Journal of Thermal Biology 115 (2023) 103594

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Potential for urban warming to postpone overwintering dormancy of temperate mosquitoes

Lydia R. Fyie [a,*], Hannah R. Tronetti [b], Mary M. Gardiner [a], Megan E. Meuti [a]

[a] *Department of Entomology, The Ohio State University, 2021 Coffey Rd, Columbus, OH, USA, 43210*
[b] *Department of Animal Sciences, The Ohio State University, 2029 Fyffe Rd, Columbus, OH, USA, 43210*

ARTICLE INFO

Keywords:
Northern house mosquito
Culex pipiens
Diapause
Urban Heat Island

ABSTRACT

Cities are generally hotter than surrounding rural areas due to the Urban Heat Island (UHI) effect. These increases in temperature advance plant and animal phenology, development, and reproduction in the spring. However, research determining how increased temperatures affect the seasonal physiology of animals in the fall has been limited. The Northern house mosquito, *Culex pipiens*, is abundant in cities and transmits several pathogens including West Nile virus. Females of this species enter a state of developmental arrest, or reproductive diapause, in response to short days and low temperatures during autumn. Diapausing females halt reproduction and blood-feeding, and instead accumulate fat and seek sheltered overwintering sites. We found that exposure to increased temperatures in the lab that mimic the UHI effect induced ovarian development and blood-feeding, and that females exposed to these temperatures were as fecund as non-diapausing mosquitoes. We also found that females exposed to higher temperatures had lower survival rates in winter-like conditions, despite having accumulated equivalent lipid reserves relative to their diapausing congeners. These data suggest that urban warming may inhibit diapause initiation in the autumn, thereby extending the active biting season of temperate mosquitoes.

1. Introduction

Urbanization changes the physical environment and creates unique climates that can influence the physiology of living organisms (Pickett et al., 2011). Urban environments have a more impervious surfaces (e.g., pavement and buildings), less vegetation and a higher prevalence of atmospheric pollutants and particulates. Each of these factors cause urban areas to have higher levels of precipitation, altered temporal patterns of humidity and higher air temperatures (Kuttler, 2008; Pickett et al., 2001). The phenomenon of increased temperatures within cities (densely populated areas characterized by a large percent of impervious surface) compared to their rural counterparts is known as the Urban Heat Island (UHI) effect (Arnfield, 2003; Oke, 1973, 1982). The UHI effect varies in intensity, ranging from a 1–2 °C difference to up to 5–10 °C depending on latitude, the surrounding biome, time of day, and season (Imhoff et al., 2010; Pickett et al., 2001).

The abundance of many insect groups is affected by UHIs (Youngsteadt et al., 2017). For example, bee abundance can decrease with increased urban temperatures (Hamblin et al., 2018), whereas the UHI effect is associated with increased abundance of the cicadas *Cyptotympana atrata* and *Hyalessa fuscata* (Nguyen et al., 2018), the scale insect *Melanaspis tenebricosa* (Dale and Frank, 2014a, 2014b), and several mosquito species (Townroe and Callaghan, 2014). Additionally, increased temperatures due to climate change and urbanization can allow disease vectors, like mosquitoes and ticks, and invasive species to expand into areas that were previously unfavorable (Alto and Juliano, 2001; Buczek et al., 2014; Cordonnier et al., 2020; Gutierrez and Ponti, 2014; Hongoh et al., 2012; Misslin et al., 2016; Musolin, 2012; Parris and Hazell, 2005).

As insects are ectotherms, high temperatures in UHIs are likely to accelerate their development and induce a wide range of physiological stress responses (Ciota et al., 2014; González-Tokman et al., 2020). The UHI effect may also alter the ability of some insects and other arthropods to persist in urban environments. For instance, while the UHI effect increases the rate of development in moth larvae and *Daphnia* (Backe et al., 2021; Brans and De Meester, 2018), increased temperatures cause significant delays in the early development of black widow spiderlings (Johnson et al., 2019). In cities, bee species with lower thermal maxima experience the largest decline in abundance (Hamblin et al., 2017). Aside from differences in thermal tolerance among species, local

---

* Corresponding author.
E-mail address: fyie.1@osu.edu (L.R. Fyie).

https://doi.org/10.1016/j.jtherbio.2023.103594
Received 14 September 2022; Received in revised form 15 May 2023; Accepted 17 May 2023

adaptation can result in intraspecific variation in the heat-tolerance of rural and urban insect populations (Diamond et al., 2017, 2018; Nguyen et al., 2020).

Changes to plant and animal physiology can result in phenological shifts in urban environments (Jochner and Menzel, 2015). Increased temperatures affect the migratory behavior of birds (Tryjanowski et al., 2013), and cause urban plants in temperate environments to flower and leaf-in earlier in spring (Meng et al., 2020; Mimet et al., 2009; Neil and Wu, 2006; Seress et al., 2018). The UHI effect also advances the springtime phenology of ants, butterflies, moths, mosquitoes, dragonflies and damselflies, enabling them to reproduce and reach maximum abundance earlier in the year (Chick et al., 2019; Diamond et al., 2014; Hajdasz et al., 2019; Townroe and Callaghan, 2014; Villalobos-Jiménez and Hassall, 2017). UHIs can also delay the onset of overwintering dormancy in temperate plants such that green leaf canopy in urban environments persists later into the autumn than rural environments (Jochner and Menzel, 2015; Melaas et al., 2016; Zipper et al., 2016). However, we do not fully understand how the UHI effect may influence the seasonal physiology of animals in the autumn. Several studies indicate that increased temperatures due to climate change allow insects to produce additional generations late in the season (Jönsson et al., 2011; Kerr et al., 2020; Stoeckli et al., 2012; Van Dyck et al., 2015). Urban warming also shifts the life history of invasive caterpillars such that they spend more time in their most cold-tolerant larval stage during autumn (Backe et al., 2021). Seasonal patterns of the incidence of vector-borne diseases are directly linked to changes in the seasonal physiology of the insects that transmit their associated pathogens (Fisman, 2012; Peffers et al., 2021). Therefore, the UHI effect could influence the seasonal biology of disease vectors and potentially extend the period of active disease transmission within cities.

