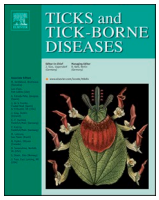
Ticks and Tick-borne Diseases 15 (2024) 102373

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis



Original article

Spatiotemporal dynamics of *Ixodes ricinus* abundance in northern Spain

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ARTICLE INFO

Keywords:
Density
Ecology
Lyme borreliosis
Modelling
Tick
Tick-borne disease

ABSTRACT

Ixodes ricinus is the most medically relevant tick species in Europe because it transmits the pathogens that cause Lyme borreliosis and tick-borne encephalitis. Northern Spain represents the southernmost margin of its main European range and has the highest rate of Lyme borreliosis hospitalisations in the country. Currently, the environmental determinants of the spatiotemporal patterns of *I. ricinus* abundance remain unknown in this region and these may differ from drivers in highly favourable areas for the species in Europe. Therefore, our study aimed to understand the main factors modulating questing *I. ricinus* population dynamics to map abundance patterns in northern Spain.

From 2012 to 2014, monthly/fortnightly samplings were conducted at 13 sites in two regions of northern Spain to estimate spatiotemporal variation in *I. ricinus* questing abundance. Local abundance of *I. ricinus* was modelled in relation to variation in local biotic and abiotic environmental conditions by constructing generalised linear mixed models with a zero-inflated negative binomial distribution for overdispersed data.

The different developmental stages of *I. ricinus* were most active at different times of the year. Adults and nymphs showed a peak of abundance in spring, while questing larvae were more frequent in summer. The main determinants affecting the spatiotemporal abundance of the different stages were related to humidity and temperature. For adults and larvae, summer seemed to be the most influential period for their abundance, while for nymphs, winter conditions and those of the preceding months seemed to be determining factors. The highest abundances of nymphs and adults were predicted for the regions of northern Spain with the highest rate of Lyme borreliosis hospitalisations. Our models could be the basis on which to build more accurate predictive models to identify the spatiotemporal windows of greatest potential interaction between animals/humans and *I. ricinus* that may lead to the transmission of *I. ricinus*-borne pathogens.

1. Introduction

Ticks are blood-feeding arthropods that can cause negative effects to their hosts, either through blood extraction (Duffy, 1983; Boulinier and Danchin, 1996; Jones et al., 2019; Debow et al., 2021) or through the transmission of pathogens that can cause disease (Ruiz-Fons et al., 2014; Gilbert, 2016; Almazán et al., 2018). *Ixodes ricinus* (Linnaeus, 1758) is the primary vector of several pathogens that cause important human diseases such as Lyme borreliosis (LB), tick-borne encephalitis (TBE) and granulocytic anaplasmosis (Gern and Humair, 2002; Mansfield et al., 2009; Rizzoli et al., 2011, 2014). It is also vector of pathogens affecting animal health, e.g., Louping ill virus (LIV), which causes a severe encephalitis in ruminants (Balseiro et al., 2012; Ruiz-Fons et al., 2014) and red grouse chicks (Gilbert, 2016). The diversity of hosts on which *I. ricinus* feeds is high (Jameson and Medlock, 2011). Larvae and nymphs can feed on over 300 recorded hosts (Kahl and Gray, 2023). In general, *I. ricinus* larvae feed on small vertebrates (micromammals, hedgehogs, birds, and lizards; Rizzoli et al., 2014), although some studies have demonstrated that they can also do so on large animals (e.g., Tälleklint and Jaenson, 1997; Gilbert et al., 2000). Nymphs feed on small and large vertebrates (Estrada-Peña et al., 2005; Ruiz-Fons et al., 2006; Rizzoli et al., 2014; Díaz et al., 2019), while the adults frequently feed on larger mammals, e.g., ungulates (Ruiz-Fons et al., 2006). In northern Spain, *I. ricinus* larvae seem to select with higher frequency small vertebrates

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https://doi.org/10.1016/j.ttbdis.2024.102373
Received 21 September 2023; Received in revised form 19 June 2024; Accepted 21 June 2024
Available online 3 July 2024

such as micromammals and birds (Estrada-Peña et al., 2005), whereas the nymphs have been found on birds (Estrada-Peña et al., 2005) and ungulates (Ruiz-Fons et al., 2006; Díaz et al., 2019) and the adults on medium and large-sized mammals (Estrada-Peña et al., 2005; Ruiz-Fons et al., 2006). However, host usage rate may vary locally depending on variations in the structure of the community of hosts because *I. ricinus* displays a generalist behaviour (Kahl and Gray, 2023). We currently do not know how the local structure of the populations of vertebrates modulate the frequency with which different stages of *I. ricinus* feed on hosts in northern Spain.

The geographic distribution of *I. ricinus* ranges from Scandinavia to the North of Africa (Noureddine et al., 2011). The northern third of the Iberian Peninsula represents the southern limit of the core distribution area of *I. ricinus* in Europe (Estrada-Peña et al., 2004). Further south in the Iberian Peninsula, the environmental favourability for the species becomes marginal and it is only present in moderate to low abundances in areas that meet the requirements for its development (e.g., Peralbo-Moreno et al., 2022). Abundance determinants may vary widely between distribution areas and within highly environmentally favourable settings (Lindgren et al., 2000), so the determinants may not necessarily be the same or have the same weight in highly favourable compared to moderate and marginally favourable environments (Estrada-Peña et al., 2004; Hampe and Petit, 2005). Needham and Teel (1991) indicated that desiccation is a crucial factor limiting the questing behaviour of ticks, and the review of knowledge gaps on the ecology of *I. ricinus* by Gray et al. (2021) suggested that water balance plays a crucial role in determining the species distribution limit. However, the spatiotemporal abundance of *I. ricinus* is influenced by a combination of biotic and abiotic factors (Randolph, 2000; Lindgren et al., 2000; Qviller et al., 2014). These factors not only influence tick survival and activity but also their abundance, which in turn depends on the host's use of space (Ruiz-Fons and Gilbert, 2010; Peralbo-Moreno et al., 2022). Ticks are highly dependant on host availability, and given that host abundance is highly variable at small spatiotemporal scales, it is expected that the finer the scale at which determinants of tick abundance are assessed, the more weight the hosts will acquire relative to other environmental parameters (Gilbert, 2010; Tagliapietra et al., 2011; Kilpatrick et al., 2017; Fernández-Ruiz and Estrada-Peña, 2020; Dumas et al., 2022). The abundance of *I. ricinus* may be also driven by other biotic environmental determinants (Estrada-Peña et al., 2005; Barandika et al., 2006; Ruiz-Fons et al., 2012). The structure and composition of the habitat is a relevant parameter shaping *I. ricinus* abundance and spatial distribution at small spatial scales (Boyard et al., 2008; Medlock et al., 2013) and the habitat also conditions host space use (Acevedo et al., 2011). In addition, the abundance of hosts can also fluctuate over time, with greater fluctuations in small vertebrates than in large ones (Smith and Lyons, 2011). Generally, the spring-summer season is when the highest number of hosts is found because of breeding, while in winter there may be higher mortality of hosts, especially micromammals, due to harsh winter temperatures and food scarcity (Andreassen et al., 2021).

Tick abundance is a crucial parameter to understand pathogen transmission dynamics (Eisen and Eisen, 2016; Cuadrado-Matías et al., 2022; O'Neill et al., 2023). Frequently, the greater the abundance of ticks, the greater the prevalence of infected ticks and higher the expected probability of a host encountering (and being bitten by) an infected tick (e.g., Ruiz-Fons et al., 2013; Cuadrado-Matías et al., 2022). However, the role that the different developmental stages of ticks play in pathogen transmission is variable and dependant on the biology of the pathogen. This, combined with the slightly different environmental requirements of the different stages of *I. ricinus* (Gray et al., 2009; Alonso-Carné et al., 2016), indicate that abundance modelling should be carried out independently for each tick, even when the inter-dependencies between these stages are modelled (e.g., Ruiz-Fons and Gilbert, 2010). Thus, understanding the determinants of the abundance of each developmental stage of *I. ricinus*, and whether their effects are similar or different for each stage is essential to understand the ecology of the species. Unravelling how environmental determinants drive the spatiotemporal variation in the abundance of *I. ricinus* stages in the southernmost limit of its core European distribution area may provide relevant knowledge that constitutes the starting point to prevent and control (re)emerging *I. ricinus*-borne diseases such as Louping ill (Balseiro et al., 2012; Ruiz-Fons et al., 2014) and Lyme borreliosis (Amores-Alguacil., et al. 2022) in the Iberian Peninsula. Thus, our main goal was to model the questing density of each stage of *I. ricinus* over time to unravel the differential effects of drivers on population dynamics and pathogen transmission.

2. Materials and methods

2.1. Study area and questing tick density estimation

From 2012 to 2014, 13 sites drawn from two different northern Spanish regions, the Basque Country (R_1; $n = 4$) and Asturias region (R_2; $n = 9$; Fig. 1), that are within the southernmost boundary of the core European distribution area of *I. ricinus*, were surveyed monthly/fortnightly to estimate questing tick density (from now on, tick density) and activity variations. The four sites surveyed in R_1 were located within the boundaries of "Sierra de Entzia" (Fig. 1), a mountain range with a high-altitude plateau (700–1200 m) located to the east of the province of "Araba" (42°48′21″N 2°20′06″W), with a surface area of 4991 ha. Sampling sites were allocated to the two main habitat types in the region, woodland (three sites) and shrubland (one site) (Appendix A). The remaining nine sites were distributed along the region known as "Sierra del Sueve", a mountain range located by the north-eastern coast of R_2 region (43°25′33″N 5°14′56″W) (Fig. 1). This mountain range spans 8112 ha extending in a southwest-northeast direction and the altitudes range from the sea level to the 1161 m at the highest peak. The study sites in this region were allocated to the southwest ($n = 5$) and northeast ($n = 4$) slopes of the mountain range (Fig. 1). However, after 2012, the number of sites was reduced to 3 per slope. The sampling sites were allocated to the three main habitat types in this region, which included woodland, shrubland, and grassland (Appendix A). Initially, there were three sites in grassland, three in shrubland and three in woodland in R_2, but after 2012 one sampling site from each habitat type was removed, leaving two sites per habitat type sampled in 2013 and 2014.

