MANUSCRIPT TITLE: Mosquito population dynamics is shaped by the interaction among larval density, season, and land use

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KEY WORDS

Aedes albopictus; Density-Dependence*;* Fitness*;* Per Capita Growth Rate*;* Seasonality*;* Life History Traits

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DATA AVAILABILITY STATEMENT

The author is still cleaning the code that support the findings of this study for publication purposes, but data and code will be openly available on Figshare if the paper is accepted.

ABSTRACT (English)

Understanding how variation in key abiotic and biotic factors interact at spatial scales relevant for mosquito fitness and population dynamics is crucial for predicting current and future mosquito distributions and abundances, and the transmission potential for human pathogens. However, studies investigating the effects of environmental variation on mosquito traits have investigated environmental factors in isolation or in laboratory experiments that examine constant environmental conditions that often do not occur in the field. To address these limitations, we conducted a semi-field experiment in Athens, Georgia using the invasive Asian tiger mosquito (*Aedes albopictus*). We selected nine sites that spanned natural variation in impervious surface and vegetation cover to explore effects of the microclimate (temperature and humidity) on mosquitoes. On these sites, we manipulated conspecific larval density at each site. We repeated the experiment in the summer and fall. We then evaluated the effects of land cover, larval density, and time of season, as well as interactive effects, on the mean proportion of females emerging, juvenile development time, size upon emergence, and predicted per capita population growth (i.e., fitness). We found significant effects of larval density, land cover, and season on all response variables. Of most note, we saw strong interactive effects of season and intra-specific density on each response variable, including a non-intuitive decrease in development time with increasing intra-specific competition in the fall. Our study demonstrates that ignoring the interaction between variation in biotic and abiotic variables could reduce the accuracy and precision of models used to predict mosquito population and pathogen transmission dynamics, especially those inferring dynamics at finer-spatial scales across which transmission and control occur.

ABSTRACT (Spanish)

Para poder predecir la distribución y abundancia de las poblaciones de mosquitos y la transmisión potencial de patógenos a humanos, es crucial comprender cómo factores abióticos y bióticos clave para el éxito reproductivo y la dinámica poblacional de los mosquitos interactúan a escalas relevantes. Sin embargo, los estudios que han investigado los efectos de variables ambientales en las características demográficas de los mosquitos han considerado su efecto de forma aislada o en experimentos de laboratorio bajo condiciones ambientales constantes que, a menudo, no reflejan lo que ocurre en el campo. Para abordar estas limitaciones, llevamos a cabo un experimento de semi-campo en Athens, Georgia, utilizando el mosquito invasor tigre asiático (*Aedes albopictus*). Seleccionamos nueve sitios que abarcaban variaciones naturales en la superficie impermeable y cobertura vegetal para explorar los efectos del microclima (temperatura y humedad) en los mosquitos. También manipulamos la densidad de larvas de tigre asiático en dos experimentos que fueron realizados en el verano y otoño. Evaluamos los efectos de la cobertura vegetal, la densidad de larvas, la temporada climática, y la interacción entre estas variables en la proporción de hembras que emergieron, el tiempo de desarrollo de las larvas, el tamaño al momento de la emergencia, y el crecimiento demográfico per cápita previsto (éxito reproductivo). Encontramos efectos significativos de la densidad de larvas, la variación de la cobertura vegetal y la estación del año en todas las variables de respuesta. Más notablemente, observamos un fuerte efecto de la interacción entre la temporada climática y la densidad de larvas en todas las variables de respuesta, incluyendo una disminución no intuitiva en el tiempo de desarrollo con el aumento de la competencia intraespecífica en el otoño. Nuestro estudio evidencia que ignorar la interacción entre variables abióticas y bióticas podría reducir la exactitud y precisión de los modelos utilizados para predecir las dinámicas de las poblaciones de

mosquitos, y por tanto, de la transmisión de patógenos. Esto, especialmente en modelos que infieren estas dinámicas a escalas espaciales más finas, en las cuales ocurre la transmisión y el control.

INTRODUCTION

Arthropod-borne viruses (arboviruses) pose significant global health and economic burdens (Espinal et al. 2019; Girard et al. 2020; Ogunlade et al. 2021). Some of the most notable arboviruses (dengue, Chikungunya, yellow fever, Ross River, and Zika) are transmitted by *Aedes* mosquitoes and often cause chronic morbidity and high mortality for people infected. *Aedes*-borne arboviruses continue to expand, which is partially attributed to increasing urbanization, climate change, and the geographical spread of *Aedes* mosquitoes (Bhatt et al. 2013; Wilder-Smith et al. 2017). To better anticipate areas and times of seasons most at risk for vector-borne pathogen transmission, target vector control efforts, and reduce the incidence of these pathogens, it is critical to understand how global change influences mosquito population dynamics.

Multiple environmental factors (abiotic and biotic) affect mosquito demography, vital rates (e.g., larval development, growth, and survival), and population dynamics. Key abiotic factors include variation in temperature, relative humidity, and precipitation, while biotic factors include variation in intra- and inter-species competition, availability of resources, and presence / density of biological enemies (predators and parasites). In addition, these environmental factors can exhibit considerable spatial heterogeneity (Cohen et al. 2016). When this spatial variation is ignored, predictions of mosquito population dynamics averaged over space or time can lead to considerable bias if the effects of these factors are nonlinear (Bozinovic et al. 2011; Martin and

Huey 2008; Ruel and Ayres 1999). As a result, mathematical models might overlook the influence of hot spots or ecological traps on mosquito population dynamics.

Given the effects that global change (e.g., urbanization and climate change) could have on the environment and thus mosquito-borne pathogen transmission and public health, numerous studies have focused on the effects of abiotic factors, especially temperature, on mosquitoes (Mordecai et al. 2019; Nik Abdull Halim et al. 2022; Reinhold et al. 2018; Shocket et al. 2020). Temperature has non-linear (unimodal) effects on mosquito traits and their ability to transmit pathogens (Mordecai et al. 2017). Shifts in temperature can alter mosquito distributions, consequently changing the spatial and temporal patterns of environmental suitability for mosquito-borne pathogen transmission (Tesla et al. 2018; Miazgowicz et al. 2020; Wimberly et al. 2020). Climate change is expected to decrease suitability for transmission in some areas while increasing it in others (Lafferty and Mordecai 2016; Ryan et al. 2021; 2023). Therefore, the incorporation of abiotic factors (e.g., temperature and precipitation) into predictive models has advanced our understanding of mosquito population dynamics (Alto and Bettinardi 2013; Evans et al. 2019; Murdock et al. 2017), pathogen transmission, and overall disease risk (Messina et al. 2016; Mordecai et al. 2013; Zhang et al. 2017).

