1) pp. 191–204.

Silaghi, C., Beck, R., Oteo, J. a., Pfeffer, M. and Sprong, H. (2016). Neoehrlichiosis: an emerging tick-borne zoonosis caused by *Candidatus* Neoehrlichia mikurensis. *Experimental & Applied Acarology* 68 (3) pp. 279–297.

Simser, J. A., Palmer, A. T., Fingerle, V., Wilske, B., Kurtti, T. J. and Munderloh, U. G. (2002). *Rickettsia monacensis* sp. nov., a spotted fever group rickettsia, from ticks (*Ixodes ricinus*) collected in a European city park. *Applied and Environmental Microbiology* 68 (9) pp. 4559–4566.

Siński, E., Pawełczyk, A., Bajer, A. and Behnke, J. M. (2006a). Abundance of wild rodents, ticks and environmental risk of Lyme borreliosis: A longitudinal study in an area of Mazury Lakes district of Poland. *Annals of Agricultural and Environmental Medicine* 13 (2) pp. 295–300.

Siński, E., Bajer, A., Welc, R., Pawełczyk, A., Ogrzewalska, M. and Behnke, J. M. (2006b). Babesia microti: Prevalence in wild rodents and *Ixodes ricinus* ticks from the Mazury Lakes District of north-eastern Poland. *International Journal of Medical Microbiology* 296 (Suppl 1) pp. 137–143.

Sínski, E., Pawelczyk, A., Bajer, A., Behnke, J. M., Siński, E., Pawełczyk, A., Bajer, A. and Behnke, J. M. (2006). Abundance of wild rodents, ticks and environmental risk of Lyme borreliosis: A longitudinal study in an area of Mazury Lakes district of Poland. *Annals of Agricultural and Environmental Medicine* 13 (2) pp. 295–300.

Skuballa, J., Oehme, R. and Hartelt, K. (2007). European hedgehogs as hosts for Borrelia spp., Germany. *Emerging Infectious Diseases Infect Diseases* 13 (6) pp. 6–7.

Skuballa, J., Petney, T., Pfäffle, M. and Taraschewski, H. (2010). Molecular detection of *Anaplasma phagocytophilum* in the European hedgehog (*Erinaceus europaeus*) and its ticks. *Vector Borne Zoonotic Diseases* 10 pp. 1055–1057.

Skuballa, J., Petney, T. N., Pfäffle, M., Oehme, R., Hartelt, K., Fingerle, V., Kimmig, P. and Taraschewski, H. (2012). Occurrence of different *Borrelia burgdorferi* sensu lato genospecies including *B. afzelii, B. bavariensis*, and *B. spielmanii* in hedgehogs (*Erinaceus* spp.) in Europe. *Ticks and Tick-borne Diseases* 3 (1) pp. 8–13.

Smith, M. H., Gardner, R. H., Gentry, J. B. and Kaufman, D. W. (1975). Density estimations of small mammal populations. In *Small mammals: their productivity and population dynamics* (eds. Golley, F. B., Petrusewicz, K., and Ryszkowski, L.), pp. 25–53. Cambridge University Press, London , New York, Melbourne.

Socolovschi, C., Mediannikov, O., Raoult, D. and Parola, P. (2009). The relationship between spotted fever group rickettsiae and ixodid ticks. *Veterinary Research* 40 (2) pp. 1–20.

Socolovschi, C., Gaudart, J., Bitam, I., Huynh, T. P., Raoult, D. and Parola, P. (2012). Why are there so few *Rickettsia conorii* -infected *Rhipicephalus sanguineus* ticks in the wild? *PLoS Neglected Tropical Diseases* 6 (6) pp. e1697.

Sonenshine, D. E. and Mather, T. N. (1994). *Ecological dynamics of tick-borne zoonoses.* (eds. Sonenshine, D. E. and Mather, T. N.) Oxford University Press, New York.

Southwood, T. R. E. and Henderson, P. A. (1989). Ecological methods. *Ecological methods*.

Spielman, A., Wilson, M. L., Levine, J. F. and Piesman, J. (1985). *Ecology of* Ixodes

dammini-*borne human babesiosis and Lyme disease.*

Špitalská, E., Štefanidesová, K., Kocianová, E. and Boldiš, V. (2012). *Rickettsia slovaca* and *Rickettsia raoultii* in *Dermacentor marginatus* and *Dermacentor reticulatus* ticks from Slovak Republic. *Experimental and Applied Acarology* 57 (2) pp. 189–197.

Spitzenberger, F. (2001). *Die Säugetierfauna Österreichs. Grüne Reihe des Bundesministeriums für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft*. Band 13. Austria Medien Service, Graz.

Stanek, G. (2005). Durch Zecken übertragbare Krankheitserreger in Mitteleuropa. *Wiener Klinische Wochenschrift* 117 (11-12) pp. 373–380.

Stanek, G. (2009). Büchse der Pandora : Krankheitserreger in *Ixodes ricinus* -Zecken in Mitteleuropa. *Wiener Klinische Wochenschrift* 121 pp. 673–683.

