Title: Mosquito species and age influence thermal performance of traits relevant to malaria transmission

Authors:

Miazgowicz, KL[1,2,3], Mordecai, EA[4], Ryan, SJ[5,6,7], Hall, RJ[1,3,8], Owen, J[1], Adanlawo, T[8], Balaji, K[1], and Murdock CC[1,2,3,8,9,10*]

Author Affiliations:

[1]Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, Georgia, United States of America.

[2]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, Georgia, United States of America.

[3]Center of Ecology of Infectious Diseases, University of Georgia, Athens, Georgia, United States of America.

[4]Biology Department, Stanford University, Stanford, California, United States of America.

[5]Quantitative Disease Ecology and Conservation (QDEC) Lab, Department of Geography, University of Florida, Gainesville, FL, United States

[6]Emerging Pathogens Institute, University of Florida, Gainesville, FL, United States.

[7]School of Life Sciences, University of KwaZulu-Natal, Durban, South Africa

[8]Odum School of Ecology, University of Georgia, Athens, Georgia, United States of America.

[9]Center for Vaccines and Immunology, University of Georgia, Athens, Georgia, United States of America.

[10]River Basin Center, University of Georgia, Athens, Georgia, United States of America.

*Corresponding Author: Courtney C. Murdock; cmurdock@uga.edu

Abstract

Models predicting disease transmission are a vital tool in the control of mosquito populations and malaria reduction as they can target intervention efforts. We compared the performance of temperature-dependent transmission models when mosquito life history traits were allowed to change across the lifespan of *Anopheles stephensi*, the urban malaria mosquito, to models parameterized with commonly derived estimates of lifetime trait values. We conducted an experiment on adult female *An. stephensi* to generate daily per capita values for lifespan, egg production, and biting rate at six constant temperatures. Both temperature and age significantly affected trait values. Further, we found quantitative and qualitative differences between temperature-trait relationships estimated based on daily rates versus directly observed lifetime values. Incorporating these temperature-trait relationships into an expression governing transmission suitability, relative $R_0(T)$, model resulted in minor differences in the breadth of suitable temperatures for *Plasmodium falciparum* transmission between the two models constructed from only *An. stephensi* trait data, but a substantial increase in breadth compared to a previously published model consisting of trait data from multiple mosquito species. Overall this work highlights the importance of considering how mosquito trait values vary with mosquito age and mosquito species when generating temperature-based environmental suitability predictions of transmission.

Key Words: Anopheles, Temperature, Malaria, Transmission, Life History, Senescence

Introduction

Despite the progress of global malaria elimination programs in reducing the incidence of human malaria, particularly *Plasmodium falciparum*, malaria remains a leading cause of morbidity and mortality among infectious diseases (1). The occurrence of multi-class drug and insecticide resistance, in addition to alterations in mosquito behavior, challenge our ability to eradicate malaria and poses the possibility of resurgence (1-6). While numerous environmental factors affect the distribution and prevalence of mosquito-borne diseases, temperature is one of the most pervasive abiotic factors affecting both mosquito and parasite vital rates (7-23). However, even though the importance of these factors is increasingly recognized, gaps remain in the current mechanistic understanding of the relationship between malaria risk and key environmental variables. Improving our understanding of the link between temperature and malaria transmission will be crucial for predicting how transmission varies geographically, seasonally, and with climate and land use change (10, 24-30).

Recent research has begun to explicitly define the relationship between temperature and vector and pathogen traits relevant to transmission across a diversity of vector-borne disease systems (24, 26, 31-37). The net effect of these traits in determining temperature-dependent transmission potential can be described by the pathogen basic reproduction number (R_0), defined as the number of secondary cases arising from a primary case given a fully susceptible population. Transmission models that define R_0 can be used to generate predictions of disease risk, inform intervention strategies, and evaluate efficacy of various disease interventions (38-43). Although it is widely accepted that the life history traits of ectotherms exhibit unimodal responses to temperature, models often assume that the temperature-trait relationships for key mosquito and parasite life history traits are linear (37, 44-48). Further, recent research that

included nonlinear, unimodal temperature-trait relationships into an R_0 model for malaria transmission predicted a lower temperature optimum, minimum, and maximum than previous estimates based on linear relationships, which better matched field observations of entomological inoculation rate (31, 32). Key biological insights from previous models are that: 1) malaria transmission is constrained in hot summer months in equatorial and tropical regions, 2) regions of the world that are currently permissive for transmission may become less environmentally suitable with future climate warming, and 3) vector control may become more difficult as temperatures in northern latitudes become more permissive and suitable seasons extend (24, 32, 49).

Despite these advances, insights from previous mechanistic R_0 models remain constrained by a lack of entomological and parasite data (31). Temperature-trait relationships for key parameters are often indirectly estimated from a limited number of studies, leading to high uncertainty around the predicted thermal limits in current malaria R_0 models (31, 32). Additionally, the parameterization of R_0 models with temperature-trait relationships aggregated from different mosquito and parasite species likely introduces error and uncertainty in R_0 estimates given the degree of inter- and intra-species variation in life history (7, 31, 32).

Further, evidence from a diversity of invertebrate systems demonstrates that organisms experience age-related changes in life history traits (50-53). These changes reflect either senescence, a decline in general physiological function with age, or a shift in energy allocation to different life history tasks as an organism ages to maximize fitness. Limited studies suggest that age modifies mosquito life history, with some evidence that mosquitoes experience reproductive senescence (54), bite more frequently as they age (55), and exhibit age-dependent survivorship (52). Yet, incorporating the combined effect of temperature and age on mosquito life history

traits has not been explicitly addressed in temperature-dependent R_0 models for malaria. Studies often use data collected over a relatively limited portion of the mosquito lifespan to estimate lifetime traits such as biting rates, total egg production, and lifespan in models of mosquito population dynamics and disease transmission (23, 24, 26, 31, 32, 35, 36). For example, a recent study characterized the extrinsic incubation period of *P. falciparum* in a cohort of *An. stephensi* mosquitoes, but used the duration of the gonotrophic cycle to approximate the daily biting rate and thus force of infection (23). If key mosquito life history traits vary with age, and temperature influences age-related changes in these traits, then precisely when these traits are measured during the lifespan of the mosquito could impact the predicted relationships between these traits and temperature as well as the predicted environmental suitability for malaria transmission.

In this study, we conducted a cohort life table experiment on the urban Indian malaria vector (*An. stephensi*) at six different constant temperatures to address the following questions: 1) does the effect of temperature on transmission change when thermal responses of traits from a single mosquito and parasite species are used, rather than aggregated from multiple mosquito and parasite species? 2) how do *An. stephensi* life history traits vary across the full spectrum of biologically relevant temperatures? 3) do life history traits that drive human malaria transmission vary with mosquito age? and if so, 4) do age-dependent changes in life history affect temperature-trait responses and the overall temperature suitability for transmission?

Materials & Methods

Life history experimental design

An. stephensi mosquitoes were reared as described in **SI_Methods**. The life history experiment was initiated three days after adult emergence to permit mating. After they were

111 presented with an initial blood meal for 15 minutes via a water-jacketed membrane feeder we
112 randomly distributed 30 host-seeking females into individual cages (16 oz. paper cup with mesh
113 top) to one of six constant temperature treatments (16°C, 20°C, 24°C, 28°C, 32°C, 36°C ± 0.5°C,
114 80% ± 5 RH, and 12L:12D photoperiod). Each individual adult cage contained an oviposition
115 site: a small petri dish that secured to the bottom of the housing, containing cotton balls to retain
116 liquid, overlaid with a filter paper for easy egg removal and counting. Individual mosquitoes
117 were offered a blood meal for 15 min each day. An individual was scored as having taken a
118 blood meal through visual verification of the abdomen immediately after the feeding period.
119 Oviposition sites were rehydrated and checked for the presence of eggs daily. We followed
120 populations of individual females in each temperature treatment until all mosquitoes had died or
121 when less than 7% of the starting population remained. At least two biological replicates were
122 performed at each temperature (n= 30 per temperature; total n= 390 individuals). Given the
123 extended duration of these experiments (~60 days), multiple blood donors were used throughout
124 each experimental replicate.