The surveys were carried out in each site by dragging the vegetation surface of different habitats (grassland, shrubland, and woodland) with a 1 × 1 m white cotton flannelette (Cohnstaedt et al., 2012; Mays et al., 2016) along a series of continuous blanket drags of variable total length per survey (40–800 m). The transects along which blanket drags were carried out in each sampling were not linear, but of variable layout, and their layout was geo-registered using handheld GPS devices. Given the random nature of the layout of each transect, except for the starting point, the latter was considered as the reference point for the estimates of environmental parameters potentially modulating *I. ricinus* abundance. Despite their random nature, transects were always conducted in their corresponding habitat. In R_1, surveys were conducted fortnightly, while in R_2 they were conducted monthly. Each blanket drag was 10 m long, and all the captured ticks per blanket drag were collected into 2 mL tubes filled with 70% ethanol and labelled independently for later tick counting and identification in the laboratory. Larvae were collected into the tubes using entomological tweezers when numbers were low and brushes for larger captures. Tick identification was performed at the species level by two of the authors, Drs. Espí and Barandika, following the establishment of common classification criteria to avoid an observer effect and according to published specific morphological identification keys (Estrada-Peña et al., 2017). Tick counts in relation to the dragged surface were employed to estimate a tick density index per tick species and stage in each of the surveys. More detailed information about the sampling sites can be found in Appendix A.

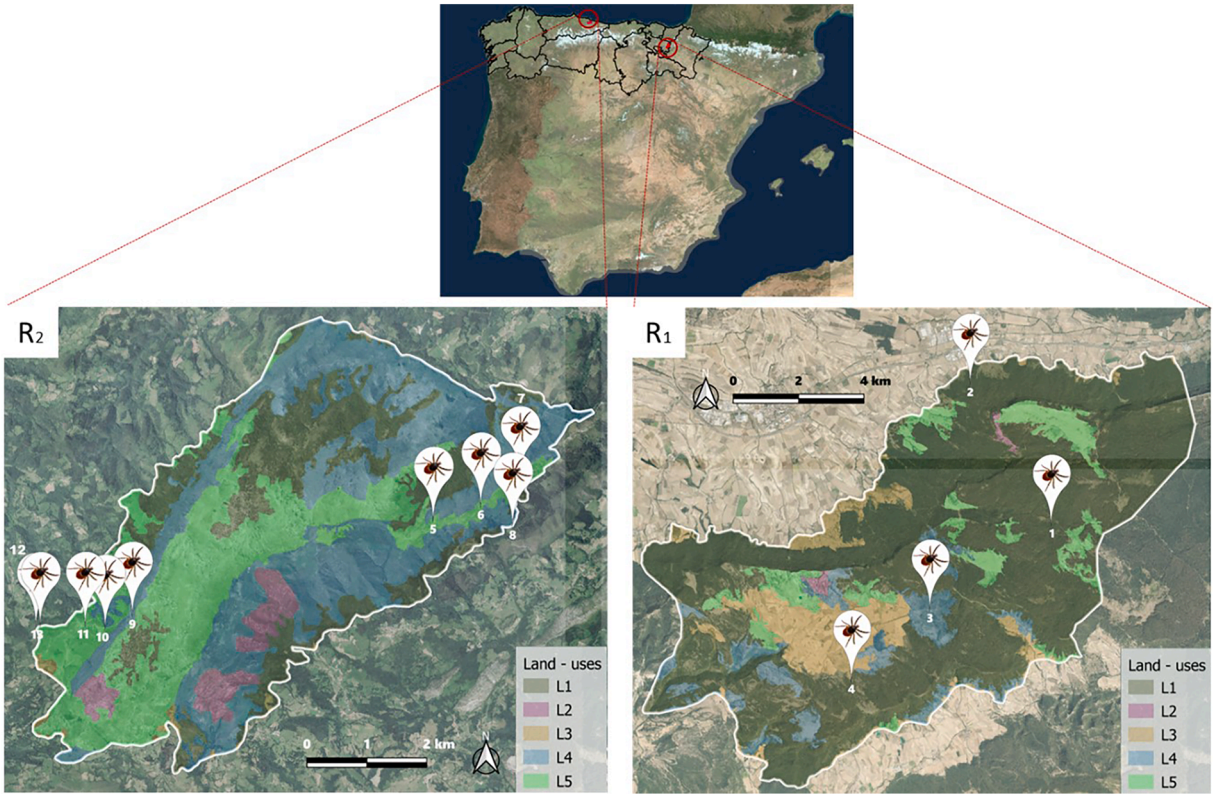

Fig. 1. Map showing the location of the two sampling areas in northern Iberian Peninsula. On the left bottom map, we represent R_2, the region surveyed in the Principality of Asturias, with its nine sampling points (5–13). On the right bottom map, we represent R_1, the region surveyed in the Basque Country, with its four (1–4) sampling points. The location and characteristics of each sampling point are given in Appendix A. The codes referring to land use are: L1 - Forest; L2 - Combination of vegetation; L3 – Agricultural land; L4 – Shrubland; and L5 - Grassland.

2.2. Environmental predictors of tick questing density

Our main goal was to model temporal tick density indices as a function of local environmental parameters potentially relevant for tick developmental rates, activity, and survival. Therefore, our response variable for modelling was the estimated number of ticks per hectare (1 hectare = 0.01 km^2) from the blanket drags conducted in each survey. Tick abundance at a given place and time depends on several factors that shape tick activity and survival in the medium-to-short time scale (James et al., 2013). To include the conditions that may have influenced both tick activity and survival in our study settings, we considered a buffer of 600 m around the starting point of each blanket drag transect per sampling site for environmental characterization. The size of the buffer was chosen according to the available information on the maximum daily host movement range for a similar region in the same geographical situation and a similar level of protection for conservation (Russo et al., 1997), so we considered that the 1.13 km^2 buffer included the conditions to which the ticks had been exposed between surveys. The selection of the environmental variables for tick density parameterization was based on their potential to modulate tick questing activity and population dynamics.

2.2.1. Abiotic predictors

The Normalized Difference Vegetation Index (NDVI) was selected because it is a proxy of soil moisture (Benedetti and Rossini, 1993), which is an indicator of the hydrological stress to which off-host ticks are exposed and that may affect their survival and, consequently, abundance (Lane et al., 1985; Needham and Teel, 1991). Furthermore, NDVI has been previously used in several tick modelling studies (Randolph, 2000; Alonso-Carné et al., 2016; Estrada-Peña et al., 2016). Land Surface Temperature (LST) is also an important factor driving tick development, activity, and survival (Lane et al., 1985; Needham and Teel, 1991). Both abiotic environmental predictors were derived from MODIS satellite information (Didan, 2015; Wan et al., 2015). The NDVI was available at a 16-day temporal scale and at a 250 m spatial resolution (Didan, 2015) and LST at a daily temporal scale and at a 1 km spatial resolution (Wan et al., 2015). We selected the information available for both predictors for the period 2011–2014. This period was selected because we estimated the following variables as predictors: i) the average/variation in NDVI/LST during the twelve months before tick capture, because *I. ricinus* takes more than a year to complete its biological cycle (Kahl and Gray, 2023), so the abiotic conditions of the previous year could have influenced *I. ricinus* density at the time of sampling; ii) the NDVI/LST average/variation during the winter (Dec-Feb) before each survey, because an overly cold or warm winter, or one with extremely variable temperatures, could compromise tick survival (Herrmann and Gern, 2013); iii) the average/variation in NDVI/LST during the summer (June-Aug) prior to each survey, as the summer could be a critical period for *I. ricinus* survival, especially in a Mediterranean-influenced area (R_1), compared to its core distribution areas in central Europe; and iv) NDVI/LST average/variation of the month before tick capture, an indicator of the recent abiotic conditions that off-host ticks had experienced and that could modulate their activity patterns. A deficit in moisture in any period could also compromise *I. ricinus* survival and modulate its activity, as they are exposed to water loss while questing in the vegetation. Further, moisture deficit can also influence the use of space by *I. ricinus* hosts and the structure of the community of hosts due to variations in resources and shelter (Grovenburg et al., 2011). All abiotic predictors were estimated for the 600 m buffer around each sample location (as performed in Peralbo-Moreno et al., 2022). In addition to average values of the abiotic predictors, we considered their variability according to the Schmalhausen law (Poh et al., 2019), a

principle that indicates that an individual or population at the limit of its distribution is very sensitive to both external and internal changes (Lewontin and Levins, 2000).

To estimate the potential cumulative effect of the average/variation in NDVI/LST on tick focal density estimates, we explored the time lagged association of abiotic conditions to the monthly dynamics of each *I. ricinus* stage by cross-correlation matrices (CCM; see Torina et al., 2023), a method to visualize lagged effects of predictors on arthropod abundance (Curriero et al., 2005). Environmental conditions have a continuous effect on organisms, and some biological processes are determined by the accumulation of certain temporal conditions and not only by conditions occurring at a given moment (Curriero et al., 2005; Gray et al., 2016). CCMs are a complex case of cross-correlations, as a second-time lag is added to the correlation. This means that not only the correlation with respect to the point in time ''t'', specified by lag 1, is calculated, but it also estimates the average of the predictor for the specified period between lags 1 and 2 (Groen et al. 2017). CCMs allowed us exploring which past time periods of a predictor were better associated to the focal tick density estimates to maximise finding best abiotic predictors of tick density and, thus, improve the predictive potential of the models. We calculated the monthly average/variation NDVI/LST up to a period of 11 months prior to tick capture (lag1 = 11, lag2 = 0) to account for the seasonal tick activity patterns within a year (Barandika et al., 2010) and included average/variation values for shorter periods. Once all the average/variation values of the abiotic parameters were calculated between lags, Spearman's rank order correlation coefficients with monthly tick density were estimated for each *I. ricinus* stage separately. The periods showing the highest correlation coefficient with each response variable were chosen for modelling. The detailed values of the correlations between time periods for each parameter can be found in Appendix B.