Stanek, G. and Reiter, M. (2011). The expanding Lyme Borrelia complex-clinical significance of genomic species? *Clinical Microbiology and Infection* 17 (4) pp. 487–493.

Stanko, M., Krasnov, B. R. and Morand, S. (2006). Relationship between host abundance and parasite distribution: Inferring regulating mechanisms from census data. *Journal of Animal Ecology* 75 (2) pp. 575–583.

Stauffer, R. C. (1957). Haeckel, Darwin and ecology. *Quarterly Review of Biology* 32 pp. 138–144.

Stehr, H. (1982). *Die Rötelmaus. Das moderne Tierlexikon. Band 9.* Verlagsgruppe Bertelsamann, Gütersloh.

Stenseth, N. C., VIljugrein, H., Jędrzejewski, W., Mysterud, A. and Pucek, Z. (2002). Population dynamics of *Clethrionomys glareolus* and *Apodemus flavicollis*: seasonal components of density dependence and density independence. *Acta Theriologica* 47 (S1) pp. 39–67.

Sting, R., Breitling, N., Oehme, R. and Kimmig, P. (2004). The occurrence of *Coxiella burnetii* in sheep and ticks of the genus *Dermacentor* in Baden-Wuerttemberg. *Deutsche Tierärztliche Wochenschrift* 111 (10) pp. 390–4.

Stoddard, S. T., Morrison, A. C., Vazquez-Prokopec, G. M., Paz Soldan, V., Kochel, T. J., Kitron, U., Elder, J. P. and Scott, T. W. (2009). The role of human movement in the transmission of vector-borne pathogens. *PLoS Negl Trop Dis* 3 (7) pp. e481.

Strle, F. and Stanek, G. (2009). Clinical manifestations and diagnosis of lyme borreliosis. *Current Problems in Dermatology* 37 pp. 51–110.

Suchomel, J., Purchart, L., Čepelka, L. and Heroldová, M. (2014). Structure and diversity of small mammal communities of mountain forests in Western Carpathians. *European Journal of Forest Research* 133 (3) pp. 481–490.

Sundell, J., Church, C. and Ovaskainen, O. (2012). Spatio-temporal patterns of habitat use in voles and shrews modified by density, season and predators. *The Journal of Animal Ecology* 81 (4) pp. 747–755.

Süss, J. (2003). Epidemiology and ecology of TBE relevant to the production of effective vaccines. *Vaccine* 21 (Suppl) pp. 19–35.

Süss, J. (2008). Tick-borne encephalitis in Europe and beyond--the epidemiological situation as of 2007. *Euro surveillance : European communicable disease bulletin* 13 (26) pp. 717–727.

Süss, J. (2011). Tick-borne encephalitis 2010: Epidemiology, risk areas, and virus strains in Europe and Asia-An overview. *Ticks and Tick-borne Diseases* 2 (1) pp. 2–15.

Süss, J. and Schrader, C. (2004). Durch Zecken übertragene humanpathogene und bisher

als apathogen geltende Mikroorganismen in Europa: Teil I: Zecken und Viren. *Bundesgesundheitsblatt - Gesundheitsforschung - Gesundheitsschutz* 47 pp. 392–404.

Süss, J., Schrader, C., Abel, U., Voigt, W. P. and Schosser, R. (1999). Annual and seasonal variation of tick-borne encephalitis virus (TBEV) prevalence in ticks in selected hot spot areas in Germany using a nRT-PCR: Results from 1997 and 1998. *Zentralblatt für Bakteriologie* 289 (5-7) pp. 564–578.

Süss, J., Fingerle, V., Hunfeld, K.-P., Schrader, C. and Wilske, B. (2004a). Tick-borne human pathogenic microorganisms found in Europe and those considered nonpathogenic. Part II: Bacteria, parasites and mixed infections. *Bundesgesundheitsblatt, Gesundheitsforschung, Gesundheitsschutz* 47 (5) pp. 470–486.

Süss, J., Schrader, C., Falk, U. and Wohanka, N. (2004b). Tick-borne encephalitis (TBE) in Germany — Epidemiological data, development of risk areas and virus prevalence in field-collected ticks and in ticks removed from humans. *International Journal of Medical Microbiology* 293 (Suppl) pp. 69–79.

Süss, J., Klaus, C., Diller, R., Schrader, C., Wohanka, N. and Abel, U. (2006). TBE incidence versus virus prevalence and increased prevalence of the TBE virus in Ixodes ricinus removed from humans. *International Journal of Medical Microbiology* 296 (Suppl) pp. 63–68.

Süss, J., Klaus, C., Gerstengarbe, F.-W. and Werner, P. C. (2008). What makes ticks tick? Climate change, ticks, and tick-borne diseases. *Journal of Travel Medicine* 15 (1) pp. 39–45.

Süss, J., Kahl, O., Aspöck, H., Hartelt, K., Vaheri, A., Oehme, R., Hasle, G., Dautel, H., Kunz, C., Kupreviciene, N., Randolph, S. E., Zimmermann, H.-P., Atkinson, B., Dobler, G., Kutsar, K., Heinz, F. X. and Steffen, R. (2010). Tick-borne encephalitis in the age of general mobility. *Wiener Medizinische Wochenschrift* 160 (3-4) pp. 94–100.

