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Seasonal and diel environment effects on host-seeking behaviour in the European tick vector, *Ixodes ricinus*

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Patterns of arthropod vector abundance and activity are key determinants of biting risk, and thus the risk of vector-borne pathogen transmission. Ticks are important vectors of animal and human pathogens worldwide. Their host-seeking activity (questing) directly influences the risk of exposure to ticks and their pathogens, but the drivers of this behaviour are poorly understood. Here, the concurrent seasonal and diel variation in *Ixodes ricinus* questing behaviour was investigated, for the first time, by blanket dragging every four hours over a 24-hour sampling cycle each month, from January to December 2022.

A predominantly nocturnal questing activity pattern was observed, moderated by seasonal moisture availability, being most evident in months with high saturation deficit. Generalised Linear Mixed Modelling predicted that the density of questing nymphs was positively associated with darkness, and negatively associated with saturation deficit, which interacted with soil temperature.

The observed seasonal questing patterns are largely consistent with published data; however this is the first study to highlight the importance of nocturnal feeding at periods of high saturation deficit.

These commonly overlooked daily rhythms and environmental moderators of tick behaviour have important implications for assessing the relative risk of exposure to ticks and their pathogens for different hosts, and for estimating abundance in scientific research and surveillance.

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1. Background

Seasonal patterns of arthropod vector abundance and activity, and diel activity patterns (variation in activity over a 24-hour cycle) are key determinants of biting risk, and thus the risk of transmission of vector-borne pathogens (e.g. [1]). Ticks are some of the most important vectors worldwide, transmitting a range of protozoal, bacterial and viral pathogens as they feed on the blood of their hosts. *Ixodes ricinus*, also known as the castor bean or sheep tick, is the most widespread and abundant tick species in the Western Palearctic [2]. The temporal risk of exposure to tick-borne pathogens (TBP), mirrors their biting activity [3–6]. To acquire a host, *I. ricinus* employs a sit-and wait strategy known as 'questing'; the ticks climb vegetation and use sensory organs on the extended foretibiae to detect chemical cues from passing hosts [7,8]. Questing activity is strongly determined by a range of climatic and microclimatic factors, particularly temperature and humidity (e.g. [9]) and, as a result, is affected by vegetation cover [10,11] and interacting effects of landscape and host abundance (as reviewed by [12]). Hence, seasonal patterns of activity can vary widely from year-to-year. Longitudinal sampling, spanning months to years, has allowed the seasonal patterns of activity in different locations and between years to be well described [3,4,8,9,13].

In comparison to seasonal patterns, the diel activity of *I. ricinus* has not been well studied and no consensus has been achieved [14–16]. It is to be expected that climate and weather patterns, mediated by habitat, will also influence daily patterns of activity, but this has proved difficult to quantify. On hill pasture in the United Kingdom, *I. ricinus* adults and nymphs were observed to be most active in the day and descended the vegetation at night [8,17]. This diel behaviour was attributed to temperatures dropping below 10 °C at night, hence ticks descending into leaflitter. In meadow habitat in Sweden, greater nocturnal activity was observed compared with daytime activity, but no discernible pattern was observed in nearby woodlands [14]. This habitat-specific behaviour was attributed to high mid-day saturation deficits in the more exposed meadow. Activity was found to be highest in the morning and evening and was highly correlated with saturation deficit [18]. Similarly fewer ticks were collected at mid-day in the UK in May, and this was associated with higher temperatures [19]. The daily pattern of activity also has also been found to vary between life-cycle stages [14,20] and over a season [16,21].

In other arthropod vector species, such as mosquitoes, where daily patterns of activity have been particularly well-studied, including factors driving and modulating biting behaviour (e.g. [22]), there is evidence that daily activity patterns influence pathogen transmission and the efficacy of control measures [23]. Variable patterns of tick questing activity therefore have potentially important epidemiological consequences. A better understanding of ecological drivers of the seasonal and daily activity patterns of *I. ricinus* is necessary to manage risk. A clearer understanding of these behaviours and how they relate to climate may allow for mitigation strategies to be put in place, such as seasonal veterinary prophylactic treatments or behaviour modification to minimise exposure during periods of peak risk. The aim of this study, therefore, was to observe the host-seeking activity of ticks throughout the day and year, and relate this to different environmental parameters.

2. Methods

(a) Study site

The study site was a 4-hectare section of unfenced deer sanctuary at Lyme Park (53.34, -2.04; Supplementary Figure 1), a site located 15 km south-east of Manchester, England. The site is on a north-west facing slope, with an elevation of 250 – 270 m. The underlying geology consists of sandstone with coal measures and soils with low permeability. There is a westerly prevailing wind and an average annual rainfall of 600 mm to 700 mm. The deer sanctuary is home to approximately 150 red deer (*Cervus elaphus*), which are free to roam around the 550-hectare park

and interact with the park's resident populations of highland cattle (*Bos taurus*) and sheep (*Ovis aries*). Within the deer sanctuary there are also rabbits (*Oryctolagus cuniculus*), badgers (*Meles meles*) and a variety of small rodents.

(b) Tick dragging - daily and seasonal questing behaviour

Initial scoping surveys of the site, conducted from June to November 2021, indicated a high tick abundance. A subset of 300 ticks from these surveys were identified morphologically [24]; all were identified as *I. ricinus*. In the main study in 2022, to quantify daily and seasonal questing behaviour of ticks, blanket dragging was conducted over a 24-hour cycle, once a month. During each 24-hour cycle, the site was sampled every four hours at 08:00, 12:00, 16:00, 20:00, 12:00 and 04:00 h (GMT). During darkness, head torches were used. At each of these 6 sampling periods within each 24-hour cycle, ten 10 m long blanket drags (transects) were conducted using a 1 x 1 m square of lightweight white cotton cloth pulled slowly over the ground (Newman et al. 2019; Salomon et al. 2020). Before each 24-hour sampling cycle, the location and orientation of each transect was randomly selected within a 0.04 km^2 subsection of the deer sanctuary using R statistical software (v.4.2.1; [25]). Each transect began at the randomised location and in the randomised orientation, except where there were obstacles, such as resting deer, whereby the closest safe path was chosen. At 5 m and 10 m of each transect, both sides of the cloth were inspected; adults and nymphs were removed using forceps and placed into an Eppendorf tube. A visual estimate of the number of larval ticks was made before they were removed and placed back into the environment. All Eppendorf tubes containing ticks were kept chilled using frozen blocks until being returned to the laboratory within 24 hours of collection. On return to the laboratory, the number of nymphs and adults collected per transect were verified. On some occasions, due to high winds and/or dark conditions, ticks escaped before being collected and these ticks were accounted for in the final numbers. A fresh blanket was used when the cloth was damp or dirty, or when a cluster of larvae was detected, to ensure no larvae were left on the cloth and that accurate recordings were taken at the next transect.

(c) Environmental variables

To relate tick questing activity to environmental covariables, at the start of each transect, the atmospheric temperature and relative humidity (RH) 10 cm above ground level were recorded (Tinytag View 2 - TV-4500: Temperature ± 0.02 °C and ± RH 0.1%; Gemini Data Loggers, UK). The soil temperature was also recorded, by placing the thermistor probe 3 cm horizontally beneath the surface (Tinytag View 2 - TV-4020 with a Thermistor Probe - PB-5001-1M5; Temperature ± 0.02 °C; Gemini Data Loggers, UK). However, no soil temperature data could be collected for August and October because the thermistor probe broke.

Illuminance was recorded 50 cm above ground level (BT-881D Digital Illuminance Light Meter: Lux ± 4% <1,000 lux, ± 5% <1,000 lux, BTMETER, UK). All artificial light sources were covered or switched off when illuminance was recorded. For sampling sessions conducted in complete darkness, with no interruption of sunrise or sunset, the lux was recorded as <1 lux. Soil moisture was taken at three points along the transect (0, 5, 10 m), using a soil moisture probe (SM150T Soil Moisture Sensor and HH150 Moisture Meter: volumetric water content ± 3%; Delta-T Devices, UK). When readings were "Out of Range" due to high water content, the upper limit of 1,600 mV was assigned. However, in January and November, soil moisture was measured by dry weight analysis of three samples of soil from each transect taken at the same depth of the moisture meter. Gravimetric dry weight was then calibrated with the Moisture Meter in December 2022. The vegetation height at three points of the transect (0, 5, 10 m) was also recorded using a 1 m metal ruler. An additional categorical system was employed to document the moisture on the vegetation; dry, light dew, heavy dew, light frost, heavy frost, wet (after rain). As this was a subjective variable, for analysis this was converted to a binary variable of dry or wet vegetation, where wet represented any form of water droplet on the vegetation. No frosts were observed.