The effect of biotic factors, however, is generally excluded from species distribution models and mechanistic models of mosquito-borne disease transmission, despite the fact that at fine scales (e.g., a backyard) biotic factors likely determine mosquito abundances (Beale et al. 2014; Ewing et al. 2019; Golding et al. 2015; Leach et al. 2016). In the field, mosquito larvae cohabitate with conspecifics and/or heterospecifics at varying densities. The competition and resource limitation that results from these interactions reduces larval nutrition and can have lasting effects on mosquito survival, reproduction, and generation time by slowing development,

reducing adult size, and lowering teneral reserves (e.g., lipids and proteins) of adult mosquitoes going through final stages of maturation) (Briegel 2003). There has been a large body of work investigating the effects of conspecific larval densities on various metrics of mosquito fitness (e.g., development rate, body size, survival, reproduction). Increases in larval densities typically decrease mosquito fitness and its components (Breaux et al. 2014; Schneider et al. 2000; Yoshioka et al. 2012); similarly, larvae reared in environments with high nutrient availability tend to emerge as larger, more fecund adults than larvae reared in low nutrient environments. Differences in mosquito body condition can also translate into differences in vector competence (Alto and Bettinardi 2013; Alto et al. 2005; 2007) and vectorial capacity (Moller-Jacobs et al. 2014; Shapiro et al. 2016).

However, there has been little investigation of the potential interactive effects of abiotic and biotic factors on mosquito population dynamics, which likely occur in the field. For example, the relationship between mosquito body size and fecundity (number of eggs produced in the first gonotrophic cycle) is modulated by temperature (Ezeakacha and Yee 2019; Reiskind and Zarrabi 2012) and food quality (Costanzo et al. 2018). Interactions in the field could be mediated by temperature-dependent growth of microbial populations, for example, that mosquitoes feed on in the juvenile environment (Ratkowsky et al. 1982), which in turn could exacerbate or dampen the effects of competition on mosquito population dynamics. Finally, most studies that have considered the interaction between abiotic (i.e., temperature, relative humidity) and biotic factors (i.e., intraspecific competition) on mosquito life history traits and population dynamics have been conducted in the lab under constant environmental conditions that may not be reflective of field environments. Instead, abiotic variables like temperature, for

example, vary diurnally, seasonally, and across space (e.g., due to variation in land use and land cover) (Wimberly et al. 2020; Murdock et al. 2017; Evans et al. 2018; 2019).

To improve our understanding of how field-relevant variation in key abiotic and biotic factors potentially interact at spatial scales relevant for mosquito fitness and population dynamics, we conducted a semi-field experiment using the Asian tiger mosquito (*Aedes albopictus*) system in Athens, Georgia during summer and fall 2017. *Ae. albopictus* is a highly invasive mosquito in the United States and other areas of the world, as well as a competent vector of arboviruses including dengue and chikungunya (Benedict et al. 2007; Bonizzoni et al. 2013; Gratz 2004; Paupy et al. 2009). We selected nine sites that spanned natural variation in impervious surface and vegetation cover, which was expected to lead to variation in the microclimate experienced by mosquitoes based on established relationships between land use, land cover, temperature, and relative humidity (Murdock et al. 2017; Wimberly et al. 2020). On these sites we also manipulated conspecific larval density. We then measured the effects of variation in land cover, larval density, time of season, and potential interactions on larval development and survival, adult size and emergence, and predicted per capita population growth (i.e., fitness) of *Ae. albopictus*. Understanding the drivers of spatial and temporal heterogeneity of *Ae. albopictus* abundance is crucial for predicting and managing its future invasive potential and understanding population dynamics of *Ae. albopictus* and other species with similar ecology (e.g., *Aedes aegypti)*, which play an important role in human arbovirus transmission.

METHODS

Experimental design

We conducted the study between June 2017 and October 2017 in Athens-Clarke County, Georgia, USA. Following previous work (Evans et al. 2018; Murdock et al. 2017), we used an impervious surface and vegetation cover map to select nine 30 x 30 m sites – three each with low (0-5%), intermediate (6-40%), and high (41-100%) areal cover of impervious surfaces, which we refer to as rural, suburban, and urban land use types respectively (Figure S1) (Evans et al. 2019; Murdock et al. 2017). Within each of the nine sites, we placed three trays in full shade separated by approximately 6-9 m. Each tray contained four wide-mouth mason jars containing 300mL of leaf infusion (see Murdock et al. 2017). Each jar was assigned a density treatment (0, 30, 60, or 120 *Ae. albopictus* first instar larvae from a laboratory colony). We covered the top of the jars with mesh netting to prevent emerging adults from escaping (and other adults from ovipositing) and staked a wire cage with a vinyl roof over the trays to the ground to prevent changes in habitat volume from rainfall, nutrients due to falling vegetation, and loss from animal disturbance.

To understand how seasonal variation in abiotic factors may alter relationships between conspecific larval density and traits governing mosquito population dynamics, we conducted this experiment in the summer (June 11 - July 25) and fall (September 8 - October 18) 2017. Jars were visited daily, at the same time of day, until no emergence was observed within the previous 24 hours. During each visit and for each jar we recorded the developmental stage of a typical mosquito (i.e., larvae, pupae, adult), number of emerged adults, and any observations that might have affected the results (e.g., fallen tree branch). If adults emerged, we collected them with a mouth aspirator, placed them into a 50 mL falcon tube labeled with the respective jar ID, and

then placed the tubes on ice. We transported mosquitoes to the laboratory and stored them at -20°C until we determined their sex (Darsie and Ward 2005) and wing length.

Microclimate variables

To measure variation in microclimate, we deployed one data logger (Monarch Instruments, Amherst, NH, USA: Radio Frequency Identification (RFID) Temperature Track-It Logger) to each tray in a separate jar filled with leaf infusion but no larvae. We also uniformly distributed six data loggers throughout each site at approximately 0.5-1.0 m above the ground and in full shade (Murdock et al. 2017). Loggers recorded air or water temperature and relative humidity every ten minutes. Microclimate data were downloaded monthly and were summarized by recording the daily minimum (T_{min}), daily maximum (T_{max}), and daily average (T_{mean}) for each logger. We also calculated the diurnal range in temperature (T_{range}) and relative humidity (R_{range}) as the difference between the observed daily minimum and maximum for each logger (e.g., $T_{range}= T_{max}-T_{min}$; $R_{range}= R_{max}-R_{min}$).

