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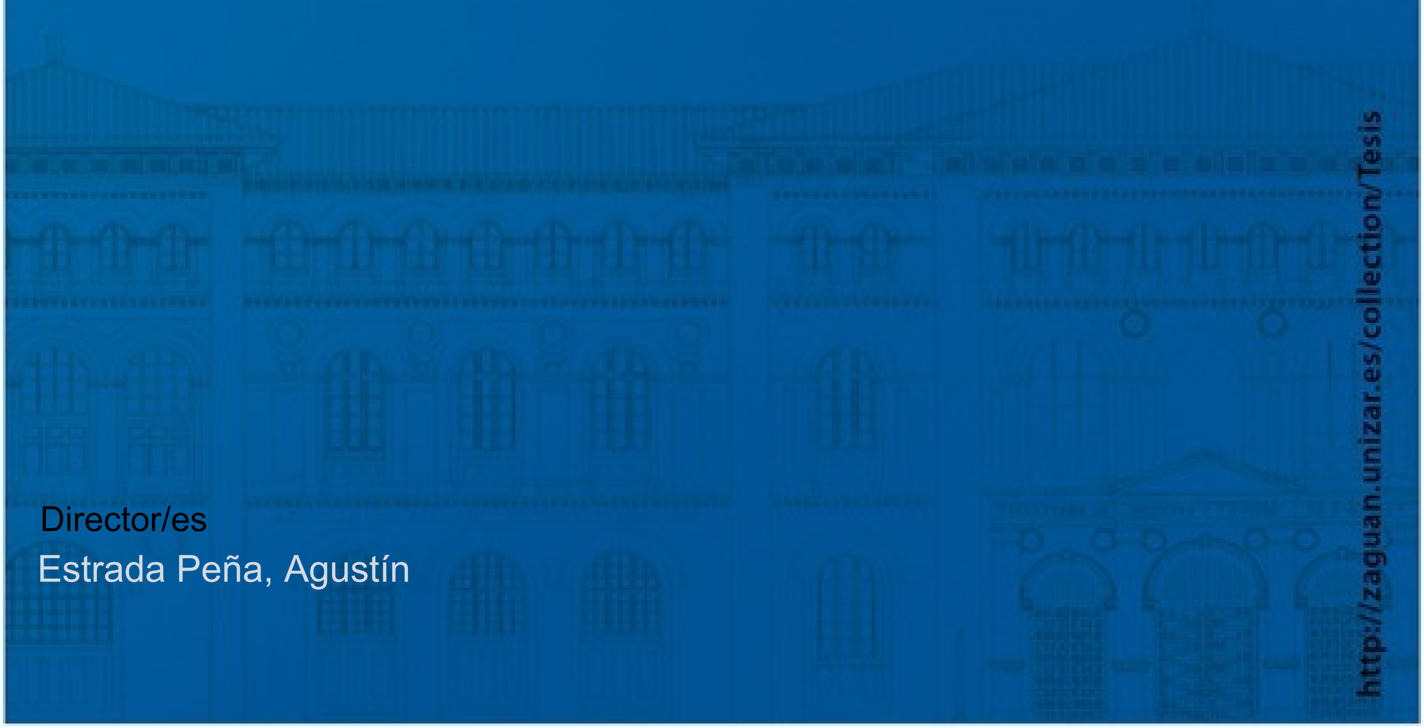
Natalia Fernández Ruiz

# The challenges to Public Health of tick - borne pathogens Los retos para la Salud Pública de los patógenos transmitidos por garrapatas



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# THE CHALLENGES TO PUBLIC HEALTH OF TICK - BORNE PATHOGENS LOS RETOS PARA LA SALUD PÚBLICA DE LOS PATÓGENOS TRANSMITIDOS POR GARRAPATAS

Autor

Natalia Fernández Ruiz

Director/es

Estrada Peña, Agustín

**UNIVERSIDAD DE ZARAGOZA**
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# The challenges to Public Health of tick - borne pathogens

# Los retos para la Salud Pública de los patógenos transmitidos por garrapatas

Natalia Fernández-Ruiz

UNIVERSIDAD DE ZARAGOZA, 2023

Director:

Dr. Agustín Estrada-Peña

# The challenges to Public Health of tick - borne pathogens

# Los retos para Salud Pública de los patógenos transmitidos por garrapatas

TESIS POR COMPENDIO DE PUBLICACIONES

Natalia Fernández-Ruiz

UNIVERSIDAD DE ZARAGOZA, 2023

Director:

Dr. Agustín Estrada-Peña

*A mi padre, mi madre y a mi hermano,*
*Por ser siempre mi punto de anclaje*

*Fue tan largo el duelo que al final,*
*Casi lo confundo con mi hogar*
(Vetusta Morla, “Cuarteles de invierno”)

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At some point during the trip, I had to change the car, and more people joined the vehicle I was driving. Alejandra Trobo came into my life by coincidence, she is my vitamin person. Patricia Manzano was sent to me by the stars; I met her at night hitchhiking, destiny knew it was the right time to send me my other half.

Along the way I stopped in different places whenever I needed a bit of fresh air, I turned off towards the A-8 and made a little stop next to María Gutiérrez just to feel it. But she never let me get too cold. When I needed warmth I would go down south with my friend Carlos Ciria, to steal a bit of light. On my way down, I would stop in a remote place, sunk in a valley, to meet my friends Mar Suárez and Juan Carlos Rodríguez, thank you for making me disconnect from work for a while. To my cousin Marileo, my cousin Ana, and my aunt Nori, for being the soundtrack of all my summers.

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# Abstract

Climate change is expected to have significant impacts on various aspects of ecosystems, including the spread and distribution of ticks. This is a subject of significant concern among researchers and Public Health experts. Tick range expansion is linked to shifts in the geographical distribution of tick populations. Rising temperatures and altered precipitation patterns can create new suitable habitats for ticks, allowing them to extend their range to regions where they were previously absent. Warmer temperatures can lead to increased tick activity and longer seasonal activity periods. Also tick populations depend on the availability of hosts, primarily small mammals and birds. Climate change can impact host species' distribution, abundance, and behavior which influence on tick populations' dynamics. Changes in the availability and movement patterns of host animals can impact the opportunities for ticks to feed and acquire pathogens. Furthermore, alterations in host behaviour, such as changes in migratory patterns, can influence the spatial dynamics of tick-borne pathogens. These changes may have several consequences.

Climate change can lead to shifts in the geographic range of tick species. As temperatures rise and suitable habitats expand, ticks may move into new regions, including higher latitudes and altitudes. This expansion can increase the potential exposure of human and animal populations to tick-borne pathogens. *Ixodes ricinus* is a vector for several human and animal tick related diseases, such as Lyme disease, tick-borne encephalitis, anaplasmosis, and babesiosis. As the ticks' distribution expands, so does the potential spread of these diseases to new areas. Areas that were previously unaffected or had low incidence rates of these diseases may become more vulnerable. Reports on similar issues are being reported form other species. The northern spread of *I.ricinus* (as well as other species) and the diseases they transmit pose significant public health challenges. Lyme borreliosis, in particular, is a well-known tick-borne illness that can lead to severe health issues if not diagnosed and treated promptly. With the expansion of tick populations and the pathogens they carry, there is an increased need for surveillance, prevention strategies, and public awareness campaigns. Warmer temperatures can accelerate tick development, leading to shorter generation times and increased reproduction rates. This can result in larger tick populations and a higher risk of pathogen transmission. Additionally, changes in precipitation patterns can impact tick survival and the availability of suitable habitats.

Addressing the impact of climate change on tick populations requires a multi-faceted approach. It involves understanding and monitoring the changing distribution of tick species, assessing the

risk of tick-borne diseases in different regions, promoting measures to reduce tick exposure (e.g., using tick repellents, wearing protective clothing, performing tick checks), and investing in research for new prevention and control methods. It is crucial to keep in mind that climate change is a complex and dynamic phenomenon with far-reaching implications for ecosystems and public health. The interplay between climate, tick populations, and tick-borne diseases is still an active area of research, and ongoing studies will provide more insights into the potential impacts in the future. Given the complex interactions between climate, ticks, and pathogens, it is challenging to predict precise outcomes. Satellite imagery can provide information on various ecological factors that influence tick distribution. For instance, models can analyse land cover types, proximity to water bodies, elevation, and vegetation indexes derived from satellite data to identify the ecological correlates of tick abundance. This knowledge can help researchers and policymakers understand the underlying factors driving tick populations and guide targeted interventions. By continuously monitoring and analysing satellite imagery, models can contribute to the development of early warning systems for tick-borne diseases. Changes in environmental factors captured by satellite data, such as vegetation density or temperature, can serve as indicators of potential increases in tick populations. Timely alerts can be sent to public health agencies to facilitate proactive measures and public awareness campaigns.

# Resumen

El cambio climático tiene importantes repercusiones en diversos aspectos de los ecosistemas, entre ellos la propagación y distribución de las garrapatas, con creciente preocupación entre investigadores y expertos en Salud Pública. La expansión de las garrapatas está vinculada a cambios en su distribución geográfica provocados por el aumento de la temperatura y la alteración de los patrones de precipitaciones, que pueden crear nuevos hábitats adecuados para las garrapatas, permitiéndoles ampliar su área de distribución a regiones en las que antes estaban ausentes. Las temperaturas más cálidas pueden aumentar la actividad de las garrapatas y alargar los periodos de actividad estacional. Además, las poblaciones de garrapatas dependen de la disponibilidad de hospedadores, principalmente pequeños mamíferos y aves. El cambio climático también puede afectar a la distribución, abundancia y comportamiento de las especies hospedadoras, lo que influye en la dinámica de las poblaciones de garrapatas afectando a la probabilidad de alimentarse y adquirir patógenos. Además, las alteraciones en el comportamiento de los hospedadores, como los cambios en las pautas migratorias, pueden influir en la dinámica espacial de los patógenos transmitidos por garrapatas. Estos cambios pueden tener varias consecuencias.

El cambio climático puede provocar cambios en el área de distribución geográfica de las especies de garrapatas. A medida que aumentan la temperatura y se amplían los hábitats adecuados, las garrapatas pueden desplazarse a nuevas regiones, incluidas latitudes y altitudes más elevadas. Esta expansión puede aumentar la exposición potencial de las poblaciones humanas y animales a patógenos transmitidos por garrapatas. *Ixodes ricinus* es un vector de patógenos para humanos y animales, como los agentes causantes de la enfermedad de Lyme, la encefalitis transmitida por garrapatas, la anaplasmosis y la babesiosis. A medida que se amplía la distribución de las garrapatas, también lo hace la posible propagación de estas enfermedades a nuevas zonas. Las zonas que antes no estaban afectadas o tenían bajas tasas de incidencia pueden volverse más vulnerables. Se están notificando casos similares de otras especies. La propagación septentrional de *I. ricinus* (así como de otras especies) y los patógenos que transmiten plantean importantes problemas de Salud Pública. La Borreliosis de Lyme, en particular, es una conocida enfermedad transmitida por garrapatas que puede provocar graves problemas de salud si no se diagnostica y trata con prontitud. Con la expansión de las poblaciones de garrapatas y los patógenos que transmiten, aumenta la necesidad de vigilancia, estrategias de prevención y campañas de concienciación pública. Las temperaturas más cálidas pueden acelerar el desarrollo de las garrapatas, acortando los tiempos de generación y aumentando las tasas de reproducción. Esto puede aumentar las poblaciones de garrapatas y el

riesgo de transmisión de patógenos. Además, los cambios en los patrones de precipitaciones pueden afectar a la supervivencia de las garrapatas y a la disponibilidad de hábitats adecuados.

Abordar el impacto del cambio climático en las poblaciones de garrapatas requiere un planteamiento polifacético. Implica comprender y vigilar la distribución cambiante de las especies de garrapatas, evaluar el riesgo de enfermedades transmitidas por garrapatas en las distintas regiones, promover medidas para reducir la exposición a las garrapatas (por ejemplo, usar repelentes de garrapatas, llevar ropa adecuada, realizar controles de garrapatas) e invertir en la investigación de nuevos métodos de prevención y control. Se hace crucial comprender el cambio climático como un fenómeno complejo y dinámico con implicaciones de gran alcance para los ecosistemas y la Salud Pública. La interacción entre el clima, las poblaciones de garrapatas y los patógenos transmitidos por garrapatas es un campo de investigación activo, y los estudios en curso proporcionarán más información sobre las posibles repercusiones en el futuro. Dadas sus complejas interacciones, resulta difícil predecir con exactitud los resultados. Las imágenes de satélite pueden aportar información sobre diversos factores ecológicos que influyen en la distribución de las garrapatas. Por ejemplo, los modelos pueden analizar los tipos de cobertura del suelo, la proximidad a masas de agua, la elevación y los índices de vegetación derivados de los datos de satélite para identificar las correlaciones ecológicas de la abundancia de garrapatas. Este conocimiento puede ayudar a investigadores y responsables políticos a comprender los factores subyacentes que determinan las poblaciones de garrapatas y orientar las intervenciones específicas. Mediante el seguimiento y análisis continuos de las imágenes de satélite, los modelos pueden contribuir al desarrollo de sistemas de alerta temprana de enfermedades transmitidas por garrapatas. Los cambios en los factores ambientales captados por los satélites, como la densidad de la vegetación o la temperatura, pueden servir de indicadores de posibles aumentos de las poblaciones de garrapatas. Las alertas oportunas pueden enviarse a los organismos de salud pública para facilitar la adopción de medidas proactivas y la realización de campañas de concienciación pública.

# Appendix

This Thesis is composed of the following publications, to be presented as a thesis by publications, as follows:

Fernández-Ruiz, Natalia, and Estrada-Peña, Agustín. "Could climate trends disrupt the contact rates between *Ixodes ricinus* (Acari, Ixodidae) and the reservoirs of *Borrelia burgdorferi* s.l.?. PloS one 15.5 (2020): e0233771. Q1; JCR: 3.7

Fernández-Ruiz, Natalia and Estrada-Peña, Agustín. "Towards new horizons: Climate trends in Europe increase the environmental suitability for permanent populations of *Hyalomma marginatum* (Ixodidae)." Pathogens 10.2 (2021): 9 Q2; JCR: 0.81

Estrada-Peña, Agustín, D'Amico, Gianluca, and Fernández-Ruiz, Natalia. "Modelling the potential spread of *Hyalomma marginatum* ticks in Europe by migratory birds." International Journal for Parasitology 51.1 (2021): 1-11. Q1 (Parasitology); JCR: 4

Estrada-Peña, Agustín, and Fernández-Ruiz, Natalia "Is composition of vertebrates an indicator of the prevalence of tick-borne pathogens?." Infection, Ecology & Epidemiology 12.1 (2022): 2025647. Q2 (Environmental science); JCR: 0.71

Fernández-Ruiz, Natalia, Estrada-Peña, Agustín, McElroy, Sharon and Morse, Kaitlyn "Passive collection of ticks in New Hampshire reveals species-specific patterns of distribution and activity." Journal of Medical Entomology 60.3 (2023): 575-589. Q1 (Veterinary miscellaneous); JCR: 2.1

Estrada-Peña, Agustín, and Fernández-Ruiz, Natalia. "An agenda for research of uncovered epidemiological patterns of tick-borne pathogens affecting human health." International Journal of Environmental Research and Public Health 20.3 (2023): 2206. Q2; JCR:0.81

# Introduction

## A background on ticks and tick-borne pathogens.

Pathogens transmitted by arthropods have gained importance in Public Health in the last few decades (Chala & Hamde, 2021). Both mosquitoes (and in a lesser degree other Diptera) and ticks are the main arthropods that have been involved in the transmission of pathogens to humans (Swei *et al.*, 2020). Mosquitoes are probably the most studied vectors owing to the importance of the diseases associated to the pathogens they transmit, like *Plasmodium*, Zika, Dengue or Chikungunya viruses (Yee *et al*., 2022). However, ticks transmit the largest variety of pathogens to humans in the northern hemisphere (Dennis & Hayes, 2002). Like Diptera, ticks inoculate infection agents through the feeding process, as they are obligate hematophagous parasites (Anderson & Magnarelli, 2008). As a consequence of the inoculation of bacteria, virus or parasites to humans, an increased incidence or prevalence of tick related diseases is being reported in animals and humans (Madison-Antenucci *et al.*, 2020). The number of tick related diseases with public health importance in Europe is increasing (Lemoine *et al*., 2022; Cuadrado-Matías *et al.*, 2022) mainly because the increased awareness and the improvement of the detection and diagnosis techniques. In animals, diseases like anaplasmosis, theileriosis or babesiosis are responsible of a serious economic burden (Antunes *et al.*, 2016). Since these diseases tend to occur in resource-poor countries, the economic losses have a higher impact on the productivity of the affected areas, resulting in for example food shortage (Renneker *et al.*, 2013). Some pathogens like *Anaplasma phagocytophilum* could produce disease in both animals and humans, while others only produce a clinical form in humans (Elhamiani Khatat *et al.*, 2017; Bente *et al.*, 2013). For instance, both the borreliosis and the tick borne encephalitis, transmitted among reservoirs and humans by ticks of the complex *Ixodes ricinus*, are being more commonly reported in northern and central parts of Europe, where the vector is prevalent (Hvidsten *et al.*, 2020). However, in southern Europe Crimean-Congo hemorrhagic fever (CCHF) or Ricketsioses, commonly circulated by the tick genera *Hyalomma*, *Dermacentor* or *Ripicephalus* (Moerbeck *et al.,* 2022; Monsalve-Arteaga *et al*., 2020). This is probably due to the observed spread or increase of abundance of some species of ticks in central and Northern Europe. In the last years, the trends in climate drove a new panorama in tick-borne pathogens and related diseases (Cunze e*t al.*, 2022). An example is the increase of records of *Hyalomma marginatum* and *Hyalomma rufipes* in central Europe (Lesiczka *et al.*, 2022; Kampen *et al*., 2007) and United Kingdom (McGinley *et al.*, 2021) with insights of a possibility of overwintering permanent populations in Central Europe in the near future (Uiterwijk *et al.*, 2021), although such “probability” has been already reported in areas of central Europe. The finding in Spain of the etiological agent of CCHF and the report of several human cases in the last few years is also a cause of concern (Juanes *et al.*, 2023). The awareness to tick and tick-borne pathogens is increasing in the society (Boulanger *et al.*, 2019). It is necessary to make a thorough revision about the evolution of ticks, main structures, biological cycle or ecological influence in order to determine the differences in the epidemiology of tick-borne pathogens.

## Origin and evolution of ticks

Molecular clocks estimated the origin of ticks in the Carboniferous period (Mans *et al.*, 2012). Since then ticks have evolved into the extant families Argasidae, Ixodidae and Nuttalliellidae, and two extinct families, namely Deinocrotonidae (Peñalver *et al.*, 2017) and Khimairidae (Chitimia-Dobler *et al.*, 2022). The main extant tick families that are considered vector of pathogens are Argasidae and Ixodidae, know also as soft and hard ticks (Hutcheson *et al*., 2021). The third extant family is only known from specimens collected on small rodents in a region of Southern Africa (Mans *et al.*, 2011). Although there is much debate regarding the taxonomic details of the family Argasidae, it is considered to include the prominent genera *Ornithodoros* (nearly 100 species) and *Argas* (almost 60 species) as well as other genera represented by a smaller number of species, like *Otobius*, *Antricola* or *Nothoaspis* (Estrada-Peña *et al.*, 2010). The family Ixodidae includes the genera *Africaniella*, *Amblyomma*, *Anomalohimalaya*, *Bothriocroton*, *Cosmiomma*, *Cornupalpatum*, *Compluriscutula*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, *Margaropus*, *Nosomma*, *Rhipicentor*, and *Rhipicephalus* (Guglielmone *et al.*, 2023). The involvement of species in these genera in the transmission of pathogens among vertebrates is far to be completely known. Studies have been conducted on a few species with interest on human health; also, it is well known that some genera are restricted to wild animals, thus remaining out of the focus of further research but in a few cases. Other species are scarce, difficult to collect and/or have a restricted distribution; its interest is not lower, but they are more difficult to collect and handle.

Both Argasidae and Ixodidae have morphological and biological differences. For example, the cuticle, scutum or mouthparts are different. While the mouthparts of hard ticks can be easily identified in dorsal and ventral positions, these parts are only visible in a ventral view in soft ticks (Sonenshine, 2005). Furthermore, soft ticks do not have a hard cuticle; they have a dorsum fully or partially covered with chitin (Nava *et al.*, 2009). The evolution of ticks and their divergence into families is not yet clear. Some researchers focus on the evolution of ticks from a functional perspective (Mans & Neitz 2004; Mans *et al.*, 2008), while others establish differences in host associations (Durden, 2006). Considering the tick-host relationships, evolution does not depend on host specificity but to a greater extent on ecological specificity, which is more relevant but difficult to assess (Cumming, 2004; Estrada-Peña *et al.*, 2023). It has now been shown how different hematophagous organisms have found different solutions to the same problem. This allows independent adaptation to vertebrate host rejection, using similar anti-defence mechanisms through convergent evolution (Mans, 2010). Not only the molecular components are susceptible to evolution, but also the interaction with pathogens. It has been shown that the *Flaviviriade* family of viruses associated to ticks have evolved in both North America and Europe, and are present in the same tick group (*Ixodes* complex of species) in different locations, suggesting that the tick-virus association is older than the separation of continents.

## An overview of the tick-transmitted pathogens.

Although the two most prominent families of ticks are blood-sucking parasites and need to ingest blood to continue the cycle, the adults of *Argasidae* feed only for some 60 minutes while hard ticks feed for days (Sonenshine & Roe, 2013). This feeding pattern is maintained during all stages of the tick cycle in both families and has an importance for the transmission of pathogens. The tick life cycle has three stages, namely larvae, nymph and adult (female and male) and the moult between them, as well as the oviposition, requires a blood meal. Hard ticks have only one nymphal stage, but soft ticks may have more than one (Sonenshine & Roe, 2013). In addition, soft ticks commonly follow an endophilic strategy for its life cycle while hard ticks can exhibit exophilic or endophilic strategies using one, two or three hosts (Sobrino *et al.*, 2012). Although most studied ticks tend to be generalist parasites, the strategy of feeding may drive to an expansion of the host range, while the endophilic behaviour tends to be associated with a smaller variety of hosts. The tick-host association represents one of the main drivers of the ecology of ticks (Mather & Ginsberg, 1994). It is known that the relative abundance of different species of hosts in an area may lead to changes in the relative abundance of ticks. This effect is most commonly observed at the local scale, because the difficulty to obtain reliable data for large territories. Central to the importance of hosts in the ticks life cycle is the specificity towards some host (Nava & Guglielmone, 2013; Esser *et al.*, 2016; Estrada-Peña *et al.*, 2023), that could be either phylogenetic or ecological. The former implies a co-evolution (and perhaps in some cases a co-speciation) while the later is based on the sharing of the habitat (Nava & Guglielmone, 2013). However, there are some discrepancies about the associations between host of different ticks stages. Vor *et al*., 2010 pinpointed that the size of the host could be one of the main drivers of specificity for the host. The paradigm is that, in general terms, the immatures of most species of ticks are associated to small mammals like rodents and Insectivora, as well as birds, while adults tend to feed in large mammals such as wild ungulates or Carnivora (Hoogstraal & Aeschlimann, 1982). However, these hypotheses ignore the behaviour of the tick (endophilic or exophilic) or the links with a range of hosts exploiting the same habitat, like *Ixodes uriae* and pelagic birds, everywhere in the world (McCoy *et al.*, 2001). It also ignores the species that are restricted to a small range of hosts (like *Ixodes frontalis* and birds using holes in trees for breeding).

As mentioned, other than a suitable source of blood for ticks, a pleiad of vertebrates may maintain and circulate pathogens by a variety of reasons, some of them not yet well understood; these vertebrates are known as reservoirs, and tick researchers are still very far to know the complete range of vertebrate involved in these associations, other than recent efforts for systematising these relationships (Cumming, 1999; Estrada-Peña & de la Fuente, 2017; Estrada-Peña *et al.*, 2014, 2015, 2016, 2020). Ticks and reservoirs establish a circulation of pathogens in foci (first hypothesized by Pavlovsky in 1944) which can be transmitted to humans. The circulation of pathogens by ticks is not only a matter of “contact with reservoirs”, since it involves molecular relationships among pathogens, vertebrates and ticks, the ecological relationships of the partners involved (sharing seasonality or habitat) or even the co-existence of the livestock and wildlife, that contributes to exacerbate the chain of casual events leading to amplification of the transmission (Bowman & Nuttall, 2008). Some vertebrate reservoirs allow the pathogens to replicate inside them without causing clinical effects. Thus, ticks feed into the reservoirs and may take the pathogen(s) that can be transmitted to humans through the saliva. The perpetuation of the pathogens throughout the tick life cycle of the tick is another point of interest. Pathogens must to be transmitted between consecutive stages of ticks, in what is known as transestadial transmission. In some cases, pathogens can be transmitted from the female to the eggs, in what is known as transovarial transmission (Estrada-Peña *et al.*, 2013). Both ways of transmission have a highly variable outcome in the “success” of transmission of the pathogen and can change even among species of vertebrates. For example, it is known that the rates of transmission of *Borrelia* to ticks by reservoirs is highly variable, and depend on the strain of the bacterium and the species of reservoirs (Hofmeester e*t al.*, 2016). The increased abundance of incompetent reservoir vertebrates could dilute the prevalence of pathogens in ticks (Keesing *et al.*, 2006) in a process that is still a subject of much debate (Huang *et al.*, 2016) and that seems to not be universal. However, it seems that the scale (or the resolution, which is the opposite) may affects the observed effects of dilution or amplification. Most important, proofs obtained from modelling point that the composition of the hosts communities may be the major the reason behind the prevalence of a tick-borne pathogen (*Borrelia* spp.) in the tick vectors in Europe (Wood & Lafferty, 2013).

Some pathogens use the tick as reservoir, without the involvement of a vertebrate. This epidemiological pattern immediately suggests an intimate co-evolution between the tick and the pathogen(s). For instance, CCHF virus uses the tick(s) as reservoirs, because the viremia in mammals is very short and is absent in birds; these epidemiological traits could not support the persistence of permanent foci of the virus (Spengler & Estrada-Peña, 2018). In the same way, the maintenance of a transovarial transmission in the bacteria of the genus *Borrelia* belonging to the recurrent fevers group, could suggest a primitive origin of these bacteria in the tick (Estrada-Peña *et al.*, 2018); it has been postulated that further adaptations to vertebrates, originated the *burgdorferi* group in which the transovarial transmission has been replaced by the presence of vertebrate reservoirs (Estrada-Peña *et al.*, 2018). It is yet unknown how the discovery of new species of *Borrelia* in Australia did affect this hypothesis, that remains valid by the time of writing.

Nonetheless the overlapping of the three actors (vertebrate-tick-pathogen) is necessary to have a permanent foci of infection (Estrada-Peña & de la Fuente, 2016). It is important to mention that there may be silent foci of circulation of pathogens, meaning that there are sites where no cases of transmission to humans exist. A typical case is the Tick-borne encephalitis virus, of which several epidemiological details are still unknown, even if it has been the object of intensive research in Central Europe in the past 50 years. Why the virus has such patchy distribution, or foci of infections "appear and disappear" in time and space is still unknown but seem to be related to the existence of a threshold below which only a restricted circulation of the pathogen could exist, human cases being anecdotical under these conditions (Van Heuverswyn *et al.*, 2023)

## The pathogens, the ticks, and the habitat.

Foci of tick-borne pathogens have an intricate structure, that is affected by external factors such as human actions (or habits) as well as by climate change. Humans actions have affected the dynamics of tick-borne diseases, in example increasing the habitat fragmentation (first reported by Estrada-Peña, 2003) or by abandoning farming lands (Estrada-Peña *et al.*, 2007; Mierzejewska *et al.*, 2015). Habitat fragmentation and the dilution effects are still a topic of debate and is a cascading effect, that can be observed in models but have not yet been conveniently demonstrated in the field. As the habitat becomes fragmented, commonly because the agricultural activities and consequent forest clearance, tick-borne pathogens tend to remain at high rates in small foci (LoGiudice *et al.*, 2003; Morlando *et al.*, 2012). Values of prevalence of the pathogens in questing ticks living in these foci have been found to increase because the lack of vertebrates that “divert” the ticks feeding them but are incompetent as reservoirs.

The abandoning of farming lands is a serious concern since such process drives to the proliferation of bush and similar short and dense vegetation, in a sequence of natural events that restore the original vegetation present in the area before the agricultural practices; these types of vegetation provide a shelter for many animals that are competent reservoirs of some pathogens. Actually, such decrease in farming activities and the rapid increase in numbers of reservoirs in these abandoned lands have been pointed out as one of the main drivers in the still ongoing epidemic of Crimean-Congo hemorrhagic fever in Turkey (Estrada-Peña *et al.*, 2010).

## The weather and climate as the main drivers (sometimes) of tick's spread.

Climate change is one of the observed causes considered to be responsible of changes of distribution of ticks, as supported by field collection. Studies are far from exhaustive because they are restricted to species affecting human health mainly in the genus *Ixodes (*Jaenson *et al.*, 2012) and to the prominent pest affecting livestock, *Rhipicephalus microplus* (Nava *et al.*, 2022). Other species have been commonly neglected, mainly because the lack of baseline data on distribution. However, other than the well known species affecting either human or animal health, like *I. ricinus*, *Ixodes scapularis*, and *R. microplus*, other species like *Dermacentor reticulatus* (Europe), *Amblyomma americanum* or *Dermacentor variabilis* (United States) are being monitored because its importance in the transmission of pathogens to either humans or animals (Grochowska *et al.*, 2020; Childs & Paddock, 2003; Duncan *et al.*, 2021). However, and this is of critical importance, while the spread of some ticks at the fringe of its distribution is being carefully recorded (because the conditions are turning more suitable for their survival, like *I. ricinus* in Scandinavia) there are no reports of their absence in sites that turned to be unsuitable because they are now warmer or drier. Actually, there is only one report, based on modelling, about the decrease of habitat suitability of some prominent ticks in South Africa (Estrada-Peña, 2003). Climate is defined as a statistical phenomenon that describes the average climate conditions for a region in a long period, while weather is the climate variables in the short period. People tend to focus on individual events of climate, ignoring the changes in the general trend (Weber, 2010) and therefore disregarding the importance of the event on the ticks life cycle. Ticks are highly influence by climate change. Warmer and shorter winters may lead to the increase of the abundance of ticks (perhaps increasing its survival or improving its development rates) as well as the survival of hosts and reservoirs (Oorebeek & Kleindorfer, 2008). This would result, in theory, into an improvement of the circulation of pathogens. However, these hypotheses lack a confirmation at the large scale, because the complexity of the topic (in some cases with dozens of species of vertebrates involved, plus large areas of a continent involving several biogeographical regions) has stopped the development of solid backgrounds explaining the survival, development and activity of ticks affecting human/animal health.

For instance, surveys conducted in Sweden between the years 1980 and 2008 have showed that permanent population of *Ixodes ricinus* did spread into higher latitudes (Jaenson *et al.*, 2012). Similar conclusions were obtained from field results carried out in Krkonoše mountains in the Czech Republic, demonstrating that the limit of populations of *I. ricinus* increased its altitude in 550 meters in the period 1990-2010 (Danielová *et al.*, 2010). There are also some examples in

North America where *I.scapularis* is moving to the southern part of Canada (Leighton *et al.*, 2012; Robinson *et al.*, 2022).

Central to the approach of this Thesis is the concept of “niche”. Several names have been used for its definition, like “environmental niche”, or “theoretical distribution” (Sillero *et al.*, 2021). Basically the concept of niche is deeply rooted in a comment by Hutchinson (1957) in the last minutes of a scientific conference, and based on the few theoretical developments developed by Grinnell at the beginning of the XXth Century. This is why it is also defined as “Grinellian” or “Hutchinsonian” niche: it refers to the set of interactions among several *abiotic* variables that define the sites where a species can expect permanent populations in the absence of catastrophic events, and without geographical barriers blocking spread. A different view is the Eltonian niche and refers to the set of *biotic* interactions among organisms, necessary for its persistence as population (Soberón, 2007). A classic example of Eltonian niche is the set of plants and pollinators, but it can also be applied to predators and press: the concept is widely used in population ecology (Phillips *et al.*, 2020).

If these concepts of niche are applied to ticks, then the Grinellian and Eltonian niches can easily been translated into the environmental conditions under which a species can persist (considering the vegetation as a modifier of climate) and the set of hosts on which a tick species prefer to feed; in other words, this would be the abiotic part of the tick’s ecology: the relationships between “environment” (*sensu lato*) and the populations of tick. Is obvious that generalist species (those with catholic feeding habits) would have a wide Eltonian niche since they could hypothetically feed on a wide range of vertebrates. However, it immediately results that different species of ticks may have different strategies of survival (like exophilic or endophilic behaviours), that may obscure the relationships between ticks and climate.

## Modelling the ticks life cycle, its presence, and its abundance.

The applications of these methods to ticks is inevitably derived to the prediction of risk, which immediately implies the knowledge of its life cycle and the modelling of the potential effects of some climate parameters on the distribution and potential risk. However, the last part of the previous sentence has been rarely addressed, and researchers seem to prefer automatic evaluations of the so-called “probability of presence” than to understand the factors governing the relationships of the ticks with its milieu. This resulted into the production of geographical maps that lack the ecological background of these relationships.

Regarding the ticks affecting human health, the first studies were carried out on the prominent species *I. ricinus*, a major pest of humans in Europe. using exclusively data obtained in laboratory incubators (“tables of life”), converted into rates of development after comparison with outdoor prevailing conditions. These pioneering studies were performed by Macleod (1934), further developed by Campbell (1948) and Milne (1950a, 1950b). These studies demonstrated that the increase of temperature, to a threshold, accelerated the development of ticks; also, the contents of water in the air (but not the rainfall) were considered as responsible of the mortality of the different development stages. Specific thresholds of temperature and humidity were established for the different stages of development of *I. ricinus* but the topic remained unvisited for several decades*.* Further studies were carried out on the topic, with data obtained from filed studies (Gardiner *et al.*, 1981).

In short, the idea behind these studies is the decomposition of the life cycle of the ticks in several steps, since the tick has several stages, using the accumulated temperature to known "when" (e.g. the approximate date) the completion of each life stage could be expected in a given area. At the same time, the saturation deficit of water or any other value displaying the amount of water in the air, is used to compute the most probable rate of mortality. Therefore, these models could show "when" and "how many" ticks can be expected in a given site at a given time; however, they systematically ignored the relative proportions (and actual abundance) of hosts and/or competent reservoirs. This resulted in a confusing approach that provides a general framework (overlapping slightly with observed actual activity) but being unable to be applied to wide areas, with an explicit aim on prevention. Similar approaches where adhered not only for the Palearctic *I. ricinus*, but for the very close Nearctic species *I. scapularis*, with an explicit simulation of the dynamics of Lyme borreliosis (Mount *et al.*, 1997). The obscure model published by the time was further replicated and improved by Gaff *et al*. (2020), but both models include a very low number of "available" hosts that contribute to the feeding of the species, which is unrealistic in many regions. Models based on the simple sum of degrees are effective and simple, but cannot be considered a serious option until the complete set of hosts are included, with their own rates of development, growth and mortality according to climate features. A much more simplistic view of these models has been carried out by Ogden and his team, in which mortality for the tick is assumed to be null because it is in equilibrium with its environment, and the contact rates with a few hosts are overwhelmingly ignored throughout all the model development (Ogden *et al.*, 2004, 2005). Pathogen transmission efficiency models were developed in conjunction with these models, that consider the probability of encountering reservoirs, ticks, and the circulation of pathogens (Ogden *et al.*, 2007), once again being focused on unrealistically low number of vertebrates and/or reservoirs of pathogens.

A different approach based on the same premises is based in what is known as $R_0$, or the threshold that could allow the presence the permanent population of ticks in an area. The value of $R_0$ has its roots on previous studies on *Plasmodium*, and is a complex value derived of the first principal component of a jacobin matrix. While its calculation is relatively simple for insects (because they have only one active biting stage) its development for ticks took several iterations, headed by Nienke Hartemink (Hartemink *et al.*, 2008) for *I. ricinus*. The cycle is divided in rows and columns that show the values of development of each "intersection" of stages, being the first principal component (i.e. the diagonal of the matrix) the one that contains the values on which $R_0$ is computed. The approach has been further used by Estrada-Peña *et al.* (2014) to demonstrate that the life cycle of *I. ricinus* can be "deconstructed" to know which part is particularly sensible to variations of climate. In any case, models based on $R_0$ are far from intuitive (but mathematically correct); its complexity has derived in a lack of consideration in the growing panoply of tools available for modelling tick risk for humans.

A second approach to modelling environments for ticks is based in what is called "mechanistic models" (Ostfeld & Brunner, 2015). They do not use the "classic" accumulative degree proportion of development, or the relative humidity as a proxy of mortality, but are based on the similarity of the niche known to be occupied by the tick and the real environment. In other words, these models compare the variables (in several dimensions) that could define the "environmental space" in which each species of tick could survive. The method, statistically simple, as been used by many researchers, who systematically adhered to the simples and wrong side of the modelling approach: the bad choice of explanatory variables. Before to develop a model, for which many software packages exist it is necessary to have a complete knowledge of the niche of the tick and therefore to select the variables that already affect its survival. This has been commonly neglected. Researchers tend to use the same set of climate variables, interpolated, that do not represent the habitat used by ticks based on the rule "one size fits all". In example, there is commonly no reference to water vapour, relative humidity, drought in the soil or any other measure of water in the air, but only rainfall, which is not correlated to the previous ones (Alonso-Carné *et al.*, 2015). Such poor selection of explanatory variables drove, in example, to the publication of "probable suitable habitat" for the tropical cattle tick, *R. microplus*, in northern Norway, near the Polar circle (Marques *et al.*, 2020).

Recent developments included the use of principal components, because the explanatory variables should not be correlated (as obtained after the orthogonal decomposition of principal components). The ENFA approach was developed and became relatively popular for some time (Hirzel *et al.*, 2002), until the hypotheses behind the method were abandoned because its ecological ambiguity. Actually, a decomposition of the explanatory variables in its principal components is probably the most counter-intuitive misuse, because it immediately implies missing the interpretation of an environmental niche before planning any modelling progress. The use of satellite recorded imagery was popularised at the end of XXth Century for ticks (Estrada-

Peña, 1998, 1999) but still based on raw measures of monthly values of data following previous studies on *Glossina* flies (Rogers *et al.*, 1997). The variables involved in the evaluation of the environmental niche were basically the ground temperature and the Normalised Difference Vegetation Index (NDVI), an index of vegetation vigour that has been correlated with the humidity of the canopy in many occasions (i.e. Stabler *et al.*, 2005).

First proposed by Scharlemann *et al.* (2008) the use of a Fourier transform of a time series of data, produces better estimates for the explanatory variables. The first programming script made publicly available for the calculation of a harmonic regression (or obtention of the Fourier transform) of satellite derived climate variables was published by Estrada-Peña and de la Fuente (2016). Immediately, three pivotal questions on the topic become obvious, namely (a) daily values of data can be obtained even if the original MODIS data had a 16-days resolution, (b) actual definitions of seasons can be summarised and, perhaps most important (c) the coefficients of the Fourier regression *are actually the explanatory variables* because they contain, in a non-correlated way, all the values necessary to "explain" a complete period of study. Further studies demonstrated the superiority of this approach to any other modelling approach (Estrada-Peña *et al.*, 2016).

The previous comments are important not only for the focus of this Thesis but also for the field of modelling. Researchers found a prolific field of publications in the "predictions" of how climate trends will affect the distribution (please note the misuse of the word "will" instead of "could"). If the procedures for modelling are not adequately and rigorously selected, as we tried to address in this Thesis, the results fall in the category of "prophecy" instead than in the field of "prediction". The choice of adequate explanatory variables is an important first step, because we need to define the environmental niche of a tick (Estrada-Peña *et al.*, 2013); but the general rule has been "one size fits all", or, in other words, the same set of interpolated climatologies "must to be" able to explain "everything" of any species of tick, which is unreliable.

The most used dataset of climate variables is known as "WorldClim" and was the first to be publicly available for research purposes. It represented the best knowledge of the climate on Earth but now has been superseded by other datasets (like PRISM, TerraClimate CRUseries, CHELSA, some of them now unavailable, or the re-assessments made under the umbrella of the project Copernicus, available at https://cds.climate.copernicus.eu/. In short, WorldClim is an interpolated map of the Earth's surface on which an estimate of climate variables is represented. This is statistically reliable for areas like central Europe or United States, in which the number or climate recording stations is high, but is meaningless in areas like Siberia or Sahara desert, in which such number is very low. In any case, the dataset was adopted as a dogma by first modellers applying to ticks such biased set of data. To note, WorldClim does not include relative humidity or any other measure of the amount of water in the air, which is well known to impact the tick ecology (Estrada-Peña *et al.*, 2013). However, researchers carelessly replaced it by the precipitation, even if it is known that both variables (rainfall and relative humidity) are no correlated (Alonso-Carné *et al.*, 2015). The modelling outcome should be expected to be as reliable as the variables introduced.

Another point of conflict is the erroneous feeling of many researchers involved in tick studies, regarding the concept of “season”, introducing concepts as “the sum of degree/days in spring” or “the deficit of water in summer”, which are variables that may have a serious impact on the tick's life cycle and thus of interest for modelling. Obviously, an interpolated climatology derives data for other purpose like (i.e.) average temperature in the *climatological* spring. This is an special point of concern, because many papers did use these “derived” data to produce predictions based on how the weather will be in a season. These researchers ignore that ticks or vertebrates do not mind about a date in calendar: seasons are an astronomical event but not a weather event. Therefore, the calculation of (i.e.) the sum of temperature in spring as it is commonly obtained has no ecological significance on the life cycle of ticks, because it includes observations made between two human-defined dates in a calendar. However, the beginning and the end of each season can be calculated over the daily series of data, just like an econometric series has inflection points, using the harmonic regression mentioned before (Estrada-Peña *et al.*, 2016). Therefore, the actual days at which each season begins or ends, can be calculated using such harmonic regression, based on solid methods derived from methods successfully applied to fields related to the stock markets or econometric data. Then, the actual sum of degrees (which is of importance to capture the rate of development of a cohort of ticks), or any combination of variables with ecological meaning can be adequately obtained. This has been reported (Estrada-Peña *et al.*, 2013, 2015) but it seems that researchers prefer a pre-tailored set of climate data without ecological meaning, without an adequate application of the importance of these data in the tick's life cycle, that could be summarized with the concept “download and use directly”.

## The biotic side of the variables affecting tick persistence.

Other researchers had emphasized the importance of the biodiversity of the community of vertebrates (hosts and reservoirs) understood as the set of organisms which have a similar distribution according to the environmental conditions, and that overlap at arable degrees over a given area (Soberón & Peterson, 2004). In other words, the concept refers to the availability of hosts (and their abundance) for tick feeding or for pathogen circulation (the competent reservoirs). Based on the above concepts, models were developed that surveyed abiotic factors including temperature and humidity, and biotic factors such as the presence of hosts, taking into account the spatial accessibility and the modelling area obtained (Peterson, 2006). The presence or absence of hosts is key in Ixodidae, and there must to be both a geographically and temporal overlap between ticks and hosts. This will determine whether there are permanent tick populations in a region; if there are also reservoirs of pathogens, there will also be foci of pathogen circulation (Peralbo-Moreno *et al.*, 2021). These biotic variables are closely related to each other, e.g. the presence of only reservoirs may increase the prevalence of the pathogen in ticks. This phenomenon usually occurs when there is habitat fragmentation, which is the reduction of an ecosystem, resulting in a reduction of species diversity in the ecosystem. In general, the area of the ecosystem is reduced, which implies that larger species no longer inhabit the ecosystem. As an example this happens in parts of Europe, where in some cases human actions fragment the habitat, leaving small mammals and rodents as the only species present, which are reservoirs of the *B. burdorgferi* complex, on which ticks feed and can perpetuate outbreaks of the bacteria as these are reservoirs. A greater biodiversity in the community with reservoirs and hosts can lead to a dilution of the pathogen as not every host is a reservoir (Tardy *et al.*, 2022). The calculation of the fragmentation of the territory is complex over large areas, so most models and projections of tick distribution do not include it in the calculations.

In any case, researchers are still far from understanding the complete set of mechanisms that govern the presence/absence and the abundance of ticks in a given area. Modelling approaches should consider both abiotic and biotic factors, or at least, the rates of contact between the ticks and the vertebrates. The complexity increases if we want to consider which species of the community are competent reservoirs, since this feature may change at the local scale. More research is necessary, based on the field studies, to be able to capture the variability happening at the level of a country or a continent. The published studies suggest that researchers can predict what could happen at the local scale, but studies on large areas tend to get diluted in the complexity of the whole system.

## The aim and methods of this Thesis: an overview.

The main objective of this thesis has been the development of tools to model the interactions of the ticks with medium-term climate, aiming to better capture the rates of contact with some key hosts (and thence the circulation of pathogens) as well as to track the trends of the impact of climate on other (presumably expansive) species of tick. Last but not least we aimed to examine new methods to track the possibility of spread of ticks coming from Africa into Europe, considering the routes of movements of their hosts and the recent changes in climate. We did also a small-scale experiment about the type and quality of data that the co-called “citizen science” can contribute to the study of the tick distribution.

While each manuscript has (as compulsory) the detailed methods and even the adequate supplementary material necessary for the duplication of the experiments, some common methods were as follow:

- Two types of climate data were systematically used, namely satellite images of the MODIS series of satellites (named “Terra” and “Aqua”) at a resolution of about 4 sq. km., and a set of interpolated climatologies called “TerraClimate”. The former is available at https://www.climatologylab.org/terraclimate.html, but also downloadable after programming from the Google Earth Engine (https://developers.google.com/earth-engine/datasets/catalog/IDAHO_EPSCOR_TERRACLIMATE). In the former, the images necessary were accessed using the R programming environment (R Core Team, 2019) using the package MODIStsp (Busetto & Ranghetti, 2016); in the later, we used a bash script for Unix, as suggested in the original website, without modifications.

- For both cases, an intensive use of scripts in the programming language R were used, either to download and pre-process the set of data (e.g. obtaining the target region from the set covering the complete Earth) or processing the series of data for decomposition into the coefficients of the harmonic regression. Every manuscript has the script purposely developed for such particular study in the supplementary material.

- Modelling was always performed using the method of Maximum Entropy (MaxEnt, developed by Shapiro *et al.*, 1995) using the Java application provided by the developers instead of other implementations available in R (e.g. the package “Wallace”). This was done only to adhere to the basic principles of the method, and to be confident that the original programming methods were used, even if in some cases they were slower than more modern implementations.

- In some cases, we were interested in the study of the contact rates of ticks with hosts, or the role of some hosts in the spread of ticks. For both cases, we intensively use the available data in the Global Bioinformatics Information Facility (GBIF, **https://www.gbif.org/**). Each manuscript in which these data were used includes the adequate acknowledgment to the used information (as compulsory for GBIF). To note, information from GBIF only includes the coordinates of observation of the queried species; therefore, the rest of information (to obtain distribution maps or to define the ecological niche of each species) was also modelled using MaxEnt. All the projected distributions of hosts were adequately subjected to a control quality and model reliability. When necessary these newly acquired data from GBIF were used to complement previous information produced by previous papers of the team and currently available freely with the doi.org/10.5061/dryad.2h3f2.

- When necessary, we used information regarding the vegetal coverage. If focused in Spain, we systematically used the “vegetation series of Iberian peninsula” by Rivas-Martínez (Rivas-Martínez,1987). If referred to the complete Europe, we used the scheme derived from LANMAP2, originally developed by the University of Vienna (Austria). Such information is not longer available on the Internet and has been removed from the official repository of the European Union and the Copernicus initiative. We do not know the reasons behind such decision (most probably waiting for an updated version) but its use was adequately acknowledged in the involved papers.

- Other details of the contact rates of ticks and hosts are mentioned in each manuscript included in this Thesis, but basically include the calculation of the potential niche sharing and thus the evaluation of such contact rates as previously published (Estrada-Peña & de la Fuente, 2016).