125 ***Statistical analysis***

126 All statistical analyses were performed using the program R (version 3.4.1). We used
127 generalized linear mixed models (GLMM) R package <*lme4::glmer()*> to estimate the effects of
128 temperature, mosquito age, and their interaction on the proportion of females that imbibed blood
129 on a given day (i.e., the number of females that took a blood meal on a given day out of the total
130 number of females alive on that day for each temperature treatment) and the mean daily egg
131 production (i.e., the number of eggs laid on a given day divided by the total number of females
132 alive on that day in a given temperature treatment) (56). Temperature, age, and their interaction
133 were included as fixed effects. Both temperature and age were included as continuous variables

that were scaled and centered. Random factors initially included block, blood donor, and mosquito individual as categorical variables. We used minimum AIC values to compare and select our final models (**SI_Table 1, SI_Table 2**). Second, we used a Log-rank test with R package <*survival::survdiff()*> on Kaplan-Meier estimates to determine if survivorship differed with temperature (57). Lastly, to determine if the daily survival rate changed across the lifespan of the mosquito, we fit a variety of survival distributions, which allow either for a constant (exponential) or variable daily mortality rate (log-normal, gamma, Gompertz, and Weibull) with R package <*flexsurv*> to the Kaplan-Meier estimates (58). AIC values were used to determine survival distribution fits. In addition, the best fitting distributions (minimum AIC) were determined at each temperature treatment separately to confirm the best-fitting survival distribution did not vary with temperature treatment (**SI_Table 3)**.

Temperature-dependent transmission potential (R_0)

We used a temperature-dependent R_0 framework parameterized from the *An. stephensi-P. falciparum* system to 1) compare predicted environmental suitability for malaria transmission to a previous $R_0(T)$ model that aimed to describe the *An. gambiae – P. falciparum* system but consisted of data aggregated from several different mosquito and parasite species (31); and 2) to evaluate the effect of age-related changes in *An. stephensi* life history on the predicted environmental suitability of *P. falciparum*. To do this, we used a common expression for R_0 derived from the Ross-MacDonald model (41, 59), which was initially expanded on in Parham & Michael (2010) to incorporate the effect of temperature on mosquito life history and thereby mosquito population size, and later modified in Mordecai et al. (2013) to approximate individual lifetime reproductive values using daily fecundity output and adult daily mortality rates. (**Equation 1, SI_Table 4**) (24, 26, 31-37, 59):

$$R_0(T)_{estimated} = \sqrt{\frac{a^{*2} bc(T) e^{-\frac{\mu^*(T)}{PDR(T)}} EFD^*(T) pEA(T) MDR(T)}{Nr\mu^*(T)^3}} \quad \text{(Eq. 1)}$$

R_0 is the expected number of new cases generated by a single infectious person or mosquito introduced into a fully susceptible population throughout the period within which the person or mosquito is infectious. R_0 components include: egg-to-adult survival probability (*pEA*), mosquito development rate (*MDR*), fecundity (*EFD;* eggs laid per female per day), biting rate (a), adult mosquito mortality rate (μ), parasite development rate (*PDR*), vector competence (*bc*; the proportion of parasite-exposed mosquitoes that become infectious), the density of humans (N), and the human recovery rate (r), with (T) indicating parameters that are dependent on environmental temperature (degrees Celsius). The host recovery rate (r) and host density (N) are assumed to be temperature independent. We label the $R_0(T)$ formulation in **Eq. 1** as 'estimated' as lifetime traits are commonly parameterized with indirect estimates based on daily rates (24, 26, 31-36).

To construct the Multi-species estimated model ($R_0(T)$Multi-species estimated) we used the thermal relationships defined in (31) and using the formulation in **Eq. 1**. To compare the Multi-species estimated model to the $R_0(T)$ model parameterized with our *An. stephesnsi* data ($R_0(T)$ *An. stephensi* estimated*)* and using the formulation in **Eq. 1**, we generated trait estimates (denoted by *) according to methods described in (13, 22, 31, 32) for biting rate (a*), lifespan (lf* as 1/ μ *), and lifetime egg production (B* as EFD*/μ *). Briefly, the inverse of the duration of the first gonotrophic cycle for each individual was used to estimate biting rate (a*). Exponential curves were fit to the tail of mosquito survivorship distributions characterized at different constant temperatures to estimate the daily mortality rate (μ*) of mosquitoes at each temperature treatment. Eggs laid per female per day (*EFD**) at each temperature was estimated

by dividing the number of eggs laid for each female in her first gonotrophic cycle by the number of days in that gonotrophic cycle. Additionally, to estimate *An. stephensi* mosquito development rate (*MDR*) and probability of egg to adult survival (*pEA*), as well as *P. falciparum* development rate (*PDR*) and vector competence (*bc*), we used data from (11) and (23). Finally, to incorporate the temperature-dependence (*T*) of each of the traits outlined above and below, we fit symmetric and asymmetric non-linear responses using Bayesian inference as described in Johnson et al. 2015 and **SI_Methods** (31).

To determine if $R_0(T)$ varies when directly observed lifetime trait values for biting rate (*a*), lifespan (*lf*), and lifetime egg production (*B*) are incorporated instead of indirect estimates of the lifetime values for these traits for *An. stephensi*, we generated the following $R_0(T)$ formulation (**Equation 2, SI_Table 4**).

$$R_0(T)_{lifetime} = \sqrt{\frac{a(T)^2 bc(T) \Upsilon(T) B(T) pEA(T) MDR(T) lf(T)^2}{Nr}} \qquad \text{(Eq. 2)}$$

Mosquito lifespan (*lf*) was defined as the total number of days a mosquito survives after being placed within her respective temperature treatment. Individual lifetime biting rate (*a*) was defined as the total number of blood meals a female takes during her lifespan (*lf*) divided by her lifespan (*lf*). Lifetime egg production (*B*) is defined as the total number of eggs laid by a female during her lifespan (*lf*). The directly observed biting rate (*a*), lifespan (*lf*), and lifetime egg production (*B*) were substituted for the indirectly estimated biting rate (a^*), lifespan ($lf^* = 1/\mu^*$), and lifetime egg production ($B^* = EFD^*/\mu^*$) in **Eq. 1**. The proportion of mosquitoes surviving the latency period, denoted as □ in **Eq. 2,** is substituted for $\exp[-\mu/PDR]$ in **Eq. 1**. To estimate □, we first fit a Gompertz distribution to survivorship data from each temperature treatment and experimental replicate. We then took the proportion of mosquitoes alive upon completion of the predicted extrinsic incubation period ($PDR_{50}(T)^{-1}$) of *P. falciparum* at each temperature. The

amount of days to reach 50% of maximum infectiousness in a mosquito population is represented by $PDR_{50}(T)^{-1}$ (23). This formulation allows us to account for age-dependent mortality in the proportion of mosquitoes surviving the latency period ( ). We then compared the relationship lifespan, biting rate, and lifetime egg production have with temperature for *An. stephensi* when these traits are directly observed (*lf*, *a*, *B*) vs. estimated (*lf**, *a**, *B**) from the data generated in this study, as well as if any observed differences translate to differences in the predicted environmental suitability for malaria transmission (R_0).

As done previously (24, 26, 31-36)), we use relative values of R_0, as opposed to absolute values, to estimate relative temperature suitability for malaria transmission, because absolute values of R_0 depend on a number of factors that vary by location and time, including humidity, breeding habitat availability, vector control, and vector – human contact rates. By rescaling $R_0(T)$ in all models to a range between 0 and 1, we can easily compare the thermal optimum and limits for relative R_0 across models. However, when adopting this relative approach, the stable transmission threshold of $R_0 > 1$ is no longer meaningful. Therefore, a conservative suitability threshold of relative $R_0(T) > 0$ is implemented where temperatures outside of this range are deemed unsuitable for transmission to occur because one or more of the component traits in $R_0(T)$ is equal to zero. Finally, sensitivity and uncertainty analyses were performed for our *An. stephensi* estimated and lifetime models as described in (31) and **SI_Methods**.

