Unravelling the phenology of Ixodes frontalis, a common but understudied tick species in Europe

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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 October-November 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 *I. 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 *I. frontalis* mostly collected under bamboo bushes.

1. Introduction

Vector-borne diseases constitute a major concern for both human and animal health. In temperate zones, hard ticks are considered to be the most important vectors in terms of diversity of pathogens, including zoonotic agents (such as *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, tick-borne encephalitis virus, *Babesia* spp.). They are 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 pathogens are complex, involving generally several host species and sometimes more than one tick species (Estrada-Peña and de la Fuente, 2014; Kurtenbach et al., 2006; Mannelli et al., 2012; McCoy and Boulanger, 2015). Therefore, the study of a particular tick-borne pathogen requires an integrated community approach, in a diversity of host 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

66   distance but also in urbanised regions that are less accessible to migrating mammals (de

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 *Ixodes*

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

81 size (Heylen et al., 2014a). *I. frontalis* is also known to be implicated in direct (*i.e.,*

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**

92 A study site was selected in spring 2014 near Nantes (47°19'N, 01°29'W) to survey

93 exophilic hard tick densities on vegetation, estimated with the drag sampling method: a

94 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

102 (Bord et al., 2014). On each transect, a 1 m^2 white flannel cloth was slowly dragged (0.5

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).

117 The ground of the seven other transects (hereafter called "Transects without bamboo":

118 n°1 to 7) was either a litter of deciduous (n°2, n°3, n°5) or coniferous needle leaves

119 (n°5), or short (<15 cm) grass (n°1, n°4, n°6, n°7)(Fig. 1).

5

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   R^2. 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.

6

144   The distribution of the different stages of *I. frontalis* was described at the study site for

145   each transect to evaluate spatial distribution. It was compared to *I. ricinus* on two types

146   of transects: with bamboo *versus* without bamboo. Comparisons were performed by

147   bilateral Fisher's exact test. All analyses were carried out in R version 3.4.1 (R Core

148   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 <mark>assumed</mark> 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.

165   All meteorological variables were correlated to *I. frontalis* larval counts, except mean

166   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 (R^2 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 x 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,

177 mean temperature over 10 days prior to tick collection explaining 53.3% of the variance

178 (Table 1, Fig. 4c and 4d, Table 2).

179 Besides *I. frontalis* larvae, for which thousands of individuals were found, only a few

180 individuals of *I. frontalis* were identified at the nymphal (n=28) and adult (n=5, all

181 males) stages, with no clear seasonality (Fig. 3b, 3c and Supplementary material I). The

182 relative rarity of those nymphal and adult stages of *I. frontalis* contrasts with the

183 abundant collection of those stages for *I. ricinus* ticks (Supplementary material II).

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 x 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

4. Discussion

202   Our study on the seasonal activity of exophilic hard ticks yielded unique information on *I.*

203   *frontalis*, an ornithophilic tick with a largely unknown ecology. To the best of our

204   knowledge, this is the first description of the phenology of the free stages of *I. frontalis.*

205   The most remarkable feature consists in an abundant population of *I. frontalis* questing

206   larvae during autumn-winter. Moreover, the distribution of *I. frontalis* appeared highly

207   aggregated, with favourable sites consisting in transects surrounded and overhung by

208   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

212   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

214   knowledge, this abundance has never been described before for *I. frontalis* questing ticks,

215   especially for the larval stage. In other studies in Europe, *I. frontalis* has been collected

216   by drag sampling, but only at the nymphal and adult stages (France: Doby, 1998; Gilot et

217   al., 1997, 1995 - Spain: Barandika et al., 2006 - Germany: Schorn et al., 2011 - Slovakia:

218   Bona and Stanko, 2013 - Italy: Ceballos et al., 2014). Therefore, *I. frontalis* questing

219   larvae may be more abundant than one would assume, due to confusion with *I. ricinus*

220   (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 m^2),

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. ricinus* could thus function as a bridging

309 species from *I. frontalis* hosts, allowing some pathogens to spill over and reach hosts

310 other than birds, including humans or domestic animals. The spatio-temporal overlap

311 between *I. ricinus* and *I. frontalis* 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

316 **Declarations of interest**

317 None.

318

319 **Acknowledgments**

335

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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 (⊙) 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).

