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KEY WORDS

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

DATA AVAILABILITY STATEMENT

The data and code that support the findings of this study are openly available on Figshare: https://figshare.com/s/93b37d27373bb0f48896.

ABSTRACT (English)

Understanding how variation in key abiotic and biotic factors interact at ecologically relevant spatial scales is crucial for predicting population dynamics, distributions, and abundances. This is especially true for vectors that transmit human pathogens. However, studies investigating the effects of environmental variation on vectors have typically 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 varied in impervious surface and vegetation cover to explore effects of natural variation in microclimate (specifically, temperature and humidity) on mosquitoes. We manipulated conspecific larval density at each site and repeated the experiment in the summer and fall to further increase the variability in temperature and humidity. We then evaluated the original design features (land cover, larval density, and season), and their interactions, on the mean proportion of females emerging, juvenile development time, size upon emergence, and estimated per capita population growth (i.e., fitness). We found significant effects of larval density, land cover, and season on all response variables, including a non-intuitive decrease in development time with increasing larval density in the fall. We repeated these analyses using the hypothesized microclimate drivers of these effects: temperature and humidity. In general, the model using the microclimate variables outperformed the model using land use and season, highlighting the roles of temperature and humidity and their interactions with density on mosquito traits and dynamics. Our study demonstrates that ignoring the interaction between variation in biotic (e.g., intraspecific competition) and abiotic (e.g., temperature and humidity) variables could reduce the accuracy and precision of models used to predict mosquito population

49 and pathogen transmission dynamics, especially those inferring dynamics at finer-spatial scales

50 across which transmission and control occur.

51

ABSTRACT (Spanish)

Es crucial comprender cómo los factores abióticos y bióticos interactúan a escalas ecológicamente relevantes para predecir la dinámica poblacional, la distribución, y la abundancia. Esto es especialmente cierto para vectores que transmiten patógenos humanos. Sin embargo, la mayoría de los estudios han considerado factores ambientales de forma aislada o en condiciones de laboratorio poco realistas. Para superar estas limitaciones, realizamos un experimento de semi-campo en Athens, Georgia, con el mosquito invasor *Aedes albopictus*. Manipulamos la densidad larval en nueve sitios que variaban en cobertura vegetal y superficie impermeable, y repetimos el experimento en el verano y otoño. Luego, evaluamos los efectos de la cobertura del suelo, la densidad larval, y la estación en la proporción de hembras adultas emergidas, el tiempo de desarrollo, el tamaño al emerger, y el crecimiento poblacional per cápita. Encontramos efectos significativos de todas las variables, así como fuertes interacciones entre estación y densidad intraespecífica de mosquitos, incluyendo una disminución no intuitiva del tiempo de desarrollo en el otoño con mayor densidad larval. Repetimos los análisis usando factores microclimáticas hipotetizados como impulsores de estos efectos: temperatura y humedad. En general, el modelo que incorpora variables microclimáticas superó al modelo basado en el uso de suelo y estacionalidad, evidenciando la importancia de la temperatura y la humedad, así como sus interacciones con la densidad larval, en las características biológicas y la dinámica poblacional de los mosquitos. Nuestros resultados demuestran que la interacción entre variación biótica (competencia intraespecífica) y abiótica (temperatura y humedad), es fundamental para mejorar la precisión de los modelos que predicen la dinámica poblacional de mosquitos y la transmisión de patógenos a escalas locales de transmisión y control.

INTRODUCTION

Three processes generally determine the distribution and abundances of organisms: environmental filtering (abiotic variables), species interactions (biotic variables), and constraints on organismal dispersal (Leibold and Chase, 2018; Menge and Sutherland, 1976; Vellend, 2016). The effects of abiotic and biotic variables on species distribution and abundances have become some of the most important issues in ecology today, especially in the context of rapid biodiversity loss (McKinney and Lockwood, 1999) climate change (Parmesan, 2006) and human-mediated dispersal (Bullock and Pufal, 2020). Multiple environmental factors (both abiotic and biotic) affect the demography, vital rates (e.g., development, growth, and survival), and population dynamics of ectothermic organisms. However, predicting the effects of abiotic and biotic variables on organismal distribution and abundance patterns is difficult because their effects on processes that determine organismal fitness and population dynamics are scale-dependent (Levin, 1992; McGill, 2010; Wiens, 1989).

It has been long hypothesized that environmental filtering is strongest at larger, more regional scales because climate variables (e.g., temperature, relative humidity, precipitation) exhibit little variation at fine-spatial scales (Houghton et al., 2002; Levin, 1992; McGill, 2010; Wiens, 1989). With the emphasis on climate warming in the ecological literature, there has been a strong focus on understanding the effects of temperature on the distribution and abundance of ectothermic organisms, as well as their traits (Bale et al., 2002; Helmuth et al., 2010; Mordecai et al., 2019; Parmesan, 2006; Pörtner and Farrell, 2008). These effects have been leveraged in species distribution models and mechanistic models to predict the current and future geographic ranges of ectothermic organisms (Amarasekare, 2024; Huey et al., 2001; Simon and Amarasekare, 2024). However, biotic variables, like intra- and inter-species competition,

availability of resources, and presence and density of biological enemies (predators and parasites), also have important effects on organismal fitness and population dynamics (Beale et al., 2014; Ewing et al., 2019; Golding et al., 2015; Leach et al., 2016). These biotic factors affect individuals and can vary among local neighborhoods; thus, the effects of these factors on species distribution and abundance patterns are hypothesized to arise at fine-spatial scales (Levin, 1992; McGill, 2010) but are often not incorporated into species distribution or mechanistic models (Cohen et al., 2016). Finally, abiotic and biotic factors can interact to affect species distributions, which could be especially relevant for organisms with complex life cycles, such as amphibians or insects, that use very different habitats across their life stages (Govindarajulu and Anholt, 2006; Indermaur et al., 2010). When this spatial variation is ignored, predictions of a given species' 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 a given species' population dynamics.