Mapping seasonal transmission range

We generated maps depicting the number of months an area is predicted to be environmentally suitable for transmission of human malaria (*P. falciparum*) to illustrate the potential impact differences in the thermal breadth among our relative $R_0(T)$ models have across a relevant landscape. We were primarily interested in comparing the amount of area predicted to

be endemically (year-round) suitable for *P. falciparum* transmission between our two *An. stephensi* relative $R_0(T)$ models incorporating either estimated or observed lifetime trait values across the current distribution of *An. stephensi*. However, we also draw comparisons between our *An. stephensi* models (*An. stephensi* estimated and lifetime) and a previously derived relative $R_0(T)$ model (Multi-species estimated), which aggregates trait data from multiple mosquito and parasite species intended to describe *P. falciparum* transmission in the *An. gambiae* system. This latter comparison serves to illustrate the differences in temperature suitability among systems. Using the median model output for each of these models, we calculated $R_0(T)$ values at 0.2˚C increments, at a 0.01 level accuracy of model output, rescaled the $R_0(T)$ values from 0-1, and plotted transmission suitability where $R_0(T) > 0$ as in Tesla et al. 2018 (35). Using the GADM global administrative boundaries data we estimated the land area with endemic (year-round) suitability within countries that span the current range for *An. stephensi*: India, Pakistan, Sri Lanka, Qatar, United Arab Emirates, and Oman (60).

Results

Temperature and age shape mosquito life history traits

A cohort life table study was used to evaluate the effect of temperature on *An. stephensi* life history traits as individuals age. Both temperature and mosquito age significantly affected the proportion of females that imbibed blood on a given day, mean daily egg production, and survivorship (**Figure 1, SI_Table 2**). However, the interaction between temperature and age did not significantly affect the proportion of females that imbibed blood on a given day or mean daily egg production (**SI_Table 2**). The proportion of females that imbibed blood on a given day was generally higher at warmer temperatures and declined as mosquitoes approached the end of

their lifespan in all temperature treatments, with this age-associated decline being most pronounced at 36°C (**Figure 1a**). Across all temperature treatments, mean daily egg production increased over time to a peak value before declining (**Figure 1a).** Peak mean daily egg production varied with temperature: peak values were higher, occurred sooner, and persisted for shorter periods of time at warmer temperatures (28°C - 36°C) compared to cooler temperatures (16°C - 24°C) (**Figure 1b**). Temperature also significantly affected survivorship (**SI_Table 2**). Survival responded unimodally to temperature, with a peak at 20°C and a decline at higher temperatures (**Figure 1b**). Finally, at all temperatures, mosquito daily probability of survival was not constant with age: a Gompertz distribution, which allows for a variable daily mortality rate, best fit the survival data at each temperature (**Figure 1c, SI_Table 2**).

Using directly observed as opposed to estimated lifetime trait values alters temperature-trait relationships

Depending on the life history trait examined (biting rate (a), lifespan (lf), or lifetime egg production (B)), using observed vs. estimated lifetime values to fit temperature-trait relationships resulted in shifts in the predicted thermal minimum (T_{min}), maximum (T_{max}), and optimum (T_{opt}) (**Figure 2a-c**, **SI_Table 5**). Temperature-trait relationships derived from estimated lifetime trait values resulted in an overall decrease in the absolute values for each lifetime trait (**Figure 2a-c**). While peak values of the temperature functions for observed lifetime biting rate (a) were approximately double (0.5 vs. 0.24) that of estimated lifetime biting rate (a*), the temperature at which these peak values occurred (T_{opt}) was 4.2°C lower for observed lifetime biting rate (**Figure 2a, SI_Table 5**). Further, the temperature-trait relationship for estimated biting rate (a*) had a substantially warmer predicted thermal minimum (T_{min}; +7.6°C) and maximum (T_{max}; +2.4°C) than that for lifetime biting rate (a) resulting in a 5.2°C reduction in the breadth of

temperatures ($T_{breadth}$) permissive for biting (**Figure 2a, SI_Table 5**). Similarly, the value for observed lifespan (*lf*) at the predicted thermal optimum (T_{opt}) was approximately twice that of estimated lifespan (*lf*;* 38.3 days vs. 16 days) (**Figure 2b, SI_Table 5**). However, in contrast to biting rate, the predicted optimum and maximum temperatures were the same for observed lifespan (*lf*) and estimated lifespan (*lf**) with only a slight 0.2°C difference in the predicted thermal minimum (**Figure 2b, SI_Table 5**). The temperature-trait relationship for observed lifetime egg production (*B*) was predicted to have a 2.2°C decrease in the T_{opt} as compared to estimated lifetime egg production (*B**), with subtle shifts in the predicted thermal minimum and maximum (**Figure 2c, SI_Table 5**). Predicted peak values were higher for observed lifetime egg production (*B*; 317.1 eggs) than estimated lifetime egg production (*B**; 225 eggs) (**Figure 2c**).

Surprisingly, the changes in temperature-trait relationships that occurred when directly observed lifetime data are used instead of indirect estimates did not yield large changes in the predicted relationship between temperature and malaria transmission across the *An. stephensi* relative $R_0(T)$ models (**Figure 2d, SI_Table 6**). There was a slight decrease from 27.6°C (*An. stephensi* estimated) to 26.6°C (*An. stephensi* lifetime) in the predicted T_{opt}, but no major differences noted in the predicted T_{min} and T_{max} across models. We did also find notable differences in the T_{min} and T_{max} of the estimated thermal relationship for the proportion of mosquitoes surviving the latency period ( ) between *An. stephensi* models, which also likely contributed to the minor shifts in relative $R_0(T)$ (**SI_Figure 4, SI_Table 7**). Finally, $R_0(T)$ was sensitive to lifespan (*lf*) and biting rate (*a*) in both *An. stephensi* models; however, the *An. stephensi* lifetime model exhibited less sensitivity to lifespan (*lf*) than the *An. stephensi* estimated model (**SI_Results**, *An. stephensi* lifetime; **SI_Figure 2** & *An. stephensi* estimated; **SI_Figure 3**).

293 ***The relationship between temperature and relative R_0 is disease system specific***

294 Integrating temperature-trait relationships from the *An. stephensi – P. falciparum* system
295 resulted in a qualitatively different temperature-relative R_0 relationship to a previously defined
296 multi-species model (31) (**Figure 2d, SI_Table 4, SI_Table 6)**. The *An. stephensi* relative $R_0(T)$
297 model parameterized with equivalent methods (*An. stephensi* estimated) displayed an increase in
298 the breadth of suitable temperatures over which $R_0 > 0$ and a decrease in the credible intervals
299 around the thermal minimum (T_{min}), maximum (T_{max}), and optimum (T_{opt}) compared to a
300 previously published model (Multi-species estimated) which was used to describe *P. falciparum*
301 transmission via *An. gambiae* (**Figure 2d, SI_Table 6**). This increase in the range of
302 temperatures that are suitable for malaria transmission results from an increase in T_{max} from
303 32.6°C (Multi-species estimated) to 36°C (*An. stephensi* estimated) and a decrease in T_{min} from
304 19°C (Multi-species estimated) to 15.6°C (*An. stephensi* estimated). The *An. stephensi* estimated
305 model also had a predicted higher T_{opt} than the previous Multi-species estimated model (T_{opt};
306 25.4°C) by 2.2°C. (**Figure 2d**, **SI_Table 6**). Finally, we found differences in the T_{opt} and T_{max} of
307 the estimated thermal relationship for the proportion mosquitoes surviving the latency period
308 (exp[$-u^*(T)/PDR(T)$]) between the Multi-species estimated and *An. stephensi* estimated models
309 (**SI_Figure 4, SI_Table 7**).

310 ***Seasonal transmission based on temperature suitability varies geographically across relative***
311 ***R_0 models***

312 To visualize the differences in model predictions, we created maps illustrating geographic
313 variation in seasonal suitability for *P. falciparum* transmission of the three relative $R_0(T)$ models
314 along with spatial descriptors of the derived maps (**Figure 3a-c, SI_Table 8**). Comparisons are
315 drawn to the Multi-species estimated model to illustrate how environmental suitability

316 predictions may vary across disease systems (**Figure 3d**). The mapped overlay of year-round
317 (12-months) environmental suitability for malaria transmission for all three models highlights the
318 broader geographic extent of temperature suitability in our *An. stephensi-P. falciparum* models,
319 most notably extending northward into India and on the Arabian Peninsula as compared to the
320 previous Multi-species estimated $R_0(T)$ model (**Figure 3d, SI_Table 8**). For example, the Multi-
321 species estimated model predicts India to contain 710,046 km^2 of endemic area, whereas our two
322 *An. stephensi* models are predicted to contain approximately 3.5 times more endemically suitable
323 area (**SI_Table 8**). Further, Qatar was predicted to be unsuitable for year-round malaria
324 transmission in the Multi-species estimated model but contained a modest area of endemic
325 transmission suitability (11,210 km^2) with our two *An. stephensi* models. In contrast, the
326 predicted endemically suitable area in Sri Lanka remained largely unaltered among models
327 predictions. In addition, there was a very subtle increase in suitability season in the northern-
328 most regions with the *An. stephensi* lifetime model compared to the *An. stephensi* estimated
329 model (**Figure 3**).

