

## Research Article

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# The drivers and consequences of unstable *Plasmodium* dynamics: a long-term study of three malaria parasite species infecting a tropical lizard

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**Abstract**

Understanding the consequences of environmental fluctuations for parasite dynamics requires a long-term view stretching over many transmission cycles. Here we studied the dynamics of three malaria parasites (*Plasmodium azurophilum*, *P. leucocyta* and *P. floridense*) infecting the lizard *Anolis gundlachi*, in the rainforest of Puerto Rico. In this malaria–anole system we evaluated temporal fluctuations in individual probability of infection, the environmental drivers of observed variation and consequences for host body condition and *Plasmodium* parasites assemblage. We conducted a total of 15 surveys including 10 from 1990 to 2002 and five from 2015 to 2017. During the early years, a lizard’s probability of infection by all *Plasmodium* species appeared stable despite disturbances ranging from two hurricanes to short droughts. Over a longer timescale, probability of infection and overall prevalence varied significantly, following non-linear relationships with temperature and rainfall such that highest prevalence is expected at intermediate climate measures. A perplexing result was that host body condition was maximized at intermediate levels of rainfall and/or temperature (when risk of infection was highest), yet we found no significant decreases in body condition due to infection. *Plasmodium* parasite species composition varied through time with a reduction and near local extinction of *P. floridense*. Our results emphasize the need for long-term studies to reveal host–parasite dynamics, their drivers and consequences.

**Introduction**

Haemosporidian parasites (*Plasmodium* and related genera) exploit thousands of vertebrate host species including mammals, squamate reptiles and birds on all warm continents (Galen *et al.*, 2018). A vexing issue in the study of human malaria focuses on prevalence patterns and their variation over time and space (Hackett, 1937). A recent study has examined >50 000 prevalence surveys of *Plasmodium falciparum* in sub-Saharan Africa since 1900 (Snow *et al.*, 2017), and found no comprehensive explanation for changes in parasite abundance. Understanding the interplay between the parasites, their hosts and the abiotic conditions of malaria infection has implications for human public health, but also for wildlife conservation. For example, a single strain of *P. relictum* caused sharp population declines of endemic birds in the Hawaiian islands (Beadell *et al.*, 2006). Also, climate change has now brought the parasites to northern latitudes and to bird species previously not infected by *Plasmodium* (Martinsen *et al.*, 2017). One pointed question focuses on the stability in prevalence patterns; that is, are the dynamics of these parasite–host systems stable through time and robust to environmental fluctuations (MacDonald, 1952)? Classic theoretical models on malaria predict temporally stable dynamics (review in Aron and May, 1982). Large environmental changes, though, may well disrupt stable endemic patterns to unpredictable outcomes (Chiyaka *et al.*, 2013).

With ongoing climate changes in temperature and rainfall patterns, could the prevalence of malaria parasites be driven into unstable patterns? Malaria parasites are vector-borne, and thus sensitive to environmental fluctuations (Harvell *et al.*, 2002; Altizer *et al.*, 2013), particularly through the effect of environmental variation on various stages of the vector and parasite life cycle (LaPointe *et al.*, 2010; Paaajmans *et al.*, 2010; Mordecai *et al.*, 2013). There is little consensus on the expected influence of climate change on the dynamics of vector-borne parasites including *Plasmodium* (Rohr *et al.*, 2011). Expected temperature increases of 1–5 °C may result in expanded malaria geographic ranges (e.g. Martinsen *et al.*, 2017), increased vector biting and parasite replication rates promoting higher transmission rates (Pascual *et al.*, 2006). However, an increase in temperature may also increase vector mortality. Therefore, the

relationship between abiotic factors, disease transmission and its ecological consequences remains an important open question in disease ecology and parasitology (Lively *et al.*, 2014).

Temporal instability could push a cascade of subtle consequences, including changes in the composition of coexisting *Plasmodium* species in the same or in closely related sympatric hosts, possibly affecting parasite virulence (Elliot *et al.*, 2002; Wolinska and King, 2009). These issues are complex and are best probed with a long-term view, analysing long data series that could allow an understanding of climate-driven changes in parasite dynamics and their consequences (Clutton-Brock and Sheldon, 2010). Most long-term studies on malaria parasites focus on those of human medical importance and are influenced by public health efforts that result in changing environments for the parasites, superimposed on natural fluctuations. Long-term studies of malaria parasites in wildlife hosts present a cleaner alternative. For example, a study characterizing bird haemosporidian parasite lineages at a millennial time frame found indications of genetic lineage switches at this time scale on Caribbean islands (Soares *et al.*, 2017). At a shorter time frame, a 17-year study of haemosporidian parasites infecting great reed warblers (*Acrocephalus arundinaceus*) found temporally stable composition of parasite lineages and no significant changes to host fitness (Bensch *et al.*, 2017). At northern latitudes, a 10-year study showed that *Plasmodium* infection of black-capped chickadees is temporally variable and associated with keratin disorder (Wilkinson *et al.*, 2016). Thus, these long-term studies focusing on avian haemosporidian systems provide invaluable insights into the dynamics of host–parasite systems, but are scarce in other hosts (but see Schall and Denis, 2013).