- Regarding the involvement of citizen science, we get enrolled in a protocol developed in New Hampshire (United States of America) by the explicit request of the main research of the study. We had no role in the design of the portal because our work was devoted to analyse and systematise all the data (literally thousands of ticks submitted to the reference laboratory), the preparation of the figures, and the writing of the manuscript itself.

- Regarding other data and methods, they escape to this condensed view of the procedures, but are completely explained (and have associated programming scripts when necessary) in all the papers included in the Thesis. All the data were made publicly accessible in the publication or though public repositories.

This Thesis has been divided in several parts that want to address key aspects in the ecology of ticks and the circulation of pathogens, as follows:

The first is aimed to disentangle the effects of the climate on the biology of two prominent species of ticks. We begin the chapter with the first joint analysis of the distribution of both ticks and some key hosts, focused on the complete distribution of *I. ricinus* in the western Palearctic. Two other studies are dedicated to ticks of the genus *Hyalomma*, in which we explored the effects of recent climate on the environmental suitability of the tick. Further on this, we proposed a preliminary assessment of the probable spread routes of *H. marginatum* due to migratory birds in their spring flights. This is an interesting study, since almost 25 species of birds (known to carry immatures of the tick) were analyzed from millions of actual records of the birds by ornithologists in the field. We approached the use of wind draught, distance and behavior of bird species to track the probable routes of spread and distance covered at weekly intervals.

The second part analyzed the importance of the species composition in the communities of vertebrate that feed *I. ricinus* and that are different according to the geographical space. We consider this an important issue since the presence and abundance of vertebrates is essential for the circulation of the pathogens transmitted by ticks. In addition, we participated in a international research together with a North American scientific group (based in New Hampshire, USA). Such study conjugated the modelling of ticks with the citizen science records, allowing to obtain the patterns of distribution of two major ticks species in Public Health (*I. scapularis* and *D. variabilis*). To reflect a summary of the current and future challenges we finished with a revision that explores the epidemiological gaps in ticks and tick borne pathogens.

# Chapter 1: "Could climate trends disrupt the contact rates between *Ixodes ricinus* (Acari, Ixodidae) and the reservoirs of *Borrelia burgdorferi* s.l.?

PLOS ONE

RESEARCH ARTICLE

# Could climate trends disrupt the contact rates between *Ixodes ricinus* (Acari, Ixodidae) and the reservoirs of *Borrelia burgdorferi* s.l.?

**Natalia Fernández-Ruiz**[1☯], **Agustin Estrada-Peña**[1,2☯*]

**1** Department of Animal Health, Faculty of Veterinary Medicine, University of Zaragoza, Zaragoza, Spain,
**2** Emerging Zoonoses Research Group, Instituto Agroalimentario de Aragón (IA2), Zaragoza, Spain

☯ These authors contributed equally to this work.
* aestrada@unizar.es

## Abstract

This study addresses the modifications that future climate conditions could impose on the transmission cycles of *Borrelia burgdorferi* s.l. by the tick *Ixodes ricinus* in Europe. Tracking the distribution of foci of a zoonotic agent transmitted by vectors as climate change shapes its spatial niche is necessary to issue self-protection measures for the human population. We modeled the current distribution of the tick and its predicted contact rates with 18 species of vertebrates known to act as reservoirs of the pathogen. We approached an innovative way for estimating the possibility of permanent foci of *Borrelia afzelii* or *Borrelia garinii* tracking separately the expected spatial overlap among ticks and reservoirs for these pathogens in Europe. Environmental traits were obtained from MODIS satellite images for the years 2002–2017 (baseline) and projected on scenarios for the years 2030 and 2050. The ratio between MODIS baseline/current interpolated climatologies (WorldClim), and the ratio between MODIS-projected year 2050 with five climate change scenarios for that year (WorldClim) revealed no significant differences, meaning that projections from MODIS are reliable. Models predict that contact rates between the tick and reservoirs of either *B. garinii* or *B. afzelii* are spatially different because those have different habitats overlap. This is expected to promote different distribution patterns because of the different responses of both groups of reservoirs to environmental variables. Models for 2030 predict an increase in latitude, mainly in the circulation of *B. garinii*, with large areas of expected permanent contact between vector and reservoirs in Nordic countries and central Europe. However, climate projections for the year 2050 predict an unexpected scenario of contact disruption. Though large areas in Europe would be suitable for circulation of the pathogens, the predicted lack of niche overlap among ticks and reservoirs could promote a decrease in permanent foci. This development represents a proof-of-concept for the power of jointly modeling both the vector and reservoirs in a common framework. A deeper understanding of the unanticipated result regarding the year 2050 is needed.

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## Introduction

Pathogens transmitted by arthropods have increasing importance in public health. One of the many examples is the expansion of *Aedes albopictus*, a mosquito vector of the etiological agents of Dengue, Zika, and Chikungunya [1]. Although mosquitoes are regarded as the most prominent vectors in human health, ticks are the arthropods that transmit the largest number of zoonotic agents in the Northern Hemisphere [2]. Some of these pathogens are tick-borne encephalitis virus (Flaviviridae), *Ehrlichia* spp., *Anaplasma phagocytophilum*, and bacteria of the genus *Borrelia*, which may cause severe clinical processes in humans [3].

Climate change is one of the factors that shape the possible changes of distribution and seasonality of vectors and the pathogens they transport to areas where they were not previously found, in what is being called (re)emerging foci of zoonoses. Climate change has been defined as, "a statistical phenomenon that describes the average climatic conditions for a region, referring to systematic and generally gradual changes in the trend, which is integrated into random climate fluctuations" [4]. Climate trends drive changes in the geographic distribution and seasonality of ticks, their vertebrate hosts, and the reservoirs of pathogens, shaping the persistence of pathogen foci [5]. Warmer and shorter winters may increase the survival of ticks, the probability of pathogen transmission, and the number of infected reservoirs [6].

Lyme borreliosis is the most frequently recorded tick-borne zoonosis in the Holarctic [7]. Approximately 26 species are recognized in the genus *Borrelia*, some of which are included in the *Borrelia burgdorferi* sensu lato (s.l.) group (Bb). Some of the most important species of the complex are *B. burgdorferi* sensu stricto (s.s.), which produces arthritis, polyneuritis, and a skin lesion called erythema migrans; *Borrelia afzelii*, which has special tropism for the skin and can lead to cutaneous lymphadenosis; and *Borrelia garinii*, which generates meningitis and inflammatory lesions in the peripheral nerves [8]. The main vector in Europe is *Ixodes ricinus*, although in other geographic areas other species of Bb can be transmitted by different tick species, such as *Ixodes pacificus* in the western United States, *Ixodes scapularis* in the eastern and central northern United States and southern Canada, or *Ixodes persulcatus*, which is distributed in Eurasia [9]. The incidence of Lyme borreliosis in the Northern Hemisphere is alarming, not only because of the severe clinical picture, but because no harmonized prevention plans are available and vector control still relies on self-protection by the human population. Surveys conducted in Sweden between 1980 and 2008 showed that permanent populations of the tick *I. ricinus* had increased in latitude, supporting climate change as one of the most influential factors in its expansion [10]. In addition, both *I. ricinus* and the pathogens it transmits are being established at higher altitudes. Systematic sampling conducted in the Krkonoše mountains in the Czech Republic indicated that the limit of altitude distribution for this tick changed from 750 m to up to 1300 m above sea level between 1990 and 2010 [11]. This spatial expansion of permanent tick populations has also been shown in other countries; a comparative study using historical data collected in Norway demonstrated an altitude increase of almost 600 m above sea level and the spread of *I. ricinus* into regions near the west coast of the country, where it was traditionally colder than currently [12]. These events do not occur only in Europe, but also in North America, where one of the most important tick species for public health, *I. scapularis*, is spreading northward, moving towards Canada and settling in large regions of southern Canada [13].

There is a demand for plausible future patterns of the range of pathogens under conditions of global environmental change, setting priorities for public health. Modeling efforts are commonly addressed to map the expected distribution of ticks based on abiotic descriptions of the environment. However, reports have demonstrated the better performance of jointly modeling both ticks and their vertebrate hosts (or pathogen reservoirs) to capture the probable distribution of health-affecting infectious agents [14]. Few other efforts have exploited process-driven models to capture the life cycle of *I. ricinus* [15–17], and only two of them addressed the potential impact of climate trends on the life cycle of *I. ricinus* without explicit modeling of the reservoir's range [16], or assuming its presence according to categories of habitat [17].

*Ixodes ricinus* parasitizes a wide range of vertebrate hosts, though only some of them can be considered reservoirs of Bb. The reservoirs of these bacteria may be birds, rodents, or insectivores. Ticks also use other hosts, such as domestic and wild ungulates, which are not reservoirs, but are essential for tick survival [18]. The different contact rates among ticks, supporting hosts, and reservoirs, exert an important effect on the prevalence of *Borrelia* spp. in ticks and are responsible for the variable local prevalence of the pathogen in ticks [19]. For a focus to persist, there must be (i) appropriate environmental conditions for the survival of the vector, (ii) an adequate density of reservoirs, and (iii) a temporal and spatial overlap between both reservoirs and vector.

The dynamics of these foci, the conditions that propitiate them, and their long-term evolution are yet unanswered but for local scales. This study aims to separately model the spatial probability of the contact rates among *I. ricinus*, its hosts, and the most important reservoirs reported for two species of *B. burgdorferi* s.l. in Europe, namely *B. afzelii* and *B. garinii*. The framework is based on a previous development aimed to evaluate the persistence of foci of pathogens through an strict definition of contact rates based on environmental features [20]. Temperature and vegetation variables obtained for the period 2002–2017 are projected to build scenarios for the years 2030 and 2050 to model the probable distribution of hosts, reservoirs, and the tick according to these scenarios, and derive the predicted contact rates. This separate modelling by species and reservoirs seeks to generate basic information on the impact of climate on Lyme borreliosis, addressing the changes in contact rates of the main actors supporting pathogen foci under the expected future climate condition.

## Material and methods

### Background

In this study, we adhered to a strict definition of the environmental niche of the tick *I. ricinus*, as well as its reservoirs and hosts [14, 20]. The environmental niche of a pathogen is not spatial, as commonly addressed, but defined as the combination of traits in an area (temperature, humidity) whose intersections allows reservoirs, vectors, and their hosts to survive, generating stable pathogens foci. The niche shapes a probability for the vector, its hosts, and the main reservoirs of Bb to coexist. We use such a definition to define contact rates among ticks, hosts, and reservoirs based on current climate data, and the projected climate for the years 2030 and 2050 based on the land surface temperature (LSTD) and the Normalized Difference Vegetation Index (NDVI). The NDVI is an estimate of the photosynthetic activity of the vegetation, but many studies have utilized this index as a proxy of the relative humidity of an area [21, 22]. In addition, these variables and their annual oscillations are the best descriptors of the environmental niche of arthropod vectors [23]. The expected distribution of each organism has been generated with the actual climate data in the period 2002–2017 recorded by the MODIS series satellites. The climate trend in this baseline period was used to build the scenarios for the years 2030 and 2050.

We adhered to a previous development that promotes the coefficients of a harmonic regression, also known as a Fourier series, representing the value of each pixel for one complete year, as the environmental traits that adequately describe the habitat for ticks [14]. We demonstrated that the use of satellite imagery improves the modeling output compared to interpolated climatologies because satellite records represent measurements on the ground surface, where ticks live [20]. This method uses only six variables (the three first coefficients of the harmonic regression for either LSTD or NDVI) to define the environmental niche. This is an improvement in the model output because it uses variables with ecological significance for the dynamics of the ticks [20] and the absence of over-fitness, a statistical issue that could produce a falsely correct model if too many descriptive variables are used [24].

## Obtaining climate data

To describe the environmental niche of the organisms involved in the transmission of Bb, climate data have been obtained from the MODIS website. Interpolated climate data have not been used because they lack moisture estimates (only precipitation is available), resulting in models of insufficient quality, probably because moisture has a higher performance than precipitation in modelling the distribution of *I. ricinus* [25]. Products from the Terra series of satellites (https://modis.gsfc.nasa.gov/data/dataprod/) were used between the years 2002 and 2017. These data have a spatial resolution of 0.05° (approximately 5,600 meters) and correspond to the products MOD11C3 (for LSTD) and MOD13C2 (for the NDVI). We chose these years because data from subsequent years (i.e., after the year 2018) were not available with scientific precision at the time of this study. For the selection, download, and processing of the data corresponding to the target territory, the "MODIStsp" library [26] was used for the R programming environment [27] that allows access to the server API of MODIS. We used monthly data from the mentioned time period between coordinates 18° O and 41.5° E, 30.7° N and 73° N.

## Climate data processing

The MODIS climate dataset was processed in five steps: (i) calculation of the average monthly values for each month in the period 2002–2017 resulting in one year of averaged monthly values, (ii) calculation of the Fourier coefficients that define LSTD and NDVI in the average year for the period 2002–2017, (iii) calculation of the temporal trend of each month in the period 2002–2017, (iv) extrapolation of that monthly trend to the years 2030 and 2050 to obtain the monthly values of LSTD and NDVI for those years, and (v) calculation of the Fourier coefficients for those new scenarios describing the projected climate environments. All of the previous steps were performed separately for the LSTD and NDVI variables. The Fourier series coefficients were calculated using an already developed, published, and open script [14].

The temporal trend of each month was calculated using the "lm" (linear model) function of R [27], taking the slope of the regression line as the indicator of the trend of LSTD or NDVI between 2002 and 2017 for each pixel. The new projected data for each month were obtained by replacing the "x" of the regression line with the new values to be calculated (months of the years 2030 or 2050). After the new monthly values were obtained, Fourier coefficients were calculated for each year.

The linear regression was subjected to a quality control to verify that the modeled climate data were kept within adequate margins of error. Comparing the output of a linear regression producing future values of LSTD and NDVI to the already available scenarios based on interpolated climatologies is complex. Both products differ, in addition to the obvious difference between the temperature recorded at the ground level or at 2 m above the ground. Moreover, interpolated climatologies have no data on NDVI, as it is a satellite-derived product only. We performed a quality control comparing the annual averaged LSTD from MODIS with the average annual temperature in WorldClim at the resolution of 0.05° [28]. We also compared the annual averaged MODIS-derived LSTD projected to the year 2050 to the average annual temperature obtained in five climate scenarios available in WorldClim, for a Representative Concentration Pathway (RCP) of 45 (HadGEM-AO, HadGEM-CC. HadGEM-ES, GFDL-CM3, and GFDL-ESM2G). A RCP is a grenhouse gas concentration (not emissions) trajectory adopted for calculation of future climate scenarios. Next, we carried out an ANOVA to determine significant differences between the ratios of the baseline periods (MODIS *vs* current WorldClim) and those of the year 2050 (MODIS *vs* climate scenarios). If significant differences are not apparent between the two periods, then the satellite-based projections of temperature follow the same trend as those produced by the atmosphere-based climate scenarios and we can assume that MODIS-derived environmental traits obtained for the year 2050 are reliable. No climate scenarios are available for the year 2030.

## Fitting the models with actual distribution data of ticks and vertebrates and obtaining projections from environmental scenarios

Several algorithms are available to obtain prediction maps of species distribution (presence/absence, not abundance) based on descriptive variables of the habitat. In this study, we used the algorithm MaxEnt (Maximum Entropy), the efficacy of which has been widely demonstrated [29–31]. Distribution models need to be "trained" with the known distribution of the species to be modeled, from which the combination of variables that define the probability of a species' presence in space is obtained. The records with the coordinates of the tick *I. ricinus*, the reservoirs of *B. burgdorferi* s.l., and the hosts of the vector were obtained from previous data that were made publicly available [16, 32]. These coordinates were already subjected to quality control at the time of publication [16]. The complete overview of the distribution of *I. ricinus* is included in S1 Fig. Briefly, the original dataset contains about 14,000 records of questing *I. ricinus* in Europe, plus around 3 million records of the 160 species of vertebrates on which the ticks has been collected feeding. The original paper [32] introduced the concept of "contact rates" as the habitat overlap between the tick and vertebrates weighted by the relative importance of each vertebrate for supporting populations of the tick, or the relative importance of each host as reservoir of *Borrelia* spp. The original dataset also provided several scripts for the R programming environment [27] allowing calculations of expected distributions, niche overlapping and contact rates between the tick and the vertebrates expected to colonize every image pixel. All the models illustrating the predicted distribution of the vector and the vertebrates were validated and discussed previously [32]. The distribution dataset of vertebrates was obtained from GBIF, and is also publicly available [32].

We included 18 species of vertebrates. The species were chosen according to a recent review as being the most important in the circulation of either *B. garinii* or *B. afzelii* [33]. Mammalian species reported to be reservoirs of *B. afzelii* and the number of records over the target territory with reliable pairs of coordinates are: *Apodemus agrarius* (612), *Apodemus flavicollis* (2,180), *Apodemus sylvaticus* (10,287), *Erinaceus europaeus* (15,062), *Lepus europaeus* (17,709), *Microtus agrestis* (2,180), and *Myodes glareolus* (54,670). Bird species, the main reservoirs of *B. garinii*, are *Cyanistes caeruleus* (122,049), *Erithacus rubecula* (104,472), *Fringilla coelebs* (153,063), *Parus major* (99,476), *Phylloscopus collybita* (93,190), *Sylvia atricapilla* (23,347), *Turdus merula* (129,003), and *Turdus philomelos* (86,230). Other vertebrates included in the study that are not recognized reservoirs of *B. burgdorferi* s.l., but important hosts for *I. ricinus*, are *Capreolus capreolus* (29,807), *Cervus elaphus* (9,199), and *Vulpes vulpes* (49,492). Vertebrate records total 1,002,028 coordinate pairs and the distribution of *I. ricinus* is represented by 14,108 points.

To calculate the environmental niche of each species, the "wallace" package [34] for R [27] was used. This library depends on the MaxEnt algorithm to obtain the expected distribution under current conditions (years 2002–2017) and project it on future scenarios. As explanatory variables, the first three coefficients of the Fourier series for LSTD and NDVI were used (six variables in total). As indicated above in the section "obtaining climate data" the environmental variables describing the niche of the ticks and the vertebrates were derived from a Fourier transform (i.e. harmonic regression) of the monthly time series of satellite images. This procedure deconstructs the complete time series into coefficients that synthetically describe the original data. It has been demonstrated that this approach produces a better modelling outcome than using interpolated climate data or averaged monthly estimates directly derived from the time series [20]. These variables describe the mean value of the variable for the considerd period of time (either LSTD or NDVI), the slope in the spring (i.e. how fast or slow is the spring rise of LSTD or NDVI) and the negative autumn slope (i.e. how fast the summer turns into lower autumn values of LSTD or NDVI). The algorithm was trained with 50% of the records of each species to be modeled, using the remaining 50% to iteratively check the confidence of the fit. The whole process was repeated 10 times for each species, randomly selecting different training and test sets, obtaining the best possible distribution model. The suitability of each model was verified using the area under the curve (AUC) of the test set, as the Akaike information criterion provides erroneous results when applied to a geographical extension [35]. The AUC compares the outcome of each model with the recorded distribution of each species, producing an evaluation of its quality in terms of similarity of the predicted *vs* its known distribution.

Predictive raster maps were produced for each species and stacked to calculate the environmental overlap of the tick with each species of vertebrate. This is calculated on a pixel by pixel rate for the complete raster, comparing the values of the probability of presence of the tick and any other vertebrate at each pixel. The calculations of the contact rates among vertebrates and the tick were obtained using an already published R script that explicitly computes the habitat overlap between each species of reservoir, vertebrate, and the tick in the range of each environmental variable, producing a value reweighted between 0–100. The script is publicly available [32].

### Projection of results into the geographic space

All data resulting from the calculations above were summarized in the biogeographical regions of the target territory. We used the LANMAP2 product [36] that describes the biogeographic characteristics of the European territory. In addition to providing a coherent definition of the territory, LANMAP2 synthesizes the region into 14 climatic domains. This procedure resulted in maps that display the projections of contact rates into an easily captured geographic space.

## Results

The difference between the MODIS- and WorldClim-derived temperature had a relative narrow range, with a greater difference in southern latitudes (Fig 1). The pattern was similar in both the baseline period and the year 2050, with smaller differences at the later time period. A pixel by pixel comparison indicated a lack of significant differences between the discrepancies in temperature in both the the baseline period and 2050 ($F = 1251.08$, $p = 0.156$), indicating that MODIS-projections to 2050 do not significantly differ from purposely developed scenarios of interpolated climate. Therefore, the regression used to build the LSTD and NDVI values for 2030 and 2050 was assumed to be realistic. No scenarios exist for 2030.

Fig 2 shows the trends in LSTD and NDVI aggregated for the European biogeographic regions extrapolated for the years 2030 and 2050 with respect to the baseline values. In general, all biogeographic regions undergo projected changes of variable magnitude, with an obvious trend in the years 2030 and 2050: increases of up to 0.5°-1° in LSTD and increases in the NDVI

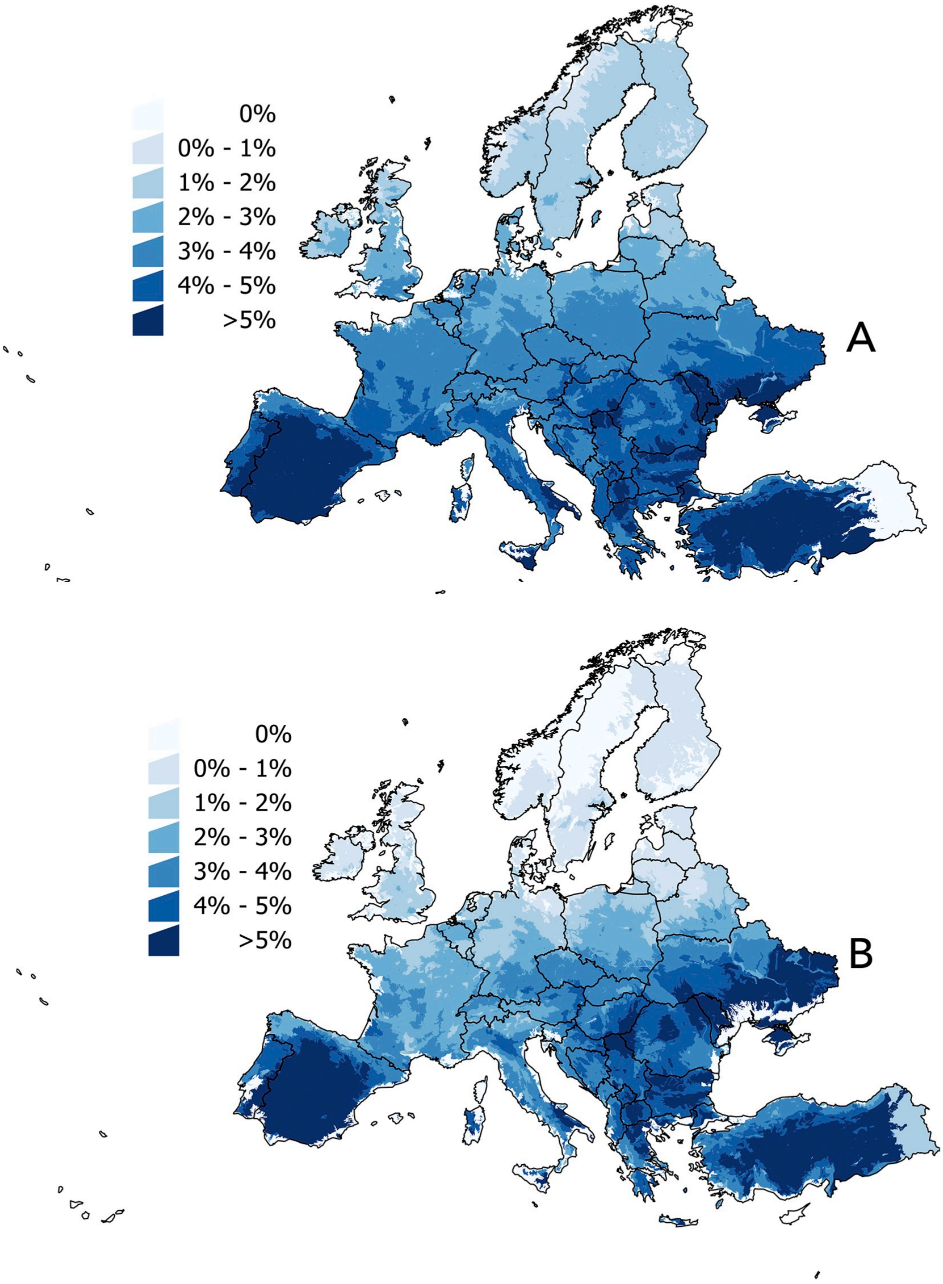

**Fig 1. Difference (in %) between satellite-derived values of annual averaged land surface temperature and the interpolated annual averaged temperature obtained from WorldClim.** (A) In the period 2002–2017. (B) In the year 2050.

https://doi.org/10.1371/journal.pone.0233771.g001

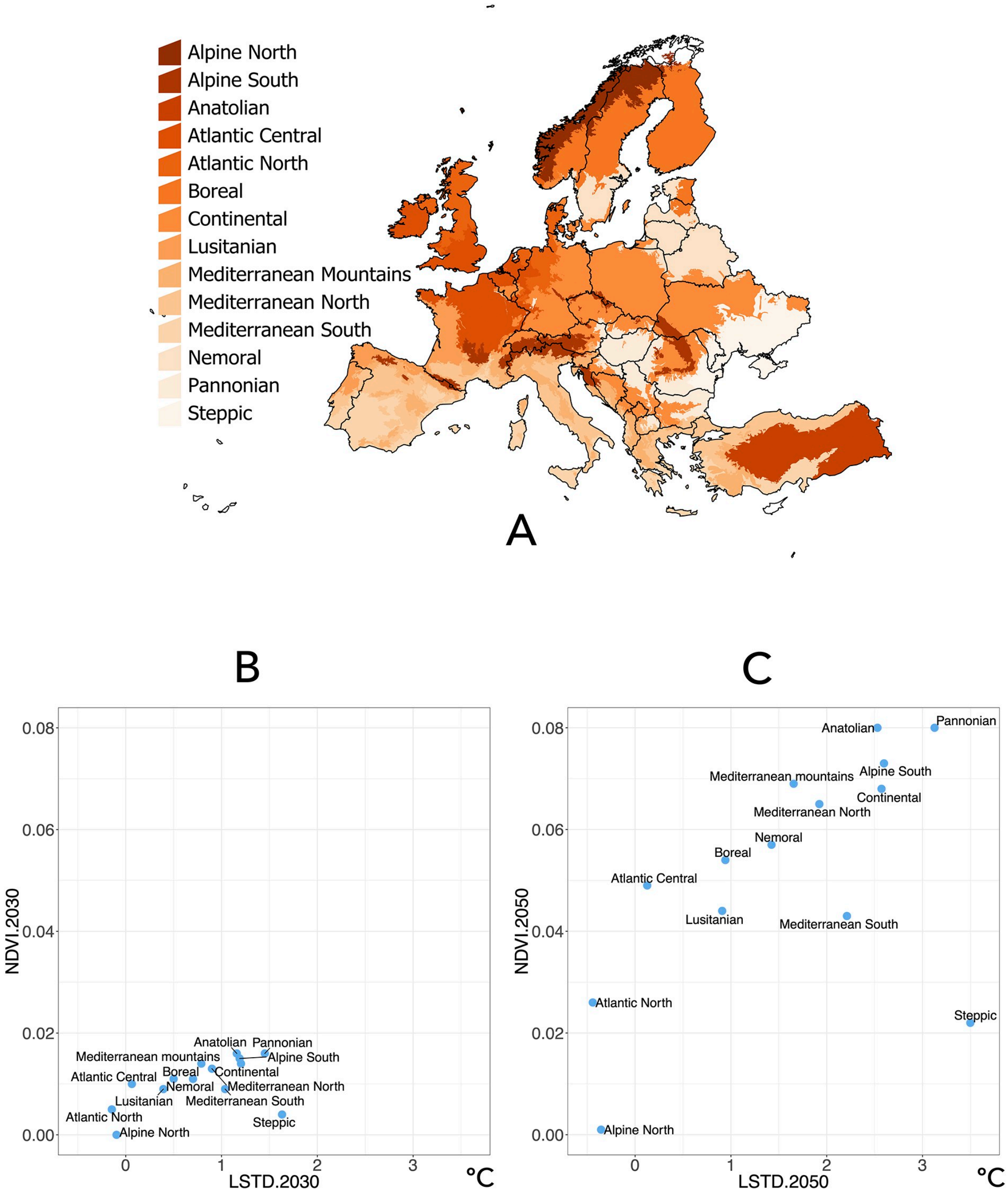

**Fig 2. The biogeographic regions of Europe used in this study with the trend of environmental traits.** (A) The geographic distribution of the biogeographic regions used in this study based on LANMAP2. (B) The projected changes in the values of the satellite-derived land surface temperature (LSTD) and the Normalized Difference Vegetation Index (NDVI) in the year 2030 compared to the baseline period (2002–2017) and obtained by time series regression. (C) The projected changes in the values of the LSTD and NDVI in the year 2050 compared to the baseline period (2002–2017) and obtained by time series regression. NDVI values are unitless and range from -1 to +1.

https://doi.org/10.1371/journal.pone.0233771.g002

of 0.02–0.08 (on a scale of -1 to 1). Note the changes in the Lusitanic, Boreal, Nemoral, Southern and Northern Mediterranean, Mediterranean, Continental, Southern Alpine, Anatolian, and Panonic regions. The climate determined for the two time periods is projected to be moderately warmer, with a longer vegetative period (growth season). An exception to this are the southern areas, which would experience a greater projected temperature increase, promoting a decrease in the green period (or higher dryness). Fig 3 projects these forecasts on the geographic context.

The raw distribution maps of every species had an AUC $> 0.72$ in every case (minimum for *Apodemus sylvaticus*, 0.72; maximum for *Ixodes ricinus*, 0.92). We do not intend to explain the behavior of each individual model for each species and how organisms use the environmental niche; our goal was to obtain the contact rates and track their trends under climate conditions expected in the near future. The predicted environmental suitability weighted by the probability of contact between *I. ricinus* and bird species for the different slices of time is included in Fig 4. In the maps, warmer colors indicate a greater probability of habitat overlap between the vector and reservoirs, which is interpreted as a higher risk of pathogen circulation.

The climate in the baseline period (Fig 4A) reflects a wide co-distribution of the vector and bird species throughout central Europe, the British Isles, southern Norway, and wide regions of central and southern Sweden. Note the wide area of high probability of contact among birds and *I. ricinus* in the strip of the Mediterranean coast of the Balkan countries. The projection of climate data to the year 2030 (Fig 4B) predicts an increase in vector-bird contact in a large territory of southern Finland and Estonia, an increase in latitude on the coast of Norway, and increased risk in eastern areas of the European territory, such as Poland, the Czech Republic, and Slovakia. However, climate projections for the year 2050 predict a decrease in areas in which the vector and birds could coexist (Fig 4C). Forecasts for that slice of time still delineate the linear relationship between contact rates and latitude. A patent decrease in the probability of overlap among birds and *I. ricinus* is predicted in the rest of Europe. In short, contact rates among *I. ricinus* and the selected species of birds would reach a maximum around the year 2030, and then decrease as the LSTD and NDVI traits promote a decrease in the probability of contact among the vector and bird reservoirs.

The modelling of the habitat sharing among *I. ricinus* and the mammalian reservoirs of *B. afzelii* (Fig 5) shows clear differences from the predictions regarding birds and *B. garinii*. The spatial projection shows wide areas of high contact rates along the Atlantic regions, central Europe, and southern Scandinavia (Fig 5A). The trend for the year 2030 predicts that changes in contact rates over time are smaller than those previously noted for birds, with a northern and eastern trend (Fig 5B). Changes were predicted mostly in the Baltic countries, areas of Eastern Europe, southern Finland, and the Balkans. Models projected into the year 2050 (Fig 5C) show a general decrease in the probability of overlap between the vector and mammalian reservoirs, though at some points, such as in Finland, the probability of contact is still higher than in the baseline period.

The modelled contact rates among *I. ricinus* and some non-reservoir hosts for the baseline period (Fig 6A) show similar predictions; maximum environmental overlap is forecasted along the Atlantic coast and in the south of the Nordic countries. The models projected into the years 2030 and 2050 (Fig 6B and 6C) predict a general decrease in the overlap between *I. ricinus* and some of its main hosts in the lower latitudes, together with an increase at higher latitude and longitude in the target territory.

## Discussion

We addressed how climate trends could impact the relationships among *I. ricinus*, the reservoirs of *B. burgdorferi* s.l. in Europe, and other vertebrates that feed the tick to capture the

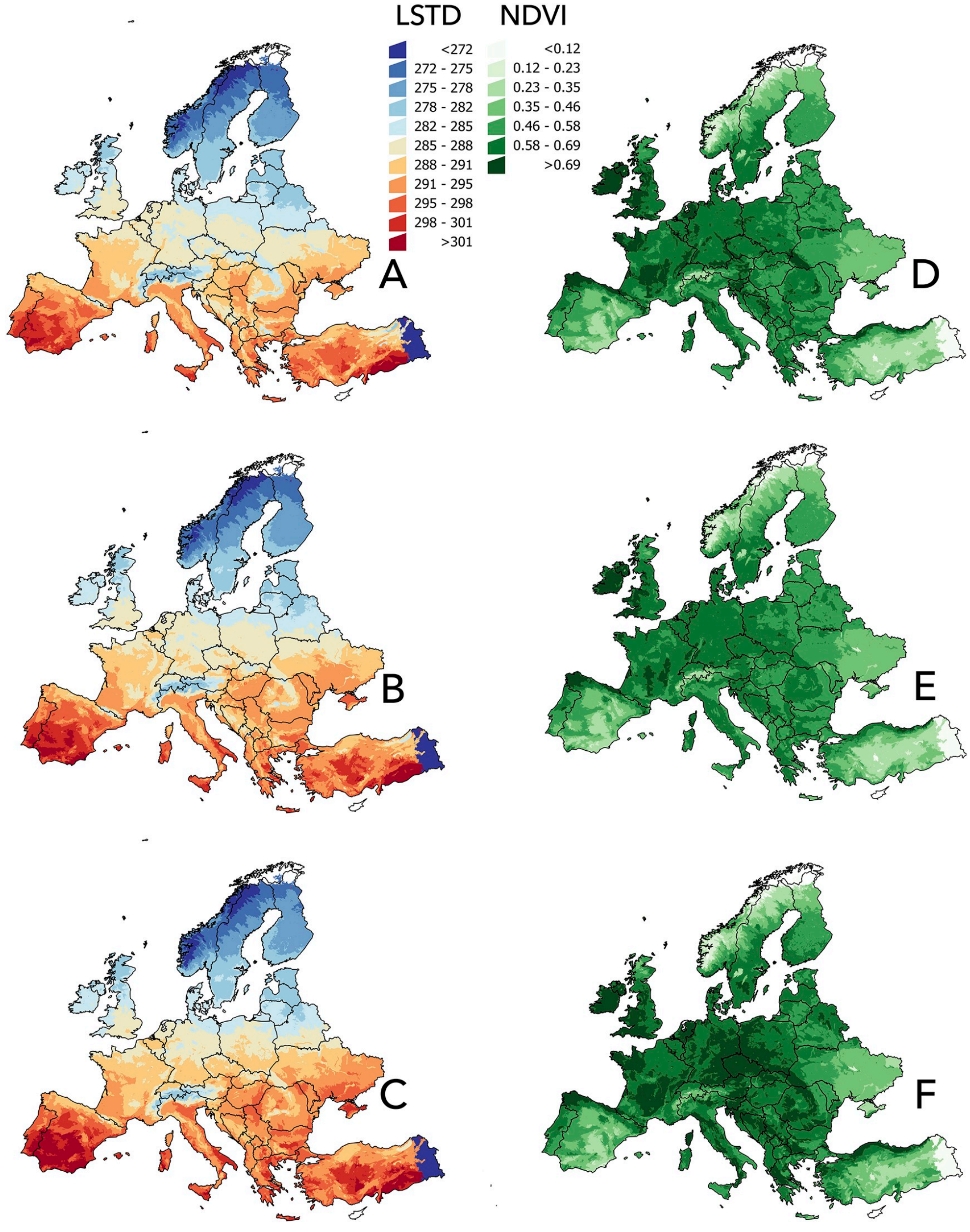

**Fig 3. Spatial projection of the satellite-derived annual average land surface temperature and Normalized Difference Vegetation Index (NDVI).** (A) Land surface temperature in the periods 2002–2017, (B) 2030, and (C) 2050. (D) NDVI in the periods 2002–2017, (E) 2030, and (F) 2050. Values of temperature are in Kelvin; values of NDVI are unitless.

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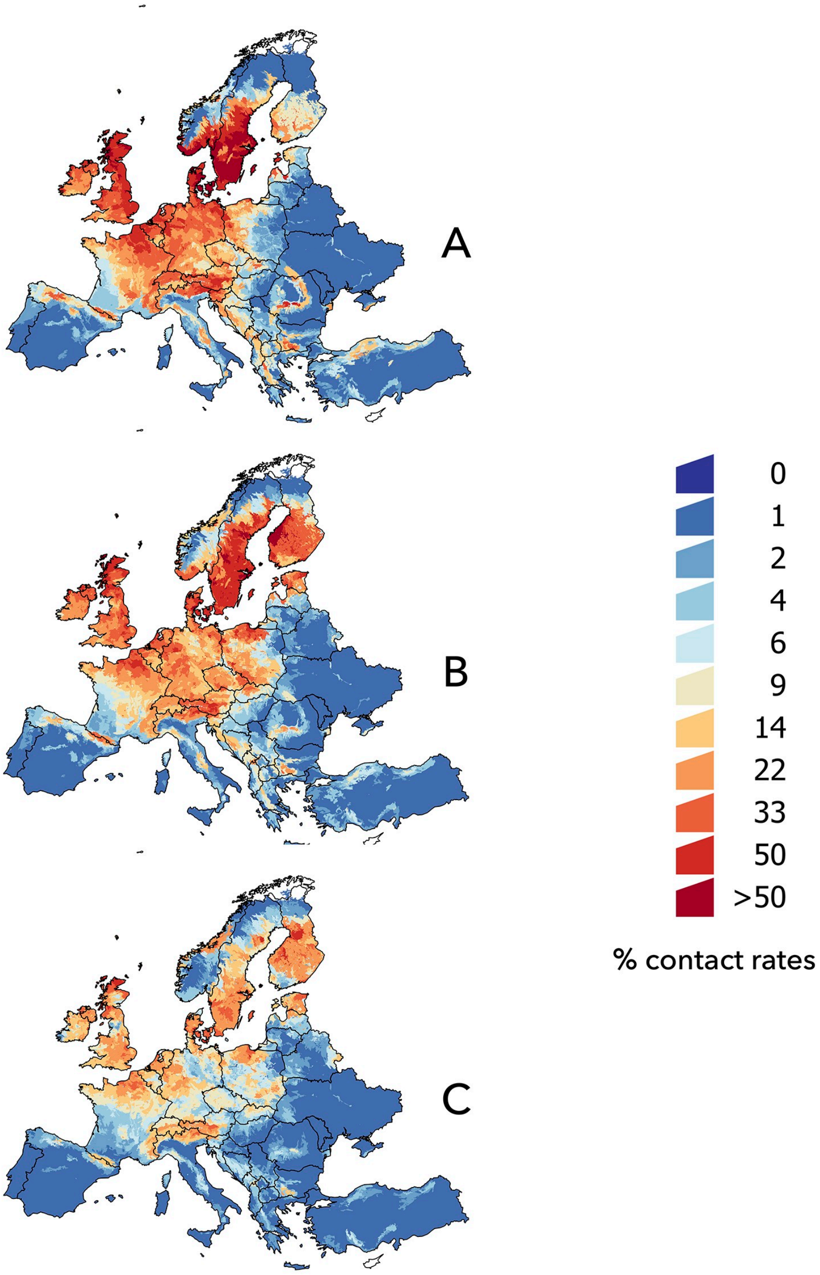

**Fig 4. The spatial distribution of the projected contact rates between *Ixodes ricinus* and avian reservoirs of *B. garinii* in the target territory.** (A) 2002–2017, (B) 2030, (C) 2050.

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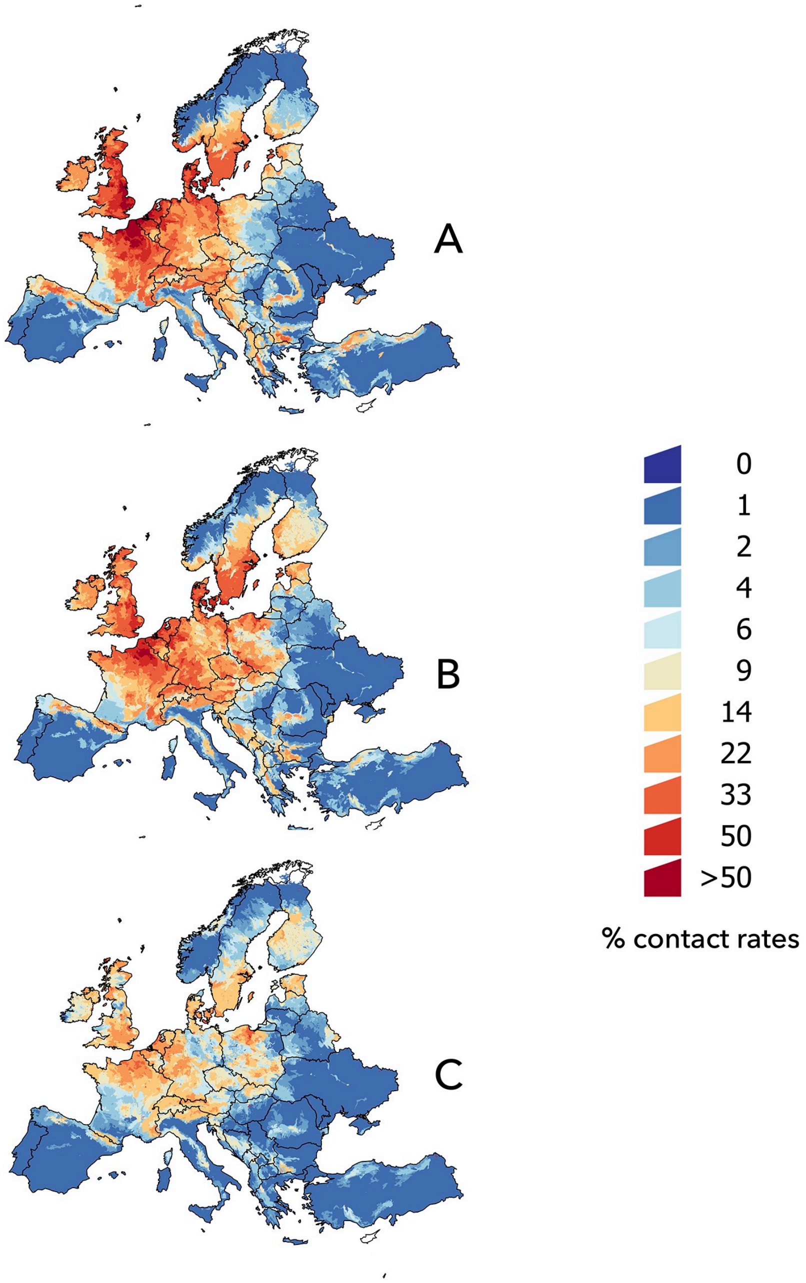

**Fig 5. The spatial distribution of the projected contact rates between *Ixodes ricinus* and mammalian reservoirs of *B. afzelii* in the target territory.** (A) 2002–2017, (B) 2030, (C) 2050.

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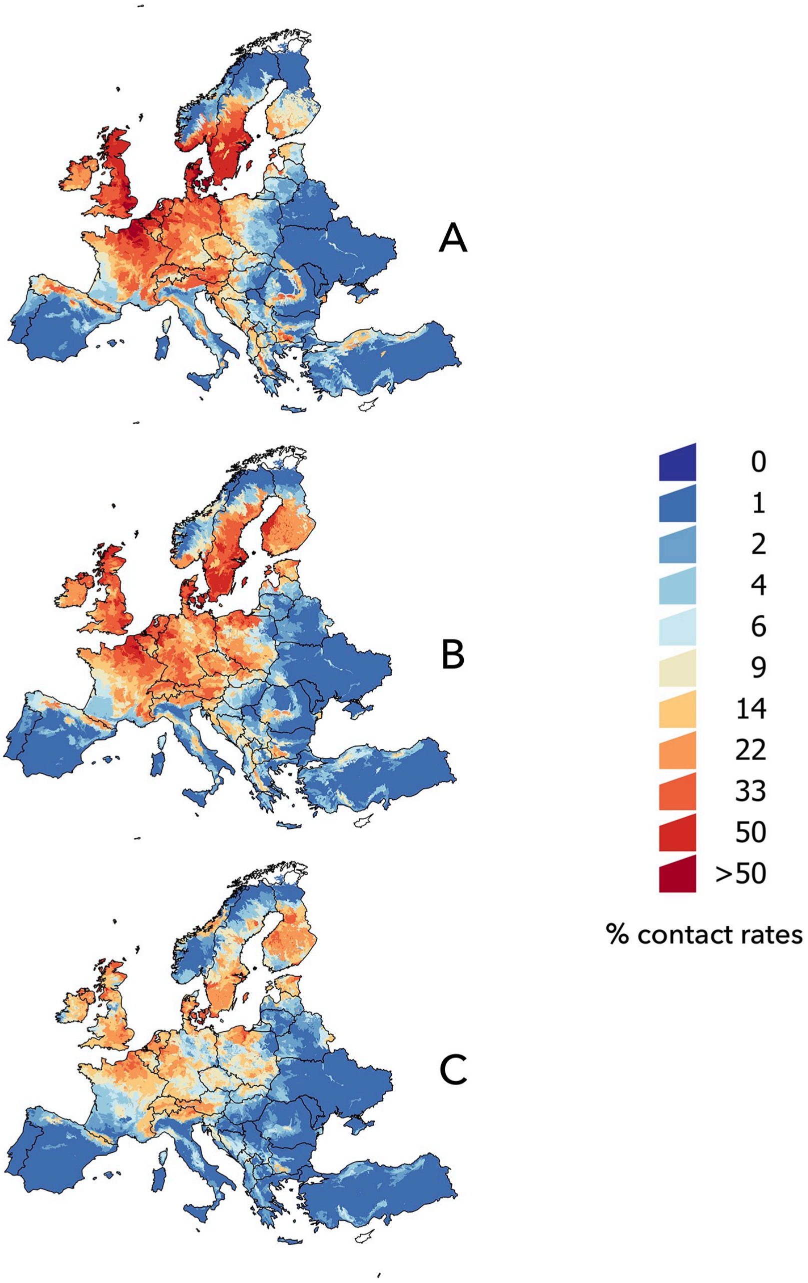

**Fig 6. The spatial distribution of the projected contact rates between *Ixodes ricinus* and the mammalian hosts that are not reservoirs of *Borrelia* spp.** (A) 2002–2017, (B) 2030, (C) 2050.

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probable contact rates of organisms circulating the pathogen. The analysis is based on previous developments that refer to the environmental niche as the basic method for evaluating these relationships [14,32]. Tick-borne pathogens persist due to a strict combination of environmental variables, which model the distribution of reservoirs that allow their circulation in permanent foci [25]. The novelty of this approach lies on the well supported concept that stable foci of a tick-borne pathogen require not only adequate environmental conditions for the vector, but also a sufficient contact rate with reservoirs [37]. Although mechanistic models have been used for years to delineate the probable distribution of ticks, they had not been used together with the expected habitat overlapping with reservoirs of a tick-borne pathogen, shaping the areas in which the pathogen could circulate. Previous modelling approaches on the impact of climate trends on ticks and risk of Lyme borreliosis addressed the changes of the life cycle of the tick under the climate conditions of future scenarios [5,13]. A recent study with similar aims [17] considered Lyme borreliosis risk driven by the presumed abundance of the vector and an estimation of the habitats preferred by vertebrates (without considering particular species), evaluating the expected changes under the conditions of future climate scenarios. That study [17] predicted that an enlarged period of tick development could lead to increased rates of transmission of *Borrelia* spp. These modelling approaches, however, look unrealistic since the "current" scenario predicted a high risk by Lyme borreliosis at peak season in areas where the tick is absent (i.e. eastern Spain) or a lack of risk in endemic countries (i.e. Ireland). In the current study, we claim that mechanistic models based on the environmental niche of the target tick, its hosts, and the *known* reservoirs of a pathogen could unveil both biotic and abiotic effects derived from climate trends, complementing process driven models [13] and producing a pixel-size picture of the presumed distribution of each organism involved in the circulation of *Borrelia* sp. Further on this, modelling separately two species of *Borrelia* with known different reservoirs provided a better understanding of the climate actions impacting the relationships between vectors and reservoirs.