330 ## Discussion

331 This comprehensive study characterized how mosquito life history traits of an urban
332 Indian malaria vector, *An. stephensi*, were jointly modified by temperature and age to affect the
333 temperature suitability for malaria transmission. This study also tailored current temperature-
334 dependent relative R_0 models to *An. stephensi* to evaluate how predictions of environmental
335 suitability for malaria transmission are influenced by: 1) the use of direct observations for
336 mosquito life history traits (e.g. lifespan, lifetime egg production, and biting rate) instead of
337 common proxies currently used in the literature to estimate these traits and 2) the inclusion of
338 *An. stephensi*-specific trait data. We found that in addition to temperature, mosquito age altered

the daily proportion of females imbibing a bloodmeal, daily egg production, and daily probability of survival. These results suggest that estimates of these life history traits characterized during a finite portion of a mosquito's lifespan may be imprecise (24, 31, 32). Importantly, we found large quantitative differences in observed lifetime trait values relative to estimates that suggest absolute transmission potential could differ with mosquito age. A failure to include the effects of mosquito age structure, for example, could have important implications for modeling approaches that predict malaria transmission dynamics. Finally, we determined that the inclusion of *An. stephensi – P. falciparum* specific data resulted in qualitatively different temperature-transmission relationships compared to a previous relative $R_0(T)$ model that used thermal responses from *An. gambiae* and other *Anopheles* and *Aedes* species, ultimately affecting predictions of regional suitability for malaria transmission (31, 32).

Research across a diversity of ectothermic organisms demonstrates that age and temperature both affect development, survival, and reproduction (50-53). In mosquitoes, limited evidence suggests that mosquitoes experience age-related changes in cuticular hydrocarbons, immune function, flight activity, insecticide resistance, biting rates, and survival (61-72). In this study, we observed a decrease in the proportion of females imbibing a blood meal and daily egg production in older *An. stephensi*. For both the proportion of females imbibing blood and survival, the rate of decline occurred faster at increasingly warmer temperatures. Previous work with *An. gambiae* showed an increase in the daily biting rate with age, which is in contrast to our findings (55). It remains unclear whether this is outcome is due to differences in senescence, allocation of resources into different life history tasks across *Anopheles* species, or nutritional conditions. Finally, the daily egg production for *An. stephensi* displayed a unimodal relationship with age, where daily egg production increased to a peak before declining. The time to reach

peak values and the subsequent decline occurred earlier in mosquitoes housed at warmer temperatures.

The temperature-sensitive age-dependent mortality rates for mosquito populations are concordant with previous work in laboratory and limited field studies (51, 73, 74). While there is some evidence that long-lived *An. gambiae* cohorts can occur in the field, it is generally assumed that mosquitoes have shorter lifespans in the field than typically observed in controlled laboratory settings (75, 76), and the same may be true for *An. stephensi*. Thus, whether mosquitoes experience senescence in the field remains an open and critical question, primarily due to the logistical difficulties of accurately aging mosquitoes, conducting mark-recapture studies, and controlling for temperature in variable environments (51).

Using direct measurements of an individual's biting rate, lifetime fecundity, and lifespan instead of common approaches to estimate these traits from truncated portions of a mosquito's life (e.g., first gonotrophic cycle only) yielded quantitatively, and in some cases qualitatively, different temperature-trait relationships. Our results suggest that previous approaches used to estimate these life history traits in the literature underestimate values for these traits across most temperatures. This could have important ramifications for predicting mosquito population dynamics as well as the effectiveness of mosquito control interventions in the field where thermal conditions vary. Further, imprecise estimates of lifespan can have a compounding effect on predictions of population dynamics and vector-borne disease transmission as it impacts estimates of total reproductive output and the amount of time a mosquito survives past becoming infectious (51, 52, 77). More effort is needed in measuring both lifespan and age-associated changes in life history traits under field settings for important mosquito disease vectors.

By using a temperature-dependent R_0 model framework we were able to explore how model parameterization of trait data (estimated vs. observed) influenced the temperature suitability for *P. falciparum* transmission. While there were substantial quantitative differences between directly measured versus estimated lifetime trait values along with qualitative differences in shape of the temperature-dependent functions for biting rate and lifetime egg production, we observed only very subtle differences in the predicted effects of temperature on the environmental suitability of malaria transmission between the *An. stephensi* estimated and *An. stephensi* lifetime models (**Figure 2d, Figure 3**). With the relative R_0 approach, absolute differences in predicted temperature-trait and temperature-transmission relationships are masked. One potential ramification of ignoring absolute differences across temperature-trait relationships is that we cannot account for variation in the intensity of malaria transmission with temperature among modeling approaches. For example, even though values of mosquito lifespan differed by up to 2-fold, the predicted temperature-relative R_0 relationship was relatively unaffected by whether the temperature-lifespan relationship was parameterized from estimated or directly observed values(**Figure 2**). These results suggest that predictions of seasonal prevalence could be improved in a modelling framework that incorporates the age-structure of mosquito populations.

Additionally, while our *An. stephensi* estimated model was sensitive to lifespan, our *An. stephensi* lifetime model was less so (**SI_Results**, **SI_Figure 2,3**). Thus, the small shifts in the predicted thermal minimum and optimum for relative R_0 to cooler temperatures in our *An. stephensi* lifetime model relative to the *An. stephensi* estimated model is primarily driven by the qualitative differences in the temperature-trait relationship between directly observed and estimated biting rate and the proportion of mosquitoes surviving the latency period (**SI_Results**,

Figure 2, **SI_Figures 2-4)**. Differences in the temperature-trait relationship for the proportion of mosquitoes surviving the latency period likely arise between models as the *An. stephensi* lifetime model accounts for mortality rates that vary with age, whereas the *An. stephensi* estimated model assumes a constant mortality rate.

Using *An. stephensi* data dramatically changed the predicted relationship between the environmental suitability of malaria transmission and temperature relative to the previously published Multi-species estimated model based largely on *An. gambiae* and *P. falciparum* (31), suggesting that the thermal limits and optima of relative $R_0(T)$ models varies across disease systems (26, 78). Specifically, we demonstrate a 3.4^{o}C decrease in the predicted thermal minimum and 3.4^{o}C increase in the thermal maximum for our *An. stephensi* estimated model, as compared to the Multi-species estimated model that used trait responses from multiple *Anopheles* and an *Aedes* species (**Figure 2d, SI_Table 4, SI_Table 6**) (32). The increase in environmental suitability at warmer temperatures could be due to differences in physiological constraints of the mosquito vectors investigated. *An. stephensi* may be selected for higher temperature tolerance, as it is found in urban areas in Asia. Thus, due to its geographical location and the urban 'heat-island effect,' this species inhabits warmer areas on average than that of the more rural *An. gambiae* (60). Further, differences in *Plasmodium* species and the method of calculating EIP could drive differences between models (79). However, this would not explain the increased suitability at cooler temperatures, which instead suggests a vector or parasite with a higher plasticity in temperature tolerance. Finally, incorporating life history data for *An. stephensi* and *P. falciparum* reduced the credible intervals around the predicted temperature-relative R_0 relationship relative to the Multi-species estimated model (**Figure 2d**) (31). In order to further refine temperature suitability predictions for the effective use in vector control and to optimally

inform public health strategies there is a strong need for additional research on temperature effects on the basic biology of disease vectors such as mosquitoes.

