

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 log<sub>10</sub> mass on log<sub>10</sub>  
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 ( $\Delta AIC = 8.58$  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 ( $\Delta AIC = 90.37$   
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 ( $\Delta AIC = 3.29$  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 *azuorhillum* 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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#### 289 LITERATURE CITED

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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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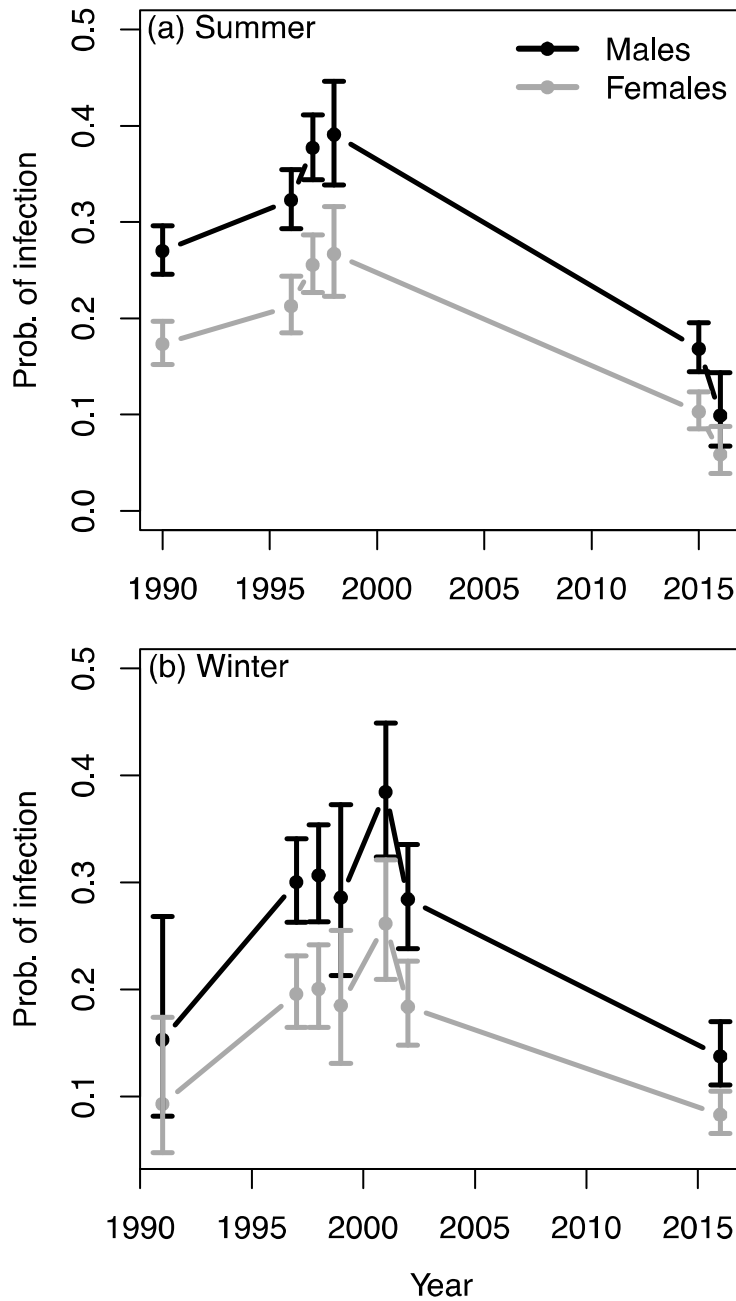
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385 Figure 1.

