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1 Unravelling the phenology of *Ixodes frontalis*, a common but understudied tick

- 2 species in Europe.
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13 Abstract:

- 14 Tick-borne diseases have a complex epidemiology that depends on different ecological
- 15 communities, associating several species of vertebrate hosts, vectors and pathogens.
- 16 While most studies in Europe are focused on *Ixodes ricinus*, the common sheep tick,
- 17 other *Ixodes* species may also be involved in the transmission or maintenance of
- 18 different pathogens. In this study, we describe for the first time the activity pattern of *I*.
- 19 *frontalis*, an understudied but widespread tick species associated with several common
- 20 bird species in Europe. Questing ixodid tick stages (larvae, nymphs and adults) of both *I*.
- 21 *frontalis* and *I. ricinus* were monitored by the drag sampling method over three years at
- 22 the same locations in Western France. Differential activities were observed depending
- 23 on *I. frontalis* life stages: nymphs and adults were present sporadically on the ground

throughout the year, while larvae exhibited a marked peak of activity around OctoberNovember with tens or even hundreds of individuals per m², followed by a slow
decrease in winter. Larvae were completely absent in summer, which contrasts with the
high numbers of *l. ricinus* larvae at this time of the year. The vegetation and the litter
where the two tick species were found also exhibited marked differences, with *l. frontalis* mostly collected under bamboo bushes.

30

31 Keywords:

Vector-borne diseases, bird ectoparasites, *Ixodes ricinus*, drag sampling, questing stages,
 population dynamics

34

35 1. Introduction

36 Vector-borne diseases constitute a major concern for both human and animal health. In 37 temperate zones, hard ticks are considered to be the most important vectors in terms of 38 diversity of pathogens, including zoonotic agents (such as *Borrelia burgdorferi* sensu 39 lato, Anaplasma phagocytophilum, tick-borne encephalitis virus, Babesia spp.). They are 40 also the vectors with the highest economical and medical impact on animals and Man (McCoy and Boulanger, 2015; Sonenshine, 1993). The transmission cycles of tick-borne 41 42 pathogens are complex, involving generally several host species and sometimes more 43 than one tick species (Estrada-Peña and de la Fuente, 2014; Kurtenbach et al., 2006; 44 Mannelli et al., 2012; McCoy and Boulanger, 2015). Therefore, the study of a particular 45 tick-borne pathogen requires an integrated community approach, in a diversity of host 46 and tick species.

47 European research has only recently started to thoroughly investigate the role of tick

48 species other than *I. ricinus* in the transmission of *I. ricinus*-associated pathogens

49 (Heylen et al., 2017b, 2014b, 2013; Jahfari et al., 2014; Obsomer et al., 2013). In Europe, 50 the generalist *I. ricinus* is the most important vector to which humans are exposed, but 51 some of the pathogens carried by this species can originate from other tick species, 52 where they may exhibit a higher prevalence (Heylen et al., 2017b; Literak et al., 2015). 53 When *I. ricinus* shares the hosts on which other tick species feed, pathogens can be 54 bridged towards humans (Bown et al., 2008; Heylen et al., 2017b). Many of the other tick 55 species within the tick community have been neglected, either because (i) they are rare 56 or difficult to collect (e.g., endophilic ticks), (ii) they are easily misidentified (Estrada-57 Peña et al., 2017a) or (iii) because they are not known to affect Man or animals of 58 interest. This lack of interest leads to probable underestimation of their presence and of 59 their real implication in pathosystems. The knowledge of basic ecological life history 60 parameters of those understudied species, including their seasonal activity patterns, is 61 crucial to understand the role of such tick species in tick-borne disease epidemiology. 62 Ticks associated with birds, such as I. frontalis or I. arboricola, are of particular interest 63 in this context because (i) they are known to carry several zoonotic pathogens such as 64 Anaplasma phagocytophilum, Borrelia, or Rickettsia spp. (Heylen et al., 2017a), (ii) birds 65 participate in the dissemination of ticks and their associated pathogens - at long distance but also in urbanised regions that are less accessible to migrating mammals (de 66 67 la Fuente et al., 2015; Heylen, 2016) - and (iii) immature stages of *I. ricinus* - the most 68 frequently reported species on humans - commonly feed on birds (Hornok et al., 2013), 69 acting as a potential bridge species with possible transfer of pathogens between birds 70 and mammals.