One of the areas in which these issues are readily apparent is in the field of mosquito ecology. Climate and land use change are rapidly altering the landscapes across which mosquitoes occur, altering the ecological relationships mosquitoes share with their environments and their vertebrate hosts. This has led to biological invasions of mosquito species and their parasites to new areas of the world (Roiz et al., 2024; Taylor et al., 2024) with consequences for the emergence of new diseases (Chala and Hamde, 2021; Lounibos, 2002; Yan et al., 2024). Understanding and predicting how future changes in climate and land use will alter mosquito dynamics and the spread of vector-borne pathogens is particularly relevant. Thus, there has been considerable investigation into the effects of temperature on mosquito distributions, population

121 dynamics, and parasite transmission (reviewed in Kraemer et al., 2019; Liu-Helmersson et al.,
122 2019; Mordecai et al., 2019; Ryan et al., 2023; Sinka et al., 2020). Recent work also suggests
123 that relative humidity can have important effects on larval traits and mosquito population
124 dynamics, possibly through effects of humidity on the surface tension of habitats (see Brown et
125 al., 2023; Huxley et al. 2025). In addition to abiotic variables, mosquitoes experience intra- and
126 inter-specific competition during their larval stage, which can affect mosquito survival,
127 reproduction, and generation time by slowing development, reducing adult size, and lowering
128 teneral reserves (e.g., lipids and proteins) of adult mosquitoes (Briegel, 2003) with significant
129 implications for mosquito fitness and vectorial capacity (Alto and Bettinardi, 2013; Alto et al.,
130 2005; Moller-Jacobs et al., 2014; Shapiro et al., 2016).

131 Currently, there has been little investigation of the potential interactive effects of abiotic
132 and biotic factors on mosquito population dynamics. Most studies that have considered the
133 interaction between abiotic (i.e., temperature, relative humidity) and biotic factors (i.e.,
134 intraspecific competition) on mosquito life history traits and population dynamics have been
135 conducted in the lab under constant environmental conditions that may not reflect field
136 environments (Evans et al., 2021; Miazgowicz et al., 2020; Tesla et al., 2018; Shapiro et al.,
137 2017). In the field, abiotic variables like temperature vary diurnally, seasonally, and across
138 space (e.g., due to variation in land use and land cover) (Evans et al., 2018, 2019; Murdock et al.,
139 2017; Wimberly et al., 2020).

140 To improve our understanding of how field-relevant variation in key abiotic and biotic
141 factors interact at spatial scales relevant for mosquito fitness and population dynamics, we
142 conducted a semi-field experiment using the Asian tiger mosquito (*Aedes albopictus*) in Athens,
143 Georgia during summer and fall 2017. *Ae. albopictus* is a highly invasive mosquito in the United

144 States and other areas of the world, as well as a competent vector of arboviruses including
145 dengue and chikungunya (Benedict et al., 2007; Bonizzoni et al., 2013). We selected nine sites
146 that varied in impervious surface and vegetation cover, and conducted an experiment across the
147 sites at two different times to leverage natural spatial and temporal variation in temperature and
148 relative humidity, and explored their effects on mosquito traits and population dynamics (Brown
149 et al., 2023; Murdock et al., 2017; Wimberly et al., 2020). During both experiments, we also
150 manipulated conspecific larval density, a biotic variable known to have large effects on metrics
151 of mosquito fitness and population growth rates (Juliano, 2009; Teng and Apperson, 2000). We
152 simultaneously monitored spatial and temporal variation in temperature and humidity. Although
153 we designed our study based upon density, land use and season, we were specifically interested
154 in the interactive effects of conspecific density, temperature and relative humidity, each of which
155 is known to affect mosquito demography in isolation (e.g., Alto and Juliano, 2001; Brown et al.,
156 2023; Mordecai et al., 2019), while controlling for other factors that might vary with season and
157 land use (e.g., leaf litter or detrital inputs). We hypothesized that temperature and humidity
158 would serve as "master variables" and would provide a more general and better predictor of
159 mosquito dynamics than land use and season. Understanding the drivers of spatial and temporal
160 heterogeneity on the dynamics of invasive species like *Ae. albopictus* is crucial for predicting
161 and managing their future invasive potential, as well as for other species with similar ecology
162 (e.g., *Aedes aegypti)* that play an important role in human arbovirus transmission.
163

164 **METHODS**

165 *Experimental design*

166 We conducted the study between June 2017 and October 2017 in Athens-Clarke County,
167 Georgia, USA. We utilized nine previously established field sites that ranged in impervious
168 surface, vegetation cover, and local microclimate (Appendix S1: Figure S1) (Evans et al., 2019).
169 Within each of the nine sites, we placed three trays in full shade separated by approximately 6-9
170 m. Each tray held four wide-mouth mason jars each containing 300 mL of leaf infusion (Evans
171 et al., 2018; Murdock et al., 2017). Three jars in each tray received a specific larval density
172 treatment (30, 60, or 120 *Ae. albopictus* first instar larvae from a laboratory colony established
173 from field collected *Ae. albopictus* in 2015, Athens, GA;; Murdock et al., 2017). We covered the
174 top of the jars with mesh netting and staked a wire cage with a vinyl roof over the trays (see
175 Murdock et al., 2017) to prevent changes in habitat volume from rainfall, the addition of
176 nutrients due to falling vegetation, and the loss of jars from animal disturbance. To understand
177 how seasonal variation in abiotic factors may alter relationships between conspecific larval
178 density and traits governing mosquito population dynamics, we conducted this experiment in the
179 summer (June 11 - July 25) and fall (September 8 - October 18) 2017. Jars were visited daily
180 and at the same time of day. Any adults that had emerged were collected and placed on ice for
181 transport back to the lab. Mosquitoes were then stored at -20°C until we determined their sex
182 (Darsie and Ward, 2005) and wing length.

