Urban woodland habitat is important for tick presence and density in a city in England

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Abstract

Urban green spaces provide an opportunity for contact between members of the public and ticks infected with pathogens. Understanding tick distribution within these areas and the drivers for increased tick density or *Borrelia* infection are important from a risk management perspective. This study aimed to generate data on tick presence, nymph density and *Borrelia* infection across a range of urban green space habitats, in order to identify those that may potentially present a higher risk of Lyme borreliosis to members of the public. Several sites were visited across the English city of Bath during 2015 and 2016. Tick presence was confirmed in all habitats surveyed, with increased

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likelihood in woodland and woodland edge. Highest nymph densities were also reported in these

habitats, along with grassland during one of the sampling years. Adult ticks were more likely to be

infected compared to nymphs, and the highest densities of infected nymphs were associated with

woodland edge habitat. In addition to Lyme borreliosis causing *Borrelia* genospecies, *Borrelia*

miyamotoi was also detected at several sites. This study adds to the growing evidence that urban

green space habitats present a public health risk from tick bites, and this has implications for many

policy areas including health and wellbeing, climate adaptation and urban green space planning.

Keywords: *Ixodes ricinus*, Lyme disease, green space, *Borrelia miyamotoi*, habitat change,

connectivity

Introduction

Urban green spaces (UGSs) are recognised as having value for improved human health and wellbeing

(HM Government, 2018; Lovell et al., 2018; Wheeler et al., 2015) as well as providing refuge for

wildlife (Natural England, 2003) and improvements to biodiversity through inclusion in ecological

networks (Defra, 2011). In terms of human health benefits, UGS can provide opportunities for

physical activity, stress reduction and social contact, as well as a reduction in exposure to

environmental stressors such as air pollution or noise and can also help reduce urban heat island

effects (Markevych et al., 2017; Twohig-Bennett and Jones, 2018). There is a general consensus that

UGS should be accessible to all, and that the design and maintenance of such spaces should involve

community participation and cross-sectoral collaboration to maximise potential benefits (Lovell et

al., 2018; WHO, 2017). There are aims to increase UGS coverage and improve green corridor

connectivity, but these spaces also provide habitat for ticks and their wildlife hosts, which could put

members of the public at risk of tick bites, Lyme borreliosis and other associated tick-borne diseases (TBD) (Rizzoli et al., 2014; Uspensky, 2014).

An increased risk of TBD is associated with natural spaces with an abundance of wildlife, where members of the public may come into contact with ticks infected with pathogens (Gray, 2002; Li et al., 2012a). Such spaces can provide ticks, such as *Ixodes ricinus,* with appropriate microclimatic conditions to survive, as well as access to hosts for a bloodmeal to support completion of the tick life cycle (Estrada-Peña, 2015). Suitable *I. ricinus* habitat is present within and at the edge of the built environment where human population numbers are higher (Hansford et al., 2017), and awareness of TBD risk may be lower (Bayles et al., 2013). This could potentially lead to an increased risk from contact with infected *I. ricinus* ticks and subsequent TBD transmission (Rizzoli et al., 2014). The assessment of the potential risk posed by TBD transmission within urban and peri-urban areas is a complex process that requires knowledge of tick presence and density, as well as tick-borne pathogen infection rates (Estrada-Peña and De La Fuente, 2014). Importantly, the potential for contact between members of the public and infected ticks (tick-bite risk) within such areas is also a key factor (Estrada-Peña and Jongejan, 1999). During recent years, an increasing number of studies have investigated the presence of *I. ricinus* within UGS, as well as the pathogens they may be infected with, to better determine public health risk (Rizzoli et al., 2014).

A key tick species found within UGS throughout Europe is *I. ricinus,* a vector of a range of pathogens important to human health including *Borrelia burgdorferi* sensu lato, the causative agent of Lyme borreliosis (Grochowska et al., 2020; Rizzoli et al., 2014; Uspensky, 2014). This tick species forms part of a complex transmission cycle, maintaining *Borrelia* bacteria in nature when feeding on reservoir hosts including birds and small mammals. Different *Borrelia* genospecies within the *Borrelia burgdorferi* s.l. group have been linked to different wildlife hosts, with *Borrelia garinii* and *Borrelia valaisiana* associated with bird or squirrel reservoirs, and *Borrelia afzelli* associated with small

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mammals (Mannelli et al., 2012; Mencke, 2013; Piesman and Gern, 2004; Tilly et al., 2008). Larger

mammals, that are particularly important for feeding adult life stages, are generally thought to be

non-competent hosts for *Borrelia* (Braks et al., 2016). It has been suggested that more diverse

communities will have a higher proportion of non-competent hosts, and if a higher proportion of

blood-meals are taken from these host, this may reduce overall transmission of *Borrelia* ('dilution

effect Wood and Lafferty, 2013). Other tick species have also been reported in UGS, including *Ixodes

hexagonus* and *Ixodes frontalis* (Heylen et al., 2019; Oechslin et al., 2017), but their public health

significance is of less importance due to lower contact rates with humans. Where *I. ricinus* has been

found in UGS, density appears to vary from 0 to over 100 nymphs per 100m^2, the higher densities

generally being associated with woodland habitat and established tick populations. A review of

Borrelia infection rates in urban areas in Europe suggests that rates can be similar to studies

conducted in the natural environment (Rizzoli et al., 2014) and that *Borrelia afzelii*, a rodent

associated *Borrelia*, is the most commonly reported genospecies (Grochowska et al., 2020). Other

Borrelia genospecies appear to be common in *I. ricinus* in UGS across Europe, however, and

dominance will likely vary depending on host species composition (Braks et al., 2016). Persistent *I.

ricinus* populations exist in urban and peri-urban areas in Europe, likely as a result of changes in

habitat, climate, wildlife populations and socioeconomic factors (Rizzoli et al., 2014; Uspensky,

2014). Microclimatic conditions required for *I. ricinus* activity include a high relative humidity

(generally above 80%) and weekly mean temperatures of 7 °C (Gray, 2002; Herrmann and Gern,

2015). The level of moisture and drying power of the air, saturation deficit, can also influence

survival, with high saturation deficit making conditions less suitable for questing ticks or the

moulting of engorged ticks (Pfäffle et al., 2013). Wildlife hosts are also required to support the

completion of the tick life cycle, by providing *I. ricinus* with a blood meal to enable moulting to the

next life stage and for females to produce eggs (Anderson and Magnarelli, 2008). Larger hosts are

generally needed to enable the establishment of *I. ricinus* populations, as they are important for

feeding adult ticks (Braks et al., 2016).

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Ixodes ricinus is the most common tick species found biting humans in the United Kingdom (UK) (Cull et al., 2018; Jameson and Medlock, 2011), and there is already evidence that this species is able to survive and bite humans and domestic animals in UGS in southern England (Greenfield, 2011; Guy and Farquhar, 1991; Hansford et al., 2017; Nelson et al., 2015). It has also been demonstrated that *Borrelia* bacteria that can cause disease in humans have been detected in UGS in England (*Borrelia afzelii, Borrelia garinii*, *Borrelia miyamotoi* and *Borrelia valaisiana*), and in some instances, *Borrelia* infection rates in nymphs within such UGS can be as high as 18.1% (Hansford et al., 2017). A small number of studies have investigated urban tick issues in the UK (Dobson et al., 2011; Hansford et al., 2015; Nelson et al., 2015), with the majority conducted in urban deer parks which may not be fully representative of typical urban green space across England.