Switaj, K., Chmielewski, T., Borkowski, P., Tylewska-Wierzbanowska, S. and Olszynska-Krowicka, M. (2012). Spotted fever rickettsiosis caused by *Rickettsia raoultii*--case report. *Przegląd epidemiologiczny* 66 (2) pp. 347–350.

Tack, W., Madder, M., Baeten, L., Vanhellemont, M., Gruwez, R. and Verheyen, K. (2012a). Local habitat and landscape affect *Ixodes ricinus* tick abundances in forests on poor, sandy soils. *Forest Ecology and Management* 265 pp. 30–36.

Tack, W., Madder, M., Baeten, L., De Frenne, P. and Verheyen, K. (2012b). The abundance of *Ixodes ricinus* ticks depends on tree species composition and shrub cover. *Parasitology* 139 (10) pp. 1273–1281.

Tadin, A., Turk, N., Korva, M., Margaletić, J., Beck, R., Vucelja, M., Habuš, J., Svoboda, P., Županc, T. A., Henttonen, H. and Markotić, A. (2012). Multiple co-infections of rodents with hantaviruses, *Leptospira*, and *Babesia* in Croatia. *Vector-Borne and Zoonotic Diseases* 12 (5) pp. 388–392.

Tagliapietra, V., Rosà, R., Arnoldi, D., Cagnacci, F., Capelli, G., Montarsi, F., Hauffe, H. C. and Rizzoli, A. (2011). Saturation deficit and deer density affect questing activity and local abundance of *Ixodes ricinus* (Acari, Ixodidae) in Italy. *Veterinary Parasitology* 183 (1-2) pp. 114–124.

Tälleklint, L. and Jaenson, T. G. T. (1994). Transmission of *Borrelia burgdorfer*i s.l. from mammal reservoirs to the primary vector of lyme borreliosis, *Ixodes ricinus* (Acari:

Ixodidae), in Sweden. *Journal of Medical Entomology* 31 (6) pp. 880–886.

Tälleklint, L. and Jaenson, T. G. (1997). Infestation of mammals by *Ixodes ricinus* ticks (Acari: Ixodidae) in south-central Sweden. *Experimental & Applied Acarology* 21 (12) pp. 755–771.

Tälleklint, L., Jaenson, T. G. and Mather, T. N. (1993). Seasonal variation in the capacity of the bank vole to infect larval ticks (Acari: Ixodidae) with the Lyme disease spirochete, *Borrelia burgdorferi*. *Journal of Medical Entomology* 30 pp. 812–815.

Talleklint-Eisen, L. and Lane, R. S. (2000). Spatial and temporal variation in the density of *Ixodes pacificus* (Acari : Ixodidae) nymphs. *Environmental Entomology* 29 (2) pp. 272–280.

Taraschewski, H. (2006). Parasiten und Wirte als Bestandteile von Ökosystemen. In *Allgemeine Parasitologie* (eds. Hiepe, T., Lucius, R., and Gottstein, H.), Thieme-Verlag.

Tast, J., Kaikusalo, A. and Järvinen, A. (2005). Population fluctuations of *Sorex araneus* at Kilpisjärvi, Finnish Lapland, as compared with rodent cycles. In *Advances in the Biology of shrews II* (eds. Merritt, J. F., Chirchfield, S., Hutterer, R., and Sheftel, B. I.), p. 454. The International society of shrew biologists, New York, NY.

Taylor, R., White, A. and Sherratt, J. (2013). How do variations in seasonality affect population cycles? *Proceedings of the Royal Society B: Biological Sciences* 280 pp. 1–12.

Tijsse-Klasen, E., Fonville, M., Reimerink, J. H., Spitzen-van der Sluijs, A. and Sprong, H. (2010). Role of sand lizards in the ecology of Lyme and other tick-borne diseases in the Netherlands. *Parasites & Vectors* 3 pp. 42.

Tilly, K., Rosa, P. A. and Stewart, P. E. (2008). Biology of Infection with *Borrelia burgdorferi*. *Infectious Disease Clinics of North America* 22 (2) pp. 217–234.

Tioli, S., Cagnacci, F., Stradiotto, A. and Rizzoli, A. (2009). Edge effect on density estimates of a radiotracked population of yellow-necked mice. *Journal of Wildlife Management* 73 (2) pp. 184–190.

Tkadlec, E. and Zejda, J. (1998). Small rodent population fluctuations: the effects of age structure and seasonality. *Evolutionary Ecology* 12 pp. 191–210.

Tonteri, E. and Jääskeläinen, A. (2011). Tick-borne encephalitis virus in wild rodents in winter, Finland, 2008-2009. *Emerging Infectious Diseases* 17 (1) pp. 1–7.

Turner, A. K., Beldomenico, P. M., Bown, K. J., Burthe, S. J., Jackson, J. a, Lambin, X. and Begon, M. (2014). Host-parasite biology in the real world: the field voles of Kielder. *Parasitology* 141 pp. 997–1017.

