

Carry-over effects of larval microclimate on the transmission potential of mosquito-borne pathogens

Michelle V. Evans^{1,2,3*}, Justine Shiau², Nicole Solano^{1,2*}, Melinda Brindley^{3,4}, John M. Drake^{1,2}, Courtney C. Murdock^{1,2,3,4,5,6}

1 Odum School of Ecology, University of Georgia, Athens, GA, USA

2 Center for the Ecology of Infectious Diseases, University of Georgia, Athens, GA, USA

3 Center for Tropical Emerging Global Diseases, University of Georgia, Athens, GA, USA

4 Department of Infectious Disease, University of Georgia, Athens, GA, USA

5 Center for Vaccines and Immunology, University of Georgia, Athens, GA, USA

6 River Basin Center, University of Georgia, Athens, GA, USA

*Corresponding Author: mvevans@uga.edu

Abstract Climate shapes the transmission of mosquito-borne pathogens through impacts on both the vector and the pathogen. In addition to direct effects of the present environment, indirect carry-over effects from previous life history stages can influence mosquito life history traits relevant to disease transmission. While this has been explored in a laboratory setting, the net effect of temperature-mediated carry-over effects due to relevant environmental variation in the larval stage is ambiguous. Here, we use data collected from a semi-field experiment investigating dengue dynamics in *Aedes albopictus* across an urban microclimate gradient and season to parameterize a mechanistic dengue transmission model. We reared *Aedes albopictus* in artificial containers across three replicate sites within three different land classes (rural, suburban, urban), characterized by the low, medium, and high proportions of impervious surface, respectively. Survival to adulthood, immature development rate, and body size were recorded daily. Emerged females were offered a dengue (serotype 2) infectious bloodmeal, kept at a constant 27 C, and assayed for infection,

dissemination, and infectiousness twenty-one days post infection. We found that survival and development rate of mosquitoes differed across season, but not land class, driven by a positive relationship of both traits with temperature. Mosquitoes reared on urban land classes and in the fall were more likely to become infected or have disseminated infections, but did not differ in infectiousness across land class or season. Incorporating carry-over effects of larval environment on measures of vector competence resulted in significantly lower predicted dengue transmission potential across land class and season, however predictions both with and without carry-over effects had a strong positive relationship with larval environmental temperature. Given the significant impact of carry-over effects on predicted transmission potential, we suggest that future mechanistic models of disease transmission include both direct and carry-over effects of environmental temperature.

1 Introduction

Climate plays an important role in the transmission of mosquito-borne pathogens, determining the geographic range of disease vectors and shaping transmission dynamics. Mosquitoes are ectotherms, and are therefore sensitive to environmental temperature, which can drive individual-level variation in character traits relevant to life history and mosquito population dynamics such as feeding rates (Delatte et al., 2009), fecundity (Yang et al., 2009), and survival (Alto and Juliano, 2001). Variation in environmental conditions can also influence traits that are relevant for pathogen transmission, such as vector competence and pathogen development within the mosquito (Lambrechts et al., 2011). However, in addition to the direct effects of the current environment, mosquito phenotype (including fitness) is shaped indirectly by the environmental conditions experienced in previous life history stages, a phenomenon known as carry-over effects (Harrison et al., 2011). Carry-over effects have been documented in a wide-range of species with complex life cycles, such as amphibians (Vonesh, 2005), migratory birds (Norris and Taylor, 2006), and insects (De Block and Stoks, 2005; Roux et al., 2015). Similarly, the mosquito life cycle is characterized by ontogenetic niche shifts, with a larval aquatic stage and an adult terrestrial stage. Based on these

studies, we reason that the thermal environment a mosquito experiences during its larval stage is likely to have lasting impacts on its adult traits, and, ultimately, on transmission potential.

There are several pathways by which carry-over effects from the larval environment might impact key adult traits that are relevant for overall fitness and disease transmission. If the larval environment is of low quality (e.g. resource scarcity, thermal stress, or crowding), individuals may experience developmental constraints that negatively impact adult fitness (Inger et al., 2010). For instance, male *Anopheles gambiae* mosquitoes reared at high-densities in the larval stage are less competitive mates than those reared at low-densities (Ng’habi et al., 2005), and female *An. stephensi* reared on a low-food diet have lower survival and fecundity than those reared on a high food diet (Moller-Jacobs et al., 2014; Shapiro et al., 2016). There are numerous studies demonstrating that variation in larval environmental temperature and nutrients significantly impact adult immune function (Muturi et al., 2012b; Price et al., 2015) and thereby the ability of adult mosquitoes to transmit arboviruses (e.g. vector competence) (Grimstad and Walker, 1991; Muturi et al., 2012a, 2011a; Alto and Bettinardi, 2013; Vantaux et al., 2016; Buckner et al., 2016). A second mechanism shaping carry-over effects can result from acclimation to a specific larval environment via trait plasticity (Monaghan, 2008). For example, *Culex* mosquitoes reduce their growth in larval environments with predator cues to avoid size-specific predation (Jourdan et al., 2016). This, in turn, decreases adult body size, with ramifications for other adults traits such as fecundity (Lounibos et al., 2002) and susceptibility to pathogens (Paulson and Hawley, 1991).

Although it is clear that temperatures at early life stages can significantly alter adult mosquito traits important for transmission, the net effect of temperature-mediated carry-over effects on overall transmission potential is ambiguous. Studies focusing solely on vector competence have found both positive (Muturi et al., 2011c) and negative (Muturi et al., 2011b) relationships between larval environmental temperature and the proportion of infectious mosquitoes. Additionally, laboratory studies designed to estimate temperature-mediated carry-over effects are typically conducted across a wide range of temperatures (e.g. with differences of 5 to 10 °C between treatments) not often experienced by mosquitoes in the wild (Cator et al., 2013). While larger treatment differences increase the likelihood of detecting temperature-mediated carry-over effects on adult traits,

they do not easily “scale-up” to explain transmission across a landscape when incorporated into temperature-dependent models of mosquito-borne disease (Pascual et al., 2006; Mordecai et al., 2017; Reiner et al., 2013). Furthermore, temperature-dependent models of mosquito-borne disease only incorporate direct effects of temperature, despite evidence that indirect carry-over effects can have large impacts on adult mosquito phenotypes. Thus, the implications of carry-over effects for mechanistic predictions of vector-borne disease remain unexplored.

In light of the above, we hypothesize that relevant environmental variation during the larval stage will have lasting impacts on adult traits that are relevant for mosquito population dynamics and pathogen transmission. To assess the implications omitting carry-over effects from mechanistic transmission models, we used data collected from a semi-field experiment in a the *Aedes albopictus*-dengue virus (serotype 2, DENV-2) system to parameterize a mechanistic dengue transmission model. We then compared model predictions when carry-over effects were incorporated relative to when they were excluded.

2 Methods

2.1 Semi-Field Experimental Design

To explore the effects of microclimate variation across an urban landscape, we used an impervious surface map (National Land Cover Database 2011 (Xian et al., 2011) to select three replicate sites ($30m \times 30m$) each of low (0-5%), intermediate (6-40%), and high (41-100%) impervious surface. Percent impervious surface is an accurate predictor of land surface temperature, particularly for urban landscapes (Yuan and Bauer, 2007), and allowed us to ensure our sites exhibited the full range of urban microclimates. To select our sites, we calculated the percent impervious surface of each $30m \times 30m$ pixel using a moving focal window of $210m \times 210m$, as the surrounding impervious surface can affect the microclimate in the pixel of interest. We then classified each pixel based on the mean impervious surface within its focal window, with 0 - 5 % representing low, 6 - 40 % representing intermediate, and 41 - 100% representing high. Because impervious surface is an effective classifier of urban land classes (Lu and Weng, 2006), we identified the sites as rural,

suburban, and urban with low, intermediate, and high impervious surface scores, respectively. Final site selection was constrained by access and permissions, however the final distribution of sites was chosen to ensure all sites were at least 2 miles from others of the same land class, and were evenly distributed across the study area (Fig. 1).

Within each site, we evenly distributed four plastic trays, each containing 100 first instar *Ae. albopictus* larvae and 1L of leaf infusion. Leaf infusion was prepared one week prior to the experiment as described in Murdock et al. (2017). Trays were screened with a fine mesh, placed in a wire cage to deter wildlife, and placed in full shade. Cages were covered with a clear plastic vinyl to keep rainwater from entering the trays. We added deionized water to trays after two weeks to prevent trays from drying up and to maintain a total water volume at 1L. We placed data loggers (Monarch Instruments: RFID Temperature Track-It logger) in vegetation next to each tray, approximately 3 feet above the ground, to collect information on the larval microclimate. Data loggers recorded instantaneous temperature and relative humidity at ten minute intervals throughout the study period.

Sites were visited daily from August 1 to September 3, 2016 and September 26 to November 8, 2016 during the summer and fall replicates, respectively, to collect emerging adult mosquitoes until all larvae emerged or died. We quantified the number of male and female mosquitoes emerging by tray per day, mosquito body size, and dengue vector competence (the proportion of mosquitoes that became infectious with dengue after receiving an artificial blood meal containing dengue virus). To estimate the effects of land class, microclimate, and season on mosquito population dynamics and transmission potential (defined as vectorial capacity), we then integrated these data into models of mosquito population dynamics and vectorial capacity.

2.2 Dengue virus *in vitro* culturing and mosquito infections

DENV-2 stock was obtained from the World Reference Center for Emerging Viruses and Arboviruses at the University of Texas Medical Branch (PRS 225 488, originally isolated from human serum in Thailand in 1974 (Vazeille-Falcoz et al., 1999)). We propagated virus by inoculating Vero (African green monkey kidney epithelial) cells with a low MOI infection. Virus-containing super-

107 natant was harvested when the cells exhibited more than 80% cytopathic effect. Supernatant was
108 cleared of cell debris by centrifugation (1000xg, 1 min), aliquoted into cryo-vials, and stored at -80
109 °C. We quantified viral titers of virus stock using TCID₅₀ assays, calculated by the Spearman-
110 Karber method (Shao et al., 2016). When mixed 1:1 with the red blood cell mixture, the final
111 concentration of virus in the blood meal was 3.540×10^6 TCID₅₀/mL.

112 Adult mosquitoes were aspirated and aggregated from each tray by day of emergence and site
113 into 16 oz. cardboard cups, returned to our ACL2 facility at the University of Georgia, and stored
114 in reach-in incubators at $27^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, $80\% \pm 5\%$ relative humidity, and a 12 h: 12 h light: dark
115 cycle. To ensure infected mosquitoes were of a similar age, mosquitoes were pooled into cohorts
116 of 4-6 days old in the summer and 4-9 days old in the fall (due to slower and more asynchronous
117 emergence rates), allowed to mate, and were fed *ad libitum* with a 10% sucrose solution. The 10%
118 sucrose solution was removed 48 hours prior to the infection and replaced with deionized water,
119 which was then removed 12-14 hours before infection to encourage higher feed rates. Infectious
120 blood meals were administered to mosquitoes through a water-jacketed membrane feeder and
121 consisted of 47% human red blood cells washed in DMEM (vol/vol), 1% sucrose(weight/vol),
122 20% FBS (vol/vol), 5 mM ATP, and 33% DMEM medium combined with 1 mL of virus stock (Shan
123 et al., 2016). Blood-fed mosquitoes were then maintained as described above for the duration of
124 the experiment.