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

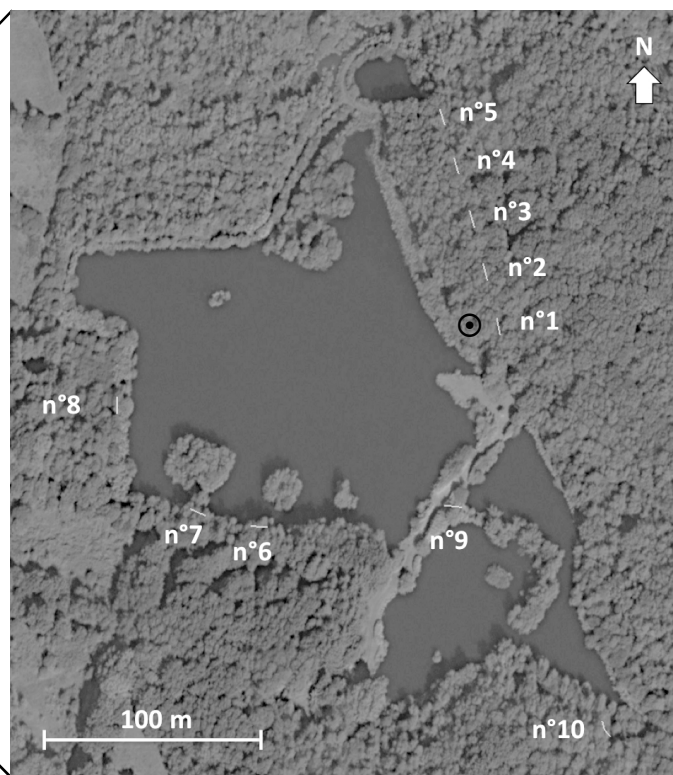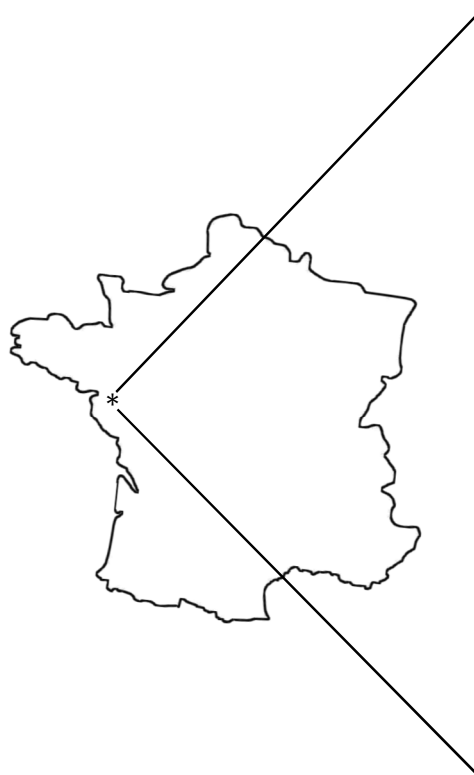549   arrows), and an internal spur on coxa I (white arrow).

550

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

552   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 Δ 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.*

563   *ricinus*. [°] and a continuous red line represents *I. frontalis*.

