Associations between weather and *Plasmodium vivax* malaria in an elimination setting in Peru: a distributed lag analysis

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ABSTRACT

Background

Plasmodium vivax (*Pv*) is the predominant malaria species in countries approaching elimination. In the context of climate change, understanding environmental drivers of transmission can guide interventions, yet evidence is limited, particularly in Latin America.

Objectives

We estimated the association between temperature and precipitation and *Pv* malaria incidence in a malaria elimination setting in Peru.

Methods

We analyzed malaria incidence data from 2021-2023 from 30 communities in Loreto, Peru with hourly weather data from the ERA5 dataset and land cover data from MapBiomas. Predictors included average weekly minimum and maximum temperature, high heat (>90th percentile mean temperature), total weekly precipitation, and heavy rain (>90th percentile total precipitation). We fit non-linear distributed lag models for continuous weather predictors and generalized additive models for binary predictors and the lookback period was 2—16 weeks. Temperature models adjusted for total precipitation; precipitation models adjusted for maximum temperature. We performed subgroup analyses by season, community type, and distance to forest edge.

Results

The median vs. lowest values of weekly average minimum temperature was associated with 2.16 to 3.93-fold higher incidence 3-16 weeks later (5-week lag incidence ratio (IR) =3.93 [95% CI 2.18, 7.09]); for maximum temperature, the association was hump-shaped across lags, with protective associations at 1-2 and 15-16 week lags and 1.07-1.66-fold higher incidence at 6-13 week lags. High heat (>27.5ºC) was associated with 1.23 to 1.37-fold higher incidence at 5--9 week lags (9-week lag IR = 1.25 [1.02, 1.53]). Associations between total precipitation and malaria incidence were hump-shaped across lags, with the strongest positive association at 750 mm of precipitation at a 9-week lag (IR=1.56; [1.27, 1.65]). Heavy rain (>186mm) was associated with 1.22–1.60-fold higher incidence at 2–10 week lags (9-week lag IR=1.23 [1.02, 1.49]).

Discussion

Higher temperatures and precipitation were generally associated with higher malaria incidence over 1–4 months.

INTRODUCTION

As countries approach malaria elimination, the proportion of malaria infections due to *Plasmodium vivax* (*Pv*) increases.[1] *Pv* presents unique challenges for malaria elimination efforts because of its ability to survive in a wide range of environments, including temperate settings, by utilizing a dormant hypnozoite stage.[2] Relapsing infections occur weeks to months after a prior infection or relapse, and serve as a significant reservoir for persistent transmission.[3] [4,5] That *Pv* infections can be latent, as well asymptomatic, sub-patent, or minimally symptomatic makes diagnosis and surveillance a challenge. Further, symptomatic infections are low density and often missed through standard diagnostics.[4,6,7]. These characteristics greatly obfuscate *Pv* transmission biology and make *Pv* harder to detect, treat, and ultimately eliminate.

Climatic and environmental factors such as temperature, precipitation, land use, and vegetation are strong drivers of malaria transmission in various endemic settings.[8] All initial *Pv* infections are environmentally mediated, and understanding these drivers is critical to interrupting the long, enduring cycles of *Pv* transmission described above. Increased precipitation can increase standing water presence, soil moisture, and humidity, which in turn increase breeding habitat for *Anopheles*,[9] the vector for malaria transmission. However, this relationship is complicated: in dry-land communities, heavy rain can flush mosquito larvae, leading to decreased mosquito populations, whereas in riverine settings such as in the Amazon, flooding from heavy rains can increase breeding ground and mosquito populations.[9] Temperature has strong effects on the capacity of the *Anopheles* vector to transmit malaria via the mosquito biting rate, mosquito abundance, parasite incubation rate, and mosquito longevity.[10,11] A schematic of the timeline of these environmental drivers of transmission is presented in Figure 1, generated from prior findings.[6,12–16] Modeling suggest *Pf* malaria transmission by various *Anopheles* peaks at 25ºC,[10,17] with substantial decreases in transmission above this thermal optimum. More recent work has supported this thermal optimum for both *Pf* and *Pv* transmission by *An. gambiae*.[11] Finally, weather could be a direct trigger for relapse infections for *Pv* infections[18], and there is other indirect evidence that relapses may be triggered by antigens in uninfected mosquito saliva (biting rate is temperature-sensitive).[3] However, there is limited research on environmental drivers of *Pv*, especially in Latin America, including in the Amazon, which is estimated to bear 5% of the world's *Pv* malaria burden.[6]

Our objective was to assess the influence of rainfall and temperature on *Pv* incidence in Loreto Region, Peru, a low transmission area key to the Peruvian government's target of malaria elimination by 2030.[19] Environmental risk factors for malaria tend to be highly localized,[20,21] and the Loreto Region has maximum temperatures between 30–35ºC thought to be above the malaria transmission thermal optimum, and daily temperature extremes are thought to impact malaria transmission[22]. To our knowledge, the thermal biology of *Pv* transmission by *An. darlingi*, the primary vector in this region, has not been well characterized in disease ecology studies.

Information about climatic drivers of *Pv* transmission is needed to inform effective tailoring and targeting of interventions for malaria elimination.

METHODS

Study design

We analyzed malaria incidence data collected from January 2021 to December 2023 in 30 communities in San Juan Bautista, Punchana, Alto Nanay, Iquitos, and Belen districts in Loreto region, Peru, an area covering 2,569 km^2 (Figure 2). This study used data collected prior to baseline for the FocaL Mass Drug Administration for Vivax Malaria Elimination (FLAME) trial in Peru (NCT05690841), and therefore communities included in this study were those that met the eligibility criteria for FLAME. The study included surveillance data from riverine communities only accessible via boat on the Itaya, Momón, and Nanay rivers and dryland communities along the Iquitos-Nauta highway. The spatial distribution of these communities is shown in Figure 2. According to a census conducted for the FLAME trial, the average population size of each community was 240 individuals (range: 31 to 807).

Peru's malaria burden is concentrated in the Loreto Region, which includes a large swath of the Peruvian Amazon.[7] Since the 1990s, rapid urbanization and deforestation in the area surrounding Iquitos, the capital of the Department of Loreto, has contributed to increased malaria burden[7], likely through increased forest edge habitat, which promotes mosquito breeding, survival, and biting[23]. While this region is approaching elimination, sustained, endemic transmission remains, with an annual incidence rate of 17.4 cases per 1000 in 2019,[19] and *Pv* malaria accounts for approximately 80% of the malaria burden.[7] Transmission typically peaks in Loreto between February and July[7] and there is a sizable asymptomatic caseload.[13] Asymptomatic infections typically go undetected and uncounted in routine malaria surveillance in Peru,[13] since only febrile individuals are tested.