*Plasmodium* infection in lizards offers a useful system to understand the interplay between environmental change and the stability of host–parasite interactions. Lizards are ectothermic hosts with life spans that allow studying multiple generations in a short period of time. Here we present a long-term study of three lizard malaria parasites (*P. azurophilum*, *P. floridense* and *P. leucocyctica*) infecting *Anolis gundlachi* in the tropical rainforest of Puerto Rico. We leverage data assembled from 1990 to 1999 (Schall *et al.*, 2000), add extensive recent sampling and reanalyse the data using statistical methods appropriate to a longer-term approach. There are several notable features for this study: the lizard life span is typically 1 year or shorter, and thus many host generations are followed in the study. The system has not been disturbed by deliberate human intervention, such as parasite control efforts, logging or human-induced land-cover change during the study period. The parasite assemblage includes three species exploiting the same host, and the dynamics of all three were followed over time. Changes in relative proportion of the *Plasmodium* species would be particularly interesting because *P. leucocyctica* infects several classes of white blood cells and the other two infect erythrocytes, with possible complex competitive interactions (Perkins, 2000). Last, a well-defined measure of body condition allows a measure of changes in host health condition over time. We seek resolution of four important questions: (1) was the parasite prevalence and risk of infection stable during the long-term study period? (2) If not, was temporal variability driven by abiotic factors (e.g. temperature and precipitation) or were there secular trends apparently independent of environmental changes [resembling the ‘autonomous’ pattern of human malaria suggested by Hackett (1937)]? (3) Was parasite species composition stable during this long-term period, or were there signs of shifts on probability of infection among species? (4) Do abiotic factors mediate the potential negative consequences (virulence) of infection to the host? This system thus offers a rare view of a malaria system in a nonhuman host – over >15 generations of the vertebrate host.

## Materials and methods

### Study system, field sampling and diagnostics

We studied the lizard *A. gundlachi* (Fig. 1a), and three species of *Plasmodium* parasites: *P. azurophilum* (Fig. 1b), *P. floridense* (Fig. 1c) and *P. leucocyctica* (Fig. 1d) at the El Verde field station located at the Luquillo Experimental Forest, in Puerto Rico (central point 18°19.263′N–65°49.146′W). Even though *P. azurophilum* infects red blood cells and *P. leucocyctica* white blood cells, these species are closely related (sister taxa) in the most recent published phylogeny of the *Plasmodium* group (Galen *et al.*, 2018). In contrast, *P. floridense* is more related to a group including *P. mexicanum* and *P. chiricahue*, which to date have not been found in Puerto Rico. The host, *A. gundlachi*, is a medium-sized lizard (mean snout–vent length (SVL) of 53.5 mm, mean mass 3.89 g) and is the most common anole in the forest understorey, reaching population sizes of 2000 ha<sup>−1</sup> (Reagan, 1992). This anole is among seven other species living at the site, but the other anoles are rarely infected (Schall *et al.*, 2000). We sampled lizards during 15 periods from 1990 to 2017: summers (May–August) 1990, 1996, 1997, 1998, 2015, 2016, 2017, and winters (January–March) 1991, 1997, 1998, 1999, 2001, 2002, 2016 and 2017.

To ensure consistency over time we replicated rigorously the protocols of field sampling and diagnostics used in the original study by Schall *et al.* (2000). Specifically, we consulted sketch maps of study sites made during the early years to sample the same sites within the forest (between 4 and 10 trails each year) and collected similar sample sizes ( $N > 650$  per survey). The experimental forest is well mapped, including a 16-ha plot with every tree identified and numbered, facilitating return to the earlier collection sites. To ensure uniform scoring of blood films, LO and MAA worked with the initiator of the early samples (JJS) with a dual-viewing microscope to become proficient in the scoring of infected lizards and identification of the parasites.

We searched for anoles on tree trunks, branches, rocks, ground and other perches. We captured lizards by hand or noose and kept them in individual bags to transport them to the laboratory where we determined sex, measured SVL, mass and sampled blood using toe clipping (Schall *et al.*, 2000). Toe clipping also ensures that individuals are not sampled more than once in a season. Captured lizards were released within 24 h after capture in the same areas where they were collected. We made thin blood smears for each individual and kept dried blood samples on filter papers for future molecular analyses. We fixed the smears using methanol (100%) and stained the slides using Giemsa stain at pH 7.5 for 50 min following the protocol by Schall *et al.* (2000). To determine if a lizard was infected we diagnosed each sample by scanning stained blood smears at 1000× for 6–10 min and identified parasite species based on morphological traits and cell class infected (Telford, 2016). To estimate host body condition, we determined the SVL and mass of each lizard.

The 15 surveys included in these analyses comprise the longest lizard–malaria study in the tropics. These surveys took place from 1990 to 2002 and additional surveys in 2015–2017, including a gap from 2002 to 2015. The data, though, are appropriate to answer our stated questions. First, each survey is an independent sample because the average life span of these lizards is ~1 year. Second, the 15 surveys sample the full range of environmental variables including years under El Niño Southern Oscillation events (extreme droughts on one extreme, and high rainfall due to hurricanes on the other) and mild years that represent average conditions. Third, the methods were rigorously consistent among samples, which prevents any potential bias due to observational variability. Therefore, the surveys are a thorough representation of the environmental conditions experienced by this host–parasite system in the long-term period.