183

184 *Microclimate variables*

185 To measure variation in water temperature, we deployed one data logger (Monarch
186 Instruments, Amherst, NH, USA: Radio Frequency Identification (RFID) Temperature Track-It

Logger) to one jar in each tray that had been filled with leaf infusion but contained no larvae. We also uniformly distributed six data loggers of the same brand throughout each site at approximately 0.5-1.0 m above the ground and in full shade to measure relative humidity. All loggers recorded microclimate variables every ten minutes with data downloaded monthly.

Mosquito life history traits

In this study, we only report female data since females determine mosquito population growth rates and pathogen transmission. To calculate the proportion of mosquito larvae and pupae that survived to emerge as adults (i.e., emergence),),) we divided the total number of females that emerged by half the total number of 1st instar larvae placed into the habitat (e.g., 15, 30, or 60 assuming half of the 1st instar larvae were female). Juvenile development time was defined as the number of days between the start of the experiment and the day an adult female emerged. One wing was removed from each individual, mounted onto a glass slide using clear nail polish, and measured with a dissecting microscope and micrometer to index body size (Packer and Corbet, 1989). To estimate the per capita growth rates of mosquito populations in response to our experimental treatments we used equation 7 from Livdahl & Sugihara (1984):

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

where N_0 is the initial number of females in a cohort (i.e., half of the density treatment), A_x is the number of females that emerged on day x, and 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, was estimated from the linear relationship between wing length and number of eggs laid from Lounibos et al. (2002):

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

In 12 cases, no females emerged from a jar (these all occurred in the fall at high density). Since removing those jars would elevate and bias r' for that treatment, we imputed their per capita growth rate by setting r' = -0.05 day^{-1}, which was just below the most negative r' observed in the other jars.

Data Analyses

We took three complementary approaches to analyze the results. First, we examined variation in water temperature and relative humidity across land use and season. To assess how daily microclimate conditions varied, we used linear mixed effects models to investigate how the daily mean, minimum, maximum, and range in air temperature (T_{mean}, T_{min}, T_{max}, T_{range}) and relative humidity (R_{mean}, R_{min}, R_{max}, R_{range}) varied with design variables.. All models included fixed effects of season, land-use type, and their interaction, and random effects of site, logger (nested within site), and day.

Second, we investigated how juvenile survival and development rate, adult size, and estimated per capita population growth (i.e., fitness) of *Ae. albopictus* varied with land use, season, and larval density. Although our putative interest was microclimate (and not land use and season per se), our sampling design was based on these categorical factors, so we started with those analyses before moving on to exploration of the effects of temperature and humidity. We also used GLMMs to determine how emergence (binomial distribution; *logit* link function), larval development rate (lognormal distribution; *log* link function), wing length (lognormal distribution; *log* link function), and per capita growth rate (Gaussian distribution; *identity* link function) varied across seasons, land-use types, and density treatments. Those models all had

232 season, land-use type, density treatment, and their interactions as fixed effects, with site and tray
233 (within site) as nested random effects. Analyses of development rate and wing length also
234 included jars (within tray) as an additional nested random effect.

235 Third, we constructed models that replaced land use and season (the design variables)
236 with temperature and humidity (the putative proximate drivers of variation mosquito dynamics).
237 To begin our analyses of the effects of microclimate on mosquito traits, we first explored the
238 correlations among the microclimate variables to avoid multicollinearity. Based on correlation
239 analyses, we included only T_{min}, T_{max}, and R_{min} in our models since they were of particular
240 biological importance to us and they were not strongly correlated to each other (Appendix S1:
241 Figure S2). We analyzed effects of the microclimate variables using GLMMs and the same
242 distributions and link functions for each response variable as described above, although in this
243 case the predictors were continuous rather than categorical.

244 Finally, to compare the use of the microclimate variables vs. season and land use for each
245 response variable, we ran three models – *null*, *base*, and *microclimate* – and used the corrected
246 Akaike Information Criterion (AIC_c) to evaluate their relative performance. The *null model* only
247 included density treatment as a fixed effect (e.g., it lacked land use and season). The *base model*
248 incorporated the original design factors (season, land-use type, density, and their interactions) as
249 fixed effects.. The *microclimate model* replaced the effects of land use and season with the
250 microclimate variables, and therefore included the fixed effects of density, along with the main
251 effects of T_{min}, T_{max}, R_{min} and their interactions with density. For each response variable, all
252 three models included the effect of density and an identical random effect structure, but they
253 varied in how they incorporated environmental variables. Microclimate variables experienced by
254 larvae in a jar were characterized by the mean environment experienced by those larvae as

255 determined by their distribution of emergence dates (i.e., for each larvae we calculated the mean
256 experienced microclimate per larvae from day 0 to its day of emergence and then averaged these
257 means across all females that emerged from the jar). In 12 cases in the fall at high density, no
258 females emerged from a jar, so the microclimate variables for those jars were imputed based on
259 emergence patterns from other jars at that site, season and density. Two sites did not have any
260 females emerge (one rural site and one suburban site); therefore, we used the emergence patterns
261 from other jars at the two other sites of the same land-use type, season, and density.