From 2005-2010, there was a large effort towards malaria elimination via the Project for Malaria Control in Andean Border Areas (PAMAFRO) program that improved case management, including through community case workers, and deployed insecticide-treated bed nets.[7] Transmission declined during the project, but the project did not achieve elimination, and transmission has since risen.[24] Following PAMAFRO, the government of Peru adopted Plan Malaria Cero (PMC) in 2017 to achieve malaria elimination. Control activities over the study period involved some reactive (RCD) and active case detection (ACD), distribution of long-lasting insecticide-treated bed nets, indoor residual spraying, and community-level chemoprophylaxis[25].

Outcome data

Our primary outcome was weekly community-level vivax malaria incidence primarily measured through passive case detection conducted by the Peruvian Ministry of Health (MINSA), though PMC activities including ACD and RCD also occurred over the study period and were recorded in surveillance data. Both physical records stored in health posts and online records compiled by MINSA were used in this analysis. Febrile patients presented for care at a health post and were tested for malaria via microscopy. According to PMC policy[25], blood smears are stained with 2% Giemsa for 30 minutes. Parasite densities are calculated from the number of asexual parasites per 200 leukocytes (or per 500, if <10 asexual parasites/200 leukocytes), assuming a leukocyte count of 8,000/µL. A blood smear is considered negative if examination of 100 high power fields does not reveal asexual parasites. Thin smears are used for parasite species identification. Slides are read by two microscopists. If there are discordant results, the results are determined by a third microscopist. Only *Pv* cases were included in the present study, and the data did not distinguish between primary infections versus relapse cases. Cases were matched to community census data to confirm community of residence, and then matched to a community centroid. Population counts per community were calculated from a census conducted from 2023, which was adjusted via interpolation for missing and/or non-participatory households.

Environmental variables

We obtained temperature and precipitation data from the ERA5-Land Hourly dataset collected by the Copernicus Climate Change Service[26]. We used the air temperature at 2 meters above the surface (K) band for all temperature variables and the total precipitation (m) band for precipitation variables. Weather variables were matched to incidence data via community centroid.

We imputed temperature or precipitation values less than 0 as missing. We aggregated hourly temperature values into the weekly minimum, mean, and maximum temperature; the average of each day's temperature range (maximum minus minimum) over a week; and total precipitation observed each week. We defined a binary indicator for high heat events coded as 1 for weeks with any days when the mean daily temperature exceeded the 90th percentile of mean daily temperatures for that year (average over all years was 27.5ºC) and 0 otherwise. Similarly, we defined a binary indicator for heavy rain events coded as 1 for weeks with any days when the total daily precipitation exceeded the 90th percentile of total daily precipitation for that year (average of all years was186mm) and 0 otherwise.

We conducted a distinct household-level analysis on distance to forest edge as a potential effect modifier of the associations between temperature and rainfall and malaria incidence. Minimum distance to forest edge was calculated from the MapBiomas Perú 2022 dataset.[27] We retained forest classes (forest, dry forest, mangrove, and flooded forest) and removed all other classes. These forest cells were transformed into vector point data for the distance calculation. For each household, we calculated the minimum Euclidean distance to the nearest forest within a 5-

kilometer (km) radius around each household. There were no households for which the minimum distance to forest edge was greater than 5km. Population density was calculated from the census data. A population density raster was created by summing up the number of individuals in each household with 1km by 1km resolution. Population density values were then extracted for each household GPS point.

Statistical analysis

We published a pre-analysis plan at https://osf.io/fgr6w. Deviations from the pre-analysis plan are listed in Table S1. All positive cases were matched to weather data. Thus, we performed a complete case analysis.

We assessed associations between malaria incidence and weather variables using distributed lag models. We chose a 2–16 week lookback period (i.e. an infection during the course of epidemiologic week 20 looked back to epidemiologic weeks 2—18) to account for potential variation in the influence of rainfall and temperature in *Pv* transmission and to account for potential impacts on relapse cases (Figure 1). For continuous exposures (weekly maximum temperature, weekly mean temperature, weekly mean temperature range, weekly minimum temperature, and total precipitation), we fit non-linear distributed lag (DL) models[28] with a log link and Poisson family using weekly malaria case counts per village as the dependent variable with an offset for log community population size. For the distributed lag cross-basis function, we specified a log function with 2 knots to allow for more flexible variation in the short term, and natural splines with 2 degrees of freedom for the non-linear predictors, which yielded the lowest AIC and BIC in model testing. All incidence ratios were calculated relative to reference weeks. For temperature predictors, weeks with the minimum observed value during the study period were used as the reference. For total precipitation, weeks with 0 mm of precipitation were used as the reference. Continuous temperature variables were adjusted for the cross-basis matrix for total precipitation, and total precipitation was adjusted for the cross-basis matrix for maximum temperature.

For binary predictors (high heat and heavy rain) we fit generalized additive models (GAMs) with a Poisson family and log link and an offset for community population size. We fit separate models at each lag from 2–16 weeks. We adjusted the high heat model for total precipitation at the concurrent lag modeled as a smooth term with $k = 3$. Similarly, we adjusted the heavy rain model for maximum temperature at the concurrent lag modeled as a smooth term with $k = 3$.

To examine the influence of other environmental factors, we conducted subgroup analyses by: distance to forest edge (above/below the median), season (rainy/dry), and community type (riverine/dry land). We discretized the distance from each household to the nearest forest edge into a binary variable for above or below median distance, and categorized months November-April as the rainy season and all other months as the dry season based on both historical precendence[7] and data for our study. For distance to forest edge models, we aggregated incidence per household-week and adjusted for 1km population density. We fit the same DL models as the main effect models on subsets of the data according to the subgroup, and non-forest models were offset for community size. We also assessed the association between the minimum distance to forest edge as both a continuous and categorical predictor and fit GAMs with Poisson distributions and log link functions.