71 In this article, we investigate seasonal activity patterns of the ornithophilic tick *lxodes* 72 frontalis, an abundant species from Europe (Estrada-Peña et al., 2017b), however with a 73 poorly-investigated phenology of its free stages and with a largely unknown ecology 74 (Heylen, 2016). *I. frontalis* is commonly found feeding on a great diversity of bird species 75 that are shared with I. ricinus (Cringoli, 2005; Doby, 1998; Martyn, 1988; Monks et al., 76 2006; Norte et al., 2012). It has also been reported to exceptionally bite humans (Cull et 77 al., 2018; Gilot et al., 1997). However, it has rarely been found questing on the ground 78 (but see Barandika et al., 2006; Bona and Stanko, 2013; Ceballos et al., 2014; Doby, 79 1998; Gilot et al., 1997, 1995; Schorn et al., 2011), possibly because it is easily 80 overlooked, especially for the larval stages that are difficult to identify due to their small size (Heylen et al., 2014a). I. frontalis is also known to be implicated in direct (i.e., 81 82 without infectious agents) paralytic and mortality phenomena in certain birds (Mans et 83 al., 2004; Monks et al., 2006), in the transmission of Chizé virus, fatal for birds (Chastel 84 et al., 1999) and on the possible contribution to the maintenance of 'Candidatus 85 Neoehrlichia mikurensis' (Movila et al., 2013), Anaplasma phagocytophilum (Jahfari et al., 86 2014) or Borrelia burgdorferi s.l. (Heylen et al., 2017a, 2013; Norte et al., 2013) in bird 87 populations. As I. ricinus may bridge I. frontalis-related pathogens towards humans, the 88 investigation of co-occurrences of both tick species in time and space, which will be 89 described in this study, is of prime importance.

90

91 **2. Material and Methods**

A study site was selected in spring 2014 near Nantes (47°19'N, 01°29'W) to survey
exophilic hard tick densities on vegetation, estimated with the drag sampling method: a
wooded private park was chosen for its high density of *I. ricinus* nymphs. Ticks were

95 collected one day per month from June 2014 to May 2017 (Supplementary material I 96 and II), during the second half of each month as regularly as possible (the collection 97 could not occur on rainy days), by 1 to 4 operators, between 13:30 and 18:00. To collect 98 questing ticks, ten marked transects of 10 m x 1 m were chosen along trails covered by 99 short grass or a leaf litter, with a minimum distance of 20 m between them (Fig. 1). Tick 100 collection was repeated 3 times consecutively on the same marked transects, in order to 101 collect more individuals and to improve accuracy in the assessment of tick abundance (Bord et al., 2014). On each transect, a 1 m² white flannel cloth was slowly dragged (0.5 102 103 m/s) along the 10 m transect (Agoulon et al., 2012): ticks were counted, removed from 104 the cloth with tweezers and stored alive (except larvae, which were stored in 70% 105 ethanol for further identification from February 2015 onwards - see below) for further 106 identification concerning stage and species according to the keys provided in Heylen et 107 al. (2014a), Hillyard (1996) and Pérez-Eid (2007). Larvae were initially only counted 108 and then discarded, without collection and identification. They were systematically 109 collected and identified under an optical microscope (100x magnification) from 110 February 2015 onwards.