This framework is based upon an explicit environmental description provided by satellite images and harmonic regression [38]. Time series of satellite data can be deconstructed into their harmonic components, which are not autocorrelated and explain the climate of a time period with a few variables. The use of interpolated climatologies [39] may be unreliable in this context because of the spatial autocorrelation of the descriptive variables, which artificially increases the statistical reliability of the models; the lack of ecological significance of some variables for ticks; the interpolated climatologies describe temperature in the air, whereas ticks live on the ground; and the lack of estimations of relative humidity or saturation deficit [40], which were replaced by the NDVI in our approach. The NDVI represents the photosynthetic vigor [41] that has been shown to be correlated with *I. ricinus* tick distribution.

The primary source of uncertainty in the current study arose from the reliability of the projected climate for 2030 and 2050. We obtained estimations of the near future climate by applying a time series regression to the baseline period of averaged monthly data (2002–2017). We acknowledge that linear regression may not completely capture the climate trends in such a large territory, as they depend on complex atmospheric interactions. Therefore, we opted to compare whether the deviations from other temperature datasets were of similar magnitude between two time periods (baseline and 2050), indicating that our projections are valid. An ANOVA unveiled a lack of significant differences between the deviations of both sets of values (baseline and 2050). Therefore, MODIS-projected values for both LSTD and NDVI in 2050 were considered reliable and robust enough to proceed.

A secondary source of uncertainty arose from the reliability of the distribution modeling of the tick and species of vertebrates that interact, producing contact rates, which depend on the extent to which explanatory variables influence the model outcomes. Every model was within the expected margins of reliability, with AUC values well over 0.7 (range 0–1). As explanatory variables are not autocorrelated, which is a feature that affects model performance [20], we consider AUC an adequate marker of model reliability. In addition, the high number of coordinates for vertebrates and the best existing *I. ricinus* dataset (more than 14,000 records, collected by specialists) convey reliability to the modeling approach.

The current study only considered the relative contributions of hosts acknowledged to have a recognized competence in the circulation of *Borrelia* spp. [33] disregarding other vertebrates that may have a local impact in the target territory. It also unaddressed features regarding the relative impact of density of vertebrate populations (i.e. deer or other wild ungulates *versus* rodents or birds) on the abundance of the tick or the persistence of *Borrelia* spp. Therefore, an unaddressed source of uncertainty depends on the relative faunal composition at the landscape scale and deserves further research within the framework outlined in this study. While we included the *most* important reservoirs of *Borrelia* spp. in the target territory, as reported [14, 33], the current study did not consider *every* possible reservoir. In some cases, it has been because the lack of a reliable number of coordinates for modelling; in others because no adequate data on reservoir abilities have been obtained. Undoubtedly, the exclussion of vertebrates that may be abundant at the local scale would bias predictions. However, such a bias should be negligible at the scale of biogeographical region, as addressed in the current study. In any case, if the proposed framework is transferred into a higher resolution, special attention to this source of uncertainty should be spacially paid.

The general overview of the expected climate shows a trend towards higher LSTD and NDVI values, with the exception of southern regions of Europe, in which the NDVI shows a tendency for higher aridity. Most important in the context of this study is the increase in LSTD and NDVI in regions of Eastern Europe and Scandinavia. The clear trend of increased NDVI in the Nordic countries is a point of interest in the epidemiology of Lyme borreliosis. Our projections are in line with previous conclusions regarding the movement of Lyme borreliosis to northern latitudes as a consequence of a longer growth season [42]. There is serious concern regarding the spread of *B. burgdorferi* s.l. into the northern latitudes in Europe [43]. This is not only the consequence of a northern spread of *I. ricinus*, which has already been recorded at 65˚N [10], but also of the joint spread of both the tick and the reservoirs of *Borrelia* spp., together with local high densities of large (non-reservoir) mammals supporting the adult ticks. The modeled forecasts agree with available field data, underlining the suitability of this modeling approach to explicitly shape the habitat overlap.

Both *B. garinii* and *B. afzelii* have already been reported to have a different distribution in Europe, which is attributed to their reservoirs' different responses to the environment [37]. This is a point of interest because the climate trends, at least for the years 2030 and 2050, are predicted to promote a divergence in the distribution of reservoirs, with a direct impact on the probable distribution of the pathogens. The projections for the year 2030 show a dramatic potential spread of *B. garinii* in large northern areas, some parts of Central Europe, and the Baltic countries. *Ixodes ricinus* has traditionally been restricted in the eastern European region by colder winter temperatures. In these areas, *I. ricinus* is replaced by *I. persulcatus*, extending through Russia to northern Japan [44]. Field studies on the dispersion of *B. garinii* towards northern latitudes [42] support a correlation between a longer growth season and the northern spread of *Borrelia* spp., but the spatial pattern of *B. afzelii* has not been explored in depth. Our forecasts correspond well with actual data on the prevalence of these bacteria in Europe [37] and support that modeling based solely on the impact of climate on the vector [17, 37] is insufficient for capturing the range of tick-borne pathogens if the distribution of hosts and reservoirs is disregarded. Our models highlight that climate trends could contribute to split the range of both species of bacteria. However, this trend could be interrupted under the climate conditions projected into 2050. The estimations for that period could disrupt the circulation of the pathogen, although isolated foci would persist. This was an unexpected result. Our estimations predict a clear disruption of the habitat sharing among the reservoirs of a pathogen and its tick vector. This does not mean an extinction of *I. ricinus* because of extreme environmental conditions, but a fracture of the finely tuned environmental overlap among reservoirs and the tick that drives the persistence of *Borrelia* spp. foci.

The capture of environmental suitability for the vector of an infectious agent, its hosts, and reservoirs over large areas is a direct, ecologically meaningful method for generating scenarios that allow epidemiological decisions to be made. Satellite pictures have enormous power to describe abiotic traits impacting ticks and the reservoirs of tick-borne pathogens. As presented here, this background cannot yet capture the high-resolution habitat fragmentation, which may generate the foci observed in the spatial distribution of Lyme borreliosis [45]. However, it is a promising tool that may supersede other approaches, providing comprehensible insight into the scenarios of future climate conditions if adequately trained. The unexpected predictions regarding the decrease in habitat sharing among *I. ricinus* and vertebrates around the year 2050, and the consequent decrease in pressure by some infectious agents deserves further research.

## Supporting information

**S1 Fig. The recorded distribution of *Ixodes ricinus* in the target area.** The figure includes about 14,000 records of all the stages of *I. ricinus* with adequare georeferencing. The vast majority of the records have been validated by local experts. However, part of the records in Finland are old (around 1970's) and would need to be re-examined. Some records of the tick in Africa may represent a different species, *Ixodes inopinatus*. All the data are available at https://datadryad.org/resource/doi:10.5061/dryad.2h3f2.
(PDF)

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## Author Contributions

**Conceptualization:** Agustin Estrada-Peña.

**Data curation:** Natalia Fernández-Ruiz, Agustin Estrada-Peña.

**Formal analysis:** Agustin Estrada-Peña.

**Investigation:** Natalia Fernández-Ruiz.

**Software:** Agustin Estrada-Peña.

**Visualization:** Agustin Estrada-Peña.

**Writing – original draft:** Natalia Fernández-Ruiz, Agustin Estrada-Peña.

**Writing – review & editing:** Natalia Fernández-Ruiz, Agustin Estrada-Peña.

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# Chapter 2: Towards new horizons: Climate trends in Europe increase the environmental suitability for permanent populations of *Hyalomma marginatum* (*Ixodidae*)

pathogens

*Article*

# Towards New Horizons: Climate Trends in Europe Increase the Environmental Suitability for Permanent Populations of *Hyalomma marginatum* (Ixodidae)

**Natalia Fernández-Ruiz [1,2,*,†] and Agustín Estrada-Peña [1,2,†]**

[1] Faculty of Veterinary Medicine, University of Zaragoza, 50013 Zaragoza, Spain; antricola@me.com
[2] Group of Research on Emerging Zoonoses, Instituto Agroalimentario de Aragón (IA2), 50013 Zaragoza, Spain
* Correspondence: natalia.ferru@gmail.com
† All authors contributed equally.

**Abstract:** Ticks and tick-borne pathogens are changing their current distribution, presumably due to the impact of the climate trends. On a large scale, these trends are changing the environmental suitability of *Hyalomma marginatum,* the main vector of several pathogens affecting human health. We generated annual models of environmental suitability for the tick in the period 1970–2018, using harmonic regression-derived data of the daily maximum and minimum temperature, soil moisture and water vapor deficit. The results demonstrate an expansion of the suitable area in Mediterranean countries, southeast central Europe and south of the Balkans. Also, the models allowed us to interpret the impact of the ecological variables on these changes. We deduced that (i) maximum temperature was significant for all of the biogeographical categories, (ii) soil humidity has an influence in the Mediterranean climate areas, and (iii) the minimum temperature and deficit water vapor did not influence the environmental suitability of the species. The conclusions clearly show that climate change could create new areas in Europe with suitable climates for *H. marginatum*, while keeping its "historical" distribution in the Mediterranean. Therefore, it is necessary to further explore possible risk areas for *H. marginatum* and its associated pathogens.

**Keywords:** *Hyalomma marginatum*; Europe; climate trends; environmental suitability

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## 1. Introduction

Major changes in ecosystems are being driven by the fast anthropogenic climate change observed in the early 21st Century [1]. Climate change is defined as "a statistical phenomenon that describes the average climatic conditions for a region, referred to systematic and generally gradual changes in the trend, which is integrated into the random fluctuations of the climate" [2]. Since empirical observations of weather are scattered over time, and memory of past events may be incomplete, climate change is not captured through personal experience. Climate change affects the dynamics of many vector-borne pathogens [3], their reservoirs and human habits, to a yet unknown scale. This may result in the (re)emergence of vector-borne diseases. While studies are diverse, probably the most easily captured effect is the alteration of the known distribution of health threatening pathogens transmitted by arthropods [4].

Ticks transmit the largest number of zoonotic agents in the Northern Hemisphere [5], and as such, are often the focus of research regarding the impact of climate change on human health. Ticks are hematophagous parasites that depend on an environmental niche of temperature and humidity ranges for their population to perpetuate; the overlap of this niche with the one preferred by many vertebrate reservoirs results in areas in which pathogens can circulate. Changes of the geographical distribution of ticks have been well documented for several species in the Nearctic [6–8] and Palearctic regions [9,10]. In short, most studies have connected the spread of ticks with warmer temperatures [11]. Interestingly, no reports seem to exist about the "decolonization" of ticks from their southern habitat as a potential consequence of environmental conditions becoming too warm or dry.

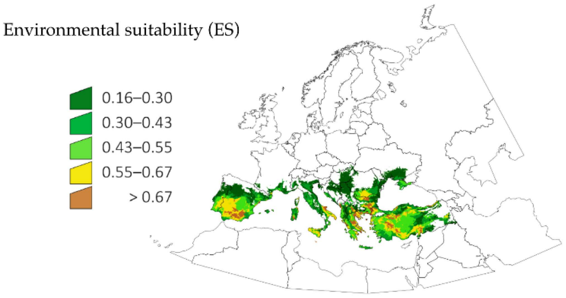
Other than continuous spread by adjacency of populations at the fringe of their environmental niches, ticks can also be introduced into a territory by the uncontrolled movements of livestock or the seasonal behavior of migratory birds. Since the pioneering work by Hoogstraal and his team [12], it has been known that the migratory routes between Africa and Europe serve as routes of dissemination of ticks belonging to the genus *Hyalomma*. Birds carry immatures of at least two species of the genus (*Hyalomma marginatum* and *Hyalomma rufipes*) that feed for several days on hosts, thus allowing transportation and further spread into northern territories. For permanent populations of *Hyalomma* to establish, it is believed that a critical threshold of temperature is necessary for tick development; this temperature provides an index of the chance of establishment. In the second decade of the 20th century, it was believed that this was a rare event, because the critical temperature threshold for molt was not reached out of the Mediterranean range of the species [13]. However, reports of adult *Hyalomma* ticks in Europe [14–18] are becoming increasingly common, suggesting that the accelerated warming affecting central Europe could impact the development of molting immature ticks, allowing adults to survive in sites where they were historically absent. A previous study based on a model of the physiological processes of the tick [19] demonstrated that the climate changes which occurred during 20th century promoted changes in the developmental rates of *H. marginatum*. This tick is considered a concern for human health, because *Hyalomma* ticks are known vectors of the Crimean–Congo hemorrhagic fever virus and *Rickettsia aeschlimannii.* The former is an arbovirus belonging to the genus *Orthonairovirus* (family Bunyaviridae) [20]. Infected patients develop a hemorrhagic fever that has a lethality ranging between 3%–50%, depending on the strain [21]. The viral agent can produce sporadic cases [22] or severe outbreaks in a large geographic area, from western China to the Middle East and in the most southeastern region of Europe [23] and most of Africa [24]. Additionally, *Rickettsia aeschlimannii* is an intracellular bacterium included in the spotted fever group [25] that is of emerging importance in regions of Europe [26]).

This study focuses on changes induced by the climate since 1970, on the expected environmental suitability (ES) for *H. marginatum* in Europe. The aim is to address the joint changes of several weather variables affecting the life cycle of the tick, their trends and their effects on the expected range of its adequate environment. We extracted the daily maximum and minimum temperature, soil surface and air water vapor deficit for each year in the target period using harmonic regressions of a new generation of re-assessed climate data. We used the largest existing dataset of records of *H. marginatum* to develop models addressing the trend of ES for the tick, and produced multiple regressions against environmental stressors to determine the variables driving the suitability for the tick according to standard bio-geographical regions of Europe.

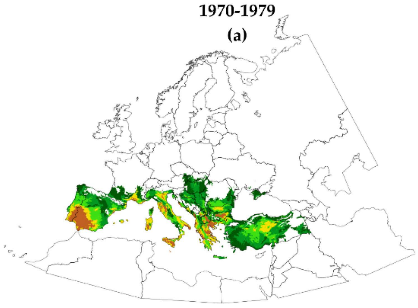
## 2. Results

Figure 1 shows the calculated changes of ES for *H. marginatum* in five time slices of 10 years for the period 1970–2018. These predictive maps are built with a combination of climate variables and the actual records of the tick in the period 1990–2006 (see Methods for a complete description of the modeling process). The figure illustrates the changes of ES in time chunks that are adequate for illustrating the spatial patterns of variation. Notable changes occurred in south coastal and near inner parts of France, with an increase of suitable area in Italy, southeastern central Europe and southern Balkans. A detailed analysis of these data showed the unexpected result that changes had occurred not only in the fringe of the distribution of *H. marginatum*, but also in zones in the currently known distribution of the tick. The increase of ES was continuous throughout the time period for large regions in Spain, France, Italy and the Aegean coasts. The obvious increase of suitable conditions in the south-western Iberian Peninsula observed between 1970–1979 (Figure 1a) and 1980–1989 (Figure 1b) is particularly striking. The period 1990–1999 (Figure 1c) shows the increase of ES in parts of central Europe, Crimean Peninsula and northeast Turkey. The complete picture indicates that, for the period 2010–2018, the most drastic changes occurred in portions of the Mediterranean coast, some large areas around southeastern central Europe and the south Balkans. Results regarding central Europe and Balkans countries are of special interest in the context of the spread of *H. marginatum*.

Figure 2 displays the changes in the trend of ES observed in selected LANMAP2 biogeographic regions of Europe, covering approximately the 80% of the territory (a selection of regions was made to improve the clarity of the presentation of this data). LANMAP2 is a general description of the biogeographic regions of Europe, and therefore, the reporting of the calculated changes according to landscape descriptions is of interest. The trend of ES was calculated by a lineal regression of its annual values in the period 1970–2018. The slope of the ES throughout this period was positive for every territory. It is noteworthy that the areas with the highest trend of ES were not those where *H. marginatum* was already established, but rather, those that are most likely to see future spread, because the environmental niche is quickly becoming suitable. Figure 2 displays the spatial trend of that slope. Major increases of ES for the tick resulted in areas of Lusitanian type climate, associated with landscape categories of pastures and permanent crops. Pannonian-, Atlantic central- and Continental-type climates experienced drastic increases in ES. A large increase in ES was predicted for Mediterranean mountains, suggesting a spread of the suitable environment of the tick into higher altitudes. Changes in climate variables are pushing *H. marginatum* to find adequate habitats further north and at higher altitudes. Interestingly, these changes are observed in different regions of Europe, meaning either an increase of ES in areas where the tick is already present or a trend toward suitability in sites where the ticks does not yet present permanent populations. It should be stressed that the presented ES trend is not an evaluation of the places that could be colonized by *H. marginatum*, but only a representation of the speed of change of climate traits relative to its environmental suitability.

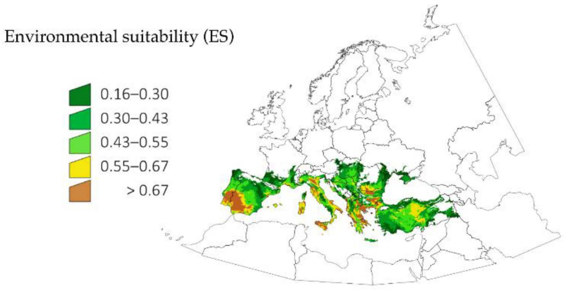


**1970-1979**
**(a)**

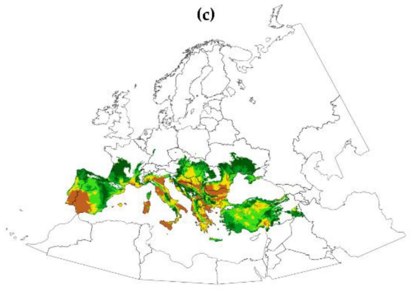


**1980-1989**
**(b)**

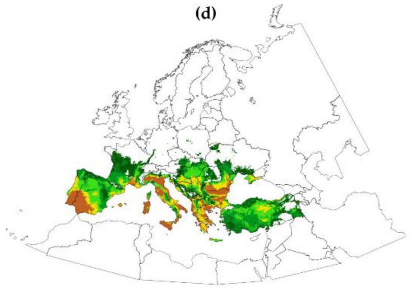
**Figure 1.** *Cont.*



**1990-1999**
**(c)**



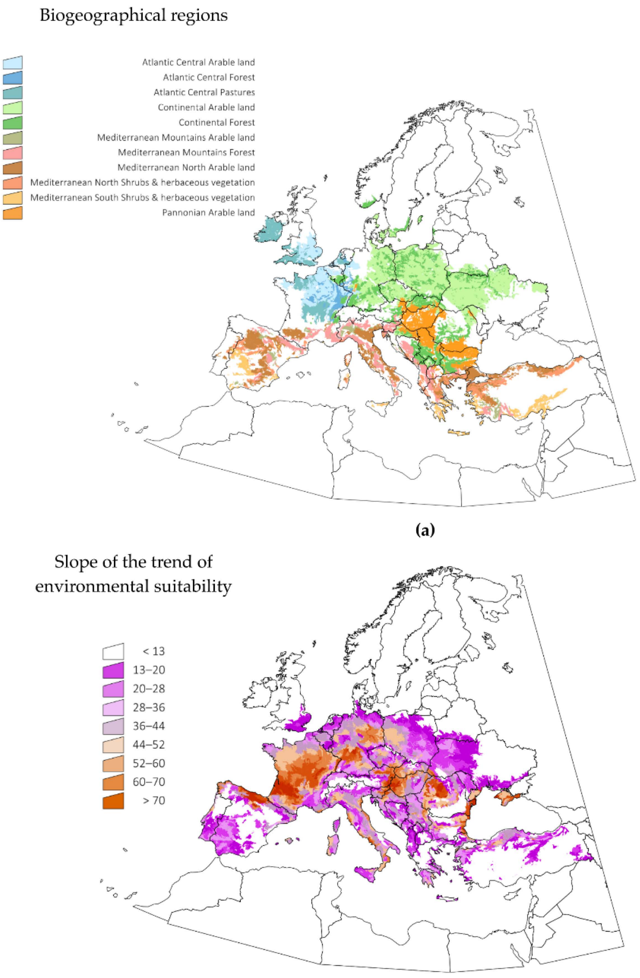
**2000-2009**
**(d)**



**2010-2018**
**(e)**

**Figure 1.** Spatial changes of environmental suitability for *Hyalomma marginatum* in five time slices covering periods of 10 consecutive years. (**a**) 1970–1979, (**b**) 1980–1989, (**c**) 1990–1999, (**d**) 2000–2009, (**e**) 2010–2018.

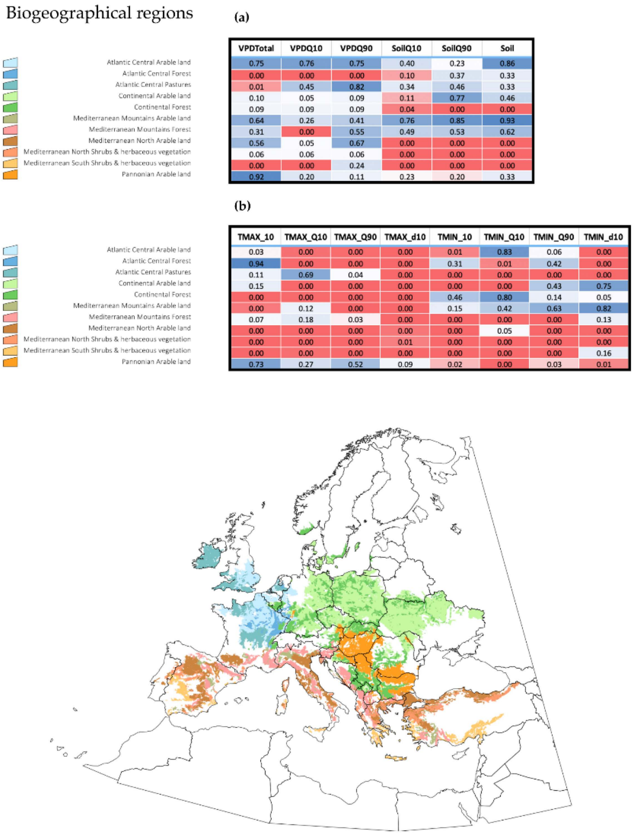
It is important to derive ecological meaning for the variables driving the changes of ES for *H. marginatum* in Europe. We obtained daily values of several explanatory variables using the complete dataset transformed via harmonic regression (see details in the Methods section) including variables related to maximum and minimum temperature, soil humidity and water vapor deficit. We calculated multiple regressions, e.g., the slopes of ES changes and of the explanatory variables, aiming to determine how both groups of slopes were correlated in the period 1970–2018. The results are displayed in Figure 3, separately for variables related to "water" and those related to "temperature". Although beyond the scope of the present discussion, it may be noted that quartile 90 temperature and the number of days above a threshold temperature of 10 °C are good indicators of the increase of ES and are the main driving variables in the northern territories of the target region. However, this is not the rule for every territory. Minimum temperature and soil humidity are highly correlated with the slope of ES in some Mediterranean habitats, suggesting that soil humidity has a role in the expansion of ES. It should be noted that minimum temperatures (i.e., quartile 10) are not behind major changes of ES in the northern territories, meaning that warmer minimum temperatures are not correlated with the observed changes in ES for *H. marginatum* at the time scale considered. Instead, the results show that the daily accumulated temperature was the variable which was best able to explain the calculated results. Water vapor deficit, a variable measured well above the ground surface, has little importance in defining the changes in ES for the target species.



**(a)**

**(b)**

**Figure 2.** The slope of the changes of environmental suitability for *Hyalomma marginatum* in the target territory in the period 1970–2018. A positive slope is not correlated with a suitable environmental for the tick (see Figure 1 for this data), but rather, only the strength of changes of the environmental variables in the period of reference. These changes are shown in (**b**) with explicit reference to the biogeographic divisions in (**a**).



(a)

| Biogeographical regions | VPDTotal | VPDQ10 | VPDQ90 | SoilQ10 | SoilQ90 | Soil |
|---|---|---|---|---|---|---|
| Atlantic Central Arable land | 0.75 | 0.76 | 0.75 | 0.40 | 0.23 | 0.86 |
| Atlantic Central Forest | 0.00 | 0.00 | 0.00 | 0.10 | 0.37 | 0.33 |
| Atlantic Central Pastures | 0.01 | 0.45 | 0.82 | 0.34 | 0.46 | 0.33 |
| Continental Arable land | 0.10 | 0.05 | 0.09 | 0.11 | 0.77 | 0.46 |
| Continental Forest | 0.09 | 0.09 | 0.09 | 0.04 | 0.00 | 0.00 |
| Mediterranean Mountains Arable land | 0.64 | 0.26 | 0.41 | 0.76 | 0.85 | 0.93 |
| Mediterranean Mountains Forest | 0.31 | 0.00 | 0.55 | 0.49 | 0.53 | 0.62 |
| Mediterranean North Arable land | 0.56 | 0.05 | 0.67 | 0.00 | 0.00 | 0.00 |
| Mediterranean North Shrubs & herbaceous vegetation | 0.06 | 0.06 | 0.06 | 0.00 | 0.00 | 0.00 |
| Mediterranean South Shrubs & herbaceous vegetation | 0.00 | 0.00 | 0.24 | 0.00 | 0.00 | 0.00 |
| Pannonian Arable land | 0.92 | 0.20 | 0.11 | 0.23 | 0.20 | 0.33 |

(b)

| | TMAX_10 | TMAX_Q10 | TMAX_Q90 | TMAX_d10 | TMIN_10 | TMIN_Q10 | TMIN_Q90 | TMIN_d10 |
|---|---|---|---|---|---|---|---|---|
| Atlantic Central Arable land | 0.03 | 0.00 | 0.00 | 0.00 | 0.01 | 0.83 | 0.06 | 0.00 |
| Atlantic Central Forest | 0.94 | 0.00 | 0.00 | 0.00 | 0.31 | 0.01 | 0.42 | 0.00 |
| Atlantic Central Pastures | 0.11 | 0.69 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Continental Arable land | 0.15 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.43 | 0.75 |
| Continental Forest | 0.00 | 0.00 | 0.00 | 0.00 | 0.46 | 0.80 | 0.14 | 0.05 |
| Mediterranean Mountains Arable land | 0.00 | 0.12 | 0.00 | 0.00 | 0.15 | 0.42 | 0.63 | 0.82 |
| Mediterranean Mountains Forest | 0.07 | 0.18 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.13 |
| Mediterranean North Arable land | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.05 | 0.00 | 0.00 |
| Mediterranean North Shrubs & herbaceous vegetation | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 |
| Mediterranean South Shrubs & herbaceous vegetation | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.16 |
| Pannonian Arable land | 0.73 | 0.27 | 0.52 | 0.09 | 0.02 | 0.00 | 0.03 | 0.01 |

**Figure 3.** Environmental variables with statistical significance in the changes of environmental suitability for *Hyalomma marginatum* in the period 1970–2018, obtained from a multiple regression among the slope of changes of suitability (dependent variable) and several environmental variables (explanatory variables). The results were calculated separately for each of various selected biogeographic areas, which cover more than 80% of the territory. Two heat maps are associated with the tables in which the "*p*" values for each variable are included: cells in red represent highly significant variables. The abbreviations of the variables are: SoilQ10, SoilQ90, and Soil (percentiles 10 and 90 of the soil moisture and the total annual soil moisture, respectively); VPDTotal (sum of the annual values of daily water vapor deficit), VPDQ10 and VPDQ90 (percentiles 10 and 90 of the of the daily water vapor deficit) in figure (**a**); and TMAX_10 (annual sum of the daily maximum temperature values exceeding of 10 °C), TMAX_Q10 and TMAX_Q90 (percentiles 10 and 90 of the of the daily maximum temperature values), TMAX_d10 (number of days in a year in which maximum temperature exceeded 10 °C); TMIN_10 (annual sum of the daily minimum temperature values exceeding of 10 °C), TMIN_Q10 and TMIN_Q90 (percentiles 10 and 90 of the of the daily minimum temperature values), TMIN_d10 (number of days in a year in which minimum temperature exceeded 10 °C) in figure (**b**).

Figure 4 displays the changes observed between the years 1970–1979 and 2010–2018, using only two variables, namely, the sum of maximum temperatures and the sum of soil water humidity; these were shown to be significantly involved in multiple regression between the slope of changes of ES and that of trends of the environmental variables. Only these two periods were considered in order to improve the visualization of the charts and provide a summary of the observed changes. The sites included were the same biogeographical categories displayed in Figure 3. Other variables were not included in the charts, and the comments below should be considered a proof-of-concept of: (a) temperature is not the only variable that changes to improve the ES for *H. marginatum*; and (b) each biogeographical region experienced changes of different weather variables, converging into the emergence of suitable niche for the tick. Trends are variable according to the region, but the general view is an increase of the temperature together with a decrease of soil humidity. Changes of soil humidity were negligible in some regions (i.e., Atlantic central regions), in which the most prominent change was the increase of temperature. Continental-type sites mostly observed a decrease of soil humidity. Changes in Mediterranean-type habitats are intermediate, and both variables seemed to act jointly. Pannonian-type habitats observed a clear increase of temperature between the two periods of time.

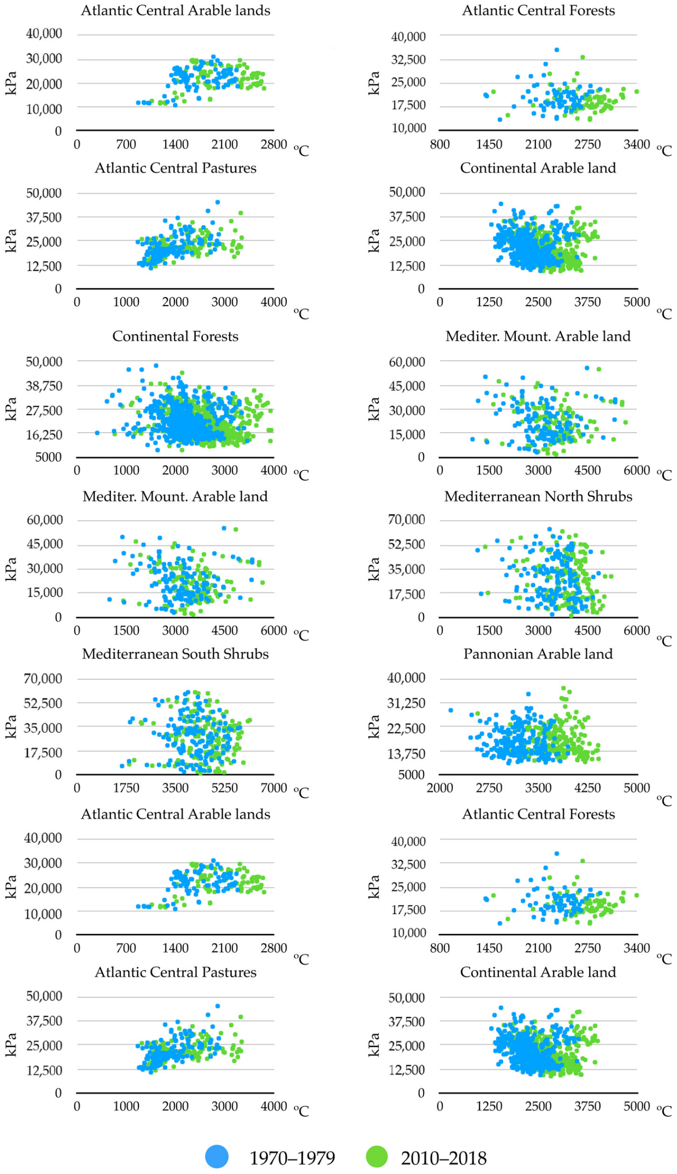


**Figure 4.** Changes in the sum of daily annual maximum temperature and of solid moisture for selected biogeographical regions. The X axis represents the sum of maximum temperature in degrees Celsius; the Y axis indicates the sum of soil relative humidity in kPa.

## 3. Discussion

We sought to evaluate the environmental suitability for *H. marginatum* in the period 1970–2018, its spatial trends and the variables that a have a stronger influence driving the slow but obvious changes observed in the target territory. We used series of climate data and geo-referenced field records of *H. marginatum*. Long series of weather data are difficult to obtain using satellite images, with the most recent beginning only in 2001, i.e., the MODIS series of Earth-orbiting satellites. Satellites have different calibration protocols and a relatively short life; therefore, it is problematic to harmonize data captured by different series of satellites, as this would not result in a long series of comparable environmental data. While we strongly support the use of satellite data for the predictive modelling of spatial distribution of organisms [27], a long series of different weather variables was necessary for this study. For this purpose, we chose the re-analyzed data of the TerraClimate repository, as they provide a long series of data extending back to 1950. As is compulsory for mechanistic modeling, this study is predicated on a set of coordinates recording the known distribution of the tick, together with a set of explanatory variables to generate the models. We previously demonstrated [27] that the use of coefficients resulting from a harmonic regression of the monthly variables produced better predictions than the use of pre-tailored weather variables. The latter provide a good description of the climate across the planet, but they cannot capture the behavior of every organism. Thus, they do not adequately explain the factors regulating the presence of permanent populations of the modeled organism. Since our methods based on harmonic regressions can produce daily values of each trait, this results in a convenient method to derive the necessary variables describing the spatial distribution. In our study, the records of *H. marginatum* were collected approximately in the period 1990–2006; as such, these data were used as the set of training variables averaged to fit the same period.

Traits regulating the colonization of *H. marginatum* have been ascribed to the effect of temperature on development stages [12]. The hypothesis of a critical threshold of accumulated temperature necessary for the spread and colonization of *H. marginatum* [12] was unequivocally confirmed by our results in most of Europe. While the TMax quartile 90 and the number of days with Tmax above a threshold of 10 °C are of importance in almost every ecological region, their impact is spatially different, alone or together with other traits. An unexpected result is the importance of the soil moisture in the driest regions of the continent (i.e., the Mediterranean-type areas). It is interesting to note that this is the area in which *H. marginatum* has been historically recorded. We interpret this result as the joint effect of several variables, with a spatially different relative importance collectively promoting stable niches in the region that is commonly colonized by the tick, resulting in the improvement of its ES.

Another unexpected result is the lack of significant results in the multiple regressions linking the trend of Tmin with ES in many of the coldest parts of the analyzed territory: minimum temperature seems not to be a limiting factor for the suitability of *H. maginatum* in central Europe; this is an area that is commonly considered to be unsuitable for the tick because of the freezing minimum temperatures. Our results show that minimum temperature, as recorded for Europe, should not be considered a factor responsible for high tick mortality, as has been widely suggested [28,29], at least in the time period considered. The trend of ES for *H. marginatum* is driven by changes in variables that have a spatially different relative importance, although the daily sum of Tmax and the number of days above the threshold of 10 °C seem to be the best explanatory variables, at least in the period of time selected in the target territory.

As expected, the water vapor deficit in the air is not a trait impacting long-term changes of the environmental niche of the tick. It is not possible to generalize this finding to other tick species, because of their different ecological requirements. Water vapor deficit, a variable measured well above the ground surface, is of little importance in delineating the changes of ES for *H. marginatum*. This underlines the importance of choosing variables with ecological meaning for every tick species, as opposed to adhering to simplistic, “one size fits all" approaches. However, this trait, together with mean or accumulated rainfall, have been largely used to model the environmental suitability for ticks. We would like to recommend a revision of previously published conclusions regarding the effect of rain or other atmospheric "water" variables on the modeling of the environmental niches of ticks. While NDVI (Normalized Derived Vegetation Index) should be applied when using satellite-derived data, a measure of soil surface moisture seems to be important when using traditional weather traits derived from re-assessments or climate recording stations.

In any case, there is no question that weather trends are giving rise to environmentally suitable conditions for *H. marginatum* in relatively large areas in Europe, a finding that is consistent with numerous reports on areas in the continent in recent years [14–18] and a previous analysis, at a rougher resolution, built on a physiological model [19] exploring developmental and mortality rates. When and where we expect that ES would increase over a critical threshold to allow permanent populations of the tick to become established is difficult to predict, because trends are spatially different. In any case, we hypothesize that environmental changes will not expand northward gradually from the fringes of the currently known tick distribution. We expect that large territories of the target region will simultaneously surpass the critical environmental threshold, and vast domains will become suitable in a few years. This hypothesis is based on the findings reported in this study, pinpointing the combined effects of several restricting variables acting together, an issue which has not been addressed before.

Both critical values of temperature and soil humidity impact the ES for *H. marginatum*, but some variables have a stronger effect according to the biogeographical region, an unexpected result of this study. The joint trend of these traits is altering the ES for the target tick into the optimum at an unprecedented rate in large areas of Europe. Nevertheless, it is necessary to stress that without the adequate density of large vertebrates necessary to feed the adult ticks, permanent populations of *H. marginatum* are highly improbable, even if adequate environmental conditions exist. This is thus a call for active surveillance of the tick, a preliminary step for the adaptation to the impact of this invasive species.

## 4. Material and methods

### *4.1. Background*

We aimed to evaluate the trends of ES of *H. marginatum* in the period 1970–2018 in a territory covering Europe, between 16°W, 28°N and 49°E, 71°N. In this study, we adhered to a strict definition of the environmental niche for *H. marginatum* without considering interactions with hosts. We aimed to evaluate the trend of the climate and the changes of the requisites for tick survival, not to predict the probability of permanent populations of the tick. The environmental niche of an organism is defined as the combination of traits in an area that draw its probability of persistence [27]. To calculate the ES for *H. marginatum*, we used: (i) a set of climate variables between the years 1970–2018; (ii) an evaluation of the ES for the tick using a maximum entropy algorithm, relying on a set of known records of the tick; and (iii) an explicit evaluation of trends in the period and target territory, as well as a solid evaluation of the most important driving variables.

### *4.2. Obtaining Climate Data*

Climate data were obtained from the TerraClimate website (http://www.climatologylab.org/terraclimate.html, accessed in January 2020). We chose four re-assessed products, at monthly intervals, namely: (i) maximum temperature (TMax) (ii) minimum temperature (TMin) (iii) water vapor deficit (WVD), and (iv) soil humidity (SH), for the period 1970–2018. These data have a spatial nominal resolution of 4 km. Water vapor deficit is measured at 2 m above the ground. According to previous studies on the ecology of the tick [30], these data should be adequate for defining the environmental niche of *H. marginatum*.

Monthly data were transformed by harmonic regression. This technique was developed by Fourier [31], enabling the decomposition of a time series into a regression defined by sine and cosine, including the first three coefficients of the harmonic regression of each environmental trait are the explanatory variables [27]. This reduces the number of variables which are necessary to describe the niche while retaining complete information about the time series, which is a fundamental in spatial modeling approaches. These coefficients synthetically describe the mean value of each variable for the considered period, the slope in the spring (i.e., how fast or slow is the spring change) and the negative autumn slope (i.e., how fast summer values turn into autumn ones). Once derived to daily values, other environmental variables can be obtained (i.e., the sum of maximum daily temperature). In short, the procedure deconstructs the complete time series into coefficients that describe the original data. It has been demonstrated that this approach produces a better modeling outcome than using interpolated climate data or averaged monthly estimates directly derived from the time series [27].

### *4.3. Model Generation*

Several algorithms are available to obtain maps of environmental suitability based on explanatory variables. In this study, we used the algorithm MaxEnt (Maximum Entropy) [32], the efficacy of which has been widely demonstrated [33]. These models need to be "trained" with the known distribution of the species to be modeled, from which the combination of variables that define the probability of a species' presence is obtained. The records with the coordinates of *H. marginatum* were obtained from a previous compilation [34] and are available at http://dx.doi.org/10.5061/dryad.2h3f2. They are also available as Supplementary Material Figure S1.

To calculate the environmental niche of each species, the "wallace" package [35] for R [36] was used. This package uses the MaxEnt algorithm to obtain the expected ES for the tick and project it onto other time chunks. As explanatory variables, we used the first three coefficients of the Fourier series for TMax, TMin, SH, and WPD. The algorithm was trained with 50% of the records of *H. marginatum*, using the remaining 50% to iteratively check the confidence of the fit. The process was repeated ten times, randomly selecting different training and test sets, obtaining the best possible distribution model. The suitability of each model was verified the use of the area under the curve (AUC) of the test set, because the Akaike information criterion provides unreliable results when applied to a geographical extension [37]. The AUC compares the outcome of each model with the recorded distribution of *H. marginatum*, producing an evaluation of the quality of the model in terms of the similarity of the predicted result with the known distribution of the modeled organism.

Since we wanted to determine the changes of ES for the target tick, we trained the models with environmental data between the years 1990–2006. This is the period for which most of the records were collected, and which should, therefore, best describe the environmental conditions driving its presence. Then, we projected the model into chunks of 10 years to obtain an overview of these changes over the time, covering the periods 1970–1979, 1980–1989, 1990–1999, 2000–2009, and 2010–2018. We also projected the trained model into annual intervals to calculate the trend of ES for *H. marginatum* (see below).

All data resulting from the above calculations were summarized into the biogeographical regions of the target territory. We used the LANMAP2 product [38] that describes the biogeographic characteristics of the European territory, synthesizing the climatic and landscape domains.

### *4.4. Other Calculations*

We asked if the change in the ES for *H. marginatum* was correlated with changes of explanatory variables with ecological meaning. It should be noted that the raw coefficients of the harmonic regression are extraordinary descriptors for modeling but lack obvious ecological meaning that can, however, be easily calculated [34]. We aimed to evaluate how the trend of ES for *H. marginatum* correlated with these ecologically meaningful variables. We produced 14 variables from the raw coefficients, namely the average, percentile 10, and percentile 90 of each variable (VPD, SH, TMax, and TMin), as well the daily sum of TMax or TMin above a threshold of 10 °C, and the number of days in a year in which TMax or TMin were above that threshold. These are traits are expected to drive the persistence of *H. marginatum*.

We calculated the slope of a lineal regression for the annual ES of *H. marginatum*, as well as the slope of a lineal regression for the annual values of each variable mentioned in the previous paragraph, done on every single pixel of each raster. We then produced a multiple regression between the ES and the environmental traits. The hypothesis assumes that if changes of slopes of both ES and variables are correlated, *p*-values of variables with statistical significance in the multiple regression will show which variables were changing at the same pace as that of ES in the period 1970–2018. It could thus be assumed that these variables are the main drivers of changes of ES for the target tick.

Multiple regressions were performed separately for each biogeographic region in the target territory, following the denominations of LANMAP2 (see above). The purpose was to demonstrate that different variables have different impacts on the trend of ES of *H. marginatum* according to the landscape features. Since there are literally dozens of biogeographical categories in LANMAP2, we selected the areas of "Atlantic Central Forests", "Atlantic Central arable land", "Atlantic Central Pastures", "Continental arable land", "Continental Forests", "Mediterranean mountains arable land", "Mediterranean mountains Forests", "Mediterranean northern shrubs", "Mediterranean southern shrubs", "Pannonian arable lands" and "Steppic shrubs" as a proof of concept. These categories cover more than 80% of the target territory.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/2076-0817/10/2/95/s1, Figure S1: The distribution of permanent populations of *Hyalomma marginatum* was obtained from literature data. To note the large numbers of collections in northern Africa, which were included in the models even if not related with the biogeographic regions of Europe.

**Author Contributions:** Conceptualization, A.E.-P. and N.F.-R.; methodology, A.E.-P. and N.F.-R.; validation, N.F.-R. and A.E.-P.; formal analysis, N.F.-R. and A.E.-P.; writing—original draft preparation, A.E.-P. and N.F.-R.; writing—review and editing, N.F.-R. and A.E.-P.; visualization, N.F.-R.; supervision, A.E.-P. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** All the tick distribution data used in this study are available under the Supplementary Materials mentioned above. The complete set of data, from which the distribution of *H. marginatum* was drawn is available at http://dx.doi.org/10.5061/dryad.2h3f2.

**Conflicts of Interest:** The authors declare no conflict of interest.

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# Chapter 3: Modelling the potential spread of *Hyalomma marginatum* ticks in Europe by migratory birds.

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# Modelling the potential spread of *Hyalomma marginatum* ticks in Europe by migratory birds

Agustín Estrada-Peña [a,b,*], Gianluca D'Amico [c], Natalia Fernández-Ruiz [a]

[a] *Department of Animal Health, Faculty of Veterinary Medicine, Zaragoza, Spain*
[b] *Instituto Agroalimentario de Aragón (IA2), Zaragoza, Spain*
[c] *Department of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania*

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ABSTRACT

This study modelled the probability of introduction of *Hyalomma marginatum* into Europe by predicting the potential migratory routes of 28 bird species and the probability to carry immatures of the tick. Flyways were modelled as a spatio-temporal feature, at weekly intervals, using satellite-derived data of temperature and vegetal phenology, together with cost surfaces derived from speed and direction of the wind (years 2002–2018). The expected period of activity of tick immatures defined the probability of ticks being carried by birds along the modelled flyways. The probability of moulting of the engorged nymphs was modelled as a linear relationship of the daily sum of temperatures after tick introduction by birds. Positive probabilities of tick introduction extend the known northern range of permanent populations to central and western France, and large portions of central Europe. The flight of birds into an area and thence the risk of introduction of *H. marginatum* is very heterogeneous, with sites receiving "waves" of different bird species at diverse times of the year. Therefore, there is not a clear period of time for introduction, as it depends on the modelled behaviour of the bird species. The probability of introduction into Baltic and Nordic countries is small. We hypothesise that conditions of a warmer climate might support permanent populations of *H. marginatum* if a high number of immatures is introduced. Active surveys in risky territories, where the tick is not yet established, are advisable for rapid intervention.

## 1. Introduction

Millions of birds migrate every year between Africa and Europe. They breed in the northern hemisphere during the summer, move south through Europe in autumn to wintering grounds in warmer African regions, and fly back north in the spring to breed again. Many species of ground-feeding birds are reported hosts of dozens of tick species. Other than the specific ticks of birds that commonly adopt a nidicolous strategy, birds can carry immature ticks of exophilic species. Birds were recognised as the main spreaders of ticks since the pioneering studies conducted by Hoogstraal and coworkers during the 1955–1961 period (Hoogstraal et al., 1961, 1964).

There is special interest in the immature ticks of the genus *Hyalomma* that are transported and spread by birds during migration. This genus has been incriminated as the vector of many pathogens threatening human health in large regions of Africa, Asia, and the Mediterranean basin. In addition to being the vector of *Rickettsia* spp. (i.e. Wallménius et al., 2014), *Hyalomma* spp. are proven vectors for the etiological viral agent of Crimean-Congo haemorrhagic fever (CCHFv; Gargili et al., 2017). Although birds are not viraemic (Ergönül, 2006), they can carry the infected ticks. This association has received increased attention in recent decades due to the massive number of birds that migrate every year. Even if they have a relatively low prevalence of ticks, they contribute to the spread of infected ticks into northern latitudes (Mancuso et al., 2019; Spengler et al., 2019). The finding of permanent foci of CCHFv in the Iberian Peninsula (Estrada-Peña et al., 2012) has fueled awareness of the probable spread of infected ticks from Africa into the European continent (Palomar et al., 2013).

In Europe, permanent populations of *H. marginatum* are restricted to the warmer parts of the Mediterranean basin. The evidence of a trend towards a warmer climate in Europe has increased recognition of this tick (together with *Hyalomma rufipes* colonising Africa and parts of Asia) as a possible invasive species. Other than weather conditions such as harsh winters which limit moulting of immatures and survival of the tick population, one of the main traits restricting tick expansion is the presence of large vertebrates

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[*] Corresponding author at: Department of Animal Health, Faculty of Veterinary Medicine, Miguel Servet 177, 50013 Zaragoza, Spain.
*E-mail address:* aestrada@unizar.es (A. Estrada-Peña).