For the association between maximum temperature and malaria incidence, we conducted two sensitivity analyses with additional adjustments. First, we adjusted for both total precipitation and minimum temperature at concurrent lags to account for potential collinearity between minimum temperature and maximum temperature (Pearson's correlation coefficient = 0.48). Second, we adjusted for minimum temperature at concurrent lags and total precipitation at 4-week delayed lags, where we only considered the effect of precipitation beginning at 6 weeks and continuing through 20 weeks. This time-delayed lag was included to account for the onset of the rainy season about 4 weeks after the highest maximum temperatures were typically observed (Figure 3).

For the association between total precipitation and malaria incidence, we conducted a sensitivity analysis where we adjusted for minimum temperature instead of maximum temperature. The Pearson's correlation coefficient for total precipitation and maximum temperature was -0.30, and thus we kept this as the main effect adjustment covariate, while the Pearson's correlation coefficient for total precipitation and minimum temperature was 0.1.

Because incidence data for the weather models was aggregated at the community level, we did not test for spatial autocorrelation or adjust for spatial clustering. All hourly to daily aggregation was performed using the Python API for Google Earth Engine on Google Colab servers. Weekly aggregation and modeling were performed in R version 4.2.1. All distributed lag models were built using the R package DLNM version 2.4.7[29]. This study was approved by the Institutional Review Board of Stanford University (72291) and by the Dirección Universitaria de Asuntos Regulatorios de la Investigación de la Universidad Peruana Cayetano Heredia (211747).

RESULTS

During the study period from January 2021 to December 2023, the rainy season generally lasted from November through April (Figure 3). Weekly cumulative precipitation ranged between 500–1750 mm during the rainy season and did not exceed 500mm in the dry season which lasted roughly from July through October.

Minimum temperature varied from 22–26ºC, and maximum temperature varied from 28–38ºC. The weekly mean temperature range varied from 4–12ºC. During the dry season, there was more variation in temperature: minimum temperature dipped below 20ºC in the early dry season, while temperature peaked during the late dry season, with maximum temperature values as high as 38ºC.

The mean threshold for high heat events across all study years was 27.5ºC, and high heat events generally occurred just prior to the rainy season. The mean threshold for heavy rain events across all study years was 186 mm, and there were more heavy rain events during the rainy season than the dry season. 2023 was an El Niño year, reflected by the low precipitation and high temperature compared to 2022 and 2021. Malaria incidence did not appear to follow a distinct seasonal trend.

Temperature

Associations between minimum or maximum temperature and malaria incidence are shown in Figure 4. Weeks with a minimum temperature of 22.5ºC (the median) were associated with 2.16–3.93-fold higher malaria incidence for lags 3–16 weeks compared to weeks with the lowest minimum temperature of 16.8ºC, with the incidence ratio peaking at lag of 5 weeks (IR = 3.93, 95% CI 2.18–7.09) (Figure 4A, 4C). Malaria incidence was approximately 3- to 4-fold higher for minimum weekly temperatures above 16.8ºC at a 5-week lag (Figure 4B).

The effect of median maximum temperature on incidence was non-linear and hump-shaped across lag time (Figure 4D). Median maximum temperature was associated with higher incidence at lags of 6–13 weeks, whereas it was associated with lower incidence at the extremes of 3 weeks and 14–16 weeks. Compared to weeks with the lowest maximum temperature (28.4ºC), weeks with a maximum temperature of 31.8ºC (the median) were associated with 66% higher malaria incidence (95 CI 1.35–2.04) at a 9-week lag (Figure 4D, 4F). The positive association between median maximum temperature and malaria incidence grew stronger as maximum temperatures increased to 32.5ºC, then plateaued and confidence intervals were wide (Figure 4E).

In a sensitivity analysis adjusting for concurrent total precipitation and minimum temperature, compared to weeks with the lowest maximum temperature, weeks with a maximum temperature of 31.8ºC (the median) were still associated with higher malaria incidence at 6–11 week lags (Figure S1). There was no association at other lags. In another sensitivity analysis adjusting for minimum temperature at concurrent lags and total precipitation at 4-week delayed lags, weeks with a maximum temperature of 31.8ºC (the median) were still associated with higher malaria incidence at 6–9 week lags (Figure S2) and were not associated with incidence at other lags.

Weeks with high heat events (one or more days when the daily mean temperature exceeded 27.5ºC) were associated with 35% lower malaria incidence at a 2-week lag and 1.23–1.37-fold higher malaria incidence at lags 5–9 weeks compared to weeks with no high heat events (Figure 5A, Table S2).

For analyses of mean temperature, compared to weeks with the lowest value (23.1ºC), weeks with a mean temperature of 25.6ºC (the median) were associated with 10–44% lower malaria incidence for lags 2–8 weeks (Figure S3A, S3C), and the association was strongest at a 3-week lag (IR = 0.66, 95% CI 0.51–0.84). The protective association was stronger at higher mean temperatures at a 3-week lag (Figure S3B).

For analyses of temperature range, compared to weeks with the narrower ranges of 4.3ºC, weeks with a temperature range of 6.5ºC (the median) were associated with 1.33- to 1.94-fold higher malaria incidence for lags 6–14 weeks (Figure S4A, S4C), with the strongest association at a 9-week lag (IR = 1.94, 95% CI 1.58–2.38). The positive association was stronger at wider mean temperature ranges at a 9-week lag (Figure S4B).

Precipitation

The association between the effect of precipitation median precipitation on incidence was non-linear and hump-shaped across lag time (Figure 6A). Compared to weeks with no rainfall, weeks with 450mm of precipitation (the median) were associated with 1.13- to 1.45-fold higher malaria incidence for lags 6–14 weeks, with the strongest association at 9 weeks (IR = 1.45, 95% CI 1.27–1.65) (Figure 6A, 6C). The association was also non-linear and hump-shaped across the range of precipitation values, with the strongest associations with malaria incidence at approximately 750mm of precipitation at a 9-week lag (Figure 6B). In a sensitivity analysis adjusting for minimum temperature instead of maximum temperature, associations were still positive but somewhat attenuated towards the null (Figure S5).

Compared to weeks without heavy rain, weeks with heavy rain events (one or more days when the daily precipitation exceeded 186mm) were associated with 1.22–1.60-fold higher malaria incidence at lags 2-10 weeks, with the strongest association at 6 weeks (IR = 1.60, 95% CI 1.33–1.94) (Figure 5B). Heavy rain was associated with 24% lower malaria incidence at 16 weeks (95% CI 0.63–0.92).