Here we report results from a study in a city in the South West of England in which we investigated tick presence, nymph density, *Borrelia* infection rates and the density of infected nymphs (DIN) across a range of habitats. Tick presence, nymph density and *Borrelia* prevalence are hypothesised to be less likely/lowest in park settings where habitat management can make the microclimate unsuitable for ticks due to lower humidity. Tick presence and high nymph density are expected to be associated with urban woodland and woodland edge habitat, as both have been shown to be important tick habitat previously in England (Hansford et al., 2017; Medlock et al., 2012). Grassland habitat, on the other hand, is hypothesised to be important for maintaining ticks, particularly in areas near woodland or woodland edge where wildlife may graze or use such spaces to move between green space patches. A range of *Borrelia* genospecies are expected to be detected as a result of the presence of birds and small mammals species in the city of Bath (Goode, 2014).

Material and Methods

Survey location

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The city of Bath is an urban area located in Somerset, England with a human population of ~89,000 (census data, 2011) and was chosen due to being based in the South West region of England where Lyme borreliosis incidence is high (Cairns et al., 2019). During 2015 and 2016, 27 sites across the city and surrounding habitat were surveyed for ticks during spring (Fig. 1; Supplementary File 1). Sites were publicly accessible green space (determined using a 1:25,000 Ordnance Survey map to identify public footpaths) and deemed to be potentially suitable for ticks (based on the presence of green space identified using Google Earth to locate green space habitat). All survey sites chosen were within a four-mile radius of the city centre (including urban fringe and some connected rural habitat). During initial field visits, survey sites were classified into one of four broad habitat categories based on habitat dominance; grassland (including wildflower meadows managed for biodiversity), park (managed primarily for use by the public, with grass cutting in place to keep the grass sward short), woodland and woodland edge. Of the four target habitats, only one was sampled at each location, even if more than one type was present. Transects surveyed in grassland were conducted away from ecotonal habitat, the area where two contrasting types of vegetation meet, as this has been found to support increased *I. ricinus* densities in grassland (Medlock et al., 2020). Transects surveyed in park habitat were conducted in open spaces, but also included habitat adjacent to hedges or under trees. Transects surveyed in woodland were completed away from edge habitat (which is where woodland edge transects were performed).

Tick collection

Ticks were collected by flagging a $1m^2$ piece of cloth over the vegetation (Milne, 1943). This is a well-recognised method for tick collection and estimated density assessments, but needs to be standardised as much as possible to allow for comparison of results (Estrada-Peña et al., 2013). All transects were sampled walking at a slow pace over a set distance of 5 m^2 to ensure ticks did not dislodge before being collected. At each survey site, approximately 60-110 x 5 m^2 transects were

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sampled over the two survey periods (spring 2015 and spring 2016). For efficiency, fewer transects were carried out at sites with high tick density (e.g. where over 50 nymphs had been collected by the time 30 transects had been completed). This was used to obtain density estimates for each area and is herein referred to as 'density'. Surveys were carried out on dry days between 10am and 5pm. Each site was visited once during May 2015 and once during May 2016, except for site 18 which was inaccessible during the second year of the study. May was chosen to coincide with increased tick density observed in studies elsewhere in England (Craine et al., 1995; Dobson et al., 2011) as well as the start of increasing reports of tick bites recorded through Public Health England's Tick Surveillance Scheme (Cull et al., 2020). Live ticks were collected into Eppendorf tubes and stored at -80°C until morphological identification to species level (Hillyard, 1996) and pathogen analysis could be carried out.
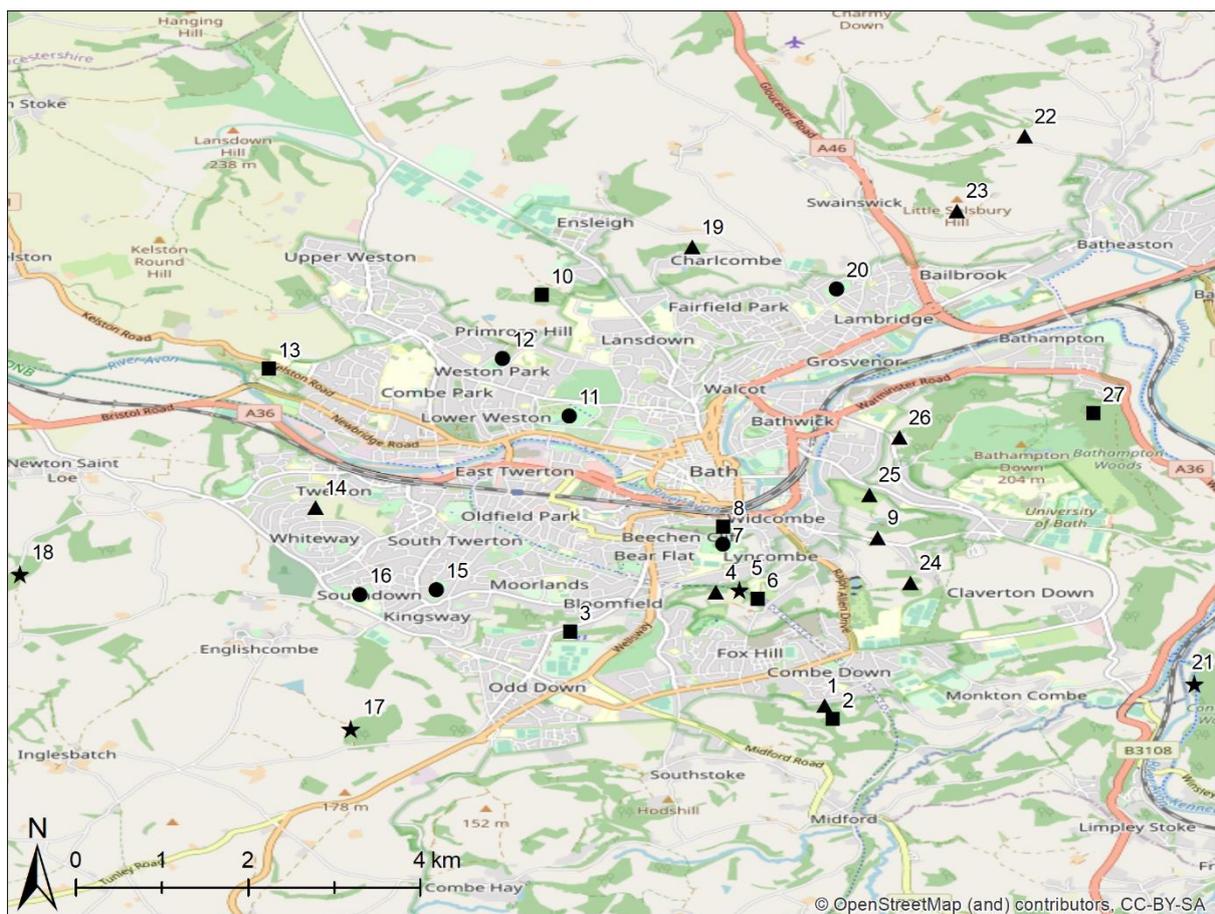
Fig. 1 – Numbered survey sites in and around the city of Bath 2015/2016; grassland represented by triangles, parks represented by circles, woodland represented by squares and woodland edge represented by stars. © OpenStreetMap (and) contributors, CC-BY-SA.

