

1 Title: The drivers and consequences of unstable *Plasmodium* dynamics: A 26-year study
2 of three malaria parasite species infecting a tropical lizard

3 Running title: Long-term drivers and consequences of parasite dynamics

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20 *Abstract.* The dynamics of vector-borne diseases are driven by interactions between
21 factors intrinsic to the parasite, its host, and environmental fluctuations. Understanding
22 these interactions requires a long-term view, especially to predict the consequences of
23 climate change on parasite dynamics. We examined temporal fluctuations in risk of
24 infection, its environmental drivers and consequences for host body condition of three
25 malaria parasites (*Plasmodium azurophilum*, *P. leucocytica*, and *P. floridense*) infecting
26 the lizard, *Anolis gundlachi*, in the rainforest of Puerto Rico. Our analyses included 13
27 surveys totaling > 8000 lizards over 26 years. During the early years of the study,
28 prevalence of all three *Plasmodium* species appeared stable despite disturbances from
29 short droughts and hurricanes. Taking the longer view, we found that the risk of infection
30 and overall prevalence vary significantly over the years, and this fluctuation was driven
31 in non-linear ways by variations in temperature and rainfall. We also found that risk of
32 infection was maximized at intermediate levels of temperature and precipitation; relative
33 proportions of the three *Plasmodium* species changed over time; and no significant
34 negative effects of parasite infection on the lizard's body condition. Overall, the results
35 emphasize the need for long-term studies to reveal host-parasite dynamics, their drivers
36 and consequences.

37 Key-words: *Anolis*, body condition, climate change, long-term disease dynamics,
38 malaria, parasite community, precipitation, Puerto Rico, temperature, virulence

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INTRODUCTION

41 Understanding the drivers and consequences of infectious disease dynamics is a
42 venerable challenge in ecology. From the first theoretical models developed in the 18th

43 century as part of smallpox control efforts to the Ross-Macdonald modeling approach
44 that describes the dynamics of malaria prevalence (Smith et al. 2012), ecology has a long
45 tradition studying long-term disease dynamics. Yet, how abiotic and biotic factors drive
46 long-term disease risk and virulence remains an open question in disease ecology (Lively
47 et al 2014).

48 Malaria parasites (*Plasmodium*) are vector-borne, and thus sensitive to
49 environmental fluctuations. While malaria prevalence seems often stable and robust to
50 environmental fluctuations, it can also change abruptly during epidemics (Schall et al.
51 2000, Chiyaka et al. 2013). Theoretical models predict stability, but only under the
52 assumption of small environmental fluctuations; severe changes can push the system
53 outside its stable equilibrium (Scheffer et al. 2001; Rohr et al. 2011). Thus, nonlinear
54 links between prevalence and environmental terms can result in unstable dynamics. A
55 current pressing issue is the possible influence of predicted climate change on malaria
56 prevalence. Unfortunately, there is little consensus on the expected influence of climate
57 change on the dynamics of vector-borne parasites including *Plasmodium* (Harvell 2009,
58 Rohr et al. 2011).

59 Two connected issues are how changes in the dynamics of vector-borne parasites
60 may be tempered both by the life history traits of the parasite itself (the "intrinsic factors"
61 of epidemiology) and interactions among players in the overall parasite-host community.
62 This story becomes circular if the parasite's prevalence drives evolutionary changes in
63 both parasite and host life histories or changes the parasite community composition
64 (Karvonen et al. 2013). Theoretical models suggest that transmission intensity plays into
65 selection for parasite virulence (Ewald 1994, Schall 2002). These changes in virulence

66 can also mediate the outcome of competition among parasite species that share the same
67 host including parasite colonization and/or extinctions, or changing individual species
68 dominance within the community (e.g. Dobson and Pacala 1992, Karvonen et al. 2013).

69 Here we present a long-term study on the dynamics of three lizard malaria
70 parasites (*Plasmodium azurophilum*, *P. floridense*, and *P. leucocytica*) infecting *Anolis*
71 *gundlachi* in the tropical rainforest of Puerto Rico---a system that was undisturbed by
72 deliberate human intervention. Schall et al. 2000 studied the long-term dynamics of this
73 lizard-malaria system from 1990 to 1999 and found stable prevalence, no temporal
74 variation in virulence and stable parasite community composition. Here we leverage
75 their data (Schall et al. 2000), add extensive recent sampling, and reanalyze the data
76 taking a longer-term view spanning 26 years to ask: (1) Is parasite prevalence and risk of
77 infection stable during the 26 years' period? (2) If not, is this temporal variability driven
78 by abiotic factors (e.g., temperature and precipitation)? (3) Was parasite species
79 composition stable during this period? (4) Was there temporal variability in parasite
80 virulence to the host?