Sub-group analyses

In a sub-group analysis by season, high heat events were protective in the dry season and associated with higher incidence in the rainy season at longer lags (Figure S6); heavy rain events were associated with higher incidence in the rainy season at up to 11-week lags, while results were generally null for the dry season (Figure S6).

We also performed a sub-group analysis by community type; 13 communities were classified as dryland communities, while 16 were riverine. High heat events were associated with higher incidence in dryland communities and lower incidence in riverine communities at longer lags (Figure S7); heavy rain events were generally associated with higher incidence in dryland communities across all lags, but lower incidence in riverine communities at longer lags (Figure S7).

At most lags, the association between malaria incidence and maximum temperature or total precipitation did not vary by a household's minimum distance to forest edge (Figure S8, S9). Shorter minimum distance to forest edge was associated with higher malaria incidence (Figure S10). For households living within 50m of forest edge, malaria incidence was 2.18-fold higher (95% CI 2.18–2.19) compared to those who lived 500m or beyond from forest edge (Figure S1A).

All main effect continuous predictor estimates can be found in Table S2. All main effect binary predictor estimates can be found in Table S3.

DISCUSSION

In this study of associations between temperature and precipitation with malaria incidence in Loreto Region, Peru, we found that higher minimum temperatures, maximum temperatures, temperature ranges, and total precipitation were all associated with higher malaria incidence compared to weeks with minimum values. Mean temperature was generally associated with lower malaria incidence in the short (2—4 weeks) and medium (5—10 weeks) term. The effect of these weather variables generally lasted over many weeks, with the longest duration and strongest associations with minimum temperature. The effect of minimum temperature also began sooner and lasted longer than that of maximum temperature or temperature range. This may suggest that cooler minimum temperatures are a limiting factor for malaria transmission. High heat events were associated with lower malaria incidence in the short term and higher malaria incidence in the medium term. Heavy rain events were generally associated with increased malaria risk in the short and medium term, and lower malaria incidence in the long term (11—16 weeks). There was strong evidence that living further away from forest edges was protective. Overall, findings suggest that higher temperatures, particularly minimum temperatures, and periods of sustained rainfall could lead to periods of increased malaria burden.

In general, our findings suggest that weather influences malaria transmission over 1-4 month lags in this region. This lasting association may be due to the relapse periodicity of *Pv* malaria. Even after typical treatment, 6-month relapse infection rates in this region ranged between 27—33%[30], but true relapse rates are likely higher because many *Pv* infections go undetected and untreated, or are treated without medical supervision, outside of study settings[31]. However, the vast majority of *Pv* infections are estimated to be relapse infections[32], and thus it is likely that increased incidence includes relapse cases – another study in this region found that PCR-confirmed *Pv* prevalence was as high as 25%, most of which were attributed to asymptomatic, low parasitemia infections.[33] Additionally, though the relapse period for *Pv* infections is poorly understood in this region, relapses from the tropical strains of *Pv* are estimated to range anywhere from 2–9 months.[16] Though strong geographic and micro-geographic differences exist in the Peruvian Amazon,[34] a study in neighboring Brazil found that the median time to the first recurrent infection was 71 days, and that treatment delays the onset of the subsequent relapse infection, while in Southeast Asia, where short latency *Pv* is also present, 90% of relapse cases occurred within 6 weeks of the initial infection.[35] The extended association with weather over many weeks could also reflect a high prevalence of asymptomatic and low parasitemia infections[4,5], which could sustain new, onward transmission well past the initial meteorological trigger.

Precipitation

We found that weeks with higher precipitation were significantly associated with higher malaria incidence at lags of 6–14 weeks. These results are generally in accordance with other studies of the effect of rainfall on *Pv* infections. A meta-analysis in Mauritania found that *Pv* incidence was highest during and after the rainy season[36] and that decreased rainfall was significantly correlated with decreased malaria burden; in temperate regions, one study in South Korea found that increased precipitation was associated with higher malaria incidence at a 10-week lag,[37] while a similar study in China also found positive associations with precipitation at lags 2–4 weeks.[38] The lasting influence of precipitation likely reflects increased breeding ground following rainfall, which could impact multiple transmission cycles.

Interestingly, heavy rain was generally associated with higher malaria incidence in the rainy season, but not in the dry season (Figure S8B). There were few heavy rain events in the dry season, thus limiting the statistical power of this analysis; however, our finding for the rainy season somewhat aligns with a hydrogeology hypothesis by Hiwas and Bretas[9], wherein heavy rain in the rainy season may flood otherwise dry areas, increasing malaria breeding ground. We also found some evidence of qualitative effect modification by community type, with heavy rain associated with lower incidence in riverine communities at longer lags and higher incidence at longer lags in dryland communities (Figure S9B). This finding contradicts the hydrogeology hypothesis[9]. In riverine communities, heavy rains may be protective if they flush and agitate otherwise slow-moving rivers and stagnant pools of water and thus disrupt mosquito larva breeding; on the other hand, in dryland communities, heavy rains may increase risk due to the accumulation of surface water in otherwise dry areas. In both cases, the effect modification is strongest at longer lags, signaling that heavy rain had long-lasting effects on the transmission cycle.

Temperature

We found that higher minimum temperatures were strongly associated with higher malaria incidence consistently across lags, while maximum temperature and high heat events were associated with higher malaria incidence in the medium term and lower incidence in the short and long term. Though the thermal optimum of the *Pv–An. darlingi* coupling has not been studied directly, there is some mixed support in the literature from other malaria transmission couplings: optimal *Pf* malaria transmission is thought to peak at 25ºC,[10,17] which generally aligns with our findings for positive associations with higher minimum temperature, which were in the range of 20-24ºC. Our finding of the strongest associations with minimum temperature are biologically plausible given that *An. darlingi* typically bite humans at nighttime[39] and daily temperatures are lowest at night.