125 For a mosquito to become infectious, arboviruses must pass through multiple tissues (i.e.
126 midgut and salivary glands) in the mosquito vector that impose significant barriers to infections
127 (Cheng et al., 2016). Therefore, we assessed mosquitoes for infection, dissemination, and infec-
128 tiousness through salivation assays and dissections (Hurlbut, 1966; Anderson et al., 2010) 21 days
129 post infection. Mosquitoes were cold anesthetized and immobilized by removing their legs and
130 wings. Wings were mounted on a glass slide to measure wing length via a dissecting scope and
131 micrometer. The proboscis of each female was then inserted into a sterile pipette tip and allowed
132 to salivate into 10-20 μL of FBS with 3mM ATP and red food coloring on a plate kept at 27°C
133 for 15 minutes, after which the salivation media was expelled into 500 μL of DMEM and stored
134 at -80°C . After salivation, we removed the head of each individual and stored the body and head

125 separately at -80 °C.

126 To determine variation in the proportion of mosquitoes that become infected (bodies positive
127 for virus), disseminated (heads positive for virus), and infectious (saliva positive for virus), we used
128 cytopathic effect (CPE) assays to test for the presence of virus in each collected tissue (Balaya
129 et al., 1969). Individual bodies and heads were homogenized in 500 µL of DMEM and centrifuged
130 at 2,500 rcf for 5 minutes. 200 µL of homogenate was added to Vero cells in a solution of DMEM
131 (1% pen-strep, 5% FBS by volume) in a 24-well plate and kept at 37 °C and 5 % CO_2 . Salivation
132 media was thawed, and plated on Vero cells as above. After 5 days, Vero cells were assessed for
133 presence of DENV-2 via CPE assays. Samples were identified as positive for virus if CPE was
134 present in the well.

135 **2.3 Mosquito body size and intrinsic growth rates (r')**

136 We calculated the per capita population growth rate (Equation 1) per tray following Livdahl and
137 Sugihara (1984):

$$r' = \frac{\ln(\frac{1}{N_0} \sum_x A_x f(\bar{w}_x))}{D + \frac{\sum_x x A_x f(\bar{w}_x)}{\sum_x A_x f(\bar{w}_x)}} \quad (1)$$

138 Where N_0 is the initial number of female mosquitoes (assumed to be 50% of the larvae, n=50),
139 A_x is the number of mosquitoes emerging on day x , D is the time to reproduction following
140 emergence (assumed to be 14 days (Livdahl and Willey, 1991)), and $f(\bar{w}_x)$ is fecundity as a
141 function of mean wing size on day x (w_x ; Equation 2). This relationship is assumed to be linear
142 and calculated via Lounibos et al. (2002):

$$f(\bar{w}_x) = -121.240 + (78.02 \times \bar{w}_x) \quad (2)$$

143 **2.4 Estimating vectorial capacity**

144 We calculated the vectorial capacity (VC ; Equation 3) for each site and season following Mordecai
145 et al. (2017):

$$VC(T) = \frac{a(T)^2 b(T) c(T) e^{-\mu(T)/EIR(T)} EFD(T) p_{EA}(T) MDR(T)}{\mu(T)^2} \quad (3)$$

Here, mosquito traits are a function of temperature, T , as described in Table 1.

Site-level VC was calculated using a combination of traits empirically measured in this study and traits estimated from thermal response models as described in Mordecai et al. (2017). The bite rate ($a(T)$), adult mosquito mortality rate ($\mu(T)$), and extrinsic incubation rate ($EIR(T)$), were calculated for mosquitoes at a constant 27 °C using temperature dependent functions from Mordecai et al. (2017). Vector competence ($b(T)c(T)$) was calculated as the proportion of infectious mosquitoes per site as found by our dengue infection assays. The number of eggs produced per female per day ($EFD(T)$) was calculated by estimating fecundity from average female wing length following Eq. 2, and then dividing this by the expected lifespan of mosquitoes ($1/\mu$). The egg-to-adult survival probability ($p_{EA}(T)$) was defined as the average proportion of adults emerging at a site. The mosquito immature development rate ($MDR(T)$) was calculated as the inverse of the mean time to emergence for female mosquitoes per site, resulting in a daily rate of development. In order to distinguish between vectorial capacity with and without carry-over effects, we constructed two models. The model without carry-over effects used mathematically estimated values for $b(T)c(T)$ and $F = EFD(T)$ based on thermal response models calculated at the adult environmental temperature (27 °C) following Mordecai et al. (2017), while the model incorporating carry-over effects used the empirically estimated values from our study for $b(T)c(T)$ and $EFD(T)$. All other parameters were the same across the two models.

2.5 Statistical Analysis

All analyses were conducted with respect to the female subset of the population, as they are the subpopulation responsible for disease transmission. In the case of data logger failure, imputed means from the site were used to replace microclimate data. Given the low intra-site variation in temperature, this assumption allowed us to include mosquito data for those trays without biasing our microclimate data. In the case of trays failing due to wildlife emptying them (two urban and one suburban in the fall replicate on experimental days 20, 22, and 20, respectively), collected

171 mosquitoes were used for infection assays, but were excluded from survival and emergence anal-
172 yses. Unless otherwise stated, all models included the interaction between predictor variables in
173 the initial fit, which were dropped based on significance ($\alpha = 0.05$). For all mixed-models, signifi-
174 cance was assessed through Wald Chi-square tests ($\alpha = 0.05$) and examination of 95% confidence
175 intervals. Pearson residuals and Q-Q plots were visually inspected for normality. When applica-
176 ble, pair-wise comparisons within each factor were conducted using Tukey multiple comparisons
177 of means, adjusting for significance with the Holm-Bonferroni method. All mixed models were fit
178 using the `lme4`(Bates et al., 2015) package in *R*(R Core Team, 2017).

179 **2.5.1 Assessing effects of land class and season on mosquito population dynamics** 180 **and transmission potential**

181 We used generalized linear mixed models to explore if microclimate (i.e. mean, minimum, max-
182 imum, and daily ranges of temperature and relative humidity), the mean proportion of adult
183 females emerging per tray, time to female emergence, female body size, the mean mosquito per
184 capita growth rate, and metrics of vector competence differed across land class and season. Fixed
185 effects in all initial models included land class, season, and the interaction, before undergoing
186 model selection based on significance testing. Site was included as a random effect in all models
187 to control for any variation inherent to the site. The effect of body size on infection dynamics was
188 also explored at the level of the individual mosquito, fitting a binomial generalized linear mixed
189 effects model including wing size as a fixed effect and site as a random effect. Finally, because
190 mosquitoes were pooled across trays within a site to estimate metrics of vector competence (bc),
191 the VC calculation was done at the site level. Thus, our analysis of land class and season on VC
192 did not require a random effect, and was tested using a two-way ANOVA.

193 **2.5.2 Assessing effects of microclimate on mosquito population dynamics and trans-** 194 **mission potential**

195 To explore whether the effects of land class and season were due to variation in microclimate,
196 we ran additional statistical analyses exploring the effects of different microclimate variables on

each response variable. In total, we measured seven microclimate variables (mean, minimum, and maximum temperature; mean, minimum, and maximum relative humidity, and daily temperature range (DTR)). However, they were all extremely correlated ($\rho > 0.9$) leading us to exclude variables from our models to reduce bias due to collinearity (Graham, 2003). To identify the microclimate variable that best explained variation in each of the above response variables, we performed model selection among seven models that included each microclimate predictor as an individual covariate, and chose the best performing model based on *AICc*. Thus, we analyzed the effect of the chosen microclimate variable on the mean proportion of adult females emerging per tray, time to female emergence, and female body size by fitting linear mixed effects models to each response variable with site included as a random factor. For metrics of vector competence, we were unable to quantify the effect of larval microclimate on the mean probability of mosquito infection, dissemination, and infectiousness at the tray level because mosquitoes were pooled by site to ensure enough adults were available for infection assays. Instead, we fit linear regressions to each response variable using the estimated mean daily temperature and relative humidity per site as predictor variables.

3 Results

Of the 3,600 first-instar larvae placed in each season, a total of 2595 and 1128 mosquitoes emerged in the summer and fall, respectively. A total of 319 female mosquitoes were force salivated, twenty per site in the summer, and varying numbers per site in the fall due to lower emergence rates (sample sizes reported in Supp. Table 1). Of this number, a total of 291 wings were mounted and measured (28 wings were damaged). Of the uninfected mosquitoes, 135 and 162 female wing lengths were measured in the summer and fall replicates, respectively.

3.1 Effects of land class and season on microclimate

We found that microclimate profiles differed significantly across both season and land class (results of significance tests reported in Supp. Table 2). Across all sites, the mean temperature was 7.73 ± 0.35 °C higher in the summer than the fall. Urban sites were significantly hotter than both

suburban and rural sites (1.26 ± 0.41 °C and 1.68 ± 0.41 °C warmer, respectively), however there was no evidence for a difference between rural and suburban land classes (0.422 ± 0.4 °C difference between land classes), a trend that persisted across seasons. The difference in minimum temperatures was similar to that found for mean temperatures, with minimum temperatures 10.62 ± 0.69 °C higher in the summer than the fall. Again, urban sites had a significantly higher minimum temperature than rural sites (2.36 ± 0.55 °C warmer). The maximum temperature did not change significantly with land class (Supp. Table 2), but was significantly different across season, with summer maximum temperatures 3.87 ± 0.64 °C higher than fall maximum temperatures. This in turn translated into more variable temperatures in the fall than the summer, with fall mosquitoes experiencing a mean DTR that was 6.65 ± 0.68 °C higher than summer mosquitoes. DTR also differed across land class, with rural sites having the largest DTR (16.50 ± 0.708 °C) compared to suburban and urban ranges, which were not significantly different from each other.

Relative humidity also differed across season and land class (Supp. Fig. 1). Mean relative humidity was 12.86 ± 0.94 % higher in summer than fall, and was lower at urban land sites compared to rural and suburban sites within a season (urban: 78.266 ± 1.79 %, rural: 86.36 ± 1.79 %, suburban: 86.51 ± 1.79 %). Similarly, the minimum relative humidity was 25.78 ± 2.50 % higher in the summer than the fall. This resulted in a larger daily relative humidity range (DHR) in the fall compared to the summer (summer: 26.85 ± 1.94 % DHR, fall: 49.89 ± 1.74 % DHR), and no difference across land class.