In addition, temperature, precipitation, wind speed, wind gusts and cloud cover were collected for each of the sampling intervals (08:00, 12:00, 16:00, 20:00, 12:00, and 04:00) at the coordinates (53.34, -2.04). from the Visual Crossing Corporation [26].

Saturation deficit (SD) was calculated using the temperature (T) and relative humidity (RH) at 10 cm (Randolph and Storey 1999). To allow a comparison of ticks questing in the day or at night, the final illuminance recordings were converted to binary measurements, where records equal to or above one lux were defined as light, and those below this threshold were defined as dark. The threshold of one lux was chosen to account for urban light pollution from nearby cities (Kyba et al. 2015), the presence of a full moon (Kyba et al. 2017) and the uncertainty in measurements. Continuous measures based on the raw illuminance data were not used in analysis as these can vary minute-by-minute, e.g., due to cloud cover.

(d) Statistical analyses

The seasonal patterns of activity of adults, nymphs, larvae were plotted and then assessed using cardidates::peakwindow R function (v.0.4.9; [27]) to identify any peaks in the time series data.

The influence of environmental factors on the density of questing nymphs was tested using generalised linear mixed effect models (GLMMs). The response variable was the number of nymph ticks collected per 10 m drag, as these are the most epidemiologically important instar stage and were observed in sufficient numbers for analysis. As is typical with ecological data, the response variable comprised count data that were positively skewed and overdispersed. Therefore, models were constructed using a negative binomial model family in R (lme4::glmer.nb v.1.1-28; [28]). To ensure that the pattern of the response variable was appropriate for the model family, each model was assessed for residual overdispersion (*overdisp_fun*; [29]). Month, and transect nested within each month, were included as random effects in the models to account for repeated sampling within each month and differences associated with each transect that were not accounted for by other model variables. Furthermore, the inclusion of these random effects helped reduce the influence of sampling month on determining the drivers of the daily changes in the density of questing nymphs.

First, univariable analyses using the GLMMs were conducted to understand the relationship between each candidate explanatory variable and the response variable. For categorical variables, post-hoc Tukey tests were used to determine the pairwise differences between the factor levels (emmeans v.1.8.1-1; [30]). The shape of the relationship was tested by comparing models built with a linear and squared variable, using the stats::anova function (v.4.1.2, test = "chisq"; [25]). To avoid collinearity effects, correlations between the continuous explanatory variables were tested using a Spearman's correlation matrix (Hmisc::rcorr v.4.7-2; [31]). If two variables had over 70% correlation, only one was taken forward. Final models were also assessed for collinearity, and a variance inflation factor (VIF) below 10 was considered acceptable (car::vif v.3.1-0; [32]).

An initial multivariable model containing non-correlated explanatory variables and interactions was constructed. All continuous variables were scaled to aid model fitting. Variables and interactions were then sequentially removed, in a manual backwards stepwise procedure, based on their contribution to the model. Comparative model performance between steps was based on the Akaike information criterion. The final model was compared to a null model containing only the random effect factor in a likelihood ratio test (stats::anova v.4.1.2 (test = "chisq"); [25]). The value of the random effects was assessed by comparing the final model with and without random effects, using likelihood-ratio tests. Vegetation moisture and soil moisture were excluded from the global GLMM models due to missing data for January and November.

3. Results

(a) Seasonal patterns of activity differed between instars

Ticks were collected every month, except in June 2022 when no sampling took place due to COVID-19 restrictions. Over 8,000 ticks were counted (Figure 1; Supplementary table 1; Supplementary figure 2); larvae were most abundant (5,034; 62.84%), followed by nymphs (2,715; 33.89%) and then adults (262; 3.27%). The three instars had different seasonal patterns of activity. Peak window analysis identified one peak in the density of questing larvae in July with a median of 10.5 larvae / 10 m^2, implying a unimodal pattern of activity (Figure 2). Two peaks were identified for nymphs; a small spring peak in April, with a median of 2 nymphs / 10 m^2, then a larger peak in November, with a median of 10 nymphs / 10 m^2 (Figure 2). A bimodal pattern of activity was also observed for adults, with a small spring peak in March and a larger peak in September (Figure 2). At least one instar stage was collected each month, with only nymphs being collected in January and only larvae in July (Figure 2).

(b) Diel activity – questing tick density was highest at night

Ticks of all three instar stages were collected at all sampling times (08:00, 12:00, 16:00, 20:00, 00:00 and 04:00). However, the daily activity of adult, nymph and larval ticks varied between months (Figure 1; Supplementary figures 3 - 5). Fewer adult ticks (n = 107) were collected at 08:00, 12:00 and 16:00 h sampling times, compared to 20:00, 00:00 and 04:00 h sampling times, from February to October (Supplementary figure 3).

Similar results were found for nymphs (Supplementary figure 4). Post-hoc Tukey tests showed the density of questing nymphs at the collection times of 08:00 and 12:00 h were significantly lower than at 20:00, 00:00 and 04:00 h (Supplementary figure 4).

The number of larvae collected varied across the year. From April to October, more larvae were collected at 20:00, 00:00 and 04:00 h sampling times, but this pattern of activity was not observed in the remaining months (Supplementary figure 5), with the highest collection (n = 258) occurring at 20:00 in July.

(i) Environmental factors – questing activity was observed under a wide range of conditions

All instar stages quested at a range of environmental conditions; between 1.6 and 40.3 °C, and between 24% RH and 100% RH (Figure 2). Nymphs were the only instar stage found in January, when the mean temperature across the 24 hour sampling period was 3.51°C [26]. Adults were first collected in February, and larvae in March when mean temperatures were 3.91 °C and 7.28 °C respectively [26]. The nymphs were found at the lowest transect temperatures (1.6 °C in February at 20:00), followed by adults (2.1 °C in February at 20:00), then larvae (3.3 °C in April at 04:00). Larvae, however, were the only instar stage found questing in July, when transect air temperatures were as high as 40.3 °C, and at relative humidities as low as 24% (Figure 2). No adults or nymphs were found questing when the mean daily temperature exceeded 25 °C. The blanket drags with the highest adult and nymph yields (denoted by darker points in Figure 2) were between 7 – 20 °C with humidities greater than 70% RH.

Visualisation of the soil data (Supplementary figure 6) suggested that there was significant variance and no meaningful relationship between measures taken using the soil moisture probe and estimates made from soil samples. Thus, the soil moisture estimates for January and November were excluded from further analyses.

