

Thermal tolerance of *Aedes aegypti* mosquito eggs is associated with urban adaptation and human interactions

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ABSTRACT

Climate change will profoundly affect mosquito distributions and their ability to serve as vectors for disease, specifically with the anticipated increase in heat waves. The rising temperature and frequent heat waves can accelerate mosquito life cycles, facilitating higher disease transmission. Conversely, higher temperatures could increase mosquito mortality as a negative consequence. Warmer temperatures are associated with urbanized areas, suggesting a need for anthropophilic mosquitoes to adapt to be more hardy to heat stress. Mosquito eggs provide an opportunity to study the biological impact of climate warming as this stage is stationary and must tolerate temperatures at the site of female oviposition. As such, egg thermotolerance is critical for survival in a specific habitat. In nature, *Aedes* mosquitoes exhibit different behavioral phenotypes, where specific populations prefer depositing eggs in tree holes and prefer feeding non-human vertebrates. In contrast, others, particularly human-biting specialists, favor laying eggs in artificial containers near human dwellings. This study examined the thermotolerance of eggs, along with larval and adult stages, for *Aedes aegypti* lineages associated with known ancestry and shifts in their host preferences. Mosquitoes collected from areas with high human density showed increased egg viability following high-temperature stress, and a similar, yet more muted effect was noted in larvae. Unlike eggs and larvae, thermal tolerance among adults showed no significant correlation based on the area of collection or human-association. This study underscores that urbanization is a major driver of egg thermotolerance, highlighting the egg stage is likely critical to mosquito survival when associated with humans and needs to be accounted for when predicting future mosquito distribution.

1. Introduction

Mosquitoes pose a public health threat due to their ability to transmit a wide range of pathogens. Dengue fever, a disease caused by a virus transmitted by *Aedes* mosquitoes, has more than quadrupled over the last decade (Bhatt et al., 2013). Zika virus has emerged rapidly since

2007, giving rise to epidemics in regions such as the South Pacific and Americas (Weaver et al., 2016). A primary vector for dengue and Zika is the yellow fever mosquito, *Aedes aegypti* (Bhatt et al., 2013; Brady and Hay, 2020). The yellow fever mosquito is a global invasive warm-weather species and thrives in urban and rural habitats across the Asian, African, and American tropics (Christophers, 1960). The globally

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distributed *Ae. aegypti* mosquitoes, mainly the 'domestic' form, *Ae. aegypti aegypti* ('Aaa'), have shifted to feed on humans (McBride, 2016). However, in the African landscape, the domestic form coexists with its wild counterpart, *Ae. aegypti formosus* ('Aaf'), a generalist that feeds on a wider range of hosts (McBride, 2016; Rose et al., 2023). Over the last few decades, *Ae. aegypti*, has spread its reach to new territories, posing a growing threat to global health (Brady and Hay, 2020).

Behavioral shifts, including host preferences and oviposition behavior, have been documented for *Ae. aegypti* (Rose et al., 2020). These behavioral shifts can be partially attributed to anthropophagy and breeding in human-associated environments (Rose et al., 2023). Multifaceted influences, such as changes in land use patterns (Swei et al., 2020), alteration of ecosystems (Vora, 2008), deforestation (Ortiz et al., 2021), urbanization (Melchiorri et al., 2018), and climate change (Reinmann et al., 2016) have increased interactions between mosquitoes and humans (Chala and Hamde, 2021). Densely populated human areas and unplanned urbanization (Kolimenakis et al., 2021), in conjunction with the heat rise due to anthropogenic effects (Mathieu and Karmali, 2016), likely promote a shift towards increased anthropophagy in mosquitoes. Moreover, urban sprawl creates ideal breeding conditions for mosquitoes by generating water-holding structures, such as discarded containers, potholes, clogged drains, and poorly maintained infrastructure, which can be exploited if mosquitoes can tolerate the increase temperature of these artificial structures (Phelan et al., 2015; Richardson et al., 2013). Additionally, dry conditions force mosquitoes to seek out water sources near human residences for breeding and hydration (Holmes and Benoit, 2019), further amplifying their interactions with humans and reliance on human-associated habitats (Rose et al., 2023). This combination of factors emphasizes the critical need to understand mosquito biology, particularly in the context of the rural-to-urban transition, the increasing preference for humans in mosquito behavior, and thermal tolerance.

To understand mosquito biology in different contexts characterized by variations in human density, a useful starting point is the egg stage. The mosquito egg is crucial for *Aedes*, as embryos can remain viable for extended periods under unfavorable conditions until water is available (Sota and Mogi, 1992). This resilience is tied to their evolutionary behavior of laying eggs on wet surfaces near water, where hatching is triggered upon eggs becoming submerged in water (Clements, 1992). Since mosquito eggs are immobile, they are particularly vulnerable to shifting environmental conditions. However, *Aedes* eggs show resilience to abiotic stress due to egg cuticle properties that allow prolonged embryo and pharate larva survival (Farnesi et al., 2017; Rezende et al., 2016; Vargas et al., 2014). Among insects, temperature stress can negatively affect egg and larval survival, larval growth, and even subsequent adult stages (Ajayi et al., 2023; Bowler and Terblanche, 2008; Rocha et al., 2017; Potter et al., 2011). *Aedes* lay eggs in diverse habitats, ranging from natural tree holes to artificial containers like discarded tires, which often expose eggs to varying levels of thermal stress (Bennett et al., 2019; David et al., 2023).

Urban environments exacerbate thermal stress by amplifying heat (Hsu et al., 2021; Tuholske et al., 2021), creating distinct thermal conditions compared to buffered natural habitats. This is even more drastic for artificial surfaces that will absorb heat more readily than natural materials (Loveday et al., 2019). This variation in habitat and temperature exposure can induce different levels of heat shock across life cycle stages, resulting in distinct biological responses in eggs, larvae, and adults (Hug et al., 2024; Couret and Benedict, 2014; Richardson et al., 2013). Survival in urban environments likely requires differences in the thermal tolerance of mosquitoes across their developmental stages compared to those in rural areas. However, little is known about whether differences in the thermal tolerance of mosquito eggs, along with larvae and adults, are related to variations in human association. Here, we test the hypothesis that *Ae. aegypti* eggs, larvae, and adults from densely populated areas and with increased human preference have a higher thermal tolerance. We examined the entire scenario from

the perspective of mosquito specialization toward humans, considering multiple indices such as mosquito ancestral origin, host preference index, and human population density that are associated with a shift towards humans (Rose et al., 2020). We assessed the individual and combined effects of each factor and collectively defined as 'human specialization syndrome' for *Ae. aegypti*. Lastly, we analyzed several temperature-and precipitation-related bioclimatic variables (Fick and Hijmans, 2017) as potential predictors to better understand the regional drivers of mosquito egg thermal tolerance.

2. Materials and methods

2.1. Mosquito collection and maintenance

Aedes aegypti mosquito colonies were established from eggs collected across western Africa using seed germination papers in ovitraps (Rose et al., 2020). For our study, colonies from five locations in Senegal (PK10, Mindin, Kedougou, Ngoye, and Thies) and two in Ghana (Kintampo and Kumasi) were selected (Table 1; Supplementary Table S1). One lab adapted strain (Gainesville) was used for baseline studies. All mosquito populations were collected between 2017 and 2018, with experiments conducted throughout 2021 to 2023. The larval experiments were conducted in 2024. The mosquito larvae were reared in plastic pans measuring 30.5 cm × 7.5 cm × 5 cm with a consistent stocking density of approximately 200 larvae per pan. The rearing medium consisted of deionized (DI) water, finely ground fish food (Tetra-min, Goldfish Flakes), and yeast extract (Difco, BD 210929). Mosquitoes were maintained in a climate-controlled facility (27 ± 2 °C; 65–70 % RH; 16-8-hours (h) light-dark cycle). The adults were kept in 30.5 cm × 30.5 cm × 30.5 cm mesh cages with access to water and 10 % sucrose solution *ad libitum*. Females (12–14 days old) were routinely blood-fed on a human host (University of Cincinnati IRB, 2021-0971) to maintain the populations, and eggs were collected in brown hardwound towels for thermal stress studies. The thermal tolerance of eggs, larvae and adults were assessed for each population (described below). A summary of the statistical analyses and modeling results is presented in Supplementary Fig. 1.

2.2. Temperature stress experimental procedure

Adults, larvae and eggs were subjected to controlled heat stress for 2 h and 6 h to assess their thermal tolerance. Experiments on seven *Ae. aegypti* populations began with preliminary studies on a laboratory-maintained Florida population [Benzon Research - Gainesville (GAI)] to establish baseline thermal parameters before analyzing African populations (Supplementary Figs. 2–4). Experiments were conducted starting at a lower temperature (25 °C) and moving 4 °C higher until the survival ceased. In case of adult thermotolerance, when examined initially between two lines collected from habitats with high differences in human population density (urban-THI and rural-MIN) to check deviation at the lower levels of temperature stress (25 °C and 29 °C), none was found; we decided to carry on with high levels of temperature stress. Before the experiment, all the biological samples were kept at the climate-controlled facility (described previously). To ensure a swift acclimation process, an additional 30 min (mins) temperature ramp procedure was implemented. The initial temperature in the heat bath was set to 29 °C, and the temperature ramp was carried out manually using a gradual transition process. Over the first 15 min, the temperature was increased (or decreased) to the midpoint between 29 °C and the target temperature. Subsequently, for the next 15 min, the temperature was further raised (or lowered) from the midpoint average to attain the target temperature. A digital thermometer (Omega™) was used to confirm the temperature during each assay. All the trials were consistently conducted at approximately the same circadian window (9.00–10.30 a.m. local time), and post-experiment, the biological replicates were moved to the climate-controlled rearing conditions for

Table 1

Information on mosquito populations - Information about the country and habitat of origin for *Aedes aegypti* populations, highlighting ecological (human population density), behavioral (host preference), genomic (ancestry), and derived “human specialization syndrome” index.