Diapause is a programmed state of developmental arrest in insects that allows them to survive unfavorable environmental conditions (Hahn and Denlinger, 2011; Koštál, 2006). In temperate environments, the physiological changes associated with diapause enable insects to survive harsh winter conditions such as low temperatures and food scarcity (Tauber et al., 1986). Diapause is particularly important for the persistence of temperate mosquitoes and the pathogens they transmit, because overwintering mosquitoes can serve as reservoirs for viruses and other pathogens (Andreadis, 2012; Denlinger and Armbruster, 2014; Eldridge, 1987). Urban warming has the potential to create fine-scale local microclimates where insects can more easily survive winter (Ravasi et al., 2018), which could lead to changes in the timing of diapause (Marshall et al., 2020). The Northern house mosquito, *Culex pipiens* (Family: Culicidae), transmits several pathogens in North America, including St. Louis encephalitis virus and West Nile virus (WNV) (Farajollahi et al., 2011; Mitchell et al., 1980). Adult females of *Cx. pipiens* enter a facultative diapause in response to short daylengths and low temperatures (Eldridge, 1968). Diapausing females accumulate lipid reserves (Onyeka and Boreham, 1987), and arrest ovarian development (Sanburg and Larsen, 1973). Diapausing females will also and stop host-seeking and biting vertebrates (Mitchell, 1983). While in diapause, females of *Cx. pipiens* are unable to utilize blood-meals for reproduction, and mosquitoes that are forced to consume vertebrate blood in laboratory settings may instead convert blood into energy reserves to survive the winter (Mitchell, 1983; Mitchell and Briegel, 1989). Higher temperatures in UHIs may postpone the initiation of diapause, thereby extending the period where mosquitoes such as *Cx. pipiens* are biting and transmitting pathogens. Previous studies demonstrate that diapause initiation was inhibited in urban flesh flies, as well as butterflies and moths that were exposed to higher temperatures and light pollution (Merckx et al., 2021; Mukai et al., 2021). However, we lack empirical evidence showing that the temperature differences caused by the UHI effect in the autumn are sufficient to interfere with diapause initiation in mosquitoes.

The objective of the present study was to examine whether relatively small differences in temperatures that are characteristic of the UHI effect in autumn will inhibit diapause initiation in *Cx. pipiens*. To test this, we conducted a laboratory experiment and exposed mosquitoes to a short-day, autumnal photoperiod at low temperatures or at the same photoperiod at slightly higher temperatures (+2 and + 4 °C) that are representative of the UHI effect in temperate environments. We then measured several phenotypic makers of diapause in this species to determine whether females had successfully entered diapause. We also measured whether UHI-reared females are as fecund as those reared in long-day, summer conditions and the long-term, overwintering survival of mosquitoes prevously reared in UHI-conditions when overwintered under the standard (18 °C) temperatureconditions. We hypothesized that relatively small increases in temperature would be sufficient to inhibit diapause initiation in *Cx. pipiens,* and result in adult female mosquitoes with large egg follicles, decreased lipid content, increased blood-feeding frequency, and decreased overwintering survival.

2. Materials and methods

2.1. Insect rearing and temperature treatments

Mosquitoes were obtained from a laboratory colony of *Culex pipiens* (Buckeye strain) established in 2013 from field-collected egg rafts in Columbus, OH. Larvae for the experiments were reared in environmental growth chambers set to a diapause-inducing photoperiod (11.5 h:12.5 h Light:Dark) and one of three temperature treatments: 20 °C, 22 °C, and 24 °C. Our previous work shows that 20 °C and the 11.5:12.5 L:D photoperiod induces diapause in this population (Fyie et al., 2021). Imhoff et al. (2010) have found that temperatures in the urban core (75–100% impervious surface area) are approximately 4 °C higher than the surrounding rural environment (areas near the urban contour with less than 25% impervious surface area) in the “northern temperate broadleaf and mixed forest” ecoregion. This ecoregion represents a distinct biome within a geographical area defined by Olson et al. (2001) and contains cities such as Columbus, OH; Baltimore, MD; and Minneapolis, MN (Imhoff et al., 2010). As our laboratory colony was established from mosquitoes collected in the suburbs of Columbus in 2013, the 4 °C increase was therefore representative of the temperature differences mosquitoes would experience between rural and urban areas. A 2 °C temperature difference was selected as a median to that extreme, and additionally matches the mean daily temperature difference between urban and rural areas near Toledo, OH from September and October (Schmidlin, 1989). Therefore, these temperature differences, while small, are ecologically relevant of the UHI effect that mosquitoes likely experience in this region.

For ovarian development, body size, and lipid content measurements, larvae from 3 to 4 batches of egg rafts (cohorts) were placed in clear plastic rearing containers with 450 mL of reverse osmosis (RO) water (27 × 15.5 × 6 cm; ~220 larvae per container; three containers per cohort, Fig. 1A). Larvae were fed ground Tetramin fish food until pupation. Pupae from each rearing container were allowed to eclose separately into cages (9-11 cages per temperature), and adults were provided cotton wicks with RO water and 10% sucrose *ad libitum*. Wet sponges were placed near the mesh of each adult cage and changed regularly to maintain a relative humidity of approximately 75% within the cage (Robich and Denlinger, 2005). Raw data for egg follicle size, body size, and lipid content from adult mosquitoes reared at 20 °C (diapause control) were previously published in Fyie et al. (2021).

For the blood-feeding, fecundity, and survival experiments, mosquito larvae were reared as described above. However, all pupae were placed in a large cage (30.5 × 30.5 × 30.5 cm) to emerge as adults (Fig. 1B and C). This allowed us to randomly and evenly divide female mosquitoes into smaller cages for subsequent experiments.

For the survival experiments, mosquitoes were obtained from a laboratory colony of *Cx. pipiens* established in 2013 from field-collected egg rafts in Columbus, OH that were outcrossed with wild type mosquitoes collected from the same geographic area in the summer of