Accurately predicting malaria transmission depends on variation in other abiotic and biotic factors, as well as socioeconomic factors that determine human exposure to infectious mosquitoes that the R_0 approach does not capture. Further, the R_0 framework employed in this work is static and does not incorporate the effect of temporal variation in daily or seasonal temperatures, as well as fluctuations in vector and host densities or disease states (e.g., susceptible, exposed, infectious, recovered). However, here we demonstrate that the predicted thermal suitability for endemic malaria transmission in Southeast Asia is more substantial than previously predicted. Additional study limitations associated with the experimental design are presented in **SI_Discussion**. Surprisingly, the predicted temperature-relative R_0 relationship and overall land area of endemic environmental suitability were only subtly affected by using common approaches to estimate mosquito lifetime traits (e.g., lifespan, lifetime egg production, and biting rate) versus directly measuring them. However, differences in the overall magnitude of these traits—as opposed to the shapes of their thermal responses—could affect transmission in ways not captured using the relative $R_0(T)$ approach. This work highlights the importance of integrating data specific to a disease system of interest and underscores the need for more basic research in the field to improve the accuracy of mechanistic transmission models.

Acknowledgements

The authors would like to thank the CURO (Center for Undergraduate Research Opportunities) program and the NSF REU PopBio (Population Biology of Infectious Diseases) program at the University of Georgia for their assistance in conducting this research. We would also like to thank Drs. Leah Johnson and Melinda Brindley for insightful discussion and Dr.

453 Marta Shocket for deriving the derivates used in the sensitivity analyses for both the *An.*
454 *stephensi* estimated and lifetime models. This work was supported in-part by the NSF GRFP
455 (Graduate Research Fellowship Program) and a NIH R01 award (1R01AI110793-01A1). EAM
456 and SJR were supported by an NSF EEID grant (DEB 1518681). EAM was also supported by a
457 Hellman faculty fellowship, a Stanford Woods Institute for the Environment – Environmental
458 Ventures Program grant, and a Terman Award.

Figure 1. Temporal Effects. *Anopheles stephensi* life history daily trait values across six temperature treatments (16°C, 20°C, 24°C, 28°C, 32°C, and 36°C) for the (**a**) proportion of females that imbibed blood, (**a**) daily egg production, and (**c**) survivorship. In **a** and **b**, colored dots represent trait values for each replicate, with smoothed averages (loess; solid line) and the trend across daily mean values (faded solid line) shown. The proportion of females that imbibed blood, is the defined as the total number of females who took a blood meal on a given day over the total number of females alive on that day. Daily egg production is defined as the total number of eggs laid on a given day over the total number of female alive on that day. In **c**, Kaplan-Meier estimates (solid line, upper and lower 95% CI: shaded area) and the best-fitting survival distribution (Gompertz; dashed line) are shown.

Figure 2. Direct Comparison of Lifetime vs. Estimated Traits. A direct comparison of temperature-trait relationships between observed lifetime trait values (black) and estimated lifetime values (blue) for (**a**) biting rate (*a*), (**b**) lifespan (*lf*), and (**c**) lifetime egg production (*B*). No data points (dots) are displayed for estimated lifetime values in **c**, as this trait is the product of the temperature-trait relationships for estimated daily egg production and estimated lifespan ($B^*(T) = EFD^*(T) \times lf^*(T)$). Comparison of the three relative $R_0(T)$ models (**d**). Each model is displayed as relative to the respective max T_{opt} upper 95% credible interval (CI) model value with mean model values (solid line) and 95% CI (faded area) across temperature shown.

Figure 3. Mapping of relative $R_0(T)$. Number of monthly pixels with $R_0(T)>0$ for (**a**) Multi-species estimated, (**b**) *An. stephensi* estimated, and (**c**) *An. stephensi* lifetime. (**d**) Mapping

481 overlay of all three models with endemic transmission (12-months suitability, deep red shading
482 in previous panels) with *An. stephensi* lifetime (bottom layer, purple), *An. stephensi* estimated
483 (middle layer, deep red), and Multi-species estimated (top layer, pink). Thus, the pink region
484 corresponds approximately to the areas where all three models predict endemic suitability, the
485 deep red reflects the additional area predicted to have endemic suitability by the *An. stephensi*
486 models, while the purple represents the additional area predicted for endemic suitability by the
487 *An. stephensi* lifetime model only.

488

References

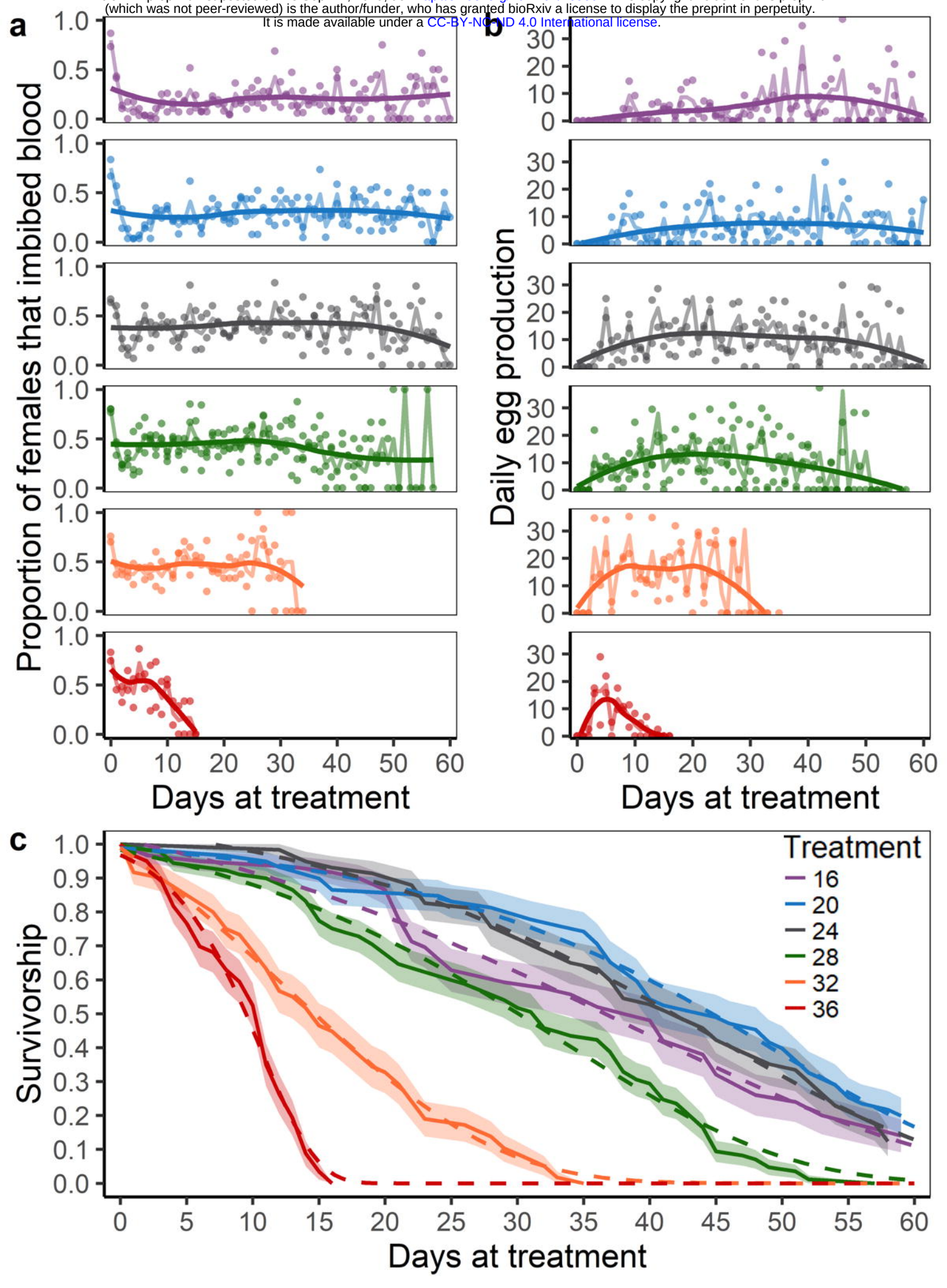
1. World Health Organization. World malaria report 2017. Geneva, Switzerland: World Health Organization; 2017. p. volumes.
2. WHO-vector-borne diseases [Available from: http://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases.
3. Blasco B, Leroy D, Fidock DA. Antimalarial drug resistance: linking Plasmodium falciparum parasite biology to the clinic. Nat Med. 2017;23(8):917-28.
4. Imwong M, Hien TT, Thuy-Nhien NT, Dondorp AM, White NJ. Spread of a single multidrug resistant malaria parasite lineage (PfPailin) to Vietnam. Lancet Infect Dis. 2017;17(10):1022-3.
5. Cisse MB, Keita C, Dicko A, Dengela D, Coleman J, Lucas B, et al. Characterizing the insecticide resistance of Anopheles gambiae in Mali. Malar J. 2015;14:327.
6. Norris LC, Main BJ, Lee Y, Collier TC, Fofana A, Cornel AJ, et al. Adaptive introgression in an African malaria mosquito coincident with the increased usage of insecticide-treated bed nets. Proc Natl Acad Sci U S A. 2015;112(3):815-20.
7. Reisen WK, Fang Y, Martinez VM. Effects of temperature on the transmission of west nile virus by Culex tarsalis (Diptera: Culicidae). J Med Entomol. 2006;43(2):309-17.
8. Chown SL, Nicolson, S. Insect physiological ecology: mechanisms and patterns. Oxford: Oxford University Press; 2004.
9. Beck-Johnson LM, Nelson WA, Paaijmans KP, Read AF, Thomas MB, Bjornstad ON. The effect of temperature on Anopheles mosquito population dynamics and the potential for malaria transmission. PLoS One. 2013;8(11):e79276.
10. Beck-Johnson LM, Nelson WA, Paaijmans KP, Read AF, Thomas MB, Bjornstad ON. The importance of temperature fluctuations in understanding mosquito population dynamics and malaria risk. R Soc Open Sci. 2017;4(3):160969.
11. Paaijmans KP, Heinig RL, Seliga RA, Blanford JI, Blanford S, Murdock CC, et al. Temperature variation makes ectotherms more sensitive to climate change. Glob Chang Biol. 2013;19(8):2373-80.
12. Paaijmans KP, Read AF, Thomas MB. Understanding the link between malaria risk and climate. Proc Natl Acad Sci U S A. 2009;106(33):13844-9.
13. Lardeux FJ, Tejerina RH, Quispe V, Chavez TK. A physiological time analysis of the duration of the gonotrophic cycle of Anopheles pseudopunctipennis and its implications for malaria transmission in Bolivia. Malar J. 2008;7:141.
14. Stratman-Thomas W. The Influence of Temperature on Plasmodium Vivax. The American Journal of Tropical Medicine and Hygiene. 1940;s1-20(5):703-15.
15. Knowles R, Basu BC. Laboratory studies on the infectivity of *Anopheles stephensi*. Journal of the Malaria Institute of India. 1943;5(1):1-29 pp.
16. Siddons LB. Observations on the influence of atmospheric temperature and humidity on the infectivity of Anopheles culicifacies giles. J Mal Inst India. 1944;5:375–88.
17. Shute PG, Maryon M. A study of human malaria oocysts as an aid to species diagnosis. Trans R Soc Trop Med Hyg. 1952(46):275–92.
18. Vaughan JA, Noden BH, Beier JC. Population dynamics of Plasmodium falciparum sporogony in laboratory-infected Anopheles gambiae. J Parasitol. 1992;78(4):716-24.
19. Eling W, Hooghof J, van de Vegte-Bolmer M, Sauerwein R, van Gemert G. Tropical temperatures can inhibit development of the human malaria parasite Plasmodium falciparum in the mosquito. Proc Exp Appl Entomol. 2001(12):151–6.
20. Bayoh M. Studies on the development and survival of anopheles gambiae sensu stricto at various temperatures and relative humidities: Durham University; 2001.