Name	Country	Habitat	Human Population Density km ²	Preference	Ancestry	Human Specialization Syndrome Index (Density + Weighted Sum of Preference + Ancestry)	BEI Resource/Other information
PK10 (PKT)	Senegal	Rural	12.29111819	-0.33436322	0.001113444	0.2769402	Rose et al. (2020)
Mindin (MIN)	Senegal	Rural	53.60547457	-0.477038214	0.017289316	0.3798573	Rose et al. (2020)
Kintampo (KIN)	Ghana	Rural	37.22657519	-0.581702248	0.031685947	0.365912	Rose et al. (2020)
Kedougou (KED)	Senegal	Rural	12.24971172	-0.522748506	0.008563421	0.3651815	Rose et al. (2020)
Ngoye (NGO)	Senegal	Urban	232.6737489	0.693053879	0.3738158	0.7917581	Rose et al. (2020)
Kumasi (KUM)	Ghana	Urban	2264.553235	-0.300140692	0.067482211	0.6279402	Rose et al. (2020)
Thies (THI)	Senegal	Urban	421.1161584	0.396234544	0.221672909	0.6934605	Rose et al. (2020)
Gainesville, Florida (GAI)	USA	Urban					Benzon Research/ Lab strain

recovery.

Adult mosquitoes (males and females) were exposed to a range of temperatures (33 ± 1 , 37 ± 1 , and 41 ± 1 °C). Eight ($n = 8$) 7–10-day old adult mosquitoes, separated by sex, were held in 50 mL perforated empty plastic vials to allow for airflow. The experiments were conducted with five replicate vials for each sex, temperature, and population. Vials were partially submerged in a water bath (ThermoFisher Scientific) and covered with polystyrene foam to maintain constant temperature throughout the experiment. Fourth instar larvae were used to assess larval thermal tolerance under 25 ± 1 , 29 ± 1 , 33 ± 1 , 37 ± 1 , and 41 ± 1 °C. Ten larvae ($n = 10$) were placed in 15 mL sterile screw-cap tubes (ThermoFisher Scientific), each containing 10 mL of DI water. Each temperature-treatment condition was replicated across five tubes. The tubes were floated in a water bath during the experiment. After the temperature stress, 5 mL DI water mixed with fish food was pipetted into the tubes and the tubes were returned to rearing conditions for survival checks at later time points. For the egg thermal tolerance study, egg batches collected from each population cage were held under rearing conditions for two weeks to ensure proper embryonation (Maiga et al., 2017). Eggs ($n = 30$ –60 per replicate on oviposition paper from multiple females with 10–12 replicates per treatment) were stored in 8 mL size plastic vials (Thornton Plastics: 2.5Udbl) for temperature stress experiment. Heat stress (25 ± 1 , 29 ± 1 , 33 ± 1 , 37 ± 1 , 41 ± 1 , and 45 ± 1 °C) was generated using a digital dry bath (Thermo Scientific™). Plastic vials were placed in dry heat blocks and covered with insulated styrofoam to ensure a constant temperature. After the thermal stress experiment, the egg vials were filled with DI water and fish food once the temperature returned to 29 °C. Two days following the experiment, 80 % ethanol was used to preserve larvae and eggshells and the vials were stored at -20 °C until viability counts.

2.3. Temporal variation in population specific heat stress responses across mosquito life stages

Proportional survival assessments for adult mosquitoes (sex-specific) and larvae were conducted at an early time point (2 h) and at a later time point (24 h) following the thermal stress. Survival was measured by counting the number of mobile individuals, and the proportion of survival was calculated as the ratio of mobile individuals (adults or larvae) to the total sample size ($n = 8$ for adults; $n = 10$ for larvae). Egg proportional survival was determined as the ratio of hatched eggs to the total number of hatched and unhatched eggs. Hatched eggs were identified by the presence of a clean cut/break of the egg cap, indicating larval emergence (Sota and Mogi, 1992; Sota, 1993; Urbanski et al., 2010; Metz et al., 2023), where unhatched eggs looked intact with no presence of larval emergence. We also counted larval numbers in the experimental units, but we observed larval cannibalism and inconsistent larval presence across vials, leading us to exclude larva from our egg survival analysis. Differences in sex-specific adult- and larval survival rates at early and late time points and egg viability across populations

for each duration and intensity of temperature stress were analyzed using generalized linear regression models with the ‘glm’ function from the lme4 R package (Chambers and Hastie, 1992). Post-hoc comparisons to identify significant population level differences were performed using the ‘emmeans’ package (default - multiple comparison Tukey methods) in R (version 4.2.3), where $p < 0.05$ were considered statistically significant (Searle et al., 1980). Results from the regression models and post-hoc tests are presented in Supplementary Table S2 (adult survival), Table S3 (larval survival), and Table S4 (egg viability). The average survival rates of *Ae. aegypti* across different life stages (sex-specific adults, larvae, and eggs) were visualized for each population under various temperature-treatment combinations. Figures were produced using R 4.2.3 and edited with Inkscape 1.3.

2.4. Evaluation of mosquito thermal tolerance and key influential factors

To examine population-specific responses, the relationship between proportional survival rates at each life stage (sex-specific adults, larvae and eggs) - and temperature was analyzed using a ‘glm’ function (Proportional survival = $m \times \text{Temperature}(x) + c$) for each stress duration. The regression coefficients (slopes or “m”) for each mosquito collection site represent the thermotolerance at each life stage. To evaluate the thermotolerance, we tested two modeling approaches - 1) using only the slope (“m”) and 2) the full linear equation (“mx + c”), and both approaches yielded identical results; so, we proceeded with the slope-only model for subsequent analyses. Next, we investigated the relation of ecological, behavioral, ancestral, and bioclimatic factors (Fick and Hijmans, 2017; Rose et al., 2020) on the thermal tolerance of *Ae. aegypti* across different life stages - adults, larvae, and eggs (Table 1; Supplementary Table S1). Our initial analysis of survival differences among populations revealed distinct patterns of thermal tolerance across mosquito life stages. Due to the significant statistical differences observed in egg emergence, modeling was focused on understanding the factors associated with egg thermal tolerance.

Ecological indices, such as local human population density within a 20 km² buffer around collection sites, were used to assess mosquito adaptation to human habitats (NationsUnited, 2019). The collection of localities exhibited a high contrast in human population density, serving as a baseline for categorizing locations as urban (>200/km²) or rural (<50/km²) (Table 1). Behavioral indices captured shifts in host preference driven by human population density, while ancestry was traced through genetic variations linked to human specialization (Rose et al., 2020). Quantitative measures of ancestral genetic heritage values and olfactometer-based host preference values (Rose et al., 2020) were used in our analysis to evaluate their influence on thermotolerance across life stages. To further investigate the climatic factors shaping egg thermotolerance, we analyzed multiple WorldClim 2 bioclimatic variables of mosquito collection localities, including annual mean temperature, precipitation, and quarterly variations in temperature and precipitation (Supplementary Table 1) (Fick and Hijmans, 2017). These analyses

provide insights into the role of climate in driving mosquito egg thermotolerance in the context of human specialization. Each population was represented by a single value for each predictor tested, and we ensured a standardized approach to assess their influence on thermal tolerance using a generalized linear mixed-effects model (GLMM) with a binomial distribution and a logit link function ('glmer'). Egg hatching outcomes were categorized as binary: "success" (hatched eggs) and "failure" (unhatched eggs). Population was included as a random effect to account for variation among sampled populations. We first assessed the individual effects of ecological, behavioral, and ancestral factors on egg thermal tolerance. Significant predictors were then incorporated into a multivariate regression model to evaluate the combined influence of ecological pressures driving mosquito specialization toward human hosts. To capture this broader ecological context, we developed a "human specialization syndrome" index, integrating ecological, behavioral, and human population density factors that facilitate mosquito adaptation. Given the potential for multicollinearity, we integrated highly correlated variables into a single index by computing a weighted sum of their regression coefficients (Carrico et al., 2015). Specifically, human specialization syndrome refers to a suite of traits, including genetic background and behavioral shifts in mosquitoes, driven by exposure to high human population densities, that collectively enhance specialization on human hosts. Additionally, we examined both temperature- and precipitation-related bioclimatic variables individually to assess their influence on egg thermal tolerance. When significant associations were detected, we further analyzed these climate variables alongside the "human specialization syndrome" continuum to determine their combined effects. To ensure a robust predictor selection process, we employed two complementary approaches: AIC-based model selection and a correlation-based approach, ensuring that interdependent variables were not redundantly included in the analysis. Finally, we visualized the effects of each significant predictor by estimating the effects of 2- and 6-h-long temperature stresses on egg thermotolerance. The data were standardized using the 'scales' package (Wickham and Seidel, 2016), enabling direct comparisons and facilitating correlations with significant predictors identified in earlier analyses. These series of analyses allowed for a holistic understanding of how the predictors influence egg thermotolerance. Even though egg thermotolerance was the main goal, proportional survival for adult and larval thermotolerance were evaluated in a binomial way within 'glm' framework due to restrictions in sample size. The association between thermotolerance of the life-stage and host preference due to ancestry and human population density was measured.

3. Results

3.1. Population level variation in temperature tolerance of mosquito life stages

In general, survival rates of adult mosquitoes declined with increasing temperatures ($F_{1,838} = 1494, p < 2.2 \times 10^{-16}$) (Fig. 1, Supplementary Fig. 2), adult females showed increased thermal tolerance compared to adult males ($F_{1,838} = 44.88, p = 3.84 \times 10^{-11}$), and mortality increased with the longer elapsed treatment time and recovery period ($F_{1,838} = 38.33, p = 9.37 \times 10^{-10}$) (Fig. 1). We observed only a few significant differences in survival among the populations when compared to the same sex at each temperature treatment ($33 \pm 1, 37 \pm 1, \text{ and } 41 \pm 1 \text{ } ^\circ\text{C}$), although urban mosquitoes showed slightly better survival (Fig. 1; Supplementary Table S2). Of interest, there was a slight increase in heat tolerance for populations from urban areas (NGO, THI, and KUM), but this was not significant when compared to the more rural populations.

Larval survival was significantly reduced with an increase in both the severity ($F_{1,698} = 836.6; p < 2.2 \times 10^{-16}$) and duration ($F_{1,698} = 7.29; p = 0.009$) of heat stress (Fig. 2, Supplementary Fig. 3). The only notable difference was at $37 \text{ } ^\circ\text{C}$ and $41 \text{ } ^\circ\text{C}$, where urban populations exhibited higher thermotolerance than mosquitoes from rural areas (Fig. 2; Supplementary Table S3). Following a $41 \text{ } ^\circ\text{C}$ temperature stress for 6 h, the highest larval survival rate was recorded for the Kumasi population (Supplementary Table S3). At this temperature, larval survival rates across most populations approached zero, particularly for all rural lines, which demonstrated negligible survival. The urban populations exhibited a much higher thermal tolerance, highlighting a contrast in thermotolerance between urban and rural mosquito populations. These findings underscore the significant impact of high-temperature exposure on larval viability and the increased thermotolerance exhibited by urban populations under extreme conditions.