111 The park was characterized by mainly deciduous trees (Quercus spp., Betula sp.), the 112 presence of two ponds, a humid peaty soil (with *Carex* spp., *Salix* sp., *Myrica gale*) and 113 several non-native plants along different parts of the trails, including bamboo 114 (*Phyllostachys* spp.). The ten transects presented marked differences in terms of habitat: 115 three transects (hereafter called "Transects with bamboo": n°8 to 10) were surrounded 116 and overhung by bamboo and presented a litter covered with bamboo leaves (Fig. 1). The ground of the seven other transects (hereafter called "Transects without bamboo": 117 118 n°1 to 7) was either a litter of deciduous (n°2, n°3, n°5) or coniferous needle leaves (n°5), or short (<15 cm) grass (n°1, n°4, n°6, n°7)(Fig. 1). 119

120 A meteorological station was installed in January 2015 in a wooded part of the study site 121 (Fig. 1), recording hourly macroclimatic data exploited at the level of the whole site: 122 temperature, hygrometry and wind speed. Saturation deficit was calculated from 123 temperature and hygrometry according to Hartmann (1994). Mean values of these four 124 meteorological factors were considered as explanatory variables for analyses, at 125 different time intervals: 0-30 days, 0-10 days and 0-5 days prior to the day of tick 126 collection. Thus, twelve meteorological variables were considered, crossing mean values 127 of four factors and three time intervals (Table 1).

128 The temporal dynamics of the different stages of *I. frontalis* was described at the level of 129 the whole study site. For the larval stage, to evaluate the influence of meteorology, the 130 relationship between tick counts and meteorological variables was considered from 131 February 2015 to May 2017 by Spearman's rank correlation, and the best relationship 132 was selected according to the rho and its p-value. After log transformation of larval 133 counts (log(larval count + 1)), because of the overdispersion of the data, a general linear 134 model was built to assess the influence of this selected meteorological variable, based on 135 R². In parallel, to evaluate the influence of regular seasonal phenomena, the relationship 136 between *I. frontalis* larval counts and day length on the day of tick collection was 137 estimated in the same manner, leading to a similar model, hereafter called "seasonal 138 model". Finally, 4 models were compared, the best one being selected according to the 139 lowest Akaike information criterion (AIC): 1) "meteorological model" (1 selected 140 meteorological variable); 2) "seasonal model" (1 variable: day length); 3) "combined 141 model" (1 meteorological variable and day length); 4) "combined model with interaction" 142 (1 meteorological variable, day length and interaction between them). The same 143 statistical process was applied to the larval stage of *I. ricinus* for comparison.

The distribution of the different stages of *I. frontalis* was described at the study site for
each transect to evaluate spatial distribution. It was compared to *I. ricinus* on two types
of transects: with bamboo *versus* without bamboo. Comparisons were performed by
bilateral Fisher's exact test. All analyses were carried out in R version 3.4.1 (R Core
Team, 2017).

149

150 **3. Results**

151 Temporal dynamics of I. frontalis

152 From September to November 2014, an increase in the density of questing larvae was 153 observed on transect n°9 (i.e., 14 larvae in September, 1230 in October, more than 3000 154 (rough count) in November and 466 in December 2014). As at that time of the study 155 larvae were assumed to belong to *I. ricinus*, they were just counted and discarded 156 without prior identification. In January 2015, larvae of transect n°9 (n=490) were kept 157 and all were identified as *I. frontalis* (Fig. 2). Since that date, larvae from the whole study 158 site were then stored and identified for the remaining 28 months of the study (from 159 February 2015 to May 2017). Larval questing seasonality of *I. frontalis* could therefore 160 be assessed for that period: a marked peak of activity of *I. frontalis* larvae was observed 161 around October-November, followed by a slow decrease in winter and by the absence of 162 larvae in summer (Fig. 3a and Supplementary material I). Assuming that most of the 163 larvae collected earlier than January 2015 on transect n°9 belonged to *I. frontalis* 164 emphasizes this observed seasonality.