Mosquito life history traits

In this study, we only report female data since females (not males) are responsible for egg production, population growth rates, and pathogen transmission. Therefore, emergence, development time, wing length, fecundity, and population growth rates all refer to female individuals (unless otherwise noted). To calculate emergence, we divided the total number of females that emerged by half the total number of 1st instar larvae placed into the habitat (assuming half of the larvae were female). Juvenile development time was defined as the day an adult female emerged (since the start of the experiment). One wing was removed from each

individual, mounted onto a glass slide using clear nail polish, and measured with a dissecting microscope and micrometer. Wings were measured from the alular notch to the apex of the wing, excluding fringe setae (Packer and Corbet 1989). Finally, we used the data collected in this experiment to estimate the per capita growth rates of mosquito populations in response to our experimental treatments by using Livdahl & Sugihara's (1984) equation 7:

$$r' = \frac{ln\left(\frac{1}{N_0}\sum_x A_x\ f(w_x)\right)}{D\ +\ \frac{\sum_x xA_x\ f(w_x)}{\sum_x A_x\ f(w_x)}} \qquad (1)$$

where N_0 is the initial number of females in a cohort (which we assume to be half the initial number of larvae in the jar (i.e., 15, 30, or 60)), A_x is the number of females that emerged on day x, D is the time to reproduction following emergence (which we assume to be 14 days: Lounibos et al. 2002). The fecundity $f(w_x)$ of females that emerged on day x, with an average wing length of w_x, is estimated from the linear relationship between wing length and number of eggs laid from Lounibos et al. (2002):

$$f(w_x) = -121.240 + (78.02\ *w_x) \qquad (2)$$

Data Analyses

To assess how daily microclimate conditions (mean, minimum and maximum temperature: T_{mean}, T_{min}, T_{max}; and relative humidity: R_{mean}, R_{min}, R_{max}) varied with season and land use, we used generalized linear mixed models (GLMMs) with lognormal distributions and *log* link functions. All models included season, land-use type, and their interaction as fixed effects with site, logger, and day as random effects. We also used GLMMs to determine how emergence, larval development rate, wing length, and per capita growth rate varied across

seasons, land use types, and density treatments. Those models all had season, land use type, density treatment, and their interactions as fixed effects, with site, tray, and jar as random effects.

All analyses were conducted in R version 4.3.3 (R Core Team 2024). Models were fit using the *glmmTMB* package (Brooks et al. 2017), and corrected Akaike Information Criterion (AICc) values were used to determine which models described the data best. One-way analysis of variance (ANOVA) was conducted for all models using the *car* package (Fox and Weisberg 2019). If models showed statistically significant effects, we performed a post-hoc analysis using Tukey's multiple pair-wise comparison of means [*emmeans* package (Lenth 2023)] to identify differences between independent variables. We assessed the scaled residuals of models for overdispersion and divergence from normality using the DHARMa package (Hartig 2022).

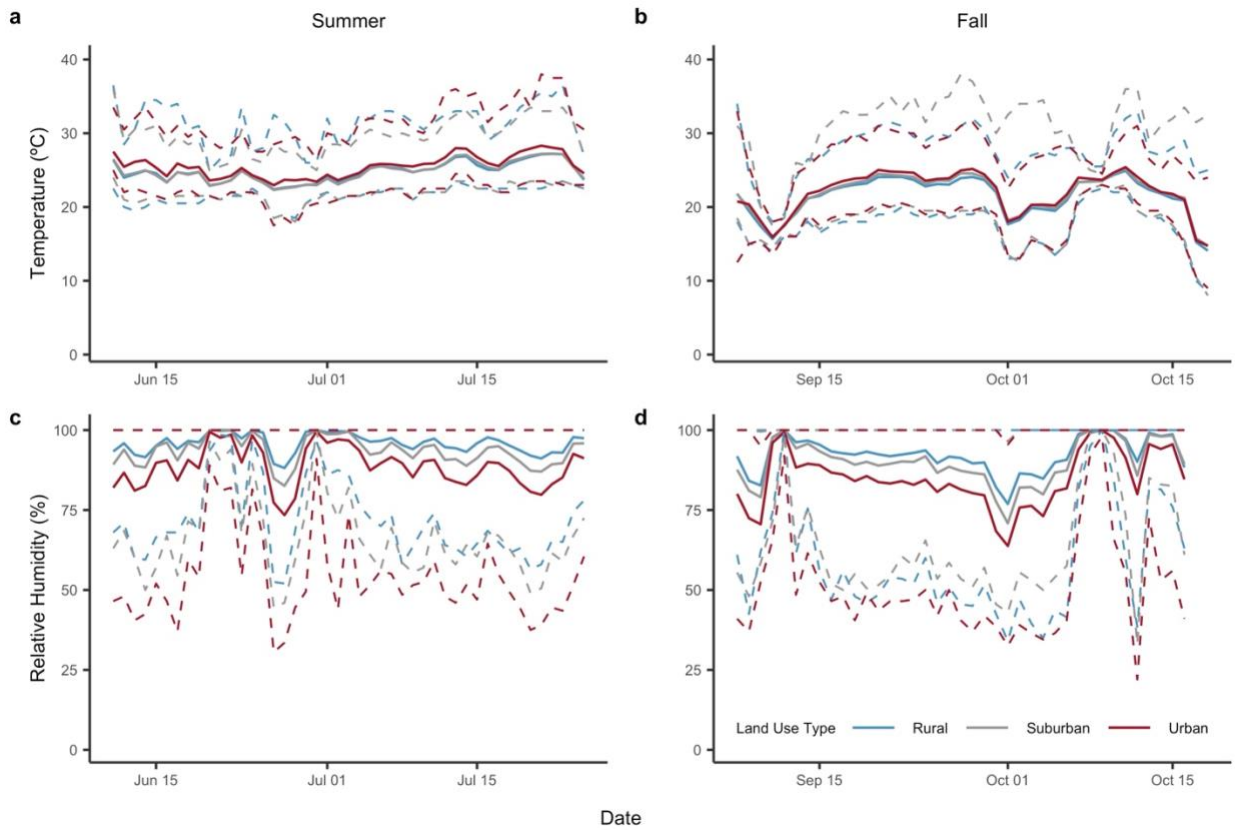

Figure 1. Temperature (ºC) (top panels) and relative humidity (%) (bottom panels) at three sites in each of three land use types (rural = blue, suburban = gray, and urban = red) in two seasons (summer 2017 [left panels] and fall 2017 [right panels]). Mean microclimates across the three land use types are in solid lines, with daily maxima and minima indicated by the dashed lines.