A clear divergence in response to temperature stress between urban and rural populations is observed for eggs, particularly at higher temperatures where urban populations show a greater positive deviation from the combined average (Fig. 3). Thermal tolerance of *Ae. aegypti* eggs showed a significant decrease in egg hatching following elevated temperature stress ($>40 \text{ } ^\circ\text{C}$) for longer duration (Fig. 3). Substantial variation occurred in the egg hatching rate among the *Ae. aegypti* populations of African origin ($F_{6,925} = 2.124, p = 0.004$) (Supplementary Table S4). As a general trend, a significant reduction in egg hatching was found as the temperature levels increased ($F_{1,930} = 24.1, p = 1.08 \times$

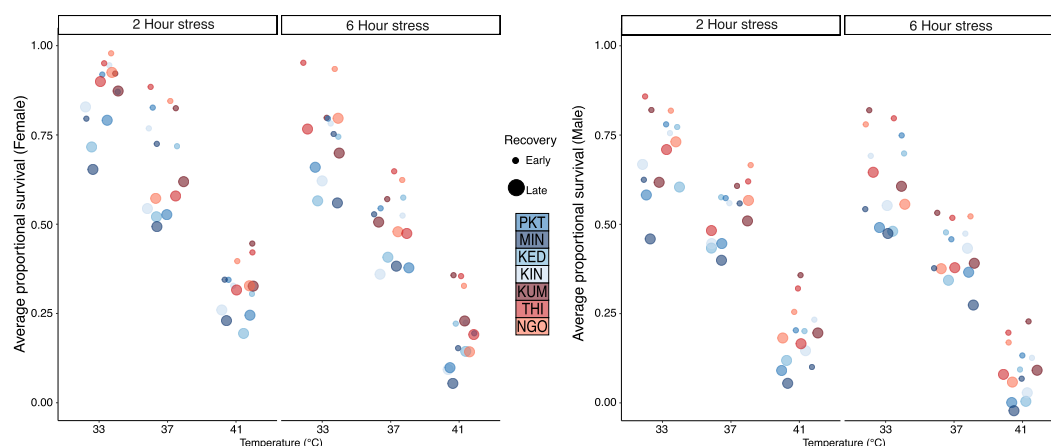


Fig. 1. Adult survival of *Aedes aegypti* populations following thermal stress. Female (left) and male (right) of *Ae. aegypti* were exposed to varying levels of temperatures for 2 h and 6 h. Following the thermal exposure, adult survival was evaluated after 2 h (smaller circles) and 24 h (larger circles). Shades of blue represent rural-origin mosquitoes and shades of red represent urban-origin populations. Statistical analyses were performed using generalized linear modeling, followed by Tukey's HSD post-hoc test when applicable.

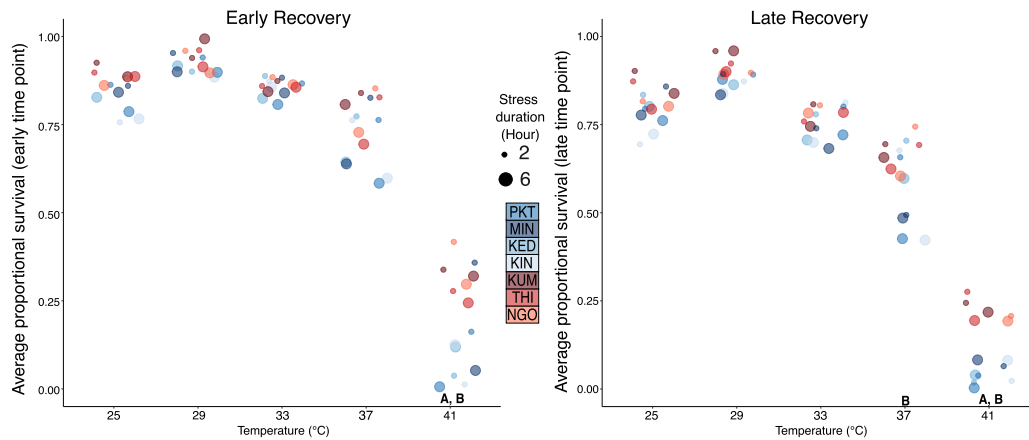


Fig. 2. Assessment of larval survival of *Aedes aegypti* populations following thermal stress. Larval survival of *Ae. aegypti* populations at an (left) early- and (right) later-time point following temperature stress for 2 h and 6 h. Shades of blue and red represent rural-and urban-origin mosquito populations, respectively. Generalized linear modeling were used for statistical comparisons, with Tukey's HSD post-hoc analysis applied where appropriate. The letters above the temperature panel (a and b) represent significant differences between urban and rural mosquito larvae temperature tolerance following 2 h (A) and 6 h (B) stress periods based on Tukey's HSD post-hoc analysis.

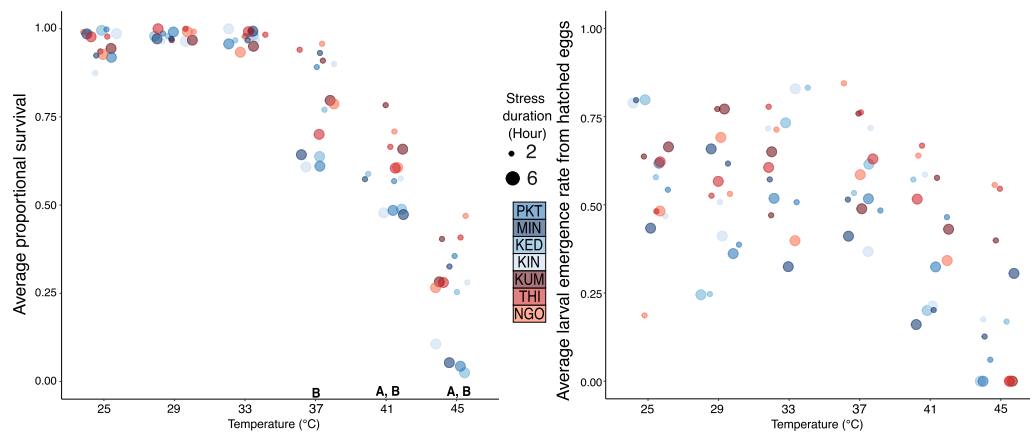


Fig. 3. Egg hatching of *Aedes aegypti* populations following thermal stress. (left) Egg hatching of *Ae. aegypti* populations after exposure to varying levels of thermal stress for 2 h and 6 h. (right) Larval emergence success from the hatched eggs following thermal stress. Shades of blue represent rural-origin mosquito populations of *Ae. aegypti*, while shades of red indicate urban-origin populations. Statistical analyses (for egg hatching) were conducted using generalized linear modeling, applying Tukey's HSD post-hoc analysis where relevant. The letters above the temperature panel (a) indicate significant differences between urban and rural mosquito egg thermotolerance after 2 h (A) and 6 h (B) of stress, determined based on Tukey's HSD post-hoc analysis.

10^{-6} ; Fig. 3, Supplementary Fig. 4). Notably, the impact was more pronounced following the exposure to >40 °C after the 2-h stress period and above 37 °C after the 6-h stress period (Supplementary Fig. 4). Following exposure to 41 °C for 2-h, the egg survival rates varied between 56.8 ± 2.58 % to 58.85 ± 4.62 % for the mosquito populations originating in areas where human population density is low ($<55/\text{km}^2$), whereas the egg hatching varied between 66.53 ± 3.38 % to 78.33 ± 2.74 % for the mosquito populations collected from areas where human density is moderate ($225\text{--}425/\text{km}^2$) to high ($>2000/\text{km}^2$) (Supplementary Table S4). Significant differences were observed between populations collected from high (urban) and low human (rural) density areas when exposed to 45 °C for 6 h ($F_{1,75} = 166$, $p < 2.2 \times 10^{-16}$); the lowest egg hatching, 2.47 ± 1.1 % was encountered for a population, Kedougou (human density $12.25/\text{km}^2$), collected from rural habitats; and the maximum egg hatching was found for Thies population, 28.06 ± 4.06 %, a moderately human dense area ($421/\text{km}^2$). We visualized larval emergence patterns from hatched eggs, irrespective of temperature stress duration, and found no discernible pattern, acknowledging potential confounding effects from larval cannibalism

(Fig. 3).

3.2. Influential factors impacting egg thermotolerance in *Ae. aegypti*

The binomial logistic regression analysis revealed both the intensity and duration of temperature stress ($p < 2.2 \times 10^{-16}$) had a strong negative impact on egg hatching success, demonstrating prolonged exposure to higher temperatures significantly reduces the likelihood of egg survival. Our results showed that a comprehensive set of factors related to specialization on human hosts and habitats (human specialization syndrome) significantly associated with egg thermotolerance (Fig. 4a–c). We dissected the components of human specialization syndrome to assess their independent and combined effects on egg thermotolerance. Egg thermotolerance significantly varied with human population density ($p = 7.309 \times 10^{-4}$), suggesting a potential trend where eggs from areas with higher human density exhibited slightly increased thermal tolerance (Fig. 4a). Both host preference ($p = 0.009$) and ancestry ($p = 0.012$) significantly related to egg thermotolerance. However, their high correlation ($r = 0.97$, $t_{(930)} = 118.62$, $p < 2.2 \times$