All meteorological variables were correlated to *I. frontalis* larval counts, except mean
wind speed. The best correlation concerned mean temperature over 10 days prior to

167 tick collection (Table 1: Spearman's rank correlation rho=-0.6721; p-value=8.955 x 168 10⁻⁵): larvae could be abundant (n>50) only when the mean temperature did not rise 169 above 12°C over the previous 10 days (R² of the corresponding meteorological 170 model=0.4693)(Fig. 4a). Day length was more correlated to larval counts (Spearman's 171 rank correlation rho=-0.8078; p-value= 2.027×10^{-7}): larvae could be abundant (n>50) 172 only when day length was below 12 h (R^2 of the corresponding seasonal model=0.7531) 173 (Fig. 4b). Meteorological and combined models (with or without interaction) exhibited a 174 higher AIC than the seasonal model: the seasonal model was therefore selected for *I*. 175 frontalis larvae, day length explaining 75.3% of the variance (Table 2). In comparison, 176 the same process led to the selection of the meteorological model for *I. ricinus* larvae, mean temperature over 10 days prior to tick collection explaining 53.3% of the variance 177 178 (Table 1, Fig. 4c and 4d, Table 2).

Besides *I. frontalis* larvae, for which thousands of individuals were found, only a few individuals of *I. frontalis* were identified at the nymphal (n=28) and adult (n=5, all males) stages, with no clear seasonality (Fig. 3b, 3c and Supplementary material I). The relative rarity of those nymphal and adult stages of *I. frontalis* contrasts with the abundant collection of those stages for *I. ricinus* ticks (Supplementary material I).

184 Spatial distribution of I. frontalis

185 *I. frontalis* stages were clustered in 3 main transects (n°8, 9, 10), with occasional

186 presence (in decreasing order of importance) on transects n°5, n°2, n°3, n°6 or n°7

187 (Supplementary material I). The distribution of *I. frontalis* was significantly different

188 from that of *I. ricinus* when considering on the one hand transects with bamboo (n°8 to

189 10) and on the other hand other transects (n°1 to 7) (Fisher's exact tests: $p < 2.2 \times 10^{-16}$

190 for larvae, p=6.9 x 10⁻⁶ for nymphs and p=0.0161 for adults)(Table 3). Considering the

191 ratio of *I. frontalis* versus *I. ricinus*, places with bamboo appeared to be more favourable 192 to *I. frontalis*, with the highest contrast for larvae (O.R.=842 (C.I.95%=[427-1905])), 193 followed by nymphs (O.R.=8 (C.I.95%=[3-31])) and adults (O.R. incalculable because of 194 the absence of *I. frontalis* adults on places without bamboo)(Table 3). On transects with 195 bamboo, *I. frontalis* larvae were predominant compared to *I. ricinus* larvae (representing 196 82% of the captures), while *I. frontalis* nymphs and adults represented respectively 2% 197 and 19% of the captures. On transects without bamboo, *I. frontalis* larvae and nymphs 198 represented respectively 0.5% and 0.3% of the captures, and adults were absent (Table 199 3).

200

201 4. Discussion

Our study on the seasonal activity of exophilic hard ticks yielded unique information on *I. frontalis*, an ornithophilic tick with a largely unknown ecology. To the best of our
knowledge, this is the first description of the phenology of the free stages of *I. frontalis*.
The most remarkable feature consists in an abundant population of *I. frontalis* questing
larvae during autumn-winter. Moreover, the distribution of *I. frontalis* appeared highly
aggregated, with favourable sites consisting in transects surrounded and overhung by
bamboo.

209 Abundance and recurrence of questing stages of I. frontalis

210 *I. frontalis* was firmly established at our study site, as evidenced by long-term presence

- 211 (up to 36 months, from June 2014 to May 2017) of the three questing developmental
- stages, with 2218 confirmed *I. frontalis* larvae (from January 2015 to May 2017:
- 213 Supplementary material I), 28 nymphs and 5 adults (males). To the best of our

knowledge, this abundance has never been described before for *I. frontalis* questing ticks,
especially for the larval stage. In other studies in Europe, *I. frontalis* has been collected
by drag sampling, but only at the nymphal and adult stages (France: Doby, 1998; Gilot et
al., 1997, 1995 - Spain: Barandika et al., 2006 - Germany: Schorn et al., 2011 - Slovakia:
Bona and Stanko, 2013 - Italy: Ceballos et al., 2014). Therefore, *I. frontalis* questing
larvae may be more abundant than one would assume, due to confusion with *I. ricinus*(Heylen et al., 2014a).