Pathogen screening

Ticks were tested for the presence of *Borrelia* spp. bacteria. Ammonium hydroxide (NH4OH; Sigma Aldrich) was used to extract DNA from individual ticks (Hansford et al., 2015). Individual ticks were placed into separate PCR tubes, with every eighth tube left empty to use as a contamination control. Once DNA extraction was completed, a sample from each tube was added to the same position on a qPCR plate. A modified pan-*Borrelia* qPCR targeting the *Borrelia* 16S rRNA gene was used to detect the presence of all *Borrelia* bacteria (primers 5'-AGC CTT TAA AGC TTC GCT TGT AG-3' (forward), 5'-GCC TCC CGT AGG AGT CTG G-3' (reverse) and probe 5'-CCG GCC TGAG AGG GTG AAC GG-BHQ1-3'; Parola et al., 2011). A QIAgility system (QIAGEN) was used to prepare PCR plates using 10 µl of ABI TaqMan Fast Universal master mix (Applied Biosystems, Waltham, MA), 1 µl of primer/probe mix (0.18 µl forward primer, 0.18 µl reverse primer, 0.05 µl probe and 0.59 µl H_2O per sample; final concentration 900 nM each primer and 250 nM probe), 4 µl PCR grade H_2O and 5 µl of sample DNA. A QuantoStudio 7 Flex real-time PCR system (Applied Biosystems) was used to perform the PCR, using a program with a hold step for 20 seconds at 95 °C, followed by 40 cycles of 3 seconds at 95 °C and 20 seconds and 60 °C. Sanger sequencing of the 5S-23S rRNA gene (primers forward 5'- GAG TTC GCG GGA GAG TAG GTT ATT GCC-3', reverse 5'- TCA GGG TAC TTA GAT GGT TCA CTT CC-3'; Alekseev et al., 2001) and BLAST analysis were used to determine *Borrelia* genospecies (Cull et al., 2021). Ticks were also tested for *Borrelia miyamotoi* using a PCR assay to detect the *flaB* gene that is specific to *B. miyamotoi* (primers 5' - AGA AGG TGC TCA AGC-3' (forward), 5'TCG ATC TTT GAA AGT GAC ATA T-3' (reverse) and probe 5'Atto647N-AGC ACA ACA GGA GGG AGT TCA AGC-BHQ2-3'; Layzell et al., 2018). DNA extraction, qPCR master mix preparation, qPCR amplification and sequencing were each carried out in separate laboratories to eliminate potential cross-contamination. Positive and

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negative controls were used to ensure that qPCR reagents were able to amplify target DNA, and to ensure false positives did not occur due to contamination. Additional extraction controls (a blank well in each row of the qPCR plate) were used to determine if any contamination between samples had occurred during the DNA extraction or PCR process.

Statistical analysis

All statistical analyses were carried out in R version 3.5.3 (R Core Development Team, 2020). We tested for the effects of habitat type and year on tick presence, nymph density, *Borrelia* prevalence and the density of *Borrelia* infected nymphs (DIN) using generalized linear mixed models (GLMM). A GLMM with a binomial error and logit link function was used to investigate tick presence (absence / presence; all tick stages) in relation to habitat type, year and their interaction. As the total distance dragged per site varied, this was also included in the model, to assess if this influenced the presence of ticks. Survey site location was included in the model (and all subsequent models) as a random effect, to account for the non-independence of observations from the same site (Harrison, 2014).

A GLMM with a negative binomial error (used due to overdispersion) and log link function was used to investigate nymph density. The number of nymphs collected per 5 m^2 was used as the response variable and habitat type, year and their interaction were included as fixed effects. Survey site was included as a random effect. Only nymphs were used for density analysis, as they have the most significant impact on human health (Kilpatrick et al., 2017) and low numbers of adults were collected.

A GLMM with a binomial error and logit link function was used to investigate *Borrelia* infection (all tick stages, infected or uninfected) in individual ticks in relation to habitat type, year and tick life stage (adults vs nymphs). Survey site was included as a random effect. There was insufficient data to investigate possible interactions between site and year.

A GLMM with a Poisson error and log link function was used to investigate the influence of habitat and year on DIN. Study site was included as a random effect. A Poisson distribution was most

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appropriate as there was no evidence of overdispersion. An offset variable in this model (the log of the total distance dragged per survey location) was used to account for uneven distance dragged at each survey location (Millins et al., 2016; Zuur et al., 2009). There was insufficient data to investigate DIN at the transect level or possible interactions between site and year. Furthermore, DIN models did not converge because of a lack of infected ticks in parks and missing values. We therefore excluded the park habitat from DIN analyses.

Significance of fixed effects was determined by comparing two nested models, with and without the factor of interest, using likelihood ratio tests (lmtest; Zeileis and Hothorn, 2002). Interactions were removed from the final models if non-significant. If the model revealed significant habitat effects or significant interaction effects between habitat and year, we used post-hoc Tukey tests (multcomp; Hothorn et al., 2008; emmeans; Lenth, 2020) to determine which habitat types, life stages or habitat types – year combinations differed from one another.

Model assumptions (overdispersion, zero-inflation) were validated using the DHARMa package (Hartig, 2019). Statistically significant terms ($p < 0.05$) are highlighted. 95% confidence intervals (CI) are presented and were calculated using the Rmisc function in R (Hope, 2013).

Results

Ixodes ricinus ticks (n=1095) were collected from 22 (81.5%) of 27 sites during the study period. Nymphs were the most common life stage (n=943, 86.1%) followed by adult males (n=84, 7.7%) and adult females (n=68, 6.2%; Table 1). No larvae were detected during the survey. Overall, tick presence (including adults and/or nymphs) was detected on 16.3% (n=439) of 2698 transects. Repeated tick presence was confirmed at 16 sites (Supplementary File 2) and within all habitat types surveyed. *Borrelia* infected ticks were found at 12 (44.4%) of 27 sites across the city, with an overall infection rate of 4.5% in all life stages, and 3.1% in nymphs (Table 1). Mean nymph density per 100 m^2 across all sites was 6.99 (95% CI 6.0-8.0) and mean DIN was 0.22 (95% CI 0.14-0.29) per 100 m^2.