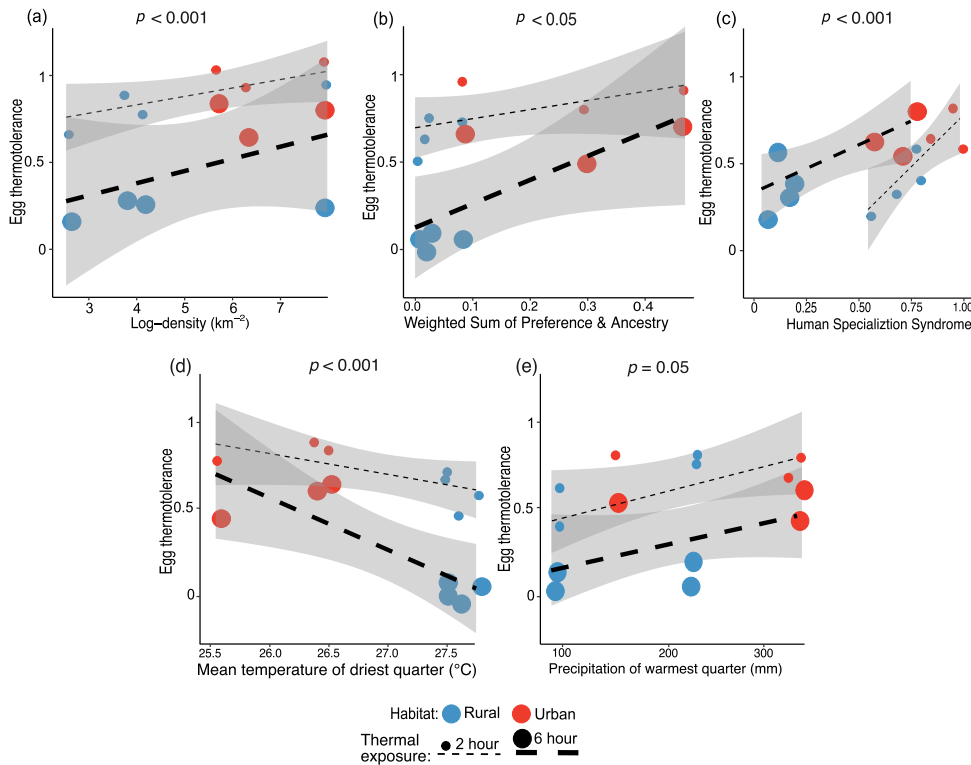


Fig. 4. Factors associated with egg thermotolerance in *Aedes aegypti*. (a) Significant relation of human population density on egg thermotolerance reveals that eggs originating from urban sites exhibit higher hatching rates ($p = 7.309 \times 10^{-4}$). (b) The joint association of host preference and mosquito ancestral origin on egg thermotolerance with a focus on the duration of thermal stress. (c) Variables in (a) and (b) combined in an index of human specialization syndrome, which fits the model better (LRT $p = 4.59 \times 10^{-8}$). (d) Mean temperature of the driest quarter shows significant negative association with egg thermotolerance ($p = 8.931 \times 10^{-4}$). (e) precipitation during the warmest quarter of the year ($p = 0.05$) had marginal significant association with egg thermotolerance. p values indicate level of significance.

10^{-16}) suggested a deeply intertwined relationship. To address this potential redundancy in combined model, we integrated host preference and ancestry into a single index based on their weighted regression coefficients, which showed a statistically significant association with egg thermotolerance ($p = 0.009$), highlighting the joint influence of these factors (Fig. 4b). The influence of human population density or the joint influence of ancestry and host preference on egg thermotolerance were found to be equally strong predictors when considered separately (Table 2). However, the composite of all three factors as “human specialization syndrome” index, i.e., ancestry, host preference (as weighted preference + ancestry) and human population density (Table 1) - improved the model fit significantly compared to any of the individual or joint models ($p = 4.59 \times 10^{-8}$, Table 2; Fig. 4c).

To assess the role of climatic factors on egg thermotolerance, we examined each of the temperature- and precipitation-related bioclimatic variables and, upon identifying significant relationships, proceeded with a step-up AIC model selection process informed by baseline correlation analysis and chi-square tests (Table 2). Among the temperature-related variables, annual mean temperature (bio1), mean temperature of driest (bio9), warmest (bio10), and coldest (bio11) quarters were found to be significantly related to egg thermotolerance. We identified the mean temperature of driest quarter of the year (bio9) as the strongest temperature-related bioclimatic factor influencing egg thermotolerance ($p = 8.931 \times 10^{-4}$; Fig. 4d). Among precipitation related variables, only precipitation of the warmest quarter (bio18) significantly associated with egg thermotolerance ($p = 0.045$; Fig. 4e). When we integrated climatic factors with human specialization syndrome components, the model did not show any significant improvement (LRT, $p = 0.47$). Incorporating climatic factors (bio9 and bio18) alongside human specialization syndrome in a multiple regression framework resulted in

a model with a slightly reduced fit compared to the model considering only human specialization traits (Table 2). Ultimately, the human specialization syndrome best explains the data with climatic factors playing a nuanced role when considered alongside human specialization traits in relation to egg thermotolerance. The comparatively moderate relationship ($\Delta AIC = 2.5$; Table 2) between climatic factors and human specialization traits warrants further exploration to better understand its impact on egg survival.

Further exploring the temporal trends of egg thermotolerance, our results indicate a strong correlation between ancestral origin of mosquito populations regarding mosquito host preference for humans and increased egg hatching rates under thermal stress in populations collected from densely populated urban habitats (Supplementary Fig. 6). Notably, the correlation between preference for human host and egg thermal tolerance was apparent when eggs were exposed to temperatures at or above 37°C . This underscores a clear and discernible effect of human specialization syndrome on egg hatching during thermal exposure of mosquitoes collected from urban and rural habitats. As the temperature level and duration of thermal exposure increased, a more pronounced association of human preference on the egg hatching index became evident (Supplementary Fig. 6). When we reanalyzed the data along an urbanization gradient to assess the association with local human population density, our results remained consistent, showing that mosquitoes from areas with higher human population density exhibited enhanced egg thermotolerance under elevated thermal stress.

Thermal tolerance across other life stages of *Ae. aegypti* was assessed, highlighting responses under elevated thermal stress and stage-specific adaptations towards human specialization. There was not a definite relationship between the adult- or larval-thermotolerance and ‘human specialization syndrome’ indices. While larval thermotolerance

Table 2

Model comparisons for predictors of survival under thermal stress: Models incorporate combinations of temperature, stress duration (time), ancestry, preference, population density, human specialization index, and bioclimatic variables. All models include a random intercept for Line. AIC values, chi square values, and significance ($\text{Pr}(>\text{Chisq})$) from likelihood ratio tests comparing each model.

Statistics	Model	Comparison	Chisq	AIC	Pr (>Chisq)
Null (or Thermotolerance) Model (Ht)	$Y \sim \text{Temp} + \text{Time} + (1 \text{Line})$			5748.1	
Preference (Hp)	$Y \sim \text{Temperature} + \text{Time} + \text{Preference} + (1 \text{Line})$	Ht vs. Hp	6.8038	5743.3	0.009096
Ancestry (Ha)	$Y \sim \text{Temperature} + \text{Time} + \text{Ancestry} + (1 \text{Line})$	Ht vs. Ha	6.3451	5743.8	0.01177
Preference & Ancestry combined (Hp_a)	$Y \sim \text{Temperature} + \text{Time} + \text{Weighted_preference_ancestry} + (1 \text{Line})$ [$\text{Weighted_pref_ancestry} < - \text{beta_preference} * \text{Preference} + \text{beta_ancestry} * \text{Ancestry}$]	Ht vs. Hp_a	6.8247	5743.3	0.008991
Density (Hd)	$Y \sim \text{Temperature} + \text{Time} + \log(\text{Density}) + (1 \text{Line})$	Ht vs. Hd	11.409	5738.7	0.0007309
Human Specialization Syndrome (Hs)	$Y \sim \text{Temperature} + \text{Time} + \text{Weighted_preference_ancestry} + \log(\text{Density}) + (1 \text{Line})$	Ht vs. Hs	33.795	5718.3	4.59E-08
Hp_a compared to Hd	Preference & Ancestry combined vs. Density	Hp_a vs. Hd	4.5843	5738.7	0
Hp_a compared to Hs	Preference & Ancestry combined vs. Human Specialization Syndrome	Hp_a vs. Hs	26.971	5718.3	2.07E-07
Hd compared to Hs	Density vs. Human Specialization Syndrome	Hd vs. Hs	22.386	5718.3	2.23E-06
Annual Mean Temperature (BIO1)	$Y \sim \text{Temperature} + \text{Time} + \text{BIO1} + (1 \text{Line})$	Ht vs. BIO1	3.9132	5746.2	0.04791
Mean Temp of Wettest Quarter (BIO8)	$Y \sim \text{Temperature} + \text{Time} + \text{BIO8} + (1 \text{Line})$	Ht vs. BIO8	2.1238	5748	0.145
Mean Temp of Driest Quarter (BIO9)	$Y \sim \text{Temperature} + \text{Time} + \text{BIO9} + (1 \text{Line})$	Ht vs. BIO9	11.025	5739.1	0.000899
Mean Temp of Warmest Quarter (BIO10)	$Y \sim \text{Temperature} + \text{Time} + \text{BIO10} + (1 \text{Line})$	Ht vs. BIO10	5.3779	5744.8	0.02039
Mean Temp of Coldest Quarter (BIO11)	$Y \sim \text{Temperature} + \text{Time} + \text{BIO11} + (1 \text{Line})$	Ht vs. BIO11	4.9234	5745.2	0.02649
Full Temp BIOs (Hx)	$Y \sim \text{Temperature} + \text{Time} + \text{BIO1} + \text{BIO9} + \text{BIO10} + \text{BIO11} + (1 \text{Line})$	Ht vs. Hx	13.764	5742.4	0.008088
BIO9 compared to significant temperatures BIOs	BIO9 vs. Full Temp BIOs	BIO9 vs. Hx	2.7392	5742.4	0.4336
Precipitation Seasonality (BIO15)	$Y \sim \text{Temperature} + \text{Time} + \text{BIO15} + (1 \text{Line})$	Ht vs. BIO15	0.1154	5750	0.734
Precipitation Wettest Quarter (BIO16)	$Y \sim \text{Temperature} + \text{Time} + \text{BIO16} + (1 \text{Line})$	Ht vs. BIO16	2.3923	5747.7	0.1219
Precipitation Driest Quarter (BIO17)	$Y \sim \text{Temperature} + \text{Time} + \text{BIO17} + (1 \text{Line})$	Ht vs. BIO17	0.9345	5749.2	0.3337
Precipitation Warmest Quarter (BIO18)	$Y \sim \text{Temperature} + \text{Time} + \text{BIO18} + (1 \text{Line})$	Ht vs. BIO18	4.0279	5746.1	0.04475
Precipitation Coldest Quarter (BIO19)	$Y \sim \text{Temperature} + \text{Time} + \text{BIO19} + (1 \text{Line})$	Ht vs. BIO19	0.0012	5750.1	0.9722
Bioclimatic influence (Hb)	$Y \sim \text{Temperature} + \text{Time} + \text{BIO9} + \text{BIO18} + (1 \text{Line})$	Ht vs. Hb	11.446	5740.7	0.00327
Combined model (Hc) = (Hs + Hb)	$Y \sim \text{Temperature} + \text{Time} + \text{Weighted_preference_ancestry} + \log(\text{Density}) + \text{BIO9} + \text{BIO18} + (1 \text{Line})$	Ht vs. Hc	35.303	5720.8	4.03E-07
Hb compared to Hc	Bioclimatic influence vs. combined model	Hb vs. Hc	23.857	5720.8	6.60E-06
Hs compared to Hc	Human Specialization vs. combined model (Hs vs. Hc)	Hs vs. Hc	1.5072	5720.8	0.4707

exhibited a weak relationship with human preference at 37 °C, this relationship became more apparent at 41 °C (Supplementary Fig. 7). Sex-specific studies revealed only adult female survival was moderately related to host preference, but few of the conditions are significant (Supplementary Fig. 8). However, for both the adult and larval cases the association was not significant and would require more populations with known host preference to determine a potential relationship between thermal tolerance and other factors.