**Fig. 1.** (A) The host *Anolis gundlachi* is infected by three malaria parasites. The figure shows examples of Giemsa-stained blood smears of (B) *Plasmodium azurophilum*, (C) *P. floridense* and (D) *P. leucocyttica*.

### Environmental variables

We compiled temperature and rainfall data from El Verde weather station records and estimated daily mean minimum, maximum, cumulative (rain) and variance of registered temperatures and rainfall 30 and 120 days prior to the sampling month (Schall *et al.*, 2000). Hurricane events occurred in September 1995 (two), July 1996 and September 1998. While Hurricane Maria affected Puerto Rico in September 2017, surveys took place in June and July. Therefore, the effect of the storm is not captured in this dataset. We did not include hurricanes *per se* in the analysis, but high rainfall marked those periods in the precipitation data.

### Analyses

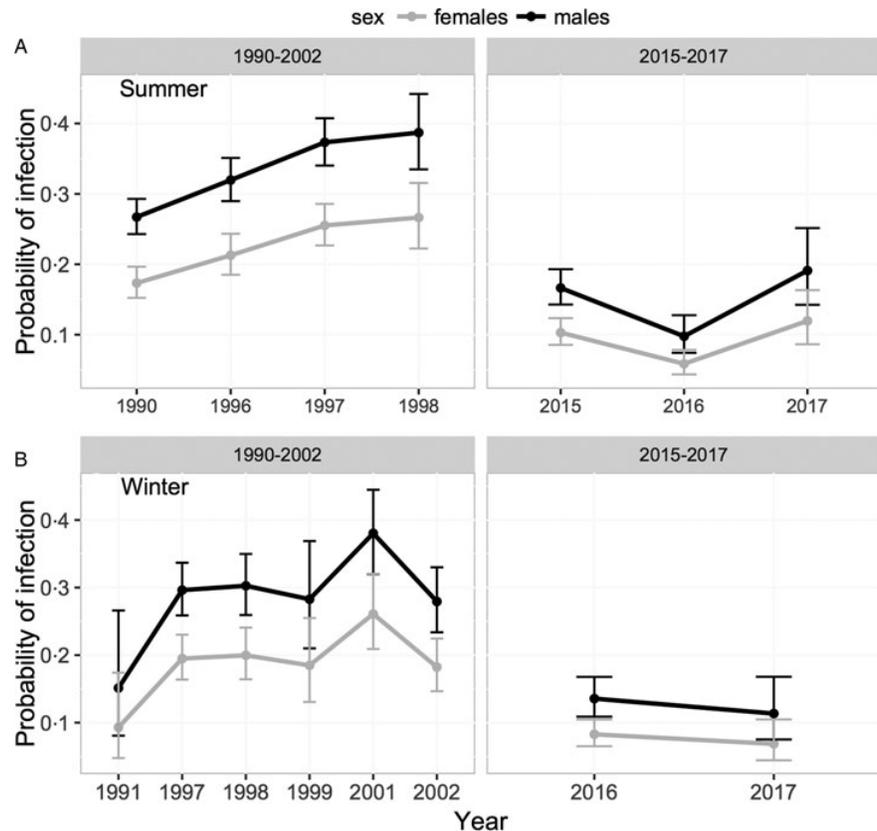
We followed Chamberlin's (1965) approach of comparing multiple working hypotheses for each one of our four research questions. This approach contrasts with the more common Popperian method in which a single alternative hypothesis is compared against a null one. The key difference between the two approaches is that the multiple working hypotheses framework allows for the possibility that more than one hypothesis may be simultaneously true (Elliott and Brook, 2007; Betini *et al.*, 2017). Here we operationalize this approach developing a list of *a priori* hypotheses for each of the questions, which are represented by a model. We compared each model – including a null (intercept only) model – using Akaike Information Criterion adjusted for small sample sizes (AICc). The most parsimonious model has the lowest AICc score. In the case of multiple models having similar low AICc scores (<2 AICc units), we use AIC weights to assess the relative contribution of each hypothesis to explain the observed relationship. If two or more models had similar AICc scores we averaged the top models by weighting each shared parameter estimate by the model's AICc weight (Burnham and Anderson, 2004). We fitted all models using maximum likelihood and conducted model selection and averaging using the AICcmodavg package in R 3.0.3 (R Core Team, 2013).

To determine if the individual probability of infection was stable through the long-term study period, we compared eight binomial models. These models (with exception of the null model) include season, sex and/or SVL as controlling covariates because Schall *et al.* (2000) found strong evidence showing that the probability of infection was higher in the summer than in

the winter season. They also found that bigger males had a higher probability of infection than smaller males or females. We compared models predicting the probability of infection as a function of (1) SVL, year and sex separately, (2) the additive effect of sex, SVL and season, (3) a similar model, but including an additive effect of year, (4) a model including sex, SVL and season with an interactive effect between sex and SVL, (5) a similar model including an additive effect of year and (6) an intercept-only model that represented the null hypothesis that none of the variables explains the observed temporal variability in probability of infection (Supplementary Table S1). If there was significant temporal variation in the dynamics, the most parsimonious model (lowest AICc score) would include the effect of sampling year.