81 METHODS

82 *Study system, field sampling, and diagnostics*

83 We sampled lizards in a rainforest at the El Verde Field Station at the Luquillo
84 Experimental Forest in eastern Puerto Rico (N 18°19.263' - W 65°49.146'). *Anolis*
85 *gundlachi* is among seven anoles at the site, but the others are only very rarely infected
86 (Schall et al. 2000). We sampled during 13 periods over 26 years: summers (May-
87 August) 1990, 1996, 1997, 1998, 2015, 2016, and winters (January-March) 1991, 1997,

88 1998, 1999, 2001, 2002 and 2016. To insure consistency over time we replicated
89 rigorously the protocols of field sampling and diagnostics used in the original study by
90 Schall et al. (2000). Specifically, we sampled the same sites within the forest (between 4
91 and 10 trails each year) and collected similar sample sizes ($N > 650$). Infection status
92 was determined by examination of stained blood smears, and parasite species scored
93 based on morphological traits and cell class infected (Telford 2016).

94 We compiled temperature and rainfall data from the El Verde weather station
95 records and estimated the daily mean minimum, maximum, and variance of registered
96 temperatures and rainfall 30 and 120 days prior to the sampling month (Schall et al.
97 2000). Hurricane events occurred in September 1995 (two), July 1996, and September
98 1998. We do not include hurricanes *per se* in the analysis, but high rainfall would mark
99 those periods in the precipitation data. To estimate host body condition— a sub-lethal
100 measure of parasite virulence—we measure the snout-vent length (SVL) and mass of each
101 lizard. Detailed field and laboratory methods and collection locations are given in the
102 Supplemental Information.

103 *Analyses*

104 To determine if the risk of infection was stable through the 26-year study period, we
105 compared eight binomial models in their ability to predict the individual probability of
106 infection. Each model represents a hypothesis describing what factors better explained
107 temporal variability in infection probability. These models (with exception of the null
108 model) included sex, season and/or SVL as a predictor because Schall et al. 2000 found
109 strong evidence showing that the probability of infection increases with body size (SVL)
110 with males having a higher probability of infection than females (sex). They also found

111 that the probability of infection was higher in the summer than in the winter (season). We
112 compared models predicting the probability of infection as a function of (1) SVL, year,
113 and sex separately, (2) the additive effect of sex, SVL and season, (3) a similar model
114 with an additive effect of year (4) a model including the same sex, SVL and season with
115 an interactive effect between sex and SVL, (5) a similar model with an additive effect of
116 year, and (6) an intercept-only model that represented the null hypothesis that neither of
117 the tested variables explains the observed temporal variability in probability of infection.
118 These models were fitted using maximum likelihood and compared using Akaike
119 Information Criterion adjusted for small sample sizes (AICc), where the most
120 parsimonious model will result in the lowest score. If there was significant temporal
121 variation in the dynamics, the most parsimonious model (lowest AICc score) would
122 include the effect of sampling year.

123 We followed a similar model selection approach to test which environmental
124 variables (i.e., rainfall and/or temperature) better predicted the proportion of infected
125 individuals. Following Schall et al. 2000, we compared models that included mean
126 rainfall 30 days and 120 days before the field sampling. We also fitted models with the
127 variance of rainfall or temperature 30 or 120 days before the field sampling (Vasseur et
128 al. 2014). To allow for a possible non-linear effect we also fitted individual and additive
129 models with a second-degree polynomial. Last, we fitted a null (intercept only) model to
130 describe the case where none of the tested variables better explains the observed patterns
131 in prevalence. All models were fitted using maximum likelihood and we used AICc to
132 select the most parsimonious model.