4. Discussion

4.1. Anthropocene and urban adaptations of mosquitoes

Understanding thermal performance concerning the life history traits of mosquitoes has provided invaluable insight into their thermal adaptations (Couper et al., 2021; Lahondère and Bonizzoni, 2022; Mordecai et al., 2019). The increase in anthropogenic activities is likely to have implications for the biology of specific mosquitoes with their response to thermal stress (Rochlin et al., 2016; Schrama et al., 2020). The thermal tolerance of mosquitoes across developmental stages has not been extensively examined. We identified that urban-associated mosquito populations exhibited a notably higher egg hatching rate than rural-associated mosquito populations following thermal stress. Overall, the egg stage exhibited a higher thermal tolerance compared to larvae and adults, likely due to the protective nature of the egg structure, which

provides a buffer against extreme temperatures. Notably, the largest variation among populations was only seen at the highest temperatures for eggs, suggesting that eggs can survive extreme heat, which may contribute to differences in mosquito population outbreaks between urban and rural regions. Furthermore, our analysis of ecological and behavioral indices indicated that egg thermal tolerance may represent a previously unrecognized part of human specialization syndrome. This thermal tolerance is robustly influenced by the key factors associated with the syndrome (host preference, genomic ancestry, and human density), and the factors are best understood as a multidimensional trait shaped by multiple interacting factors. Interestingly, our findings suggest that moisture parameters, such as precipitation seasonality and precipitation levels during the driest months do not exert a discernible impact on the thermal tolerance of *Ae. aegypti* eggs. Increased larval thermal tolerance is also noted in the urban mosquito populations, but much more muted than the eggs. In contrast, adult thermal tolerance has very limited significant differences or no discernible trends among the populations of *Ae. aegypti*. Our study provides a foundation to determine the intricate interplay between ecological factors, heat stress, and mosquito egg thermal tolerance.

4.2. Adult mosquito survival and thermal tolerance under varying temperatures

Assessing adult mosquito survival under different temperatures

provides insight into their thermal tolerance and adaptability (Ware-Gilmore et al., 2023; Zani et al., 2005). In this study, several populations of *Ae. aegypti* were characterized to evaluate their temperature tolerance and assess variations in survival. Overall, we found variations in survival rates between males and females, with a consistent decline in survival with temperature increases. However, we detected only a few significant differences in survival between mosquito populations collected from rural and urban habitats, particularly among the female *Ae. aegypti*. We did note that populations from urban areas have a generally higher thermal tolerance than those from rural areas, but this was not significant in relation to factors such as ancestry or human population density. It is likely that if *Ae. aegypti* was collected over a larger geographic range or if substantially more populations were collected, significant differences would have been observable in adults, as seen in another *Aedes* species, *Ae. albopictus* (Carlassara et al., 2024). Although adult thermal tolerance can be masked when individuals from distinct climatic backgrounds inhabit similar thermal environments over time. Tropical and temperate *Drosophila melanogaster*, residing within the experimental thermal range of 11–32 °C, showed converging survival rates, with no significant differences observed between the two populations (Trotta et al., 2006). The fruit flies exhibited thermal plasticity in developmental rate, body size, and fertility; however, distinct adaptive responses following heat stress were not prominent among flies from different origins (Trotta et al., 2006). Critical insights into thermal tolerance across various *Drosophila* species reveal how thermal limits and performance curves can differ based on habitat temperature. The evolutionary constraints of thermal plasticity observed in adult *Drosophila* populations suggest potential parallels in mosquitoes, where both thermal tolerance and behavioral adaptations may enhance resilience to thermal stress (MacLean et al., 2019). The survival of an organism in nature is contingent upon factors such as season and geographical location (Rosewell and Shorrocks, 2008), and laboratory measurements may not align with survival in the wild (Moioń et al., 2020), as adults can effectively behaviorally thermoregulate by moving to a more favorable microhabitat to avoid warm periods. We demonstrated that female mosquitoes exhibited higher survival rates compared to male mosquitoes, a pattern common in arthropods (Bodlah et al., 2023; Benoit et al., 2011). Thermoregulatory behavior, such as engaging in evaporative cooling during the blood-feeding process to counter the rapid temperature increase, may contribute to the sex-specific difference in survival (Benoit et al., 2011; Lahondère and Lazzari, 2012). Furthermore, thermal tolerance differences may be influenced by the size disparity between female and male mosquitoes, where females are slightly larger than their male counterparts (Holmes and Benoit, 2019). While our experiment did not directly assess the impact of bloodmeals or size disparity on adult thermotolerance, it is possible that over time colony maintenance conditions influenced this phenomenon.

4.3. Larval thermal tolerance shows effects of human association at temperatures near the upper limit

The behavioral plasticity of adults complements the thermal resilience of earlier life stages, with habitat-specific thermal adaptations shaping survival strategies across mosquito life stages. Larval thermal tolerance in *Ae. aegypti* demonstrates pronounced effects at temperatures near the upper critical limit. The distinct responses observed in *Ae. aegypti* populations at extreme temperatures (>40 °C) underscore the role of their ecological habitats on thermal adaptation during immature stages. The container-breeding *Ae. aegypti* is more closely linked with densely populated urban environments (Reiskind and Lounibos, 2013; Rodrigues et al., 2015). These habitats expose the species to increased temperatures, erratic water availability, and pollution, which likely exerts stronger selection pressures for traits like heat tolerance and suitability for artificial breeding sites (Rodrigues et al., 2015). Over time, these challenges may have strengthened *Ae. aegypti*'s resistance to environmental stressors, especially in urban settings. Furthermore,

molecular studies have revealed significant upregulation of heat shock proteins in *Ae. aegypti* larvae following exposure to heat stress, particularly in conditions where temperatures regularly exceed 37 °C (Arun et al., 2017). This molecular response aligns with observed survival rates in *Ae. aegypti* larvae under extreme heat events (Gratz, 2004). Furthermore, the buffering effect of certain habitats, such as shaded microclimates and abundance of water containers shield larvae and eggs from temperature extremes if the females deposited the eggs in a favorable location (Couper et al., 2023). The larval thermotolerance peaked at 29 °C, though we consistently observed lower survival at 25 °C - a somewhat unexpected trend. During rearing, the stacked plastic pans with closed lids may have created a thermal gradient toward the upper bound of colony maintenance temperature, potentially impacting survival. Subtle stress responses at 25 °C could also contribute to developmental delays and metabolic inefficiencies, ultimately affecting late (24 h) survival (Reinhold et al., 2018). It is also interesting that a similar, albeit weaker, trend was observed in egg survival. This might indicate a common underlying mechanism, such as temperature-dependent metabolic rates affecting multiple life stages, where optimum egg and larvae survival occur between 27 and 29 °C.

4.4. Egg survival of mosquitoes under temperature stress in relation to urbanization and contrasting oviposition sites

The egg stage is immobile and confined to the site of deposition, suggesting exposure to high temperatures could occur and reduce egg viability. Among other insects, no differences in thermal tolerance were noted in the different lineages of eggs for the tobacco hornworm, *Manduca sexta* (Potter and Woods, 2012) and there has been very little focus on this topic in other arthropod systems (Hilker et al., 2023; Ajayi et al., 2023). Our study revealed significant differences in the egg-hatching rates for *Ae. aegypti* based on their origin of collection (areas with high and low human population density) when exposed to heat stress. Larval presence was excluded from our egg viability analysis due to cannibalism, which limits our ability to fully assess the downstream effects of temperature stress on eggs. Microclimatic variations within urban and rural environments, dry season intensity, and precipitation regimes can impact mosquito egg survival following heat stress (Mogi et al., 1996). A sparsely vegetated urban site can reach surface temperatures up to 11 °C higher than the surrounding countryside (Mohajerani et al., 2017), due to increased artificial surfaces along with a lack of vegetation (Moss et al., 2019; Paschalis et al., 2021). The variability in available materials for mosquito egg deposition across different habitats significantly contributes to the observed differences in egg hatching among collection sites. Thermally absorbing and conductive materials such as rubber, plastic, and concrete with water holding capacity are abundant in a densely populated area, and mosquitoes prefer these substances for egg laying (Seng and Jute, 1994). In contrast, in wild areas or rural areas with lower human density, mosquitoes typically prefer for tree holes or natural habitats to lay eggs (Suganthi et al., 2014). Notably, during hot summer days or exposure to direct sunlight, live wood remains cooler due to multiple processes (e.g. evaporative cooling) compared to other substances (Moss et al., 2019). Moreover, driven by the increased opportunity of biting human hosts and the availability of alternative water sources, mosquitoes tend to move towards densely populated habitats (Fernández-Grandon et al., 2015). This shift necessitates greater environmental stress tolerance, as seen in other terrestrial arthropods adapting to urban areas (Diamond et al., 2018; Harvey et al., 2023), and aligns with differences in mosquito abundance and thermotolerance between urban and rural areas (Lim et al., 2021), suggesting that urban mosquitoes also have heat tolerance for their eggs. Overall, we show that heat stress, specifically above 37 °C, significantly reduced *Ae. aegypti* egg hatching, and the populations from urban areas with increased human preference demonstrated greater thermal tolerance compared to counterparts from more rural areas with a reduced human preference.