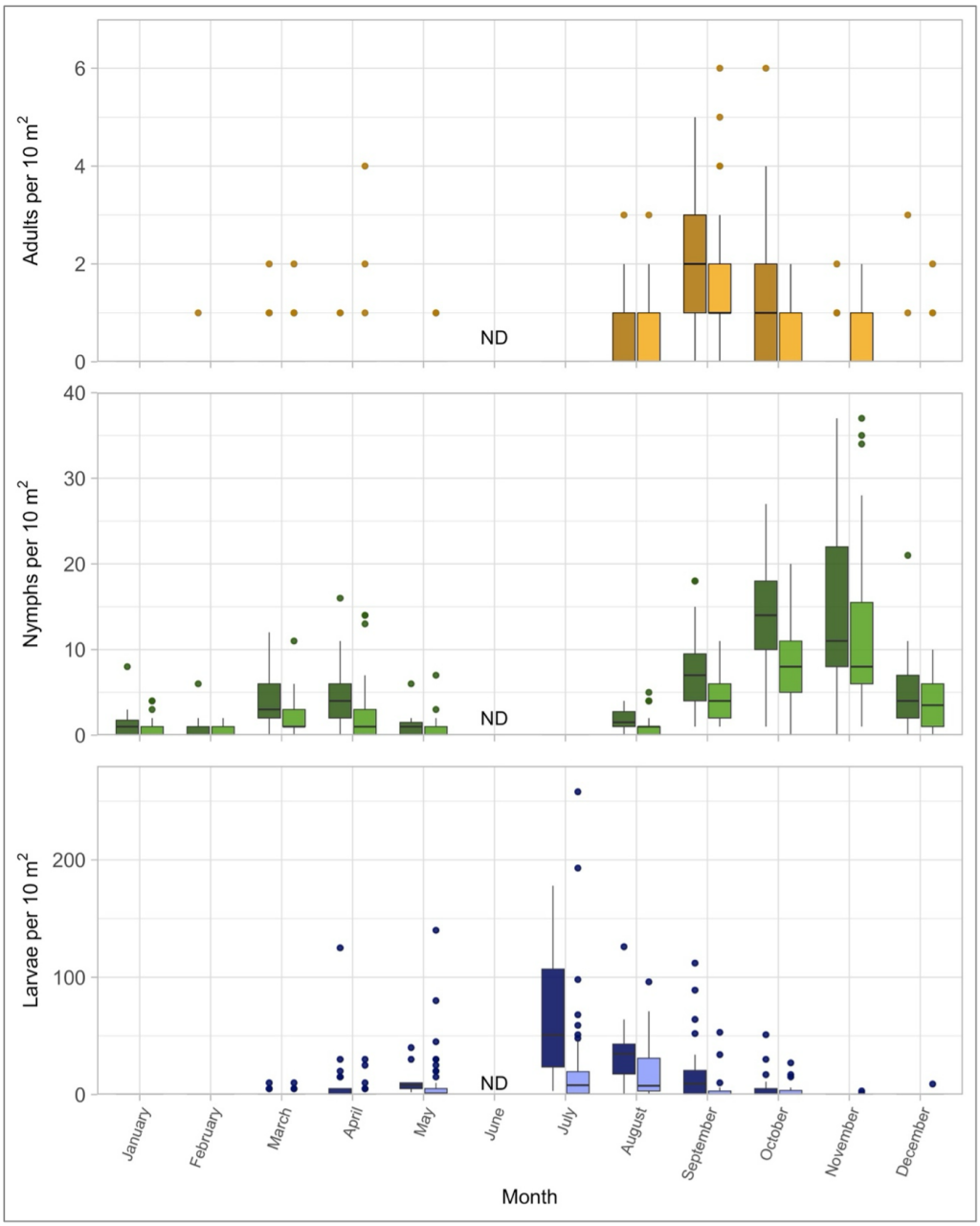

Figure 1. The density of questing of adults, nymphs and larvae, counted in light and dark conditions for each sampling month. The darker colour (left bar) represents ticks counted where light levels were under one lux, and the lighter colour (right hand bar) represents light levels over one lux. Boxes represent the interquartile range of the data, with a line at the median value. Whiskers extend to the minimum and maximum values, with outliers (points more than 1.5 times the interquartile range above the third quartile (upper limit of the box) or below the first quartile (lower limit of the box)) represented by filled circles.

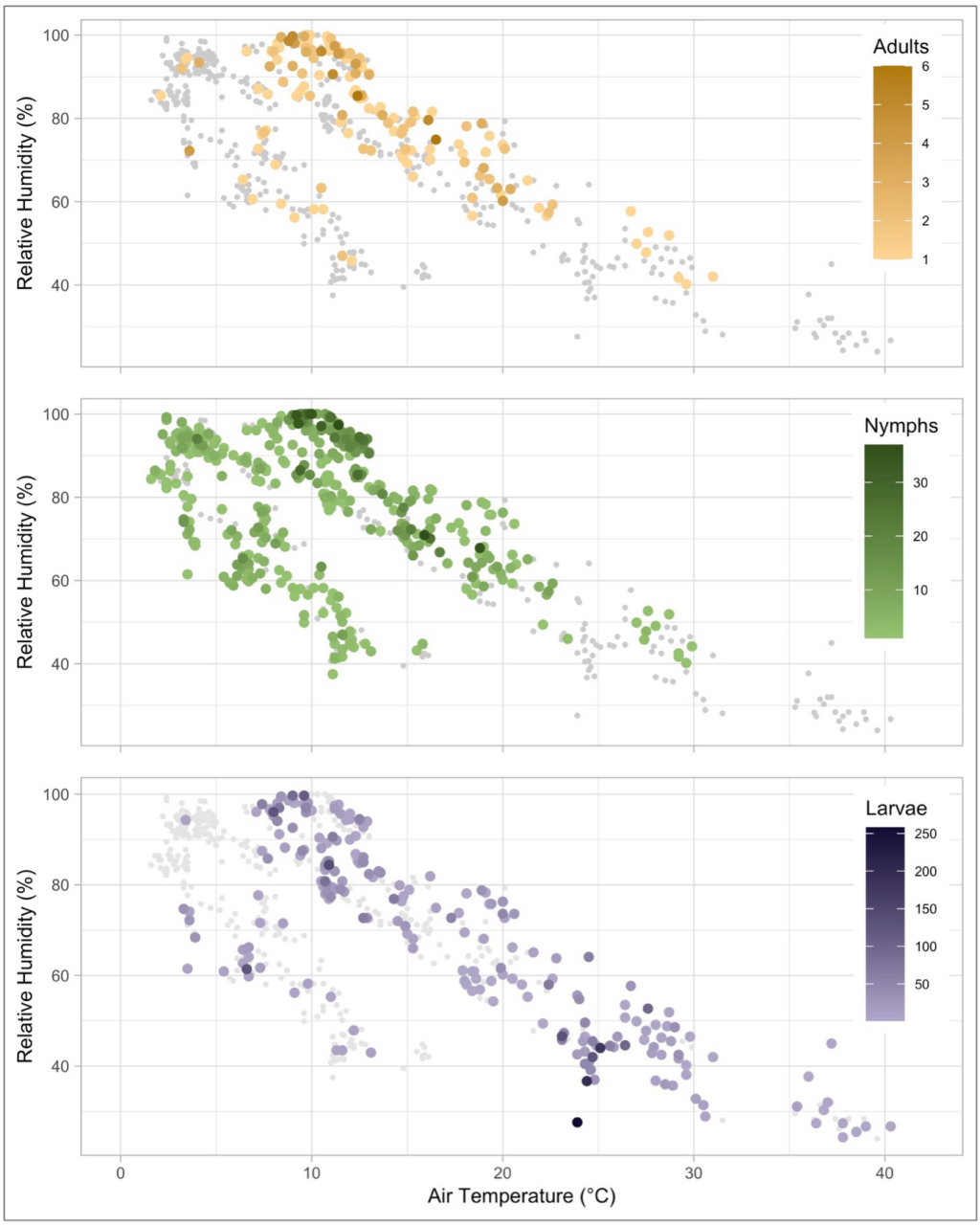

Figure 2. The density of questing adults, nymphs and larval ticks collected per 10 m^2 of tick dragging under different environmental conditions. The temperature and relative humidity were recorded at 10 cm above ground level. Coloured circles represent the different life stages of ticks collected, with darker colours reflecting greater density of ticks, and smaller grey circles representing the sampled conditions where that life stage was absent.

(ii) Environmental factors – questing nymph density is associated with saturation deficit and light levels

Univariable analysis of the influence of individual environmental factors on the density of questing nymphs was performed using GLMMs (Supplementary table 2). The model performed significantly better when temperature was included as a squared variable (chi^2= 5.55; df = 1; P = 0.018) and predicted an initial negative relationship between the density of questing nymphs and air temperature. Conversely, there was a positive relationship with humidity, whereby the density of questing nymphs was greater at higher humidities. These results relate to the predicted negative relationship between the density of questing nymphs and the saturation deficit. Nymph activity also varied with vegetation moisture; the density of questing nymphs was greater on wet vegetation, compared to dry. A median density of 4 nymphs / 10 m^2 was found on wet vegetation conditions compared to 2 nymphs / 10 m^2 in dry conditions. Soil moisture (mV) was also positively correlated with density of questing nymphs, with more nymphs predicted to be active at higher (wetter) soil moistures. Conversely, wind speed, vegetation height and soil temperature were not significantly associated with the density of questing nymphs (Supplementary table 2).

The initial multivariable GLMM, prior to backwards stepwise selection, included variables with correlation coefficients below 0.7 (Supplementary figure 7); saturation deficit, light level, soil temperature, vegetation height, wind speed, and meteorological interactions (Supplementary table 3). The final multivariable GLMM, after backwards stepwise selection, included light level, saturation deficit and an interaction between saturation deficit and soil temperature as fixed effects, and month with nested transects as random effects (Table 1). The final model predicted that density of questing nymphs was greater under dark conditions compared to light, and at lower saturation deficits (Figure 1; Supplementary figure 8). The same observation was made for larvae, and this night-time activity was most dominant in July when a median of 50.5 larvae/ 10 m^2 was found at night versus 8 larvae / 10 m^2 in the day. No strong daily pattern of activity with relation to light and dark could be shown with adults due to their low numbers.

Table 1. Generalised linear mixed model multivariable analysis of the relationship between different environmental factors and the density of questing nymphs in Lyme Park, UK, in 2022. Month and transect number within each month were included as random effects. Model coefficient estimate, standard error (SE), Z score and P value are presented. Significant results are highlighted in bold.