133 To assess changes in composition of the three *Plasmodium* species through time

134 we compared 10 multinomial logit models in their ability to predict the individual
135 probability of being healthy, or infected by one of three *Plasmodium* parasites (four
136 categories; co-infections were not considered). This modeling approach is an extension
137 of a logistic regression for multinomial response variables. These models included (1) the
138 single effect of sex, SVL, or year; (2) models considering the additive and interactive
139 effect of year and SVL; (3) a model considering the additive effect of sex and SVL; (4)
140 the additive effect of these three variables, with either interactions of year with SVL and
141 sex. We also fitted a null (intercept-only) model that represents the case where any of
142 these variables explains the probability of getting infected by either of the three
143 *Plasmodium* parasites. The models were fitted using maximum likelihood using the nnet
144 package and compared using AICc.

145 To estimate body condition (a proxy for sub-lethal parasite virulence to the host;
146 Schall and Pearson 2000) we used the residual index R_i (Cox and Calsbeek 2015). We
147 calculated this index using the residuals of the linear regression of log₁₀ mass on log₁₀
148 SVL. We made a separate analysis for each sex, and for each year for which body mass
149 and SVL data were available (1996, 1997, 1998, 2015, and 2016). Lizards with positive
150 residuals are heavier than average (better body condition), while lizards with negative
151 residuals are skinnier relative to their SVL than average (Schall et al. 2000). To test for
152 differences in body condition due to infection state or time, we used a two-way ANOVA,
153 where the two factors were year and infection state (infected vs. non-infected). All
154 analyses were done in R 3.0.3 (R Development Core Team 2016).

155 RESULTS

156 *Long-term dynamics and its environmental drivers*

157 A total of 8055 *Anolis gundlachi* lizards were sampled over the 26-year period.
158 The most parsimonious model explaining the variability in the risk of infection in this
159 period included the additive effect of sex, SVL, season, year, and the interaction between
160 sex and SVL ($\Delta\text{AIC} = 8.58$ between this model and the next best; Table S1). For both
161 summer and winter samples, the risk of infection was relatively stable for the early years,
162 dropping significantly during the 2015–2016 period (Fig. 1, Table S2). Overall
163 prevalence dropped \sim half for males and $>$ half for females from 1990–2002 vs. 2015–
164 2016 (Fig. S1).

165 The most parsimonious model explaining temporal variations in the proportion of
166 infected individuals included the additive quadratic effect of mean maximum daily
167 temperature and mean daily rainfall through 120 days before the sampling ($\Delta\text{AIC} = 90.37$
168 between this model and the next best; Table S3). The risk of infection followed a
169 nonlinear response to temperature at a variety of rainfall levels (Fig. 2, Table S4).
170 Maximal predicted risk of infection was maximized at a minimum temperature of $\sim 26^\circ$
171 and rainfall of 9.62 mm.

172 *Long-term changes in parasite species composition*

173 The most parsimonious model explaining the probability of an individual being
174 healthy, or infected by *P. azurophilum*, *P. leucocytica* or *P. floridense* included the
175 additive effect of SVL, sex and year ($\Delta\text{AIC} = 3.29$ between this model and the next best;
176 Table S5). This model predicts a decrease in the probability of infection of all
177 *Plasmodium* parasites after 2002 (Fig. 3). Thus, the drop in prevalence during the most
178 recent sample periods was not due to only one species of parasite declining, but an
179 overall decline. *P. azurophilum* was consistently the most common species, and remained

180 at a similar proportion of all infections throughout the 26-year period. *P. floridense* and
181 *P. leucocytica* changed their relative dominance, but this apparently was due to a secular
182 decline in *P. floridense* over the entire study period. This model predicted no differences
183 in the partial relationship between SVL and probability of infection by the different
184 *Plasmodium* parasites (Fig. S4). Whereas the model predicts little differences between
185 sexes in the probability of getting infected by *P. azurophilum*, the probability of getting
186 infected by *P. floridense* and *P. leucocytica* is predicted to be greater in females (Fig.
187 S5).