3.2 Direct effects of land class, season, and microclimate on population growth

The total proportion of adult females emerging per tray was significantly higher in summer than fall ($\chi^2 = 71.54.32$, $p < 0.001$), but did not differ across land class (Fig. 3A). There was a strong positive relationship between mean daily temperature and larval survival to emergence by tray (Table 2, $t = 8.09$, $p < 0.001$), a possible explanation for the difference in larval survival between the summer and fall replicates. The mean rate of larval development per tray (defined as the inverse of the time to emergence) was significantly different between summer and fall (Fig. 3B,

249 $\chi^2 = 588.04$, $p < 0.001$), with daily development rates of $0.074 \pm 0.002 \text{ day}^{-1}$ and 0.0387 ± 0.002
250 day^{-1} , respectively. Similarly, there was a significant positive relationship between temperature
251 and larval development rate (Table 2, $t = 6.89$, $p < 0.001$).

252 **3.3 Carry-over effects of land class, season, and microclimate on pop-** 253 **ulation growth**

254 We did not observe a significant effect of land class or season on uninfected mosquito wing size,
255 with the null model receiving the lowest $AICc$. After incorporating the number of adult females
256 emerging per day, the date of emergence, and their body size into the per capita growth rate
257 equation (Equation 1), we found that the estimated per capita growth rate was higher in the
258 summer season than the fall season ($\chi^2 = 217.58$, $p < 0.001$), and was positive for both seasons
259 suggesting the possibility of population growth across both seasons (Fig. 3C). There was no
260 evidence for a difference in population growth across land class or temperature.

261 **3.4 Carry-over effects of land class, season, and microclimate on vec-** 262 **tor competence**

263 We found that both land class and season did significantly impact the probability of a mosquito
264 becoming infected and disseminating dengue infection (Fig. 4, Supp. Table 1). In general,
265 mosquitoes in the fall had higher a higher probability of infection and dissemination than those
266 in the summer, and urban mosquitoes had a lower probability of infection than suburban and
267 rural mosquitoes (Supp. Table 1). The probability of becoming infectious did not differ across
268 land class, nor season, despite the higher probability of mosquito infection and dissemination in
269 the fall and on suburban and rural sites. This suggests that the ability of virus to penetrate the
270 salivary glands differs in adults reared in the summer vs. the fall and across land class, with a
271 higher proportion of dengue infected mosquitoes becoming infectious in the summer and on urban
272 sites (Supp. Table 1, $\chi^2 = 13.65$, $p < 0.001$). Finally, we also found the probability of infection
273 to decline with increasing body size ($z = -2.18$, $p = 0.0289$), although there was no evidence for

274 a relationship between body size and the probability of dissemination or infectiousness.

275 Differences in metrics of vector competence across land class and season were driven by a
276 strong relationship with microclimate. Across the range of temperatures in our study, we found
277 that infection and dissemination rates decreased with increasing mean daily temperatures, while
278 there was no relationship between infectiousness and mean daily temperature (Table 2).

279 **3.5 Integrating direct and carry-over effects into estimates of trans-** 280 **mission potential**

281 When calculating VC without the inclusion of carry-over effects, VC was higher in the summer
282 than the fall ($\chi^2 = 35.84$, $p < 0.001$), however this trend disappeared when carry-over effects were
283 included. In the summer season, there was a trend for VC to increase with increasing urbanization,
284 that is, urban sites had higher predicted vectorial capacity than suburban, which had a higher
285 value than rural. This trend was not significant, however, given the small sample size ($n=9$) and
286 the disproportional impact of having no infectious mosquitoes at one site, resulting in a value
287 of $VC = 0$ for one sample. Further, we found that calculated vectorial capacity increased with
288 temperature for both models, although the increase was more pronounced when not accounting for
289 carry-over effects (Fig. 5, $\beta = 0.77$ and $\beta = 3.80$ for models with and without carry-over effects,
290 respectively). When comparing VC calculations with and without carry-over effects, we found
291 that including carry-over effects decreased the expected vectorial capacity overall by an average
292 of 84.89 ± 2.86 %.

293 **4 Discussion**

294 Mathematical models of mosquito-borne disease rarely include the larval stage of the life cycle
295 (Reiner et al., 2013), and of those that do, few include the influence of carry-over effects on
296 important mosquito life-history traits (but see Roux et al. 2015). This is likely due, in part,
297 to the lack of empirical studies parameterizing the carry-over effects of the larval environment in
298 mosquito-pathogen systems (Parham et al., 2015), most of which are laboratory studies conducted

299 across a wider range of temperatures than that seen in the field. Here, we demonstrate that fine-
300 scale differences in larval microclimate generate carry-over effects on adult vector competence
301 and fecundity, resulting in variation in mosquito population dynamics and transmission potential
302 across an urban landscape and across season, driven by differences in microclimate.

303 We found evidence of an urban heat island effect across the landscape of Athens, GA, with
304 temperatures in urban sites warmer relative to rural and suburban sites, between which there
305 were no differences. This finding validates our original classification of land classes based on
306 impervious surface, although it suggests that it is only at intermediate to high levels of impervious
307 surface ($> 40\%$) that the urban heat island effect manifests. When comparing relative humidity
308 across land class within a season, warmer urban sites had lower relative humidity than rural
309 and suburban sites. This is not surprising, as temperature and relative humidity are negatively
310 correlated, given that warmer air has a lower capacity to hold water. However, we did find
311 relative humidity to be higher in the summer than the fall. We believe this was caused by a
312 drought during the fall replicate, during which our study site received no rainfall as compared to
313 47.50 mm of rainfall during the summer replicate (Georgia Automated Environmental Monitoring
314 Network, <http://www.georgiaweather.net/>), resulting in lower atmospheric moisture during the
315 fall replicate.

316 The subtle heterogeneity in microclimate we observed resulted in significantly different pre-
317 dicted population growth rates through its effects on larval survival, development rates, and female
318 body size. We found adult female emergence to be higher in the summer than the fall due to a
319 strong positive relationship between daily mean temperature and the proportion of female adults
320 emerging. This could be because the daily mean temperatures (25.43°C) across all sites in the
321 summer were closer to the predicted thermal optimum for *Ae. albopictus* for the probability of
322 egg to adult survival (p_{EA} ; $24\text{--}25^{\circ}\text{C}$) (Mordecai et al., 2017) than in the fall (17.69°C). While
323 this result contrasts with patterns observed in a previous study on these field sites that found
324 lower adult emergence in the summer relative to the fall (Murdock et al., 2017), it is likely due to
325 seasonal differences in microclimate and timing of when these two studies were conducted (sum-
326 mer and fall replicates in June-July and September-October in 2015 versus August-September and

327 September-November in 2016). We also observed more rapid larval development rates (*MDR*) in
328 the summer relative to the fall, and on warmer urban sites in the fall only. Again, this is likely due
329 to the strong positive relationship observed between *MDR* and mean larval temperature, as the
330 metabolic rate of mosquitoes and this trait have been shown to increase with warming tempera-
331 tures (Delatte et al., 2009; Mordecai et al., 2017). Surprisingly, we found no effect of land class or
332 season on uninfected female mosquito body size, in spite of the difference in temperatures across
333 season. Following allometric temperature-size relationships of ectotherms, warmer larval temper-
334 atures lead to smaller bodied mosquitoes (Angilleta et al., 2004; Kingsolver and Huey, 2008). Our
335 results are contrary to many laboratory studies that have found cold rearing temperatures result
336 in large bodied mosquitoes (*Ae. albopictus* (Reiskind and Zarrabi, 2012), *Culex tarsalis* (Dodson
337 et al., 2012), *Anopheles gambiae* (Koella and Lyimo, 1996)).

338 There could be several explanations for why we did not observe an effect of larval environmental
339 temperature on adult body size. Nutrient availability and quality has been shown to mediate the
340 relationship between temperature and body size (Farjana et al., 2011). The majority of the above
341 laboratory studies rear larvae on high quality food sources, such as fish food or liver powder. The
342 leaf infusion used in our experiment relied on yeast and naturally colonizing microorganisms such
343 as bacteria, both of which grow more slowly at low temperatures (Ratkowsky et al., 1982), likely
344 constraining growth of larvae. Indeed, Lounibos et al. (2002) provided leaf litter as a nutrient
345 source, and found a positive relationship between temperature and male *Ae. albopictus* body size.
346 However, diet quality may not be the only explanation for our positive temperature-body size
347 relationship. A similar study on these sites in 2015 found a negative relationship between larval
348 temperature and adult *Ae. albopictus* body size (Murdock et al., 2017) when reared on a similar
349 leaf infusion as used in this experiment. There were, however, several key differences between the
350 2015 study and our study. First, the 2015 study was conducted in July and September rather than
351 August and October, which resulted in cooler temperature profiles for the 2016 study relative to
352 the 2015 study. As with many other mosquito life history traits (Mordecai et al., 2017, 2013),
353 *Ae. albopictus* body mass has a non-linear relationship with temperature across the range of 20
354 - 30 °C (Muturi et al., 2011c). Assuming body size scales similarly, the 2016 study would fall

355 nearer the lower extreme of the thermal function resulting in smaller bodied mosquitoes at colder
356 temperatures. Second, while we initially provided larvae similar amounts of food as the 2015
357 study, our larvae were reared in trays instead of bell jars, which had higher surface to volume
358 ratio and experienced more evaporation overall. Thus, increased evaporation on warmer sites and
359 during the summer could concentrate the amount of food provided per larvae over time resulting
360 in larger bodied mosquitoes with increasing larval temperatures.

361 When larval survival, mosquito development rates, and body size at emergence were combined
362 into a mosquito population dynamic model, we found significant influences of land class, season,
363 and microclimate on the per capita growth rate of mosquito populations. Overall, mosquito per
364 capita growth rates were higher in the summer relative to the fall due to higher adult daily
365 emergence and more rapid mosquito development rates. Unlike the summer, where land class
366 did not significantly affect mosquito population growth rates, urban sites were predicted to have
367 higher population growth rates than suburban and rural sites in the fall due to higher larval
368 survival and development rates. Other studies have found mosquito population growth rates to
369 vary with increasing urbanization (Li et al., 2014), deforestation (Afrane et al., 2007), and with
370 season (Murdock et al., 2017). Our study, however, suggests the effects of microclimate variation
371 with land class on mosquito population dynamics can be dependent on coarser climactic patterns
372 such as seasonality.