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on which the adults can feed (Vial et al., 2016). Nonetheless, the increasing density of wild ungulates and leporids in some parts of Europe, which are known hosts for adults and immatures of *H. marginatum*, respectively, could favour the persistence of this tick population. The spread of *H. marginatum* in northern latitudes is related not only to its introduction in areas with suitable weather allowing the completion of its life cycle, but it is strongly dependent on a minimum threshold of introduced ticks. That threshold density of moulting ticks should be able to produce a population of adults capable of mating and surviving the winter.

Previous studies have reported immature stages of *H. marginatum* on migratory birds in the United Kingdom (Jameson et al., 2012) and central Europe (Capek et al., 2014), a previously unnoticed population of *H. marginatum* adults in southern France (Vial et al., 2016), questing adults in Germany (Kampen et al., 2007; Chitimia-Dobler et al., 2016) and Austria (Duscher et al., 2018), and isolated adults feeding on local hosts in the United Kingdom (Hansford et al., 2019). Subsequently, concerns were raised regarding the impact of climate trends on the ability of imported immature ticks to moult and survive in central and northern Europe (Gale et al., 2012). It is imperative to evaluate the probability of introduction of immature *H. marginatum* by birds (the most commonly reported way in which the tick spreads) and to know the northern limit at which these introduced populations could moult into questing adults. The emphasis is on prevention of tick-borne diseases affecting humans or animals. This knowledge could guide the surveys and control efforts in countries in which there is a high probability of *H. marginatum* introduction.

This study focuses on modelling the migratory flyways of several species of birds reported as hosts of the immatures of *H. marginatum* and evaluates the potential spatial overlap of stopovers of the travelling birds with areas where ticks are permanently present. This overlap in bird migration and immature *H. marginatum* activity is necessary to allow the birds to pick up the questing immatures. We also assess the main routes of potential entry of birds from Africa, the time of the year they use flyways over Europe, and the probability of introduction of *H. marginatum* into the northern latitudes of the target territory.

## 2. Material and methods

### 2.1. Workflow of the study

This study modelled the probability of spreading *Hyalomma* ticks throughout Europe during the migratory flights of 28 bird species that are recognised as hosts of *Hyalomma* immatures. Our approach used: (i) a set of records of the birds, geo-referenced and with a date of observation, (ii) environmental features to define weekly habitat suitability for both birds and ticks, (iii) models of the flyways for birds at weekly intervals, (iv) calculation of the probability of birds carrying and spreading ticks, and (v) calculation of the probability of immatures moulting once they drop off the hosts. The study did not aim to model the density of ticks, the probability of finding adequate hosts at the sites of introduction, nor survival of the tick population during winter. The schematic workflow is included in the Fig. 1.

### 2.2. Collection of bird observation data

Given the difficulty in obtaining national datasets of birds observations with homogeneous consistency and reliability, we used the eBird database (http://ebird.org, last accessed December 2018) as the source of bird observations. The eBird observational dataset (EOD) contains the occurrence of species defined as a record of a particular bird taxon in a particular place at a particular time (Soberón and Peterson, 2009). These data provide a valuable set of information that can be used to address a variety of biological questions (Sullivan et al., 2014).

The current list of birds recorded as hosts of *Hyalomma* spp., based on published reports, includes 85 species. Of these, we queried EOD for the distribution data of 28 species that (i) have been reported as hosts of the immatures of *H. marginatum*, (ii) are sedentary or migratory, and (iii) have partial or complete migratory movements involving Africa, the Mediterranean basin, and northern Europe. The species are *Acrocephalus arundinaceus, Acrocephalus scirpaceus, Alauda arvensis, Anthus campestris, Anthus pratensis, Anthus trivialis, Lullula arborea, Luscinia luscinia, Luscinia megarhynchos, Luscinia svecica, Monticola saxatilis, Motacilla flava, Oenanthe oenanthe, Phoenicurus phoenicurus, Phylloscopus collybita, Phylloscopus sibilatrix, Phylloscopus trochilus, Saxicola rubicula*, *Saxicola rubetra, Sitta europaea, Sylvia atricapilla, Sylvia borin, Sylvia cantillans, Sylvia communis, Sylvia curruca, Turdus merula, Turdus philomelos,* and *Turdus viscivorus*. Other birds that are recorded as hosts of the *Hyalomma* immatures have a recorded distribution in EOD restricted to small parts of Europe (i.e. *Sylvia nisoria* or *Sylvia hortensis*). Few records exist for these species to model reliable habitat suitability models. These and other similar species were not included in the study. Only bird species for which at least 100 different observation points per week were available in EOD, regardless of the flock size, were included in our modelling. A total of 3,379,240 records met the inclusion criteria and were used for further mapping protocols.

Data obtained from EOD were observations of birds, not points defining a specific migratory route. We approached this issue by predicting the environmental suitability for each bird species at weekly intervals. We thus assumed that birds have a probability of moving from any spatial point in the week W to any point in week W + 1 according to the environmental suitability in both periods, weighted as explained in Section 2.4.

### 2.3. Collection and assessment of climate data

We approached the modelling of the distribution of each species of bird, within the coordinates 18°00′ W and 41°35′ E, 30°50′ N and 73°00′ N, using satellite-derived land surface temperature daytime data (LSTD) and the Normalised Difference Vegetation Index (NDVI). Products from the Terra series of MODIS satellites (https://modis.gsfc.nasa.gov/data/dataprod/) have been used for the period 2002–2018. These data have a spatial resolution of 0.05° (approximately 5,600 m) and correspond to the products MOD11C3 (for LSTD) and MOD13C2 (for the NDVI). For the selection, download and processing of the data corresponding to the target territory, the "MODIStsp" library (Busetto and Ranghetti, 2016) was used for the R programming environment (R Core Team, 2017; R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/) that allows access to the API server of MODIS. The combination of LSTD and NDVI has produced better results to model the environmental suitability for ticks (Estrada-Peña et al., 2016) or for birds (i.e. Schloss et al., 1999; Lin et al., 2008). Averaged (2002–2018) data were finally generated at weekly intervals.

### 2.4. Modelling the weekly distribution of species of birds

We adhered to spatio-temporal exploratory models (STEMs) to assess the changes in distribution of birds along their migratory movements. This procedure has been considered as highly suitable for birds (i.e. Fink et al., 2010) since it simultaneously deals with both spatial and temporal variation in the explanatory variables. Observational data for each bird species were used to model the distribution of birds individually at weekly intervals, training the

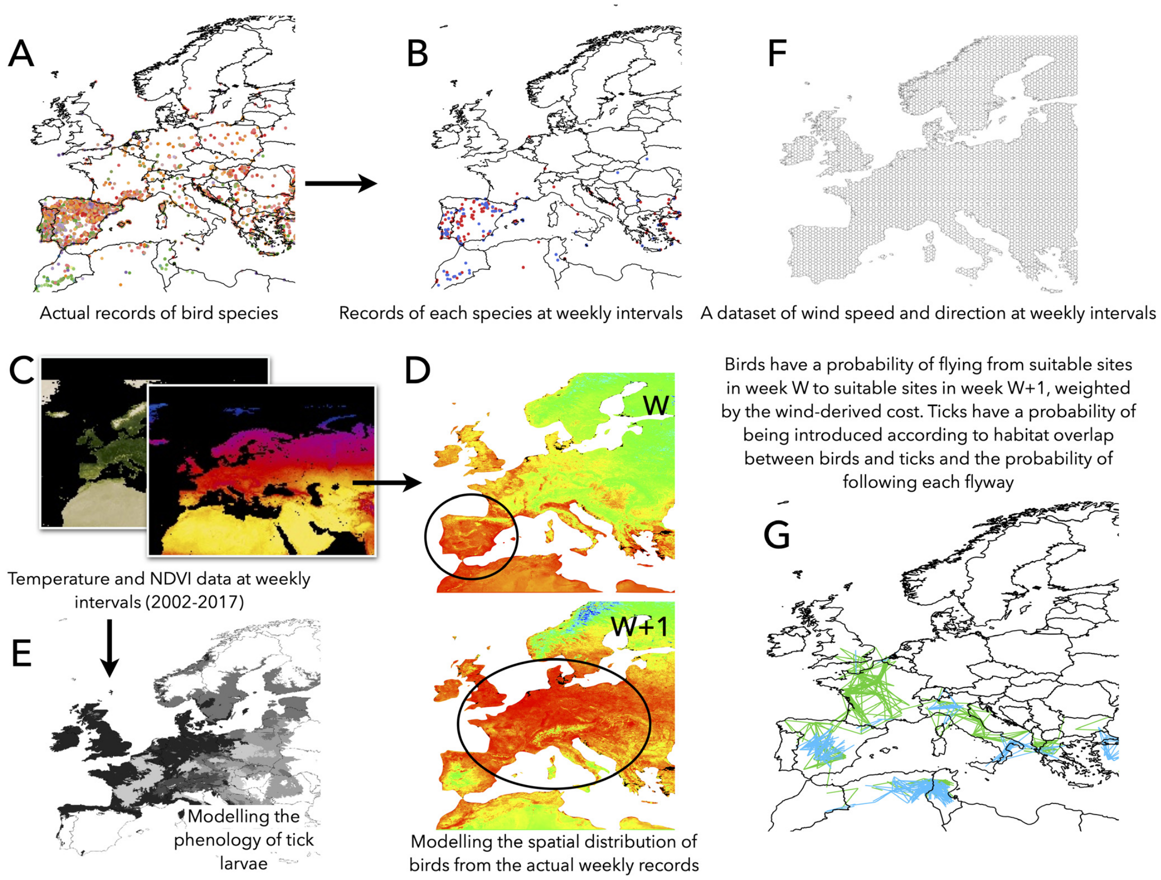


**Fig. 1.** The workflow of the study (the images are for illustrative purposes, not based on actual data). (A) The coordinates of observations of 28 species of birds with specific mention to date were obtained from the eBird dataset and adjusted to weekly intervals (the colours of the dots represent the hypothetical temporal distribution of the data). (B) Coordinates of observations at weekly intervals were extracted separately for each bird species. (C) Environmental traits including land surface temperature at daytime (LSTD) and the normalised difference vegetation index (NDVI) were obtained from the MODIS-Terra satellite series to produce an average year at weekly intervals for the period 2002–2018. (D) Spatio-temporal models were used to predict the regions where each bird species is expected to be present at week W. The models detect the zones of change between two consecutive weeks (W and W + 1, roughly marked as circles in D) and extract the coordinates of positive environmental suitability for each bird. (E) Accumulated weekly values of LSTD predict the probability of activity of larvae of *Hyalomma marginatum* at week W (shades of grey are only illustrative, and not actual). (F) A dataset of gridded observations at weekly intervals of wind speed and direction is used to produce cost friction surfaces at the pressure level of 850 hPa. (G) The most probable flyways linking the points where each bird species is predicted to be present at weeks W and W + 1 are calculated, as a probability derived from wind, environmental suitability and the length of each flyway, including the probability of carrying larvae of *H. marginatum*.

models with the environmental data of LSTD and NDVI for each week.

We chose the Stem package (Cameletti, 2015; Stem: Spatio-temporal models in R. R package version 1.0. https://CRAN.R-project.org/package=Stem) for R to develop models trained with the reported distribution of the bird species containing coordinates and date, including at least 100 different observations per species/week. The Expectation-Maximisation (EM) algorithm used to develop the models and to perform spatial predictions for each bird/week is explained by Bilmes (1998) and Amisigo and van de Giesen (2005). Briefly, environmental variables (weekly LSTD and NDVI) were used together with the actual records split by species/week to train one model of each bird species in each week. The Kalman smoother and the EM algorithm were further used to estimate the missing values, i.e. "where the bird should be". The suitability of each species/week model was verified using a criterion called "area under the curve" (AUC). It has been shown that the Akaike information criterion provides erroneous results when applied to a geographical extension (Velasco and González-Salazar, 2019). AUC values were calculated with the package "pROC" (Robin et al., 2011) for R.

### 2.5. Obtaining and processing wind data: modelling bird migratory routes

Results from spatio-temporal modelling are raster images, each pixel displaying the probability of the presence of a bird species. In short, modelling "expands" the number of records available from EOD and explains how environmental suitability changes at weekly intervals. The assumption is that birds can fly from pixels in week W to pixels in week W + 1 with a probability which is the product of the environmental suitability in both weeks. It is thus explicitly assumed that birds will have a higher probability of flying from and to pixels with higher environmental suitability, weighted by the cost of flight, which we assumed was the wind speed and direction.

Once the probability of the presence of each bird species was modelled at weekly intervals, the most probable flyways connecting sites of presence in two consecutive weeks were calculated. We simplified the migratory flyways as lines linking sites that represent suitable habitats at week W (departure) and week W + 1 (destination). This is a distance-constrained shortest path problem (Ahuja et al., 1993; Miller and Shaw, 2001) where the coordinates for departure-destination are the sites with suitable habitat for each bird species in two consecutive weeks (W and W + 1). The framework finds the optimal pathway that maximises the probability of coping with a cost surface generated by wind speed and direction every two consecutive weeks, considering that flocks of birds are limited by a maximum daily flight capability (Erni et al., 2005). The R script for calculations is included in the supplementary "Research Data" (see Section 2.7).

Wind data were obtained from the UERRA (Uncertainties in Ensembles of Regional Reanalyses) for Europe on pressure levels, available at http://cds.climate.copernicus.eu (accessed February 2nd, 2019). We used 6 hourly data for the complete 2002–2018 period at a pressure level of 850 hPa. The raster images covering every 6 h period were transformed into weekly averages with a script in R using the packages "raster" (Hijmans, 2019; raster: Geographic Data Analysis and Modelling. R package version 3.0-2. https://CRAN.R-project.org/package=raster) and "rgdal" (Bivand et al., 2019; rgdal: Bindings for the 'Geospatial' Data Abstraction Library. R package version 1.4-4. https://CRAN.R-project.org/package=rgdal). We prepared friction surfaces representing the cost of flying from suitable sites in two consecutive weeks using the packages rWind (Fernández-López and Schliep, 2019) and gDistance (van Etten, 2018; gdistance: Distances and Routes on Geographical Grids. R package version 1.2-2. https://CRAN.R-project.org/package=gdistance) for R. Friction surfaces represent the "cost of flying" following each potential flyway between any two points of raster images displaying suitable habitat. The script for R handling the wind surfaces is available as part of the "Research Data" associated to this paper (see Section 2.7). It is important to note that each species of bird has different flight patterns which are further blurred by weather traits and the fat condition of each specimen. Therefore, we opted for a conservative strategy, assuming a fixed maximum distance of 700 km of flight in 1 week (Ellegren, 1993; Alerstam et al., 2007; Pennycuick et al., 2013). Once friction cost by wind was assigned to every flyway linking coordinates for consecutive weeks, we weighted the result by its length: the longer the flyway, the lower the weight in the monotonic range 0–100. The flyway between two coordinates is not a binary parameter (yes/no) but a probability of flight according to its length and its cost. A total of 432,493 flight legs in the calendar weeks 6–21 were included in analyses. It was decided that by week 21 (first week of June) the birds are already in their breeding sites (Yohannes et al., 2009). No further effort was made to continue modelling after week 21.

### 2.6. Modelling the activity of tick immatures and the moult of engorged nymphs

Birds have different potentials to carry ticks. It would ideally be necessary to know the densities of both birds and questing immature ticks at each site to derive a reliable probability of contact between hosts and ticks. However we derived the probability of contact as a linear product of the predicted environmental suitability for both organisms, since it has been demonstrated that environmental suitability correlates well with abundance (i.e. Mora et al., 2011; Weber et al., 2017). We therefore chose to predict the overlapping of larval tick activity and bird presence in a site. If the probabilities of ticks' and birds' presence are high, then there is a high probability that the birds carry ticks. Since it is known that larvae plus nymphs feed on birds for an average time of 21 days (Hoogstraal, 1979), it is possible to calculate the probability of birds carrying ticks to any pixel as a function of the time of flight. In other words, a bird will have a probability of introducing ticks in a territory after following its flyways for three consecutive weeks (21 days of tick feeding), after the bird has been in a territory where the tick was active.

We predicted the phenology of *H. marginatum* larvae to determine the probability of ticks-bird contacts at weekly intervals. We used a previously validated model of the life cycle of *H. marginatum* (Estrada-Peña et al., 2011) to assess the probability of larvae being recruited into the active population of ticks, which is a function of accumulated temperature. The probability of each previously modelled flyway, derived from its length and cost, was further weighted by the probability that larvae are active. This provides the probability of birds carrying ticks to a territory.

Finally, we evaluated the portions of the target territory that could allow the moulting of engorged nymphs once those dropped off birds. According to Emelianova (2006) [Ecology of the tick genus *Hyalomma* Koch 1844 (Acarina: Ixodidae) in Central Caucasus and adjacent territories. In Russian, PhD Thesis, Stavropol], the approximate sum of temperatures necessary for the moult of engorged nymphs of *H. marginatum* is around 660 °C above a threshold temperature of 15 °C which is necessary to activate the moulting process. We modelled the probability of moulting of engorged nymphs in the target territory by a simple sum of degree days after the date of the predicted drop off moving birds. We described the result as a probability, i.e. places with a sum of temperature >660 °C (above the threshold of 15 °C) will have a probability of 100%, monotonically decreasing as the temperature decreases. The recursive sum of temperatures was stopped either when 660 °C was reached (probability of moult = 100%) or when the mean daily temperature dropped below the threshold of 15 °C (probability of moult calculated as a percent of the limit of 660 °C).

### 2.7. Data accesibility

Scripts in R and the individual flyways of each species of birds considered in this study (in shapefile format) are available at https://data.mendeley.com/datasets/ymtnk5rpf9/1 with doi https://doi.org/10.17632/ymtnk5rpf9.1. A general "Readme" file explains the contents of the complete dataset.

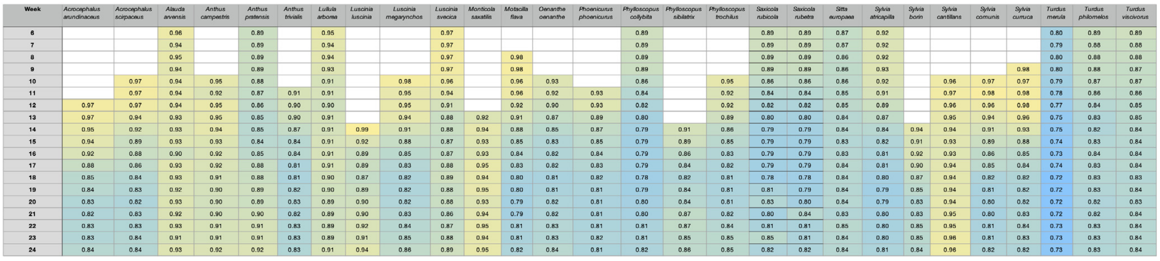
## 3. Results

### 3.1. Outcomes of STEM models

Spatio-temporal modelling of the environmental suitability for 28 species of birds in Europe provided reliable results. Fig. 2 includes the AUC parameter of the models for each species of bird and week, using both LSTD and NDVI as explanatory variables. The minimum AUC in the whole dataset was 0.716 for *T. merula* in week 18 (meaning for 71.6% of correct classifications in the poorest model). This procedure was thus considered reliable to track the departure and destination points on which to build the probable flyways of the birds.

### 3.2. Bird potential to carry ticks

Fig. 3 summarises two important features of the bird flights and the probability of spread of immatures of *H. marginatum*. They refer to the average probability of introduction (Fig. 3A) and the median week at which birds were predicted to be present in a territory (Fig. 3B). The probability of introduction is highest in northern Africa (west to Tunisia) and the complete Mediterranean coast of Europe because the movements of the birds between the two



| Week | *Acrocephalus arundinaceus* | *Acrocephalus scirpaceus* | *Alauda arvensis* | *Anthus campestris* | *Anthus pratensis* | *Anthus trivialis* | *Lullula arborea* | *Luscinia luscinia* | *Luscinia megarynchos* | *Luscinia svecica* | *Monticola saxatilis* | *Motacilla flava* | *Oenanthe oenanthe* | *Phoenicurus phoenicurus* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | | | 0.96 | | 0.89 | | 0.95 | | | 0.97 | | | | |
| 7 | | | 0.94 | | 0.89 | | 0.94 | | | 0.97 | | | | |
| 8 | | | 0.95 | | 0.89 | | 0.94 | | | 0.97 | | 0.98 | | |
| 9 | | | 0.94 | | 0.89 | | 0.93 | | | 0.97 | | 0.98 | | |
| 10 | | 0.97 | 0.94 | 0.95 | 0.88 | | 0.91 | | 0.98 | 0.96 | | 0.96 | 0.93 | |
| 11 | | 0.97 | 0.94 | 0.92 | 0.87 | 0.91 | 0.91 | | 0.95 | 0.94 | | 0.96 | 0.92 | 0.93 |
| 12 | 0.97 | 0.97 | 0.94 | 0.95 | 0.86 | 0.90 | 0.90 | | 0.95 | 0.91 | | 0.92 | 0.90 | 0.93 |
| 13 | 0.97 | 0.94 | 0.93 | 0.95 | 0.85 | 0.90 | 0.91 | | 0.94 | 0.88 | 0.92 | 0.91 | 0.87 | 0.89 |
| 14 | 0.95 | 0.92 | 0.93 | 0.94 | 0.85 | 0.87 | 0.91 | 0.99 | 0.91 | 0.88 | 0.94 | 0.88 | 0.85 | 0.87 |
| 15 | 0.94 | 0.89 | 0.93 | 0.93 | 0.84 | 0.84 | 0.91 | 0.92 | 0.88 | 0.87 | 0.93 | 0.85 | 0.83 | 0.85 |
| 16 | 0.92 | 0.88 | 0.90 | 0.92 | 0.85 | 0.84 | 0.91 | 0.89 | 0.85 | 0.87 | 0.93 | 0.84 | 0.82 | 0.84 |
| 17 | 0.88 | 0.86 | 0.93 | 0.92 | 0.88 | 0.81 | 0.91 | 0.89 | 0.83 | 0.88 | 0.95 | 0.83 | 0.82 | 0.83 |
| 18 | 0.85 | 0.84 | 0.93 | 0.91 | 0.88 | 0.81 | 0.90 | 0.87 | 0.82 | 0.89 | 0.94 | 0.80 | 0.81 | 0.82 |
| 19 | 0.84 | 0.83 | 0.92 | 0.90 | 0.89 | 0.82 | 0.90 | 0.89 | 0.82 | 0.88 | 0.95 | 0.80 | 0.81 | 0.81 |
| 20 | 0.83 | 0.82 | 0.93 | 0.90 | 0.89 | 0.83 | 0.89 | 0.90 | 0.82 | 0.88 | 0.93 | 0.79 | 0.82 | 0.81 |
| 21 | 0.82 | 0.83 | 0.92 | 0.90 | 0.90 | 0.82 | 0.89 | 0.90 | 0.83 | 0.86 | 0.94 | 0.79 | 0.82 | 0.81 |
| 22 | 0.83 | 0.83 | 0.93 | 0.91 | 0.91 | 0.83 | 0.89 | 0.92 | 0.84 | 0.87 | 0.95 | 0.81 | 0.83 | 0.81 |
| 23 | 0.83 | 0.84 | 0.91 | 0.91 | 0.91 | 0.83 | 0.89 | 0.91 | 0.85 | 0.88 | 0.94 | 0.81 | 0.83 | 0.82 |
| 24 | 0.84 | 0.84 | 0.93 | 0.92 | 0.92 | 0.83 | 0.91 | 0.94 | 0.86 | 0.89 | 0.95 | 0.82 | 0.84 | 0.81 |

| Week | *Phylloscopus collybita* | *Phylloscopus sibilatrix* | *Phylloscopus trochilus* | *Saxicola rubicola* | *Saxicola rubetra* | *Sitta europaea* | *Sylvia atricapilla* | *Sylvia borin* | *Sylvia cantillans* | *Sylvia comunis* | *Sylvia curruca* | *Turdus merula* | *Turdus philomelos* | *Turdus viscivorus* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 0.89 | | | 0.89 | 0.89 | 0.87 | 0.92 | | | | | 0.80 | 0.89 | 0.89 |
| 7 | 0.89 | | | 0.89 | 0.89 | 0.87 | 0.92 | | | | | 0.79 | 0.88 | 0.88 |
| 8 | 0.89 | | | 0.89 | 0.89 | 0.86 | 0.92 | | | | | 0.80 | 0.88 | 0.88 |
| 9 | 0.89 | | | 0.89 | 0.89 | 0.86 | 0.93 | | | | 0.98 | 0.80 | 0.88 | 0.87 |
| 10 | 0.86 | | 0.95 | 0.86 | 0.86 | 0.86 | 0.92 | | 0.96 | 0.97 | 0.97 | 0.79 | 0.87 | 0.87 |
| 11 | 0.84 | | 0.92 | 0.84 | 0.84 | 0.85 | 0.91 | | 0.97 | 0.98 | 0.98 | 0.78 | 0.86 | 0.86 |
| 12 | 0.82 | | 0.92 | 0.82 | 0.82 | 0.85 | 0.89 | | 0.96 | 0.96 | 0.98 | 0.77 | 0.84 | 0.85 |
| 13 | 0.80 | | 0.89 | 0.80 | 0.80 | 0.84 | 0.87 | | 0.95 | 0.94 | 0.96 | 0.75 | 0.83 | 0.85 |
| 14 | 0.79 | 0.91 | 0.86 | 0.79 | 0.79 | 0.84 | 0.84 | 0.94 | 0.94 | 0.91 | 0.93 | 0.75 | 0.82 | 0.84 |
| 15 | 0.79 | 0.89 | 0.85 | 0.79 | 0.79 | 0.83 | 0.82 | 0.91 | 0.93 | 0.89 | 0.88 | 0.74 | 0.83 | 0.84 |
| 16 | 0.79 | 0.86 | 0.83 | 0.79 | 0.79 | 0.83 | 0.81 | 0.92 | 0.93 | 0.86 | 0.85 | 0.73 | 0.84 | 0.84 |
| 17 | 0.79 | 0.84 | 0.82 | 0.79 | 0.79 | 0.84 | 0.81 | 0.90 | 0.94 | 0.85 | 0.84 | 0.74 | 0.83 | 0.84 |
| 18 | 0.78 | 0.82 | 0.81 | 0.78 | 0.78 | 0.84 | 0.80 | 0.87 | 0.94 | 0.82 | 0.82 | 0.72 | 0.83 | 0.83 |
| 19 | 0.79 | 0.84 | 0.81 | 0.81 | 0.79 | 0.84 | 0.79 | 0.85 | 0.94 | 0.81 | 0.82 | 0.72 | 0.83 | 0.84 |
| 20 | 0.80 | 0.84 | 0.81 | 0.83 | 0.80 | 0.84 | 0.79 | 0.83 | 0.94 | 0.80 | 0.82 | 0.72 | 0.82 | 0.83 |
| 21 | 0.80 | 0.87 | 0.82 | 0.80 | 0.84 | 0.83 | 0.80 | 0.83 | 0.95 | 0.80 | 0.83 | 0.72 | 0.82 | 0.84 |
| 22 | 0.81 | 0.87 | 0.84 | 0.81 | 0.81 | 0.85 | 0.80 | 0.85 | 0.95 | 0.81 | 0.84 | 0.73 | 0.83 | 0.84 |
| 23 | 0.81 | 0.85 | 0.85 | 0.85 | 0.81 | 0.84 | 0.80 | 0.85 | 0.96 | 0.81 | 0.83 | 0.73 | 0.83 | 0.84 |
| 24 | 0.82 | 0.86 | 0.85 | 0.82 | 0.82 | 0.84 | 0.81 | 0.84 | 0.96 | 0.82 | 0.82 | 0.73 | 0.83 | 0.84 |

**Fig. 2.** The Area under the curve (AUC) values of each model by bird species and week. Cold colours account for the lower values of AUC (minimum: 0.72) and warm colours account for higher AUC values (maximum: 0.99). Colours are intended to summarise the numerical results, from cold (low values of AUC) to warm (high values of AUC).

land masses occur mainly at the peak of larval activity (Fig. 3B). It is noteworthy that this probability of introduction remains high for most areas in the Iberian Peninsula, southern France, the southern Alps, Balkan countries and western Turkey. Modelling the routes of migratory birds revealed portions of central Europe were susceptible to the introduction of *H. marginatum* immatures. Mapping of the expected averaged weekly flight of birds (Fig. 3B) showed that northern Africa, the Iberian Peninsula, south and western France and the United Kingdom are reached by birds in the first weeks of the year. Other regions of Europe receive the birds later in the year, with an obvious latitudinal and longitudinal gradient (the more northern and eastern, the later their arrival).

The modelling results indicate that (i) "waves" of different bird species may reach the same site in different weeks and (ii) flyways differ according to the time of year and do not necessarily begin in Africa and always continue northwards. Some areas of the target territory may receive several waves of ticks in a year, carried by different species of birds with different probabilities of introduction of immature *H. marginatum*. Figs. 4 and 5 display the weekly probability of introduction of the tick trough the flyways of birds between weeks 6 and 21, which are further summarised in the Fig. 6. The complete set of files displaying the results before, for each species of bird at weekly intervals are available as part of the "Research Data" associated with this paper (see Section 2.7). The western route linking northern Africa with western Europe and the United Kingdom is one of the earliest flyways that could introduce *H. marginatum* into northern latitudes. Between weeks 11 and 13, the model predicted a positive risk of tick introduction into western France, Belgium, the Netherlands, and south-eastern United Kingdom. After the week 14, the flyways through the Iberian Peninsula decreased, with yet another period of spread through much of central Europe. This wave of birds reaches central France, eastern Austria, Slovakia and the Czech Republic. Most of the Balkans territory is predicted to be affected by two waves of migrating birds, mainly from Turkey and nearby territories, the first around weeks 15–17 and the second starting from weeks 19–21.

### 3.3. Prediction of the territory suitable for Hyalomma moulting

Prediction of the spatial probability of moult of immature ticks in the target territory is included in the Fig. 7. It is important to note the estimation does not indicate how many ticks could moult or the survival rate of ticks after moulting (i.e. density of questing adult ticks). It is only an assessment of the territories where the accumulated temperature (averaged for 2002–2018) is enough to allow engorged nymphs to moult after ticks drop off arriving birds. Probability rates higher than 65% were recorded over large territories in northern Africa and Mediterranean Europe, including Turkey. Predictions show large areas adequate for moulting in southern and western France, Balkan countries, Hungary and Romania, and extending well into Ukraine and Crimea. Other small and patchy sites where moulting could be completed were observed in central Europe.

## 4. Discussion

This study modelled the migratory routes of 28 passerine bird species reported as hosts of immature *H. marginatum* to infer the probable northern limit of introduction of this tick species in Europe. The framework did not aim to uncover the ecological basis of bird migration, but to track the importance of tick introduction by birds with a focus on human health. We intended to provide a tool which was able to capture the probability of introduction of the tick from sites with permanent populations to areas where the tick is being recognised as a growing risk (Kampen et al., 2007; Jameson et al., 2012; Capek et al., 2014; Vial et al., 2016; Duscher et al., 2018; Chitimia-Dobler et al., 2016; Hansford et al., 2019). A large dataset of birds' observations and satellite-derived abiotic traits consisting of temperature and vegetation phenology averaged for the period 2002–2018 was used in this model. Cost surfaces using the wind direction and speed were used to presume the most probable flyways of birds at weekly intervals. We did not include *H. rufipes*, a tick whose immatures also parasitize birds following the route Africa-Europe, due to the lack of a model of its life cycle and the scarcity of data on bird distribution in Africa. It would thus be necessary to extend this framework to Saharan Africa, for which reliable data are largely unavailable.

An individual follow-up of each bird could be addressed using data from ring recoveries or national survey schemes. However, after a preliminary assessment of the available data, we realised (i) the scarcity of data in northern Africa, (ii) the paucity of information over large parts of Europe and Africa, (iii) the pervasive noise resulting from scattered information obtained mainly by ring recoveries from dead animals, and (iv) the difficulties in obtaining valid data from several countries. We are aware that the dataset used in this study has a bias, but the modelling approach should be considered a proof-of-concept rather than the complete European landscape of bird migration and possible tick spread. Common species, those that are of particular interest, or those actively studied for a variety of reasons, would be over-represented in these datasets. Also, countries with tradition of bird watching would produce more records than others (birds cannot be recorded if an ornithologist is not present to record it). On the other hand, only inhabited areas of northern Africa (away from desert regions) contributed a few observations, introducing a further bias. Thus, we opted for a conservative approach using the eBird database. Even this curated dataset has a bias because a val-

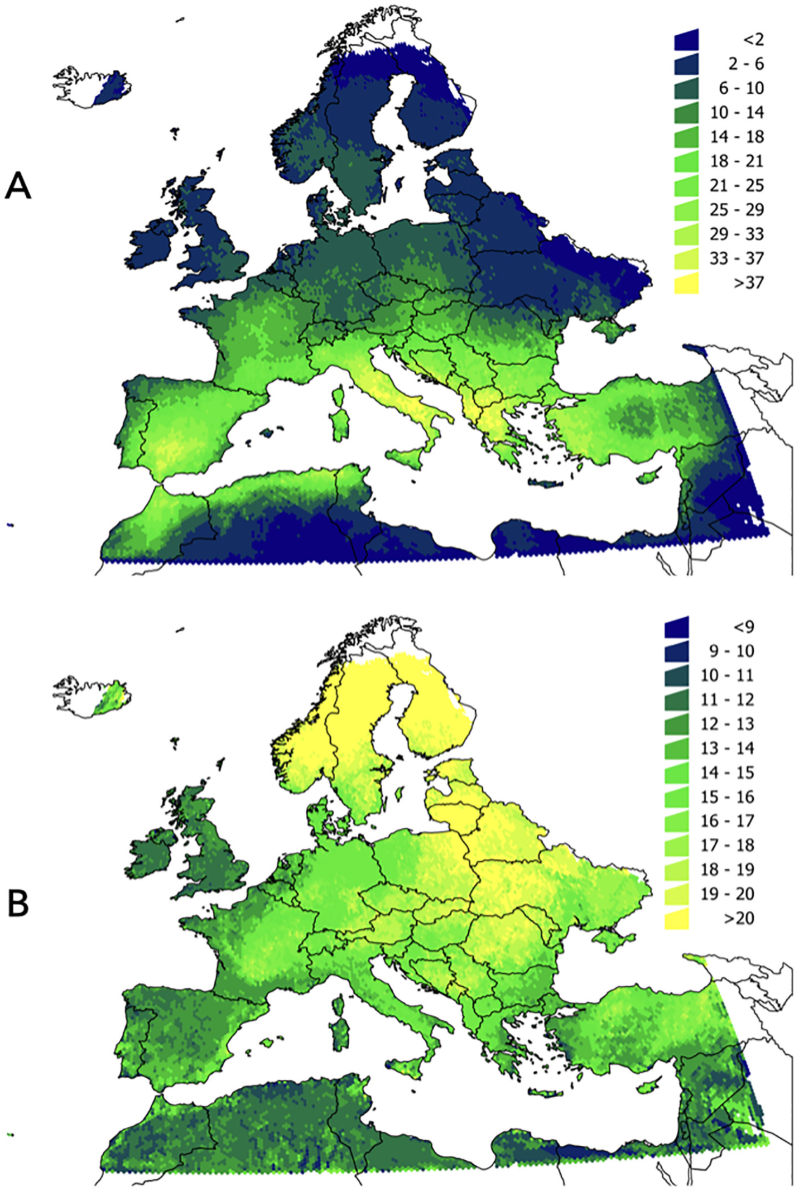


**Fig. 3.** The results of modelling the flyways of birds and introduction of *Hyalomma marginatum* immatures. (A) The probability of introduction of *H. marginatum* immatures through the flyways of 28 species of migratory birds in Europe. (B) The median week at which birds stop over a territory, averaged for the 28 species included in the model.

idation of the identification is not carried out. The use of more than 3 million observations involving 28 species should minimise such bias. We considered only bird species with a minimum of 100 records per week, aiming to output solid estimations from the habitat suitability modelling. Furthermore, not every species of bird recorded as a host for immatures of *H. marginatum* has been included in the study due to the paucity of reliable data. We thus focused on some birds, looking for an adequate threshold of records and dates of observation. Larger species might introduce ticks into Europe by direct flyways linking sub-Saharan Africa with Europe in a non-stop trip (Hoogstraal et al., 1961; Hoogstraal, 1979).

The departure and arrival points of bird migratory pathways are calculated from habitat suitability maps generated weekly from satellite-derived environmental features, resulting in a probability of presence of the bird. Migratory species show quick changes in the inter-weekly habitat suitability, from which a pattern of movement can be captured. However, slightly changing environmental suitability is predicted for sedentary birds and, therefore, the algorithm selecting the shortest routes between suitable sites chooses the nearest sites. Each species of bird has different flying strategies and copes with flight costs in different ways. For example, larger birds could fly faster or better resist the force of an inconvenient wind. The sources of variability are

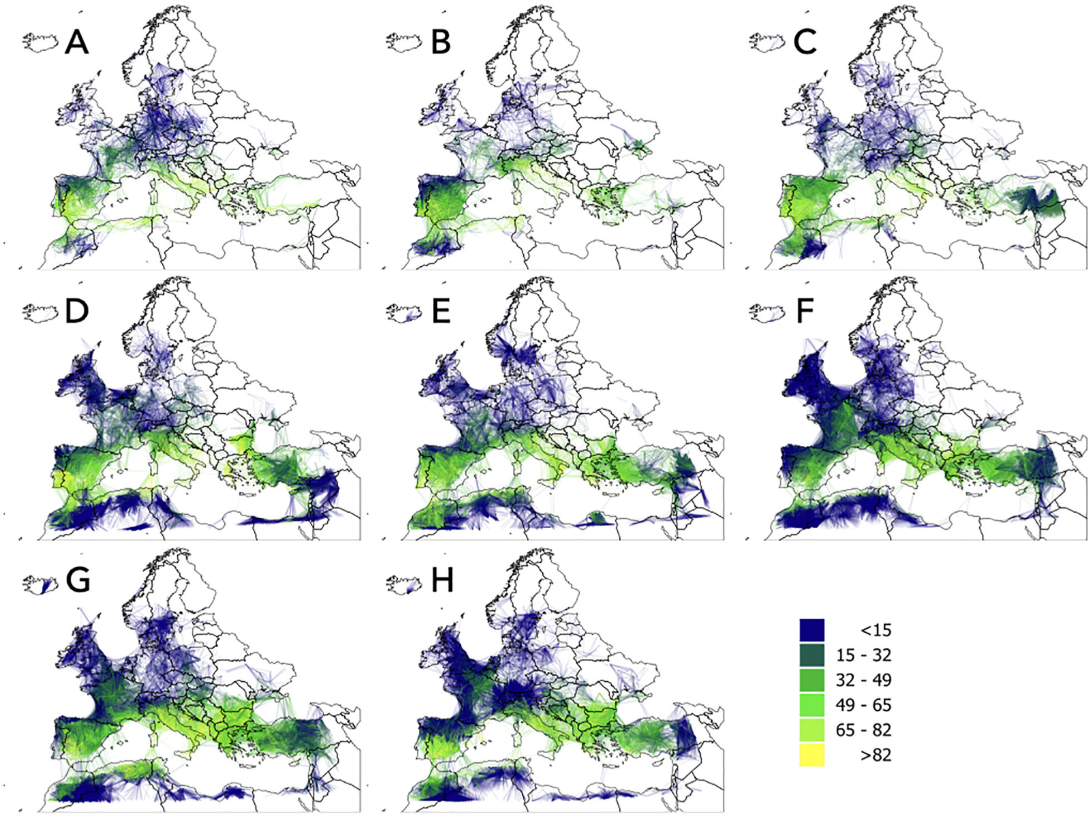


**Fig. 4.** The predicted flyways of birds shown as weeks of the year, displaying the probability of introduction of *Hyalomma marginatum* immatures. (A–H) Weeks 6 to 13, respectively.

even larger: fully fuelled birds could better manage the energy consumption needed to travel. We are aware that some bird species have permanent populations that overwinter in Europe and that the migrant populations from Africa join them in spring. Flight speed is not only derived from allometric traits, i.e. weight and size, but has a deep phylogenetic root (Alerstam et al., 2007). We thus used a simplified modelling strategy, in which the individual contribution of each bird to the spread of the tick is ignored, favouring an ensemble perspective, assuming that birds fly between two points, fully fuelled, with a probability that depends on a wind-derived, distance-dependent, friction cost surface. Additionally, the weather may change among years, driving a different tick life cycle pattern or an unusual bird migration pattern (Downs and Horner, 2008).

Climate variables used in previous studies of habitat suitability for birds varied considerably. Some studies have employed only the mean annual precipitation and temperature values (e.g. Peterson, 2003; Seoane et al., 2003). Araujo et al. (2005) used the temperature of the coldest and warmest months, and July–September precipitation, in addition to annual values. Other studies have focused on the relationships between birds and climate of spring or early summer (Forsman and Mönkkönen, 2003; Virkkala et al., 2005). We addressed the issue of spatial modelling using satellite-recorded information of LSTD and NDVI, as tailored sets of environmental variables at weekly intervals, resulting from a complete year, averaged for 2002–2018. The values of the AUC, an index measuring the reliability of the habitat classification (Fielding and Bell, 1997) by bird and calendar week, are indicative for models that classify the habitat with a reliability higher than 71% in the poorest instance. Our results match with field observations such as (i) the main route from northern Africa to the Iberian peninsula (Palomar et al., 2016), (ii) the approximate dates of migration over Italy (Spina and Volponi, 2008) or over the United Kingdom (Wernham et al., 2002), or (iii) the multiple flyways with different origins that converge in Hungary (Hornok et al., 2016).

It is not possible to include in the modelling framework the individual contribution of each bird as a carrier of ticks due to the paucity of data including the parasitic load of each species. This is because ticks do not have a normal distribution on hosts (Randolph et al., 1999), because a poor physiological state of the host could produce heavier infestations (Tschirren et al., 2007), or the existence of small sites with high tick density that are frequently visited by birds and undetected by this framework. We indirectly approached this by using the contact rates between birds and ticks. These are derived from the probabilities of presence of the bird and the tick at each pixel, as well as the predicted activity of tick larvae at weekly intervals. These estimations output a probability for birds carrying ticks flying among sites in consecutive weeks, and thence the probability of introduction of engorged nymphs in a territory. This study did not consider the mortality rate of ticks in winter. Its calculation relies on estimations of the

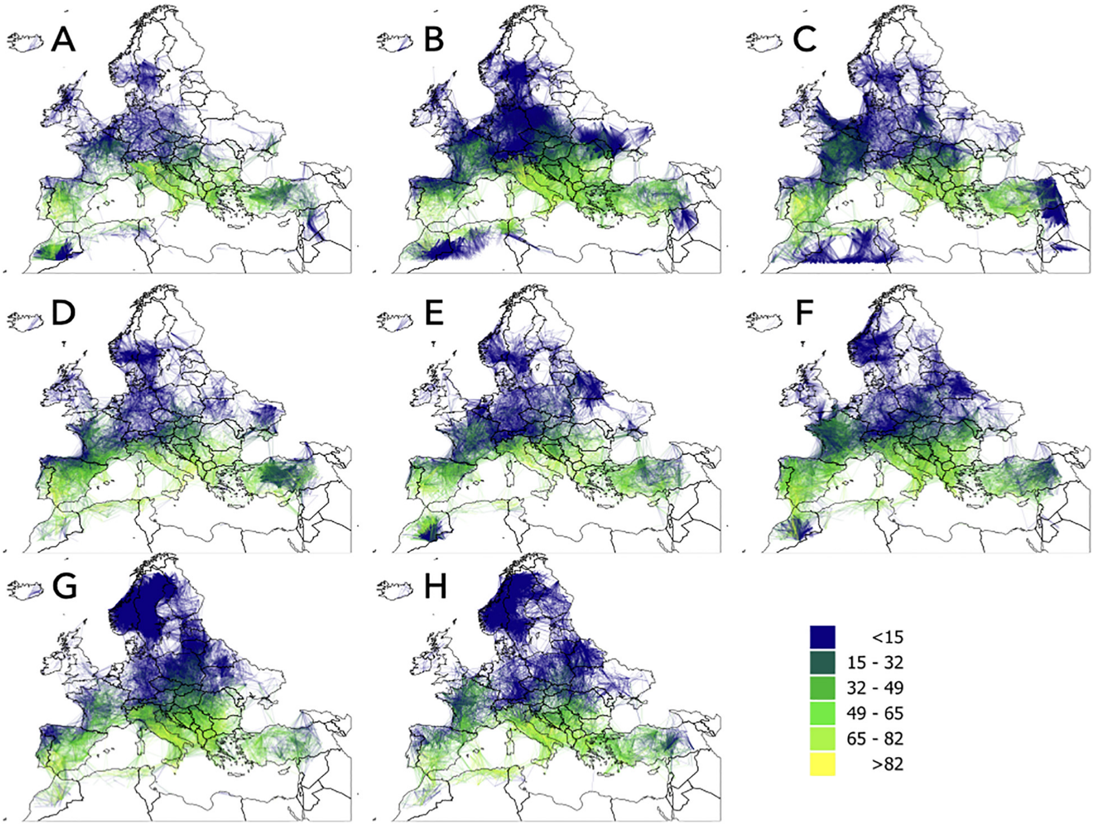


**Fig. 5.** The predicted flyways of birds shown as weeks of the year, displaying the probability of introduction of *Hyalomma marginatum* immatures. (A–H) Weeks 14 to 21, respectively.

ground temperature, the buffering effect of the vegetation, and the metabolic rates of ticks, information currently unavailable. Different studies have mentioned "observations" stating that "high numbers of ticks can be killed as a consequence of harsh winters in the Russian steppes" (summarised by Hoogstraal, 1979). While this has become a kind of dogma shaping the establishment of permanent populations of *Hyalomma*, an empirical estimation remains unaddressed.

The importance of building future climate scenarios did not escape our attention. This could be addressed by time series analysis of the satellite imagery, producing likely future conditions; such products should be validated against the available general circulation models. However, it is statistically difficult to match projections from satellite records with the general circulation models issued under scenarios of $CO_2$ emissions. Some studies have addressed this issue (i.e. Sobrino and Julien, 2013) but there is still no direct way to compare results from different modelling approaches, and validation is essential. Furthermore, it is impossible to estimate speed and direction of wind in the future. Further research on the topic is required, which would contribute to a better understanding of the reasons behind the invasive events of *Hyalomma* spp. reported in Europe. Do they constitute a trend towards northern latitudes or is part of circumstantial evidence augmented by increased awareness?

Although the probability of introduction of *H. marginatum* into northern latitudes is still low, its probable longer survival due to a warmer climate, its heterogeneous introduction, the presence of more than one immature stage on each bird, and the clustering of migratory birds in small areas, makes local and temporal establishment of *Hyalomma* ticks not unlikely in central Europe. It is important to note that the risk of newly established permanent populations of this invasive tick is not only related to its introduction. A minimum number of engorged immatures must be introduced, and after successful moulting, both males and females must meet on large hosts and produce viable offspring capable of surviving the winter. However, responding to a notification of finding of *Hyalomma* ticks by performing regional active surveys would enable the timely investigation of a potential introduction, and even the local eradication of a freshly established population.