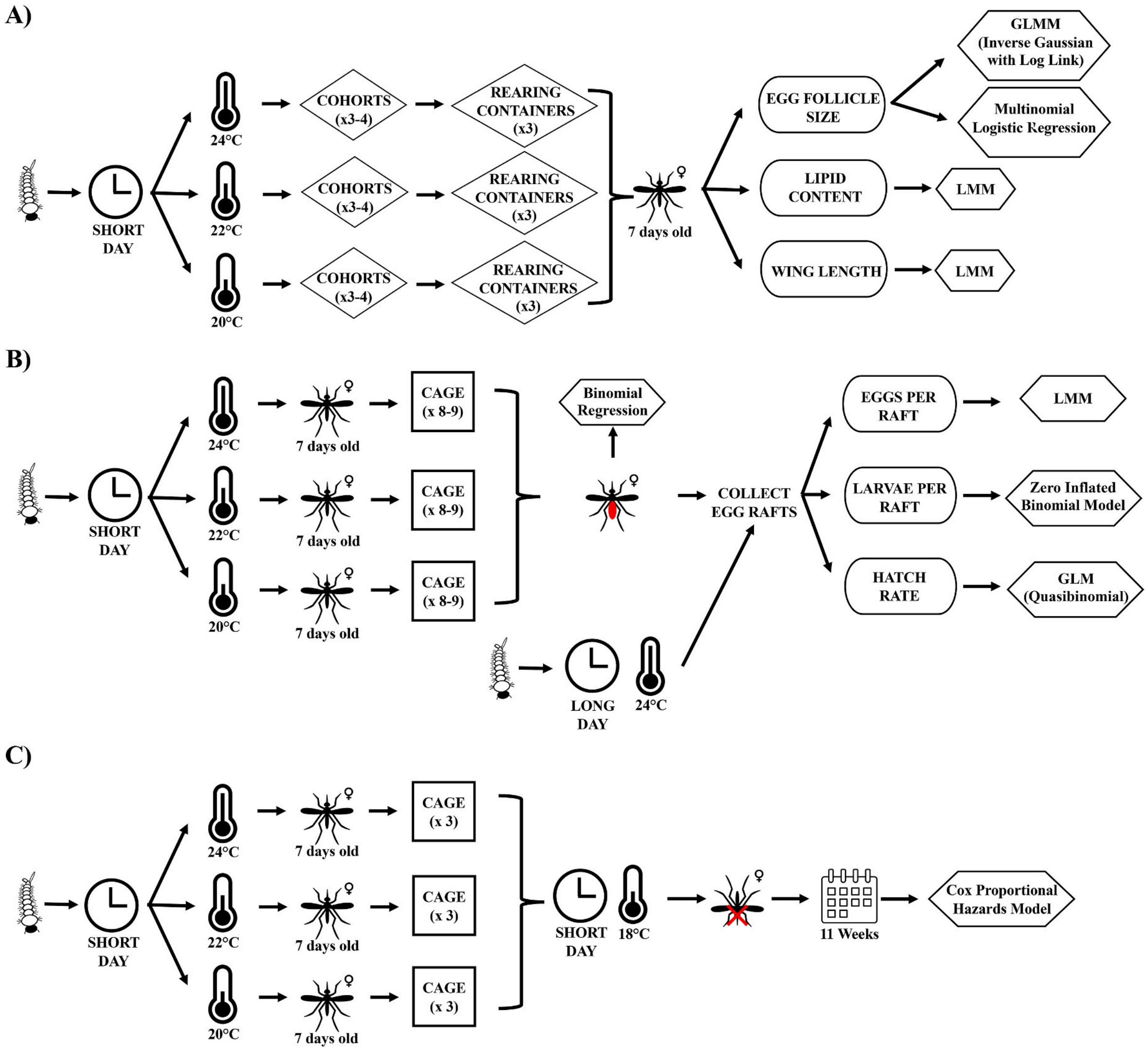

Fig. 1. Schematic describing rearing conditions and experimental design for differences in (a) ovarian development, lipid content, and body size; (b) blood-feeding proclivity and fecundity; and (c) survival. The specific model used to analyze each variable is included in the diagram. GLMM = Generalized Linear Mixed Model; LMM = Linear Mixed Model.

2021. This was done to bolster the colony's genetic diversity. Larvae and adults were reared in the same conditions as described above.

We also assessed phenotypic differences between non-diapausing females and mosquitoes that were exposed to autumnal photoperiods and low (20 °C) and higher temperatures (22 °C and 24 °C). For these comparisons, a set of larvae from the colony outcrossed in 2021 were reared in summer-like conditions (24 °C, 16:8 h L:D, LD24, Fig. 1B). The LD24 group of mosquitoes allowed us to qualitatively compare phenotypic differences between non-diapausing females and mosquitoes that were exposed to autumnal photoperiods and low (20 °C) and higher temperatures (22 °C and 24 °C). However, due to potential differences in genetics after outcrossing, the LD24 group was only included in statistical analyses to assess differences in the fecundity of mosquitoes reared in short day conditions at 22 °C and 24 °C. For these analyses, the fecundity of the non-diapausing, LD24 mosquitoes was used as a control because mosquitoes reared in short day conditions at 20 °C entered diapause and did not produce any offspring.

2.2. *Egg follicle measurements*

Approximately seven days after adult eclosion, ovaries were dissected from females (14-20 per cage, 168-233 total females for each temperature treatment) in 0.9% saline solution (Fig. 1A). The egg follicles were separated with a dissecting needle and measured at 200-fold magnification with an inverted microscope (Nikon). The average egg follicle size was determined by averaging the lengths of 10 egg follicles for each female. Females were classified into one of three categories: "diapause" if the average egg follicle length was <75 μm; "nondiapause" if the average egg follicle size was >90 μm; and "intermediate" if the average egg follicle size was between 75 and 90 μm (Meuti et al., 2015).

2.3. *Blood-feeding assays*

Four days after peak adult emergence, female mosquitoes were randomly sorted into separate cages (35-50 females per cage, 8-9 cages per temperature, Fig. 1B). Sucrose sources were removed ~24 h prior to blood-feeding. Six to nine days after peak adult emergence, mosquitoes were offered a blood-meal consisting of chicken blood spiked with of 0.1 M ATP (~40 μL ATP solution/3 mL of blood) with a Hemotek artificial membrane feeding system (Blackburn, UK) 2 h prior to the onset of scotophase (lights off). After the 2 h feeding period, blood-fed females were identified by the presence of a distended abdomen and blood visible in gut (Sella stage 2; Detinova, 1962; Martínez-de la Puente et al., 2013). Females that had not taken a blood-meal were removed from the cage and counted to determine the proportion of blood-fed females in each cage (number blood-fed/[number blood-fed + number non-blood-fed]).

2.4. Fecundity measurements

Blood-fed females were left in their cages, and a 10% sucrose solution was placed back into the cages. Four days after feeding, oviposition water was placed in cages that contained at least one blood-fed female (6-9 cages per temperature treatment, Fig. 1B). As each female produces a single egg raft (Mpho et al., 2002), we could determine the number of eggs and larvae produced by individual females within each treatment. All egg rafts were collected and placed in individual cups, photographed using at 20× magnification (Leica Microsystems), and then returned to the maternal temperature condition to hatch. The number of eggs in each raft and the number of hatched larvae were subsequently counted and the corresponding hatch rate was calculated (larvae hatched/total eggs in each respective raft) as previously described (Huck et al., 2021).

2.5. Lipid content and body size

One week after adult emergence, the body size of the female mosquitoes was obtained using previously described protocols (Mpho et al., 2000) by measuring the wing length of a subset of 5–15 female mosquitoes used in ovarian dissections per cage (59-68 total females per temperature treatment, Fig. 1A). Photos were taken at 20× magnification (Leica Microsystems) and wing lengths were measured as previously described (Fyie et al., 2021).

The average lipid content was measured in 4–8 females per cage (60-68 total females per temperature, Fig. 1A) using Vanillin-Phosphoric acid assay protocols as previously described (Van Handel, 1985), with minor adjustments that enabled the use of small volumes of mosquito lipid content to be measured using a microplate reader as previously described (Meuti et al., 2015). After determining the lipid content in each individual mosquito, the values were standardized by calculating the ratio of lipid to lean mass (total mass-total lipid content) as previously described (Huck et al., 2021).