373 Our results agree with laboratory studies in other arboviral systems (chikungunya (Westbrook
374 et al., 2010; Adelman et al., 2013), yellow fever (Adelman et al., 2013), and Rift Valley fever (Turell,
375 1993)) that found cool larval environmental temperatures to enhance arbovirus infection relative
376 to warmer larval environments. Studies on the *Ae. albopictus*-dengue virus system have also
377 found that low larval temperatures enhance mosquito susceptibility to virus infection, although
378 this was dependent on larval nutrition (Buckner et al., 2016) and the stage of the infection (i.e.
379 mid-gut vs. dissemination vs. saliva) (Alto and Bettinardi, 2013). While we found infection and
380 dissemination to decrease with increasing temperatures, there was no effect of temperature on viral
381 presence in the saliva. Our findings suggest that carry over effects due to microclimate variation
382 across land class and season affects the overall efficiency of dengue infections. Thus, even though

383 a smaller proportion of mosquitoes reared on urban sites and in the summer became infected and
384 disseminated infection, these mosquitoes were more likely to become infectious. This, in turn,
385 resulted in no net difference in the proportion of mosquitoes that ultimately become infectious
386 across land class and season and suggests that later stages of viral infection (i.e. salivary gland
387 penetration) may be differentially impacted by larval environmental temperature than earlier
388 stages (i.e. midgut escape). Other studies that have measured mosquito body size in relation
389 to infection status have similarly found that smaller bodied mosquitoes are more susceptible to
390 infection (Muturi et al., 2011b; Alto and Bettinardi, 2013; Alto et al., 2005, 2008).

391 Current statistical and mechanistic models of vector-borne disease prediction focus primarily
392 on the direct effects of environmental variables on mosquito densities and disease transmission
393 and rarely include the effects of the larval stage (Mordecai et al. 2017, see Reiner et al. 2015 for
394 a review of models in the *P. falciparum* system). Even fewer consider the lasting impact of this
395 stage on adult traits relevant for fitness and disease transmission (Ezeakacha, 2015). We find that
396 when carry over effects are not incorporated, mechanistic models overestimate the effects of key
397 environmental drivers (e.g. temperature) on vector-borne disease transmission. The relatively
398 small differences in temperature across our study site (less than 1.5 °C) resulted in a two-fold
399 difference in predicted vectorial capacity when omitting carry-over effects. While overall trans-
400 mission potential still exhibited a positive relationship with increasing temperature, the inclusion
401 of carry-over effects dampened this effect.

402 Past studies of carry-over effects have been primarily lab based, allowing for detailed study
403 of the mechanisms of how carry-over effects impact mosquito life history traits (Alto et al., 2008,
404 2005), but limiting extrapolation to real world conditions. However, many of these studies are con-
405 ducted across wide environmental gradients at constant temperatures that are not characteristic
406 of field conditions. Like these laboratory studies, we found carry-over effects to be pervasive, with
407 important implications for mosquito population dynamics and potential disease transmission, de-
408 spite the subtle differences in microclimate observed across land class and season. Thus, we would
409 expect these phenomena to have an even larger impact in more urbanized areas, particularly
410 megacities, with larger seasonal and spatial microclimate ranges (Peng et al., 2012).

411 Additionally, carry-over effects are not simply limited to microclimate, and have been observed
412 as a result of variation in larval nutrition (Moller-Jacobs et al., 2014), intra- and inter-specific den-
413 sities (Reiskind and Lounibos, 2009; Alto et al., 2005, 2008), and predation (Roux et al., 2015)
414 in mosquito systems. Abiotic and biotic factors will likely interact to influence carry over effects
415 (Buckner et al., 2016; Muturi et al., 2011a, 2012a; Muturi and Alto, 2011; Muturi et al., 2010), and
416 how abiotic and biotic factors shape carry-over effects could be scale-dependent. For example, bi-
417 otic processes are thought to be relatively more important at local geographic scales, while abiotic
418 processes tend to dominate at regional geographic scales in predictive models of species distribu-
419 tions (Cohen et al., 2016). There is also evidence to suggest that the magnitude of the interaction
420 between abiotic and biotic processes on carry over effects is scale-dependent (Leisnham et al.,
421 2014). Future exploration of the scale-dependent contribution of different environmental factors,
422 their interactions, to carry-over effects is needed to improve models of mosquito distributions,
423 population dynamics, and disease transmission.

424 In conclusion, we found fine-scale variation in microclimate to shape mosquito population dy-
425 namics and the transmission potential of mosquito-borne diseases both through direct impacts on
426 larval survival and development rates, and indirectly through carry-over effects on vector compe-
427 tence and fecundity. Our study suggests that more empirical work in the lab and field is needed to
428 better characterize carry over effects associated with relevant environmental drivers. The interac-
429 tion between the larval and adult environments, mediated by carry-over effects, could have complex
430 consequences for adult phenotypes and fitness for mosquitoes as well as other organisms. Given
431 the devastating impact of disease in other species with complex life histories (e.g. chytridiomycosis
432 in amphibians), the role of carry-over effects in disease transmission may be an important, though
433 understudied, mechanism that must be better understood to control disease spread. Thus, incor-
434 porating relationships between carry-over effects and organismal life-history traits into statistical
435 and mechanistic models will lead to more accurate predictions on the distributions of species,
436 population dynamics, and the transmission of pathogens and parasites.

437 **5 Acknowledgements**

438 We thank members of the Murdock and Brindley labs for discussion and technical support con-
439 ducting viral assays. We thank Diana Diaz, Abigail Lecroy, and Marco Notarangelo for assistance
440 in the field and lab. M.V.E was funded by the University of Georgia (Presidential Fellowship) and
441 the National Science Foundation Graduate Research Fellowship (Grant No. NUMBER). N.S. was
442 funded by the National Science Foundation Research for Undergraduates (Grant No.). M.B. was
443 supported by FILL IN. J.M.D. was funded by FILL IN. CCM was funded by FILL IN.

444 **6 Contributions**

445 M.V.E, J.M.D, and C.C.M designed the study; M.V.E, J.S. and N.S. collected the data; M.V.E.
446 and M.B. cultured virus and conducted CPE assays; M.V.E, J.M.D, and C.C.M analyzed the data;
447 M.V.E., J.M.D, and C.C.M prepared the tables and figures; M.V.E., J.S., N.S., M.B., J.M.D, and
448 C.C.M drafted the manuscript. All authors gave final approval for publication.

449 **7 Data accessibility**

450 All data and code used in analyses are available on figshare (DOI).

References

- Zach N. Adelman, Michelle A. E. Anderson, Michael R. Wiley, Marta G. Murreddu, Gladys Hazitha Samuel, Elaine M. Morazzani, and Kevin M. Myles. Cooler Temperatures Destabilize RNA Interference and Increase Susceptibility of Disease Vector Mosquitoes to Viral Infection. *PLOS Neglected Tropical Diseases*, 7(5):e2239, May 2013. ISSN 1935-2735. doi: 10.1371/journal.pntd.0002239.
- Yaw A. Afrane, Goufa Zhou, Bernard W. Lawson, Andrew K. Githeko, and Guiyun Yan. Life-table analysis of *Anopheles arabiensis* in western Kenya highlands: effects of land covers on larval and adult survivorship. *The American Journal of Tropical Medicine and Hygiene*, 77(4): 660–666, October 2007. ISSN 0002-9637.
- Barry W Alto and David Bettinardi. Temperature and dengue virus infection in mosquitoes: Independent effects on the immature and adult stages. *The American Journal Of Tropical Medicine And Hygiene*, 88(3):497–505, March 2013. ISSN 1476-1645. doi: 10.4269/ajtmh.12-0421.
- Barry W Alto and Steven A Juliano. Temperature Effects on the Dynamics of *Aedes albopictus*(Diptera: Culicidae) Populations in the Laboratory. *J Med Entomol*, 38(4):548–556, July 2001. doi: 10.1603/0022-2585-38.4.548.
- Barry W. Alto, L. Philip Lounibos, Stephen Higgs, and Steven A. Juliano. Larval Competition Differentially Affects Arbovirus Infection in *Aedes* Mosquitoes. *Ecology*, 86(12):3279–3288, 2005. ISSN 0012-9658.
- Barry W Alto, Michael H Reiskind, and L Philip Lounibos. Size alters susceptibility of vectors to dengue virus infection and dissemination. *Am. J. Trop. Med. Hyg.*, 79(5):688–695, October 2008.
- Sheri L. Anderson, Stephanie L. Richards, and Chelsea T. Smartt. A Simple Method for Determining Arbovirus Transmission in Mosquitoes. *Journal of the American Mosquito Control Association*, 26(1):108–111, March 2010. ISSN 8756-971X. doi: 10.2987/09-5935.1.

477 Michael J Angilleta, Todd D Steury, and Micheal W Sears. Temperature, growth rate, and body
478 size in ectotherms: Fitting peice of a life-history puzzle. *Integrative and Comparative Biology*,
479 44:498–509, January 2004.

480 S Balaya, SD Paul, LV D’Lima, and KM Pavri. Investigations on an outbreak of dengue in Delhi
481 in 1967. *Indian J. Med. Res.*, 57(4):767–774, 1969.

482 Douglas Bates, Martin Mächler, Ben Bolker, and Steve Walker. Fitting linear mixed-effects models
483 using lme4. *Journal of Statistical Software*, 67(1):1–48, 2015. doi: 10.18637/jss.v067.i01.

484 Eva A Buckner, Barry W Alto, and L Philip Lounibos. Larval Temperature–Food Effects on Adult
485 Mosquito Infection and Vertical Transmission of Dengue-1 Virus. *J Med Entomol*, 53(1):91–98,
486 January 2016. doi: 10.1093/jme/tjv145.

487 Lauren J Cator, Shalu Thomas, Krijn P Paaijmans, Sangamithra Ravishankaran, Johnson A
488 Justin, Manu T Mathai, Andrew F Read, Matthew B Thomas, and Alex Eapen. Characterizing
489 microclimate in urban malaria transmission settings: A case study from Chennai, India. *Malaria*
490 *Journal*, 12(1):1–1, March 2013. doi: 10.1186/1475-2875-12-84.

491 Gong Cheng, Yang Liu, Penghua Wang, and Xiaoping Xiao. Mosquito defense strategies against
492 viral infection. *Trends in parasitology*, 32(3):177–186, March 2016. ISSN 1471-4922. doi:
493 10.1016/j.pt.2015.09.009. URL <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4767563/>.

494 Jeremy M Cohen, David J Civitello, Amber J Brace, Erin M Feichtinger, C Nicole Ortega, Jason C
495 Richardson, Erin L Sauer, Xuan Liu, and Jason R Rohr. Spatial scale modulates the strength
496 of ecological processes driving disease distributions. *PNAS*, 113(24):E3359–E3364, June 2016.
497 doi: 10.1073/pnas.1521657113.

498 Marjan De Block and Robby Stoks. Fitness Effects from Egg to Reproduction: Bridging the Life
499 History Transition. *Ecology*, 86(1):185–197, 2005. ISSN 0012-9658.

500 H Delatte, G Gimonneau, A Triboire, and Didier Fontenille. Influence of Temperature on Immature
501 Development, Survival, Longevity, Fecundity, and Gonotrophic Cycles of *Aedes albopictus*,

502 Vector of Chikungunya and Dengue in the Indian Ocean. *J Med Entomol*, 46(1):33–41, January
503 2009.

504 Brittany L. Dodson, Laura D. Kramer, and Jason L. Rasgon. Effects of larval rearing temperature
505 on immature development and West Nile virus vector competence of *Culex tarsalis*. *Parasites*
506 *& Vectors*, 5(1):199, September 2012. ISSN 1756-3305. doi: 10.1186/1756-3305-5-199.