4.5. Egg thermotolerance as an aspect of mosquito specialization towards humans

Alongside temperature stress, the interplay of urbanization, microclimate variation, and other potential ecological factors are likely to have an influential role in shaping the survival of the populations (Erraguntla et al., 2021; Rochlin et al., 2016). Although bioclimatic patterns had less pronounced effects on egg thermotolerance, the mean temperature of the driest quarter of the year showed a relationship to egg viability. Interestingly, we found that certain rural areas experience approximately 1 °C higher mean temperatures compared to urban areas. We believe this disconnect between the egg thermal tolerance occurs as the air temperature recording was used in our analysis due to availability. Surface temperature, specifically the local sites where females oviposit, more accurately captures temperatures that eggs will be exposed to; however, such data are less readily available. As mentioned previously, urbanized structures and artificial breeding sites (discarded tires) are likely to be hotter than the air temperature due to their heat absorption and retention capacity compared to tree holes that can buffer these effects (Parker et al., 2024; de Jesús Crespo and Rogers, 2021). Variations in egg stage for other insects across different habitats can significantly impact subsequent life stages and the overall fitness of a population (Fisher et al., 1990). While bioclimatic data provided useful insights, the spatial resolution may not be fine scaled enough to fully capture urban effects on microclimatic conditions that impact egg thermotolerance. Surface temperatures at oviposition sites, such as discarded tires or tree holes, likely differ from recorded air temperatures and could play a more direct role in shaping egg survival patterns.

Genetic diversity and habitat adaptation greatly influence the biology of mosquitoes (Couper et al., 2021; Rose et al., 2023). In the African landscape, mosquito populations display admixture between the domestic human-specialist form ('Aaa') and forest form ('Aaf') (Rose et al., 2020). The proportion of 'Aaa'-like ancestry (admixture) is the main driver that influences traits such as host preference (Rose et al., 2023). Although 'Aaa' is traditionally associated with domestic habitats, not all 'Aaa' populations are necessarily urban, and not all urban populations are heavily skewed towards 'Aaa' form (Rose et al., 2020, 2023). Populations from the highly human-dense area of Kumasi (Ghana), have a genetic background that is predominantly 'Aaf', but with substantially more 'Aaa' ancestry than is observed in nearby rural populations (Rose et al., 2020). Interestingly, our study indicates a high thermal tolerance in the eggs collected from the Kumasi. Given the association of 'Aaa'-like ancestry with egg thermal tolerance, increased 'Aaa' ancestry in specific loci may control thermal tolerance relative to genome-wide levels, or could reflect variation in thermal tolerance within 'Aaf', beyond the differences observed between the two forms. Our study supports the previous research that suggests genetic variations and local adaptations influence the physiological responses of insects to temperature fluctuations (González-Tokman et al., 2020; Sternberg and Thomas, 2014). The link between ancestry and thermal tolerance suggests that higher thermal tolerance of eggs is a trait, along with an increased preference for humans, that has allowed an anthropophilic shift from a more generalist nature. Numerous cases exist of mosquitoes flourishing in densely populated human environments while yet favoring non-human hosts (Honnen and Monaghan, 2017). Nonetheless, for *Ae. aegypti*, the fundamental dynamics remain unclear, especially concerning the existence of any behavioral, physiological, or morphological trade-offs in their host preferences between humans and non-humans. Our research indicates that shifts in thermal tolerance due to urbanization may be a critical factor in allowing a closer association with humans. Furthermore, the prevalence of ovipositional locations and the availability of blood meals in urban settings may significantly influence these trade-offs. It is important to highlight that the populations examined are primarily characterized by 'Aaf' ancestry, with some admixture from 'Aaa'. The rising pressures of the Anthropocene may be facilitating the divergence of these two lineages, where 'Aaa' has

an ever-increasing human preference with an associated increase in thermal tolerance.

4.6. Conclusions

Our study represents one of the first extensive analyses of temperature stress on *Aedes* mosquito adults, larvae and eggs of distinct origin and how genetic variations, local adaptations, or other factors influence the physiological responses of *Ae. aegypti* to temperature fluctuations. We found that the egg-hatching rates following heat stress were positively impacted by increasing human density, which suggests that egg thermotolerance could be critical for mosquito survival during climate change and rural-to-urban transitions. However, while temperature fluctuations significantly impacted larval development and adult survival, the effects of urbanization and other environmental factors were less pronounced. The process of urbanization, encompassing rapid urban growth, unplanned expansion, and human population density, unquestionably shapes mosquito populations (Carlassara et al., 2024; Kolime-nakis et al., 2021), leading to differential resilience of mosquito populations in the face of abiotic extremities. A limitation of our studies is that only seven populations were used, and these mosquitoes were held in the lab for three-five years before assessment. Studies with more populations and that were analyzed after a limited period in the lab (1–2 years) may identify effects of urbanization and human specialization in all stages (e.g. adults). As adults are mobile, behavioral changes, such as observed differences in daily rhythms (Ajayi et al., 2024), could increase thermotolerance, but would have not been evaluated by our assays. Notably, the largest variation among populations was only seen at the highest temperatures for eggs, suggesting that eggs can survive extreme heat, potentially driving differences in mosquito population outbreaks between urban and rural regions. To further understand these patterns, future research should investigate more thermal treatments (heat hardening, fluctuating thermal regimes, etc.) and the joint influence of temperature and humidity on egg survival for a longer duration, shedding light on the adaptive potential of mosquito populations in a changing climate. These comprehensive studies will be essential for establishing mosquito egg viability under varying conditions to predict hatching patterns and mosquito population dynamics.

CRedit authorship contribution statement

Souvik Chakraborty: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Emily Zigmond:** Writing – review & editing, Resources, Investigation, Formal analysis, Data curation. **Sher Shah:** Writing – review & editing, Resources, Investigation, Formal analysis, Data curation. **Diya Dayal:** Resources, Investigation, Data curation. **Massamba Sylla:** Resources. **Jewelna Akorli:** Resources. **Sampson Otoo:** Resources. **Noah H. Rose:** Writing – review & editing, Resources. **Carolyn S. McBride:** Writing – review & editing. **Peter A. Armbruster:** Writing – review & editing, Resources. **Joshua B. Benoit:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary tables and figures

Supplementary tables and figures to this article can be found online at <https://doi.org/10.1016/j.jtherbio.2025.104167>.

Data availability

Data to this article can be found online at <https://zenodo.org/records/15065947>

