Spatiotemporal activity of the *Ixodes ricinus* tick in England

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Authors Declaration

I declare that the work in this thesis was carried out in accordance with the regulations of the University of Liverpool. The work is original except where indicated by special reference in the text and no part of the dissertation has been submitted for any other degree.

Any views expressed in the thesis are those of the author and in no way represent the University of Liverpool.

The thesis has not been presented to any other University for examination either in the United Kingdom or overseas.

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Abstract

The *Ixodes ricinus* tick is an opportunistic, generalist species, capable of feeding on multiple hosts. It is the vector of several pathogens of human and veterinary concern. In the UK, this tick is the primary vector of *Borrelia burgdorferi sensu lato,* the causative bacterial agent of Lyme borreliosis. In recent years, the number of laboratory confirmed cases of Lyme borreliosis has increased in the UK, potentially due to an increased awareness of the disease or possibly of a change in the distribution or epidemiology of the pathogen.

The aim of this research was to investigate the spatiotemporal activity of this tick and identify key weather and microclimate related variables which influence seasonal increases in its host seeking behaviour.

This was achieved by deploying a comprehensive field monitoring strategy where multiple sites were monitored for *I. ricinus* activity over a number of years. The seasonality of *I. ricinus* activity was analysed alongside localised weather data to determine if specific weather related cues were associated with increases and declines in host seeking behaviour. Additionally, *I. ricinus* specimens collected from different locations during the study were analysed for the presence of *Borrelia burgdorferi sensu lato*.

Peak *I. ricinus* nymphal questing activity in spring was found to be associated with seven consecutive days of mean temperature of ≥7 °C, while an increase in potential evapotranspiration towards 25 mm/week was associated with a decline in host seeking activity following spring peak.

Investigations of *Borrelia burgdorferi sensu lato* infection in *I. ricinus* specimens, confirmed the widespread geographical distribution of this bacterial complex, and the presence of several genospecies of human pathogenic potential.

Chapter 1: The importance of Tick-borne Pathogens, Ixodes ricinus as a vector, and overview of PhD aims.

1.1 An introduction to Tick-borne pathogens: diverse, adaptive and global.

Ticks represent a formidable pathogen vector, second only to the mosquito in terms of global human disease, acting as the main vector of arthropod borne pathogens in the temperate northern hemisphere (de la Fuente *et al*., 2008). From a veterinary perspective, tick borne disease (TBD) can result in severe annual losses in livestock, leading to decreased yield and contributing to massive socio-economic stress for farming communities, particularly in the developing world.

Evolutionary adaptations over millions of years, since at least the upper Cretaceous (90–94 mya) (Klompen & Grimaldi, 2001) have resulted in three distinct tick families (see Table 1.1) and over 900 species worldwide. Many species occupy specific niche environments, completing their entire life cycle in the nest/burrow of a host, or indeed, on a single host. Others are more opportunistic, with the ability to feed on a diverse array of hosts; sometimes feeding on a different host during each phase of its life cycle, as is the case with *Ixodes ricinus*.

The tick's significance as a vector for such a diverse range of pathogens is intrinsically linked to their haematophagous parasitic lifestyle. Some of the key factors in determining their success as a vector include: ***i*** a propensity to feed on the blood of mammalian, avian and reptilian hosts ***ii.*** the tendency of some species to feed on several hosts in one life time ***iii***. relative longevity compared to other arthropods - some species completing their life cycle in less than 1 year (weeks for some) and others taking up to four years ***iv.*** hardiness and the capacity to adapt their behaviour in response to environmental cues ***v.*** the ability to remain attached for long periods of time (perhaps days or more than a week in the case of *Ixodidae*), aided by complex mechanisms to evade host immune responses.

Table 1.1 Described tick families. Adapted from (Mans *et al.*, 2011; Parola & Raoult, 2001).

	Tick Families		
	Ixodidae	*Argasidae*	*Nuttalliellidae*
Distribution	Global	Global	Southern Africa
Described species	703	200	1
Defining characteristics	Presence of a hardened scutum on the dorsal surface of all life stages. Apical facing mouth parts. Prolonged feeding of all life stages – days or weeks.	Absence of scutum; possess leathery integument. Ventrally facing mouth parts. Generally, more rapid feeding-minutes/hours.	Possess a partially hardened pseudo-scutum and leathery integument. Apically facing mouth parts. Feeding habits and life cycle unknown.

As well as competent pathogen vectors, some species of tick also pose a threat to human and veterinary health through toxic paralysis. Tick borne paralysis, of varying degrees, has been reported from 69 tick species globally (Gothe & Neitz, 1991). *Ixodes holocyclus*, commonly referred to as the Australian paralysis tick, is responsible for thousands of cases of tick borne paralysis in companion animals and farm animals each year (Stone & Aylward, 1987). The neurotoxin produced by a single female tick when feeding, is capable of killing a large dog (Stone & Wright, 1981). Human cases do occur, with a number of fatalities reported in the early 20th century (Hall-Mendelin *et al.*, 2011).

More recently, ticks have also been identified as the instigators of complex allergic reactions. Tick bite induced mammalian meat allergy was first reported in 2007 (van Nunen *et al.* 2007); it is associated with a sensitisation to the carbohydrate allergen, galactose-α-1,3-galactose (α-Gal). This oligosaccharide is present in all mammals, with the exception of Old World Monkeys and Apes. It is also present in the gastrointestinal tract of some ticks. Transfer of α-Gal from tick to human can trigger an immune response in humans, leading to α-Gal hypersensitivity - later consumption of mammalian products containing α-Gal has been implicated in (sometimes sever) delayed allergic reactions (Chung *et al.*, 2008; Commins *et al.*, 2009). This allergy has been associated with bites from*; Ixodes ricinus* (Europe), *Ixodes holocyclus* (Australia) and *Amblyomma americanum* (U.S.A) but has also been reported from South Africa, Costa Rica, Korea and Japan (van Nunen, 2015), suggesting the implication of many more tick species.

From an animal health and economic perspective, the damage caused by ticks penetrating the skin can lead to infection, impacting on animal condition and causing direct damage to animal hides (ref), all potentially resulting in health decline in the animal and economic losses for the farmer.

In recent years, the burden of tick borne disease (TBD) appears to be expanding globally, aided in part by; globalisation and the resultant movement of livestock, land use change, climate change and changes in wildlife distribution (Dantas-Torres, 2015; Gray, 2008; Medlock *et al.*, 2013; Ostfeld & Brunner, 2015). Improved epidemiological and diagnostics surveillance have advanced identification of novel or re-emerging pathogens of veterinary and human significance (see Table 1.2), although the actual burden of disease varies greatly depending on the pathogen in question.

Few other vectors can boast the diversity of pathogens transmitted by ticks. Globally, they act as vectors for range of bacterial (including rickettsial), viral and protozoan pathogens. The evolutionary interface shared by ticks and pathogens has resulted in a complex relationship which has been determined in some instances as beneficial to both tick and pathogen (José de la Fuente *et al.*, 2016). For example, studies in *Ixodes scapularis*, have demonstrated that infection with *A. phagocytophilum,* results in the upregulation of certain proteins which promote tick survival in cold environments (Neelakanta *et al.*, 2010). In turn, the suppression of the host inflammation and immune response by compounds in the tick saliva, help facilitate the transfer of the pathogen from tick to host, so called saliva-assisted transmission (SAT) (Kazimírová & Štibrániová, 2013; Nuttall & Labuda, 2004).

1.1.2 Tick-borne viruses

Globally, ticks have been confirmed as vectors of approximately 160 described viruses (Nuttall, 2014), comprising of 38 viral species (Labuda & Nuttall, 2004) across six viral families; *Flaviviridae, Bunyaviridae, Orthomyxoviridae, Rhabdoviridae, Reoviridae,* and *Asfarviridae*. This impressive diversity suggests an ancient co-evolution and multiple, independent events of infection/transfer across several viral lineages (Brackney & Armstrong, 2016). The majority of tick-borne viruses (TBVs), with the exception of African swine fever virus, are RNA viruses and therefore have the capacity to mutate faster, resulting in a more rapid evolution than DNA viruses. The ability of viruses to survive and replicate in tick cells, as well as vertebrate cells, is key to their perpetuity, rooted in their apparent non-detrimental impact on tick cells (Nuttall, 2009).

Even though many TBVs have been identified, the mechanisms by which viruses infiltrate and disseminate in tick cells are still quite poorly understood. The initial process of vertical transmission from host to tick through the uptake of infected host blood results in viral interaction with the mid-gut barrier of the tick. Viral manipulation of gut-epithelial cell proteins and potential interaction with the tick's own gut microbiome may play a role in successful viral dissemination (Narasimhan & Fikrig, 2015). Once the tick-gut barrier has been traversed, the virus can then migrate to other tissues within the tick (Booth *et al.*, 1991). Once viral infection has been established, the tick remains infected throughout its life and so becomes a potential vector, facilitating the distribution of zoonotic infection.

Within tick populations, transovarial transmission is known to occur for several viruses examples (Gonzalez *et al*., 1992; Rehacek, 1962). Additionally, co-feeding, where infection may be transferred directly from tick to tick whilst they are feeding adjacently on the same host, has been demonstrated for some viral infections e,g, tick-borne encephalitis virus (Labuda *et al*., 1997; Randolph, Gern, & Nuttall, 1996). Both scenarios play an important role in the spatial and temporal maintenance of some viruses in tick populations and consequently, are important for the propagation of these viral pathogens in animal and human systems.

Both soft bodied and hard bodied ticks are known to transmit TBVs but those of public and veterinary health concern are generally transmitted by hard bodied ticks, (*Ixodidae*). Examples of TBVs of concern to public and veterinary health include:

Crimean-Congo Haemorrhagic Fever (CCHF): The primary tick vector is the *Hyalomma* genus but direct contact with infected blood or organs also represents a substantial proportion of cases. CCHF causes severe haemorrhagic disease in humans and has a mortality rate of 10-40% (WHO, 2013). The virus is endemic in much of Asia, Africa, the Balkans and the Middle East. It was detected in Spain for the first time in 2010, in *Hyalomma lusitanicum* ticks collected from wild red deer (*Cervus elaphus*) (Estrada-Peña *et al.*, 2012). Subsequently, a tick bite acquired human infection was reported in Spain in 2016 (ECDC, 2016), resulting in fatality; the first such case in Western Europe.

Tick-borne encephalitis virus (TBEV): The main vectors are *Ixodes ricinus* and *Ixodes persulcatus*, but infection can also be acquired from unpasteurized milk products. TBEV is endemic in much of Europe, Russia, China (Wu *et al.*, 2013) and Japan (Takashima, 1998); between 5000 and 12000 cases are reported annually (WHO, 2016). It has been classed as an emergeing disease in parts of northern Europe (Csángó *et al.*, 2004). The virus is divided into Siberian, Far Eastern and European viral subtypes. The large majority of human infections (70- 95%) are believed to be asymptomatic, but this varies with subtype, as do disease symptoms and mortality rate; 1-2% for the European subtype, 8% for the Siberian subtype and over 20% for the Far-Eastern subtype (Gritsun et al., 2003; Pettersson *et al.*, 2014). Interestingly, although one of the primary vectors is abundant in the UK, the virus itself is not endemic.

Kyasanur Forest Disease virus (KFDV): The main vector is *Haemaphysalis spinigera*, but transmission has also been associated with species of *Hyalomma, Ixodes*, *DermacentoR*, *Argas* and *Rhipicephalus* genera. KFDV was first described in 1957 in the Karnataka state of Southern India (Work *et al.*, 1959). Approximately 400 to 500 human cases are reported annually, with associated symptoms ranging from haemorrhagic fever to neurological manifestations; mortality is reported at 3-10% (Shah *et al.* 2018). The virus is endemic in rodent populations and causes mass die-offs in some local simian species - these act as sentinel species, triggering vaccination of all humans in a 5km radius of a positive simian or indeed, human case (**Kiran *et al.*2015**). Initially confined to four states in the south, outbreaks have recently occurred outside of this range; deforestation and human expansion in to once forested habitats are thought to play a role (Sadanandane *et al.*, 2017).

Powassan virus (POWV): The main vectors are *Ixodes cookei*, *Ixodes marxi* and *Ixodes scapularis*. POWV is related to TBEV and West Nile virus but has only been reported in the United States and Russia. In a similar clinical progression to these viruses, it can lead to encephalitis, of which approximately 10% of reported cases are fatal (CDC, 2015). Seventy-five cases have been reported in the Northern and Great Lake region of the United States in the past decade and cases appear to be on the increase (Piantadosi *et al*., 2016). It is uncertain if this increase is due to enhanced awareness or a natural advancement due to ecological factors. Genomic analysis suggest that the US strain emerged from a common ancestor in eastern Russia approximately 500 years ago (Pesko *et al.*, 2010). It is speculated that the virus travelled eastwards into the US with trade in animals furs (Mansfield *et al.*, 2017).

Louping Ill virus (LIV): The main vector is *Ixodes ricinus*. Also related to TBEV, LIV was known to cause severe morbidity and mortality in sheep across the UK and Ireland for many years, isolation of the virus came in 1929 (Greig *et al.*, 1930). A decline in profits from sheep farming has almost certainly lead to decreased surveillance and records of this virus in the UK and Ireland, however its impact on grouse numbers in the north of England and Scotland have resulted in serious economic losses in recent decades (Hudson *et al.*, 1995, Sotherton *et al.*, 2017). LIV is zoonotic but rarely reported in humans, with transmission generally only associated with farming, abattoir and veterinary sectors (Davidson, Williams, & Macleod, 1991). From 1934 to 2014, 45 human cases were reported in the UK (Jeffries *et al*., 2014).

African swine fever virus (ASFV): The primary vectors of this DNA virus are members of the *Ornithodoros* genus of soft ticks (Plowright *et al.*, 1969), in contrast to most TBVs which are transmitted by hard ticks. It presents asymptomatically in bush pigs and warthogs, but is associated with severe haemorrhagic disease in domestic and wild pigs - varying from low to 100% mortality depending on the isolate involved (Blome *et al.*, 2012). It is an excellent example of pathogen species jump, as well as the global transit of a TBV via a wildlife/livestock interface. It was initially endemic in domestic pigs in several African countries, then much of the Iberian Peninsula and Sardinia, eventually spreading to the Americas (Labuda & Nuttall, 2004) through movement of animals. Once infection is established, the virus can be shed in the excrement of domestic pigs, maintaining its transmission cycle in a domestic setting (Guinat *et al.*, 2016).

1.1.3 Protozoan Infections

Protozoan infections are caused by single-celled eukaryotic organisms which infect the blood or tissues of a host. Three closely related genera, *Babesia*, *Theileria* and *Cytauxzoon*, often referred to as piroplasms (Yabsley & Shock, 2013) pose a pathogenic risk to wildlife, companion animals and livestock, some being responsible for huge economic losses in farming globally, specifically in the cattle industry (Pereira *et al.*, 2016).

Babesia: The main vector are *Ixodes* ticks. There are approximately one hundred described species of *Babesia*, some of which cause disease in wildlife, livestock and humans (Homer *et al.*, 2000). The infection is considered emerging in many countries, with more than 50% of cases reported in literature since 1985 (Jongejan & Uilenberg, 2004).

Often referred to as the ‘tick-borne malaria’, due to the similarity in the parasitic process and resulting symptoms, the *Babesia* parasite infects host erythrocytes. Progression through its life cycle causes the erythrocytes to rupture but pathogenicity varies between species (Yabsley & Shock, 2013).

Babesia divergens is endemic in many European cattle populations; indeed, the parasite is recognised as one of the foremost pathogens of cattle globally, causing Redwater Fever (Zintl *et al.*, 2003), and is generally present wherever the vector and bovine hosts are present.

Of further veterinary concern, *Babesia canis* can cause moderate to severe illness in dogs. A cluster of *B. canis* infections in untraveled dogs was reported in the UK in winter of 2015/16, the first such apparent endemic cluster detected in the UK (Hansford *et al.* 2016; Phipps *et al.,* 2016; Wright, 2018). This geographically restricted foci of infection were associated with the *Dermacentor reticulatus* tick; a species found in isolated populations in the south of England, West Wales and Essex, the latter being where this infection was reported (Hansford *et al.* 2018). In addition to *B. canis, several other Babesia* species are associated with canine babesiosis in Europe, including *B. gibsoni, B. vulpes, B vogeli* (Solano-Gallego et al., 2016).

Human cases of babesiosis are rare but when they do occur, they are often associated with severe disease, particularly in immunocompromised patients (Hunfeld *et al.,* 2008; Morch *et al.,* 2015). Sporadic human cases have been reported in Australia, Africa and South America (Tonnetti et al., 2017). The *Babesia* species most associated with human infection in the United States is *Babesia microti*; most infections present asymptomatically but serious disease and mortality of approximately 21% can occur in immunocompromised patients (Carpi et al., 2016). In Europe human cases have been associated with *B. divergens, B. microti* and *B. venatorum (EU1).*

***Theileria*:** Several genera of *Ixodes* ticks, including *Amblyomma, Haemaphysalis, Hyalomma* and *Rhipicephalus* act as vectors for multiple species of *Theileria*. *Theileria* has a complex in host lifecycle which involves infection of host leukocytes with sporozoites. Once infections is established, the organism uses the hosts own immune system to further infiltrate host cells, including erythrocytes (Pérez *et al*,. 2014). Infection can cause illness in wild and domestic bovidae, as well as equids and small ruminants.

Key species which contribute to huge global losses in cattle, include *T. parva* and *T. annulata*. *T. parva (*main vector: *Rhipicephalus appendiculatus)* causes East Coast Fever (ECF), which occurs throughout eastern central and southern Africa, and is another example of agriculture aided pathogen transfer, finding its way from its natural host, the African Cape buffalo (*Syncerus caffer*) to non-native imported cattle (Jongejan and Uilenberg, 2004). The infection is one of the most devastating TBDs of cattle in Sub-Saharan Africa (Minjauw and McLeod, 2003), present in at least twelve countries. Herd mortality can reach 80% in some regions where control measures are hampered by conflict or poverty (Malak *et al.*, 2012; Marcellino *et al.*, 2012).

Advancements have been made in recent years and eradication of the infection in South Africa has been achieved through a strict movement of cattle policy, slaughter of infected cattle and careful wildlife management. A vaccine has also been developed and has been licensed in several African countries (Nene *et al.*, 2016).

T. annulate (main vector: *Hyaloma* genus) causes Tropical theileriosis (TT), also referred to Mediterranean theileriosis causes disease in cattle, domestic and wild water buffalo and yaks across the Mediterranean, northern Africa, the Middle East, India and China. While in most cases, pathogenicity is considered less severe than that of ECF (Morrison, 2015), TT is still capable of causing serious disease and is responsible for considerable economic losses.

T. lestoquardi, also transmitted by *Hyalomma* ticks, is responsible for moderate to severe disease in sheep and goats across the Mediterranean, Middle East and Asia; as are *T. Iowenshuni* and *T. uilenbergi,* transmitted by *Haemaphysalis* ticks, in China (Yin *et al*., 2008).

T. equi, transmitted by several tick genera, primarily, *Dermacentor*, *Rhipicephalus* and *Hyalomma,* causes equine prioplasmosis in domestic and wild equids; the disease also being associated with *Babesia caballi* (OIE, 2009). This infection is widespread, causing disease and loss of equids, globally. Although, not endemic in the UK, it is present in eastern Europe and in many countries from which horses are imported. With the expanding distribution of *Dermacentor* (Hansford *et al.* 2018) and potential importation of *Hyalomma* species (Handford *et al.* 2019), it remains to be seen whether this piroplasm infection will one day establish in the UK.

In addition to the severity of disease, cattle and equids which recover from Theileriosis usually become carries of infection, thereby maintaining the infection in local tick populations (OIE, 2009).

***Cytauxzoon* spp**., following a similar cellular infection progression to other piroplasmida, causes Cytauxzoonosis wild and domestic felines. *Cytauxzoon felis,* transmitted by *Dermacentor variabilis* and *Amblyomma americannum,* is specifically associated with progressive disease and high mortality in domestic cats (Sherrill & Cohn, 2015). It was first reported in the United States, the bobcat (*Lynx rufus*) acting as its natural host and was thought to be confined to the south eastern states; however, it is expanding its range, and has recently been described in Brazil. Other *Cytauxzoon* species have been reported globally, as has associated disease, European species have been detected in native wild cat species and in domestic cats throughout the continent (Carli *et al.,* 2012; Criado-Fornelio, 2004; Nentwig *et al.,* 2018), resulting in varying in pathogenicity (Wang *et al*., 2017). The disease has not been described in the UK and is there is no evidence of association with human disease.

1.1.4 Bacterial Pathogens

Ticks have been recognised as vectors of human bacterial pathogens since the start of the 20th century (Parola & Raoult, 2001). Since then several key bacterial pathogens have been described in both humans and livestock, maintained by wildlife hosts. In addition, bacterial-like rickettsial pathogens are generally associated with ticks, mites, lice and fleas; the agent being passed to humans through the saliva or faeces of the parasite (Nicholson *et al., 2017*). With the onset of advanced sequencing methods, many new species of rickettsia have been identified in recent years. As of 2013, 26 novel species had been reported globally (Parola et al., 2013), within which, multiply sub-species have been proposed (Nieri-Bastos *et al*., 2018).

Rocky Mountain Spotted Fever (RMSF): Main vectors include various Ixodidae species but particularly *Dermacentor variabilis* and *Dermacentor andersoni,* and more recently *Rhipicephalus sanguineus* (Demma *et al.,* 2005). In the US and Canada it is associated with *R. rickettsia* and is the most common tick-borne rickettsial infection associated with humans (Dantas-Torres, 2007). It is thought to be maintained and amplified in nature by small mammals such as ground squirrels (*Spermophilus* spp.) and rabbits (*Sylvilagus* spp.) (Niebylski *et al.,*1999) and is maintained in the tick population by transovarial and transstadial transmission.

Mediterranean Spotted Fever (MSF): Main vector is *Rhipicephalus sanguineus,* has been well documented in Europe, as well as in Africa, India and parts of the Middle East, since the early 20th century. It is caused by *Rickettsia conorii* and is considered endemic in the Mediterranean region. While domestic dogs are a common host of *Rh. sanguineus*, it is believed that they are only a transient reservoir of the pathogen (Rovery, Brouqui, & Raoult, 2008). The disease itself is considered relatively benign but severe cardiac, neurologic and renal complication and occasionally fatality has been reported (Rossio *et al*., 2015). In addition to MSF, at least five novel spotted fever rickettsiae have been described in Europe over the past decade: *Rickettsia helvetica, Rickettsia monacensis, Rickettsia massiliae, Rickettsia raoultii and Rickettsia sibirica mongolitimonae* (Kuscu *et al*., 2017) have been identified in various *Ixodes* ticks (including *I. ricinus*), and have been associated with mostly mild MSF-like illness (Portillo *et al.*, 2015). It has been suggested that migratory passerine_birds could play a role in the maintenance and dispersal of these various rickettsial agents of human concern across Europe (Elfving *et al*., 2010).

The significance of tick-borne rickettsial disease in humans in the UK is unknown but widespread incidence of *R. helvetica* and *R. raoultii* was reported for the first time in *Ixodes ricinus* and *Dermacentor reticulatus* ticks in 2011(Tijsse-Klasen *et al.*, 2011), suggesting the potential for Spotted fever type infections in the UK.

Table 1.2 Tick borne pathogens of human and veterinary concern found in Europe. Adapted from (Dantas-Torres *et al*., 2012), Socolovschi *et al*., 2009, Deardorff *et al*., 2013).

Tick-borne Pathogens of Human and Veterinary Health Significance detected in Europe – Some Key Examples

Disease	Pathogen	Primary Vectors	Distribution
Bacterial Agents			
Human granulocytic anaplasmosis	*Anaplasma phagocytophilum*	*Haemaphysalis concinna,* *Haemaphysalis punctata,* *Ixodes ricinus,* *Rhipicephalus bursa*	Mainland Europe, Scandinavia, Ireland,UK
Lyme borreliosis	*Borrelia burgdorferi s.l.*	*Ixodes ricinus,* *Ixodes persulcatus,*	Mainland Europe, Ireland, Scandinavia, UK
Mediterranean spotted fever	*Rickettsia conorii*	*Rhipicephalus sanguineus,* *Rhipicephalus turanicus*	Europe
Protozoan Agent			
Babesiosis	*Babesia divergens,* *B. microti*	*Ixodes ricinus,* *Dermacentor reticulatus*	Europe, Scandinavia, UK
Viral Agent			
Crimean–Congo haemorrhagic fever	*Nairovirus*	*Genus Hyalomma*	Mainland Europe
Tick-borne encephalitis	*Flavivirus*	*Ixodes persulcatus, Ixodes ricinus, Haemaphysalis concinna, Haemaphysalis punctata*	Mainland Europe, Scandinavia
Louping ill	*Flavivirus*	*Ixodes ricinus*	Ireland, UK

Anaplasma phagocytophilum is considered an emerging infection in humans, first reported in the mid-1990s in the mid-western United States (Chen *et al.*, 1994), having being initially described as a pathogen of cattle, goats and sheep in Europe (Stuen, 2007). It has since been reported in humans in Canada (Parkins *et al.*, 2009) and across Europe (Thomas *et al.*, 2009). The bacterium is maintained in wildlife and serological investigations suggest that it has a complex distribution of reservoir hosts (Foley *et al.*, 2008). *Ixodes* ticks maintain the cycle of transmission, *Ixodes scapularis* acting as a primary tick vector in the eastern US and *Ixodes pacificus* in the western US. *I. ricinus* acts as the main vector in Europe (Sonenshine and Roe, 2014), likewise in the UK, although here, there is evidence that *I. trianguliceps* is involved in maintaining endemic infection in certain wildlife hosts (Bown *et al.*, 2006).

The infective agent invades and colonises host neutrophils and can result in human granulocytic anaplasmosis (HGA). Initial symptoms are quite non-descript; cough, fever, chills, muscle pain and infection may resolve itself in healthy patients. However, renal and neurological complications can manifest and death can occur, particularly in immunocompromised patients (Ismail, Bloch, & McBride, 2010). Humans cases in Europe are described as infrequent but are likely under reported (Medlock *et al.*, 2018); in the US, incidence rates have increased since the disease became notifiable in 1999, with approximately 6000 cases reported in 2017 (CDC, 2019)

Several other species of *Anaplasma* impact on veterinary health throughout the world; *Anaplasma marginale,* causing bovine anaplasmosis, is responsible for economic losses in the cattle industry in Africa, Asia, Australia, South and Central America, southern Europe, and the USA (Yang et al., 2017). At least 20 species of tick, primarily from genera *Dermacentor* and *Rhipicephalus* have been identified as vectors of *A. marginale* (Rar & Golovljova, 2011).

Likewise, *Anaplasma ovis, is* responsible for losses in sheep and goats, the main cause of ovis and caprine anaplasmosis in tropical and subtropical countries. In recent years the zoonotic potential of *A. ovis* was raised when a variant was isolated in a patient in Cyprus (Chochlakis *et al.*, 2010)

Candidatus Neoehrlichia mikurensis is considered an emerging bacterial infection in humans, causing sever systemic inflammatory syndrome in immunocompromised patients. It has been isolated from febrile patients in both Europe (Sanogo et al., 2003) and Asia (Li et al., 2012), detected in rodents in South Korea (Jha et al., 2018), Japan (Kawahara et al., 2004), and has been detected in multiple European rodent and mammal species (Portillo A. *et al,* 2018). In Europe the pathogen has been isolated from questing *I. ricinus,* thought to be its primary vector but has also been detected in feeding *I. hexagonus, I. trianguliceps and I. frontalis* (Blaňarová *et al*., 2016; Jahfari *et al.*, 2017; Krücken *et al.*, 2013; Movila *et al,* 2013) ticks from across Europe. Several studies have been conducted but Ca. N. mikurensis has yet to be detected in UK ticks.

Due to its relatively recent classification as a human pathogen in 2010 (Welinder-Olsson *et al.*, 2010), and unsuccessful attempts at culturing, a comprehensive understanding of the mechanism of pathogenesis or epidemiology remains unclear.

1.1.5 *Borrelia burgdorferi sensu lato*

The *Borrelia* genus contains spirochetes transmitted by ticks or lice and can be classified into two major groups **-** the relapsing fever group and the *Borrelia burgdorferi sensu lato (s.l.)* complex. In addition, several intermittent clades exist which seem to sit outside both groups. With advancements in genomic technology, the structure of these groups is currently under debate (Barbour *et al.* 2017; Margos *et al.* 2018). *Borrelia burgdorferi s.l.* is a globally diverse complex *of spirochete bacteria*, *some of which are* associated with Lyme borreliosis (also known as Lyme disease). To date, human Lyme borreliosis has been diagnosed in more than eighty countries (Scott et al., 2017a). The genospecies contained within this group are closely related to each other but are associated, to some extent, with different host species, and for pathogenic genospecies, different clinical presentations. The majority of human Lyme borreliosis being attributed to just three genospecies (van Dam *et al.,* 1993).

The first genospecies linked to human disease was in late 1970s - a cluster of arthritis cases in children in Lyme, Connecticut were eventually tied to exposure to the *Ixodes scapularus* tick. The pathogen in question was named *Borrelia burgdorferi* (Burgdorfer *et al.*, 1982) after the researcher who identified it, Wilhelm "Willy" Burgdorfer. Since then at least twenty three genospecies and genomospecies have been identified (see Table 3), all transmitted by *Ixodes* ticks (Scott *et al.*, 2017).

B. burgdorferi sensu stricto (s.s) remains the genospecies most associated with human disease in the United States and Canada but is also associated, to a lesser extent, with human infection in Europe. *Borrelia afzelii* and *Borrelia garinii* account for most of the human cases in Europe and the UK. In recent years however, a newly described genospecies, *B. mayonii* has been confirmed as the cause of human disease in the United States (Pritt *et al.,* 2016), while several others have been isolated from a small number or single Lyme borreliosis patients in Europe; *Borrelia bissettii, Borrelia lusitaniae*, *Borrelia spielmanii* and *Borrelia valaisiana* (Kurtenbach et al., 1998) (Collares-Pereira *et al.,* 2004; Diza, *et al.,* 2004; de Carvalho, *et al.,* 2008; Rudenko, *et al.,* 2009). These genotypes remain in the minority of diagnosed cases of Lyme borreliosis and there remains debate on their role in human illness (Margos et al., 2017). However, as laboratory genomic capabilities continue to advance and clinical diagnosis abilities improve, there is little doubt that additional human disease causing genotypes will be described.

Table 1.3 *Borrelia* genospecies and distribution. *Indicates confirmed association with human infection. ** Isolated from single cases of Lyme borreliosis. Adapted from: (Casjens *et al*., 2011; Kingry *et al*., 2016; Mannelli *et al*., 2012; Stanek & Reiter, 2011)

Borrelia Genospecies Distribution		
Eurasia	**Americas**	**Both New and Old World**
*B. afzelii**	*B. americana*	*B. burgdorferi sensu stricto(s.s.) **
*B. bavariensis**	*B. andersonii*	*B. bissettii ***
B. japonica	*B. californiensis*	*B. carolinensis*
B. lusitaniae	*B. kurtenbachii*	
B. sinica	*B. mayonii**	
*B. spielmanii***		
B. tanukii		
B. turdi		
*B.valaisiana***		
B. yangtze		
*B. garinii**		
B. finlandensis		

Borrelia spirochetes are maintained in wildlife systems, many genospecies adapting to infect a specific host (e.g. *Borrelia spielmanii* in the dormouse (*Muscardinus*)) or host group (e.g. *B. garinii* in birds (Richter *et al*, 2011)). The spirochetes are transferred with a blood meal when the tick feeds on an infected host. Once spirochetes are ingested by the tick they migrate to the gut of the tick and remain there, aided by the presence of two outer surface proteins (OspA and OspB), which facilitate their ability to attach and survive in the tick gut epithelia cells (Neelakanta *et al*., 2007). When the tick feeds, the prolonged ingestion of blood causes a cascade of changes in the regulation of the spirochete surface proteins - amongst them, OspA and OspB are down regulated and OspC is upregulated (Schwan *et al.*, 1995). OspC is believed to be involved in migration of the bacteria to the tick salivary gland and has been demonstrated to display antiphagocytic properties which aide in evading the host immune defences (Carrasco *et al.*,2015). This host immunity avoidance tactic is one of many which allow the bacteria to infiltrate host cells.

In regions where multiple genospecies circulate in the wildlife population, several may be present simultaneously in infected ticks. In recent years it has become apparent that a wide diversity of subtypes within many *Borrelia* genospecies also exists, further complicating the epidemiology of this this bacterial complex (Rudenko *et al.*, 2011).

Table 1.4 Confirmed human pathogenic *Borrelia* genospecies known to circulate in the UK.

Genospecies	**Associated reservoir host**	**Associated symptoms**
B. burgdorferi senu stricto (s.s)	Both rodents and birds	Arthritis
B. afzelii	Rodents – various	Skin manifestations e.g. acrodermatitis chronica atrophicans
B. garinii	Terrestrial and sea birds	Neuroborreliosis

1.2.1 Lyme borreliosis

Different genospecies of *Borrelia* are associated with different clinical presentations in humans (see Table 1.4) and onset of symptoms can vary from within two days to one month of a tick bite. The pathogenicity and progression of disease seem to depend on a variety of factors, with some seropositive patients apparently showing no sign of disease and others displaying a range of symptoms (G. Wang *et al.*, 2002;

Wormser *et al.,* 2008). Disease progression can start with early localised infection at the bite site, sometimes represented by an erythema_migrans (EM) rash. This inflammatory response to the bacterial spirochetes migration from the bite occurs in some but not all cases (Schutzer *et al.,* 2013). As the infection progresses, early to mid-stage manifestations may include nonspecific symptoms, such as, fatigue, stiffness, fever and muscle pain. In later dissemination of the bacteria through the body, more system or organ specific manifestations may occur. Neuro-borreliosis, which represents 10-15% of Lyme patients (Halperin, 2008, Zajkowska, 2007) can present as, meningoradiculitis, encephalomyelitis (Sindic *et al*., 1987), facial palsy (Clark et al., 1985), meningitis (Pachner & Steere, 1985) and in rare occasions, stroke (Almoussa *et al.,* 2015). Further late stage manifestations can include Lyme Carditis (Steere *et al.,* 1980) which presents in 0.3 to 4% of untreated adult Lyme patients in Europe and 1.5 to 10% in the United States (Scheffold *et al.*, 2015). Lyme arthritis is associated more frequently with *Borrelia burgdorferi s.s.* infection and can range from occasional bouts of joint pain, to prolonged joint arthritis, to presentation of chronic erosive arthritis (Steere *et al.,* 1987).

With such a myriad of potential symptoms and with many Lyme borreliosis confirmed patients failing to report a tick bite, diagnosis can be extremely difficult, further hampered by a lack of diagnostic tools (D'Alessandro *et al.*, 2017). In cases where diagnosis is confirmed, it is usually done so with a combination of clinical presentation, patient history (i.e. likely exposure to tick bite) and serological evidence (Scheffold *et al.*, 2015). Early stage antibiotic treatment is recommended on initial diagnosis and usually clears infection (Wormser *et al.,* 2003, Wormer *et al.,* 2006) without further complications. However, late or missed diagnosis can result in an established infection, potentially resulting in a range of complex symptoms, the severity of which may depend on many factors, such as potential co-infection with other pathogens, the immune system status of the patient and the genospecies involved in infection.

Lyme borreliosis is the most common tick borne disease in Europe, with approximately 85000 cases reported annually (ECDC, 2015). However, vast variation in incidence rates exists between countries; ranging from 312/100,000 in Slovenia to 0.001in Italy (Sykes & Makiello 2017). Since the introduction of enhanced Lyme borreliosis surveillance in the UK in 1997, almost 7000 laboratory confirmed cases have been identified. Mean cases per 100,000 of population have increase steadily during this period, from 0.38 per 100,000 of total population in 1997-2001, to 2.2 per 100,000 per total population in 2015 (PHE Zoonoses UK Report 2015). This increase probably represents an artefact of increased awareness and surveillance rather than an increase in the actual prevalence of the disease. However, it is estimated 1,000 – 2,000 cases go unreported in England and Wales each year due to diagnosis and treatment based on the clinical presentations alone.

1.2.2 *Ixodes ricinus* as a vector of *Borrelia burgdorferi* s.*l.*

The success of this expansive bacterial complex partially lies in its ability to infect and replicate in many reservoir host species, including rodents, mammals and birds. However, its association with some of the most prevalent and opportunistic of *Ixodes* tick vectors ensures the spatial and temporal maintenance in the environment. The hard bodied tick *Ixodes ricinus* has long been recognised as the vector of many pathogens of veterinary and medical importance, including *Borrelia* (Jongejan & Uilenberg, 2004). This truly opportunistic tick is known to feed on approximately 300 different animal species (Anderson, 1991). Generalist feeding habits, free living life stages and a wide distribution has contributed to *I. ricinus* being the primary vector of *Borrelia burgdorferi s.l.* in Europe.

A number of key wildlife hosts play a role in the maintenance of *Borrelia* in the UK, some acting as amplification hosts (Mannelli *et al.*, 2012). Song birds are known to play an important role in maintenance and distribution (Heylen, 2016) – reflected by the often high prevalence of *B. garinii* compared to other genotypes (Saint-Girons *et al*., 1998). Ground foraging birds are seen as particularly important; Common Blackbirds (*Turdus merula*), Song Thrushes (*Turdus philomelos*), Dunnocks (*Prunella modularis*), European Greenfinches (*Carduelis chloris*) and Chaffinches (*Fringilla coelebs*) have been shown to carry large numbers of larval and nymphal ticks, as well as harbouring high rates of *B. garinii* infection (James *et al.*, 2011). Terrestrial species

Other ticks are capable of transmitting and may indeed play a role in the maintenance of *Borrelia* in wildlife systems. *Ixodes uriae*, for example, has been established as a vector of *Borrelia* in seabird colonies. This species generally completes its entire life cycle in the nest of its avian host so is less likely to come into contact with humans, except perhaps with hunters and ornithologists. However, this species certainly contributes to the migration of the pathogen globally (Olsen *et al.*, 1995). Likewise, it has been suggested that *Ixodes hexagonus* (hedgehog tick) may play an important role in maintaining the pathogen in UK wildlife populations (Jahfari *et al.*., 2017). However, once again, due to the nidicolous life cycle of this tick (confined primarily in the nests of hedgehogs), its importance in the human transmission cycle is thought to be minimal.

The key to *I. ricinus* success as a *Borrelia* vector seems to be greatly influenced by its ecology, life cycle and multi-host feeding habits.

1.3.1 *Ixodes ricinus* life-cycle

I. ricinus is a three-host tick, whose life cycle consists of four stages; egg, larval stage, nymphal stage and adult. This life cycle can take between 1.5 and 4.5 years to complete (Milne, 1949). The length of time taken to complete the entire cycle is dependent on the time of year when the larval and nymphal stage moult and when the adults emerge from their final moult, coupled with each stage's success in finding a suitable host. Laboratory studies have demonstrated the ability of unfed larval and nymphal *I. ricinus* to feed 13-15 months after fasting. The same study, revelled that unfed females and males survived for at least 21 months under laboratory conditions (Macleod, 1932).

Feeding time for each life stage varies - once hatched, the larvae, which are generally confined to the hatching site, seek a small host, usually a small mammal or bird. The larva feeds for 3 to 5 days, when fully engorged it will detach and retreat to the vegetation undergrowth in preparation for moulting into the nymphal stage. When suitable environmental conditions prevail, the nymph emerges to seek a blood meal. Being slightly more robust that the larva, the nymph has the ability climb further up vegetation and so will likely attach to larger hosts e.g. foraging squirrels, deer, pheasants, dogs. If it is successful in attaching to a host, it will feed for 4 to 6 days. On detachment, the nymph will also retreat to the moist undergrowth where it will enter a developmental phase, emerging as an adult female or male.

Once emerged, adults also have the ability to climb high vegetation if necessary, so will tend to encounter and as a result feed on larger hosts. If it is successful in finding a host, the male will feed intermittently whilst focusing on its primary objective of finding and mating with a female, usually found on the same host. There is evidence to suggest that post-mating males will aide female feeding by attaching close to the site where the female is attached and releasing immunomodulatory saliva proteins, allowing the female to feed more effectively (Wang *et al.*, 1998, Rechav and Nuttall, 2000). The adult female will feed for 7 to 11 days, obtaining nutrients to nourish developing eggs (if mated). Once fully engorged, the female detaches and retreats to the undergrowth, here she will begin to digest this huge blood meal and oocyte maturation will begin (Bowman and Nuttall, 2008). She will seek a suitable moist habitat to deposit her (up to 2000) eggs (Milne, 1944) before exhausting all energy reserves and die.

These brief interactions with hosts account for only a small proportion of *I. ricinus* interaction with its surrounding environment. MacLeod (1939) noted that “the tick spends only some 18 days in the parasitic phase out of a total life-span of some 500-1,500 days”. The majority of its time is actually spent in either: developmental periods of behavioural diapause which are hormonally regulated (Gray *et al.*, 2016), periods of so called ‘quiescence’ (Lees and Milne,1951) which is influenced by environmental factors, or in host seeking periods, known as ‘questing’, which are also greatly influenced by environmental factors (Milne, 1950).

During periods of diapause or quiescence, *I. ricinus* retreats to the vegetation mat layer, while host seeking usually occurs in the upper vegetation layer (for nymphs and adults especially). The questing process involves the tick climbing into the upper vegetation, here it outstretches its front legs, on which the sensory Haller’s organ is located, and waits for a host to brush past. If it manages to latch onto a host, *I. ricinus* will attempt to attach; using its serrated hypostome to pierce the skin of its host and anchor itself in place so that feeding can commence. It will remain attached until feeding is complete; in some circumstances where detachment has occurred before feeding is complete, for example, if blood flow has stopped or the host has manually removed the tick, *I. ricinus* will seek another host in order to complete its blood meal.

The triggers for these retreat or host seeking periods are a complex combination of biotic and abiotic factors. Investigating these cues may allow for predictions of when *I. ricinus* is at its most active, how seasonal interactions with hosts might influence pathogen dynamics, and how changing environmental factors such as climate and vegetation cover might influence *I. ricinus* distribution

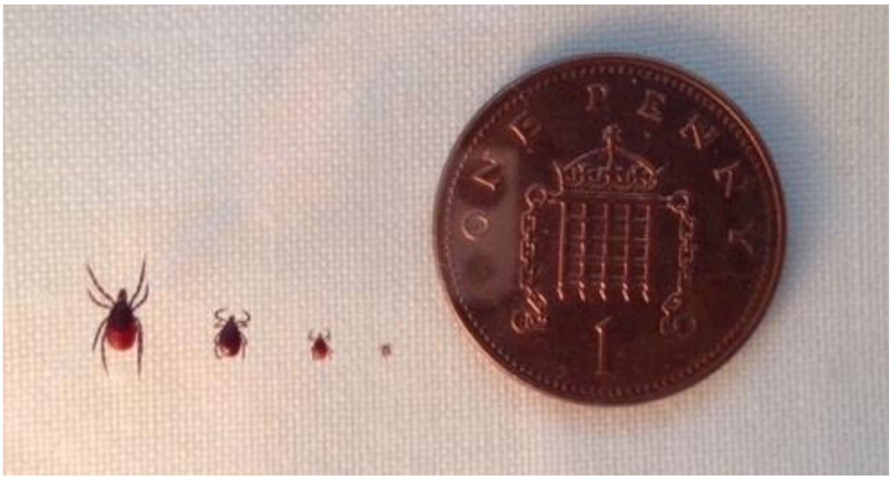


Fig 1.1 Unfed *Ixodes ricinus* (l to r): female, male, nymph, larva. (L. McGinley, 2015)

1.3.2 Role of humidity in *I. ricinus* survival and behaviour

Questing for a host represents a significant risk of desiccation for *I. ricinus.* As a member of the Ixodidae family of ticks, they are distinguished by the presence of a sclerotized, protective scutum on their dorsal surface which is relatively impermeable to water. However, even compared to other species of the same family, *I. ricinus* seems particularly sensitive to water loss - losing water 10 to 15 times more rapidly than *Dermacentor andersoni* in controlled studies (Lees, 1946). Further studies have demonstrated that dehydrated *I. ricinus* will not drink free standing water when offered (Kahl & Alidousti, 1997) and that osmoregulation is in fact primarily controlled by the salivary glands (Rudolph & Knulle 1974). The maintenance of water balance in *I. ricinus* is not only important during challenging periods of environmental exposure but also when ingesting large volumes of blood over prolonged periods during feeding (Knulle & Rudolph, 2013).

The salivary glands allow *I. ricinus* to absorb water vapour directly from the atmosphere when certain conditions prevail i.e. when they are dehydrated to a certain critical level - the critical equilibrium humidity (CEH), and when the relative humidity of the atmosphere exceeds a certain critical value (Kaufman, 2010). MacLeod (1939) approximated that this critical value for the unfed *I. ricinus* tick to be > 80% and 85% for the engorged tick. However, the energy reserves of the tick may impact on this critical level, with ticks which have been actively questing for prolonged periods (20-30 days), only obtaining homeostasis in water saturated air (Lees, 1946). The longer it takes to find a host, the more times *I. ricinus* will have to descend and re-ascend the vegetation – this process in itself leading to further loss of moisture through respiratory loss (Rudolph & Knülle,1979).

This extreme sensitivity to moisture loss and constant struggle to maintain water balance naturally impacts on the behaviour of *I. ricinus.* The urge to find a host will compel *I. ricinus* to leave the moisture rich vegetation mat layer but the role of temperature in desiccation will drive it back to this moisture rich sanctuary.

1.3.3 Role of temperature in the survival and behaviour of *I. ricinus*

Temperature has long been recognised as a primary influence on *I. ricinus* activity, not only in its activity but also in limiting the tick's geographic range (Gray *et al.,* 2009). Low temperatures towards freezing point appear to instigate an environmental diapause, stemming from an inability to complete metabolic tasks. High temperatures approaching 25°C increase the drying power of the surrounding air (the saturation deficit) initiating a cessation of questing activity (Perret *et al.,* 2000; Randolph &

Storey, 1999). While changes in winter day length undoubtedly trigger diapause in *I. ricinus,* it is thought that increased day length alone is not enough to initiate increased questing activity and must also be accompanied by an increase in temperature (Randolph, 2004)

Various studies have indicated approximations to threshold questing temperature for *I. ricinus* - weekly mean daily maximum temperature above 7°C has been demonstrated as significant for the initiation of questing in nymphs and adults (Perret *et al.,* 2000), whereas a weekly mean daily maximum of 10°C has been demonstrated for larvae (Randolph, 2004). However local tick populations have been shown to demonstrate adaptations to temperature. Gilbert and colleagues (2014) demonstrated that *I. ricinus* from colder climates tended to quest at cooler temperatures while the reverse was true for specimens from warmer climate. This apparent adaptation to local climate suggests that *I. ricinus* has the potential to adapt to changing climate. Indeed, rising temperatures may have a knock on effect on altering the distribution of vegetation and wildlife, which in turn potentially creates new habitats for ticks (Medlock *et al.,* 2012).

1.3.4 Role of photoperiod on *I. ricinus* behaviour.

All life stages of *I. ricinus* possess photoreceptor cells which run along the side of the coxa (Perret, 2003). Axons connecting these cells form the optic nerve and are believed to be involved in the processing of light and shade (Belozerov, 1982). The ability to differentiate between shade and light allows the tick to not only sense changes in day length but also in the detection of shadows from passing host (Lees, 1951). Perret and colleagues demonstrated that a decline in light levels was enough to initiate movement in *I. ricinus* and did not necessarily have to be accompanied by changes in relative humidity or temperature (Perret, 2003). This study showed that most walking was undertaken by *I. ricinus* during darkness, nymphs walking up to approximately 10m at night and suggested that this nocturnal activity may increase the chances of finding a host due to an increase of rodents and small mammals at night.

Photoperiod itself has been shown to have a regulatory influence on the transition of

I. ricinus (and other *Ixodes* spp.) through its life stages (Belozerov, 2002). Simulated lab experiments have also demonstrated that incremental decreases in ‘day length’ have an influence on *I. ricinus*, which enters diapause at approximately 13 hours of day length (Sonenshine &Roe, 2014). Such experimental results suggest that photoperiod also plays a critical role in the onset of diapause (Gray *et al.,* 2016b).

1.3.5 Influence of ground cover and vegetation on *I. ricinus*

The dependency of *I. ricinus* on a moisture rich habitat for its survival is reflected in the landscapes it tends to inhabit. Milne (1944) observed that the geological composition of landscape (of Northern England) and its capacity for natural drainage impacted to some extent on tick abundance, whereas soil type and pH did not seem to correlate with tick distribution. Through these studies, Milne categorised habitats, surveyed vegetation types and sampled tick distribution accordingly. He concluded that tick “density distribution is correlated to the thickness or 'roughness' of the vegetation layer”, more specifically he associated the thickness of the mat layer i.e. the layer of decaying vegetation found next to the soil, with a denser population of ticks. He referred to this mat layer as the “prime controlling factor” in tick distribution and linked this observation back to MacLeod's (1934) laboratory findings on the importance of humidity to tick survival, stating that “it is the perennial dampness under a rough vegetation layer which is the controlling (tick) factor”. More recent studies have also pointed to the importance of geological, as well as topographical features of the landscape(Medlock *et al.,* 2008).

I. ricinus is found throughout the UK in a range of habitats but is predominately associated with moorland, heathland, grassland, rough upland (Pietzsch et al., 2005a) and woodland – woodland, generally thought to be of particular importance (Medlock *et al.*, 2012; Tack *et al.*, 2012).This distribution is not accidental and is intrinsically linked to the moisture rich, insulated ground layer associated with these habitats. Woodland habitats may also support a more diverse host distribution (Dobson & Randolph, 2011; Medlock *et al.,* 2012). The vegetation layer propagates and maintains a relatively stable microclimate, rich in moisture, shaded from the sun and offers protection from extremes in temperature (Randolph & Storey, 1999). Therefore, changes in vegetation structure and distribution can greatly influence the abundance of *I. ricinus,* both by altering the host composition of the landscape and the microclimate

1.3.6 *Ixodes ricinus* - host dynamics

Due to their limited lateral movements, *I. ricinus* dispersal and, to a large extent survival and reproductive capacity is determined by host availability (Lees, 1948; Milne, 1948). The larvae in particular do not tend to move horizontally over large distances and often remain close to where they have emerged from the egg (Medlock *et al.,* 2013). They are therefore often found in large clusters, lying in wait for a suitable host, usually a small mammal, rodent or bird, although larvae will attach to any viable host animal. Species such as the Yellow-necked mouse (*Apodemus flavicollis*), Wood mouse (*Apodemus sylvaticus*) and bank vole (*Myodes glareolus)* have been shown to support large numbers of larval *I. ricinus* in UK woodland habitats (Cull *et al.*, 2017). Lagomorphs, such as the European Hare (*Lepus europaeus*), Mountain Hare (*Lepus timidus)* and rabbit *(Oryctolagus cuniculus)* play an important role in maintaining adult and juvenile *I. ricinus* populations (Tallekint & Jaenson 1993), whilst also maintaining the *Borrelia* infection cycle (Rizzoli *et al.,* 2014).

Larger mammals and often, humans act as the primary hosts for nymphs, as this life stage tends to quest higher on the vegetation than larvae, as such they may bypass hosts confined to the lower vegetation levels (Craine *et al.,* 1995). Because of their small size and therefore the ability to go undetected on the host, nymphs in particular are believed to be a key vector for *B. burgdorferi* in humans (Jaenson et al., 2009). Adult ticks are often associated with larger mammals and grazers which have the body mass to support them. Historically, sheep played a key role in the maintenance and distribution of *I. ricinus* in the UK, particularly in upland and agricultural areas. Much research was carried out in the earlier part of the 20th century on the host potential of sheep, due mainly to issues with livestock disease such as Louping Ill. Milne (1948) noted that in rough hill or moorland, sheep were the primary tick host, estimating that over 90% of female ticks were supported by sheep flocks in a given area. However, this dynamic may have changed in recent years, due to changes in farming methods, land management and the use of acaricides.

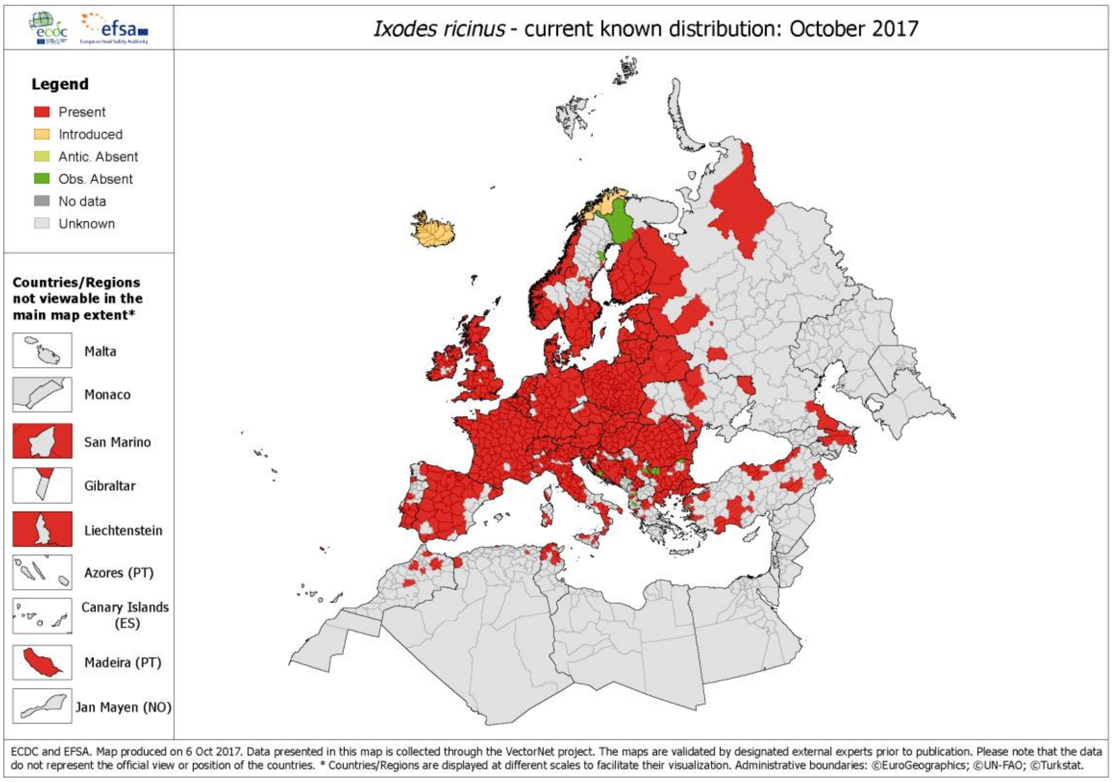
Deer are considered an important host for *I. ricinus* - increased deer populations throughout the US (Ogden *et al.*, 2005), Europe (Jaenson & Lindgren, 2011) and the UK (Dobson & Randolph, 2011b) have been associated with changes in tick distribution. In Sweden, an increase in roe deer numbers, coupled with generally milder winters (Lindgren *et al.,* 2000) was linked to the northward expansion of *I. ricinus* (Jaenson *et al.,* 2012), bringing TBEV with it. In the UK, red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) are estimated to be at their highest numbers since the Mesolithic period and have expanded into ranges where they were not previously recorded (Ward, 2005). Additionally, introduced species such as Chinese Water Deer (*Hydropotes inermis),* Fallow Deer (*Dama dama*), Muntjac Deer (*Muntiacini*) and Sika Deer (*Cervus nippon*) are also expanding their range in the UK (The Great British Deer Survey 2016) and offer a new population of grazers on which ticks can feed.

In terms of *Borrelia* infection, deer themselves are not considered competent hosts for *B. burgdorferi* (Kurtenbach *et al.,* 2002); however they do play a key, yet complex role in *I. ricinus* distribution (Medlock *et al.,* 2013). On the one hand, these large mammals offer greater feeding opportunities for *I. ricinus* and so reduce tick questing duration times, leading to a decreased potential for human/tick interaction. Deer may also serve as a 'mop' for *Borrelia* infection – causing a dilution effect and disrupting the *Borrelia* transmission cycle, although studies into this theory have resulted in contradictory findings (Gray *et al.*, 1992, Rizzoli *et al.,* 2002, James *et al.*, 2013, Werden *et al.,* 2014). It is the case however that more large mammals provide ample feeding opportunities for a greater numbers of ticks, especially adult ticks, facilitating a progression through the ticks life cycle (Scharlemann *et al.,* 2008) whilst also providing a method of range dispersal (Medlock, *et al.,* 2013)

1.3.7 Changes in the seasonality and distribution of *I. ricinus*

The influence of microclimate, weather and host distribution on *I.ricinus* populations and pathogen distribution has been emphasized in recent years, with notable shifts recorded in Europe and Scandinavia. Changes in the northerly limit of *I. ricinus* in Sweden have been attributed to a warming climate (Jaenson & Lindgren, 2011), coupled with a dramatic rise in deer numbers attributed in part, to decline of deer predators (Lindstorm *et al.,* 1994). This shift in the tick vector has been correlated with a steady increase in TBEV cases in Sweden over the past two decades (Jaenson *et al.,* 2012).

A four-year study conducted in the Czech Republic revealed a shift in *I. ricinus* upper distribution limit, from 700-800m to 1100m above sea level (m.a.s.l.) in a 20-year period (Materna *et al.,* 2005). Changes in altitude distribution have also been observed in Bosnia and Herzegovina, with *I. ricinus* distribution shifting from 800m.a.s.l. (or less) in the 1950s to 1190m.a.s.l. in 2010 (Omeragic, 2011). In Slovakia too, an increase in altitude range of *I. ricinus* has been observed, from 800m.a.s.l. to approximately 1200m.a.s.l. (Medlock *et al..,* 2013).



ECDC and EFSA. Map produced on 6 Oct 2017. Data presented in this map is collected through the VectorNet project. The maps are validated by designated external experts prior to publication. Please note that the data do not represent the official view or position of the countries. * Countries/Regions are displayed at different scales to facilitate their visualization. Administrative boundaries: ©EuroGeographics; ©UN-FAO; ©Turkstat.

Fig 1.2 Map of current *I. ricinus* distribution in Europe. ECDC,2017.

This expansion has not just been limited to *I. ricinus* – similar advancements have been recorded in *Dermacentor reticulatus* ticks. In Slovakia, an altitudinal shift in *D. reticulatus* has been linked to an overall 1°C increase in mean air temperature between 1980 and 2004 (Bullova, 2009), as well as changes in land use. In the United States, changes in the abundance, distribution and seasonal activity of *Ixodes scapularis* and *Ixodes pacificus* (vectors for *Borrelia burgdorferi* and other pathogens) have been linked to changes in land use, host abundance, weather and climate (Eisen *et al.,*2016). Such changes have been suggested as the primary driving force behind the expansion beyond endemic ranges of several TBDs in recent decades (Laaksonen *et al.,* 2017; Lovrich *et al.,* 2011; Kugeler *et al.,* 2015). The emergence of Lyme borreliosis in Canada has been attributed to the expansion of *I. scapularis* from one single geographic area (Long Point, Ontario on the Lake Erie shoreline) (Barker *et al.,* 1992) to more densely inhabited eastern and central provinces (Ogden *et al*., 2009). The expansion of this tick vector is also thought to be linked, in part, to warming climate (Ogden *et al.,* 2014).

1.4.1 Future directions: the aims of this PhD research

Changes in climate, land use and host distribution can clearly have an impact on tick populations and their associated pathogens. Several researchers have emphasised the need for further study on these driving factors, emphasising specifically, research gaps in; ***i.*** seasonal dynamics of *I. ricinus*; ***ii.*** drivers of 'questing' initiation in *I. ricinus;* ***iii.*** the impacts of changing climate on *I. ricinus* seasonality and distribution, and ***iv.*** the influence of ecological factors on the presence/absence of *I. ricinus* in endemic areas (Gray, 1991; Gray, 2008; Gray *et al.,* 2016b; Medlock *et al.*, 2008).

The research presented here aims to build on the work which has gone before, much of which has been referenced throughout this chapter, and to address some of these knowledge gaps. Specifically, research will focus on: ***i.*** the influence of localised weather variables on the seasonality and initiation of *I. ricinus* questing behaviour; ***ii.*** the role of landcover type on the presence/absence and density of *I. ricinus;* ***iii****.* the temporal and spatial distribution of *Borrelia burgdorferi s.l.* in England, with specific reference to landcover type.

Developing a better understanding of *I. ricinus* activity and the prevalence of

Borrelia burgdorferi s.l. will allow us to better address the public health concerns surrounding *I. ricinus* in England and Wales, and in doing so, develop better strategies to mitigate public risk of tick bites and TBD.

1.4.2 Chapter overview

Chapter 1: The importance of Tick-borne pathogens, *Ixodes ricinus* as a vector and overview of PhD aims. This chapter aims to outline the importance of ticks as a pathogen vector and document the role they play in the transmission of pathogens of human and veterinary significance. Special attention is given to the *Borrelia burgdorferi s.l.* bacterial complex and its primary vector, *Ixodes ricinus*.

Chapter 2: Material and Methods. This chapter details the materials and methods used in the execution of field work, laboratory investigations and statistical analysis throughout the project. It describes the broad study set up of the three key studies involved i.e. temporal investigation of *I. ricinus* questing activity, spatial investigation of *I. ricinus* questing activity, and presence of *Borrelia burgdorferi s.l.* in questing *I. ricinus.* Details of study-specific methods e.g. chapter-specific statistical analysis is further detailed in each chapter.