2.6. Survival

Mosquitoes were reared from larvae as previously described. Approximately seven days after adult emergence, diapausing (20 °C) and UHI-reared (22 °C and 24 °C) females were transferred into smaller cages (40 females per cage, 3 cages per temperature treatment, Fig. 1C). Adults from each of the three temperature treatments were then placed in a common incubator set to a photoperiod of 8:16 (L:D) and 18 °C and all food sources were removed. This photoperiod, temperature, and lack of food were used to simulate overwintering conditions for diapausing *Cx. pipiens*. A relative humidity of approximately 75% was maintained by placing a wet sponge at each cage and wrapping in clear plastic (Robich and Denlinger, 2005). To further prevent mosquitoes from desiccating, adults were provided RO water *ad libitum*. Survival was measured every 2–3 days for the first 6 weeks, and then once a week for the remaining weeks by counting and removing dead females from the cage. The total number of dead females each week was recorded for analysis. The survival experiment was concluded at 11 weeks (12 weeks after female emergence) as this represents roughly 3 months in diapause and the length of time that diapausing females are kept in our laboratory.

2.7. Data analysis

All statistical analyses were performed in R 4.0.4 using generalized linear models (R Foundation for Statistical Computing, Vienna, Austria). Wing length, lipid content, egg follicle size, number of eggs per raft, and number of larvae hatched per raft were analyzed for normality using an Anderson-Darling test (*nortest* package; Gross and Ligges, 2015). A logarithmic transformation was applied to the ratio of lipid to lean mass to fit normality assumptions (Fig. 1). For all analyses, simple linear models were used to detect outliers which were removed from the statistical analyses and graphical representations of the data (*car* package; Fox and Weisberg, 2019). For data transparency, summary statistics and graphical visualization of all data including outliers where they were removed are presented as supplementary data (Fig. S1, Table S1). We were unable to transform all other data to normality, and thus used generalized linear models with appropriate distributions for the data. For all models, type II analysis-of-variance was performed to generate likelihood ratio test statistics (*car* package; Fox and Weisberg, 2019), and an alpha level of 0.05 was used to determine significance.

Due to the bimodal distribution of the data, differences in egg follicle size were analyzed using a Generalized Linear Mixed-Effect model with an inverse Gaussian distribution and log link as previously described with cage as a random effect (lme4 package; Bates et al., 2015; Fyie et al., 2021, Fig. 1), and post-hoc pairwise comparisons were examined using Tukey's test (*multcomp* package; Hothorn et al., 2008). Differences in diapause status (as classified by egg follicle size) were analyzed using a multinomial logistic regression (*nnet* package; Venables and Ripley, 2002, Fig. 1) and pairwise comparisons were tested using estimated marginal mean contrasts (*emmeans* package; Lenth, 2021).

Differences in the female proclivity to blood-feed and number of egg rafts laid per blood-fed females were analyzed using a binomial logistic regression and post-hoc comparisons were tested using Tukey's test (*multcomp* package; Hothorn et al., 2008, Fig. 1). Photos of egg rafts that were not suitable for counting were removed (i.e. parts of the raft were obscured, 7 out of 397 rafts) and rafts with a calculated hatch rate greater than 1 were also removed from the analysis (11 out of 397 total collected rafts, n = 379 remaining egg rafts that were analyzed). The number of eggs per raft were analyzed using Linear Mixed-Effect Models (*lme4* package; Bates et al., 2015, Fig. 1) with temperature as a fixed effect and cage as a random effect. Due to zero-inflation in the data and overdispersion, differences in the number of larvae produced per female were tested using a zero-inflated negative binomial model (*pscl* package; Zeileis et al., 2008, Fig. 1). As the data were highly variable, comparisons of hatch rate by temperature and photoperiodic condition were analyzed using a Generalized Linear Model with a quasibinomial distribution (Fig. 1). Post-hoc pairwise comparisons for the number of eggs/raft and hatch rate were analyzed using general linear hypotheses with Tukey's test in the *multcomp* package (Hothorn et al., 2008) and using estimated marginal means in the *emmeans* package for larvae number (Lenth, 2021).

Comparisons of significant differences in wing length and lipid content by treatment were analyzed using Linear Mixed-Effect Models (*lme4* package; Bates et al., 2015) with temperature as a fixed effect and cage as a random effect (Fig. 1). Differences in survival times between mosquitoes reared at different temperatures were analyzed using a Cox proportional hazards regression model (*survival* package; Therneau, 2020; Therneau and Grambsch, 2000, Fig. 1). To account for possible variation between cages, cage identity was included in the model as a clustered variable. We found evidence of non-proportional hazards, which can be explained by variation in the risk of death over time (Zhang et al., 2018). To address this, we included a time-dependent coefficient with the interaction of time and rearing temperature (time interval cut at 5 observation weeks when female mosquitoes were 6 weeks old; *timedep* vignette in the *survival* package; Therneau, 2020). Post-hoc pairwise contrasts of the hazard ratio for each temperature over time were analyzed using estimated marginal means with Tukey p-value adjustments (*emmeans* package; Lenth, 2021).

3. Results

3.1. Ovarian development

Exposure to higher temperature stimulated with ovarian development in females of *Cx. pipiens* (Fig. 2). Female mosquitoes that were reared at higher temperatures had larger average egg follicle size (Fig. 2A; Table 1, $p < 0.0001$). Average egg follicle size was 28% larger in females reared at 22 °C (Mean ± SE; 68.15 ± 1.13 μm) than those reared at 20 °C (Mean ± SE; 54.64 ± 0.87 μm) and this difference was significant (Table 1, $p < 0.0001$). Similarly, the average egg follicle size of females reared at 24 °C was 38% larger than those reared at 20 °C (Mean for 24 °C ± SE; 75.40 ± 1.31 μm; Table 1, $p < 0.0001$). Additionally, three individuals (one reared at 22 °C and two at 24 °C) exhibited egg follicles larger than 200 μm, indicative of autogenous egg development. These individuals were identified as outliers and are not included in the egg follicle size analysis but are included in Fig. S1.