In conclusion, we modelled the probability of introduction of *H. marginatum* into Europe as an average for the period 2002–2018. The model relied on the potential migratory routes of 28 species of birds calculated as a spatio-temporal feature using satellite-derived data of temperature and photosynthetic activity, cost surfaces made from speed and direction of winds, and the expected period of activity of tick immatures. Positive probabilities of introduction and nymphal moult overlap with the known range of the

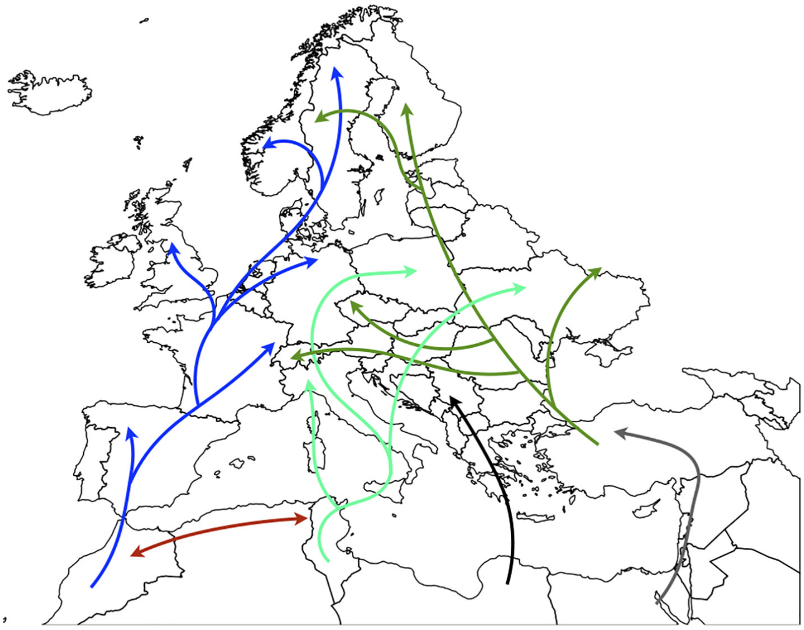


**Fig. 6.** A schematic overview of the main flyways of birds over European territory. The colours are random and indicate different routes and sources of birds that are active in different weeks of the year. The scheme is a generalisation of the Figs. 4 and 5.

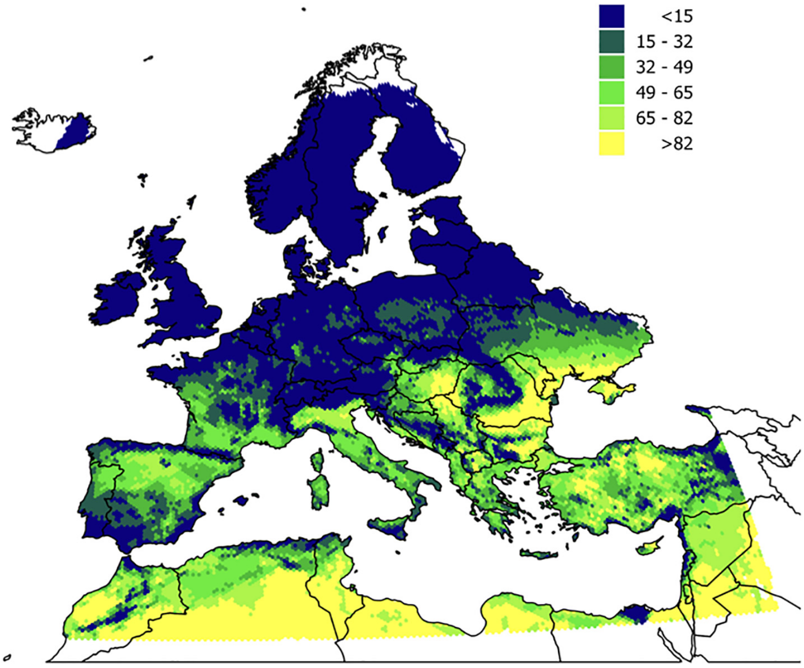


**Fig. 7.** The probability of moult of engorged nymphs of *Hyalomma marginatum* in the target territory. The probability is calculated according to a fixed limit of accumulated temperature of 660 °C above a threshold temperature of 15 °C. For example, if a site has a daily *mean* temperature of 20 °C, the engorged nymphs would moult in 33 days after introduction by birds. These sites will have a probability of moult of 100%. Probabilities lower than 100% are calculated by the ratio between the real accumulated temperature and the threshold of 660 °C.

tick, but they extend into central and western France, and large portions of central Europe. Monitoring of *Hyalomma* is advisable in European countries where it is continuously introduced but not yet endemic.

## Acknowledgements

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# Chapter 4: Is composition of vertebrates an indicator of the prevalence of tick-borne pathogens?.

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# Is composition of vertebrates an indicator of the prevalence of tick-borne pathogens?

Agustín Estrada-Peña & Natalia Fernández-Ruiz

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RESEARCH ARTICLE

# Is composition of vertebrates an indicator of the prevalence of tick-borne pathogens?

Agustín Estrada-Peña[a,b] and Natalia Fernández-Ruiz[a,b]

[a]Department of Animal Health. Faculty of Veterinary Medicine, University of Zaragoza, Zaragoza, Spain; [b]Instituto Agroalimentario de Aragón (Ia2), Zaragoza, Spain

**ABSTRACT**

Communities of vertebrates tend to appear together under similar ranges of environmental features. This study explores whether an explicit combination of vertebrates and their contact rates with a tick vector might constitute an indicator of the prevalence of a pathogen in the quest for ticks at the western Palearctic scale. We asked how 'indicator' communities could be 'markers' of the actual infection rates of the tick in the field of two species of *Borrelia* (a bacterium transmitted by the tick *Ixodes ricinus)*. We approached an unsupervised classification of the territory to obtain clusters on the grounds of abundance of each vertebrate and contact rates with the tick. Statistical models based on Neural Networks, Random Forest, Gradient Boosting, and AdaBoost were detect the best correlation between communities' composition and the prevalence of *Borrelia afzelii* and *Borrelia gariniii* in questing ticks. Both Gradient Boosting and AdaBoost produced the best results, predicting tick infection rates from the indicator communities. A ranking algorithm demonstrated that the prevalence of these bacteria in the tick is correlated with indicator communities of vertebrates on sites selected as a proof-of-concept. We acknowledge that our findings are supported by statistical outcomes, but they provide consistency for a framework that should be deeper explored at the large scale.

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## Introduction

Communities are sets of organisms that tend to appear together in ecosystems, from microbes [i.e. 1] to higher vertebrates or plants. Members of a community interact and can colonize a range of environmental conditions, with species exhibiting different relative abundances. Interactions among species provide a view of the community in equilibrium; this is the 'optimal composition and size' of a community under a given set of (a)biotic constraints. Other than the use of communities of organisms as bioindicators of the health of ecosystems [2], they can be used to assess the quality of human health and the predicted future response to changes in climate [3]. Hawkins et al. [4] stated that the determinants of local biodiversity and variation of organisms are a central aim of modern ecology, and that a basic set of temperature, rainfall, or evapotranspiration 'can account for much of the variation in plant and animal species diversity across space.' [4].

It has been recognized that parasites influence host communities in different ways [5,6] but the opposite view, i.e. how the vertebrates' community affects parasites, has been less addressed [i.e., 7, 8]. There are excellent examples, generally performed on the regional scale, about the impact of the community structure of vertebrates on the faunal composition of parasites [9,10]. Regarding the effects of vertebrates' communities on the circulation of vector-borne pathogens, it has been demonstrated that the relative composition of communities may influence the patterns of tick-borne pathogen infection rates [summarized by 11]. As an example, it has been pointed out that some carnivores may play only a small role in the circulation of the tick-borne pathogen *Borrelia* spp, but they are able to influence the density of small mammals and birds by a predator–prey cascade of effects [13] on the rodents and birds that are reservoirs of the pathogen. The composition of the vertebrates affects the entire complex of vertebrates-ticks-pathogens [14,15].

The issue surrounding tick-borne pathogens revolves around the questions of: (i) whether there is a combination of vertebrates behind the range of existing field observations of prevalence of a tick-borne pathogen, (ii) whether it is a reproducible finding for each ecosystem, and (iii) how the alterations of these natural communities may affect the circulation of the tick(s) or the pathogen(s). There are obvious logistical issues in conducting large-scale

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**CONTACT** Agustín Estrada-Peña aestrada@unizar.es Miguel Servet 177, Zaragoza 50013, Spain Department of Animal Health, Faculty of Veterinary Medicine, University of Zaragoza,

Supplemental data for this article can be accessed here.

tick surveys [14]. By way of example, a large meta-study was carried out on the reservoir capacities of some well-studied reservoirs of tick-borne bacteria of the genus *Borrelia*, correlating the abundance of *I. ricinus* and the prevalence of *B. burgdorferi* s.l. with morphological and physiological traits of the animals, concluding that 'few vertebrate species dominate the *B. burgdorferi* s.l. life cycle' [16]. Other studies have conducted research on the significance of different vertebrates in the transmission rates of tick-borne pathogens [i.e. 17, 18].

A pending issue in this field of research is the translation of an explicit composition of vertebrates into an epidemiologically coherent indicator for the circulation of a tick-borne pathogen on a large scale. It must be noted that some vertebrates just feed the tick, while others contribute to the circulation of pathogens (acting as reservoirs). The rate of ticks feeding on reservoirs is a spatially variable feature generated by the community of available vertebrates that could dramatically change the prevalence of a pathogen in ticks. It depends upon the relative abundance of each vertebrate and its 'attractiveness' to the tick, plus the status of the vertebrate as a reservoir or not. The idea of the importance of vertebrates' communities on the complex patterns of dilution or amplification of prevalence of *B. burgdorferi* s.l. in ticks has been discussed and summarized by 19. These authors enumerated the issues regarding the impact of vertebrates' composition on the infection rates of tick-borne pathogens and wrote 'combined with data on host feeding utilization, infection prevalence and duration and magnitude of infectiousness [the data on hosts and reservoirs] could be used to make predictions of nymphal infection prevalence across space or time.'

The impact of the joint contribution of a community of vertebrates on the prevalence of a tick-borne pathogen has been systematically neglected for large regions. The relative composition of a community of vertebrates changes along spatial scales as a response to gradients of environmental conditions that also impact contact rates with ticks. Knowledge of the effects of the faunal composition of vertebrates across a geographical gradient on tick-borne pathogens would provide an unparalleled framework, helping to evaluate the relative importance of vertebrates' composition on the circulation of tick-borne pathogens. Such a description of an 'indicator community' could then be expected to describe the prevalence of a pathogen in questing ticks.

This study aims first to classify the territory of the western Palearctic into clusters, using the known distribution of 165 species of vertebrates reported as hosts for the tick *I. ricinus*, in a method known as bioregionalization. Each cluster results from similar presence and abundance patterns of a set of vertebrate's species, statistically different from those found in other clusters. We calculated both the species richness and the phylogenetic diversity of the communities per cluster. We further correlated the composition of the communities against the prevalence of either *Borrelia afzelii* or *Borrelia garinii*, two major pathogens transmitted by that tick, as a proof-of-concept, using data about questing nymphs of *I. ricinus* at the European scale. The purpose is not to predict infection rates in ticks in the territory, but to demonstrate that an indicator community exists, correlating with the prevalence of a tick-borne pathogen even on a large scale. The main novelty of our approach is the building of spatial regions according to the vertebrates' composition, thus proposing a method to de-correlate the animals' assemblages with the abiotic factors.

## Material and methods

### *Background*

Following the concepts provided by [20], we refer to the exercise of obtaining spatial units with a similar composition of vertebrates as 'bioregionalization'. It is a classification technique. This study focuses on the characterization of clusters that have a similar composition of vertebrates in the western Palearctic, weighted by contact rates with the tick *I. ricinus* [21]. As a proof-of-concept, we outlined the indicator community in selected clusters of the target region against the infection rate of two species of *B. burgdorferi* s.l. in questing *I. ricinus* nymphs, as previously compiled and reported [22].

### Compiling the reported distribution of vertebrates and *I. ricinus* in the target territory

We collected information about the recorded distribution of the vertebrate hosts for *I. ricinus* in Europe, with coordinates, originally published by Estrada-Peña and de la Fuente (2016) (data available at https://datadryad.org/stash/dataset/doi:10.5061/dryad.2h3f2). These data produced the maps of the predicted distribution of vertebrates, also available in the same link. We updated the outcome of that previous analysis only for the species of vertebrates for which the number of records reported had increased by more than 10% since the date of the previous publication (2016) using new records from GBIF (https://www.gbif.org, last accessed March 2020). Since the proof-of-concept of this study uses the distribution of the tick *I. ricinus*, vertebrates were selected to include only those species hosting the tick (166 species reported). We acknowledge that this does not reflect the complete distribution of *all* the vertebrates in the western Palearctic, but rather, those that have an impact on the circulation of the selected pathogens. In total, we handled more than 3 million geo-referenced records of vertebrates and more than 14,000 records of *I. ricinus*.

### *Mapping the distribution of vertebrates*

For calculations of the predicted distribution of both the tick and vertebrates recorded as hosts or reservoirs of *Borrelia,* we used a series of monthly values of temperature, soil humidity, and water vapor deficit between the years 1980 and 2018, from the TerraClimate repository (http://www.climatologylab.org/terraclimate.html, last accessed March 2020). The complete time series was summarized as the monthly average of each variable. Each set of average monthly values was subjected to harmonic regression. The use of harmonic regression coefficients has been previously validated [23] since they are free of the frequent issues of spatial correlation and multicollinearity between layers. Harmonic regression produces the best fit for seasonal variability of each variable, and each regression curve has several coefficients. We used the first three coefficients of the harmonic regression for each climate variable as explanatory variables for predictive mapping (total: 9 explanatory variables).

We independently modeled the presence of each species using the niche modeling algorithm MaxEnt integrated in the 'dismo' package [24] for R [25]. Models were developed with linear and quadratic features, using a variable number of background points (10,000–100,000), 10 replicates per species were modeled, and 70% of points for training purposes. We used cross-validation to compare the resulting models. The variable number of background points was proportional to the number of actual records. This strategy was implemented because some species have many records in the target region, while others are poorly represented. Each model was replicated 100 times, partitioning the data into replicate folds, with each fold used in turn to test the model. The regularization multiplier was set to 1.

We evaluated the performance of the models using the Boyce index implemented in the 'ecospat' package 26 for R [25]. We did not use the usual index of the Area Under the Curve (AUC) since it has received criticism as being affected by background area. Considering that we are dealing with vertebrates that may have a relatively restricted distribution, the ratio between the size of the background and the actual vertebrate's distribution may have an impact on modeling [27]. Rotllan-Puig and Traveset 28 commented on the rationale behind the Boyce index that varies between – 1 and +1. The only species of vertebrate that had poor modeling values according to the Boyce index was *Luscinia luscinia*, which was dropped from the final dataset, resulting in the total of 165 distribution maps. However, this new modeling exercise did not change the original conclusions on the predicted distribution (maximum change <1%, recorded for *Capreolus capreolus*; values of change for the remaining species well below the 0.5%); therefore, we continue considering the maps of Estrada-Peña and de la Fuente (2016) as a valid picture of the predicted distribution of vertebrates and *I. ricinus* over the target territory. The final maps intended for bioregionalization (see point 2.4) display the expected distribution of each vertebrate and its environmental suitability (translated here as 'abundance').

We then explicitly addressed the contact rates between each vertebrate and *I. ricinus* as reported [21], calculating the overlap of habitat throughout the complete target territory on a pixel-by-pixel basis. This was done in two steps, first using the function 'pno' (predicted niche occupancy) in the package 'phyloclim' [29] for R, following the concepts by [30], on which the function 'niche.overlap' calculates the percent of the environmental niche that is shared by any pair of species. The result represents a measure of the amount of habitat shared by any tick-vertebrate combination [21].

To note, we did not consider the actual contribution of each species of vertebrates to support the feeding of the tick because 'tick preferences' to feed on different vertebrates, obtained from field or laboratory data, as summarized by [16]. We think that reliable field data are available for only the most surveyed vertebrates; they are thus unavailable for more than 90% of the species included in this study. Therefore, the inclusion of the empirical data on host's preferences by the tick for the few available species would introduce a distorting variable because it could not be applied to every pair of associations tick-vertebrate. The tick preferences towards each vertebrate were obtained from two previous reports, derived from the centrality index of a network analysis on published records of *I. ricinus* on hosts [21]; the raw files to build the networks are available at https://datadryad.org/stash/dataset/doi:10.5061/dryad.2h3f2.

### *Clustering the distribution of vertebrates into spatial units*

A stack consisting of 165 layers of vertebrates' raster maps was used for an unsupervised classification of the territory. The purpose is to classify the territory into clusters based on the abundance of each vertebrate and the contact rates with the ticks by pixel. We applied an unsupervised classification that used the aforementioned stack of maps but did not supply any response data (that is, we did not identify any pixel as belonging to a particular class). This technique is useful when we have no prior knowledge of the study area. We used the k-means clustering algorithm to process a set of maps that resulted in the bioregionalization. To implement a k-means classification algorithm, the target number of regions (k) was determined by maximizing the cluster validity index. The Calinski–Harabasz Variance Ratio Criterion (VRC) 31 was used to measure within-group and between-group dispersions. The classification produced a set of areas representing a unique combination of vertebrates' species, their abundance (from modeling), and contact rates with the tick. Clusters of the same category are statistically inseparable, and clusters belonging to different categories are statistically different. The optimal number of categories for the target territory was 36, although two of them were returned as 'empty clusters' due either to the absence of vertebrates or of *I. ricinus*.

### Measuring the phylogenetic diversity of the vertebrates

We asked if the phylogenetic diversity of each vertebrates' community is correlated with certain traits of the tick presence or abundance (modeled). The Open Tree of Life is an online phylogenetic tree of life that is updated by adding published and curated phylogenetic trees of any organism. The project integrates these new trees as they are published into the mega-tree hosted by the website. It is thus possible to query the complete mega-tree and a subset of a number of species or other taxa ranks to prepare an *ad hoc* synthetic tree. These subset trees are not ultrametric, and it is necessary to calculate the branch length between any pair of species to have the phylogenetic measures of interest. We used data from the Open Tree of Life (https://tree.opentreeoflife.org, last accessed March 2021) for phylogenetic calculations, accessing its API using the 'rotl' package [32] for R.

We calculated the phylogenetic diversity (PD) and species richness (SR) existing in each cluster of the target territory using the package 'picante' [33] for R. While SR is a pure count of species, PD estimates the amount of phylogenetic variability in a cluster using the sum of the lengths of the branches of the phylogenetic tree of vertebrates present in that cluster. For the main calculations, we used only the generic name of each vertebrate to improve the solidness of the outcome. Supplemental material 1 includes the phylogenetic trees of the vertebrates' species together with the use of these hosts by *I. ricinus* and includes a 'readme' file (in PDF format) with information about all the files. Supplemental material 2 includes these same phylogenetic trees of vertebrates' species together with the spatial context of the target area.

### Proof-of-concept: discriminating the prevalence of Borrelia spp. in questing ticks using the composition of communities

We aimed to demonstrate that, at a rough scale of landscape, there is an indicator community of vertebrates that could describe the infection rates of *B. afzelii* and *B. garinii* in questing ticks. We chose these two bacteria because they are widely distributed in Europe, they have different vertebrate reservoirs (in general terms, birds for *B. garinii*, rodents for *B. afzelii*), and reports point to a role of community composition on the infection rates 34. It is not possible to develop these calculations for the complete target territory (due to lack of data on prevalence in ticks), or to apply the hypothesis to the *points* (coordinates) in which *Borrelia* spp. have been reported. Therefore, we covered the target territory with a hexagonal tessellation with a radius of 0.25°, selecting only those cells in which data of prevalence in ticks of either *B. afzelii* or *B. garinii* (or both) have been published. The choice of the diameter is not unintentional: higher cell sizes blurred the results (many species of vertebrates in the same cell) and smaller cell sizes commonly overfitted the models (too few species of vertebrates present in the cell). Data on the distribution of *Borrelia* spp. in Europe were obtained from [22], accounting for the prevalence of the pathogens in questing ticks. This produced 549 records for *B. garinii*, 555 records for *B. afzelii*, and 319 cells of the different clusters further used for modeling exercises (Figure 1)]. No limitations on the sample size of ticks were introduced as constraints in the selection, aiming to increase the number of sites to test.

The dataset on prevalence rates of *Borrelia* in ticks has been used as published [22]. In short, the dataset was compiled from scientific literature, between the years 1990 and 2017. All the details about the bibliographical query are described in the original study [22]. To summarize, only data from molecular detection of the pathogen(s) on questing nymphs of *I. ricinus* were used. We excluded every report of ticks collected while feeding, since it is not possible to ascertain if the DNA of the pathogen was already in the tick or was acquired with the blood meal. We also excluded field data on larvae or adults, the former because there is no transovarial transmission of these pathogens and the larvae hatch free of them; the latest because adults are more difficult to collect, and surveys tend to produce consistently a fewer number of specimens. As mentioned, only molecular tests (qPCR, RT-PCR) were used; other tests, like dark field microscopy or xenodiagnoses (i.e., infection of naïve hosts allowing ticks collected in the field to feed), while useful in its own context, are not comparable among them, thus biasing the statistical comparisons.

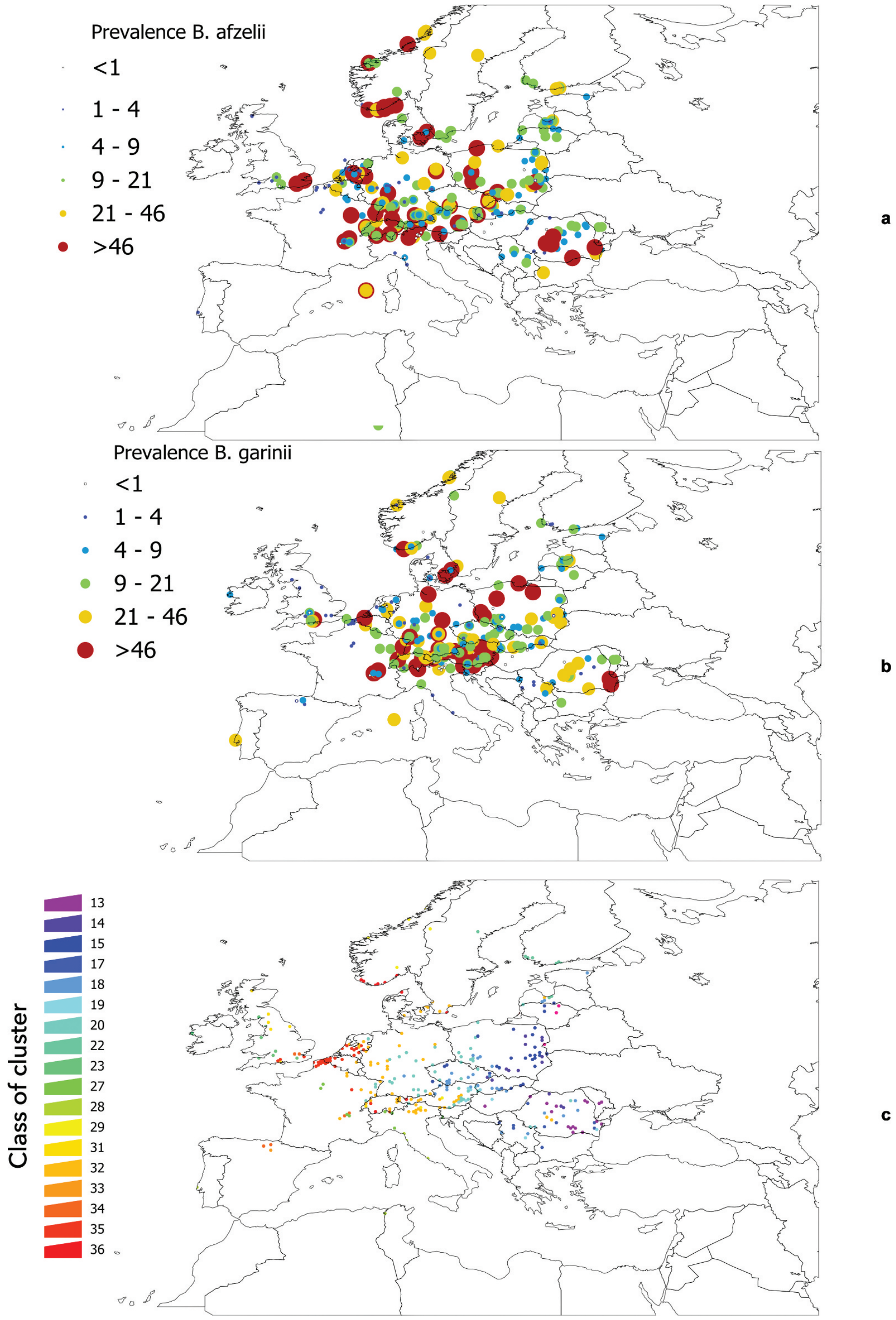

**Figure 1.** General background of distribution of *Borrelia* spp. in western Palearctic and the individual sets of clusters used for further analyses. a: The coordinates of records of *B. afzelii* in questing nymphal *I. ricinus* as reported in the published literature. b: The coordinates of records of *B. garinii* in questing nymphal ticks as reported in the published literature. For both a and b, compilation finished in the year 2018. Color and size of the dots mean for the reported prevalence. c: The sites used for statistical evaluations between the communities of vertebrates and infection rates of *Borrelia* spp. were calculated. The color of each point (which is actually an hexagon whose diameter is 0.25º) corresponds to the correlative numbering of the clusters obtained from the bioregionalization of vertebrates.

The purpose of modeling is two-fold, namely (i) to explain the prevalence of *Borrelia* spp. in questing for nymphal ticks and (ii) to delineate the indicator community that shapes the recorded infection rates in ticks. Modeling was done in the Orange Programming Environment (which is freely available from https://orangedatamining.com).

### *Modeling the vertebrates' communities that might drive the prevalence of Borrelia in the vector*

We purposely chose sites of the same cluster category, displaying differences in infection rates with other clusters and with a minimum of 15 independent surveys of questing ticks. We ultimately selected clusters 32 and 20 for *B. afzelii* (average reported prevalence in questing nymphs 10.5% and 22.4%, respectively) and clusters 35 and 20 for *B. garinii* (average reported prevalence in questing nymphs 7% and 15%, respectively). It was difficult to find clusters with lower prevalence of *Borrelia* spp. (i.e., lower than 7%) because the scarcity of these records in published literature (less than seven different surveys) compromising the quality of the statistical outcomes. It was impossible to select a significant number of clusters with a reported prevalence of '0.' The mere lack of reporting at a site (thus prevalence = 0) could mean that surveys have never been conducted at that site.

All algorithms for model development were available in the Orange programming environment. Supplemental material 3 includes the scripts using a graphical interface for repeating the modeling exercises or issuing new ones under different conditions. The ecological meaning of the calculations is also shown as separate charts in the body of the text. We used four different modeling approaches to correlate a given combination of vertebrates with the prevalence of *Borrelia* spp. in questing ticks: (i) Neural Networks, (ii) Random Forest, (iii) Gradient Boosting, and (iv) AdaBoost. All are algorithms of 'regression and classification' that operate on numerical data to obtain a response. Neural networks are comprised of node layers, containing an input layer, one or more hidden layers, and an output layer. Each node connects to another with a weight and a threshold. If the output of the node is above the specified threshold value that node is activated, sending data to the next layer of the network. The specific combination of 'on-off' nodes provides the solution [35]. For Neural Networks, we used 100 neurons per hidden layer, the ReLu algorithm, the Adam solver and 200 iterations. Random Forest, an ensemble learning method developed by 36, builds a set of decision trees. Each tree is developed from a bootstrap sample from training data. For each individual tree, an arbitrary subset of attributes is drawn from which the best attribute for the split is selected. The final model is based on the majority of votes from individually developed trees in the forest [37]. For Random Forests, we included 10 trees (i.e., the number of decision trees will be included in the forest), five trees to be split, which specifies the number of attributes that are arbitrarily drawn at each node at every step of the tree's development, without balance of classes, and three replicates of each model. Gradient boosting [38] is a method for creating an ensemble that starts by fitting an initial model (e.g., a tree or linear regression) to the data. A second model is then built, focusing on improving predictions where the first model performs poorly. The combination of these two models is expected to be better than either model alone. The process is then repeated many times, each successive model attempting to correct for the shortcomings of the combined boosted ensemble of all previous models. For Gradient Boosting, we used 100 trees with a learning rate of 0.1. AdaBoost (short for 'adaptive boosting') is a machine-learning algorithm, formulated by 39,that uses learning algorithms and iteratively tries to improve the solution in an adaptive way (tweaking weak learners in favor of those instances misclassified by previous classifiers.]. For AdaBoost, we used 50 estimators, a learning rate of 1, the SAMME.R classification algorithm and a linear regression lost function.

### *Detection of indicator communities and infection rates by Borrelia.*

The modeling algorithms mentioned in the previous point are addressed to predict the prevalence of the pathogen in the questing tick using the abundance and contact rates of the tick vector with the vertebrates. Here, we explicitly asked for the 'indicator community': the subset of vertebrates that better explains these infection rates, removing species that have little or no significance in the outcome. Our purpose is not to state the individual roles of each vertebrate, but rather, the effects of the whole community on the observed prevalence of the pathogen(s) in ticks. We used a 'rank filter' based on RReliefF [a method originally developed by 40]. The filter employs a stand-alone modeling algorithm to extract the set of candidate subsets of vertebrates that contribute most to the modeling results. In other words, the rank filter extracts variables with the highest impact on the results and promotes them as the 'best set of vertebrates' that are behind the observed prevalence of *Borrelia* spp. in questing ticks.

As mentioned, Supplemental data 3 includes the scripts for Orange and the input files necessary for reproducing the complete set of calculations explained in section 2.6 of Methods. Interested readers should have a basic knowledge of Orange programming environment to reproduce the results.

## Results

### Clusters of vertebrates and *I. ricinus* in western Palearctic follow a gradient of climate

The unsupervised classification of the territory using the k-means algorithm produced 36 clusters, shown in Figure 2. Note that clusters are defined by the composition of 165 species of vertebrates and their relative abundance weighted by contact rates with *I. ricinus*. Clusters 1 and 2 were restricted to the coldest mountain regions of Scandinavia and either the vertebrate species targeted in the study or the tick are predicted to be absent for these areas. According to the clustering methods, areas with the same color in Figure 3 have a more similar vertebrate composition/contact rates within them than among other areas. Clusters have similar communities of vertebrates to the level of significance $p = 0.05$. All clusters depicted in Figure 2 are statistically different from the faunal composition of other clusters in terms of vertebrate communities. Clusters of the same color may be spatially separated by other clusters.

### High contact rates of tick and vertebrates are concentrated in clusters with highest phylogenetic diversity

Figure 3 represents the calculated environmental suitability/contact rates for vertebrates in clusters of the target region. The heatmap also includes two dendrograms: one for vertebrates (linking those that tend to appear together) and one for clusters (linking those that tend to have similar vertebrate composition). Note that there is a clear gradient of spatial variability. Of interest (comparing Figures 2 and 3) is the poor variability of some territories, including mainly desert areas of northern Africa and contiguous Asia, and the higher species diversity in others. These patterns are not only affected by the 'abundance' of each vertebrate but also by the contact rates with *I. ricinus*, showing low values in clusters where niche overlap between tick and vertebrates is low. Note that many clusters in the territory (grouped mainly in north, central, and Western Europe) are predicted to carry large fractions of the complete set of vertebrates in this study, suggesting both a substantial contact with *I. ricinus* in these areas.

We calculated the PD and SR of each cluster (Figures 4 and 5). The values of both indexes do not perfectly overlap because species richness is not the same as phylogenetic diversity: areas of high SR may have a low PD because existing vertebrates are phylogenetically close. In general terms, most of the western Palearctic has a PD higher than 10 (a value considered high), meaning there is a wide range of potential, *phylogenetically distant* hosts for *I. ricinus*. Most of Central Europe, the Baltic countries, and southern Scandinavia, as well as parts of northern Spain and other mountain ranges (i.e., in Italy or Romania) displayed a high PD, suggesting large communities of phylogenetically separated vertebrates that could interact with the tick. Further on this, the highest environmental suitability for *I. ricinus* overlaps the territories with the highest PD of vertebrates ($R^2$: 0.897, F: 1287.14, $p < 0.05$). Values of SR and PD tend to attenuate in the eastern range of the map and in northern Africa. Since the contact rate of the tick with the vertebrates is part of

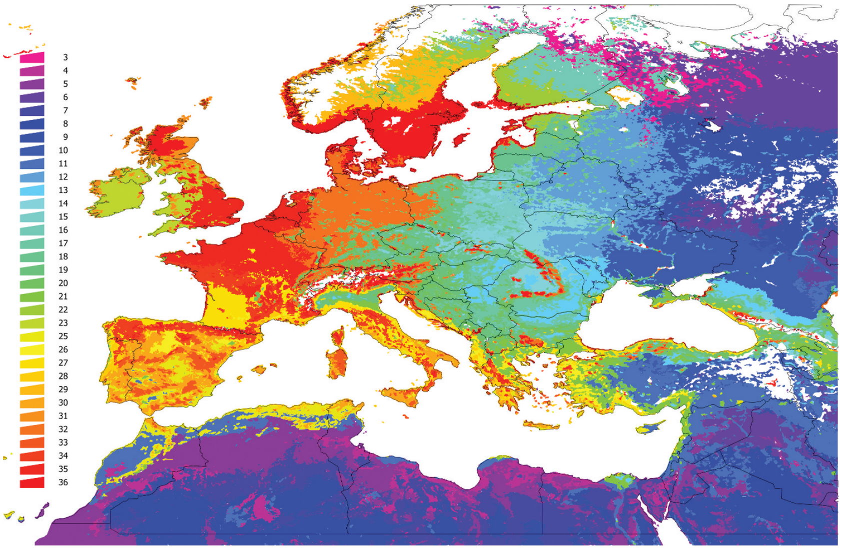

**Figure 2.** Clustering and the regions resulting from bioregionalization of the expected distribution of vertebrates in the target region, the expected distribution of *Ixodes ricinus* and its niche overlap. The map was obtained using an unsupervised classification using k-means on the raster maps of the predicted distribution of 165 species of vertebrates and *I. ricinus* and calculating the predicted niche occupancy of every pair of combinations vertebrate – tick. The unsupervised classification returned 36 categories, of which the 1 and 2 are in northern Scandinavia, western Russia, and eastern Turkey, where *I. ricinus* is absent. We kept the remaining 34 categories. Colors of the figure are random.

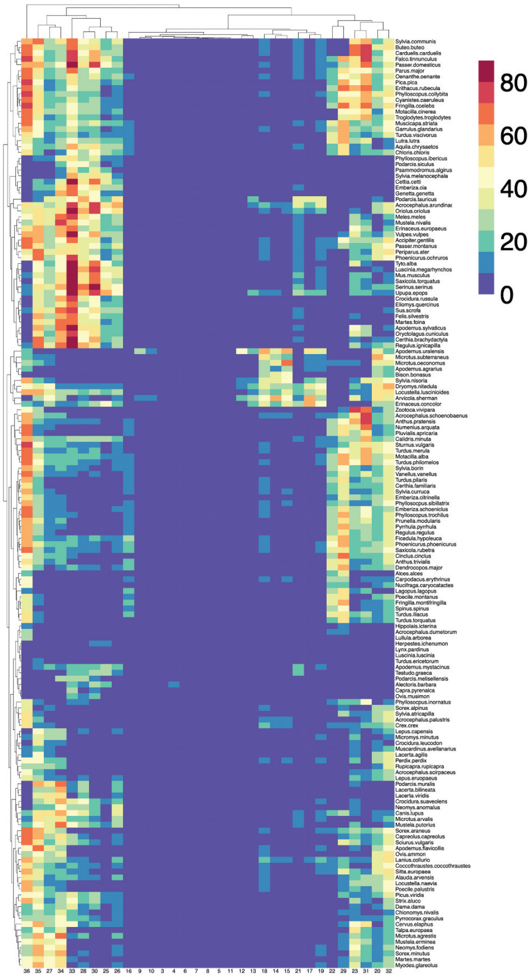

**Figure 3.** A heatmap representing the abundance of vertebrates, weighted by the contact rates with *Ixodes ricinus* in the western Palearctic at each cluster of the territory. The values in the heatmap show two dendrograms, one for the vertebrates that tend to appear together (left of the figure) and the other for sites that tend to support similar fauna of vertebrates (top of the figure). Specific names for every vertebrate are included, even if the phylogenetic tree of vertebrates has been made using only generic denominations.

our strict definition of communities, such attenuation of values should be expected because *I. ricinus* is predicted to be mostly absent from the mentioned regions.

Correlations between the prevalence of *B. afzelii* or *B. garinii* with the PD or SR of each cluster were far from significant (PD: $R^{2:}$ 0.0009 for *B. afzelii* and 0.0012 for *B. garinii*; SR: $R^{2:}$ 0.0014 for *B. afzelii* and 0.0125 for *B. gariniiI*; p > 0.8 in both cases) indicating that the prevalence of the pathogens is not only correlated with the contact rates of *I. ricinus* with *any* vertebrate. Therefore, the mere co-existence of large populations of the tick and a high number of available vertebrates, is not a hallmark for the circulation of the chosen pathogens. This suggests that the pathogens could be linked to peculiar combinations of vertebrates feeding the tick.

### *Proof-of-concept: detecting the communities driving the prevalence of B. afzelii and B. garinii in selected clusters*

We asked whether indicator communities exist as the best index of infection rates by either *B. afzelii* or *B. garinii* in *I. ricinus*. This is not to evaluate whether each cluster resulting from bioregionalization carries a *unique indicator species* of vertebrate shaping high or low values of prevalence of *Borrelia* spp. in questing ticks. The goal is to find communities displaying statistically solid relationships with the patterns of prevalence in ticks. This analysis cannot be done on single sites belonging to a given cluster but rather by using *all* sites belonging to the same category of clusters. With these cautionary words, we first concluded that the infection rates in questing ticks are statistically different among the clusters, as detected by an ANOVA test (*B. afzelii*: F: 77.08; p < 0.0001; *B. garinii*: F: 1042.3; p < 0.0001).

The ecological meaning of the proof-of-concept is schematized in Figure 6. It represents the communities of vertebrates in the three different clusters selected for testing by the modeling algorithms (whose spatial distribution is shown in the accompanying maps) expressing the contact rates with *I. ricinus* corrected by the hosts preferences, and the prevalence of *Borrelia* spp. reported in questing nymphal ticks. At a first view, it is difficult to observe a pattern. The task of the algorithms is twice: (i) evaluate the combinations of vertebrates' species to obtain a better correlation with tick prevalence, and (ii) deduce a better combination of vertebrates' species that produces that outcome and display it in a reduced space of principal components. To note, vertebrates 'compete' for the tick, and it may have high contact rates with a non-reservoir vertebrate (therefore 'diluting' the circulation of the pathogen) or with a prominent reservoir. The modeling must 'remove the noise' leaving only the most important vertebrates whose joint contribution describes the field findings.

### *The performance of the models: explaining the prevalence of Borrelia spp. through the vertebrates' composition.*

The results of the modeling algorithms for each cluster and both *B afzelii* and *B. garinii* are included in Table 1. Several algorithms displayed high reliability,

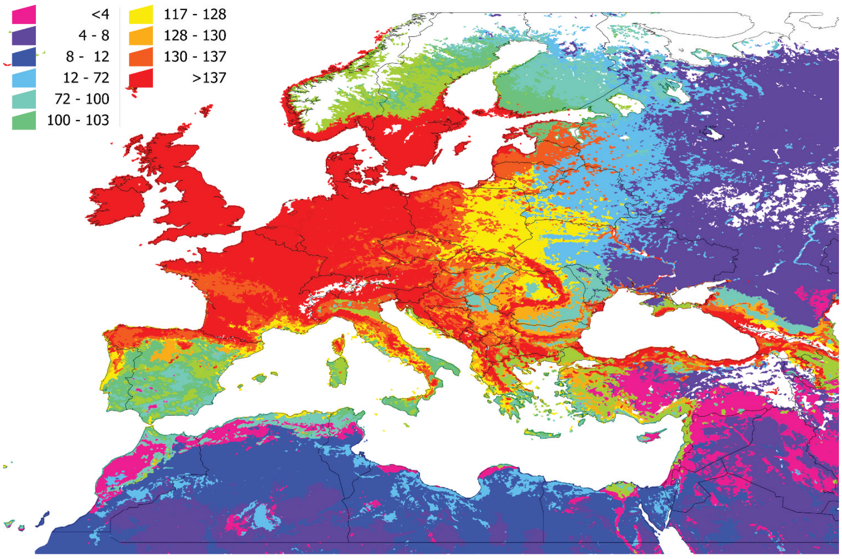

**Figure 4.** Species richness of vertebrates in the target territory. The value must be interpreted as the number of vertebrates reported as hosts of *I. ricinus* that are expected to be present in the territory and available for the tick because they share portions of the environmental niche.

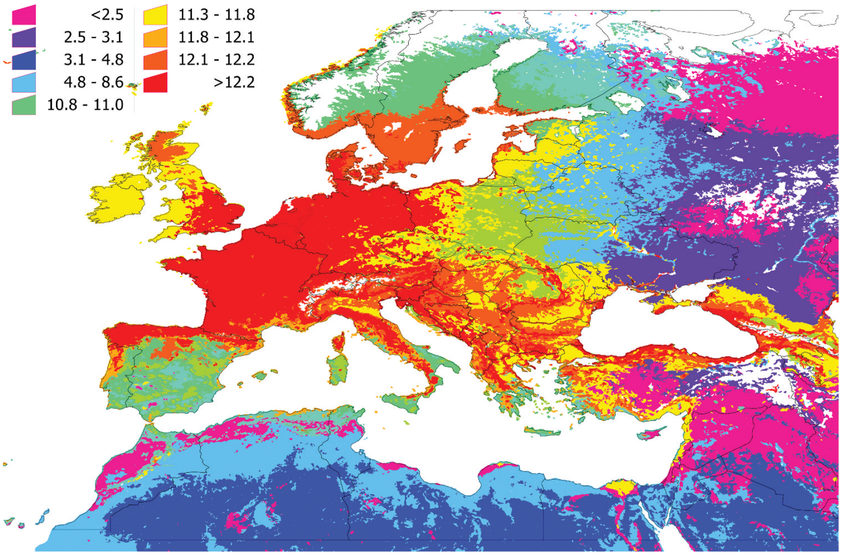

**Figure 5.** The phylogenetic diversity of the vertebrates in the target territory. The value must be interpreted as the phylogenetic diversity of the vertebrates reported as hosts of *I. ricinus* that are expected to be present in the territory, measured by the method of Faith. Supplementary Research Data contains the details of the spatial distribution of each cluster, and the use of portions of the phylogenetic tree of the vertebrates by the tick in each cluster.

accounting for the effect of the combination of some species on the prevalence of *Borrelia* spp. in questing ticks. Some of the algorithms systematically demonstrated a poorer ability to discern the faunal composition linked to the prevalence.

Modeling results for the clusters selected as of high or low prevalence (bold typeface in Table 1) clearly supports the fact that there is an indicator community of vertebrates that results in good correlations with infection rates by *Borrelia* spp. in *I. ricinus*. Gradient Booster provided the best algorithm, with an $R^2$ value of more than 0.9 for each tested condition. Figures 7 and 8 expand the ecological explanation of the proof-of-concept and summarize the resulting communities of vertebrates for each condition (cluster+pathogen), separated in the reduced space of principal components after the ranking algorithm. Although these results came

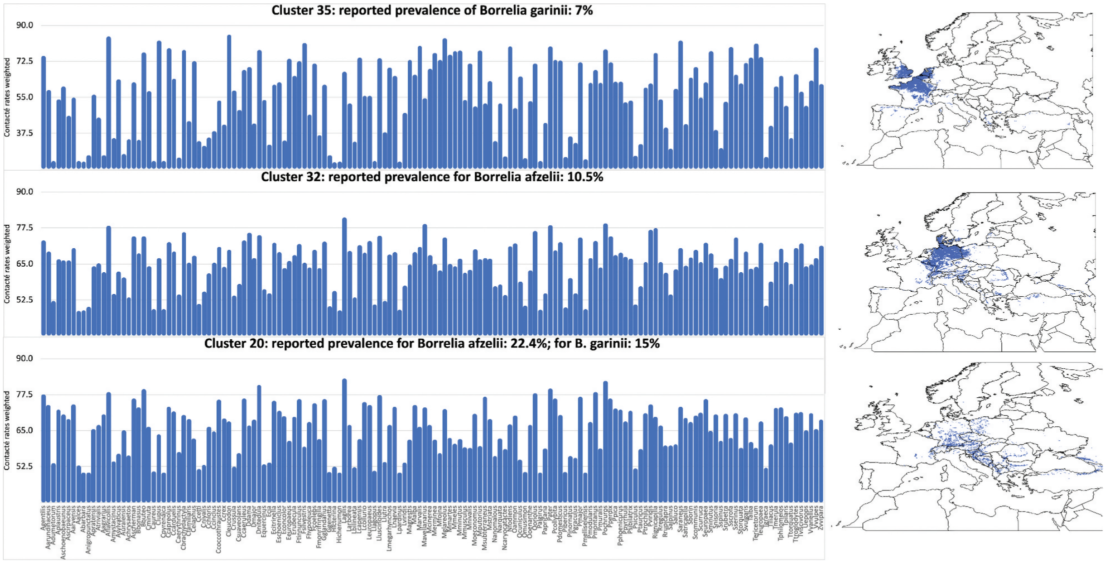

**Figure 6.** The modelled suitability of each species of vertebrate included in this study, weighted by the contact rates with the tick *I. ricinus* and the preferences of the tick for each host (histograms). Only data for areas included in the categories 20, 32 and 35 are shown, since they represent the most contrasting sites regarding prevalence of *Borrelia* spp. in the target territory. These ar ethe sites that were subjected to modeling. A small map at right shows the spatial extension of these territories. All these data were obtained from Supplemental material 3.

**Table 1.** Outcome of the modeling algorithms between the vertebrates' community and the reported prevalence of *Borrelia afzelii* and *Borrelia garinii* in questing nymphs of *Ixodes ricinus* ticks, including clusters 12 to 36, for which there are available data. Cluster (left column) is a consecutive numbering of the unsupervised classification carried out on the target territory as shown in Figure 1(c). The column 'prevalence' indicates the averaged reported prevalence of either *B. afzelii* or *B. garinii* together with the number of reported surveys in that cluster in parentheses. Each other column indicates the percent of correct classification of the prevalence of *Borrelia* in questing ticks by the regression and classification algorithms, separately for the two pathogens tested.

| Cluster | Prevalence: *B. afzelii* | Neural network | Random forest | Gradient boosting | AdaBoost | Prevalence: *B. garinii* | Neural network | Random forest | Gradient boosting | AdaBoost |
|---|---|---|---|---|---|---|---|---|---|---|
| 12 | 10.55 (3) | Only 3 surveys | | | | 5.00 (3) | Only 3 surveys | | | |
| 13 | 11.18 (17) | 0.625 | 0.481 | 1 | 0.997 | 5.38 (13) | 0.607 | 0.464 | 1 | 1 |
| 14 | 16.58 (14) | 0.666 | 0.338 | 1 | 0.947 | 6.02 (14) | 0.816 | 0.359 | 1 | 1 |
| 15 | 14.1 (26) | 0.522 | 0.564 | 1 | 0.999 | 6.83 (26) | 0.837 | 0.639 | 1 | 0.995 |
| 17 | 9.21 (11) | 0.613 | 0.761 | 1 | 0.999 | 10.5 (11) | 0.985 | 0.927 | 1 | 1 |
| 18 | 8.93 (32) | 0.778 | 0.589 | 1 | 0.993 | 8.46 (32) | 0.801 | 0.737 | 1 | 0.998 |
| 19 | 3.62 (10) | 1 | 0.601 | 1 | 1 | 14.03 (10) | 0.899 | 0.855 | 1 | 0.998 |
| 20 | **10.56 (44)** | **0.811** | **0.791** | **1** | **0.988** | **18.61 (44)** | **0.562** | **0.691** | **1** | **0.998** |
| 22 | 13.45 (9) | 1 | 0.377 | 1 | 1 | 8.83 (9) | 1 | 0.597 | 1 | 1 |
| 23 | 7.04 (8) | 0.994 | 0.778 | 1 | 0.999 | 2.67 (8) | 1 | 0.743 | 1 | 1 |
| 27 | 3.81 (8) | 1 | 0.426 | 1 | 1 | 8.94 (8) | 0.999 | 0.422 | 1 | 1 |
| 28 | 1.09 (5) | 0.999 | 0.133 | 1 | 0.555 | 5.58 (5) | 0.994 | 0.186 | 1 | 1 |
| 29 | 21.45 (8) | 1 | 0.487 | 1 | 1 | 20.19 (8) | 1 | 0.543 | 1 | 0.989 |
| 31 | 6.18 (8) | 1 | 0.887 | 1 | 1 | 1.06 (8) | 0.999 | 0.777 | 1 | 1 |
| 32 | **22.39 (64)** | **0.443** | **0.655** | **0.999** | **0.999** | 15.5 (64) | 0.598 | 0.716 | 1 | 0.994 |
| 33 | 0 (1) | Only 1 survey | | | | 1.4 (1) | Only 1 survey | | | |
| 34 | 12.53 (7) | 1 | 0.25 | 1 | 1 | 8.4 (7) | 1 | 0.455 | 1 | 1 |
| 35 | 11.13 (33) | 0.866 | 0.685 | 1 | 0.982 | **7.21 (33)** | **0.941** | **0.713** | **1** | **0.998** |
| 36 | 23.08 (11) | 0.890 | 0.448 | 1 | 0.998 | 12.17 (11) | 0.998 | 0.535 | 1 | 1 |

from pure modeling, there is agreement between the species included as most/less prominent in each indicator community, and the observed field rates of prevalence of *Borrelia* spp.