Chapter 3: Temporal *Ixodes ricinus* activity at a woodland site in southern England - an exploration of four years of data. With the aim of better understanding when increased *I. ricinus* nymphal questing occurs, when peak questing density is reached and when post-spring questing decline occurs, four years of almost continuous weekly *I. ricinus* activity data collected from a woodland site in the south of England was examined. The timing and window for which peak activity transpired was examined for each year alongside microclimate, vegetation and local weather variables. In particular, several meteorological variables i.e. weekly mean temperature, potential evapotranspiration and precipitation were investigated to identify potential associations with the commencement of increased nymphal questing activity in spring leading to peak activity, followed by a decline in activity. Regression analysis was used to investigate the influence of local weather on weekly nymphal questing activity, as well as the influence of microclimate and vegetation height/type on questing nymphal density.

Chapter 4: Spatiotemporal *Ixodes ricinus* activity from selected sites across England. A continuation of the work presented in Chapter 3, the aim of this chapter is to apply the same techniques of examining when increases in nymphal questing occurred in spring and how local weather and microclimate might influence commencement and decline in *I. ricinus* nymphal questing. In this chapter, nymphal questing data from six different sites located throughout England, recorded over a two-year period, is analysed alongside region specific meteorological variables, and site-specific microclimate and vegetation data.

By comparing the questing activity between sites, analysing how this may vary, and how this increased activity is influenced by weather and microclimate, specific cues associated with increased questing are identified. These cues will be used in further attempting to predict when peak nymphal questing occurs.

Chapter 5: Spatial dynamics of *Ixodes ricinus* activity - an exploration of six different landcover types. The aim of this chapter is to investigate how seasonal *I. ricinus* density varies across different landcover types, specifically: broadleaf woodland, coniferous woodland, calcareous grassland, improved grassland, heathland and acid grassland. Understanding how landcover influences the presence, absence and density of questing *I. ricinus* will help inform targeted public health tick awareness.

Chapter 6: Spatiotemporal dynamics of *Borrelia burgdorferi s.l.* - an exploration of landcover type, regional and seasonal dynamics. This chapter presents data on the presence of *Borrelia burgdorferi s.l* in a proportion of *I. ricinus* nymphs collected from each of the temporal survey sites (Chapters 3 and 4) and the spatial survey sites (Chapter 5). The presence of different genospecies of the *Borrelia burgdorferi s.l.* complex is presented, together with a statistical analysis of prevalence across landcover types.

Chapter 7: Summary discussion and conclusions. This chapter is a general discussion on research findings, including how these might inform a UK public health narrative on *Ixodes ricinus* ticks and Lyme borreliosis.

Chapter 2: Materials and Methods

2. 1 Survey sites

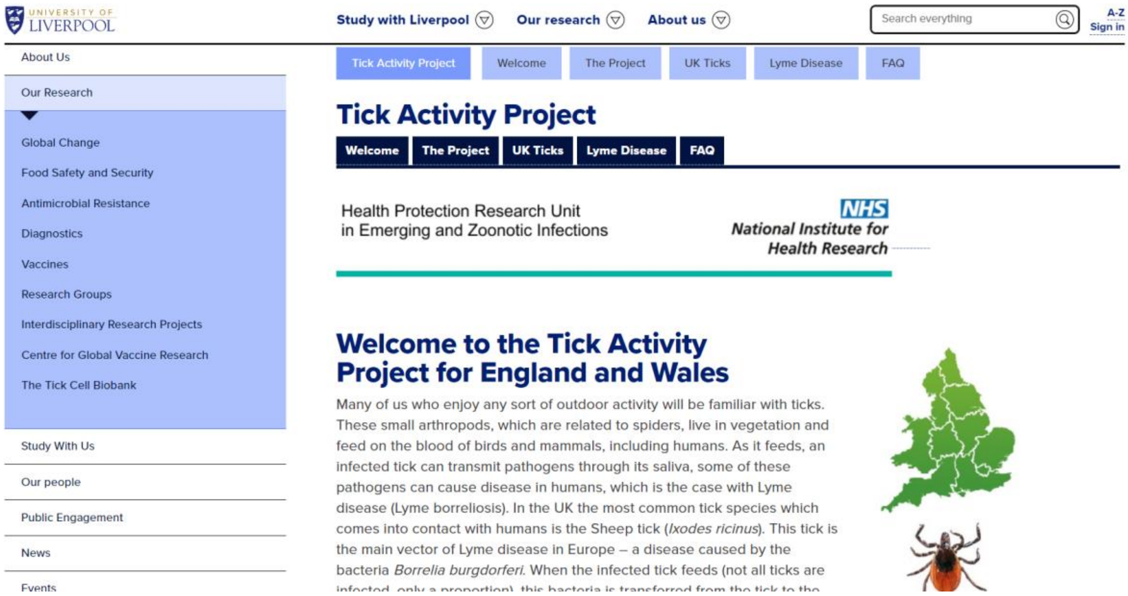
Several different survey sites were used throughout this project, specific to the aims of each research question. The temporal component of the study involved a citizen science study, where volunteers conducted regular tick surveys throughout England over a two-year period; in addition, the author conducted weekly studies at two woodland sites in Wiltshire. The spatial study involved repeated surveying by the author of different sites within different habitat types over two seasons.

2.1.1 Survey site selection: Temporal study

The aim of the temporal study was to investigate the seasonal questing activity of the *Ixodes ricinus* nymph, particularly the relationship between the commencement of peak questing activity in spring, and the decline of questing activity following peak. This was investigated at several sites throughout England, questing data collected was analysed alongside on-site microclimate and environmental variables, and local 5 km gridded weather data.

In an effort to cover as large a geographical scale as possible, volunteer surveyors from across England and Wales were invited to engage in the study. Prior to the inclusion of volunteers on the project, an application for ethical approval was filed with the University of Liverpool, which detailed the field activities to be undertaken by volunteers. The application was approved on the basis that volunteers would be fully briefed on the project and on the potential hazards of working in the field.

A project webpage was set up which detailed the project aims, volunteer involvement, project contact details, as well as information on *I. ricinus* and Lyme disease (Fig2.1). This website was hosted on the University of Liverpool, Institute of Global Health website: https://www.liverpool.ac.uk/infection-and-global-health/research/zoonotic-infections/tick-activity-project/



Tick Activity Project

Health Protection Research Unit
in Emerging and Zoonotic Infections

National Institute for
Health Research

Welcome to the Tick Activity Project for England and Wales

Many of us who enjoy any sort of outdoor activity will be familiar with ticks. These small arthropods, which are related to spiders, live in vegetation and feed on the blood of birds and mammals, including humans. As it feeds, an infected tick can transmit pathogens through its saliva, some of these pathogens can cause disease in humans, which is the case with Lyme disease (Lyme borreliosis). In the UK the most common tick species which comes into contact with humans is the Sheep tick (*Ixodes ricinus*). This tick is the main vector of Lyme disease in Europe – a disease caused by the bacteria *Borrelia burgdorferi*. When the infected tick feeds (not all ticks are infected, only a proportion), this bacteria is transferred from the tick to the

Fig 2.1 The Tick Activity Project webpage, containing project background, details of the project and point of contact.

Various interest groups throughout England and Wales were contacted, including The Wildlife Trust, The National Trust, The Forestry Commission, Lyme Disease Association, as well as, regional conservation groups and entomology societies. Each group was provided with details of the website as invited to disseminate the project and website details to potential volunteers. Those interesting in participant in the project were invited to contact the project coordinator (the author). On first contact, potential volunteers were provided with information on the project and provided with pre-prepared materials on the project, which consisted of the following documents: A Field Study Participation Information; a Call for Volunteers; a detailed Study Protocol; A Tick Identification Help Sheet (all of which were compiled by the author) see the end of the Appendix section for copies of these documents.

On reading the documents, if the volunteer wished to take part in the study they were asked to sign a volunteer consent form to ensure that they had read all of the safety information detailed in the provided documents. Confirmed volunteers were then sent a volunteer pack which contained all of the equipment required to conduct filed surveys; this contained the following: one wooden pole, 3 poly cotton 1 m^2 flags, a pair of watch makers fine tipped forceps, a supply of screw topped tubes containing 300 µl of 70 % ethanol, clip board, thermohygrometer, measuring tape, first aid kit, Tick Twister™ tick removal tool, postage and envelopes for the return of ticks, a supply of survey sheets and survey protocol.

Volunteers were asked to conduct a fortnightly survey for *I. ricinus* activity at an appropriate field site. Volunteers monitored the same ten transects using the flagging method (described below), questing tick specimens were collected and recorded; in addition, environmental variables: temperature (°C), relative humidity (%RH), vegetation height (cm) and type were also recorded at time of survey. Surveys were to be conducted fortnightly, at each site from March to November, over a two-year period (2015 and 2016).

In total, 35 of those who contacted the project coordinator agreed to take part in the study and were supplied with the appropriate documentation and field kit. Of these, seven participants excused themselves from the study prior to survey commencement. For the each of the study years, six volunteers conducted one to three surveys, seventeen conducted 4 to 9 surveys and five volunteers conducted >10 surveys (Table 2.1).

Of the sites which produced <10 surveys per year, seven of these recorded zero Qt and cessed surveys in year one of the study

An additional site was surveyed weekly by the author (Cholderton), this site produced 94 surveys across the two-year period. The Bentley Wood site was surveyed on the same day as the Cholderton site; this site was analysed separately as a longitudinal data set (Chapter 3) as four years weekly data of *I. ricinus* activity had been collected there. However, the 2015/2016 survey data from the Bentley Wood site was also included with Cholderton data in regression analysis in Chapter 4.

In the interest of constructing a reasonable time series of questing nymph data against which meteorological variables could be compared, only sites which consistently found ticks and conducted >10 surveys in any given year were included in the final analysis (Chapter 4)). Each of these sites conducted at least ten surveys in any given year, with the exception of the Northumberland site which conducted surveys in 2015 only (Table2.1). Throughout the temporal analysis (described in Chapter 4), these are the only sites which are used and referred to.

Table 2.1 All sites which agreed to take part in the study; details of site location, coordinates, period in which surveys were conducted, total surveys conducted and totals *I. ricinus* ticks collected. The top six sites (highlighted by the black boarder) were the sites used in the final analysis. *surveyed in 2015.

Site Location	**Lat long Coordinates**	**Period of inclusion in the study/Active period**	**Total surveys**	**Total Questing collected**
Sidbury, Devon	50.7265, -3.2099	March - November 2015 April - October 2016	27 19	135 105
Hurt Wood, Surrey	51.1748, -0.4222	March - September 2015 March - November 2016	15 18	93 119
Hutton Roof, Cumbria	54.1941, -2.6952	April - October 2015 March - September 2016	10 13	192 139
Colt Crag Northumberland	55.095, -2.1149	April - October 2015 -	11	632
Richmond Park, Greater London	51.4526, -0.2842	April - November 2015 February - December 2016	21 32	503 688
Cholderton, Wiltshire	51.1715, -1.7053	January - December 2015 January - December 2016	49 45	356 217
Wytham Woods Oxford (woodland)	51.7711, -1.3384	March - August 2015 -	8	3
Wytham Woods, Oxford (grassland)	51.7711, -1.3384	March - August 2015 -	8	8
Bartley Heath Hampshire	51.2764,0.9542	April - July 2015 March - December 2016	7 13	344 194
Daneway Banks Gloucestershire	51.7305,2.0925	March - October 2015 March - October 2016	9 10	25 16
Berkhamsted Hertfordshire	51.7681, -0.5419	April - June 2015	8	0

		-		
Kirkby Moor Lincolnshire	53.1481, -0.1730	March - July 2015 -	6	0
Isle of Axholme Lincolnshire	53.6261, -0.8550	April 2015 -	1	15
Ness Botanic Gardens, Merseyside	53.2720, -3.0448	April - Jul 2015 -	7	0
Brooklands Farm Dorset	50.7550, -2.4740	March - July 2015 -	4	18
Penshurst Estate Kent	51.1881, 0.1967	March - Oct 2015 -	8	0
Crediton Devon	50.7550, -2.4740	March - April 2015 -	2	11
Seaton burn Northumberland	55.0624, -1.6465	April -July 2015 -	6	1
Ramsey Island Pembrokeshire	51.8668, -5.3429	April -August 2015 -	7	74
Staindrop, Durham	54.5781, -1.8157	April - July 2015	6	0
Ruthin, Denbighshire	53.0642, -3.3066	April - July 2015	9	0
Normandy, Surrey	51.2397, -0.6746	April - May 2015	3	17
Velmean Common Hampshire	51.2690, -0.8210	April - August 2015	5	55
Cockermouth Cumbria	54.6615, 3.3613	June - July 2015 -	3	0
Windsor Great Park Berkshire	51.4620, -0.6147	July - September 2015 -	3	0
Greyfield Wood Somerset	51.3224, -2.5167	July - September 2015 March - September 2016	3 7	65 280
Thetworth Forest, Norfolk	52.5589, 0.8367	April - September 2016	6	43
Talerddig, Wales	52.5822, -3.6053	April – July 2016	5	0
Havergate Island Norfolk	52.0734,1.5260	April - June 2016	3	22

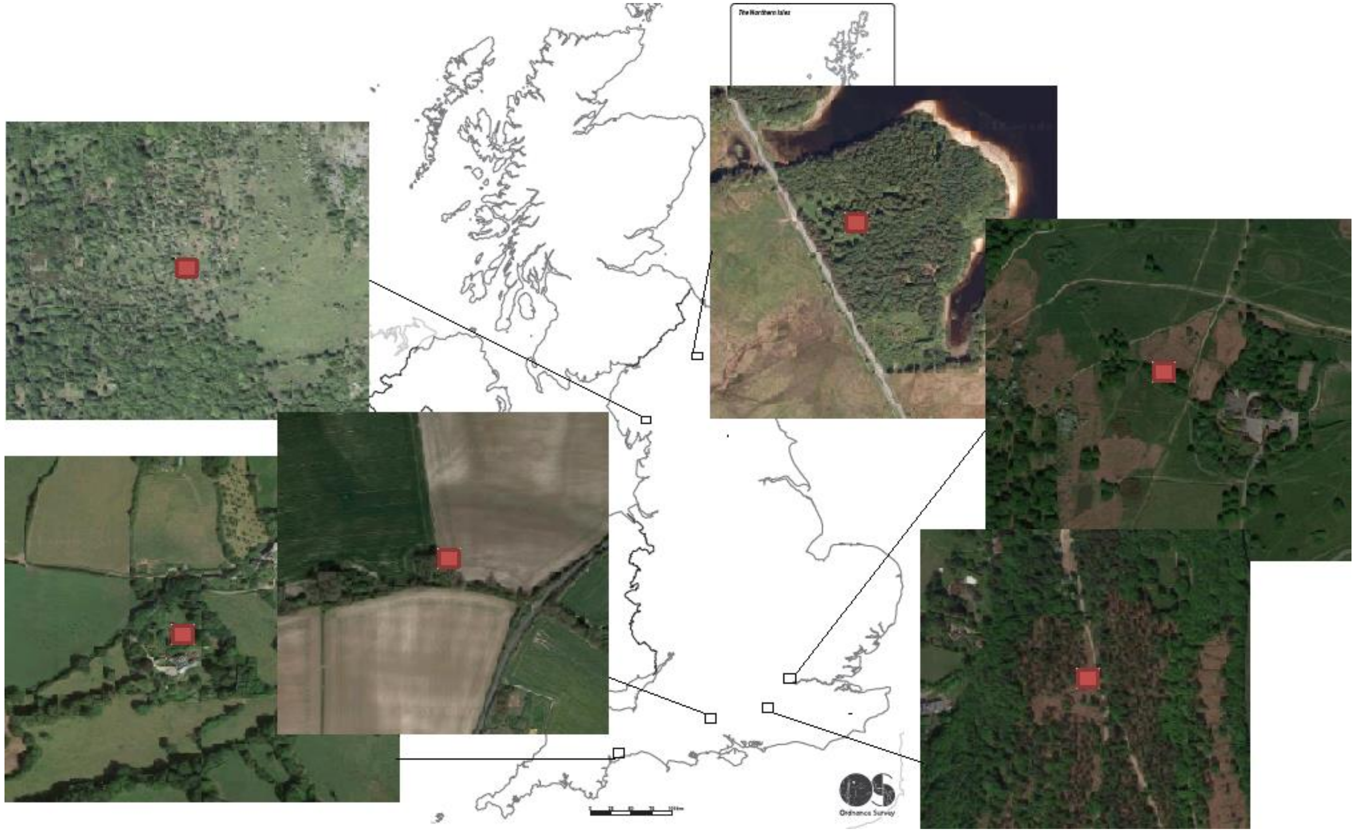


Fig 2.2 The locations and image of the six sites used in the temporal analysis of peak *I. ricinus* activity. Ordnance Survey Maps; Imagery ©2018,Google

2.1.2 Survey site selection: Bentley Wood

The fact that *Ixodes ricinus* is commonly associated with woodland, both broadleaf and coniferous (as detailed in Chapter 1 and 5), infers that woodland sites are most appropriate for the intensive study of *I. ricinus,* and the environmental factors which regulate its questing activity.

Bentley Wood, a mixed woodland located in the south of England (51°04'N 1°39'W), frequented by dog walkers, horse riders and wildlife enthusiasts, was selected for this study. The woodland consists of 665 hectares of mixed woodland and is noted as a biological Site of Special Scientific Interest due to its diversity of rare butterfly species.

This particular site was chosen for study due to its accessibility, public use and previously established knowledge of high *I. ricinus* densities (Medlock *et al.,* 2012). Surveys commenced in March 2013 and still continue on a weekly basis, however, the data set analysed for the purpose of this study focused on March 2013 to December 2016. Surveys conducted from March 2013 until September 2014 were carried out by the Medical Entomology and Zoonose Ecology group at Public Health England, Porton Down. Surveys from October 2014 onwards were conducted by the author. Surveys were conducted weekly, except in the case of extreme weather (e.g. rain or excessive windy days) or logistical issues (e.g. lack of transport).

Fig 2.3 The location of Bentley Wood and satellite image of the linear survey site within the woodland. Baseline map of UK provided by the Ordinance survey; satellite image provided by Google maps, 2018.

A linear area, running adjacent to a walking path, was selected for the survey area. Within this, ten 10 m transects of vegetation were measured. Since *I. ricinus* horizontal movements are limited (Perret *et al.*, 2003), a gap of ten metres was left between each survey transect to ensure that sampling in one transect did not affect sampling in another. Throughout the study period, the site was visited and surveyed once a week, on days which were dry, and between the hours of 10:00 and 17:00.

Fig 2.4 Each individual transect (T1 – T10) which was surveyed during the study. Photos are of spring vegetation. LMcGinley, May 2016

2.2 Spatial study

The aim of the spatial study was to investigate the presence/absence and density of questing *I. ricinus* nymphs and adults, the presence/absence of larvae in six different land cover types.

2.2.1 Spatial Study: site selection

Based on geographical and ecological knowledge of the area region, an area which traversed part Wiltshire and Hampshire in the south of England was selected as the study site. This area was chosen as it provided a range of different landcover types, and allowed for regular site visits in spring, summer and autumn 2016.

ArcGIS software (version 10.2) was used for all mapping, extraction of slope, aspect, elevation data, and randomised site selection process. Landcover types were investigated using the CEH Landcover layer (LCM2000). CEH landcover layer in ArcGIS, the village of Redlynch was chosen as a centre point, around which a buffer zone of radius 8 km was created; from this zone, survey sites were selected. Landcover such as marshland and arable were excluded from the survey area due inaccessibility and unsuitability as a tick habitat (Estrada-Peña, 2000).

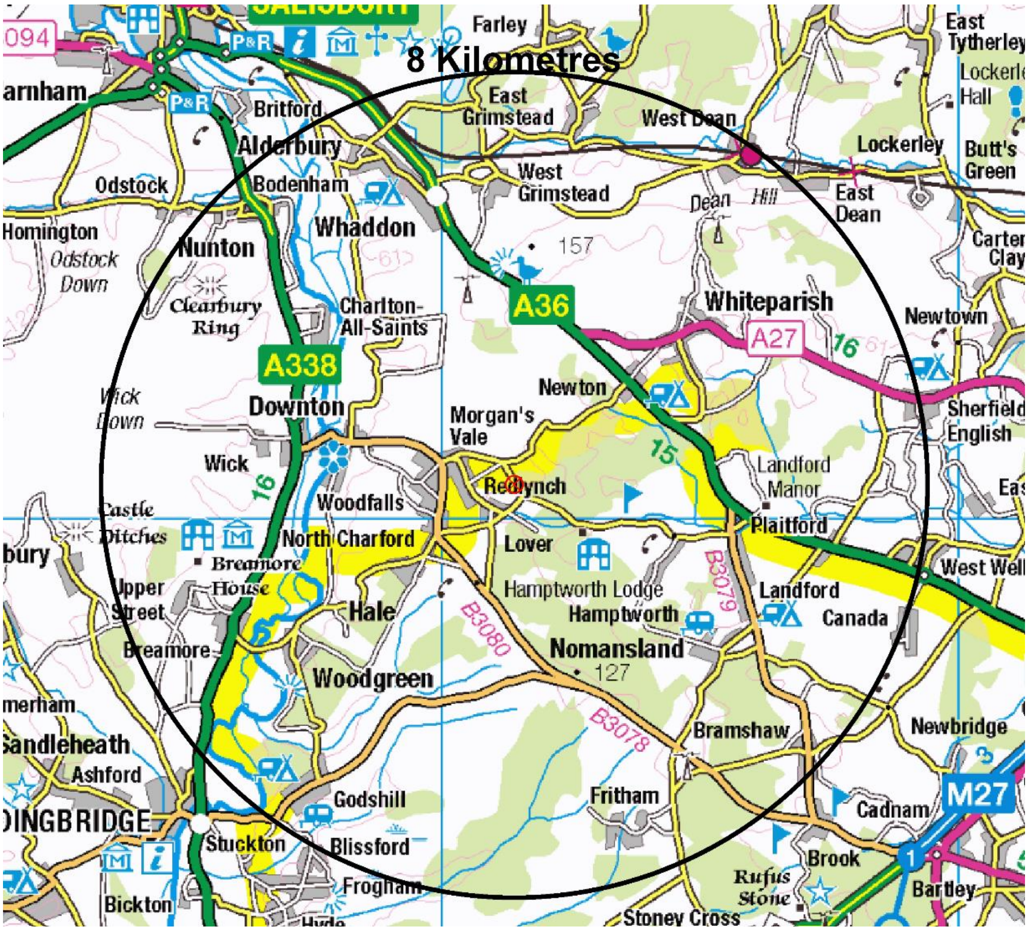


Fig 2.5 The area used (contained within the circular buffer zone of radium 8 km) from which spatial study sites were selected at random. Google Maps (2015).

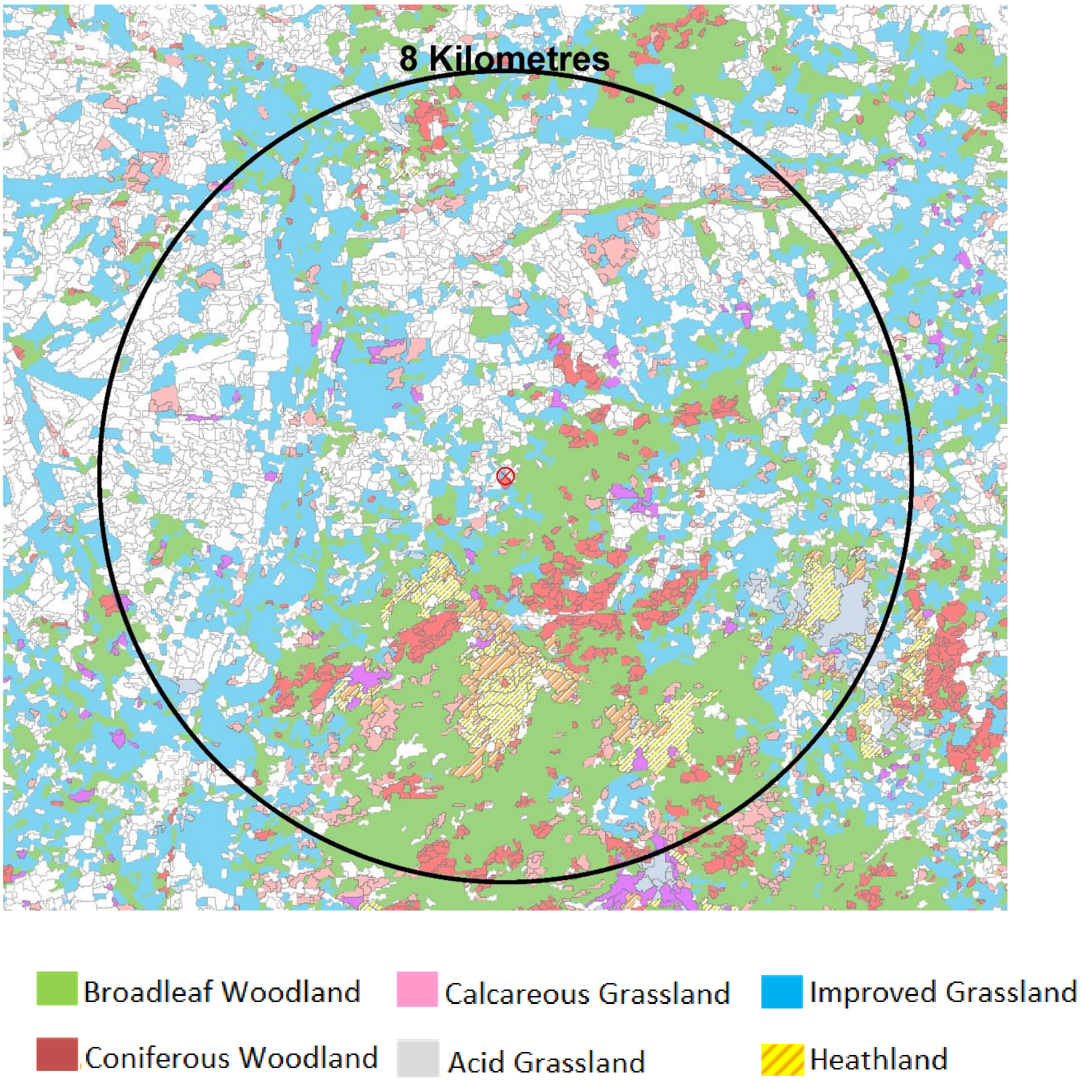


Fig 2. 6 The area selected as the spatial study: (above) OS map of the area chosen from which spatial sites were selected; (below) CEH landcover layer (ArcGIS software) of the same area. 24 sites from six different land cover types were selected for study from this 8 km radius zone.

Six landcover types: Broadleaf Woodland (BL), Coniferous Woodland (CN), Calcareous Grassland (CL), Improved Grassland (IM), Heathland (HL) and Acid Grassland (AC) were selected for tick sampling due to their dominance in the UK landscape, their accessibility to the public for outdoor activities, and for their potential to accommodate tick hosts.

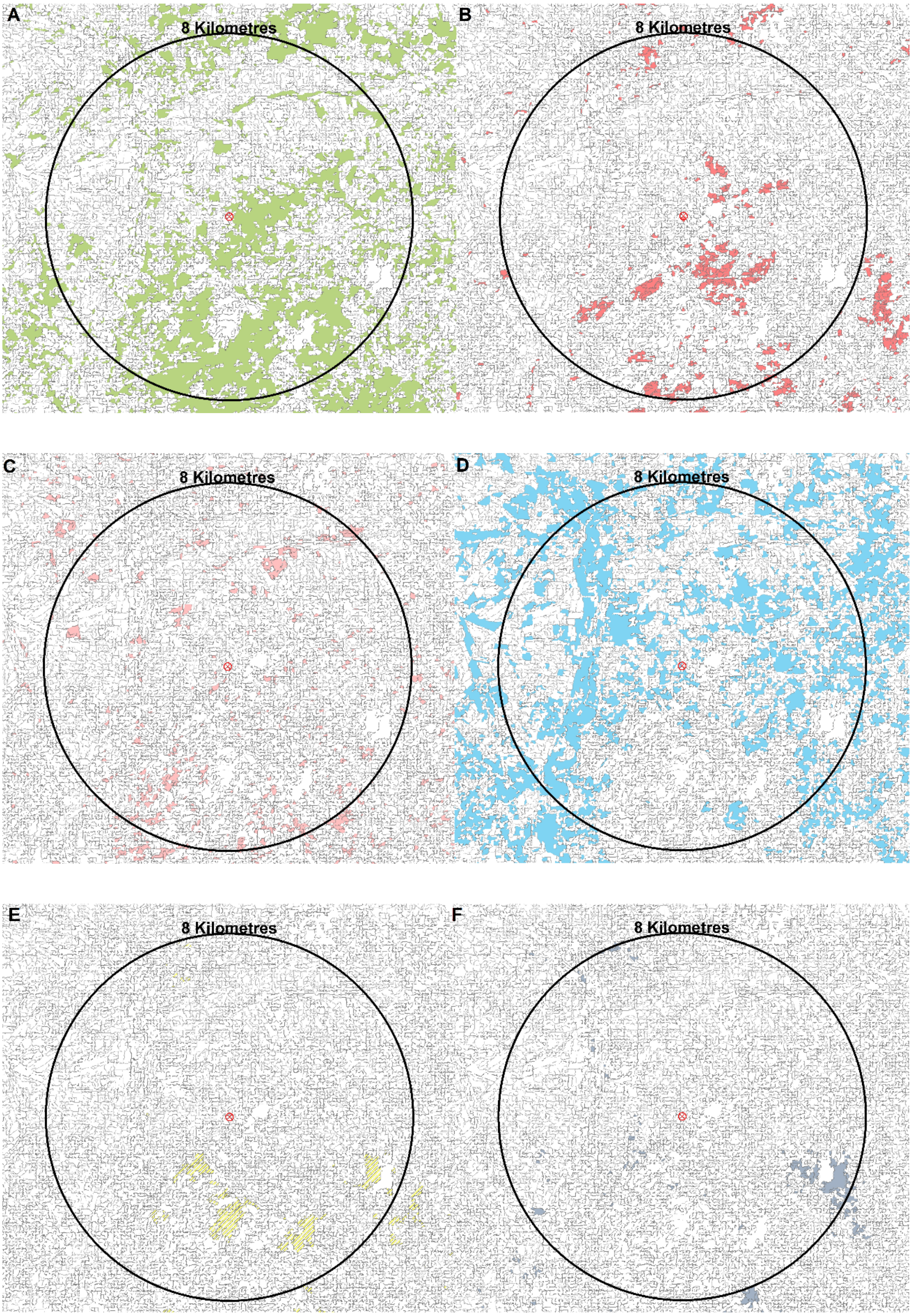


Fig 2. 7 The individual landcover layers for spatial site selection: **A.** Broadleaf woodland, **B.** Coniferous woodland, **C.** Calcareous grassland, **D.** Improved grassland, **E.** Heathland, **F.** Acid grassland. Four sites were selected from each of these layers for study.

ArcGIS software was used to randomly generate potential survey points within each selected land cover type. Maps were transferred onto a portable Juno® Handheld and each site was ground-truthed to confirm the landcover type and site accessibility before surveying commenced.

If a site was deemed inaccessible at the ground-truthing stage (e.g. private property or dangerous to access), a substitute site was chosen as close as possible to this original point. A total of four survey sites for each of the six selected land cover types were included in the final study (Table 2.1)

Table 2.2 Landcover identification, site name, coordinates, aspect, slope and elevation, 50metre DTM resolution. Geographical data was extracted using ARCGIS software.

Site No.	Site ID	Site name	Lat/long coordinates	Aspect	Slope (degrees)	Elevation (m a.s.l.)
Broadleaf Woodland (Fig2.7)						
1	BL1	E. Grimstead	51.051, -1.693	Flat (-1)	0	61
2	BL2	Whiteparish	51.03, -1.643	SW (202.5 - 247.5)	2.82	147
3	BL3	Mean Wood	51.008, -1.629	SE (112.5 - 157.5)	3.85	54
4	B4	Eyeworth Pond	50.931, -1.677	SE (112.5 - 157.5)	2.23	85
Coniferous Woodland (Fig2.8)						
5	CN1	Barnell Copse	50.996, -1.643	NE (22.5 - 67.5)	0.61	60
6	CN2	Peaked Wood	50.973, -1.670	NE (22.5 - 67.5)	0.20	50
7	CN3	Godshill	50.952, -1.720	S (157.5 - 202.5)	6.32	94
8	CN4	Franchises Common Wood	50.960, -1.667	N (0 - 22.5)	1.54	54
Calcareous Grassland (Fig2.9)						
9	CL1	Pepperbox Hill Carpark adjacent	51.021, -1.699	S (157.5 - 202.5)	2.63	143
10	CL2	Pepperbox hill meadow	51.021, -1.694	S (202.5 - 247.5)	4.47	127
11	CL3	Pepperbox Hill Nr. Grimstead Rd.	51.028, -1.686	N (292.5 - 337.5)	7.77	140
12	CL4	Pepperbox Hill Bridleway	51.021, -1.703	S (112.5 - 157.5)	3.69	141
Improved Grassland (Fig 2.10)						
13	IM1	E. Grimstead	51.050, -1.694	S (157.5 - 202.5)	2.15	60
14	IM2	Farley	51.063, -1.681	N (0 - 22.5)	1.81	70
15	IM3	Britford	51.050, -1.767	E (67.5 - 112.5)	0.57	43
16	IM4	Britford Lower Rd.	51.049, -1.776	N (22.5 - 67.5)	0.84	49
Heathland (Fig 2.11)						
17	HL1	Telegraph Hill	50.948, -1.674	N (22.5 - 67.5)	2.07	122
18	HL2	Bramshaw	50.945, -1.667	Flat (-1)	0	127
19	HL3	Woodgreen B3078	50.944, -1.742	S (157.5 - 202.5)	0.91	57
20	HL4	Woodgreen, Lady's Mile	50.945, -1.745	S (112.5 - 157.5)	8.68	78
Acid Grassland (Fig 2.12)						
21	AC1	Canada Common	50.958, -1.593	NW (292.5 - 337.5)	2.56	40
22	AC2	Copythorne	50.958, -1.579	SW (202.5 - 247.5)	1.47	42
23	AC3	Penn Common	50.949, -1.602	NE (22.5 - 67.5)	0.83	41
24	AC4	Wellow	50.969, -1.597	N (337.5 - 360)	4.34	39

Fig 2. 8 Broadleaf Woodland sites (L to R 1 to 4): BL1, BL2, BL3, BL4.

Fig 2. 9 Coniferious Woodland sites (L to R 5 to 8): CN1, CN2, CN3, CN4.

Fig 2. 10 Calcarious Grassland sites (L to R 9 to 12): CL1, CL2, CL3, CL4.

Fig 2. 11 Improved Grassland sites (L to R 13 to 16): IM1, IM2, IM3, IM4.

Fig 2. 12 Heathland sites (L to R 17 to 20): HL1, HL2, HL3, HL4.

Fig 2. 13 Acid Grassland sites (L to R 21 to 24): AC1, AC2, AC3, AC4.

2.3 Sampling of questing ticks: temporal study, including Bentley Wood

The following method was used for the Bentley Wood survey (as reported in Chapter 3) and all temporal field surveys (as reported in Chapter 4). All surveys were undertaken when the weather was dry i.e. not raining and vegetation was not saturated, and in day time hours, between the hours of 10am and 5pm.

Once a site was selected, ten 10 m stretches of vegetation were measured, leaving a gap of 10 m in between each one. These same ten transects were surveyed for the duration of the study. The ‘flagging’ method (Rulison, 2013)was used to measure questing *I. ricinus* densities; this method consisted of running a 1m^2 poly-cotton flag which was attached to a pole, across the selected 10m transect of vegetation whilst walking at approximately 0.5 m/second (Agustín Estrada-Peña *et al*, 2013). The host mimicking effect of the flag brushing against the vegetation acting as a cue for questing ticks, which if present, would attempt to attach to the flag (Fig2.14a).

Several other methods (Dobson *et al.*, 2011) of tick collection were considered including ‘dragging’, however, the flagging method was chosen because of its efficiency in collecting *I. ricinus* (Dantas-Torres *et al.*, 2013), whilst not requiring the surveyor to walk directly through the survey area, thereby reducing tick bite risk. The flagging technique is used to estimate the density and therefore, activity of questing *I. ricinus* nymphs and adults in the 10 m² transect area; it did not take account of ticks in diapause or states of quiescence. Therefore, this method was used as a measure of questing *I. ricinus*, not a measure of the entire population present in the vegetation (Estrada-Peña *et al.* 2013).

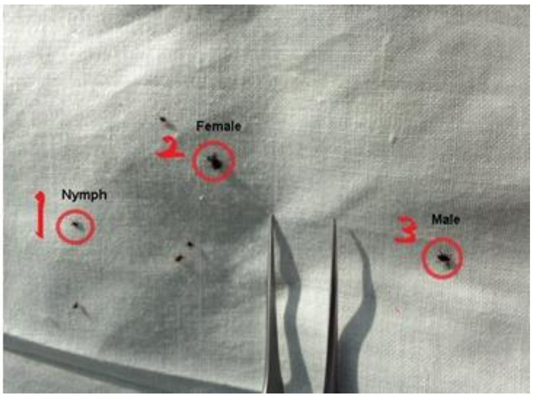
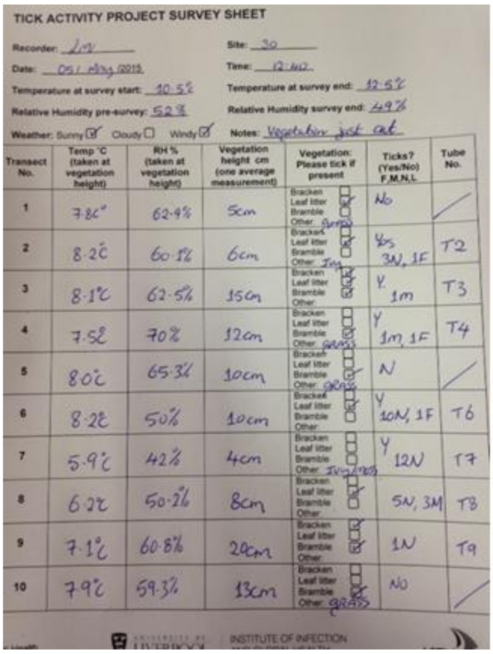


Fig 2.14a Conducting a survey for questing ticks using the flagging technique. **Fig 2.14b** (L to R) A nymph, female and male attached to the flag. L. McGinley 2015

At the end of 10m, the flag was gently turned over and any ticks were removed into a collection tube (Fig 2.15b). Ticks from Bentley Wood and the Cholderton site (surveyed by the author) were transferred into screw top tubes, a single tube was used per transect and labelled with the site name, survey date and transect number; these were transferred to -40°C freezer within an hour of survey.

Ticks collected by volunteers at all temporal survey sites were placed in tubes containing 70%ethanol, killing the contained ticks and preserving them until they were returned to the lab. The number of questing nymphs (Qn), female (Qf) and male (Qm) ticks, and the presence of larval ticks were also noted on a specifically designed survey sheet (Fig 2.14a). During data recording and analysis, the number of questing female and male ticks were summed to give the number of total questing adults (Qa), total questing ticks (Qt) were the sum of Qn and Qa.



TICK ACTIVITY PROJECT SURVEY SHEET

Recorder: LM
Site: 30
Date: 05/May/2015
Time: 12:40
Temperature at survey start: 10.5°C
Temperature at survey end: 12.5°C
Relative Humidity pre-survey: 52%
Relative Humidity survey end: 49%
Weather: Sunny ☑ Cloudy ☐ Windy ☑
Notes: Vegetation just cut

Transect No.	Temp °C (taken at vegetation height)	RH % (taken at vegetation height)	Vegetation height cm (one average measurement)	Vegetation: Please tick if present	Ticks? (Yes/No) F,M,N,L	Tube No.
1	7.8C°	62.4%	5cm	Bracken ☐ Leaf litter ☑ Bramble ☐ Other: Grass	No	/
2	8.2°C	60.1%	6cm	Bracken ☐ Leaf litter ☑ Bramble ☐ Other: Ivy	Yes 3N, 1F	T2
3	8.1°C	62.5%	15cm	Bracken ☑ Leaf litter ☑ Bramble ☑ Other:	Y 1m	T3
4	7.5°C	70%	12cm	Bracken ☐ Leaf litter ☐ Bramble ☑ Other: GRASS	Y 1m, 1F	T4
5	8.0°C	65.3%	10cm	Bracken ☐ Leaf litter ☐ Bramble ☑ Other: GRASS	N	/
6	8.2°C	50%	10cm	Bracken ☐ Leaf litter ☑ Bramble ☐ Other:	Y 10N, 1F	T6
7	5.9°C	42%	4cm	Bracken ☐ Leaf litter ☐ Bramble ☐ Other: Ivy/grass	Y 12N	T7
8	6.2°C	50.2%	8cm	Bracken ☐ Leaf litter ☑ Bramble ☐ Other:	5N, 3M	T8
9	7.1°C	60.8%	20cm	Bracken ☑ Leaf litter ☐ Bramble ☑ Other:	1N	T9
10	7.9°C	59.3%	13cm	Bracken ☐ Leaf litter ☐ Bramble ☑ Other: GRASS	NO	/

Fig 2.15a Completed survey sheet. **Fig 2.15b** (L to R) *I. ricinus* adult male, nymphs and larvae in collection tubes. L. McGinley 2015

A number of environmental variables and observations were also recorded at time of survey: temperature (°C) and relative humidity (RH %) at the start and end of survey; visual observations on weather and the presence of wildlife. For each 10 m transect surveyed, temperature (°C) and RH% were measured using a handheld thermometer-hygrometer; both variables were recorded at tick questing height. Vegetation height was measured at three different points along the 10 m transect (start, mid-point and end) and an average calculated and recorded for that transect. The three most common vegetation types visible in each transect were also recorded.

Total questing tick (Qt) density for each survey (total survey area of 100 m^2) was obtained by summing the total number of questing nymphs (Qn) and total number of questing adults (Qa) collected per survey. The presence of larvae was noted but they were not collected. The geometric mean of Qa, Qn and Qt was derived for each survey (100 m^2) from summing those collected from the ten individual transects. Peak Qn activity was defined as the maximum number of Qn collected for a given survey.

To standardise and compare between survey years, the commencement of questing for each year was defined as the first pre-peak survey for which the number of Qn was approximately 25% of maximum peak questing densities for that year. Decline of questing was defined as the first post-peak survey for which density of Qn observed, was less than or equal to 25% of the maximum peak for that year. Studies from Eisen *et al.*, (2002) and Hauser *et al.*, (2018), have both defined onset and decline of peak in a similar fashion but using a 10% of peak threshold. Considering the data collected during this study, 10% of nymphal density did not seem appropriate as this would often indicate peak onset as starting from the first survey of the year. A threshold of 25% provided a stronger differentiation between survey weeks which provided single tick specimens and those for which an increase in activity could be distinctly identified.

2.3.1 Sampling of questing ticks: spatial study

To investigate the seasonal variation in *I. ricinus* density between landcover types, four sites were selected from each of the six different landcover types. Four different sites were chosen to reduce bias and ensure that each landcover type was represented by several independent sites. Each of these sites was surveyed for questing ticks three times in spring 2016, and three times in autumn 2016. Sites were surveyed three times to allow independent data collection at multiple time points (Fuller, 1999), whist maintaining a realistic survey strategy i.e. the author could logistically conduct a maximum of three visits to each of the 24 sites within each season. Each site was also surveyed twice in summer 2016; these summer surveys were used to collect additional ticks for *Borrelia* analysis and were not included in the final landcover type analysis.

In spring, all surveys were conducted from mid-April to mid-May (weeks sixteen to week nineteen); in autumn, surveys were conducted from late September to mid-October (week thirty-eight to week forty-one); in summer all surveys were conducted from mid-July to early August (week twenty-eight to thirty-one).

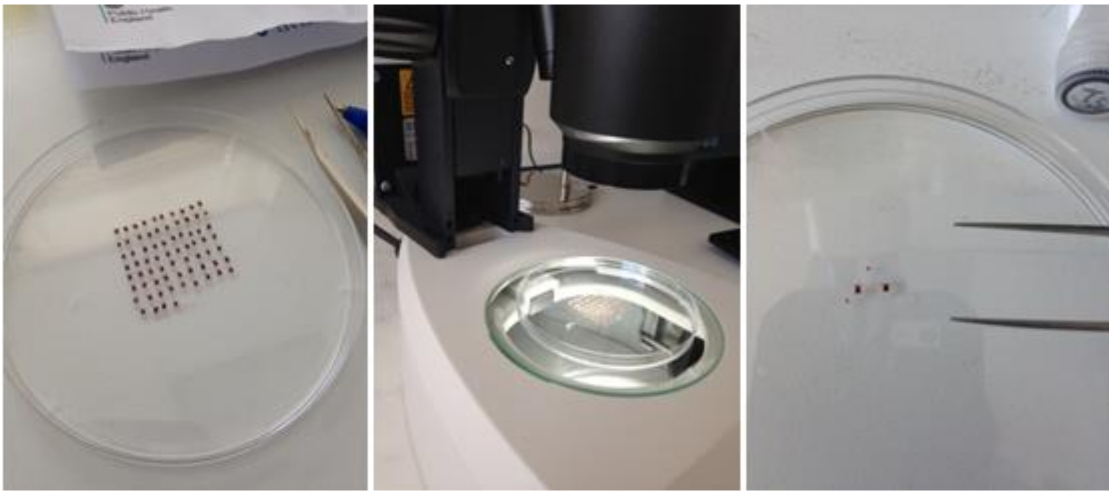
Within each landcover type, surveys were separated by a minimum of five days and a maximum of ten days. During each on-site survey, thirty 5 m^2 transects were surveyed using the flagging technique, with an additional fifteen transects flagged if no questing ticks were collected in the initial thirty transects. The presence or absence of larvae was noted but were not included in the calculations of questing ticks because of their tendency to cluster and therefore not considered representative of questing ticks.

The mean density of Qn per 5 m^2 was calculated for each survey replicate by dividing the total number of Qn collected at that site by the number of $5m^2$ drags conducted.

2.4.1 Tick identification

For both temporal and spatial studies, all ticks collected were identified to species level by microscopy. On return from field surveys, tubes containing ticks collected by the author were frozen at -80°C and identified in batches at a later date.

Prior to identification tubes corresponding to each survey were removed from the freezer - ticks were removed from individual tubes (a tube at a time to avoid freeze-thawing) and placed on a petri dish. Adults and nymphs were separated into rows on the petri dish, dorsal side up and checked using a microscope.



(L to R) **Fig 2.16a** Nymphs ready for identification on a petri dish **Fig 2.16b** Microscopic Identification **Fig 2.16c** Nymphs and larvae on a petri dish ready for identification. L McGinley 2015

A tick identification key (Hillyard, 1996) was used to confirm key identifying features of each individual tick (Fig 2.12). Both dorsal and ventral aspects of each tick were examined (Fig 2.14)

Fig 2.17 Dorsal view of an *I. ricinus* nymph (above) and ventral view (below). L. McGinley 2015

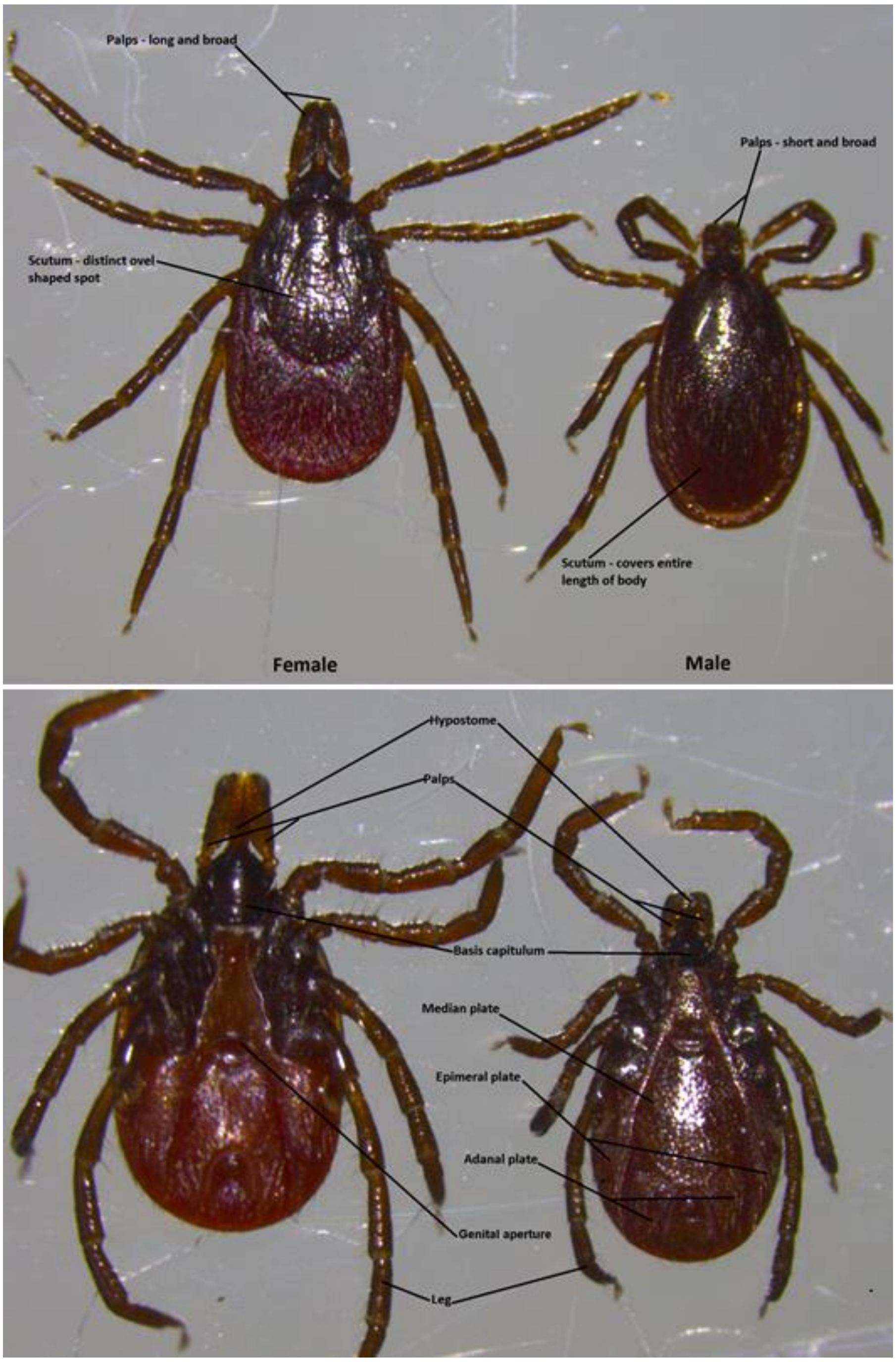


Fig 2.18 (Top) Dorsal view of an adult female (L) and adult male (R) and ventral view (Lower) with some key identifying features. L McGinley 2015

The number of nymphs and adults were checked against the accompanying survey sheet (for both temporal and spatial studies) and any corrections were made to the survey e.g. if a nymph had been recorded as an adult male or if a tick identified as a species other than *I. ricinus*. Ticks confirmed as *I. ricinus* were returned to a new tube, labelled with site identification, transect number (if recorded) and date collected. Once identified, ticks from the spatial study, together with those collected from Bentley Wood and Cholderton were returned to -80°C storage. Ticks confirmed as another species were removed from the study cohort and stored separately, these accounted for <0.5% of ticks collected.

An identical procedure was used for the identification of ticks collected by volunteers; however, these ticks arrived in 70 % ethanol. These ticks remained stored in 70 % ethanol for the duration of the project, once identified they were returned to the 70 % ethanol tubes and stored in a locked safety cabinet at room temperature (20-22°C).

2.4.2 Recording of climate and weather variables

Microclimate and weather variables which were previously reported as potentially influential to the questing activity of *I. ricinus,* were recorded throughout the study. Temperature (°C) and relative humidity (% RH) were recorded at time of survey for each of the temporal and spatial studies. Temporal sites recorded temperature at vegetation height for each individual transect surveyed, while spatial study sites recorded temperature once just as surveying commenced.

Long term climate and weather data was supplied by the UK Met Office. Some variables of importance to questing *I. ricinus* activity were calculated from those variables collected on site and from those supplied by the UK Met Office; these included saturation deficit (SD) and potential evapotranspiration (PET). Saturation deficit is a measure of the drying power of the air (Kalra & Parkash, 2016), and has previously been reported as influential to *I. ricinus* activity (Perret *et al.,* 2000; Perret *et al.,* 2003). Potential evapotranspiration (PET) was used as a proxy for vapour pressure deficit. PET is defined as the “maximum quantity of water capable of being evaporated in a given climate from a continuous expanse of vegetation covering the whole ground and well supplied with water. It includes evaporation from the soil and transpiration from the vegetation from a specific region in a specific time interval, expressed as depth of water. This variable has previously been used as a proxy for vapour pressure deficit in tick abundance research (Ruiz-Fons et. al 2012) and was investigated in this study as a predictor of cessation in *I. ricinus* activity.

2.4.3 Recording of onsite microclimate variables

For all sites surveyed by the author, including all spatial sites and temporal survey sites located at Bentley Wood and Cholderton, the Rotronic HP21 HygroPalm RH/Temp Meter to measure temperature and RH (%). At Bentley Wood, temperature measurements were recorded from the commencement of surveys in 2013, however, the recording of RH (%) did not commence at this site until mid-2015, as such, analysis using on-site RH (%) data for Bentley Wood is confined to 2016.

At all temporal sites, volunteers measured on-site temperature and RH (%) using an Omega pen theromohygrometer. This different model was used as a result of cost considerations, as it had to be supplied to multiple sites.

All equipment calibrated and tested before use in the field.

Table 2.4 Equipment used to measure onsite microclimate variables for spatial and temporal studies

Equipment specifications		
Equipment name	**Supplier**	**Specifications**
Rotronic HP21 HygroPalm RH/Temp Meter	Tempcon	Integral sensors Range of application -10 to 60 °C / 0 to 100 % RH Accuracy at 23°C ±5 K: 1 % RH Adjusted at 23 °C and 10, 35, 80 %rh Service interface (UART)
Handheld Thermohygrometre	Omega	Omega pen theromohygrometer Range of application -2 to 40 °C / 0 to 100 % RH

Where recorded, on-site temperature and RH (%) were used to calculate the saturation deficit for each site/transect. The following equation was used to calculate saturation deficit (SD) (Perret *et al.*, 2000):

$$SD = \left(1 - \frac{RH}{100}\right) 4.9463e0.0621 * T$$

Where:

SD = saturation deficit in millimetres of mercury (Hg)

RH = relative humidity (%) T = Temperature (°C)

2.4.4 UKCP09 gridded observation datasets

The UK Met Office supplied UKCP09 gridded observational datasets for each of the temporal survey sites and the Bentley site. The following daily data were provided at a 5km resolution: daily mean temperature (Tmean °C), daily minimum temperature (Tmin °C), daily maximum temperature (Tmax °C), and daily precipitation temperature (mm). This data was supplied for Jan 1960 to December 2016.

The calculation for long term climate averages used the 1961 to 1990 data set for Bentley Wood and for each of the temporal survey sites. This dataset was used as the climate average reference, against which anomalies for survey years were calculated. These anomalies informed how Tmean °C and precipitation for survey years related to historical Tmean °C and precipitation for those 5 km grids in which the study sites were located.

The daily maximum and minimum temperature were used to calculate potential evapotranspiration for each of the temporal survey sites, this was calculated using the Hargreaves equation:

$$\mathrm{PET} = 0.0023 * 0.408 * \mathrm{Ra} * \left(\frac{\mathrm{Tmax} + \mathrm{Tmin}}{2} + 17.8\right) * \mathrm{sqrt}(\mathrm{Tmax} - \mathrm{Tmin})$$

where:

Ra = Incoming solar energy (MJ.m-2.day-1)

Hargreaves equation was used for this calculation due to a lack of data for soil humidity.

The UKCP09 data set was also used to calculate the number of seasonal frost days for each temporal survey site. A frost day is defined as any day for which daily minimum temperature dropped below 0°C.

This data set was also used to investigate the relationship between different thresholds for weekly mean temperature at each of the temporal sites, in relation to the initiation and cessation of peak *I. ricinus* questing activity. A seven-day running temperature mean was calculated for each site, and this was plotted alongside questing nymph (Qn) activity for each temporal site. Correlations between initiation of peak, actual peak, and cessation of questing nymph activity against seven-day running Tmean (ºC) were investigated for each site.

Correlations between Qn and weekly accumulated PET, as well as weekly precipitation were also investigated in this manner. Analysis of these variables against Qn activity for different sites was undertaken to investigate any correlation between sites of when initiation, peak and cessation of peak Qn questing occurred. Such correlations (if detected) could potentially be used to predict in which weeks Qn questing might reach its peak. The definition of ‘commencement’ of peak questing *I. ricinus* activity, ‘peak’ questing activity and the ‘decline’ of peak questing activity is detailed in Chapter 3.

2.5 Spatial and seasonal prevalence of *Borrelia burgdorferi sensu lato*

The bacterial complex *Borrelia burgdorferi sensu lato* contains several genospecies which are pathogenic to humans and are associated with the manifestation of Lyme borreliosis. To frame the Bentley Wood, temporal and spatial investigations in a public health context, a portion of the ticks collected at each site were analysed for the presence of several genospecies of *Borrelia burgdorferi sensu lato*.

2.5.1 *Borrelia* DNA extraction and amplification

A proportion of the *I. ricinus* nymphs obtained from each of the temporal and spatial sites where individually tested for the presence of *Borrelia* DNA. A DNA extraction process described by Jahfari *et al.* 2012, was used for all tick extractions. All nymphs collected by the author had been stored at -80°C prior to extraction, all nymphs collected by volunteers had been stored in 70% ethanol; all nymphs were considered unfed and DNA was extracted as such. All manipulations were carried out in a fume hood.

Individual nymphs were placed in a PCR microtube with 100µl of ammonium hydroxide (NH_4OH). A total of 82 ticks were processed at a time, each strip of tubes had one tube allocated as an extraction control tube (containing 50µl of PCR grade H_2O), to monitor for potential cross-contamination of tubes. With lids closed tightly, tubes were placed in a 100°C heating block and boiled for 20 minutes (+/- 1 minute). Tubes were removed and centrifuged for 1 minute and then placed pack in the 100°C heating block for 15 minutes (+/- 1 minute), lids on each tube were opened to allow the evaporation of NH_4OH from each tube. Following this 15 minute incubation, lids were closed, and tubes removed from the heating block and centerfield. Following the NH_4OH evaporation process, the remaining 50µl (approximately) of lysates was placed at 4°C overnight and then at -80C ° until *Borrelia* PCR screening was carried out.

2.5.2 *Borrelia* DNA amplification

A pan-Borrelia qPCR modified from that described by Parola *et al*. 2011 and validated by the Rare and Imported Pathogens Lab (RIPL) at PHE, Porton, was used for the detection of *Borrelia* DNA. This assay was designed to detect several pathogenic *Borrelia* genotypes. Primers designed to target and amplify the of a 148-bp fragment of a 16S RNA–encoding region were used:

Primers:

Bor16S3F: 5'-AGC CTT TAA AGC TTC GCT TGT AG-3'

Bor16S3R: 5'-GCC TCC CGT AGG AGT CTG G-3'

Bor16S3 Probe: 5'-6FAM- CCG GCC TGA GAG GGT GAACGG-BHQ1 3'

The PCR was conducted using the TaqMan® Fast Universal Master Mix (Applied Biosystems); the following was used per reaction;

5 ul 20x primer/probe mix from 100 uM stocks.

0.18 ul Forward primer (10 uM)

0.18 ul Reverse primer (10 uM)

0.05 ul Probe

0.59 ul PCR grade H_20

10 ul ABI TaqMan Fast Universal master mix

1 ul 20x primer probe mix

4 ul PCR grade H_20

15 µl of prepared master mix and 5 µl of template DNA was used in the reaction. A positive control (*Borrelia burgdorferi* B31 strain) and negative control (PCR grade H_2O) was run alongside each batch of extracted tick DNA.

The PCR assay was processed on the QuantStudio Flex7 and analysis was carried out using Quantstudio realtime PCR software v1.

All positive samples with a cycle threshold (Ct value) level of log-based fluorescence <36 (≈10–20 copies of spacer) were considered positive for the presence of *Borrelia* DNA; these were subsequently sequenced to obtain the specific genospecies.

2.5.3 *Borrelia* DNA sequencing

Samples which tested positive for *Borrelia* DNA on the initial pan-*borrelia* screening assay where prepared for sequencing; the sequencing process would clarify the specific genospecies present.

In preparation for sequencing, a second PCR assay was used to amplify the 148-bp fragments, developed from Alekseev *et al.*, 2001, modified and validated by the Rare and Imported Pathogens Lab (RIPL) at PHE, Porton.

Primers:

B5S borseq: GAGTTCGCGGGAGAGTAGGTTATTGCC

23S bor seq: TCAGGGTACTTAGATGGTTCACTTCC

The PCR was conducted using the Platinum® Taq DNA Polymerase (Inverotgen)); the following was used per reaction;

5µl 10x platinum Taq Buffer

1µl 10mM dNTPs

1.5µl 50mM MgCl2

2µl 10mM B5S borseq

2µl 10mM 23S borseq

0.2µl Platium taq

33.3µl H2O

45 µl of prepared master mix and 5 µl of template DNA was used in the reaction. A positive control (cultured *Borrelia burgdorferi* B31 strain) and negative control (PCR grade H_2O) was run alongside the samples.

The PCR was processed using the Applied Biosystems Veriti® Thermal Cycler, under the following conditions:

94°C for 5 minutes.

10 cycles:

94°C for 20 seconds (denaturation)

70°C for 30 seconds (annealing), lowering by 1°C per cycle.

72°C for 30 seconds (elongation)

40 cycles:

94°C for 20 seconds

60°C for 30 seconds

72°C for 30 seconds.

Final extension: 72°C for 7 minutes

Hold: 4 ºC

DNA purification was carried out using the Qiagen QIAquick PCR Purification Kit (Qiagen, 2008).