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Habitat	Total ♂; mean per 100 m² (95% CI)	Total ♀; mean per 100 m² (95% CI)	Total N; mean per 100 m² (95% CI)	Total ticks; mean per 100 m² (95% CI)	*Borrelia* positive nymphs / tested; % (95% CI)
Grassland	40; **0.76** (0.49-1.03)	33; **0.62** (0.36-0.89)	197; **3.72** (2.88-4.57)	270; **5.11** (4.11-6.11)	5/186; **2.7%** (0.04-5)
Park	2; **0.06** (0-0.15)	2; **0.06** (0-0.15)	8; **0.25** (0.06-0.44)	12; **0.38** (0.15-0.60)	0/7; **0%** (0)
Woodland	16; **0.45** (0.19-0.71)	18; **0.51** (0.25-0.76)	263; **7.39** (5.88-8.89)	297; **8.34** (6.72-9.97)	11/247; **4.5%** (1.9-7.1)
Woodland Edge	26; **1.80** (0.96-2.64)	15; **1.04** (0.49-1.59)	475; **32.87** (25.69-40.06)	516; **35.71** (28.10-43.32)	12/471; **2.5%** (1.1-3.9)
Overall	84; **0.62** (0.47-0.78)	68; **0.50** (0.36-0.64)	943; **6.99** (6.0-8.0)	1095; **8.12** (7.05-9.18)	28/911; **3.1%** (2-4.2)

Table 1 – Ticks collected during spring 2015 and spring 2016 in the city of Bath the total collected and mean density per 100 m² and 95%CI of each life stage and percentage of nymphs infected with *Borrelia*. ♂ = male, ♀ = female, N = nymph

Predictors for presence of I. ricinus

Habitat was a significant predictor of tick presence (χ^2 (3) = 16.34, p < 0.001). Tick presence was significantly more likely in woodland and woodland edge compared to park (Fig. 2; Table 2). Tick presence was more likely in woodland edge compared to grassland and in grassland compared to park, but this was only borderline significant (Fig. 2; Table 2). Ticks were more likely to be found in woodland edge compared to woodland, and in woodland compared to grassland, but this was not significantly different (Fig. 2; Table 2). Tick presence was less likely during 2015, but this was only borderline significantly different to 2016 (χ^2 (1) = 2.98, p = 0.084) and the interaction between habitat and year was also not significant (χ^2 (3) = 3.88, p = 0.274).
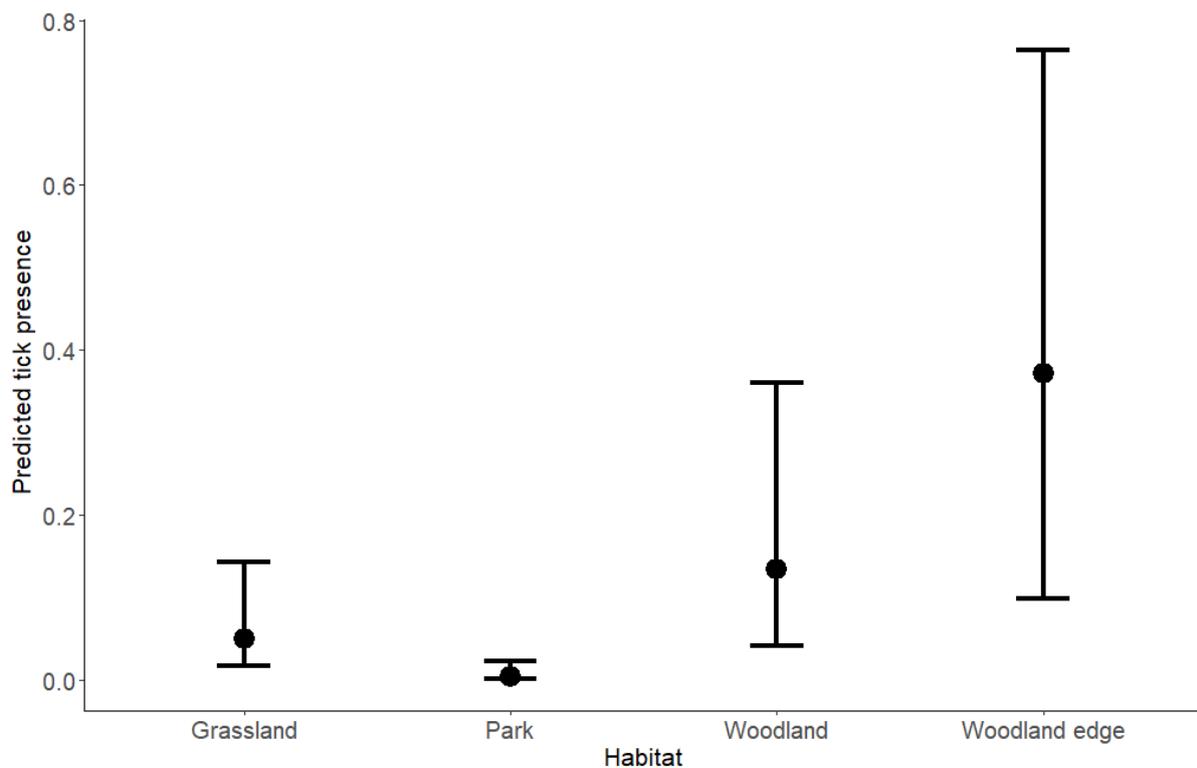

Fig. 2 – Predicted tick presence in urban green space habitat in 2015 and 2016 showing tick presence is significantly more likely in woodland and woodland edge compared to park habitat.

Predictors of density of I. ricinus

Mean nymph density per 100 m^2 across all sites was 6.99 (95% CI 6.0-8.0). We observed a borderline significant interaction effect between year and habitat on nymph density (χ^2 (3) = 7.50, p = 0.057).

Most nymphs were collected in woodland edge (mean/100 m^2 =32.87; 95% CI 25.69-40.06) and woodland (mean/100 m^2 =7.39; 95% CI 5.88-8.89), with fewer nymphs collected in grassland (mean/100 m^2 =3.73; 95% CI 2.88-4.57) and park habitats (mean/100 m^2 =0.25; 95% CI 0.06-0.44) (Table 1). The difference in nymph density was significantly higher in woodland and woodland edge compared to park habitat during both years (Table 2). Density was less consistent in grassland, being significantly higher compared to park habitat in 2016 only (Table 2).

Model	Pairwise contrasts	Estimate	SE	z	p
Tick presence	_G/P_	2.66	1.07	2.49	0.061
	_W/G_	1.07	0.86	1.25	0.591
	_WE/G_	2.41	1.05	2.31	0.095
	_W / P_	3.74	1.13	3.31	**0.005**
	_WE / P_	5.08	1.29	3.95	**<0.001**
	_WE/W_	1.34	1.09	1.23	0.603
Nymph density 2015	_G/P_	2.11	1.19	1.78	0.279
	_W/G_	1.13	0.95	1.19	0.629
	_WE/G_	2.71	1.12	2.42	0.073
	_W / P_	3.24	1.24	2.61	**0.045**
	_WE / P_	4.81	1.38	3.68	**0.003**
	_WE/W_	1.58	1.16	1.35	0.530
Nymph density 2016	_G / P_	4.23	1.50	2.81	**0.025**
	_W/G_	1.35	0.94	1.43	0.478
	_WE/G_	2.63	1.12	2.34	0.088
	_W / P_	5.58	1.55	3.60	**0.002**

	_WE / P_	6.86	1.67	4.11	**<0.001**
	_WE/W_	1.28	1.17	1.09	0.694
DIN	_W/G_	1.30	1.06	1.23	0.435
	_WE / G_	2.87	1.20	2.40	**0.043**
	_WE/W_	1.57	1.11	1.42	0.331