We followed a similar model selection approach to test which environmental variables (i.e. rainfall and/or temperature) better predict the proportion of infected individuals through time. We compared 35 models that included various combinations of mean rainfall 30 days and 120 days before the field sampling (Schall *et al.*, 2000). These also included models with the variance of rainfall or temperature 30 or 120 days before the field sampling (Vasseur *et al.*, 2014). To allow for a possible non-linear effect we also fitted individual and additive models with a second-degree polynomial. Last, we fitted a null (intercept only) model to describe the case where none of the tested variables better explains the observed patterns in prevalence (Supplementary Table S3).

To assess the relationship between environmental factors, infection status and body condition we estimated body condition using the residual index  $R_i$  (Cox and Calsbeek, 2015). We calculated this index using the residuals of the linear regression of  $\log_{10}$  mass on  $\log_{10}$  SVL. Lizards with positive residuals are heavier than average (better body condition), while lizards with negative residuals are skinnier relative to their SVL than average (Schall and Pearson, 2000). We made a separate analysis for each sex, including data from the years for which body mass and SVL data were available (1996, 1997, 1998, 2015, 2016 and 2017). Previous studies show that *A. gundlachi* lizards are particularly sensitive to maximum environmental temperatures (Huey and Webster, 1976; Hertz *et al.*, 1993) and cumulative rain (Schall and Pearson, 2000). Therefore, we compared 14 models that predicted variability in body condition as a function of maximum temperature 30 days of the census, cumulative rain 6 months before the survey, infection state and their additive combinations. To account for potential non-linear effects, we also compared



**Fig. 2.** The predicted partial relationship between probability of infection of *A. gundlachi* by three malarial parasites and time. This probability of infection is relatively constant in the early years but decreases in 2015–2017. Points represent mean estimates and the bars represent 95% CIs.

models that incorporated a second-degree polynomial effect of temperature and rainfall. We also compared a null (intercept-only) model that represents the case where none of these variables explains variability in body condition (Supplementary Tables S5 and S7).

To assess changes in composition of the three *Plasmodium* species through time we compared 10 multinomial logit models in their ability to predict the individual probability of being non-infected or infected by one of three *Plasmodium* parasites (four categories; co-infections were not considered because they were infrequent). This modelling approach is an extension of a logistic regression for multinomial response variables. We included sex and SVL in the models as controlling variables because these may influence the probability of infection by different *Plasmodium* species. We compared models including (1) the single effect of sex, SVL or year; (2) models considering the additive and interactive effect of year and SVL; (3) a model considering the additive effect of sex and SVL and (4) the additive effect of these three variables, with either interactions of year with SVL, or sex. We also fitted a null (intercept-only) model that represents the case where none of these variables explains the probability of getting infected by any of the three *Plasmodium* parasites (Supplementary Table S10). The models were fitted using maximum likelihood applying the `nnet` package in R (Venables and Ripley, 2013).

## Results

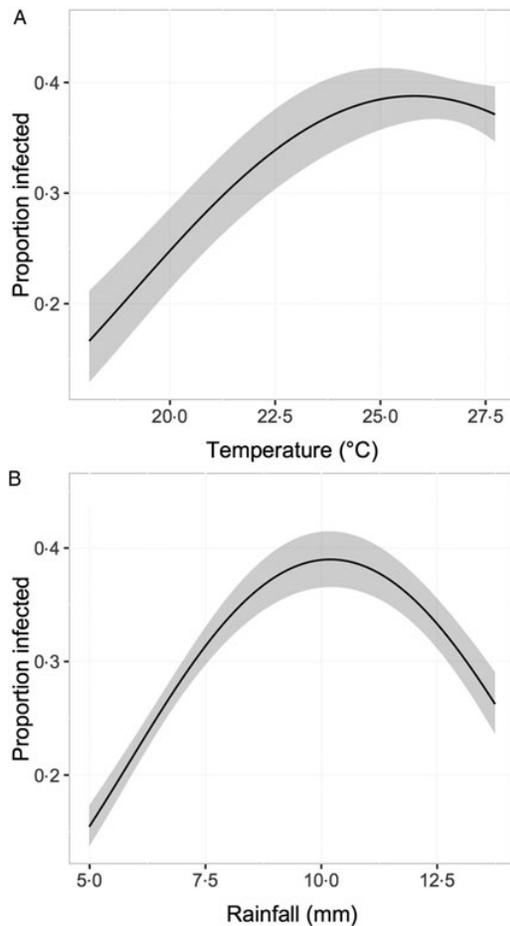
### Long-term dynamics in probability of infection

A total of 8991 *A. gundlachi* lizards were sampled in 15 surveys from 1990 to 2017. The most parsimonious model explaining variability in the individual probability of infection included the additive effect of sex, SVL, season, year and the interaction

between sex and SVL ( $\Delta\text{AICc} = 10.31$  between this model and the next best,  $\text{AICc}$  weight = 0.99; Supplementary Table S1). In the summer, the probability of infection and prevalence were relatively stable from 1990 to 1998 (Supplementary Fig. S1) with an estimated individual probability of infection fluctuating between 0.28 and 0.38 for males and between 0.17 and 0.27 for females. The probability of infection decreased during the 2015–2017 period to 0.10–0.19 for males, and 0.06–0.12 for females (Fig. 2A, Supplementary Table S2). In the winter, the probability of infection and prevalence was low in 1991 (Supplementary Fig. S1; average probability of infection of 0.15 in males, and 0.09 in females). Then it increased and remained relatively stable from 1997 to 2002 with an individual probability of infection fluctuating between 0.28 and 0.38 in males and between 0.19 and 0.26 in females. This apparent stability was disrupted in 2016–2017 where the probability of infection decreased (average probability of infection of 0.11–0.14 in males, and 0.07–0.08 in females; Fig. 2B, Supplementary Fig. S1 and Table S2).