188 *Long-term changes in the body condition due to infection*

189 The body condition index (BCI) of females in the summer did not vary
190 significantly by infection state ($F_{1,753} = 3.84$, $P = 0.05$), year ($F_{3,753} = 0.039$, $P = 0.99$) or
191 their interaction ($F_{3,753} = 0.56$, $P = 0.64$; Fig. S6). We found a similar pattern in males in
192 the summer season where BCI did not vary significantly by infection state ($F_{1,1357} =$
193 0.042 , $P = 0.84$), year ($F_{3,753} = 0.001$, $P = 1.0$) or their interaction ($F_{3,753} = 0.530$, $P =$
194 0.66 ; Fig. S6). The pattern in the winter season resembles that of the summer where BCI
195 for females did not vary significantly by infection state ($F_{1,356} = 3.01$, $P = 0.084$), year
196 ($F_{2,356} = 0.12$, $P = 0.89$), or their interaction ($F_{1,356} = 1.36$, $P = 0.24$; Fig. S6). BCI for
197 males in the winter did not vary significantly by infection state ($F_{1,752} = 0.42$, $P = 0.52$),
198 year ($F_{2,752} = 0.01$, $P = 0.99$), or their interaction ($F_{2,752} = 0.36$, $P = 0.70$; Fig. S6).

199 DISCUSSION

200 We assembled a long-term data set spanning 26 years to explore the dynamics of three
201 malaria parasites and their vertebrate host. This is one of the longest of such studies on a

202 natural system of a vector-borne disease infecting a nonhuman host (see also Schall and
203 St. Denis 2010). Our results show that risk of infection for the anole by *Plasmodium*
204 parasites varied little early in the study despite several short droughts and hurricane
205 events. Prevalence of all three *Plasmodium* species, though, declined substantially during
206 the most recent years. Overall, variation in risk of infection was associated with
207 fluctuations in temperature and rainfall four months before each sample period, but the
208 relationship with temperature was nonlinear such that maximum risk of infection is
209 predicted at an intermediate temperature and rainfall. The recent drop in risk of infection
210 coincided with a severe drought in Puerto Rico. During the study, one species, *P.*
211 *azuorophilum* was consistently the most common parasite, but one of the others, *P.*
212 *floridense*, declined slowly. Finally, we found that body condition did not fluctuate
213 significantly over time for both summer and winter periods and was not significantly
214 different between non-infected and infected individuals. Overall, these patterns were not
215 apparent during the early years of the study; thus, a long-term approach was needed to
216 reveal the true response of the parasite dynamics to environmental changes.

217 The delayed link between rainfall and temperature with parasite prevalence (120
218 days) most likely is a consequence of shifting vector abundance and biting behavior.
219 Unfortunately, the identity of vector(s) of the three parasite species is not known, but *P.*
220 *floridense* is known to infect *Culex* mosquitoes in Florida (Klein et al. 1987). During the
221 severe 2015 drought we noted an overall dry aspect to the forest, with little standing
222 water in puddles, *Heliconia* flowers or epiphytic bromeliads, which could have restricted
223 breeding locations for mosquitoes. Most striking, despite the general effect of variation
224 in temperature and humidity over the 26-year period, no dramatic effect on prevalence,

225 nor risk of infection, was seen after short environment disruptions from dry periods or
226 severe hurricanes during the first 10 years of the study period. Schall et al. (2000) noted
227 that the hurricanes caused severe physical damage to the forest, including massive
228 defoliation. Thus, the drop in risk of infection in the later samples could indicate that the
229 2015 drought was of sufficient duration to shift the system to a new dynamic state.

230 But, what caused the differences in parasite prevalence for the summer vs. winter
231 seasons? Rainfall is greater in the summer, which increases potential habitats for vector
232 breeding increasing the rate of vectors to hosts. Also, summer is the mating season for *A.*
233 *gundlanchi* anoles when lizards are more active and defending territories (Reagan 1992,
234 Gorman and Licht 1974). If the vectors are daytime active, the lizards could be more
235 exposed to vector bites. Alternatively, the lower prevalence in winter may be only
236 apparent if the parasite reduces reproduction in the lizard blood if vector activity ceases.
237 Using a PCR-based method to detect very low-parasitemia infections and cryptic
238 infections were more common in the winter season (unpublished data).