Following purification, the DNA concentration of each sample was determined using the Nano Drop ™ Nucleic Acid Quantification process. Once DNA concentration were determined, samples were diluted as necessary, plated with the appropriate primers and sent to the Genomic Services and Development Unit at PHE Colindale for Sanger sequencing.

Once sequences were returned, each was entered into the NCBI’s (National Centre for Biotechnology Information) BLAST database (Basic Local Alignment Search Tool) and matched to those *Borrelia* sequences already held in the database

2.6 Statistical analysis and data visualisation

2.6.1 Statistical analysis in R

All statistical analysis and data visulisation was carried out using R statistical software R version 3.4.0 (R Development Core Team, Vienna, Austria). The development environment Rstudio version 1.0.153 (RStudio Team 2016). RStudio: Integrated Development for R was used to run R software.

The following extension packages were used in data manipulation, analysis and visualisation:

- bbmle version 1.0.20: Tools for General Maximum Liklihood estimation
- car version 3.0.0: Companion to applied regression
- data.table version 1.11.2: An extension of Data.frame
- dbplyr version 1.2.2: A dplyr back end for databases
- DHARMa version 0.2.0: Residual diagnostics for hierarchical (multi-level/mixed) regression models
- Dplyr version 0.7.5: A grammer of data manipulation
- Dunn.test version 1.3.5 Dunn's Test for multiple comparisons using rank sums
- forecast version 8.3: Forecasting functions for time series and linear models
- FSA version 0.8.20: Simple fisheries stock assessment method
- ggplot2 version 2.2.1 The grammar of graphics
- ggpubr version 0.1.7: ggplot based publication ready plots
- ggrepel version 0.8.0: automatically position non-overlapping test labels with ggplot2
- glmmTMB version 0.2.2.0: Generalized linear mixed models using template model builder
- lme4 version 1.1-17: linear mixed-effects models using 'Eigen' and S4
- magrittr version 1.5: a forward-pipe operation
- MASS version 7.3-50: Support functions and datasets for Venables and Ripley's MASS
- nlme version 3.1-131: Linear and nonlinear mixed effects models

- RColorBrewer version 1.1-2: ColorBrewer Palettes
- Reshape2 version 1.4.3: Flexibly reshape data
- Scales version 0.5.0: Scales functions for visualizations
- Stats4 version 3.4.1: Statistical functions using S4 classes
- wesanderson version 0.3.6: Wes Anderson colour pallet

2.6.2 Statistical tests and processes

A number of statistical tests were used for various aspects of the analysis; the following are a list of tests used throughout

- ANOVA: a parametric test used to compare the mean of multiple continuous variables i.e. used to compare temperature data from multiple sites.
- Dunn's Test: a non-parametric post-hoc test on multiple comparisons using rank sums. This was used following the Kruskal-Wallis test to determine the significance of variables such as vegetation height on questing tick densities.
- Kruskal-Wallis test: a non-paramedic equivalent of the one-way ANOVA. Carried out on two or more groups with no assumptions on the homogeneity of variance or normal distributions. This test was used when comparing different groups of variables (with non-normal distribution) i.e. questing tick density per transect, vegetation height on-site, weekly precipitation from different survey sites or different seasons.
- Paired t-test: used to test continuous variables with approximated normal distributions i.e. when comparing temperature data collected from the same sites using two different methods or temperature data from the same site from different study years.
- T-test: used to compare independent continuous data from two group's i.e. comparing weekly temperature data from two different sites.
- Turkey test: a parametric post-hoc test used following an ANOVA to determine the difference between groups i.e. the differences between temperature data from different sites.

2.6.3 Statistical models used

A number of statistical models were used to determine the significance of various environmental and meteorological data on tick presence/absence and questing densities.

Generalized Linear Mixed Models (GLMMs) were used to examine the effect of variables such as temperature, precipitation, relative humidity, saturation deficit, vegetation height and type on questing tick densities. GLMMs were used because of their ability to deal more effectively with data which is not necessarily normally distributed, may be grouped by site or season and measured on multiple occasions. These models allowed the incorporation of a random effect which helps account for spatial or temporal repetition of sampling. Due to the distribution of tick data, a negative binomial error distribution was used in all GLMMs.

For presence absence data, binomial-response generalised linear models (GLMs) were used to account for the binary nature of the data.

All models were initially constructing using all variables (the full model) and a drop process used for the least significant variable until the model which fitted the data best was obtained, the model selection process was based on the AIC (Akaike Information Criteria) value. All models were tested for uniformity, over-dispersion, spatial and temporal autocorrelation and zero-inflation.

2.7 Data storage

All tick survey data was initially recorded on paper and they transferred in to Excel 2010. Paper copies were maintained by the author.

All personal contact information of volunteers was maintained by the author and stored securely on a PHE encrypted laptop; no paper copies containing personal information were maintained.

Climate data from the UKCP09 was extracted to and stored in Excel file format. All data for analysis in R was stored as a csv file format.

All files were version controlled and backed up to the PHE network, in addition, non-confidential data was backed up to external hard drives. On completion of the project data will remain stored on the PHE network for use in further analysis by the Medical Entomology and Zoonoses Ecology team and their collaborators.

Chapter 3: Temporal *Ixodes ricinus* activity at a woodland site in southern England: an exploration of four years of data.

3.1 Introduction

Ixodes ricinus questing activity is strongly influenced by the biotic and abiotic factors of its environment. This tick is extremely sensitive to fluctuations in temperature and the amount of moisture in the air. Laboratory and field studies have been used to demonstrate the critical importance of both factors in the regulation of its questing behaviour (Lees, 1946; Milne, 1950; Perret *et al*., 2000; Randolph, 2004).

The act of host seeking, known as 'questing', results in the tick leaving the undergrowth, climbing the vegetation to more exposed and a potentially more variable microclimate. Studies have pointed to daily maximum air temperatures of 7°C for nymphal and adult *I. ricinus* , and 10°C for the less robust larval stage, as important thresholds for the commencement of questing activity (Macleod, 1936; Perret *et al*., 2000; Randolph, 2004); exposure to lower temperatures is assumed to have a negative impact on tick metabolism (Clark, 1995). Daily maximum temperatures approaching 20°C have been associated with a decline in questing activity (Qviller *et al.,* 2014). Exposure to higher temperatures, as a result of prolonged questing results in the abandonment of questing and a return to the vegetation layer to rehydrate; repetition of this process degrades energy reserves and decreases the chance of the tick attaining a blood meal (Randolph, 2004).

Several field studies investigating the dynamics of questing behaviour, temperature, humidity, and other factors including precipitation and day length, (Daniel *et al*., 2015; Del Fabbro *et al.*, 2015;Knap *et al.*, 2009) have suggested a complex relationship. Daniel *et al*., (2015) concluded that temperature (near ground), relative humidity and day length were good statistical predictors of nymphal activity; Del Fabbro *et al.,* (2015) reported precipitation (monthly mean), followed by temperature to be a crucial factor in *I. ricinus* abundance; Knap *et al.,* (2009 found that increased saturation deficit was linked to a decline in questing activity. However, a common obstacle in the assessment of these factors on tick questing is the lack of weekly longitudinal data, often due to the logistical effort required to maintain continuous monitoring. As such, studies to assess these factors over multiple years are extremely rare, and so gaps remain in our knowledge regarding the microclimate-driven triggers of *I. ricinus* questing behaviour in the UK.

This UK study is unique as it uses weekly survey data of questing *I. ricinus* activity across a four year period from one location, in which questing tick data, as well as environmental and microclimate variables were collected.

Assessing the timing and intensity of questing tick activity has important consequences for public health; public exposure to *I. ricinus* increases tick bite risk, which in turn, increases exposure to a range of pathogens (Walk *et al.*, 2009). In the UK, the tick-borne pathogen of primary concern is *Borrelia burgdorferi s.l.*, the causative agent of Lyme borreliosis. Lyme borreliosis follows a seasonal trend in laboratory confirmed cases and recent data confirms a rise in laboratory confirmed cases (PHE, 2018). Questing nymphs (Qn) are seen as a particular concern due to their small size and ability to feed undisturbed for long periods (Robertson *et al.*, 2000; Wilhelmsson *et al.*, 2013). As such, the ability to use environmental and weather based variables to potentially predict when commencement of peak questing activity, particularly nymphal activity, might occur could have positive implications for tick-bite/Lyme borreliosis mitigation.

This chapter will investigate the first steps toward this, in identifying possible weather related cues which might be used in predicting commencement of spring questing *I. ricinus*, and the decline in questing immediately following peak activity.

In particularly, the commencement of Qn activity in spring and the decline following peak activity in relation to mean temperature (°C), potential evapotranspiration (PET mm/week) and precipitation (mm/week) will be investigated. In addition, the influence of ground level microclimate and vegetation variables will be investigated for their association with Qn questing activity and density.

3.2 Materials and methods

3.2.1 Questing *I. ricinus* density and peak analysis

Please refer to Chapter 2, section 2.2 for details on site selection and tick collection at Bentley Wood.

Total questing tick (Qt) density for each survey (total survey area of 100 m^2) was obtained by summing the total number of questing nymphs (Qn) and total number of questing adults (Qa) collected per survey. The presence of larvae was noted but they were not collected, this was due to the tendency of larvae to cluster and therefore not represent true questing activity. The geometric mean of Qa, Qn and Qt was derived for each survey (100 m^2) from summing those collected from the ten individual transects.

Peak Qn activity was defined as the maximum number of Qn collected for a given survey. To standardise and compare between survey years, the commencement of questing for each year was defined as the first pre-peak survey for which the number of Qn was approximately 25% of maximum peak questing densities for that year. Decline of questing was defined as the first post-peak survey for which density of Qn observed, was less than or equal to 25% of the maximum peak for that year. Studies from Eisen *et al.,* 2002 and Hauser *et al.,* 2018, have both defined onset and decline of peak in a similar fashion but using a 10% of peak threshold. Considering the data collected during this study, 10% of nymphal density did not seem appropriate as this would often indicate peak onset as starting from the first survey of the year. A threshold of 25% provided a stronger differentiation between survey weeks which provided single tick specimens and those for which an increase in activity could be distinctly identified.

3.2.2 On-site environmental variables and microclimate data

Please refer to Chapter 2, section 2.4 for details of microclimate data collection and the use of longitudinal meteorological data.

In all survey years, for each 10m transect temperature (T, °C) was recorded at questing height, using a handheld, digital thermohygrometer. In addition, relative humidity (RH %) was recorded for each 10m transect, from mid-2015 onwards. Therefore, analysison RH and saturation deficit are confined to 2016. The arithmetic mean T and RH values per survey were calculated from the 10 individual transect readings. Vegetation height (cm) was measured along three points of each 10m transect (beginning, mid and end point) and an average height was recorded for each 10m transect. Details of the most dominant vegetation type in each transect was also recorded. To differentiate between longitudinal data, each of these variable will be referred to throughout the chapter as ‘on-site’ or ‘time of survey’ variables

3.2.3 Investigation of peak questing activity and meteorological data

Meteorological data for the 5km grid square in which the Bentley Wood survey site is located (coordinates: 51°02’42.0”N, 51°02'N 1°40'W), was provided by the UK Met Office from the UKCP09 dataset (Hollis & McCarthy, 2017). Daily mean temperature (Tmean °C), daily minimum temperature (Tmin °C), daily maximum temperature (Tmax °C), precipitation (mm) were supplied for all survey years. These measurements are referred to throughout the chapter as ‘weekly grid’ temperature (weekly Tmean °C, weekly Tmin °C and weekly Tmax °C) and weekly precipitation (mm). The same data was supplied for the 1961-1990 climatology; this dataset was used as the climatology baseline, against which, anomalies for survey years were calculated. Potential evapotranspiration (PET mm/wk) was calculated as described in Chapter 2. Based on previous studies which indicated 7°C as an important threshold for *I. ricinus* nymphal questing (Macleod, 1936; Perret et al., 2000; Randolph, 2004), a seven day/weekly continuous occurrence of Tmean $\geq$7°C was investigating in relation to increased nymphal questing activity.

3.2.4 Statistical analysis

All statistical analysis was carried out using the statistical software R version 3.4.0 (R Development Core Team, Vienna, Austria).

A variety of statistical tests were used to investigate the relationship between *I. ricinus* questing and recorded microclimate, weather and vegetation variables recorded at Bentley Wood; due to the normal distribution of annual temperature data, paired Student’s t-test were used to investigate the difference between temperature records; due to the non-normal distribution of Qn data, the Kruskal-Wallis test was used to investigate differences in tick questing densities, followed by post hoc pairwise Wilcox test. Analysis of the commencement, peak and cessation of Qn activity across all years was investigated by comparison of time-series plots. Finally, generalized linear mixed models (GLMMs) were used to investigate the significance of microclimate and environmental variables on the abundance of Qn. The glmmTMB package (Brooks *et al.* 2018) was selected for GLMM analysis because of its ability to deal with zero inflated count data. Three separate negative binominal GLMM models were constructed; GLMM1: to investigate weather and climate variables at an individual survey level across all four years, weekly mean temperature, precipitation, potential evapotranspiration and cloud cover, as well as the 1, 3 and 7 day lagged effects of temperature, precipitation and potential evapotranspiration were investigated using this model. GLMM2**:** to investigate microclimate and vegetation variables at an individual transect level. Temperature, vegetation height and type, all recorded at time of survey were investigated. GLMM3: to investigate additional microclimate variables recorded on-site, specific to 2016 i.e. temperature at time of survey, relative humidity and saturation deficit. 2016 was the only year were RH (%) was collected on-site for the entire year and so it was the only year for which this this variable could be considered in the analysis, likewise for saturation deficit which is calculated using RH (%).

3.3 Results

3.3.1 *Ixodes ricinus* annual questing activity: 2013 to 2016

During the four-year study period, 183 surveys and a total of 1830m^2 transects resulted in the collection of 1884 Qn and 259 Qa (Table 3.1). Significant intra-annual variation was observed in both Qn (H = 41.9, 3 d.f., p = 4 x10^{-9}) and Qa (H = 56.50015, 3 d.f., p = 3.3 x 10^{12}) densities, this was true of all years, with the exception of nymphal activity in 2013 and 2015 (Wilcox pairwise comparison = 0.68) and adult activity in 2014 and 2015 (Wilcox pairwise comparison = 0.9).

Qn densities of zero were recorded in a total of 35 surveys over the four-year period; a maximum annual Qn density of 96 Qn/100m^2 was recorded in May 2015 (week 21). Qa densities of zero were recorded in a total of 101 surveys, with a maximum of 19 Qa/100m^2 recorded in May 2013 (week 21).

For each year of the study, a distinctive peak in Qn activity i.e. maximum Qn density/100m^2, was observed in the first half of the year (**Fig 3.1**). The timing of this peak occurred between week 14 (April) and week 24 (June) for all years, however, its intensity varied between years. The greatest density of Qn/100m^2, observed in 2015 was four times that of that recorded in 2016 and more than two and a half times greater than that recorded in 2014.

In 2014, and to a lesser extent in 2016, a secondary autumnal peak in Qn activity was observed at the end of October (week 44). This later peak was attributed to just two surveys in 2014 and one survey in 2016. In both of these years exhibited reduced Qt densities in comparison with other survey years, as such, this secondary Qn peak could only be considered an actual peak in relation to the low numbers of Qn observed in the spring of 2014 and 2016, but not when compared to Qn densities of 2013 and 2015.

Table 3.1 Details of the total number of 10m^2 transects surveyed for each year, the percentage of transects in which Qn or Qa were observed; the percentage of weeks for which transects were positive for Qn and Qa; the total count of Qa and Qn, as well as the mean and 95 %confidence intervals {CI}are presented.

Year	**Survey weeks**	**Total transects**	**% Qa +ve transects**	**% Qa +ve weeks**	**Total Qa**	**Qa mean[CI] /10m^2**	**% Qn +ve transects**	**%Qn +ve weeks**	**Total Qn**	**Qn mean [CI] /10m^2**
2013	39	390	18.2%	62%	118	1.6 [0.9, 2.4]	39.5%	77%	575	5.4 [3.1, 9]
2014	50	500	9.8%	50%	66	0.8 [0.5, 1.2]	31.4%	80%	312	3.5 [2.4, 4.9]
2015	49	490	9.8%	34%	62	0.7 [0.4, 1.1]	39.6%	73%	780	6.9 [4.5, 10.2]
2016	45	450	2.9%	20%	13	0.2 [0.07,0.3]	24.9%	62%	217	2.2 [1.3, 3.4]

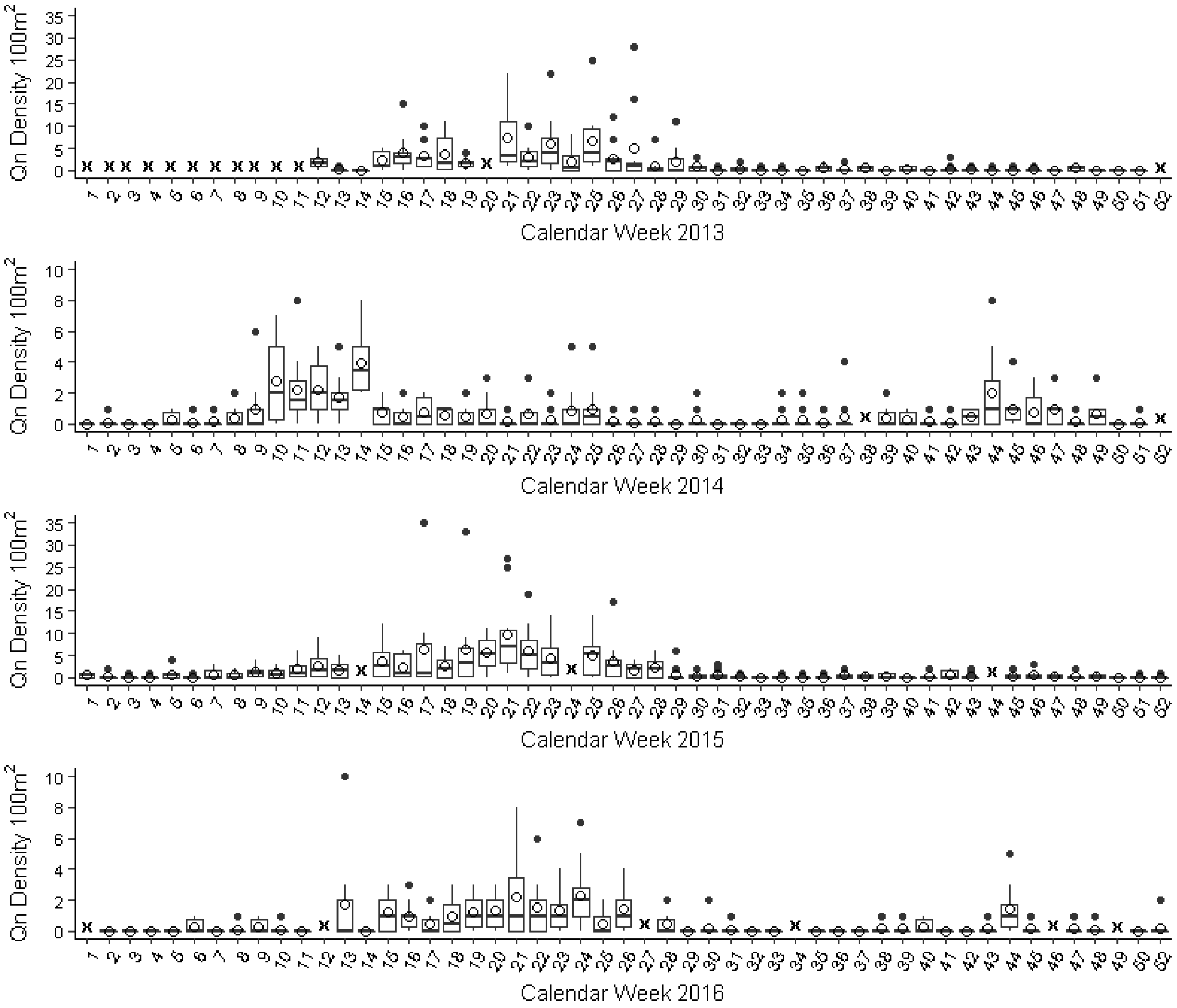


Fig 3.1 Weekly Qn count data per 100m^2 for each survey year. Each box describes a single survey of 10 transects. Boxes present the interquartile range of transect results: outliers are represented by a solid black circle, the median is represented by a black line and the mean number of Qn collected during that survey is represented by an open black circle. Weeks for which no surveys were conducted are represented by an x. Note the scale difference between plots to highlight distribution variation in each year.

Qa activity also exhibited an annual increase in activity, which displayed a similar pattern of accumulation to Qn activity, in that increased questing occurred during the spring months; although, activity continued into summer in three of the four years (2013 to 2015). Similarly to Qn the intensity of this increased activity varied between years, however, Qa densities always remained less than that of Qn in any given year (Fig 3.3). In 2016, the final study year, the maximum Qa density/10m^2 transect never exceeded more than a single Qa. It should be noted that as this study continued in 2017 and 2018, Qn remained at approximately 2016 levels, while Qa numbers recovered beyond 2016 numbers but remained below pre-2016 numbers.

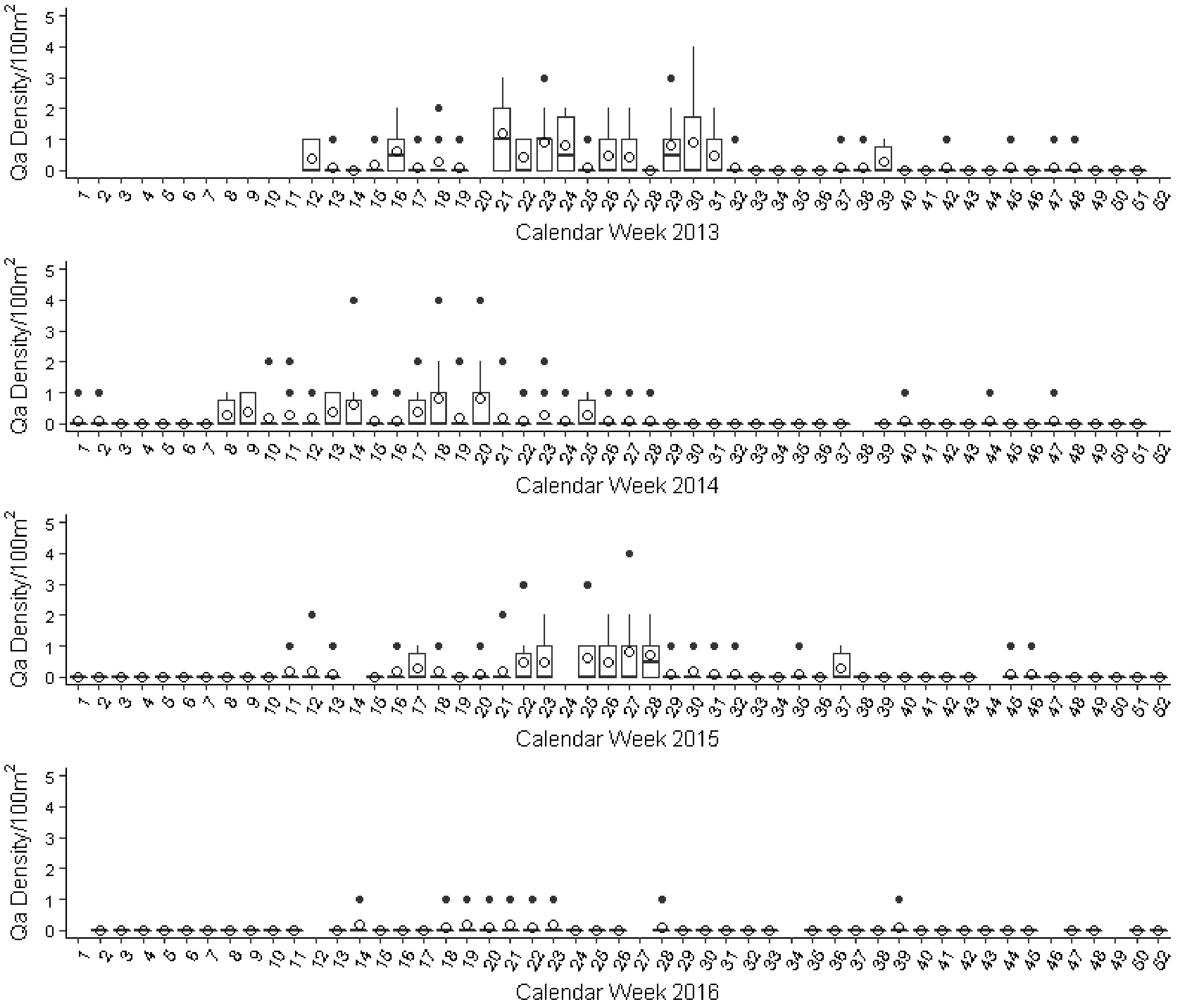


Fig 3.2 Weekly Qa count data per 100m^2 for each survey year. Each box represents a single survey – outliers are represented by a solid black dot, the median is represented by a black line and the mean number of Qn collected during that survey is represented by a black ring. Weeks for which no surveys were conducted are represented by an

3.3.2 *I. ricinus* activity – annual seasonal trends

When surveys commenced in March 2013 (week 12), *I. ricinus* were already active, being collected in the first survey. Qn densities per survey (100m^2) varied on the weeks approaching peak questing, which occurred in late May (week 21). In 2015, peak Qn activity also occurred in week 21. Notably, these years exhibited the highest nymphal densities recorded during the entire study period. In comparison, 2014 and 2016 observed Qn densities were much reduced (Fig 3.1).

An early onset of initial questing activity was observed for 2014, commencing in week 9 (late February) and peaking in week 14 (early April), much earlier than in other years. Not only was this year characterised by an earlier commencement in Qn but the duration for which increased activity was sustained was shorter than in other years, with the peak Qn densities declining sharply after the maximum peak in week 14 (early April).

Of all study years, 2016 produced the fewest Qt and the fewest number of Qn positive surveys (Table 3.1). In contrast with other years, the first survey which recorded the appearance of a Qn did not occur until early February (week 6). From late March onwards, low densities of Qn were collected consistently until the end of July, peaking in early June (week 24). Following the end of July, however, just eleven of the remaining twenty surveys observed Qn.

In summary, 2013 and 2015 were characterised by higher *I. ricinus* Qn densities, particularly during the spring peak which occurred from week 15 to week 27 (mid-April to early July) in 2013 and week 12 to week 26 (late March to late June) in 2015. In both years, a unimodal pattern in Qn questing was observed, with peak Qn density occurring in week 21 (April to early July).

2014 was characterised by lower Qn densities and a bimodal pattern of Qn questing activity. The initial higher intensity spring increase in activity ran from week 8 to week 14 (late February to early April), peaking in week 14. The second autumn period of increased activity ran from week 44 to 45 (late October to early November).

2016 was characterised by the lowest tick densities of the four year period, although, an increase in spring questing activity was present, as in previous years. In addition, an autumn peak occurred, attributed to a single survey, coinciding with the secondary peak in 2014 activity (week 44, late October).

Qa activity followed a similar pattern of activity to Qn for all years (Fig 3.2); 2013 exhibited a period of increased activity between week 16 and week 27 (mid-April to early July), peaking in week 21(end of May). In 2014 increased Qn activity occurred from week 8 (late February), peaking in both weeks 18 and 20 (late April and early May). 2015 was characterised by a broken period of increased activity from week 11 until week 28 (mid-March to early July), with peak occurring in week 27(early July). 2016 exhibited the lowest Qn density of the four year study period. A broken period of increased Qn activity was observed from week 14 to week 23 (early April to early June), with single Qa specimens being collected on two other occasions (week 28 and 39, July and September) for the remainder of that yea

3.3.3 Commencement and decline of peak questing nymph activity in relation to 5 km grid variables

The timing of the commencement and decline of peak Qn activity for each year of the study was investigated in relation to weekly grid temperature (weekly Tmean °C), weekly precipitation (mm/week) and evapotranspiration (PET) (Fig 3.3).

In 2013, surveys did not start until late March, so no comment can be made of Qn activity before this point. With the exception of 2016, for all study years i.e. 2014 and 2015, the commencement of peak Qn activity, commencement being defined as the week for which the number of Qn recorded/100m^2 was ≥ 25% of the maximum Qn/100m^2 (peak), occurred the week before Tmean exceeded 7°C for more than seven consecutive days. In 2016, both commencement of Qn activity and seven consecutive days of Tmean at 7˚C occurred in the same week. In all year's actual peak Qn occurred after the 7 °C threshold had been reached (red dashed line in Fig 3.3). In 2014, the 7 °C Tmean threshold occurred earlier than in other year, as did peak *I. ricinus* activity.

Declines in Qn activity appeared to coincide with an increase in PET towards 25 mm/week, his was true of all years with the exception of 2014, for which commencement, peak and decline in activity were confined to a seven week period, all of which occurred before PET reached 22mm/wk. In 2013 and 2015, the week in which maximum PET was recorded, occurred immediately prior to the decline of peak Qn activity; decline was defined as the week for which the number of Qn recorded/100m^2 was ≤ 25% of the maximum Qn/100m2 (peak). In 2014 and 2016, peak Qn activity exhibited immediate decline in the week following the peak, in both years this occurred a few weeks before PET had reached its maximum for those years (>35mm/wk).

The influence of both the 7˚C weekly Tmean threshold and weekly PET in relation to Qn activity was further indicated in with mixed model analysis (Table3.6). Both the effect of mean temperature seven days prior to survey and the PET on week of survey were described as significant factors in the density of Qn/100m^2.

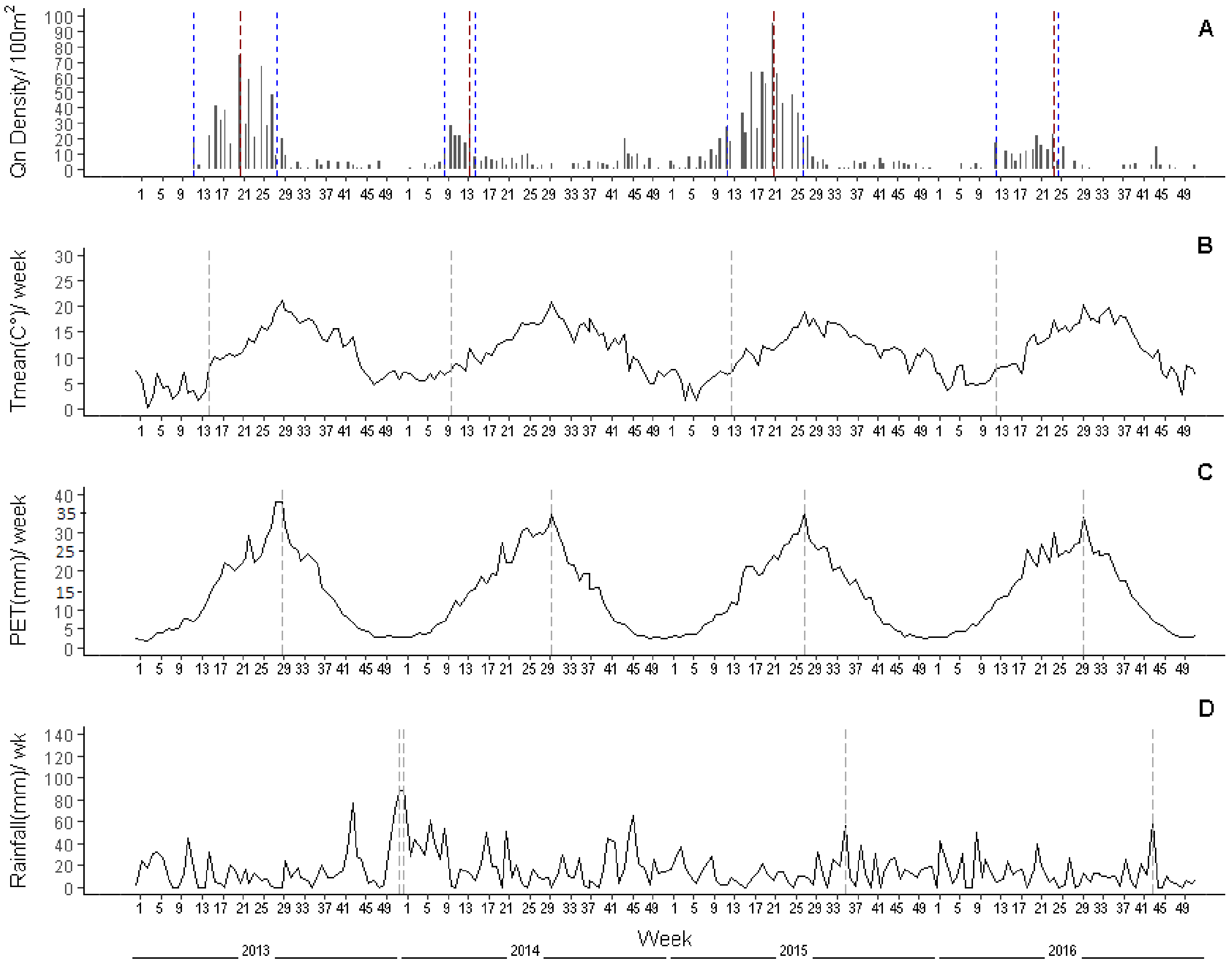


Fig 3.3 Time series over the four year study period comparing: **A.** Qn/100m2, red dashed line represents peak activity, blue dashed lines represent week of 25% of maximum peak; **B.** weekly Tmean (°C)*, grey dashed line represents first week of Tmean at≥7°C for seven consecutive days: **C.** weekly potential evapotranspiration (PET), week of maximum PET (mm/week);**D** weekly rainfall, grey dashed line represents week of maximum rainfall (mm/week)*. *obtained from 5 km gridded UKCP0

3.3.4 Climate observations during the study period

Meteorological data for the 5 km grid (UK Met Office UKCP09 data) in which Bentley Wood is located, indicated a cold start to the initial months of 2013, with twenty-four frost days (day of Tmin <0°C) recorded across January and February. In spring (March, April, May), twenty-six frost days were recorded; this represented the greatest number of annual frost days recorded during the study (Table 3.2). Overall, 2014 recorded the least number of frost days across all years of the study, with just eighteen in total, over a third less than the preceding year. Spring of 2014 recorded just four frost days, additionally, the date for which the temperature exceeded 7°C for more than seven consecutive days occurred earlier than in any of the other study years, occurring in the first week of March (week 10).

Table 3.2 Number of frost days calculated for each season and year of the study. Data calculated from the UKCP09 5km gridded data. Winter relates to December of the previous year, plus January and February of the current year; spring refers to March, April, May; summer refers to June, July, August; autumn refers to September, October, and November.

Number of seasonal frost days					
Year	**Winter**	**Spring**	**Summer**	**Autumn**	**Total**
2013	34	26	0	5	65
2014	11	4	0	3	18
2015	35	3	0	3	41
2016	16	15	0	5	36

With just 3 frost days recorded in spring, 2015 had the fewest spring frost days of all study years. However, January, February and the previous December, had the second highest number of frost days; twenty two in total. Of the four year study period, the second greatest numbers of spring frost days were recorded in 2016. Sixteen frost days, one more than that in spring were recorded in January and February.

Anomaly analysis (relative to 1961-1990 baseline) for this 5km area, revealed 2013 had a slightly below average Tmean for the months of February, April and May, and almost 2°C below average for March (Fig 3.4). Although, the previous winter months (November and December 2012) exhibited above average temperatures. In 2014, all months exceeded temperature norms, except for August, which was slightly below average. 2015 exhibited higher than average temperature for spring and early summer months, and well above average for the months of November and December; with a mean temperature (± SE) of 10.7°C ± 0.3°C for the latter, twice that of other study years. All months of 2016 exhibited above average or comparative temperatures to the climate normal other than May and July, which was slightly below average.

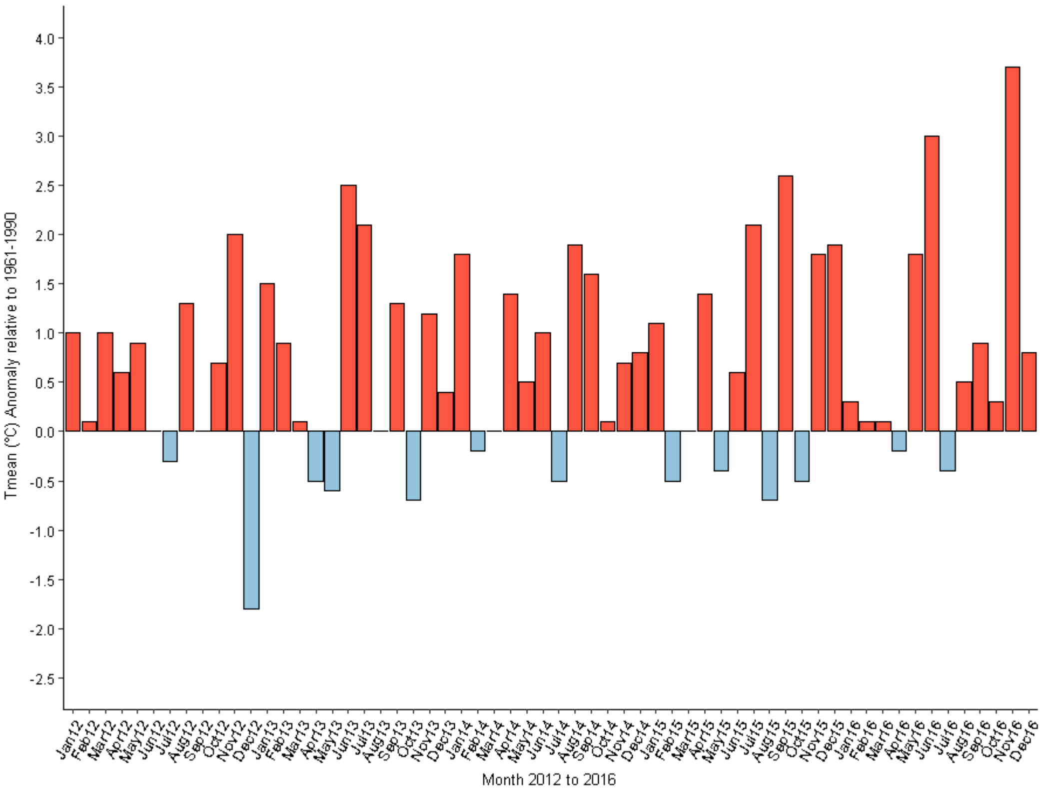


Fig 3.4 Standardized temperature anomalies (relative to 1961 to 1990) for the 5km grid in which Bentley Wood is located. Anomalies for each year of the study plus the year prior to the study are shown.

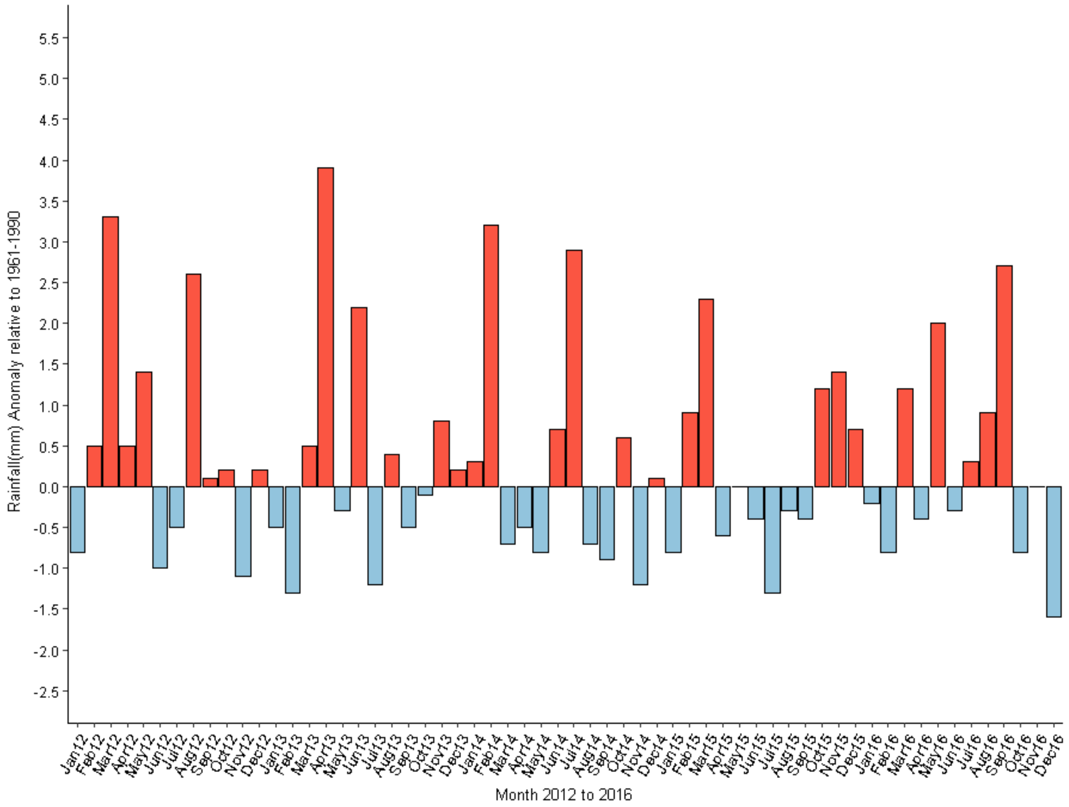


Fig 3.5 Standardized precipitation anomalies relative to 1961 to 1990 for 5km grid in which Bentley Wood is located. Anomalies for each year of the study plus the year prior to the study are shown.

Precipitation in 2013 was relatively similar to the 1961-1990 climatology, with just slightly below average precipitation for most months, with the exception of October and December which exhibited higher than average precipitation (**Fig 3.5**). This high rainfall continued into January and February of 2014, a total of 411.8mm was recorded; more than double that for this period in any of the other years. This excessive precipitation was also reflected in the anomaly analysis, revealing these early months of 2014 as well above the 30 year climate average. Precipitation was also above average for April and May, resulting in the highest recorded rainfall of the four year period.

3.3.5 Seasonal *Ixodes ricinus* nymph density in relation to 5 km grid variables

Examination of time series plots for each year of the study did highlight some potentially significant relationships between increased densities of Qn with increased daily Tmean (°C) towards 7°C in 2015 and 2016. 2014 was somewhat of an anomality both in Qn activity and weather; increased Qn questing commenced earlier, as did peak and decline, there was also an earlier onset of daily Tmean (°C) ≥7°C, coupled with a low number of frost days in the preceding months. This coincided with an earlier commencement of nymphal questing than that observed in other years.

In all years, actual peak Qn was recorded after the occurrence of the first week of Tmean (°C) ≥7°C; however, in most years commencement in nymphal questing had already occurred before this threshold 7°C was reached.

An increase in PET (mm/week) approaching ≥25 mm/week coincided with a decline in Qn activity in all years, with the exception of 2014. However, a post summer decline in PET did not coincide with a secondary increase in questing.

On examination of time series plot, precipitation did not appear to directly influence Qn densities. Although, maximum rainfall recorded for 2013 was recorded in the final week of the year and was subsequently followed by the week of maximum precipitation recorded in 2014; this higher than average rain fall did coincide with a low Qn densities in spring 2014.

Generalized linear mixed models (GLMM) with a negative binomial error distribution were used to investigate the effect of various weekly meteorically and lagged meteorically variables (Table 3.3) on Qn density. To account for spatial and temporal autocorrelation, site and survey date were included as random effects. Model selection was based on Akaike Information Criteria (AIC). Models were tested for over/under dispersal, and spatial and temporal autocorrelation.

Table 3.3 The list of variables considered in GLMM1, which was used to investigate the significance of weekly and lagged meteorological variables on questing nymphs (Qn) densities.

List of weekly meteorological variables considered in the initial GLMM1
All variables were obtained from or calculated using 5 km grid UKCP09 data
Weekly mean temperature (°C)
Weekly minimum temperature
Weekly maximum temperature
Weekly mean precipitation (mm)
Weekly potential evapotranspiration
1 day lag daily mean temperature (°C)
3 day lag daily mean temperature (°C)
7 day lag daily mean temperature (°C)
1 day lag daily min temperature (°C)
3 day lag daily min temperature (°C)
7 day lag daily min temperature (°C)
1 day lag daily max temperature (°C)
3 day lag daily max temperature (°C)
7 day lag daily max temperature (°C)
1 day lag mean precipitation (mm)
3 day lag mean precipitation (mm)
7 day lag mean precipitation (mm)
1 day lag mean PET (mm/week)
3 day lag mean PET (mm/week)
7 day lag mean PET (mm/week)

Table3.4 GLMM1 The effect of long term weather and climate variables on questing nymph (Qn) densities across all years of the study: parameter estimates of fixed effects from a generalised linear mixed model (GLMM) –those effects indicated as significant are highlighted. Analysis based on weekly survey data.

GLMM1 output				
Coefficients	Estimate	Std. error	Z-value	p-value
Intercept	2.619	0.313	8.347	< 2e-16
Weekly Tmean (°C)	-0.049	0.059	-0.827	0.408
Weekly potential evapotranspiration (mm)	0.116	0.014	8.517	**< 0.001**
7-day lag of weekly 5km grid Tmean (°C)	-0.192	0.046	-4.169	**<0.001**
3-day lag of weekly 5km grid Tmean (°C)	0.045	0.041	1.097	0.273
1-day lag of weekly 5km grid precipitation (mm)	-0.100	0.027	-3.722	**<0.001**

Across all years, significance of a one day lagged effect for precipitation on Qn activity was indicated from mix model analysis (Table 3.4). When examining the actual rainfall recorded for the week of survey, surveys recording >25 Qn/100m^2 all occurred on weeks of <25mm of rainfall.

A 7-day lag in Tmean (°C) was also found to be significant ($P < 0.001$), which supports the potential influence of increased temperature in spring and its influence on Qn densities.

PET (mm/week) was also found to be significant which may support its further use as a predictive to in the decline of Qn following spring peak.

3.3.6 Seasonal *Ixodes ricinus* nymphal density in relation to on-site microclimate variables and vegetation

No Qn were recorded at on- site temperatures of <4 °C across all years, and < 6% of total Qn for any given year were observed at temperatures of 4°C to 6 °C. Just two surveys recorded a temperature of below 5 °C of the entire study; one in January 2013, which recorded no questing ticks at 4 ⁰C; the other, in January 2014 produced three Qn at 4 ⁰C, one in each of three transects. In 2013 and 2014 over 80% of Qn were observed at temperature between 7 ⁰C and 21 ⁰C; this was true of over 60% of Qn in 2015 and 2016 (Fig 3.6).

The highest recorded on-site temperature was 31°C, recorded in July 2014 and again in July 2016. Qn were observed on both of these occasions; three in 2014 and two in 2016. On both occasions, Qn were identified in one of two transects.

Surveys which recorded the greatest number of Qn i.e. greater than 50Qn/100m^2 were all conducted on days where temperatures ranged between 14°C and 22°C (Fig 3.6). However, outliers of 49 Qn at 26°C (June 2015) and 23 Qn at 28°C (June 2016) were also recorded.

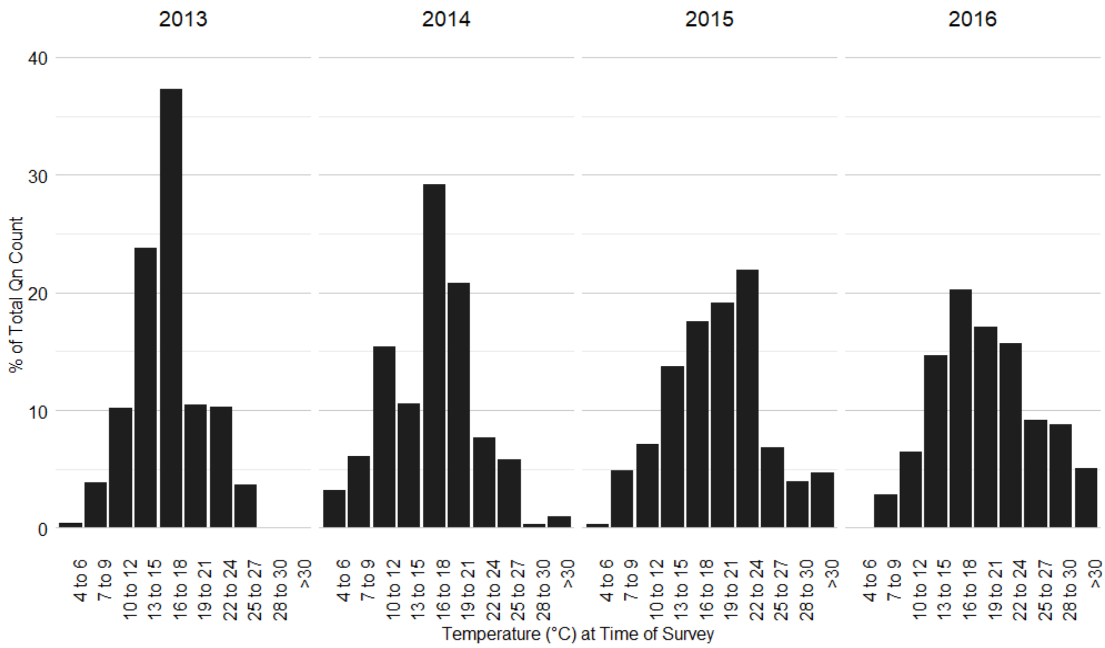


Fig 3.6 Percentage of total questing nymphs (Qn) counts from individual transects, observed at different on-site temperature (°C) ranges. Temperature at time of survey refers to that recoded for each individual transect at time of survey.

The relationship between Qn activity and on-site temperature (recorded by handheld monitor) on the weeks leading up to peak Qn activity i.e. the greatest density of Qn collected/100m²) was investigated (using Spearman's correlation) for each study year. A positive correlation was identified between on-site temperature and Qn for this period for all study years; 2013: $r_s = 0.89$, P = 0.002; so too in 2014; $r_s = 0.87$, P = 0.003; 2015: $r_{s\,=}$ 0.7, P = 0.01, and in 2016; $r_s = 0.61$, P = 0.04.

GLMMs outputs investigating the influence of onsite variables (Table 3.5), also indicated the significance of onsite Tmean (z value = 4.905, P <0.001) on Qn densities.

The importance of relative humidity (RH%) on Qn was investigated for 2016. 85% of Qn were recorded at RH of between 41% to 90% (Fig 3.7). Qn were still detected at lower densities on days of RH of <40%; 14% of all Qn recorded in 2016 were recorded at RH of 21% to 40% (Fig 3.8).

However, GLMM analysis for 2016 data did not find RH (%) as a significant influence on Qn activity.

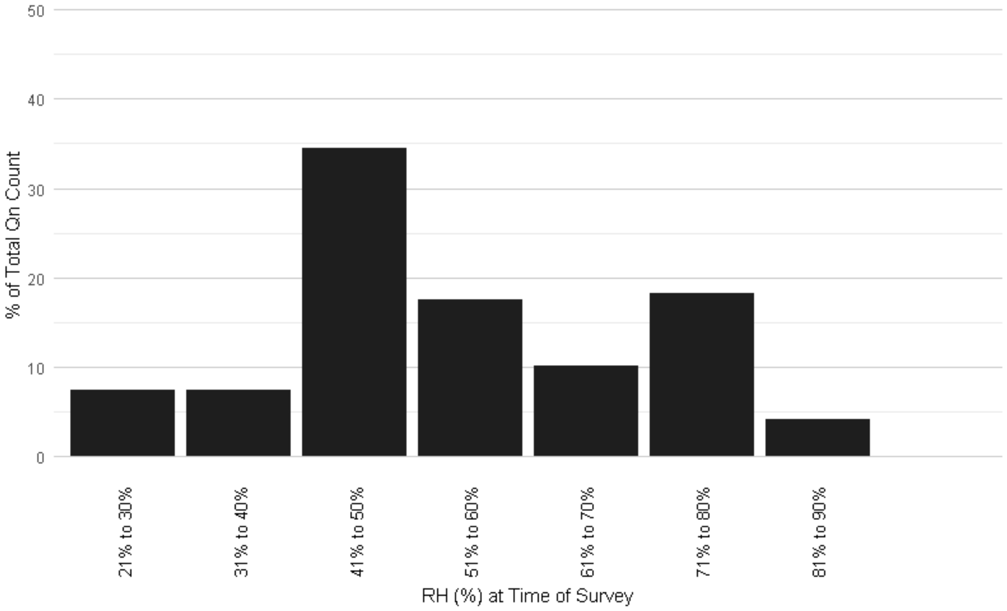


Fig 3.7 Percentage of total questing nymphs (Qn) counts from individual transects, observed at different on-site relative humidity (%) ranges. RH (%) at time of survey refers to that recoded for each individual transect at time of survey.

On-site temperature and relative humidity were used to calculate saturation deficit at the time of survey. For 2016, 85% of Qn were observed at saturation deficits of <12 mmHg. Qn were observed at saturation deficits of 13 mmHg up to 24mmHg but this accounted for just 15% of all Qn observed in 2016 (Fig 3.9).

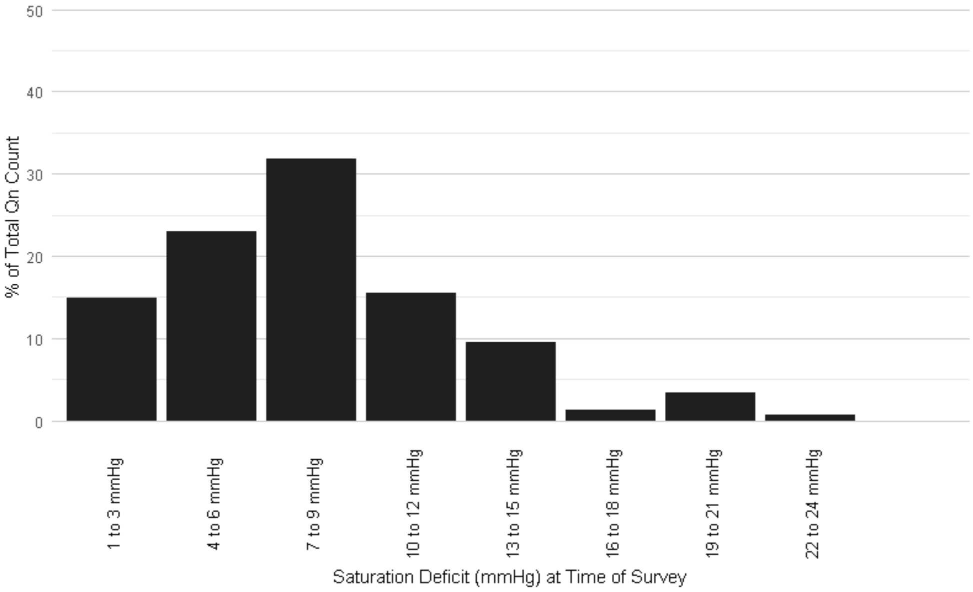


Fig 3.8 Percentage of total questing nymphs (Qn) counts from individual transects, observed at different on-site saturation deficit (mmHg) ranges. Saturation deficit at time of survey refers to that

calculated for each individual transect from the temperature (°C) and relative humidity (%) recorded at time of survey.

Vegetation at the site followed a consistent pattern of seasonal growth, culminating in maximum vegetation heights observed in mid-summer. Management of the survey area and mowing of the transects occurred at several points throughout the study. The predominant vegetation types varied between transects and season, but generally consisted of a mix of grass, bramble, bracken, nettles and leaf litter. At a transect level, glmm analysis indicated a significant negative relationship between vegetation height and Qn activity, however, no specific vegetation types were identified as significant (**Table 3.5**)

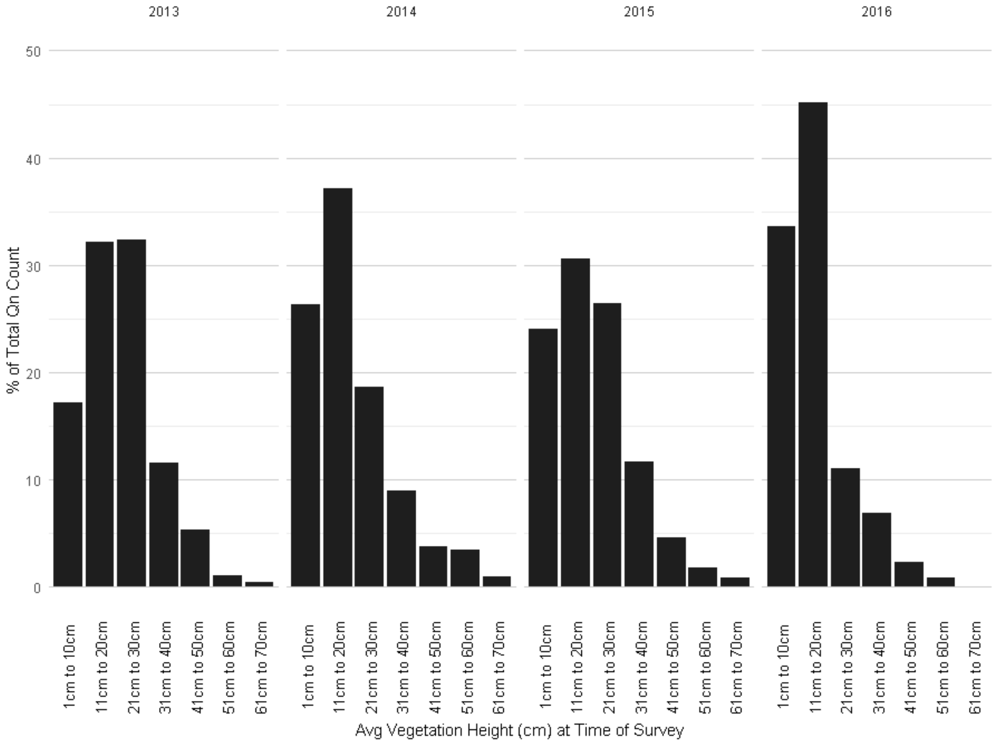


Fig 3.9 Percentage of total questing nymphs (Qn) counts from individual transects, observed at different on-site vegetation height (cm) ranges. Avg vegetation at time of survey refers to the average vegetation height calculated from 3 measures of vegetation height within each individual transect at time of survey**.**

Table 3.5 The list of variables considered in GLMM2, which was used to investigate the significance of microclimate and vegetation variables (recorded at time of survey) in relation to questing nymphs (Qn) densities.

List of weekly microclimate variables considered in the initial GLMM2
All variables were recorded on-site at time of survey
Temperature (°C) at time of survey
Vegetation height
Vegetation: bramble
Vegetation: grass
Vegetation: leaf litter
Vegetation: nettles
Vegetation: exposed ground/short grass

Table 3.6 GLMM2: The effect of on-site variables on Qn densities across all years of the study: parameter estimates of fixed effects from a generalised linear mixed model (GLMM) –those effects indicated as significant are highlighted. Analyses based on individual transect data.

GLMM2 output				
Coefficients	Estimate	Std. error	Z-value	p-value
Intercept	-1.882	0.772	-2.439	0.015
Temperature (°C) at time of survey	0.079	0.016	4.905	**<0.001**
Vegetation height	-0.036	0.005	-7.053	**<0.001**
vegetation: Bramble	0.419	0.735	0.571	0.568
vegetation: grass	0.488	0.720	0.678	0.498
vegetation: leaf litter	-0.457	0.739	-0.617	0.537
vegetation: nettles	0.504	0.8066	0.625	0.531
vegetation: exposed ground/short grass	0.644	1.382	0.466	0.641

Table 3.7 The list of variables considered in GLMM3, which was used to investigate the significance of microclimate variables (recorded on site at time of survey) on questing nymphs (Qn) densities in 2016 only.

List of weekly microclimate variables considered in the initial GLMM3
All variables were recorded on-site at time of survey
2016 survey Mean temperature (°C) at time of survey
2016 survey Mean RH (%)
2016 survey Saturation deficit (mmHg)
2016 survey Vegetation height(cm)
2016 survey Dominant vegetation type: Bramble; Grass; Ivy; Leaf; Nettle; Saplings

Table3.8 The effect of on-site variables on Qn densities 2016 only: parameter estimates of fixed effects from a generalised linear mixed model (GLMM) - those effects indicated as significant are highlighted. Analysis based on weekly survey data, recorded at time of survey.

GLMM3 output				
Coefficients	Estimate	Std. error	Z-value	p-value
Intercept	-1.607	1.37	-1.173	0.241
Temperature (°C) at time of survey 2016	0.138	0.058	2.372	**0.018**
Relative humidity (%) 2016	-0.025	0.019	-1.339	0.181
Saturation deficit 2016	-0.122	0.078	-1.567	0.117

3.4 Discussion

The aim of this study was to investigate possible key weather related cues which might be used in predicting commencement of spring questing *I. ricinus*, and the decline in questing immediately following peak activity. In addition, influence of ground level microclimate and vegetation variables were investigated for their association with Qn questing activity and density.

The study highlighted the seasonal and annual variation in Qn density that can occur in a single habitat, in particular, the variation in intensity and timing of spring peak Qn activity. For each year of the study, a distinctive peak in Qn activity i.e. maximum Qn density/100m^2, was observed in the first half of the year, with a lower intensity secondary peak occurring in both 2014 and 2016.

The first weekly occurrence of a Tmean of ≥ 7°C coincided with an overall increase in Qn densities in all years but commencement of this increased activity appeared to occur before this temperature threshold had been reached. This suggests that a threshold temperature of 6°C or 6.5°C may be more appropriate for potential prediction of increased Qn activity. PET (mm/week) approaching 25mm/week appeared to coincide with a post-spring peak decline in Qn densities in all but one year (2014). Regression analysis suggested that PET, a 7-day lag in Tmean (°C) and a 1-day lag in precipitation were found to have significant effects on Qn densities. Temperature and vegetation height recorded at time of survey were also found to have a significant effect on Qn density.