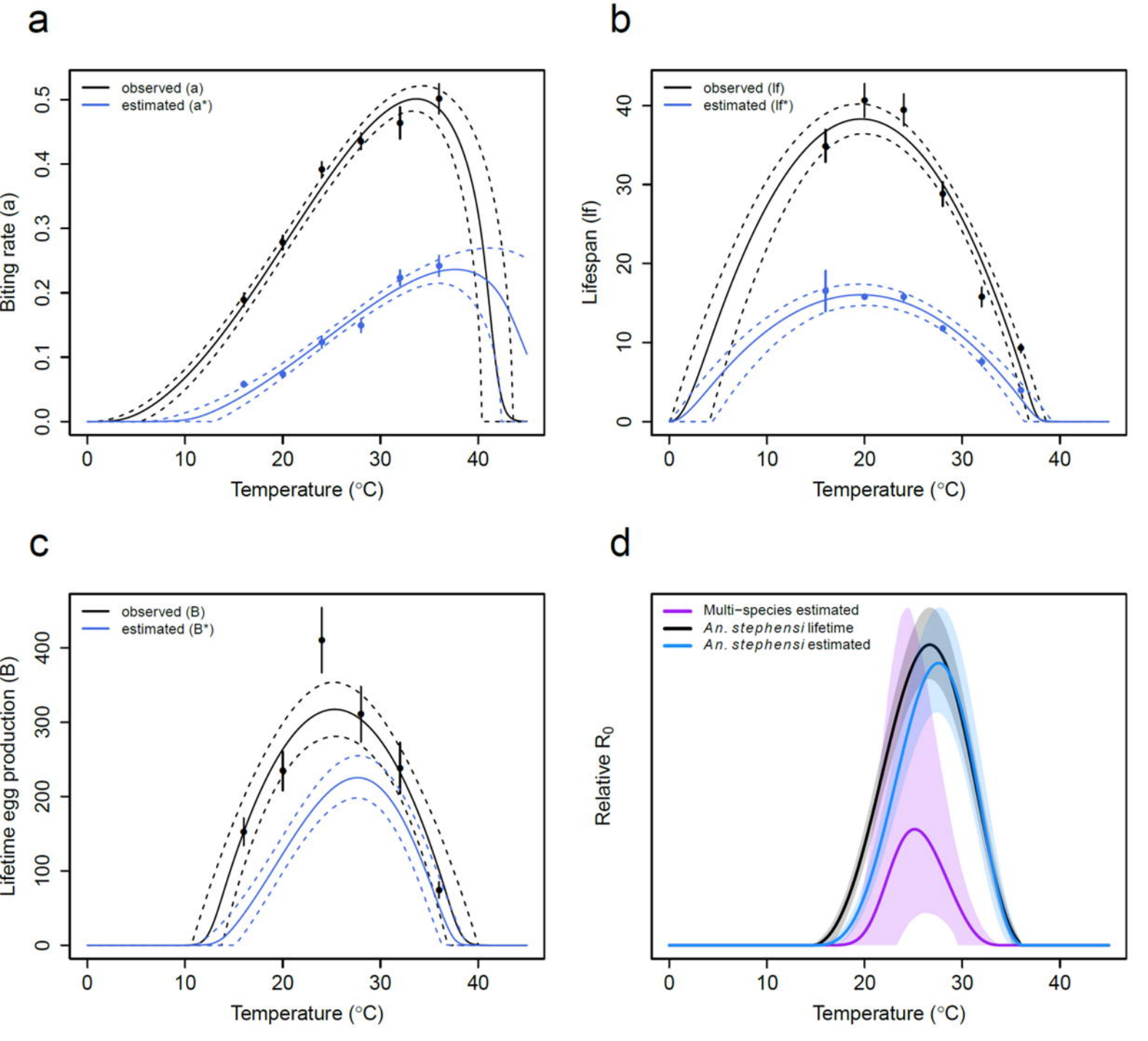
21. Bayoh MN, Lindsay SW. Effect of temperature on the development of the aquatic stages of Anopheles gambiae sensu stricto (Diptera: Culicidae). Bull Entomol Res. 2003;93(5):375-81.
22. Delatte H, Gimonneau G, Triboire A, Fontenille D. Influence of temperature on immature development, survival, longevity, fecundity, and gonotrophic cycles of Aedes albopictus, vector of chikungunya and dengue in the Indian Ocean. J Med Entomol. 2009;46(1):33-41.
23. Shapiro LLM, Whitehead SA, Thomas MB. Quantifying the effects of temperature on mosquito and parasite traits that determine the transmission potential of human malaria. PLoS Biol. 2017;15(10):e2003489.
24. Ryan SJ, McNally A, Johnson LR, Mordecai EA, Ben-Horin T, Paaijmans K, et al. Mapping Physiological Suitability Limits for Malaria in Africa Under Climate Change. Vector Borne Zoonotic Dis. 2015;15(12):718-25.
25. Fischer D, Thomas SM, Suk JE, Sudre B, Hess A, Tjaden NB, et al. Climate change effects on Chikungunya transmission in Europe: geospatial analysis of vector's climatic suitability and virus' temperature requirements. Int J Health Geogr. 2013;12:51.
26. Mordecai EA, Cohen JM, Evans MV, Gudapati P, Johnson LR, Lippi CA, et al. Detecting the impact of temperature on transmission of Zika, dengue, and chikungunya using mechanistic models. PLoS Negl Trop Dis. 2017;11(4):e0005568.
27. Kraemer MU, Sinka ME, Duda KA, Mylne AQ, Shearer FM, Barker CM, et al. The global distribution of the arbovirus vectors Aedes aegypti and Ae. albopictus. Elife. 2015;4:e08347.
28. Siraj AS, Santos-Vega M, Bouma MJ, Yadeta D, Ruiz Carrascal D, Pascual M. Altitudinal changes in malaria incidence in highlands of Ethiopia and Colombia. Science. 2014;343(6175):1154-8.
29. Rueda LM, Brown TL, Kim HC, Chong ST, Klein TA, Foley DH, et al. Species composition, larval habitats, seasonal occurrence and distribution of potential malaria vectors and associated species of Anopheles (Diptera: Culicidae) from the Republic of Korea. Malar J. 2010;9:55.
30. Scranton K, Amarasekare P. Predicting phenological shifts in a changing climate. Proc Natl Acad Sci U S A. 2017;114(50):13212-7.
31. Johnson LR, Ben-Horin T, Lafferty KD, McNally A, Mordecai E, Paaijmans KP, et al. Understanding uncertainty in temperature effects on vector-borne disease: a Bayesian approach. Ecology. 2015;96(1):203-13.
32. Mordecai EA, Paaijmans KP, Johnson LR, Balzer C, Ben-Horin T, de Moor E, et al. Optimal temperature for malaria transmission is dramatically lower than previously predicted. Ecol Lett. 2013;16(1):22-30.
33. Shocket MS, Ryan SJ, Mordecai EA. Temperature explains broad patterns of Ross River virus transmission. Elife. 2018;7.
34. Taylor RA, Mordecai EA, Gilligan CA, Rohr JR, Johnson LR. Mathematical models are a powerful method to understand and control the spread of Huanglongbing. PeerJ. 2016;4:e2642.
35. Tesla B, Demakovsky LR, Mordecai EA, Ryan SJ, Bonds MH, Ngonghala CN, et al. Temperature drives Zika virus transmission: evidence from empirical and mathematical models. Proc Biol Sci. 2018;285(1884).
36. Tesla B, Demakovsky LR, Packiam HS, Mordecai EA, Rodriguez AD, Bonds MH, et al. Estimating the effects of variation in viremia on mosquito susceptibility, infectiousness, and R0 of Zika in Aedes aegypti. PLoS Negl Trop Dis. 2018;12(8):e0006733.
37. Parham PE, Michael E. Modeling the effects of weather and climate change on malaria transmission. Environ Health Perspect. 2010;118(5):620-6.
38. Smith DL, McKenzie FE, Snow RW, Hay SI. Revisiting the basic reproductive number for malaria and its implications for malaria control. PLoS Biol. 2007;5(3):e42.
39. Eikenberry SE, Gumel AB. Mathematical modeling of climate change and malaria transmission dynamics: a historical review. J Math Biol. 2018;77(4):857-933.