2.2.2. Habitat predictors

Ixodes ricinus is more frequently found in forested areas than in grasslands or shrublands because woodlands provide more stable soil moisture conditions (e.g., Ruiz-Fons and Gilbert, 2010). The habitat also conditions host space use (Acevedo et al., 2011), so the structure of the habitat forces variations in host availability for the ticks that could result in local variation in tick density. Woodland is also frequently used by shelter-seeking hosts, both during the night or in adverse conditions (Godvik et al., 2009; Laguna et al. 2021). Also, in periods of pasture scarcity, woodlands can provide food resources to herbivorous vertebrates, i.e., large ungulates (Bobrowski et al. 2020). Land use variables were estimated as the proportion (%) within the 600 m buffer of each of the four land uses: i) woodland (L1); ii) shrubland (L2); iii) herbaceous grassland (L3); and iv) cropland (L4). Land uses were derived from the CORINE database (European Environment Agency EEA, 2018) at a 1:100,000 spatial resolution.

2.2.3. Host availability predictors

The availability of domestic vertebrates for ticks was approached by estimating livestock density for each 600 m buffer using the information at a municipality scale of the national 2009 livestock census of the Spanish National Statistics Institute (https://www.ine.es). The initial step involved georeferencing the information by assigning a unique code to each municipality. Subsequently, we computed the density of each livestock species (sheep, goat, cattle, horse and pig) per municipality. Thereafter, for each livestock species of interest, we calculated the weighted average of the species density for each of the 600 m buffers around survey sites. For each buffer, the weighted average density of each livestock species was estimated as a function of the proportion of the surface of each municipality within the buffer. For example, if 100% of the buffer area was within a single municipality, that buffer was assigned the average livestock density values of that municipality. For modelling purposes, we selected the average density of heads of domestic ruminants (cattle, sheep, and goats) because these are the most relevant domestic species hosting *I. ricinus* in the study area (Barandika et al., 2010).

To estimate the availability of important wild hosts for *I. ricinus* adults (also for nymphs, and perhaps also for larvae; Tälleklint and Jaenson, 1997; Gilbert et al., 2000) in the study area, (Ruiz-Fons and Gilbert, 2010; Peralbo-Moreno et al., 2022; O'Neill et al., 2023), we gathered the results of the spatial prediction of wild ruminant abundance (red deer – *Cervus elaphus* and roe deer – *Capreolus capreolus*) performed at the European scale (ENETWILD-consortium et al. 2022) and projected at a 10 × 10 km spatial resolution. The Eurasian wild boar is not a relevant host to *I. ricinus* in northern Spain (Ruiz-Fons et al., 2006) and was thus excluded from the analysis. No information is available to estimate the population status of small vertebrates (micromammals, hedgehogs, lizards, or birds) in the study area, which could be hosts of *I. ricinus* immature stages. Even though our study is focused on the temporal variation in tick density, we could not estimate host density or space use variation at the temporal scale of the study because no information is available, so the proxies employed as host availability for the ticks were the best available for our goals.

2.2.4. Tick questing density autocorrelations

The local density of any *I. ricinus* stage can be largely determined by the density of its previous stage, e.g., the density of nymphs in vegetation is partially a result of the previous density of larvae and the rate of larvae that have successfully moulted into nymphs (O'Neill et al., 2023). Thus, each stage may be density-dependant on its previous stage at local scales; larvae depend on adults reproducing and laying eggs, nymphs on larvae, and adult on nymphs. Thus, for each specific stage model, we considered its own previous density (as ticks may persist alive for long periods of time in vegetation (Lees, 1946), and the density of its previous stage with two purposes: i) to explore inter-stadial density-dependant effects with field data; and ii) to deal with the possible problem of temporal autocorrelation in our data (Shumway and Stoo, 2000).

All variables estimated are displayed in Table 1.

2.3. Tick questing density models

We standardized all continuous predictors to reduce variability in the measurement scales using the 'scale' function in R (version 4.1.2). Following the methodology outlined in Zuur et al. (2010), we conducted an initial data exploration to check for imbalances, examine records, explore the relationship between response variables and predictors, and select the appropriate response distribution. To prevent the undesirable effects of collinearity on model parameterization, we excluded some of the highly correlated predictors using correlation matrices estimated with the 'cor' function of R. When a correlation was equal to or greater than |0.7|, one of the two variables was excluded based on ecological criteria of their expected influence on *I. ricinus* population dynamics.

We parameterized density models for the different developmental stages of *I. ricinus* separately using the 'glmmTMB' R package (Brooks et al., 2017). Due to high levels of overdispersion in the response variables and an excess of zeros in the data (Zuur et al., 2009), we used generalized linear mixed-effects models (GLMMs) with a zero-inflated negative binomial distribution ('nbinom2' family) and separate logit and log link functions for the zero-inflation and conditional models, respectively. Zero-inflated models consist of two parts: the zero-inflated binomial process, which models the probability of a zero value, and the conditional count process, which models densities using a negative binomial GLM (Zuur et al., 2009). The response variables were the estimated density of *I. ricinus* adults, nymphs, and larvae per hectare. Since the density index is a continuous value and the distributions on which the density models are based only admit integer values, the tick density was rounded to the integer value. Due to the structure of our experimental design, the sampling site nested with the region was considered a random variable in the models, since each of the 13 points where ticks were sampled periodically belong only to one of the two

Table 1
Variables considered as potential predictors of *Ixodes ricinus* questing larval/nymphal/adult density along with their mean and standard deviation values. Superscripts indicate the different factors into which the predictors were grouped for the variance partitioning procedures. Predictors that were included from the model parameterization are marked in bold text. The NDVI and LST data used in the estimation of the predictors were those recorded in the period 2011–2014. The spatial scale of the variables was 250 × 250 m pixels for NDVI, 1 × 1 km pixels for LST, 1:100,000 for land use variables, 10 × 10 km pixels for wild host abundance index, and the municipality for domestic ruminant density.

Codes	Descriptions	Mean	SD
site[r]	Sampling site (13 categories: S_1-S_{13})	–	–
region[r]	Study region (2 categories: R_1 & R_2)	–	–
nms[a]	Average summer NDVI	0.78	1e-03
nvs[a]	Summer NDVI variance	2e-03	2e-06
nmw[a]	Average winter NDVI	0.53	0.01
nvw[a]	Winter NDVI variance	0.03	4e-04
nma[a]	Average annual NDVI	0.67	2e-03
nva[a]	Annual NDVI variance	0.02	3e-04
lms[a]	Average summer LST (°C)	24.65	2.16
lvs[a]	Summer LST variance (°C)	14.21	2.11
lmw[a]	Average winter LST (°C)	8.81	1.77
lvw[a]	Winter LST variance (°C)	17.36	9.89
lma[a]	Average annual LST (°C)	18.30	1.02
lva[a]	Annual LST variance (°C)	59.30	19.41
lagnmad[a]	Average NDVI of the 9 months before sampling (*I. ricinus* adult model)	0.68	0.02
lagnvad[a]	NDVI variance of months 3–9 before sampling (*I. ricinus* adult model)	0.01	9e-05
laglmad[a]	Average LST of months 4–5 before sampling (°C) (*I. ricinus* adult model)	16.21	5.99
laglvad[a]	LST variance of months 7–11 before sampling (°C) (*I. ricinus* adult model)	15.65	8.11
lagnmn[a]	Average NDVI of months 2–11 before sampling (*I. ricinus* nymph model)	0.66	2e-03
lagnvn[a]	NDVI variance of months 4–7 before sampling (*I. ricinus* nymph model)	0.01	1e-04
laglmn[a]	Average LST of month 8 before sampling (°C) (*I. ricinus* nymph model)	16.45	7.17
laglvn[a]	LST variance of month 7 before sampling (°C) (*I. ricinus* nymph model)	16.76	14.32
lagnml[a]	Average NDVI of the month before sampling (*I. ricinus* larva model)	0.68	0.02
lagnvl[a]	NDVI variance of months 4–6 before sampling (*I. ricinus* larva model)	0.01	2e-04
laglml[a]	Average LST of months 5–7 before sampling (°C) (*I. ricinus* larva model)	16.02	6.05
laglvl[a]	LST variance of months 2–5 before sampling (°C) (*I. ricinus* larva model)	15.37	7.49
wild[b]	Average red/roe deer predicted abundance index	1.15	0.16
cat[b]	Average domestic ruminant (cattle, sheep & goats) density (heads/hectare)	0.16	0.05
m[c]	Month (12 categories: Jan-Dec)	–	–
dadir_p[d]	Questing density of adult *I. ricinus* in the month before sampling (ticks/hectare)	84.86	211.95
dnir_p[d]	Questing density of *I. ricinus* nymphs in the month before sampling (ticks/hectare)	4365.65	9685.13
dlir_p[d]	Questing density of *I. ricinus* larvae in the month before sampling (ticks/hectare)	7226.46	43,696.97
l1[e]	Woodland cover (%)	0.41	0.32
l2[e]	Shrubland cover (%)	0.29	0.27
l3[e]	Herbaceous grassland cover (%)	0.27	0.24
l4[e]	Cropland cover (%)	0.04	0.09

[a] abiotic environmental factor.
[b] host biotic factor.
[c] abiotic factor of the month.
[d] tick biotic factor.
[e] biotic land use factor.
[r] random effects.

large study regions in which this work is framed. The same group of variables was used for both the zero-inflated and conditional parts of the model. Final models were obtained using a forward stepwise procedure manually calculated based on the Akaike Information Criterion (AIC) (Burnham and Anderson, 2004). The model with the lowest AIC was considered to be most parsimonious and models separated by two AIC units or less were considered to have similar support. The residuals as well as their distribution were explored with the R package 'DHARMa' (Hartig and Hartig, 2017).

2.4. Deviance partitioning

When explaining the variation in a response variable using model predictors, the explanatory variables can be clustered into different groups, known as factors. Using this quantitative method, we can estimate the relative importance of each factor and their joint effects (Borcard et al., 1992). For this purpose, we developed partial models for each of the five considered factors - (a) abiotic environmental factor, (b) biotic host factor, (c) abiotic month factor, (d) biotic tick factor, and (e) abiotic land-use factor - considering the variables that were included in the final density model of each tick stage. The factor to which each variable belongs is specified in Table 1. Deviance partitioning procedures were applied to the final model outputs to account for deviance explained by each independent factor (potentially a, b, c, d, and e in this case), and the overlaid effect between factors. For applications and further estimation details see Alzaga et al. (2009) and Peralbo-Moreno et al. (2022).