RESULTS

Microclimate

Five of the 54 temperature loggers placed in larval environments failed (three in the summer and two in the fall), and 36 of the 108 loggers placed in terrestrial environments failed or were stolen (16 in the summer and 20 in the fall). However, microclimate variation across season and land use types was well represented by the remaining loggers and failure rates were roughly equivalent across sites. Diurnal temperature and relative humidity metrics all varied significantly with season (Table S1). Summer temperatures (i.e., mean daily T_{min}, T_{mean}, and T_{max}) were higher than fall temperatures (Figure 1, Tables 1 & S1). Relative humidity (mean daily R_{min}, R_{mean}, and R_{max}) was also greater in the summer than fall (although R_{max} was only significantly greater in summer urban sites) (Figure 1, Tables 1 & S1). Seasonal variation in diurnal ranges for each abiotic factor also occurred. Mosquitoes in the summer experienced lower fluctuations throughout the day in temperature (T_{range}) and relative humidity (R_{range}) than in the fall (Table 1). Finally, we observed significant effects of land use on diurnal temperature and relative humidity metrics (Table S1). Urban sites had significantly warmer daily average minimum temperatures (T_{min}) than rural sites overall (Table 1) and were less humid than suburban and rural sites (i.e., lower R_{min}, R_{mean}, and R_{max}: Table 1). As a result, urban sites experienced a wider diurnal range in relative humidity (R_{range}) than rural sites (Table 1).

In addition to the main effects of season and land use on mosquito microclimate variables, we observed a significant interaction between season and land use on average daily T_{max} and T_{range} (Table S1). T_{max} was lower in suburban sites in the summer, but higher in the fall, compared to rural and urban sites (Table 1). Urban sites also had marginally lower average diurnal temperature ranges (T_{range}) than rural sites, but only in the fall season (Table 1). We did

not observe an interactive effect of season and land use on metrics of relative humidity (Table S1).

Emergence

Of the 5670 first instar *Ae. albopictus* placed into the field during each season, a total of 3156 (56%) mosquitoes (males and females combined) successfully emerged during the summer while only 1233 (38%) mosquitoes (males and females combined) emerged in the fall. We observed significant main effects of season and conspecific density on the proportion of female first instar larvae that emerged as adults (i.e., survived through the larval and pupal stages; Table S2). Females began emerging earlier in the summer trial (day 9) than in the fall trial (day 13), and there was a significantly higher proportion of first instar larvae that survived to emerge as adult females from the low density treatment relative to medium and high density treatments (Table 2).

We also observed significant interactions between season and density as well as land use type and density on adult female emergence (Table S2). For example, there was no significant difference in the survival of female larvae in the low-density treatments in the summer and fall; however, the negative effects of increasing conspecific density on the larval survival was much greater in the fall (Figure 2a, Table 3). Further, the proportion of females that emerged from larval habitats in each conspecific density treatment varied across land use, with the proportion of females that emerged at low conspecific densities being highest in suburban sites. Habitats with intermediate conspecific densities on urban sites experienced the highest survival. Finally, for larval habitats with high conspecific densities, rural sites had the highest juvenile survival (Figure 3).

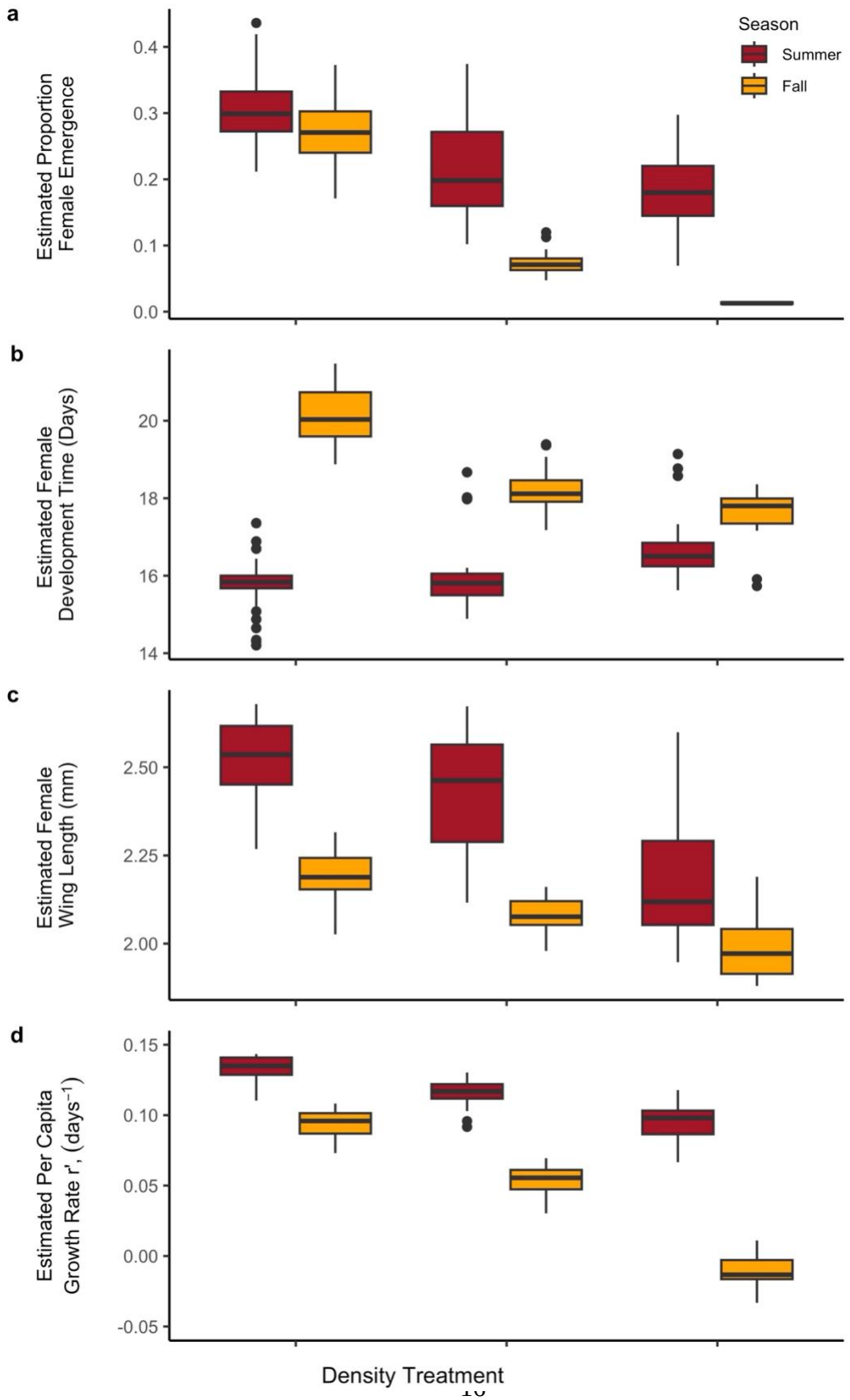
a
Estimated Proportion Female Emergence
0.4
0.3
0.2
0.1
0.0
Season
Summer
Fall
b
Estimated Female Development Time (Days)
20
18
16
14
c
Estimated Female Wing Length (mm)
2.50
2.25
2.00
d
Estimated Per Capita Growth Rate r', (days⁻¹)
0.15
0.10
0.05
0.00
-0.05
Density Treatment