564

565   **Fig. 4.** Relationship between <mark>mean</mark> 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)
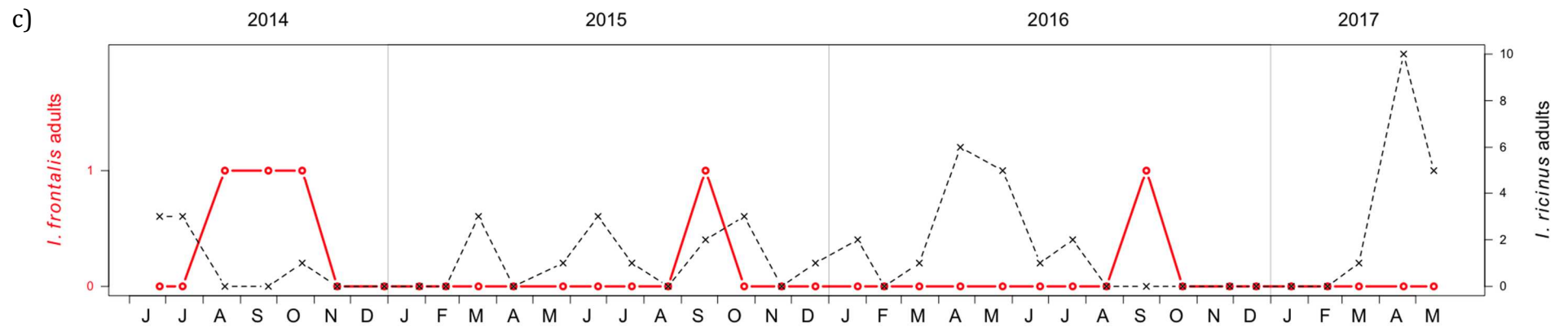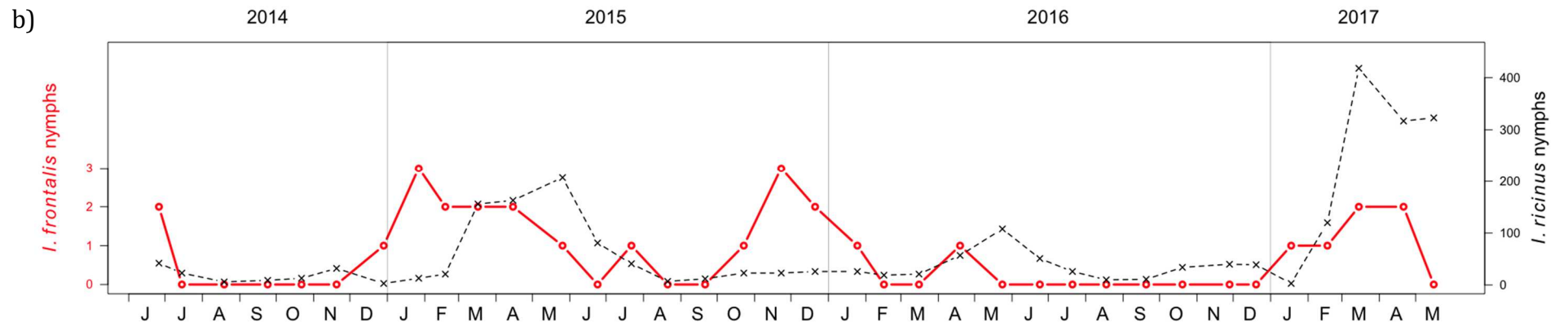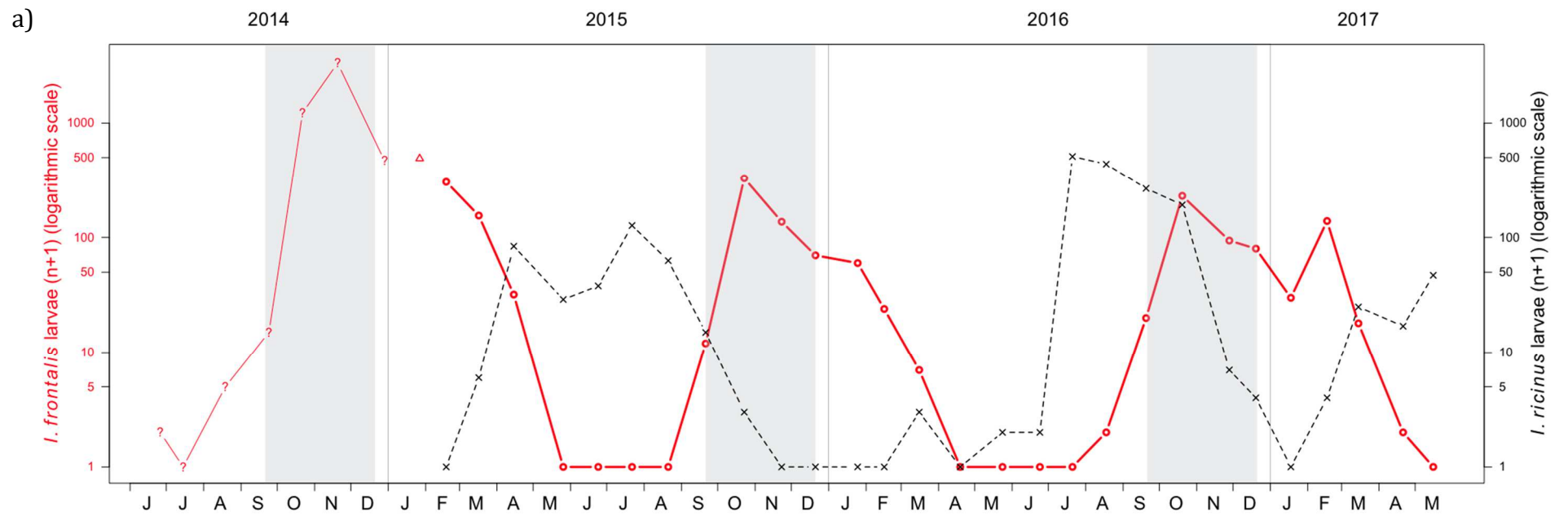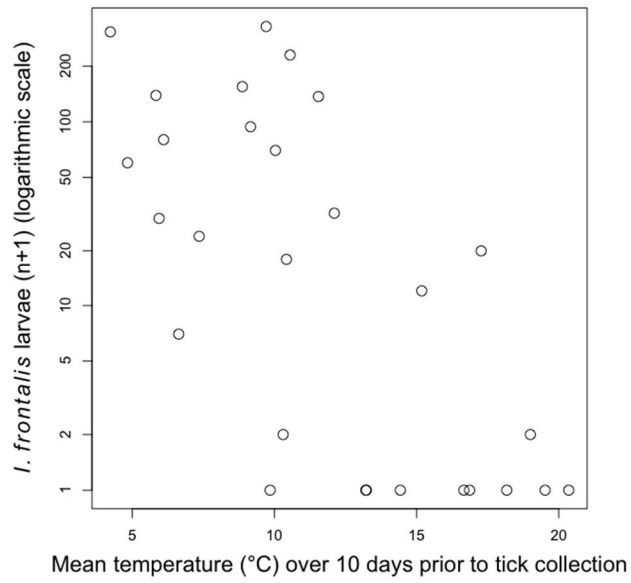
573   corresponds to the absence of larva.