262 All analyses were conducted in R 4.5.1 (R Core Team, 2025). Code to run analyses and
263 create figures can be found on Figshare (Solano, 2026). Models were fit using the *glmmTMB*
264 package (Brooks et al., 2017), and compared by AIC_c using the *bblme* package (Bolker and R
265 Development Core Team, 2023). Type III analysis of variance (ANOVA) was conducted for all
266 models using the *car* package (Fox et al., 2024). The *emmeans* package was used to obtain
267 means and confidence intervals after adjusting for random effects (Lenth et al., 2025). We
268 assessed model fit using the scaled residuals from the *DHARMa* package (Hartig et al., 2024).
269

270 **RESULTS**

271 *Microclimate*

272 Five of the 54 aquatic temperature loggers failed (three in the summer and two in the
273 fall), and 36 of the 108 terrestrial loggers failed or were stolen (16 in the summer and 20 in the
274 fall). Despite these losses, failure rates were roughly equivalent across sites and microclimate
275 variation across season and land use types was well represented by the remaining loggers. Water
276 temperature (i.e., mean daily T_{min}, T_{mean}, and T_{max}) and relative humidity (mean daily R_{min}, R_{mean},
277 and R_{max}) metrics all varied significantly with season, land use, and their interaction (Appendix

278 S1: Figure S3, Tables S1 and S2), demonstrating that land use and season captured significant
279 spatiotemporal variation in mosquito-relevant microclimates.
280
281 *Emergence*
282 Of the 5670 first instar *Ae. albopictus* placed into the field during each season, a total of
283 3156 mosquitoes (56% of the starting population) successfully emerged as adults during the
284 summer while only 1233 mosquitoes (38%) emerged in the fall (emergence is equivalent to
285 survival through the larval and pupal stages). Assuming that the initial frequency of females was
286 50%, there were significant main effects of season and conspecific density on the proportion of
287 female first instar larvae that survived to emergence, with greater survival in the low-density
288 treatment relative to medium- and high- density treatments (Figure 1a, Appendix S1: Table S3).
289 There also were interactions between season and density (Figure 1a) as well as land-use type and
290 density (Appendix S1: Figure S4, Table S3). For example, there was little difference in the
291 survival of female juveniles in the low-density treatments in the summer and fall; however, the
292 negative effects of increasing conspecific density on juvenile survival was much greater in the
293 fall (Figure 1a). Further, female juveniles at low conspecific densities survived best in suburban
294 sites, while those at intermediate densities survived best in urban sites and those at high densities
295 survived best in rural sites (Appendix S1: Figure S4).
296 Some of these effects likely arose through the influence of the microclimate variation
297 across land use and season. For example, the microclimate model substantially outperformed the
298 null model ($\Delta AIC_c = 393$; Table 1), with the probability of female emergence (juvenile survival)
299 decreasing with increasing density and increasing with increasing temperature (T_{min}) and relative
300 humidity (R_{min}). In addition to these main effects, we observed interactions of both microclimate

301 variables with density (Figure 2a): as density increased, the positive effect of temperature (i.e.,
302 T_{min}) and relative humidity (R_{min}) increased in magnitude (Figure 2a). However, the base model
303 outperformed the microclimate model ($\Delta AIC_c = 6.3$; Table 1), suggesting that the temperature
304 and humidity variables did not capture as much of the spatiotemporal drivers of juvenile survival
305 as did the original design variables (land use and season).
306
307 *Female Development Time*
308 Both season and conspecific larval density significantly affected the average amount of
309 time required for adult female emergence (Table 1, Appendix S1: Table S3), with females on
310 average developing more quickly in the summer (day 9 mean emergence date) relative to the fall
311 (day 13 mean emergence date) and in the low-density treatment (Figure 1b, Appendix S1: Figure
312 S4b). However, a significant interaction between season and larval density demonstrates that the
313 main effect of density is being driven by the relationship between mean female development
314 time (time to emergence) and larval density in the summer (Appendix S1: Figure S4, Table S3).
315 Females reared in the low-density treatment were the fastest to develop in the summer but were
316 the slowest to develop in the fall (Figure 1b).
317 These effects on development time were better predicted using the microclimate variables
318 ($\Delta AIC_c > 30$ for the microclimate model compared to both the null and base models).
319 Development time decreased with increasing daily T_{min}, and this effect was strongest for
320 mosquitoes developing in low density environments (Figure 2b). In contrast to the other life
321 history parameters, there was little evidence to suggest a role of relative humidity on
322 development time (Table 1).
323

Wing Length

We observed significant main effects of season and density on female wing length (Appendix S1: Figure S4, Table S3). 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 1c, Appendix S1: Figure S4). The observed variation in wing length was better predicted by the microclimate model compared to the null or based models (Table 1: $\Delta AIC_c > 18$). Increased temperature (reflected in both T_{min} and T_{max}) increased (Figure 2c, Table 1). Additionally, we observed a significant interaction between density and daily R_{min}, with increases in relative humidity resulting in increased wing size at low and medium densities but not at high density (Figure 2c, Table 1).

Estimated 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 estimated per capita population growth rate, r' (Equation 1, Figure 1d, Appendix S1: Table S3). In general, the estimated per capita growth rate was greater in the summer relative to the fall and was greater at low densities than at high densities (Figure 1d). A significant interaction between season and density was observed due to greater declines in the estimated per capita growth rate with increasing conspecific density in the fall relative to the summer (Figure 1d, Appendix S1: Tables S3). 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 1d, Appendix S1: Figure S4). These effects may have arisen through the effects of T_{min} and R_{min}, both of which were positively associated with increased per capita growth (Figure 2d, Table 1), although both

347 microclimate variables also exhibited significant interactions with density. For example, the
348 effect of increasing T_{min} was greater on larvae living at high densities and more muted for those
349 living at lower densities (Figure 2d).
350
351 *Effects of microclimate on mosquito performance*
352 In presenting our results, we initially emphasized the effects of our base model because
353 our study was designed with respect to land use and season. However, for two of the four
354 response variables (development time and wing length), the model based on microclimate
355 performed better than the base model by a considerable degree (e.g., $\Delta AIC_c > 18$: Table 1), and
356 the microclimate and base models performed similarly for per capita population growth rate
357 ($\Delta AIC_c=1.1$). In contrast, the base model performed better than the microclimate model for
358 emergence ($\Delta AIC_c=6.3$). The null model, which excluded abiotic variables, always performed
359 the poorest (Table 1; $\Delta AIC_c > 80$). In general, the microclimate models showed that mosquito
360 performance increased with increasing warming temperature (T_{min} or T_{max}) and increasing
361 minimum relative humidity (R_{min}), although the magnitude of these effects often depended on
362 density (Table 1, Figure 2). Thus, the microclimate effects captured the observed variation in
363 mosquito traits as effectively as season and land use and further highlighted the interactions of
364 biotic (density) and with abiotic (microclimate) variables.
365
366 **DISCUSSION**
367 To date, the majority of studies investigating the effects of various abiotic and biotic
368 factors on mosquito fitness, population dynamics, vector competence, and transmission potential
369 have explored the effect of varying a single environmental variable such as temperature (e.g.,