3.4.1 *Ixodes ricinus* nymphal and adults questing densities

In each year of the study, the density of Qn was greater than the density of Qa, although, the density of both in any given year varied substantially. In 2013 a greater density (mean Qn [CI]:5.4/10m^2 [3.1, 9]) of questing *I. ricinus* ticks was recorded than in 2014 (3.5/10m^2 [2.4, 4.9], however, Qn densities exceeded Qa densities in both years, by a factor of five. The greatest density of questing *I.* ricinus of the study was recorded in 2015 (6.9/10m^2 [4.5, 10.2], with Qn densities exceeding Qa by a factor of twelve. In 2016, overall questing *I. ricinus* densities were at their lowest (2.2/10m^2 [1.3, 3.4]) of the four year study, and Qn densities were almost seventeen times greater than Qa. It is worth noting that as this site continued to be monitored weekly throughout 2017 and 2018, Qa numbers did recover from 2016.

The fact that overall Qn densities were higher than that of Qa in this open woodland, is not hugely surprising, and has been observed in several other studies which have used a similar sampling technique in the UK (Randolph *et al.* 2002) and in Europe (Cayol *et al.*, 2017). Given that the process of developing to adult stage requires survival through nymphal stage, plus the acquisition of and successful feeding on a secondary host, fewer nymphs would likely survive and progress to adult stage.

Due to the complex interaction of factors influencing *I. ricinus* populations, it is difficult to ascertain an exact explanation for the variation in the densities from year to year, or the ratio between Qn and Qa densities in any given year. Multiple factors, including some out-with the scope of this study, play an important role in questing tick densities, and to some extent, on the temporal questing pattern. Host abundance is certainly one of these factors (Hofmeester *et al.* 2017, Jaenson *et al.* 2012; Ruyts *et al.* 2018). The availability of large host such as deer provides a large body mass for all stages of the tick to feed. Although, deer abundance was not investigated as part of this study; Roe Deer (*Capreolus capreolus*), Fallow Deer (*Dama dama*), Reeves Muntjac (*Muntiacus reevesi*) and Red Deer (*Cervus elaphus*) have all been confirmed in the area of study (Harris & Linham 2017), and deer have been observed on multiple occasions during field surveys (personal observation). The presence of deer, no doubt, contributes to sustaining the tick population within the woodland, as well as acting as a mechanism of distribution. Deer do roam freely in this area so it is difficult to attribute the inter-annual fluctuation of ticks, on deer abundance.

Small mammal and rodent abundance are of benefit to the larval and nymphal *I. ricinus* stages – these potential hosts inhabit the vegetation undergrowth, and are more easily accessible to the less mobile, larval stage. While the intra-annual small mammal density for this particular study period is unknown, the abundance of several small mammal species and their interaction with *I. ricinus* in woodland nearby has previously been described by Cull *et al.* 2017. In this study, several rodents species were reported at this site, primarily; yellow necked mice (*A. flavicollis*), wood mice (*A. sylvaticus*) and bank voles (*Myodes glareolus*). *A.*

flavicollis exhibited the highest infestation rates, and larval stages representing 98% of ticks removed from all species.

These factors, particularly rodent abundance, can themselves, be influenced by factors such as mast years; years of a bumper harvest of nuts/berries produced in woodlands (Schnurr *et al.* 2002); and land management strategies (Millins *et al.* 2017), such as fencing and biodiversity management (Gilbert *et al.* 2012). Fluctuations in host populations, impacts, not only tick densities but also play an important role in pathogen dynamics. This interaction is discussed in more detail in Chapter 4.

3.4.2 Initiation and decline of nymphal questing activity – seasonal trend

The temporal pattern of *I. ricinus* questing has been shown to vary with geographical location; the influence of local weather, and microclimate, no doubt, playing an important role. The wide distribution of this species, longitudinally from the Iceland (19.5° W) to the Ural Mountains (45° E), and laterally from Norway (66° N) to North Africa (30^0 S) (Alfredsson et al., 2017; Pietzsch et al., 2005a; Soleng et al., 2018), has results in the observation of multiple questing patterns. Bimodal patterns have been observed in Russia (Korotkov *et al.* 2015), central Europe (Daniel *et al.*, 2015), and have previous been reported in England (Randolph *et al.*, 2002). Unimodal peaks have been reported in Ireland (Gray, 2008), Wales (Steele & Randolph, 1985) and Algeria (Yousfi-Monod & Aeschlimann, 1986), albeit at different times of the year. Both patterns can be temporally diverse, depending on local microclimate and weather, which can in turn be influenced by factors such as elevation and aspect (Qviller *et al*., 2014).

In the study presented here, a relatively consistent unimodal pattern in spring Qn activity was observed for each year of the study. Each year exhibited an increase in Qn activity in late February/early March (week 9 to 13), which continued into spring and peaked in mid-spring or early summer (between week 14 and week 24). For three of the survey years; 2013, 2015 and 2016, the pinnacle of Qn activity occurred within the same four week period: week 21 to week 24 (end of May to mid-June). In this case the earlier years also exhibited a much greater intensity in Qn numbers, while 2016 exhibited a much reduced level of questing activity – the lowest of the four year study. Conversely, 2014 Qn numbers began to rise in week 9 (end of February), reaching peak in week 14 (early April). Again, the intensity and indeed, the duration of this peak was much reduced, compared to 2013 and 2015. It is interesting that both years which exhibited the lowest density of spring Qn, exhibited a secondary questing in autumn. Based on this study, the mechanisms behind this are not quite clear.

The specific timing of the peak in each year seems to be, at least in part, driven by the increased temperature in the early spring. As reported in the results section, only two surveys recorded an on-site temperature of less than 5°C, and Qn were recorded on just one of these occasions; three Qn at 4°C. The contrast between these surveys may lie in the fact that light snow was observed in the 2013 survey, suggesting a wind chill factor; whereas damp vegetation was observed in the 2014 survey, suggesting lack of frost.

Greater Qn numbers (n=50Qn/100m$^{2)}$ were observed in surveys recording an on-site temperature of greater than 10°C; with 14°C to 22 °C being associated, for the most part, with higher densities of Qn. Statistically significant positive correlations between on-site Tmean and Qn densities were determined for the onset weeks of increased activity. On-site temperature at time of survey was also found to have a positive significance (P <0.001) from the multivariate analysis conducted (Table 3.6, GLMM2). These results concur with previous studies relating seasonal temperature to increased Qn density (Kiewra *et al.* 2014; Perret *et al.* 2000; Qviller *et al.* 2014).

The fact that the onset of Qn activity in 2014 commenced much earlier than the other study years, could in part, be related to the fact that 2014 recorded the least number of frost days; much lower than 2013 and 2015, and similar to that of 2016. Likewise, mean weekly temperatures for 2014 also exceeded climate normal for all months of 2014 other than August (Fig 3.4). The increase in weekly Tmean towards 7 °C also occurred earlier in this year than in any other study year.

I. ricinus can survive acclimatized short term exposure to extremely low temperatures of -14.4C° to -18.9°C , however, the accumulated exposure to temperatures of -10°C is detrimental to the survival of unfed nymphs and fed nymphs in diapause (Dautel & Knülle, 1997). Evidence suggests that there is a level of evolutionary adaptation to localised climatic conditions and that nymphs may adapt to quest at temperatures consistent with their home range (Tomkins et al., 2014) and perhaps, specific microclimate .

In all years, the distinctive peak was followed by a sharp decline in Qn numbers, which did not recover to previous intensity; although, a slight increase in Qn numbers was observed in one survey conducted in late October, for both 2014 and 2016. High summer temperatures certainly increase the risk of desiccation and questing has been reported to cease completely at temperature approaching 35°C (MacLeod, 1935), driving questing ticks into the ground level vegetation to rehydrate and escape excessive temperatures. On the few occasions where Qn were recorded at temperatures exceeding 30°C (July 2014 and 2016), they were collected in shaded transects, out of direct sunlight. This indicates that while temperature may exceed optimal levels for *I. ricinus*, it may still attempt to quest if it is protected from the direct intensity of the sunlight. Investigation of peak *I. ricinus* activity and PET indicated a relationship between max PET and a decline in Qn questing. PET approaching 25-35mm/week, appeared consistent with sharp decline in Qn densities (Fig 3.3). PET is calculated from 5 km gridded temperature and so is correlated with maximum annual temperature, but its calculation also considers other factors such as day length.

The summer dip in numbers may also be the result of unsuccessful questing of the spring cohort of ticks, energy levels depleted before a new host is acquired. Indeed, previous studies have suggested that a small secondary peak in autumn activity (Randolph, 2002), may represent a second cohort of Qn emerging from moulted larvae which overwintered and fed in spring, rather than the re-emergence of the same cohort of Qn active in spring (Perret *et al.*, 2004). Conversely, in situations where host abundance is high, it could be the case that a dip in summer Qn densities is a result of successful questing and feeding, resulting in the spring cohort of Qn already entering developmental diapause before the onset of summer.

Factors other than temperature were also highlighted in the multivariate analysis; weekly precipitation was shown to have a less substantial but still significant negative relationship with nymphal questing densities. Given that laboratory observations have illustrated the negative impact of desiccation on *I. ricinus,* and other field studies have found RH% (Perret *et al.,*2000), and in some cases increased soil humidity (Medlock *et al*., 2008), to be a significantly positive influence on all stages of *I. ricinus*, this is perhaps a contradictory observation. While a previous study in England reported the negative effects of precipitation on *I. ricinus* host attachment (Robertson *et al*., 2000); all surveys for the study presented here were conducted on dry days, therefore, inability to attach to the flag should not have impact on Qn observed. Potentially, excessive rain may have an impact on the emergence of *I. ricinus* form periods of diapause, or more likely have an impact on its immediate environment; flooding areas or damping down vegetation, thereby inhibiting the ability to quest. Days of heavy precipitation also tend to be cooler in the UK which could return to the relationship with Qn and temperature

Vegetation too, had a significant negative relationship with questing nymph activity. This was often observed in the field – when vegetation was excessively high, few questing nymphs were found. This could potentially be due to the physiology of smaller nymphal ticks; a combination of persevering energy by negating the excessive excursion of climbing high, only to descend again if under treat of desiccation. Vegetation height is linked to temperature and precipitation, however, and as such, it can be difficult to separate the potential influence of these three factors. Higher vegetation may indeed impact on the efficiency of the survey process, as the flag fails to come into sufficient contact with questing ticks (Dobson *et al.* 2011, Medlock *et al.* 2012).

In terms of tick bite risk management, vegetation is the obvious contender for alteration during a land management intervention. Previous studies at this woodland on the management of woodland rides suggests that very low vegetation was not constant with high Qn numbers; Qn densities increasing with vegetation height to the point when the excessive height of the vegetation interfered with the survey process (Medlock *et al.* 2012).

Concluding remarks

The influence of weather and microclimate on *I. ricinus* population distribution, TBD transmission dynamics and temporal questing behaviour, is an area of great importance, especially with the current threat of climate change. The impact of changing climate on habitat and host movement has been well documented and has the potential to greatly alter the distribution of *I. ricinus* and its associated pathogens (Alkishe et. al., 2017; Ostfeld & Brunner 2015).

The influence of temperature in particular, could have substantial impacts on the seasonal patterns of nymphal questing activity and in turn, on the host feeding dynamic. Previous studies have demonstrated the propensity of questing nymphs to remain in the lower levels of the vegetation layer when exposed to higher temperatures, leading to increased feeding on smaller ground dwelling hosts, such as rodents (Randolph & Storey 1999). Such behaviour would alter *Borrelia burgdorferi s.l.* infection dynamics, potentially increasing exposure of questing nymphs to infected rodent and birds (the main reservoir host for *Borrelia burgdorferi s.l.* in the UK), thereby maintaining temporal and spatial transmission dynamics at a local level. Decreased nymphal questing at on-site temperatures above 22°C, observed during this study, supports this theory. Although, several caveats i.e. host abundance leading to successful/unsuccessful nymphal questing, could also play a role.

In terms of more wide ranging impacts on seasonal questing trends; warming winters and increased mean ground temperature in spring, could contribute to the earlier questing onset in nymphs (Medlock *et al.*, 2013). All years of this study exhibited higher temperatures in December and January when compared to long-term climate averages, and perhaps the low number of frost days in 2014, coupled with an earlier peak in nymphal questing could be an indication of this phenomenon. However, continued long term study is required before such conclusions can be verified.

In terms of more immediate implications, while peak questing activity poses a greater risk of tick bite to the public, and contributes to *Borrelia burgdorferi s.l.* transmission dynamics, it’s important to note that Qn were active at this site all year round. Because of the high resolution of this data set, Qn activity could be monitored on a weekly basis and were observed to be active in all months. Over the course of the four year study period, a survey was conducted on every week of the year; across all years, just one consistently (week 50, in early December) failed to produce questing ticks. This emphasises the importance of being tick aware at all times when visiting, not only this particular woodland, but all areas with the potential to maintain *I. ricinus* populations.

In an effort to mitigate tick bite risk, further investigations are required of potential management strategies focusing on the management of vegetation height along path edges - impacting not only on the moisture retaining ability of the underlying ground layer but also the shading of potential questing ticks from direct sunlight, thereby reducing the habitat suitability for Qn.

The findings presented here could also be used as a basis for predicting peak and decline in spring peak Qn activity based on threshold mean weekly temperatures approaching 7°C and potential evapotranspiration approaching 25mm/week. The next step in this process is to use these indicators to investigate the subsequent surveys conducted at Bentley Wood (years 2017 and 2018).

Chapter 4: Spatiotemporal *Ixodes ricinus* activity from selected sites across England

4.1 Introduction

Ixodes ricinus is the most common tick in the UK, its historical distribution being attributed in part, to the presence and movement of sheep between farmland (Milne, 1943). In more recent times, the continued expansions, and maintenance of populations has, again in part, been associated with changes in land use and the expansion of deer populations (Gray *et al.*, 1998). The presence and abundance of *I. ricinus* can vary substantially across landscapes, owing to a combination of habitat suitability and host availability. While field abundance studies (Dobson *et al.,* 2011b; Greenfield, 2011; Millins *et al.,* 2016), tick bite records (Cull *et al.,* 2018) and historical investigations (Pietzsch *et al.,* 2005b) have confirmed the distribution and to some extent, the seasonal patterns of *I. ricinus* in the UK, there have been few studies to investigate its spatiotemporal activity on a large geographic scale.

In the UK the nymphal life stage of this tick is of particular interest to human health because of its capacity to transmit *Borrelia burgdorferi s.l.* and, unlike the adult life stage, it's potential to feed unnoticed for longer periods due to its smaller size (Robertson *et al.,* 2000a; Wilhelmsson *et al.,* 2013). A better understanding of seasonal trends in nymphal questing behaviour, particularly in relation to periods of peak questing activity, and hence, increased tick bite risk, would allow better targeted, public health driven, tick awareness campaigns. Additionally, identifying weather related cues associated with the onset and decline of peak questing activity could be used to develop future predictive models based on regional weather, which could benefit both human and veterinary health.

A complex combination of factors can influence the intensity and timing of peak nymphal questing activity. A key component; microclimate can vary considerably between habitats, influenced by geographical features such as aspect, elevation, soil type, as well as type and structure of vegetation (Goldstein *et al*., 2018; Medlock *et al*., 2012; Mejlon & Jaenson, 1997; Qviller *et al*., 2014; Tomkins *et al*., 2016).

In Chapter 3, the temporal activity of *I. ricinus* was discussed in relation to a single woodland site, over a four year period. In this chapter, the temporal activity, specifically in relation to the commencement of activity and the decline of activity following peak *I. ricinus* nymphal questing, is examined for several sites throughout England, over a two year period.

The purpose of this study was threefold; ***i.*** to investigate the annual temporal pattern of *I. ricinus* activity in six locations, across a two year period. In particular the study focused on commencement of activity and decline of activity following peak nymphal questing and investigated the effects of local weather on these events; ***ii.*** to investigate the influence of microclimate variables i.e. temperature, relative humidity and saturation deficit on *I. ricinus* nymphal questing patterns in different regions of England and explore how these variables are related to local weather data; ***iii.*** to investigate if patterns of questing nymph activity observed in Bentley Wood (the four year Wiltshire woodland study) were comparable with different regions and habitats across England.

4.2 Methods

4.2.1 Tick collection and analysis

Site selection is described in Chapter 2, section 2.3 as is tick survey technique and recording of microclimate and weather variables.

Questing ticks (Qt) were collected at each site using the flagging method, as described in Chapter 2 (section 2.2.). Both questing adults (Qa) and questing nymphs (Qn) were counted and recorded; the presence of larvae in transects was noted but not counted. Analysis focused predominantly on Qn densities.

Peak nymphal questing activity was defined as the maximum nymphal density recorded for that year. In an effort to standardise and compare the commencement and decline of Qn activity at each site, the commencement of peak questing activity was defined as the first survey for which Qn densities were recorded at 25% of the total densities of the maximum. Decline in Qn activity was defined as the first survey following peak Qn for which Qn densities were 25% of the maximum recorded for that year. Studies from Eisen *et al.,* (2002) and Hauser *et al,.* (2018) have both defined onset and decline of peak in a similar fashion but using a 10% of peak threshold. Considering the data collected during this study, 10% of nymphal density did not seem appropriate as this would often indicate peak onset as starting from the first survey of the year. A threshold of 25% provided a stronger differentiation between survey weeks which provided single tick specimens and those for which an increase in activity could be distinctly identified.

4.2.2 Statistical analysis

All statistical analysis was carried out using the statistical software R version 3.4.0 (R Development Core Team, Vienna, Austria).

A variety of statistical tests were used to investigate the significance of various relationships. Differences between 5 km gird temperature records were analysed using a paired t-test, as temperature data were normally distributed. Differences between 5 km grid rainfall records were analysed using non-paramedic Kruskal-Wallis test as this data was not normally distributed. Differences between Qt densities were also analysed using the non-paramedic Kruskal-Wallis test, as both Qn and Qa densities were non- normal and highly skewed.

Due to a miscommunication in the early months of the study, some sites did not record on-site RH (%) for part or all of 2015, as such; analysis of RH (%) and saturation deficit were restricted to 2016 only. On site temperature and vegetation height were recorded for and included in the analysis of both years.

The relationship between 5 km weekly weather data and the commencement and post-peak decline of Qn activity was investigated by comparison of time series plots. Finally, a generalized linear mixed model (GLMM) **GLMM1:** was used to assess the effect of weekly meteorological variables on Qn density at an individual survey level. Explanatory variables were: 5 km grid weekly Tmean (°C), Tmin (°C) and Tmax (°C), weekly precipitation (mm/week), weekly PET (mm/week). In this model, 1 day, 3 day and 7 day lagged effects of each of these variables was also investigated.

Due to limitations of the data for some sites, models were constructed using data from the Wiltshire (Cholderton) site and London site, in addition to the 2015/2016 Bentley Woods site data; the combination of these data provided the most comprehensive data set for which GLMMs could be constructed. The Bentley Wood site had been surveyed on the same day as the Cholderton site. These three datasets (Cholderton, London and Bentley) were used in both models.

The glmmTMB (v. 0.2.2) package (Brooks *et al.* 2018) was selected for GLMM analysis because of its ability to deal with zero inflated count data, over dispersion, as well as spatial and temporal autocorrelation. The DHARMa (v. 0.2.0) package was used to interpret model residuals and model fit.

4.3 Results

4.3.1 Initially, thirty-five volunteers confirmed interest in participating in the project; of these, seven participants excused themselves from the study prior to survey commencement. For the each of study year, six volunteers conducted one to three surveys, seventeen conducted 4 to 9 surveys and five volunteers conducted > 10 surveys for each year of the study. An additional site was surveyed weekly by the author; this site produced 94 surveys across the two year period.

Of the sites which produced <10 surveys per year, seven of these recorded zero Qt and ceased surveys in year one of the study; a further two sites recorded in excess of 250 Qt in any one year, and the remainder recorded <75 Qt in any one year (Appendix. Table A.4.1)

In the interest of constructing a reasonable time series of Qn data against which, meteorological variables could be compared, only sites which conducted >10 surveys in any given year were included in the final analysis (Table 4.1). Each of these sites conducted at least ten surveys in any given year, with the exception of the Northumberland site which conducted surveys in 2015 only.

Table 4.1 Sites which produced >10 surveys for each survey year and which were included in the analysis presented here. *Northumberland was surveyed in 2015 only.

County	Site name	Coordinates	Habitat	Active survey months
Devon	Sidbury	50.72652, -3.20997	Grassland, interspersed with coniferous trees	March - November 2015 April - October 2016
Surrey	Hurt Wood	51.17486, -0.42221	Open path in mixed woodland area	March - September 2015 March - November 2016
Cumbria	Hutton Roof	54.19416, -2.69522	Limestone grassland with woodland scrub	April - October 2015 March - September 2016
Northumberland	Colt Crag	55.095, -2.11496	Coniferous wooded area on edge of reservoir	April - October 2015 -
Greater London	Richmond Park	51.4526, -0.28427	Mixed acid grassland with ancient oak	April - November 2015 February - December 2016
Wiltshire	Cholderton	51.17158, -1.70539	Deciduous wooded path surrounded by arable	January - December 2015 January - December 2016

4.3.2 Questing nymph and adult *I. ricinus* densities

During the two year study period, a total of 2932 Qn and 340 Qa were collected across all six sites listed in Table 4.1. The frequency at which surveys were conducted varied between sites, however, some sites consistently produced a greater percentage of Qt positive transects compared to others (Table 4.2).

The Northumberland site conducted the second lowest number of surveys for 2015 but produced the greatest number of Qn (42.4[CI: 25.1, 71.2 Qn/100^2]) per 100 m^2, with over 90% of transects producing Qn in that year. The only other northern site, based in Cumbia, produced the second greatest percentage of Qn positive transects in 2015. In 2016, this site produced the greatest number of Qn per 100 m^2 (8.7 [6.1, 12.2] Qn/100^2) from the fewest number of surveys conducted that year.

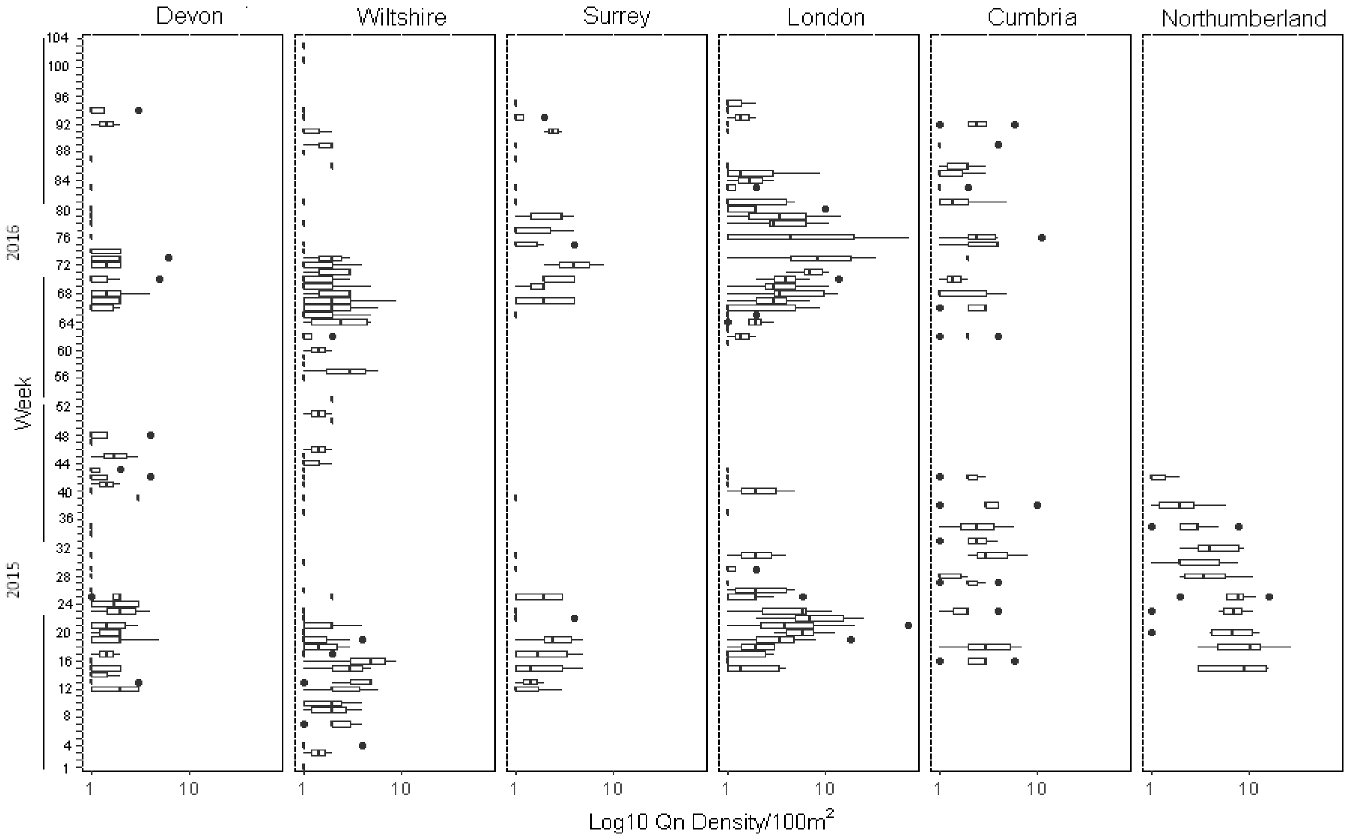
Overall, Richmond Park in London produced the greatest number of Qt of any site; recording 686 Qn and 320 Qa from a total of thirty-two surveys in 2016. In contrast, Wiltshire produced the fewest Qn/100m^2 in both study years; 2.5 [CI: 1.5, 3.7 Qn/100m^2] in 2015 and 2.2 [CI: 1.4, 3.2]Qn/100m^2 in 2016.

All sites surveyed also produced Qa, however, less percentage Qa positive transects were identified in comparison with Qn positive transects, this was true of all years and all sites (Table 4.2).

Table 4.2 Details presented: total number of weeks surveyed by each site; total number of 10m^2 transects surveyed annually at each site; the average number of weeks between each survey; the percentage of transects in which Qn or Qa were observed; the percentage of weeks for which Qa and Qn were observed; the total count of Qn and Qn observed at each site, as well as the mean and confidence intervals. Note: S20 was not surveyed in 2016.

Site	**No. of survey weeks**	**Surveys start/end date**	**Avg no. weeks between surveys**	**Total transects surveys**	**% Qn +ve transects**	**% Qn +ve weeks**	**Total Qn**	**Qn mean [CI]/10m^2**	**% Qa +ve transects**	**% Qa +ve weeks**	**Total Qa**	**Qa mean[CI]/10m^2**
2015												
Devon	27	20Mar - 28Nov	1.5	270	29%	96%	125	3.7 [2.6,4.9]	4%	33%	10	0.3 [0.1, 0.5]
Surrey	15	21Mar - 29Sep	1.8	150	28%	77%	78	2.9 [1.3, 5.7]	7%	47%	15	0.6 [0.2,1.2]
Cumbria	10	18Apr - 20Oct	2.6	100	63%	100%	168	5.9 [4.5,7.8]	19%	70%	24	0.9 [0.2, 2.1]
Northumberland	11	15Apr - 18Oct	2.6	110	91%	100%	594	42.4[25.1,71.2]	25%	82%	38	2.7[1.4,4.6]
Greater London	21	09Apr - 29Nov	1.6	210	50%	91%	475	8.7[4.3,16.8]	25%	67%	28	1 [0.6, 1.6]
Wiltshire	49	02Jan - 23Dec	1	490	27%	94%	296	2.5 [1.5,3.7]	11%	45%	60	0.9 [0.6,1.2]
2016												
Devon	19	03Apr - 18Oct	1.5	190	28%	79%	83	2.7 [1.4,4.6]	11%	58%	22	0.8 [0.4,1.4]
Surrey	18	10Mar - 08Nov	1.8	180	29%	83%	104	3.4 [1.8,6.1]	8%	56%	15	0.7 [0.3,1.1]
Cumbria	13	11Mar - 04Oct	2.3	130	45%	100%	131	8.7 [6.1,12.2]	6%	54%	8	0.5 [0.2,0.9]
Northumberland	-	-	-	-	-	-	-	-	-	-	-	-
Greater London	32	14Feb - 11Dec	1.3	320	43%	84%	686	7.3[3.9,12.9]	11%	56%	37	0.7 [0.4,1.2]
Wiltshire	45	04Jan - 19Dec	1.1	450	23%	67%	192	2.2 [1.4, 3.2]	13%	56%	83	1.1 [0.6,1.6]

Fig 4.1 Weekly Qn ($\log_{10}$) per $100m^2$ for each survey site included in analysis. In each boxplot, each box describes a single survey of 10 transects. Boxplot boxes present the interquartile range of transect results; outliers are represented by a black circle; the median is represented by a black line.



4.3.3 Peak questing nymphs activity – seasonal and spatial trends

For both years of the study a spring peak in Qn densities was observed at all sites. The week in which peak activity occurred differed for all sites between years; occurring during a six week window for all sites in 2015, between week 16 and 21. In 2016 this increased Qn activity persisted slightly later, during a nine week window, between week 16 and 28 (Table 4.3). Three sites; Surrey, London and Cumbria exhibited a later peak in 2016 than in 2015.

In both study years, Qn activity peaked earliest at Cholderton in Wiltshire, occurring on the same week in mid-April for both years. Devon and London both exhibited just a one to two week difference between years, while Cumbria and Surrey exhibited a difference of six and eight weeks respectively, between years. Notably, these two sites, together with the Northumberland site, were located at greatest elevation above sea level (Fig 4.3c).

No clear temporal-latitudinal trend in Qn peak activity emerged for either study year (Fig 4.3a). In 2015, each of the more westerly sites reached peak Qn activity earlier than the most easterly site (London), however, this trend was not repeated in 2016 (Fig 4.3b).

Table 4.3 Details the date and week for which peak Qn density was recorded at each site. Latitude/longitude coordinates and the elevation (meters above sea level) are also displayed.

County	**Lat/Lon co-ordinates**	**Elevation**	**Qn Peak Week 2015**	**Qn Peak Week 2016**
Devon	50.72652, -3.20997	87m	19 (07May15)	18(03May16)
Wiltshire	51.17158, -1.70539	99m	15 (16Apr15)	15 (14Apr16)
Surrey	51.17486, -0.42221	228m	19 (14May15)	27 (08Jul16)
Greater London	51.4526, -0.28427	36m	21 (21May15)	23 (12Jun16)
Cumbria	54.19416, -2.69522	185m	18 (07May15)	24 (17Jun16)
Northumberland	55.095, -2.11496	210m	18 (07May15)	-

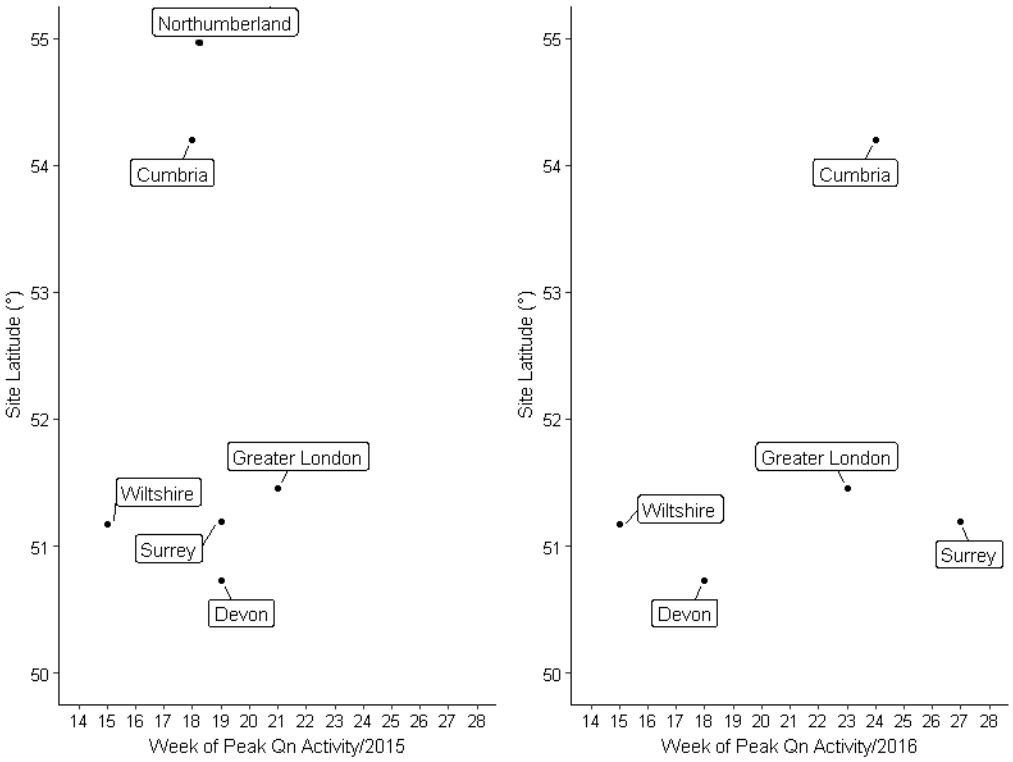


Fig 4.2a Details the week of peak Qn activity for each site and each year of study in relation to site latitude. Sites are represented by their latitudinal coordinates on the y-axis and the week at which peak Qn activity was observed on the x-axis.

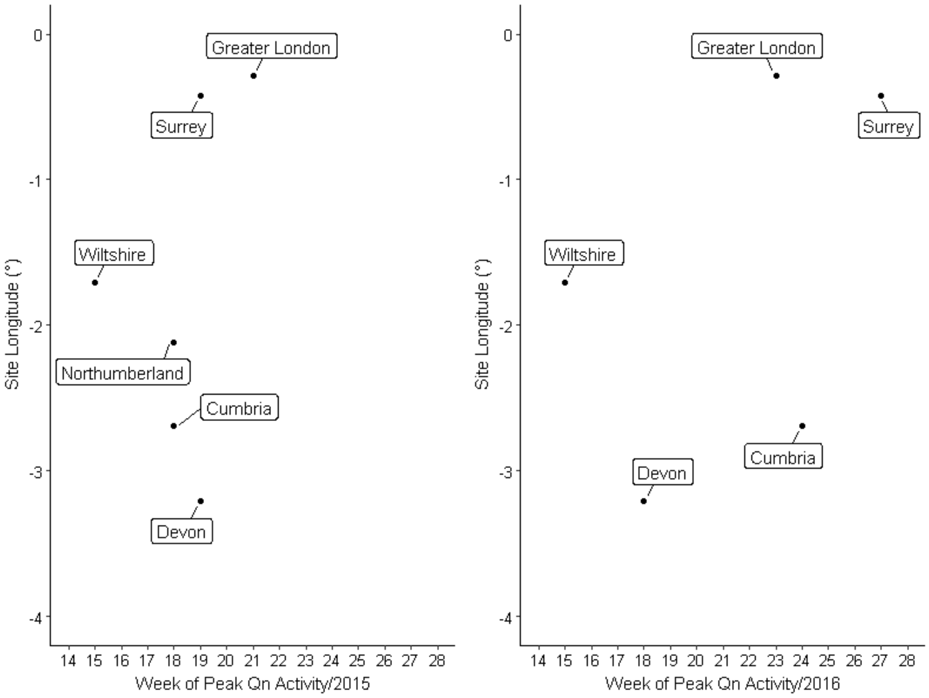


Fig 4.3b Details the week for which peak Qn activity for each site and each year of study in relation to site longitude. Sites are represented by their longitudinal coordinates on the y-axis and the week at which peak Qn activity was observed on the x-axis.

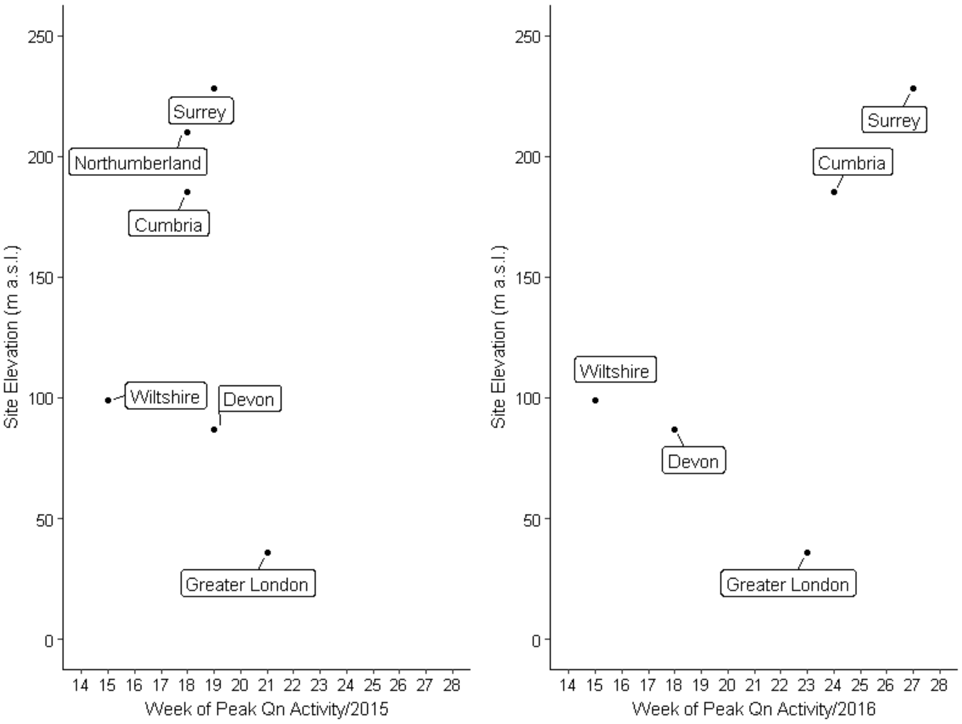


Fig 4.3c Details the week for which peak Qn activity for each site and each year of study in relation to site elevation (meters above sea level). Sites are represented by their elevation above sea level (m a.s.l.) on the y-axis and the week at which peak Qn activity was observed on the x-axis.

A unimodal pattern of Qn activity was exhibited for both years at the London site, with no notable peak in the autumn months.

The Northumberland site exhibited a peak in spring, with Qn remaining active through the summer and into autumn, however, no secondary peak was observed prior to the halt of surveys in week 42 (mid - October).

For all other sites an additional increase in Qn activity was observed in autumn (Fig 4.2). A secondary autumn peak, almost equivalent to that of spring was observed at the Cumbria site. This secondary peak occurred in week 37 (early September) of 2015 and in week 40 (early October) of 2016. The Devon site also exhibited a secondary peak in Qn activity between weeks 42 and 47 (mid-October to mid-November) in 2015, and week 41 (early October) in 2016. In both cases for this site, the density of Qn was less than the initial spring peak but >25% of the initial spring peak.

In Surrey a secondary peak was observed in 2016, in weeks 39 and 40 (late September). In the same year, in week 37 (early September), an increase in Qn activity was also observed at Cholderton in Wiltshire. (Please refer to Appendix; p. Fig A4.1 to 4.6 for actual Qn counts per week).

4.3.4 Commencement and decline of peak Qn activity in relation to weekly mean temperature, precipitation and evapotranspiration.

As with Bentley Wood analysis (presented in Chapter 3), the timing of commencement, peak and decline in Qn activity was investigated for each site in relation to weekly 5 km grid temperature (weekly Tmean °C), weekly 5 km grid precipitation (rainfall mm/week) and evapotranspiration (PET mm/week). In particular, the relationship between a weekly threshold temperature of 7°C and the commencement of increased Qn activity was investigated, as was the relationship between maximum PET and declined Qn activity.

For all sites, other than London and Wiltshire, nymphal questing was already underway when surveys began.

The commencement of increased (25% of maximum Qn density) Qn activity for the majority of sites occurred before the first week for which 7 °C was reached. Peak Qn activity occurred at all sites after this 7 °C threshold had been reached.

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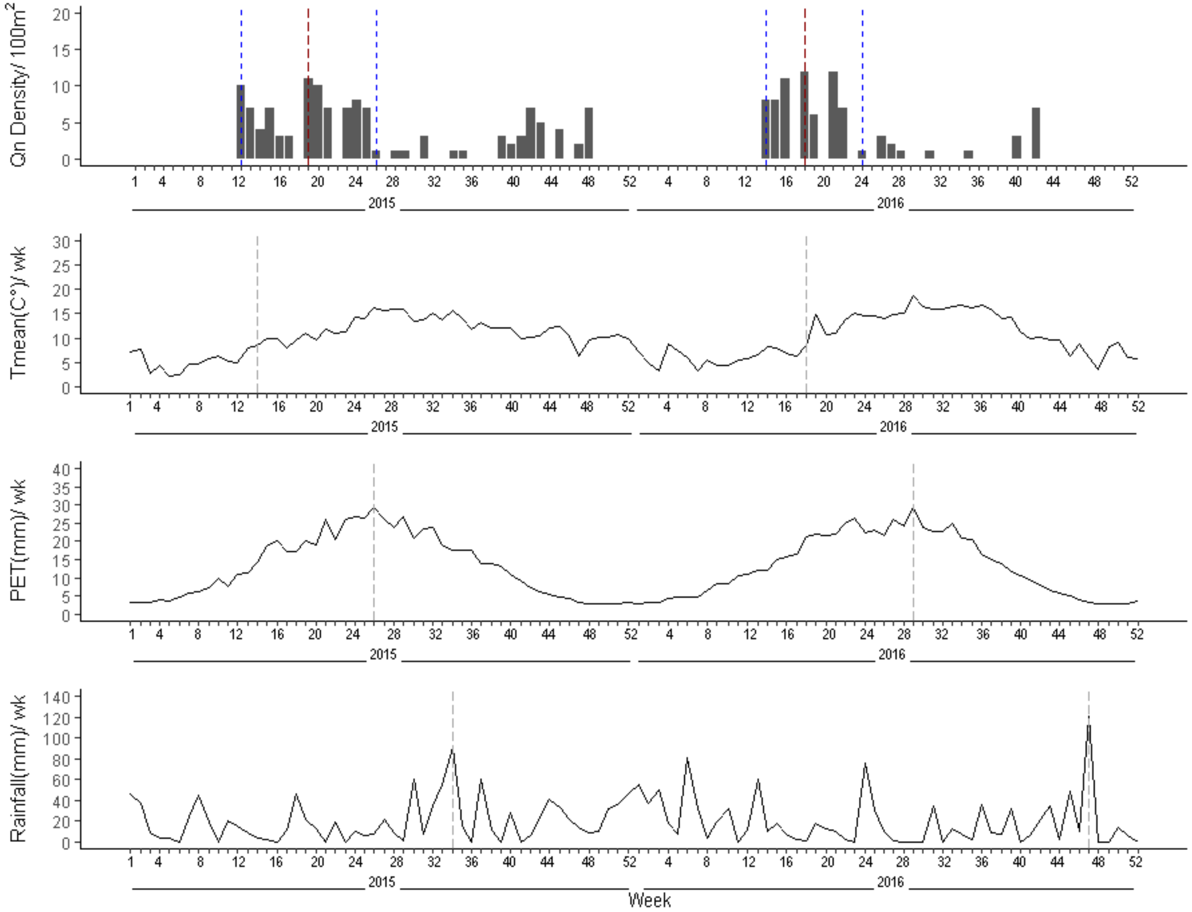
Fig 4.4A Devon Time series of Qn/100m^2 over the two year study period, compared to weekly Tmean (°C), weekly precipitation (obtained from 5 km gridded UKCP09 data) and potential evapotranspiration (PET). Red dashed line, week of peak Qn activity; blue dashed line, week for which 25% of maximum peak Qn); grey dashed line (Tmean plot), first week of Tmean at >7°C for seven consecutive days; (PET plot), week of maximum PET (mm); (precipitation plot), week of maximum precipitation.



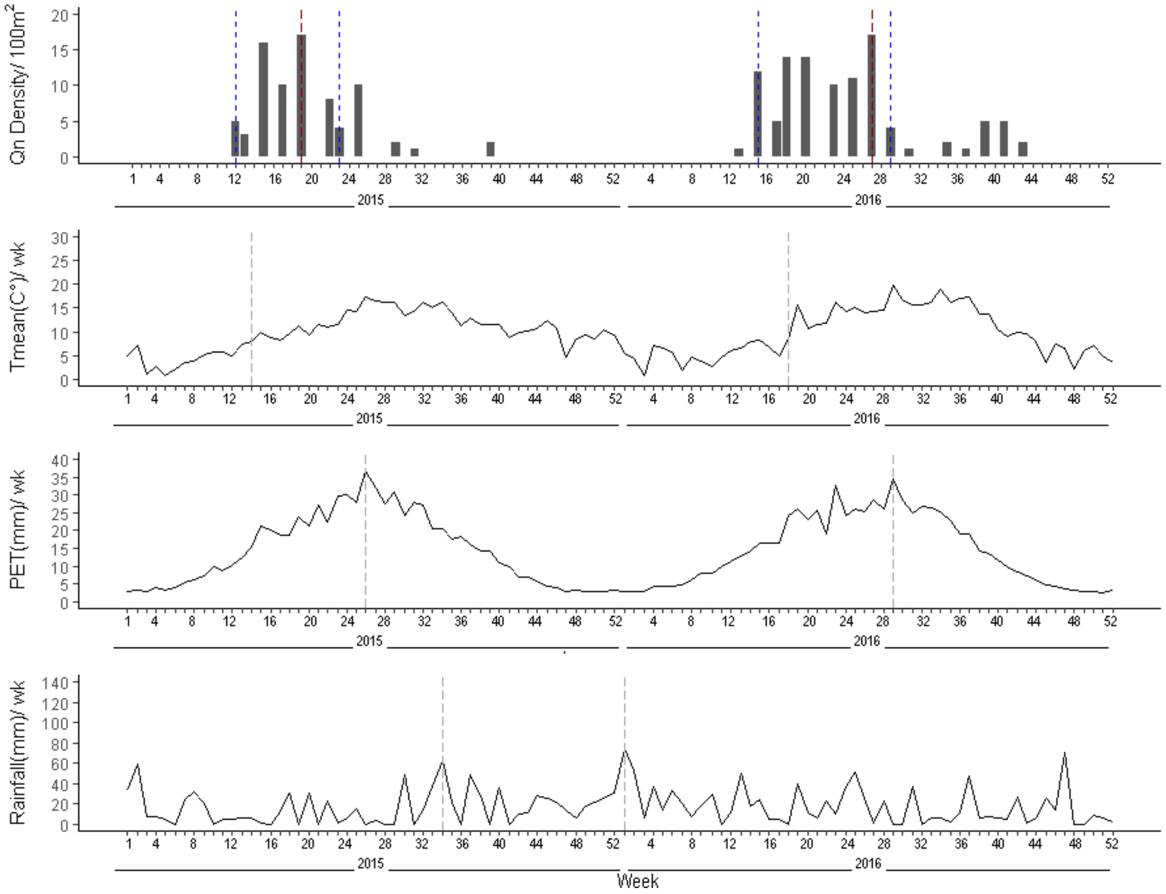
4.3.4.1 Devon

Surveys at Devon (Fig 4.4A) were conducted from week 12 (mid- March) of 2015, at this stage, ticks were already active and 25% of peak Qn activity had already been reached. This was also true of 2016; the first survey of the year produced a Qn density, just 10% less than that of peak Qn density which occurred five weeks later. For each year, the increase of Qn activity coincided with a prolonged period of warming which resulted in a daily Tmean (°C) of ≥7°C for seven consecutive days, preceding peak Qn in both 2015 and 2016.

Maximum potential evapotranspiration (PET) coincided with the decline in Qn densities for both years.

Maximum precipitation for this particular 5 km grid occurred outside the survey period; occurring in the final weeks of years, week 52 and week 46, respectively

Fig 4.4B Surrey Time series of Qn/100m^2 over the two year study period, compared to weekly Tmean (°C), weekly precipitation (obtained from 5 km gridded UKCP09 data) and potential evapotranspiration (PET). Red dashed line, week of peak Qn activity; blue dashed line, week for which 25% of maximum peak Qn); grey dashed line (Tmean plot), first week of Tmean at >7°C for seven consecutive days; (PET plot), week of maximum PET (mm); (precipitation plot), week of maximum precipitation.



4.3.4.2 Surrey

Surrey (Fig 4.4B) also conducted its first survey of 2015 on week 12 (late March). At this point, an increase in Qn activity was already observed and continued, reaching maximum density in week 20. A decline in Qn activity, below 25% of maximum peak, was observed from week 24 (early June); surveys conducted from week 29 (early July) produced Qn densities of <12% of the maximum peak for that year.

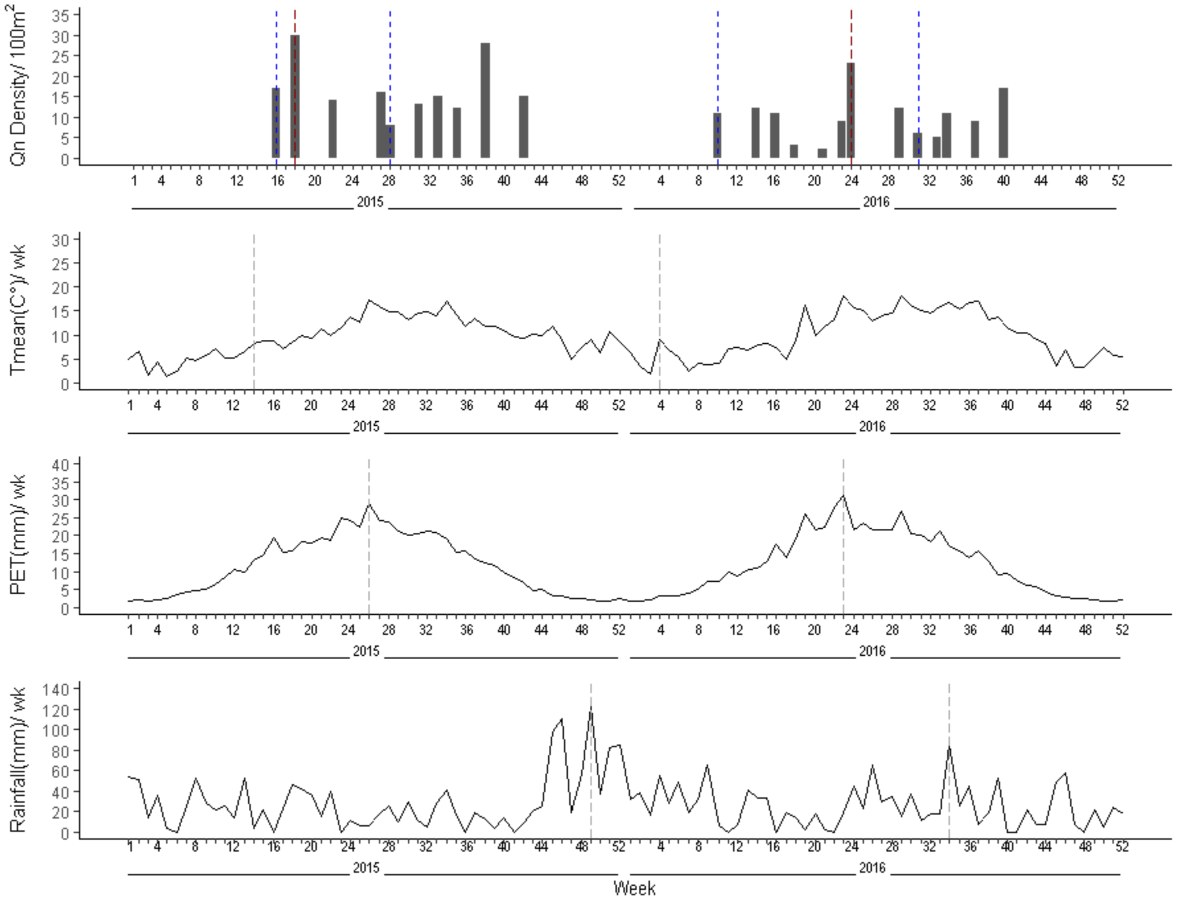
In 2016, peak Qn was observed in week 27 (early July); this was preceded by four survey weeks where Qn densities exceeded 25% of the peak Qn density for that year. A decline in questing activity was observed from week 29 (late July) onwards, however, a slight secondary peak, exceeding 25% of spring peak density, was observed in week 40 and 42 (late September and early October).

Increased Qn activity in spring coincided with increased Tmean (°C) for both years, however, Qn densities exceeding 25% of peak spring Qn activity were observed prior to the first week of Tmean exceeding a 7°C threshold. This first week where Tmean exceeded 7°C, occurred earlier in 2015 (week 14) than in 2016 (week 18); peak Qn activity also occurred earlier in 2015, eight weeks earlier than in 2016.

The decline in questing activity coincided with maximum PET in 2015. In 2016, maximum PET occurred in week 29, immediately following peak Qn activity; this coincided with a sharp decline in Qn activity.

Maximum precipitation occurred in week 34 of 2015, when questing densities were already in decline. In 2016, maximum precipitation occurred prior to surveys commencing.

Fig 4.4C Cumbria Time series of Qn/100m^2 over the two year study period, compared to weekly Tmean (°C), weekly precipitation (obtained from 5 km gridded UKCP09 data) and potential evapotranspiration (PET). Red dashed line, week of peak Qn activity; blue dashed line, week for which 25% of maximum peak Qn); grey dashed line (Tmean plot), first week of Tmean at >7°C for seven consecutive days; (PET plot), week of maximum PET (mm); (precipitation plot), week of maximum precipitation.



4.3.4.3 Cumbria

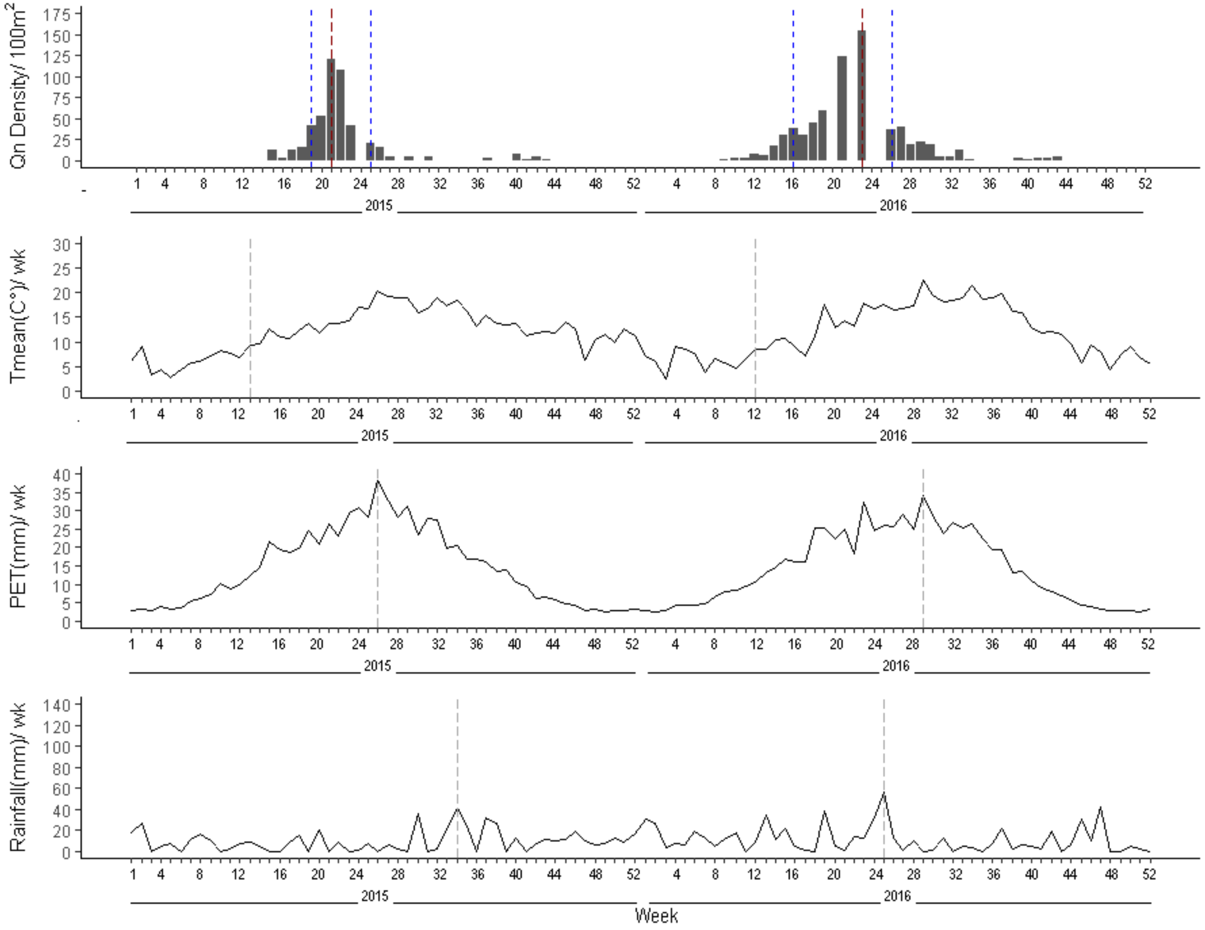
In both 2015 and 2016, Qn densities at the Cumbrian site (Fig 4.4C) had already reached >25% of the peak Qn spring density. This peak occurred in week 18 (early May) of 2015, however, notably, a secondary peak occurred in week 38 (mid-September), the density of which was just 7% less than that of spring.

The occurrence of first week of Tmean exceeding 7°C in 2015 preceded peak Qn activity. A decline in Qn activity appeared to coincide with the occurrence of maximum PET for that year.

In 2016, peak Qn activity was observed in mid-June, seven weeks later than in 2015; a secondary peak was also observed in September (week 40). The first week of Tmean exceeding 7°C occurred in late January – much earlier than for any other site or year. This early increase in weekly Tmean was followed by four weeks of weekly Tmean below 5°C; however, the first surveys for this year were carried out after this period.

Two peaks PET did initially appear to coincide with a sharp decline in Qn activity in the weeks preceding peak, in contrast with other site and other years, maximum PET occurred the week before peak Qn activity. A drop just below 25mm/week appeared to co-inside with peak Qn activity.

Fig 4. 4D London Time series of Qn/100m^2 over the two year study period, compared to weekly Tmean (°C), weekly precipitation (obtained from 5 km gridded UKCP09 data) and potential evapotranspiration (PET). Red dashed line, week of peak Qn activity; blue dashed line, week for which 25% of maximum peak Qn); grey dashed line (Tmean plot), first week of Tmean at >7°C for seven consecutive days; (PET plot), week of maximum PET (mm); (precipitation plot), week of maximum precipitation.



4.3.4.4 London

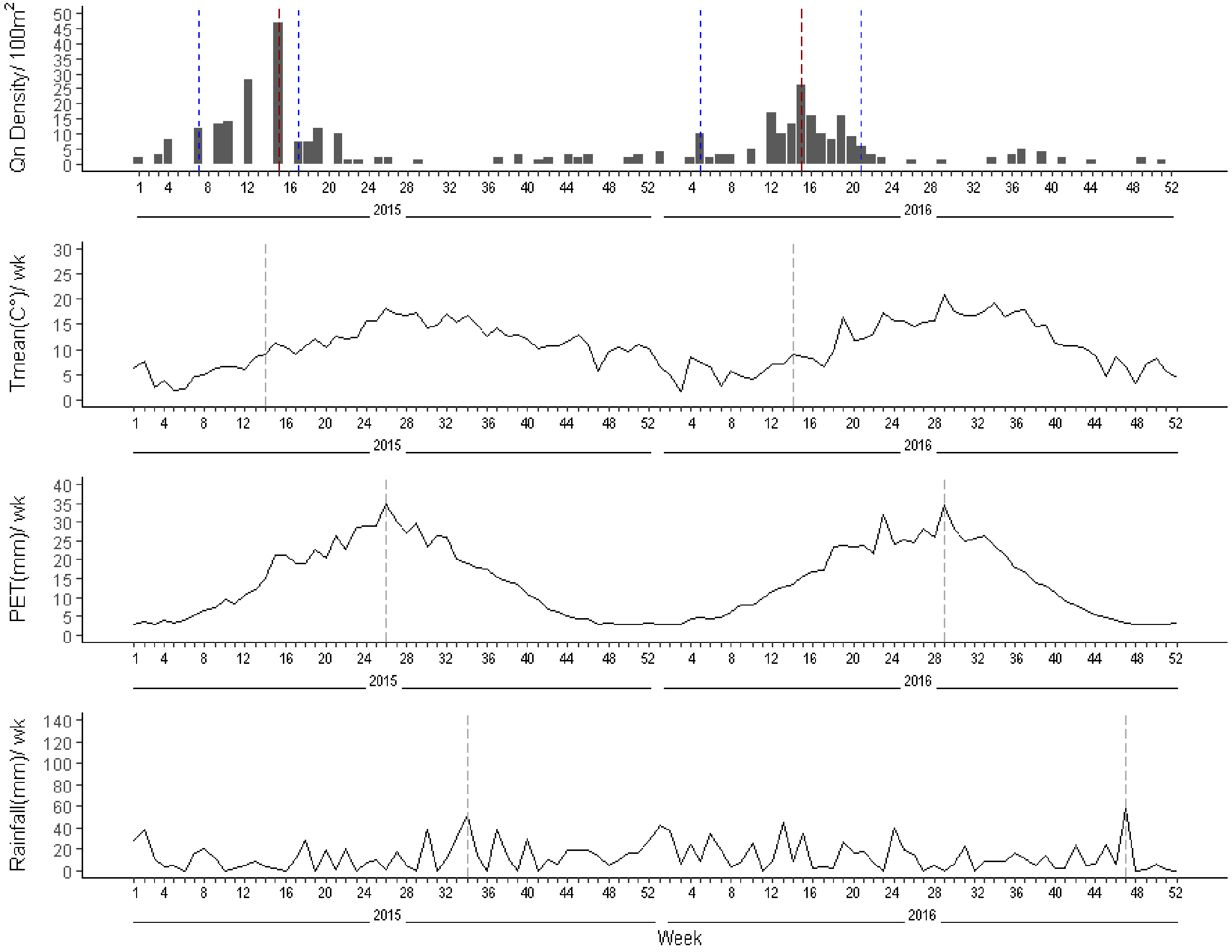
The first survey at London (Fig 4.4D) was carried out on week 15 (early April) of 2015. An increase in activity, exceeding 25% of peak, was observed in early May, occurring on week 19, increasing again in week 20 and reaching maximum Qn density in week 21. The subsequent survey in week 22 also exhibited a high Qn density, just 10% less than that of peak density. A steady decline in Qn densities was observed from week 23 onwards, with all survey weeks following this, exhibiting less than 18% of the spring peak.