Predictor	Estimate	SE	Z	P
Intercept	-0.063	0.528	-0.119	0.905
Saturation deficit (mmHg)	-0.907	0.204	-4.435	**<0.001**
Light level (Dark)	0.207	0.098	2.110	**0.035**
Saturation deficit (mmHg):Soil temperature (^oC)	0.391	0.133	2.935	**0.003**

4. Discussion

This study has quantified the daily activity of *I. ricinus* over complete 24-hour periods and throughout the year – whereas previous studies have omitted night or winter observations [14,16]. A daily rhythm of nocturnal activity was observed consistently, with some variation throughout the year. This is consistent with observations of ticks in open habitats in other European regions [14,20] and a reduction in questing activity at mid-day has been observed elsewhere under both field [19,33] and experimental conditions [18]. Such daily rhythms have also been documented in other parasites and vectors, and can arise due to endogenous or exogenous factors (reviewed by [34]). The study presented here sought to identify the environmental factors driving seasonal and diel patterns of activity in ticks.

In laboratory experiments, negative phototaxis has been observed [7,35], as well as darkness-induced mobility under a range of abiotic conditions [7,36]. Furthermore, in the field in Richmond Park, UK, tick questing was negatively associated with light intensities [33]. There are some apparent inconsistencies between studies observing the daily behaviour of ticks, but these may be explained by other environmental covariates influencing microclimate. In Hungary, more ticks were observed in the three hours after sunrise compared to before sunrise, which led the authors to speculate whether light could initiate questing as there was no correlation with other environmental variables such as temperature [16]. However, the latter study's plots were shaded by trees. A comparison of daily questing activity of *I. ricinus* at two sites found that the nocturnal questing behaviour in the meadow habitat was not replicated in the forested habitat, and that the availability of all stages was negatively correlated with temperature [14]. Therefore, in the meadow habitat, higher midday temperatures and saturation deficits may have initiated desiccation avoidance behaviours, while the more climatically stable forest environment permitted more consistent questing behaviours over the 24-hour periods [14]. In open woodlands in the Netherlands, there was no significant difference in the number of larvae or adults questing in the day vs night, but significantly more nymphs were collected at night [20], and more dominant nocturnal activity was observed in plots with less vegetation coverage, implying a more exposed microclimate [20]. Furthermore, experimental observations support a nocturnal pattern of activity, driven by saturation deficits [18,19].

These interpretations of previous observations of ticks throughout Europe are consistent with the current analyses. Although tick activity was significantly associated with light levels in our univariable analyses, the influence of light levels was moderated by other environmental conditions in the final multivariable model, where the daily activity of ticks was also negatively associated with saturation deficit, which interacted with soil temperature. Ixodid tick mortality is highly dependent on water loss, which is regulated by the atmospheric saturation deficit [37]. At night, the cooler temperatures and higher relative humidities, hence lower saturation deficits, may provide more less desiccating conditions for questing [16,18,20]. This moderating effect of saturation deficit is evident in our observed seasonal patterns of tick activity. There was no obvious seasonal shift from an exclusively diurnal to nocturnal pattern of activity here, but in the months where there was little variation in the saturation deficit throughout the day, such as January, February and December, the nocturnal pattern of activity was less evident. In contrast, in months where midday saturation deficits were high, such as July, there was a dominant pattern of nocturnal activity.

Olfactory and kinetic cues caused by a high density of nocturnal rodent hosts have been suggested as drivers for tick behaviour [7,8], and there are diverse examples of other parasites whose activity patterns coincide with host availability, thus maximising transmission opportunities (reviewed by [34]). At Lyme Park, small rodents and deer were present, but not recorded and hence a correlation between host density and activity and tick questing cannot be established. However, the nocturnal patterns of activity observed in ticks, corresponds to nocturnal patterns in small mammals in the UK [38] and the nocturnality observed in deer in areas of high human activity [39,40]. Consequently, both climatic conditions and host activity may influence the daily behavioural patterns of ticks.

The results of the present study suggest that during warm weather, there is greater risk of tick exposure at night and around dawn, when saturation deficits are lower, especially in the presence of dew. This could have important epidemiological considerations. For example, in the summer there are increased evening recreational activities such as camping, which would increase human exposure. Similarly, it is advised that in warm weather, pets should be exercised during the cooler parts of the day, including early morning and late in the evening, which could coincide with higher densities of questing ticks [19].

The seasonal pattern of tick activity observed in Lyme Park resembles patterns previously recorded for *I. ricinus* in the UK [3,4,41,42]. The relatively high numbers of questing nymphs and adults observed in spring is likely to be due to the emergence of ticks from behavioural quiescence as temperatures rise [3,43,44], while the autumnal peak in activity is likely to represent attempted feeding of the newly moulted cohort of ticks. The often low autumn peak seen in the UK has been attributed to cooler summer temperatures delaying development [3]. However here, spring-fed juvenile ticks may have fed and moulted into their next instar stage by autumn increasing the observed density of questing ticks between September and November. The low numbers of questing ticks commonly reported in mid-summer is likely to be driven to the environmental conditions; if the saturation deficit rises above an estimated 4.4 mmHg, *I. ricinus* exhibits positive geotropism behaviours to avoid desiccation [3,9,35]. Other longitudinal studies have observed this bimodal pattern of tick activity in regions, altitudes and years where there is high moisture stress during the mid-summer period [9,44–46].

Although the larval pattern of activity followed the typical distribution observed throughout the UK, the timing of the peak activity was unexpected as it coincided with high saturation deficits exceeding 40 mmHg, which is well above the threshold considered suitable for larval questing [3,9,10,35]. The reason for this is unknown, but it is it possible that the behaviour was driven by starvation [47,48] leading to questing under sub-optimal conditions.

Beyond challenging our assumptions on the lower temperature thresholds for tick activity, the observations reported here have important implications for tick ecology research, since biases introduced by the timing and conditions of tick sampling may be underappreciated. The density of questing ticks was lower at daytime sampling times typically represented in tick surveys (08:00, 12:00 and 16:00) compared to atypical sampling times (20:00, 00:00 and 04:00); up to eight times more larvae were collected outside of typical sampling hours. It should therefore be noted that the increase in the recorded density of questing ticks at night is substantial, considering that one could expect more type one errors at night (false negatives) due to the small size of ticks, especially larvae (1 – 2 mm), rendering them difficult to see under artificial light [21]. Therefore, it is likely that current blanket dragging practices severely underestimate the active tick population. Furthermore, there was a higher density of questing nymphs when the vegetation retained water droplets, which were often caused by dew. The presence of dew on vegetation may increase the relative humidity and improve the microclimate to favour questing [10]. This was an unusual finding, as tick sampling is seldom performed during or after rain due to wet vegetation [16,33]. Therefore, the current standard sampling protocols may need to be adjusted to encompass sampling with wet vegetation (both dew and rain induced), to extend our understanding of tick behaviour under these conditions. Given that sampling throughout the night has practical, logistical and safety limitations simply including early morning and late evening sampling would help to increase the accuracy of abundance estimates [19].

Due to the dependency of tick development and activity on climatic factors, it is likely that there will be seasonal changes in the patterns of tick activity with climate change [49]. Any increase in the synchrony of larval and nymphal questing activity is of concern due to co-feeding between the instars contributing to direct pathogen transmission [50,51]. If nocturnal questing is driven by the avoidance of desiccating midday conditions, then nocturnal patterns of activity may become more extreme, further increasing the opportunity for co-feeding between uninfected larvae and infected nymphs. However, if such changes in environmental conditions are at odds

with circadian rhythms (which are, as yet, unexplored in ticks), then non-linear patterns of change in activity may arise, such as bimodal diel activity.

Further work is required to investigate the environmental and chronobiological drivers of host-seeking behaviour in ticks, and a recently-published guide signposts researchers new to this expanding field of research [52]. Experiments and observations should be repeated at multiple sites, or using ticks sourced from multiple sites, since there is evidence for intraspecific variation of tick questing behaviour based on climate of origin [53], and include a range of species, since the rate of water loss is species-specific, possibly due to differences in cuticle permeability and spiracle morphology influencing transpiration rates [54]. Additionally, nutrition has been shown to influence host-seeking behaviour in mosquitoes [22], thus further work should also strive to incorporate nutrition status, where possible. For example, assessing the lipid content and age of the tick when collected, may provide insight into how starvation influences behaviour, but requires a large sample size. Finally, the influence of pathogens on tick fitness has been identified in a modelling study as a key parameter driving transmission tick-borne pathogen transmission dynamics [55]. Thus, investigating the infection status of the tick would provide greater insight into how this influences their environmental tolerance [56–58].

5. Conclusion

The seasonal and nocturnal activity of ticks observed in this study, moderated by saturation deficit, has generated new insights into the drivers of tick behaviour and tick bite exposure risk, and provides a foundation for further work. Our results, interpreted in the context of previous work, evidence that both abiotic factors, such as the saturation deficit and vegetation moisture, and biotic factors, including host abundance, influence the daily and seasonal patterns of tick activity. Furthermore, our observations challenge recognised thermal limits of tick activity, indicating a potential risk of year-round exposure to ticks in temperate regions such as the UK, with elevated risk during the night and at dawn, especially when daytime conditions result in high saturation deficit. This has implications for those managing risk of exposure to tick bites.