221 Phenology of I. frontalis

222 In our study, questing adults and nymphs were present sporadically throughout the year. 223 The larval stage, however, showed a marked seasonality, with an onset of questing 224 activity in September, followed by a peak in October-November, a slow decrease in 225 winter and an absence of activity in summer. Current literature reports only on the 226 phenology of host-feeding individuals in the wild (Doby, 1998; Norte et al., 2015, 2012). 227 Doby (1998) described the feeding activity of *I. frontalis* (by the time synonymized with 228 « *Ixodes pari* ») on blackbirds (*Turdus merula*) in France. The three parasitic life stages 229 were found constantly between late autumn and early winter, but not during summer. 230 The studies in Portugal by Norte et al. (2015, 2012) found that feeding activity takes 231 place between October and March. Larval activity peaks in November and secondary 232 peaks are observed in February or August, whereas nymphal peak activity is found in 233 December. The activity pattern of those bird-feeding ticks is consistent with the 234 autumnal peak of questing larvae found at our site (Fig. 3).

235 *Life cycle duration of* I. frontalis

236 The understanding of the entire life cycle duration of *I. frontalis* is still debated. Doby 237 (1998) suggested a one-year life cycle, based on the developmental durations of ticks 238 obtained from experimental bird infestations. Also Heylen (unpublished results) 239 suggests that the life cycle may take only one year, if ticks have the opportunity to feed 240 soon after moulting. He monitored ticks in a breeding colony that was initiated for 241 vector-competence experiments (Heylen et al., 2017b, 2014b) and morphological 242 analysis (Heylen et al., 2014a). Still, if ticks have to wait for the host or enter diapause, 243 the life cycle will probably take much longer. A one-year life cycle is compatible with our 244 field data, where a clear seasonality is observed for the questing larvae (Fig. 3a), which 245 could correspond to the interval between two generations. However, as Doby (1998) 246 found the three stages on hosts at the same time (between late autumn and early 247 winter), it could suggest a longer life cycle. Further controlled monitoring studies under 248 quasi-natural conditions are clearly needed to deduce the real life cycle duration of *I*. 249 *frontalis*, by measuring the tick development duration of each stage and their longevity 250 between blood meals.

251 Determinants of the phenology of questing stages of I. frontalis

252 We found that day length, rather than meteorological conditions, showed the strongest 253 correlation with larval counts of *I. frontalis*: a higher abundance of larvae was observed 254 on short days, suggesting a main influence of regular annual determinants. On the 255 contrary, we found that the questing activity of the larval stage of *I. ricinus* was mainly 256 driven by temperature, with an increase in the population in warm conditions. Indeed, 257 questing behaviour in this species is known to be influenced by meteorological 258 conditions - at least for the nymphal stage (Cat et al., 2017; Jensen, 2000; Kiewra et al., 259 2014; Perret et al., 2003, 2000; Schulz et al., 2014; Walker, 2001). Because I. frontalis

260 larvae are active during autumn and winter, we assume that the determinant for the 261 phenology of this tick species may be different from *I. ricinus*. We hypothesize that the 262 autumn-winter peak of *I. frontalis* larvae may correspond to the feeding of female ticks 263 on birds after bird reproduction (*i.e.*, in summer) during a short period. This would 264 induce a synchronization of egg development, with a fast increase in the larval 265 population, as we observe. It would be consistent with the observation in the UK of a 266 peak in August-September of tick-related syndrome cases on birds due to *I. frontalis* 267 adult females (Monks et al., 2006), even if the situation may be different in other parts of 268 the distribution area of *I. frontalis* (such as *I. ricinus*, showing a different phenology in 269 western versus central Europe: Kurtenbach et al., 2006). For I. ricinus, the feeding of 270 females is known to occur during a long spring period (Randolph 2004), leading to a less 271 synchronized egg development and hence perhaps a greater influence of temperature on 272 larval emergence in summer. However, for *I. frontalis*, alternative hypotheses such as 273 egg or larval diapause or delayed egg hatching cannot be excluded before conducting 274 additional investigations.