2.5. Temporal overlap between stages

Estimating the temporal overlap of questing activity between different stages of *I. ricinus* can help determine whether different stages may feed simultaneously on shared hosts, leading to potential co-feeding pathogen exchange between stages (Randolph et al., 1996; Barandika et al., 2010). The relevance that this non-systemic transmission pathway may have on the ecology of some pathogens in northern Spain should be the subject of future studies. To test for temporal overlap between *I. ricinus* stages, we calculated overlap indices for pairs of stages with the 'overlap' package in R (Ridout and Linkie, 2009). The day of the year when each sampling took place was assigned and this temporal variable was rescaled to radians, so that the day of the year is scaled between 0 and 2pi, with the first days of the year being closer to 0 and the last days closer to 2pi. We used the 'overlapEst' function in 'overlap' package to estimate the value of overlap between pairs of stages, taking the 'Dhat4' value as the most accurate one, following the indications of Rovero and Zimmermann (2016) as the sampling size was bigger than 75.

Tick density was re-scaled between 0 and 1 to avoid problems caused by the large differences in density of *I. ricinus* stages. The 'bootCI' function was used to estimate the confidence intervals after 10,000 Bootstrap replicates. We selected the confidence interval value provided by 'basic0' in the 'bootCI' function to control for Bootstrap biases. As the overlap values are purely descriptive, the 'compareCkern' function of the 'activity' package in R (Rowcliffe and Rowcliffe, 2023) with 10,000 replicates was used to calculate the statistical significance level for the overlaps between stages.

2.6. Internal model validation

The temporal and spatial predictive capacity of the selected models was estimated using multiple methods. To internally assess the predictive performance of our models, we used the train-test split methodology to part the dataset randomly into a 70% training set for model parameterization and a 30% test set (Schratz et al., 2019). Predictions were carried out using the 'predict' function of 'car' package in R (Fox and Weisberg, 2019). We evaluated the association between observed and predicted density in the test dataset using calibration plots and correlation tests. Calibration plots were generated by dividing the predicted tick density in the test dataset into nine percentile bins and plotting the mean predicted density for each percentile against the mean observed

density in the same percentile bin. We repeated the split-train-test procedure 100 times and estimated the mean and confidence interval of the correlation values between predicted and observed densities on the test datasets to assess the overall predictive performance of the models. We also internally tested the predictive capacity of the models by splitting the data set into two years for model calibration while the remaining year was left for the validation. This was done to test the predictive ability of the model in other years, so we use the same model regardless of the temporal partition of the database.

2.7. External model validation

For external validation of tick density models, we used a dataset of tick questing densities estimated from questing tick surveys performed between 2003 and 2008 following the same approach employed in our study (Barandika et al., 2008, 2010; Ruiz-Fons et al., 2012). Data from 373 blanket drag transects were collected from 10 different sites in R_1; Barandika et al. (2008) presented the results of 191 monthly surveys carried out between 2003 and 2005 and Barandika et al. (2010) presented the results of 182 surveys performed between 2006 and 2008. The remaining 12 registers were collected at specific times of the year in 2004 in 12 sampling sites of R_2 (Ruiz-Fons et al., 2012). We calculated the same predictors for this dataset that were considered for modelling with our 2012–2014 data; we standardized these variables using mean and standard deviation from the calibration data set. We also applied the transformation of tick numbers per site into density values (ticks/hectare). The model that was calibrated with our own data was employed to predict over external data, so we estimated the Spearman's correlation indices between predicted and observed density values in the points remaining after applying Multivariate Environmental Similarity Surfaces (MESS) analysis (see Section 2.8 for details on MESS analysis).

2.8. Spatiotemporal model projection

After internal and external validation, we projected the models for each *I. ricinus* stage to the region of North-western Spain in which the species can be frequently observed or it is permanently established (Ruiz-Fons et al., 2006; Remesar et al., 2019; Černý, et al., 2020). The models were projected into a 1 × 1 km spatial resolution grid. We applied a MESS analysis using the 'modEVA' package in R (Barbosa et al., 2013). The MESS analysis considers the maximum and minimum values of each variable of the calibration model dataset and compares this range with the value of the validation data set. In the case of a value falling within the ranges in which the model was parameterized, that value can be used to project our model, whereas if the locality has a value that falls outside the ranges it cannot be used because it is ignored whether values outside the bounded ranges respond in the same way as those used to calibrate the model. For further information see Elith et al. (2010). The three models were projected for each month using 2013 as reference, and region '2′ and point '8′ were randomly selected as the reference for random effects.

2.9. Checking temporal correlations of predicted and observed values

Once the projection of the models was carried out, we calculated whether the temporal prediction of the models varied significantly from the temporal pattern of densities observed in our data using a similar methodology as for the temporal overlap between *I. ricinus* stages (Section 2.5). The only difference to the temporal overlap analysis of our original dataset was that, due to the huge differences in sample size, to standardize the overlap curves, the mean value of the prediction for each month was compared with the monthly mean of our original data. In addition, the consistency in the model's ability to detect areas of high or low tick density over the months was estimated. For this purpose, we calculated the standard deviation of the predicted values of the same grid for the different months. As the prediction was standardised between 0 and 1, a high standard deviation would indicate that the model predicts unevenly high and low tick densities over the months, while a low standard deviation indicates a constant prediction value. Only those grids selected by the MESS where predicted values were available for the two stages in the comparison were considered for correlation estimations.

3. Results

3.1. Tick diversity and questing density: descriptive results

A total of 70,187 ixodid ticks belonging to 8 species was collected in the 13 study sites, including 49,474 larvae, 19,677 nymphs, and 1036 adults (Table 2). In terms of density, *Ixodes ricinus* was the predominant species in all its developmental stages in both regions. Tick communities were heterogeneous between the study regions but similar at sites within the same region (Appendix C). We found the greatest diversity for adult ticks, with seven of the eight species occurring in R_1 and six of the eight species occurring in R_2. We found differences in the second most abundant species between the two study regions, being *Haemaphysalis punctata* in R_1 and *Haemaphysalis concinna* in R_2 (Table 2).

Regarding *I. ricinus*, the estimated density of larvae was three times higher in R_2 in comparison to R_1, fifteen times higher for nymphs and two times higher for adults (Table 2), with the ratio L:N:A being 56:7:1 in R_1 and 77:63:1 in R_2. We observed significant variations in the density of the different developmental stages of *I. ricinus* between study years (Fig. 2). In general, a higher density was recorded in 2013, and it was also higher in 2014 in comparison to 2012 (all significance values at $p <$ 0.01 for Tukey's test). Annual differences in density were observed for all stages of *I. ricinus* and in the two study regions with few exceptions. We found no differences in adult *I. ricinus* density between 2013 and 2014 in R_1, and we found more larvae in 2012 than in 2014 also in this region. The phenology of the *I. ricinus* stages varied both between years and study regions. Larval activity peaked around the summer months (June-August) in a constant unimodal pattern and activity peaks occurred later in the year in R_2 (July-August) than in R_1 (June-July).

Table 2
Tick species per developmental stage (L: larva; N: nymph; A: adult) collected in four sites in R_1 ("Sierra de Entzia") and nine sites in R_2 ("Sierra de El Sueve"). The table displays the sum of the ticks collected (No.) across the surveys performed from 2012 to 2014 and the average questing tick density (D; ticks/hectare).

Species	Stage	R_1		R_2	
		No.	D	No.	D
Ixodes ricinus	L	20,338	2995.12	19,273	9075.14
	N	2517	404.00	15,905	6134.59
	A	361	56.90	251	97.09
Haemaphysalis punctata	L	6898	945.00	398	208.03
	N	434	65.53	169	52.55
	A	145	23.05	19	6.67
Haemaphysalis concinna	L	0	0.00	2200	1005.24
	N	0	0.00	652	254.24
	A	0	0.00	86	31.45
Rhipicephalus bursa	L	0	0.00	367	102.07
	N	0	0.00	0	0.00
	A	2	0.27	16	4.32
Dermacentor reticulatus	L	0	0.00	0	0.00
	N	0	0.00	0	0.00
	A	45	7.03	31	8.59
Haemaphysalis inermis	L	0	0.00	0	0.00
	N	0	0.00	0	0.00
	A	42	7.03	18	7.30
Dermacentor marginatus	L	0	0.00	0	0.00
	N	0	0.00	0	0.00
	A	19	2.88	0	0.00
Haemaphysalis sulcata	L	0	0.00	0	0.00
	N	0	0.00	0	0.00
	A	1	0.14	0	0.00

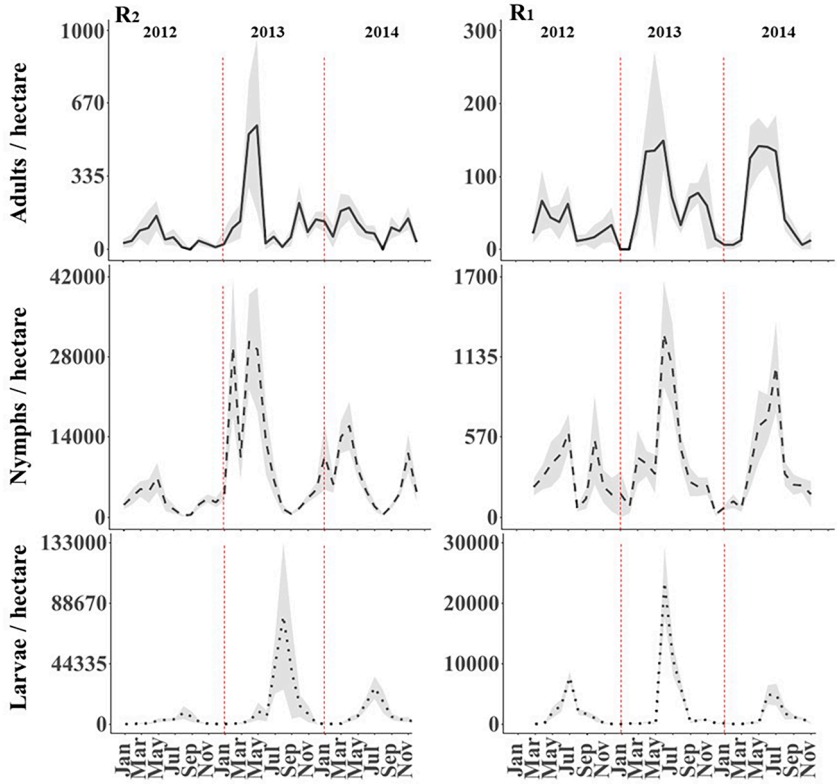

Fig. 2. Monthly questing density of each *Ixodes ricinus* stage (Adults in top charts, nymphs in middle charts and larvae in bottom charts) throughout the three years of the study in both study regions (R_2 in left charts and R_1 in right charts). The grey-shaded areas represent the standard deviation from the monthly average value. The scale of the Y-axis is not homogeneous between regions/stages for an appropriate visualization.