In 2016, surveys started in week 7, with the first Qn observed from week 9 (late February). At week 17 (late-April) and increase in Qn activity was observed, with peak Qn activity noted in week 24 (mid-June); steady decline in Qn activity (below 25% of peak) was observed from week 26 (late June).

In both years, weekly Tmean reached >7°C for the first time, prior to the commencement in Qn activity. Maximum PET, which were higher at this site than any other, did occur slightly after Qn decline was observed.

In 2015, maximum precipitation was recorded in week 34, several weeks after the decline in Qn activity, however, in 2016; maximum precipitation was recorded the week after peak Qn activity, unfortunately, no surveys were conducted on this week so the impact of this increased rainfall on Qt densities is unknown.

Fig 4. 4E Wiltshire Time series of Qn/100m^2 over the two year study period, compared to weekly Tmean (°C), weekly precipitation (obtained from 5 km gridded UKCP09 data) and potential evapotranspiration (PET). Red dashed line, week of peak Qn activity; blue dashed line, week for which 25% of maximum peak Qn); grey dashed line (Tmean plot), first week of Tmean at >7°C for seven consecutive days; (PET plot), week of maximum PET (mm); (precipitation plot), week of maximum precipitation.



4.3.4.5 Wiltshire

Surveys at Cholderton (Fig 4.4E) in Wiltshire took place from the first week of 2015 and 2016, running almost weekly for each year. As such, and unlike other sites, Qn activity in the pre-spring period was observable. Qn were observed in three of four weeks of January 2015, be it at densities less than 7% that of peak. An increase in Qn densities exceeding 25% of peak Qn activity was first noted in week 7 (mid-February). Peak Qn activity was observed in week 15 (mid-April), followed by a sharp decline (<15% of peak) in activity in week 17. From week 21(mid-May), observed Qn densities fell below 10% of the spring peak; Qn activity did not recover for the remained of the year.

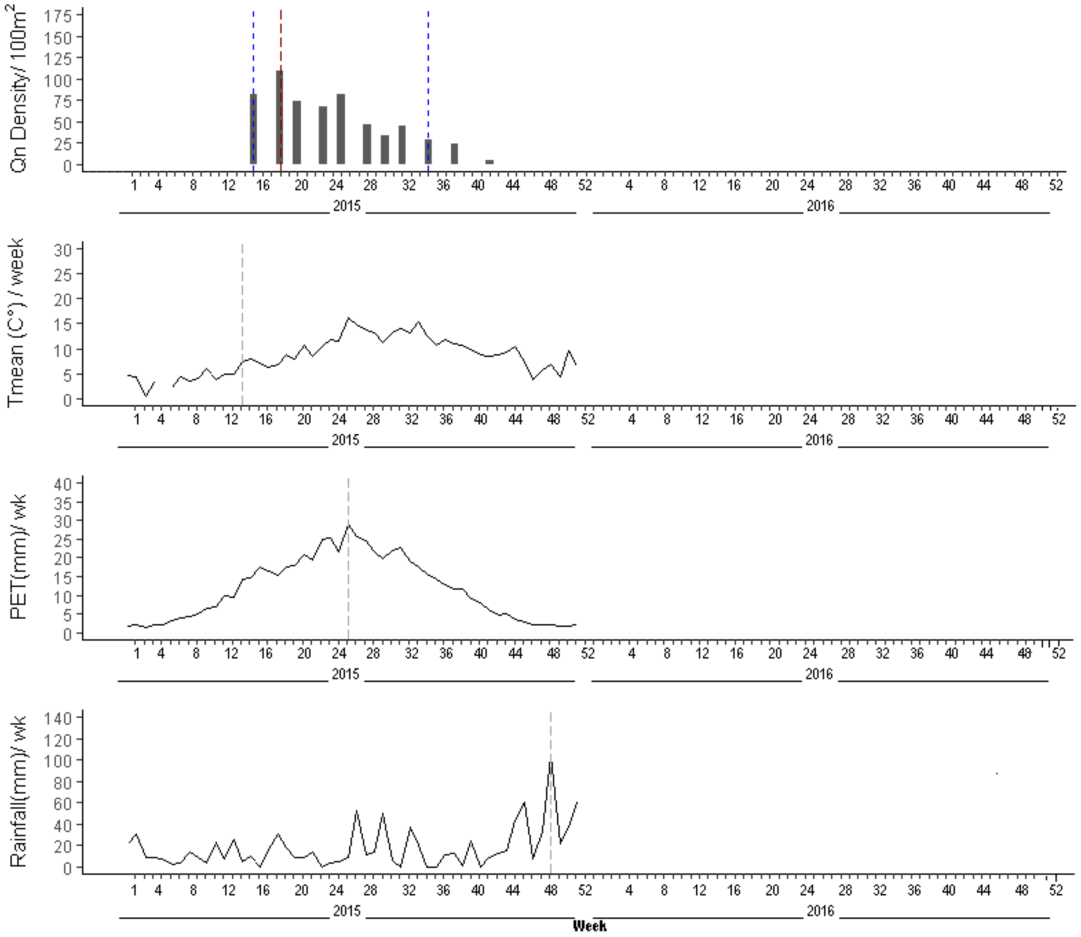
The first week were weekly Tmean exceeded 7°C (week 14) did occur before peak Qn activity was reached, however, increased Qn activity was observed for six weeks prior to this temperature threshold being reached.

In 2016, Qn activity was observed from the second week of January, although, an increase equating to 25% of peak activity was not observed until week 5 (early- February); following this, Qn densities fell below 20% of peak until week 12 (mid-March) with peak Qn activity observed in week 15 (end of April). Following week 23 (early June) Qn densities remained below 20% of peak Qn for the remainder of the year.

Maximum PET occurred well after peak Qn activity in both years, however, increases in PET of >25mm/week did appear to coincide with decreases in Qn densities in both years.

Precipitation reached a maximum on the last week of 2015 and week 24 of 2016, however Qn activity had already declined at this point.

Fig 4. 4F. Northumberland* Time series of Qn/100m^2 over the two year study period, compared to weekly Tmean (°C), weekly precipitation (obtained from 5 km gridded UKCP09 data) and potential evapotranspiration (PET). Red dashed line, week of peak Qn activity; blue dashed line, week for which 25% of maximum peak Qn); grey dashed line (Tmean plot), first week of Tmean at >7°C for seven consecutive days; (PET plot), week of maximum PET (mm); (precipitation plot), week of maximum precipitation. ***surveyed in 2015 only**



4.3.4.6 Northumberland

Northumberland (Fig 4.4F) was surveyed in 2015 only. When surveys started on week 16 (mid-April) Qn activity was already 75% of the maximum recorded for that year. Qn densities remained above 25% of spring peak densities until late august, declining further in the remaining two surveys of the year.

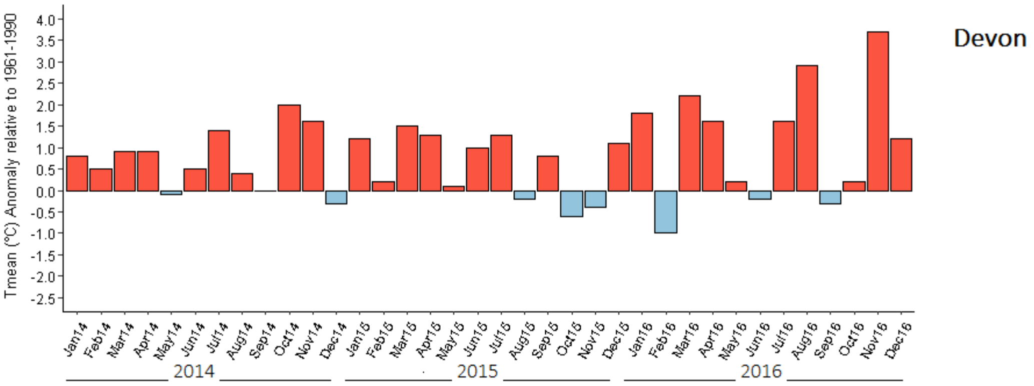
The first week of Tmean (°C) remaining above 7°C had already occurred before surveys commenced. Maximum PET did coincide with a 45% drop in Qn activity in week 26 (late June). Maximum precipitation occurred in early December, by which time, all surveys had been completed.

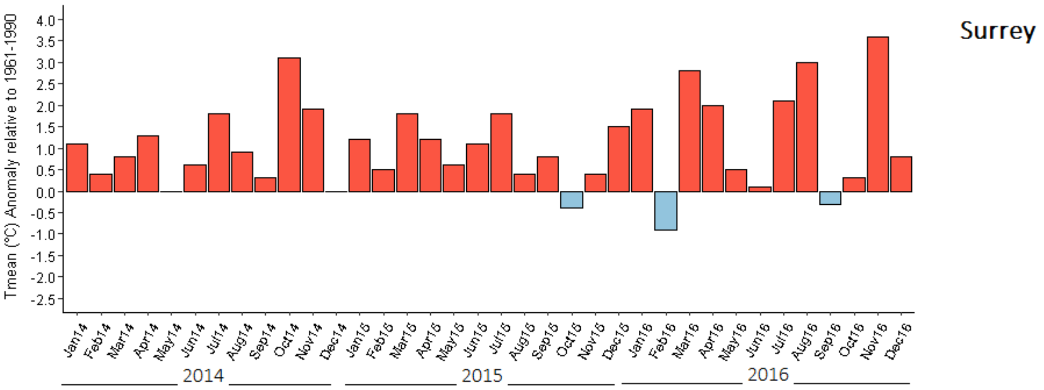
4.3.5 Climate observations relating to the study period

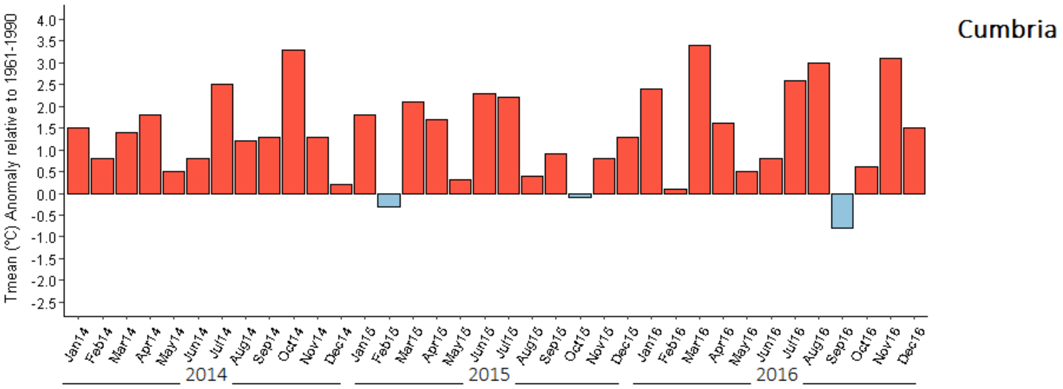
4. 3.5.1 Temperature and precipitation anomalies

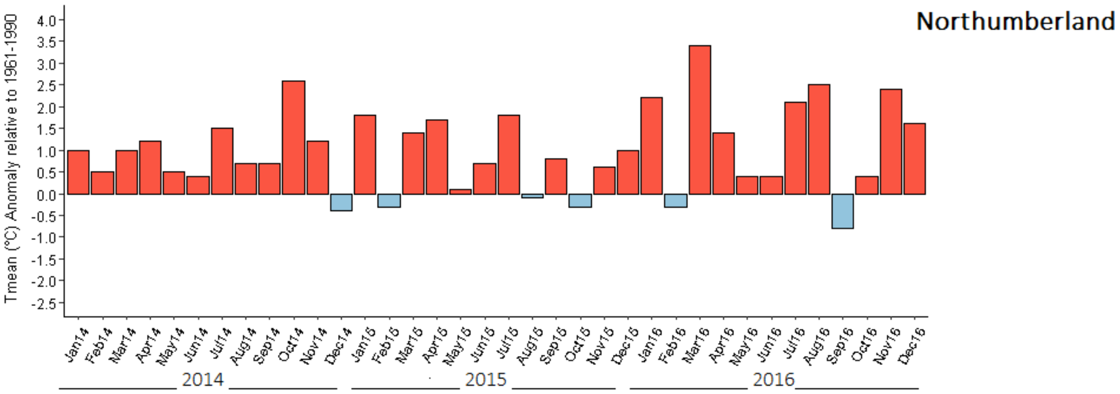
When compared to historical climate averages (1961 to 1990), there was a general positive trend in weekly mean temperature (°C). For each site, and for each year of the study, at least nine of twelve months exhibited a positive Tmean anomaly. Key months for *I. ricinus* questing activity were consistently warmer than average for every site (Fig 4.5). October of both years was consistently cooler than average at all sites, and February of 2016 was constantly cooler at all sites with the exception of Cumbria.

Precipitation anomalies were more variable across months for all years; however, in 2015, above average precipitation was recorded during key periods of spring Qn activity for sites Surrey and Wiltshire, all other sites for 2015 exhibited below average precipitation in spring. In 2016, positive spring anomalies, for at the majority of spring months, were exhibited at all sites (Fig 4.6).


Devon
Tmean (°C) Anomaly relative to 1961-1990
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Jan14 Feb14 Mar14 Apr14 May14 Jun14 Jul14 Aug14 Sep14 Oct14 Nov14 Dec14 Jan15 Feb15 Mar15 Apr15 May15 Jun15 Jul15 Aug15 Sep15 Oct15 Nov15 Dec15 Jan16 Feb16 Mar16 Apr16 May16 Jun16 Jul16 Aug16 Sep16 Oct16 Nov16 Dec16
2014
2015
2016


Surrey
Tmean (°C) Anomaly relative to 1961-1990
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Jan14 Feb14 Mar14 Apr14 May14 Jun14 Jul14 Aug14 Sep14 Oct14 Nov14 Dec14 Jan15 Feb15 Mar15 Apr15 May15 Jun15 Jul15 Aug15 Sep15 Oct15 Nov15 Dec15 Jan16 Feb16 Mar16 Apr16 May16 Jun16 Jul16 Aug16 Sep16 Oct16 Nov16 Dec16
2014
2015
2016


Cumbria
Tmean (°C) Anomaly relative to 1961-1990
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Jan14 Feb14 Mar14 Apr14 May14 Jun14 Jul14 Aug14 Sep14 Oct14 Nov14 Dec14 Jan15 Feb15 Mar15 Apr15 May15 Jun15 Jul15 Aug15 Sep15 Oct15 Nov15 Dec15 Jan16 Feb16 Mar16 Apr16 May16 Jun16 Jul16 Aug16 Sep16 Oct16 Nov16 Dec16
2014
2015
2016


Northumberland
Tmean (°C) Anomaly relative to 1961-1990
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Jan14 Feb14 Mar14 Apr14 May14 Jun14 Jul14 Aug14 Sep14 Oct14 Nov14 Dec14 Jan15 Feb15 Mar15 Apr15 May15 Jun15 Jul15 Aug15 Sep15 Oct15 Nov15 Dec15 Jan16 Feb16 Mar16 Apr16 May16 Jun16 Jul16 Aug16 Sep16 Oct16 Nov16 Dec16
2014
2015
2016

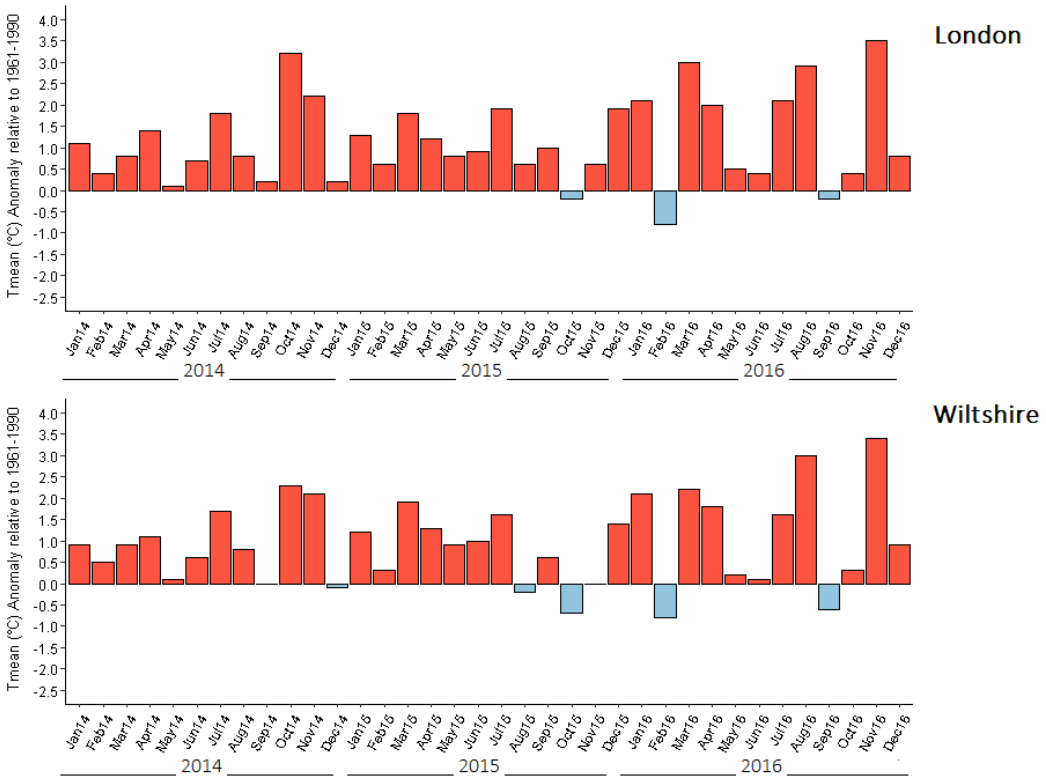
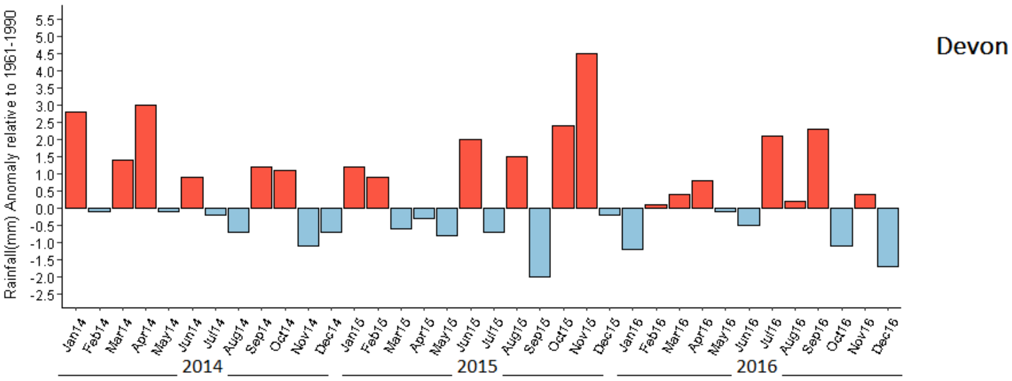
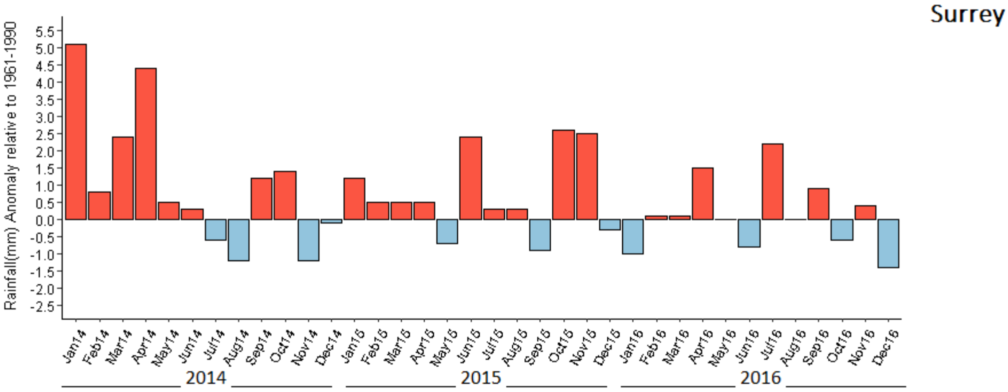
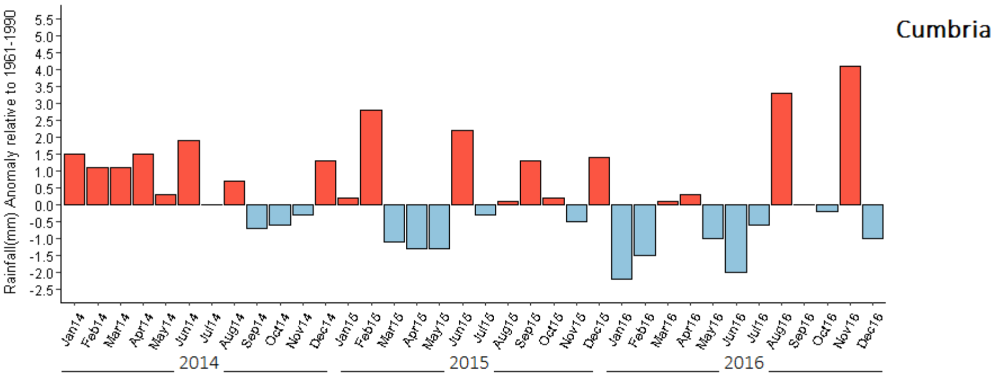
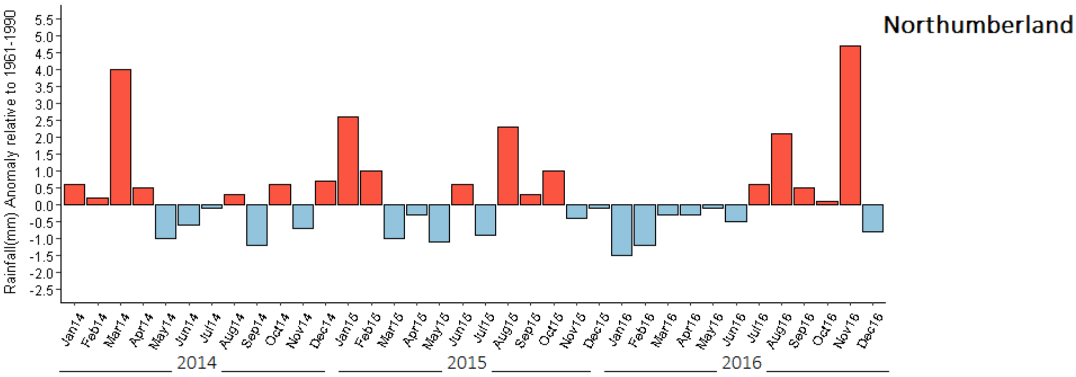


Fig 4.5 Standardized Temperature (°C) anomalies (relative to 1961 to 1990), for the 5 km grid in which each site was located. From top to bottom: Devon, Surrey, Cumbria, Northumberland, London, and Wiltshire. Anomalies for the years of study and the year prior to study are shown.


Devon
Rainfall(mm) Anomaly relative to 1961-1990
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Jan14 Feb14 Mar14 Apr14 May14 Jun14 Jul14 Aug14 Sep14 Oct14 Nov14 Dec14
Jan15 Feb15 Mar15 Apr15 May15 Jun15 Jul15 Aug15 Sep15 Oct15 Nov15 Dec15
Jan16 Feb16 Mar16 Apr16 May16 Jun16 Jul16 Aug16 Sep16 Oct16 Nov16 Dec16
2014
2015
2016


Surrey
Rainfall(mm) Anomaly relative to 1961-1990
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Jan14 Feb14 Mar14 Apr14 May14 Jun14 Jul14 Aug14 Sep14 Oct14 Nov14 Dec14
Jan15 Feb15 Mar15 Apr15 May15 Jun15 Jul15 Aug15 Sep15 Oct15 Nov15 Dec15
Jan16 Feb16 Mar16 Apr16 May16 Jun16 Jul16 Aug16 Sep16 Oct16 Nov16 Dec16
2014
2015
2016


Cumbria
Rainfall(mm) Anomaly relative to 1961-1990
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Jan14 Feb14 Mar14 Apr14 May14 Jun14 Jul14 Aug14 Sep14 Oct14 Nov14 Dec14
Jan15 Feb15 Mar15 Apr15 May15 Jun15 Jul15 Aug15 Sep15 Oct15 Nov15 Dec15
Jan16 Feb16 Mar16 Apr16 May16 Jun16 Jul16 Aug16 Sep16 Oct16 Nov16 Dec16
2014
2015
2016


Northumberland
Rainfall(mm) Anomaly relative to 1961-1990
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Jan14 Feb14 Mar14 Apr14 May14 Jun14 Jul14 Aug14 Sep14 Oct14 Nov14 Dec14
Jan15 Feb15 Mar15 Apr15 May15 Jun15 Jul15 Aug15 Sep15 Oct15 Nov15 Dec15
Jan16 Feb16 Mar16 Apr16 May16 Jun16 Jul16 Aug16 Sep16 Oct16 Nov16 Dec16
2014
2015
2016

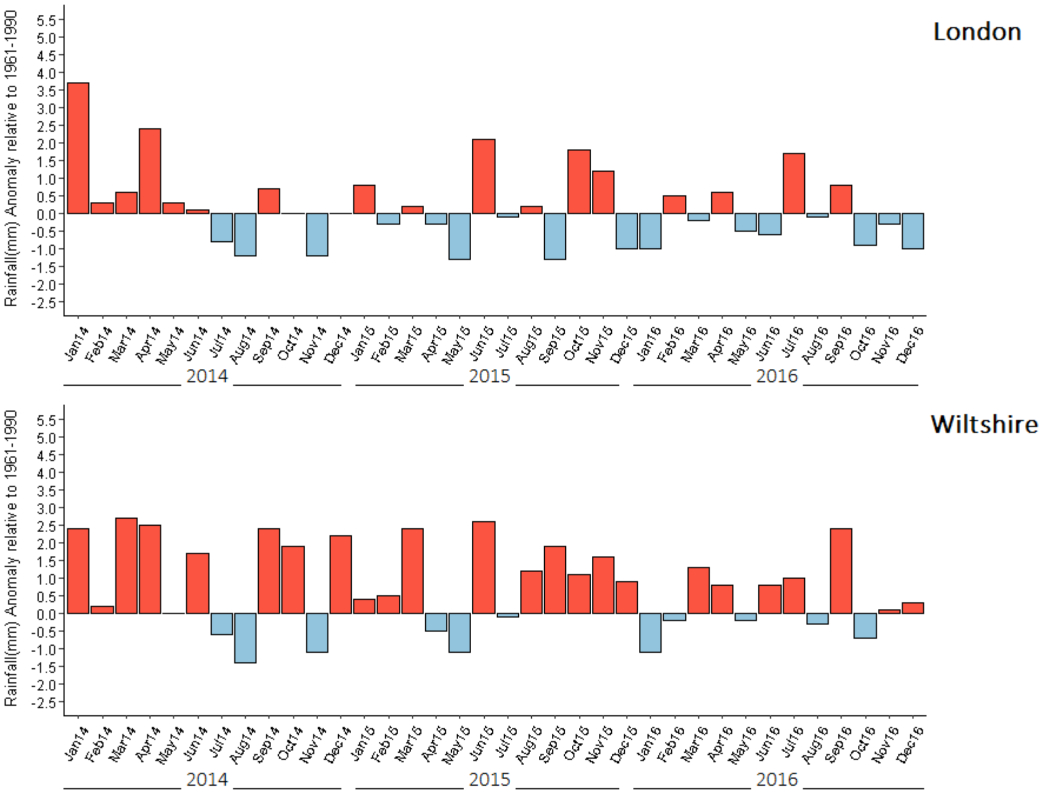


Fig 4.6 Standardized Precipitation (mm) anomalies (relative to 1961 to 1990), for the 5 km grid in which each site was located. From top to bottom: Devon, Surrey, Cumbria, Northumberland, London, and Wiltshire. Anomalies for the years of study and the year prior to study are shown.

4. 3.5.2 Temperature and precipitation between sites

All sites which were surveyed in both survey years, exhibited the same general trend in weekly Tmean (°C) (Fig 4.7). None of the sites exhibited significant inter-annual difference in weekly Tmean. However, significant differences between Tmean were exhibited between individual sites for both 2015 ($P < 0.001$) and 2016 ($P < 0.001$).

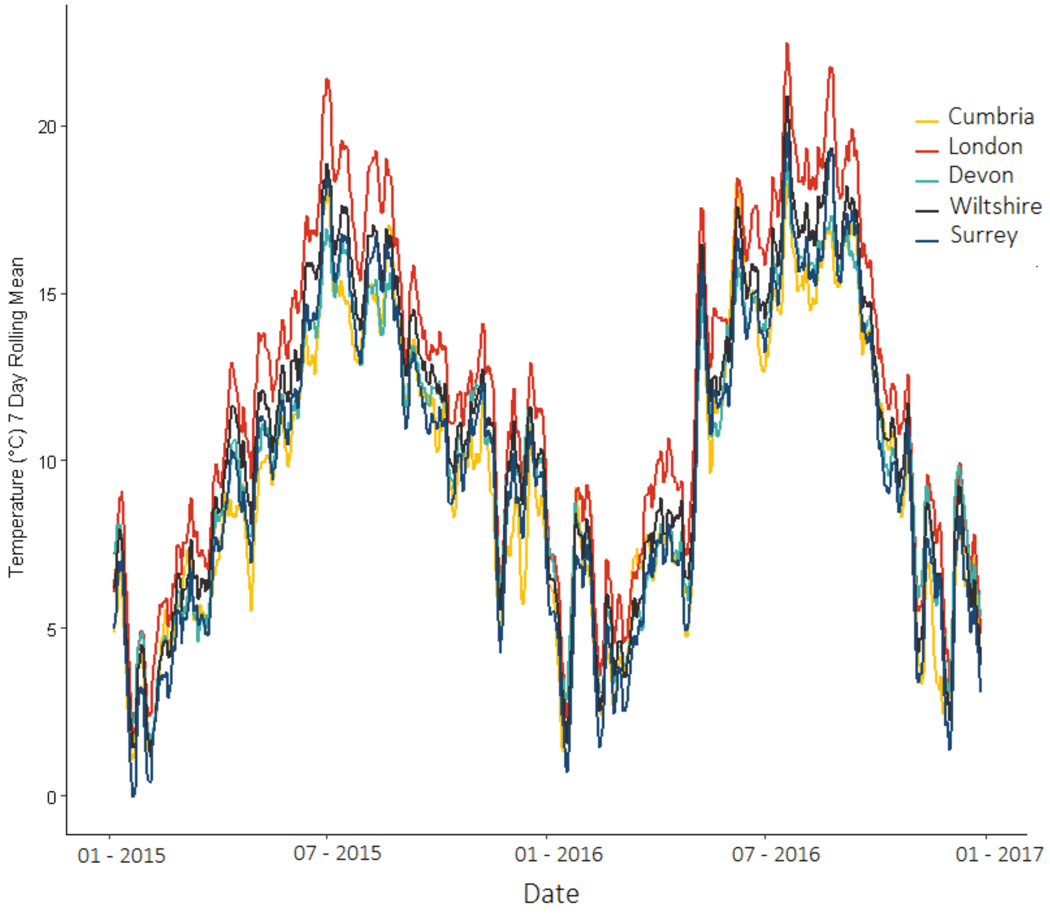


Fig 4.7 7-day smoothed weekly Tmean (°C) for all sites which were surveyed across both study years. Data obtained from UKCP09 weekly 5km gridded data.

Annual rainfall trends were more erratic, however, none of the sites exhibited significant inter-annual difference in mean weekly rainfall (mm) (Fig 4.8). Significance differences were observed between sites in both 2015 (P <0.001) and 2016(P <0.001)

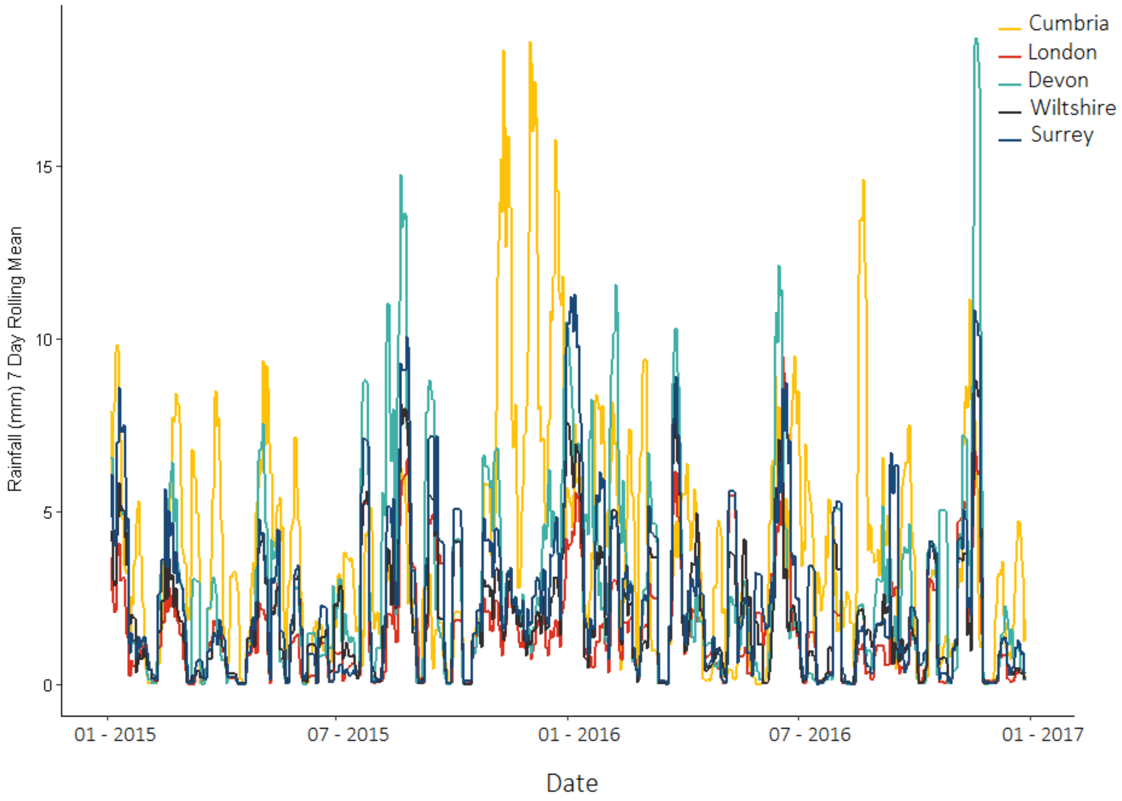


Fig 4.8 7-day smoothed weekly rainfall (mm) for all sites which were surveyed across both study years. Data obtained from UKCP09 weekly 5km gridded data.

4.3.5.3 Frost days

For both years, all sites exhibited a greater number of frost days (days of Tmin <1°C) in winter (January, February and December of the previous year) than in any other season (Fig 4.9). Surrey recorded the most winter frost days, however, the Northumberland site, recorded the most overall, across all seasons, with a total of 61.

When compared to 2016, all southern sites i.e. Devon, Wiltshire, Surrey and London recorded fewer frost days in spring 2015, during weeks where *I. ricinus* activity was beginning to increase. The fewest number of frost days for both years, were recorded at the London site.

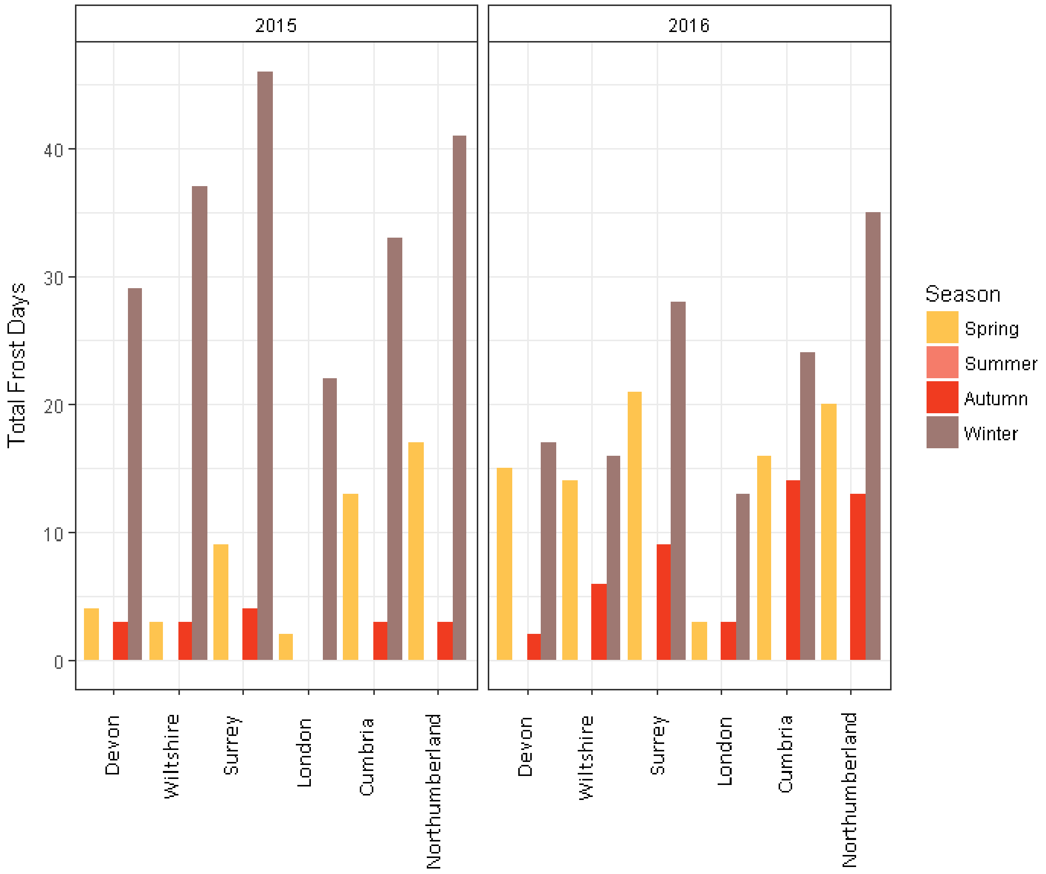


Fig 4.9 Number of frost days which occurred at each site, for each year of the study. Frost days were calculated from 5 km grid data for each site. Spring (March, April, May); summer (June, July, August), autumn (September, October, November) and winter (Jan, February and December of the previous year) are represented individually. Sits are listed (l to right) from the most southerly to the most northerly.

4.3.5.4 Questing nymph densities: impact of meteorological variables

Generalized linear mixed models (GLMM) with a negative binomial error distribution were used to investigate the effect of various weekly meteorically and lagged meteorically variables (Table 4.4) on Qn density. To account for spatial and temporal autocorrelation, site and survey date were included as random effects. Model selection was based on Akaike Information Criteria (AIC). Models were tested for over/under dispersal, and spatial and temporal autocorrelation

Table 4.4 GLMM1: The effect of long term weather and climate variables on Qn densities across all years of the study: parameter estimates of fixed effects from a generalised linear mixed model (GLMM) –those effects indicated as significant are highlighted. Analysis based on weekly survey data.

List of weekly meteorological variables considered in the initial GLMM1
All variables were obtained from 5 km grid UKCP09 data
Weekly mean temperature (°C)
Weekly minimum temperature
Weekly maximum temperature
Weekly mean precipitation (mm)
Weekly mean potential evapotranspiration
1 day lag daily mean temperature (°C)
3 day lag daily mean temperature (°C)
7 day lag daily mean temperature (°C)
1 day lag daily min temperature (°C)
3 day lag daily min temperature (°C)
7 day lag daily min temperature (°C)
1 day lag daily max temperature (°C)
3 day lag daily max temperature (°C)
7 day lag daily max temperature (°C)
1 day lag mean precipitation (mm)
3 day lag mean precipitation (mm)
7 day lag mean precipitation (mm)
1 day lag mean PET (mm/week)
3 day lag mean PET (mm/week)
7 day lag mean PET (mm/week)

Table 4.5 GLMM1: Final model. The effect of microclimate variables on Qn densities in 2016 only: parameter estimates of fixed effects from a generalised linear mixed model (GLMM) - those effects indicated as significant (P-value = <0.05) are highlighted. Analysis based on weekly survey data

Coefficients	Estimate	Std. error	Z-value	P-value
Intercept	1.25242	0.49119	2.550	0.01078
Weekly 5km grid PET	0.06233	0.01944	3.206	**<0.001**
Weekly T minimum 5km grid	-0.07890	0.04987	-1.582	0.1
1-day lag of weekly 5km grid PET	0.38865	0.15327	2.536	**<0.01**
7-day lag of weekly 5km grid min T (°C)	-0.10075	0.03500	-2.879	**<0.001**

The final model indicated the significance of weekly PET (mm/week); 1-day lag in PET (mm/week) and 7-day lag effect of weekly minimum temperature (°C) on Qn densities.

4.3.6 Questing nymph densities: impact of microclimate and environmental variables

For each site, total percentages of observed Qn from individual transects were compared to temperature (°C), RH (%) and vegetation height (cm) recorded at time of survey for each of those transects. This was carried out for temperature and vegetation height for both years of the study; however, due to gaps in the recording of RH (%) at some of the sites in 2015, only data for 2016 was used in comparisons between percentage of total Qn counts and RH (%), and for saturation deficit (mmHg).

4.3.6.1 On-site temperature at time of survey

None of the study sites recorded temperatures of below 4 °C at time of survey (Fig4.10). In fact, only the Wiltshire and London sites recorded temperatures of <7 °C during survey. At the Wiltshire site, temperatures of <7 °C occurred in four survey weeks in spring 2015, and in five survey weeks in 2016; Qn were observed on six of these occasions. At the London site, onsite temperatures of < 7 °C occurred twice in spring 2016; no Qn were observed on either of these occasions.

For all sites in both 2015 and 2016, an increased percentage of Qn were observed at temperatures exceeding 7°C, with five of the six sites (Table 4.6), recording more than 60% of total Qn at temperatures between 13°C to 24°C.The exception to this was the London site in 2016 which observed over 60% of total Qn at temperatures between 19°C to 30°C.

Table 4.6 The number of occasions temperature (°C) within the listed ranges was recorded at time of survey for individual transects at each site in 2015 and 2016. A colour gradient, light to dark, is used to indicate increased frequency of listed temperature ranges. * Northumberland was only surveyed in 2016

Temperature (°C) ranges	Devon	Surrey	Cumbria	London	Wiltshire	Northumberland*
1°C to 3°C	0	0	0	0	0	0
4°C to 6°C	0	0	0	9	38	0
7°C to 9°C	10	19	0	18	145	0
10°C to 12°C	54	8	1	5	124	10
13°C to 15°C	121	43	29	74	205	2
16°C to 18°C	119	65	33	76	129	9
19°C to 21°C	106	71	62	92	135	25
22°C to 24°C	40	58	49	100	119	30
25°C to 27°C	6	36	26	79	34	20
28°C to 30°C	1	13	16	40	1	7
31°C to 33°C	0	7	10	13	0	3
34°C to 36°C	0	0	0	3	0	2
≥37°C	0	0	0	0	0	2

At temperatures above 24 °C, no observations of Qn were made in the southerly sites of Devon and Wiltshire, even though, temperatures in excess of this were recorded at both sites. Qn were recorded at each of the other sites at temperatures of ≥ 30 °C, although, these observations accounted for <11% of total observed Qn density in any given study year.

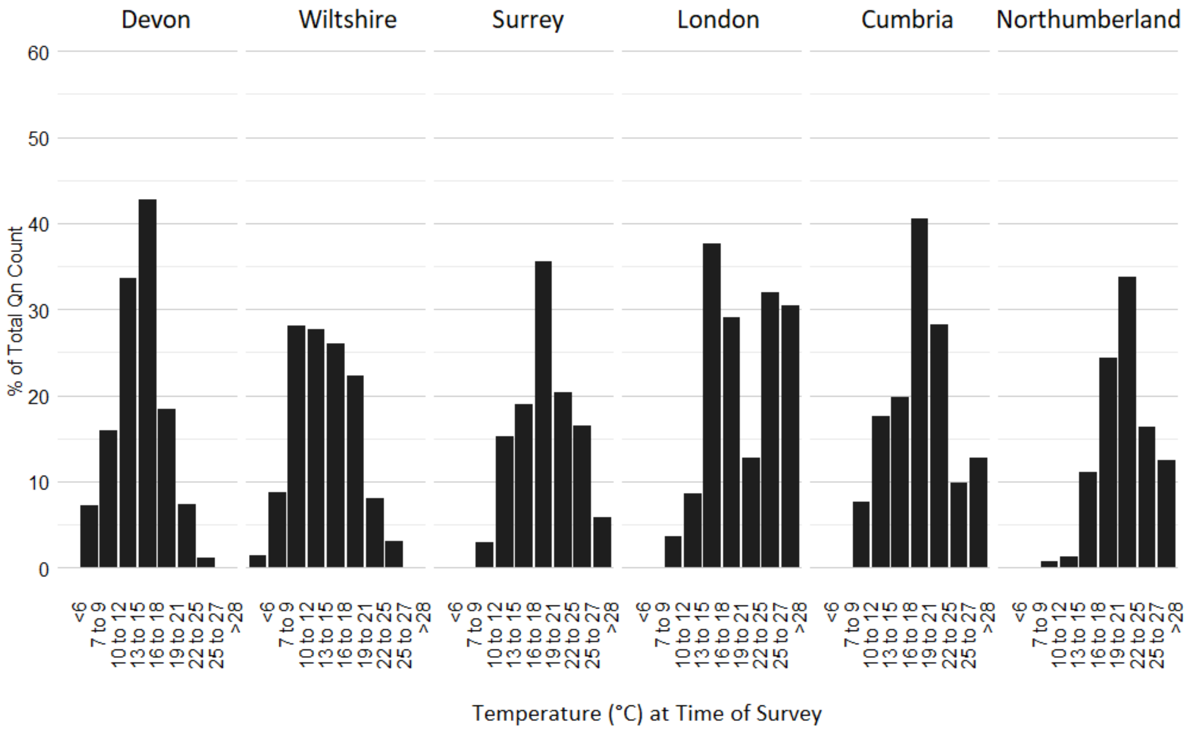
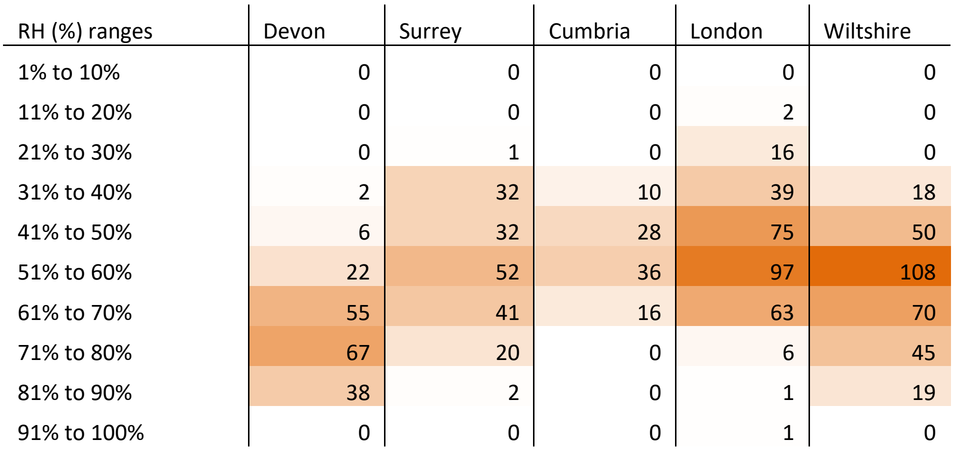


Fig 4.10 Percentage of total Qn counts from individual transects, observed at different temperature (°C) ranges combined across both study years. Temperature refers to that recorded on site at time of survey.

4.3.6.2 On-site relative humidity at time of survey

In 2016, none of the sites recorded RH (%) below 10% and just one survey in late April recoded a RH (%) of less than 20% - this survey was conducted at the London site on a day where temperature at time of survey exceeded 30°C. Almost 90% of transects from all sites recorded RH (%) between 41% and 90% (Table 4.6).

Table 4.7 The number of occasions RH (%) within the listed ranges was recorded at time of survey for individual transects at each site in 2016. A colour gradient, light to dark, is used to indicate increased frequency of listed relative humidity ranges.



RH (%) ranges	Devon	Surrey	Cumbria	London	Wiltshire
1% to 10%	0	0	0	0	0
11% to 20%	0	0	0	2	0
21% to 30%	0	1	0	16	0
31% to 40%	2	32	10	39	18
41% to 50%	6	32	28	75	50
51% to 60%	22	52	36	97	108
61% to 70%	55	41	16	63	70
71% to 80%	67	20	0	6	45
81% to 90%	38	2	0	1	19
91% to 100%	0	0	0	1	0

Qn were recorded at RH (%) of <20% but only in small numbers, just 2% of total Qn collected at the London site. The greatest percentages of Qn recorded at this site were recorded at RH (%) of 55-60%. In fact, for the majority of sites, a greater percentage of total Qn were recorded at RH (%) between 51% and 70%, the exception being Surrey, which recorded, albeit marginally, a greater percentage total of Qn at 31% to 40% (Fig. 4.11)

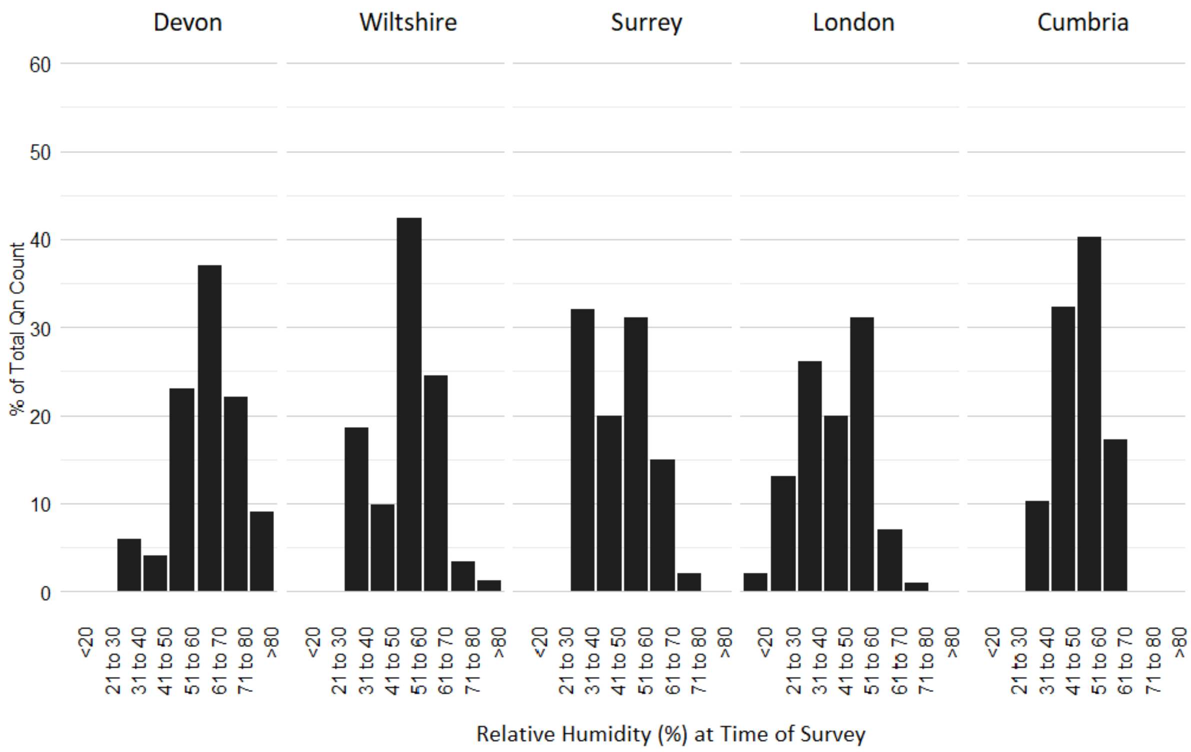


Fig 4.11 Percentage of total Qn counts from individual transects, observed at different relative humidity (%) ranges. RH (%) refers to that recorded on site at time of survey.

4.3.6.3 On-site saturation deficit at time of survey

For over 90% of transects surveyed, a saturation deficit of < 12 mmHg was recorded. Surrey, Cumbria, London and Wiltshire did record saturation deficit above 13mmHg, with London recording a maximum of 27mmHg on four occasions (Table 4.8).

Table 4.8 The number of occasions saturation deficit (mmHg) within the listed ranges was recorded at time of survey for individual transects at each site in 2016. A colour gradient, light to dark, is used to indicate increased frequency of listed saturation deficit ranges.

Saturation deficit (mmHg) ranges	Devon	Surrey	Cumbria	London	Wiltshire
1 to 3 mmHg	69	20	0	21	61
4 to 6 mmHg	88	53	21	81	134
7 to 9 mmHg	30	66	49	91	79
10 to 12 mmHg	3	24	9	53	34
13 to 15 mmHg	0	6	5	23	2
16 to 18 mmHg	0	6	3	11	0
19 to 21 mmHg	0	4	2	5	0
22 to 24 mmHg	0	1	1	5	0
25 to 27 mmHg	0	0	0	4	0
>27 mmHg	0	0	0	0	0

For all sites, with the exception of London, over 60% of Qn were recorded at a saturation deficit of <6mmHg; zero Qn were recorded at saturation deficits of >21mmHg at any of these sites. The London site exhibited greater variability, with Qn recorded from 1mmHg to a maximum of 33 mmHg, however, over half of Qn at this site were still recorded at ≤ 12 mmHg (Fig 4.12)

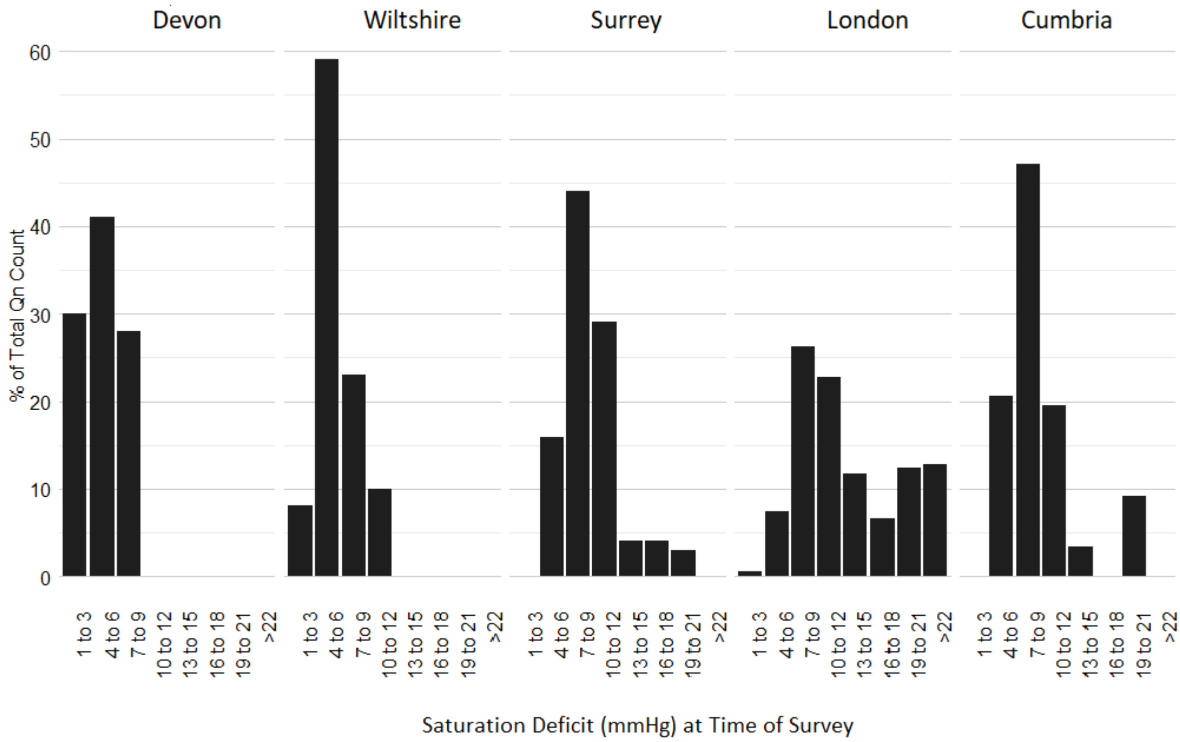
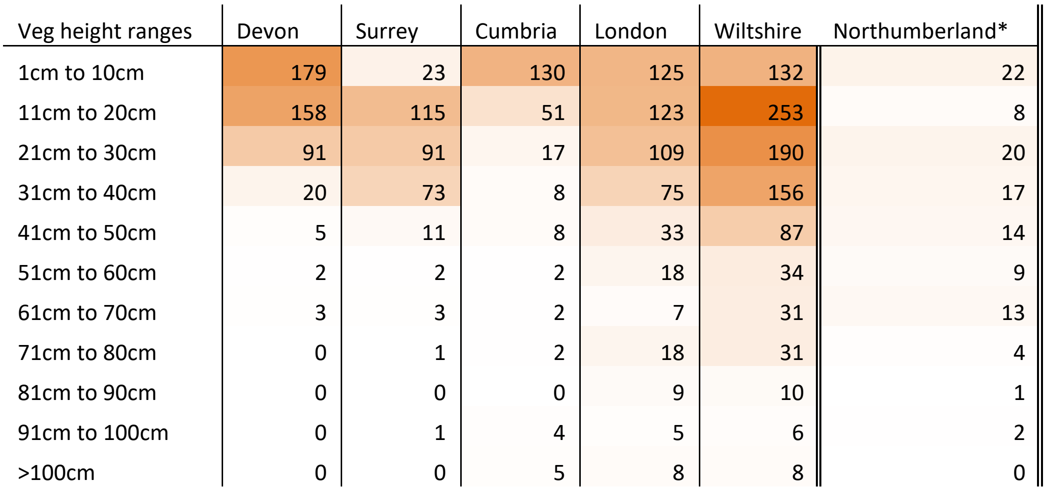


Fig 4.12 Percentage of total Qn counts from individual transects, observed at different saturation deficit (mmHg) ranges. Saturation deficit was calculated based on temperature and relative humidity at time of survey.

4.3.6.4 On-site vegetation at time of survey

Vegetation composition and height varied between sites depending on habitat, season, and in some cases land management.

Table 4.9 The number of occasions vegetation height (cm) within the listed ranges was recorded at time of survey for individual transects at each site in site in 2015 and 2016. A colour gradient, light to dark, is used to indicate increased frequency of listed vegetation height ranges. * Northumberland was only surveyed in 2016



Veg height ranges	Devon	Surrey	Cumbria	London	Wiltshire	Northumberland*
1cm to 10cm	179	23	130	125	132	22
11cm to 20cm	158	115	51	123	253	8
21cm to 30cm	91	91	17	109	190	20
31cm to 40cm	20	73	8	75	156	17
41cm to 50cm	5	11	8	33	87	14
51cm to 60cm	2	2	2	18	34	9
61cm to 70cm	3	3	2	7	31	13
71cm to 80cm	0	1	2	18	31	4
81cm to 90cm	0	0	0	9	10	1
91cm to 100cm	0	1	4	5	6	2
>100cm	0	0	5	8	8	0

Across all sites, Qn were observed in greater densities at vegetation height of <40 cm (Fig 14.13); below this threshold, accounted for between 75% to 100% of Qn observations across all sites and years. Qn were still observed at vegetation heights of greater than 50 cm but this account for <5% of total observed at all sites, with the exception of the Northumberland site, for which 19% of Qn were observed heights greater than 50cm.

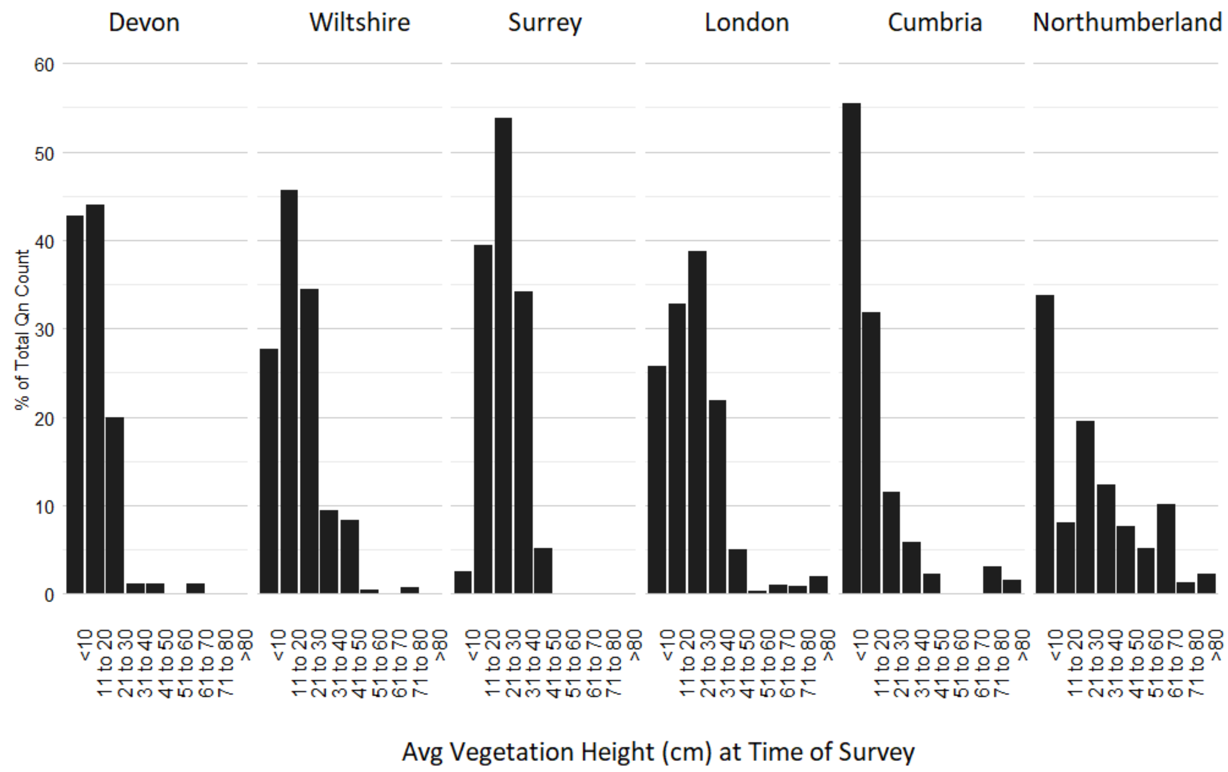


Fig 4.13 Percentage of total Qn counts from individual transects, observed at different vegetation height (cm) ranges combined across both study years. Vegetation refers to that recoded on site at time of survey.