Turni, H. (2005a). Gelbhalsmaus *Apodemus flavicollis* (Melchior, 1834). In *Die Säugetiere Baden-Württembergs. Band 2* (eds. Braun, M. and Dieterlen, F.), pp. 229–237. Ulmer, Stuttgart (Hohenheim).

Turni, H. (2005b). Waldspitzmaus *Sorex araneus* Linnaeus, 1758. In *Die Säugetiere Baden-Württembergs. Band 2* (eds. Braun, M. and Dieterlen, F.), pp. 48–55. Ulmer Verlag, Stuttgart.

Turni, H. (2005c). Waldmaus *Apodemus sylvaticus* (Linnaeus, 1758). In *Die Säugetiere Baden-Württembergs. Band 2* (eds. Braun, M. and Dieterlen, F.), pp. 238–243. Ulmer Verlag, Stuttgart (Hohenheim).

Uilenberg, G. (2006). *Babesia*-A historical overview. *Veterinary Parasitology* 138 (1-2) pp. 3–10.

Ulrich, R. G., Heckel, G., Pelz, H.-J., Wieler, L. H., Nordhoff, M., Dobler, G., Freise, J.,

Matuschka, F.-R., Jacob, J., Schmidt-Chanasit, J., Gerstengarbe, F.-W., Jäkel, T., Süss, J., Ehlers, B., Nitsche, A., Kallies, R., Johne, R., Günther, S., Henning, K., Grunow, R., Wenk, M., Maul, L. C., Hunfeld, K.-P., Wölfel, R., Schares, G., Scholz, H. C., Brockmann, S. O., Pfeffer, M. and Essbauer, S. S. (2009). Rodents and rodent associated disease vectors: the network of "rodent carrying pathogens" introduces itself. *Bundesgesundheitsblatt, Gesundheitsforschung, Gesundheitsschutz* 52 (3) pp. 352–369.

Untersteiner, H. (2007). *Statistik - Datenauswertung mit Excel und SPSS*. 2nd ed. Facultas Verlags- und Buchhandels AG, Wien, Österreich.

Václav, R., Ficová, M., Prokop, P. and Betáková, T. (2011). Associations between coinfection prevalence of *Borrelia lusitaniae, Anaplasma* sp., and *Rickettsia* sp. in hard ticks feeding on reptile hosts. *Microbial Ecology* 61 (2) pp. 245–253.

van Duijvendijk, G., Sprong, H. and Takken, W. (2015). Multi-trophic interactions driving the transmission cycle of *Borrelia afzelii* between *Ixodes ricinus* and rodents: a review. *Parasites & Vectors* 8 (1) pp. 643–654.

van Duijvendijk, G., Coipan, C., Wagemakers, A., Fonville, M., Ersöz, J., Oei, A., Földvári, G., Hovius, J., Takken, W. and Sprong, H. (2016). Larvae of *Ixodes ricinus* transmit *Borrelia afzelii* and *B. miyamotoi* to vertebrate hosts. *Parasites & Vectors* 9 (1) pp. 97–103.

van Overbeek, L., Gassner, F., Van Der Plas, C. L., Kastelein, P., Nunes-Da Rocha, U. and Takken, W. (2008). Diversity of *Ixodes ricinus* tick-associated bacterial communities from different forests. *Federation of European Microbiological Societies* 66 (1) pp. 72–84.

Vassallo, M., Paul, R. and Perez-Eid, C. (2000). Temporal distribution of the annual nymphal stock of *Ixodes ricinus* ticks. *Experimental & Applied Acarology* 24 pp. 941–949.

Vayssier-Taussat, M., le Rhun, D., Buffet, J. P., Maaoui, N., Galan, M., Guivier, E., Charbonnel, N. and Cosson, J. F. (2012). *Candidatus* Neoehrlichia mikurensis in bank voles, France. *Emerging Infectious Diseases* 18 (12) pp. 2063–2065.

Vázquez, D. P., Poulin, R., Krasnov, B. R. and Shenbrot, G. I. (2005). Species abundance and the distribution of specialization in host – parasite interaction networks. *Journal of Animal Ecology* 74 (5) pp. 946–955.

Viro, P. and Niethammer, J. (1982). *Clethrionomys glareolus* (Schreber, 1780) - Rötelmaus. In *Handbuch der Säugetiere Europas. Band 2/I, Nagetiere II* (ed. Niethammer, J. and Krapp, F.), pp. 109–146. Akademische Verlagsgesellschaft, Wiesbaden.

Vogel, P. (1995a). *Apodemus flavicollis* (Melchior, 1834). In *Die Säugetiere der Schweiz: Verbreitung, Biologie und Ökologie. Band 103* (ed. Hausser, J.), pp. 274–278. Birkäuser Verlag, Basel.

Vogel, P. (1995b). *Apodemus sylvaticus* (L., 1758). In *Die Säugetiere der Schweiz: Verbreitung, Biologie und Ökologie. Band 103* (ed. Hausser, J.), pp. 268–273. Birkhäuser Verlag, Basel.