Regarding *B. afzelii* (Figure 7(a)), sites belonging to cluster 20 (high prevalence of *Borrelia* reported) have as most contributing vertebrates the Yellow-necked mouse (*Apodemus flavicollis*) and the Bank vole (*Myodes glareolus*). Some Insectivora, ungulates like the Red deer (*Cervus elaphus*), the Chamois (*Rupicapra rupicapra*) and the Mountain sheep (*Ovis ammon*), carnivores, and a few birds are

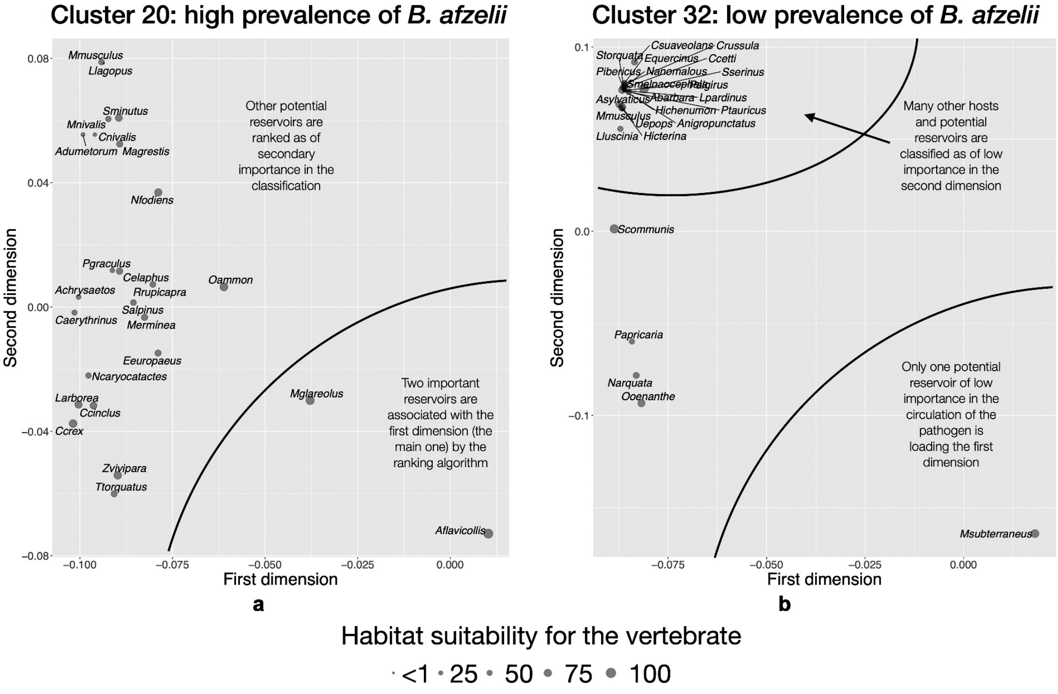

**Figure 7.** The indicator community of vertebrates projected on the reduced space in two areas of the target territory reporting different rates of infection by *B. afzelii* in questing nymphs of *I. ricinus.*

shown as secondary components of the indicator community in that cluster. However, the community defining sites of cluster 32 (low prevalence) is poorer and dominated by the European pine vole (*Microtus subterraneus*), and other species of rodents (i.e., *Mus* spp., *Apodemus* spp.), and Insectivora (i.e., *Crocidura*

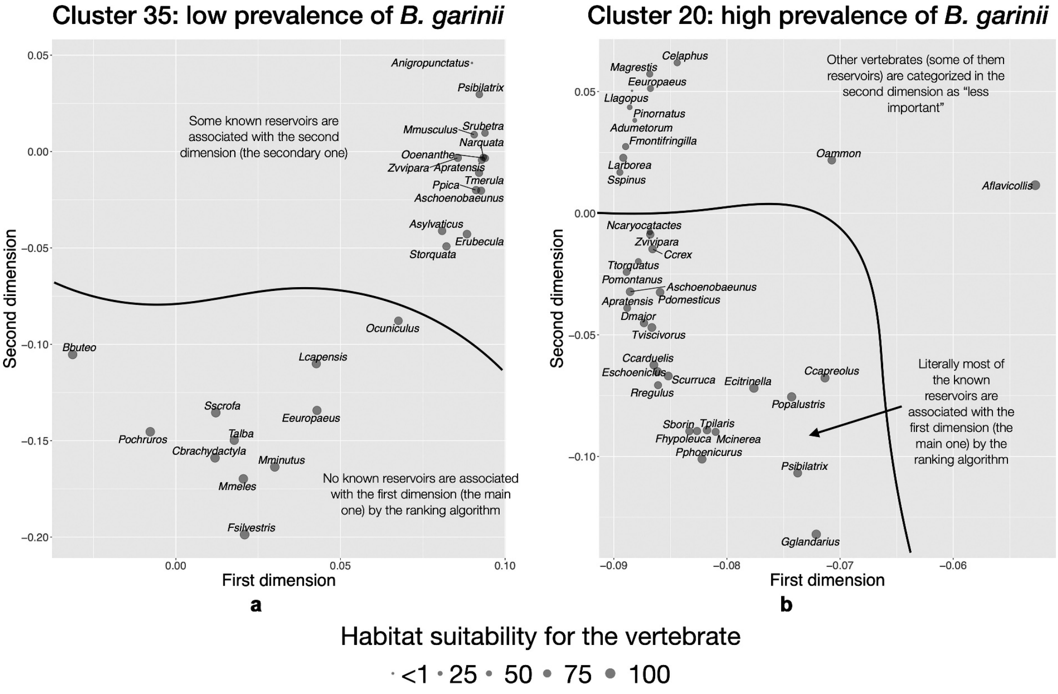

**Figure 8.** The indicator community of vertebrates projected on the reduced space in two areas of the target territory reporting different rates of infection by *B. garinii* in questing nymphs of *I. ricinus.*

spp.) that appear distant from the main set of dominant species and closely grouped among them (Figure 7(b)). There is a strong joint occurrence of these secondary species with a low contribution to the reported prevalence of the pathogen in the tick.

A different indicator community was detected for *B. garinii* (Figure 8). Sites reporting low-to-medium prevalence (cluster 35, Figure 8(a)) have several species of mammals, which rank prominently as the main species driving the results of modeling. The only bird among these dominant species is the short-toed treecreeper (*Certhia brachydactyla*). Other species of birds, which are reservoirs of the bacterium, form a community that ranks second in the cluster definition (top of the chart), and that group together, out of the main group of dominant vertebrates. Sites belonging to cluster 20 (highly reported infection rates by *B. garinii* in questing ticks, Figure 8B) are dominated by birds (which are the reservoirs of the pathogen). Some ungulates and Insectivora are part of the community. It is of interest to note that the Chamois, *Capreolus capreolus*, is detected by the modeling algorithms as a prominent part of the indicator community (bottom of the chart) but the Red deer, *Cervus elaphus*, (top of the chart) is not.

Summarizing, the indicator community of sites with high prevalence of *B. afzelii* has a large component of Rodentia and Insectivora (its reservoirs), with birds located in secondary positions. An inverse situation has been detected at sites with low-medium prevalence by the pathogen. Clusters with high prevalence of *B. garinii* are dominated by their reservoir birds, with a significant increase in rodents in clusters in which prevalence is smaller. Carnivora and Ungulata are always secondary members of the indicator community because of their role as tick feeders, contributing to the population of ticks, but not reservoirs.

## Discussion

We demonstrated that a bioregionalization of the western Palearctic can be built with an epidemiological focus on tick-borne pathogens, based on the distribution and abundance of hundreds of species of vertebrates and the contact rates with the tick *I. ricinus*. The classification of modeled distribution maps resulted in clusters reflecting specific combinations of vertebrates and different contact rates with the tick vector. This could provide a strict determination of the impact of changing climate conditions on the predicted distribution of both the tick and the vertebrates, and thus the contact rates and the resulting epidemiological consequences. Previous approaches have aimed at evaluating the background behind the prevalence of *Borrelia* spp. in ticks, considering only abiotic features (Estrada-Peña et al., 2011). The effects of the variability of vertebrates on the infection rates of a tick-transmitted pathogen have never been addressed at anything beyond the regional scale, based on the gold standard based on field or laboratory protocols calculating the prevalence of questing nymphs. However, this study proposed a statistical procedure that establishes the impact of the communities of vertebrates on the infection rates of *Borrelia spp*. in questing *I. ricinus* nymphs, and that is well correlated with the recorded situation in the target territory [22]. This study is a proposal, open to major improvements, that pinpoints an area to be addressed also for other tick-borne pathogens.

The relative importance of several vertebrates in the epidemiology of Lyme borreliosis in Europe has been addressed in literally dozens of studies: not only the key role of rodents or birds as reservoirs of some species [i.e., 16, 41, 42] but also the dual role played by some taxa like large ungulates on the amplification/dilution of the pathogen [i.e., 43, 44]. In the USA, research has been partly focused on the life history traits of different vertebrates, aiming to find a correlate of their contributions to the infection rates of ticks by *B. burgdorferi* s.l. 45 The list of references above is far from complete but provides an appraisal of open debates on the topic. To note, the evaluation of the effect of the vertebrates' communities on infection rates by *Borrelia* spp. in questing ticks has already been proposed by Mysterud et al. (2019a) but using field experiments.

Normally, only a few species of hosts in foci of tick-transmitted pathogens are studied in the field, most likely due to the impressive logistical issues involved in such surveys or because of the difficulty in collecting scarce vertebrates or protected species [46]. One of the major issues in conducting separate modeling of individual species of organisms is that they may interact in different ways [47,48]. The strategy 'predict first, then cluster' as adhered to here, seems to be a good method when modeling groups of co-occurring organisms [20]. Interactions among species are already included in datasets from which the predicted maps are derived: if two or more species compete for a resource and one ends up displaced by the competition phenomena, a lack of records of the affected species will be noticed when the competing species is present.

We observed that higher contact rates of *I. ricinus* with the vertebrates are well correlated for sites in which the phylogenetic diversity of vertebrates is high. This is an important finding since the phylogenetic diversity of an area could be important when species differ in their contribution to the support of the populations of ticks and pathogens. Thus, the phylogenetic composition rather than the list of species in an ecosystem could be of particular importance for understanding the always complex epidemiology of tick-borne pathogens. According to Webb et al. [49], the interpretation is that 'the more distantly related two species are, the greater the likelihood that they differ ecologically' (summarized by Cadotte et al. [50]). At sites in which phylogenetic diversity is high, the tick has literally dozens of vertebrates to feed upon since every site in the environmental niche of vertebrates is suitable for the tick. We state that the system, under these conditions, may be highly redundant: the absence of a few key species is replaced by the presence of others that could not be significant under a different community composition.

The concept of indicator species has deep ecological roots in multivariate statistics (Legendre et al. [51]). According to Legendre and De Cáceres [52], a species is 'an indicator of a group of sites if the indicator value of the species is the highest for that group of sites and is statistically significant at a preselected significance level.' Our study was based on that concept (revisited by De Cáceres et al. [53]) but aimed to pinpoint indicator *communities* of vertebrates instead of single species. These indicator communities are expected to change in both space and time because of their intrinsic requirements, the availability of resources, and the occasional replacement of species due to trends of climate [54,55]. We previously demonstrated that there are highly significant statistical differences in the infection rates of ticks among sites, for both *B. afzelii* and *B. garinii* 56. However, climate shapes the occurrence of vertebrates and delineates the gradients of contact of the co-occurring species with the vector. This is the intricate niche epidemiology of *Borrelia* spp. that has been already elaborated, using a network analysis [21].

We acknowledge the gaps in this study. Some issues may affect the calculations, such as the sample size of surveyed questing ticks and reported prevalence, the season of the year when the survey was done, local vegetation conditions or landscape fragmentation, or even the method for detecting *the bacterium* (e.g., either pools of ticks or specimens processed individually). Our study also ignored the specific contributions of individual vertebrates' species to the feeding of the tick or transmission of the pathogen. This is an important point since these data are commonly obtained in field or laboratory protocols [11,57]. A method to evaluate the individual importance of each vertebrate in the circulation of *Borrelia*, aimed to replace the field surveys, and that is based on the entire network of relationships among vertebrates and the tick has been already proposed [21]. This procedure corresponds well with the identification of keystone taxa in ecological studies [58]. Since we are looking for indicator communities, the centrality of the network derived from the matrix of interactions among partners looks like the obvious value to reflect the tick preferences for a host [following 58]. However, no studies have yet linked both field-derived and network-derived data given the paucity of data for many vertebrates. We consider that the validation of the network approach is a necessary step before taking a deeper dive into the reservoir capacity of tick-borne pathogens by vertebrates. The key concept is, 'how does the combination of several vertebrates affect transmission rates to a single vector?' At least for *Borrelia* spp., factors allowing speciation are commonly linked to the phylogeny of the reservoir 59,60–63) which fully supports our selection of species and comments on reservoirs.

Results are consistent with the epidemiology of the pathogen, circulating only among some of the vertebrates that feed the tick vector. It is nevertheless of interest to note that the ranking of the vertebrates of each indicator community matches the current knowledge of the most common reservoirs of tested species of *Borrelia*, and points to the dual role played by Carnivora or Ungulata [12]. Most importantly, variability of the communities, in either species composition or abundance, is detected as key factors shaping infection rates in ticks, even if recognized reservoirs are present, but ranked as less important in the community. We think that results are compatible with the knowledge of the ecology of *Borrelia* spp. and their reservoirs. There is still considerable room for improvement of the methods, linking network approaches with spatial modeling and ranking algorithms as well as the basic assumptions, but results are encouraging.

It did not escape our attention that the highest infection rates of both species of *Borrelia* in the three selected groups of clusters have always been found in a more fragmented area (compare, i.e. the spatial fragmentation of clusters in Figure 6). The effect has not been conceptualized in our models since we focused on a purely biological approach regarding the community composition of vertebrates, and because the scale of work would not allow to capture these fine differences. However, the effect has been mentioned in the literature on the topic and even pinpointed as one of the most important effects of preliminary assessments on the distribution of *Borrelia* spp. in Europe 56. On a small scale, the importance of landscape structure has been pointed out as affecting prevalence of some species of *Borrelia*, most probably because of the impact on the diversity of vertebrates' communities [64]. The scale of our study cannot outline or reject these field studies, but this is an open field that deserves interesting findings when compared with the probable relative rarefaction of some key vertebrates.

We aimed for an ecologically sound and radically different approaches to explain the infection rates of a tick-borne pathogen in the vectors: could a bioregionalization including contact rates among ticks and vertebrates be correlated with the prevalence of *Borrelia* spp. in ticks? Our analysis added an extra dimension that may be of interest to the study of the dynamics of tick-borne pathogens. We anticipate that a wide-open field of research remains ahead of this view: just to cite the example of Tick-borne encephalitis (TBE) that is also transmitted by the same tick and observes a puzzling pattern of distribution, seasonality and (re)emergence of foci [i.e., 65]. We hope this approach can provide innovative ways to approximate the complex epidemiology of many tick-borne pathogens using a synthetic background [42,44,56,66–68].

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## Disclosure statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Author contributions

AEP conceptualized the work, calculated the networks, wrote the first versions of the draft, outlined the figures, obtained, and processed climate data, programmed the scripts in Orange, and wrote some of the R scripts for calculation of habitat suitability. NFR did part of the literature search and updated existing data on climate; she prepared several figures from scripts in R and wrote parts of the draft document. Both authors wrote and agreed on the final version of the manuscript.

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# Chapter 5: Passive collection of ticks in New Hampshire reveals species-specific patterns of distribution and activity.

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Vector-Borne Diseases, Surveillance, Prevention

# Passive collection of ticks in New Hampshire reveals species-specific patterns of distribution and activity

**Natalia Fernández-Ruiz[1,2], Agustín Estrada-Peña[1,2], Sharon McElroy[3], Kaitlyn Morse[3,*]**

[1]Department of Animal Health, Faculty of Veterinary Medicine, University of Zaragoza, 50013 Zaragoza, Spain, [2]Instituto Agroalimentario de Aragón (IA2), Zaragoza, Spain, [3]BeBop Labs, Tick Project, NH 03268, USA [*]Corresponding author, mail: kaitlyn@beboplabs.org

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Ticks and tick-borne diseases are increasing in the United States, including New Hampshire (NH). We report on the findings of an ongoing free crowdsourcing program spanning four years within NH. The date of tick's submission was recorded along with species, sex, stage, location they were collected (translated into latitude and longitude), the activity the individual was doing when the tick was found, and host species. A total of 14,252 ticks belonging to subclass Acari, family Ixodidae and genera *Ixodes*, *Dermacentor*, *Amblyomma*, and *Haemaphysalis* was recorded from the period 2018–2021 throughout NH. A total of 2,787 *Ixodes scapularis* and 1,041 *Dermacentor variabilis*, were tested for the presence of *Borrelia* sp. (Spirochaetales: Spirochaetaceae), *B. burgdorferi sensu lato*, *B. miyamotoi*, *B. mayonii*, *Babesia microti* (Piroplasmida: Babesiidae), *Anaplasma phagocytophilum* (Rickettsiales: Anaplasmataceae), *Francisella tularensis* (Thiotrichales: Francisellaceae), and *Rickettsia rickettsii* (Rickettsiales: Rickettsiaceae) by PCR. For the *I. scapularis* ticks tested, the pathogen prevalence was 37% *B. burgdorferi s.l.* 1% *B. miyamotoi*, 6% *A. phagocytophilum*, and 5% *Ba. microti*. Only one *D. variabilis* resulted positive to *F. tularensis.* We created state-wide maps informing the differences of ticks as detailed by administrative divisions. *Dermacentor variabilis* peaked in June and *I. scapularis* peaked in May and October. The most reported activity by people with tick encounters was while walking/hiking, and the least was biking. Using the reported distribution of both species of ticks, we modeled their climate suitability in the target territory. In NH, *I. scapularis* and *D. variabilis* have distinct patterns of emergence, abundance, and distribution. Tick prevention is important especially during April–August when both tick species are abundant and active.

*Key words: Ixodes scapularis*, *Dermacentor variabilis*, community science, tick borne pathogens, climate modeling

## Introduction

Surveillance of disease and vectors of disease is crucial to ensure human and pet safety. Ticks serve as vectors for more than 13 unique human tick-borne illnesses caused by 18 different pathogens in the United States, and there are even more tick-borne pathogens that affect pets and/or livestock (CDC 2015). Throughout the United States, the Centers for Disease Control and Prevention (CDC) receives 30,000 reports of clinical Lyme disease, caused by *Borrelia burgdorferi sensu lato*, but estimations point to 8–12 times underreporting with the actual numbers estimated at 476,000 cases (Kugeler et al. 2021), with the highest numbers concentrated in New England (Schiffman et al. 2018). There is also a concern for other tick-borne diseases that are far less accurately tracked as compared to *B. burgdorferi* (Rochlin and Toledo 2020). To make matters worse, the beginning and end of tick activity/seasons changes according to weather conditions and extends with warmer autumns and winters, making it harder to pinpoint at-risk regions and periods (Wikel 2022). There are numerous records demonstrating that some tick species are spreading to the northern parts of the United States (Eisen and Eisen 2018, Sonenshine 2018, Wikel 2022). Most commonly, climate has been pinpointed as one of the main drivers behind such spread (Ogden et al. 2021), but the presence and abundance of hosts or the landscape features are also important features to account for the spread of ticks (Pfäffle et al. 2013, Talbot et al. 2019).

The distribution of ticks over large territories has been commonly explored by field surveys, which are complex and expensive in nature or conducted at limited temporal or spatial scales. To overcome this hurdle, it has become common to involve community scientists in the surveillance of ticks. Community science, collectively known as the altruistic participation of volunteers in scientific work, is becoming an important ground for several studies dealing with the passive surveillance of the distribution of an organism. This is commonly called 'public participation in scientific research', 'passive surveillance'. or 'community-based monitoring'. One of the main problems regarding community science is the assessment of the data quality, leading to questioning how good is the data gathered by participants (Cronje et al. 2011, Wiggins et al. 2011, Kosmala et al. 2016, Eisen and Eisen 2021). Issues arose when participants are asked to perform similar tasks as the researchers, producing some data for which they are not prepared (like species identification), even after an introductory course. Community science has been used for passive surveillance of ticks consistently for over one decade and helped to identify the spread of ticks and transmitted pathogens (Ogden et al. 2006, Gasmi et al. 2019, Nelder et al. 2019) or using animals as sentinels (Lee et al. 2019). The validity of passive surveillance has been improved by the multiple applications for mobile devices like smartphones (Hamer et al. 2018, Fernández et al. 2019) that complement the reporting of a parasite with a picture of the specimen and/or the possibility of the mobile device to track its position (an earlier review is available by Madder et al. 2012). To note, in most cases a cell phone does not have enough magnifying power to provide a clear picture of a feeding immature tick, that is commonly identified by a specialist under a stereomicroscope. Thus, the best passive tick surveillance gathers three key pieces of information (Eisen and Eisen 2021, Poh et al. 2022), namely (i) an accurate identification of the tick by specialists, and if necessary, the further processing of the tick(s) for carried pathogen(s); (ii) a basic set of questions prepared by the researcher(s) to gather as much information as possible from the participants; and (iii) coordinates of collection, together with minor details of the record. Although, the influence of socio-economic strata in the altruistic participation, or mistakes in geo-localization remain pertinent drawbacks (Eisen and Eisen 2021).

Several tick species affecting humans have been reported in northeastern United States. Of these, some of the most reported in the region are *Ixodes scapularis* and *Dermacentor variabilis* (Dergousoff et al. 2013, Hahn et al 2016, Sonenshine 2018, Duncan et al. 2021, Wikel 2022). These species increase their spatial range by a variety of reasons, including (but not restricted to) climate trends, changes in land use, and/or (re)colonization by wildlife. Climate trends have been proposed as one of the main traits affecting the distribution of the ticks. Efforts are addressed to capture their environmental niche and project the results into the territory (Alkishe et al. 2021). Spatial modeling can thus predict the probability of the presence of a tick (Laniak et al. 2013). The environmental niche is defined by using records of tick coordinates linked with variables like temperature and the amount of water in the ground or the air and matching the known distribution of the species with the preferred range of the environmental variables (Estrada-Peña et al. 2013).

New Hampshire (NH) is a northeastern state of United States and one of the places with highest incidence of Lyme disease, Anaplasmosis, and Babesiosis in USA, as reported by the CDC (2021). This study aims to analyze the ticks submitted to BeBop Labs (https://www.beboplabs.org), a non-profit organization that is filling in the gaps of ticks and tick-borne disease surveillance within NH. We summarize the ticks collected from humans, pets, and other domestic animals, pinpointing conclusions about the distribution and activity period(s) of the ticks, the most common situations of risk, the pathogens carried as detected by PCR, and a preliminary modeling of the two most reported species, namely *I. scapularis* and *D. variabilis*.

## Methods

### Tick Submission and Identification

Ticks started to be collected by residents in NH, in June 2017 through a free program. The program was advertised through partnerships with Plymouth State University, the University of NH, NH camps, and similar organizations, as well as through local newspaper articles and social media. Submitted ticks were received by postal mail along with a questionnaire. Since the program is still ongoing, this study reports the data between the years 2018 and 2021. In 2017, there were fewer ticks submitted, and the accompanying questionnaire was different. Due to the SARS-CoV-2 pandemic in 2020, the participation could have also been affected, but the program was still running. Ticks were received live, dead, taped to paper, or in alcohol, but immediately upon receipt ticks were kept dehumidified and at room temperature until identification and pathogen testing, a method that has been shown to preserve DNA (Bonnet et al. 2010). For each tick, we recorded the date of collection, and we identified species, sex, stage, location in which the ticks were collected (specific as town and street address, and/or latitude and longitude), the activity that the host was doing when the tick was found (for human hosts), if the tick was crawling or biting, and the species of host (i.e., human, dog, etc.). Although each submission was annotated with the exact date of finding and/or removal, weekly intervals (together with a mention to the month and the season) provided a better overview of the period of tick activity. Tick species identification was based on published identification keys (Clifford et al. 1961, Brinton et al. 1965, USDA 1976, Keirans et al. 1978, 1998a, Yunker et al. 1986, Durden et al. 1996). Within the results, we have also included 111 ticks obtained by shared information from Ticknology, Fort Collins, CO, for the year 2019, and the publicly available tick submission information from MedZu, Inc., Tick Report, Amherst, MA (formerly within University of Massachusetts Amherst Laboratory of Medical Zoology) for the complete period (years 2018–2021) available at: https://www.tickreport.com/stats. Thus, some species of ticks were also identified using PCR targeting specific genes from individual tick species and performed by these laboratories. We focused our analyses on *I. scapularis* and *D. variabilis*, the most prominent tick species received, yet we also identified a few other tick species (see below, Results, Table 1) and they were not analyzed further.

**Location of tick.** Participants recorded location to the best of our knowledge to where the tick was found. Explanations of the activity done just before noticing the bite of the tick, including details like 'biking between A and B (several kilometers)' could not provide reliable coordinates of the exact place of bite. Therefore, we included the coordinates as submitted. All the results regarding the number of ticks submitted and identified were mapped at the level of the administrative perimeter of each town in NH, as provided by GRANIT, the statewide repository of geographical information (https://www.granit.unh.edu, last accessed Dec 2021). We adopted this mapping procedure because the ZIP codes are too broad, sometimes overlapping, and not contiguous to accurately represent the spatial variability of the collected ticks.

In viewing the use of the natural vegetation areas over the State, as available in the US Environmental Protection Agency (http://www.epa.gov/wed/pages/ecoregions.htm, last accessed Dec 2021),

**Table 1.** The total number of submitted ticks by year in the period 2018–2021 by volunteer participants in New Hampshire

| Year | *Ixodes scapularis* | *Dermacentor variabilis* | *Ixodes cookei* | *Ixodes marxi* | *Ixodes pacificus* | *Ixodes* spp. | *Amblyomma americanum* | *Dermacentor albipictus* | *Dermacentor* spp. | *Haemaphysalis longicornis* | Unidentified | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2018 | 516 | 1,090 | 4 | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 1,614 |
| 2019 | 1,314 | 4,407 | 5 | 2 | 1 | 0 | 3 | 2 | 1 | 0 | 14 | 5,749 |
| 2020 | 1,141 | 878 | 5 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 14 | 2,041 |
| 2021 | 1,286 | 3,501 | 3 | 0 | 0 | 0 | 1 | 2 | 9 | 1 | 45 | 4,848 |
| Total | 4,257 | 9,876 | 17 | 4 | 1 | 1 | 7 | 5 | 10 | 1 | 73 | 14,252 |

we determined these regions are divided into three hierarchical levels. Level I is the coarsest level, dividing North America into 15 ecological regions. Level II divides the continent into 50 regions. At Level III, there are 181 ecological regions for North America. We used the level III, just to realize that there are only 3 of these ecological regions over New Hampshire, therefore the vegetation areas were too large to provide a meaningful interpretation of the results.

We used the index Moran's *I* to estimate two different values of spatial autocorrelation, namely (i) the number of ticks received from each administrative division in NH, and (ii) the number of residents in each division. Low values of Moran's *I* (near −1) typically mean randomness of data or no spatial association, high values (near +1) are indicative of clustering thus consistent with a spatial association. A high clustering of the number of specimens received according to administrative divisions may be indicative of (i) there is a patchy suitable habitat or (ii) the participants submitting ticks are highly clustered in the space: We also carried out a linear correlation to check if there is a relationship between the number of people living in each administrative division and the number of ticks submitted, which would be indicative of the simple rule 'more ticks from a site because more residents in such site'. Moran's *I* was calculated in R (R Core Team 2022) using the code provided by Brundson and Comber (2022) for the book 'An Introduction to Spatial Analysis in R'; code is available on Internet (https://bookdown.org/lexcomber/brunsdoncomber2e/) and is not part of the original book.

**Tick hosts.** Due to the wide variety of hosts reported by the correspondents, we separated them by humans, pets (dogs and cats), farm animals (cattle, horses, sheep, goat), deer, other, and blank (no response given). Other refers to inanimate objects such as couch, counter, wall, or floor. We disregarded the status of the tick (i.e., feeding or crawling on the host) and considered that each one represents a record. Most likely, the discovery of a crawling tick was because of an increased awareness of the person; thus, preventing the bite.

Total numbers of ticks are presented in all tables, percent of total is calculated by dividing the individual number by total multiplied by 100. Statistical tests are elaborated further in the results section and performed using Microsoft Excel, typically a one-tailed homoscedastic T-test to assess differences in average tick numbers.

### Detection of Pathogens

All life stages of *I. scapularis* and *D. variabilis* were tested for the presence of the most common tick-borne pathogens reported in New England (i.e., Rounsville et al. 2021). Ticks were tested by fee for service laboratories, following each laboratories' protocols (Dykstra et al. 2020). The ticks collected in 2018–2020 were tested by both Ticknology, Fort Collins, CO and MedZu, Inc., Tick Report, Amherst, MA (formerly within University of Massachusetts Amherst Laboratory of Medical Zoology) and ticks collected in 2021 were tested by MedZu. Ticknology adhered to the following protocol: ticks were transferred to 1.5 ml microcentrifuge tubes before resuspension in Buffer ATL (Qiagen) and homogenized manually according to the manufacturer's instructions. *Borrelia* species, *B. burgdorferi s. l., B. miyamotoi, B. mayonii, Babesia microti,* and *Anaplasma phagocytophilum* were detected in two multiplex TaqMan PCR assays targeting different genes. *Dermacentor variabilis* was also tested for *Francisella tularensis* and *Rickettsia rickettsii.* One multiplex also targeted *I. scapularis* actin, which acts as a positive control for the DNA purification and PCR reaction. Both positive and negative controls for each PCR target were also performed with each 96 well plates representing ~3% of the total tests performed. MedZu protocol is published in numerous reports (Xu et al. 2016, 2019, 2021, Dykstra et al. 2020, Milholland et al. 2021) and is as follows. Briefly, DNA was extracted from each tick using the Epicenter Master Complete DNA and RNA Purification Kits (Epicenter Technologies, Madison, WI) following the manufacturer's protocols. The selected pathogens were detected by a multiplex TaqMan PCR assay targeting different genes (Xu et al. 2016, 2019) in 20 µl reaction volumes using Brilliant II qPCR Master Mix (Agilent, La Jolla, CA). Xu et al. 2016 describe the cycling conditions 10 min at 95°C with 40 15 s cycles at 95°C and 1 min at 60°C as well as the primers for *B. microti* tubulin (Forward GATTTGGAACCTGGCACCATG, Reverse AATGACCCTTAGCCCAATTATTTCC) and *A. phagocytophilum* MSP2 (Forward ATGGAAGGTAGTGTTGGTTATGGTATT, Reverse TTGGTCTTGAAGCGCTCGTA). Xu et al. (2019) reiterated those primers and further provided sequences to differentiate *I. scapularis* (Forward TGCGTTTTCTTTGAGCAAATGCACGAG, Reverse GTACGGGATTTTCCACAAACGGTATCCA) from *I. pacificus* (Forward CTCGGAGCAAGTACGGAGGTAG, Reverse TTTCCACAAAACGGTCGCCATC) and the detection of *B. burgdorferi s.l.* ospA (Forward ATAGGTCTAATATTAGCCTTAATAGCAT, Reverse AGATCGTACTTGCCGTCTT) and *B. miyamotoi* glpQ (Forward GACATAGTTCTAACAAAGGACAATATTCC, Reverse TCCGTTTTCTCTAGCTCGATTGG). The protocols of submission followed in this study precluded the preparation of samples of ticks of the same species and sex to prepare pools and calculate the Minimum Infection Rate (MIR) as already done in other studies (Burket et al. 1998, Wójcik-Fatla et al. 2011).

## Ticks According to Human Activities

We received a variety of responses regarding the activity that the host was doing when the tick was found because we allowed for an open-text question. To compile responses, we first disregarded any responses to the activity section on any host other than humans. Then we categorized responses together by (i) identifying and defining the categories as they appear in the questionnaires, (ii) generating a list that recompiles all the activities written by the participants into a smaller set of categories, and (iii) we totaled all years together to achieve higher numbers and analyzed by the percent of total number of tick species versus human activity category and in a heat map with number of ticks per human activity category during weeks of the year. We summarized the categories below:

1. **Field and Wilderness:** Deep contact with fields beyond a backyard and not on trails, including camping and anything with the world 'field' in it. We consider these activities the deepest contact of humans with the wild areas. Examples: archery, birding, bug catching, bushwhack hiking, camping, campfire, field games, geocaching, haying, hunting, orienteering, picking wild blueberries, surveying, or walking through woods or long grass.
2. **Walking/Hiking:** Walking along designated trails within a rural setting, not urbanized (see category 5) or with pets (see category 7). Examples: hiking on a trail, nature walk, rock climbing, walking on trails, trail work, walking across backyard or woods, trail running.
3. **Backyard activities:** Defined as activities done within a backyard of a home or a workplace. Examples: chilling in backyard, clearing brush, fence repair, gardening, home repairs, landscaping, masonry, mowing lawn, at a park, planting or harvesting garden, playing in yard, raking leaves, recess, splitting wood, weeding, yardwork.
4. **Biking:** Moving fast along a trail on an exposed vehicle. Examples: biking, four-wheeling.
5. **Urbanized:** Activities within an urbanized setting, including within shared public spaces. Examples: 9-square, baseball, basketball, getting mail, loading car, picking up trash along road, playground, ropes course, tennis, unloading groceries, walking along road.
6. **Indoor:** The participants indicated that the tick was noticed indoors most likely after any kind of other unreported or unnoticed previous activities. Defined as found inside a building, including anything eating related such as having breakfast, lunch, or dinner. Examples: art, bathroom, in bed, in cabin, changing clothes, classes, cleaning, dinner, driving, eating, in house, laundry, office work, playing music, reading, resting, school, showering, sitting, sleeping, tick checks, and watching TV.
7. **Animal related:** Defined as activities with animals including pets. Examples: barn chores, beekeeping, dog walk, farming, feeding pig, hiking with dogs, raking goat pen, and tending chickens.
8. **Water related:** Activities around water. Examples: boating, fishing, gold panning, kayaking, sitting on beach, and swimming.
9. **Eliminated:** Responses such as AP, arm, back, belly, biting, ear, hair, hand, neck, NP, on clothes, S, shoulder, under pants, and wrist were not considered an activity.
10. **Blank.** No activity recorded.

## Modeling

For modeling the potential distribution of *I. scapularis* and *D. variabilis* in NH we used the coordinates provided by the participants when submitting the ticks. As explanatory variables, we used the first three coefficients of a harmonic regression of the mean annual temperature, soil humidity, and atmospheric water vapor deficit between the years 1980 and 2018. Climate data were obtained from the TerraClimate public repository (http://www.climatologylab.org/terraclimate.html, last accessed Mar 2021). The procedure of the harmonic regression was proposed by Estrada-Peña et al. (2014) as a method to decompose the series of monthly climate values, improving time resolution and retaining the ecological meaning. These authors also demonstrated that the coefficients of the regression are the best explanatory variables (i.e., the model predictors) of the environmental suitability of a territory for a tick, because they represent average annual values, their seasonality, and the amplitude for each season. Many coefficients could be calculated, but in practical terms, the first three provide an adequate reliability for outlining the climate suitability. Complete details and examples can be found in the original publication (Estrada-Peña et al. 2014).

Together with the series of monthly environmental variables, including mean temperature, soil humidity, and water vapor deficit between 1980 and 2018, the coordinates for both *I. scapularis* and *D. variabilis* were used to train the model. These coordinates may not always correspond with the actual site of collection, and some of them may represent the coordinates of a persons' home. In any case, given the area of New Hampshire and the high number of ticks received, we decided to ignore the negligible bias produced by these probably incorrectly allocated records. Anyway, we should expect more tick samples submitted from areas where either the tick is abundant and/or where the human population is high. We addressed this issue by (i) removing the repeated coordinates in modeling (i.e., many submissions from the same pair of coordinates) and (ii) calculating if there is a significant higher number of ticks received from areas with larger population. We did a simple linear regression (and calculated its significance, *P*) using Microsoft Excel between the population of each administrative division in NH against the number of ticks submitted from that division, assuming that the effort for tick detection and submission was the same in every territory.

We independently modeled the presence of each tick species using the niche modeling algorithm MaxEnt integrated in the 'dismo' package (Hijmans et al. 2020) for R (R Core Team 2022). Models were developed with linear and quadratic features, using 10,000 background points (in which the ticks have not been reported). Each model was replicated 100 times per species, using 70% of points for training purposes, removing duplicate coordinates, and selecting the best model regarding the value of the Boyce's index reported to perform better that the commonly used Area Under the Curve AUC (Hijmans et al. 2020). Cross-validation was used to compare the resulting models, partitioning the data into replicate folds, with each fold used in turn to test the model. The regularization multiplier was set to 1. Finally, the results from the models were plotted following the administrative divisions of New Hampshire, to follow actual tick records as mentioned before.

## Results

### Tick Species Identified (Years 2018–2021)

We received a total of 14,252 ticks in the period 2018–2021, collected in 259 different sites throughout NH (Table 1). The most submitted species of ticks were *I. scapularis* at 4,257 and *D. variabilis* at 9,876 specimens, together totaling 14,133. *Dermacentor variabilis* was approximately twice as abundant as *I. scapularis* and statistically different at $P$-value 0.08 for one-tailed homoscedastic T-test. A few specimens of *Amblyomma americanum* (7), *I. cookei* (17), *I. marxi* (4), *I. pacificus* (1) D. *albipictus* (5), and one *Haemaphysalis longicornis*, as well as 10 undetermined *Dermacentor* spp. were also submitted. A total of 73 ticks were damaged enough to prevent its identification. The life stages are indicated only for *I. scapularis* and *D. variabilis* in Table 2.

We received a total of 3,568 adults (~84%), 382 nymphs (~9%), 16 larvae (~less than 1%), and 291 unrecorded life stages (~7%) *I. scapularis* (Table 2). We received a total of 8,589 adults (~87%), 3 nymphs (~less than 1%), 1 larva (~less than 1%), and 1,283 unrecorded life stage (~13%) *D. variabilis* (Table 2). For all years, most ticks were collected on humans, representing 892 *I. scapularis* and 5,709 *D. variabilis* totaling 6,601 ticks. *Dermacentor variabilis* were found on humans 5 times more than *I. scapularis* ($T = 4{,}844.22$; $P = 0.06$ for one-tailed homoscedastic T test). We also received 4,247 ticks without a recorded host, including 2,295 *I. scapularis*, (including most of the nymphal *I. scapularis*) and 1,952 *D. variabilis* ($T = 1{,}108.19$; $P = 0.46$). Up to 972 *I. scapularis* and 1,795 *D. variabilis* were found on pets ($T = 2{,}219.54$; $P = 0.24$), 29 *I. scapularis* or 97 *D. variabilis* on farm animals ($T = 2{,}988.01$; $P = 0.17$); most of these were adult ticks. Only 7 *I. scapularis* were found on other hosts, as compared to 323 *D. variabilis* ($T = 4{,}777.75$; $P = 0.065$) indicating the unique questing habits of *D. variabilis* are on inanimate objects. Only adult *I. scapularis* were found on deer. Looking at the numbers of ticks from both Table 1 and 2, a human host is more likely to encounter a *D. variabilis* ticks than *I. scapularis* ($T = 5{,}6779.44$; $P = 0.06$) (including finding them in a general human environment, the 'other hosts'), but having a pet ($T$: 5,118.29; $P = 0.24$) results in about an equal chance of encounter for both species of ticks.

### Tick Distribution

Ticks were collected primarily in the southern parts of the state (Fig. 1) showing a clear contrast with the probable absence of both *I. scapularis* and *D. variabilis* in the northern region of the state. We also noticed there is also a small cluster of submissions in the upper middle of the state where the project started.

The number of *I. scapularis* and *D. variabilis* were aggregated at the spatial level of administrative divisions (Fig. 2). The total number of mapped *I. scapularis* is 3,642; the value for total *D. variabilis* is 6,494. The values in the map reflect the sum of ticks received from each administrative division. This provides a better pattern for capturing the distribution and abundance of the ticks than the pure dot or the aggregation of data at the level of county.

Other than the general rule of their absence in the northern regions, both *I. scapularis and D. variabilis* are noticed at variable numbers and unique clusters. *Ixodes scapularis* seems to prefer areas near the south and coastal parts of the state, but also reach high abundance in sites of central parts of the state, as well as being more abundant on the western border of NH. Although *D. variabilis* is found more northern than *I. scapularis* it is also absent for the most northern regions of the state. Some *D. variabilis* were submitted from the southern and southeastern NH, yet the highest number of specimens were received from the central parts of the state into the western border. The central cluster of *D. variabilis* is more prominent than for *I. scapularis*. Values of Moran's I (*I. scapularis*: −0.204, $P < 0.01$; *D. variabilis*: −0.228, $P = 0.09$) confirmed that both species have a random distribution with no spatial association. Values of Moran's I for the population of administrative were also clearly negative (−0.399, $P = 0.07$). The correlation between the population of each administrative division of NH and the total number of ticks (including both species) submitted is low ($R^2$: 0.401, $P = 0.22$). Thus, ticks were received from sites where they are more abundant, or awareness is higher, not from sites where a larger human population (and thus more participants) resides.

### Seasonal Activity of Ticks

The weekly activity of submitted *I. scapularis* or *D. variabilis* is presented in Fig. 3. It was not possible to separate stages and activity because most samples were adults (probably because the larger and noticeable size); we considered that extrapolations of lack of immatures' in this context could be statistically risky. Activity patterns, considering all stages together, were consistent among years, but abundance varied for each species and for each year. The highest abundance of *I. scapularis* concentrated in weeks 13–31, or April–June and autumn to winter, from weeks 40–49, or September–November. Looking closely at the differences from 2018 to 2021, the time that increasing numbers of *I. scapularis* were collected progressively gets earlier each year. In 2018, *I. scapularis* submissions began at week 16–17, 2019 at week 15, and 2020 and 2021 at week 13. While *I. scapularis* activity was bimodal, the seasonal activity of *D. variabilis* had only one major peak corresponding to approximately week 21 with a total period of submitted ticks from week 16 to 31 or April through August. Submissions varied by year, but every other year more *D. variabilis* were submitted. Looking at these four years of data, it appears *D. variabilis* comes out in much larger numbers every other year. The numbers of *D. variabilis* collected in 2018 and 2020 were just less than 1,000 and in 2019 and 2021 were greater than 3,000. In any case, weeks 16–31 or April–August should be considered of high risk for humans in NH to encounter both *I. scapularis* and *D. variabilis* ticks since this time of the year concentrates the maximum activity of both most prominent tick species.

Human activities associated with the finding of *I. scapularis* (890 records) or *D. variabilis* (5,709 records) ticks (a total of 6,599 records) are depicted in Fig. 4. Figure 4A depicts the percentage of the total ticks recorded associated with a human activity category for each tick species for each human activity category. Figure 4B

**Table 2.** The hosts from which *I. scapularis* and *D. variabilis* received were found, arranged by years, and separated as adults, nymphs, or unknown stages

| Host | 2018 | | | | | | 2019 | | | | | | 2020 | | | | | | 2021 | | | | | | Total # |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I. scapularis | | | D. variabilis | | | I. scapularis | | | D. variabilis | | | I. scapularis | | | D. variabilis | | | I. scapularis | | | D. variabilis | | | |
| | Nymph | Adult | Unk. h | Nymph | Adult | Unk. h | Nymph | Adult | Unk. h | Nymph | Adult | Unk. h | Nymph | Adult | Unk. h | Nymph | Adult | Unk. h | Nymph | Adult | Unk. h | Nymph | Adult | Unk. h | |
| Humans[a] | 7 | 269 | 1 | 0 | 24 | 578 | 25 | 149 | 0 | 1 | 2,702 | 28 | 7 | 221 | 0 | 0 | 572 | 2 | 19 | 139 (54 with dog)[g] | 1 | 0 | 1705 (19 with dog)[g] | 78 | **6,601** |
| Pets[b] | 0 | 25 | 0 | 0 | 30 | 61 | 1 | 188 | 2 | 0 | 682 | 4 | 3 | 219 | 0 | 0 | 106 | 0 | 3 | 530 | 1 | 0 | 842 | 70 | **2,767** |
| Farm animals[c] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 55 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 25 | 0 | 0 | 38 | 0 | **1,26** |
| Deer[d] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 62 | 0 | 0 | 0 | 0 | **62** |
| Other[e] | 0 | 0 | 1 | 0 | 0 | 13 | 0 | 2 | 0 | 0 | 72 | 0 | 0 | 2 | 0 | 0 | 41 | 0 | 0 | 2 | 0 | 0 | 197 | 0 | **330** |
| Blank[f] | 0 | 0 | 213 | 0 | 3 | 381 | 191 (5 larva) | 691 | 56 | 1 | 803 | 59 | 47 (7 larva) | 621 | 14 | 1 larva | 148 | 4 | 79 (4 larva) | 365 | 2 | 1 | 546 | 5 | **4,247** |
| Total | **7** | **294** | **215** | **0** | **57** | **1,033** | **222** | **1,034** | **58** | **2** | **4,314** | **91** | **64** | **1,063** | **14** | **1** | **871** | **6** | **105** | **1177** | **4** | **1** | **3,347** | **153** | **14,133** |
| Grand totals | 516 | | | 1,090 | | | 1,314 | | | 4,407 | | | 1,141 | | | 878 | | | 1,286 | | | 3,501 | | | |

[a] Total 892 *I. scapularis* and 5,709 *D. variabilis* found on humans for all years.

[b] Total 972 *I. scapularis* and 1795 *D. variabilis* found on pets for all years.

[c] Total 29 *I. scapularis* and 97 *D. variabilis* found on farm animals for all years.

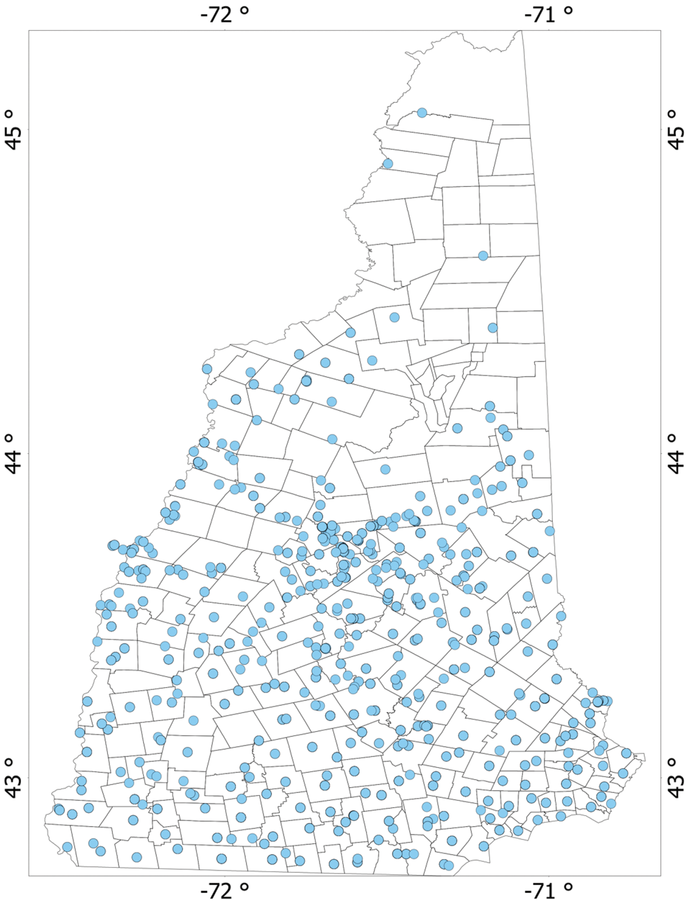
[d] Total 62 *I. scapularis* and 0 *D. variabilis* found on deer for all years.