239 Our study adds to the growing evidence that the relation between prevalence of
240 malaria parasites and climatic measures is complex, and often nonlinear (Mordecai et al.
241 2013, Garamszegi 2011). Adding to the complexity is the role of spatial scale (Wilkinson
242 et al. 2016). For example, in the *Sceloporus occidentalis-Plasmodium mexicanum* system
243 in California, when looking at the relationship between landscape features and infection
244 prevalence, the type of ground cover (grass, rocks, and leaf litter) affected the probability
245 of capturing infected or healthy lizards (Eisen and Wright, 2001). Note that changes in
246 temperature and rainfall could alter microhabitat quality, such as production of leaf litter
247 and ground cover. Thus, suggestions that simple rising temperatures during climate

248 change will increase prevalence of malaria parasites ignore the more complex story.
249 Indeed, our data suggest anole's risk of infection by malaria parasites may decrease in
250 current climate change scenarios.

251 The El Verde lizard malaria system has an added interest because we could follow
252 through time the relative proportions of three species of *Plasmodium* that infect a single
253 lizard host. Despite the major drop in the probability of infection during the later sample
254 periods, and the major disruption of the forest by hurricanes in the early years, *P.*
255 *azuophilum* remained at a constant higher proportion of infections. *P. floridense*
256 gradually changed over the years and switched proportion of infections with the third
257 species, *P. leucocytica*. What could account for this secular change? These two species
258 infected different cell classes (erythrocytes for *P. floridense* and several classes of white
259 blood cells for *P. leucocytica*), and therefore are not likely to be competitors for within-
260 host habitats, and are unlikely to cross-react with the immune system. The competitive
261 interactions of malaria parasite species that exploit the same host are poorly studied (but
262 see Schall and Bromwich, 1994). We suspect the explanation falls to the biology of the
263 vectors, either different insect host species or how parasites may compete within a single
264 vector.

265 Body condition is used as a proxy of the energetic state of lizards and their fitness
266 (Cox and Calsbeek 2015). Our results are consistent with previous studies that found
267 little evidence of a relationship between lizard health and parasite infection in the
268 Caribbean (Schall and Pearson 2000; Schall and Staats 2002). This may be explained by
269 two non-mutually exclusive explanations. First, parasitaemia of Caribbean *Plasmodium*
270 species is lower than those of California or Africa, which may result in a lower rate of

271 blood cells turnover. Second, low virulence could be the result of lower transmission
272 intensity as predicted by the theory of virulence evolution (e.g., Ewald 1994).

273 We conclude that the most significant finding of this study is the value, even the
274 necessity, of a long-term approach (> 20 yrs) to understanding the ecology of host-
275 parasite systems. Studying the *A. gundlachi* - *Plasmodium* spp. system over decades
276 allowed us to gain a better picture of the patterns and possible mechanisms behind
277 prevalence changes, shifts in the blood parasite community, and the effects on the body
278 condition of the hosts. The influence of environmental variables, exactly the kind
279 expected with ongoing climate change, became apparent only after taking this long-term
280 approach.

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288

289 LITERATURE CITED

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361

362 **Figure captions:**

363 **Fig 1.** Risk of infection of *Anolis gundlachi* by three malaria parasite species over time
364 at a rainforest in eastern Puerto Rico. Risk of infection is higher in the summer vs. winter
365 months. Note how the risk of infection is relatively constant in the early years, but
366 decreases in 2015–2016. Dots are the infection probability estimates and bars represent
367 the standard errors.

368 **Fig 2.** Predictions of the most parsimonious model explaining the temporal variation in
369 the proportion of infected *Anolis gundlachi* by malaria parasites. This model predicted
370 the proportion of infected individuals by the additive effect of squared mean daily rainfall
371 and mean daily temperature 120 days before sampling. Lines represent estimated means
372 and shaded areas 95% confidence intervals.

373 **Fig 3.** Partial predictions from the most parsimonious multinomial model showing a
374 general decrease through time in the risk of infection of *Anolis gundlachi* by three
375 *Plasmodium* parasites. The model predicts a decrease in the probability of infection by *P.*
376 *floridense* in the last three censuses compared to *P. leucocytica*. Dots represent the
377 infection probability estimates by each *Plasmodium* species and the bars represent 95%
378 confidence intervals. The letters “w” and “s” following the year describes the winter of
379 summer season respectively.