Nymphs were captured in the vegetation almost year-round in both regions (Fig. 2), with higher winter density in R_2 than in R_1. The activity pattern was mainly unimodal (except in 2012) in R_1 with a spring-summer peak around June-July and only a second peak was observed in 2012 in October. In contrast, the pattern was mainly bimodal in R_2, with a high peak around February-May (variable between years) and a lower density peak in November-December. The patterns of activity of adult *I. ricinus* were homogeneous in both regions and bimodal, but the winter activity was slightly higher in R_2 than in R_1. Adult densities peaked mainly in spring-to-summer with a second peak by mid-autumn (October-November). The activity periods of both adults and nymphs were longer in both regions than those of larvae.

3.2. Determinants of tick questing density

The monthly density of adult *I. ricinus* was slightly positively correlated with lagged values of NDVI from the capture month up to nine months earlier ($r_s(9,9) = 0.16$) and negatively correlated with NDVI variation between months three and nine prior to the capture ($r_s(9,3) = -0.10$). For the nymphs, the highest correlation with NDVI was observed for the period between three and eleven months before the capture ($r_s(11,2) = 0.59$) while the highest correlation coefficient with the variation in NDVI occurred in the period between months five and seven before tick capture ($r_s(7,4) = -0.41$). Larvae were strongly positively correlated with the NDVI in the month of capture ($r_s(0,0) = 0.58$) and also positively correlated with NDVI variation in the period between months five and six before the capture ($r_s(6,4) = 0.38$). Average LST for the month five prior to capture showed the highest correlation coefficient ($r_s(5,4) = -0.17$) with the density of adult *I. ricinus* while the highest correlation with LST variation was observed with average values of months eight to eleven before the capture ($r_s(11,7) = 0.21$). For the nymphs, highest correlations were observed for both LST ($r_s(8,8) = 0.40$) and LST variation ($r_s(7,7) = 0.28$) for a period of eight and seven months from the month of capture. Finally, only slight maximum correlation coefficients were observed for *I. ricinus* larvae density with LST values between months seven and six prior to the capture ($r_s(7,5) = -0.61$) and with LST variation of the period between months three and five before the capture ($r_s(5,2) = 0.30$).

The predictors that were chosen for modelling are displayed in Table 1. The zero-inflated part of the model determines the likelihood of ticks being absent, so a positive estimate indicates a higher likelihood of tick absence. Conversely, positive estimates in the conditional part of the model denote a positive influence on tick density. Concerning the zero-inflated part of the model that best fitted *I. ricinus* adults' density (Table 3), we found differences in the probability of zero counts between months; the probability of a zero was statistically significant and higher in August compared to January (the reference category). Summer variations in NDVI and LST and the average summer LST were negatively related to the probability of zero *I. ricinus* adults with statistical significance, whereas the average LST between four and five months before adults' capture favoured the absence of them on vegetation. In the conditional part of the density model, we found almost no statistically significant differences between January and the other months; the significance value was marginally significant ($p = 0.05$) in April, during the major peak of adults observed. Variation in summer LST was slightly positively related to density with a statistical significance, whereas the average summer LST was negatively associated with the density of adult *I. ricinus*.

The zero-inflated part of the selected model (Table 4) for *I. ricinus* nymphs indicated a statistically significant higher probability of finding them in June and November compared to January. The LST variation seven months before nymphs capture and the winter NDVI variation were also negatively related to the absence of *I. ricinus* nymphs. Nevertheless, in the conditional part of the density model, the density of

Table 3
Predictors included within the best fitted *Ixodes ricinus* adults' questing density model (GLMM with a zero-inflated negative binomial distribution and a logit link function for zero-inflation model and a log link for conditional model). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Predictors shown as abbreviations include: i) m: month; ii) nvs: Summer NDVI variance; iii) laglmad: Average LST of months 4–5 before sampling; iv) lvs: Summer LST variance; and v) lms: Average summer LST.

	Conditional model				Zero-inflated model			
Predictor	B	SE	Z	P	β	SE	Z	P
Intercept	4.67	0.40	11.54	***	0.38	0.80	0.48	0.63
m[a]								
February	0.17	0.31	0.53	0.60	0.75	0.66	1.13	0.26
March	0.23	0.28	0.82	0.41	−0.07	0.67	−0.11	0.91
April	0.68	0.35	1.92	0.05	−0.14	0.85	−0.16	0.87
May	0.42	0.43	0.99	0.32	0.75	1.03	0.73	0.47
June	0.29	0.47	0.62	0.54	1.28	1.10	1.17	0.24
July	0.06	0.43	0.15	0.88	1.53	1.01	1.52	0.13
August	−0.38	0.37	−1.01	0.31	2.20	0.89	2.48	*
September	0.15	0.29	0.51	0.61	0.79	0.68	1.16	0.24
October	0.26	0.26	1.04	0.30	−0.49	0.60	−0.81	0.42
November	0.28	0.26	1.05	0.29	−0.40	0.59	−0.68	0.50
December	0.22	0.26	0.82	0.41	−0.65	0.59	−1.09	0.27
nvs	−0.11	0.07	−1.63	0.10	−0.42	0.20	−2.16	*
laglmad	−0.20	0.14	−1.41	0.16	0.95	0.34	2.76	**
Lvs	0.19	0.07	2.89	**	−0.41	0.17	−2.41	*
lms	−0.18	0.08	−2.16	*	−0.93	0.21	−4.44	***

[a] The reference category for month is January.

Table 4
Predictors included within the best fitted *Ixodes ricinus* nymphs' questing density model (GLMM with a zero-inflated negative binomial distribution and a logit link function for zero-inflation model and a log link for conditional model). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Predictors shown as abbreviations include: i) m: month; ii) laglvn: LST variance of month 7 before sampling; iii) lagnvn: NDVI variance of months 4–7 before sampling; iv) lvw: Winter LST variance; v) dlir_p: Density of *I. ricinus* larvae in the month before sampling; and vi) nvw: Winter NDVI variance.

	Conditional model				Zero-inflated model			
Predictor	β	SE	Z	P	β	SE	Z	P
Intercept	6.85	0.56	12.25	***	−1.71	0.59	−2.90	**
m[a]								
February	0.62	0.25	2.48	*	0.03	0.73	0.05	0.96
March	0.55	0.22	2.45	*	−18.55	3253.79	−0.01	1.00
April	1.01	0.23	4.42	***	−18.78	3866.54	−0.00	1.00
May	0.81	0.23	3.59	***	−18.12	2001.13	−0.01	0.99
June	0.60	0.24	2.50	*	−1.66	0.82	−2.03	*
July	0.39	0.24	1.60	0.11	−0.39	0.72	−0.54	0.59
August	−0.59	0.26	−2.32	*	0.91	0.71	1.28	0.20
September	−0.67	0.24	−2.78	**	−0.60	0.76	−0.79	0.43
October	−0.04	0.22	−0.18	0.86	−1.59	0.84	−1.90	0.06
November	0.45	0.23	1.99	*	−1.84	0.92	−2.00	*
December	−0.07	0.25	−0.29	0.77	−0.16	0.71	−0.23	0.82
laglvn	−0.00	0.04	−0.01	0.99	−0.61	0.27	−2.29	*
lagnvn	−0.12	0.05	−2.27	*	0.36	0.20	1.82	0.07
lvw	−0.23	0.07	−3.25	**	0.24	0.17	1.40	0.16
dlir_p	0.02	0.05	0.44	0.66	0.07	0.10	0.73	0.47
nvw	0.07	0.05	1.26	0.21	−0.64	0.19	−3.41	***

[a] The reference category for month is January.

I. ricinus nymphs from February to June and in November were higher than in January, while they were lower in August and September. The variation of the NDVI between four and seven months before nymphs capture and the winter LST variation were instead negatively associated with the density of *I. ricinus* nymphs.

The zero-inflated part of the model for *I. ricinus* larvae (Table 5) showed that the lowest probability of absence occurred from April to November with respect to January. The density of domestic ruminants and average summer LST were also negatively related to *I. ricinus* larvae absence. In the conditional part of the density model, *I. ricinus* larvae were more abundant from March to November compared to January and with higher values of the average summer LST.

The exploration of models' residuals showed no specific pattern (Appendix D).

3.3. Differential effects of factors on I. ricinus questing density

Deviance partition analyses showed different levels of explanation of the factors included in the final models for the three stages of *I. ricinus* (Fig. 3). Not all the factors explained part of the deviance of the final models, although month and environmental factors were included in all three final models. The tick density factor (previous density of larvae) was only explanatory in the model for *I. ricinus* nymphs while the host factor was only selected for the larvae model. The biotic land-use factor was not included in any final model. For all three developmental stages of *I. ricinus*, the month factor was the most explanatory, followed by the environmental factor of abiotic conditions. We also found some relevant values of deviance explained by the overlaid effects of month and the environmental factors. These factors are clearly related, so *I. ricinus* abundance cannot be explained by any of these factors separately. Some of the intersections between factors in the larvae model showed convergence problems in partial models for those groups of variables, so

Table 5
Predictors included within the best fitted *Ixodes ricinus* larvae questing density model (GLMM with a zero-inflated negative binomial distribution and a logit link function for zero-inflation model and a log link for conditional model). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Predictors shown as abbreviations include: i) m: month; ii) cat: Average domestic ruminant density; and iii) lms: Average summer LST.