[e] Other indicates inanimate objects such as couch, counter, wall, and floor. Total 7 *I. scapularis* and 323 *D. variabilis* found on other for all years.

[f] Blank indicates a host was not given. Total 2,295 *I. scapularis* and 1,952 *D. variabilis* for all years.

[g] Host response indicated ticks were found on both dog and human.

[h] Unk: Unknown, referring to an unrecorded life stage.



1

**Fig. 1.** The spatial distribution of the sites in New Hampshire from which ticks were submitted for this study, according to the coordinates provided by the participants. The dots only indicate occurrence but not abundance. In some cases, specimens were submitted without coordinates but with the name of a town. In these cases, coordinates of the town were obtained and plotted. The map shows where participants have concentrated as well as the sites in the northern parts of the State from where no ticks were submitted.

and C maps the number of ticks, *I. scapularis* in Fig. 4B and *D. variabilis* in Fig. 4C along a heat map with the associated human activity category by week of the year. There was a significant portion of ticks submitted to our project that were not associated with a human activity (category 10), corresponding to 23% *D. variabilis* and most of the *I. scapularis* or 41% (Fig. 4A). Despite this observation, the group 'walking/hiking' (category 2) is associated with the highest tick encounters. Respectively, 24% of *I. scapularis* (Fig. 4A) occurring throughout weeks 16–25 and even higher tick numbers in the weeks 39–48 (fall season) were found while 'walking/hiking' (category 2, Fig. 4B). Twenty-five percent of *D. variabilis* were collected while 'walking/hiking' (category 2, Fig. 4A), and the peak was observed within weeks 16–31 (Fig. 4C). The next highest-risk activity is related to 'backyard activities' (category 3) for which we found 23% likelihood of finding *D. variabilis* from weeks 17–30 and 17% *I. scapularis* from weeks 14–25. It is twice as likely to find *D. variabilis* than *I. scapularis* while performing activities in fields and wilderness (category 1, 6%–2%, respectively), urbanized (category 5, 3%–1.6%, respectively), or indoors (category 6, 13%–6.5%, respectively). In activities around water (category 8) people were twice as likely to find *I. scapularis* (3.7%) than *D. variabilis* (1.5%). It is necessary to note that biking (category 4) is the category with the lowest encounter for finding both *D. variabilis* and *I. scapularis*. A low percentage of ticks, mostly *D. variabilis* were not able to be categorized by participants.

## Pathogens Detected in the Submitted Ticks

The search for pathogens was focused on the most common tick-borne pathogens reported in New England. We tested 2,787 *I. scapularis* and 1,041 *D. variabilis*. About 1,191 *I. scapularis* were positive for a pathogen (~43% *I. scapularis* carried a pathogen) and only 1 *D. variabilis* was positive for *F. tularensis* in 2019 (~0.1% *D. variabilis* carried a pathogen). Results for the pathogens harbored by *I. scapularis* are included in Table 3. In total for all years and including nymphal and adult stages, *Borrelia* spp. was found in 38% of the tested *I. scapularis* (Table 3). To note, *B. mayonii* and *R. rickettsii* were not found in any tick. The complex of species *B. burgdorferi s.l.*, has been recorded in 37% of the processed *I. scapularis*. The recurrent–fever agent, *B. miyamotoi* has been found in a scarce 1% of processed ticks (37 specimens). On the other hand, both *A. phagocytophilum* and *Ba. microti* had low prevalence values,

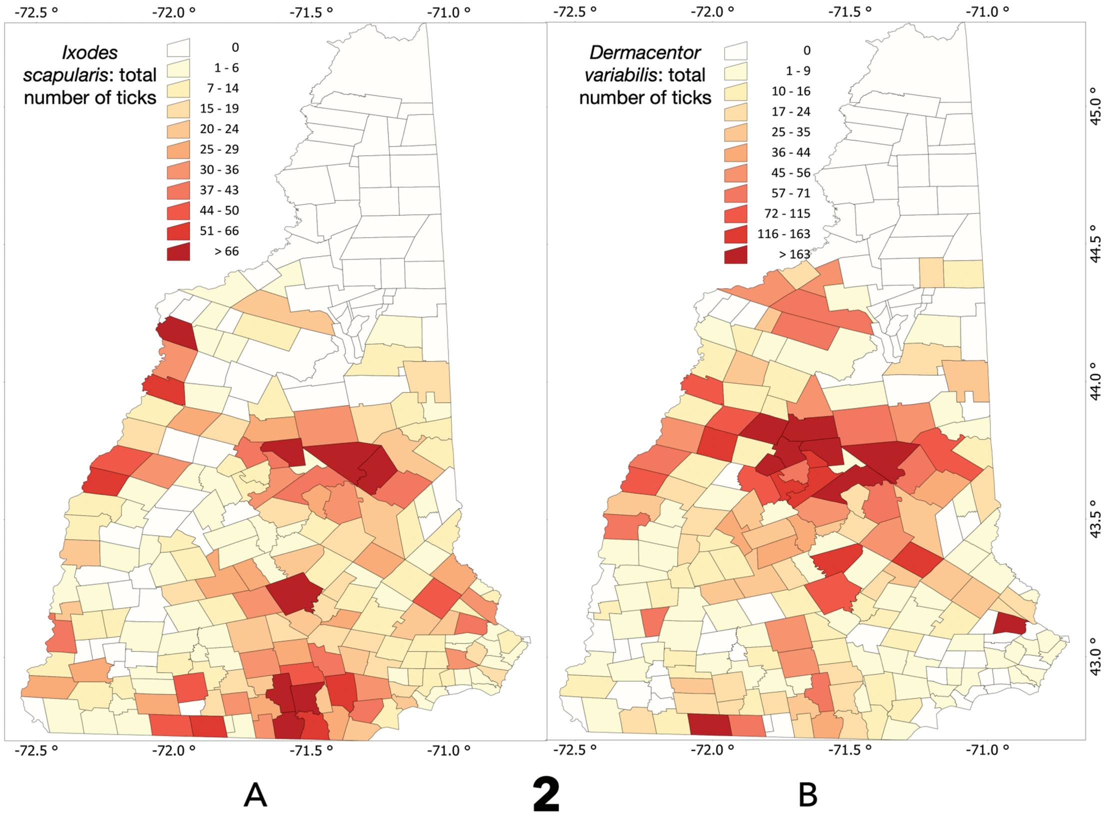


**Fig. 2.** The distribution of ticks mapped in New Hampshire according to the number of submitted specimens in the period 2018–2021 at the level of 'city boundaries'. The distribution of *I. scapularis* (Fig. 2A), with a total number of 3,642. The distribution of *D. variabilis* (Fig. 2B), with a total number of 6,494. The legend reflect the total number of specimens received from each administrative division.

around 5%–6% of the *I. scapularis* processed (169 and 148, respectively). We also found that out of all *I. scapularis* collected through 2018–2021, 7% (204 ticks) were co-infected by any pair, triple, or quadruple combination of the pathogens mentioned. We also included in Table 3 the breakout of pathogen detection among adult and nymph *I. scapularis* ticks per season of each year 2018–2021. In the combined totals, including all years, adult ticks carried on average for all years 42% *Borrelia* spp., 41% *B. burgdorferi s.l.*, 1% *B. miyamotoi*, 7% *A. phagocytophilum*, and 6% *Ba. microti*. Nymphs carry on average 18% *Borrelia* spp., 17% *B. burgdorferi s.l.*, 1% *B. miyamotoi*, 4% *A. phagocytophilum*, and 3% *Ba. microti*.

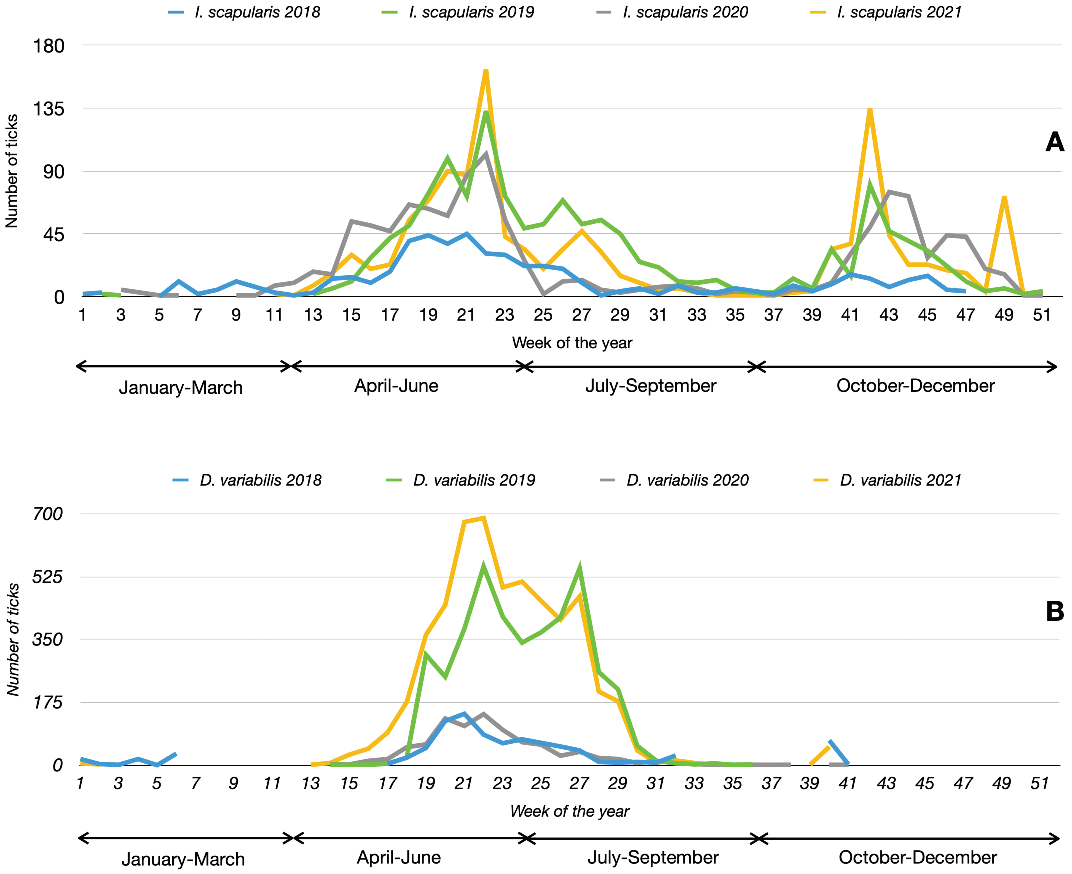
### Modeling

We modeled the distribution of both *I. scapularis* and *D. variabilis* using temperature, soil moisture, and air water vapor deficit (Fig. 5). The results obtained reflect the expected environmental suitability for both species of ticks on a scale of 0–100 based on the probability of presence. Both presence models provided a Boyce's index greater than 0.8 (0.82 for *I. scapularis*, 0.88 for *D. variabilis*) meaning for good predictive results in the modeling protocols.

The model for *I. scapularis* predicts that the tick should be absent in northern NH, with probabilities of presence being higher in the southeastern part of the state according to a north-south gradient of increasing suitability (Fig. 5). Interestingly, there are two clear areas of high suitability, namely the southern area, and the center of the State. A completely different picture has been obtained for *D. variabilis* (Fig. 5) meaning that both species may share some portions of the habitat, but do not completely overlap in NH. *Dermacentor variabilis* is also predicted to be absent in the north of the state, but it could find a suitable weather more northern than *I. scapularis*.

## Discussion

The current study used community science to address the identification of ticks submitted by people in the state of New Hampshire (USA) following an advertised program through news and social media, resulting in a total of 14,293 ticks, in the period 2018–2021, of which 6,599 had a 'human activity' associated (according to returned questionnaires). The predominant ticks submitted were *I. scapularis* and *D. variabilis*, but additional species were also collected The identification of a single *I. pacificus* is also negligible and unusual as *I. pacificus* is more common to the western United States (Hahn et al. 2016, Xu et al. 2019, Porter et al. 2021), perhaps introduced from traveling, a matter not included in our questionnaire. According to Sonenshine (2018) the records of *A. americanum* in NH far exceed its northern distribution range, but the few specimens submitted preclude further conclusions about the spread of this tick into northern latitudes; these records were probably casual introductions carried by wildlife, since some species of birds may be hosts of this tick (Allan et al. 2010) or translocation from



**3**

**Fig. 3.** The seasonal pattern of abundance of *I. scapularis* (Fig. 3A) and *D. variabilis* (Fig. 3B) in New Hampshire at a time resolution of one week, with indications of the month of the year, based on the number of each specimen submitted by the participants. Different lines indicate the different years 2018–2021.

traveling participants. Yet, *A. americanum* is frequently identified in the state of Maine, as it has a more active state surveillance program than NH (Keirans et al. 1998b, Lado et al. 2020).

Prior research from passive surveillance studies is that they primarily focus on one species of tick (*I. scapularis*) and/or one pathogen (*B. burgdorferi s.l.*) (Ogden et al. 2018, Porter et al. 2019, 2021, Eisen and Paddock 2020). Yet, our research primarily focused on both *I. scapularis* and *D. variabilis* because their overwhelmingly high relative abundance in the submitted samples. When we began this project in 2017, the communication campaign and the number of ticks received was considerably fewer than for the other years. We also consider that results from the year 2020 could be affected in some way because of the ongoing SARS-CoV-2 pandemic. This is impossible to verify, but the number of ticks submitted in 2020 is slightly lower than for other years. Even if we consider these challenges, we can pinpoint tick distribution across large regions (but see Eisen and Eisen 2021, for the pros and cons of the method) and we think that the number of ticks is high enough to establish conclusions. In collecting and tracking patterns of both *I. scapularis* and *D. variabilis*, each species has unique seasonality and distribution patterns, and pathogen's prevalence as compared to each other. Thus, the analysis of both species next to each other helps make our results novel, helping people to better prevent themselves from tick encounters.

We received significantly more *D. variabilis* than *I. scapularis*, which is supported by other studies reporting *D. variabilis* as the predominant *Dermacentor* spp. within the northern part of the United States (Dergousoff et al. 2013, Boorgula et al. 2020, Duncan et al. 2021). Both *D. variabilis* and *I. scapularis* have different periods of activity in our surveys. The adults of *D. variabilis* have a unimodal cycle, extending its activity in the target region approximately in the weeks 16–31 and showing only one major peak, most commonly around week 21. A higher number of submissions of *D. variabilis* versus *I. scapularis* happened in 2019 and 2021. We consider the reduction in *D. variabilis* submissions in 2018 and 2020 because *D. variabilis* follows a 2-yr cycle, the first year with immatures feeding on small wild animals (going unnoticed by contributors), the second with adults biting humans and other hosts. If another factor would affect the submitting efforts, the observed decrease would also be mirrored by data on *I. scapularis,* a fact not detected in our series of data. For both tick species, we found that small differences may occur between consecutive years because many factors, including (but not exclusive to) the weather of the previous autumn-winter, the spring rise of temperature, the abundance of hosts, and probably the composition of vertebrates' communities. *Dermacentor variabilis* is also larger than *I. scapularis*, thus it is easier to find crawling or biting.

The bimodal activity of *I. scapularis* has been already reported on many occasions, although it is commonly driven by the dominant

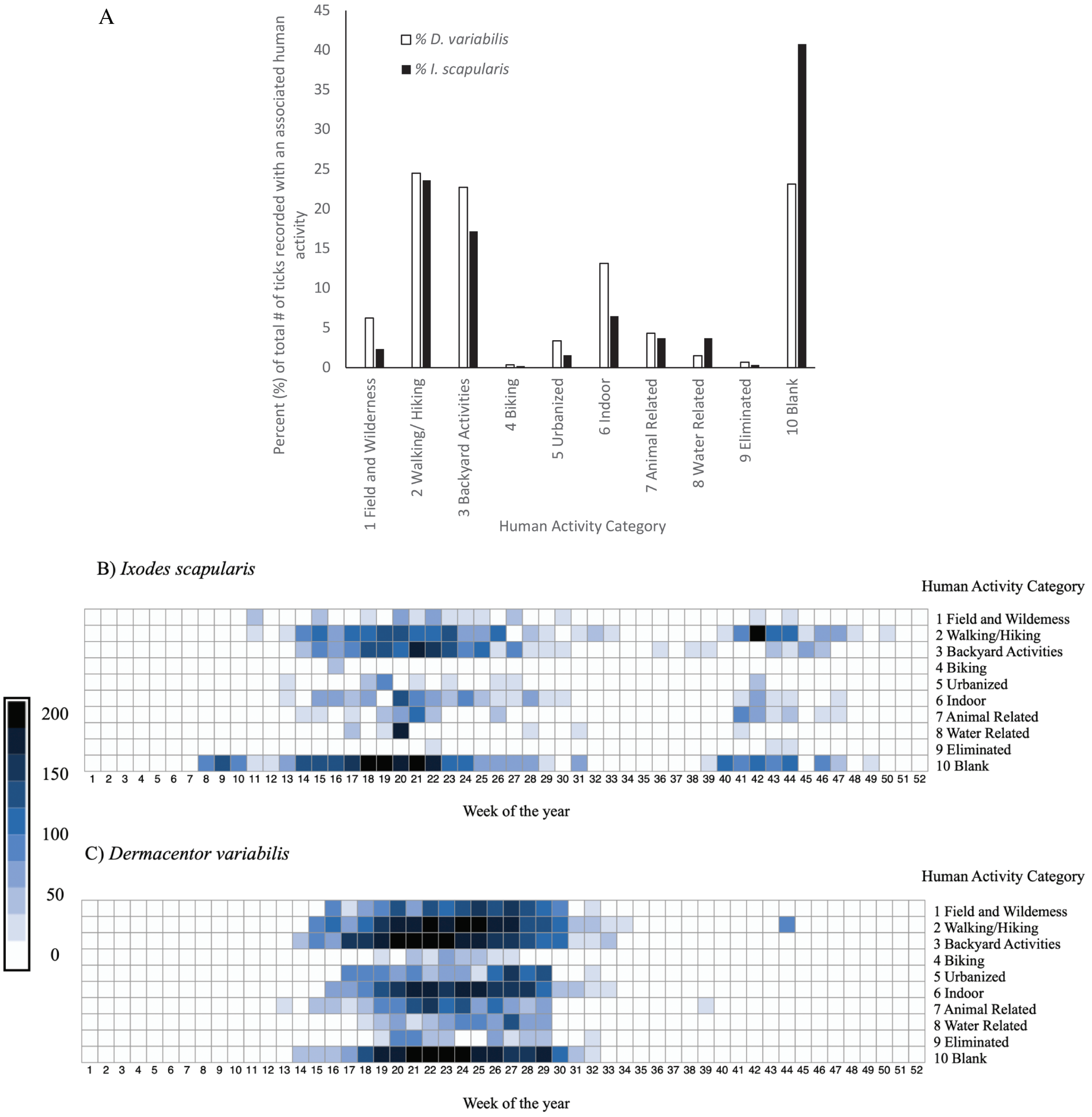


**Fig. 4.** Totaled ticks found per human activity over years 2018–2021. Figure 4A depicts the percent of *I. scapularis* (black) and *D. variabilis (white)* found for each associated category of human activity. Figure 4B and C depicts heat maps for the number of *I. scapularis* (Fig. 4B) and *D. variabilis* (Fig. 4C) shown according to the week of the year and the associated category of human activity. Scale is from 0 to 200 darker shades representing more numbers of ticks.

climate (Ogden et al. 2018). In our results, the total number of submissions of *I. scapularis* did not experience large changes among years. The autumn-winter peak did have a larger variability in the number of submitted specimens of *I. scapularis* than during other seasons of the year, as already demonstrated for other regions (Ostfeld et al. 1996, Ogden et al. 2006). Our short series of data did not confirm an expansion of the activity period of *I. scapularis,* but it is indicated that this species is active all year round with clear peaks of more abundance. Both species of ticks have overlapping peaks in late spring and early summer. People should take preventive measures, and routinely perform tick checks during the months of April through August due to high tick abundance.

Human activity influences the risk of an individual to be bitten by a tick. We aimed to address the most basic classification of the literally hundreds of activities reported by participants. Salkeld et al. (2019) also manifested the difficulty in relating a human activity with the tick bite; however, a different pattern emerged from the one noted for *I. scapularis* by Mead et al. (2018) or Porter et al. (2019) who pointed out a higher number of contacts in people's yards, in contrast with the low number of exposures detected in forest-associated recreations. This large difference of results may be due to different methods of classification of the human activities, since Mead et al. (2018) only reported 'outdoors in public spaces', which includes several of our categories. This is a complex topic because

**Table 3.** Prevalence of the five pathogens found in adult and nymph *I. scapularis* ticks in New Hampshire by season

| Agent[a] | | Number of positive ticks (% positive) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2018 | | | 2019 | | | 2020 | | | 2021 | | | |
| | | Spring[b] | Fall[b] | Total[c] | Spring[b] | Fall[b] | Total[c] | Spring[b] | Fall[b] | Total[c] | Spring[b] | Fall[b] | Total[c] | Grand totals (% positive) |
| Total Tested # of ticks | Total | **390** | **121** | **512** | **616** | **284** | **901** | **486** | **287** | **773** | **232** | **367** | **601** | 2,787 |
| | **Adult** | **281** | **10** | **292** | **459** | **214** | **674** | **455** | **260** | **715** | **158** | **341** | **501** | |
| | **Nymph** | **5** | **2** | **7** | **124** | **41** | **165** | **31** | **20** | **50** | **73** | **21** | **94** | |
| *Borrelia* | Total | 159 (41) | 35 (29) | **195 (38)** | 217 (35) | 111 (39) | **328 (36)** | 182 (37) | 131 (46) | **313 (40)** | 84 (36) | 149 (41) | **235 (39)** | 1071 (38) |
| | Adult | 118 (42) | 4 (40) | **123 (42)** | 189 (41) | 95 (44) | **284 (42)** | 175 (38) | 126 (48) | 301 (42) | 73 (46) | 142 (42) | 217 (43) | |
| | Nymph | 3 (60) | 0 (0) | **3 (43)** | 17 (14) | 8 (20) | **25 (15)** | 7 (23) | 5 (25) | 12 (24) | 10 (14) | 6 (29) | 16 (17) | |
| *Borrelia burgdorferi sensu lato* | Total | 157 (40) | 32 (26) | **190 (37)** | 214 (37) | 109 (38) | **323 (36)** | 177 (36) | 129 (45) | **306 (40)** | 81 (40) | 139 (35) | **222 (37)** | 1041 (37) |
| | Adult | 116 (41) | 3 (30) | **120 (41)** | 187 (41) | 93 (43) | **280 (42)** | 170 (37) | 124 (48) | **294 (41)** | 72 (46) | 134 (39) | **208 (42)** | |
| | Nymph | 3 (60) | 0 (0) | **3 (43)** | 16 (43) | 8 (20) | **24 (15)** | 7 (23) | 5 (24) | **12 (24)** | 9 (12) | 5 (24) | **14 (15)** | |
| *Borrelia miyamotoi* | Total | 6 (2) | 3 (2) | **9 (2)** | 6 (1) | 2 (1) | **8 (1)** | 7 (1) | 5 (2) | **12 (2)** | 2 (1) | 6 (2) | **8 (1)** | 37 (1) |
| | Adult | 6 (2) | 3 (30) | **6 (2)** | 5 (1) | 2 (1) | **7 (1)** | 7 (2) | 5 (2) | **12 (2)** | 1 (1) | 4 (1) | **5 (1)** | |
| | Nymph | 0 (0) | 0 (0) | **0 (0)** | 1 (1) | 0 (0) | **1 (1)** | 0 (0) | 0 (0) | **0 (0)** | 1 (1) | 1 (5) | **2 (2)** | |
| *Anaplasma phagocytophilum* | Total | 26 (7) | 6 (5) | **32 (6)** | 21 (3) | 18 (6) | **37 (4)** | 26 (5) | 16 (6) | **43 (6)** | 22 (9) | 35 (10) | **57 (9)** | 169 (6) |
| | Adult | 23 (8) | 0 (0) | **23 (8)** | 15 (3) | 16 (7) | **31 (5)** | 23 (5) | 16 (6) | **39 (5)** | 19 (12) | 34 (10) | **53 (11)** | |
| | Nymph | 0 (0) | 0 (0) | **0 (0)** | 4 (3) | 2 (5) | **6 (4)** | 3 (10) | 1 (5) | **4 (8)** | 2 (3) | 0 (0) | **2 (2)** | |
| *Babesia microti* | Total | 30 (8) | 5 (4) | **35 (7)** | 25 (4) | 11 (4) | **36 (4)** | 18 (4) | 24 (8) | **42 (5)** | 12 (5) | 23 (6) | **35 (6)** | 148 (5) |
| | Adult | 24 (9) | 1 (10) | **25 (9)** | 20 (4) | 3 (4) | **29 (4)** | 18 (4) | 24 (9) | **42 (6)** | 10 (6) | 21 (6) | **31 (6)** | |
| | Nymph | 0 (0) | 0 (0) | **0 (0)** | 4 (3) | 1 (3) | **5 (3)** | 0 (0) | 0 (0) | **0 (0)** | 2 (3) | 2 (10) | **4 (4)** | |
| Coinfected[d] | Total | 37 (9) | 7 (6) | **44 (9)** | 30 (5) | 14 (5) | **44 (5)** | 23 (5) | 29 (10) | **52 (7)** | 24 (10) | 40 (11) | **64 (11)** | 204 (7) |

[a] *B. mayonii* was not detected in any tick.

[b] Spring includes January through July and Fall includes August through December. The grand totals for Spring and Fall include all stages i.e., larvae and unknown are included.

[c] Grand totals for each year include stage as indicated or if total all stages including larvae and unknown, as well as all months including blanks.

[d] Coinfection is defined as any tick that contains more than one identified pathogen (if tick is positive for both *Borrelia* and *B. burgdorferi* or *Borrelia* and *B. miyamotoi* that is only one identified pathogen).

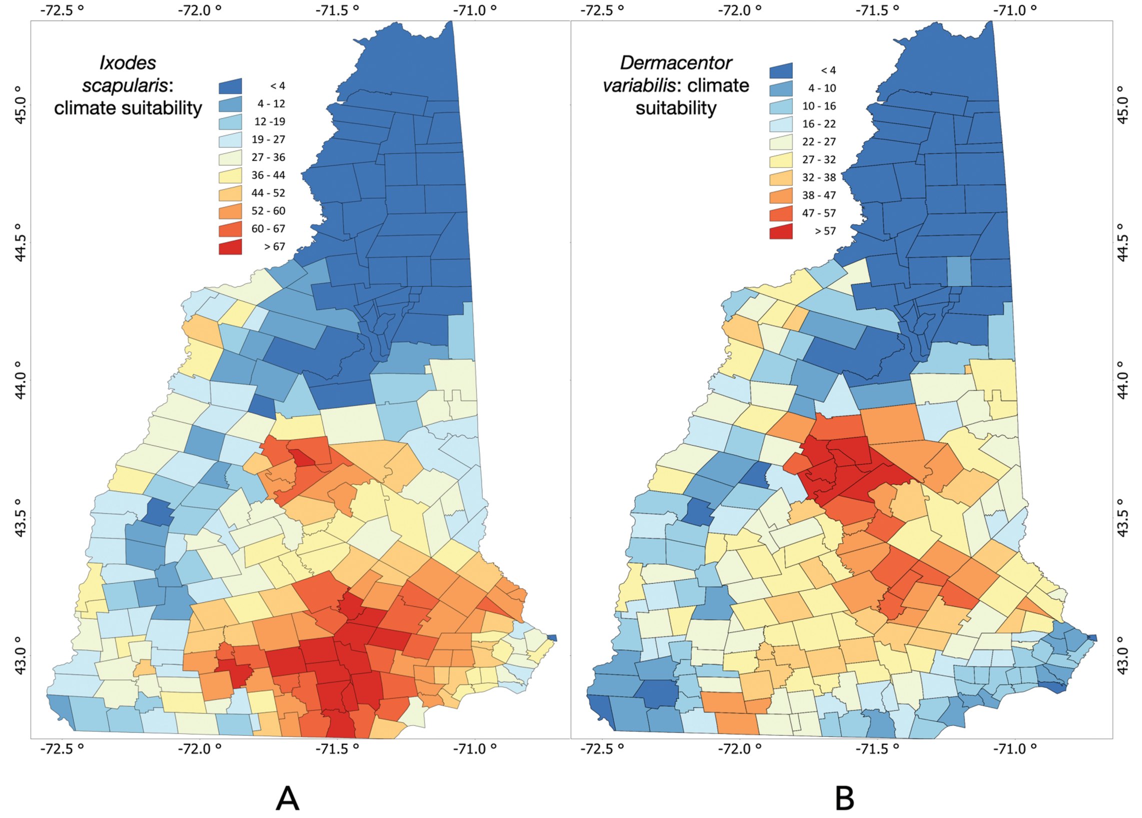


**Fig. 5.** Predicted distribution of ticks within New Hampshire based on a weather-dependent model. *I. scapularis* (Fig. 5A), and D. *variabilis* (Fig. 5B), mapped as probabilities from 0 to 100 as indicated by the legends.

the time elapsed between the tick bite and its finding affects what an individual records as the activity. Similar to the reports, within this project we did observe a higher tick encounter for human activities associated with walking/ hiking and backyard activities as supposed to activities in an urbanized location or around water. The smallest number of tick encounters was associated with human bicycling.

Our reported prevalence results of *Borrelia* spp., *B. burgdorferi s.l., B. miyamotoi*, *B. mayonii, A. phagocytophilum*, and *Ba. microti* harbored by *I. scapularis* are not uncommon in comparison with other published reports in the region (Schulze et al. 2013, Johnson et al. 2017, Sánchez-Vicente et al. 2019) or for the complete country (Porter et al. 2021). The detection of up to 37% of ticks positive to the DNA of *B. burgdorferi s.l.* is far higher than the relatively low values found for *Ba. microti* or *A. phagocytophilum*, although the rise of *Ba. microti* has been reported (Diuk-Wasser et al. 2014). Similar percentages of *I. scapularis* containing *B. burgdorferi s.l.* have also been previously observed (Little et al. 2019, Xu et al. 2019). *Borrelia miyamotoi* was present in 1% of the tested ticks. The co-infection of ticks with more than one pathogen was 7%, in line with other reports (Benach et al. 1985, Sánchez-Vicente et al. 2019, Milholland et al 2021). These findings support the idea of NH as an area of high risk for *Borrelia* spp., a fact to be considered when planning and implementing informative alerts to the public. *Dermacentor variabilis* was tested for *F. tularensis* and *R. rickettsii*, pathogens commonly found in this tick, but analyses yielded only 1 out of 1,041 tested *D. variabilis*.

An interesting point noticed in this study is the lack of submissions of both *I. scapularis* and *D. variabilis* from the northern parts of the state. Reasons for the lack of submissions from northern regions may include (a) the poor impact of our project advertisements in the northern region, (b) a lower population in the northern parts of the state decreasing the probabilities of participation, (c) the lack of important roads in the region, preventing humans to move easily among different areas of this part of the state, then impeding the contact with ticks, or (d) the actual absence of these ticks in the area. Additionally, with our passive surveillance protocol we would expect to receive more specimens from areas with higher awareness. Yet, since we demonstrated that the distribution of ticks received is random, there is no correlation with the total population of the State, and the climate-predicted distribution of both species is different, it reduces the limitations of this protocol.

We created maps predicting the tick distributions in NH based on submissions, that were primarily associated with temperature, soil moisture, and atmospheric water vapor deficit. We did not include data on vegetation as an explanatory variable (Winter et al. 2021), because of the scale of the mapping (meters). Although produced at a different resolution, and using different explanatory variables, our map of the probability of suitable habitat for *I. scapularis* in NH fits well with the results obtained by Diuk-Wasser et al. (2010). Similar studies have also shown the effect of climate change on Lyme disease within the USA (Moore et al. 2014, Monaghan et al. 2015, Couper et al. 2021) or northeastern parts of the country (Little et al. 2019, Elias et al. 2021). The mapping efforts suggest that modeling habitat suitability for tick vectors may contribute better to epidemiological models of transmission of tick-borne pathogens. We evaluated if more ticks were received from more populated sites, a situation that could distort the purely environmental modeling; we however rejected that hypothesis as there was no correlation at the scale of the State. This precludes any *substantial* biasing effect. Then, predictive modeling using only environmental variables could be able to effectively separate the weather factors affecting tick distribution and removing the biasing effects of the human side.

The combination of active surveys, community science, and predictive mapping may be a compelling source of information, increasing the knowledge about the distribution of *I. scapularis* and *D. variabilis*; thus, improving tick bite and tick-borne disease prevention campaigns. We demonstrated that passive surveillance of ticks may be an excellent tool *if paired* with active surveys; otherwise, some 'background noise' (linked to sites of higher awareness) may be present in the data obtained. This passive surveillance data is extremely important, considering the scale (a complete state of USA), the fraction of costs, and the continuously updated set of data as submitted by the participants. Even with the constraints mentioned in this study, data obtained by volunteer participation may be an excellent source for tick-borne pathogens detection, even if only pinpointing the relative importance of each organism in the complete target territory.

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## Conflict of Interest

None declared.

## Ethical Approval

All the correspondents participated voluntarily in the study, submitting ticks by mail. No personal information was used to report our findings and this information is considered the intellectual property of BeBop Labs.

## Author Contributions

K.M. and S.M. monitored the complete program, including advertising, collection of funds, reception of ticks and doing the necessary steps for tick classification and pathogen detection. A.E.P. and N.F.R. elaborated the statistics, carried out the modeling, prepared some figures, and co-wrote the draft of the manuscript. All the authors wrote, read, and agreed on the final version of the manuscript.

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# Chapter 6: An agenda for research of uncovered epidemiological patterns of tick-borne pathogens affecting human health.

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*Opinion*

# An Agenda for Research of Uncovered Epidemiological Patterns of Tick-Borne Pathogens Affecting Human Health

**Agustín Estrada-Peña [1,2,*] and Natalia Fernández-Ruiz [1,2]**

[1] Department of Animal Pathology, University of Zaragoza, 50013 Zaragoza, Spain
[2] Instituto Agroalimentario de Aragón (IA2), 50013 Zaragoza, Spain
* Correspondence: aestrada@unizar.es

**Abstract:** The panorama of ticks and tick-borne pathogens (TBP) is complex due to the many interactions among vertebrates, vectors, and habitats, occurring at different scales. At a broad spatial range, climate and host availability regulate most tick processes, including questing activity, development, and survival. At a local scale, interactions are obscured by a high indeterminacy, making it arduous to record in field surveys. A solid modelling framework could translate the local/regional empirical findings into larger scales, shedding light on the processes governing the circulation of TBP. In this opinion paper, we advocate for a re-formulation of some paradigms in the research of these outstanding cycles of transmission. We propose revisiting concepts that faced criticisms or lacked solid support, together with the development of a conceptual scheme exploring the circulation of TBP under a range of conditions. We encourage (i) an adequate interpretation of the niche concept of both ticks and vertebrate/reservoir hosts interpreting the (a)biotic components that shape the tick's niche, (ii) an assessment of the role played by the communities of wild vertebrates on the circulation of pathogens, and (iii) the development of new approaches, based on state-of-the-art epidemiological concepts, to integrate findings and modelling efforts on TBP over large regions.

**Keywords:** ticks; reservoirs; pathogens; climate gradients; communities of vertebrates; epidemiological relationships

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## 1. Introduction

Ticks are prominent vectors of pathogens affecting human health; some of them are shared with pets and/or livestock, while some others are maintained in nature in complex epidemiological cycles [1]. These cycles tend to involve uncounted wild animals and result in intricate patterns of relationships between ticks and competent reservoirs, behind the driving forces of climate and the features of the landscape [2]. An enormous amount of information has been gained through numerous field surveys in many countries, supporting a complex picture of epidemiological relationships. However, research on ticks and tick-borne pathogens (TBP) still miss a solid body of science that could integrate the findings obtained at local/regional scales and project them into a larger area. Modelling approaches seem to be necessary for this purpose if we aim to capture the large area patterns.

The importance of wild vertebrates as hosts for the different stages of ticks and/or acting as reservoirs of TBP is well known [3]. However, scale matters in such research, and therefore, local/regional patterns are difficult to translate into the large view. The driving causes observed behind an epidemiological pattern are not simply "translated" to broader regions, since it is known that the relative composition of communities of wild vertebrate impact the circulation of TBP [4], their prevalence in questing ticks and, consequently, the risk to humans. An integrative framework covering *large regions at high resolution*, aimed at capturing, understanding, and preventing the transmission of TBP, is necessary for the development of protection approaches for human health. Obvious logistic issues prevent coordinated and simultaneous surveying efforts in several countries; these coordinated surveys could provide a comparable set of results suitable for building an integrated modelling that include the impact of weather, vegetation, landscape, deforestation, and the spatially and phylogenetically variable importance of the prevailing vertebrates [5,6]. We believe that the reformulation of some principles behind the epidemiology of TBP affecting human health is necessary. Studies in this direction have begun to emerge [7–10], improving the identification of ticks and TBP with molecular methods and consolidating techniques allowing for intercomparisons of field studies; they remain the building blocks of any integrative approach. However, studies on ticks have seldom incorporated views from fields of ecology, such as the importance of exploited organisms (hosts), modelling methods, or network analyses. Nowadays, information about the geographical distribution of wild hosts for ticks or competent reservoirs for TBP is massively available, as are datasets containing information about the traits of vertebrates that could influence their ability to circulate TBP [11,12]. Weather data are routinely captured by a cloud of earth-orbiting satellites at an unexpected resolution, very adequate for producing large area estimations of the factors affecting tick and vertebrate physiology. High-resolution maps of vegetation and distribution of vertebrates are available for many regions of the world [13].

This opinion paper aims to propose new ways to consider some of the factors affecting the circulation of TBP (schematized in Figure 1), looking for an agenda for research embedded within the notion of "a community ecology of landscapes" [14]. We aim to elaborate on the current perspectives of the components of the epidemiology of TBP, suggesting new approaches, proposing the rethinking of long-standing concepts, and promoting new methodological approaches to these studies. We are not enumerating actual examples that need a different focus. Instead, we aim to build a proposal for research, looking at concepts and not at cases or patterns occurring at regional scales. Our view of TBP and human health aims to be global, prioritizing topics of research, being confident that we are scratching just the tip of the iceberg.

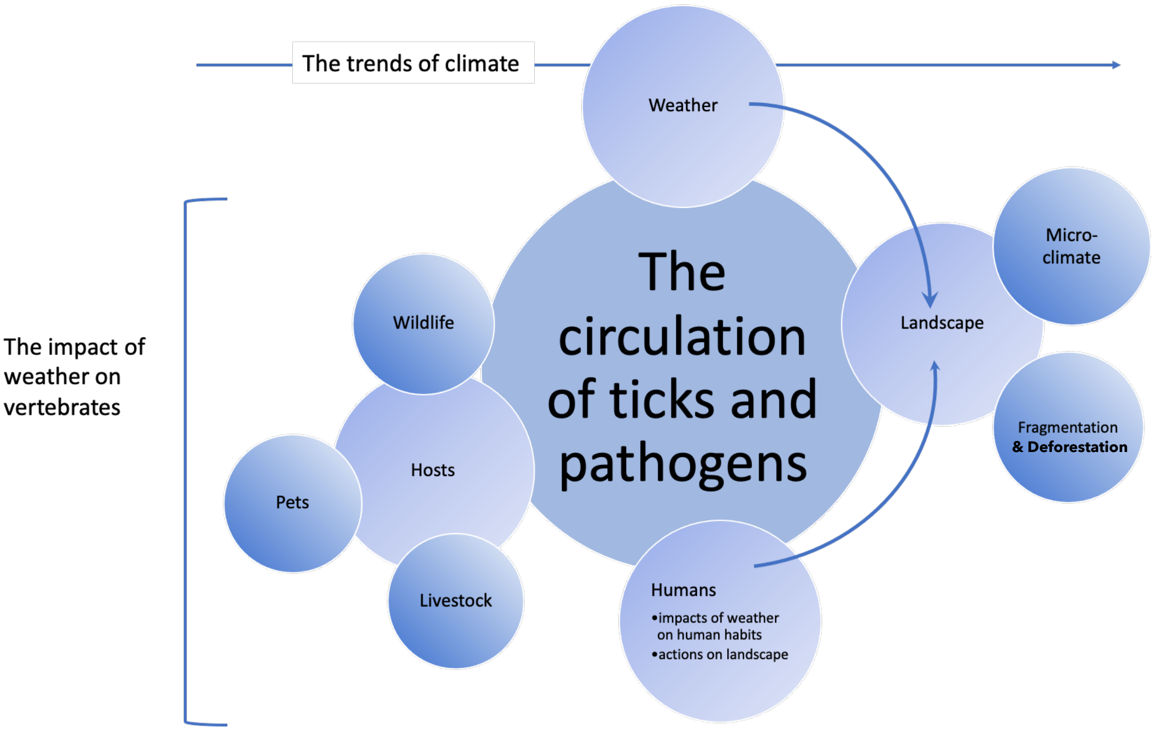


**Figure 1.** A schematic view of the traits affecting the circulation of tick-borne pathogens. We aimed to represent the main factors that affect the physiology of ticks (survival, development), their involvement

with vertebrates carrying pathogens, and their contact with humans while transmitting these pathogens. The "trends of climate" included on top is a generalist term that refers to the many variations (natural or human induced) that are being observed, and that affect the other components. In example, "livestock" may result affected in many ways by the effects of changing climate and, indirectly, have an impact on ticks and transmitted pathogens. In the same way, "humans" may affect the landscape in many ways, which tend to be geographically different (i.e., urbanization). In any case, the chart intends to be only a generalist overview of processes affecting the transmission of pathogens by ticks.

## 2. The Weather and the Ticks

The weather is the main driver of the physiology of ticks, influencing the activity and survival of questing stages or the development and mortality of moulting ones. The weather also regulates other features of the habitat where ticks thrive, such as vegetal coverage or the presence or abundance of hosts, in part. Therefore, weather drives most of the actions impacting the life cycle of ticks (some other actions, such as deforestation or habitat fragmentation, tend to be human driven). Modelling exercises were soon addressed to capture either the seasonal component of the life cycle, or more recently, to address the probability of presence, also called species distribution models (SDMs). A few researchers have explored other solid approaches to the circulation of TBP, such as the calculation of $R_0$ for the tick's life cycle and hence, the circulation of TBP [15,16]. The SDMs may produce results on the probable distribution of a species if based on a correct selection of variables [17–19]. Resulting maps are informative for citizens (i.e., indicating hazardous areas), but research on the topic needs a statistically tractable background. We would address how weather could affect tick's life cycle only after exploring the conclusions of the mathematical definition of the environmental niche. Modelling the potential distribution of TBP from only the presence/absence data of ticks, or from clinical records in humans, may be unreliable.

Every organism tracks a gradient of restricting variables allowing its persistence in the absence of competitors or predators [20]. Some approaches to tracing the tick environmental niche are based on the presumed capacity of some spatially interpolated variables, averaged for several years, that presumably explain the ecology of every species of tick in every corner of their distribution ranges. The prevailing "dogma" in scientific reports is that ticks' "suitable habitat" can be deduced from a number of explanatory variables (that sometimes lack ecological meaning, such as the reduction to Principal Components). In this view, results could be immediately plotted into a geographic map displaying the *actual* tick distribution (which is actually a probability of its *potential distribution* derived from the matching with climate variables). We show a basic explanation of this concept in Figure 2, using only two hypothetical environmental variables and also including the importance of vertebrate hosts and climate, aiming to simplify the concept to reinforce the view.

Efforts devoted to comparing the basic components of climate that allow the colonization of an area by ticks over large areas are limited [21]. In any case, the field of ticks suffers from the issues already identified regarding the application of SDMs to the representation of their environmental niche [22], namely (i) the "blind" selection of explanatory variables, without an expert assessment of the ecological importance of each variable, (ii) the tendency to map the potential distribution of a tick without considering the availability of vertebrates (resulting in lack of data about TBP circulation, as we will show later), and (iii) the ***unreliable*** projections of these models into future scenarios without considering climatic, statistical, and/or ecological arguments contrary to its use that have already been analysed [23–28]. If the models made with the current climate conditions may be unreliable, how to be confident about the future projections? [26,28]. Further on this, species with widely distributed populations (e.g., *Ixodes ricinus* in Europe) have different limiting factors according to the range considered (Figure 3). Thus, the building of one single model intended to capture the potential distribution of the species, may not reproduce the local limiting variables that affect the spread or survival of a species. As far as we know, this has been explored only for the tick *Rhipicephalus decoloratus* in Africa [29], which does not affect humans. Physiological models, as developed for mosquitoes [30], are still unavailable for ticks. These modelling approaches apply the principles of thermodynamics to organisms, deriving models of processes and their physiological consequences [31]. Physiological models are based on mass and energy gains or losses of the studied organisms and are considered a quantitative leap in the modelling of the distribution and seasonality of mosquito-borne pathogens. Similar models should be addressed for ticks, being probably more complex because of the existence of several stages of the tick's life cycle that probably react differently to stressors.

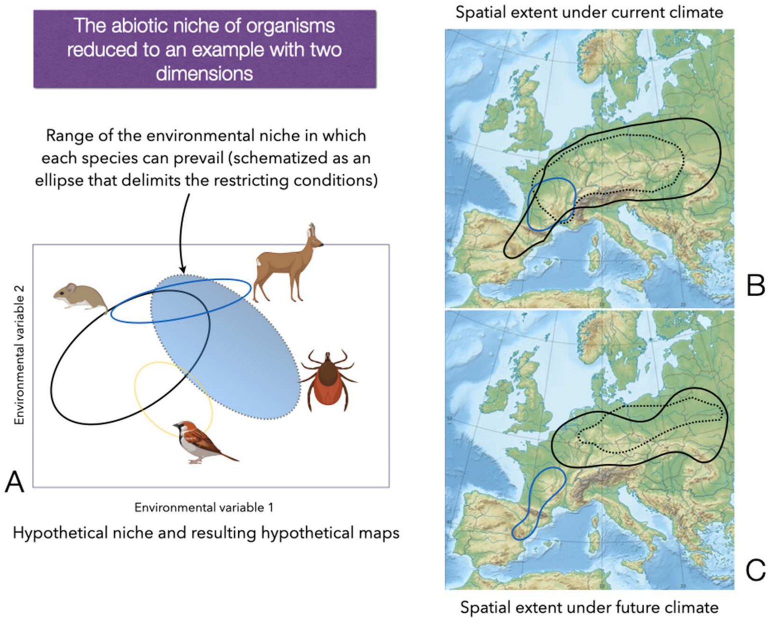


**Figure 2.** Representation of the concept of "environmental niche". We illustrate (**A**) a simplified representation of the hypothetical environmental niches of one species of tick and several species of vertebrates, with only two hypothetical variables (axes X and Y). In this view, organisms could have permanent populations in the area inside each ellipse, which "delimits" the area of suitable traits. Areas of overlap among ellipses correspond to areas of "niche overlap" or habitat sharing without geographical barriers. The shared area is thus proportional to the contact rates of a tick with different vertebrates. The suitable niche is commonly translated into maps displaying portions that could fit inside these suitable conditions according to the current climate (contours in (**B**)) or using scenarios of the future climate (hypothetical illustration in (**C**)). Parts of the illustration were created with BioRender.com. Original map of Europe downloaded under licence CC from Wikipedia (https://commons.wikimedia.org/wiki/File:Europe_relief_laea_location_map.jpg accessed on 12 March 2022).