275 Spatial distribution of questing stages of I. frontalis

276 Some transects exhibited frequent high densities of *I. frontalis*, especially for the larval 277 stage. Despite being distant from each other, the transects shared the same physical 278 characteristics with bamboo surrounding and overhanging the ground. The literature 279 reports that the physical characteristics of the collection sites for questing *I. frontalis* are 280 very similar to biotopes where *I. ricinus* can be found (Ceballos et al., 2014; Cringoli et al., 281 2005; Gilot et al., 1997, 1995; Schorn et al., 2011). To our knowledge, the presence of I. 282 frontalis in bamboo bushes has never been described before. At our study site, we 283 suspect that the abundance of *I. frontalis* under bamboo is a consequence of bird

284 attendance. Bamboo may be a favourable place for birds to roost overnight, difficult to 285 reach for predators. However, bamboo does not seem favourable for bird nesting, 286 because of the lack of solid forked branches, hindering the attachment of nests. Thus, we 287 hypothesize that the timing of detachment of engorged female ticks under bamboo is 288 distinct from the reproduction period of birds, even if we have no data on the bird host 289 species involved for the maintenance of the population of *I. frontalis* at our study site. *I.* 290 *frontalis* is known to infest a wide range of birds, because virtually any bird species is 291 submitted to parasitism when it walks on the ground (Doby, 1998; Hornok et al., 2016). 292 A good candidate species could be the blackbird (*Turdus merula*), which has been 293 observed at the site. It presents both the highest infestation prevalence and the highest 294 tick burden in the literature (Doby, 1998; Norte et al., 2015). This point could be studied 295 in the future, along with potential pathogen transmission consequences.

296 Conclusion

297 We have demonstrated that I. frontalis and I. ricinus have different phenologies and 298 occupy distinct habitat types at our study site. Nevertheless, we have also shown that 299 they can co-occur both in time and space (even at a very limited spatial scale of 10 m²), 300 indicating a possibility for those two tick species to parasitize the same host individuals 301 and thus to share pathogens. We thus strongly encourage researchers to systematically 302 identify the larval stage of hard ticks found on the ground. Even if *I. ricinus* is the most 303 common species collected in Europe with the drag sampling method, *I. frontalis* may 304 have been underestimated, especially during autumn and winter. A pathogen such as 305 *Borrelia turdi*, belonging to the *Borrelia burgdorferi* s.l. group, can have a prevalence of 306 14.8% in some *I. frontalis* populations (Literak et al., 2015) and the vector competence 307 of I. ricinus for this Borrelia species has been recently demonstrated experimentally

308	(Heylen et al., 2017b). The generalist tick <i>I. ricinus</i> could thus function as a bridging
309	<mark>species from <i>I. frontalis</i> hosts, allowing</mark> some pathogens to spill over and reach hosts
310	other than birds, including humans or domestic animals. The spatio-temporal overlap
311	between <i>I. ricinus</i> and <i>I. frontalis</i> may also be influenced by the currently observed
312	climate change and thus influence the epidemiology of the tick-borne diseases
313	associated with those ticks, an issue that should be investigated through dedicated
314	studies.
315	
216	Declarations of interest
210	Decial ations of interest

- 317 None.
- 318

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536	Legends to figures
537	Fig. 1. Geographical position (*) of the study site in France (left). Aerial photograph of
538	the observatory with position (\odot) of the meteorological station (center). Two transects,
539	illustrating transects without (n°1) or with (n°9) bamboo (right).
540	
541	Fig. 2. Distinction of Ixodes frontalis (a) and Ixodes ricinus (b) at the larval stage (light
542	microscopy).
543	
544	Ixodes frontalis larvae (a) have a marked protuberance on the front margin of the basis
545	of the capitulum, between palp and hypostome (black arrow), external spurs on coxa I, II
546	and III (grey arrows), and an internal spur on coxa I (white arrow).