### Environmental drivers of temporal variability

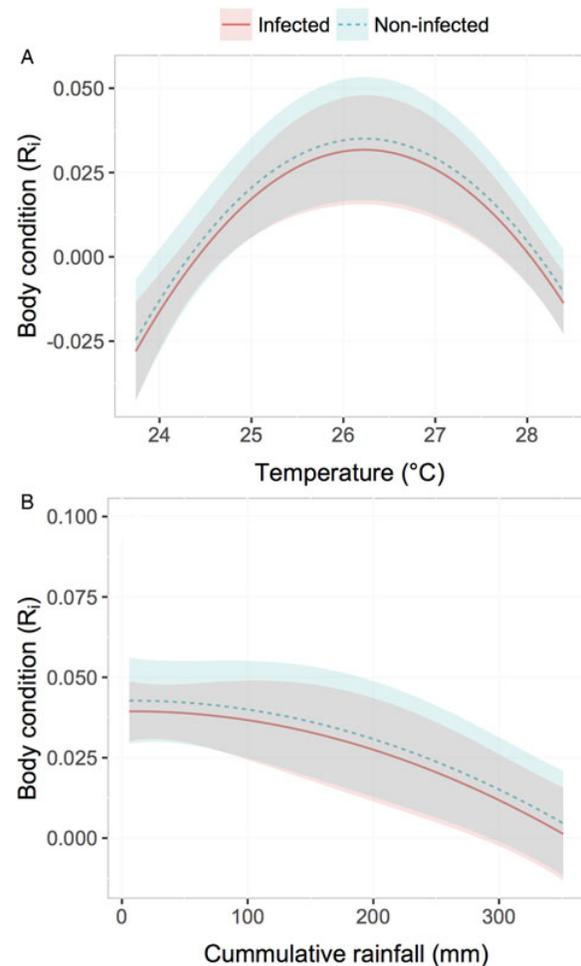
The most parsimonious model explaining temporal variations in the proportion of infected individuals through time included the additive quadratic effect of mean maximum daily temperature and mean daily rainfall through 120 days before the sampling ( $\Delta\text{AICc} = 25.72$  between this model and the next best,  $\text{AICc}$  weight = 1; Supplementary Table S3). The probability of infection followed a nonlinear response with temperature and rainfall. The proportion of infected individuals increased with increasing temperature and maximized at  $\sim 26^\circ\text{C}$  after which the relationship decelerates (Fig. 3A, Supplementary Table S4). The proportion of infected individuals maximized at an intermediate measures of rainfall ( $\sim 10$  mm; Fig. 3B, Supplementary Table S4).



**Fig. 3.** Predictions of the most parsimonious model explaining temporal variation in the proportion of infected *A. gundlachi* by malaria parasites. This model predicted the proportion of infected individuals by the additive effect of squared (A) mean maximum temperature and (B) mean daily rainfall 120 days before sampling. Lines represent estimated means and shaded areas 95% CIs.

#### Environment-mediated effects of infection on body condition

The most parsimonious model explaining the relationship between body condition ( $R_i$ ) of females and abiotic variables included a quadratic additive effect of mean daily maximum temperatures and cumulative rain 1 month before the survey ( $\Delta\text{AICc} = 0.49$  between this model and the next best,  $\text{AICc}$  weight = 0.56; Supplementary Table S5). Yet, the following best model, which included the same variables in addition to the infection state, had also similar explanatory power ( $\text{AICc}$  weight = 0.44). Therefore, we conducted model averaging between these two models (Supplementary Table S6). This average model predicts a non-linear partial relationship between body condition and temperature where body condition is maximized at  $\sim 26^\circ\text{C}$  (Fig. 4A). This model also predicts a slow decrease in body condition with increasing cumulative rainfall (Fig. 4B, Supplementary Table S6). While average body condition was higher in non-infected females, the confidence intervals (CIs) overlapped suggesting lack of difference between the two groups. The most parsimonious model explaining these relationships for males included the effect of cumulative rain a month before the survey ( $\Delta\text{AICc} = 0.34$  between this model and the next best,  $\text{AICc}$  weight = 0.47; Supplementary Table S7). The following best model had a similar explanatory power included the same variable in addition to the infection state ( $\text{AICc}$  weight = 0.39). Therefore we conducted model averaging between these two models (Supplementary