	Conditional model				Zero-inflated model			
Predictor	β	SE	Z	P	β	SE	Z	P
Intercept	5.15	0.60	8.54	***	2.37	0.65	3.65	***
m[a]								
February	−0.17	0.72	−0.23	0.82	0.34	0.83	0.42	0.68
March	1.98	0.67	2.98	**	−0.25	0.73	−0.34	0.73
April	1.79	0.57	3.15	**	−2.08	0.68	−3.06	**
May	2.97	0.57	5.24	***	−2.65	0.68	−3.89	***
June	3.93	0.54	7.24	***	−5.06	0.80	−6.36	***
July	3.81	0.54	7.10	***	−5.77	0.87	−6.61	***
August	3.46	0.54	6.43	***	−4.36	0.72	−6.06	***
September	2.41	0.55	4.39	***	−4.03	0.72	−5.56	***
October	1.82	0.55	3.34	***	−3.47	0.69	−5.06	***
November	1.25	0.56	2.23	*	−2.54	0.68	−3.72	***
December	−0.38	0.61	−0.63	0.53	−0.65	0.72	−0.89	0.37
cat	0.49	0.31	1.57	0.12	−0.69	0.30	−2.33	*
lms	0.44	0.13	3.53	***	−1.21	0.22	−5.40	***

[a] The reference category for month is January.

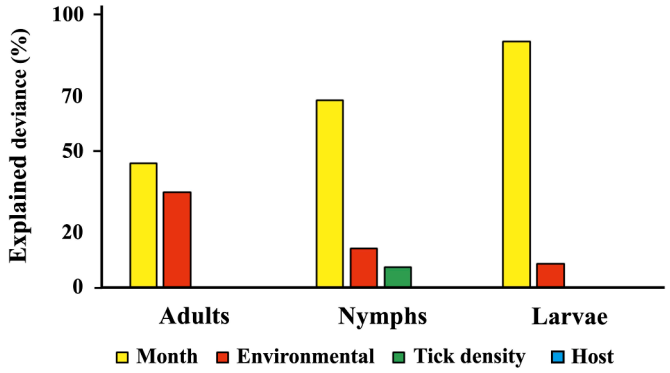

Fig. 3. Bar chart showing the proportion of the deviance explained by the pure effect of each factor in the questing density models for each *Ixodes ricinus* stage.

NA values of explained deviance were provided. All interaction values are displayed in Appendix E.

3.4. Temporal overlapping between developmental stages

After performing the overlap analysis between pairs of developmental stages of *I. ricinus*, we found that there were statistically significant differences between some stages. The overlap value (and 95% CI) between adults and nymphs was 0.82 (± 0.02) and the significance level ($p = 0.296$) indicated no significant difference in the temporal pattern of adult and nymphal *I. ricinus* density in our study area. In contrast, the overlap values were 0.38 (± 0.03) between adults and larvae and 0.26 (± 0.03) between nymphs and larvae; in both cases, the significance value of the overlap was $p < 0.001$. Therefore, the differences in the temporal density pattern between these stages were statistically significant in our study area. Both adults and nymphs had a major peak of activity in spring and a minor peak in autumn, while larvae had an activity peak in summer (Fig. 4).

3.5. Predictive capacity of the models

The correlation test between observed and predicted density values on test datasets showed Pearson correlation coefficients of 0.50 for adults, 0.65 for nymphs, and 0.59 for larvae. Calibration plots of all these correlations are shown in Appendix F.

The validation of the model using two of the three study years for calibration and the remaining one for prediction provided Pearson correlation coefficients between 0.543 and 0.392 for adults, between 0.674 and 0.379 for nymphs, and between 0.632 and 0.473 for larvae. The combination of train-test years that offered the best correlation coefficient varied for each developmental stage, with no constant pattern of prediction between years and stages (see Appendix G).

The external validation of the model was carried out using 385 monthly registers from other studies. However, the number of registers on which we could project the model after applying the MESS was considerably reduced. A total of 104, 105, and 128 samples were used to validate the models for adults, nymphs, and larvae, respectively, obtaining Pearson correlation coefficients of 0.173, 0.277, and 0.292, respectively (Appendix H).

3.6. Spatiotemporal projection of the models

Concerning the temporal variation of model prediction accuracy for the phenology of each *I. ricinus* stage with respect to observations, we found high levels of overlapping in all three stages (Appendix I). The overlap value (and the 95% CI) for adults was 0.90 (± 0.09), with a significance level of $p = 0.621$, which means that there is no significant difference in the temporal pattern of density between our original data and the projection of the model. We found similar results for nymphs and larvae. The overlap value for nymphs was 0.91 (± 0.08) with $p = 0.461$, while it was 0.75 (± 0.22) with a $p = 0.481$ for the larvae, however the peak of activity predicted for larvae is slightly deviated from the overall trend of our data. This informs us that our models can adequately predict the periods of activity for each stage of development.

Regarding the spatial projection capacity of the models to identify high and low-density grids consistently over time, the average standard deviation (and dispersion values) of the predicted abundance (rescaled between 0 and 1) of the adults was 0.094 (± 0.044), 0.126 (± 0.055) for nymphs, and 0.056 (± 0.035) for larvae. This informs us that the models robustly predict high- and low-density areas over time and that high and low density predicted areas for each *I. ricinus* stages does not change over time.

The spatial projection of the model for adult *I. ricinus* (Fig. 5; Appendix J) showed higher predicted densities around mountain systems in the region, including 'Montes de León', the Cantabrian Mountains, the Iberian System and the Pyrenees (https://www.ign.es), as well as large areas of 'Galicia' and the eastern coast of the Cantabrian Sea. As the projection of the adult tick model contains much uncertainty, the results should be carefully examined and interpreted with caution. In the case of nymphs, the model predicts higher densities mainly in coastal areas,

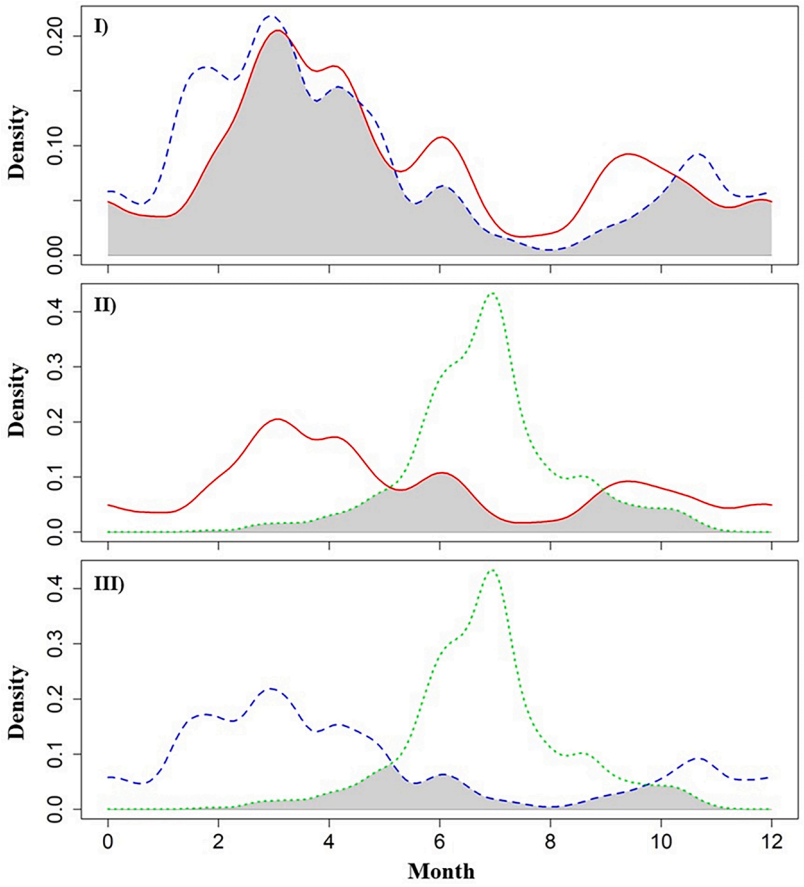

Fig. 4. Temporal overlap of paired *Ixodes ricinus* stages questing density per month: I) adults-nymphs; II) adults-larvae; and III) nymphs-larvae. The phenology of adults is shown in solid-red, that of nymphs in dashed-blue and that of larvae in dotted-green. The overlapping period is indicated by the grey-shadowed region in the charts.

especially in regions such as 'Galicia', 'Asturias', and the Basque Country, where environmental conditions are mild. However, there are also some mountainous areas where the model predicts high densities, such as 'Montes de León' and the Cantabrian Mountains. We only observe large areas of high uncertainty in some areas of 'Galicia' and in the southern zone of 'Burgos'. Similarly, the larvae model presents many valid projection areas, however, a general pattern of high density of *I. ricinus* larvae was predicted for some areas of Atlantic Ocean climate influence in the Basque Country and Navarre mainly. However, all these areas are of high uncertainty, so the conclusions should be taken with caution. The models for adults and nymphs in Fig. 5 are projected for the month of May, while the model for larvae is projected for the month of August for better visualization of spatial results. The projections for each month, along with their corresponding MESS analysis, can be found in Appendix J.

4. Discussion

This study provides novel insights to the major determinants of questing *I. ricinus* density in the southernmost European limit of its core distribution area and into its spatiotemporal dynamics. Our findings accurately describe the main drivers for the different developmental stages of *I. ricinus* in northern Spain and a predictive framework that could help in better estimating spatiotemporal changes in their densities. Our models may represent the starting point for building more accurate predictive models to identify the spatiotemporal windows of abundance of the different stages of *I. ricinus*. Accurate models could enable early warnings to be issued to local human populations at risk of exposure to *I. ricinus*, e.g., hunters, hikers, or bikers, reducing the rate of tick bites and, perhaps, the transmission of tick-borne pathogens.