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Figure 2. Mixed effects model prediction of the (**a**) the proportion of female *Ae. albopictus* larvae that emerged (i.e., survival probability), (**b**) mean female *Ae. albopictus* development time (days), (**c**) mean wing length (mm), and (**d**) per capita intrinsic growth rate, r' ($days^{-1}$) for each density treatment (low = 30 larvae, medium = 60 larvae, high = 120 larvae) across two seasons (summer = red, fall = orange). Each box represents the interquartile range (25th to 75th percentile), with the median (black horizontal line), whiskers extend to the extremes or 1.5 times the interquartile range beyond the interquartile (whichever is smaller), and the dots indicate any values beyond the whiskers.

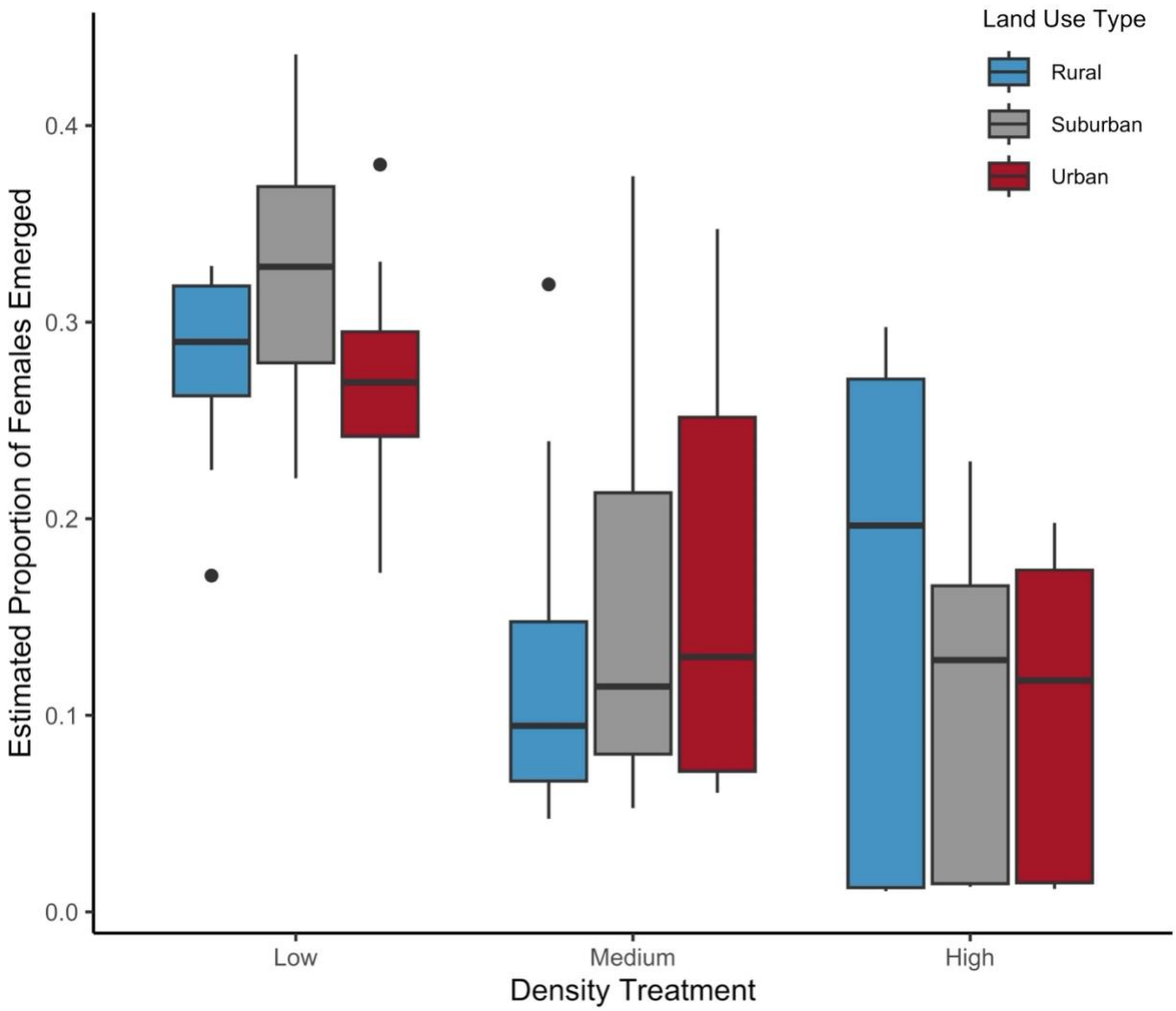

Figure 3. Mixed effects model prediction of the proportion of female *Ae. albopictus* larvae that emerged from each density treatment (low = 30 larvae, medium = 60 larvae, high = 120 larvae) across each land use type (rural = blue, suburban = gray, urban = red). Each box represents the interquartile range (25th to 75th percentile), with the median (black horizontal line), whiskers extend to the extremes or 1.5 times the interquartile range beyond the interquartile (whichever is smaller), and the dots indicate any values beyond the whiskers.

Female Development Time

Both time of season and conspecific larval density significantly affected the average amount of time required for adult female emergence (Table S2), with females developing more quickly in the summer relative to the fall and from the low-density treatment (Tables 2 & S2, Figure 2b). However, a significant interaction between season and larval density demonstrates that the main effect of density is being driven by the relationship between mean female development time (time to emergence) and larval density in the summer. Females reared in the low-density treatment were the fastest to develop in the summer but were the slowest to develop in the fall (Figure 2b, Table 3).