507 Nnaemeka F Ezeakacha. *Environmental impacts and carry-over effects in complex life cycles: the*
508 *role of different life history stages*. PhD thesis, University of Southern Mississippi, 2015.

509 T Farjana, N Tuno, and Y Higa. Effects of temperature and diet on development and interspecies
510 competition in *Aedes aegypti* and *Aedes albopictus*. *Medical and Veterinary Entomology*, 26
511 (2):210–217, July 2011. doi: 10.1111/j.1365-2915.2011.00971.x.

512 Michael H. Graham. Confronting Multicollinearity in Ecological Multiple Regression. *Ecol-*
513 *ogy*, 84(11):2809–2815, November 2003. ISSN 1939-9170. doi: 10.1890/02-3114. URL
514 <http://onlinelibrary.wiley.com/doi/10.1890/02-3114/abstract>.

515 P. R. Grimstad and E. D. Walker. *Aedes triseriatus* (Diptera: Culicidae) and La Crosse virus.
516 IV. Nutritional deprivation of larvae affects the adult barriers to infection and transmission. *J.*
517 *Med. Entomol.*, 28(3):378–386, May 1991. ISSN 0022-2585.

518 Xavier A. Harrison, Jonathan D. Blount, Richard Inger, D. Ryan Norris, and Stuart Bearhop.
519 Carry-over effects as drivers of fitness differences in animals. *J. Anim. Ecol.*, 80(1):4–18, January
520 2011. ISSN 1365-2656. doi: 10.1111/j.1365-2656.2010.01740.x.

521 H. S. Hurlbut. Mosquito salivation and virus transmission. *The American Journal of Tropical*
522 *Medicine and Hygiene*, 15(6):989–993, November 1966. ISSN 0002-9637.

523 Richard Inger, Xavier A. Harrison, Graeme D. Ruxton, Jason Newton, Kendrew Colhoun, Gud-
524 mundur A. Gudmundsson, Graham McElwaine, Matthew Pickford, David Hodgson, and Stuart
525 Bearhop. Carry-over effects reveal reproductive costs in a longdistance migrant. *J. Anim. Ecol.*,
526 79(5):974–982, 2010. ISSN 0021-8790.

Jonas Jourdan, Jasmin Baier, Rdiger Riesch, Sven Klimpel, Bruno Streit, Ruth Mller, and Martin
Plath. Adaptive growth reduction in response to fish kairomones allows mosquito larvae (*Culex*
pipiens) to reduce predation risk. *Aquatic Sciences*, 78(2):303–314, April 2016. ISSN 10151621.
doi: 10.1007/s00027-015-0432-5.

Joel G. Kingsolver and Raymond B. Huey. Size, temperature, and fitness: Three rules. *Evol Ecol*
Res, 10(2):251–268, 2008. ISSN 1522-0613.

J. C. Koella and E. O. Lyimo. Variability in the relationship between weight and wing length of
Anopheles gambiae (Diptera: Culicidae). *Journal of Medical Entomology*, 33(2):261–264, March
1996. ISSN 0022-2585. WOS:A1996TX07900013.

L Lambrechts, K P Paaijmans, T Fansiri, L B Carrington, L D Kramer, M B Thomas, and T W
Scott. Impact of daily temperature fluctuations on dengue virus transmission by *Aedes aegypti*.
PNAS, 108(18):7460–7465, May 2011. doi: 10.1073/pnas.1101377108.

Paul T Leisnham, Shannon L LaDeau, and Steven A Juliano. Spatial and Temporal Habitat Seg-
regation of Mosquitoes in Urban Florida. *PLoS ONE*, 9(3), January 2014. doi: 10.1371/jour-
nal.pone.0091655.

Yiji Li, Fatmata Kamara, Guofa Zhou, Santhosh Puthiyakunnon, Chunyuan Li, Yanxia Liu, Yanhe
Zhou, Lijie Yao, Guiyun Yan, and Xiao-Guang Chen. Urbanization Increases *Aedes albopic-*
tus Larval Habitats and Accelerates Mosquito Development and Survivorship. *PLoS Neglected*
Tropical Diseases, 8(11):e3301–12, November 2014. doi: 10.1371/journal.pntd.0003301. URL
<http://dx.plos.org/10.1371/journal.pntd.0003301>.

Todd P Livdahl and George Sugihara. Non-Linear Interactions of Populations and the Importance
of Estimating Per Capita Rates of Change. *The Journal of Animal Ecology*, 53(2):573–580, June
1984. doi: 10.2307/4535.

Todd P Livdahl and Michelle S Willey. Prospects for an Invasion: Competition between *Aedes*
albopictus and Native *Aedes triseriatus*. *Science*, 253:189–191, January 1991.

552 L P Lounibos, S Suarez, Z Menendez, N Nishimura, R L Escher, S M O’Connell, and J R Rey.
 553 Does temperature affect the outcome of larval competition between *Aedes aegypti* and *Aedes*
 554 *albopictus*? *J. of Vec. Eco.*, 27(1):86–95, June 2002.

555 Dengsheng Lu and Qihao Weng. Use of impervious surface in urban land-use classifica-
 556 tion. *Remote Sensing of Environment*, 102(1):146–160, May 2006. ISSN 0034-4257. doi:
 557 10.1016/j.rse.2006.02.010.

558 Lillian L. Moller-Jacobs, Courtney C. Murdock, and Matthew B. Thomas. Capacity of mosquitoes
 559 to transmit malaria depends on larval environment. *Parasites & Vectors*, 7:593, 2014. ISSN
 560 1756-3305. doi: 10.1186/s13071-014-0593-4.

561 Pat Monaghan. Early growth conditions, phenotypic development and environmental change.
 562 *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, 363(1497):1635–1645, May 2008. ISSN 0962-8436,
 563 1471-2970. doi: 10.1098/rstb.2007.0011.

564 Erin A. Mordecai, Krijn P. Paaijmans, Leah R. Johnson, Christian Balzer, Tal Ben-Horin, Emily
 565 de Moor, Amy McNally, Samraat Pawar, Sadie J. Ryan, Thomas C. Smith, and Kevin D.
 566 Lafferty. Optimal temperature for malaria transmission is dramatically lower than previously
 567 predicted. *Ecol. Lett.*, 16(1):22–30, January 2013. ISSN 1461-0248. doi: 10.1111/ele.12015.

568 Erin A. Mordecai, Jeremy M. Cohen, Michelle V. Evans, Prithvi Gudapati, Leah R. Johnson,
 569 Catherine A. Lippi, Kerri Miazgowicz, Courtney C. Murdock, Jason R. Rohr, Sadie J. Ryan,
 570 Van Savage, Marta S. Shocket, Anna Stewart Ibarra, Matthew B. Thomas, and Daniel P.
 571 Weikel. Detecting the impact of temperature on transmission of Zika, dengue, and chikungunya
 572 using mechanistic models. *PLOS Neglected Tropical Diseases*, 11(4):e0005568, April 2017. ISSN
 573 1935-2735. doi: 10.1371/journal.pntd.0005568.

574 Courtney C. Murdock, Michelle V. Evans, Taylor D. McClanahan, Kerri L. Miazgowicz, and
 575 Blanka Tesla. Fine-scale variation in microclimate across an urban landscape shapes variation
 576 in mosquito population dynamics and the potential of *Aedes albopictus* to transmit arboviral

577 disease. *PLOS Neglected Tropical Diseases*, 11(5):e0005640, May 2017. ISSN 1935-2735. doi:
578 10.1371/journal.pntd.0005640.

579 Ephantus J Muturi and Barry W Alto. Larval Environmental Temperature and Insecticide Ex-
580 posure Alter *Aedes aegypti* Competence for Arboviruses. *Vector-Borne and Zoonotic Diseases*,
581 11(8):1157–1163, August 2011. doi: 10.1089/vbz.2010.0209.

582 Ephantus J. Muturi, Katie Costanzo, Banugopan Kesavaraju, Richard Lamp-
583 man, and Barry W. Alto. Interaction of a pesticide and larval competition
584 on life history traits of *Culex pipiens*. *Acta Tropica*, 116(2):141–146, Novem-
585 ber 2010. ISSN 0001-706X. doi: 10.1016/j.actatropica.2010.07.003. URL
586 <http://www.sciencedirect.com/science/article/pii/S0001706X10001981>.

587 Ephantus J. Muturi, Katie Costanzo, Banugopan Kesavaraju, and Barry W. Alto. Can Pesti-
588 cides and Larval Competition Alter Susceptibility of *Aedes* Mosquitoes (Diptera: Culicidae)
589 to Arbovirus Infection? *Journal of Medical Entomology*, 48(2):429–436, March 2011a. ISSN
590 0022-2585. doi: 10.1603/ME10213.

591 Ephantus J Muturi, Chang-Hyun Kim, Barry W Alto, May R Berenbaum, and Mary A Schuler.
592 Larval environmental stress alters *Aedes aegypti* competence for Sindbis virus. *Trop Med Int*
593 *Health*, 16(8):955–964, May 2011b. doi: 10.1111/j.1365-3156.2011.02796.x.

594 Ephantus J. Muturi, Richard Lampman, Katie Costanzo, and Barry W. Alto. Effect of temperature
595 and insecticide stress on life-history traits of *Culex restuans* and *Aedes albopictus* (Diptera:
596 Culicidae). *J. Med. Entomol.*, 48(2):243–250, March 2011c. ISSN 0022-2585.

597 Ephantus J. Muturi, Millon Blackshear, and Allison Montgomery. Temperature and density-
598 dependent effects of larval environment on *Aedes aegypti* competence for an alphavirus. *J. Vector*
599 *Ecol.*, 37(1):154–161, June 2012a. ISSN 1948-7134. doi: 10.1111/j.1948-7134.2012.00212.x.

600 Ephantus J Muturi, Alive Nyakeriga, and Millon Blackshear. Temperature-mediated differential
601 expression of immune and stress-related genes in *Aedes aegypti* larvae. *Journal of the American*
602 *Mosquito Control Association*, 28(2):79–83, July 2012b.

603 Shinichi Nakagawa and Holger Schielzeth. A general and simple method for obtaining
604 r^2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 4
605 (2):133–142, 2013. ISSN 2041-210X. doi: 10.1111/j.2041-210x.2012.00261.x. URL
606 <http://dx.doi.org/10.1111/j.2041-210x.2012.00261.x>.

607 Kija R. Ng’habi, Bernadette John, Gamba Nkwengulila, Bart GJ Knols, Gerry F. Killeen, and
608 Heather M. Ferguson. Effect of larval crowding on mating competitiveness of *Anopheles gambiae*
609 mosquitoes. *Malaria Journal*, 4:49, 2005. ISSN 1475-2875. doi: 10.1186/1475-2875-4-49.

610 D. Ryan Norris and Caz M Taylor. Predicting the consequences of carry-over effects for mi-
611 gratory populations. *Biology Letters*, 2(1):148–151, March 2006. ISSN 1744-9561. doi:
612 10.1098/rsbl.2005.0397.