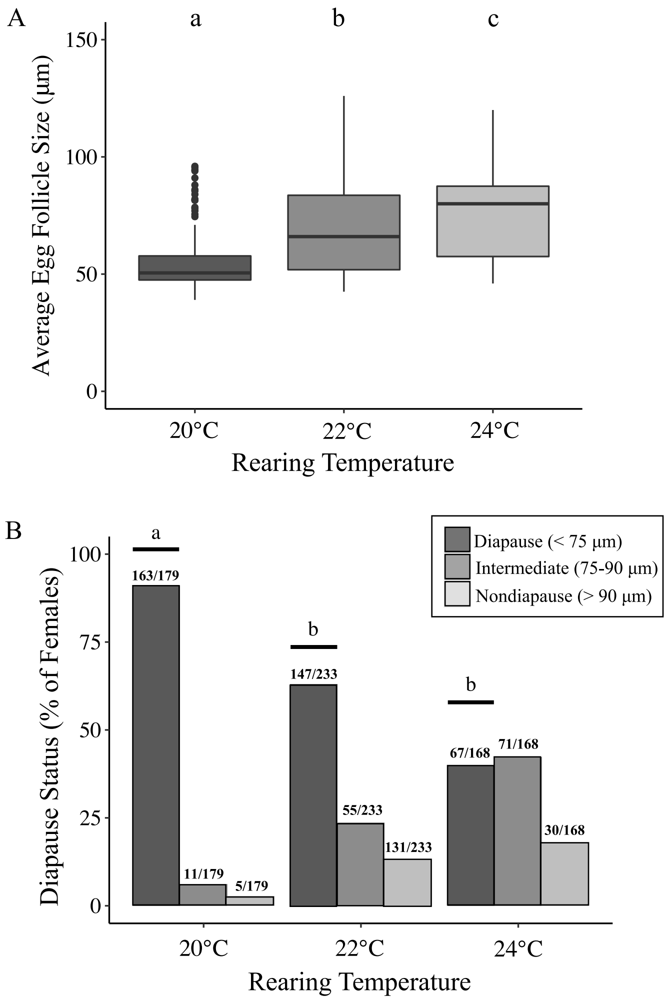

Fig. 2. Exposure to higher temperatures stimulates reproductive development reduces the proportion of females in diapause. (A) UHI temperatures (22 °C and 24 °C) increased average egg follicle size. Significant pairwise differences are indicated by different letters (Tukey Post-Hoc, $p < 0.05$). (B) Percent of individuals in diapause decreased as temperatures increased. Individuals were considered to be in diapause if average egg follicle length was <75 μm, in a nondiapause state if average egg follicle length was >90 μm, and an intermediate state if average egg follicle length was between 76 and 89 μm. The number of individuals in each diapause status are indicated above the bars. Significant pairwise differences in proportion of females in each diapause state are indicated by different letters (EMMs Contrasts, $p < 0.05$). For both panels: 20 °C (n = 179 females), 22 °C (n = 233 females), 24 °C (n = 168 females).

Table 1

Summarization of generalized linear models examining how exposure to increased temperatures influence various phenotypes relating to diapause in *Culex pipiens*. Post-hoc pairwise comparisons are included where the main effect was significant.

Model Parameters	Test Statistic, P value
Average Egg Follicle Size	
(a) Temperature	χ^2 = 72.39, **p < 0.0001**
1) 22 °C	t = 6.17, **p < 0.0001**
2) 24 °C	t = 8.20, **p < 0.0001**
(a) Tukey Contrasts	
1) 22 °C–20 °C	z = 6.17, **p < 0.001**
2) 24 °C–20 °C	z = 8.20, **p < 0.001**
3) 24 °C–22 °C	z = 2.66, **p = 0.022**
Diapause Status	
(a) Temperature	χ^2 = 111.28, **p < 0.001**
1) Intermediate	
a) 22 °C	z = 4.904, **p < 0.001**
b) 24 °C	z = 7.76, **p < 0.001**
2) Nondiapause	
a) Intercept	z = 7.68, **p < 0.001**
b) 22 °C	z = 3.89, **p < 0.001**
c) 24 °C	z = 5.32, **p < 0.001**
(a) Estimated Marginal Means	
1) Diapause	
a) 20 °C/22 °C	t ratio = 6.44, **p = 0.002**
b) 20 °C/24 °C	t ratio = 5.69, **p = 0.003**
c) 22 °C/24 °C	t ratio = 1.27, p = 0.461
2) Intermediate	
a) 20 °C/22 °C	t ratio = −1.75, p = 0.264
b) 20 °C/24 °C	t ratio = −4.41, **p = 0.011**
c) 20 °C/24 °C	t ratio = −1.52, p = 0.349
3) Nondiapause	
a) 20 °C/22 °C	t ratio = −2.17, p = 0.156
b) 20 °C/24 °C	t ratio = −3.94, **p = 0.018**
c) 22 °C/24 °C	t ratio = −0.32, p = 0.950
Blood-Feeding Frequency	
(a) Temperature	χ^2 = 131.91, **p < 0.0001**
1) 22 °C	z = 4.47, **p < 0.0001**
2) 24 °C	z = 9.11, **p < 0.0001**
(b) Tukey Contrasts	
1) 22 °C–20 °C	z = 4.47, **p < 0.001**
2) 24 °C–20 °C	z = 9.11, **p < 0.001**
3) 24 °C–22 °C	z = 8.18, **p < 0.001**
Egg Raft Production Rate	
(a) Temperature	χ^2 = 21.35, **p < 0.001**
1) 22 °C	z = 3.08, **p = 0.002**
2) 24 °C	z = 3.83, **p = 0.0001**
(b) Tukey Contrasts	
1) 22 °C–20 °C	z = 3.08, **p = 0.005**
2) 24 °C–20 °C	z = 3.83, **p = 0.0003**
3) 24 °C–22 °C	z = 1.37, p = 0.339
Number of Eggs per Raft	
(a) Temperature	χ^2 = 6.33, p = 0.097
1) 22 °C	z = 2.31, **p = 0.021**
2) 24 °C	z = 2.46, **p = 0.014**
2) Long Day 24 °C	z = 2.50, **p = 0.013**
Number of Larvae per Raft	
(a) Temperature	χ^2 = 1.46, p = 0.482
1) 24 °C	z = −0.62, p = 0.539
2) Long Day 24 °C	z = 1.053, p = 0.292
Hatch Rate (Larvae/Eggs per Raft)	
(a) Temperature	χ^2 = 6.43, **p = 0.040**
1) 24 °C	z = 1.65, p = 0.099
2) Long Day 24 °C	z = 0.173, p = 0.863
(b) Tukey Contrasts	
1) 24 °C–22 °C	z = 1.65, p = 0.218
2) Long Day 24 °C–22 °C	z = 0.17, p = 0.983
3) Long Day 24 °C–24 °C	z = −2.41, **p = 0.040**
Wing Size	
(a) Temperature	χ^2 = 1.00, p = 0.606
2) 22 °C	z = −0.51, p = 0.613
3) 24 °C	z = −1.00, p = 0.317

(*continued on next page*)

Table 1 (*continued*)

Model Parameters	Test Statistic, P value
Total Lipid Content/Total Lean Mass	
(a) Temperature	$\chi^2 = 1.38$, $p = 0.501$
2) 22 °C	$z = -0.34$, $p = 0.734$
3) 24 °C	$z = -1.15$, $p = 0.251$

Temperature also altered the proportion of individuals in diapause (average egg follicles <75 μm), nondiapause (>90 μm), or in an intermediate state (75–90 μm; Fig. 2B, Table 1, $p < 0.0001$). Although nearly all females reared at 20 °C were in diapause (N = 163/179 females), significantly fewer females reared at 22 °C (N = 147/233 females; Table 1; p = 0.002) and 24 °C (N = 67/168 females; Table 1; p = 0.003) were in diapause. However, only the 24 °C treatment had significantly more females that were in an intermediate state (N = 71/168 females; Table 1, p = 0.011) and nondiapause state (N = 30/168 females; Table 1, p = 0.018) relative to the 20 °C treatment (N = 11/179 females in an intermediate state). In contrast, there is no significant difference in percentage of females that were in an intermediate or nondiapause state relative to those reared at 20 °C (N = 5/179 females in a nondiapause state) and 22 °C (N = 55/233 and 31/233 females respectively; Table 1, $p > 0.05$).