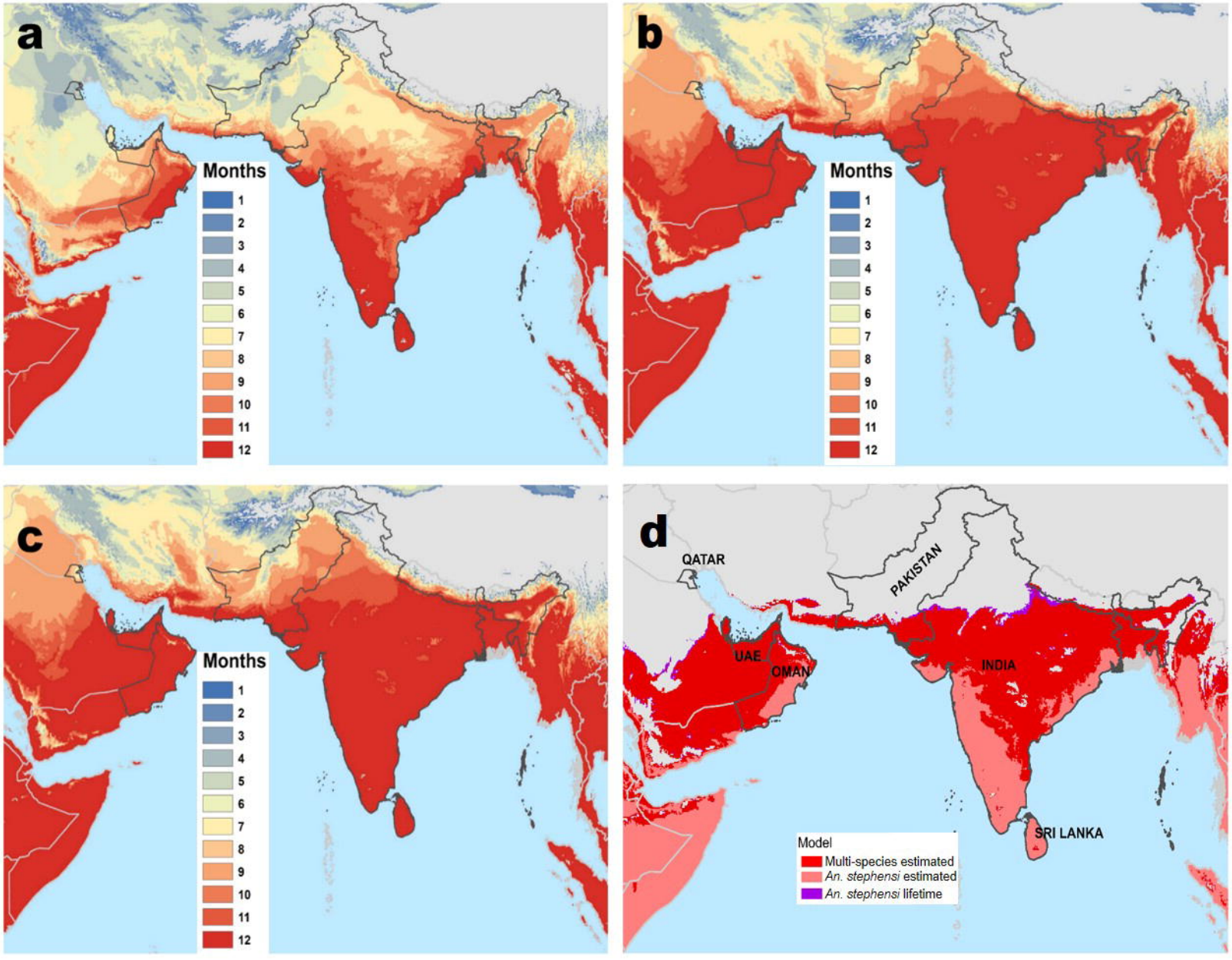
583 40. Koella JC. On the use of mathematical models of malaria transmission. Acta Trop. 1991;49(1):1-
584 25.
585 41. Macdonald G. The epidemiology and control of malaria. London, New York: Oxford University
586 Press; 1957. 201 p p.
587 42. Reiner RC, Jr., Perkins TA, Barker CM, Niu T, Chaves LF, Ellis AM, et al. A systematic review of
588 mathematical models of mosquito-borne pathogen transmission: 1970-2010. J R Soc Interface.
589 2013;10(81):20120921.
590 43. Smith DL, Battle KE, Hay SI, Barker CM, Scott TW, McKenzie FE. Ross, macdonald, and a theory
591 for the dynamics and control of mosquito-transmitted pathogens. PLoS Pathog. 2012;8(4):e1002588.
592 44. Gething PW, Van Boeckel TP, Smith DL, Guerra CA, Patil AP, Snow RW, et al. Modelling the
593 global constraints of temperature on transmission of Plasmodium falciparum and P. vivax. Parasit
594 Vectors. 2011;4:92.
595 45. Martens WJM, Jetten TH, Focks DA. Sensitivity of malaria, schistosomiasis and dengue to global
596 warming. Clim Chang. 1997;35:145-56.
597 46. Craig MH, Snow RW, le Sueur D. A climate-based distribution model of malaria transmission in
598 sub-Saharan Africa. Parasitol Today. 1999;15(3):105-11.
599 47. Alonso D, Bouma MJ, Pascual M. Epidemic malaria and warmer temperatures in recent decades
600 in an East African highland. Proc Biol Sci. 2011;278(1712):1661-9.
601 48. Ermert V, Fink AH, Jones AE, Morse AP. Development of a new version of the Liverpool Malaria
602 Model. I. Refining the parameter settings and mathematical formulation of basic processes based on a
603 literature review. Malar J. 2011;10:35.
604 49. Murdock CC, Sternberg ED, Thomas MB. Malaria transmission potential could be reduced with
605 current and future climate change. Sci Rep. 2016;6:27771.
606 50. Leips J. Insect Models of Immunosenescence. Handbook on Immunosenescence2009. p. 87-105.
607 51. Ryan SJ, T. Ben-Horin, and L. R. Johnson. Malaria control and senescence: the importance of
608 accountingfor the pace and shape of aging in wild mosquitoes. Ecosphere. 2015;6(170).
609 52. Styer LM, Carey JR, Wang JL, Scott TW. Mosquitoes do senesce: departure from the paradigm of
610 constant mortality. Am J Trop Med Hyg. 2007;76(1):111-7.
611 53. Leips J, Gilligan P, Mackay TF. Quantitative trait loci with age-specific effects on fecundity in
612 Drosophila melanogaster. Genetics. 2006;172(3):1595-605.
613 54. McCann S, Day JF, Allan S, Lord CC. Age modifies the effect of body size on fecundity in Culex
614 quinquefasciatus Say (Diptera: Culicidae). J Vector Ecol. 2009;34(2):174-81.
615 55. Gary RE, Jr., Foster WA. Effects of available sugar on the reproductive fitness and vectorial
616 capacity of the malaria vector Anopheles gambiae (Diptera: Culicidae). J Med Entomol. 2001;38(1):22-8.
617 56. Bates D, Machler M, Bolker BM, Walker SC. Fitting Linear Mixed-Effects Models Using lme4. J
618 Stat Softw. 2015;67(1):1-48.
619 57. Therneau TM, Grambsch PM. Modeling survival data : extending the Cox model. New York:
620 Springer; 2000. xiii, 350 p. p.
621 58. Jackson CH. flexsurv: A Platform for Parametric Survival Modeling in R. J Stat Softw.
622 2016;70(8):1-33.
623 59. Dietz K. The estimation of the basic reproduction number for infectious diseases. Stat Methods
624 Med Res. 1993;2(1):23-41.
625 60. Kiszewski A, Mellinger A, Spielman A, Malaney P, Sachs SE, Sachs J. A global index representing
626 the stability of malaria transmission. Am J Trop Med Hyg. 2004;70(5):486-98.
627 61. Brei B, Edman JD, Gerade B, Clark JM. Relative abundance of two cuticular hydrocarbons
628 indicates whether a mosquito is old enough to transmit malaria parasites. J Med Entomol.
629 2004;41(4):807-9.