Table 2 – Tukey's test between fixed effects for all models. Estimates are for the first level listed in the pairwise contrasts. G= grassland, P = park, W= woodland, WE= woodland edge

Predictors of Borrelia infected ticks and DIN

Ticks testing positive for *Borrelia* were collected from 12 (44.4%) of 27 sites surveyed (Supplementary File 2). Of 1059 ticks (83 ♂, 65 ♀ and 911 nymphs) tested, 48 (4.5% 95% CI 3.3-5.7) were positive, including eight males (9.6% 95% CI 3.3-15.9), 12 females (18.5% 95% CI 11.7-25.3) and 28 (3.1% 95% CI 2.0-4.2) nymphs. *Borrelia* infection differed significantly between tick life stages (χ^2 (1) = 23.1, p <0.001), with adults (males and females) being significantly more likely to be infected with *Borrelia* compared to nymphs (χ^2 (1) = 5.1, p <0.001). Overall infection was 4.9% (22/445 ticks tested; 95% CI 2.9-6.9) in 2015 and 4.2% in 2016 (26/614 ticks tested; 95% CI 2.6-5.8). This difference between years was not statistically significant (χ^2 (1) = 0.01, p = 0.983). Similarly, infection in nymphs was 3.9% (16/413 nymphs tested; 95% CI 2.1-5.7) in 2015 and 2.4% (12/498 nymphs tested; 95% CI 1.1-3.7) in 2016. Across all tick life stages, the likelihood of *Borrelia* infection did not differ significantly between habitat types (χ^2 (3) = 1.98, p = 0.577).

DIN was highest in woodland (mean/100 m^2 = 0.33; 95% CI 0.14-0.52) and woodland edge (mean/100 m^2 = 0.82; 95% CI 0.36-1.28), followed by grassland (mean/100 m^2 = 0.10; 95% CI 0.01-0.19) and park (0 per 100 m^2). However, the effect of habitat type on DIN was only borderline significant (χ^2 (2) = 5.85, p = 0.053). Post-hoc contrasts revealed that DIN was significantly higher in

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woodland edge compared to grassland (Table 2), but no other pairwise comparisons were statistically significant (Table 2). DIN did not differ significantly per year (χ^2 (1) = 0.04, p = 0.841).

Borrelia genospecies

Borrelia garinii accounted for 40.9% of all *Borrelia* positive specimens in 2015 (n=9/22), followed by *B. valaisiana* (9.1%; n=2/22) and *B. afzelii* (n=1/22; 4.5%). *Borrelia valaisiana* was the most commonly detected genospecies in 2016 (n=9/26; 34.6%), followed by *B. afzelii* (5/26; 19.2%) and *B. garinii* (n=3/26; 11.5%). Sequence data could not be obtained for 10 of the qPCR positive samples from 2015, and from nine of the positive samples from 2016 (Supplementary File 3). In woodland habitat, *Borrelia garinii* was the most detected genospecies (8/17; 47.1%), followed by *B. valaisiana* (4/17; 23.5%) and *B. afzelii* (1/17; 5.9%). In woodland edge habitat, *Borrelia valaisiana* was the most detected genospecies (5/18; 27.8%), followed by *B. garinii* (4/18; 22%) and *B. afzelii* (3/18; 16.7%). In grassland habitat, *Borrelia valaisiana* and *B. afzelii* were the most common genospecies detected (3/12; 25% each). *Borrelia* bacteria were detected in one female collected from Park habitat (site 7) during 2016. Sequence data could not be obtained from this specimen, and the remaining 11 ticks collected from park habitats tested negative.

Borrelia miyamotoi was detected in four nymphs and one female (0.38% of all ticks tested) from woodland, woodland edge and grassland habitats. This included repeated detection of infected nymphs at a central woodland site (site 6) during both years, and co-infection in a female infected with *B. afzelii*, and a nymph infected with *B. valaisiana*.

Discussion

Ixodes ricinus infected with *Borrelia* bacteria can be found in a variety of habitats within and around the city surveyed, but overall, DIN appears to be low (less than one infected nymph per 100 m^2).

Ticks were more likely to be present in woodland and woodland edge habitat, which also had the highest nymph densities. In accordance with other UK studies which have included UGS (Dobson et al., 2011; Greenfield, 2011; Guy and Farquhar, 1991; Hansford et al., 2017, 2015; Nelson et al., 2015; Sorouri et al., 2015; Vollmer et al., 2011), tick presence and nymph density varied between habitat types and a range of *Borrelia* genospecies important to human health were detected. The results provide further evidence that members of the public using UGS within and around cities may be at risk from tick-bites, adding further weight to the growing data now published in over 20 countries across Europe (Borşan et al., 2020; Cekanac et al., 2010; Christova et al., 1998; Corrain et al., 2012; Didyk et al., 2017; Fingerle et al., 1999; Gray et al., 1999; Guy and Farquhar, 1991; Heylen et al., 2019; Hornok et al., 2014; Junttila et al., 1999; Koci et al., 2007; Krčmar et al., 2014; Li et al., 2012b; Lommano et al., 2012; Makenov et al., 2019; Mäkinen et al., 2003; Pangrácová et al., 2013; Pet'ko et al., 1997; Plch and Basta, 1999; Reis et al., 2011; Reye et al., 2010; Sen et al., 2011; Vogelgesang et al., 2020; Wielinga et al., 2006; Žygutienė et al., 2008).

The repeated presence of males, females and nymphs in grassland, woodland and woodland edge suggests that a range of UGS habitats could potentially support persistent tick populations. We did not detect any larvae, but surveys were not timed to coincide with peak larval activity. Larvae may have been absent more generally, if adult females were not able to find suitable larger hosts to feed on and complete their life cycle by laying eggs. It is unlikely that microclimate conditions were unsuitable for larvae, particularly in woodland or woodland edge habitats which supported highest nymph densities. All tick life stages of *I. ricinus* have been collected from a park in central London (Richmond Park) and UGS in Salisbury (Hansford et al., 2017), suggesting that other UGS in England likely support tick population establishment (Greenfield, 2011; Nelson et al., 2015), as has been reported elsewhere in Europe (Borşan et al., 2020; Bukowska et al., 2003; Corrain et al., 2012; Hercík et al., 2007; Heylen et al., 2019; Junttila et al., 1999; Oechslin et al., 2017; Pejchalová et al., 2007; Reye et al., 2010; Schicht et al., 2012; Szekeres et al., 2017; Tappe et al., 2014; Vaculová et al., 2019).

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Nymphs of *I. ricinus* are the most important tick life stage for Lyme borreliosis transmission (Kilpatrick et al., 2017) and were the most common life stage collected in this study. Nymph density varied significantly between habitats from 0.25 per 100 m^2 in parkland habitat up to 32.87 per 100 m^2 in woodland edge habitat. Overall mean density of nymphs across all survey locations (6.99 per 100 m^2) was lower compared to those reported in studies at an urban deer park in Richmond Park, London (26.11 per 100 m^2; (Nelson et al., 2015), higher than previous studies in a smaller urban city in Salisbury, South West England (2.63 per 100 m^2; (Hansford et al., 2017) and similar to studies across a number of urban and peri-urban sites in Poland which reported overall nymph density of 5-10 per 100 m^2 (Kowalec et al., 2017; Welc-Falęciak et al., 2014). Such differences are likely being driven by differences in tick host populations or habitat composition.