370 Agyekum et al., 2022; Liu-Helmersson et al., 2014; Monteiro et al., 2007), conspecific density
371 (e.g., Hawley, 1985; Reiskind and Lounibos, 2009; Sauers et al., 2022), or food resources (e.g.,
372 Levi et al., 2014; Telang and Wells, 2004). Further, these studies are often conducted in
373 laboratory environments under relatively constant conditions. However, mosquito populations in
374 the field will be subject to the effects of many different environmental variables that fluctuate
375 diurnally and seasonally and likely interact. Previous work in the *Ae. albopictus* system suggests
376 that subtle variation in microclimate in the field can translate into significant variation in
377 mosquito life history traits, population dynamics, and arbovirus transmission potential (Evans et
378 al., 2018; 2019; Murdock et al., 2017; Wimberly et al., 2020). Our experimental study builds on
379 these results by showing that season and land use (via their effects on temperature and relative
380 humidity) can interact with variation in key biotic variables (conspecific density) to affect
381 metrics of mosquito fitness. The results from this study have broad implications for our
382 understanding and ability to predict the effects of current and future environmental variation on
383 mosquito fitness and population dynamics, and the environmental suitability for mosquito-borne
384 disease transmission.
385
386 **Land use affects microclimate with subtle effects on metrics of mosquito fitness and**
387 **population dynamics**
388 Like previous studies conducted in the same region (Athens, Georgia) (Evans et al., 2018;
389 Murdock et al., 2017; Wimberly et al., 2020), urban sites had higher minimum temperatures than
390 rural sites and were drier than non-urban land use types. This is consistent with the Urban Heat
391 Island (UHI) effect, where nighttime temperatures (analogous to T_{min}) are 1-2.5ºC higher
392 (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, hydrologically permeable land covers re-emit less heat (e.g., forests, shrublands, wetlands), have higher evapotranspiration rates, and often provide ample shading resulting in lower temperatures and higher relative humidity than urban areas (Bowler et al., 2010; Brown et al., 2023; Huang et al., 1987; Jabbar et al., 2023). Despite the variation in microclimate observed across land use, this variation was small (Appendix S1: Figure S2) and for the most part did not significantly affect components of mosquito fitness or estimated per capita growth rates within a given season (Appendix S1: Figure S4). This is not necessarily surprising as 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 (Oke, 1973; Peng et al., 2011; Zhou et al., 2017). We did observe effects of land use on the relationship between conspecific density and juvenile mosquito survival (i.e., emergence), suggesting the effects of intra-specific competition could potentially be modified across an urban gradient. However, while significant, the effects of the interaction between land use and density on metrics of mosquito fitness were relatively small (Appendix S1: Figure S4), likely because variation in microclimate (e.g., T_{min} and R_{min}) was not large across Athens within a given season (Appendix S1: Figure S3).

Seasonal variation in microclimate affects 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. As a result, we observed strong seasonal effects on juvenile survival, the juvenile

development time, adult body size, and estimated per capita growth rates. Mosquitoes are ectothermic, and their 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 *Ae. albopictus* juvenile survival and mosquito development rates are maximized at ~25ºC and ~35ºC, respectively (Mordecai et al., 2017). Thus, it is unsurprising that we generally saw faster juvenile development times and increased adult female emergence in the warmer summer field trial (with average temperatures ranging from 25-25.7ºC) than the cooler fall (with temperatures ranging from 21.1-21.6ºC) (Figures 1, 2, Appendix S1: Figure S3).

Seasonal variation in microclimate shapes the relationship between intra-specific competition and metrics of mosquito fitness and population dynamics

In general, our results support previous research that shows decreasing temperature or increasing conspecific larval densities result in decreased juvenile survival, smaller adults upon emergence, and decreased per capita growth rates (Lord, 1998; Sauers et al., 2022; Walker et al., 1991). However, our results also highlight 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, we observed a negative effect of increasing conspecific larval density on juvenile survival (i.e., the proportion of adult females emerging). However, this trend was much stronger during the fall trial (Figure 1a). This could be due to a hurricane that occurred at the beginning of the fall trial (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

439 temperatures in the fall were <15ºC for three consecutive days. These temperatures, which can
440 result in high *Ae. albopictus* larval and pupal mortality (reviewed in Waldock et al., 2013),
441 combined with the additional stress of developing under high intra-specific competition, could
442 synergistically increase juvenile mortality. This interactive effect of temperature and density on
443 juvenile survival is further supported by our microclimate analyses, which demonstrated that
444 effects of cooler temperatures were more severe at higher densities (Figure 2a).