3.2. *Blood-feeding proclivity*

Exposure to higher temperatures induced blood-feeding in females of *Cx. pipiens* (Fig. 3; Table 1, $p < 0.0001$). Mosquitoes reared at 24 °C were significantly more likely to blood-feed than those reared at 20 °C (Table 1, $p < 0.0001$). We found that 48.68% ± 4.21% (Mean ± SE) of females reared at 24 °C took a blood-meal while only 2.08% ± 0.71% (Mean ± SE) of females reared at 20 °C did. At 22 °C, 12.97% ± 2.76% (Mean ± SE) of females took a blood-meal, which was also significantly higher than the percentage of diapausing females that were reared at 20 °C that took a blood meal (Table 1, $p < 0.0001$).

3.3. *Fecundity*

At least some females reared at all temperatures that took a blood-meal laid egg rafts (Table 2). Temperature significantly influenced whether blood-fed females laid egg rafts (Fig. 4A, Table 1, $p < 0.0001$) such that females that took a blood-meal and were reared at 22 °C and 24 °C were more likely to lay eggs than those reared at 20 °C (Table 1, p = 0.005 and p = 0.0003 respectively). We then compared the reproductive output of all females reared at both UHI temperatures (22 °C and 24 °C) to the fecundity of diapausing and non-diapausing mosquitoes that were reared at long days (16:8 L:D) and 24 °C. Females reared at short days and elevated UHI temperatures were as fecund as non-diapausing mosquitoes in all metrics we examined (Fig. 4). The number of eggs in each raft did not differ between any of the temperatures and rearing conditions (Fig. 4B; Table 1, p = 0.097). None of the egg rafts produced by females reared at 20 °C produced larvae, and so this temperature treatment was removed from larval output and hatch rate analyses. Temperatures and photoperiod did not affect the number of larvae per female (Fig. 4C; Table 1, p = 0.482), but did affect the hatch rate, or number of larvae that hatch per eggs laid by an individual female (Fig. 4D Table 1, p = 0.040), such that egg rafts produced by females reared in short day conditions at 24 °C have a significantly higher hatch rate than those reared in long day conditions at the same temperature (66.9 ± 3.6% and 57.0 ± 2.8% [Mean ± SE], respectively; Table 1, p = 0.040).

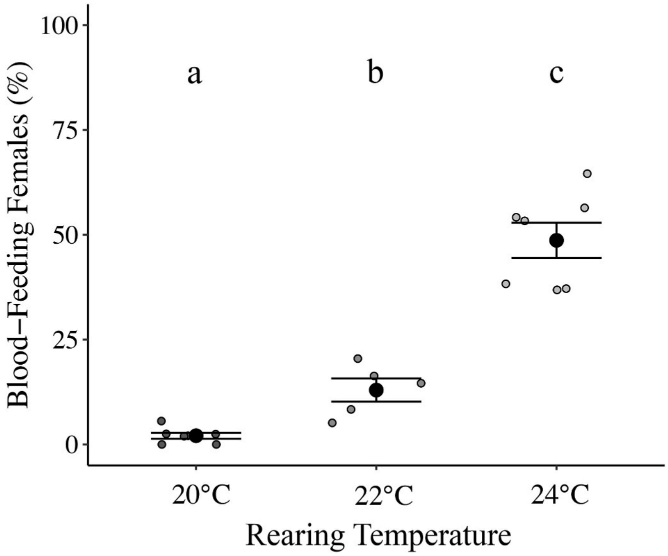

Fig. 3. Females exposed to UHI temperatures are more likely to take a blood-meal. Individual cages containing 35 to 50 female mosquitoes are shown in gray dots. Mean ± SE are shown in black. Significant pairwise comparisons are indicated with different letters (Tukey Post-Hoc, $p < 0.001$).

Table 2
Rates of egg rafts laid per female blood-fed at each rearing temperature.

	Total Egg Rafts/Total Blood-Fed Females	Percent Blood-fed females Producing Egg Rafts per Cage (Mean ± SE) (N = Number of Cages)
20 °C	2/12	5.55% ± 5.55% (N = 6)
22 °C	39/54	75.26 ± 8.70% (N = 6)
24 °C	114/148	81.19% ± 4.58% (N = 7)

3.4. *Mosquito size and lipid content*

The size of female mosquitoes, as measured by wing length, did not differ across the temperatures (Table 1, p = 0.606). Temperature also did not alter lipid accumulation (Fig. 5A; Table 1, p = 0.501). The total percentage of lipid (μg) to lean mass (μg) of females reared at 20 °C, 22 °C, and 24 °C were 11 ± 1%, 12 ± 1% μg, and 10 ± 1% respectively (Mean ± SE).

3.5. *Survival*

Long-term survival in winter-like conditions (18 °C, 8:16 L:D, no food) differed between females that were initially reared in simulated UHI temperatures (22 °C and 24 °C) and those that were initially reared at diapause-inducing temperatures (20 °C; Fig. 5B; Wald test = 310.4, df = 4, $p < 0.001$). From 1 to 5 weeks after the removal of food, the mortality rate for females that were initially reared at 24 °C was 35 times higher than those reared at 20 °C (Table 3, $p < 0.001$). However, the mortality rate for individuals initially reared at 22 °C was not significantly different than that of mosquitoes reared at 20 °C when both groups were exposed to 18 °C (Table 3, p = 0.128). Although females initially reared at 22 °C had similar mortality rates for the first five weeks that they were exposed to 18 °C and the absence of food, their mortality rate for the next six weeks was 6 times greater than diapausing females that had been initially reared at 20 °C (Table 3, $p < 0.001$). The survival rates of mosquitoes that were initially reared at 22 °C and 24 °C that were both exposed to 18 °C without food were significantly different for the first time-block (2–6 weeks old: EMMs, z ratio = −4.77, $p < 0.001$), but during not the second time-block (6–12 weeks old, EMMs, z ratio = 2.40, p = 0.157).

4. Discussion

Previous studies demonstrate that both photoperiod and temperature regulate diapause in *Cx. pipiens* (Eldridge, 1968; Sanburg and Larsen,