Von Loewenich, F. D., Geißdörfer, W., Disqué, C., Matten, J., Schett, G., Sakka, S. G. and Bogdan, C. (2010). Detection of "*Candidatus* Neoehrlichia mikurensis" in two patients with severe febrile illnesses: Evidence for a European sequence variant. *Journal of Clinical Microbiology* 48 (7) pp. 2630–2635.

Vorou, R. M., Papavassiliou, V. G. and Tsiodras, S. (2007). Emerging zoonoses and vector-

borne infections affecting humans in Europe. *Epidemiology and Infection* 135 (8) pp. 1231–1247.

Walker, D. H. and Ismail, N. (2008). Emerging and re-emerging rickettsioses: endothelial cell infection and early disease events. *Nature Reviews MIcrobiology* 6 (5) pp. 375–386.

Watts, C. H. S. (1968). The foods eaten by wood mice (*Apodemus sylvaticus*) and bank voles (*Clethrionomys glareolus*) in Wytham Woods , Berkshire. *Journal of Animal Ecology* 37 (1) pp. 25–41.

Wikel, S. K. (1996). Host immunity to ticks. *Annual Review of Entomology* 41 (66) pp. 1–22.

Wikel, S. K. and Bergman, D. (1997). Tick-host immunology: significant advances and challenging opportunities. *Parasitology Today* 13 (10) pp. 383–389.

Williams, S. C. and Ward, J. S. (2010). Effects of Japanese barberry (Ranunculales: Berberidaceae) removal and resulting microclimatic changes on *Ixodes scapularis* (Acari: Ixodidae) abundances in Connecticut, USA. *Environmental Entomology* 39 (6) pp. 1911–1921.

Wilske, B., Busch, U., Eiffert, H., Fingerle, V., Pfister, H.-W., Rössler, D. and Preac-Mursic, V. (1996). Diversity of OspA and OspC among cerebrospinal fluid isolates of *Borrelia burgdorferi* sensu lato from patients with neuroborreliosis in Germany. *Medical Microbiology and Immunology* 184 (4) pp. 195–201.

Wilson, K., Bjørnstad, O. N., Dobson, A. P., Merler, S., Poglayen, G., Read, a F. and Skorping, A. (2001). Heterogeneities in macroparasite infections: patterns and processes. In *The Ecology of Wildlife Diseases* (eds. Hudson, P. J., Rizzoli, A., Grenfell, B., Heesterbeek, T. H., and Dobson, A. P.), pp. 6–44. Oxford University Press, New York.

Wirtz, H.-P. (2001). Zecken als Krankheitsüberträger: Was tun bei einem Stich? *Biologie in unserer Zeit* 31 (4) pp. 229–238.

Wodecka, B., Rymaszewska, A. and Skotarczak, B. (2014). Host and pathogen DNA identification in blood meals of nymphal *Ixodes ricinus* ticks from forest parks and rural forests of Poland. *Experimental and Applied Acarology* 62 pp. 543–555.

Wölfel, R., Essbauer, S. and Dobler, G. (2008). Diagnostics of tick-borne rickettsioses in Germany: A modern concept for a neglected disease. *International Journal of Medical Microbiology* 298 (Suppl 1) pp. 368–374.

Wood, S. S. N. (2006). *Generalized Additive Models: An Introduction with R.* CRC Press.

Woolhouse, M. E., Dye, C., Etard, J. F., Smith, T., Charlwood, J. D., Garnett, G. P., Hagan, P., Hii, J. L., Ndhlovu, P. D., Quinnell, R. J., Watts, C. H., Chandiwana, S. K. and Anderson, R. M. (1997). Heterogeneities in the transmission of infectious agents: implications for the design of control programs. *Proceedings of the National Academy of Sciences of the United States of America* 94 (1) pp. 338–342.

Xu, G., Fang, Q. Q., Keirans, J. E. and Durden, L. A. (2003). Molecular phylogenetic analyses indicate that the *Ixodes ricinus* complex is a paraphyletic group. *The Journal of Parasitology* 89 (3) pp. 452–457.

Zahler, M. and Gothe, R. (1997). Evidence for the reproductive isolation of *Dermacentor marginatus* and *Dermacentor reticulatus* (Acari: Ixodidae) ticks based on cross-breeding, morphology and molecular studies. *Experimental & Applied Acarology* 21 (10-11) pp. 685–696.

Zilber, L. A. (1939). Spring (spring-summer) epidemical tick-borne encephalitis. *Akademii*

Nauk Seriia Biologicheskaia (Moskva) 2 pp. 9–37. (in Russian).

Zimmermann, P. and Brinkmann, S. (2006). *Hardtwald zwischen Graben und Karlsruhe*. Regierungspräsidium Karlsruhe, Referat 56 “Naturschutz und Landschaftspflege.”

Zuk, M. and McKean, K. A. (1996). Sex differences in parasite infections: patterns and processes. *International Journal for Parasitology* 26 (10) pp. 1009–1024.