Wing Length

We observed significant main effects of season and density on female wing length (Table S2). Females reared in the summer had longer wings compared to the fall, and females developing in the high conspecific larval treatment had significantly shorter wings than mosquitoes from the low-density treatment (Figure 2c). We did not observe a significant interaction between these two factors on female wing size (Table S2).

Per Capita Population Growth Rate

Integrating these responses revealed strong effects of season, conspecific larval density, and the interaction between these two factors on the per capita population growth rate, r' (Figure 2d, Table S2). In general, the per capita growth rate was greater in the summer relative to the fall and at higher conspecific densities (Table 2). A significant interaction between season and density was observed due to greater declines in per capita growth rate with increasing

conspecific density in the fall relative to the summer (Figure 2d, Table 3). Additionally, we observed negative per capita growth rates at the highest conspecific density in the fall, while all other combinations of factors led to positive projected per capita growth (Figure 2d).

DISCUSSION

To date, the majority of studies investigating the effects of various abiotic and biotic factors on mosquito fitness, population dynamics, vector competence, and transmission potential investigate the effect of varying a single environmental variable such as temperature (e.g., Liu-Helmersson et al. 2014; Agyekum et al. 2022; Monteiro et al. 2007), conspecific density (e.g., Hawley 1985; Sauers et al. 2022; Reiskind and Lounibos 2009), and food resources (e.g., Levi et al. 2014; Telang and Wells 2004). Further, these studies are often conducted in laboratory environments across a range of constant conditions that explore a wider range of environmental conditions than what might be experienced in the field. However, mosquito populations in the field will be subject to the effects of many different environmental variables that fluctuate diurnally and seasonally, and likely interact. Previous work in the *Ae. albopictus* system suggests that microclimate variation in the field (rather than coarser environmental manipulations in the lab) can translate into significant variation in mosquito life history traits, population dynamics, and arbovirus transmission potential (Evans et al. 2018; 2019; Murdock et al. 2017; Wimberly et al. 2020). Our experimental study builds on these results by showing that variation in microclimate conditions (temperature and relative humidity) with season and land use can interact with variation in key biotic variables (conspecific density) to affect metrics of mosquito fitness (larval survival and development time, female size at emergence, and per capita growth rates). The results from this study have broad implications for our understanding and

ability to predict the effects of current and future environmental variation on mosquito fitness, population dynamics, and the environmental suitability for mosquito-borne disease transmission.

The effects of land use on microclimate, metrics of mosquito fitness, and population dynamics

Similar to previous studies conducted in the same region (Athens, Georgia) (Wimberly et al. 2020; Murdock et al. 2017; Evans et al. 2018), urban sites had higher minimum temperatures than rural sites and were drier than non-urban land use types. This is consistent with the Urban Heat Island (UHI) effect, where nighttime temperatures (analogous to T_{min}) are 1-2.5ºC higher (Hibbard et al. 2017) than surrounding areas due to the higher percentage of impervious surfaces in urban areas that absorb, store, and re-emit heat (e.g., asphalt and concrete). However, in non-urban areas, pervious materials that absorb yet do not re-emit heat (e.g., mulch, soil, vegetation), have higher evapotranspiration rates, and provide ample shading resulting in lower temperatures and higher relative humidity than urban areas (Oke 1982; Bowler et al. 2010; Huang et al. 1987; Jabbar et al. 2023). Despite the variation in microclimate observed across land use, this variation for the most part did not affect metrics of mosquito fitness or predicted per capita growth rates. This is somewhat different from previous studies in this system (Murdock et al. 2017; Evans et al. 2018) and others (Waldock et al. 2013; Li et al. 2014) that found microclimate variation across land use to significantly affect the proportion of adults emerging, mosquito development rate, and the ability of mosquitoes to transmit dengue-2 virus. However, this is not necessarily surprising as the previous effects observed were subtle and likely due to the fact that Athens-Clarke County is a small city in Georgia, with less variation in impervious surface cover, vegetation cover, and ambient temperatures than seen in larger cities in the U.S. and worldwide

(Peng et al. 2011; Oke 1973; Zhou et al. 2017; Imhoff et al. 2010). We did observe effects of land use on the relationship between conspecific density and the proportion of adult females emerging, suggesting the effects of intra-specific competition could potentially be modified across an urban gradient. However, while significant, these effects were subtle.

The effects of seasonality on microclimate, metrics of mosquito fitness, and population dynamics

Seasonal variation in microclimate was much larger than what we observed across land use, with temperature and relative humidity being higher during the summer field trial relative to the fall. Our finding of a higher relative humidity in the summer compared to the fall is consistent with a previous study in the same system (Evans et al. 2018), probably due to higher levels of precipitation during the summer. As a result, we observed strong seasonal effects on the proportion of females emerging from the larval environment, the juvenile development time, their body size upon emergence, and predicted per capita growth rates from larval habitats. This is not unexpected considering mosquitoes are ectothermic, and trait performance has been shown to have non-linear relationships with temperature variation across a diversity of mosquito species (reviewed by: Mordecai et al. 2019). For example, laboratory work conducted under a range of constant temperatures predicts that *Ae. albopictus* larval survival and mosquito development rates should be optimized at ~25ºC and ~35ºC, respectively (Mordecai et al. 2017). Thus, it is unsurprising that we generally saw faster larval development times and increased adult female emergence in the warmer summer field trial (with temperatures ranging from 25-25.7ºC) than the cooler fall (with temperatures ranging from 21.1-21.6ºC) across our sites.

As a result of these seasonal effects on metrics of mosquito fitness, we also observed higher per capita growth rates in the summer relative to the fall. These results support the findings of previous work in this system, that found similar seasonal trends in adult female emergence, juvenile development times on these sites, and predicted mosquito population dynamics (Evans et al. 2018; 2019, but see Murdock et al. 2017 which saw reduced female emergence in the summer relative to the fall overall). Interestingly, despite warmer summer temperatures and the increased juvenile development times in the summer relative to the fall, mosquitoes emerged at larger sizes. This is counter to what would be expected from previous work in this system (Murdock et al. 2017, but see Evans et al. 2018 that found no effect of season on wing size) and other studies (Ezeakacha and Yee 2019; Lounibos et al. 2002; Briegel and Timmermann 2001), but could be due to seasonality modifying the effect of conspecific density on the juvenile development time and larval survival (proportion of adult females emerging).