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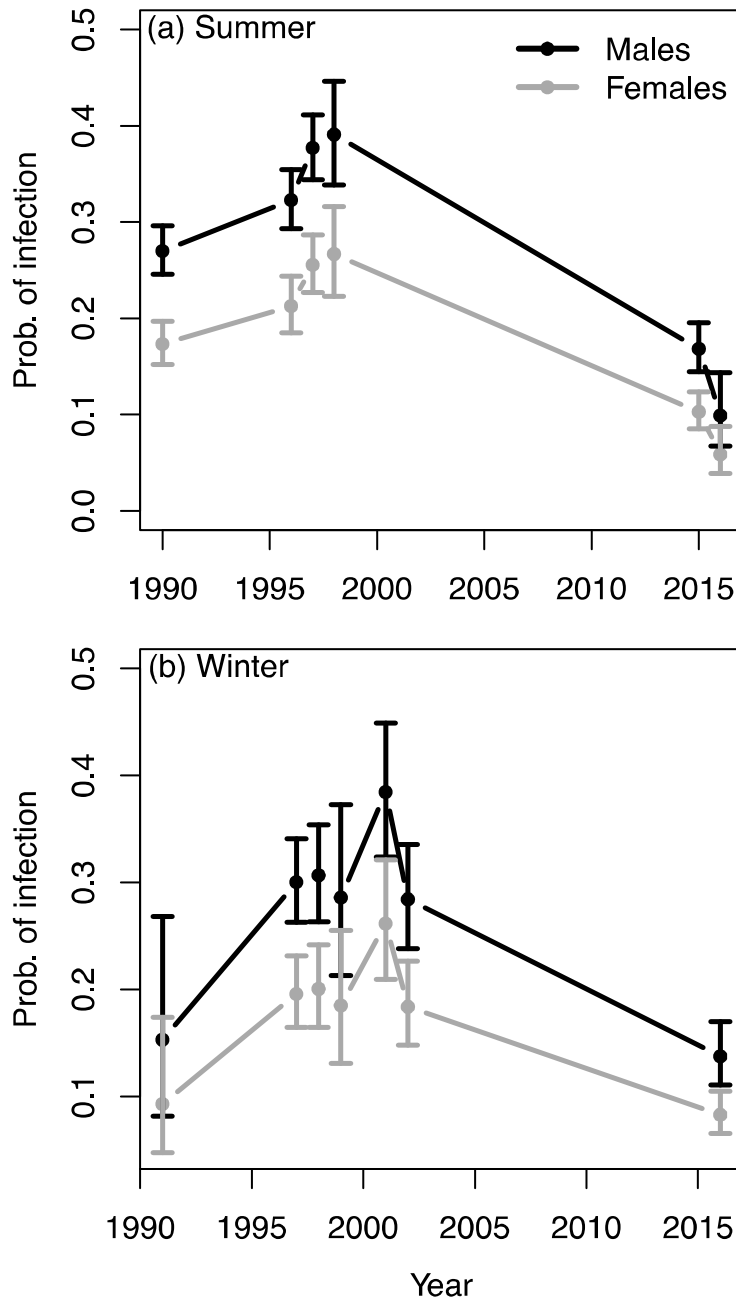
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385 Figure 1.