7 APPENDIX

Appendix A

Small mammal sampling form used for capture-mark-recapture live trapping. Session indicates the combination of month and year of sampling, Tag (1-2-3) = day of sampling, Zeit (M-A) indicates morning (M) or afternoon (A) sampling. Patch= sampling grid (1 to 3), Falle= trap number (1-75), Maus Nr.= individual small mammal number, Recap (+/-)= capture status of small mammal (new or previously captured), Re-recap= recapture within a sampling interval, Art= species, Sex (f/m)= gender (f=female, m= male), Alter (j/sa/a)= age class (j= juvenile, sa= subadult, a= adult), reproductive= status of sexual activity (+/-), Gewicht (g)= weight of the individual, Zecken (+/-)= ticks present or absent.

FANGBOGEN Kleinsäuger										Anwesende:		
Habitat:										Datum:		
Session:												
Tag (1-2-3) Zeit (M-A)	Patch Nr.	Falle Nr.	Maus Nr.	Recap (+/-)	Re-Recap	Art	Sex (f/m)	Alter (j/sa/a)	reproduktiv	Gewicht (g)	Bemerkungen:	Zecken (+/-)

Tick burden on small mammal species. All tick species and all small mammal species are included. Table A shows the infestation of A. flavicollis mice with all tick species and instars found on A. flavicollis at the four sites during the sampling period from 2012 to 2014. n refers to the total number of individuals collected at the respective site and the respective year. % gives the proportion of the collected ticks for the respective host, site and year, compared to the overall number collected in total. Table B provides the same information as table A for M. glareolus. Table C gives the total number of tick species and instars collected on the minor small mammal species, and provides the proportions (%) of tick species and instars by site and year

A		2012					2013					2014				
A.flavicollis		**AW**	**HW**	**MB**	**SW**	**total**	**AW**	**HW**	**MB**	**SW**	**total**	**AW**	**HW**	**MB**	**SW**	**total**
examined	**n**	150	163	184	127	624	82	61	69	37	249	284	107	151	80	622
D. reticulatus	n	0	18	5	0	23	0	52	0	0	52	1	12	13	0	26
larvae	%	0	4.2	1.2	0.0	5.3	0.0	12.1	0.0	0.0	12.1	0.2	2.8	3.0	0.0	6.0
D. reticulatus	n	0	4	0	0	4	0	55	0	0	55	0	3	0	0	3
nymphs	%	0	0.7	0.0	0.0	0.7	0.0	10.1	0.0	0.0	10.1	0.0	0.6	0.0	0.0	0.6
I. acuminatus	n	1	9	21	0	31	1	0	0	0	1	0	0	0	1	1
larvae	%	2.6	23.7	55.3	0.0	81.6	2.6	0.0	0.0	0.0	2.6	0.0	0.0	0.0	2.6	2.6
I. acuminatus	n	0	2	0	0	2	3	0	0	0	3	0	0	0	0	0
nymphs	%	0.0	15.4	0.0	0.0	15.4	23.1	0.0	0.0	0.0	23.1	0.0	0.0	0.0	0.0	0.0
I. ricinus	n	1212	977	1518	636	4343	935	1404	1196	285	3820	1058	1532	2329	1110	6029
larvae	%	6.8	5.5	8.6	3.6	24.5	5.3	7.9	6.8	1.6	21.6	6.0	8.6	13.1	6.3	34.0
I. ricinus	n	6	6	1	4	17	17	44	62	18	141	14	7	19	22	62
nymphs	%	2.0	2.0	0.3	1.3	5.6	5.6	14.4	20.3	5.9	46.2	4.6	2.3	6.2	7.2	20.3
I. trianguliceps	n	0	2	0	1	3	0	0	0	0	0	0	0	0	0	0
larvae	%	0.0	4.0	0.0	2.0	6.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
I. trianguliceps	n	0	0	0	2	2	0	0	1	1	2	0	1	0	1	2
nymphs	%	0.0	0.0	0.0	8.0	8.0	0.0	0.0	4.0	4.0	8.0	0.0	4.0	0.0	4.0	8.0
I. trianguliceps	n	0	0	0	0	0	0	0	0	6	6	0	0	0	0	0
females	%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	85.7	85.7	0.0	0.0	0.0	0.0	0.0
prevalence	**%**	94.0	87.1	94.0	82.7	89.9	92.7	98.4	85.5	100.0	93.2	79.2	94.4	96.0	88.8	87.1
mean	$\bar{x}$ ±	8.1 ±	6.2 ±	8.4 ±	5.1 ±	7.1 ±	11.7 ±	25.5 ±	18.2 ±	8.4 ±	16.4 ±	3.8 ±	14.5 ±	15.6 ±	14.2 ±	9.8 ±
abundance	**SD**	8.3	7.7	8.4	7.6	8.1	11.3	24.8	22.7	8.2	19.5	4.2	16.1	19.2	21.2	15.2
mean	$\bar{x}$ ±	8.6 ±	7.2 ±	8.9 ±	6.1 ±	7.9 ±	12.6 ±	25.9 ±	21.3 ±	8.4 ±	17.6 ±	4.8 ±	15.4 ±	16.3 ±	16 ± 21.8	11.3 ±
intensity	**SD**	8.3	7.8	8.3	7.9	8.2	11.3	24.8	23.1	8.2	19.7	4.2	16.2	19.3		15.7
range	**Min-Max**	0 - 51	0 - 66	0 - 60	0 - 60	0 - 66	0 - 47	0 - 108	0 - 108	0 - 31	0 - 108	0 - 27	0 - 86	0 - 130	0 - 116	0 - 130