We were surprised to find that higher maximum temperatures in the range of 30-34ºC and high heat events (>27ºC) were associated with increased incidence at medium lags and lower incidence at short lags. Our maximum temperature findings were attenuated, but robust in sensitivity analyses. High heat events may be correlated with other longer-term weather variations, such as higher maximum temperature for prolonged periods, that affect incidence. High temperatures can reduce the vector biting rate and survival rate, which may explain the negative associations at shorter lags. However, high maximum temperatures may trigger *Pv* relapse and increase the reservoir of active infections that contribute to onward malaria transmission, explaining the positive association at medium lags. The positive association between higher maximum temperature and high heat and malaria at medium lags conflicts with previous disease ecology literature supporting a thermal optimum for *Anopheles-Pf* malaria transmission at 25ºC with an upper limit of 32.6ºC,[10] and validated *Pf* malaria transmission declines above 28ºC[17] in Africa. Our result does have some support in the literature: a study in neighboring regions of the Amazon with similar maximum temperatures (26.8–35.2ºC) found that a 1ºC increase in maximum temperature was associated with higher incidence at 1- and 2-month lags.[40] Generally, however, *Anopheles* life stages related to transmission (i.e., biting rate, larva survival, fecundity, adult survival) are thought to decline at the median maximum temperature range in our study (30–34ºC),[11] indicating that vector activity is unlikely to be the mechanism driving this positive association. Further research on the thermal biology of *Pv–An. darlingi* in tropical settings are needed to elucidate the influence of temperature on malaria transmission in this region. However, it is also possible that microclimates and small-scale ecological and topographical variation in our study sites tempered high temperatures and their associated negative impacts on transmission, since our weather data was at 11km resolution and did not capture temperature variation more finely (i.e. in shaded forest areas, close to water bodies)

One alternative explanation is that associations with maximum temperature primarily reflect relapsing *Pv* infections, which could be triggered by temperature itself or co-infections that are more common at higher temperatures. Extrinsic triggers of *Pv* relapse include co-infections that result in host inflammation, subsequent primary *Pv* infections, seasonal changes in sunlight and temperature, and mosquito bites and their associated immune responses[16,41]. In our study site, dengue is increasingly common,[42] and it is well-documented that dengue transmission is positively associated with maximum temperatures similar to those in our study[43–45] and has a higher thermal optimum (29ºC) and upper limit (34.5ºC) than malaria.[10] If high heat events trigger *Pv* relapse, symptoms would likely appear within 2 weeks, yet we found positive associations with maximum temperature at lags of 6–11 weeks. It is possible that high temperatures activate the hypnozoite and increase the reservoir of active infectious hosts, potentially leading to onward transmission and multiple cycles of infection, explaining the delayed positive association.

Relationships with high heat varied by season and community type. At lags of 3—4 months, high heat was associated with higher malaria incidence in the dry season but lower malaria incidence in the rainy season. High heat events may dry up surface water in the dry season, reducing vector breeding, while they may lead to stagnation of water bodies in the rainy season, promoting breeding. Similarly, we found that high heat was associated with higher malaria incidence in dryland areas, but lower malaria incidence in riverine areas at lags of 3—4 months. In general, the dryland communities in our study site were more peri-urban and more densely populated; considering dengue transmission is generally thought to thrive in more urban, densely populated communities,[46,47] it is possible that elevated incidence reflects higher relapse rates due to co-infection with diseases like dengue.

Limitations

Our study had several limitations. We were unable to distinguish between initial and relapse infections in our incidence data; we would expect that weather would influence initial and relapse cases over different lag periods, but our analysis was not able to investigate this. However, using distributed lag models allowed us to investigate associations over a 4-month period, including potential relapse cases. A follow-up study using genomic methods to differentiate relapse and initial infections could shed light on how weather influences primary vs. relapse infections. Our outcome data also only covered a 3-year period, preventing this analysis from studying longer climatic trends in incidence in this region. The study period also included two years (2021 and early 2022) where control efforts for the COVID-19 pandemic likely limited malaria transmission as well. We were also unable to control for PMC activities nor interventions over the study period.

Regarding the weather data, one of our study years (2023) was an El Niño year; a study capturing a full El Niño-La Niña cycle could better elucidate associations with incidence along these more variable climate cycles. While we selected ERA-5 Land remote sensing data for its temporal coverage, its 11 km spatial resolution prevented identification of small-scale microclimates, i.e. those created my forest cover and topographical features, and thus did not capture small-resolution variations that likely have a large effect on mosquito breeding and survival habitat[48]. Additionally, publicly available surface water data did not reflect our ground observations of surface water in the study site, so we did not include it in this analysis. Further research that considers mediation by surface water could further shed light on the complicated dynamics between precipitation and malaria incidence in this region. One such complication that our study did not resolve is the concentration-dilution hypothesis[9] for malaria transmission, which posits that heavy rain in riverine communities can cause flooding and increase standing water presence, leading to increased incidence, while the same heavy rain can flush dryland communities and lead to decreased incidence. Our sub-group analyses by distance to forest edge, community type, and season were also likely limited in statistical power; these findings could be more conclusive with a longer look back period. Furthermore, our binary predictor analyses modeled individual lags separately and did not account for time autocorrelation, limiting comparisons between adjacent lags and with DL models. This study was also correlational, and inferred relationships with individual weather variables may be confounded by collinearity and correlation with other weather variables, and thus makes it difficult to tease out individual direct effects.

Conclusions

In our study of malaria incidence in Loreto Region, Peru, we observed generally positive associations with higher temperatures and higher rainfall for extended lag periods beginning 2–3 weeks after symptom onset and enduring for about 1–4 months. Our findings indicated that the coupled transmission and relapse cycle of *Pv–An. darlingi* may have more complicated associations with higher temperatures than other malaria parasite – vector pairings, a critical finding in the face of climate change and global warming. These findings provide critical context to ongoing malaria elimination efforts, since apparent successes or failures of malaria interventions may be due in part to long-lasting effects of weather on initial infection and relapse.

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Figure 1. Timeline of main hypothesized mechanisms of the impact of temperature and precipitation on *Plasmodium vivax* infection. Time estimates are generalized and were generated from previous findings.