630 62. Hugo LE, Kay BH, Eaglesham GK, Holling N, Ryan PA. Investigation of cuticular hydrocarbons for
631 determining the age and survivorship of australasian mosquitoes. Am J Trop Med Hyg. 2006;74(3):462-
632 74.
633 63. Hillyer JF, Schmidt SL, Fuchs JF, Boyle JP, Christensen BM. Age-associated mortality in immune
634 challenged mosquitoes (Aedes aegypti) correlates with a decrease in haemocyte numbers. Cell
635 Microbiol. 2005;7(1):39-51.
636 64. Chun J, Riehle M, Paskewitz SM. Effect of mosquito age and reproductive status on melanization
637 of sephadex beads in Plasmodium-refractory and -susceptible strains of Anopheles gambiae. J Invertebr
638 Pathol. 1995;66(1):11-7.
639 65. Li J, Tracy JW, Christensen BM. Relationship of hemolymph phenol oxidase and mosquito age in
640 Aedes aegypti. J Invertebr Pathol. 1992;60(2):188-91.
641 66. Nayar JK, Sauerman DM, Jr. A comparative study of flight performance and fuel utilization as a
642 function of age in females of Florida mosquitoes. J Insect Physiol. 1973;19(10):1977-88.
643 67. Mourya DT, Hemingway J, Leake CJ. Changes in enzyme titres with age in four geographical
644 strains of Aedes aegypti and their association with insecticide resistance. Med Vet Entomol.
645 1993;7(1):11-6.
646 68. Rowley WA, Graham CL. The effect of age on the flight performance of female aedes aegypti
647 mosquitos. J Insect Physiol. 1968;14(5):719-28.
648 69. Kaufmann C, Collins LF, Brown MR. Influence of Age and Nutritional Status on Flight
649 Performance of the Asian Tiger Mosquito Aedes albopictus (Diptera: Culicidae). Insects. 2013;4(3).
650 70. Mnyone LL, Kirby MJ, Mpingwa MW, Lwetoijera DW, Knols BG, Takken W, et al. Infection of
651 Anopheles gambiae mosquitoes with entomopathogenic fungi: effect of host age and blood-feeding
652 status. Parasitol Res. 2011;108(2):317-22.
653 71. Koella JC, Boete C. A genetic correlation between age at pupation and melanization immune
654 response of the yellow fever mosquito Aedes aegypti. Evolution. 2002;56(5):1074-9.
655 72. Pigeault R, Nicot A, Gandon S, Rivero A. Mosquito age and avian malaria infection. Malar J.
656 2015;14:383.
657 73. Afrane YA, Zhou G, Lawson BW, Githeko AK, Yan G. Effects of microclimatic changes caused by
658 deforestation on the survivorship and reproductive fitness of Anopheles gambiae in western Kenya
659 highlands. Am J Trop Med Hyg. 2006;74(5):772-8.
660 74. Christiansen-Jucht C, Erguler K, Shek CY, Basanez MG, Parham PE. Modelling Anopheles gambiae
661 s.s. Population Dynamics with Temperature- and Age-Dependent Survival. Int J Environ Res Public
662 Health. 2015;12(6):5975-6005.
663 75. Lehmann T, Dao A, Yaro AS, Adamou A, Kassogue Y, Diallo M, et al. Aestivation of the African
664 malaria mosquito, Anopheles gambiae in the Sahel. Am J Trop Med Hyg. 2010;83(3):601-6.
665 76. Adamou A, Dao A, Timbine S, Kassogue Y, Yaro AS, Diallo M, et al. The contribution of aestivating
666 mosquitoes to the persistence of Anopheles gambiae in the Sahel. Malar J. 2011;10:151.
667 77. Bellan SE. The importance of age dependent mortality and the extrinsic incubation period in
668 models of mosquito-borne disease transmission and control. PLoS One. 2010;5(4):e10165.
669 78. Mordecai EA, Caldwell JM, Grossman MK, Lippi CA, Johnson LR, Neira M, et al. Thermal biology
670 of mosquito-borne disease. Ecol Lett. 2019.
671 79. Ohm JR, Baldini F, Barreaux P, Lefevre T, Lynch PA, Suh E, et al. Rethinking the extrinsic
672 incubation period of malaria parasites. Parasit Vectors. 2018;11(1):178.

673




a
observed (a)
estimated (a*)
Biting rate (a)
0.0
0.1
0.2
0.3
0.4
0.5
0
10
20
30
40
Temperature (°C)
b
observed (lf)
estimated (lf*)
Lifespan (lf)
0
10
20
30
40
0
10
20
30
40
Temperature (°C)
c
observed (B)
estimated (B*)
Lifetime egg production (B)
0
100
200
300
400
0
10
20
30
40
Temperature (°C)
d
Multi−species estimated
An. stephensi lifetime
An. stephensi estimated
Relative R0
0
10
20
30
40
Temperature (°C)


a
Months
1
2
3
4
5
6
7
8
9
10
11
12
b
Months
1
2
3
4
5
6
7
8
9
10
11
12
c
Months
1
2
3
4
5
6
7
8
9
10
11
12
d
QATAR
PAKISTAN
UAE
OMAN
INDIA
SRI LANKA
Model
Multi-species estimated
An. stephensi estimated
An. stephensi lifetime