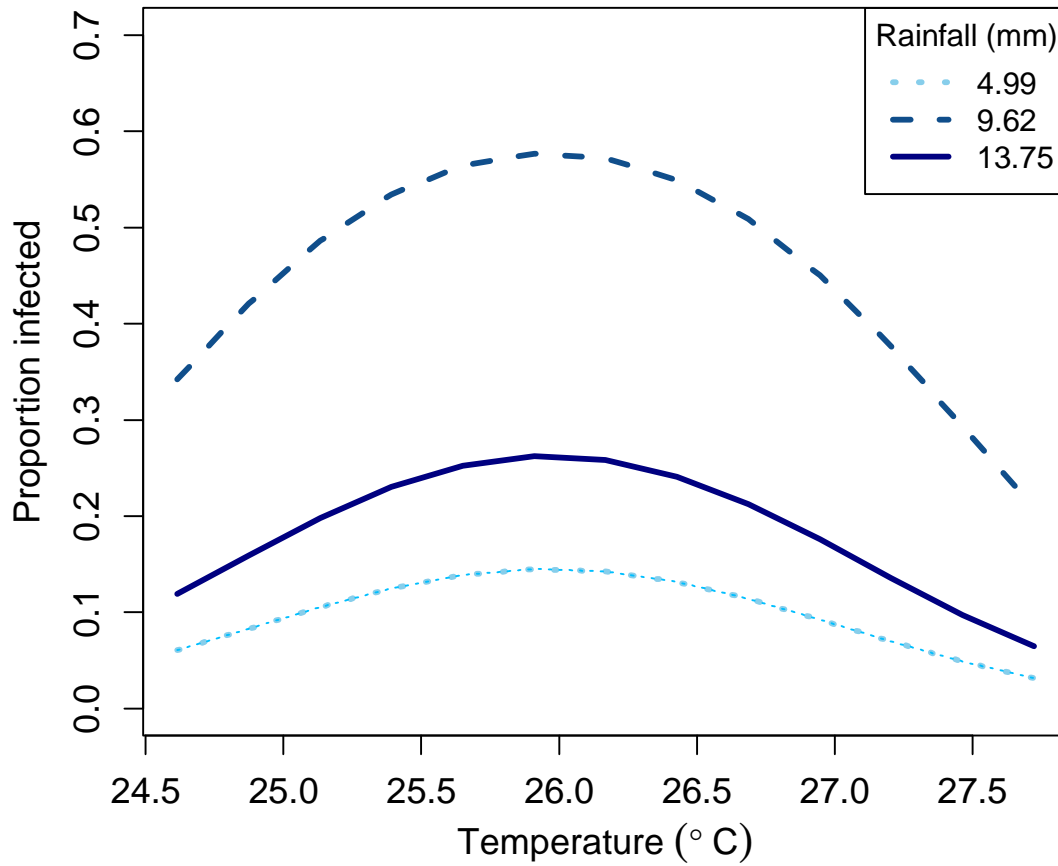


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389 Figure 2



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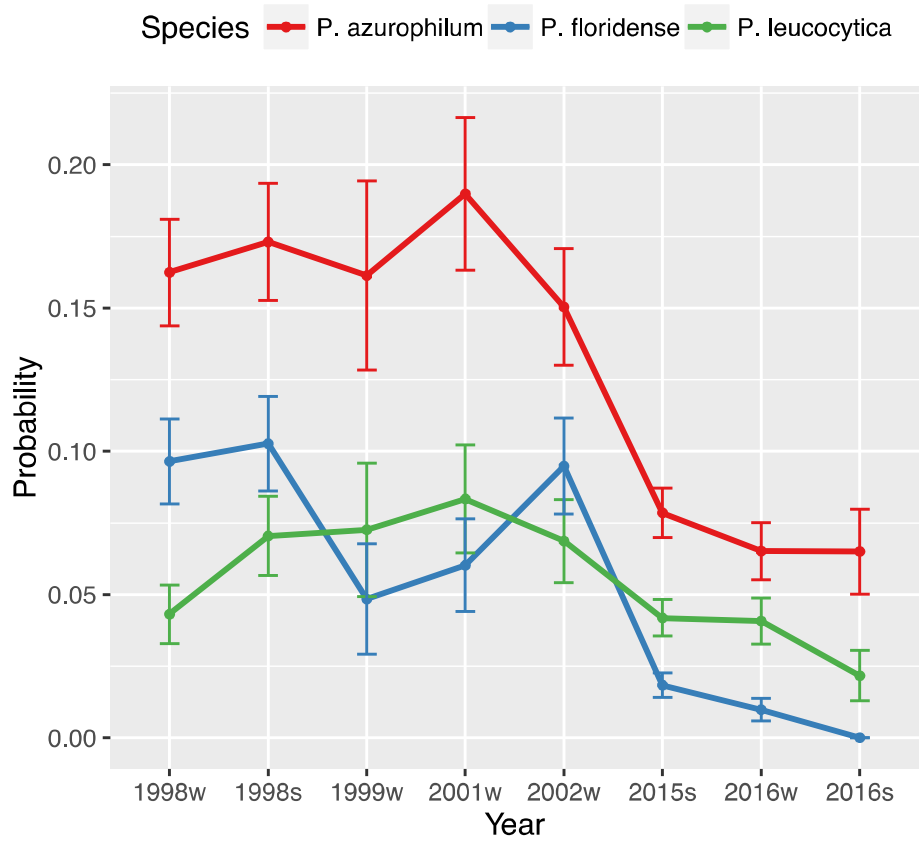
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