References

- Ajayi, Oluwaseun M., Oyen, Kennan J., Davies, Benjamin, Finch, Geoffrey, Piller, Benjamin D., Harmeyer, Alison A., Wendeln, Katherine, Perretta, Carlisle, Rosendale, Andrew J., Benoit, Joshua B., 2023. Egg hatching success is influenced by the time of thermal stress in four hard tick species. *J. Med. Entomol.* <https://doi.org/10.1093/jme/tjad142>. October.
- Ajayi, O.M., Susanto, E.E., Wang, L., Kennedy, J., Ledezma, A., Harris, A.C., Smith, E.S., Chakraborty, S., Wynne, N.E., Sylla, M., Akorli, J., Otoo, S., Vinauger, C., Benoit, J. B., 2024. Intra-species quantification reveals differences in activity and sleep levels in the yellow fever mosquito. *Aedes aegypti*. *Med. Vet. Entomol.* 38 (4), 482–494.
- Arun, Sivan, Ananganallur Nagarajan, Shriram, Nagarajan, Muruganandam, Ramanathan, Thamizhmani, 2017. Expression of heat shock proteins (HSPs) in *Aedes aegypti* (L) and *Aedes albopictus* (skuse) (Diptera: Culicidae) larvae in response to thermal stress. *Acta Trop.* 167 (March), 121–127.
- Bennett, Kelly L., Gómez Martínez, Carmelo, Almanza, Alejandro, Rovira, Jose R., Owen McMillan, W., Enriquez, Vanessa, Barraza, Elia, et al., 2019. High infestation of invasive *Aedes* mosquitoes in used tires along the local transport network of Panama. *Parasites Vectors* 12 (1), 264.
- Benoit, Joshua B., Lopez-Martinez, Giancarlo, Patrick, Kevin R., Phillips, Zachary P., Krause, Tyler B., Denlinger, David L., 2011. Drinking a hot blood meal elicits a protective heat shock response in mosquitoes. *Proc. Natl. Acad. Sci. U. S. A.* 108 (19), 8026–8029.
- Bhatt, Samir, Gething, Peter W., Brady, Oliver J., Messina, Jane P., Farlow, Andrew W., Moyes, Catherine L., Drake, John M., et al., 2013. The global distribution and burden of dengue. *Nature* 496 (7446), 504–507.
- Bodlah, Muhammad Adnan, Iqbal, Javaid, Ashiq, Aneela, Bodlah, Imran, Jiang, Shoulin, Ahmad Mudassir, Muhammad, Rasheed, Muhammad Tariq, Fareen, Ammara Gul E., 2023. Insect behavioral restraint and adaptation strategies under heat stress: an inclusive review. *Journal of the Saudi Society of Agricultural Sciences* (February). <https://doi.org/10.1016/j.jssas.2023.02.004>.
- Bowler, Ken, Terblanche, John S., 2008. Insect thermal tolerance: what is the role of ontogeny, ageing and senescence? *Biol. Rev. Camb. Phil. Soc.* 83 (3), 339–355.
- Brady, Oliver J., Simon, I. Hay, 2020. The global expansion of dengue: how *Aedes aegypti* mosquitoes enabled the first pandemic arbovirus. *Annu. Rev. Entomol.* 65 (1), 191–208.
- Carlassara, Martina, Khorramnejad, Ayda, Oker, Helen, Bahrami, Romina, Lozada-Chávez, Alejandro Nabor, Vittoria Mancini, Maria, Quaranta, Stefano, Bod, Melanie J.A., Lahondère, Chloé, Bonizzoni, Mariangela, 2024. Population-specific responses to developmental temperature in the arboviral vector *Aedes albopictus*: implications for climate change. *Glob. Change Biol.* 30 (3), e17226.
- Carrico, Caroline, Gennings, Chris, Wheeler, David C., Factor-Litvak, Pam, 2015. Characterization of weighted quantile sum regression for highly correlated data in a risk analysis setting. *J. Agric. Biol. Environ. Stat.* 20 (1), 100–120.
- Chala, Bayissa, Hamde, Feyissa, 2021. Emerging and Re-emerging vector-borne infectious diseases and the challenges for control: a review. *Front. Public Health* 9 (October), 715759.
- Chambers, John M., Hastie, Trevor, 1992. *Statistical Models in S*. Wadsworth & Brooks/Cole Advanced Books & Software.
- Christophers, S., 1960. *Aedes Aegypti* (L.) the Yellow Fever Mosquito: its Life History, Bionomics and Structure. The Syndics of the Cambridge University Press, Bentley House, London, p. 200.
- Clements, A.N., 1992. *The Biology of Mosquitoes: Development, Nutrition and Reproduction*. CABI.
- Couper, Lisa I., Farner, Johannah E., Caldwell, Jamie M., Childs, Marissa L., Harris, Mallory J., Kirk, Devin G., Nova, Nicole, et al., 2021. How will mosquitoes adapt to climate warming? *eLife* 10 (August). <https://doi.org/10.7554/eLife.69630>.
- Couper, Lisa I., Farner, Johannah E., Lyberger, Kelsey P., Lee, Alexandra S., Mordecai, Erin A., 2023. Mosquito thermal tolerance is remarkably constrained across a large climatic range. *Proceedings of the Royal Society B*. <https://doi.org/10.1098/rspb.2023.2457>.
- Couret, Jannelle, Benedict, Mark Q., 2014. A meta-analysis of the factors influencing development rate variation in *Aedes aegypti* (Diptera: Culicidae). *BMC Ecol.* 14 (1), 3.
- David, Mariana R., Maciel-de-Freitas, Rafael, Petersen, Martha T., Bray, Daniel, Hawkes, Frances M., Mandela Fernández-Grandon, G., Young, Stephen, Gibson, Gabriella, Hopkins, Richard J., 2023. *Aedes aegypti* oviposition-sites choice under semi-field conditions. *Med. Vet. Entomol.* 37 (4), 683–692.
- Diamond, Sarah E., Chick, Lacy D., Perez, Abe, Strickler, Stephanie A., Zhao, Crystal, 2018. Evolution of plasticity in the city: urban acorn ants can better tolerate more rapid increases in environmental temperature. *Conservation Physiology* 6 (1), coy030.
- Erraguntla, Madhav, Dave, Darshit, Zapletal, Josef, Myles, Kevin, Adelman, Zach N., Pohlentz, Tyler D., Lawley, Mark, 2021. Predictive model for microclimatic temperature and its use in mosquito population modeling. *Sci. Rep.* 11 (1), 18909.
- Farnesi, Luana C., Vargas, Helena C.M., Valle, Denise, Rezende, Gustavo L., 2017. Darker eggs of mosquitoes resist more to dry conditions: melanin enhances serosal cuticle contribution in egg resistance to desiccation in *Aedes*, *Anopheles* and *Culex* vectors. *PLoS Neglected Trop. Dis.* 11 (10), e0006063.
- Fernández-Grandon, G. Mandela, Gezan, Salvador A., Armour, John A.L., Pickett, John A., Logan, James G., 2015. Heritability of attractiveness to mosquitoes. *PLoS One* 10 (4), e0122716.
- Fick, Stephen E., Hijmans, Robert J., 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas: new climate surfaces for global land areas. *Int. J. Climatol.: A Journal of the Royal Meteorological Society* 37 (12), 4302–4315.
- Fisher, Ian J., Bradshaw, William E., Chris, Kammeyer, 1990. Fitness and its correlates assessed by intra- and interspecific interactions among tree-hole mosquitoes. *J. Anim. Ecol.* 59 (3), 819.
- González-Tokman, Daniel, Córdoba-Aguilar, Alex, Dáttilo, Wesley, Lira-Noriega, Andrés, Sánchez-Guillén, Rosa A., Villalobos, Fabricio, 2020. Insect responses to heat: physiological mechanisms, evolution and ecological implications in a warming world. *Biol. Rev. Camb. Phil. Soc.* 95 (3), 802–821.
- Gratz, N.G., 2004. Critical review of the vector status of *Aedes albopictus*. *Med. Vet. Entomol.* 18 (3), 215–227.
- Harvey, Jeffrey A., Tougeron, Kevin, Gols, Rieta, Heinen, Robin, Abarca, Mariana, Abram, Paul K., Basset, Yves, et al., 2023. Scientists' warning on climate change and insects. *Ecol. Monogr.* 93 (1). <https://doi.org/10.1002/ecm.1553>.
- Hilker, Monika, Salem, Hassan, Fatouros, Nina E., 2023. Adaptive plasticity of insect eggs in response to environmental challenges. *Annu. Rev. Entomol.* 68 (January), 451–469.
- Holmes, Christopher J., Benoit, Joshua B., 2019. Biological adaptations associated with dehydration in mosquitoes. *Insects* 10 (11). <https://www.ncbi.nlm.nih.gov/pubmed/31661928>.
- Honnen, Ann-Christin, Monaghan, Michael T., 2017. City-dwellers and country folks: lack of population differentiation along an urban-rural gradient in the mosquito *Culex pipiens* (Diptera: Culicidae). *J. Insect Sci.* 17 (5), 107.
- Hsu, Angel, Sheriff, Glenn, Chakraborty, Tirthankar, Diego, Manya, 2021. Disproportionate exposure to urban heat island intensity across major US cities. *Nat. Commun.* 12 (1), 2721.
- Hug, David O.H., Kropf, Alida, Amann, Marine O., Koella, Jacob C., Verhulst, Niels O., 2024. Unexpected behavioural adaptation of yellow fever mosquitoes in response to high temperatures. *Sci. Rep.* 14 (1), 3659.
- Jesús Crespo, Rebeca de, Rogers, Rachel Elba, 2021. Habitat segregation patterns of container breeding mosquitoes: the role of urban heat islands, vegetation cover, and income disparity in cemeteries of new orleans. *Int. J. Environ. Res. Publ. Health* 19 (1), 245.
- Kolimenakis, Antonios, Heinz, Sabine, Wilson, Michael Lowery, Winkler, Volker, Yakob, Laith, Michaelakis, Antonios, Papachristos, Dimitrios, Richardson, Clive, Horstlick, Olaf, 2021. The role of urbanisation in the spread of *Aedes* mosquitoes and the diseases they transmit-A systematic review. *PLoS Neglected Trop. Dis.* 15 (9), e0009631.
- Lahondère, Chloé, Bonizzoni, Mariangela, 2022. Thermal biology of invasive *Aedes* mosquitoes in the context of climate change. *Current Opinion in Insect Science* 51 (June), 100920.
- Lahondère, Chloé, Lazzari, Claudio R., 2012. Mosquitoes cool down during blood feeding to avoid overheating. *Curr. Biol.* CB 22 (1), 40–45.
- Lim, Ah-Young, Cheong, Hae-Kwan, Chung, Yeonseung, Sim, Kisung, Kim, Jong-Hun, 2021. Mosquito abundance in relation to extremely high temperatures in urban and rural areas of incheon metropolitan city, South Korea from 2015 to 2020: an observational study. *Parasites Vectors* 14 (1), 559.
- Loveday, Jane, Loveday, Grant, Byrne, Joshua J., Ong, Boon-Lay, Morrison, Gregory M., 2019. Seasonal and diurnal surface temperatures of urban landscape elements. *Sustainability* 11 (19), 5280.
- MacLean, Heidi J., Sørensen, Jesper G., Kristensen, Torsten N., Loeschcke, Volker, Beedholm, Kristian, Kellermann, Vanessa, Overgaard, Johannes, 2019. Evolution and plasticity of thermal performance: an analysis of variation in thermal tolerance and fitness in 22 *Drosophila* species. *Phil. Trans. Roy. Soc. Lond. B Biol. Sci.* 374 (1778), 20180548.
- Maïga, H., Yamada, H., Bimbile-Somda, N.S., 2017. Guidelines for routine colony maintenance of *Aedes* mosquito species. IAEA Physical and Chemical. <https://www.iaea.org/resources/manual/guidelines-for-routine-colony-maintenance-of-aedes-mosquito-species-version-10>.
- Mathieu, K., Karmali, M., 2016. Vector-borne diseases, climate change and healthy urban living: next steps. *Canada Communicable Disease Report = Relevé Des Maladies Transmissibles Au Canada* 42 (10), 219–221.
- McBride, Carolyn S., 2016. Genes and odors underlying the recent evolution of mosquito preference for humans. *Curr. Biol.* CB 26 (1), R41–R46.
- Melchiorri, Michele, Florczyk, Aneta J., Freire, Sergio, Schiavina, Marcello, Pesaresi, Martino, Kemper, Thomas, 2018. Unveiling 25 Years of planetary urbanization with remote sensing: perspectives from the global human settlement layer. *Remote Sens.* 10 (5), 768.
- Metz, Hillery C., Miller, Alexandra K., You, Janet, Akorli, Jewelna, Avila, Frank W., Buckner, Eva A., Kane, Philomina, et al., 2023. Evolution of a mosquito's hatching behavior to match its human-provided habitat. *Am. Nat.* 201 (2), 200–214.