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Competing Interests. RW has previously received funding from MSD Animal Health UK.

Authors' Contributions. Conceptualisation; MN, HRV. Methodology; MN, RW, BLM, HRV. Formal analysis; MN, HRV. Investigation; MN, HRV. Resources; MN, HRV. Writing - original draft; MN, HRV. Writing - review & editing; MN, RW, BLM, HRV. Visualisation; MN. Supervision; RW, BLM, HRV. Project administration; BLM, HRV. Funding Acquisition; RW, HRV.

Data Accessibility. All data generated by this study have been deposited in the VecDyn database [59] for open use: https://vectorbyte.crc.nd.edu/vecdyn-detail/951. The dataset is under embargo until acceptance of this manuscript for publication.

Use of Artificial Intelligence (AI) and AI-assisted technologies. No AI nor AI-assistive technologies were used in the preparation of this manuscript.

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6. Supplementary Materials

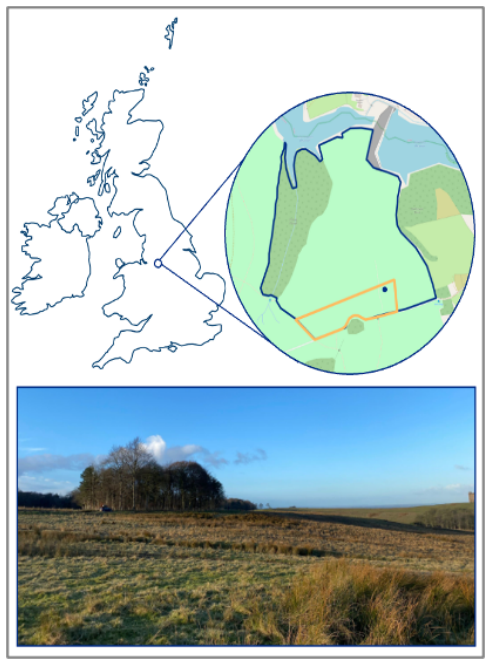

Figure 1. The location of the study site in the United Kingdom, with the deer sanctuary at Lyme Park outlined in blue, and the 0.04km2 sampling zone is in orange (OpenStreetMap, 2024). The bottom image represents the habitat of the study region, the photograph was taken from the blue dot presented in the study region map.

Table 1. The total number of larvae, nymphs and adults collected within the 24-hour sampling session each month. For larvae, these approximate total numbers due to their small size.

Month	Start Date	Larvae	Nymphs	Adults
January	13-01-2022	0	56	0
February	11-02-2022	0	28	1
March	21-03-2022	40	191	13
April	10-04-2022	310	200	9
May	21-05-2022	597	47	3
July	18-07-2022	1,823	0	0
August	08-08-2022	1,362	72	32
September	13-09-2022	649	357	116
October	03-10-2022	240	672	60
November	12-11-2022	4	837	20
December	03-12-2022	9	255	8

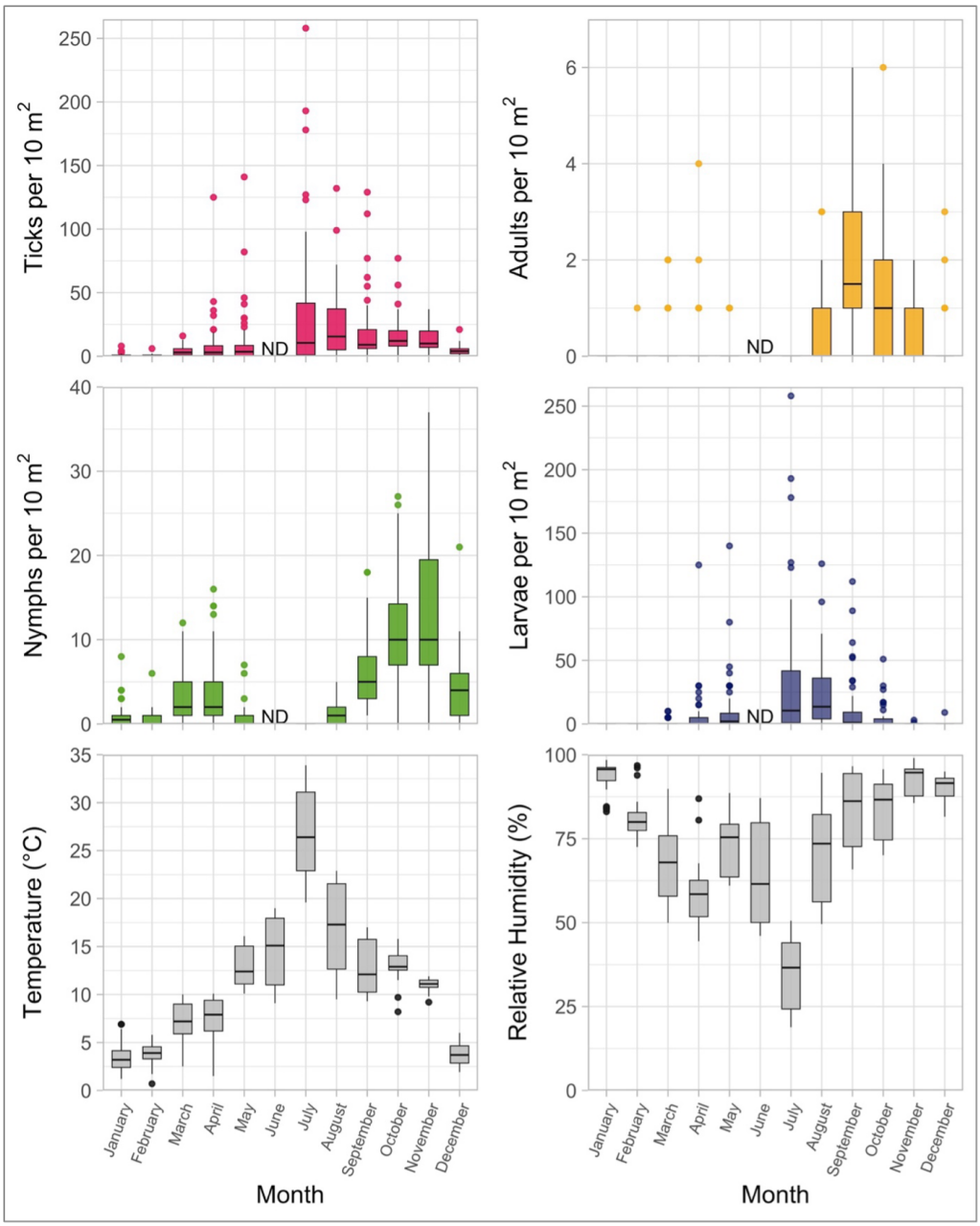

Figure 2. The density of questing ticks collected by blanket dragging at each transect in the 24-hour sampling cycles from January – December 2022 at Lyme Park, UK, and the air temperature and relative humidity during each sampling interval, taken from Visual Crossing (Visual Crossing Corporation, 2024). ND refers to no data, where no sampling took place in the month of June. Boxes represent the interquartile range of the data, with a line at the median density of ticks collected. Whiskers extend to the minimum and maximum values, with outliers represented by filled circles.