B

		2012					2013					2014				
M. glareolus		**AW**	**HW**	**MB**	**SW**	**total**	**AW**	**HW**	**MB**	**SW**	**total**	**AW**	**HW**	**MB**	**SW**	**total**
examined	**n**	155	77	93	172	497	213	18	42	11	284	266	64	117	13	460
D. reticulatus	n	2	127	3	0	132	0	9	0	0	9	59	97	3	0	159
larvae	%	0.5	29.5	0.7	0.0	30.6	0.0	2.1	0.0	0.0	2.1	13.7	22.5	0.7	0.0	36.9
D. reticulatus	n	1	172	0	0	173	2	130	0	0	132	8	155	4	0	167
nymphs	%	0.2	31.7	0.0	0.0	31.9	0.4	23.9	0.0	0.0	24.3	1.5	28.5	0.7	0.0	30.8
I. acuminatus	n	0	3	0	0	3	0	0	0	0	0	2	0	0	0	2
larvae	%	0.0	7.9	0.0	0.0	7.9	0.0	0.0	0.0	0.0	0.0	5.3	0.0	0.0	0.0	5.3
I. acuminatus	n	0	1	0	0	1	0	0	0	0	0	7	0	0	0	7
nymphs	%	0.0	7.7	0.0	0.0	7.7	0.0	0.0	0.0	0.0	0.0	53.8	0.0	0.0	0.0	53.8
I. ricinus	n	290	125	150	153	718	687	118	158	70	1033	337	229	310	46	922
larvae	%	1.6	0.7	0.8	0.9	4.1	3.9	0.7	0.9	0.4	5.8	1.9	1.3	1.8	0.3	5.2
I. ricinus	n	3	2	0	0	5	22	4	13	1	40	10	11	4	0	25
nymphs	%	1.0	0.7	0.0	0.0	1.6	7.2	1.3	4.3	0.3	13.1	3.3	3.6	1.3	0.0	8.2
I. ricinus	n	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
females	%	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
I. trianguliceps	n	0	1	4	9	14	2	0	0	0	2	0	1	1	0	2
larvae	%	0.0	2.0	8.0	18.0	28.0	4.0	0.0	0.0	0.0	4.0	0.0	2.0	2.0	0.0	4.0
I. trianguliceps	n	0	3	0	1	4	0	0	1	0	1	0	0	1	0	1
nymphs	%	0.0	12.0	0.0	4.0	16.0	0.0	0.0	4.0	0.0	4.0	0.0	0.0	4.0	0.0	4.0
I. trianguliceps	n	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
females	%	0.0	0.0	0.0	14.3	14.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
prevalence	**%**	69.0	85.7	74.2	50.6	66.2	77.0	88.9	85.7	90.9	79.6	54.5	82.8	75.2	76.9	64.3
mean	$\bar{x}$ ±	1.9 ±	5.6 ±	1.7 ±	1 ± 1.3	2.1 ± 4	3.3 ± 4	14.6 ±	4.1 ± 4.1	6.5 ±	4.3 ± 7.1	1.6 ± 3	7.7 ± 7.6	2.8 ± 3.3	3.5 ± 4.8	2.8 ± 4.6
abundance	**SD**	2.4	8.5	1.7				21.3		7.1						
mean	$\bar{x}$ ±	2.8 ±	6.6 ±	2.3 ±	1.9 ±	3.2 ±	4.3 ± 4.1	16.4 ±	4.8 ± 4	7.1 ±	5.4 ± 7.6	2.9 ±	9.3 ± 7.5	3.7 ± 3.3	4.6 ± 5	4.3 ± 5.1
intensity	**SD**	2.4	8.8	1.6	1.2	4.6		21.9		7.2		3.6				
range	**Min-Max**	0 - 12	0 - 40	0 - 7	0 - 6	0 - 40	0 - 25	0 - 74	0 - 18	0 - 18	0 - 74	0 - 27	0 - 29	0 - 18	0 - 18	0 - 29