Nymph density was significantly higher in woodland edge and can be explained by the ecotonal habitat present, which has previously been shown to increase the risk of tick-borne disease transmission (Killilea et al., 2008; Pfäffle et al., 2013; Rizzoli et al., 2014). Both urban and rural studies elsewhere in England have reported similar findings (Dobson et al., 2011; Hansford et al., 2017; Medlock et al., 2020, 2012). Nymph density reached almost one nymph per metre in one location (site 17). Such densities are commonly reported in rural studies in Exmoor or Dartmoor in England (Dobson et al., 2011). The similar densities detected in Bath are likely a reflection of the location of three of the four woodland edge sites which were outside of the city boundaries with greater connectivity to rural green space. Urban woodland edge within the city boundary (site 5) however, had the second highest nymph density across the study locations (47 nymphs per 100 m^2) and was comparable to some densities (31-136 nymphs per 100 m^2) reported in Richmond Park (Dobson et al., 2011) and a number of rural mainland sites in Scotland (Millins et al., 2018). One interesting finding from one of the sites was the presence of questing *I. ricinus* on the edge of a

tarmac pathway, where ivy, leaves and other vegetation had collected on top of gravel. The potential importance of ivy in urban green space has already been highlighted elsewhere (Hansford et al., 2017; Hornok et al., 2014), but can be emphasised again here, where very little mat layer was present. This challenges the view that a deep mat layer is required for tick presence and high densities in some UGS (Greenfield, 2011).

Clearly, urban and peri-urban woodland habitats are important for ticks and could be key areas for elevated Lyme borreliosis risk. Nymph density in woodland was higher compared to a previous study of smaller urban woodland habitats in Salisbury, England (Hansford et al., 2017) and some peri-urban woodland sites in Germany (6.6-7.71 nymphs/100 m^2; (Hauck et al., 2020; Maetzel et al., 2005) but lower compared to larger urban woodland sites in France and Slovakia where over 20 nymphs/100 m^2 have been reported (Chvostáč et al., 2018; Marchant et al., 2017; Vourc'h et al., 2016). The highest nymph densities in woodland were reported at sites 6, 13 and 27 on the edge of the city boundary, directly adjacent to rural habitats. All appeared to have a wider range of tree species as well as a thicker understory, compared to the other woodland sites surveyed; suggesting location, connectivity and composition may be important predictors of nymph density.

Ticks were present in almost all grassland habitat surveyed, with nymph density higher than in park habitat, but lower compared to woodland edge. Another study in England (Hansford et al., 2017) reported similar low densities at grassland sites (less than 1 nymph per 100 m^2), but this wasn't consistent at all grassland sites in our study. Particular grassland habitats (sites 1, 4, 24 and 26) had higher densities, possibly a result of increased connectivity to wider rural habitat, which would provide ease of access for wildlife and subsequent tick introduction (Uspensky, 2014). The only grassland site (site 14) without any ticks detected is in the west of the city surrounded by housing/built environment. This site is a recently sown meadow that is one example of several across Bath being used to improve biodiversity across the city. Tick presence at this site may occur

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once the meadow is established, when sward height is sufficient for *I. ricinus* survival (Greenfield, 2011; Medlock et al., 2012, 2008) and wildlife start to utilise the space.

Park habitat had significantly lower nymph density compared to most of the other habitats surveyed in 2015, and all habitat surveyed in 2016. The change in 2016 may have been a result of increased habitat management across park habitat in Bath earlier that year, which aimed to keep grass short and less suitable for tick survival. Ticks were detected in two of six parks surveyed (sites 7 and 12), where overall nymph density was low (fewer than 0.25 nymphs collected per 100 m^2). The same density was reported by Hansford et al., (2017) in park habitat in Salisbury and similar densities (between to 0.21-0.28/100 m^2) were reported in central public parks in Helsinki, Finland (Sormunen et al., 2020), Cluj-Napoca, Romania (Borşan et al., 2020) and Moscow, Russia (Klemola et al., 2019; Makenov et al., 2019). Interestingly, both parks with tick presence in Bath were either adjacent to woodland or directly connected to rural grassland. Other studies in Belgium, the Czech Republic, the Netherlands, Poland and Slovakia have also reported fewer ticks in parks compared to surrounding rural habitat, particularly in locations enclosed by the built environment (Heylen et al., 2019; Sytykiewicz et al., 2012; Vaculová et al., 2019; Venclíková et al., 2014; Wielinga et al., 2006). All other parks in this study were isolated, being cut off from rural habitat or larger UGS by road networks and housing.

Apparent absences of ticks from similarly isolated park habitat have also been reported in Belgium, Germany and London (Heylen et al., 2019; Maetzel et al., 2005; Nelson et al., 2015). Connectivity cannot be the only factor however, as tick presence was detected at a park that is centrally located with mowed grass and large, individual trees offering both open space and shaded areas for members of the public to enjoy (site 7). Ticks, including a female infected with *Borrelia*, were only found in areas where grass had been left to grow, including under trees and alongside hedgerows. This suggests that shorter grass within park habitats is unsuitable for ticks, likely a result of

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unsuitable microclimatic conditions that are required for tick survival (Cekanac et al., 2010; Hansford et al., 2017; Kubiak et al., 2019). Habitat management such as maintaining grass at shorter levels in UGS could be a useful tool for reducing *I. ricinus* survival in targeted, higher risk areas (Cekanac et al., 2010; Dobson et al., 2011; Kowalec et al., 2017; Maetzel et al., 2005; Medlock et al., 2012). Although low tick density was recorded, the repeated presence of ticks within park areas with high footfall puts local residents and visiting members of the public potentially at risk from ticks bites, in areas where tick encounters might be unexpected (Bayles et al., 2013).

Overall *Borrelia* infection in nymphs (3.1%) was similar to those reported in other urban study sites in England (2.8-3.6%) (Dobson et al., 2011; Hansford et al., 2015; Nelson et al., 2015), urban sites in the Czech Republic and Ukraine (2.4-3.7%) (Didyk et al., 2017; Plch and Basta, 1999) as well as some rural study sites in England and Scotland (2.4-2.6%) (Bettridge et al., 2013; Kurtenbach et al., 1998; Lambert et al., 2019; Layzell et al., 2018; Millins et al., 2018; Vollmer et al., 2011). Some woodland sites (site 2 and 27) had the highest *Borrelia* infection rates (15.4 and 8.3% respectively), both being similar to those reported elsewhere in English peri/urban woodland (7.7-19%; (Hansford et al., 2017; Vollmer et al., 2011), and the latter being consistent with infection rates reported in the same area previously (7.7%; Vollmer et al., 2011). This suggests that in some urban areas, *Borrelia* infected ticks are indeed established and infection rates are perhaps stable over long periods. Urban woodland in the Czech Republic, France, Germany, Poland and Slovakia similar had similar prevalence (7-15.5%) (Chvostáč et al., 2018; Marchant et al., 2017; Michalik et al., 2003; Pejchalová et al., 2007; Stańczak et al., 2004; Szekeres et al., 2017; Vourc'h et al., 2016). *Borrelia* bacteria were not detected in over 100 nymphs collected at two sites (grassland and woodland and sites 1 and 13) with high nymph density. *Borrelia* infection rates may have been too low to detect in this sample, competent *Borrelia* hosts may be absent from this area, or larger hosts such as deer, cattle and sheep are possibly reducing *Borrelia* infection rates as a result of the dilution effect (Wood and Lafferty, 2013). The presence of *Borrelia* infected ticks did not appear to differ significantly between habitats or years,

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but DIN was significantly higher in woodland edge. Low numbers of ticks collected within urban park habitat means additional data are required to explore the public health risk of ticks in these important green space habitats in southern England.