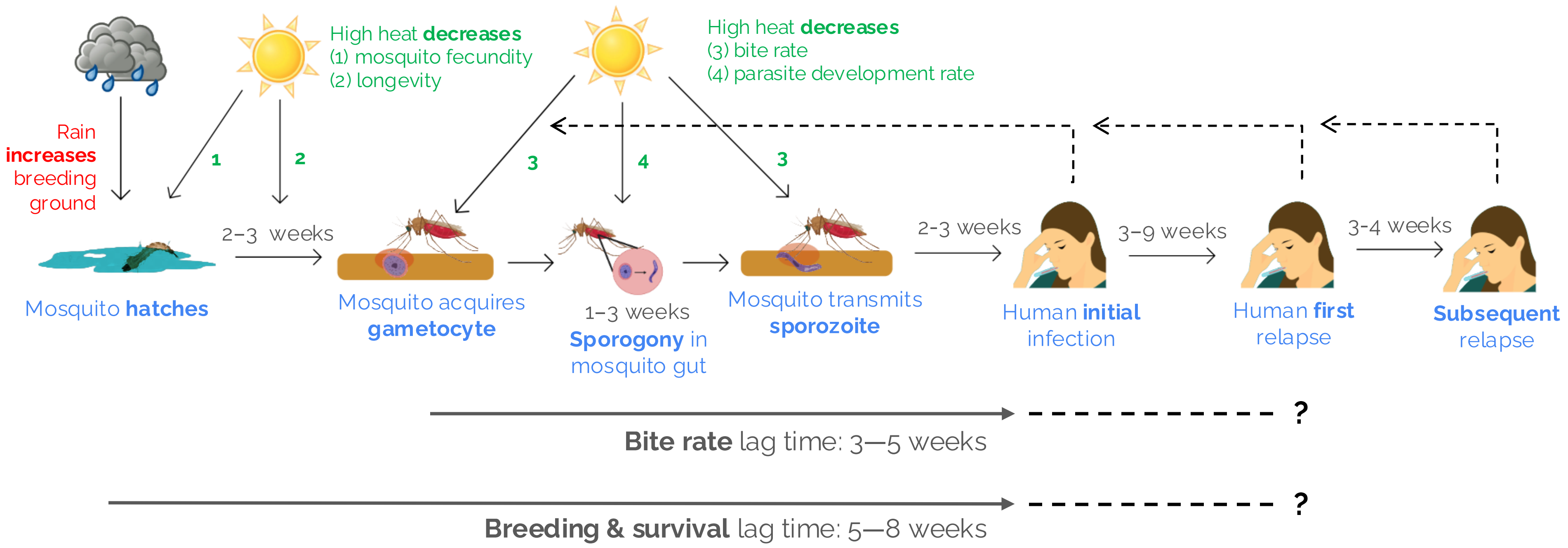
Figure 2. Study area map. Dark green areas indicate forest cells. Circles with black outlines represent the 30 study communities, colored by annual malaria incidence per 1000 individuals. The blue river to the right of Iquitos is the Amazon River.

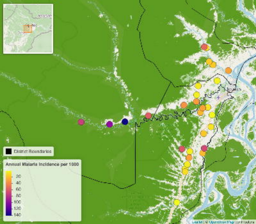
Figure 3. Weather and malaria incidence trends over the study period. All measures are aggregated weekly. The blue dots in plot A mark weeks with a heavy rain event, defined as one or more days when the daily precipitation total exceeded the 90th percentile, which averaged 186mm over the study period. The orange dots in plot B mark weeks with a high heat event, defined as one or more days when the mean temperature exceeded the 90th percentile. Shaded blue regions represent the rainy season, defined as November–April. In plot B, the darker orange line tracks the maximum temperature observed each week, and the lighter orange line tracks the minimum temperature observed each week.

Figure 4. Association between temperature and malaria incidence. The associations were fit using distributed lag non-linear models. Plots A–C show the association for minimum temperature, and plots D–F show the association for maximum temperature. For all plots, incidence ratios are relative to reference weeks with the minimum observed predictor value. Plots A and D show the association between temperature and malaria incidence at the median predictor value compared to the reference. For minimum temperature, the reference is 16.8ºC; for maximum temperature, the reference is 28.4ºC. For minimum temperature, the median is 22.5ºC. For maximum temperature, the median is 31.8ºC.

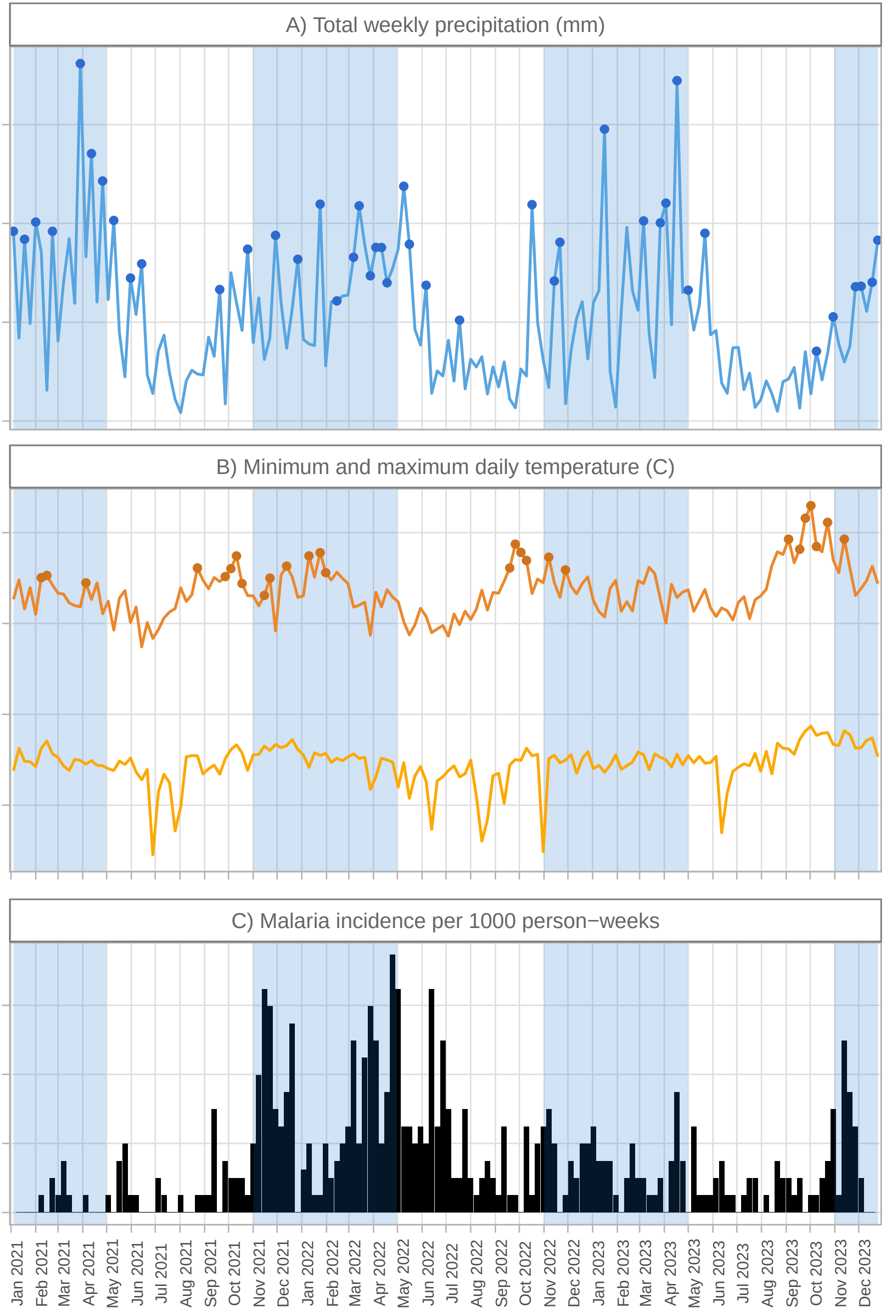
Figure 5. Association between binary predictors and malaria incidence. Incidence ratios were calculated for weeks with the event relative to weeks without the event. The average high heat threshold over the study period was 27.5ºC. The average heavy rain threshold over the study period was >186 mm of precipitation in one day. High heat estimates at each lag were adjusted for total precipitation at the same lag. Heavy rain estimates at each lag were adjusted for maximum temperature at the same lag.