4.1. Considerations regarding the methodological approach

It is important to recognize that no sampling method is perfect and choosing the most suitable method depends on the study's objectives, considering the advantages and limitations of each approach (Cuadrado-Matías et al., 2024). Sampling frequency varied amongst the sites, with three out of 13 sites only being sampled in 2012. Furthermore, fortnight sampling was performed in several surveys in R_1 in contrast to monthly sampling in R_2, although this variation was controlled by the models' structure (mixed nested effects) and, therefore, did not affect the validity of the modelling results.

An important limitation of our study was the lack of information on temporal variations in host availability for different stages of *I. ricinus*. We assumed that host availability for *I. ricinus* adults would be a crucial determinant for the density of questing larvae, nymphs, and adults because several studies have shown the important role that ungulates play as amplifiers of *I. ricinus* (Adamska, 2020; Ruiz-Fons and Gilbert, 2010; Fabri et al. 2021), but this effect may be more noticeable spatially than temporally with the available data (Peralbo-Moreno et al., 2022). At the local scale, host availability for adults and nymphs of *I. ricinus* may vary over time despite minimal inter-annual fluctuations in host density (Rosà et al., 2019) due to variations in the frequency of space use by these animals. However, the variables that we were able to construct with the available information cannot inform about seasonal variation in host abundance. In addition, larvae of *I. ricinus* seem to select small vertebrates for feeding in the study area according to available studies (Estrada-Peña et al., 2005), so that densities of questing nymphs may be

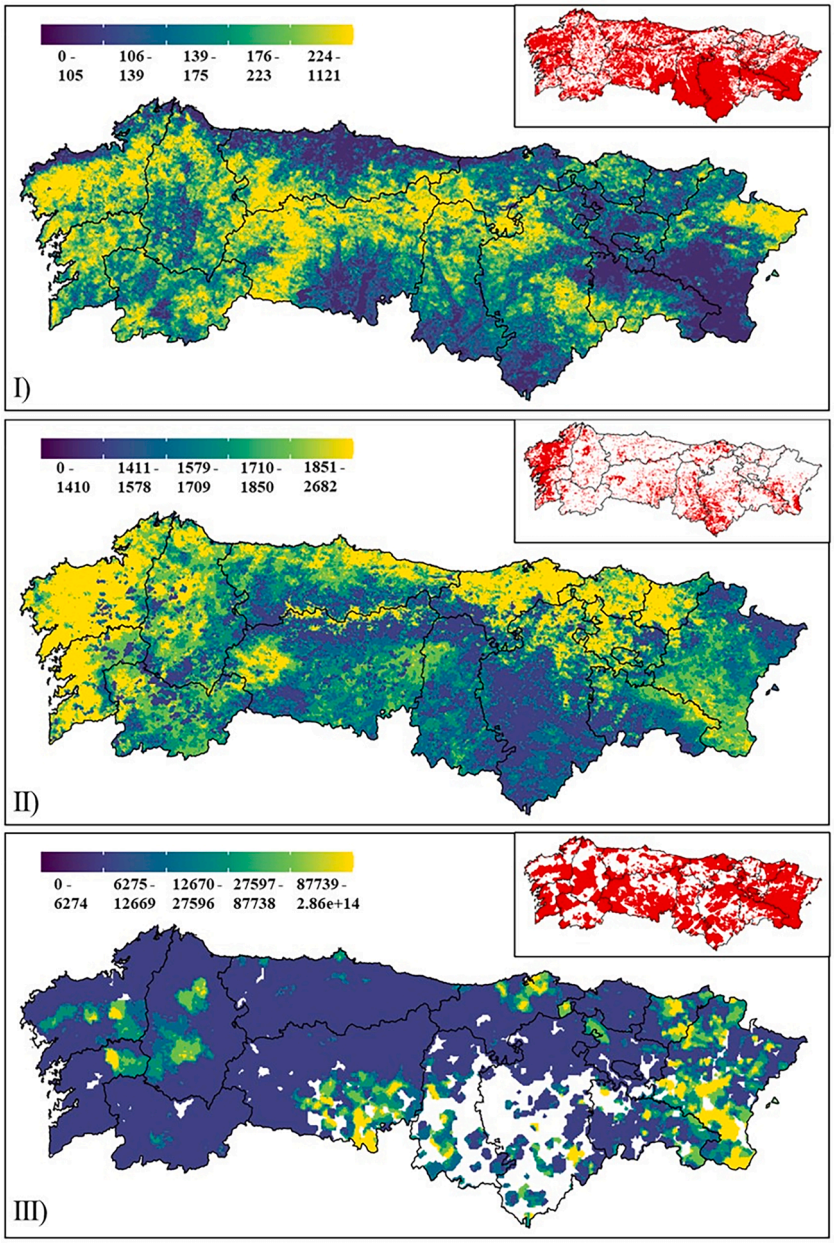

Fig. 5. Maps showing the projection of the questing density models for each stage of *I. ricinus* to northwestern Iberian Peninsula: I) adults; II) nymphs; III) larvae. Lighter colors indicate higher predicted density (ticks/hectare), while darker colors indicate lower density, and density categories were established using percentiles. The models for adults and nymphs are projected for the month of May, while the model for larvae is projected for the month of August, as they are the main months of activity for each stage. Blank areas on the larvae map are due to lack of information in the livestock census for those municipalities. The map in the upper right corner shows (in red) the areas where the MESS analysis indicates high uncertainty in the model for that prediction.

strongly conditioned by local abundance and temporal variations in the availability of these hosts (O'Neill et al., 2023). Large vertebrates, such as ungulates, may play an important role as hosts for *I. ricinus* larvae in northern Spain, as it has been reported in other regions of Europe (e.g. Tälleklint and Jaenson, 1997). But perhaps, to properly evidence this role, it is necessary to consider the abundance of ungulates at the period of peak larval activity, which we were unable to estimate.

Even though our model has good temporal coverage, its spatial projection capability may be limited due to the low number of sampled points ($N = 13$). However, we are confident that the model can provide reliable projections to areas with similar conditions to those in our study and we relied on the MESS analysis to identify areas with higher uncertainties in predictions. Fitting the models with new density input data in the future would significantly improve their predictive accuracy at small spatiotemporal scales.

4.2. Tick diversity and I. ricinus questing density and activity patterns

The findings largely confirm our expectations regarding the structure of the community of exophilic ticks in northern Spain, as previously reported by Ruiz-Fons et al. (2006), Barandika et al. (2010), and Remesar et al. (2019). The most abundant tick species was *I. ricinus*, for which the highest abundances are reported in northern Spain (Barandika et al., 2010; Remesar et al., 2019) where suitable moisture and temperature conditions support large populations (Ruiz-Fons et al., 2006, 2012). Outside northern Spain, *I. ricinus* is only locally abundant in areas of mainland Spain with moisture-enriched microclimates (Peralbo-Moreno et al., 2022).

Other abundant tick species were *Haemaphysalis punctata* in R_1 and *Haemaphysalis concinna* in R_2 (Table 2). The higher abundance of *H. concinna* in R_2 might be attributed to its requirement for high moisture levels and low temperatures. In contrast, *H. punctata* appears to be more tolerant to changing environmental conditions (Estrada-Peña et al., 2013), and thus it was found in the two study regions. Both species were identified as carriers of different pathogens (Barandika et al., 2008; Portillo et al., 2008; del Cerro et al., 2022), including some species of the genus *Borrelia*.

The causes for the notable differences in *I. ricinus* density specifically between the two study regions were not explored in this study and most probably are due to differences in both abiotic and biotic environmental conditions between a purely Atlantic climate influenced region (R_2; Cfb climatic region - temperate climate with no dry season - according to the Köppen-Geiger climate classification; Chazarra-Bernabé et al., 2022) and an Atlantic-to-Mediterranean climatic transition region (R_1; Csb climatic region - temperate climate with dry summer). Differences in abiotic environmental conditions between the two regions, in relation to mean values and variation in different time periods of NDVI and LST, can be clearly seen in the maps included in Appendix K. Spatial variations in information on wild hosts of *I. ricinus* are available at ENETWILD-consortium et al. (2022). The activity patterns of adult *I. ricinus* were similar between regions, while those of the immatures differed, which may clearly shape regional variations in abiotic environmental favourability. Whereas adults are more tolerant to changes in environmental conditions, allowing them to remain active for longer periods of time, even in less favourable conditions, the immatures are only active within the most favourable and, thus, shorter periods (Gray et al., 2009). Estrada-Peña et al. (2004) showed a unimodal peak of larval density in summer and a decreasing rate of development towards autumn. It would be expected that only those larvae that are active in spring-summer and successfully find a host would reach a sufficiently rapid rate of development in the summer conditions to moult into nymphs. The nymphal peak observed in autumn in Estrada-Peña et al. (2004), as well as in our study sites, is probably the result of the sum of the unfed spring nymphs re-emerging after a summer diapause and the nymphs emerging from larvae that fed in spring-to-summer. Nymphs emerging at the spring peak (April-June) may feed and moult to adults rapidly in summer, adding unfed adults to those that did not find a host in spring and, together, giving rise to the autumn adult peak (Estrada-Peña et al., 2004; Barandika et al. 2010; this study). Adult females feeding during the spring would lay their eggs in a period of high developmental rate, while those feeding in autumn would not give rise to larvae until mid-following spring when development rates begin to increase, which together would drive the summer larval peak observed by Estrada-Peña et al. (2004) and us. Larvae need a certain accumulation of degree-days to accelerate their development and hatch from eggs (Alonso-Carné et al., 2016). Barandika et al. (2010) found higher hydrological stress in continental sites when compared to the more coastal ones, as we have observed in our own study. However, variations in winter temperatures (milder in areas of Atlantic climatic influence) and hydrological stress would explain that, in more northern latitudes of Spain, the seasonality in nymph activity is less pronounced and that even the unimodality in larvae activity disappears in some sites and years (see Barandika et al., 2010). Thus, the developmental cycle of *I. ricinus* in the North of Spain would be quite homogeneous with slight local variations with respect to that described by Estrada-Peña et al. (2004).