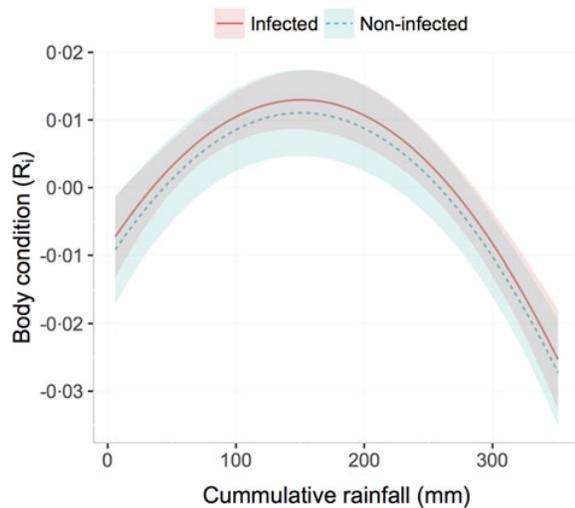


**Fig. 4.** Partial predictions from the most parsimonious model describing the relationship between *A. gundlachi* females body condition and (A) mean daily maximum temperature, and (B) cumulative rain during the month previous to the survey. Red shaded area represents 95% CI for infected individuals, the blue shaded area represents 95% CI for the non-infected individuals and the grey shaded area represents overlap between them. This area of overlap indicates that the predicted relationship was similar between infected and non-infected females.

Table S8). This average model predicted that male's body condition maximized at intermediate levels of cumulative rain ( $\sim 152$  mm of rain in a month; Fig. 5). While average body condition was predicted higher for infected individuals, the CIs overlapped with those predicted for non-infected individuals suggesting lack of difference in body condition between the two groups (Fig. 5).

#### Long-term changes in parasite species composition

The most parsimonious model explaining the probability of an individual either being infected or non-infected by *P. azurophilum*, *P. leucocytica* or *P. floridense* was best described by the additive effect of SVL, sex and year ( $\Delta\text{AICc} = 9.02$  between this model and the next best,  $\text{AICc}$  weight = 0.99; Supplementary Table S9). This model predicts a decrease in the probability of infection of all *Plasmodium* parasites after 2001. The probability of infection by *P. azurophilum* increases to the levels before 2001 in 2017 (Fig. 6, Supplementary Table S10). The most common species was consistently *P. azurophilum*. The remaining parasite species, *P. floridense* and *P. leucocytica* changed their relative dominance, but this apparently was due to a secular decline in *P. floridense* over the entire study period (Fig. 6, Supplementary Table S10).



**Fig. 5.** Partial predictions from the most parsimonious model describing the relationship between *A. gundlachi* males' body condition and cumulative rain during the month previous to the survey. Red shaded area represents 95% CI for infected individuals, the blue shaded area represents 95% CI for the non-infected individuals and the grey shaded area represents overlap between them. This area of overlap indicates that the predicted relationship was similar between infected and non-infected females.

This model predicted no differences in the partial relationship between SVL and probability of infection by the different *Plasmodium* parasites (Supplementary Fig. S3). Whereas the model predicts no difference between sexes in the probability of getting infected by *P. azurophilum* or *P. floridense*, the probability of becoming infected by *P. leucocyttica* was greater in females (Supplementary Fig. S4).

## Discussion

We assembled a long-term dataset including 15 surveys conducted from 1990 to 2017 to identify the environmental drivers of *Anolis-Plasmodium* temporal dynamics and their consequences for host body condition and parasite species composition. Our results show that the probability of infection for the anole by *Plasmodium* parasites varied little early in the study and declined substantially in 2015–2016. This drop in infection probability coincided with a severe drought in Puerto Rico. However, the long-term approach allowed detection of subtler, but important patterns. Overall, temporal variation in probability of infection was associated with fluctuations in temperature and rainfall 4 months before each sampling period. The relationship with both environmental variables was nonlinear predicting maximum probability of infection at intermediate rainfall and a decelerating relationship with increasing maximum temperature. Similarly, this long-term analysis showed that host body condition maximized at the intermediate levels of maximum temperature and/or rainfall. Moreover, the optimal temperature for female's body condition was very similar to the optimal temperature for transmission (26 °C), suggesting that the environmental conditions that maximize host health may also catalyse parasite transmission. During the study period, *P. azurophilum* was consistently the most common parasite while *P. floridense* declined to almost local extinction. The study by Schall *et al.* (2000), which analysed the first decade of data, concluded that the dynamics were relatively stable over time because these patterns were not apparent during the early years of the study. Therefore, a longer-term approach (>10 years) was needed to

reveal the response of the parasite dynamics to environmental changes and its consequences.

The delayed link between rainfall and temperature with parasite prevalence (120 days) most likely is a consequence of shifting vector abundance. During the severe 2015 drought, we noted an overall dry aspect to the forest, with little standing water in puddles, *Heliconia* flowers, or epiphytic bromeliads that could have restricted breeding locations for mosquitoes. Most strikingly, despite the general effect of variation in temperature and humidity over the study period, no dramatic effect on prevalence was seen after short environmental disruptions from dry periods or severe hurricanes in the 1990s. Hurricanes caused severe physical damage to the forest including massive defoliation (Reagan and Waide, 1996; Schall *et al.*, 2000). Thus, the drop in the probability of infection in the later samples could indicate that the 2015 drought was of sufficient duration and strength to shift the system to a new dynamic state. Substantial theory and empirical evidence support the conclusion that natural ecological systems can experience such alternative stable states (Petraitis, 2013).

This new stable state may be the consequence of shifts in the dominance of polymorphic forms of each species as a result of co-evolution of the host and its parasites. Strain theory has a long history in human malaria research recognizing that these parasite species are highly polymorphic, often resulting in a collection of almost discrete forms within species (Gupta and Day, 1994; McKenzie *et al.*, 2008). The severe drought of 2015 may have shifted dominance patterns favouring strains that can still spread under these generally unfavourable conditions at the expense of having low transmission rates. A non-mutually exclusive alternative hypothesis includes a decrease in favourable environmental conditions for vector transmission.