4.4 Discussion

A continuation of the work presented in Chapter 3, the aim of this chapter is to apply the same techniques of examining when increases in nymphal questing occurred in spring and how local weather and microclimate might influence commencement and decline in *I. ricinus* nymphal questing. In this chapter, nymphal questing data from six different sites located throughout England, recorded over a two-year period, was analysed alongside region specific meteorological variables, and site-specific microclimate and vegetation data.

By comparing the questing activity between sites, analysing how this may vary, and how this increased activity is influenced by weather and microclimate, specific cues associated with increased questing are identified. These cues will be used in further research to attempt temporal predictions of when peak nymphal questing might occur on an year to year basis.

4.4.1 Influence of weather on Ixodes ricinus questing patterns

Previous studies have exhibited the limited questing of *I. ricinus* nymphs at temperatures below 5 °C (Qviller *et al.,* 2014) and the importance of a weekly mean maximum temperature of 7°C in the initiation of increased questing activity (Clark, 1995; Macleod, 1936b; J. L. Perret *et al.*, 2000; S. Randolph, 2004).

A maximum peak in Qn activity was observed in all sites in spring, occurring between week 16 and 21 in 2015, and between week 16 and 28 in 2016. At the London site, a unimodal pattern of questing was observed in each year. At the Northumberland site, following an initial peak, Qn remained active into summer, declining in autumn. In Cumbria too, Qn remained activate at higher densities into summer and for both years, exhibiting an autumn peak almost equal to that in spring. Devon exhibited a bimodal pattern of activity in both years; Surrey and Wiltshire exhibited unimodal activity in 2015 and bimodal activity in 2016.

The commencement of increased Qn activity (measured as 25% of maximum spring peak) occurred prior to the first occurrence of seven consecutive days of 7 °C at sites in Devon, Surrey and Wiltshire in both years, and after seven consecutive days of 7°C at the Cumbia, London and Northumberland sites. However, maximum peak Qn activity occurred after this threshold had been reached at all sites.

However, the lag between this 7°C Tmean threshold being reached and peak Qn occurring did vary greatly between sites; from 1 week in Cholderton (Wiltshire) to 8 weeks in London (2015), and in 2016, from 1 week in Cholderton to 20 weeks in Cumbria.

Whereas the commencement of increased activity proved difficult to relate unequivocally to a the 7°C Tmean threshold, and the lag between 7°C and actual peak activity did vary, actual peak Qn activity did consistently occurred after this threshold in spring, both at each of the sites documented here and at the Bentley Wood site across all study years. This phenomenon could certainly be manipulated to develop potential predictive models to estimate the timing of maximum nymphal questing activity at geographically distinct site. However, careful consideration would be required on the lag between 7°C Tmean threshold and Qn peak and what might cause this. Many site-specific factors may have attributed to this lag, such as microclimate, land management and host abundance.

A decline in Qn activity did appear to coincide with max PET of 25 to 30 mm/week for all sites. The analysis of post-peak decline in Qn questing in relation to PET revealed that declines in Qn densities coincided with an increase in PET towards 25mm/week. Whilst an arbitrary cut-off of 25 % of peak was used to indicate the point at which increased questing commenced and declined, dips and increases in Qn densities, both pre and post peak, seemed to coincide with increases and decreased in PET.

Relating the complex behaviour of *Ixodes ricinus* nymphs, which are so sensitive to changes in temperature and humidity within their immediate habitat, to weather data calculated at a 5 km grid resolution, is fraught with difficulty (Estrada-Peña *et al.*, 2016). However, regression analysis did indicate that PET (mm/week) for the week of survey and 1-day lag of PET (mm/week) were significant for Qn densities. A 7-day lag of weekly minimum temperature was also found to be significant in this regression analysis, however, weekly mean temperature was not.

A relationship between weeks of maximum precipitation and Qn activity was not apparent. For each year of the study, maximum precipitation occurred out-with the spring question period, with the exception of London for which maximum rainfall occurred on the same week as peak Qn activity. Whereas the Bentley study (Chapter 3) indicated that a 1-day lag in rainfall had a negative association with Qn density, no such relationship was identified here. Previous studies (Kiewra *et al.*, 2014) have indicated that days of previous rain fall can have a negative influence on Qn questing behaviour. The abundance and frequency of rain fall which contribute to maintaining relative humidity and saturation deficit at ground level, however, may also interfere with the collection of Qt, with damp vegetation resulting in a dampened flag and a likelihood of decreased tick attachment. Therefore, it is difficult to ascertain if rainfall has a true negative impact on questing, especially with lack of evidence from this study.

Given these findings, specifically in relation to the significance of PET (mm/week), plus given the absence of higher resolution data, the window between seven consecutive days of 7 °C and PET of 20-25 mm/week, could be used as a basis of prediction of a window of weeks for which commencement and decline of peak nymphal questing is likely to occur.

It was also noted that for both years of the study, all sites exhibited higher than average weekly mean temperatures (°C) when compared to historic climate averages for that 5Km grid. If this trend continues, the temporal dynamics of questing *I. ricinus* may shift, potentially resulting in changes in *borrelia* infection dynamics and resulting in an extended window of tick-bite risk.

4.4.2 Influence of microclimate on *Ixodes ricinus* density and questing patterns

The influence of changes in temperature, relative humidity and resulting saturation deficit, on the questing behaviour of *I. ricinus* is well documented from early laboratory and field studies (Lees & Milne, 1950; McLeod, 1935). As already discussed, temperatures of below 5 °C have been demonstrated to inhibit questing behaviour, whilst temperatures above 7 °C coincided with increased questing activity, to a temperature of 15-17 °C were questing started to decline (Qviller *et al.,* 2014).

However, temperature does not act in isolation, the threat of desiccation in questing *I. ricinus* results in the tick descending back into the vegetation in order to rehydrate. Therefore, the RH (%) of the tick's immediate environment, in combination with temperature, regulates its questing behaviour. Some studies suggest a saturation deficit of greater than 4.4 mm, results in diminished nymphal questing (Perret *et al.,* 2000), however, further studies suggest that while questing may be reduced, high saturation deficit levels of 15-20 mm may be survived by the tick but may induce a period of quiescence (Randolph & Story, 1999).

In this study, over 90 % of all saturation deficit recorded were ≤ 12 mmHg. For all sites, the greatest Qn densities were recorded at saturation deficits of <12 mmHg, however, London did record over 20% of its Qn at situation deficits above 19 mmHg). In addition, it produced several Qn records at saturation deficit approaching 30mmHg (31°C at 28%RH) in spring 2016. Finding Qn at such high saturation deficit thresholds appears counterintuitive given this ticks susceptibility to desiccation, however, if nymphs are capable of surviving limited periods at high temperatures/low RH(%), perhaps it is beneficial to remain questing to the limit of survival. There may also be an element of adaptation to home habitat microclimate which could result in *I. ricinus* from different regions adapting to questing at slightly lower or higher temperatures in their area or even specific microhabitat (Tomkins *et al.,* 2014).

Concluding remarks

This study identified significant longitudinal weather variables which could be associated with maximum spring questing *I. ricinus* nymphal densities. The significance of seven consecutive days of Tmean of 7ºC prior to peak Qn activity and PET on decline of this activity could certainly be investigated for use in predictive models to give an indication of when peak Qn densities might occur. Knowing when this increased Qn activity occurs would have positive implication for tick awareness and public health messaging and reduction of tick bite risk. Such a tool could be expanded to inform local authorities, who could then disseminate the information through social media, thereby engaging with potential high risk groups e.g. ramblers, dog walkers, as well as increase general tick awareness amongst the public.

As this study compliments that performed at Bentley Wood, where weekly data on tick activity continues to be collected to this day, the next step is to use data from the Bentley Wood site which was continuously collected in 2017/18, to investigate if a simple model based on weekly Tmean threshold and PET (mm) can predict commencement, peak and decline of Qn densities following spring time peak for 2017/2018 nymphal questing data.

Chapter 5: The spatial dynamics of *Ixodes ricinus* activity - an exploration of six different landcover types.

5.1 Introduction

Ixodes ricinus ability to establish and persist at a location is largely determined by host availability and habitat suitability (Estrada-Peña, 2001; Hofmeester *et al.,* 2017; Medlock *et al.*, 2008). This ticks dependence on moisture availability which allows rehydration between periods of questing, and development, restricts its ability to establish in a moisture free environment. Equally, a humid habitat with few viable hosts equally prevents establishment. As a result, *I. ricinus* is often associated with certain key habitats, particularly those which may be buffered from harsh fluctuations in temperature and contain a dense moisture rich vegetation layer, such as broadleaf woodland or bracken covered heathland. However, UK human tick bite data (Cull *et al.,* 2018) and historical records (Pietzsch *et al.,* 2005) suggest a wide UK distribution of *I. ricinus*, which may suggest establishment in a range of habitats. *I. ricinus* habitat associations has implications not only for the potential exposure to tick bites but also exposure to potential pathogens, particularly *Borrelia burgdorferi s.l*, the presence of which is determined by wildlife hosts. Therefore, an understanding of habitat associated *I. ricinus* densities is important for better understanding of human tick bite risk and *Borrelia* exposure.

This chapter describes a comprehensive, cross seasonal study of *I. ricinus* presence/absence and density in six different landcover types throughout spring and summer 2016. The aim of this study was to (***i*). investigate the presence/absence and density of questing *Ixodes ricinus* in different landcover types; (***ii.)***identify the specific landcover factors which may influence changes in the presence/absence or density of *Ixodes ricinus* nymphs and (***iii.)*** investigate seasonal changes in presence/absence and abundance of questing nymphs.

5.2. Materials and Methods

Materials and Methods – please refer to Chapter2 for details on selection of site, survey technique and statistical analysis. A brief description will be given here.

5.2.1 Spatial Survey sites

A total of 24 sites were selected from six different land cover types, four sites from each land cover.

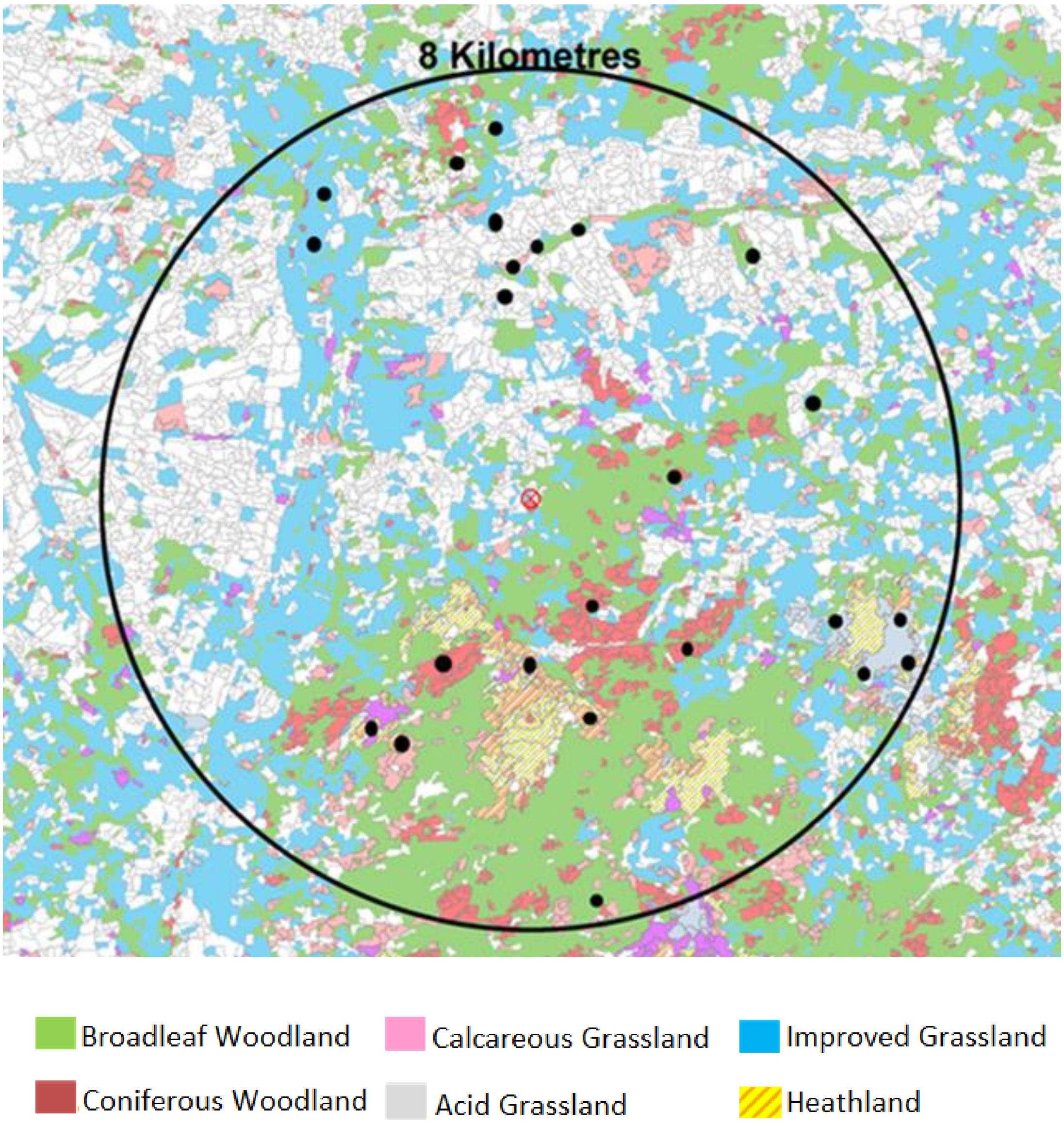


Fig. 5.1 Landcover map generated by ArcGIS software; the 8km radius buffer zone is displayed, dots represent the individual randomised survey sites with in each of the six different landcover types. The white areas are excluded areas of arable or urban landcover.

Table 5.1 Landcover identification, site name, coordinates, aspect, slope and elevation, 50metre DTM resolution. Geographical data was extracted using ARCGIS software.

Site No.	Site id	Site name	Lat/long	Aspect	Slope (degrees)	Elevation (m a.s.l.)
1	Broadleaf1	E. Grimstead	51.051, -1.693	Flat (-1)	0	61
2	Broadleaf2	Whiteparish	51.03, -1.643	SW (202.5 - 247.5)	2.82	147
3	Broadleaf3	Mean Wd	51.008, -1.629	SE (112.5 - 157.5)	3.85	54
4	Broadleaf4	Eyeworth Pond	50.931, -1.677	SE (112.5 - 157.5)	2.23	85
5	Coniferous1	Barnell Copse	50.996, -1.643	NE (22.5 - 67.5)	0.61	60
6	Coniferous2	Peaked Wd	50.973, -1.670	NE (22.5 - 67.5)	0.20	50
7	Coniferous3	Godshill	50.952, -1.720	S (157.5 - 202.5)	6.32	94
8	Coniferous4	Franchises Common Wd	50.960, -1.667	N (0 - 22.5)	1.54	54
9	Calcareous1	Pepperbox Hill Carpark adjacent	51.021, -1.699	S (157.5 - 202.5)	2.63	143
10	Calcareous2	Pepperbox hill meadow	51.021, -1.694	S (202.5 - 247.5)	4.47	127
11	Calcareous3	Pepperbox Hill Nr. Grimstead Rd.	51.028, -1.686	N (292.5 - 337.5)	7.77	140
12	Calcareous4	Pepperbox Hill Bridleway	51.021, -1.703	S (112.5 - 157.5)	3.69	141
13	Improved1	E. Grimstead	51.050, -1.694	S (157.5 - 202.5)	2.15	60
14	Improved2	Farley	51.063, -1.681	N (0 - 22.5)	1.81	70
15	Improved3	Britford	51.050, -1.767	E (67.5 - 112.5)	0.57	43
16	Improved4	Britford Lower Rd	51.049, -1.776	N (22.5 - 67.5)	0.84	49
17	Heath1	Telegraph Hill	50.948, -1.674	N (22.5 - 67.5)	2.07	122
18	Heath2	Bramshaw	50.945, -1.667	Flat (-1)	0	127
19	Heath3	Woodgreen B3078	50.944, -1.742	S (157.5 - 202.5)	0.91	57
20	Heath4	Woodgreen, Lady’s Mile	50.945, -1.745	S (112.5 - 157.5)	8.68	78
21	Acid1	Canada Common	50.958, -1.593	NW (292.5 - 337.5)	2.56	40
22	Acid2	Copythorne	50.958, -1.579	SW (202.5 - 247.5)	1.47	42
23	Acid3	Penn Common	50.949, -1.602	NE (22.5 - 67.5)	0.83	41
24	Acid4	Wellow	50.969, -1.597	N (337.5 - 360)	4.34	39

5.2.2 Statistical analysis

For each site tick density was expressed as the total number of questing ticks collected per 5m^2.

All statistical analysis was carried out using the statistical software R version 3.4.0 (R Development Core Team, Vienna, Austria).

The density of Qn and Qa per 5m^2 from each site and landcover type was highly skewed and exhibited a non-normal distribution, as such these data were analysed using the non-parametric Kruskal Wallis test followed by post-hoc analysis using the Dunn Test, with a Bonferroni adjustment: Temperature, relative humidity, vegetation height and saturation deficit were analysed in the same manor. Results from the Kruskal Wallis test, where reported as: (H(df) = P-value); where H is the test statistic, under the null hypothesis, the chi-square distribution approximates the distribution of H; df refers to degrees of freedom i.e. the number of groups being analysed – 1; and the P-value i.e. the probability of the significance statistic being that extreme or more so if the null hypnotises is true.

The post-hoc Dunns test reports a Z- value and P-value for each variable and tests for significance between pairs of groups.

To account for the repeated measure nature of the study, negative binomial generalized linear mixed models (GLMM) were used to investigate the significance of landcover and on-site environmental variables i.e. temperature (°C), RH (%), saturation deficit and vegetation height, on the questing tick density.

Two generalized linear mixed models (GLMMs) were constructed: GLMM1: to investigate the significance of landcover, temperature (°C), RH (%), saturation deficit and vegetation height on Qn density. GLMM2: to investigate the significance of landcover, temperature (°C), RH (%), saturation deficit and vegetation height on Qa density.

Due to the binary nature of presence/absence data, binomial-response generalised linear models (GLMs) with bias-reduction were used to investigate the presence/absence data. There GLMs were constructed; GLM1: to investigate the influence of landcover and environmental variables on the presence of Qn; GLM2: to investigate the influence of these same variables on Qa; GLM3: to investigate the influence of these same variables on the presence of larvae.

5.3. Results

5.3.1 Density of *I. ricinus* in different landcover types

All sites produced at least one questing nymph at some point during the study, however, the number of transects in which ticks were found varied between landcover type, as did the density of Qn and Qa (Fig5.2).

A total of 2355 5m^2 transects, i.e. 11,775 metres, were surveyed in spring. In total 1765 questing *I. ricinus*; 109 females, 119 males and 1537 nymphs were collected (Table 5.1). Qn were present in 65% of transects in broadleaf woodland, 59% of transects in coniferous woodland, 22% of transects in calcareous grassland, 18% of transects in heathland, 4% of transects in acid grassland and 3% of transects in improved grassland.

Larvae were detected on at least one occasion at all broadleaf woodland sites surveyed, at two of the coniferous woodland sites and at two of the calcareous grassland sites. Larvae were not observed at the improved grassland, heathland or acid grassland sites during the spring survey period.

Table5.2 Total questing nymphs (Qn), females (Qf), males (Qm) and adults (Qa) collected per landcover type. Mean Qn and Qa density (per 5m^2) with 95% confidence intervals [CI] per land cover type is shown. Note: Qn mean and 95%CI based on (log$_{10}$+1).

Landcover Type	Number of 5m^2 transects	Total metres surveyed	Total Qn	Detransformed Qn mean [CI] /per 5m^2	Total Qa	Detransformed Qa mean [CI] /per 5m^2
Spring						
BL	360	1800	667	1.17 [1.34, 1.02]	109	0.2 [0.24, 0.15]
CN	360	1800	478	0.89 [1.01, 0.77]	60	0.1 [0.15, 0.09]
CL	375	1875	264	0.30 [0.38, 0.23]	43	0.07 [0.09, 0.04]
IM	435	2175	15	0.02 [0.04,-0.01]	0	0.0
HL	360	1800	98	0.18 [0.22, 0.13]	14	0.03 [0.04, 0.01]
AC	465	2325	15	0.02 [0.03, 0.01]	0	0.0
Autumn						
BL	360	1800	97	0.19 [0.23, 0.15]	20	0.04 [0.05, 0.02]
CN	360	1800	130	0.25 [0.30, 0.20]	25	0.05 [0.07, 0.03]
CL	405	2025	12	0.02 [0.03, 0.01]	15	0.03 [0.04, 0.01]
IM	510	2550	1	0	0	0.0
HL	390	1950	54	0.09 [0.12, 0.06]	26	0.04 [0.06, 0.03]
AC	495	2475	4	0.004 [0.01, 0]	1	-

In autumn, a total of 2520 5m transects, i.e. 12,600 metres were surveyed. A total of 391 questing *I. ricinus*; 51 females, 37 males and 303 nymphs were collected in autumn. Qn were present in 26% of transects in coniferous woodland, 22% of transects in broadleaf woodland, 11% of transects in heathland, 3% of transects in calcareous grassland, 0.6% of transects in acid grassland, and 0.2% of transects in improved grassland.

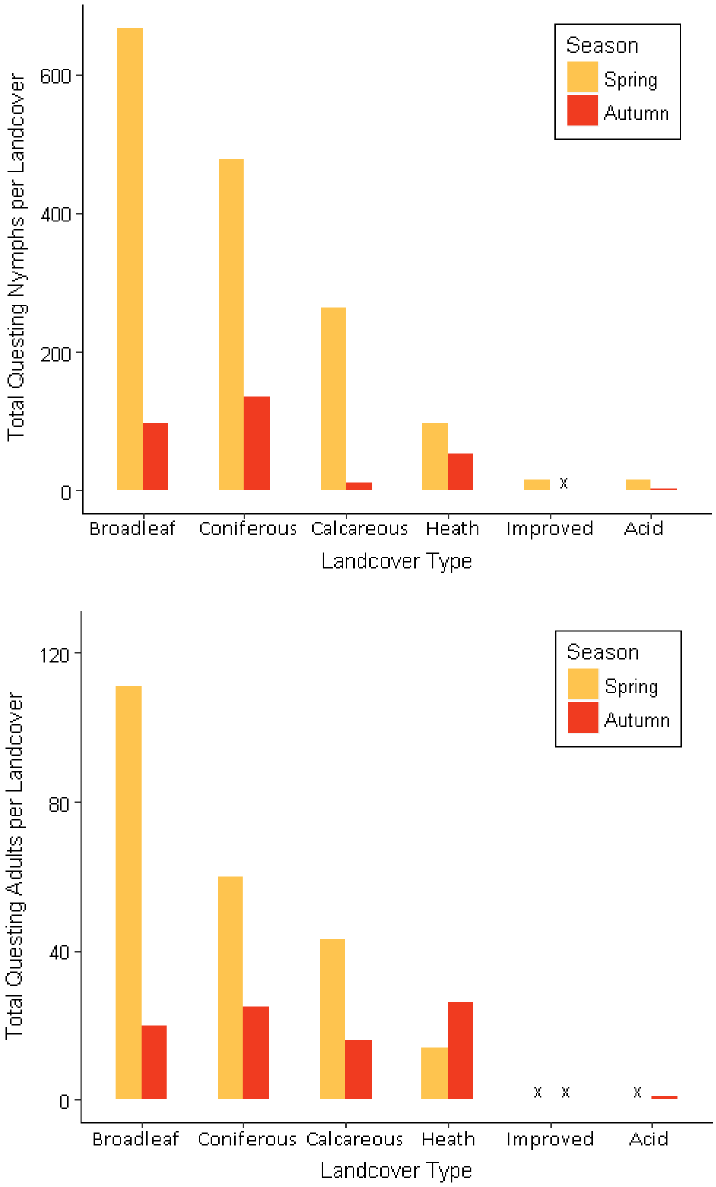


Fig 5.2 Total questing nymphs (top) and adults (lower) collected per landcover type during the spring and autumn survey periods. Note: x denotes that no ticks were observed in that particular landcover type during that season.

Larvae were present in at least one transect at all of the broadleaf woodland, coniferous woodland and calcareous grassland sites during the autumn surveys. Significantly more woodland transects were positive for larvae in autumn rather than spring, especially in coniferous woodland. Calcareous grassland however, exhibited fewer larval positive transects in autumn. In contrast to spring, larvae were recorded in the heathland, improved grassland and acid grassland sites, albeit, a low number of positive transects (Fig.5.3)

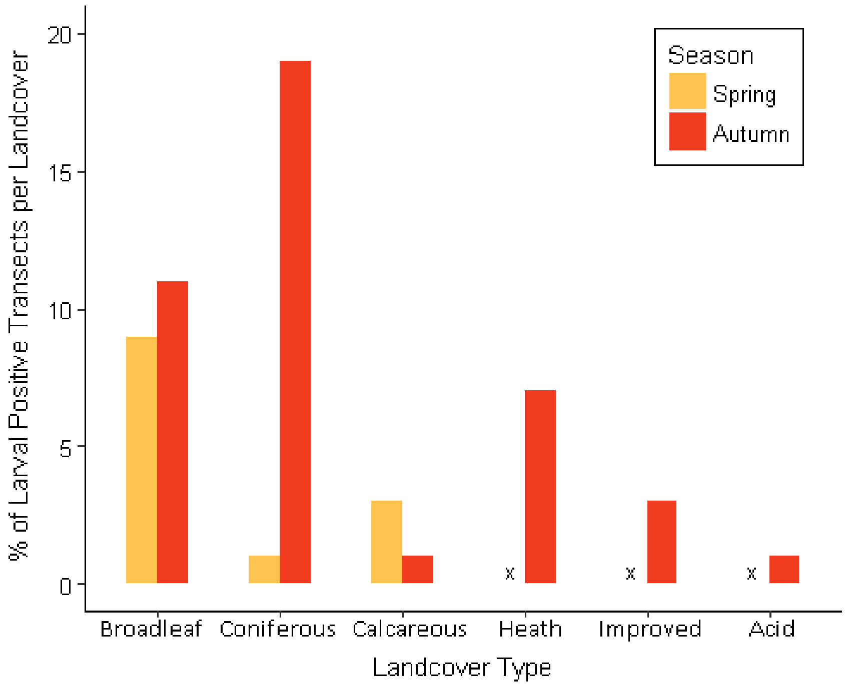


Fig 5.3 Percentage of $5m^2$ transects which were positive for larvae for each different landcover type in both spring and autumn.

5.3.2 Variability in questing *I. ricinus* density between landcover types

Overall, a statistical significance difference in Qn densities between landcover types was observed in spring (H(5df) = 47.6, p-value < 0.001). The greatest number of Qn per 5m^2 was observed in broadleaf woodland (1.7/5m^2 [1.34, 1.02]); this was not statically significant from that of coniferous woodland (z statistic = 2.4, p-value = 0.13). Both woodland sites differed significantly from each of the grassland and heathland sites (Table 5.3) which all produced ≤0.3 Qn/5m^2.

Table 5.3 Statistical comparison using Kruskal – Wallis test of spring Qn densities across different land cover types. Additional pairwise comparisons using Dunn test show the Dunn z-statistic and p-value for each pairwise test.

Comparison of Qn densities across landcover types in spring (Kruskal-wallis test)					
Kruskal-Wallis H = 47.6 On 5 degrees of freedom, p-value <0.001					
Post hoc analysis (Dunn test) comparison by group					
Z statistic p-value	Acid Grassland	Broadleaf Woodland	Calcareous Grassland	Coniferous Woodland	Heathland
Broadleaf Woodland	-20.6 <0.001	-	-	-	-
Calcareous Grassland	-6.1 <0.05	13.8 <0.05	-	-	-
Coniferous Woodland	-18.1 <0.001	2.4 0.1	-11.4 <0.05	-	-
Heathland	-4.7 <0.05	14.9 0.05	1.3 0.9	12.6 <0.001	-
Improved Grassland	-0.06 1.0	20.2 0.001	5.9 <0.001	17.7 <0.001	4.6 <0.001

Spring Qa densities also proved significantly different between landcover types overall (H (5df) = 57.1, p-value <0.001). Once again, broadleaf exhibited the greatest density of Qa/5m^2, followed by coniferous woodland. Individual post-hoc pairwise comparisons revealed no statistically significant difference between woodland Qa densities or indeed, heathland, calcareous grassland and woodland Qa densities, all of which exhibited questing adults (Fig 5.4). No adult ticks were observed in acid or improved grassland sites during the spring survey period.

Table 5.4 Statistical comparison using Kruskal – Wallis test of spring Qa densities across different land cover types. Additional pairwise comparisons using Dunn test show the Dunn z-statistic and p-value for each pairwise test.

Comparison of Qa densities across landcover types in spring (Kruskal-wallis test)					
Kruskal-Wallis H = 57.1 On 5 degrees of freedom, p-value <0.001					
Post hoc analysis (Dunn test) comparison by group					
Z statistic p-value	Acid Grassland	Broadleaf Woodland	Calcareous Grassland	Coniferous Woodland	Heathland
Broadleaf Woodland	-3.8 <0.01	-	-	-	-
Calcareous Grassland	-2.6 0.07	1.2 1.0	-	-	-
Coniferous Woodland	-5.2 <0.001	-2.3 1.0	-2.5 0.09	-	-
Heathland	-4.9 <0.001	-0.9 1.0	-2.2 0.2	0.4 1.0	-
Improved Grassland	0.2 1.0	3.9 <0.001	2.8 <0.05	5.4 <0.001	5.1 <0.001

In autumn, coniferous woodland exhibited the greatest density of Qn per transect (0.2/5m^2 [0.23, 0.15]), however, this was not significantly from that of broadleaf woodland (H = -2.3, p-value = 1.0). While heathland exhibited fewer Qn than both woodland land types, this was not revealed to be a statistically significant (Table 5.5). Each of the grassland landcover types produced ≤0.02 Qn/5m^2; improved grassland producing just a single Qn from the entire 2550 m sampled over the autumn period.

Table5.5 Statistical comparison using Kruskal – Wallis test of autumn Qn densities across different land cover types, Additional pairwise comparisons using Dunn test show the Dunn z-statistic and p-value for each pairwise test.

Comparison of Qn densities across landcover types in autumn (Kruskal-wallis test)					
Kruskal-Wallis H = 51.4 On 5 degrees of freedom, p-value <0.001					
Post hoc analysis (Dunn test) comparison by group					
Z statistic p-value	Acid Grassland	Broadleaf Woodland	Calcareous Grassland	Coniferous Woodland	Heathland
Broadleaf Woodland	-10.6 <0.001				
Calcareous Grassland	-1.1 1.0	9.2 <0.01			
Coniferous Woodland	-13.1 <0.001	-2.3 0.2	-11.5 <0.01		
Heathland	-5.1 0.07	5.4 1.0	-3.8 1.0	7.7 0.8	
Improved Grassland	0.2 1.0	10.9 <0.001	1.3 1.0	13.4 <0.001	5.3 <0.05

Similarly, Qa densities were also greater in coniferous woodland during the autumn surveys, although did not differ significantly from broadleaf woodland or indeed, from heathland or calcareous grassland (Table 5.6). Acid and improved grassland did not yield Qa during the autumn surveys.

Table5.6 Statistical comparison using Kruskal – Wallis test of autumn Qa densities across different land cover types. Additional pairwise comparisons using Dunn test show the Dunn z-statistic and p-value for each pairwise test.

Comparison of Qa densities across landcover types in autumn (Kruskal-wallis test)					
Kruskal-Wallis H = 27.5 On 5 degrees of freedom, p-value = <0.001					
Post hoc analysis (Dunn test) comparison by group					
Z statistic p-value	Acid Grassland	Broadleaf Woodland	Calcareous Grassland	Coniferous Woodland	Heathland
Broadleaf Woodland	-3.1 <0.05				
Calcareous Grassland	-2.2 0.4	0.9 1.0			
Coniferous Woodland	-3.4 <0.01	-0.2 1.0	-1.2 1		
Heathland	-3.0 <0.05	0.1 1.0	-0.8 1	0.4 1.0	
Improved Grassland	0.3 1.0	3.4 <0.01	2.5 0.2	3.7 <0.01	3.3 <0.05

5.3.3 Seasonal *I. ricinus* density variability within landcover types

The following section describes the inter-site variability in *I. ricinus* density, tables detailing Qn and Qa densities per 5 m^2 for each individual site can be found in the Appendix (Table A5.1 to A5.6).

5.3.3.1 Broadleaf Woodland

During spring, questing *I. ricinus* were present in 69% of transects surveyed in broadleaf woodland. Surveys conducted in this landcover type during this period, displayed the greatest density of Qn (1.17/5 m^2 [1.34, 1.02]) of the entire study. Qa densities were also greater (0.2/5 m^2 [0.45, 0.15]) than any other landcover type. The presence of larvae was also consistent, found during all three survey replicates, at three of the broadleaf sites, and on one occasion at a fourth broadleaf site.

In autumn, questing *I. ricinus* were collected in just 25% of broadleaf woodland transects. Whilst the density of Qn and Qa fell dramatically, larvae were present at each of the four sites across all three survey replicates.

In spring, Broadleaf site three (BL3) exhibited greater density of Qn per 5 m^2 in each of its three survey replicates than any of the other sites (Fig 5.4), however, overall there was not a statistically significant difference between sites (P-value = 0.6).

Autumn exhibited a significantly lower Qa density/5 m^2 than spring (P-value <0.01). All survey replicates produced at least one nymph during this period, however, the maximum mean recorded in any site being <0.5 Qn per 5m^2. The number of Qa showed little across site variation during autumn (p = 0.7)

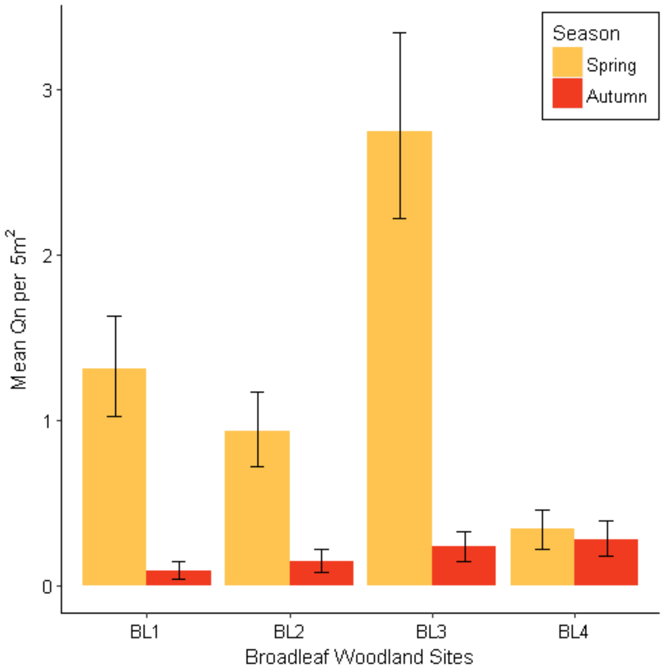


Fig 5.4 The contrast between spring and autumn mean Qn densities /5 m² for each broadleaf site. The 95% confidence intervals around the mean/5 m^2 are also displayed.

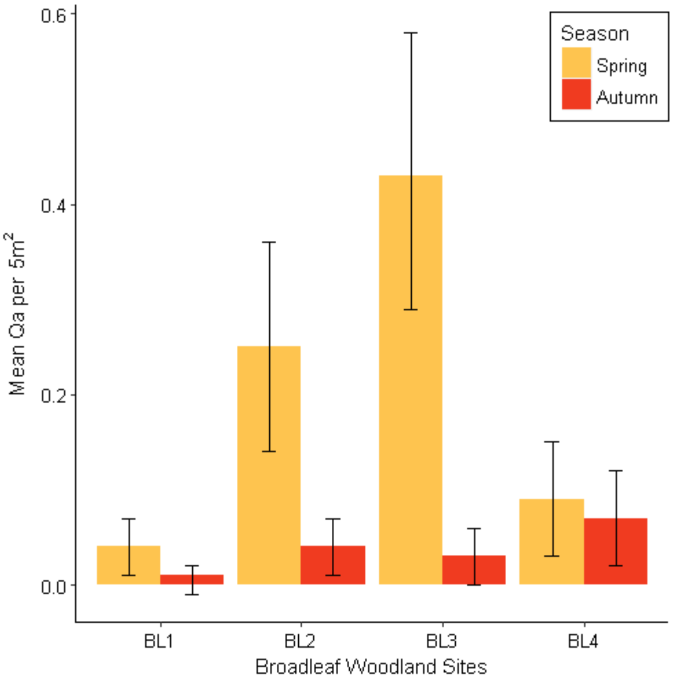


Fig 5.5 The contrast between spring and autumn mean Qa densities /5 m² for each broadleaf site. The 95% confidence intervals around the mean/5 m^2 are also displayed.

5.3.3.2 Coniferous woodland

During spring surveys, *Ixodes ricinus* were present in 62% of coniferous woodland transects, making it the second highest prevalence landcover type; with a Qn density of 0.9/5 m^2 [1.01, 0.8] and Qa density of 0.1/5 m^2 [0.15, 0.09]. The numbers of Qn collected between sites varied but not significantly so (P-value = 0.5). In spring, larvae were recorded for just one survey replicate at three of the four sites (CN1, CN2 and CN3); the fourth site did not yield any larvae during the spring surveys.

Autumn surveys resulted in half as many positive transects, with just 31% positive for questing *I. ricinus*. A significant (P <0.001) fall in the number of Qn collected across all sites was observed in autumn, were overall Qn numbers were reduced by almost one third, compared to spring.

Eleven of the twelve surveys conducted across all coniferous sites during the autumn season produced larvae.

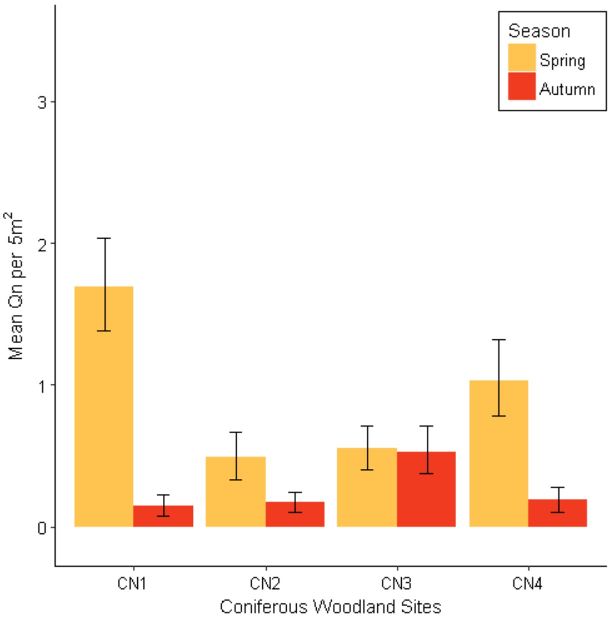


Fig 5.6 The contrast between spring and autumn mean Qn densities /5 m² for each coniferous site. The 95% confidence intervals around the mean/5 m^2 are also displayed.

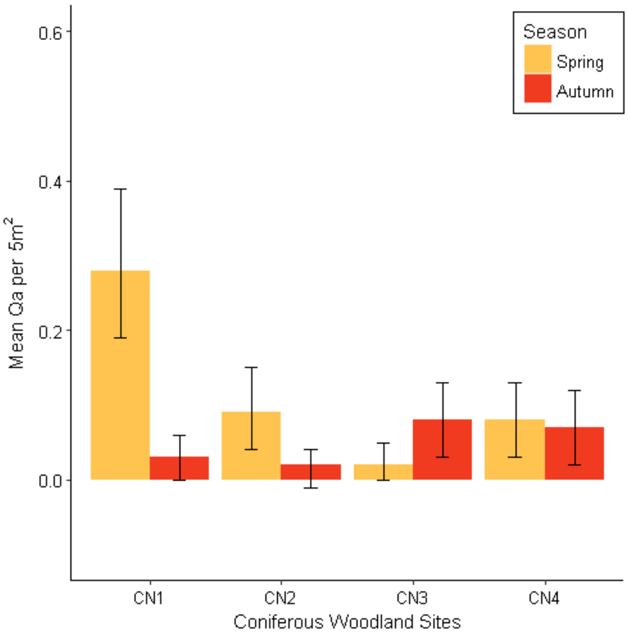


Fig 5.7 The contrast between spring and autumn mean Qa densities /5 m² for each coniferous site. The 95% confidence intervals around the mean/5 m^2 are also displa

5.3.3.3 Calcareous grassland

During the spring surveys, calcareous grassland site one (CL1) produced substantially more nymphs than any of the other sites in this category ($P < 0.01$). In total 56% of transects were positive for *I. ricinus* in CL1, compared to 30% of CL4, 8% of CL3 and 5% of transects in CL2.

All survey replicates for CL1 exhibited a mean Qn density of > 1.5/ 5 m^2; mean per 5m^2 ranged from, 1.53 to 3.67 Qn/5 m^2 for all survey replicates at this specific site. Qa density was also significantly higher at this site, with replicates CL2-CL4 ranging from just 0.23 Qa/5m^2 to 0.53 Qa/5m^2 (Qa mean per 5 m^2).

During spring surveys, larvae were present in all three survey replicates at CL1, in just one replicate in CL2, and were absent at both CL3 and CL4.

A significant drop in Qn/5m^2 was observed between spring and autumn ($P < 0.01$), in fact, overall just 6% of calcareous grassland transects were positive for questing *I. ricinus* during autumn surveys. Although all sites exhibited low Qt numbers during autumn, in a stark contrast to spring, CL1 produced the fewest Qn/5 m^2 compared to other calcareous sites; a Qn density of 0.008/5m^2 in autumn compared with 2.5 Qn/5 m^2 in spring.

CL1 also displayed a substantial drop in the number of Qa compared to spring to autumn; 2.5 Qn per 5 m^2 in spring and 0.008 Qn per 5m^2 in autumn. In contrast, CL2 exhibited an increase in nymphal density from 0 Qn/ 5 m^2 to 0.08 Qn/ 5m^2.

Larvae were detected in two survey replicates at CL1, once at CL2 and CL4, and not at all at CL3.

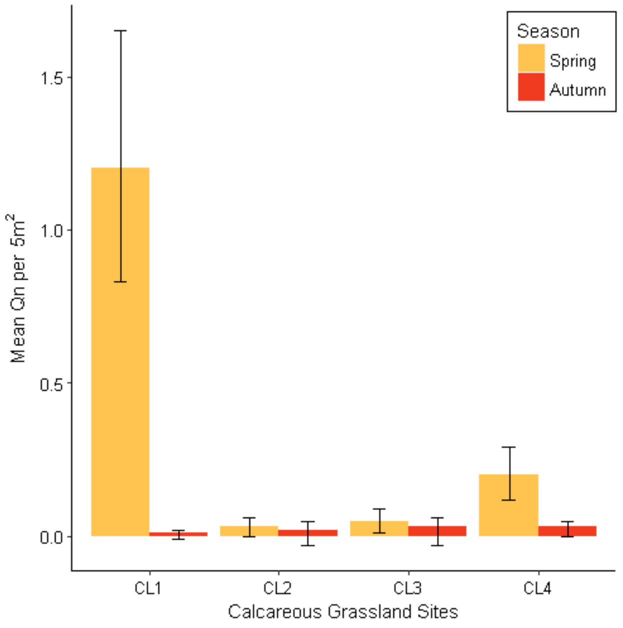


Fig 5.8 The contrast between spring and autumn mean Qn densities /5 m² for each calcareous site. The 95% confidence intervals around the mean/5 m^2 are also displayed.

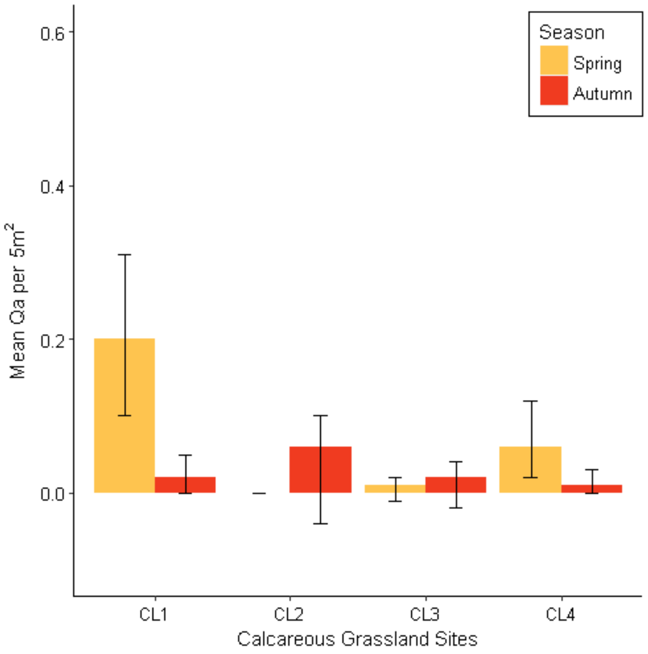


Fig 5.9 The contrast between spring and autumn mean Qa densities /5 m² for each calcareous site. The 95% confidence intervals around the mean/5 m^2 are also displayed.

5.3.3.4 Improved Grassland

Overall, improved grassland exhibited the lowest percentage positive transects for questing *I. ricinus* in spring; of the four sites, IM2 exhibited the highest percentage of just 10%, followed by IM1, at 4%; <1% of transects surveyed in IM3 and IM4 were positive for questing *I. ricinus*.

Although variation was observed, no significant difference in Qn densities was detected between improved grassland sites ($P = 0.3$). IM2 produced the greatest density of Qn/m^2; 0.008/5 m^2 . IM1 produced just 0.04 Qn/ m^2; both IM3 and IM4 produced just a single Qn for all spring surveys.

No Qa were observed at improved grassland sites in spring, nor were larvae.

Autumn surveys also resulted in low Qn densities, with only one site (IM1) producing a single positive transect and a single Qn. All other sites yielded zero questing Qn or Qa during autumn.

Larvae were detected at two of the four sites in autumn - on all three survey occasions at IM1 and on just one occasion at IM2.

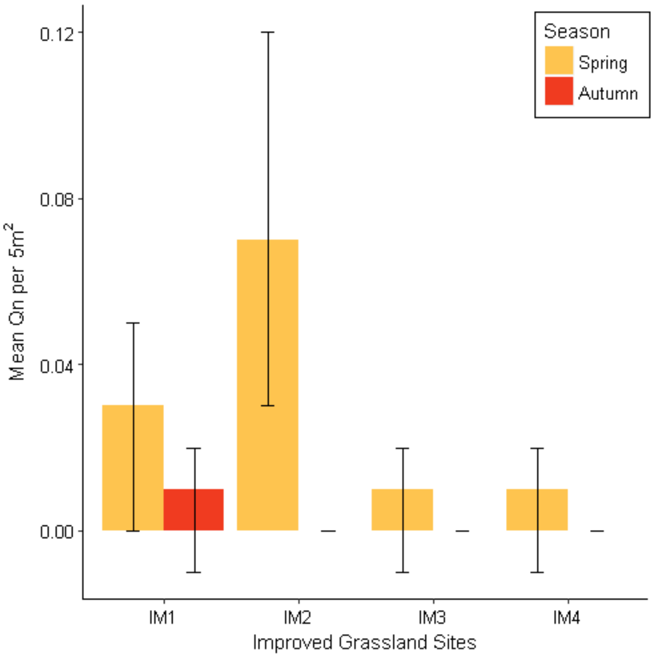


Fig 5.10 The contrast between spring and autumn mean Qn densities /5 m² for each calcareous site. The 95% confidence intervals around the mean/5 m^2 are also displayed.

5.3.3.5 Heathland

In spring the percentage of heathland positive Qt transects ranged from 10% to 32% between sites. A significant difference in Qn/5m ^{2} between sites ($P<0.05$), specifically between site HL4 and HL1 ($P<0.05$) was observed. HL1 had the density of Qn/ 5 m^2 (0.42 Qn/ 5m^2 in spring; HL2 and HL4 yielded half as many nymphs per site, both producing an overall mean (per 5m²) Qn density of <0.2 Qn/5 m^2.

In spring, Qa density showed little overall variation across HL1, HL2 and HL3, however, variation was observed between site replicates. No adults were detected at HL4 during the spring surveys. Additionally, no larvae were detected across any of the heathland sites during spring surveys.

In autumn, fewer Qn were detected at each of the heathland sites, in comparison to spring, with an overall mean Qn per 5m² of 0.138 ± 0.02, almost half that of spring. Similar to spring, H1 and H3 yielded greater number of Qn, compared to H2 and H4.

Qa densities increased slightly across all sites in autumn, with the exception of H2, which showed a decrease in Qa/5m^2. Larvae were identified at eight of the twelve heathland sites in autumn

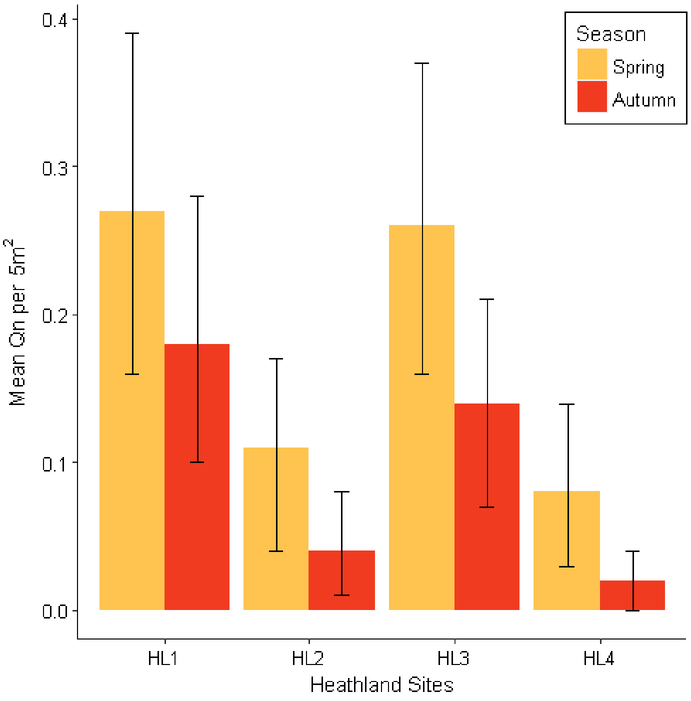


Fig 5.11 The contrast between spring and autumn mean Qn densities /5 m² for each heathland site. The 95% confidence intervals around the mean/5 m^2 are also displayed.

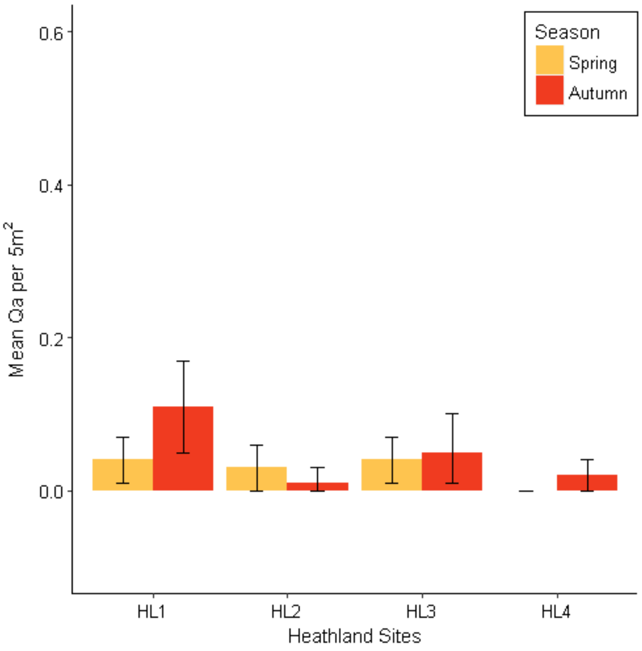


Fig 5.12 The contrast between spring and autumn mean Qa densities /5 m² for each heathland site. The 95% confidence intervals around the mean/5 m^2 are also displayed.

5.3.3.6 Acid Grassland

In terms of overall questing *I. ricinus* density, acid grassland yielded the lowest overall density of Qn/5m^2 during the spring survey period (0.03 Qn/5m^2). Qn numbers were low across all sites, with only AC1and AC3 producing Qt on all three survey replicates; site AC2 and sAC4 produced nymphs on just one of the three survey replicates.

No Qa were observed on acid grassland sites in spring, nor were any larvae detected.

In autumn just a single nymph was detected at each of the four survey sites, resulting in an overall mean of 0.003 Qn/5m^2. In addition, a single female was detected at AC1 during the first survey of the season – none of the other sites yielded Qn during autumn.

Larvae were detected on one survey occasion at three of the four sites (AC1, AC3 and AC4) but not at all at AC2.

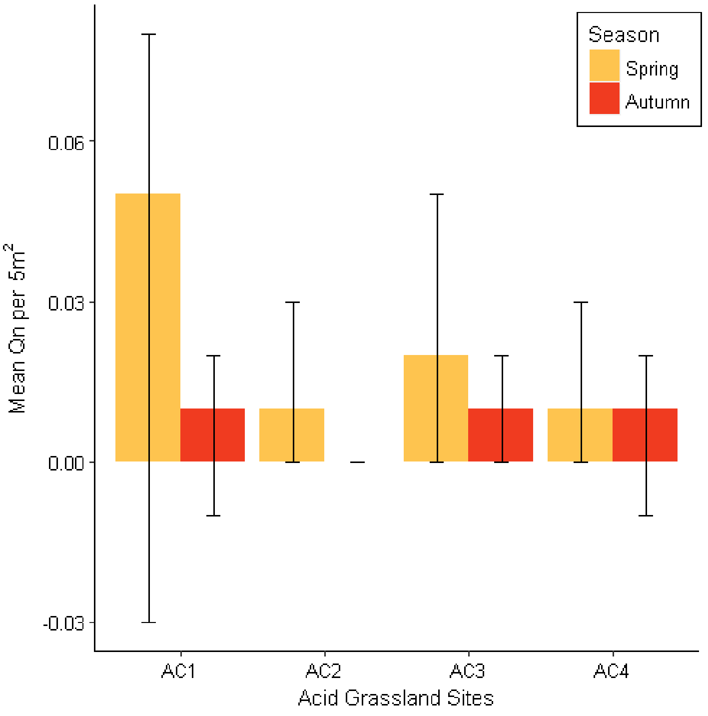


Fig 5.13 The contrast between spring and autumn mean Qn densities /5 m² for each acid grassland site. The 95% confidence intervals around the mean/5 m^2 are also displayed.

5.3.4 Environmental variables across landcover types

5.3.4.1 Temperature

There was no overall significant difference in temperature between landcover types in spring (H (df:5) = 1.9, P-value = 0.8), with overall mean temperature recorded near questing height ranging from 17.5-19.3 °C for all landcover types. Acid grassland displayed the highest overall mean of 19.8 °C, and broadleaf the lowest; 17.5 °C (Fig 5.15).

In autumn too, no significant difference in temperature was observed between landcover types (H (df 5): 10.5, P-value = 0.06). Overall mean temperature ranged from 16.6 °C for improved grassland to 19.1 °C for calcareous grassland. (Fig 5.14).

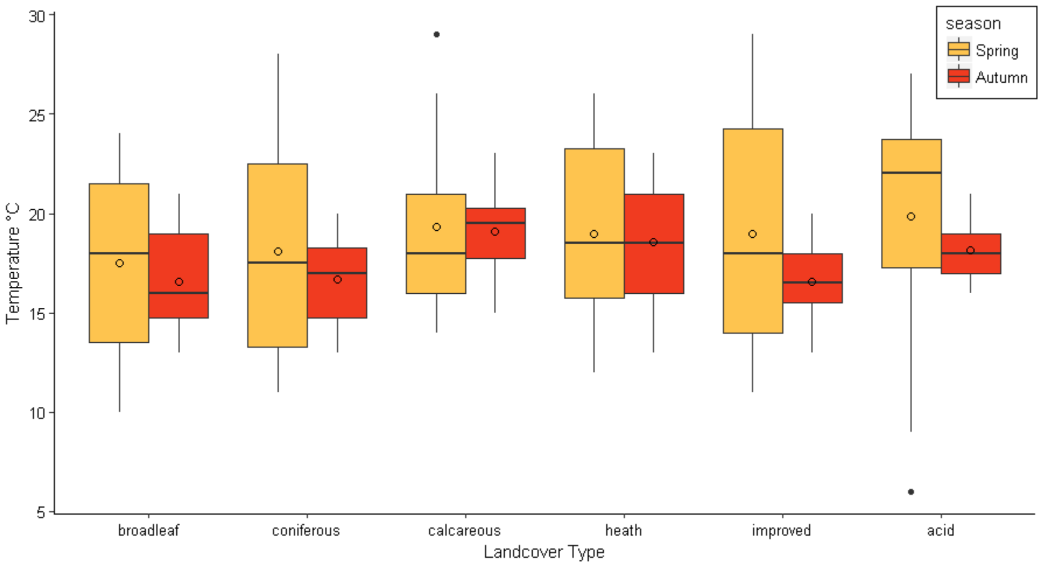


Fig 5.14 Seasonal temperature (°C) variation across different landcover types. Each box describes temperatures recorded at all surveys for a particular season. Boxes and whiskers present the interquartile range of recorded temperature at each site. The median is represented by a black line with each box, the mean temperature per landcover is represented by an open circle, and outliers are represented by a solid black circle.

In spring, none of the individual sites within each landcover type exhibited an overall significant difference in temperature at time of survey; however, broadleaf, heathland and acid grassland all exhibited significant temperature differences between surveys replicates. Broadleaf (P <0.05) survey temperatures ranged from a lowest record of 10 °C to a high of 24 °C. Improved grassland survey replicates (P <0.05) recorded a low of 14 °C and a highest temperature of 29 °C. Heathland recorded a lowest temperature of 12 °C to 26 °C over the course of the surveys, and acid grassland (<0.01) recorded the lowest temperature of the entire spring survey, 6 °C on the final survey at site AC4.

Similarly, in autumn no overall significant difference in temperature was observed between landcover types, however, during this period, significant intra-site differences in temperature were recorded sites in all landcover sites and across survey replicates.

In relation to nymphal density, larger densities/per transect of *I. ricinus* nymphs were recorded at temperatures of between 14 -23°C. However, temperature did not prove a significant factor in the models constructed in the study (Table 5.9, Table 5.10). The highest temperature recorded during surveys was 29°C; this was in calcareous grassland (site CL2), a single questing nymph was recorded during this survey. This temperature was also recorded at an improved grassland site in spring IM4; no *I. ricinus* were recorded on this occasion however. The lowest temperature recorded during the surveys period was 6°C; this was recorded on acid grassland in spring at AC4, no Qt were recorded during this survey. The lowest temperature at which a survey was conducted and *I. ricinus* was detected was 9°C, in acid grassland during spring.

5.3.4.2 Relative Humidity

Overall, mean RH (%) was significantly higher in autumn than in spring, this was the case across all landcover types (Fig.5.15). Overall, broadleaf woodland landcover differed significantly (P < 0.001) from a mean RH of 40 % in spring to and mean of 74 % in autumn; coniferous woodland recorded a mean RH of 35 % in spring and an overall mean of 66 % in autumn (P <0.001); calcareous grassland exhibited a mean RH of 35 % in spring and 63 % in autumn (P <0.001); improved grassland exhibited 46% mean RH in spring and 68% in autumn; heathland recorded a springtime mean of 37% RH, and 57% in autumn; finally, acid grassland exhibited a mean RH of

41% in spring and 55% in autumn. Intra-site variation was observed, however, all sites within each landcover recorded a lower RH in spring than in autumn.