A range of *Borrelia* genospecies with the potential to cause disease in humans were detected during this study. *Borrelia garinii* and *Borrelia valaisiana*, both thought to be linked to enzootic transmission cycles involving birds and grey squirrels (Millins et al., 2015), were the most commonly detected during this study. These genospecies were also the most commonly reported in other peri-/urban locations in the south of England (Hansford et al., 2017; Kurtenbach et al., 2001; Vollmer et al., 2011), Belgium, Czech Republic, France, Germany, Ireland, Moldova and Slovakia (Basta et al., 1999; Chvostáč et al., 2018; Heylen et al., 2019; Kirstein et al., 1997; Koci et al., 2007; Kybicová et al., 2017; Maetzel et al., 2005; Marchant et al., 2017; May et al., 2015; Pejchalová et al., 2007; Reis et al., 2011). The majority of ticks testing positive came from woodland or woodland edge habitat, both key habitats for birds in urban areas. This may partly explain the dominant genospecies present (Vaculová et al., 2019), but it may also be a result of spill over from rural enzootic transmission cycles occurring outside the city, particularly as the dominant genospecies reported in southern England is also *B. garinii* (Cull et al., 2021). *Borrelia garinii* has been linked to neuroborreliosis (Sprong et al., 2018) which may not always present with a typical bullseye rash (Steere et al., 2016). This may delay symptom recognition and diagnosis amongst an urban population, who may be less tick or Lyme borreliosis aware (Bayles et al., 2013), and this could potentially lead to Lyme borreliosis complications (Steere et al., 2016). In addition, the newly reported human pathogen *B. miyamotoi* was also detected across a range of habitat types, in similar levels to those previously reported in England (Hansford et al., 2017; Layzell et al., 2018). This justifies further research and possibly increased awareness raising in urban settings, if this potential pathogen was found to be causing clinical disease.

The main aim of this study was to detect ticks in UGS, establish which habitats might be important for ticks, and to detect the circulation of *Borrelia* pathogens important to human health. Several limitations of the study have been identified and must be considered alongside the results presented above. Some survey locations were up to four miles from the city centre, and results within these locations may have been influenced by differences in connectivity between green space (Estrada-Peña, 2002). Results from these locations may not be fully representative of UGS within town or city boundaries and may potentially overestimate risk. Additionally, only one habitat type was chosen to survey within each survey location, even if other habitats were present. Both factors could potentially influence tick presence, establishment or *Borrelia* infection rates. Surveying a single city during spring and repeating for two consecutive years provides a good indicator of tick presence and *Borrelia* infection, but could have been improved with seasonal surveys, and additional cities to increase sample sizes. This would allow for the results to be generalised to wider UGS in southern England, particularly in park habitat where fewer ticks were collected but where visitor numbers can be high. A final consideration must be given concerning tick studies using flagging or dragging to estimate tick densities and make comparisons, due to individual surveyor differences in the execution of these methods. Caution must therefore, be taken when comparing results between studies (Nyrhilä et al., 2020).

Conclusions

This study confirms the presence of *Borrelia* infected *I. ricinus* in a range of well-established UGS, adding peri-urban grassland and urban woodland to the existing list of habitats with potential for elevated densities of nymphs that may be infected with *Borrelia*. Previously, only urban deer parks and woodland edge habitat had been identified as having elevated nymph densities or *Borrelia* infection rates in England (Dobson et al., 2011; Hansford et al., 2017). Such areas could present a risk of Lyme borreliosis transmission if visiting members of the public are not expecting a tick encounter or being bitten by infected ticks. This is particularly important to consider alongside the promotion

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of the human health benefits of UGS, and the societal and environmental benefits of improving urban biodiversity. Fewer ticks were found in park habitat where vegetation was managed, e.g. grass cutting, and this presents some opportunities for future management strategies. Higher nymph densities were associated with wooded areas or other habitat types that appeared to have better connectivity to other urban or surrounding rural green space. Initiatives to improve UGS connectivity and increase green space coverage may have a significant impact on tick presence, nymph density and *Borrelia* infection rates. Further investigation into urban landscape and patch level metrics (particularly in central park habitats with tick presence), such as those used by Heylen et al., (2019), could help further define urban Lyme borreliosis risk in England and indeed across other UGS in Europe where *I. ricinus* ticks are commonly detected. Such areas could be targeted for control or interventions, particularly as many urban areas are already managed as part of existing green space strategies.

Supplementary File 1 – Site location numbers, habitat type, coordinates and descriptive notes on survey locations in the city of Bath

Site No.	Habitat type	X	Y	Notes
1	Grassland	51.35676	-2.34816	Combe Down short grassland, bordered by woodland and hedgerows, which forms part of a connected network of green space at the city boundary to the south.
2	Woodland	51.35535	-2.34726	Combe Down, ancient deciduous woodland with ancient herb layer and minimal shrub layer, which forms part of a connected network of green space at the city boundary to the south.
3	Woodland	51.36455	-2.37474	Odd Down (The Tumps), deciduous woodland with dense herbage and climbers, adjacent to a cricket pitch and surrounding by housing and road networks.
4	Grassland	51.36869	-2.35950	Lyncombe Vale, short grassland, bordered by woodland which forms part of a network of connected green space adjacent to the dismantled railway (now a cycle path/walkway that runs

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				through the city). Bounded by housing and road networks.
5	Woodland Edge	51.36895	-2.35697	Lyncombe Vale deciduous woodland edge which forms part of a network of connected green space adjacent to the dismantled railway (now a cycle path/walkway that runs through the city). Bounded by housing and road networks.
6	Woodland	51.36798	-2.35513	Lyncombe Vale, deciduous ancient woodland with dense stands of wild garlic, which forms part of a network of connected green space adjacent to the dismantled railway (now a cycle path/walkway that runs through the city). Bounded by housing and road networks.
7	Park	51.37377	-2.35880	Alexandra Park, amenity green space with short grass, bordered by ornamental trees, adjacent to site 8 and surrounded by housing and road networks.
8	Woodland	51.37559	-2.35871	Beechen Cliff, deciduous ancient woodland with wild garlic and defined path network, adjacent to site 7 and surrounded by housing and road networks.
9	Grassland	51.37446	-2.34260	Widcombe short grassland, bordered by mature hedgerow, adjacent to Smallcombe wood on the eastern edge of the city, separated from wider rural space by B roads.
10	Woodland	51.39999	-2.37770	Primrose Hill, deciduous amenity woodland used for education, adjacent to arable fields on the northern edge of the city, separate from wider rural space by B roads.
11	Park	51.38724	-2.37487	Victoria Park, amenity green space with short grass, scattered veteran and young trees, central city location surrounded by built environment and road networks.
12	Park	51.39330	-2.38182	Weston Park, amenity green space with short grass, longer grass margins and bordered by trees/shrubs, situated on the northern edge of the city close to Primrose Hill, connected to arable fields.
13	Woodland	51.39219	-2.40623	Newbridge, deciduous woodland with dense herbage, adjacent to the River Avon and bounded by an A road.
14	Grassland	51.37762	-2.40139	Twerton short grassland with isolated hawthorn bushes, bounded by the build environment and a network of roads.