Seasonality shapes the relationship between intra-specific competition and metrics of mosquito fitness and population dynamics

The effects of increasing conspecific larval density on larval survival, larval development time, size upon emergence, and predicted per capita growth rates have been documented extensively to date. In general, our results support previous research that shows increasing conspecific larval densities result in decreased juvenile survival, smaller adults upon emergence, and decreased per capita growth rates (Walker et al. 1991; Sauers et al. 2022; Lord 1998). However, our study highlights a critical and novel role of seasonality and land use in modifying the effects of conspecific larval density on mosquito fitness and population dynamics.

In both the summer and fall trial, we observed a negative effect of increasing conspecific larval density on larval survival (i.e., the proportion of adult females emerging). However, this trend was much stronger during the fall trial. This could be due to a hurricane that occurred at the beginning of the study (day three) when larvae were in their most vulnerable stage (i.e., first instar) and/or due to cooler fall temperatures being below the thermal optimum for *Ae. albopictus* larval survival (~25ºC, Mordecai et al. 2017). For example, mean temperatures in the fall were <15ºC for three consecutive days. These temperatures, which can result in high *Ae. albopictus* larval and pupal mortality (reviewed in Waldock et al. 2013), combined with the additional stress of developing under high intra-specific competition, could work synergistically to increase juvenile mortality.

Contrary to our hypothesis and the multitude of prior studies (e.g., Sauers et al. 2022; Lord 1998; Ezeakacha and Yee 2019), larvae developed faster with higher conspecific densities in the fall. Several factors could explain this result. First, the timing of larval mortality is known to affect the amount of resources available to the surviving larvae (Neale and Juliano 2019; McIntire and Juliano 2018; Evans et al. 2022). If mortality of early instar larvae was elevated by cooler fall temperatures and high intra-specific densities, the surviving larvae would have experienced reduced intra-specific competition and increased access to food resources, both of which allow larvae to develop faster (Evans et al. 2022; Neale and Juliano 2019; Teng and Apperson 2000). Stage-specific overcompensation and the Hydra effect have been detected in *Ae. albopictus* when early larval mortality occurred (McIntire and Juliano 2018). Another possible explanation may be survival bias. Since very few females survived in the fall from the medium-density treatment (7.2%) and even fewer from the high-density treatment (1.2%), the results are biased to those few individuals that did survive; these survivors might have been

individuals of higher fitness quality allowing them to persist and develop in more competitive conditions. A third explanation could be that larvae facultatively alter their development rate based on a stressor such as low temperatures and/or low quantity of nutritional resources due to increased competition. While not demonstrated before in *Ae. albopictus*, previous work in the *Ae. triseriatus* and *Ae. communis* systems have shown larvae can speed up development rates in environments subject to evaporation (Chodorowski 1969; Juliano and Stoffregen 1994).

Based on the extensive literature documenting relationships between mosquito wing size (i.e. size upon emergence) with variation in conspecific density (Alto et al. 2005; Lord 1998; Evans et al. 2021) or temperature (Kingsolver and Huey 2008; Rueda et al. 1990), we observed unexpected, seasonal effects on these relationships. While wing size upon emergence decreased with increasing conspecific density as previous work has shown, this effect was stronger in the warmer summer than the cooler fall. Temperature effects have also been well studied with warmer temperatures resulting in smaller wings and body size–"hotter is smaller"–and cooler temperatures resulting in larger wings and body size–"cooler is bigger" hypothesis (Ezeakacha and Yee 2019; Kingsolver and Huey 2008; Rueda et al. 1990). Our results contradict the "cooler is bigger" hypothesis since mosquitoes developing in the summer had larger wings than those reared in the fall. Only a few studies have demonstrated smaller wing lengths at cooler temperatures (Reiskind and Zarrabi 2012; Lounibos et al. 2002). The result could be attributed to slower microbial growth in the fall (due to cooler temperatures) limiting the amount of food available to larvae combined with anabolism being less efficient at low temperatures (Reiskind and Janairo 2018). Thus, females in the summer may have access to more microbially rich habitats regardless of larval density treatment as well as a greater capacity to incorporate nutrients into tissue, allowing them to develop more quickly in the summer and emerge as larger

adults. Finally, like development time, mosquito size upon emergence converges at high densities regardless of season, suggesting that high amounts of intra-specific competition could eventually outweigh seasonal variation in abiotic factors such as temperature and relative humidity on mosquito body size and condition.

In general, while per capita growth rates declined as larval conspecific densities increased (see also: Lord 1998; Edgerly and Livdahl 1992; Reiskind and Lounibos 2009) we found the per capita growth rates in the summer to be higher and less affected by intra-specific competition than in the fall. This is likely because mosquitoes in the summer (relative to the fall) survived better, developed more rapidly, and emerged as larger adults with higher predicted fecundity despite increases in intra-specific competition. Most studies to date have considered the effects of temperature or conspecific density on growth rates in isolation, without considering the possible interaction. Those that do consider the effects of temperature on the relationship between intra-specific competition and per capita growth rates have generally found intra-specific competition to intensify as temperatures warm (Evans et al. 2021; Teng and Apperson 2000), which is not what we observed in this study.

CONCLUSION

This study has several important implications. It is generally thought that variation in biotic factors (e.g., intra-specific and inter-specific competition, quality and quantity of habitat, biological enemies) will vary more and thus exert their effects on organismal fitness and population dynamics at fine-spatial scales relative to abiotic factors (e.g., temperature, precipitation, relative humidity) that have more regional effects (Wiens 1989; McGill 2010; Russell et al. 2006; Sandel and Smith 2009). This study demonstrates that while variation in

abiotic factors at fine-spatial scales (e.g., microclimate) had relatively little effect, we do see important effects of season, which represents variation in abiotic factors but at a longer temporal scale. The experimentally imposed variation in density (a biotic factor) had much greater effects on mosquito traits and interacted with the abiotic factors. Thus, ignoring the interaction between variation in biotic and abiotic variables could reduce the accuracy and precision of models used to predict mosquito population and disease transmission dynamics, especially those inferring dynamics at finer-spatial scales across which transmission and control occur. By not accounting for these interactions, mismatches in model predictions could result in unexpected variation in mosquito densities spatially and seasonally, as well as unexplained carry-over effects of how these variables interact to affect mosquito fitness and vectorial capacity (Moller-Jacobs et al. 2014; Shapiro et al. 2016; Alto et al. 2007).

AUTHOR CONTRIBUTIONS

CCM designed the experiment. NS, ECH, CWH, PMN, AMS, and JWW conducted fieldwork for the study. NS conducted the statistical analyses, with guidance from CCM and CWO. NS drafted the manuscript with guidance from CCM and CWO. All authors were involved in reviewing and editing the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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Table 1. Daily mean microclimate values (95% confidence intervals) across seasons (summer and fall) and land use type (rural, suburban, urban). Temperature (ºC) data were collected from data loggers placed in the larval environment, while relative humidity (%) data were collected from loggers placed <1m above ground.