We observed large temporal overlaps in nymph and adult activity in the study area (Fig. 4) that may have potential implications for the exchange of pathogens when they feed together on a host (Ogden et al. 1997), and it is usual to find *I. ricinus* nymphs and adults co-feeding on wild ungulates (Carpi et al., 2008; Voordouw, 2015). According to our findings, we hypothesize that the co-feeding exchange of pathogens between *I. ricinus* adults and nymphs may perhaps pose an added pathway to increase pathogen prevalence in questing adult ticks, which could result in an increased pathogen transmission rate to susceptible hosts. The transmission potential for *I. ricinus*-borne pathogens between cohorts of *I. ricinus* nymphs and larvae seems negligible according to the low overlapping activities of both stages in our study area. This finding contrasts with those of central and eastern Europe where this transmission pathway is a major driver of TBEV prevalence and transmission risk to humans (Randolph, 2011). However, demonstrating pathogen transmission between co-feeding *I. ricinus* adults and nymphs requires appropriate experimental studies, as the role of this transmission route remains unclear for pathogens such as *B. burgdorferi* sensu lato (Cutler et al., 2021).

4.3. Determinants of I. ricinus questing density

The modelling results indicate that a multifactorial analysis is the most accurate approach for modelling the temporal density of exophilic ticks at small spatiotemporal scales (Tables 3–5). This is because all factors, including environmental conditions, host availability, seasonality, and density-dependant effects, as well as their interactions, play crucial roles in explaining the variance in tick density over space and time (Gilbert, 2010; Kilpatrick et al., 2017; Peralbo-Moreno et al., 2022).

The best fitted models for the three *I. ricinus* stages highlight the relevance of the seasonality in their activity as major determinant of density, which is undoubtedly connected to the environmental determinants of both development and host-seeking behaviour as the results of the deviance partition analysis indicate. The slightly more limited activity period of the nymphs in comparison to the adults and the markedly limited summer activity of the larvae were indicated by the high influence of the study month on their density when compared to the adults.

Our findings suggest that summer conditions play an important role in modulating adult *I. ricinus* density in contrast to winter conditions that were not found to be influential, indicating that the summer season is the limiting period for the activity of adults and that pathogen transmission risks from adult bites is at its lowest rate in the summer in northern Spain. This result matches with the distribution limits of the species, as further south from the core distribution it is only found sporadically in areas characterized by mild summer temperatures and high humidity conditions, as evidenced in Peralbo-Moreno et al. (2022).

High variation in environmental abiotic conditions (especially in winter) affected nymph density. The higher the variability in winter temperatures and in the variability of NDVI between four and seven months before capture, the lower the density of *I. ricinus* nymphs. As previously observed (Dautel and Knülle, 1997; Estrada-Peña et al., 2012), extreme events can jeopardize the survival of organisms by temporarily exceeding the species' tolerance limits. This is especially so in the case of an area where environmental constraints are limiting for the species because environmental ranges probably are around the tolerance limits of the species (Sexton et al., 2009: Poh et al., 2019). The less extreme winter conditions in R_2 are reflected in higher abundances of nymphs in winter, while in R_1, and especially in years when winter temperature and NDVI drop sharply, we find a drop in peak activity (Appendix L). As mentioned in Medlock et al. (2013), snow cover can enhance the survival of ticks during winter by preventing repeated cycles of freezing and thawing. This aligns with the findings suggested by our model, indicating that rapid fluctuations in winter temperatures primarily impact the more vulnerable stages of ticks, such as nymphs. Additionally, it is important to consider that these extreme conditions also affect the hosts upon which ticks feed. Smaller vertebrates, which seem to be relevant hosts for *I. ricinus* larvae in our study area (Estrada-Peña et al., 2005), tend to be more susceptible to these conditions compared to larger mammals, which are relevant hosts for nymphs and essential hosts for adults (Estrada-Peña et al., 2005; Barandika et al., 2010).

Although the parameters defining host availability for *I. ricinus* larvae and nymphs in this study were not the most appropriate, the

slight positive effect on larval density of local density of domestic ruminants suggests the dependence of larvae on the feeding success of adult females from whose eggs they originate (see Appendix M). This also applies to adults and nymphs, as the density of domestic ruminants also played an important role in their models, but due to problems arising from the complexity of the models we had convergence problems when selecting these variables. The success of adult females in finding a host to feed on is a crucial parameter in larval density (O'Neill et al., 2023) and this success is strongly linked to the availability of large animals (Gilbert, 2010; Kilpatrick et al., 2017; Peralbo-Moreno et al., 2022). Getting data on the temporal variation in host availability affecting each *I. ricinus* stage could greatly improve future studies and allow us to better understand how modulating host availability could result in changing *I. ricinus* density. The observed positive influence of summer soil temperature on *I. ricinus* larvae density clearly fits the observed summer peak (Alonso-Carné et al., 2016) and fits with the requirements of larvae on accumulated temperatures to hatch from eggs and accelerate their development (Estrada-Peña et al., 2004). The high activity of *I. ricinus* larvae in summer despite the high hydrological stress in this season of the year indicates that their abundance is highly dependant on temperature because the temperature conditions necessary for their development within eggs only occur in summer in northern Spain (Appendix L).

4.4. Model projections

Model predictions applied to values beyond the calibration limits of the models may lead to erroneous results and conclusions. The application of MESS makes it possible to identify areas where model predictions are more or less accurate, allowing for more or less confident discussions about the spatial projection of the models. Although the external validation of the models does not provide very high correlation values given the sample size we used, it should be noted that the models can discern between high- and low-density areas.

The projection of the adult density model generally indicates that coastal areas and the southernmost low inland areas are areas of low favourability (Fig. 5a). However, the central projection zone, comprising numerous mountainous areas, exhibits a higher predicted adult density. This information contrasts with other studies conducted in the northernmost regions of the species distribution (Medlock et al., 2013; Qviller et al., 2013), where the density of *I. ricinus* tends to decrease with increasing altitude (Gilbert, 2010) likely due to the contrasting effect of the primary limiting factor, temperature, at each distributional boundary. As altitude increases, the temperature decreases, and the required degree-days for development and moulting may take longer periods of time or not be met at all (Gray et al., 2021) (Appendix K). It is expected that this effect of altitude also occurs in our study area, so perhaps these observations only result from the high uncertainty in the prediction of the adult model. Contrary to adults, the nymph model predicts higher densities in several coastal areas with lower uncertainty, where the temperature is more constant (see Appendix K for further information about temperature in the study area).

The projection of the models for adults and nymphs indicates several areas in "Galicia" (despite the high uncertainty in this area), "Asturias", and the Basque Country as areas of high *I. ricinus* abundance. These areas have the highest rate of Lyme borreliosis hospitalisations in Spain and also have the highest increase in the rate of Lyme hospitalisations in recent years (Bonet Alavés et al., 2016; Amores-Alguacil et al., 2022).

The model for larvae identified patches of the study region with high summer temperatures as of high abundance. However, its predictions are the least reliable of the three models, so these need to be improved for better accuracy in the future.

5. Conclusions

- The environmental factors that influence the spatiotemporal questing abundance of *I. ricinus* vary within the distribution range of the species. In the north of the Iberian Peninsula, the most limiting factors of the abundance of questing adults and nymphs are associated with temperature, especially summer temperature for adults, and with variation in soil moisture and temperature conditions for nymphs. However, larvae are positively influenced by summer temperatures, probably because higher summer temperatures are essential for their development, allowing larvae to hatch in early summer from eggs laid by females in late spring and subsequently seek hosts throughout the summer.
- In the north of the Iberian Peninsula, there is a greater temporal overlap of seasonal activity between adults and nymphs of *I. ricinus* ticks compared to larvae. This may perhaps have implications for the ecology of *I. ricinus*-borne pathogens that should be considered in future research approaches. The low seasonal overlap in larval and nymphal activity indicates that the likelihood of non-systemic co-feeding transmission of pathogens between these stages should be low.
- Our models are accurate in predicting the seasonality in the activity of the different life stages of *I. ricinus* in the north of the Iberian Peninsula. Moreover, their spatial predictive accuracy is good although limited. Therefore, they may constitute the basis for the development of models that are able to predict with very high reliability the spatiotemporal windows of higher *I. ricinus* abundance. Such models would help warning the human population at risk of interaction with these ticks in a pre-emptive manner and could perhaps reduce the risk of *I. ricinus*-borne pathogen transmission.

Funding

This study is the result of funding by the Spanish Ministry for the Science and Innovation/Spanish Research Agency (MCIN/AEI/10.13039/501100011033/) and EU Regional Development Fund (EU-RDF) through projects CGL2017–89,866-R, PID2019–111699RB-I00 & PID2022–141906OB-C21, and by the Regional Government of Castilla-La Mancha and the EU European Social Fund (EU-ESF) through project SBPLY/19/180501/000321. We also acknowledge funding by MCIN/AEI/10.13039/501100011033 and by the European Union "Next Generation EU"/PRTR. Recovery, Transformation and Resilience Plan - Next Generation EU" through the TED2021–132599B-C21/22 project, and by "Instituto de Salud Carlos III", MCIN/AEI/10.13039/501100011033 and EU-Next Generation EU funds through "CIBER-Consorcio Centro de Investigación Biomédica en Red (CB 2021/13/00083)". AP-M would like to thank the MCIN/AEI, EU-ESF and the University of Castilla-La Mancha (UCLM) for funding support through contract 2019-PREDUCLM-10932. Open Access funding provided by the CRUE-CSIC agreement with Elsevier.

CRediT authorship contribution statement

Alfonso Peralbo-Moreno: Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Alberto Espí:** Writing – review & editing, Resources, Project administration, Investigation, Funding acquisition, Data curation. **Jesús F. Barandika:** Writing – review & editing, Resources, Project administration, Investigation, Funding acquisition, Data curation. **Ana L. García-Pérez:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Pelayo Acevedo:** Writing – review & editing, Writing – original draft, Validation, Supervision, Conceptualization. **Francisco Ruiz-Fons:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

Acknowledgments

We are grateful to Asturias and Basque Country Regional Governments for facilitating the permits for tick surveys. We greatly appreciate the constructive criticism provided by three anonymous reviewers and the managing editor of Ticks and Tick-borne Diseases (Dr. Olaf Kahl), which has undoubtedly resulted in a significant improvement in the presentation of our study in a clear and concise manner to readers.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2024.102373.

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