Figure 6. Association between total precipitation and malaria incidence. The association was fit using a distributed lag non-linear model. For all plots, incidence ratios are relative to reference weeks with 0mm of precipitation. Plot A shows the association between precipitation and malaria incidence at the median predictor value, 450mm, compared to the reference.


High heat decreases
(1) mosquito fecundity
(2) longevity
High heat decreases
(3) bite rate
(4) parasite development rate
Rain increases breeding ground
1
2
3
4
3
2–3 weeks
2-3 weeks
3–9 weeks
3-4 weeks
1–3 weeks
Mosquito hatches
Mosquito acquires gametocyte
Sporogony in mosquito gut
Mosquito transmits sporozoite
Human initial infection
Human first relapse
Subsequent relapse
?
Bite rate lag time: 3—5 weeks
?
Breeding & survival lag time: 5—8 weeks



District Boundaries
Annual Malaria Incidence per 1000
20
40
60
80
100
120
140
Leaflet | © OpenStreetMap contributors



A) Total weekly precipitation (mm)
0
500
1000
1500
B) Minimum and maximum daily temperature (C)
20
25
30
35
C) Malaria incidence per 1000 person−weeks
0.0
0.5
1.0
1.5
Jan 2021
Feb 2021
Mar 2021
Apr 2021
May 2021
Jun 2021
Jul 2021
Aug 2021
Sep 2021
Oct 2021
Nov 2021
Dec 2021
Jan 2022
Feb 2022
Mar 2022
Apr 2022
May 2022
Jun 2022