N

n°5

n°4

n°3

n°2

n°1

n°8

n°7   n°6

n°9

n°10

100 m

n°1

n°9

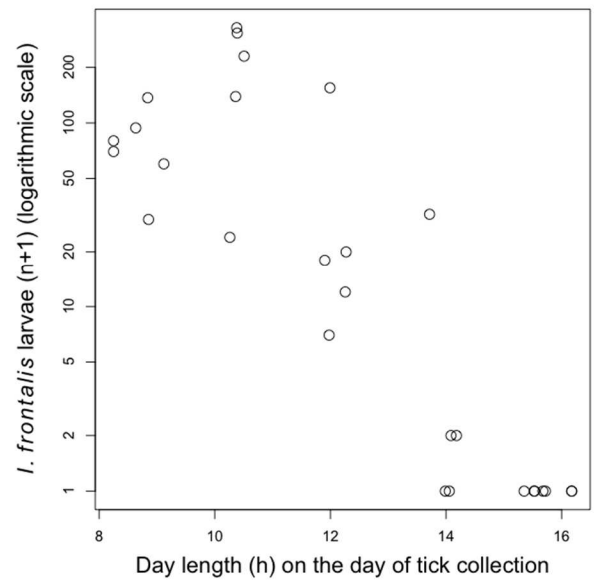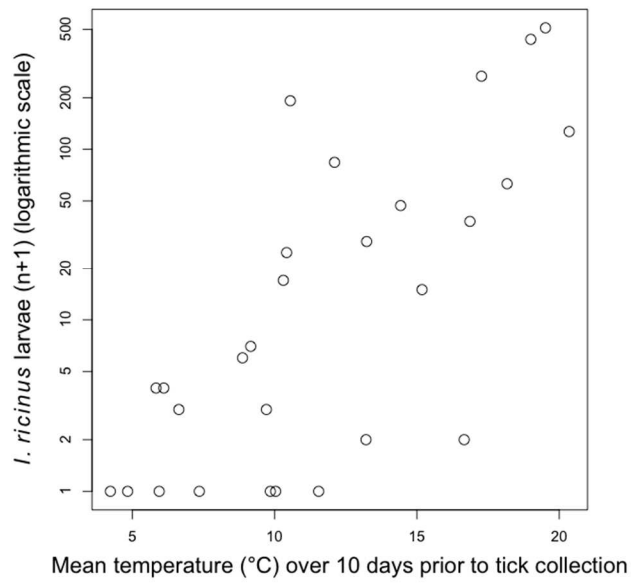a)   b)
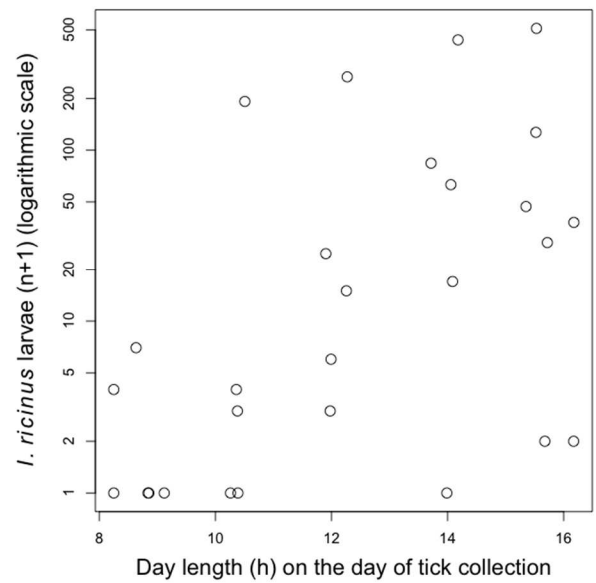
0.1 mm