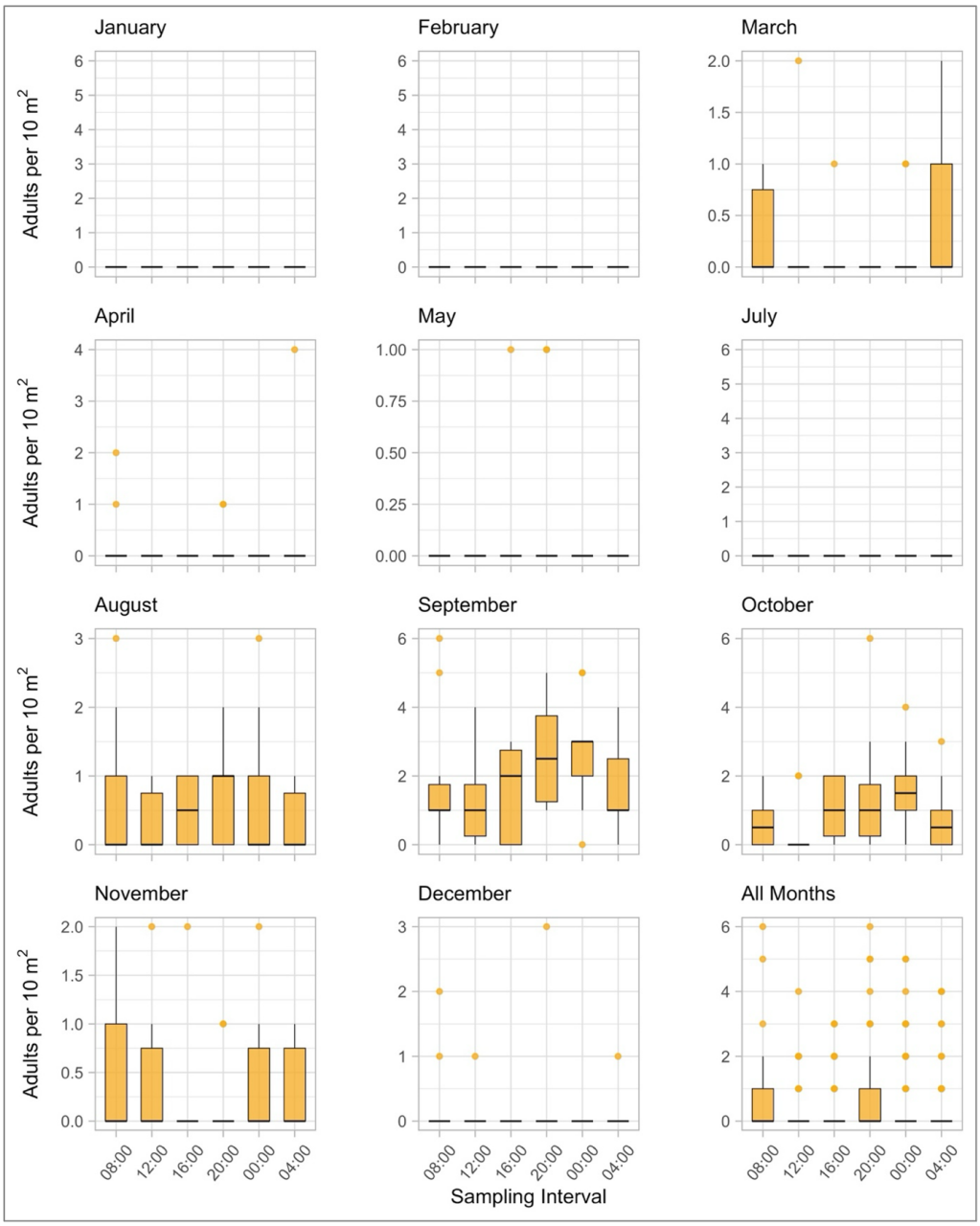

Figure 3. The density of questing adult ticks collected per 100 m^2 of blanket dragging at each of the sampling periods [08:00, 12:00, 16:00, 20:00, 12:00, 04:00 (Greenwich Mean Time)] for individual sampling months, and a total of all months. Boxes represent the interquartile range of the data, with a line at the median value. Whiskers extend to the minimum and maximum values, with outliers represented by filled circles.

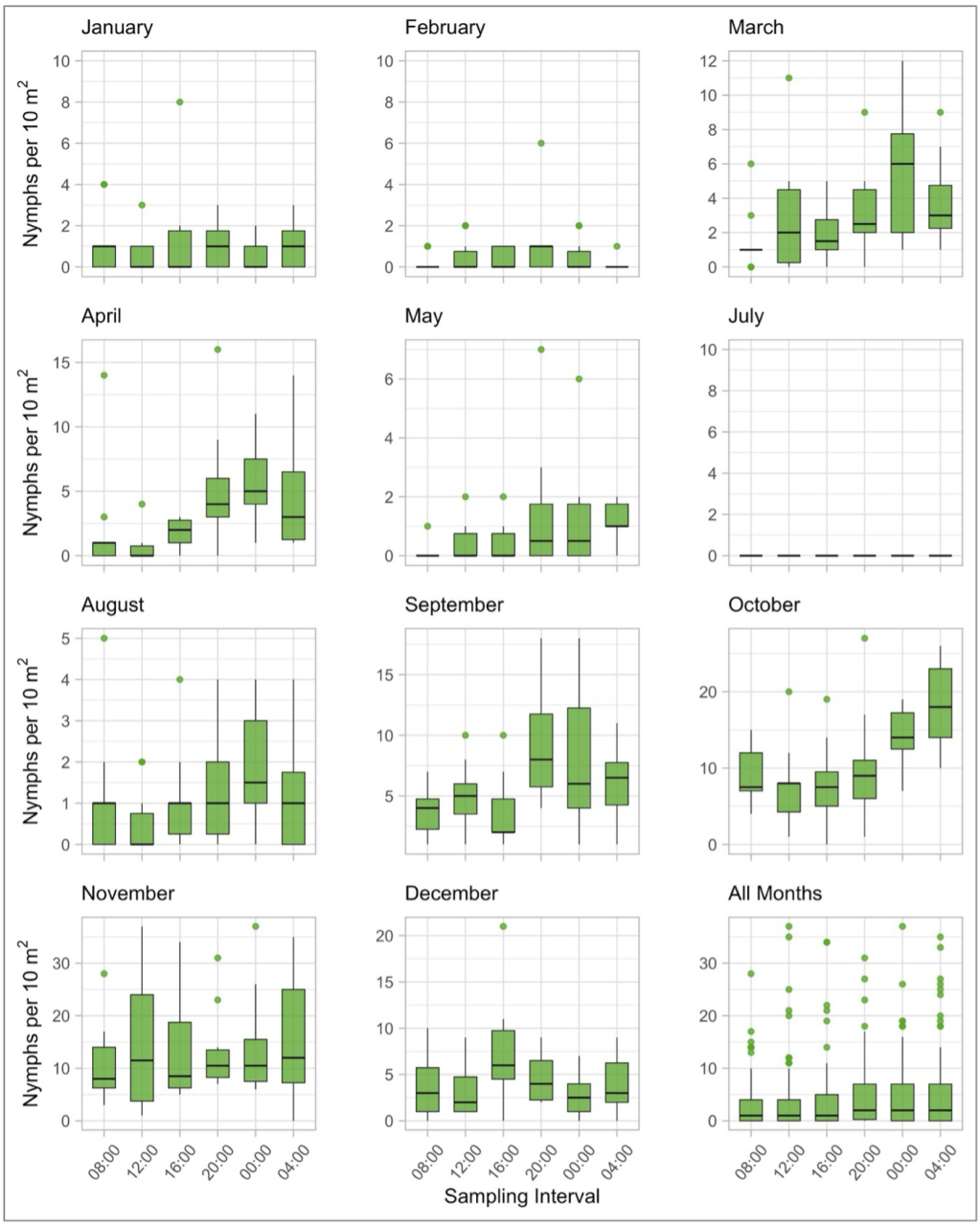

Figure 4. The density of questing nymphal ticks collected per 100 m^2 of blanket dragging at each of the sampling periods [08:00, 12:00, 16:00, 20:00, 12:00, 04:00 (Greenwich Mean Time)] for individual sampling months, and a total of all months. Boxes represent the interquartile range of the data, with a line at the median value. Whiskers extend to the minimum and maximum values, with outliers represented by filled circles.