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15	Park	51.36894	-2.38874	Southdown Park, amenity green space, rough grass slope, surrounding by the built environment and separated from adjacent arable fields by a B road.
16	Park	51.36841	-2.39674	Kingsway Park, amenity green space, open grassy field, surrounded by the built environment and network of roads.
17	Woodland Edge	51.35431	-2.39764	Middle Wood, deciduous woodland adjacent to track, just outside the city of bath, surrounded by rural habitat and sparse B roads.
18	Woodland Edge	51.37058	-2.43225	Whistling Copse, deciduous woodland edge with ferns, adjacent to track, just outside the city of bath, surrounded by rural habitat and sparse B roads.
19	Grassland	51.40506	-2.36199	Charcombe short grassland, bordered by woodland, on the northern edge of the city surrounded by arable fields and separated from the wider rural environment by B roads.
20	Park	51.40060	-2.34689	Alice Park, amenity green space with short grass and isolated ornamental trees, surrounded by housing and road networks.
21	Woodland Edge	51.35908	-2.30945	Conkwell deciduous woodland adjacent to track, next to the Kennett & Avon canal and adjacent to wider rural green space with a network of B roads (cut off from wider rural habitat with an A road).
22	Grassland	51.41674	-2.32719	Chilcombe Bottom short grassland, bordered by woodland, close to site 23.
23	Grassland	51.40887	-2.33431	Little Solsbury Hill short grassland with areas of hawthorn scrub, adjacent to a woodland to the north of the city and surrounded by rural habitat.
24	Grassland	51.36969	-2.33917	Short grassland with areas of hawthorn scrub, part of the Bath Skyline and located at The priory. Part of a network of greenspace to the east of the city, separated from wider rural space with A and B roads.
25	Grassland	51.37896	-2.34346	Short grassland adjacent to woodland, part of the Bath Skyline and located at Dolmeads. Part of a network of greenspace to the east of the city, separated from wider rural space with A and B roads.
26	Grassland	51.38499	-2.34033	Short grassland with scattered tree, part of the Bath Skyline and located at Bathwick Part of a network of greenspace to the east of the city,

				separated from wider rural space with A and B roads.
27	Woodland	51.38750	-2.31999	Bathampton, deciduous woodland, part of the Bath Skyline and connected to a large woodland, but separated from wider rural space by A roads.

Supplementary File 2 - Ticks collected and tested for *Borrelia burgdorferi* sensu lato across the city of

Bath during 2015-2016.

Habitat	Site ID	No. 5m² transects	Ticks collected (tested)				Nymphs per 100 m² mean (95% CI)	*Borrelia* infection nymphs (95% CI)	DIN per 100 m² mean (95% CI)
			♂	♀	N	Total			
Grassland	1	106	12	15	42 (41)	69 (68)	7.92 (4.25-11.60)	0/41	0
	4	105	16	12 (10)	33 (32)	61 (58)	6.29 (3.53-9.04)	3.1% 1/32 (0-9.0)	0.19 (0-0.57)
	9	105	3	1	3	7	0.57 (0-1.22)	0/3	0
	14	106	0	0	0	0	0	0	0
	19	105	0	0	3	3	0.57 (0-1.22)	0/3	0
	22	107	1	1	0	2	0	0	0
	23	105	0	0	1	1	0.19 (0-0.57)	0/1	0
	24	107	4 (3)	1	22 (13)	27 (17)	4.11 (1.89-6.33)	0/13	0
	25	105	0	0	4	4	0.76 (0-1.68)	0/4	0
	26	106	4	3	89	96	16.79 (10.82-22.76)	4.5% 4/89 (0.2-8.8)	0.76 (0.03-1.48)
Park	7	105	1	2	6 (5)	9 (8)	1.14 (0.09-2.19)	0/5	0
	11	111	0	0	0	0	0	0	0
	12	105	1	0	2	3	0.38 (0-0.91)	0/2	0
	15	107	0	0	0	0	0	0	0
	16	106	0	0	0	0	0	0	0
	20	106	0	0	0	0	0	0	0
Woodland	2	105	1	1	15 (13)	17 (15)	2.86 (1.30-4.42)	15.4% 2/13 (0-35.0)	0.44 (0-1.01)
	3	105	0	4	7 (6)	11 (10)	1.33 (0.36-2.30)	0/6	0
	6	90	3	2	46 (42)	51 (47)	10.22 (6.29-14.16)	4.8% 2/42 (0-11.3)	0.49 (0-1.15)
	8	105	1	3	10	14	1.90 (0.76-3.05)	0/10	0

10	108	0	0	1	1	0.19 (0-0.55)	0/1	0
13	90	7	1	94 (91)	102 (99)	20.89 (12.78-29.00)	0/91	0
27	109	4	7	90 (84)	101 (95)	16.51 (11.38-21.65)	8.3% 7/84 (2.4-14.2)	1.37 (0.40-2.34)
Woodland Edge 5	63	11	5 (4)	148 (145)	164 (160)	46.98 (32.01-61.96)	3.4% 5/145 (0.1-6.3)	1.6 (0.23-2.96)
17	60	13	9	272	294	90.67 (66.69-114.65)	2.2% 6/272 (0.1-3.9)	1.99 (0.45-3.53)
18	61	1	1	53	55	17.38 (10.60-24.15)	1.9% 1/53 (0-5.6)	0.31 (0-0.97)
21	105	1	0	2 (1)	3 (2)	0.38 (0-0.91)	0/1	0
All sites NA	2698	84	68	943 (911)	1095 (1059)	6.99 (6.00-8.00)	3.1% 8/911 (2.0-4.2)	0.22 (0.14-0.29)

Supplementary File 3 - *Borrelia burgdorferi* sensu lato genospecies detected in each habitat per year

across the city of Bath 2015-2016

Habitat	Year	*Borrelia afzelii*			*Borrelia garinii*			*Borrelia valaisiana*			Untyped		
		♂	♀	N	♂	♀	N	♂	♀	N	♂	♀	N
Grassland	2015	0	1	0	0	0	0	0	0	2	0	3	1
	2016	1	1	0	0	0	0	0	0	1	0	1	1
Park	2015	0	0	0	0	0	0	0	0	0	0	0	0
	2016	0	0	0	0	0	0	0	0	0	0	1	0
Woodland	2015	0	0	0	0	1	5	0	0	1	0	1	2
	2016	1	0	0	1	0	1	0	1	2	0	1	0
Woodland Edge	2015	0	0	1	0	0	3	0	0	0	0	0	1
	2016	0	0	2	0	1	0	1	0	4	4	0	1
Total	2015-2016	2	2	3	1	2	9	1	1	10	4	7	6

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