The identity of the vectors that transmit *Plasmodium* among *Anolis* lizards in Puerto Rico is not yet known. Two of our findings may suggest that there may be more than one dipteran species transmitting the parasite. First, if there were a unique vector species transmitting the three *Plasmodium* parasites we may find similar infection patterns among species. However, our results show that the probability of infection of female lizards is greater for *P. leucocyttica* and *P. floridense*, but there is no difference between sexes for *P. azurophilum* (Supplementary Fig. S3). This difference is not likely to be the result of similar natural history traits between the parasites species. The latest *Plasmodium* phylogeny shows *P. leucocyttica* and *P. azurophilum* as sister taxa and *P. floridense* more related to a different group (Galen *et al.*, 2018). Second, we found different patterns of temporal variation in the probability of infection among parasite species (Fig. 6). This may be the result of different responses to climate variability by multiple vector species. Our research group is currently testing mosquitoes for the presence of the three *Plasmodium* species infecting the lizards in El Verde. To date, in the field station we have only detected these parasites in *Culex* mosquitoes (Acevedo, M, unpublished), which are also the most common mosquito genus at the site (Yee, D, unpublished). Moreover, *P. floridense* is known to infect *Culex* mosquitoes in Florida (Klein *et al.*, 1987). Yet, amplifying *Plasmodium* from a mosquito shows that the vector fed on an infected lizard, but does not necessarily imply vector competence. Therefore, more thorough work is still needed to identify the vector(s).

Why are males more likely to get infected than females? Higher rates of infection in males are common in many species (Roberts *et al.*, 2001). Ecological and physiological mechanisms have been proposed to explain this pattern. While the ecological mechanisms explaining this pattern in Puerto Rican anoles are not yet understood, we know that males can defend territories that are two times larger than females (Losos, 2011). To defend their territories males move larger distances increasing their

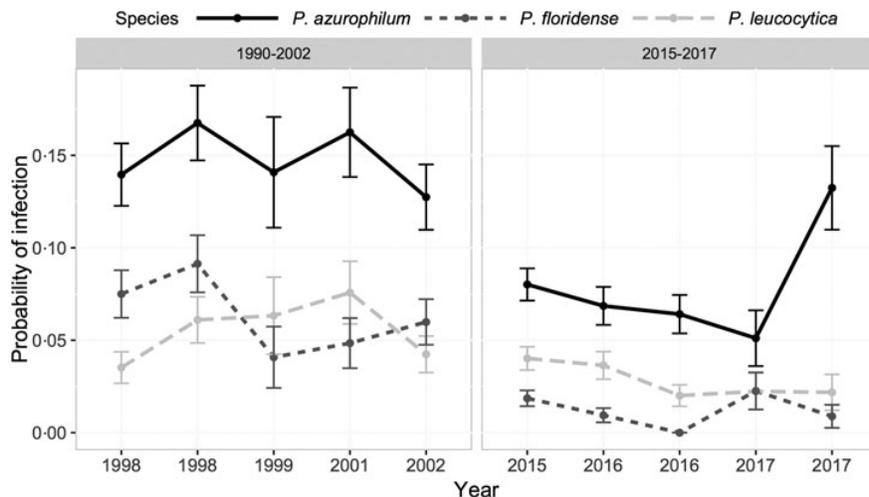


Fig. 6. Partial predictions from the most parsimonious multinomial model showing a general decrease through time in the probability of infection of *A. gundlachi* by three *Plasmodium* parasites. The model predicts a secular decrease in the probability of infection by *P. floridense*. Dots represent the infection probability estimates for each *Plasmodium* species and the bars represent 95% CIs.

exposure to infectious vector bites by experiencing a larger diversity of vector habitat. Physiology may also play an important role. Males are characterized by having higher testosterone levels that have been shown to suppress immune responses against protozoan infections in many species (Wunderlich *et al.*, 1988; Zuk and McKean, 1996).

What caused the differences in parasite prevalence for the summer vs winter seasons? Rainfall is greater in the summer, which increases potential habitats for vector breeding, increasing the ratio of vectors to hosts. Also, summer is the mating season for *A. gundlachi* when lizards are more active and defending territories (Gorman and Licht, 1974; Reagan, 1992). If the vectors are daytime active, the lizards could be more exposed to vector bites. Alternatively, if the parasite reduces reproduction in the blood when vector activity ceases, the lower prevalence in winter may only be apparent. There is no evidence that lizards can clear an infection. Also, using a polymerase chain reaction-based method to detect very low-parasitemia infections showed cryptic infections were more common in the winter season (Vardo *et al.*, 2005).