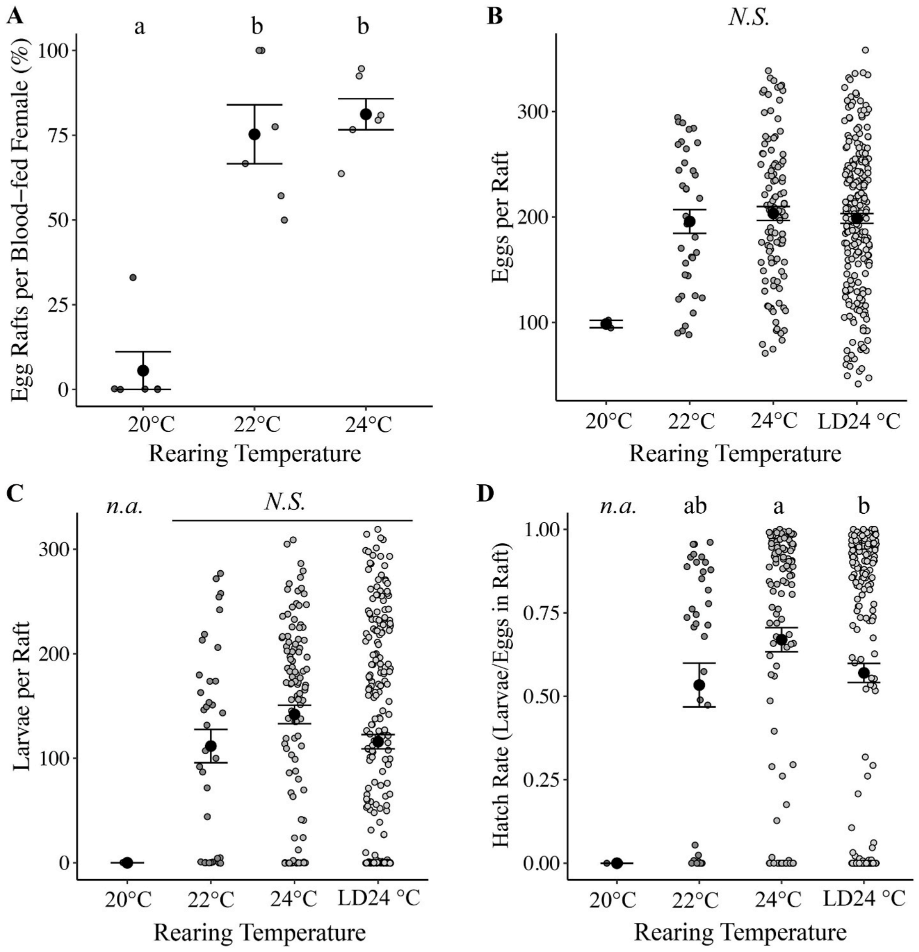

Fig. 4. Females exposed to UHI temperatures are as fecund as non-diapausing mosquitoes (LD24). The reproductive output from individual females (egg rafts) are shown in gray dots and the mean ± SE is designated in black. For all panels, post-hoc significant differences between groups are designated with different lowercase letters, and non-significant differences are indicated by *N.S.* on the figure (Panels A, B, and D: Tukey Post-Hoc, Panel C: EMM Contrasts; α = 0.05). For panels C and D, egg rafts collected from females reared at 20 °C were removed from the analysis as they did not produce any larvae, indicated by *n.a.* on the figure. LD24 females were reared in summer-like, diapause-averting conditions (16:8 Light:Dark, 24 °C).

1973; Spielman and Wong, 1973). Our results are consistent with these foundational studies. For example, Eldridge (1968) found that both short photoperiods and low temperatures are necessary for the suppression of ovarian development and blood-feeding in *Cx. pipiens*, such that females reared at 25 °C had a high percentage of developing ovaries and blood-feeding. However, our study additionally shows that small increases in temperature are sufficient to inhibit diapause. We also found that females reared in elevated temperatures not only fail to suppress ovarian development but are as fecund as long day-reared, nondiapausing females. As other researchers have observed that several species of insects are able to produce additional generations in response to climate change (Kerr et al., 2020; Stoeckli et al., 2012), this suggests that higher temperatures associated with UHIs have the capacity to postpone diapause initiation. Further, urban warming, in combination with other elements of the urban environment like light pollution, appears to inhibit diapause induction in the autumn in both flesh flies and lepidopterans (Merckx et al., 2021; Mukai et al., 2021). Our results highlight that temperature increases alone may be sufficient to inhibit diapause initiation in insects.

This study shows that exposure to slightly higher temperatures caused female mosquitoes to be more likely to blood-feed (Fig. 3), lay eggs, and produce larvae (Fig. 4). Our results indicate that the UHI effect may increase vector-borne pathogen transmission by extending the amount of time mosquitoes are actively biting and thereby prolong seasonal cycles of pathogen transmission. Previous studies also demonstrate that elevated temperatures alter mosquito phenology (Murdock et al., 2017; Reisen et al., 2010). For example, higher winter temperatures lead to earlier springtime *Culex* activity in California (Reisen et al., 2010) and urban warming is expected to enhance the ability of the tiger mosquito, *Aedes albopictus,* to transmit pathogens in the autumn (Murdock et al., 2017). We found that mosquitoes reared in slightly elevated temperatures were able to produce larvae. Therefore, higher temperatures in cities have the potential to increase vector-borne pathogen transmission in the autumn by increasing mosquito proclivity to blood-feed when West Nile virus is most prevalent (Kilpatrick et al., 2006).

Although the diapause state in *Cx. pipiens* consists of both reproductive arrest and lipid accumulation, individuals that are exposed to simulated-UHI temperatures in the presence of short days became reproductively active (Fig. 4) while simultaneously accumulating nutrient reserves (Fig. 5). Although we were not able to statistically analyze lipid accumulation between the UHI-reared females and non-diapausing, long-day females because of differences in their genetic backgrounds and slight differences in the larval feeding protocol, UHI-reared females appear to have slightly higher lipid content than non-diapausing females (Table S2). We found no differences in the fecundity of UHI-exposed females relative to long-day reared, non-diapausing females, indicating that females exposed to short days and high temperatures were somehow able to simultaneously allocate resources to both reproduction and lipid accumulation. These results raise interesting questions about paradigms of metabolic trade-offs during diapause, which may have implications for long-term fitness in these populations (Hahn and Denlinger, 2007).

Diapause is an alternative developmental pathway that is generally considered to be energetically costly, with its own distinct metabolic requirements and demands (Hahn and Denlinger, 2007; Harvey, 1961; MacRae, 2010). Insects that enter adult reproductive diapause invest energy in survival at the expense of reproduction (Hahn and Denlinger,