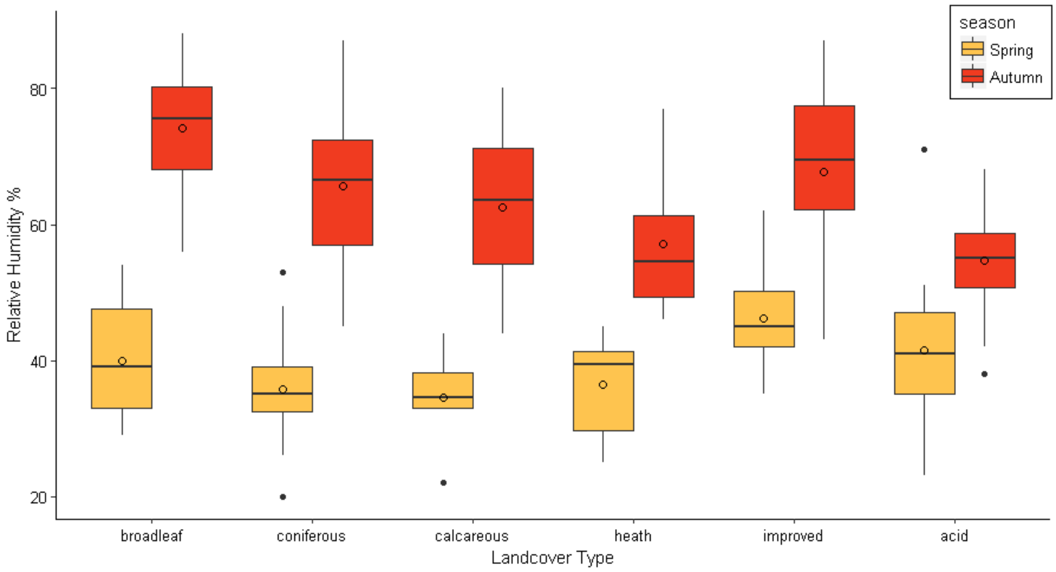


Fig 5.15 Seasonal relative humidity (%) variation across different landcover types. Mean landcover type RH represented by circle, median represented by line. Each box describes temperatures recorded at all surveys for a particular season. Boxes and whiskers present the interquartile range of recorded temperature at each site. The median is represented by a black line with each box, the mean temperature per landcover is represented by an open circle, and outliers are represented by a solid black circle.

Less variation in RH at individual sites occurred in spring compared to autumn (Fig 5.15), with the exception of AC4 (acid grassland site 4). In spring, improved grassland sites displayed the highest RH values (44-51%), closely followed by heathland (36-37%), acid grassland (35-49%), broadleaf woodland (35-45%), coniferous woodland (34-37%) and calcareous grassland (30-38%).

Broadleaf woodland sites displayed the highest relative humidity in autumn (70-83%), followed by improved grassland (61-81%), coniferous woodland (59-73%), calcareous grassland (50-70%), heathland (48-66%) and acid grassland (50-59%).

The lowest RH at which nymphs were collected was 20%, recorded at coniferous woodland site CN4 in spring, 1.07Qn/5m^2 were collected in this survey. The highest RH recorded during a survey was 88%, recorded at broadleaf site BL2 in autumn, 0.17 Qn/ 5m^2 were collected during this survey.

In subsequent models (Table 5.6), RH was only found to be significant in the presence of larval *I. ricinus*.

5.3.4.3 Saturation Deficit

Saturation deficit was calculated from on-site measurements of temperature and RH, consequently, it was lower in autumn than in spring across all landcover types (Fig 5.16). The mean saturation deficit for each individual site was also greater in spring than in autumn, as with RH and temperature, less variation occurred in autumn.

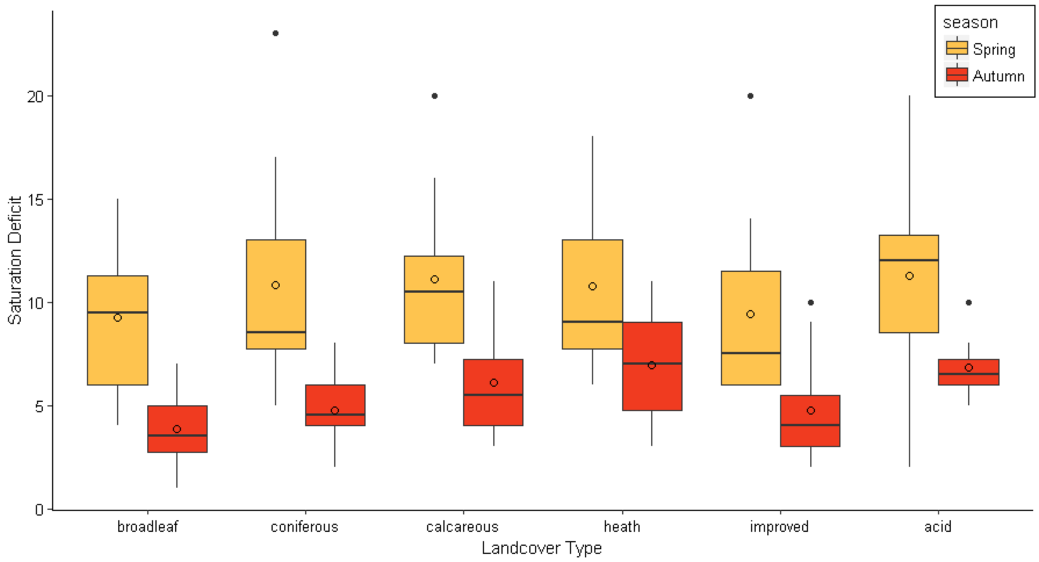


Fig 5.16 Seasonal saturation deficit variation across different landcover types. Mean saturation deficit per landcover type is represented by circle, median represented by line. Each box describes temperatures recorded at all surveys for a particular season. Boxes and whiskers present the interquartile range of recorded temperature at each site. The median is represented by a black line with each box, the mean temperature per landcover is represented by an open circle, and outliers are represented by a solid black circle.

In line with temperature and relative humidity readings, the lowest calculated saturation deficit of 1mmHg occurred at broadleaf site BL2 in autumn (equivalent to a temperature of 14°C and RH of 88%); a high of 20 mmHg was calculated for site CN4 (temperature of 28°C and RH of 20%).

Some intra-site variation in saturation deficit did occur in calcareous grassland and heathland; however, all other landcover types exhibited uniformity of lower saturation deficit in autumn compared to spring.

5.3.4.4 Vegetation Height

Overall, mean vegetation height was greater in autumn across all landcover types (Fig 5.18), but significantly so in broadleaf woodland ($P < 0.01$) and calcareous grassland ($P < 0.05$).

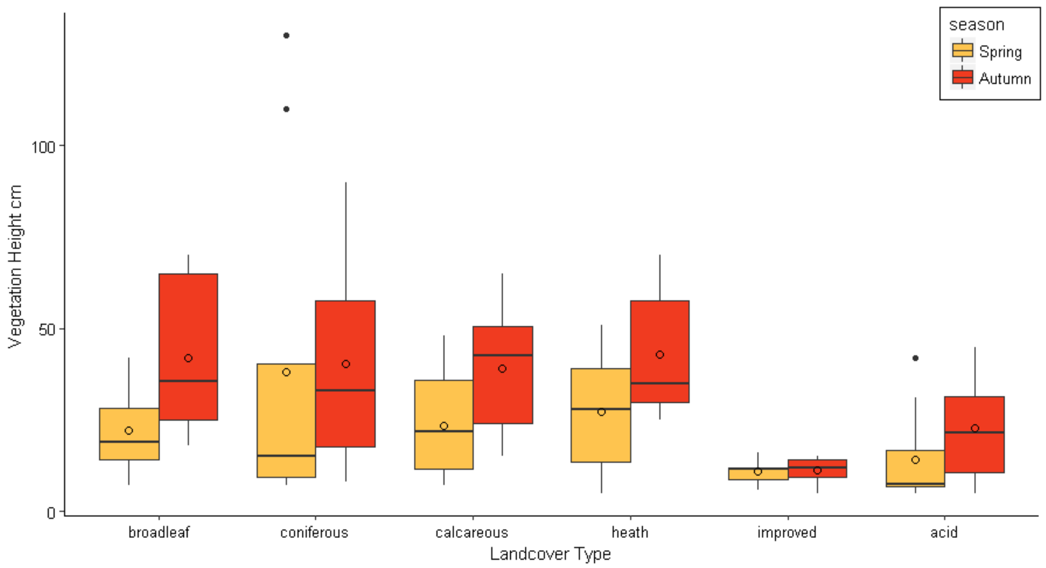


Fig 5.17 Seasonal vegetation height variation across different landcover types. Mean vegetation height per landcover type is represented by circle, median represented by line. Each box describes temperatures recorded at all surveys for a particular season. Boxes and whiskers present the interquartile range of recorded temperature at each site. The median is represented by a black line with each box, the mean temperature per landcover is represented by an open circle, and outliers are represented by a solid black circle.

Although variation in vegetation height was observed across different landcover types in spring, none proved significantly so (Fig 5.17).

In autumn, improved grassland proved significantly different to all other landcover types, with the exception of acid grassland (P =1.0); broadleaf (P <0.001), coniferous (P <0.01), calcareous (P <0.01) and heathland (P <0.001).

In terms of nymphs, larger densities per transect were recorded at vegetation heights of between 13-22cm. The lowest vegetation height of 5cm was recorded at several two sites; once in heathland site HL4, and once in acid grassland AC1, both during spring surveys. Vegetation height of 5cm was also recorded in autumn at height was also been recorded at acid grassland AC2 and also at improved grassland site IM1. The heathland site HL4 had recently been mown in an attempt to manage overgrowth on an adjacent bridleway. AC1 and AC2 were continuously grazed by a combination of cattle, sheep, wild ponies and donkeys. The improved grassland sites had been mown for hay in the weeks previous to the autumn surveys. Qn were recorded on each of these occasions.

The highest vegetation recorded at any individual site was 130cm, recorded at coniferous woodland site CN4; a Qn density of $1.93/5m^2$

5.3.5 Factors contributing to *Ixodes ricinus* presence/absence – model output

Logistic regression was used to investigate the effect of different landcover types, as well as the environmental variables recorded at each site i.e. temperature, relative humidity, vegetation height and saturation deficit on the presence/ absence of Qn, Qa and larvae. Three Generalised Linear Models (GLMs) were constructed; **GLM1**: to determine the significance of these multiple parameters on the presence/absence of Qn; **GLM2**: to determine the significance of these parameters on the presence/absence of Qa, and **GLM3**: to determine the significance of these parameters on the presence/absence the three stages of larvae.

Each GLM used a binomial distribution error, a logit function and an error reduction; each model was constructed using the brglm package (v.0.6.1) (Kosmidis, 2017) in R statistical software (v. 3.4.0).

Table5.6 Output from GLM1, GLM2 and GLM3; For each of the explanatory variable the regression coefficient, standard error (SE), z-value (test statistics) and the p value (indicting the probability of significance) are given. Variables which have been calculated as significant are highlighted

	Estimate (SE)	z-value	p-value
Output from individual GLMs			
GLM1	Estimate (SE)	z-value	p-value
Y = nymph presence			
Intercept	2.96 (1.72)	1.7	0.08
Broadleaf Woodland	4.94 (1.64)	3.01	**0.003**
Calcareous Grassland	1.78 (0.74)	0.74	**0.015**
Coniferous Woodland	4.93 (1.7)	2.89	**0.004**
Heathland	2.75 (0.89)	3.06	**0.002**
Improved Grassland	-0.65 (0.63)	-1.03	0.303
Temperature	-0.04 (0.06)	-0.75	0.454
Relative humidity	-0.03 (0.02)	-1.87	0.062
Vegetation height	-0.028 (0.02)	-1.7	0.089
GLM2			
Y = adult presence			
Intercept	-1.9 (1.69)	-1.13	0.258
Broadleaf Woodland	4.5(1.03)	4.36	**1.33x10^{-5}**
Calcareous Grassland	2.88(0.94)	3.07	**0.0022**
Coniferous Woodland	4.47 (1.02)	4.36	**1.31x10^{-5}**
Heathland	3.01(0.94)	3.21	**0.0013**
Improved Grassland	-1.11 (1.66)	-0.67	0.504
Temperature	-0.02 (0.059)	-.0.41	0.683
Relative humidity	-0.007(0.01)	-0.48	0.627
GLM3			
Y = Larvae presence			
Intercept	-7.09(1.89)	-3.79	**0.00001**
Broadleaf Woodland	4.47(0.99)	4.54	**5.64x10^{-6}**
Calcareous Grassland	1.34 (0.80)	1.67	0.096
Coniferous Woodland	2.84(0.85)	3.34	**0.0008**
Heathland	1.69(0.81)	2.09	0.037
Improved Grassland	-0.51(0.87)	-0.59	0.56
Temperature	0.089(0.06)	1.46	0.14
Relative humidity	0.076(0.02)	4.24	**2.28x10^{-5}**
Vegetation height	-0.016 (0.01)	-1.45	0.147

5.3.5.1 Questing nymphs

Four of the six landcover types identified as having a significant positive effect on the presence of Qn; ***i***. broadleaf woodland ($P < 0.01$), ***ii.*** coniferous woodland ($p = 0.0038$) and ***iii.*** heathland ($P < 0.01$) were determined to have a highly significant influence. A marginally significant effect was identified in ***iv.*** calcareous grassland ($P < 0.05$). None of the other model parameters i.e. improved grassland, acid grassland, temperature, relative humidity and vegetation height, emerged as significant for the presence/absence of Qn.

5.3.5.2 Questing adults

GLM2: several parameters emerged as significant for the presence of Qa*:* ***i.*** broadleaf woodland ($P < 0.001$) and ***ii.*** coniferous woodland ($P < 0.001$), both emerged as highly significant, whilst; ***iii***. calcareous grassland ($P < 0.01$) and ***iv.*** heathland ($P< 0.001$) were reported to have a significant effect. None of the additional model parameters i.e. improved grassland, acid grassland, temperature or relatively humidity emerged as significant in the presence/absence of Qa.

5.3.5.3 Larvae

GLM3: A highly significant positive impact on the presence of larvae at a site was attributed to the following parametres: ***i.*** broadleaf woodland ($P < 0.001$) ***ii.*** coniferous woodland ($P < 0.001$) and ***iii.*** relative humidity ($P < 0.001$). Heathland ($P <0.05$) was also found to be significant. Acid grassland ($P < 0.001$) was found to be highly significant in determining a negative effect on the presence on larvae. All other parameters in the model i.e. calcareous grassland, improved grassland, temperature and vegetation height did not emerge as significant terms in the presence/absence of *I. ricinus* larvae.

5.3.5.4 Factors contributing to the density of *Ixodes ricinus*

Generalised Linear Mixed Models (GLMMs) were used to assess the effects of each different landcover type, as well as temperature, relative humidity, saturation deficit and vegetation height on the density of Qn and Qa. Two individual GLMMS were used; **GLMM1**: to assess the effect of the above variables on the density of Qn, and **GLMM2**: to assess the effect of the above variables on the density of Qa.

Table5.7 Output from GLMM1 and GLMM2; For each of the explanatory variable the regression coefficient, standard error (SE), z-value (test statistics) and the p value (indicting the probability of significance) are given. Variables which have been calculated as significant are highlighted.

Output from individual GLMMs			
GLMM1	Estimate (SE)	z-value	p-value
Y = nymph density			
Intercept	-0.29 (0.44)	-0.66	0.51
Broadleaf Woodland	3.66 (0.58)	0.58	**<0.001**
Calcareous Grassland	2.01 (0.59)	3.42	**<0.001**
Coniferous Woodland	3.53 (0.58)	6.12	**<0.001**
Heathland	2.04 (0.5)	3.5	**<0.001**
Improved Grassland	-0.299 (0.64)	-0.45	0.65
GLMM2			
Y = adult density			
Intercept	-3.27(1.05)	-3.12	0.002
Broadleaf Woodland	4.78(1.7)	4.47	**<0.001**
Calcareous Grassland	3.9(1.09)	3.59	**<0.001**
Coniferous Woodland	4.39(1.07)	4.11	**<0.001**
Heathland	3.62	1.09	**<0.001**
Improved Grassland	-17.6	6.9	0.99

5.3.5.5 Questing nymphal density

GLMM1, four of the six landcover types identified as having a significant positive effect on the density of *Qn*; ***i***. broadleaf woodland (P <0.001), ***ii.*** coniferous woodland (P <0.001) and ***iii.*** heathland (P <0.001) were determined to have a highly significant influence on Qn density. None of the other model parameters i.e. improved grassland, acid grassland, temperature, relative humidity and vegetation height, emerged as significant for of Qn density.

5.3.5.6 Questing adult density

GLMM2: several parameters emerged as significant for the presence of Qa*:* ***i.*** broadleaf woodland (P <0.001) and ***ii.*** coniferous woodland (P <0.001), both emerged as highly significant, whilst; ***iii***. calcareous grassland (P <0.001) and ***iv.*** heathland (P <0.001) were reported to have a significant effect. None of the additional model parameters i.e. improved grassland, acid grassland, temperature or relatively humidity emerged as having a significant effect on Qa density.

5.4 Discussion

The aim of this study was to investigate the density of questing *I. ricinus* in six different landcover types and examine the influence of landcover associated environmental variables i.e. temperature, relative humidity, saturation deficit and vegetation height on both the presence/absence and density of questing *I. ricinus*.

Broadleaf woodland was associated with the highest density of Qn/5 m^2 and Qa/5 m^2 in spring, followed by coniferous woodland, calcareous grassland and heathland; improved grassland and acid grassland both recorded the lowest density of Qn and Qa/5m^2. In autumn, however, the highest densities of Qn/Qa/ 5 m^2 were recorded in coniferous woodland, followed by broadleaf woodland, heathland, calcareous grassland, acid grassland and improved grassland.

Broadleaf woodland, coniferous woodland, heathland and calcareous grassland were assessed as having a significant effect on the presence of both Qn and Qa; broadleaf woodland, coniferous woodland, heathland as significant in the presence of larvae, as was relative humidity. Acid grassland appeared to have a significantly negative effect on the presence of larvae.

Broadleaf woodland, coniferous woodland, heathland were assessed as having a significant positive on the density of Qn and; these variables, with the addition of calcareous grassland were also determined as significant to Qa density.

5.4.1 Landcover and *Ixodes ricinus*

Due the specific physiology of the *Ixodes ricinus* tick, namely in relation to its propensity to quest in the open environment, as well as the limiting effect of desiccation on the questing process, the importance of habitat to this ticks survival and propagation is paramount (Lees, 1946; Milne, 1950; Perret et al., 2000; Randolph, 2004).

Certain vegetation types, and land-cover compositions can act as a buffer to unfavourable weather conditions (Gehlhausen et al., 2000), creating more stable microclimate for developing and questing *I. ricinus*. Dense woodland canopy can protect from intense heating and drying power of the sun. Likewise, thick sward or compacted ground level vegetation e.g. leaf litter, can act to stabilise ground temperatures. Additionally, dense ground-level vegetation contributes to maintaining high humidity; this humidity-rich refuge is vital to *I. ricinus,* allowing it to rehydrate following periods of unsuccessful questing (Gaede & Knulle, 1997; Kahl & Alidousti, 1997; Rudolph & Knulle, 1974) and providing a stable environment during periods of development and inactivity.

The importance of these factors is reflected in the results described here; the positive association of woodland habitat, both broadleaf and coniferous, is undoubtedly linked to these factors. However, the association is not so clear cut. The variation observed at the different sites within these higher Qn/Qa density landcover types, suggests the potential nuances associated with higher *I. ricinus* densities. For example, broadleaf woodland site BL3 exhibited 8 times more Qn than BL4, and over twice as many as BL1 and BL2 in spring; in terms of temperature and humidity, this site did not exhibit a significant difference compared to the other sites. Compared to site BL1 and BL2 vegetation was shorter and comparable to BL3, however no significant variation was detected. In autumn, this site did not differ significant between other sites in the number of Qn/Qn observed, all exhibiting reduced questing densities in comparison to spring. Therefore, it is difficult to say with certainty why this specific woodland produced more Qn and Qa than the other 3 sites. Perhaps the influence of specific hosts played a role, as suggested in other studies (Boyard *et al.,* 2008; Perez *et al.,* 2016). A more diverse woodland, supporting a range of small mammals, birds and larger grazers would result in an abundance of potential hosts for *I. ricinus*. This site did consistently report the presence of larvae on all three survey replicates, suggesting an established population, and with an abundance of Qn, potentially hints at ample supply of ground dwelling birds/rodents associated with larval feeding (Kurtenbach *et al.,* 1995). Perhaps a lack of larger mammals results in Qn and Qa questing for extended periods at this site, unable to find a host and so more were available to collect during survey.

The fact that coniferous woodland overtook broadleaf woodland in autumn surveys, producing greater densities of Qn and Qa per/5m^2 is also curious. Broadleaf woodland, particularly those populated with birch (ref) and oak (Tack *et al.,* 2012), or mixed woodland (ref), are associated with increased densities of *I. ricinus.* Again, it is difficult to say with certainty why this might be the case. Coniferous site CN1, in particular demonstrated higher densities of Qn and Qa that other coniferous sites. Environmental variables recorded at this site (temperature, relative humidity, and consequently saturation deficit, as well had vegetation height) did exhibit less variation than other coniferous sites, this potentially resulted in a more stable questing environment, resulting in *I. ricinus* questing for longer periods, hence more likely to be collected.

Calcareous grassland sites CL2, CL3 and CL4 were quite consistent in questing tick densities across both seasons; however, CL1 exhibited significantly higher Qn and Qa in spring. This result is more than likely influenced by the presence of a tree line at this site (personal observation), although not investigated here. Vegetation height was also significantly lower at this site in comparison to the others ($P < 0.05$).

Heathland showed no significant variation in Qa and Qn densities between sites, with the exception of HL4 which underwent vegetation management in spring, which most likely impacted on questing behaviour. Overall heathland did exhibit a stable mean temperature over spring and autumn, and did exhibit a low relative humidity, similar to that of acid grassland. However, its comparably (to woodland sites) low numbers of Qn and Qa, could also be the result of an abundance of birds and grazers at each of the sites (personal observation); potentially, more ticks had already found a host, resulting in fewer questing ticks available for survey.

In comparison to other landcover types, lower densities of Qn and Qa were recorded at improved and acid grassland. Both of these land types did exhibit significant variation around the mean temperature, most likely an artefact of the open nature of these landcover types, and a combination of semi-managed or grazed landscape. All acid grassland sites in particular, supported an abundance of hosts, specifically sheep, dogs, equids and birds (personal observation); however, perhaps this exposed nature of the landscape, results in an unstable, less buffered environment for ticks. Likewise, these sites exhibited lower vegetation, in comparison with other sites, providing a less sheltered and perhaps less moisture rich ground layer in which to develop/rehydrate.

A curious observation during this study is the apparent inverse relationship between RH in spring and Qn densities. All landcover types and sites exhibited a lower percentage RH in spring when compared to autumn, however, Qn and Qa were recorded at greater densities in all land cover types in spring. Laboratory studies have suggested that nymphal questing decreases at > 4.4 mmHg (Perret *et al.*, 2000), however, Qn were collected in high densities from saturation deficits of > 9mmHg. This was also observed in the temporal study (as described in Chapter 4). Perhaps in the field, 4.4 mmHg is too conservative a cut-off as other environmental factors may influence the ticks ability to quest at slightly higher saturation deficits.

Concluding Remarks

This study, reaffirmed previously published studies which infer a positive association between woodland and higher questing *I. ricinus* densities; however, it also suggests that coniferous woodland can provide just as suitable a habitat for questing *I. ricinus* as broadleaf or mixed woodland. The intra-site variation within landcover types suggests a complex dynamic, perhaps not just bound by a single environmental factor. The role of small mammal cycles, particularly in woodland, as well as habitat connectivity, require further study in relation to higher seasonal *I. ricinus* questing densities. In terms of human tick bite risk, the influence of high tick density landscapes (such as woodland) in close proximity to low density landscapes (such as acid grassland) required further research. Likewise, investigations on how tick bite risk compares between low density tick landcover types with high numbers of visitor and high density landscapes with few visitors.

Chapter 6: The spatial-temporal dynamics of *Borrelia* infected *Ixodes ricinus* nymphs

6.1 Introduction

Ixodes ricinus is the primary vector of *Borrelia burgdorferi* s.*l*., the causative bacterial agent of Lyme borreliosis in the UK. In the past two decades, an increase in Lyme borreliosis has been reported across much of Western Europe (Sykes & Makiello, 2016), including in the UK. The most recent case figures from England and Wales report an increase of 445 laboratory confirmed cases (1579 cases increased from 1134) from 2016 to 2017 (Public Health England, 2018). While this increase may, in part, be an artefact of increased surveillance, better awareness amongst the public and health care professionals, and changes to case definitions, there is also the possibility of a genuine change in pathogen distribution and disease incidence rates.

Several human pathogenic genospecies of the bacterial complex are known to circulate throughout the UK, each associated with different clinical symptoms; *B. afzelii;* associated with skin manifestations, *B. garinii;* linked with neuroborreliosis; *B. burgdorferi (sensu stricto)* which is associated with arthritis and *B. valaisiana,* which has been isolated from a single neuroborreliosis patient (Casjens *et al.,* 2011; Kingry *et al.,* 2016; Kurtenbach *et al.,* 1998; Mannelli *et al.,* 2012; Stanek & Reiter, 2011).

The bacterial complex circulates in specific wildlife populations and is transmitted to the tick whilst it feeds, thereby maintaining a continuous transmission cycle. Rodents act as a reservoir host for *B. afzelii,* birds for *B. garinii* and *B. valaisiana, while B. burgdorferi* s. *s.* is maintained by both rodent and avian species (Hanincova *et al.* 2003; Heylen, 2016; Kurtenbach *et al.,* 1998). Therefore, the wildlife composition of a landscape has a direct impact, not only on the prevalence of *Borrelia burgdorferi s.l.,* but also the genospecies in circulation.

As such, studies on the density of *I. ricinus* and the prevalence of *Borrelia burgdorferi* s.*l.* in specific habitats, as well as specific regions, can be useful in determining infection risk and localised genospecies specific symptoms.

Previous UK studies on the distribution and prevalence of *Borrelia burgdorferi* s.*l.*, have focused on various habitat types, woodland in Scotland and in northern England, (Bettridge *et al.*, 2013; James *et al.*, 2014; Millins *et al.*, 2016); recreational lands (Nelson *et al.*, 2015), and in urban parks (Hansford *et. al.*, 2017) This chapter presents data from the cross seasonal stratified survey (Spatial study presented in Chapter 5), investigating the prevalence of *B. burgdorferi* s.*l* six landcover types in the south west of England, and also from eight sites across England (Temporal study presented in Chapter 4). While the initial purpose of both Spatial and Temporal studies was to collect data on *I. ricinus* activity, investigating the presence of *B. burgdorferi s.l.* from the Qn collected in these studies was considered an important public health question, contributing to the existing knowledge and providing direction for future study

6.2 Materials and Methods

A proportion of the *I. ricinus* questing nymphs (Qn) obtained from each of the temporal and spatial sites were individually tested for the presence of *Borrelia burgdorferi s.l.* DNA. Only Qn were analysed due to their association with human tick bites and Lyme borreliosis infection (Robertson *et al.,* 2000; Wilhelmasson *et al.,* 2013). Additionally, a proportion of larvae from Cumbria were also analysed. This site had yielded a high density of larval *I. ricinus* and the on-site volunteer had collected a proportion of these. In all 70 larvae were analysed; where Qn were tested individually, ten larvae were pooled into individual tubes and processed identically to nymphs.

Qn from the Bentley Wood site were included, plus an additional site in Hampshire which was surveyed as part of the regional study in 2015/2016 but had insufficient survey numbers to be included in that analysis. This site did however produce high densities of Qn and as such, a proportion of these Qn were included in *Borrelia* analysis.

Where possible, if a high density of Qn had been collected, a randomised selection of a minimum 50 Qn were selected for pathogen testing. For those sites with ≤ 50 Qn, all available Qn were tested. For the landcover study sites in the SW of England, Qn from spring and autumn were tested; for the temporal study, due to a lower density of ticks in autumn at all sites, only ticks from spring were tested.

The DNA extraction, amplification and sequencing process is described in Chapter 2, section 2.5.1.

6.2.1 Statistical analysis

All statistical analysis was carried out using R statistical software R version 3.4.0 (R Development Core Team, Vienna, Austria). 95% binominal confidence intervals were calculated for individual landcover and temporal study sites.

To investigate whether the proportion of *Borrleia* infected Qn were the similar across landcover types, the Kruskal Wallis test was used to detect a significance between the proportion of Qn detected between landcover types. If this test detected significance, it was followed up by a Dunn test which tested the pair-wise significance between individual landcover types. The same method was used for the temporal sites.

6.3 Results

6.3.1 Spatial study: *Borrelia* across different landcover types

The density of *I. ricinus* varied between landcover types and sites (as presented in Chapter 4). Where possible, a minimum of 50 randomised Qn per landcover site were tested for the presence of *Borrelia burgdorferi s.l.* For sites that produced <50, all Qn collected at that site were tested. In total, 876 nymphs (604 for spring and 272 from autumn) were analysed for the presence of *Borrelia burgdorferi s.l.* The bacterial DNA was detected in 4.2% of samples; a total of 37 nymphs (29 from spring and 8 from autumn).

All *Borrelia* positive samples were from the following landcover types: broadleaf woodland, coniferous woodland, calcareous grassland and heathland (Table 6.1). The pathogen was not detected in improved or acid grassland; however, fewer ticks were collected from these landcover types, resulting in a statistically inadequate number for pathogen analysis (n<17 in spring and n<3 in autumn). A significant difference in the proportion of infected Qn across landcover types was observed in both spring (H(5df) = 20.08, P-value =0.001) and autumn (H(5df) =12.9, P-value <0.05) (Table 6.2 & Table 6.3).

All pathogenic strains associated with Lyme borreliosis and known to circulate in the UK, with the exception of *B. burgdorferi s.s.,* were identified in the analysis. For all infected Qn, across site and season, *B. garinii* accounted for 40.5%, *B. valaisiana* for 24.3%, *B. afzelii* for 8.1%, and 27% could not by typed to genospecies level and were reported as untyped.

Table 6.1. Number of Qn tested and number of Qn positive (%) for *Borrelia burgdorferi s.l* listed by landcover type and season. Breakdowns of individual genospecies per landcover type are highlighted.

Landcover Type	Qn tested	*Borrelia burgdorferi* s.l. +ve Qn (%:95% CI)	*B. garinii* +ve (%: 95% CI)	*B. afzelii* +ve (%: 95% CI)	*B. valaisiana* +ve (%: 95 CI)	Not typed (%: 95% CI)
Spring 2016						
Broadleaf Wood	194	12 (6.2%: 3,10)	5 (41.6%: 14, 70)	1 (8.3%: 0,24)	4 (33.3%: 7,60)	2 (16.6%: 0,38)
Coniferous Wood	200	11 (5.5%: 2,9)	5 (45.5%: 16,75)	1 (9.1%: 0,26)	1 (9.1%:0,26)	4 (36.4%: 8,65)
Calcareous Grass	89	5 (5.6%: 1,10)	2 (40%: 0,83)	0	0	3 (60%: 17,100)
Improved Grass	15	0	0	0	0	0
Heathland	89	1 (1.1%: 0,3)	1 (100%)	0	0	0
Acid Grass	17	0	0	0	0	0
Autumn 2016						
Broadleaf Wood	92	5 (5.4%: 1,10)	1 (20%: 0,55)	1 (20%: 0,55)	3 (60%:17, 100)	0
Coniferous Wood	110	3 (2.7%: 0,6)	1 (33.3%: 0,87)	0	1 (33.3%: 0,87)	1 (33.3%:0,87)
Calcareous Grass	12	0	0	0	0	0
Improved Grass	2	0	0	0	0	0
Heathland	53	0	0	0	0	0
Acid Grass	3	0	0	0	0	0

Table6.2 Statistical comparison using Kruskal – Wallis test of proportion of infected Qn across different land cover types in spring 2016. Additional pairwise comparisons using Dunn test show the Dunn z-statistic and p-value for each pairwise test.

Comparison of the proportion of *Borrelia burgdorferi s.l.* +ve Qn across landcover types in spring (Kruskal-wallis test)					
Kruskal-Wallis H = 20.08 On 5 degrees of freedom, p-value =0.001					
Post hoc analysis (Dunn test) comparison by group					
Z statistic **p-value**	Acid Grassland	Broadleaf Woodland	Calcareous Grassland	Coniferous Woodland	Heathland
Broadleaf Woodland	**-3.-17** **<0.05**	-	-	-	-
Calcareous Grassland	-1.82 0.51	1.35 1.0	-	-	-
Coniferous Woodland	**-2.81** **<0.05**	0.35 1.0	-0.99 1.0	-	-
Heathland	-0.51 1.0	2.66 0.6	1.31 1.0	2.31 0.15	-
Improved Grassland	0.0 1.0	**3.17** **0.011**	1.82 0.51	**2.82** **0.04**	0.51 1.0

Table 6.3 Statistical comparison using Kruskal – Wallis test of proportion of infected Qn across different land cover types in autumn 2016, Additional pairwise comparisons using Dunn test show the Dunn z-statistic and p-value for each pairwise test.

Comparison of the proportion of *Borrelia burgdorferi s.l.* +ve Qn across landcover types in autumn (Kruskal-wallis test)					
Kruskal-Wallis H = 12.88 On 5 degrees of freedom, p-value <0.05					
Post hoc analysis (Dunn test) comparison by group					
Z statistic **p-value**	Acid Grassland	Broadleaf Woodland	Calcareous Grassland	Coniferous Woodland	Heathland
Broadleaf Woodland	**-2.21** **<0.013**	-	-	-	-
Calcareous Grassland	0.0 0.5	2.21 0.01	-	-	-
Coniferous Woodland	**-2.18** **<0.05**	0.04 0.48	-2.18 <0.05	-	-
Heathland	0.0 0.5	2.21 0.01	0.0 0.5	2.18 0.02	-
Improved Grassland	0.0 0.5	**2.22** **0.01**	0.0 0.5	**2.18** **<0.5**	0.0 0.5

6.3.1.1 Broadleaf Woodland

The highest prevalence of *Borrelia burgdorferi s.l.* was detected in broadleaf woodland; this was the case in both spring and autumn. In spring, *B. garinii* was most prevalent, however, in autumn, *B. valaisiana* accounted for the majority of infections (Fig 6.1).

Between individual sites (Appendix Table A5.1), during spring, BL3 accounted for 41.7% of all *Borrelia burgdorferi* s.*l.* infected Qn, followed by BL4 (33.3%), BL2 (16.7%) and BL1 (8.3%).

B. valaisiana and *B. garinii* the only genospecies identified at BL3; B. *afzelii, B. valaisiana and B. garinii* were detected at BL4. Only *B. garinii* was identified at BL2, while the single positive Qn from BL1 was untyped.

In autumn, the number of Qn available for testing was significantly lower compared to spring (P <0.01), however, BL3 and BL4 still had the highest prevalence of *Borrelia burgdorferi* s.*l. B. afzelii* (33.3%) and *B. valaisiana* (66.6%) were both detected at BL3, *B. garinii* (50%) and *B. valaisiana* (50%). No infected Qn were detected at of the other two broadleaf sites in autumn.

6.3.1.2 Coniferous Woodland

In spring, coniferous woodland had a *Borrelia burgdorferi* s.*l.* prevalence of 5.5%, this contrasted with 2.7% in autumn. All three common genospecies were detected in spring but only *B. garinii* and *B. valaisiana* were detected in autumn.

Between sites in spring, CN3 accounted for 64% of the *Borrelia* positive Qn detected in coniferous woodland sites, with *B. garinii* being the dominant genospecies (57%), and *B. afzelii* accounting for 14% of positive Qn. 28.5% of positive ticks at this sites were not typed to a specific genospecies. Site CN1 accounted for 27% of *Borrelia* positive Qn, *B. garinii* and *B. valaisiana* were detected. CN2 accounted for 9% of *infected* Qn but the specific genospecies could not be typed. No infected Qn were detected at CN4 in spring.

In autumn, CN1, CN3 and CN2 produced an equal prevalence of *Borrelia burgdorferi* s.*l*. (33.3%); only *B. valaisiana* was detected at CN3, only *B. garinii* at CN3 and the *Borrelia* infection detected at CN2 could not be typed to a specific genospecies.

6.3.1.3 Calcareous Grassland

In spring, *Borrelia burgdorferi* s.*l.* was detected at just two calcareous grassland sites; CL1, which accounted for 40% of all calcareous positive Qn, and CL4 which accounted for 60%. Only *B. garinii* was detected at CL1.Positives from CL4 could be typed to a specific genospecies. In autumn, none of the calcareous grass sites produced infected Qn.

6.3.1.4 Heathland

Borrelia burgdorferi s.*l.* was detected at just one heathland site in spring; a single tick which accounted for 9% of all of those tested from site H4 was infected with *B. garinii*. No infected Qn were detected at heathland sites in autumn; this was also true of calcareous grassland, improved grassland and acid grassland sites.

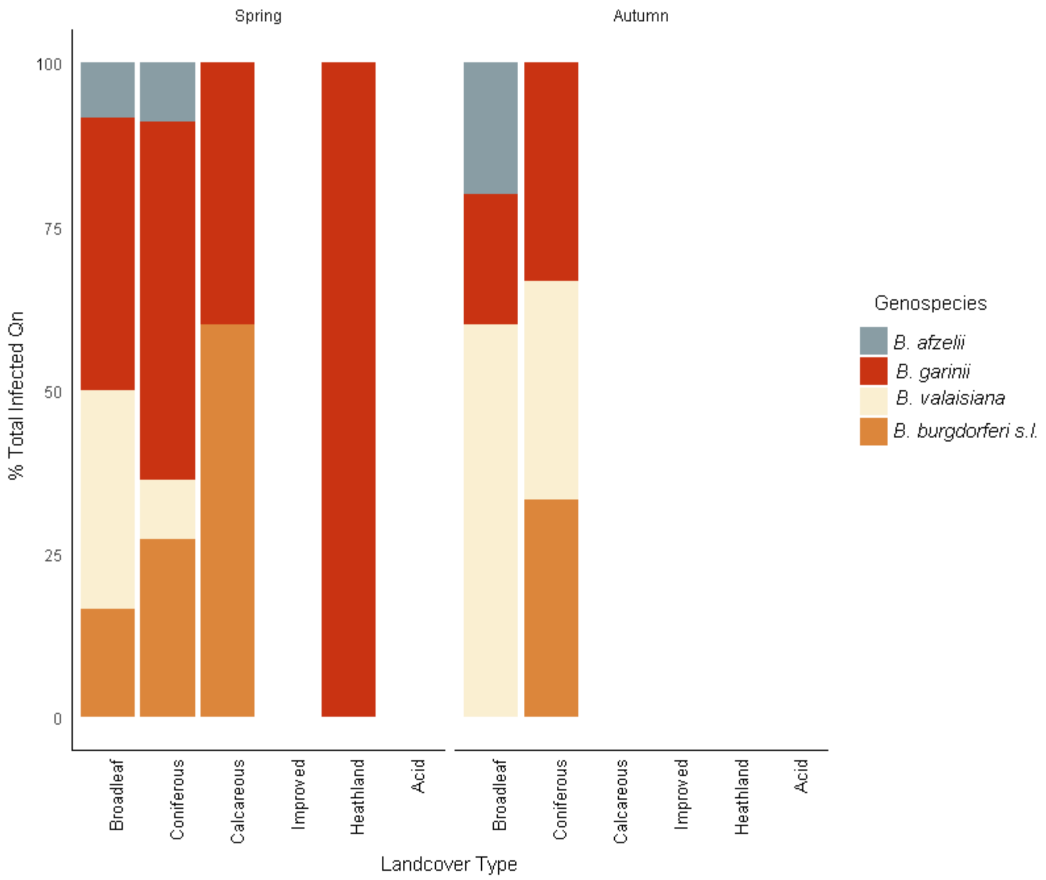


Fig 6.1 Describes the % genospecies of each genospecies detected at each of the six spatial study sites in spring and summer 2016. *B. burgdorferi s.l.* is noted for those which could not be typed to a particular genospecies. Note: no infected questing nymphs (Qn) were detected at improved or acid grassland; none were detected at heathland or calcareous grassland in autumn.

6.3.2 Regional study: *Borrelia* across different geographical locations

The number of *I. ricinus* collected at each of the temporal site varied, (as presented in Chapter 4) both between sites and between years. Where possible, a minimum of 50 Qn per site were tested for the presence of *Borrelia burgdorferi s.l.* and sequenced to obtain the genospecies. A total of 1232 Qn from all sites were tested for the presence of *Borrelia burgdorferi s.l.* DNA, these included all sites presented in Chapter 3 plus an additional site in Hampshire. No significant difference in the proportion of infected Qn was observed between sites (H (df: 7) = 7, P-value >0.05), or indeed between sites across years (H (df: 6) = 6, P-value >0.05).

6.3.2.1 Bentley Wood

A total of 300 Qn from Bentley Wood were tested for the presence of *Borrelia burgdorferi s.l.* From spring 2014, a 2% prevalence was detected from 100 Qn; in 2015 prevalence of 4% was detected from 200 Qn, and in 2016, a prevalence of 10% was detected in 50 Qn. *B. garinii, B, afzelii* and *B. valaisiana* were detected at this woodland; the latter detected only in 2016, while *B. garinii* and *B. afzelii* were both detected in 2015 (Table 6.2).

6.3.2.2 Devon

A total of 100 Qn were tested for the presence of *Borrelia burgdorferi s.l;* 50 Qn from 2015 and 50Qn from 2016. A prevalence of 4% was detected in 2015, with only *B. garinii* confirmed. In 2016, 8% of Qn were found to be infected; both *B. valaisiana* and *B. garinii* were confirmed (Fig 6.2).

6.3.2.3 Cholderton

50 Qn were tested from both 2015 and 2016; a prevalence of 4% was detected in 2015 but none of these could be identified to genospecies. In 2016, a prevalence of 10% was detected; 20% of these were identified as *B. garinii*, the remainder were untyped.

6.3.2.4 Surrey

In 2015, 2% of the 50 Qn tested positive for the presence of *Borrelia burgdorferi* s.*l.* DNA, consisting of a single nymph which could not be typed to genospecies. In 2016, 4% of Qn were found to be infected; both *B. garinii* and *B. valaisiana* were confirmed.

6.3.2.5 London

100 Qn were analysed from both study years; 6% of Qn were found to be infected in 2015, half of these were found to be *B. afzelii*, while the remainder were untyped. In 2016, 2% of Qn tested positive for the bacterial DNA, all of these were confirmed as *B. afzelii.*

Table 6.2 Number of Qn tested and number of Qn positive (%) for Borrelia burgdorferi s.l listed by temporal site and year. Breakdowns of individual genospecies per landcover type are highlighted'

Site	Qn tested	*Borrelia burgdorferi* s.l. +ve Qn (%)	*B. garinii* +ve (%)	*B. afzelii* +ve (%)	*B. valaisiana* +ve (%)	Untyped
2014						
Bentley Wood	100	2 (2%: 0,5)	-	-	-	2 (100%)
2015						
Bentley Wood	150	6(4%: 1,7)	2(33.3%: 0,71)	1(16.6%: 0,46)	2(33.3%: 4,71)	1(16.6%: 0,46)
Devon	50	2 (4%: 0,9)	1(50%: 0,100)	-	-	1(50%: 0,100)
Cholderton	50	2(4% : 0,9)	-	-	-	2(100%)
Surrey	50	1(2%: 0,4)	-	-	-	1(100%)
London	100	6(6%: 1,11)	-	3(50%: 10,90)	-	3(50%: 10,90)
Cumbria	49	1(2%: 1,6)	1(100%)	-	-	-
Northumberland	97	1(1%: 1,5)	-	-	-	1(100%)
Hampshire	50	2(4%: 1,6)	-	-	1(50%: 0,100)	1(50%: 0,100)
2016						
Bentley Wood	50	5(10%: 1,18)	-	-	2(40%: 0,83)	3(60%: 17,100)
Devon	50	4(8%: 0,16)	1(25%: 0,67)		2(50%: 1,99)	1(25%: 0,67)
Cholderton	50	5(10%: 2,18)	1(20%:0,55)	-	-	4(80%: 45,100)
Surrey	46	2(4.4%:0,10)	1(50%: 0,100)			1(50%: 0,100)
London	100	2(2%)	-	2(100%)	-	-
Cumbria	39*	3 (7.7%)	1(33.3%:0,100)	1(33.3%:0,100)		1(33.3%: 0,87)
Northumberland	-	-	-	-	-	-
Hampshire	50	-	-	-	-	-

6.3.2.6 Cumbria

Just 49 Qn were available for testing from 2015; 2% of these of these were found to be infected with *B. garinii*. In 2016, 39 Qn were available for testing, both *B. afzelii* and *B. garinii* were detected. Additionally, a high density of larvae had been collected from this site in Jul 2016; a proportion of these were also analysed for the presence of *Borrelia burgdorferi* s.*l.* DNA. One the pooled collection of ten larvae were found to be infected, this was confirmed as *B. afzelli.* All of the larvae tested were assumed to be unfed and none appeared engorged when identified by microscopy. Therefore, it assumed that the larvae were infected transovarially.

6.3.2.7 Northumberland

This site was surveyed in 2015 only and 97 Qn were available for testing; of these 2% were infected, although, genospecies could not be confirmed by sequencing.

6.3.2.8 Hampshire

Qn were also tested from this additional site which was surveyed as part of the temporal study. From those 50 Qn tested from 2015, 4% of Qn were found to be infected, *B. valaisiana* was confirmed by sequencing. None of the Qn analyses in 2016 were found to be infected.

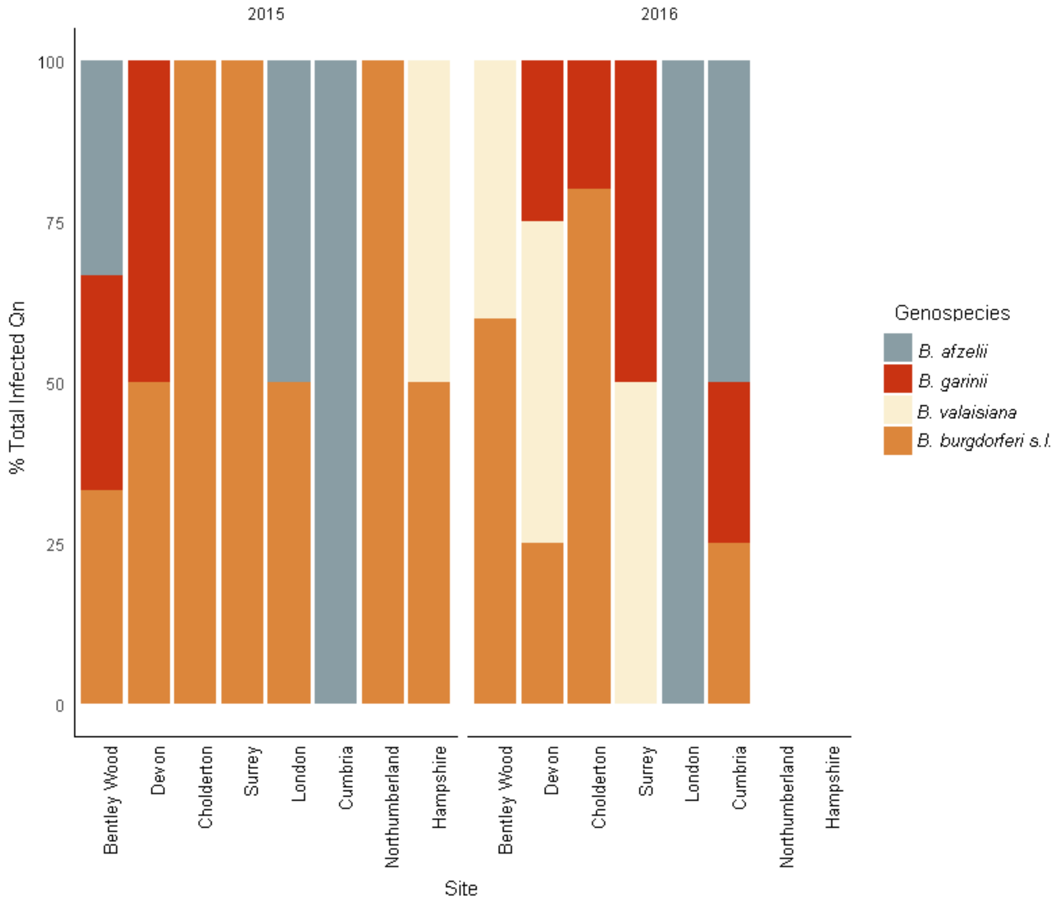


Fig 6.2 Describes the % genospecies of *Borrelia burgdorferi* detected at each of the six temporal sites in 2015 and 2016. Genospecies detection from analysis carried out on questing nymphs (Qn) from collected in 2015/2016 from Bentley Wood and Hampshire are also included. *B. burgdorferi s.l.* is noted for those which could not be typed to a particular genospecies.

6.4. Discussion

6.4.1 *Borrelia* prevalence in *I. ricinus* nymphs: spatial study

Borrelia burgdorferi s.*l.* was detected in broadleaf woodland, coniferous woodland, calcareous grassland and heathland. No infected Qn were detected in improved or acid grassland, however, these landcover types had a low density of Qn in comparison with other landcover types.

In spring, Broadleaf woodland was found to have the highest proportion of infected Qn (6.2%: 95% CI: 3, 10%), followed by calcareous grassland (5.6%: 95% CI: 1, 10%), coniferous woodland (5.5%:95% CI: 2, 9%) and heathland (1.1%: 95% CI: 0, 3%); in autumn, infected Qn were identified in broadleaf woodland (5.4%:1,10%) and coniferous woodland (3%: 95% CI: 0, 6%) only. *B.garinii, B. afzelii* and *B. valaisiana* were detected in both woodland landcover types, only *B. garinii* was confirmed in heathland and calcareous grassland. Across the entire study, of those successfully sequenced, B. garinii was the most prevalent genospecies, followed B. valaisiana for and B. afzelii.

The prominence of *Borrelia burgdorferi* s.*l.* in broadleaf woodland over other landcover types has previous been documented, both in European and in UK studies. This is not entirely surprising given the integral influence of temperature and moisture availability on *I. ricinus* questing behaviour and survival. The influence of specific vegetation types (as reported in Chapter 4) which are often ubiquitous within UK broadleaf woodlands, provide an ideal habitat for *I. ricinus*; buffering from extreme fluctuations in temperature, whilst provide a stable moisture rich ground layer. Likewise, this environment provides shelter and feeding opportunities for a host of rodents, small mammal, birds and larger grazers. This mix of potential *Borrelia burgdorferi* reservoir hosts, coupled with larger mammals which may support and increased density of feeding ticks, providing the ideal situation, in which a continuous transmission cycle may perpetuate. However, several studies have reported low densities of *I. ricinus*, coupled with a low prevalence of B. burgdorferi, or in some cases where it appears absent completely in some broadleaf woodland sites (Grassner *et al.,* 2011; Bettridge *et al*. 2013). This finding was supported here by the variation between the four broadleaf sites, which from a habitat standpoint, all appeared equally capable of supporting high *I. ricinus* densities, as well as Borrelia reservoir hosts.

Whereas some studies have reported a significant difference in *Borrelia burdorferi* prevalence between broadleaf and coniferous woodlands, that was not the case during the spring study reported here. However, whereas infected nymphs were detected in all of the broadleaf sites, just three of the four coniferous sites produced infected ticks. Additionally, one site in particular exhibited a prevalence of 14% (Appendix Table A 6.1), almost twice that of the other sites combined.

The dominance of individual sites in both Qn density and in *Borrelia* infection was also noted across calcareous grassland sites. CL1 exhibited a greater density of questing *I. ricinus*, likely due to the presence of an extensive tree line and scrub on the periphery on the site (personal observation); however, CL4 which consisted of a track of calcareous grassland surrounded by arable lands and bordered by a motorway, exhibited a comparatively fewer Qn, but *Borrelia* prevalence almost 4 times that of CL1. This is a curious finding given the apparent cut-off nature of the site and potentially fewer blood meal hosts. However, the cut-off nature of this site may attribute to an intensification of *Borrelia* reservoir hosts, resulting in a transmission loop, where a small population of infected e.g. rodents infect new cohort of *I. ricinus* each year, which in turn, infect new generations of rodents and small mammals.

The lower density of ticks in heathland, improved grassland and acid grassland may reflect the more exposed nature of these sites and potentially the lack of buffering to extremes of heat and cold, making them more susceptible too desiccation. Heathland and acid grassland sites certainly supported an abundance of birds and large grazers (personal observation) but few Qn were observed, particularly at improved and acid grassland sites. One Qn from a single heathland site was found to be infected with *B. garinii;* given that fewer than 50 Qn were collected from each of the heathland sites, it is difficult to interpret significant findings from this result; however, that fact that all heathland sites supported populations of horses, donkeys, cattle and in some cases, sheep, there may be an element of a ‘dilution effect’ occurring.

While this study did not have to the scope to investigate mammal diversity of or fragmentation at each of the sites (Ehrmann *et al.,* 2018), it is likely that these factors play an influential role in *I. ricinus* density and on the density of infected nymphs.

6.4.2 *Borrelia* prevalence in *I. ricinus* nymphs: temporal study

The majority of these sites were located in the south of England, with two sites located in the north of England; one in the northeast (Northumberland) and one in the Northwest (Cumbria). All, with the exception of Northumberland, were surveyed on at least 10 occasions in 2015 and 2016. The frequency of surveys did vary between sites (as presented in Chapter 3), however no significant difference in the proportion of infected Qn was detected between sites, or for individual sites between years. All sites surveyed in 2015, produced at least one infected Qn; the same was true of 2016, with the exception of the Hampshire site which did not produce any infected Qn in 2016.

The mix of geographical locations and habitats reinforces previous findings which suggest that human pathogenic genospecies of the *B. burgdorferi* s.*l* circulate throughout the UK, possibly at very low prevalence and in a range of different habitats (Bettridge *et al.,* 2013; Hansford *et al.,* 2017; Layzell et al., 2018).

In this study, Bentley Woods was unique in that *B. garinii*, *B afzelii* and *B. valaisiana* were all detected there in 2015. Cumbria and Devon were the only other sites where more than one of these three genospecies were detected; *B. garinii* and *B. afzelii* in Cumbria, and *B. garinii* and *B. afzelii* in Devon. The presence of *B. afzelii* in both Cumbria and Devon, as well as in Richmond park, suggests a wider distribution than previously thought (Kurtenbach *et al.,* 1998). All other sites reported just one of these three genospecies; however, almost half of the *B. burgdorferi* s.*l* DNA extracts were left uncategorised to a single genospecies, which might suggest the presence of a *Borrelia* genospecies which could not be fully sequenced with the primers used in this study. This will require further investigation, specifically for *Borrelia miyamotoi,* a genospecies associated with relapsing fever which has been previously detected in the south of England (Hansford *et al.,* 2015; Layzell *et al.,* 2018).

The detection of *B. afzelii* in larvae is an interesting finding as this would suggest transovarial transmission, which is considered rare (Mannelli *et al.*, 2012). However, given that the larvae did not appear to have fed, it is likely that this is the case. This genospecies has been detected in larvae previously (Rijpkema & Bruinink 1996) and has been found to successfully transmit the bacteria (Duijvendijk, 2016).

Concluding remarks

This study confirmed the presence of human pathogenic *B. burgdorferi* s.*l* genospecies across four difference landcover types and demonstrated the diversity in genospecies across these landcover types, as well as, between different sites within them. Additionally, pathogenic genospecies were detected at eight different sites throughout England, confirming the wide distribution of *B. burgdorferi* s.*l* in England, albeit at a potentially very low prevalence. The bacterial complex was also confirmed in larvae collected from the field.

The study highlighted that high Qn density did not necessarily ensure high *Borrelia burgdorferi s.l* infection in questing ticks and affirmed the complexity of this pathogen cycle.

The ecological diversity of reservoir species drives the diversity of *B. burgdorferi* s.*l* genospecies in circulation; the abundance and distribution of reservoir hosts is likely influenced by habitat fragmentation, ecological land management, development and agriculture. Likewise, the augmentation of local wildlife populations through culling or indeed, introduction e.g. of game birds, is likely to have an impact in circulating genospecies, as well as questing *I. ricinus* density.

As case numbers of Lyme borreliosis continue to increase in the UK, it is important that health professional and the public are aware of the potential risk of infection from an *I. ricinus* tick bite, not just from nymphs and adults but also potentially larvae. Likewise, it’s important to raise awareness that while some regions of the UK or certain landcover types might be considered more high risk, it is possible to acquire a tick bite and *Borrelia burgdorferi* s.*l* infection from anywhere there is a viable environment to support *I. ricinus* and wildlife hosts.

Chapter 7: General discussion and concluding remarks

7.1 Introduction

The global burden of tick borne disease has become of increasing significance in recent decades and are likely to continue to do so (Medlock & Leach 2015; Paules *et al.,* 2018). The evolutionary adaptability of ticks to a wide range of environments and hosts has resulted in a diverse array of over 900 species, some of which are generalist parasites with extended, multi-host life cycles. This ability to feed on multiple host species and retain infection throughout their life cycle has resulted in ticks (as a whole) having the ability to transmit a greater diversity of pathogens than any other arthropod vector (Jongejan & Uilenbery, 2004).

Globally, recent decades have seen a monumental shift in environmental change; the augmentation and degradation of landscapes for agriculture and development, as well as the impacts of climate change, have all influenced the distribution of tick hosts, and resulted in changes to tick habitats. Consequently, changes in the distribution of TBD has been noted throughout the globe; the expansion of Tick Borne Encephalitis in Europe, the emergence of Crimean-Congo haemorrhagic fever in Spain (Negredo *et al.,* 2017; ECDC, 2018), repeated outbreaks and expansion of Kyasanur Forest Disease in southern India (Shah *et al.,* 2018), the expansion of Lyme borreliosis (Hickling *et al.,* 2018; Eddens *et al.,* 2018) in the United States, as well as the emergence of multiple tick-borne rickettsiae (Paddock *et al.,*2016).

In the UK, the discovery of previously undetected tick-borne pathogens such as *Borrelia miyamotoi* (Hansford *et al.,* 2015) and spotted fever group rickettsiae (Klasen *et al.,* 2011) reflect the many unknowns of TBD. While the implication (if any) on human health of newly detected pathogens has yet to be established, the TBD of primary concern in the UK continues to be Lyme Borreliosis.

Public Health England (PHE) have recently updated the confirmed laboratory case records, these figures show an increase of >30% on the previous year, to 2.7 mean annual case rate /100,000 population (PHE, 2018). While this increase still leaves the UK with a lower rate than much of Europe (ECDC, 2016), it is still concerning.

In an effort to better understand the dynamics of *I. ricinus*, the primary vector of the causative agent of Lyme Borreliosis, this project was established with the aims of: ***i.*** building on existing data sets on the longitudinal activity of *I. ricinus* across England*;* ***ii.*** determining the relationship between the seasonality of *I. ricinus* questing activity, microclimate and local weather variables; ***iii.*** investigating the density of *I. ricinus* across different landcover types; ***iv.*** investigating the presence of *Borrelia burgdorferi s.l.* across the different study sites included in this project.

7.2 Building on existing data sets on the longitudinal activity of *I. ricinus* across England

This project continued the collection of longitudinal data on *I. ricinus* seasonality first started by the Medical Entomology and Zoonoses Ecology group (PHE, Porton) at Bentley Wood in 2013. It also established similar longitudinal study sites across England. These data sets complimented each other in that both collected data on the seasonality of *I. ricinus* and on the microclimate and vegetation at the site. While the more comprehensive Bentley Woods (presented in Chapter 3) data set allowed the seasonality of *I. ricinus* to be investigated alongside microclimate and longitudinal weather variables over a four year continuous weekly period, the other temporal sites (as presented in Chapter 4) allowed comparisons to be made across England on *I. ricinus* seasonality in relation to weather and microclimate. While collection of data at the sites across England lasted for the duration of this project, the collection of data at Bentley Wood continues and will be used to further investigate outcomes from this project.

In addition, this dataset has provided a spatially and temporally diverse 'biobank' of tick specimens which continue to be used in a variety of additional projects, including pathogen and genetic diversity studies

7.3 Seasonal questing of *I. ricinus* in relation to microclimate and local weather variables

Studies at both Bentley Wood and the other temporal sites provided insight on the relationship between nymphal *I. ricinus* questing densities, microclimate and local variables. While the influence of factors such as temperature, relative humidity and saturation deficit on this tick species had been well documented in the lab and in some field studies (Daniel *et al.*, 2015; Del Fabbro *et al.*, 2015 Knap *et al.*, 2009; Lees, 1946; Milne, 1950; Perret *et al.*, 2000; Randolph, 2004; Tomkins *et al.*, 2014), a comprehensive UK weekly data set such as that from Bentley, had not been investigated previously.

The primary focus on the commencement of increased activity in spring, the maximum spring peak in activity and the post-peak decline in *I. ricinus* nymphal activity was because of the association of the nymphal life stage with Lyme borreliosis infection (Robertson *et al.,* 2000a; Wilhelmsson *et al.*, 2013) and the seasonality of Lyme borreliosis infection in the UK which peaks from June to August, most likely after a lag from tick bite and diagnosis to confirmation (Public Health England, 2017). With the latest figures on Lyme borreliosis case data exhibiting an increase (PHE, 2018), mitigation of tick-bite exposure in the public, particularly during this increased period of high risk is priority. Therefore, identifying environmental or weather based cues which influence nymphal questing at this time and could potentially be used to predict it in future years was a key focus of this project. Basing the hypothesis on previous studies which indicated 7ºC as an important temperature in the instigation of questing (Macleod, 1936b; Perret *et al.,* 2000; Randolph *et al.,* 2004), the investigation of a weekly (consecutive seven day) Tmean threshold of 7 ºC (calculated from 5 km grid data) association with increased nymphal questing was carried out. For both Bentley Wood and the other temporal sites, initiation of questing often preceded the first occurrence of seven consecutive days of 7 ºC but preceded actual maximum peak spring questing. While predictive models to test if this temperature threshold could actually forecast peak nymphal questing were not developed as part of this project, the insight gained from this study and the additional data collected at Bentley Wood (from 2017 onwards) will be used for this purpose.

Similarly, the duration of spring peak nymphal questing and its decline were investigated using potential evapotranspiration. A threshold of 25 to 30mm/week appeared to coincide with a decline in spring nymphal questing. Again, while predictive models based on potential evapotranspiration were not developed as part of this study, this variable has previously been used successfully to model tick activity dynamics (Garcia-Martí *et al.*, 2017) and will be used in addition with the 7 ˚C threshold with the larger Bentley Wood data set (2013 – 2018).