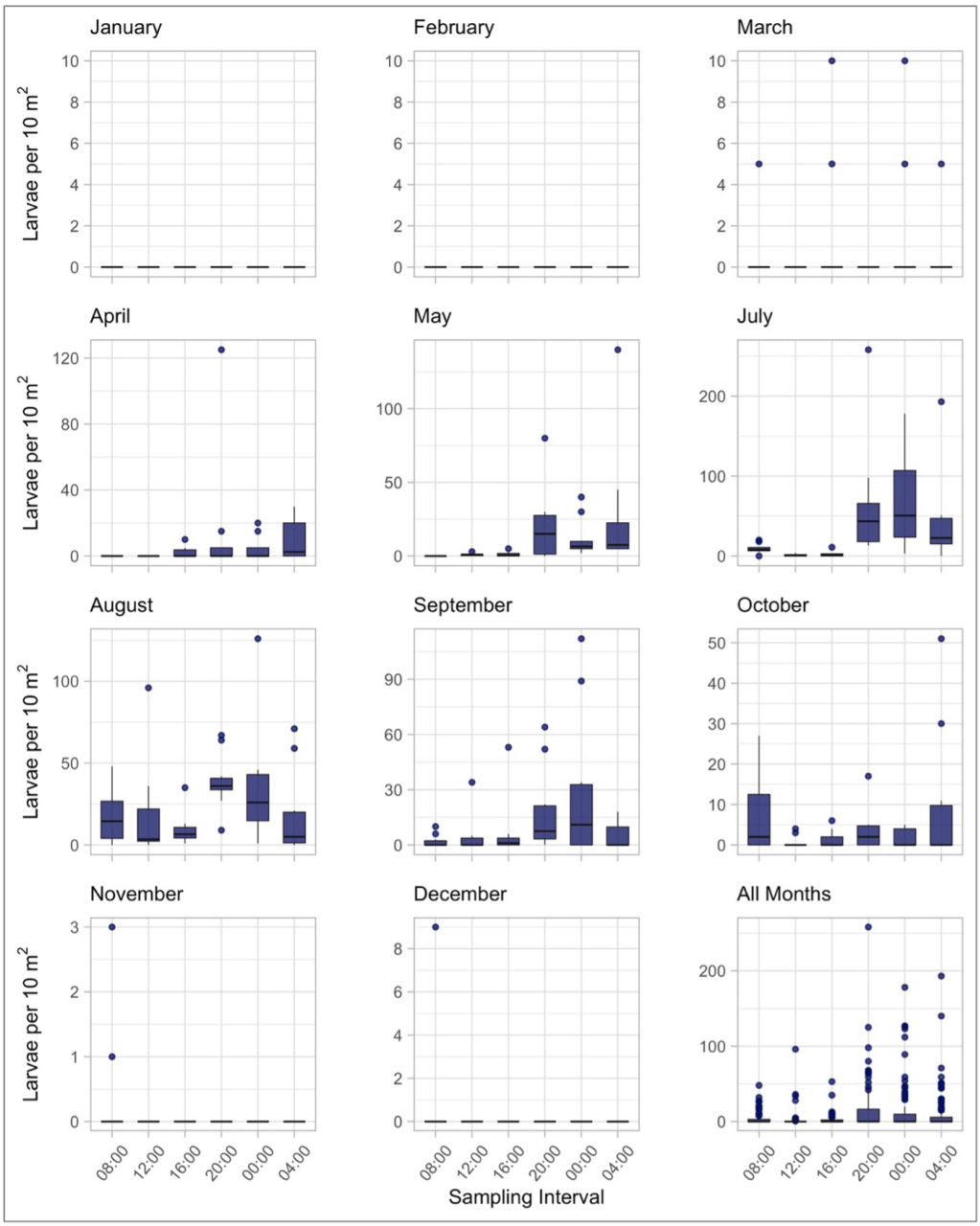

Figure 5. The density of questing larval ticks collected per 100 m^2 of blanket dragging at each of the sampling periods [08:00, 12:00, 16:00, 20:00, 12:00, 04:00 (Greenwich Mean Time)] for individual sampling months, and a total of all months. Boxes represent the interquartile range of the data, with a line at the median value. Whiskers extend to the minimum and maximum values, with outliers represented by filled circles

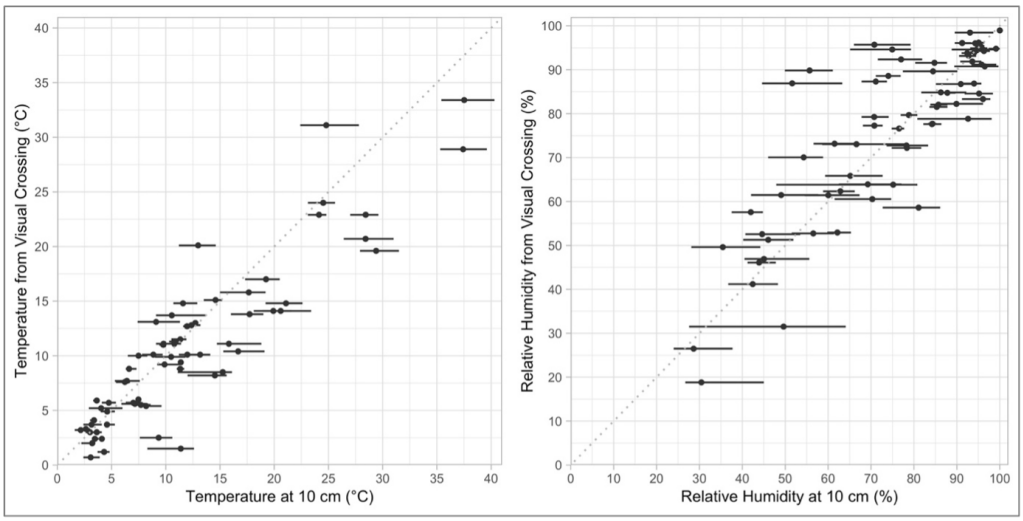

Figure 6. The temperature and relative humidity recorded 10 cm above ground level at each transect, with the Tinytag and the recording provided by Visual Crossing (Visual Crossing Corporation, 2024). Black circles and whiskers represent the mean and range of the 10 ground-level recordings plotted against the Visual Crossing recording for that sampling interval. The dashed abline represents perfect correspondence between the two data sets.

Table 2. Results of the generalised linear mixed model univariable analyses of the relationship between different environmental factors and the density of questing nymphs in Lyme Park, UK, in 2022. Month and transect number within each month were included as random effects for each univariable analysis. Model coefficient estimate, standard error (SE), Z score and P value are presented. For sampling interval, multiple comparisons between levels were performed with Tukey post-hoc analysis. Significant results are highlighted in bold.

Predictor		**Estimate**	**SE**	**Z**	**P**
Temperature (^{o}C)	Intercept	0.250	0.562	0.445	0.657
	First term	-11.35	2.627	-4.32	**<0.005**
	Second term	7.165	2.995	2.392	0.0167
Soil temperature (^{o}C)	Intercept	0.702	0.611	1.150	0.250
		-0.042	0.028	-1.510	0.130
Relative humidity (%)	Intercept	-1.525	0.552	-2.760	0.006
		0.024	0.003	6.990	**<0.005**
Saturation deficit (mmHg)	Intercept	0.701	0.450	1.560	0.120
		-0.108	0.018	-5.980	**<0.005**
Wind speed (km / h)	Intercept	0.134	0.572	0.230	0.810
		0.012	0.014	0.900	0.370
Vegetation height (cm)	Intercept	0.195	0.560	0.350	0.727
		0.003	0.003	1.357	0.175
Vegetation moisture	Wet	0.499	0.596	0.840	0.402
	Dry	-0.350	0.098	-3.560	**<0.005**
Soil moisture (mV)	Intercept	-0.110	0.619	-0.180	0.859
		0.001	0.000	2.750	**0.006**
Light level	Light	0.073	0.540	0.134	0.893
	Dark	0.486	0.078	6.193	**<0.005**
Sampling interval	08:00 vs 12:00	-0.008	0.142	-0.057	1.000
	08:00 vs 16:00	-0.192	0.139	-1.378	0.741
	08:00 vs 20:00	-0.564	0.135	-4.178	**<0.005**
	08:00 vs 00:00	-0.589	0.135	-4.371	**<0.005**
	08:00 vs 04:00	-0.550	0.135	-4.067	**<0.005**
	12:00 vs 16:00	-0.184	0.139	-1.318	0.775
	12:00 vs 20:00	-0.556	0.135	-4.109	**<0.005**
	12:00 vs 00:00	-0.581	0.135	-4.302	**<0.005**
	12:00 vs 04:00	-0.542	0.135	-4.000	**<0.005**
	16:00 vs 20:00	-0.372	0.132	-2.819	0.055
	16:00 vs 00:00	-0.397	0.132	-3.011	**0.031**
	16:00 vs 04:00	-0.358	0.132	-2.704	0.074
	20:00 vs 00:00	-0.025	0.127	-0.193	1.000
	20:00 vs 04:00	0.015	0.127	0.114	1.000
	00:00 vs 04:00	0.039	0.127	0.307	1.000

Figure 7. Correlations between candidate independent variables for the GLMM. Colours indicate the direction and strength of the correlation. Values represent Spearman's ρ. SD = saturation deficit. AT = air temperature. ST = soil temperature. VH = vegetation height. WS = wind speed?????????MADDIE PLEASE CHECK?????????. RH = relative humidity.

Table 3: Results of the backwards stepwise procedure for generating the final generalised linear mixed model multivariable analysis of the relationship between different environmental factors and the density of questing nymphs in Lyme Park, UK, in 2022. Month and transect number within each month were included as random effects for each univariable analysis. Model coefficient estimate, standard error (SE), Z score and P value are presented.