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547 *Ixodes ricinus* larvae (b) have a straight front margin of the basis of the capitulum,

548 between palp and hypostome (black arrow), small external spurs on coxa I and II (grey

549 arrows), and an internal spur on coxa I (white arrow).

550

Fig. 3. Temporal distribution of questing stages of *Ixodes frontalis* and *Ixodes ricinus*.

a) larvae, b) nymphs, c) adults.

553

554 For larvae, the scale is logarithmic, and 1 was added to the counts (n): the baseline («1» 555 on the Y axes) corresponds to the absence of larva. Note that larvae collected before 556 27/01/2015 were not identified, but (1) those collected on transect n°9 until 557 29/12/2014 are represented by «?» as they were presumed to belong to *I. frontalis* (see 558 text and Supplementary Material I and II), (2) and those collected on transect n°9 on 559 27/01/2015 are represented by \triangle as they were confirmed for the first time as *I. frontalis*. 560 From 18/02/2015 to 16/05/2017, all the transects were considered because larval 561 identification was systematic. For larvae only, shaded grey areas highlight the 562 seasonality of *I. frontalis* during autumn seasons. ^x and a dashed black line represents *I. ricinus*. ° and a continuous red line represents *I. frontalis*. 563 564 565 Fig. 4. Relationship between mean temperature over 10 days prior to tick collection (a, 566 c) or day length (b, d) and *Ixodes frontalis* (a, b) or *I. ricinus* (c, d) larval counts (n+1). 567 568 The relationship between mean temperature (over 10 days prior to tick collection) and 569 *Ixodes frontalis* or *I. ricinus* larval counts (on the day of tick collection) was considered 570 from February 2015 to May 2017 (28 dates) for the whole observatory. The same set of 571 data was used for the influence of day length. To represent larval counts on a 572 logarithmic scale, 1 had to be added to the counts (n): the baseline (« 1 » on the Y axes) 573 corresponds to the absence of larva.









b)

Mean temperature (°C) over 10 days prior to tick collection

a)

Day length (h) on the day of tick collection

Table 1

Relationship between *Ixodes frontalis* or *Ixodes ricinus* larval counts and meteorological variables or day length, attested by Spearman's rank correlation.

a) Ixodes frontalis

	0-30 days*	0-10 days*	0-5 days*
Mean hygrometry	p=0.003	p=0.008	p=0.008
	rho=0.534	rho=0.494	rho=0.488
Mean temperature	p=0.002	p<0.001	p<0.001
	rho=-0.565	rho=-0.672	rho=-0.645
Mean wind speed	p=0.657	p=0.407	p=0.597
	rho=-0.088	rho=-0.163	rho=-0.104
Mean saturation	p=0.001	p<0.001	p<0.001
deficit	rho=-0.597	rho=-0.623	rho=-0.616
Day length (on the day of tick collection)		<u>p<0.001</u> rho=-0.808	

b) Ixodes ricinus

-						
	0-30 days*	0-10 days*	0-5 days*			
Mean hygrometry	p=0.002	p=0.010	p=0.048			
	rho=-0.560	rho=-0.4798062	rho=-0.377			
Mean temperature	p<0.001	<u>p<0.001</u>	p<0.001			
	rho=0.668	rho=0.717	rho=0.680			
Mean wind speed	p=0.108	p=0.039	p=0.030			
	rho=-0.310	rho=-0.392	rho=-0.412			
Mean saturation	p<0.001	p<0.001	p=0.002			
deficit	rho=0.704	rho=0.599	rho=0.553			
Day length (on the day of tick collection)		p=0.004 rho=0.527				

* prior to the day of tick collection

For each meteorological factor and time interval prior to tick collection, and for day length on the day of tick collection, the relationship with larval counts was estimated by Spearman's rank correlation, with indication of p-value (p) and rho (rho). Cells in grey indicate a significant correlation ($p \le 0.05$). For each meteorological factor, the cell in bold indicates the most relevant time interval to be considered. The cell with the best correlation is underlined.