613 P E Parham, J Waldock, G K Christophides, D Hemming, F Augusto, K J Evans, N Fefferman,
614 H Gaff, A Gumel, S LaDeau, S Lenhart, R E Mickens, E N Naumova, Richard S Ostfeld, P D
615 Ready, M B Thomas, J Velasco-Hernandez, and E Michael. Climate, environmental and socio-
616 economic change: Weighing up the balance in vector-borne disease transmission. *Philosophical*
617 *Transactions of the Royal Society B: Biological Sciences*, 370(1665):20130551–20130551, Febru-
618 ary 2015. doi: 10.1098/rstb.2013.0551.

619 M. Pascual, J. A. Ahumada, L. F. Chaves, X. Rod, and M. Bouma. Malaria
620 resurgence in the East African highlands: Temperature trends revisited. *Proceed-*
621 *ings of the National Academy of Sciences of the United States of America*, 103(15):
622 5829–5834, April 2006. ISSN 0027-8424. doi: 10.1073/pnas.0508929103. URL
623 <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1416896/>.

624 S L Paulson and W A Hawley. Effect of body size on the vector competence of field and laboratory
625 populations of *Aedes triseriatus* for La Crosse virus. *Journal of the American Mosquito Control*
626 *Association*, 7(2):170–175, June 1991. ISSN 8756-971X.

627 Shushi Peng, Shilong Piao, Philippe Ciais, Pierre Friedlingstein, Catherine Ottle, François-Marie
628 Bréon, Huijuan Nan, Liming Zhou, and Ranga B. Myneni. Surface Urban Heat Island Across

629 419 Global Big Cities. *Environ. Sci. Technol.*, 46(2):696–703, January 2012. ISSN 0013-936X.
630 doi: 10.1021/es2030438.

631 David P. Price, Faye D. Schilkey, Alexander Ulanov, and Immo A. Hansen. Small mosquitoes,
632 large implications: Crowding and starvation affects gene expression and nutrient accumulation
633 in *Aedes aegypti*. *Parasites & Vectors*, 8:252, 2015. ISSN 1756-3305. doi: 10.1186/s13071-015-
634 0863-9.

635 R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for
636 Statistical Computing, Vienna, Austria, 2017. URL <https://www.R-project.org/>.

637 D. A. Ratkowsky, J. Olley, T. A. McMeekin, and A. Ball. Relationship between temperature and
638 growth rate of bacterial cultures. *J. Bacteriol.*, 149(1):1–5, January 1982. ISSN 0021-9193,
639 1098-5530.

640 Robert C. Reiner, T. Alex Perkins, Christopher M. Barker, Tianchan Niu, Luis Fernando Chaves,
641 Alicia M. Ellis, Dylan B. George, Arnaud Le Menach, Juliet R. C. Pulliam, Donal Bisanzio,
642 Caroline Buckee, Christinah Chiyaka, Derek A. T. Cummings, Andres J. Garcia, Michelle L.
643 Gatton, Peter W. Gething, David M. Hartley, Geoffrey Johnston, Eili Y. Klein, Edwin Michael,
644 Steven W. Lindsay, Alun L. Lloyd, David M. Pigott, William K. Reisen, Nick Ruktanonchai,
645 Brajendra K. Singh, Andrew J. Tatem, Uriel Kitron, Simon I. Hay, Thomas W. Scott, and
646 David L. Smith. A systematic review of mathematical models of mosquito-borne pathogen
647 transmission: 1970–2010. *J. R. Soc. Interface*, 10(81):20120921, April 2013. ISSN 1742-5689,
648 1742-5662. doi: 10.1098/rsif.2012.0921.

649 Robert C. Reiner, Matthew Geary, Peter M. Atkinson, David L. Smith, and Peter W. Gething.
650 Seasonality of *Plasmodium falciparum* transmission: A systematic review. *Malar. J.*, 14(1),
651 December 2015. ISSN 1475-2875. doi: 10.1186/s12936-015-0849-2.

652 M. H. Reiskind and L. P. Lounibos. Effects of intraspecific larval competition on adult longevity
653 in the mosquitoes *Aedes aegypti* and *Aedes albopictus*. *Med. Vet. Entomol.*, 23(1):62–68, March
654 2009. ISSN 1365-2915. doi: 10.1111/j.1365-2915.2008.00782.x.

655 Michael H. Reiskind and Ali A. Zarrabi. Is bigger really bigger? Differential responses to temper-
656 ature in measures of body size of the mosquito, *Aedes albopictus*. *Journal of Insect Physiology*,
657 58(7):911–917, July 2012. ISSN 0022-1910. doi: 10.1016/j.jinsphys.2012.04.006.

658 Olivier Roux, Amélie Vantaux, Benjamin Roche, Koudraogo B. Yameogo, Kounbobr R. Dabiré,
659 Abdoulaye Diabaté, Frederic Simard, and Thierry Lefèvre. Evidence for carry-over effects of
660 predator exposure on pathogen transmission potential. *Proc. R. Soc. B*, 282(1821):20152430,
661 December 2015. ISSN 0962-8452, 1471-2954. doi: 10.1098/rspb.2015.2430.

662 Chao Shan, Xuping Xie, Antonio E Muruato, Shannan L Rossi, Christopher M Roundy, Sasha R
663 Azar, Yujiao Yang, Robert B Tesh, Nigel Bourne, Alan D Barrett, Nikos Vasilakis, Scott C
664 Weaver, and Pei-Yong Shi. An Infectious cDNA Clone of Zika Virus to Study Viral Virulence,
665 Mosquito Transmission, and Antiviral Inhibitors. *Cell Host and Microbe*, pages 1–23, May 2016.
666 doi: 10.1016/j.chom.2016.05.004.

667 Qiang Shao, Stephanie Herrlinger, Si-Lu Yang, Fan Lai, Julie M. Moore, Melinda A. Brindley, and
668 Jian-Fu Chen. Zika virus infection disrupts neurovascular development and results in postnatal
669 microcephaly with brain damage. *Development*, 143(22):4127–4136, November 2016. ISSN
670 0950-1991, 1477-9129. doi: 10.1242/dev.143768.

671 Lillian L M Shapiro, Courtney C Murdock, Gregory R Jacobs, Rachel J Thomas, and Matthew B
672 Thomas. Larval food quantity affects the capacity of adult mosquitoes to transmit human
673 malaria. *Proc. B*, 283(1834):20160298–8, July 2016. doi: 10.1098/rspb.2016.0298.

674 Michael Turell. Effect of environmental temperature on the vector competence of *Aedes tae-*
675 *niorhynchus* for Rift Valley fever and Venezuelan equine encephalitis viruses. *Am. J. Trop.*
676 *Med. Hyg.*, 49(6):672–676, 1993.

677 Amélie Vantaux, Thierry Lefèvre, Anna Cohuet, Kounbobr Roch Dabiré, Benjamin Roche, and
678 Olivier Roux. Larval nutritional stress affects vector life history traits and human malaria
679 transmission. *Sci. Rep.*, 6(36778):1–10, November 2016. doi: 10.1038/srep36778.

680 M. Vazeille-Falcoz, L. Mousson, F. Rodhain, E. Chungue, and A. B. Failloux. Variation in oral
681 susceptibility to dengue type 2 virus of populations of *Aedes aegypti* from the islands of Tahiti
682 and Moorea, French Polynesia. *The American Journal of Tropical Medicine and Hygiene*, 60
683 (2):292–299, February 1999. ISSN 0002-9637.

684 James R. Vonesh. Sequential predator effects across three life stages of the African tree frog,
685 *Hyperolius spinigularis*. *Oecologia*, 143(2):280–290, March 2005. ISSN 0029-8549, 1432-1939.
686 doi: 10.1007/s00442-004-1806-x.

687 Catherine J. Westbrook, Michael H. Reiskind, Kendra N. Pesko, Krystle E. Greene, and L. Philip
688 Lounibos. Larval Environmental Temperature and the Susceptibility of *Aedes albopictus* Skuse
689 (Diptera: Culicidae) to Chikungunya Virus. *Vector-Borne and Zoonotic Diseases*, 10(3):241–
690 247, September 2010. ISSN 1530-3667. doi: 10.1089/vbz.2009.0035.

691 G. Xian, C. Homer, J. Dewitz, J. Fry, N. Hossain, and J. Wickham. Change of impervious surface
692 area between 2001 and 2006 in the conterminous united states. *Photogrammetric Engineering
693 and Remote Sensing*, 77(8):758–762, 2011.

694 H. M. Yang, M. L. G. Macoris, K. C. Galvani, M. T. M. Andrighetti, and D. M. V. Wan-
695 derley. Assessing the effects of temperature on the population of *Aedes aegypti*, the vec-
696 tor of dengue. *Epidemiol. Infect.*, 137(8):1188–1202, August 2009. ISSN 0950-2688. doi:
697 10.1017/S0950268809002040.

698 Fei Yuan and Marvin E Bauer. Comparison of impervious surface area and normalized difference
699 vegetation index as indicators of surface urban heat island effects in Landsat imagery. *Remote
700 Sens. Environ.*, 106(3):375–386, 2007. doi: 10.1016/j.rse.2006.09.003.

701 List of Figures

702	1	Map of study sites in Athens, GA, with inset illustrating location of Athens-Clarke	
703		County (black outline) in the state of Georgia. Symbols represent land classes	
704		(square: rural, circle:suburban, triangle: urban). Colors represent the amount of	
705		impervious surface within the 210m focal area of each pixel, as illustrated on the	
706		color bar on the bottom.	31
707	2	Microclimate differed significantly across both season and land class. Date ranges	
708		with green and orange background represent the summer and fall trials, respectively.	
709		The solid line represents the mean temperature across trays in each land class. The	
710		dotted lines represent the mean minimum and maximum temperatures across trays	
711		in each land class.	32
712	3	Female larval a) survival rate, b) development rate and c) population growth rate	
713		across the summer and fall trials and three land classes.	33
714	4	Infection dynamics (infected, disseminated, and infectious mosquitoes) across land	
715		class and season. Bars represent mean and standard errors of raw data across sites	
716		(n=3 per treatment).	34
717	5	The calculated vectorial capacity by site across individual mean temperature prior	
718		to infection assays (a). The incorporation of carry-over effects reduces the expected	
719		vectorial capacity at sites, although the effect is lessened at cooler temperatures.	
720		Inset charts on the right indicate calculated vectorial capacity without carry-over	
721		effects (b), with carry-over effects (c), and the percent difference due to the incor-	
722		poration of carry-over effects (d). Error bars represent standard error.	35
723	6	Supplementary Figure 1. Relative humidity across both replicates.	36