445 Contrary to our initial hypothesis and many prior studies (e.g., Ezeakacha and Yee, 2019;
446 Sauers et al., 2022), larvae appeared to develop faster with higher conspecific densities in the
447 cooler fall (Figures 1b, 2b). Several factors could explain this result. First, the timing of
448 juvenile mortality is known to affect the amount of resources available to the surviving larvae
449 (Evans et al., 2022; McIntire and Juliano, 2018; Neale and Juliano, 2019). If mortality of early
450 instar larvae was elevated by cooler fall temperatures and high intra-specific densities, the
451 surviving larvae would have experienced reduced intra-specific competition and increased access
452 to food resources, both of which allow larvae to develop faster (Evans et al., 2022; Neale and
453 Juliano, 2019; Teng and Apperson, 2000). Stage-specific overcompensation and the Hydra
454 effect have been detected in *Ae. albopictus* when early larval mortality occurred (McIntire and
455 Juliano, 2018). Another possible explanation may be survival bias or frailty. Very few females
456 survived in the fall from the medium-density treatment (7.2%) and even fewer from the high-
457 density treatment (1.2%). If the few individuals that did survive were of higher quality (e.g.,
458 competitive ability), then their subsequent development time might be expected to be greater
459 than observed in the low-density treatment. A third explanation could be that larvae facultatively
460 alter their development rate based on a stressor such as low temperatures and/or low quantity of
461 nutritional resources due to increased competition. While not demonstrated before in *Ae.*

462 *albopictus*, previous work in the *Ae. triseriatus* and *Ae. communis* systems have shown larvae
463 can speed up development rates in environments subject to evaporation (Chodorowski, 1969;
464 Juliano and Stoffregen, 1994).

465 Our results contrast with the extensive literature documenting relationships between
466 mosquito wing size (i.e. size upon emergence) with variation in conspecific density (Alto et al.,
467 2005; Evans et al., 2021; Lord, 1998) or temperature (Kingsolver and Huey, 2008; Rueda et al.,
468 1990). Warmer temperatures have been shown to typically result in smaller wings and body size
469 – "hotter is smaller"– and cooler temperatures typically result in larger wings and body size –
470 "cooler is bigger" hypothesis (Alto and Juliano, 2001; Ezeakacha and Yee, 2019; Kingsolver and
471 Huey, 2008; Rueda et al., 1990). Our results contradict these hypotheses. Instead, we
472 consistently found that wing length increased with an increase in the minimum temperature,
473 although the slope of this relationship was less for mosquitoes developing at high conspecific
474 densities (Figure 2c). Only a few studies have demonstrated smaller wing lengths at cooler
475 temperatures (Lounibos et al., 2002; Reiskind and Zarrabi, 2012). This result could be attributed
476 to slower microbial growth in the fall and mosquito anabolism being less efficient (due to cooler
477 temperatures) (Reiskind and Janairo, 2018). Thus, with females in the warmer summer
478 experiencing more microbially rich habitats regardless of larval density treatment as well as a
479 greater capacity to incorporate nutrients into tissue, facilitating more rapid adult female
480 emergence and larger body sizes in the summer. Finally, like development time, mosquito size
481 upon emergence converges at high densities regardless of season, suggesting that high amounts
482 of intra-specific competition could eventually outweigh the influence of variation in abiotic
483 factors such as temperature and relative humidity.

484 In general, while growth rates declined as larval conspecific densities increased (see also:
485 Edgerly and Livdahl, 1992; Lord, 1998; Reiskind and Lounibos, 2009) and increased at higher
486 temperatures (Alto and Juliano, 2001), we found the estimated per capita growth rates at higher
487 temperatures (e.g., in the summer) were less affected by intra-specific competition than at lower
488 temperatures (e.g., in the fall). This intriguing result suggests that mosquito populations might
489 be less strongly regulated by density-dependent processes as temperatures increase on the
490 landscape Most studies to date have considered the effects of temperature or conspecific density
491 on growth rates in isolation, without considering the possible interaction. Those that have
492 considered the interaction between temperature and intra-specific competition on per capita
493 growth rates have generally found intra-specific competition to intensify as temperatures warm
494 (Evans et al., 2021; Teng and Apperson, 2000), which is not what we observed in this study.
495 However, we did not account for possible effects of temperature and relative humidity on adult
496 longevity or on size-specific fecundity, which could potentially modify our estimates of the per
497 capita population growth. Given the interest in vector-pathogen dynamics in response to global
498 change, interactions between temperature and density dependence warrant further investigation.
499

500 **CONCLUSION**

501 This study has several important implications. It is generally thought that biotic factors
502 (e.g., intra-specific and inter-specific competition, quality and quantity of habitat, biological
503 enemies) will vary more at fine spatial scales, driving local variation in organismal fitness and
504 population dynamics, whereas abiotic factors will dominate regional effects because they vary at
505 larger spatial scales (McGill, 2010; Russell et al., 2006; Wiens 1989). Our study demonstrates
506 that while variation in biotic factors (e.g., intra-specific competition) and abiotic factors (e.g.,

temperature and relative humidity) are both influential, they interact to affect mosquito population dynamics at local scales. Ignoring the interaction between variation in biotic and abiotic variables could reduce the accuracy and precision of models (no matter their scale) used to predict species distributions and population dynamics, as well as the transmission dynamics of mosquito-borne pathogens. By not accounting for these interactions, mismatches in model predictions could result in unexpected spatial and temporal variation in species densities and host-parasite interactions, especially for organisms with complex life cycles like mosquitoes (Moller-Jacobs et al., 2014; Shapiro et al., 2016). While our results demonstrate the critical role of abiotic factors like temperature and relative humidity that are linked to variation in land use and season, future work should incorporate other aspects of land use and seasonality, such as habitat quality that arises through variation in detrital inputs and precipitation, which will affect the strength of intraspecific competition and potentially the interaction with abiotic variables.