Meteorological variables, day length and larval counts were considered from February 2015 to May 2017 (28 dates) for the whole observatory.

Table 2

Summary of general linear models explaining log-transformed *Ixodes frontalis* and *Ixodes ricinus* larval counts (log(larval count+1))

		Ixodes frontalis						Ixodes ricinus							
		Estimate	SE	t	р		R ²	AIC	Estimate	SE	t	р		R ²	AIC
Meteorological model	Intercept Mean temperature (0-10 days)°	6.18125	0.81965 0.06507	7.541 -4.795	< 0.001 < 0.001	***	0.4693	110.1175	-1.36635 0.31930	0.73859 0.05864	-1.850 5.445	0.0757 < 0.001	•	0.5328	104.2857
Seasonal model	Intercept Day length#	11.11442 -0.69772	0.98589 0.07835	11.274 -8.905	< 0.001 < 0.001	***	0.7531	88.69496	-2.5494 0.3997	1.6302 0.1296	-1.564 3.085	0.1300 < 0.01	**	0.268	116.8585
Combined model	Intercept Mean temperature (0-10 days)° Day length#	10.91245 -0.04765 -0.63600	1.03358 0.06595 0.11640	10.558 -0.723 -5.464	< 0.001 0.477 < 0.001	***	0.7388	90.11617	-1.14317 0.33178 -0.03000	1.37854 0.08796 0.15525	-0.829 3.772 -0.193	0.4148 < 0.001 0.8483	***	0.4962	106.2439
Combined model with interaction	Intercept Mean temperature (0-10 days)° Day length# Interaction	7.29429 0.29308 -0.33442 -0.02665	3.36400 0.30869 0.29100 0.02359	2.168 0.949 -1.149 -1.130	< 0.05 0.3519 0.2618 0.2698	*	0.7416	90.66578	-2.81454 0.48917 0.10931 -0.01231	4.59050 0.42124 0.39709 0.03219	-0.613 1.161 0.275 -0.382	0.5460 0.2570 0.7850 0.7060		0.4784	108.0738

° prior to the day of tick collection.

on the day of tick collection.

For each explanatory variable, the parameter estimate, the standard error (SE), the *t* value (test statistics) and the *p* value (significance codes: 0 '***' 0.001 '*' 0.01 '*' 0.05 '.' 0.1 ' 1) are given.

For the whole model, R² and AIC are given.

For *Ixodes frontalis*, the seasonal model was selected as the best model according to AIC (in bold).

For Ixodes ricinus, the meteorological model was selected as the best model according to AIC (in bold).

Table 3

Habitat preference of *Ixodes frontalis* compared to *Ixodes ricinus* on two types of transects: with bamboo *versus* without bamboo.

a) cumulative larval counts*

* Larvae were systematically identified on each transect from 18/02/2015 to 16/05/2017 (last 28 dates): only these dates were considered.

Cumulative larval counts (last 28 dates: 18/02/2015 to 16/05/2017)	Transects with bamboo (n°8, n°9, n°10)	Transects without bamboo (n°1, n°2, n°3, n°4, n°5, n°6, n°7)				
Ixodes frontalis	1720	8				
Ixodes ricinus	376	1487				
b) cumulative nymphal counts						
Cumulative nymphal counts	Transects with bamboo	Transects without bamboo				
(36 dates: 26/06/2014 to 16/05/2017)	(n°8, n°9, n°10)	$(n^{\circ}1, n^{\circ}2, n^{\circ}3, n^{\circ}4, n^{\circ}5, n^{\circ}6, n^{\circ}7)$				
- Ixodes frontalis	24	4				
Ixodes ricinus	1088	1436				
c) cumulative adult counts						
Cumulative adult counts	Transects with bamboo	Transects without bamboo				
(36 dates: 26/06/2014 to 16/05/2017)	(n°8, n°9, n°10)	(n°1, n°2, n°3, n°4, n°5, n°6, n°7)				
- Ixodes frontalis	5	0				
Ixodes ricinus	22	32				