a)

b)

c)

d)

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

b) *Ixodes ricinus*

	0-30 days*	0-10 days*	0-5 days*
Mean hygrometry	**p=0.002** **rho=-0.560**	p=0.010 rho=-0.4798062	p=0.048 rho=-0.377
Mean temperature	p<0.001 rho=0.668	**p<0.001** **rho=0.717**	p<0.001 rho=0.680
Mean wind speed	p=0.108 rho=-0.310	p=0.039 rho=-0.392	**p=0.030** **rho=-0.412**
Mean saturation deficit	**p<0.001** **rho=0.704**	p<0.001 rho=0.599	p=0.002 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≤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					R^2	AIC	*Ixodes ricinus*					R^2	AIC
		Estimate	SE	*t*	*p*				Estimate	SE	*t*	*p*			
Meteorological model	Intercept	6.18125	0.81965	7.541	< 0.001	***	0.4693	110.1175	**-1.36635**	**0.73859**	**-1.850**	**0.0757**	.	**0.5328**	**104.2857**
	Mean temperature (0-10 days)°	-0.31205	0.06507	-4.795	< 0.001	***			**0.31930**	**0.05864**	**5.445**	**< 0.001**	***		
Seasonal model	Intercept	**11.11442**	**0.98589**	**11.274**	**< 0.001**	***	**0.7531**	**88.69496**	-2.5494	1.6302	-1.564	0.1300		0.268	116.8585
	Day length#	**-0.69772**	**0.07835**	**-8.905**	**< 0.001**	***			0.3997	0.1296	3.085	< 0.01	**		
Combined model	Intercept	10.91245	1.03358	10.558	< 0.001	***	0.7388	90.11617	-1.14317	1.37854	-0.829	0.4148		0.4962	106.2439
	Mean temperature (0-10 days)°	-0.04765	0.06595	-0.723	0.477				0.33178	0.08796	3.772	< 0.001	***		
	Day length#	-0.63600	0.11640	-5.464	< 0.001	***			-0.03000	0.15525	-0.193	0.8483			
Combined model with interaction	Intercept	7.29429	3.36400	2.168	< 0.05	*	0.7416	90.66578	-2.81454	4.59050	-0.613	0.5460		0.4784	108.0738
	Mean temperature (0-10 days)°	0.29308	0.30869	0.949	0.3519				0.48917	0.42124	1.161	0.2570			
	Day length#	-0.33442	0.29100	-1.149	0.2618				0.10931	0.39709	0.275	0.7850			
	Interaction	-0.02665	0.02359	-1.130	0.2698				-0.01231	0.03219	-0.382	0.7060			

° 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, R^2 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 (36 dates: 26/06/2014 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	24	4
Ixodes ricinus	1088	1436

c) cumulative adult counts

Cumulative adult counts (36 dates: 26/06/2014 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	5	0
Ixodes ricinus	22	32