	Summer			Fall		
	Rural	Suburban	Urban	Rural	Suburban	Urban
Temperature (℃)						
T_{min}	22.5 (22.0, 22.9)[a]	22.7 (22.2, 23.2)[ab]	23.3 (22.8, 23.7)[b]	17.5 (17.1, 17.9)[c]	18.2 (17.8, 18.7)[d]	18.4 (18.0, 18.8)[d]
T_{mean}	25.0 (24.5, 25.6)[a]	25.1 (24.5, 25.6)[a]	25.7 (25.2, 26.3)[a]	21.1 (20.6, 21.6)[b]	21.3 (20.8, 21.8)[b]	21.6 (21.2, 22.1)[b]
T_{max}	28.7 (27.4, 29.9)[a]	28.3 (27.0, 29.5)[a]	29.2 (28.0, 30.5)[a]	25.7 (24.6, 26.8)[b]	26.4 (25.2, 27.6)[b]	25.3 (24.2, 26.4)[b]
T_{range}	6.37 (5.26, 7.47)[a]	5.65 (4.51, 6.80)[a]	6.10 (5.03, 7.18)[a]	8.00 (6.93, 9.08)[a]	8.17 (7.02, 9.32)[a]	6.69 (5.62, 7.77)[b]
Relative Humidity (%)						
R_{min}	80.8 (74.9, 87.1)[a]	75.7 (70.4, 81.5)[ab]	68.0 (63.4, 73.0)[b]	70.1 (65.1, 75.4)[c]	68.9 (63.8, 74.5)[cd]	61.4 (57.0,66.2)[d]
R_{mean}	96.0 (92.9, 99.3)[a]	93.9 (90.8, 97.0)[a]	88.2 (85.5, 91.1)[b]	92.1 (89.1, 95.2)[c]	90.3 (87.3, 93.5)[c]	84.5 (81.7, 87.3)[d]
R_{max}	100.0 (99.0, 101.0)[a]	100.0 (99.0, 100.9)[a]	98.8 (97.9, 99.7)[a]	99.6 (98.7, 100.6)[a]	99.3 (98.3, 100.3)[a]	97.4 (96.5, 98.3)[b]
R_{range}	19.8 (15.5, 24.2)[a]	24.6 (20.4, 28.9)[ab]	31.0 (27.0, 35.0)[b]	28.8 (24.6, 33.1)[c]	29.4 (24.9, 33.8)[c]	34.6 (30.4, 38.9)[c]

* Letters represent significant differences as measured by Tukey's multiple comparison of means, adjusting for significance with the Holm-Bonferroni method.

Table 2. Mean demographic trait values [female emergence (%), female development time (days), female wing length (mm), intrinsic growth rate] (95% confidence intervals) across seasons (summer and fall), land use type (rural, suburban, urban), and larval density treatment (30 = low, 60 = medium, 120 = high).

	Female Emergence (%)	**Development Time (days)**	**Wing Length (mm)**	**Per Capita Growth Rate ($days^{-1}$)**
Season				
Summer	22.6 (19.9, 25.4)[a]	16.1 (15.5,16.7)[a]	2.40 (2.35, 2.45)[a]	0.114 (0.109, 0.119)[a]
Fall	6.6 (5.33, 8.05)[b]	18.5 (17.6, 19.4)[b]	2.09 (2.03, 2.15)[b]	0.045 (0.040, 0.051)[b]
Land Use Type				
Rural	12.3 (9.7, 15.6)[a]	17.4 (16.3, 18.6)[a]	2.24 (2.16, 2.33)[a]	0.078 (0.070, 0.086)[a]
Suburban	13.2 (10.5, 16.6)[a]	17.2 (16.2, 18.4)[a]	2.23 (2.15, 2.31)[a]	0.082 (0.074, 0.090)[a]
Urban	12.0 (9.3, 15.3)[a]	17.0 (15.9, 18.2)[a]	2.24 (2.16, 2.32)[a]	0.078 (0.071, 0.087)[a]
Density Treatment				
Low	28.9 (25.2, 32.9)[a]	17.8 (17.1, 18.5)[a]	2.35 (2.29, 2.40)[a]	0.113 (0.108, 0.119)[a]
Medium	12.4 (10.5, 14.6)[b]	17.0 (16.3, 17.7)[b]	2.28 (2.22, 2.33)[b]	0.085 (0.079, 0.090)[b]
High	4.8 (3.7, 6.3)[c]	16.9 (16.0, 18.0)[b]	2.10 (2.03, 2.17)[c]	0.041 (0.035, 0.047)[c]

***** Letters represent significant differences as measured by Tukey's multiple comparison of means, adjusting for significance with the Holm-Bonferroni method.

Table 3. Generalized Linear Mixed Model results of the interaction between season (summer vs. fall) and larval density treatment (30 = low, 60 = medium, 120 = high) on demographic rates. Cells represent mean values (95% confidence intervals).

	Density Treatment	Female Emergence (%)	Development Time (days)	Wing Length (mm)	Per Capita Growth Rate ($days^{-1}$)
Summer	Low	30.9 (26.1, 36.1)[a]	15.7 (15.0, 16.4)[a]	2.53 (2.46, 2.60)[a]	0.133 (0.126, 0.140)[a]
	Medium	20.6 (17.2, 24.4)[b]	15.9 (15.2, 16.5)[a]	2.49 (2.42, 2.56)[a]	0.115 (0.108, 0.123)[b]
	High	17.6 (14.9, 20.8)[c]	16.7 (16.0, 17.3)[b]	2.20 (2.14, 2.26)[b]	0.093 (0.086, 0.101)[c]
Fall	Low	27.0 (22.5, 32.0)[d]	20.1 (19.3, 21.1)[c]	2.18 (2.12, 2.25)[d]	0.094 (0.087, 0.101)[d]
	Medium	7.2 (5.6, 9.1)[e]	18.2 (17.3, 19.2)[d]	2.09 (2.02, 2.15)[e]	0.054 (0.047, 0.061)[e]
	High	1.2 (0.7, 1.9)[f]	17.2 (15.6, 19.0)[b]	2.00 (1.90, 2.11)[e]	–0.011 (-0.021, -0.002)[f]

* Letters represent significant differences as measured by pairwise comparison using Tukey's multiple comparison of means, adjusting for significance with the Holm-Bonferroni method.