Our study adds to the growing evidence that the relationship between malaria transmission and climatic measures is complex and often nonlinear (Garamszegi, 2011; Mordecai *et al.*, 2013). The observed patterns could be the result of complex interactions between variation in environmental conditions and its effects on various stages in the parasite life cycle. Variations in temperature and rainfall can affect host susceptibility, which is particularly important in ectothermic hosts such as anoles. Our results showing a non-linear effect of environmental fluctuation and anole body condition partially supports this hypothesis. Environmental fluctuations can also affect other variables predicted by the Ross-Macdonald theoretical model that influence transmission such as vector abundances, biting rates, the probability of infection from the host to vector and from the vector to host, vector death rates, incubation periods and recovery rates (Smith *et al.*, 2012). Adding to this complexity is the role of spatial scale. For example, in the *Sceloporus occidentalis*-*P. mexicanum* system in California, when studying the relationship between landscape features and infection prevalence, the type of ground cover (grass, rocks and leaf litter) influenced the probability of infection (Eisen and Wright, 2001). Note that changes in temperature and rainfall could alter microhabitat quality, including the production of leaf litter and ground cover. Therefore, suggestions that rising temperatures alone will increase the prevalence of malaria parasites ignore the more complex story.

Temperature and rainfall also mediated *Anolis* body condition. Measures of body condition are commonly used as a proxy of the energetic state of lizards and their fitness (Peig and Green,

2009; Cox and Calsbeek, 2015). The host *A. gundlachi* is a thermoconformer-shaded forest specialist whose metabolic performance depends closely on temperature (Huey and Webster, 1976). We found that females had an optimal body condition at an environmental temperature of  $\sim 26^\circ\text{C}$ , which is consistent with the experimental voluntary maximum temperature tolerated by the species (Hertz *et al.*, 1993). The non-linear relationship between *A. gundlachi*'s body condition and rainfall likely reflects the conflict between the levels of rainfall that promote abundance of food resources and periods of extremely heavy precipitation that result in physiological stress for the lizards. The optimal temperature for female body condition was similar to optimal temperature for transmission. This result suggests support for the theoretical prediction that environmental conditions mediate the interaction and potential co-evolution between the host and parasites (Elliot *et al.*, 2002; Wolinska and King, 2009). Because the parasite reproduces at the expense of the host resources, having ambient temperatures that promote optimal host body condition will likely result in optimal resources for the parasite to exploit for within-host reproduction (Seppälä *et al.*, 2008). Optimal temperatures may also mediate other mechanisms that promote transmission. For example, increasing temperatures may result in higher gametocyte production, but if production increases beyond a certain threshold it may ultimately decrease vector survival (assuming virulence to the vector). Also, temperature may mediate transmission-blocking immune responses or processes related to competition among co-infecting parasites (Mideo and Day, 2008). Field experiments will be a natural follow-up to confirm these observational results.

We found no significant difference in body condition between the infected and non-infected individuals. This is consistent with previous studies that found little evidence of a relationship between male lizard health and parasite infection in the Caribbean (Schall and Pearson, 2000; Schall *et al.*, 2002), but contrasts with studies of lizard malaria in California or Africa (Schall, 2002). This pattern in lizards may be explained by three non-mutually exclusive explanations. First, virulence to male hosts can be expressed in other traits that may not be captured by a body-condition index such as changes in aggressive behaviour or stamina. Second, parasitemia of Caribbean *Plasmodium* species is lower than those of California or Africa (Schall pers obs.), which may result in a lower rate of blood cell turnover. Third, low virulence could be the result of lower transmission intensity as predicted by the theory of virulence evolution in the host-parasite systems that have co-evolved for many generations (Ewald, 1995). The pattern in avian malaria is similar in which

some host species *Plasmodium* infection has negligible effects on body condition – mostly attributed to low parasitemia (e.g. Granthon and Williams, 2017), but there are significant negative effects in other species leading to dramatic increases in mortality (e.g. van Riper et al., 1986).

The El Verde lizard malaria system has an added interest because we could follow through time the relative proportions of three species of *Plasmodium* that infect a single lizard host. Despite the major drop in the probability of infection during the later sample periods, and the major disruption of the forest by hurricanes in the early years, *P. azurophilum* remained at a constant higher proportion of infections. *P. floridense* gradually decreased over the years almost approaching local extinction. What could account for this secular change? *P. floridense* and *P. leucocyta* infect different cell classes (erythrocytes for *P. floridense* and several classes of white blood cells for *P. leucocyta*), and therefore are not likely to be competitors for within-host habitats. Yet, *P. floridense* would be competing with *P. azurophilum* – the most dominant species – for erythrocytes. The competitive interactions of malaria parasite species that exploit the same host are poorly studied (but see Schall and Bromwich, 1994). Here, the gradual drop over in the prevalence of *P. floridense* over both hurricane and drought conditions is particularly perplexing. We suspect the explanation falls to the biology of the vectors, either different species or how parasites may compete within a single vector. Similar parasite species turnovers have been observed in long-term studies of bird malaria. For instance, *Plasmodium* and *Haemoproteus* lineages in Caribbean birds show significant temporal variation at the scale of decades, which suggests frequent parasite's local colonization and extinction events (Fallon et al., 2004).

The most significant finding of this study is the value, even the necessity, of a long-term approach to understand the ecology of host-parasite systems. In our study, the cycle of parasites between hosts likely follows an annual pattern because the lizards live ~1 year. Studying the long-term dynamics of the *A. gundlachi*-*Plasmodium* system allowed us to gain a better picture of the patterns and possible mechanisms behind prevalence changes, shifts in the blood parasite assemblage and the effects on the body condition of the hosts. The influence of environmental variables became apparent only after taking this long-term approach.

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