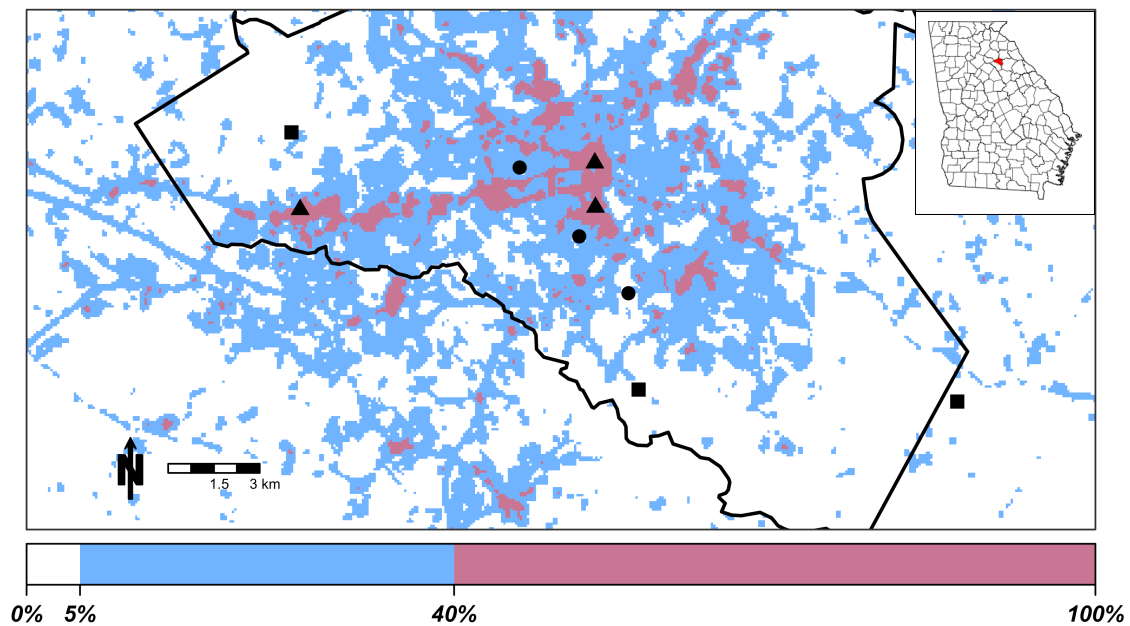


Figure 1: Map of study sites in Athens, GA, with inset illustrating location of Athens-Clarke County (black outline) in the state of Georgia. Symbols represent land classes (square: rural, circle: suburban, triangle: urban). Colors represent the amount of impervious surface within the 210m focal area of each pixel, as illustrated on the color bar on the bottom.

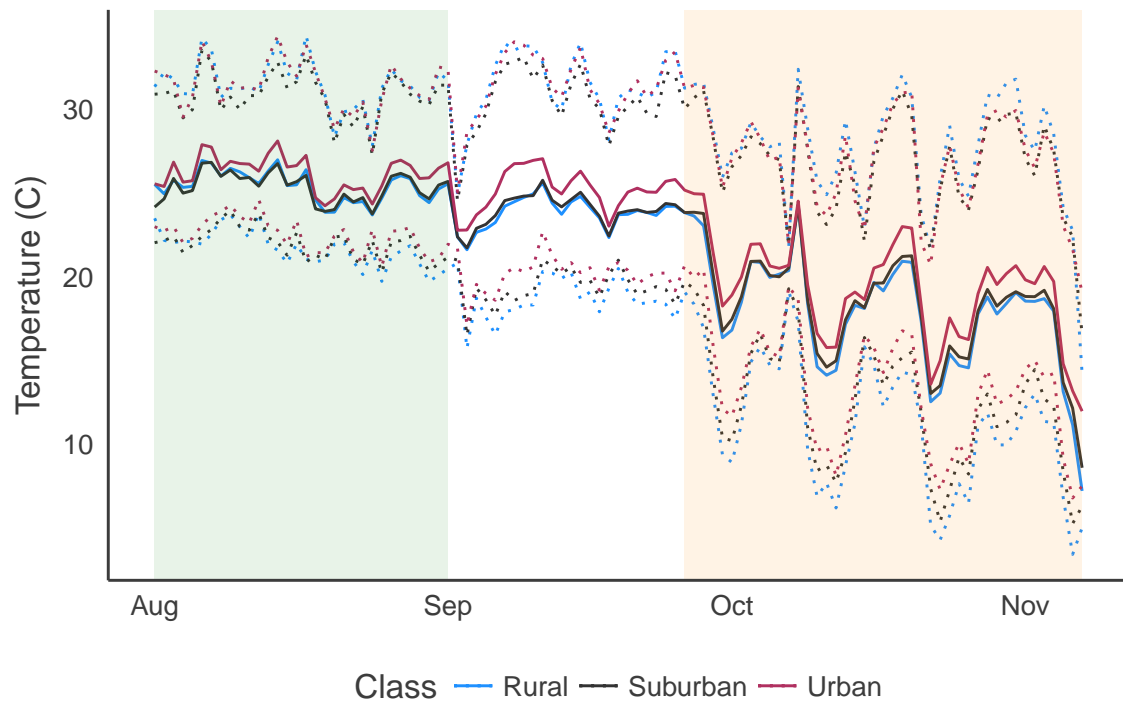


Figure 2: Microclimate differed significantly across both season and land class. Date ranges with green and orange background represent the summer and fall trials, respectively. The solid line represents the mean temperature across trays in each land class. The dotted lines represent the mean minimum and maximum temperatures across trays in each land class.

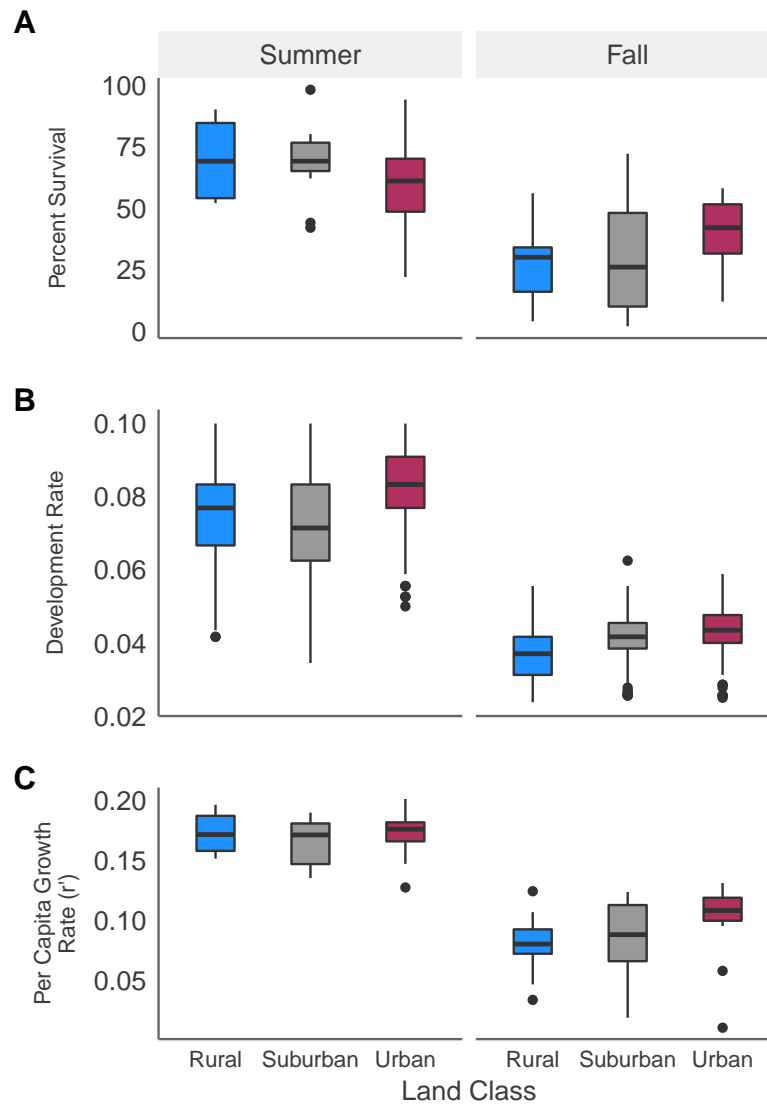


Figure 3: Female larval a) survival rate, b) development rate and c) population growth rate across the summer and fall trials and three land classes.

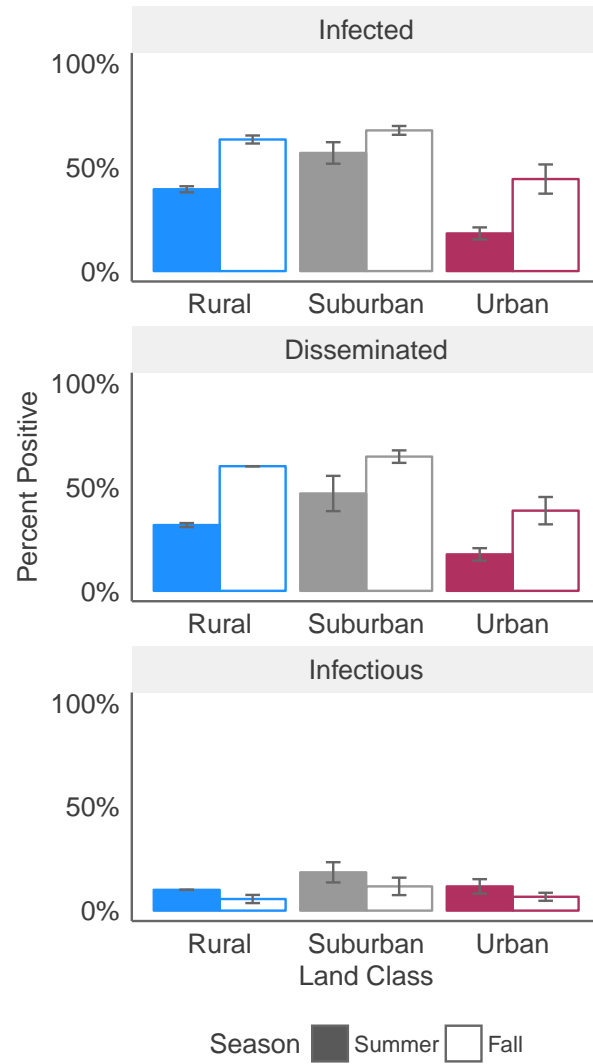


Figure 4: Infection dynamics (infected, disseminated, and infectious mosquitoes) across land class and season. Bars represent mean and standard errors of raw data across sites ($n=3$ per treatment).