CONTRIBUTIONS

CCM designed the experiment. NS, ECH, CWH, PMN, AMS, and JWW conducted fieldwork. NS led, and GRJ assisted with 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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542

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Table 1. Results from Generalized Linear Mixed Effects Models testing the effects of larval density treatment (30 = low, 60 = medium, 120 = high), land-use type (rural, suburban, urban), and/or microclimate (T_{min}, T_{max}, R_{min}) on mosquito traits across summer & fall seasons. The mosquito traits were analyzed using three model structures: (1) the Null model – main effect of larval density (but no other fixed effect); (2) the Base model – main effects of season, land use, and larval density and their interactions; and (3) the Microclimate model – main effects of larval density, water temperature (T_{min}, T_{max}) and relative humidity (R_{min}) and the interactions between density and T_{min}, T_{max}, and R_{min}. Experimental site and tray (nested in site) were included as random factors in all models, and jar (nested in tray) was included for development time and wing length models. ΔAIC_c values, degrees of freedom, and statistically significant fixed effects are shown. Significance is denoted as $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***). The best model(s) based on $\Delta AIC_c \leq 2$ is indicated in bold.

Response Variable	Model	ΔAICc	df	Significant Predictors
Emergence	Null	399.3	5	Density***
	Base	**0**	**20**	**Density***, Season:Density***, Landuse:Density***
	Microclimate	6.3	14	Density***, Density:Tmin***, Density:Tmax*, Density:Rmin***
Development Time	Null	92	7	Density*
	Base	30.5	22	Season***, Density**, Season:Density*,
	Microclimate	**0**	**16**	**Density*, Tmin***, Density:Tmin***
Wing Length	Null	80.4	7	Density***
	Base	18.7	22	Season***, Density***
	Microclimate	**0**	**16**	**Density***, Tmin***, Tmax*, Rmin***, Density:Rmin***
Intrinsic Growth Rate (r')	Null	215.4	6	Density***
	Base	**0**	**21**	**Season***, Density***, Season:Density***
	Microclimate	**1.1**	**15**	**Density***, Tmin***, Rmin*, Density:Tmin***, Density:Rmin***

871 **FIGURE CAPTIONS**

872 **Figure 1.** Results of mixed-effects model for *Ae. albopictus:* (**a**) the proportion of female larvae
873 that emerged (i.e., survival probability), (**b**) female development time (days), (**c**) female wing
874 length (mm), and (**d**) estimated per capita intrinsic growth rate, r' (day^{-1}), for each density
875 treatment (low = 30 larvae, medium = 60 larvae, high = 120 larvae) across two seasons (summer
876 = red, fall = orange). Horizonal and vertical lines give the mean +/- 95% CI, and the dots
877 indicate raw values [based on females in (b), (c), and jars for (a), (d)] adjusted for random
878 effects.

879

880 **Figure 2.** Results of mixed-effects model for *Ae. albopictus:* (**a**) the proportion of female larvae
881 that emerged (i.e., survival probability), (**b**) female development time (days), (**c**) female wing
882 length (mm), and (**d**) estimated per capita growth rate, r' (day^{-1}), for each of the three density
883 treatments (low = 30 larvae, medium = 60 larvae, high = 120 larvae), as influenced by minimum
884 temperature (T_{min}, ºC), and minimum relative humidity (R_{min}, %). Dots indicate raw values
885 [based on females in (b), (c), and jars for (a), (d)] adjusted for random effects. Black lines give
886 the predicted effects of T_{min} for each density treatment.