Jul 2022
Aug 2022
Sep 2022
Oct 2022
Nov 2022
Dec 2022
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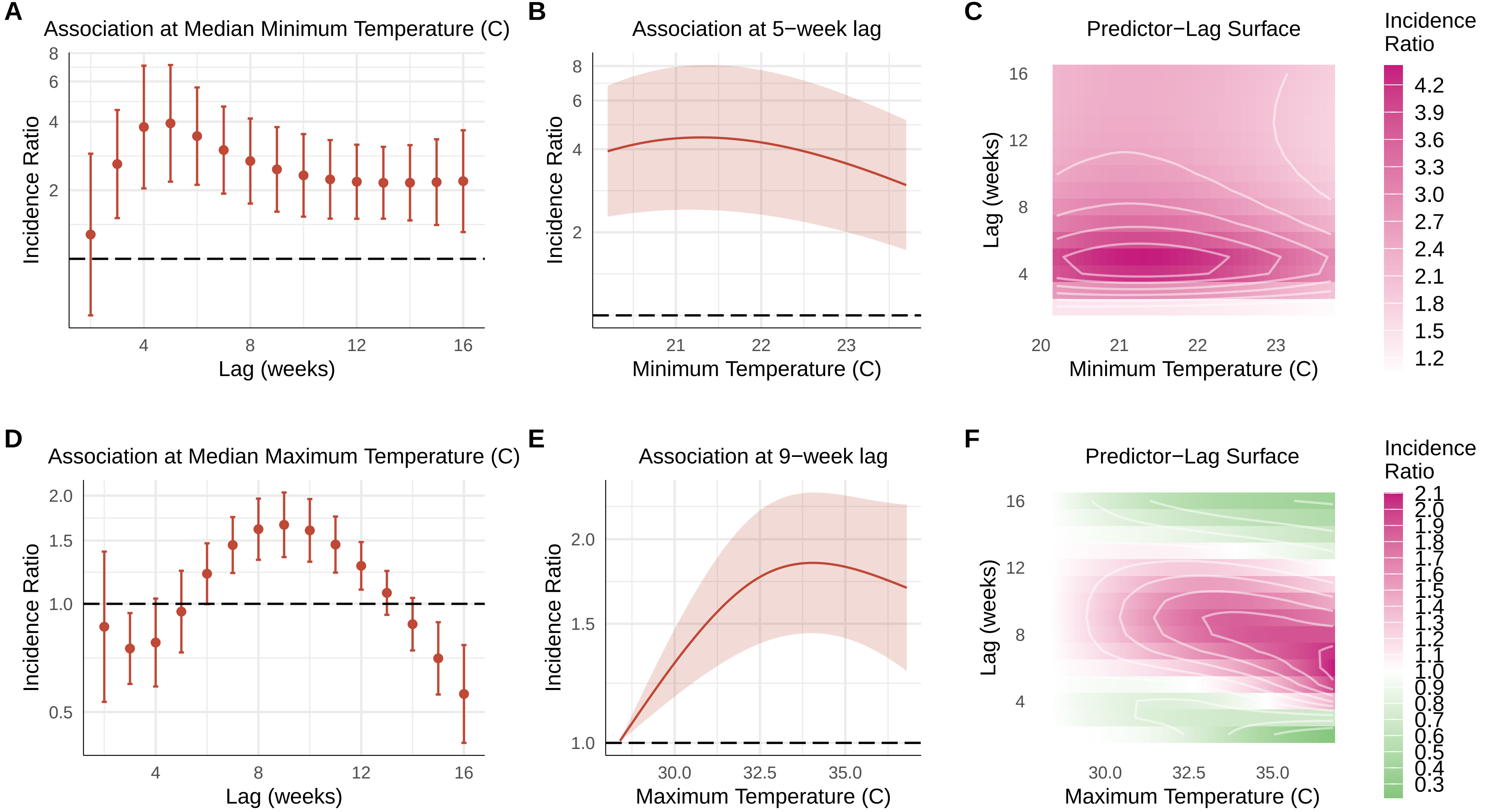
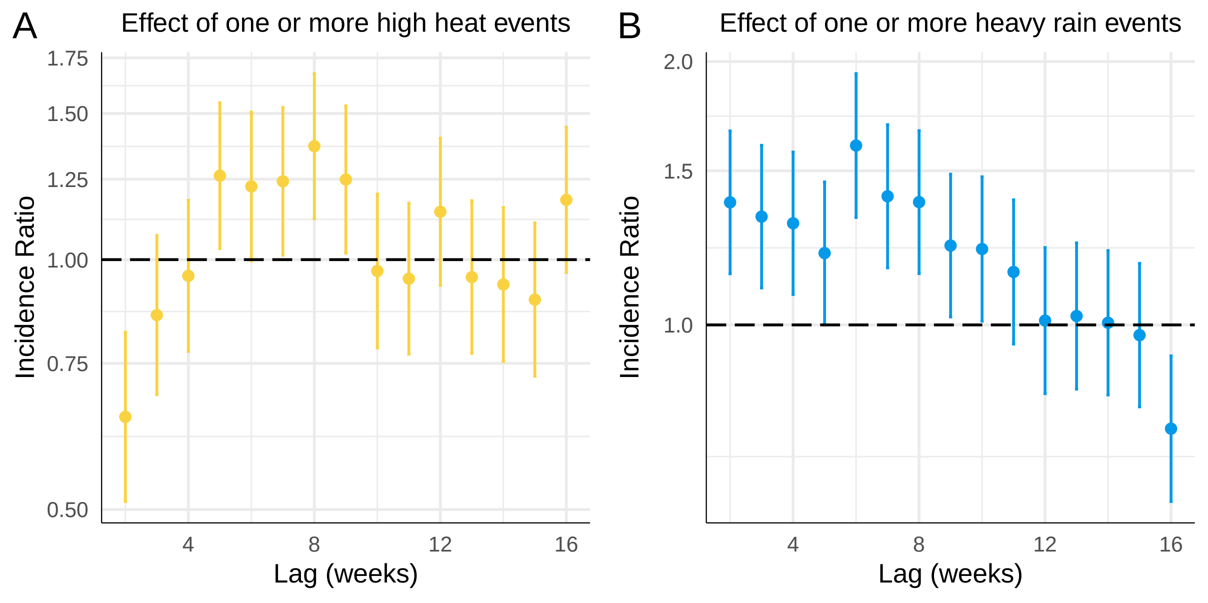
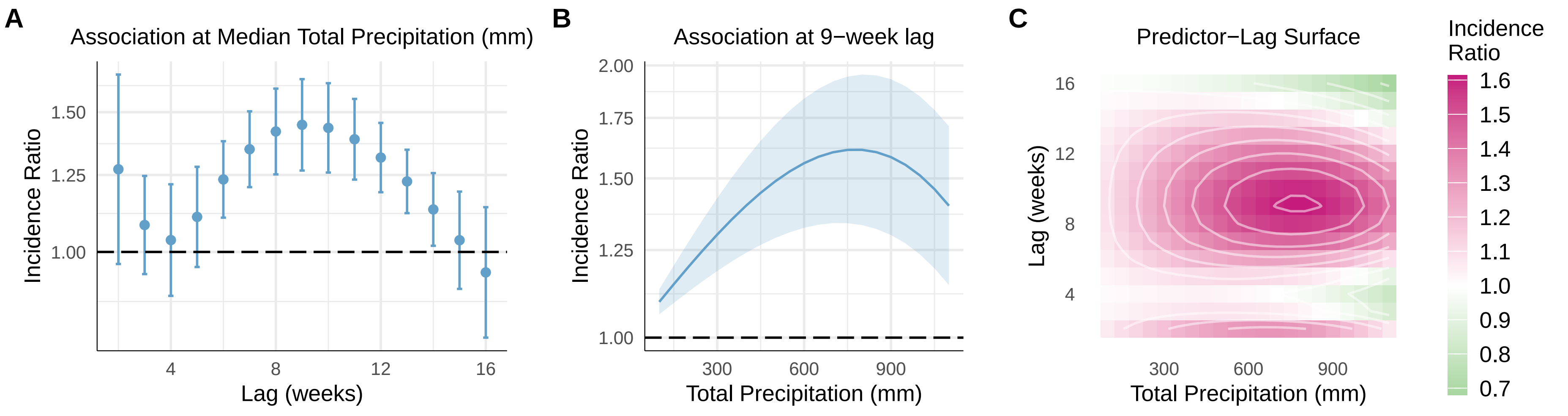
Oct 2023
Nov 2023
Dec 2023



A
Association at Median Minimum Temperature (C)
Incidence Ratio
Lag (weeks)
B
Association at 5−week lag
Incidence Ratio
Minimum Temperature (C)
C
Predictor−Lag Surface
Lag (weeks)
Minimum Temperature (C)
Incidence Ratio
D
Association at Median Maximum Temperature (C)
Incidence Ratio
Lag (weeks)
E
Association at 9−week lag
Incidence Ratio
Maximum Temperature (C)
F
Predictor−Lag Surface
Lag (weeks)
Maximum Temperature (C)
Incidence Ratio


A
Effect of one or more high heat events
Incidence Ratio
1.75
1.50
1.25
1.00
0.75
0.50
4
8
12
16
Lag (weeks)
B
Effect of one or more heavy rain events
Incidence Ratio
2.0
1.5
1.0
4
8
12
16
Lag (weeks)


A
Association at Median Total Precipitation (mm)
Incidence Ratio
1.50
1.25
1.00
4
8
12
16
Lag (weeks)
B
Association at 9−week lag
Incidence Ratio
2.00
1.75
1.50
1.25
1.00
300
600
900
Total Precipitation (mm)
C
Predictor−Lag Surface
Lag (weeks)
16
12
8
4
300
600
900
Total Precipitation (mm)
Incidence Ratio
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7