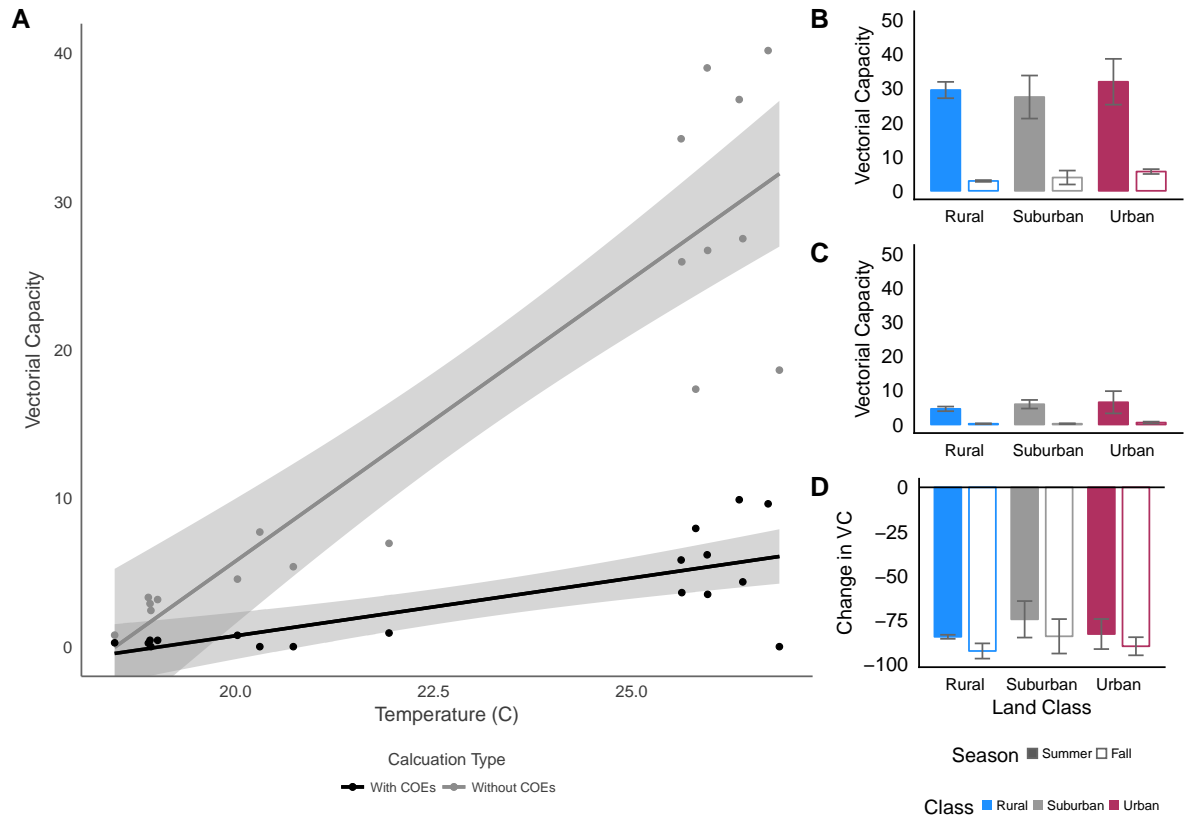


Figure 5: The calculated vectorial capacity by site across individual mean temperature prior to infection assays (a). The incorporation of carry-over effects reduces the expected vectorial capacity at sites, although the effect is lessened at cooler temperatures. Inset charts on the right indicate calculated vectorial capacity without carry-over effects (b), with carry-over effects (c), and the percent difference due to the incorporation of carry-over effects (d). Error bars represent standard error.

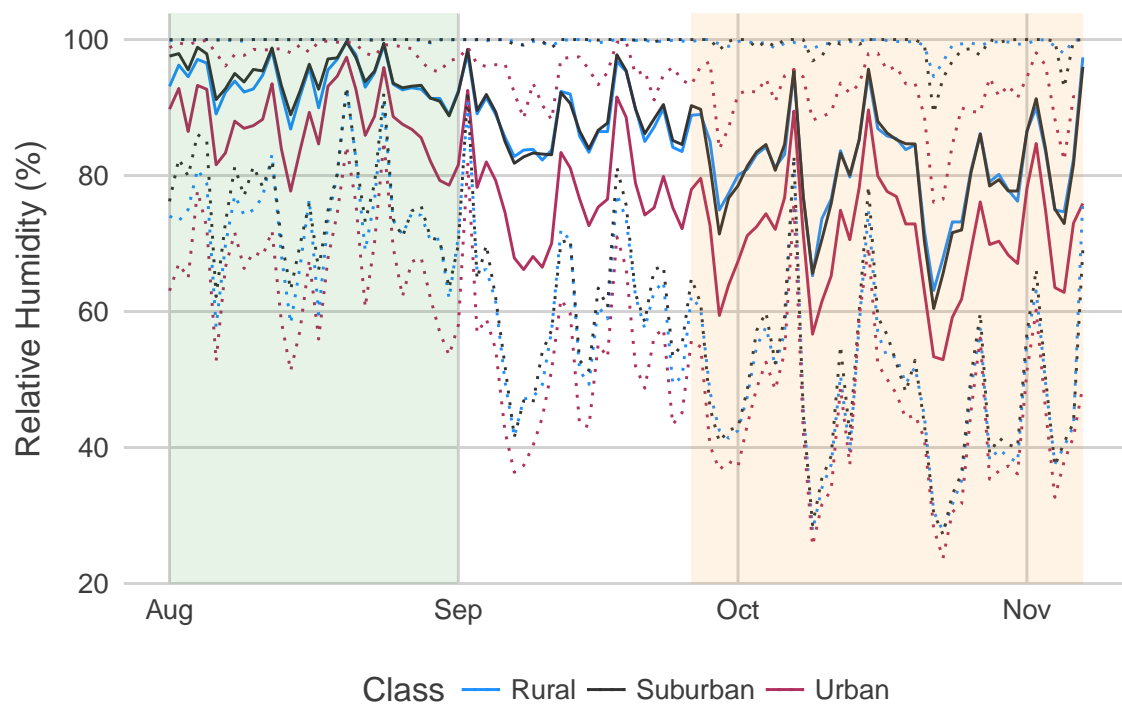


Figure 6: Supplementary Figure 1. Relative humidity across both replicates.

724 List of Tables

725	1	Sources of parameters used in the VC equation. Parameters sourced from Mordecai	
726		et al. (2017) were mathematically estimated at a constant temperature of 27 °C.	
727		Parameters that included carry-over effects are starred.	38
728	2	Relationship between microclimate variables and population and infection dynamics	
729		of mosquitoes. Linear mixed effect models were used to determine the effect of tem-	
730		perature on survival, development, population growth rate, and vectorial capacity,	
731		with site as a random effect, while generalized linear models with logit-link func-	
732		tions were used in the calculation of virus dynamics. Conditional R^2 values for linear	
733		mixed models were calculated via Nakagawa and Schielzeth (2013). Superscripts	
734		represent significance as calculated by Wald Chi-square tests with Holm-Bonferroni	
735		corrections ($*p < 0.5$, $**p < 0.01$, $***p < 0.001$).	39
736	3	Supplemental Table 1. The efficiency rates of infection (mosquitoes with dengue	
737		positive bodies), dissemination (infected mosquitoes with dengue positive heads)	
738		and infectiousness (infected mosquitoes with dengue positive saliva) across season	
739		and land class. Raw numbers of positive samples are shown with percentages in	
740		parentheses.	40
741	4	Supplemental Table 2. Chi-square values (subscripts represent degrees of freedom)	
742		resulting from linear mixed models analyzing effect of land class and season on	
743		microclimate variables. Superscripts represent significance as calculated by Wald	
744		Chi-square tests with Holm-Bonferroni corrections ($*p < 0.5$, $**p < 0.01$, $***p <$	
745		0.001).	41

Parameter	Definition	Without carry-over effects	With carry-over effects
$a(T)$	Per-mosquito bite rate	Mordecai et al. 2017	Mordecai et al. 2017
$b(T)c(T)^*$	Vector competence	Mordecai et al. 2017	Current Study
$\mu(T)$	Adult mosquito mortality rate	Mordecai et al. 2017	Mordecai et al. 2017
$EIR(T)$	Extrinsic incubation rate (i.e. inverse of extrinsic incubation period)	Mordecai et al. 2017	Mordecai et al. 2017
$EFD(T)^*$	Number of eggs produced per female mosquito per day	Mordecai et al. 2017	Current Study
$p_{EA}(T)$	Egg-to-adult survival probability	Current Study	Current Study
$MDR(T)$	Mosquito immature development rate	Current Study	Current Study

Table 1: Sources of parameters used in the VC equation. Parameters sourced from Mordecai et al. (2017) were mathematically estimated at a constant temperature of 27 °C. Parameters that included carry-over effects are starred.

Response Variable	β_{Temp}	β_{RH}	$\beta_{Temp \times RH}$	R^2
Survival	2.716***	-	-	0.601
Development Rate (day^{-1})	0.016***	0.00382***	-0.00014***	0.743
Per Capita Growth Rate (r')	0.0127***	-	-	0.787
Infection (Body)	-0.129***	0.0376*	-	0.586
Dissemination (Head)	-0.0667***	0.0160*	-	0.591
Infectiousness (Saliva)	-	0.0459	-	0.155
Vectorial Capacity (VC)	0.779***	-	-	0.548

Table 2: Relationship between microclimate variables and population and infection dynamics of mosquitoes. Linear mixed effect models were used to determine the effect of temperature on survival, development, population growth rate, and vectorial capacity, with site as a random effect, while generalized linear models with logit-link functions were used in the calculation of virus dynamics. Conditional R^2 values for linear mixed models were calculated via Nakagawa and Schielzeth (2013). Superscripts represent significance as calculated by Wald Chi-square tests with Holm-Bonferroni corrections (* $p < 0.5$, ** $p < 0.01$, *** $p < 0.001$).

Season	Land Class	No. tested	No. infected (%)	No. disseminated (%)	No. infectious (%)
<i>Summer</i>					
	Rural	56	22 (39)	19 (48)	6 (15)
	Suburban	57	32 (56)	26 (81)	10 (31)
	Urban	51	10 (20)	10 (100)	7 (70)
<i>Fall</i>					
	Rural	50	32 (64)	30 (94)	3 (9)
	Suburban	43	28 (65)	25 (89)	3 (11)
	Urban	51	10 (20)	10 (100)	7 (70)

Table 3: Supplemental Table 1. The efficiency rates of infection (mosquitoes with dengue positive bodies), dissemination (infected mosquitoes with dengue positive heads) and infectiousness (infected mosquitoes with dengue positive saliva) across season and land class. Raw numbers of positive samples are shown with percentages in parentheses.

	Min. Temp.	Mean Temp.	Max. Temp.	DTR	Min. RH	Mean RH	DHR
Land Class (χ^2_2)	12.40**	16.16***	3.71	8.23*	9.93**	22.91***	0.85
Season (χ^2_1)	1809.77***	1320.55***	362.39***	549.30***	838.43***	745.35***	755.49***
Land Class x Season (χ^2_2)	6.6*	3.21	1.13	11.79**	3.77	11.12**	28.57***

Table 4: Supplemental Table 2. Chi-square values (subscripts represent degrees of freedom) resulting from linear mixed models analyzing effect of land class and season on microclimate variables. Superscripts represent significance as calculated by Wald Chi-square tests with Holm-Bonferroni corrections (* $p < 0.5$, ** $p < 0.01$, *** $p < 0.001$).