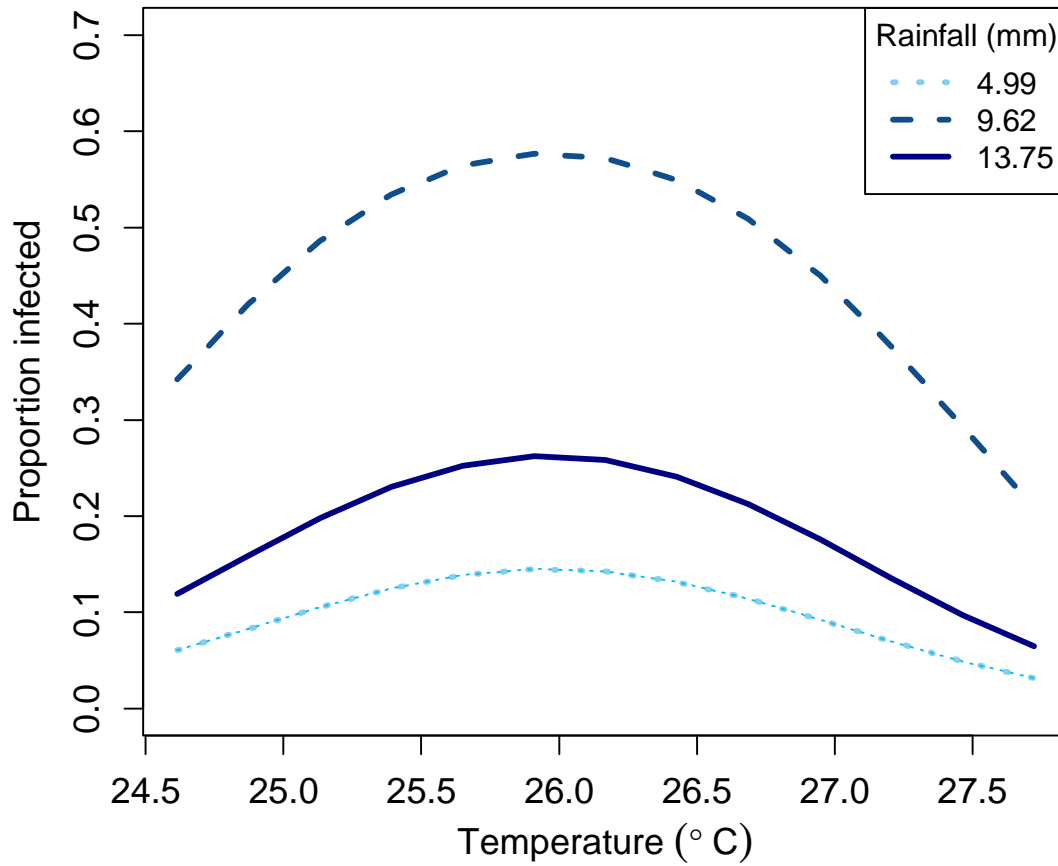


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389 Figure 2



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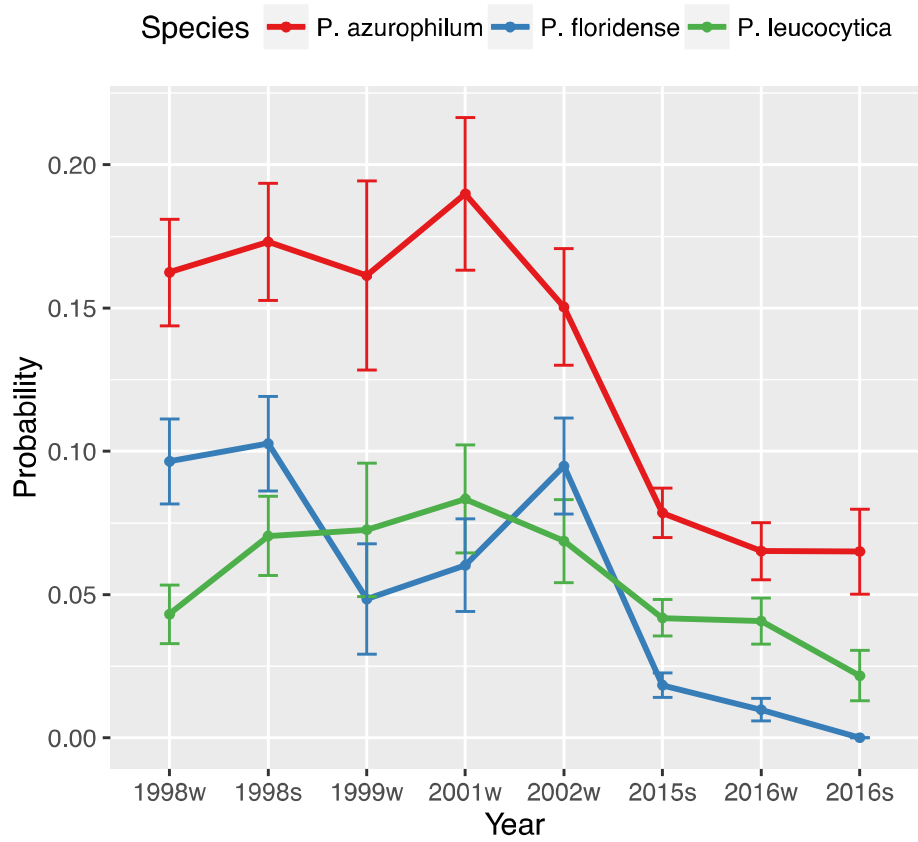
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396 Figure 3



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