We can foresee several topics requiring additional research regarding the climate and the environmental niche of ticks, which we would like to propose for future research, as follows:

(i) The tick's environmental niche is a hypervolume with a mathematical definition. A hypervolume is the intersection of the gradient of several climate variables that define the climate comfort for the tick. The capture and mathematical definition of such a niche for species affecting human health greatly improves calculations of risk and design of major strategies for protecting human health from TBP. To note, studies purely devoted to the evaluation of $R_0$, the distribution of host and reservoirs, should also evaluate such a niche. The translation of these data to a map would result in an informative tool, but not in an instrument of decision. We do think that the interpretation of the findings obtained through the analysis of the limiting variables experienced by the tick is a priority.

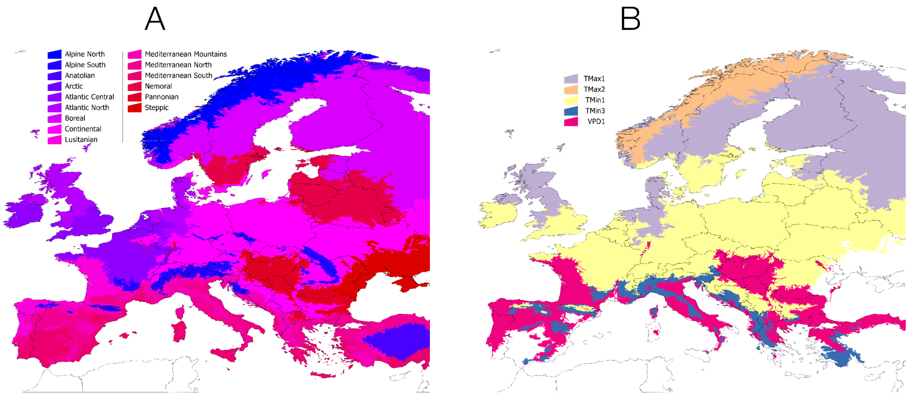


**Figure 3.** The tick *Ixodes ricinus* has geographically dependent different responses to environmental features. In (**A**), the climate regions of Europe are included with its standard denomination. A total of 11,349 records of the tick was used to develop an environmental suitability model (described in [31,32]). In the case of the illustration in (**B**), we aimed to pinpoint the most limiting variable for tick presence/absence. We did choose as explanatory variables the three first coefficients of a harmonic regression, obtained as explained in [31–33]. TMAX, TMIN and VPD refer to maximum temperature, minimum temperature, and vapour pressured deficit, respectively. The numbers next to each variable (1, 2, 3) refer to the annual average (1), the index describing the beginning of the spring (2), or the beginning of autumn (3) from the harmonic regression. See [33–35] for complete explanation of the methods that are not the focus of this paper.

(ii) There is a tendency to use the climate variables "as available" on the Internet without further verification or transformation. This is not a criticism of existing climate datasets, but a call of attention for epidemiologists working on ticks and TBP. For example, the seasons of the year are commonly calculated following astronomical dates; however, ticks do not adhere to an artificial construct that lacks an ecological background. Wettest, warmest, or driest quarters are calculated in a similar way. For example, studies [35] could not find "evidence supporting the superiority of the [current] calculation approach". The superior performance of an approach based on a harmonic regression of monthly series of climate data, allowing the calculation of daily weather values has been demonstrated and validated [32] and scripts for calculations are freely available [33]. It is important to validate these methods or to provide solid variables that (a) have ecological meaning for ticks, (b) are not self-correlated, and (c) have been selected after an evaluation of the impact of the weather on the modelled tick.

(iii) Species with large distributions may have "regional strains", adapted to the prevailing climate, an extreme demonstrated so far for a few species [28,36]. The training of one single model, later projected to the full range of the species seems to not be the best modelling approach. On the other hand, an "-omics" approach to these hypothetical "races" or "strains" could probably provide key details regarding the transmission of TBP. Local strains of ticks, adapted to the prevailing climate, could gain contact with key reservoirs, circulating strains of a pathogen. As far as we know, this extreme has never been addressed in depth.

(iv) Field surveys [37,38] proved that the trends of climate are pushing some tick species out of their "historical" limits or altering their periods of questing on hosts [39]. Nevertheless, studies of the *combined* impacts of the climate, the probable density and distribution of the hosts (that are affected also by the climate), and the landscape transformations, are available only for a few medically significant tick species. We think this is a promising field of research because of the need for a solid framework over which to build a new epidemiological approach.

## 3. Vertebrates: The Neglected Component of the Tick Niche

The vertebrate hosts are essential for the parasitic way of life, and they constitute the "biotic side" of the tick ecological niche. Solid field studies support the notion that the relative abundance of key vertebrates is a decisive factor affecting the circulation of some TBP (i.e., [39]). A community of vertebrates can be defined as a group of *co-occurring* species that share a similar gradient of *environmental conditions*, therefore overlapping their niches in a variable portion (see Figure 2). Communities of species result from environmental filtering, biotic filtering (competition or predation between pairs of species), and other processes such as dispersal, temporal variability, and ecological drift [40–42]. If we consider the large scale, such as a continent, a community shows compositional spatial gradients as a response to the climate, food availability, shelter, landscape, vegetation composition, competitors, and/or predators [41]. Communities have relative proportions of species, resulting in a modulating effect of the tick load and supporting the variability and prevalence of TBP in some cases [43] or the circulation of different strains of the same species of pathogens [44,45], because the communities may lack in a variable proportion the necessary competent reservoirs.

Such communities exhibit a *phylogenetic diversity* derived from the mixture of species; we consider such diversity the driving force behind the selective circulation of TBP [46,47]. Therefore, the *relative* composition of vertebrates in a community may lead to drastic changes of its ability to either support tick feeding or circulate pathogens. We encourage to not correlate the community of vertebrates with its richness of species. Instead, the phylogenetic diversity of the whole community, the degree of habitat sharing with the ticks, and the preferences of parasitism of the ticks towards each species of vertebrate, would provide a more balanced view of the epidemiology of TBP [44]. It has been stated that "biotic interactions and environmental filtering shape tick host communities distinctively between specific regions" and "host community composition is an important factor determining the persistence of tick-borne pathogens" [4,48]. A high phylogenetic diversity of vertebrates in a region could allow different species of TBP to be transmitted by a variable number of tick species [44,48] or by one generalist vector, feeding on a large variety of vertebrates. A low phylogenetic diversity of the community of vertebrates (e.g., the dominance of a few species) would probably drive the dominance of one or a few TBP. Changes in the vertebrate's phylogenetic diversity may result from natural or human actions on the landscape, such as changes in culture patterns, deforestation, or habitat fragmentation [48]. A synthesis seems to be necessary, but how to translate important findings from local surveys to the continental scale is yet unknown. A change of paradigm aiming to a unifying framework is a necessary move forward.

How does climate influence the patterns of the phylogenetic diversity and relative abundance of hosts for ticks or competent reservoirs for pathogens over large regions? How do generalist ticks adapt to changing combinations of hosts? These questions have been only superficially addressed but are of pivotal importance in the prediction of future scenarios of TBP transmission and we aimed to summarize in Figures 4 and 5. Changes of climate may affect the interactions among ticks and vertebrates, promoting changes in the rates of contact between ticks and reservoirs, with the consequent variations in the transmission rates of TBP [7]. If the prevailing climate promotes a change in the phylogenetic composition of reservoirs, some vertebrates involved in TBP circulation may no longer share the habitat with the tick vector. On the other hand, new reservoirs could be over-represented increasing the transmission rates of a TBP (see the hypothetical examples in Figures 4 and 5). Since vertebrates have different capacities to harbour and transmit the pathogens to feeding ticks, and ticks may show preferences to some vertebrates as hosts, such re-organization of the community could change the epidemiological status of a TBP in the area.

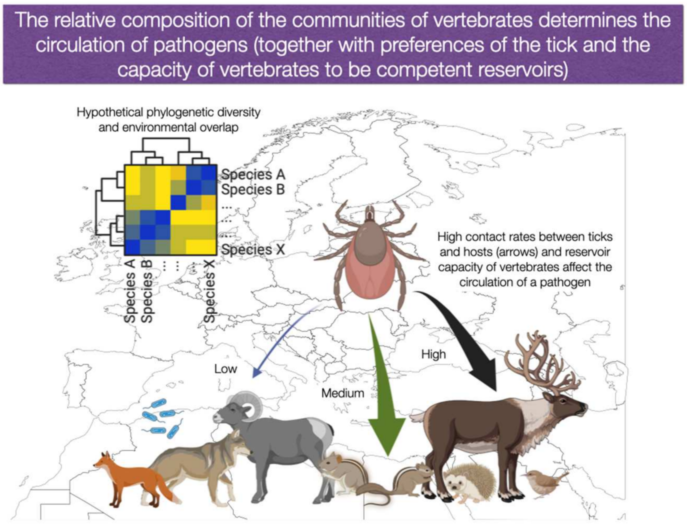
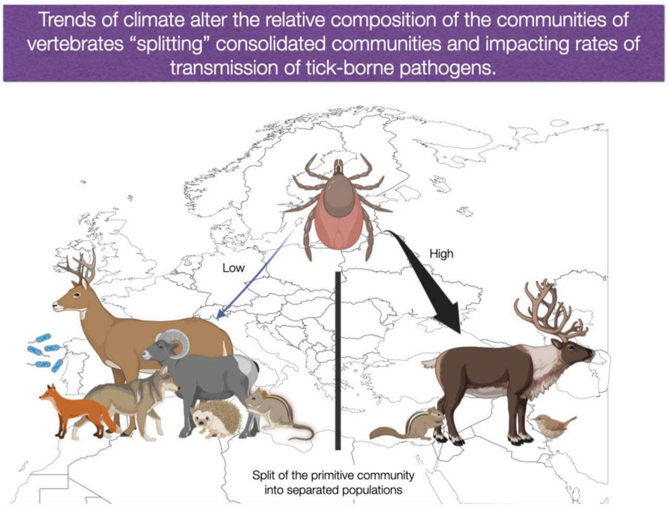


**Figure 4.** Wild vertebrates belong to communities of different relative composition according to the environmental features, such as climate or vegetation. Ticks have preferences for feeding on different species of vertebrates; in turn, vertebrates have different competence supporting the transmission of pathogens (reservoir capacity). The figure shows a hypothetical community of vertebrates that has a value of phylogenetic diversity and share portions of the habitat; only a few vertebrates hypothetically reservoir a pathogen (left). The transmission of TBP is, in part, due to the traits of each vertebrate and the contact rates of tick vectors with competent reservoirs. In the figure, arrows of different colours and sizes intend to illustrate hypothetical different contact rates among ticks and vertebrates. Thus, the species composition, the relative abundance of each vertebrate, the capacity to reservoir a pathogen, and the pressure of tick bites will shape the permanent circulation of pathogens. Parts of the illustration were created with BioRender.com (accessed on 1 November 2022).

In this context, we consider necessary to introduce the concept of "keystone vertebrates" [49], which would contribute to feeding most of the ticks and/or spreading specific TBP. One of the most important challenges in this approach is to understand how losing these keystone vertebrates may subsequently lead to a loss of interactions between the *pathogen* and the tick *vector*. However, the number of vertebrate species examined is still low, and many species of ticks are probably more generalist than previously suspected [50]. Studies on the topic [44,51,52] concluded that the networks of ticks, vertebrates, and pathogens tend to be redundant: the same tick species interacts with several groups of vertebrates; therefore, the lack of one or few vertebrates would do not drive to the exhaustion of ticks and/or TBP in the area.

Another key challenge in the epidemiology of TBP is to predict how the communities of ticks and vertebrates simultaneously respond to spatiotemporal variation in abiotic conditions [53–56]. The integration of the *simultaneous* modelling of the niche for both vertebrates and ticks is a challenge that would provide a privileged view of the contact rates between vectors and reservoirs. However, for multi-species data sets, fitting many models is required, which makes interpretation challenging and computationally requesting. Multi-species modelling should ideally address the two most important drawbacks of the results produced by environmental models, namely the incorrectly identified organisms and the under-representation of some species because the low probability to observe them (to note, the model should deal with hundreds of species of vertebrates at the continental scale, obtained from compiled datasets that would inevitably contain errors of identification). Joint species modelling evaluates simultaneously the environmental suitability of several species, accounting for phenomena of competition; they can accommodate for effects derived from host's phylogenetic composition and effects of climate on joint distributions [54–56]. A model that accounts for species-to-species associations can be expected to be superior in predicting community-level features [57,58], and therefore, its effects on the circulation of pathogens, on ticks, and its response to stressing variables could be adequately measured. Hierarchical modelling of communities [59] could be of great help in the task.



Trends of climate alter the relative composition of the communities of vertebrates "splitting" consolidated communities and impacting rates of transmission of tick-borne pathogens.

**Figure 5.** Climate is an essential driver of the reservoir composition of tick-borne pathogens.The situation schematized in Figure 4 is subjected to change because the climate could segregate established communities of vertebrates. Such split of communities could be the outcome of the different tolerance to the climate of the species of vertebrates involved. The split would contribute to changes in the epidemiology of tick-borne pathogens because competent reservoirs could change its density in the newly established communities. The relative abundance of competent reservoirs and contact rates with the tick vector would be different for each group of vertebrates in the new environmental gradient, resulting in new epidemiological patterns. Parts of the illustration were created with BioRender.com (accessed on 1 November 2022).

We consider this a field to have plenty of opportunities for exploring unexpected sources of variability regulating the epidemiological chains of TBP, generating hypotheses about how environmental filtering of vertebrates influence both ticks and TBP. Some topics that we consider necessary to address in a near future are outlined below:

(i) It is necessary to continue generating knowledge on tick-hosts associations in the context of the community [60]. Other than prevalence values of *single* pathogens on *each* vertebrate, the focus should be on how a tick species could be "allocated" among the available vertebrates in the community, along the gradients of climate, landscape, and phylogenetic diversity of communities.
(ii) There is a growing need for sharing of available datasets of tick distribution data, not restricted only to the medically prominent species. Predictive models of possible occurrence need to be trained with accurate and reliable records and coordinates, which are mainly available for a few medically important tick species.
(iii) Researchers on ticks and TBP imperiously need to know the estimated distribution of vertebrates in large areas. Good knowledge exists for countries such as the United States (through the GAP program: https://www.usgs.gov/programs/gap-analysis-project, last accessed on 1 November 2022), but this is lacking for most other countries. We urge to the preparation of digital atlases of distributions (actual and potential) of tick hosts and TBP reservoirs, together with their contributions to the circulation

of TBP. The analysis of the resulting communities and the impact of phylogenetic relationships on the TBP circulation is a foreseeable outcome.

(iv) We consider it of importance to analyse how changes in the circulation of TBP could happen under several restrictive variables, including both the direct effects of the climate, and the *indirect* effects derived of the lack of co-occurrence among vertebrates. Carefully designed field surveys in near sites exhibiting large changes of habitat (i.e., culture areas versus forest, etc.) or even an exhaustive bibliographic search could provide data, at least for the most studied species of ticks.

(v) Although it may seem far from the focus of this paper, the study of the impact of the abiotic gradients of the landscape (climate, fragmentation, deforestation, competence phenomena) on the composition of the vertebrate community is a fundamental step towards a better understanding of the epidemiology of TBP. These studies are an urgent need for epidemiologists, stakeholders, and decision makers.

## 4. Analysing the Tick–Host–Pathogen Relationships

The most direct way of collecting information about the relationships among ticks, vertebrates, humans, and pathogens is through field surveys or laboratory protocols. These methods aim to establish the real potential of either a tick as a vector or of a vertebrate as a reservoir; they are pivotal for any further analysis. These studies have been complemented by comparisons between the empirically observed prevalence of TBP in questing ticks and species-specific biological traits of vertebrates (e.g., size, diet, dispersal, offspring) or the ticks trying to reach a consensus on vectorial or reservoir abilities of ticks or hosts, respectively [50]. Recently, network constructs have been demonstrated to show many features of the associations of communities [51,52].

We ponder that the scarcity of data for many species of ticks can prevent a consolidated framework that ideally would integrate all these data. We proposed that a network construct could adequately represent the relationships among ticks, vertebrates, and pathogens; the examples studied thus far [45,46,51,52] have shown that tick-derived networks are recursive, resilient, coevolved structures (to note, co-evolution is not the same that co-speciation). Recursivity confers strength to networks of ticks, vertebrates, and pathogens [61]. A network could explain the resilience to disturbance of the tick–vertebrate–pathogen interactions, contributing to understanding the impact on the transmission rates of TBP given a certain disturbance. With all the recognized challenges [62,63], networks could be based on the contact rates of vertebrates and ticks as driven by environmental niche constraints. The conclusions obtained from the networks should be validated against the actual prevalence of various TBP under different field conditions. If tested adequately, these methods could result in a *holistic* view of the biotic relationships and the vectorial abilities of ticks assembled from many local studies (see Figure 6).

Notably, any network is based on the number of co-occurrences between pairs of organisms [64]. Imbalanced information would influence the observed interactions. The relative abundances of competent reservoirs, the phylogenetic diversity of the community, and the contact rates with target tick(s) would depend on an adequate input for any further calculation. All these tools already exist, and the next move forward should explore how both worlds (field ecology and modelling approaches of ticks and hosts) could complement each other, according to some basic requirements:

(i) We firmly support a modelling approach of the expected environmental niche of vertebrates and tick(s) to fill gaps in not yet surveyed areas and obtain an indirect estimation of the contact rates using networks. Indexes of interactions obtained from these models could be calculated for ecological regions, allowing comparison among results from different regions, and testing its usefulness.

(ii) An ecoregionalization of large areas, such as a continent, seems to be necessary for prospective studies of tick-borne pathogens [65]. Such division of the territory should be based on key habitat features (such as temperature, water deficit, forest density, etc.) known to affect both ticks and vertebrates.

(iii) Ecological studies on vertebrates suggest that while keystone species' effects on an organism's persistence are intuitive, their manifestation in complex natural communities is context-dependent and difficult to predict [66]. An empirical evaluation of this concept using a network of tick-vertebrate-pathogens in a target area has never been performed.

(iv) One important challenge in the epidemiology of TBP is to understand whether the network constructs could explain which attributes of the vertebrates regulate their relative competence as reservoirs in situations simulating real communities. This is another promising field of research, bringing together both field and in silico approaches.

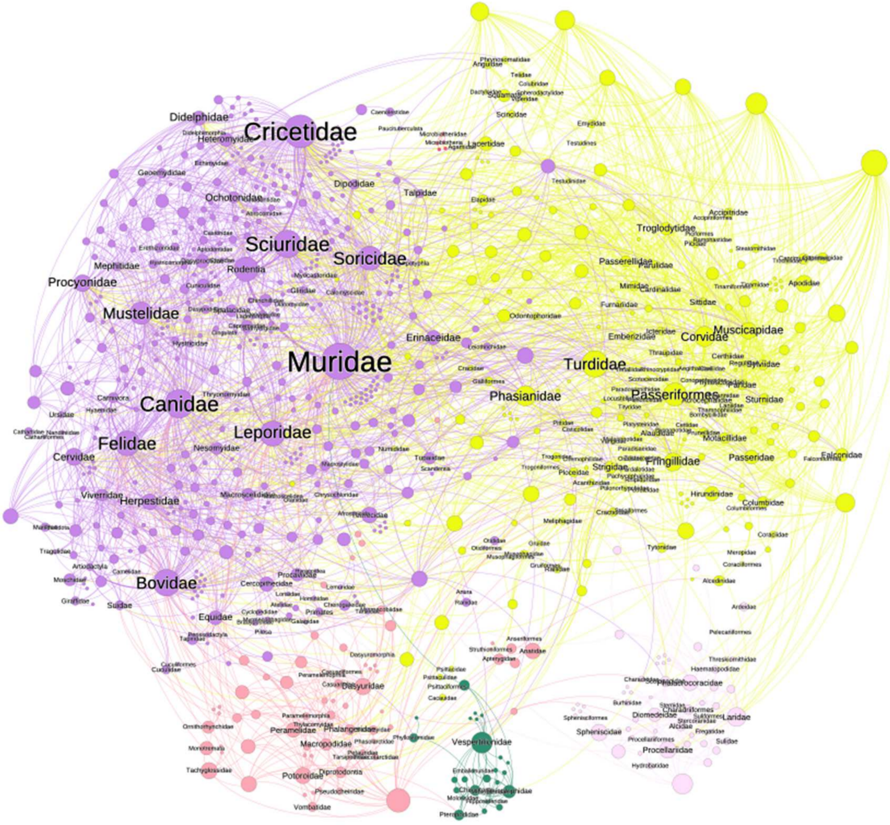


**Figure 6.** Tools to extract information on the circulation of TBP. Methods used for ecological studies could help to better capture the circulation of TBP. The network displayed shows the reported hosts of every species of the tick genus *Ixodes* in the world (compiled by Alberto A. Guglielmone, INTA, Rafaela, Argentina). Nodes (circles) are either species of ticks or families of vertebrates, and their size is proportional to their importance in the network; the labels of the tick species have been removed to improve the figure's clarity. Lines (links) represent the use of a host by a tick. The colours correspond to clusters, groups of tick-vertebrates that tend to appear together more frequently than with others; this is a property of the network known as "modularity". The complete network is available as Supplementary Material, allowing unlimited on-screen zoom. Such a network could be upgraded with links to pathogens circulated by combinations of vertebrates and ticks and with specific mention to the ecological region. Different indices can be obtained from a network, describing the importance of ecological interactions and co-occurrence events under different scales and abiotic conditions.

## 5. Conclusions

We firmly believe that studies about ticks and transmitted pathogens need a major refocus. We favour a deep integration of available geo-referenced data with explicit mention of the hosts and the prevalence of pathogens detected together with adequate reporting of the status of the ticks (questing or feeding). Linking the epidemiology of TBP with the ecology of wild animals, the impact of the landscape on tick survival, and the effects of the habitat structure on the relative abundance of competent reservoirs are topics open for future research. Modelling the tick's probable distribution requires homogenization of methods adhering to reliable rules, including a strict definition of an adequate set of variables retaining ecological meaning. For example, a reduction of explanatory variables via principal components reduces collinearity but loses any biological information carried by the original variables. This method (which is statistically correct) favours the production of maps but lacks any biological message.

We propose to explore new approaches, such as joint modelling of communities of vertebrates and contact rates with a target tick, together with a statistical background aimed at converting the outcome of such modelling into epidemiological information. It is necessary to continue with field sampling, improving the already growing available information on the topic; these studies represent the most valuable source for validating other statistically based developments. We propose alternative methods that could be applied to large ranges, supported by records of the vertebrate presence and further modelling of their distribution, together with evaluations of phylogenetic and functional diversity, linked to a network construct as the best way to obtain meaningful indices of tick–host relationships.

How best to achieve this remains an open question deeply related to the way we consider metacommunity-structuring patterns. However, using already existing modelling frameworks, strengthening capacity-building, and encouraging research teams already involved in building ecological knowledge around ticks is a logical step forward. It is just a change of scale.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijerph20032206/s1, Figure S1: **The network of the reported hosts of every species of the tick genus *Ixodes* in the world**. Nodes (circles) are either species of ticks or families of vertebrates, and their size is proportional to their importance in the network; the labels of the tick species have been removed to improve the figure's clarity. Lines (links) represent the use of a host by a tick. The colours correspond to clusters, groups of tick-vertebrates that tend to appear together more frequently than with others; this is a property of the network known as "modularity".

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# Discussion

This study has addressed untangle problems derived from the modelling of the distribution of ticks and their transmitted pathogens. This compendium of publications shows the enormous complexity involved in the accurate development of models that could predict the circulation of pathogens, and thus the potencial foci, aiming at prevention. The list of publications includes models for species of the genera *Ixodes*, *Hyalomma* and *Dermacentor*, which are responsible for the transmission of many pathogens with Public Health relevance, such as CCHF virus, the *B. burdorgferi* bacterial complex or the bacteria of the genus *Rickettsia*. Throughout these studies, several combined approaches have been used. Mathematical methods such as algorithms of maximum entropy, neural networks or decision trees have been tested in different procedures, integrated into different databases of climate, as well as the recorded distribution of vertebrates and ticks.

The facts mentioned above should call the attention of researchers regarding the complex relationships operating on the “ticks move”. A complete modelling approach including all the aspects governing the epidemiology of tick-borne pathogens has been never achieved; it seems that we are still far of such integrative approach, other than general modelling overviews over large areas, or more detailed approaches focused on local or regional scales. In both cases, the complete associations of the involved partners (climate, vegetation, dominant vertebrates, infectivity of reservoirs, seasonal dynamics of both ticks and hosts) are missing, most probably because the complexity obtaining these features and their interactions in field studies.

Our proposed approaches for the different tick species capture the distribution of ticks both retrospective and prospective. Besides, we highlighted the importance of climate in tick ecology, which greatly influences the presence or absence of ticks. The publications selected for this Thesis include different modelling methods and climate datasets, exploring several alternatives of interaction between them and the tick ecology. For instance, large parts of the northern Palearctic are covered by ice, snow and/or clouds for large periods in winter; this results in a lack of data in the satellite-derived information for a period of the year that is critical for the survival of ticks (Fick & Hijmans 2017). Furthermore these models have been combined with the most recent dataset on the distribution of ticks in the target region (Estrada-Peña *et al.*, 2017), together with an updated dataset of records of 162 species of vertebrates over the territory, openly available in GBIF (**www.gbif.org**). The use of actual records ensure the accuracy of the environmentally mediated distribution of ticks and vertebrates. Nevertheless the sampling pressure do not always cover all the sites. Therefore, we explored the capacities of citizen science for providing ticks, through the use of an existing network in the state of New Hampshire (USA), an area with high awareness on tick-borne pathogens. We focused on the reliability of the records, as well as the bias derived from the geographical gaps in tick submission.

The starting point of the modelling chain is the type of climate data series being used. Most long-term climate data series are interpolated. This type of data has an inherent redundancy that may introduce noise in modelling resulting from internal statistical issues of the datasets, like self-correlation. However, the development of technology has led to more accurate satellite climate data, and algorithms that can remove the clouds or artefacts distorting the real data. Although satellite data are better, they are only available at adequate resolution since 2001 (Fick & Hijmans, 2017) which makes the data series shorter; prior to these new series of satellite images, the highest resolution available was around 64 km$^2$. In this study, either satellite-derived or interpolated datasets were subjected to methods based on harmonic regression to obtain daily values of climate variables. By breaking down time series satellite data into its harmonic components, which are not self-correlated and provide a concise representation of climate during a specific timeframe, the selected framework can explain the climate using only a few variables, which simplifies modelling and increases accuracy. Additionally, estimations of relative humidity or saturation deficit are absent in these data and are instead substituted by the Normalised Difference Vegetation Index (NDVI). The NDVI captures the photosynthetic vigour (Julien *et al.*, 2006), which has been observed to correlate well with the distribution of *I. ricinus* ticks, one of the first models we obtained.

The utilization of interpolated climatologies could be unreliable in this context due to the spatial autocorrelation of descriptive variables, which artificially enhances the statistical reliability of the models. Moreover, some variables lack ecological significance for ticks, and the interpolated climatologies describe air temperature while ticks inhabit the ground. The tick's environmental niche is a hypervolume with a mathematical definition. In this context, an hypervolume is the intersection of the gradient of several climate variables that define the climate comfort for the tick. The capture and mathematical definition of such a niche for species affecting human health greatly improves calculations of risk and design of major strategies for protecting human health from tick-borne pathogens. To note, studies devoted to the evaluation of $R_0$, should include the distribution of host and reservoirs as part of the niche (Ogden *et al.*, 2014).

The translation of hypervolume-derived data into a map would result in an informative tool, but not in an instrument of decision. We do think that the interpretation of the findings obtained through the analysis of the limiting variables experienced by the tick is a priority. The higher efficiency of a method based on a harmonic regression of monthly series of climate data allows the calculation of daily weather values has been demonstrated and validated; scripts for calculations are freely available (Estrada-Peña & Estrada-Sánchez, 2014). Further studies on the habitat suitability for *I. ricinus* based on these newly proposed approaches could not find “evidence supporting the superiority” of calculations based on “classic” approaches (Estrada-Peña & de la Fuente, 2016). It is important to either validate these methods or to provide solid variables that have ecological meaning for ticks, that are not self-correlated, and  selected after an evaluation of the impact of the weather on the modelled tick. Species with large distributions may have “regional strains”, adapted to the prevailing climate, an extreme demonstrated so far for a few species (Nolzen *et al.*, 2022; Gilbert *et al.*, 2014) but that still is impossible to include in modelling strategies. Local strains of ticks, adapted to the prevailing climate, could gain contact with key reservoirs, circulating strains of a pathogen. Field surveys (Jore *et al.*, 2011; Jaenson *et al.*, 2012) proved that the trends of climate are pushing some tick species out of their “historical” limits or altering their periods of questing for hosts (Rosa & Pugliese, 2007). Nevertheless, studies of the combined climate's impacts, the probable density and distribution of the hosts (that are affected also by the climate), and the landscape transformations, are available only for a few medically significant tick species.

The reliability of distribution modelling for ticks and the species of vertebrates they interact with determines the contact rates among ticks and vertebrates and introduces an additional source of uncertainty. This uncertainty is influenced by the degree to which explanatory variables impact the outcomes of the models. However, all models performed for this Thesis remained within the expected range of reliability, with values consistently exceeding 0.7 (on a scale between 0 and 1). The modelling approach benefits from a large number of coordinates of vertebrates recorded as hosts for the tick(s) and the utilization of the most comprehensive *I. ricinus* dataset available (comprising over 14,000 records collected by experts), which contributes to the overall reliability of the models. However, modelling the potential distribution of tick-borne pathogens from only the presence/absence data of ticks, or from clinical records in humans, may be unreliable (Estrada-Peña & de la Fuente, 2017) because silent foci of pathogen circulation may exist remaining undetected.

The effect of temperature on the developmental stages has been identified as a key factor regulating the colonization of central Europe by genera like *Hyalomma* as well as for the northern spread of *I. ricinus*. Our results unequivocally confirm the hypothesis of a critical threshold of accumulated temperature required for the spread and colonization of *ticks* in most parts of its range (Hoogstraal, 1979) providing that suitable hosts are available. Our studies pinpointed the importance of temperature maximum quartile 90 and the number of days with a maximum temperature over a threshold of 10°C. This resulted evident across almost all ecological regions, although the impact varied spatially, either individually or in combination with other traits.

Surprisingly, soil moisture emerged as a significant factor for the spread of *Hyalomma* even in the driest regions of the continent, particularly under a Mediterranean climate regime, where historical records of *H. marginatum* exist. We interpreted this result as the combined influence of multiple variables, with varying relative importance in different locations, creating stable niches that facilitate tick colonization and contribute to enhance the environmental suitability. Another unexpected finding was the lack of significant results in multiple regressions linking the minimum temperature with environmental suitability in many colder regions of the target territory. Contrary to common assumptions, minimum temperature did not appear to be a limiting factor for the suitability of *H. marginatum* in central Europe. Despite the freezing minimum temperatures in this area, they cannot be statistically considered the primary factor responsible for high tick mortality, as previously suggested (Berezin *et al.*, 1969; Kondratenko *et al.*, 1975). As expected, the water vapor deficit in the air had not a significant impact on the long-term changes of the environmental niche of the tick: it is replaced in models by the humidity of the ground. However, it is important to note that this finding may not be generalized to other tick species, as their ecological requirements may differ.

The water vapor deficit, measured at a considerable distance above the ground surface, plays a minor role in delineating changes in the environmental suitability for *H. marginatum.* This highlights the significance of selecting variables with ecological relevance for each tick species, rather than relying on simplistic approaches already tailored for other purposes (Estrada-Peña *et al.*, 2016). Nevertheless, mean or accumulated rainfall, along with the NDVI have often been used to model the environmental suitability for ticks Estrada-Peña *et al.*, 2016). We recommend reevaluating previous conclusions regarding the impact of rainfall or other atmospheric water variables on the modelling of tick environmental niches. While NDVI should be applied when utilizing satellite-derived data, the measurement of soil surface moisture appears to be important when employing traditional weather traits obtained from reassessments or climate recording stations.

Every organism tracks a gradient of restricting variables allowing its persistence in the absence of competitors or predators (Barve *et al.*, 2011). Some approaches to tracing the tick environmental niche are based on the presumed capacity of spatially interpolated variables, averaged for several years, that presumably explain the ecology of every species of tick in every corner of their distribution ranges. The prevailing “dogma” in scientific reports is that ticks’ “suitable habitat” can be deduced from a number of explanatory variables (that sometimes lack ecological meaning, such as a reduction to principal components). In this view, results could be immediately plotted into a geographic map displaying the actual tick distribution which is actually a probability of its potential distribution derived from the matching with climate variables.

Efforts to compare the fundamental climate components that facilitate tick colonization across large areas are limited, as noted by Dumas *et al.* (2022). However, the field of tick research faces challenges when applying species distribution modelling to understand their environmental niche, as highlighted by Dormann (2017). These challenges include the selection of explanatory variables without expert evaluation of their ecological significance, overlooking the importance of vertebrate availability in mapping tick distribution and the circulation of tick-borne pathogens, and the unreliable nature of projecting these models into future scenarios without considering relevant climatic, statistical, and ecological factors (Austin, 2022; Ashcroft *et al.*, 2011; Bedia *et al.*, 2013; Jetz *et al.*, 2019; Nolzen *et al.*, 2022).

There are many other ecological factors playing a part in the distribution and spreading of ticks and tick borne pathogens. Another part of this Thesis utilized a modelling approach to trace the migratory paths of 28 species of passerine birds known to host immature *H. marginatum* ticks. The objective was to infer the potential northern extent of the introduction of this tick species in Europe. The focus was not to explore the ecological aspects of bird migration but rather to track the significance of tick introduction by birds. The aim was to develop a tool capable of assessing the likelihood of tick introduction from regions with established tick populations to areas where the tick is increasingly recognized as a risk (Kampen *et al.*, 2007; Jameson *et al.*, 2012; Capek *et al.*, 2014; Vial *et al.*, 2016; Duscher *et al.*, 2018; Chitimia-Dobler *et al.*, 2016; Hansford *et al.*, 2019).

There is a particular interest in the immature ticks of the genus *Hyalomma*, which are transported and dispersed by birds during their migration. This genus has been identified as a carrier of various pathogens that pose a threat to human health in extensive regions of Africa, Asia, and the Mediterranean basin. Among these pathogens, *Hyalomma* spp. have been implicated as vectors for *Rickettsia* spp. (Wallménius *et al.*, 2014) and the causative viral agent of CCHF (Gargili *et al.*, 2017). While birds themselves are not viremic (Ergönül, 2006), they can harbour infected ticks. This association has gained significant attention in recent years due to the substantial number of birds migrating annually. Although the prevalence of ticks on birds is relatively low, they contribute to the dissemination of infected ticks into higher latitudes (Mancuso *et al.*, 2019; Spengler *et al.*, 2019). The identification of permanent CCHF foci in the Iberian Peninsula (Estrada-Peña *et al.*, 2012) has raised concerns about the potential spread of infected ticks from Africa to the European continent (Palomar *et al.*, 2013).

The results of the modelled pathways of birds flying from Africa to Europe suggest that different species of birds may arrive at the same site in distinct "waves" during different weeks, and their flight paths vary depending on the time of year. These flight paths do not always originate in Africa and continue northward. Certain areas within the target region may experience multiple waves of tick infestations in a year, carried by various bird species with varying probabilities of introducing immature *H. marginatum* ticks. For example, the western route connecting northern Africa with western Europe and the United Kingdom is one of the earliest flyways that could potentially introduce *H. marginatum* into northern latitudes.

Due to the scarcity of data regarding the parasitic load of each bird species, it is not feasible to incorporate the individual contribution of each bird as a carrier of ticks into the modelling framework. This is because ticks are not uniformly distributed among hosts, and factors such as the host's physiological condition or the presence of small sites with high tick density (Randolph *et al.*, 1999), which are frequently visited by birds but undetected by our framework, can influence infestation levels (Tschirren *et al.*, 2007). To address this, we indirectly approached the issue by considering contact rates between birds and ticks, a concept we developed in the initial paper of this PhD work.

Although the likelihood of *H. marginatum* reaching northern latitudes is currently low, several factors increase the potential for its establishment in central Europe, including its probable longer survival in a warmer climate, the heterogeneous nature of its introduction, the presence of multiple immature stages on each bird, and the clustering of migratory birds in small areas. It is important to emphasize that the risk of establishing permanent populations of this invasive tick is not solely determined by its introduction. We also recognize the importance of constructing future climate scenarios, which could be achieved through the analysis of satellite imagery time series (as discussed in Chapter 1 of this work) to predict likely future conditions. However, statistically aligning projections from satellite records with general circulation models based on $CO_2$ emission scenarios poses challenges. While some studies have addressed this issue, such as Sobrino and Julien (2013), there is still no direct method to compare results from different modelling approaches, and validation remains crucial. Additionally, estimating wind speed and direction in the future, which clearly impact the flights of the small birds carrying immature stages of *Hyalomma*, is impossible. Therefore, further research on this topic is undoubtedly necessary.

As previously mentioned, the presence of overlapping interactions between ticks, hosts, and reservoirs is essential for the circulation of pathogens. These interactions between different species contribute to the formation of a community. Vertebrate communities often coexist in similar environmental conditions, which is particularly relevant in the context of tick-borne pathogens. Not all vertebrates can serve as reservoirs for these pathogens, thence the name of “competent reservoirs”. In example, a badger may carry *Borrelia garinii* but cannot transmit it to biting ticks (Margos, pers. comm.) even if they are competent vectors. Therefore, it is a reservoir, but not a *competent* reservoir. In addition, it has been observed that the diversity of vertebrates that a tick feeds on can either diminish or enhance the transmission of a particular pathogen. In Chapter 4, we explored whether a specific combination of vertebrates and their interaction rates with tick vectors could serve as an indicator of pathogen prevalence in questing ticks within the western Palearctic region. The aim was to determine how these "indicator" communities could act as markers for actual prevalence of two species of bacteria of the *Borrelia burgdorferi* group (previously mentioned as bacteria transmitted only by the tick species *I. ricinus*) in Europe.

The methods of this study were based on an unsupervised classification of the territory using the presence/absence of vertebrates in the field, as available at GBIF. These methods were implemented to identify clusters of vertebrates based on their abundance and contact rates with ticks. These clusters represent groups of species that tend to appear together more frequently than with other vertebrates. Various classification algorithms, including Neural Networks, Random Forest, Gradient Boosting, and AdaBoost, were employed to develop models, trained using data on vertebrate communities and the prevalence of *Borrelia spp.* bacteria in ticks. The results indicated a correlation between the prevalence of these bacteria in questing *I. ricinus* ticks and specific communities of vertebrates in the selected study sites. This study pinpointed not only the key role of rodents or birds as reservoirs of some species of bacteria (Hofmeester *et al.*, 2018; Heylen *et al.*, 2013; Mysterud *et al.*, 2019a) but also the dual role played by some taxa in the amplification/dilution of the pathogen (Fabri *et al.*, 2021; Mysterud *et al.*, 2019b). Research in the USA has focused on studying the life history traits of vertebrates to understand their impact on tick infection rates caused by *B. burgdorferi* s.l (Moore *et al.*, 2014). However, due to logistical challenges and limited access to certain species, only a few host species are typically studied in areas with tick-transmitted pathogens (Brunner *et al.*, 2008). Studying individual species separately overlooks their potential interactions (Baquero & Tellería, 2000; Mouchet *et al.*, 2015). A more effective approach involves predicting and clustering groups of co-occurring organisms, considering the existing interactions among species (Hill *et al.*, 2020). The methods develop to analyse, quantify and display the communities of vertebrates at a given resolution (not higher than 4 km.) help identify instances where competition among species leads to the displacement of critical taxa, as observed through the absence of records in the presence of competing species in predictive maps.

Results support the known epidemiology of the pathogen, indicating that it circulates among specific vertebrates that serve as hosts for the tick vector. The ranking of vertebrates within each indicator community aligned with the identified reservoirs of *Borrelia* spp., highlighting the importance of Carnivora or Cetarthiodactyla in its circulation (Hofmeester *et al.*, 2018). The variability in species composition and abundance within the communities resulted in a significant importance in shaping tick infection rates, even when recognized reservoirs are present but ranked lower. The findings were consistent with existing knowledge of *Borrelia* spp. ecology and reservoirs. The study advocated for using modelling approaches to assess the potential range of vertebrates and ticks in unexplored areas, filling knowledge gaps through network analysis. This can enable the comparison of results across ecological regions evaluating the practical implications of prevention. Future studies on tick-borne pathogens, should be conducted under the recommended ecological regionalization based on key habitat features like temperature, water deficit, and forest density, particularly for large areas like continents.

We finished this compendium of publications with a study based on the concepts of community science (Kullenberg & Kasperowski, 2016) addressing the identification of ticks submitted by citizens in the state of New Hampshire (USA). The citizen science project followed a publicized program through various news and social media platforms, resulting in a total of 14,293 ticks being collected between 2018 and 2021 main species reported *I. scapularis* and *D. variabilis*. Out of these, 6,599 ticks were associated with a category of human activities based on the returned questionnaires. During the initial stages of the project in 2017, the communication campaign and tick submissions were relatively low compared to subsequent years. Additionally, we acknowledge that the data from 2020 there affected by the ongoing SARS-CoV-2 pandemic where tick submissions in 2020 were slightly lower than in other years. Despite these challenges, we believe that the abundance of ticks obtained is sufficient to draw meaningful conclusions, and we were able to identify tick distribution patterns across large regions showing both advantages and disadvantages of method, as discussed in Eisen and Eisen (2021). The study focused on collecting and analyzing data on the patterns of distribution and seasonality of both *I. scapularis* and *D. variabilis*, including their unique features, together with the prevalence of several pathogens. By examining both species together, novel insights appeared that can aid people in effectively preventing tick encounters based on influenced by human activities. We aimed to address the broad classification of various activities reported by participants. Similar to Salkeld *et al.* (2019), we encountered difficulties in linking specific human activities to tick bites. However, our findings differed from other studies (Mead *et al.*, 2018; Porter *et al*. 2019) regarding *I. scapularis*, as we observed a higher number of tick encounters in people's yards compared to forest-associated recreational areas. These discrepancies in results may be attributed to differences in the classification methods of human activities. For example, Mead *et al.* (2018) only reported “outdoors in public spaces”, while we used a wide set of categories of outdoor activities in our study. It is important to acknowledge that reporting accuracy can be affected by the time elapsed between the tick bite and its discovery.

Such study has demonstrated the effectiveness of passive tick surveillance when complemented by active surveys in order to obtain more accurate models due to the employ of more available data in more places. We believe this study contains a promising field of research because of the need for a solid framework over which to build a new epidemiological approach for ticks-borne pathogens. Furthermore combining active surveys, community science, and predictive mapping had the potential to serve as a valuable source of information, enhancing our understanding of the distribution of ticks. This can greatly contribute to the effectiveness of tick bite prevention campaigns and the management of tick-borne diseases. Using the submitted data, we generated maps predicting tick distributions in New Hampshire. These predictions primarily relied on variables such as temperature, soil moisture, and atmospheric water vapor deficit. Due to the scale of mapping (meters), we did not include vegetation data as an explanatory variable, in line with Winter *et al.* (2021). Despite the differences in resolution and explanatory variables used, our map depicting the probability of suitable habitat for *I. scapularis* in target region aligned with similar studies (Diuk-Wasser *et al.*, 2010). Other studies have also demonstrated the impact of climate change on Lyme disease across the USA (Moore *et al.* 2014, Monaghan *et al.* 2015, Couper *et al.* 2021) and specifically in northeastern regions of the country (Little *et al.,* 2019, Elias *et al.,* 2021). These mapping efforts highlight the potential of modelling tick vector habitat suitability to enhance epidemiological models for tick-borne pathogen transmission. Therefore, modelling based solely on environmental variables can effectively isolate the weather factors influencing tick distribution while minimizing the influence of human factors.

# Conclusi sons

Following the completion of this Thesis, a number of conclusions have been drawn.

1. The modelling for ticks and tick-borne pathogens needs for a significant shift of paradigm. We advocate for a comprehensive integration of geo-referenced data, specifically highlighting the hosts and reservoirs, as well as the prevalence of detected pathogens, along with accurate reporting on the status of ticks (whether questing or feeding).

2. We suggest exploring the connections between the circulation of tick-borne pathogens and the ecology of wild animals, considering the influence of landscape on tick survival, and the impact of habitat structure on the abundance of reservoirs capable of transmitting diseases. The interrelation between them will be a crucial area for future research.

3. To accurately model the probable distribution of ticks, it is essential to establish standardized methods that adhere to reliable guidelines. This includes a precise definition of a suitable set of variables that retain ecological significance for each species and probably for different geographic areas. While reducing collinearity through the use of principal components can facilitate the production of maps, it may result in the loss of biological information carried by the original variables. Therefore, although statistically valid, this method lacks a biological message; we consider it should be avoided.

4. It is compulsory to exploring novel approaches, such as joint modelling of vertebrate communities and contact rates with specific tick species, accompanied by a statistical framework to convert the outcomes of such modelling into epidemiological information. Field sampling should continue, with an emphasis on enhancing the existing wealth of information on the subject. These studies serve as valuable sources for validating other statistically driven advancements. Additionally, we suggest alternative methods that can be applied over large geographic ranges, supported by data on vertebrate presence, and incorporating distribution modelling along with assessments of phylogenetic and functional diversity.

5. The best way to accomplish these goals remains an open question closely tied to how we perceive metacommunity-structuring patterns. However, leveraging existing modelling frameworks, strengthening capacity-building efforts, and encouraging research teams already involved in developing ecological knowledge related to ticks represent a logical and progressive step forward. It simply entails a change in scale.

# Conclusiones

Tras la realización de esta Tesis, se han extraído una serie de conclusiones.

1. La modelización de la distribución de las garrapatas y los patógenos transmitidos por ellas requiere un cambio significativo de paradigma. Se aboga por una integración exhaustiva de datos georreferenciados, destacando específicamente los pertenecientes a hospedadores y reservorios, así como la prevalencia de los patógenos detectados, junto con una información precisa la distribución de las garrapatas (ya sea en la vegetación o alimentándose).

2. Se sugiere explorar las conexiones entre la circulación de patógenos transmitidos por garrapatas y la ecología de los animales silvestres, considerando la influencia del paisaje en la supervivencia de las garrapatas y el impacto de la estructura del hábitat en la abundancia de reservorios capaces de transmitir patógenos. La interrelación entre ambos será un área esencial para futuras investigaciones.

3. Para modelizar con precisión la distribución probable de las garrapatas, es necesario establecer métodos estandarizados que se atengan a directrices fiables. Esto incluye una definición precisa de un conjunto adecuado de variables que conserven su importancia ecológica para cada especie y, probablemente, para distintas zonas geográficas. Aunque la reducción de la colinealidad mediante el uso de componentes principales puede facilitar la elaboración de mapas, puede dar lugar a la pérdida de información biológica transportada por las variables originales. Por lo tanto, aunque estadísticamente válido, este método carece de mensaje biológico se considera que debería evitarse.

4. Es obligatorio explorar enfoques novedosos, como la modelización conjunta de comunidades de vertebrados y tasas de contacto con especies de garrapatas, acompañada de un marco estadístico para convertir los resultados de dicha modelización en información epidemiológica. El muestreo de campo debe continuar, haciendo hincapié en la mejora de la abundante información existente sobre el tema. Estos estudios sirven como fuentes valiosas para validar otros avances basados en la estadística. Además de métodos alternativos que puedan aplicarse en grandes áreas geográficas, con el apoyo de datos sobre la presencia de vertebrados, e incorporando la modelización de la distribución junto con evaluaciones de la diversidad filogenética y funcional.

5. La mejor manera de lograr estos objetivos sigue siendo una cuestión abierta estrechamente ligada a cómo se perciben los patrones de estructuración de las metacomunidades. Sin embargo, aprovechar los marcos de modelización existentes, reforzando los esfuerzos de capacitación. Animando a los equipos de investigación ya implicados en el desarrollo de

conocimientos ecológicos relacionados con las garrapatas que continúeme con lógica y precisión para lograr cambio a gran escala de la modelización.

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