The difficulties posed by the use of 5 km resolution weather data for vector activity and mapping studies have been well documented (Estrada-Peña *et al.*, 2016) and there are certainly limitations to the use of this data in such studies. Microclimate or very high-resolution weather data would be a more accurate predictor of *I. ricinus* activity, however, until such time when higher resolution whether data is available, or microclimate can be monitored remotely with better accuracy, current studies such as that presented here can be useful as an indicator of activity dynamics.

7.4 Density of *I. ricinus* across different landcover types

While *I. ricinus* is known to be well distributed throughout the UK and is associated with different habitat types (Cull et al., 2018; Pietzsch et al., 2005b), often the public perception is that ticks are only found in woodland or bracken habitat (professional observation). As discussed in Chapter 1 (1.3.5) and researched in Chapter 3 and 4, the factors which determine *I. ricinus* survival and ability to quest i.e. suitable temperature range and an adequately moisture rich environment, can be a driver of landcover suitability. Likewise, vegetation structure, type and abundance can determine host availability and so have an impact on tick density.

A study of the spatial dynamics of *I. ricinus* densities across different landcover types was carried out to better inform our understanding of the influence of landcover on *I. ricinus* ecology, and to inform public health information on the distribution of *I. ricinus* across different landcover types. As presented in Chapter 5, six different cover types were investigated and multiple sites in each were surveyed several times across spring and autumn.

As presented from previous studies (Medlock *et al.*, 2012; Tack *et al.*, 2012), woodland had a strong association with high densities of *I. ricinus*; in this study, broadleaf woodland in spring, and more so coniferous woodland in autumn exhibited the highest tick densities. However, calcareous grassland and heathland also emerged as highly statistically significant ($P< 0.05$) for both the presence of and density of Qn and Qa. The presence and abundance of larvae was also investigated in this study, with presence significantly associated with broadleaf and coniferous woodland and heathland.

Host abundance studies were outside the scope of this study, but it is likely that the results of this study is a reflection of a combination of availability of hosts at each of these landcover types, coupled with the weather and local microclimate conditions. Woodland offers more dense vegetation which can act as a buffer, maintaining moisture and to some extent, a less variable temperature. More exposed grassland habitats may not offer as much in the way of foraging and shelter for potential hosts, leading to fewer bloodmeal options for ticks. The presence of larvae at heathland and woos land sites supports this theory, in that fewer large mammals are present to support mating adult ticks at the grassland sites, resulting in fewer larvae being depositied at these sites. Likewise, the statistically significant (< 0.001) influence of RH(%) on the presence of larvae but not on the other life stages may also enforce the reasoning that more exposed grassland habitats may not be sufficiently moisture rich to support thriving I. ricinus populations.

7.5 *Borrelia burgdorferi s.l.* prevalence in *I. ricinus* populations

Several human pathogenic genospecies of *Borrelia burgdorferi s.l.* are known to circulate in the UK. However, awareness amongst the public and healthcare professional on the presence of the pathogen seems to vary, with many unaware the Lyme borreliosis occurs in the UK or that it is restricted to specific locations (Lorenc *et al.*, 2017).

Findings presented in Chapter 6, reflect the widespread distribution of *Borrelia burgdorferi s.l.* and highlight the spatial and seasonal presence of *B. garinii*, *B. afzelii* and *B. valaisiana* throughout the sites studied. However, high densities of *I. ricinus* do not necessarily imply a high prevalence of infection; the localised presence of blood meal hosts will have a direct impact on the *Borrelia burgdorferi* genospecies circulating in a specific area.

The isolation of *B. afzelii* from larvae demonstrates the relatively rare occurrence of transovarial *Borrelia burgdorferi s.l.* transmission and highlights the potential risk of disease transmission from larval bites. This study once again highlights the fact that *Borrelia burgdorferi s.l.*, while circulating in relatively low levels in *I. ricinus* populations, it is still widespread in distribution. These findings emphasize the case for tick awareness as a public health too and highlight the fact that Lyme borreliosis can be contracted from exposure to ticks bites in a range of different habitats and geographical locations.

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Appendix

Chapter 4

Additional sites which signed up to the temporal study but were not included in the final analysis because <10 surveys were completed in one or both years.

Table A.4.1 Additional sites which were enrolled in the temporal spatial study, as detailed in chapter 4. These sites were not included in the final analysis due to lack of data. This table details the location, the number of surveys conducted, and the number of questing ticks recorded at each sites.

Site location	Lat long?	Period of inclusion in the study/Active period	Number of surveys completed prior to departing from the study	Number of questing ticks collected as part of the study
Wytham Woods, Oxford (woodland)	51.7711, -1.33848	March - August 2015 -	8	3
Wytham Woods, Oxford (grassland)	51.7711, -1.33848	March - August 2015 -	8	8
Bartley Heath, Hampshire	51.27649,0.95428	April - July 2015 April - September 2016	7	344
Daneway Banks, Gloucestershire	51.73054,2.092515	March - October 2015 April - September 2016	9	25
Berkhamsted, Hertfordshire	51.768148, -0.541930	April - June 2015 -	8	0
Kirkby Moor, Lincolnshire	53.148165, -0.173079	March - July 2015 -	6	0
Isle of Axholme, Lincolnshire	53.626100, -0.855015	April 2015 -	1	15
Ness Botanic Gardens, Merseyside	53.272060, -3.044801	April - Jul 2015 -	7	0
Brooklands Farm, Dorset	50.755051, -2.474096	March - July 2015 -	4	18
Penshurst Place Estate, Kent	51.18813, 0.19672	March - Oct 2015 -	8	0
Crediton, Devon (private farm)	50.755051, -2.474096	March - April 2015 -	2	11
Seaton burn, Northumberland	55.062424, -1.64654	April - July 2015 -	6	1
Ramsey Island, Pembrokeshire	51.866856, -5.342978	April - August 2015 -	7	74
Staindrop, Durham	54.578104, -1.815705	April - July 2015	6	0
Ruthin, Denbighshire	53.064292, -3.306638	April - July 2015	9	0
Normandy, Surrey	51.239722, -0.674649	April - May 2015	3	17
Velmean Common, Hampshire	51.26904, -0.82109	April - August 2015	5	55
Cockermouth, Cumbria	54.661564, 3.361388	June - July 2015 -	3	0
Windsor Great Park, Berkshire	51.462061, -0.61475237	July - September 2015 -	3	0

Greyfield Wood, Somerset	51.32245, -2.51677	July - September 2015 March - September 2016	3 7	65 280
Norfolk	52.55899, 0.83675	April - September 2016	6	43
Talerddig, Wales	52.582214, -3.605361	2015		0
Havergate Island, Norfolk	52.07346,1.52600	2016		
Sandwell Valley	-	-	0	0
Dartmoor	-	-	0	0
Winstanley Wood, Greater Manchester	-	-	0	0
Rainton Meadows	-	-	0	0
Redmires	-	-	0	0
The Mears, Exmouth	-	-	0	0
Boultham Mere, Lincolnshire	-	-	0	0

Chapter 4: Boxplots of actual Qn count data for each of the temporal sites

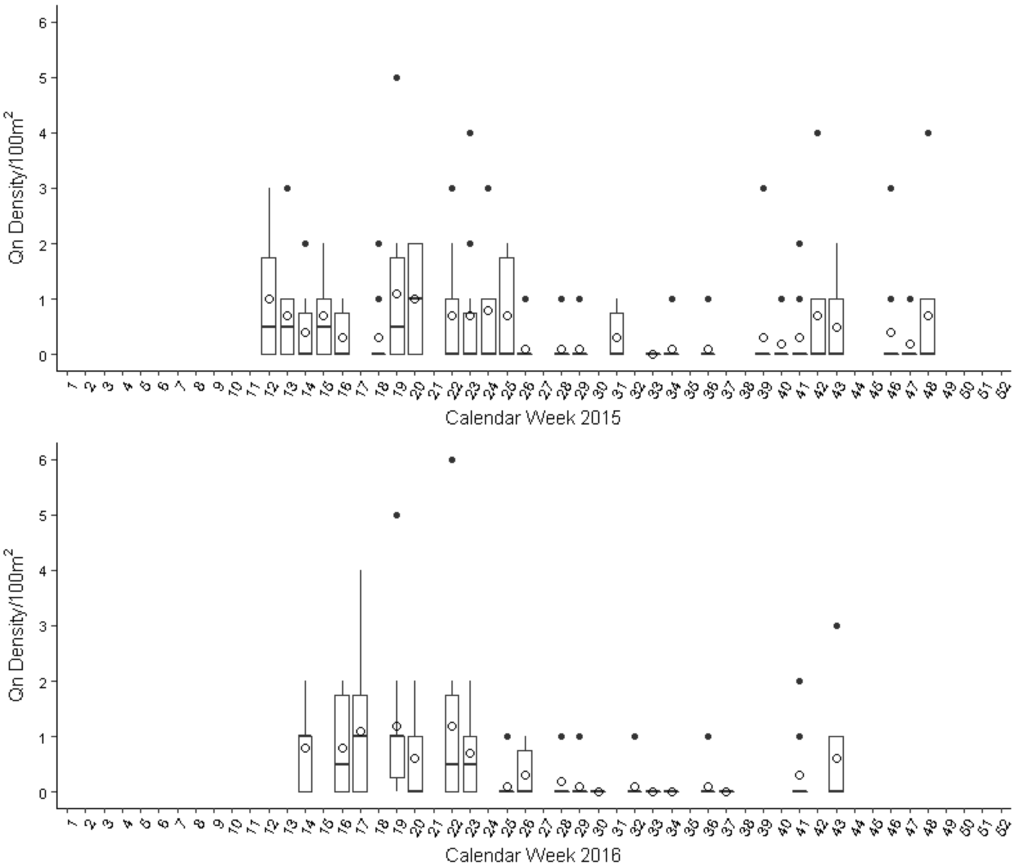

Fig A4.1 Devon; weekly Qn count data per 100m^2 for each survey year. Each box describes a single survey of 10 transects. Boxes present the interquartile range of transect results; outliers are represented by a black circle, the median is represented by a black line and the mean number of Qn collected during that survey is represented by an open circle.

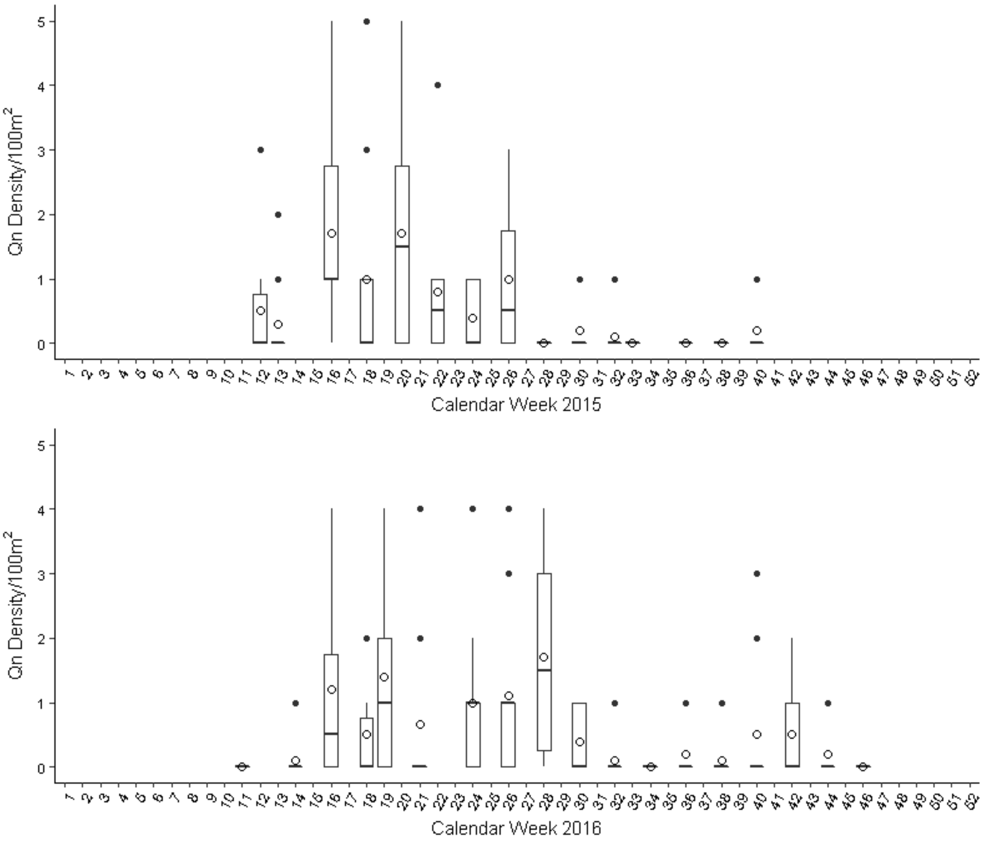

Fig A 4.2 Surrey; weekly Qn count data per 100m^2 for each survey year. Each box describes a single survey of 10 transects. Boxes present the interquartile range of transect results; outliers are represented by a black circle; the median is represented by a black line and the mean number of Qn collected during that survey is represented by an open circle.

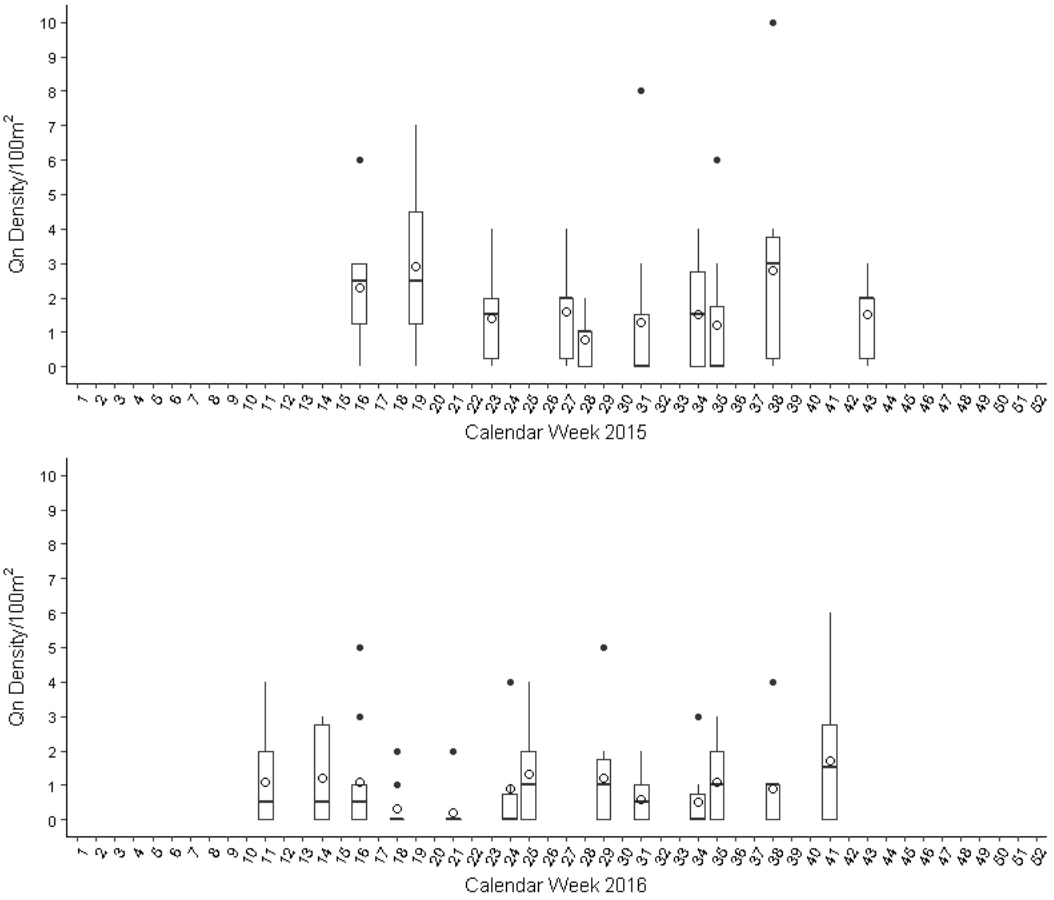

Fig A 4.3 Cumbria; weekly Qn count data per 100m^2 for each survey year. Each box describes a single survey of 10 transects. Boxes present the interquartile range of transect results; outliers are represented by a black circle, the median is represented by a black line and the mean number of Qn collected during that survey is represented by an open circle.

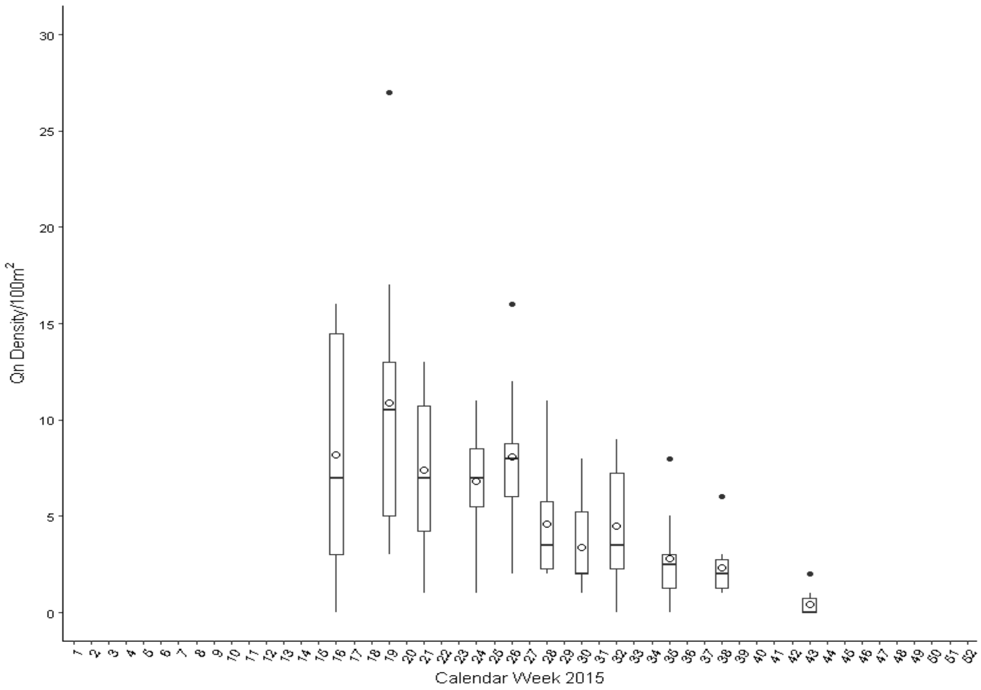

Fig A 4.4 Northumberland; weekly Qn count data per 100m^2 for each survey year. Each box describes a single survey of 10 transects. Boxes present the interquartile range of transect results; outliers are represented by a black circle, the median is represented by a black line and the mean number of Qn collected during that survey is represented by an open circle.

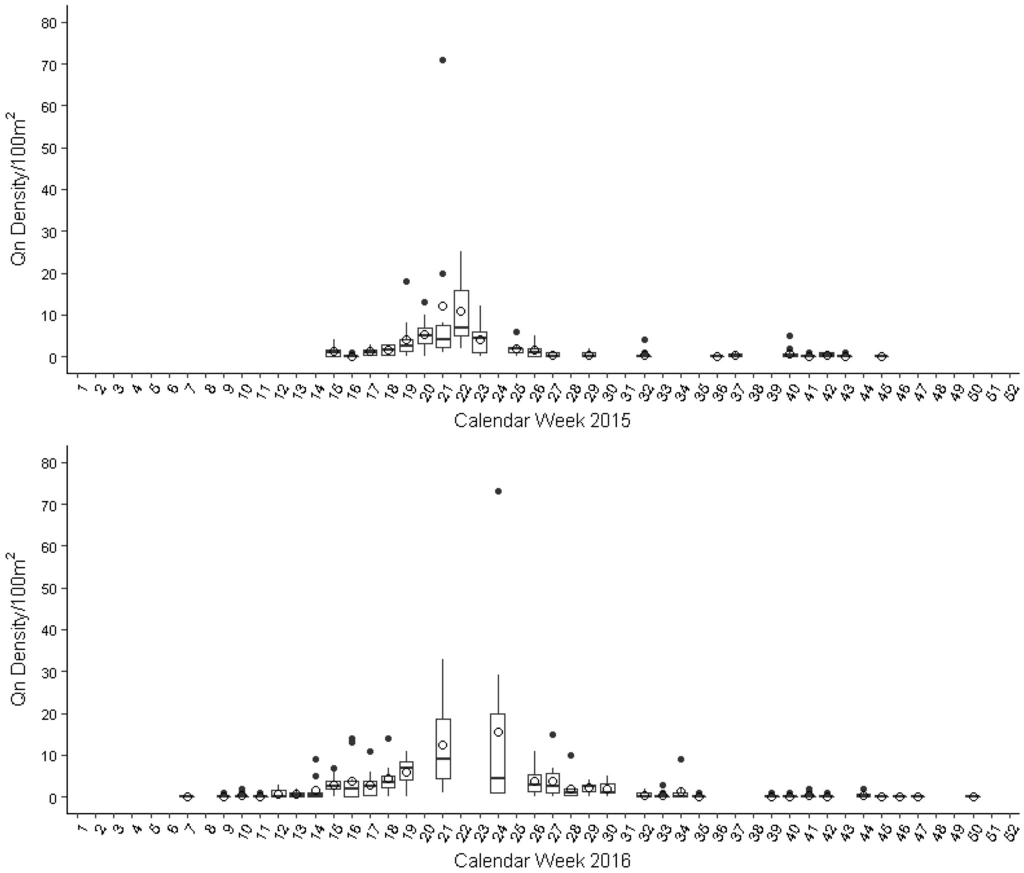

Fig A 4.5 Greater London; weekly Qn count data per 100m^2 for each survey year. Each box describes a single survey of 10 transects. Boxes present the interquartile range of transect results; outliers are represented by a black circle, the median is represented by a black line and the mean number of Qn collected during that survey is represented by an open circle.

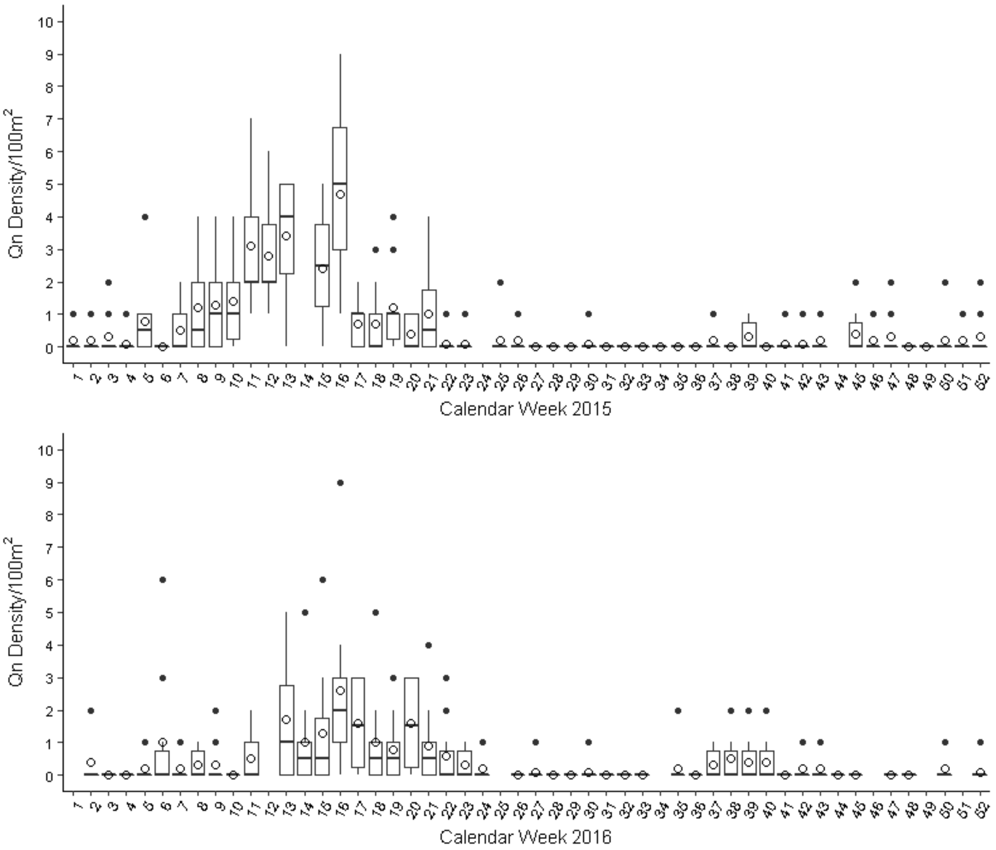

Fig A4.6 Wiltshire; weekly Qn count data per 100m^2 for each survey year. Each box describes a single survey of 10 transects. Boxes present the interquartile range of transect results; outliers are represented by a black circle, the median is represented by a black line and the mean number of Qn collected during that survey is represented by an open circl

Chapter 5 Table A5.3 Individual survey records for each of the four broadleaf woodland sites.

Landcover site ID	Survey Replicate	Date	Number of 5m Transects	Qf	Qm	Tmean °C	RH%	Saturation deficit (mmHg)	Vegetation height avg(cm)	Total Adults per site	Larva present y/n	Qn	Total Qn per site	Nymph Density per 5m (SE)
Spring														
Broadleaf 1	1	19Apr16	30	0	1	14	47	6	38		n	79		2.63 ± 0.46
Broadleaf 1	2	26Apr16	30	1	1	12	40	6	40		n	38		1.27 ±0.21
Broadleaf 1	3	07May16	30	1	1	24	49	11	42	5	y	48	165	1.60 ±0.35
Broadleaf 2	1	20Apr16	30	9	10	17	42	8	15		y	52		1.73 ±0.26
Broadleaf 2	2	27Apr16	30	5	7	15	34	8	18		y	32		1.07 ±0.24
Broadleaf 2	3	06May16	30	2	2	23	29	15	18	35	y	29	113	0.97 ±0.18
Broadleaf 3	1	19Apr16	30	7	14	19	30	11	20		y	184		6.13 ±1.07
Broadleaf 3	2	27Apr16	30	3	7	10	54	4	22		y	64		2.13 ±0.39
Broadleaf 3	3	06May16	30	14	12	23	30	14	25	57	y	98	346	3.27 ±0.44
Broadleaf 4	1	21Apr16	30	1	1	21	35	12	7		y	20		0.67 ±0.17
Broadleaf 4	2	28Apr16	30	5	3	11	51	5	8		y	13		0.43 ±0.11
Broadleaf 4	3	06May16	30	0	2	21	38	11	12	12	y	10	43	0.33 ±0.12
Autumn														
Broadleaf 1	1	21Sep16	30	0	0	17	87	2	70		y	1		0.03 ±0.03
Broadleaf 1	2	04Oct16	30	0	1	15	87	2	65		y	7		0.23 ±0.09
Broadleaf 1	3	12Oct16	30	0	0	14	75	3	43	1	y	4	12	0.13 ±0.06
Broadleaf 2	1	22Sep16	30	1	2	21	63	7	65		y	5		0.17 ±0.07
Broadleaf 2	2	03Oct16	30	0	1	16	69	4	41		y	9		0.3 ±0.11
Broadleaf 2	3	09Oct16	30	0	1	14	88	1	47	5	y	5	19	0.17±0.08
Broadleaf 3	1	24Sep16	30	0	0	19	69	5	25		y	5		0.17 ±0.07
Broadleaf 3	2	03Oct16	30	1	1	16	65	5	27		y	12		0.4 ± 0.13
Broadleaf 3	3	12Oct16	30	0	2	13	76	3	28	4	y	13	30	0.43 ± 0.11
Broadleaf 4	1	23Sep16	30	2	3	19	56	7	18		y	9		0.3 ±0.1
Broadleaf 4	2	28Sep16	30	3	0	20	78	4	25		y	13		0.43 ±0.13
Broadleaf 4	3	09Oct16	30	0	2	15	78	3	20	10	y	14	36	0.47 ±0.13

Chapter 5 Table A5.4 Individual survey records for each of the four coniferous woodland sites.

Landcover site ID	Survey Replicate	Date	Number of 5m Transects	Tmean °C	RH%	Saturation deficit(mmHg)	Vegetation height avg(cm)	Qf	Qm	Qa	Larva present y/n	Nymphs per survey	Total Qn per site	Qn Density per 5m (SE)
Spring														
Coniferous1	1	20-Apr-16	30	16	36	8	15	5	1		N	78		2.6 ±0.46
Coniferous1	2	27-Apr-16	30	11	34	7	15	6	7		N	54		1.8 ±0.22
Coniferous1	3	03-May-16	30	15	38	8	16	5	11	35	Y	66	198	2.2 ±0.37
Coniferous2	1	20-Apr-16	30	19	35	11	12	3	2		N	32		1.07±0.28
Coniferous2	2	26-Apr-16	30	11	42	6	10	1	4		Y	32		1.07±0.30
Coniferous2	3	06-May-16	30	24	26	16	17	1	1	12	N	7	71	0.23±0.09
Coniferous3	1	21-Apr-16	30	22	35	12	8	1	0		N	32		1.07±0.23
Coniferous3	2	28-Apr-16	30	11	48	5	7	2	0		Y	24		0.8 ±0.14
Coniferous3	3	06-May-16	30	25	28	17	8	0	0	3	N	12	68	0.4 ±0.13
Coniferous4	1	26-Apr-16	30	14	34	8	130	1	2		N	58		1.93±0.63
Coniferous4	2	06-May-16	30	28	20	23	110	3	1		N	32		1.07±0.27
Coniferous4	3	13-May-16	30	21	53	9	110	0	3	10	N	51	141	1.7 ±0.28
Autumn														
Coniferous1	1	22-Sep-16	30	19	71	5	17	1	1		Y	6		0.2 ±0.09
Coniferous1	2	27-Apr-16	30	18	87	2	18	1	0		Y	4		0.13±0.06
Coniferous1	3	05-Oct-16	30	13	61	4	36	1	0	4	Y	10	20	0.33±0.12
Coniferous2	1	24-Sep-16	30	20	77	4	26	0	1		Y	4		0.13±0.09
Coniferous2	2	05-Oct-16	30	17	54	6	45	0	1		Y	6		0.2 ±0.07
Coniferous2	3	13-Oct-16	30	13	83	2	51	0	0	2	N	3	13	0.1 ±0.06
Coniferous3	1	23-Sep-16	30	17	58	6	9	0	2		Y	22		0.73±0.2
Coniferous3	2	04-Oct-16	30	17	69	4	15	0	1		Y	18		0.6 ±0.16
Coniferous3	3	10-Oct-16	30	14	50	6	14	5	2	10	Y	30	70	1.0 ±0.23
Coniferous4	1	24-Sep-16	30	20	68	6	80	0	0		Y	5		0.17±0.11
Coniferous4	2	05-Oct-16	30	17	45	8	90	4	0		y	6		0.2 ±0.07
Coniferous4	3	13-Oct-16	30	15	65	4	85	3	2	9	y	16	27	0.53±0.16

Chapter 5 Table A 5.5 Individual survey records for each of the four calcareous sites

Landcover site ID	Survey Replicate	Date	Number of 5m Transects	Tmean °C	RH%	Saturation deficit (mmHg)	Vegetation height avg(cm)	Qf	Qm	Qa	Larva present y/n	Nymphs per survey	Total nymphs per site	Qn Density per 5m (SE)
Spring														
Calcareous1	1	13-Apr-16	30	26	35	16	10	4	3		y	71		2.37±0.73
Calcareous1	2	20-Apr-16	30	16	44	7	12	9	7		y	110		3.67±0.97
Calcareous1	3	27-Apr-16	30	18	22	12	20	5	5	33	y	46	227	1.53±0.45
Calcareous2	1	13-Apr-16	30	29	33	20	7	0	0		y	3		0.1 ±0.06
Calcareous2	2	20-Apr-16	30	19	34	10	7	0	0		n	2		0.07±0.05
Calcareous2	3	27-Apr-16	45	16	22	11	12	0	0	0	n	0	5	0
Calcareous3	1	20-Apr-16	30	18	43	8	24	1	0		n	2		0.07±0.05
Calcareous3	2	27-Apr-16	30	14	33	8	25	0	0		n	1		0.03±0.03
Calcareous3	3	03-May-16	30	18	37	9	35	0	0	1	n	3	6	0. 1 ±0.06
Calcareous4	1	13-Apr-16	30	24	42	13	38	5	3		n	9		0.3 ±0.1
Calcareous4	2	20-Apr-16	30	20	34	11	42	0	0		n	11		0.37±0.12
Calcareous4	3	27-Apr-16	30	14	35	8	32	0	1	9	n	6	26	0.2 ±0.09
Autumn														
Calcareous1	1	21-Sep-16	45	20	80	3	15	0	0		y	0		0
Calcareous1	2	03-Oct-16	45	18	58	6	15	2	0		y	0		0
Calcareous1	3	09-Oct-16	30	16	71	4	26	2	0	4	n	1	1	0.03±0.03
Calcareous2	1	21-Sep-16	30	20	72	5	40	1	0		n	0		0
Calcareous2	2	03-Oct-15	30	19	52	8	40	1	0		n	1		0.03±0.03
Calcareous2	3	09-Oct-16	30	17	69	4	25	3	3	8	y	2	3	0.07±0.05
Calcareous3	1	21-Sep-16	30	21	70	5	45	0	0		n	2		0.07±0.05
Calcareous3	2	03-Oct-16	30	20	55	7	45	1	0		n	2		0.07±0.05
Calcareous3	3	09-Oct-16	30	15	75	3	45	0	0	1	n	1	5	0.03±0.03
Calcareous4	1	21-Sep-16	45	23	50	10	50	0	0		y	0		0
Calcareous4	2	03-Oct-16	30	22	44	11	65	0	0		n	2		0.07±0.05
Calcareous4	3	09-Oct-16	30	18	55	7	52	1	1	2	n	2	4	0.07±0.05

Chapter 5 Table A 5.6 Individual survey records for each of the four improved grassland sites

Landcover site ID	Survey Replicate	Date	Number of 5m Transects	Tmean °C	RH%	Saturation deficit(mmHg)	Vegetation height agv(cm)	Qf	Qm	Qa per site	Larva present y/n	Qa	Total Qn per site	Qn Density per 5m (SE)
Spring														
Improved1	1	19-Apr-16	30	14	41	7	6	0	0		n	3		0.1 ±0.06
Improved1	2	27-Apr-16	45	11	42	6	11	0	0		n	0		0
Improved1	3	07-May-16	30	24	50	11	8	0	0	0	n	1	4	0.03±0.03
Improved2	1	19-Apr-16	30	12	46	6	12	0	0		n	5		0.17±0.07
Improved2	2	27-Apr-16	30	14	43	7	15	0	0		n	1		0.03±0.03
Improved2	3	07-May-16	30	26	42	14	16	0	0	0	n	3	9	0.1 ±0.06
Improved3	1	25-Apr-16	30	18	44	8	9	0	0		n	1		0.03±0.03
Improved3	2	07-May-16	45	25	46	13	12	0	0		n	0		0
Improved3	3	13-May-16	45	18	62	6	12	0	0	0	n	0	1	0
Improved4	1	25-Apr-16	30	16	53	6	10	0	0		n	1		0.03±0.03
Improved4	2	07-May-16	45	29	35	20	7	0	0		n	0		0
Improved4	3	13-May-16	45	21	51	9	12	0	0	0	n	0	1	0
Autumn														
Improved1	1	21-Sep-16	30	18	87	2	5	0	0		y	1		0.03±0.03
Improved1	2	04-Oct-16	45	16	79	3	12	0	0		y	0		0
Improved1	3	12-Oct-16	45	13	77	3	8	0	0	0	y	0	1	0
Improved2	1	26-Sep-16	45	16	76	3	14	0	0		y	0		0
Improved2	2	05-Oct-16	45	18	54	7	10	0	0		n	0		0
Improved2	3	12-Oct-16	45	16	65	5	12	0	0	0	n	0	0	0
Improved3	1	26-Sep-16	45	17	83	2	15	0	0		n	0		0
Improved3	2	03-Oct-16	45	20	43	10	15	0	0		n	0		0
Improved3	3	12-Oct-16	45	14	65	4	12	0	0	0	n	0	0	0
Improved4	1	26-Sep-16	45	18	69	5	8	0	0		n	0		0
Improved4	2	03-Oct-16	45	19	44	9	10	0	0		n	0		0
Improved4	3	12-Oct-16	45	14	70	4	15	0	0	0	n	0	0	0

Chapter 5 Table A 5.7 Individual survey records for each of the four heathland sites

Landcover site ID	Replicate number	Date	Number of 5m Transects	Tmean °C	RH%	Saturation deficit(mmHg)	Vegetation height avg(cm)	Qf	Qm	Qa per site	Larva present y/n	Qn per survey	Qn per site	Qn Density per 5m (SE)
Spring														
Heath1	1	21-Apr-16	30	15	42	7	25	0	0		n	11		0.37±0.13
Heath1	2	28-Apr-16	30	16	36	8	15	2	2		n	13		0.43±0.16
Heath1	3	06-May-16	30	26	29	18	26	0	1	5	n	14	38	0.47±0.18
Heath2	1	21-Apr-16	30	17	41	8	30	0	0		n	8		0.27±0.11
Heath2	2	28-Apr-16	30	14	40	7	42	2	2		n	1		0.03±0.03
Heath2	3	06-May-16	30	24	30	16	38	0	0	4	n	6	15	0.2 ±0.11
Heath3	1	21-Apr-16	30	19	45	9	42	2	0		n	16		0.53±0.14
Heath3	2	28-Apr-16	30	20	28	12	51	1	0		n	1		0.03±0.03
Heath3	3	06-May-16	30	23	39	12	38	2	0	5	n	17	34	0.57±0.14
Heath4	1	21-Apr-17	30	18	43	9	5	0	0		n	7		0.23±0.08
Heath4	2	28-Apr-16	30	12	40	6	6	0	0		n	3		0.1 ±0.1
Heath4	3	06-May-16	30	24	25	17	9	0	0	0	n	1	11	0.03±0.03
Autumn														
Heath1	1	23-Sep-16	30	21	55	8	25	5	2		y	4		0.13±0.06
Heath1	2	04-Oct-16	30	17	77	3	30	1	1		y	8		0.27±0.14
Heath1	3	10-Oct-16	30	16	65	5	33	2	3	14	y	14	26	0.47±0.15
Heath2	1	23-Sep-16	30	21	47	10	35	1	0		n	3		0.1 ±0.07
Heath2	2	04-Oct-16	30	17	74	4	25	0	0		n	3		0. 1 ±0.06
Heath2	3	10-Oct-16	45	16	54	6	35	1	0	2	n	1	7	0.02±0.02
Heath3	1	23-Sep-16	30	22	53	9	50	3	2		n	3		0.1 ±0.06
Heath3	2	04-Oct-16	30	20	57	7	45	0	0		y	4		0.13±0.06
Heath3	3	10-Oct-16	30	13	60	4	29	2	0	7	y	11	18	0.37±0.13
Heath4	1	23-Sep-16	30	23	46	11	65	1	2		y	0		0
Heath4	2	04-Oct-16	45	21	50	9	70	0	0		y	0		0
Heath4	3	10-Oct-16	30	16	47	7	65	0	0	3	y	3	3	0.1±0.06

Chapter 5 Table A 5.8 Individual survey records for each of the four acid grassland sites.

Landcover site ID	Replicate number	Date	Number of 5m Transects	Tmean °C	RH%	Saturation deficit(mmHg)	Vegetation height avg(cm)	Qf	Qm	Qa per site	Larva present y/n	Qn per survey	Qn per site	Qn Density per 5m (SE)
Spring														
Acid1	1	26-Apr-16	30	12	37	7	5	0	0		n	1		0.03±0.03
Acid1	2	06-May-16	30	22	35	13	7	0	0		n	5		0.17±0.07
Acid1	3	13-May-16	45	23	47	11	7	0	0	0	n	1	7	0.02±0.02
Acid2	1	26-Apr-16	45	19	23	13	5	0	0		n	0		0
Acid2	2	06-May-16	30	22	35	13	8	0	0		n	2		0.07±0.05
Acid2	3	13-May-16	45	23	47	11	6	0	0	0	n	0	2	0
Acid3	1	26-Apr-16	45	9	45	5	13	0	0		n	1		0.02±0.02
Acid3	2	06-May-16	45	27	36	17	12	0	0		n	1		0.02±0.02
Acid3	3	13-May-16	30	26	45	14	7	0	0	0	n	2	4	0.07±0.05
Acid4	1	26-Apr-16	45	6	71	2	42	0	0		n	0		0
Acid4	2	06-May-16	45	27	25	20	31	0	0		n	0		0
Acid4	3	13-May-16	30	22	51	9	28	0	0	0	n	2	2	0.07±0.05
Acid1	1	28-Sep-16	30	21	65	6	5	1	0		y	0		0
Acid1	2	05-Oct-16	30	17	57	6	5	0	0		n	1		0.03±0.03
Acid1	3	11-Oct-16	45	18	54	7	7	0	0	1	n	0	1	0
Acid2	1	28-Sep-16	45	19	64	6	25	0	0		n	0		0
Acid2	2	05-Oct-16	45	18	50	7	28	0	0		n	0		0
Acid2	3	11-Oct-16	30	16	54	6	22	0	0	0	n	1	1	0.03±0.03
Acid3	1	28-Sep-16	45	19	68	5	12	0	0		y	1		0.02±0.03
Acid3	2	05-Oct-16	45	17	42	8	35	0	0		n	0		0
Acid3	3	11-Oct-16	45	17	51	7	18	0	0	0	n	0	1	0
Acid4	1	28-Sep-16	45	21	56	8	45	0	0		y	0		0
Acid4	2	05-Oct-16	30	19	38	10	45	0	0		n	1		0.03±0.03
Acid4	3	11-Oct-16	45	16	57	6	45	0	0	0	n	0	1	0

Table A 6.2 *Borrelia burgdorferi s. l.* prevalence by survey site and season

Landcover	**Qn tested**	***Borrelia burgdorferi s.l.*** **+ve nymphs (%)**	***B. garinii*** **+ve (%)**	***B. afzelii*** **+ve (%)**	***B. valaisiana*** **+ve (%)**	**Untyped**
Spring						
Broadleaf Woodland						
BL1	50	1 (2%)	0	0	0	1 (2%)
BL 2	50	2 (4%)	1 (2%)	0	0	1 (2%)
BL 3	50	5 (10%)	2 (4%)	0	3 (6%)	0
BL4	44	4 (9%)	2 (4.5%)	1 (2.3%)	1 (2.3%)	0
Coniferous Woodland						
CN1	50	3 (6%)	1 (2%)	0	1 (2%)	1 (2%)
CN2	50	1 (2%)	0	0	0	1 (2%)
CN 3	50	7 (14%)	4 (8%)	1 (2%)	0	2 (4%)
CN4	50	0	0	0	0	0
Calcareous Grassland						
CL 1	50	2 (4%)	2 (4%)	0	0	0
CL2	6	0	0	0	0	0
CL3	6	0	0	0	0	0
CL4	27	3 (11%)	0	0	0	3()
Improved Grassland						
IM1	4	0	0	0	0	0
IM 2	9	0	0	0	0	0
IM3	1	0	0	0	0	0
IM 4	1	0	0	0	0	0

Heathland						
HL1	30	0	0	0	0	0
HL 2	14	0	0	0	0	0
HL3	34	0	0	0	0	0
HL4	11	1 (9%)	1 (9%)	0	0	0
Acid Grassland						
AC1	7	0	0	0	0	0
AC2	4	0	0	0	0	0
AC3	4	0	0	0	0	0
AC4	2	0	0	0	0	0
Autumn						
Broadleaf Woodland						
BL1	11	0	0	0	0	0
BL 2	18	0	0	0	0	0
BL3	28	3 (10.7%)	0	1(3.6%)	2(7.1%)	0
BL4	35	2 (5.7%)	1(2.9%)	0	1 (2.9%)	0
Coniferous Woodland						
CN1	20	1 (5%)	0	0	0	1 (5%)
CN2	12	0	0	0	0	0
CN3	50	1 (2%)	0	0	1 (2%)	0
CN4	28	1 (3.6%)	1 (3.6%)	0	0	0
Calcareous Grassland						
CL1	1	0	0	0	0	0
CL2	3	0	0	0	0	0
CL3	4	0	0	0	0	0
CL4	4	0	0	0	0	0
Improved Grassland						
IM1	2	0	0	0	0	0
IM2	0	-	-	-	-	-
IM3	0	-	-	-	-	-
IM4	0	-	-	-	-	-
Heathland						
HL1	28	0	0	0	0	0

HL 2	7	0	0	0	0	0
HL3	15	0	0	0	0	0
HL4	3	0	0	0	0	0
Acid Grassland						
AC1	1	0	0	0	0	0
AC 2	1	0	0	0	0	0
AC3	0	-	-	-	-	-
AC4	1	0	0	0	0	0

Tick Activity Project

Call for Volunteers

I am conducting research into tick activity and Lyme disease risk and I am looking for help from volunteers who are interested in conducting a **once fortnightly field survey**, to monitor tick activity.

The project

Public Health England and University of Liverpool's Institute for Infection and Global Health are jointly supporting a project focused on **understanding tick activity and Lyme disease risk**. The Sheep Tick, *Ixodes ricinus* is the primary host of *Borrelia burgdorferi sensu lato*, the causative agent of Lyme disease in the UK. Not all ticks carry the bacteria but an infected tick can transmit the bacteria through biting. In recent years there has been an **increase in both reported tick activity and confirmed Lyme disease cases** throughout the UK.

This project aims to **monitor *Ixodes ricinus* ticks** at numerous field sites throughout England and Wales, through this we hope to determine the seasonal pattern of *Ixodes ricinus* and how this varies across different habitats and climate regions. The hope is that from this data we will be able to develop a **forecasting tool** for tick exposure risk and in doing so, **increase awareness** on the dangers of tick bites.

The **data you collect will feed into a network of survey data from sites across England and Wales**, giving unique insight into tick activity and tick bite risk, at both a **national** and **community level**. Your contribution will be much appreciated and you will be able to check the overall progress of the project through the project website:

https://www.liv.ac.uk/infection-and-global-health/research/zoonotic-infections/tick-activity-project/

What the field survey will entail

Volunteers will be asked to **survey one field site, fortnightly from late February or early March until the end of November .**The survey involves running a 1mx1m cotton flag which is attached to a pole, over low lying vegetation - the ticks sense the vibration of the flag and latch on.

The flag is turned over (please see the photos below) and the ticks are removed into a tube using tweezers - the number of ticks and the temperature are recorded on a survey sheet. This is done in **10 x 10m transects** (section of vegetation) and **I will send out everything required,** from the flag and pole to handheld thermometer and tweezers. The ticks collected are sent to me using a prepaid envelop, where they will be identified and preserved for future testing

Get involved

If you are interested in volunteering or want more information on the project, please feel free to get in touch at: Liz.McGinley@Liverpool.ac.uk or call: 01980 616938

Tick Activity Project - Field survey participation and information.

The Tick Activity Project is currently recruiting volunteers who are interested in conducting a weekly of fortnightly survey for ticks at a designated site in their locality. If you think you might be interested in conducting field surveys for the project then please read the project outline below, this outlines the projects research aims and details what the field work entails.

If you have any questions regarding the information supplied here then please do get in touch with the project coordinator – contact details are at the end of the document.

The project

Public Health England and University of Liverpool's Institute for Infection and Global Health are jointly supporting a project focused on **understanding tick activity and Lyme disease risk**. The Sheep Tick, *Ixodes ricinus* is the primary host of *Borrelia burgdorferi*, the causative agent of Lyme disease in the UK. The tick transmits this Lyme disease causing bacteria through biting and in recent years there has been an **increase in both reported tick activity and confirmed Lyme disease cases** throughout the UK.

This project aims to **monitor ticks** at numerous field sites throughout England and Wales, through this we hope to determine the seasonal pattern of *Ixodes ricinus* ticks and how this varies across the these regions with changing weather and climate. The hope is that from this data we will be able to develop a **forecasting tool** for tick exposure risk and in doing so, **increase public awareness** on the dangers of tick exposure and Lyme disease risk.

What the field survey will entail

We are looking for **volunteers** who are willing to **survey one field site, weekly or fortnightly from late February to the Late November**. Ticks spend most of their life in the vegetation, either in the mat or leaf litter where it is humid enough for them to survive, but when conditions are favourable they climb vegetation in search for a blood meal from a passing host animal (this behaviour is known as questing). This project is interested in **monitoring questing ticks** i.e. those looking for an animal or person to attach to. The technique used is a simple flagging technique, which involves running a 1mx1m cotton flag over the surface of low vegetation, then checking the flag for and collecting any ticks which may have attached. Temperature and vegetation height will also be measured and recorded at the site. Please refer to the project protocol (Version1 19Jan14) for detailed instruction on survey technique.

Why take part?

The data you collect will feed into a **unique and important study of tick distribution**, the outcome of which will have **a real impact on public awareness** of the dangers of tick bites and more specifically, Lyme disease. Your contribution will be much appreciated and you will be able to check on how your data is being used and on the overall progress of the project through the project website.

Equipment and Costs

All **equipment and training will be provided**. Volunteers are not expected to incur any financial costs to themselves. If any equipment requires replacing then please get in touch with the project co-ordinator.

Risks

Like all outdoor activity, field surveying involves some risk. It is important that you understand and are comfortable with the protocol involved – please refer to the Health and Safety document (Version1 30Jan14) for full instructions on how to mitigate potential risks in the field. Below are potential risks associated with this field survey.

1. Surveying alone – inform someone where you are going, take a mobile phone and be aware of your coordinates if an incident arises (you can find your location grid coordinates here: www.getamap.co.uk). Be aware of the location of your local A&E. Ideally, have a buddy when you are surveying.

2. Check the weather before you set out –If there is a chance of adverse weather then reschedule your survey for another day. If weather conditions become adverse during the survey then abandon it, making a note on the survey sheet. Use sun protection and keep hydrated.

3. Wear appropriate clothing – light coloured clothing is recommended for tick surveying, as if you do happen to get a tick on your clothes it will be easier to spot. Wear sturdy, walking boots or wellies – with trousers tucked in to them to prevent tick bites.

4. Be aware of low hanging branches, uneven ground or exposed roots.

5. Cover any cuts or scratches with a waterproof bandage.

6. When surveying, if you encounter any aggressive or what you perceive to be a dangerous animals, abandon the survey and leave the site.

7. If you are bitten by a tick remove it immediately by grasping the tick as close to the skin as possible and pulling upwards slowly and firmly using a fine, pointed tweezers. Alternatively use a tick removal tool - you will find one of these in your volunteer pack, carry this with you and use according to the manufactures instructions. Apply antiseptic to the bite area and monitor for several weeks for any changes. Contact your GP if you feel unwell over the following weeks and let them know that you were bitten by a tick. Report the bite to the project coordinator/student researcher, who will advise you further.

What if I have to or want to leave the survey team

You can of course leave the project at any time without reason, we would appreciate if you could let the project coordinator know of your decision and return your field kit.

If you have any question or issues during the project

The Tick Activity Project will encourage communication with volunteers through the project website and associated social media; you can also contact the project coordinator at any time by email or phone. If there is an issue that you feel needs to be addressed by the Research Governance Officer then please contact them at: ethics@liv.ac.uk – provide the project name or description and the name of the project coordinator (in this case the student researcher).

Confidentiality

While the data you collect as part of the field survey will be analysed and shared with the public – any contact details for field volunteers will be kept on the secure University of Liverpool system – the project coordinator will not share these details with other parties.

<u>Contact details - Project coordinator/student researcher:</u>

Liz McGinley

Medical Entomology & Zoonoses Ecology

Microbial Risk Assessment & Behavioural Science

Emergency Response Department

Health Protection & Medical Directorate

Public Health England

Porton Down

Salisbury SP4 0JG

Liz.McGinley@Liverpool.ac.uk Liz.McGinley@phe.gov.uk

Tick Activity Project

Surveying protocol for *Ixodes ricinus* ticks

1. Health and safety precautions

Do not survey if:

- There is a risk of **adverse weather** conditions e.g. thunder and lightning.
- If there are dangerous or **aggressive animals** present.

Always:

- wear appropriate clothing – **sturdy shoes** or boots, **light coloured clothing**, and trousers should be tucked into socks or boots.
- **Check yourself** after being in the countryside for the presence of ticks.
- If you are bitten by a tick **remove it immediately** by grasping the tick as close to the skin as possible and pulling upwards slowly and firmly using a tweezers or tick removal tool (you will find one of these in your volunteer pack). Apply **antiseptic** to the bite area and **monitor** for several weeks for any changes. Contact your **GP** if you feel unwell over the following weeks and let them know that you were bitten by a tick. **Report the bite** to the project coordinator, who will advise you further.

2. Equipment you will need:

Pole, clips and flag

Thermometer

Survey sheet

1m ruler for measuring vegetation

Tweezers

Tubes and marker pens

Tick removal device

3. When to survey

• Try to survey in optimum conditions e.g. **dry days** (ticks are less active in cold, wet weather and windy weather makes surveying more difficult)

• When the vegetation is dry e.g. not morning/evening if dew is present or when it is raining – the best time is **between 10:30am and 3pm**.

4. Attach the 1m x 1.2m cloth to the pole by wrapping the longer length around the pole until it measures 1m x 1m (approx. 3ft 3in x 3ft 3in) and secure with 2 clips.

5. Pick your defined area for survey – this is the same area that you will survey each time. An ideal location would be **woodland**, **grassland** or **heathland** where there is likely to be an abundant small mammal population and deer and/or grazers present. It is always useful to identify a landmark for your starting point e.g. a tree, so that you return to the same starting each time you survey.

- Sites should be **easily accessible** and safe for you to visit.

- When you first begin, decide where your **10 x 10m transects** will be – you can measure out this distance before you start and count how many paces it takes you to walk this distance with the flag, this will make it easier for you the next time you survey.

- Transects do not need to be in a line but a minimum gap of **10m** (approx.33ft) **should be left between transects**.

- Each transect should be **monitored for changes** that may affect surveying e.g. **removal of vegetation** between surveys, **presence of brambles** which may catch on the drag or vegetation over 1m high. All of these can affect the efficacy of the survey method being used **so it is important that you record this**. If any of these changes occur during surveying, make sure to note it down on the back of the survey sheet.

6. Take a **temperature** and **humidity reading** at approx.1.25m (approx.4ft) from the ground, before you start and at the end of your survey. Record **vegetation type**, **height** and **weather** details, **site name** and **surveyors name** on your survey sheet. There is also room for additional notes e.g. grass recently cut.

7. Make sure the **flag is in contact with the surface of the vegetation** (see below), any questing ticks will sense the vibrations of the flag against the vegetation and assuming it’s a potential host, they will latch on to the flag. Try to avoid areas with excessive brambles, to avoid catching the flag on the vegetation as this can dislodge any collected ticks.

8. Continue dragging the flag across the vegetation for a distance of 10m - if it is breezy, keep the flag in contact with the vegetation using your free hand.

9. Once you have completed 10m, turn the flag over with minimal disturbance – you don’t want to knock off any ticks which have attached.

10. Check the flag for ticks. When the flag is turned over initially, any tick attached may not be moving – often it is easier to wait a few seconds to notice the distinctive **crawling movement** of the tick across the flag. Larvae are smaller than a poppy seed, with 6 legs, light brown in colour and glisten slightly in the light. Nymphs are dark in colour with 8 legs and about the size of a pin head. Adult males are dark brown/black, resembling a spider but with a slightly elongate body. Females are the largest, similar to a male but have a two tone appearance; reddish brown/dark brown.

11. Remove the **nymph** and **adults ticks** with the tweezers provided and place in the bottom of the tube provided – **record the presence of larvae** on the survey sheet, **you do not need to collect the larvae** – brush them off the flag before moving to your next transect. Label the tube with the transect number (e.g.T4) and date. Seal the tube tightly between transects and be careful when reopening as the ticks may crawl to the top of the tube. **Record the tube number and number of ticks** on the survey sheet. If there are no ticks, please mark so on the survey sheet - negative data is also extremely useful to us

12. Take a **temperature reading for the transect at vegetation height** and record on the box provided on the survey sheet**.**

13. Leave 10m between where your last transect ended and where your next begins - repeat the process again. You will need to complete **10 transects in total**.

14. Double check that you are happy that all the data on the survey sheet is correct and that your tube is labelled with the transect number and date.

15. To **return the tube**, place it in the box provided and place this in the bag, surrounding it with the absorbent material within – seal the bag and place in the padded envelope provided. Post to the project coordinator.

If you have any questions regarding surveying technique, please don’t hesitate to **contact** me: Liz.McGinley@Liverpool.ac.uk

Tick Activity Project

Health and Safety

The most important thing when carrying out a field survey of any type is your own safety. Taking the time to consider the potential risks before you begin the survey and thinking about ways you can avoid or remove those risks.

Here is a list of things to consider before embarking on your survey – please read and make sure you are happy with these before you start.

8. If possible survey in pairs – if some unforeseen circumstances arises e.g. you become ill, it is better to have a buddy with you. Having someone to help you with the survey will also cut the time it takes to conduct it.
9. Inform someone of where you are going and of how long you intend to be. If possible inform the site manager by email or phone e.g. if you are on a Wildlife trust or Forestry Commission managed site.
10. Take a mobile phone with you and be aware of your nearest A&E location – you may check your grid reference of where you are on (www.getamap.co.uk).
11. Check the weather before you set out – as stated in the protocol, the best time to survey for ticks is on dry days between the hours of 10:30am and 3pm. If there is a chance of adverse weather then reschedule your survey for another day. If weather conditions become adverse during the survey then abandon it, making a note on the survey sheet.

12. Use sun protection in the summer and keep hydrated.

13. Wear appropriate clothing – light coloured clothing is recommended for tick surveying, as if you do happen to get a tick on your clothes it will be easier to spot.

14. Wear appropriate footwear – sturdy, walking boots or willies – with trousers tucked in to them to prevent tick bites.

15. Be aware of low hanging branches, uneven ground or exposed roots.

16. Cover any cuts or scratches with a waterproof bandage.

17. When surveying, if you encounter any aggressive or what you perceive to be a dangerous animals, abandon the survey and leave the site.

18. If you are bitten by a tick remove it immediately by grasping the tick as close to the skin as possible and pulling upwards slowly and firmly using a tweezers or tick removal tool (you will find one of these in your volunteer pack). Apply antiseptic to the bite area and monitor for several weeks for any changes. Contact your GP if you feel unwell over the following weeks and let them know that you were bitten by a tick. Report the bite to the project coordinator/student researcher, who will advise you further.

Health and Safety regarding the handling of 70% ethanol

70% ethanol is used in the preservation of the tick specimens. The amount contained in each tube is a very small amount (less than 0.6ml); even so there are several safety points to note when handling ethanol:

PRODUCT IDENTIFICATION: Ethanol

PHYSICAL & CHEMICAL PROPERTIES

Appearance: transparent, colourless with mild odour.

Solid/liquid/gas: Liquid

Aqueous pH: neutral

HAZARD IDENTIFICATION

Main Hazard: flammable

Health Effects –

Eyes: Can be irritating to eyes

Skin: Can be irritating to skin

Ingestion: Toxic: danger of very serious side effects through inhalation, in contact with skin and if swallowed.

Inhalation: Vapours may cause drowsiness and dizziness

HANDLING & STORAGE

Handling: Keep away from sources of ignition. All electrical equipment must be fireproofed. Wash hands and face toughly after working with material. Contaminates clothing should be removed and washed before re-use.

Storage: various plastics, glass.

FIRST AID MEASURES

Eyes: Irrigate thoroughly with water for at least 10mins, if irritation persists, obtain medical attention.

Skin: Wash off with water and soap. Remove contaminated clothing and wash before re-use. In extreme/severe cases obtain medical attention.

Ingestion: wash out mouth thoroughly with water. Obtain medical attention.

Inhalation: remove from exposure, rest and keep warm. In extreme/severe cases obtain medical attention.

FIRE FIGHTING MEASURES

Extinguisher medium: Water spray, dry power or vaporising liquids

Unsuitable extinguishing medium: foams without an alcohol resistant polymer.

_ACCIDENTAL RELEASE

Personal precautions: Disperse vapour by thorough ventilation of the area. Wash any spillages on skin with water and soap. Remove any contaminated equipment.

Environmental precautions: Adverse ecological effects cannot be excluded in the event of improper handling or disposal.

Spillage: small amounts (less than 500ml): absorb on an inert absorban (e.g. BDH spillage absorption granules), wash site of spillage thoroughly with water and detergent.

STABILITY & REACTIVITY

Stable? Yes

Conditions to avoid: Avoid sources of ignition, naked flames, extreme heat, do not smoke and enclosed/non-ventilated areas.

Materials to avoid: oxidizing agents, alkali oxides, nonmetallic halides, anhydrides/acids, ethylene oxide, fluorine, hydrides, mercury compounds, silver compounds. The possibility of reaction with other substances cannot be excluded.

Hazardous decomposition products: N/A

EXPOSURE CONTROLS/PERSONAL PROTECTION

Engineering control measures: N/A

Respiratory protection: Avoid inhalation – use in well ventilated areas.

Hand protection: Avoid contact with skin, if a spillage occurs, wash exposed area with water and soap.

Eye protection: Avoid contact with eyes, if contact occurs, irrigate with water for at least 10mins.

Ingestion – Do not ingest – keep away from food, beverages.

TOXICOLOGICAL INFORMATION (Health effects)

Routes of exposure:

After ingestion – rapid absorption, damage to mucous membranes.

Contact with skin: degreasing effect on the skin, possibly followed by secondary inflammation.

On absorption: dizziness, inebriation,

Contact with eyes: may irritate.

Acute effects: On absorption: paralysis, cyanosis, narcosis, respiratory paralysis.

Chronic effects: prolonged or repeated skin contact may cause dermatitis.

Special considerations: flammable

ECOLOGICAL INFORMATION

Mobility: N/A. Persistance/degradability: good. Bioaccumulation: low (Log Pow <2)

DISPOSAL CONSIDERATIONS: The UK Environmental Protection Act 1990 applies