C		2012					2013					2014				
D. reticulatus LL	n	AW	HW	MB	SW	total	AW	HW	MB	SW	total	AW	HW	MB	SW	total
S. araneus	4	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.9%	0.0%	0.9%
S. minutus	26	0.0%	6.0%	0.0%	0.0%	6.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
D. reticulatus NN																
S. araneus	8	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	1.1%	0.0%	0.0%	1.1%	0.0%	0.4%	0.0%	0.0%	0.4%
S. minutus	1	0.0%	0.2%	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
I. ricinus LL																
A. sylvaticus	84	0.0%	0.1%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.3%	0.0%	0.0%	0.3%
M. agrestis	30	0.2%	0.0%	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
S. araneus	683	0.4%	0.7%	0.5%	0.0%	1.7%	0.0%	0.1%	0.2%	1.1%	1.4%	0.0%	0.1%	0.5%	0.1%	0.8%
S. minutus	50	0.1%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.2%
I. ricinus NN																
A. sylvaticus	5	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	1.6%	0.0%	0.0%	1.6%
M. agrestis	1	0.3%	0.0%	0.0%	0.0%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
S. araneus	5	0.0%	0.7%	0.0%	0.0%	0.7%	0.0%	0.7%	0.3%	0.0%	1.0%	0.0%	0.0%	0.0%	0.0%	0.0%
S. minutus	4	0.0%	0.3%	0.0%	0.0%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	1.0%	1.0%
I. trianguliceps LL																
S. araneus	9	0.0%	0.0%	2.0%	0.0%	2.0%	0.0%	2.0%	0.0%	14.0%	16.0%	0.0%	0.0%	0.0%	0.0%	0.0%
S. minutus	20	0.0%	0.0%	2.0%	16.0%	18.0%	0.0%	0.0%	8.0%	2.0%	10.0%	0.0%	0.0%	0.0%	12.0%	12.0%
I. trianguliceps NN																
S. araneus	6	0.0%	4.0%	0.0%	0.0%	4.0%	0.0%	0.0%	4.0%	16.0%	20.0%	0.0%	0.0%	0.0%	0.0%	0.0%
S. minutus	7	0.0%	4.0%	4.0%	0.0%	8.0%	0.0%	0.0%	4.0%	4.0%	8.0%	0.0%	4.0%	0.0%	8.0%	12.0%

Appendix C

Materials and equipment

Field

Microclimate data

Microclimate data logger (SDI-Log Data Logger)	UP GmbH
HygroClip 2 sensors	Rotronic

Tick collection and identification

Flag	In-house production
Falcon™ Tubes (15ml, 50ml)	BD Biosciences
Forceps (Biologie No.5)	Dumont
Petri dishes	Greiner Bio-one GmbH
Rotilabo®-safety microcentrifuge tubes (PP 2.0ml)	Roth
Reaction tubes (1.5ml)	Sarstedt

Small mammal capture and marking

Small mammal live traps	Longworth
Micro Tattoo System	Fine Science Tools
Ketchum Green Tattoo Paste	Ketchum, Manufacturing Inc.
Sterican needles (27G x ½“, 0.4 x 12 mm)	B. Braun Melsungen AG
Hypodermic needle (30G x ½“, 0.3 x 13 mm)	BD MicrolanceTM 3, BD
Micro-Line Spring Scale, metric (60 g, 100g)	Pesola AG

Laboratory

Tick homogenization

Disposable nitril gloves	Asid Bonz
Disposable latex gloves	VWR
Eppendorf Reference pipette (0.5-10µl, 10-100µl, 100-1000µl)	Eppendorf
Safe-lock tubes (0.5ml, 1.5ml, 2.0ml)	Eppendorf
Eppendorf Multipette 4780	Eppendorf
Multi-Tip pipette tips (10ml) Combitips advanced	Eppendorf
Steel beads (3mm Durchmesser)	VWR
MEM Earle (1x) cell culture medium w 2.2g/l NaHCO3 w stable glutamine	Biochrom AG

Nucleic acid purification

96well plate	Greiner Bio-one GmbH
Safe-lock tubes (0.5ml, 1.5ml, 2.0ml)	Eppendorf
Multi channel pipette (50-1200µl)	Biohit
NucliSens® easyMag™ Disposables	BioMérieux
NucliSens® easyMag™ Extraction Buffer 1, 2, 3	BioMérieux
NucliSens® easyMag™ Lysis Buffer	BioMérieux
NucliSens® easyMag™ Magnetic Silica	BioMérieux

Pipette tips, with filter (50-1200µl)	Biohit
Water (PCR clean)	
PCR	
Glass caplillaries (20µl)	Roche
PCRtubes (0.2ml)	Biozym
Radiator block with Centrifuge Adapters	Roche
LightCycler® DNA Master HybProbe	Roche
Primer	TibMol
Probes	TibMol
SuperScript III One-Step RT-PCR Kit with Platinum® Taq	Invitrogen
iProof High Fidelity DNA Polymerase	Bio-Rad
Gel-Electrophoresis	
Agarose Standard	Roth
Rotiphorese TBE buffer	Roth
Water	
Midori Green DNA stain	Nippon Genetics
Laboratory Equipment	
Vibration mill MM400	Retsch
Cooling centrifuge 5804R and 5417R	Eppendorf
Mini centrifuge 5415D	Eppendorf
NucliSense® easyMag™	BioMérieux
LightCycler® 1.0	Roche
LightCycler®1.5	Roche
GeneAmp® PCR System 9700	Applied Biosystems
PCR-Cycler C 1000TM Thermal Cycler	Bio-Rad
ChemiDoc XRS gel documentation system	Bio-Rad