Figure 1

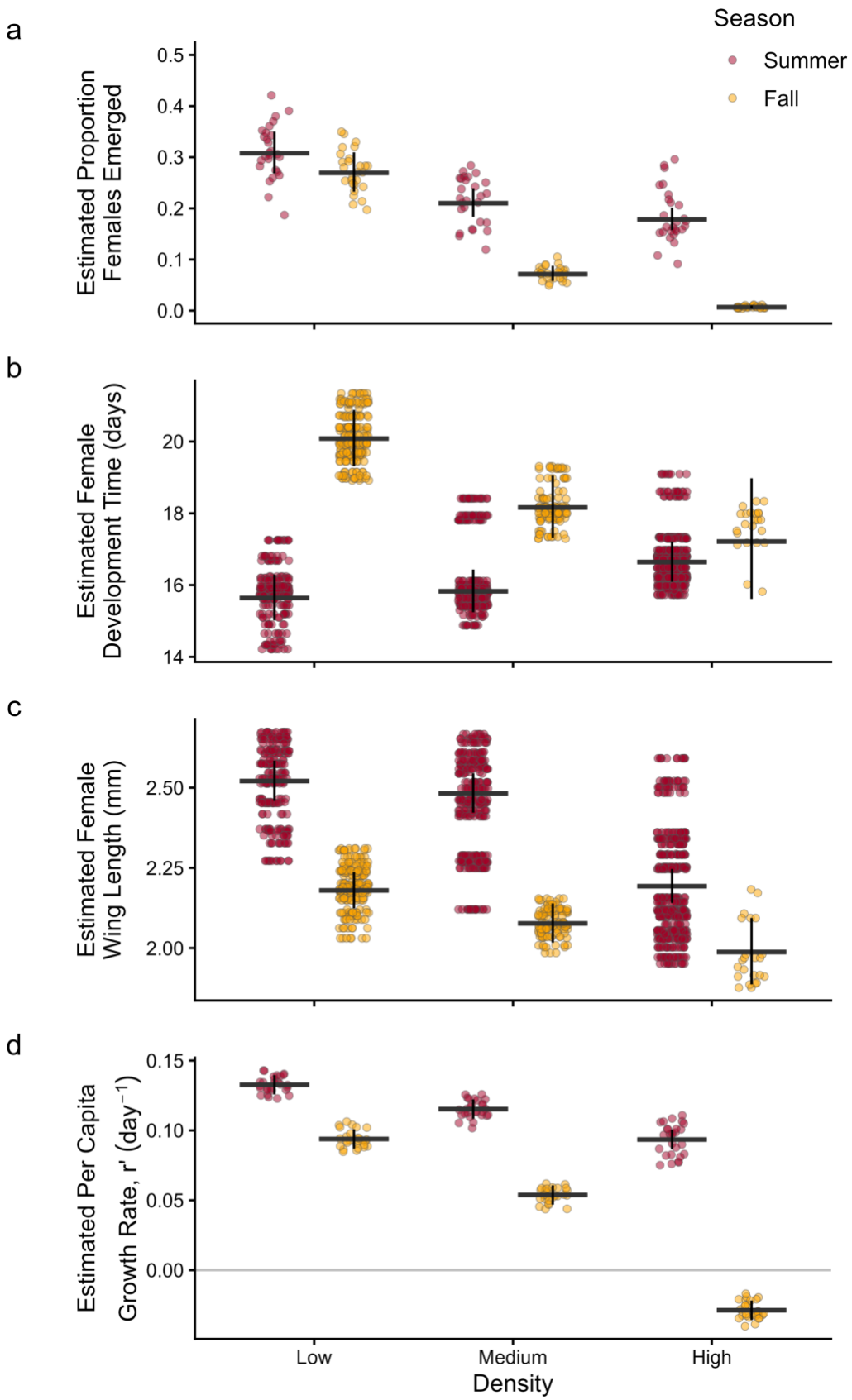
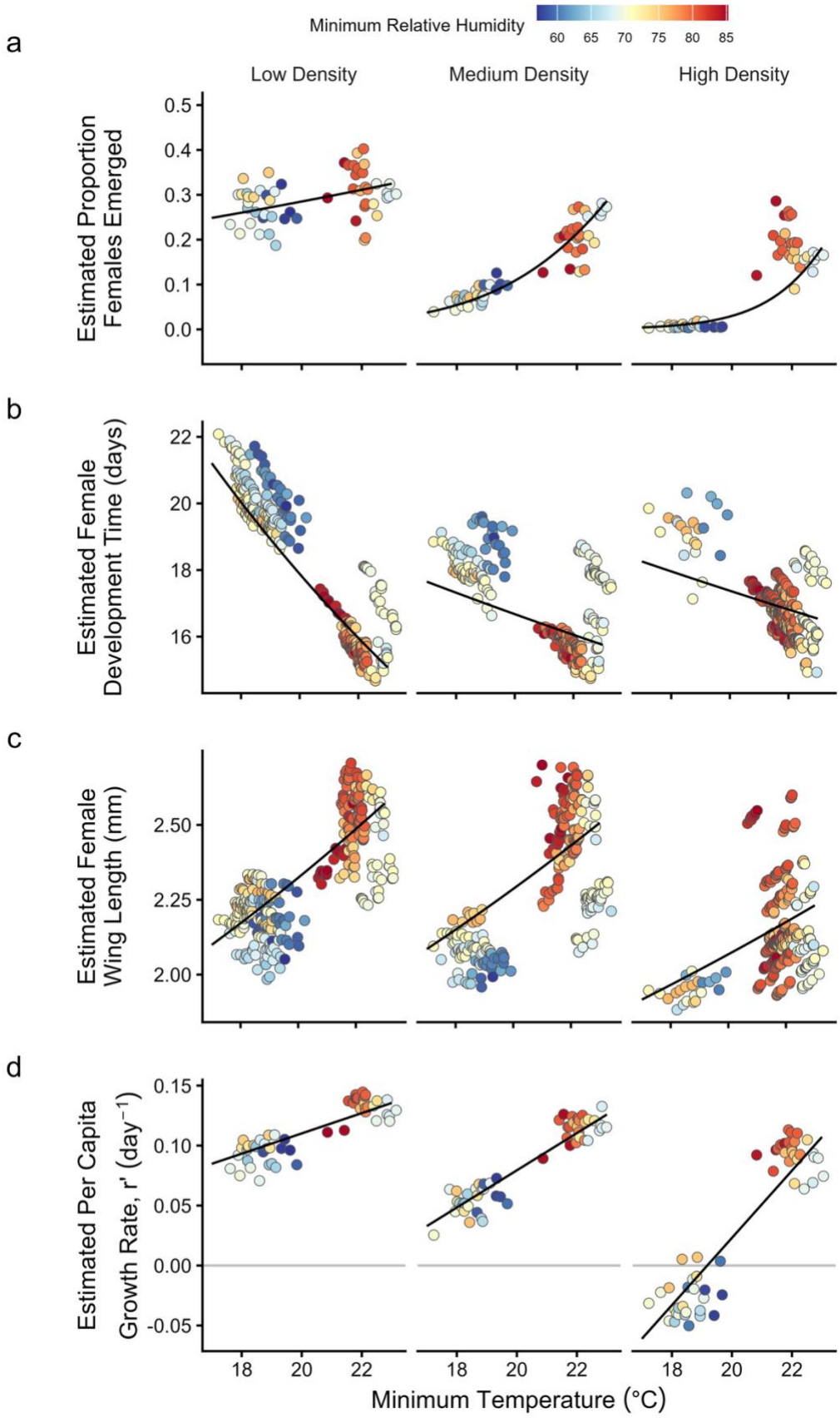

a
Season
Summer
Fall
Estimated Proportion Females Emerged
0.5
0.4
0.3
0.2
0.1
0.0
b
Estimated Female Development Time (days)
20
18
16
14
c
Estimated Female Wing Length (mm)
2.50
2.25
2.00
d
Estimated Per Capita Growth Rate, r' (day⁻¹)
0.15
0.10
0.05
0.00
Low
Medium
High
Density

Figure 2


Minimum Relative Humidity
60
65
70
75
80
85
a
Low Density
Medium Density
High Density
Estimated Proportion Females Emerged
0.5
0.4
0.3
0.2
0.1
0.0
b
Estimated Female Development Time (days)
22
20
18
16
c
Estimated Female Wing Length (mm)
2.50
2.25
2.00
d
Estimated Per Capita Growth Rate, r' (day⁻¹)
0.15
0.10
0.05
0.00
-0.05
18
20
22
Minimum Temperature (°C)