- Mogi, M., Miyagi, I., Abadi, K., Syafruddin, 1996. Inter- and intraspecific variation in resistance to desiccation by adult *Aedes* (stegomyia) spp. (Diptera: Culicidae) from Indonesia. *J. Med. Entomol.* 33 (1), 53–57.
- Mohajerani, Abbas, Bakaric, Jason, Jeffrey-Bailey, Tristan, 2017. The urban heat island effect, its causes, and mitigation, with reference to the thermal properties of asphalt concrete. *J. Environ. Manag.* 197 (July), 522–538.
- Motoñ, Mateusz, Dampc, Jan, Kula-Maximenko, Monika, Zebrowski, Jacek, Moloñ, Agnieszka, Dobler, Ralph, Roma, Durak, Skoczowski, Andrzej, 2020. Effects of temperature on lifespan of *Drosophila melanogaster* from different genetic backgrounds: links between metabolic rate and longevity. *Insects* 11 (8). <https://www.ncbi.nlm.nih.gov/pubmed/32722420>.
- Mordecai, Erin A., Caldwell, Jamie M., Grossman, Marissa K., Lippi, Catherine A., Johnson, Leah R., Neira, Marco, Rohr, Jason R., et al., 2019. Thermal biology of mosquito-borne disease. *Ecol. Lett.* 22 (10), 1690–1708.
- Moss, Joseph L., Doick, Kieron J., Smith, Stefan, Shahrestani, Mehdi, 2019. Influence of evaporative cooling by urban forests on cooling demand in cities. *Urban For. Urban Green.* 37 (January), 65–73.
- Nations, United, 2019. *World Population Prospects 2019*, vol. 141. Department of Economic and Social Affairs.
- Ortiz, Diana I., Piche-Ovares, Marta, Romero-Vega, Luis M., Wagman, Joseph, Troyo, Adriana, 2021. The impact of deforestation, urbanization, and changing land use patterns on the Ecology of mosquito and tick-borne diseases in Central America. *Insects* 13 (1). <https://doi.org/10.3390/insects13010020>.
- Parker, Allison T., Murray, Bridget A., Miller, Amber L., 2024. Mosquito species distribution in tires in rural and urban landscapes across northern Kentucky. *J. Am. Mosq. Control Assoc.* 40 (4), 182–185.
- Paschalis, Athanasios, Chakraborty, T.C., Faticchi, Simone, Meili, Naika, Manoli, Gabriele, 2021. Urban forests as main regulator of the evaporative cooling effect in cities. *AGU Advances* 2 (2). <https://doi.org/10.1029/2020av000303>.
- Phelan, Patrick E., Kaloush, Kamil, Miner, Mark, Golden, Jay, Phelan, Bernadette, Silva III, Humberto, Taylor, Robert A., 2015. Urban heat island: mechanisms, implications, and possible remedies. *Annu. Rev. Environ. Resour.* 40 (1), 285–307.
- Potter, Kristen A., Goggy, Davidowitz, Woods, H. Arthur, 2011. Cross-stage consequences of egg temperature in the insect *Manduca sexta*. *Funct. Ecol.* 25 (3), 548–556.
- Potter, Kristen A., Woods, H. Arthur, 2012. No evidence for the evolution of thermal or desiccation tolerance of eggs among populations of *Manduca sexta*. *Funct. Ecol.* 26 (1), 112–122.
- Reinhold, Joanna M., Lazzari, Claudio R., Lahondère, Chloé, 2018. Effects of the environmental temperature on *Aedes aegypti* and *Aedes albopictus* mosquitoes: a review. *Insects* 9 (4). <https://doi.org/10.3390/insects9040158>.
- Reinmann, Andrew B., Hutyra, Lucy R., Trlica, Andrew, Olofsson, Pontus, 2016. Assessing the global warming potential of human settlement expansion in a mesic temperate landscape from 2005 to 2050. *Sci. Total Environ.* 545–546 (March), 512–524.
- Reiskind, M.H., Lounibos, L.P., 2013. Spatial and temporal patterns of abundance of *Aedes aegypti* L. (*Stegomyia aegypti*) and *Aedes albopictus* (skuse) [*Stegomyia albopictus* (skuse)] in southern Florida. *Med. Vet. Entomol.* 27 (4), 421–429.
- Rezende, Gustavo L., Martins Vargas, Helena Carolina, Moussian, Bernard, Cohen, Ephraim, 2016. Composite eggshell matrices: chorionic layers and sub-chorionic cuticular envelopes. In: Cohen, Ephraim, Moussian, Bernard (Eds.), *Extracellular Composite Matrices in Arthropods*. Springer International Publishing, Cham, pp. 325–366.
- Richardson, Kelly M., Hoffmann, Ary A., Johnson, Petrina, Ritchie, Scott R., Kearney, Michael R., 2013. A replicated comparison of breeding-container suitability for the dengue vector *Aedes aegypti* in tropical and temperate Australia: breeding-container suitability for *Aedes aegypti*. *Austral Ecol.* 38 (2), 219–229.
- Rocha, S., Kerdelhué, C., Ben Jamaa, M.L., Dhahri, S., Burban, C., Branco, M., 2017. Effect of heat waves on embryo mortality in the pine processionary moth. *Bull. Entomol. Res.* 107 (5), 583–591.
- Rochlin, Iliya, Faraji, Ary, Ninivaggi, Dominick V., Barker, Christopher M., Marm Kilpatrick, A., 2016. Anthropogenic impacts on mosquito populations in north America over the past century. *Nat. Commun.* 7 (December), 13604.
- Rodrigues, Marianni de Moura, Marques, Gisela Rita Alvarenga Monteiro, Serpa, Lúcia Leandro Nunes, Arduino, Marylene de Brito, Voltolini, Júlio Cesar, Barbosa, Gerson Laurindo, Andrade, Valmir Roberto, de Lima, Virgínia Luna Castor, 2015. Density of *Aedes aegypti* and *Aedes albopictus* and its association with number of residents and meteorological variables in the home environment of dengue endemic area, são paulo, Brazil. *Parasites Vectors* 8 (1), 115.
- Rose, Noah H., Badolo, Athanase, Sylla, Massamba, Akorli, Jewelna, Otoo, Sampson, Gloria-Soria, Andrea, Powell, Jeffrey R., White, Bradley J., Crawford, Jacob E., McBride, Carolyn S., 2023. Dating the origin and spread of specialization on human hosts in *Aedes aegypti* mosquitoes. *eLife* 12 (March). <https://doi.org/10.7554/eLife.83524>.
- Rose, Noah H., Sylla, Massamba, Badolo, Athanase, Lutomia, Joel, Ayala, Diego, Aribodor, Ogechukwu B., Ibe, Nnenna, et al., 2020. Climate and urbanization drive mosquito preference for humans. *Curr. Biol.* 30 (18), 3570–3579.e6.
- Rosewell, J., Shorrocks, B., 2008. The implication of survival rates in natural populations of *Drosophila*: capture-recapture experiments on domestic species. *Biol. J. Linn. Soc.* 32 (4), 373–384. Linnean Society of London.
- Schrama, Maarten, Hunting, Ellard R., Beechler, Brianna R., Guarido, Milehna M., Govender, Danny, Nijland, Wiebe, van 't Zelfde, Maarten, Venter, Marietjie, van Bodegom, Peter M., Gorsich, Erin E., 2020. Human practices promote presence and abundance of disease-transmitting mosquito species. *Sci. Rep.* 10 (1), 13543.
- Searle, S.R., Speed, F.M., Milliken, G.A., 1980. Population marginal means in the linear model: an alternative to least squares means. *Am. Statistician* 34 (4), 216–221.
- Seng, C.M., Jute, N., 1994. Breeding of *Aedes aegypti* (L.) and *Aedes albopictus* (skuse) in urban housing of sibu town, sarawak. *Southeast Asian J. Trop. Med. Publ. Health* 25 (3), 543–548.
- Sota, Teiji, 1993. Response to selection for desiccation resistance in *Aedes albopictus* eggs (Diptera: Culicidae). *Appl. Entomol. Zool* 28 (2), 161–168.
- Sota, T., Mogi, M., 1992. Interspecific variation in desiccation survival time of *Aedes* (stegomyia) mosquito eggs is correlated with habitat and egg size. *Oecologia* 90 (3), 353–358.
- Sternberg, Eleanor D., Thomas, Matthew B., 2014. Local adaptation to temperature and the implications for vector-borne diseases. *Trends Parasitol.* 30 (3), 115–122.
- Suganthi, P., Govindaraju, M., Thenmozhi, V., Tyagi, B., 2014. Survey of mosquito vector abundance in and around tribal residential areas. *Journal of Entomology and Zoology Studies* 2 (December), 233–239.
- Swei, Andrea, Couper, Lisa I., Coffey, Lark L., Kapan, Durrell, Shannon, Bennett, 2020. Patterns, drivers, and challenges of vector-borne disease emergence. *Vector Borne Zoonotic Dis.* 20 (3), 159–170.
- Trotta, Vincenzo, Calboli, Federico C.F., Ziosi, Marcello, Guerra, Daniela, Pezzoli, Maria C., David, Jean R., Cavicchi, Sandro, 2006. Thermal plasticity in *Drosophila melanogaster*: a comparison of geographic populations. *BMC Evol. Biol.* 6 (August), 67.
- Tuholske, Cascade, Kelly, Caylor, Funk, Chris, Verdin, Andrew, Swaney, Stuart, Grace, Kathryn, Peterson, Pete, Evans, Tom, 2021. Global urban population exposure to extreme heat. *Proc. Natl. Acad. Sci. U. S. A.* 118 (41). <https://doi.org/10.1073/pnas.2024792118>.
- Urbanski, Jennifer M., Benoit, Joshua B., Robert Michaud, M., Denlinger, David L., Armbruster, Peter, 2010. The molecular physiology of increased egg desiccation resistance during diapause in the invasive mosquito, *Aedes albopictus*. *Proc. Biol. Sci.* 277 (1694), 2683–2692.
- Vargas, Carolina Martins, Helena, Farnesi, Luana Cristina, Martins, Ademir Jesus, Valle, Denise, Rezende, Gustavo Lazzaro, 2014. Serosal cuticle formation and distinct degrees of desiccation resistance in embryos of the mosquito vectors *Aedes aegypti*, *Anopheles aquasalis* and *Culex quinquefasciatus*. *J. Insect Physiol.* 62 (March), 54–60.
- Vora, Neil, 2008. Impact of anthropogenic environmental alterations on vector-borne diseases. *Medscape Journal of Medicine* 10 (10), 238.
- Ware-Gilmore, Fhallon, Novelo, Mario, Sgrò, Carla M., Hall, Matthew D., McGraw, Elizabeth A., 2023. Assessing the role of family level variation and heat shock gene expression in the thermal stress response of the mosquito *Aedes aegypti*. *Phil. Trans. Roy. Soc. Lond. B Biol. Sci.* 378 (1873), 20220011.
- Weaver, Scott C., Costa, Federico, Garcia-Blanco, Mariano A., Ko, Albert L., Ribeiro, Guilherme S., Saade, George, Shi, Pei-Yong, Vasilakis, Nikos, 2016. Zika virus: history, emergence, biology, and prospects for control. *Antivir. Res.* 130 (June), 69–80.
- Wickham, H., Seidel, D., 2016. Scales: scale functions for visualization. R Package Version, 1.0.0. <https://cran.r-project.org/web/packages/scales/scales.pdf>.
- Zani, Peter A., Swanson, Sarah E.T., Corbin, Drew, Cohnstaedt, Lee W., Agotsch, Marci D., Bradshaw, William E., Holzapfel, Christina M., 2005. Geographic variation in tolerance of transient thermal stress in the mosquito *Wyeomyia smithii*. *Ecology* 86 (5), 1206–1211.