Model	Predictor	Estimate	SE	Z	P
Model One	(Intercept)	-0.064	0.533	-0.119	0.905
	Saturation Deficit	-0.965	0.215	-4.484	<0.001
	Light level (dark)	0.242	0.283	0.853	0.394
	Vegetation height	0.026	0.041	0.626	0.532
	Soil temperature	0.171	0.159	1.076	0.282
	Wind speed	0.051	0.076	0.669	0.503
	Saturation Deficit : Light level (dark)	0.089	0.554	0.161	0.872
	Saturation Deficit : Soil temperature	0.403	0.145	2.775	0.006
	Saturation Deficit : Wind speed	0.115	0.187	0.615	0.539
	Light level (dark) : Soil temperature	-0.044	0.131	-0.333	0.739
	Light level (dark) : Wind speed	-0.050	0.115	-0.431	0.666
	Soil temperature : Wind speed	-0.024	0.078	-0.310	0.756
Model Two	(Intercept)	-0.062	0.531	-0.116	0.908
	Saturation Deficit	-0.968	0.214	-4.516	<0.001
	Light level (dark)	0.199	0.102	1.948	0.051
	Vegetation height	0.026	0.041	0.636	0.525
	Soil temperature	0.175	0.156	1.121	0.262
	Wind speed	0.051	0.075	0.676	0.499
	Saturation Deficit : Soil temperature	0.403	0.145	2.779	0.005
	Saturation Deficit : Wind speed	0.112	0.186	0.603	0.546
	Light level (dark) : Soil temperature	-0.048	0.128	-0.379	0.705
	Light level (dark) : Wind speed	-0.055	0.111	-0.496	0.620
	Soil temperature : Wind speed	-0.025	0.078	-0.324	0.746
Model Three	(Intercept)	-0.048	0.524	-0.093	0.926
	Saturation Deficit	-0.968	0.215	-4.511	<0.001
	Light level (dark)	0.198	0.102	1.939	0.052
	Vegetation height	0.027	0.041	0.644	0.520
	Soil temperature	0.188	0.151	1.249	0.212
	Wind speed	0.055	0.074	0.746	0.456
	Saturation Deficit : Soil temperature	0.393	0.142	2.770	0.006
	Saturation Deficit : Wind speed	0.098	0.181	0.543	0.587
	Light level (dark) : Soil temperature	-0.059	0.124	-0.473	0.636
	Light level (dark) : Wind speed	-0.055	0.111	-0.496	0.620
Model Four	(Intercept)	-0.057	0.530	-0.107	0.915
	Saturation Deficit	-0.968	0.214	-4.525	<0.001
	Light level (dark)	0.206	0.101	2.043	0.041
	Vegetation height	0.028	0.041	0.678	0.497
	Soil temperature	0.180	0.150	1.200	0.230
	Wind speed	0.057	0.074	0.764	0.445
	Saturation Deficit : Soil temperature	0.411	0.136	3.026	0.002
	Saturation Deficit : Wind speed	0.107	0.180	0.593	0.553
	Light level (dark) : Wind speed	-0.045	0.109	-0.414	0.679
Model Five	(Intercept)	-0.055	0.529	-0.104	0.917

	Saturation Deficit	-0.969	0.214	-4.531	<0.001
	Light level (dark)	0.198	0.099	2.001	0.045
	Vegetation height	0.029	0.041	0.702	0.482
	Soil temperature	0.177	0.150	1.182	0.237
	Wind speed	0.050	0.072	0.694	0.487
	Saturation Deficit : Soil temperature	0.416	0.135	3.075	0.002
	Saturation Deficit : Wind speed	0.147	0.150	0.978	0.328
Model Six	(Intercept)	-0.063	0.534	-0.118	0.906
	Saturation Deficit	-0.965	0.214	-4.506	<0.001
	Light level (dark)	0.205	0.099	2.077	0.038
	Vegetation height	0.030	0.041	0.739	0.460
	Soil temperature	0.175	0.150	1.169	0.243
	Saturation Deficit : Soil temperature	0.409	0.135	3.029	0.002
	Saturation Deficit : Wind speed	0.093	0.128	0.723	0.470
Model Seven	(Intercept)	-0.062	0.528	-0.117	0.907
	Saturation Deficit	-0.960	0.214	-4.484	<0.001
	Light level (dark)	0.199	0.098	2.021	0.043
	Vegetation height	0.028	0.041	0.681	0.496
	Soil temperature	0.135	0.139	0.974	0.330
	Saturation Deficit : Soil temperature	0.390	0.133	2.925	0.003
Model Eight	(Intercept)	-0.063	0.527	-0.120	0.905
	Saturation Deficit	-0.961	0.214	-4.489	<0.001
	Light level (dark)	0.202	0.098	2.061	0.039
	Soil temperature	0.123	0.138	0.896	0.370
	Saturation Deficit : Soil temperature	0.391	0.133	2.940	0.003
Final Model	(Intercept)	-0.063	0.528	-0.119	0.905
	Saturation Deficit	-0.907	0.204	-4.435	<0.001
	Light level (dark)	0.207	0.098	2.110	0.035
	Saturation Deficit : Soil temperature	0.391	0.133	2.935	0.003

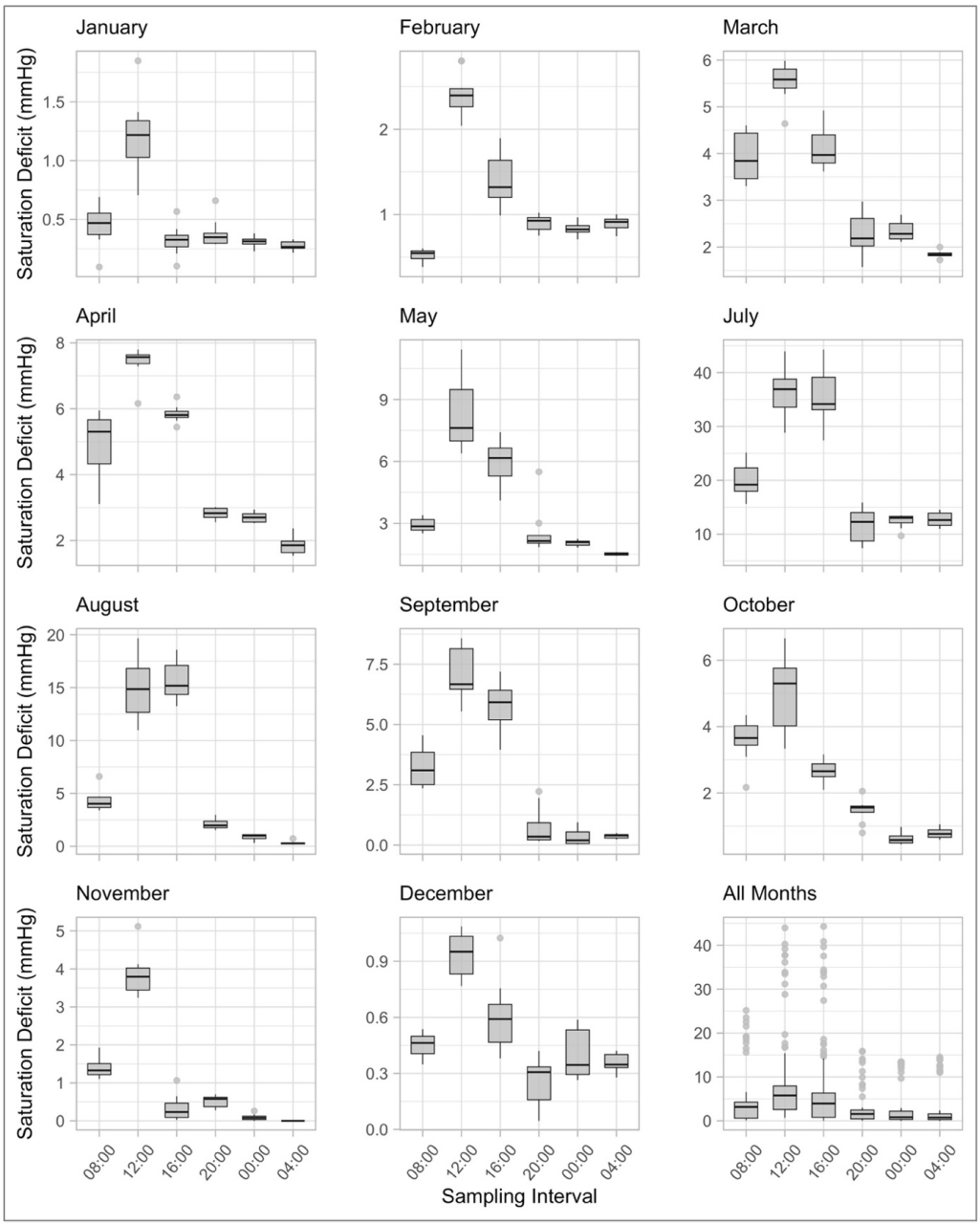

Figure 8. The saturation deficit (mmHg) at each transect in each of the sampling periods [08:00, 12:00, 16:00, 20:00, 12:00, 04:00 (Greenwich Mean Time)] for individual sampling months, and all months combined. Boxes represent the interquartile range of the data, with a line at the median value. Whiskers extend to the minimum and maximum values, with outliers represented by filled circles.