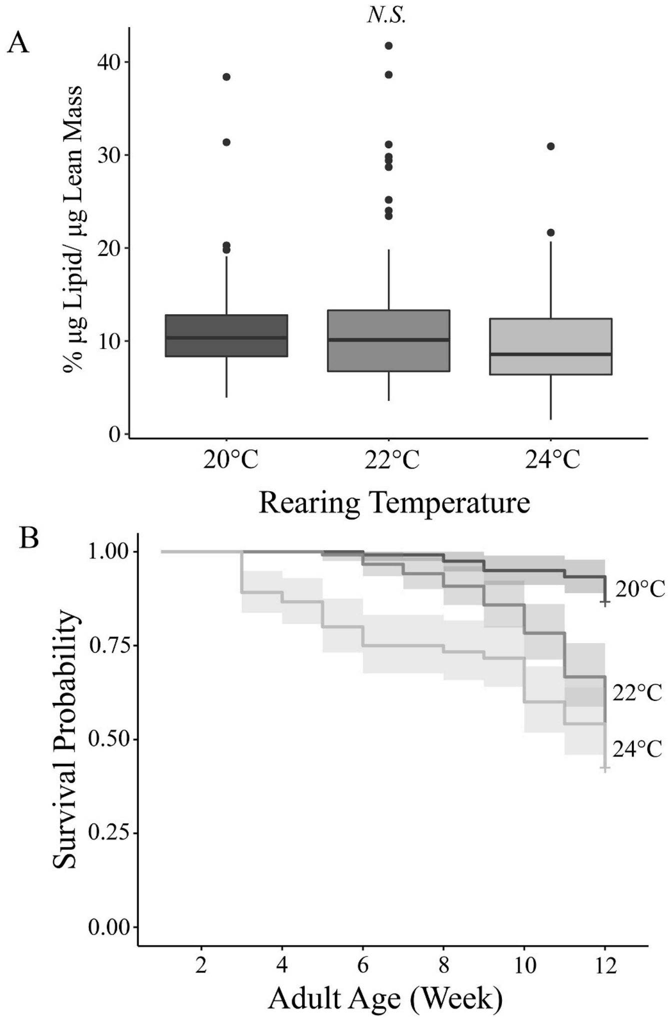

Fig. 5. Female mosquitoes exposed to UHI temperatures (22 °C and 24 °C) accumulate equivalent lipid reserves, but do not survive as long as diapausing females (20 °C). (A) Proportion of lipid to lean mass of each female did not differ significantly among female mosquitoes reared at different temperatures (LMM, $p = 0.501$); 20 °C ($n = 66$ females), 22 °C ($n = 65$ females), 24 °C ($n = 60$ females). Non-significant differences are indicated by *N.S.* on the figure. (B) Kaplan Meier survival curves for female mosquitoes reared under low (20 °C) and UHI temperatures (22 °C and 24 °C) that were transferred to short day conditions at 18 °C (Wald Test, $p < 0.001$); $n = 120$ females for each temperature. 95% Confidence Intervals are shown shaded around each survival curve.

Table 3
Output for individual variables in Cox proportional hazard regression model. For temperature, 20 °C was the reference level. The Wald statistic for each variable is provided by the z-value. Time was split at 6 weeks old (5 weeks of observation) for time-dependent interactions.

	Regression Coefficient	Robust SE	Hazard Ratio (HR) (95% CI)	z-value	p-value
Temp (22 °C) x Time (2–6 wks old)	1.39	0.92	4.02 (0.67-24.18)	1.52	0.128
Temp (24 °C) x Time (2–6 wks old)	3.55	0.84	34.83 (6.73-180.18)	**4.23**	**<0.001**
Temp (22 °C) x Time (7–12 wks old)	1.78	0.11	5.95 (4.78-7.42)	**15.91**	**<0.001**
Temp (24 °C) x Time (7–12 wks old)	1.45	0.15	4.28 (3.20-5.70)	**9.88**	**<0.001**

2011). Diapausing females of *Cx. pipiens* undergo a metabolic shift away from blood-feeding toward sugar-feeding by downregulating genes associated with blood meal digestion and upregulating genes associated with lipid accumulation (Robich and Denlinger, 2005; Sim and Denlinger, 2009). However, in our study, mosquitoes that were exposed higher temperatures and short days were able to digest and utilize blood-meals for reproduction while accumulating high lipid reserves in preparation for overwintering. Although ovarian development and lipid accumulation are seen in tandem in diapausing *Cx. pipiens*, there is evidence that different aspects of their molecular biology may regulate these to two phenotypes separately (Chang and Meuti, 2020; Sim et al., 2015; Sim and Denlinger, 2008, 2009). Photoperiod is the primary cue for diapause induction in this species because it is consistent across years (Denlinger and Armbruster, 2016; Peffers et al., 2021). However, temperature may differentially influence reproductive development and nutrient acquisition, but future studies are needed to uncover whether higher temperatures in cities impact the molecular regulation of diapause *Cx. pipiens* in the autumn. Interestingly, while females reared in elevated temperatures developed their ovaries (Fig. 2) and ingested blood-meals (Fig. 3), truly non-diapausing females reared in long-day, summer-like conditions (L:D: 16:8, 24 °C) appear to have larger egg follicles and a higher blood-feeding frequency (Table S2).

To survive prolonged periods without food diapausing insects in temperate environments must suppress their metabolic rate to preserve energy reserves acquired before diapause (reviewed by Hahn and Denlinger, 2011). Individuals that fail to accumulate sufficient reserves or otherwise inappropriately use energy will either die or opt to terminate diapause early (Hahn and Denlinger, 2011). Energy reserves for diapausing insects are highly restricted (Sinclair, 2015). For example, diapausing females of *Cx. pipiens* lose about 80% of their accumulated lipid reserves and the primary cause of mortality during diapause is premature depletion of these reserves (Onyeka and Boreham, 1987). Metabolic depression, which can reduce the rate at which nutrient reserves are consumed, is nearly universal during diapause and helps overwintering insects persist in the absence of food (Hahn and Denlinger, 2011; Sinclair, 2015). We found that despite acquiring equivalent lipid reserves, female mosquitoes that were reared at higher temperatures as larvae and young adults were not able to survive as long compared to those reared in diapause-inducing conditions when all three groups were subjected to the same low temperature (18 °C) and short-day photoperiodic conditions (L:D 8:16; Fig. 5B). This could be because of differences in the metabolic rates of the mosquitoes, or because of differences in the utilization of lipid reserves. Evidence suggests that *Cx. pipiens*, like other insects, exhibit some level of metabolic depression during diapause (Rozsypal et al., 2021; Zhou and Miesfeld, 2009). Diapausing females of *Cx. pipiens* do not catabolize their lipid reserves until approximately 49 days into diapause, instead slowly catabolizing glycogen beforehand (Zhou and Miesfeld, 2009). Diapausing females also suppress genes associated with fatty acid oxidation in early diapause and upregulate them in late diapause (Sim and Denlinger, 2009). Dynamic long-term monitoring of not only metabolic rate, but also metabolite reserves and gene expression in UHI-exposed females may elucidate how and why UHI-exposed females were not able to survive as long as females reared at lower temperatures. Regardless, our data suggest that although urban female mosquitoes may be able to take advantage of higher autumn temperatures and produce an additional generation, the UHI effect may also represent a developmental trap that prevents urban insects from surviving winter conditions (Kerr et al., 2020; Van Dyck et al., 2015). Notably, UHI-exposed mosquitoes did survive longer than nondiapausing mosquitoes suggesting that the UHI-exposed females accumulated sufficient lipid reserves for short-term survival (Table S2, Fig. S2).

Our results suggest that the UHI effect can postpone diapause initiation for temperate mosquitoes, and open several potential avenues for future research. First, our present study used consistent temperature regimes. However the UHI effect is not consistent over space or time and varies in intensity across large spatial scales due to differences in the surrounding landscape (Imhoff et al., 2010) and temporally such that temperature differences are commonly more extreme at night than during the day (Acero et al., 2013; Huang et al., 2008; Saaroni et al., 2000; Schatz and Kucharik, 2014). While we accounted for spatial variations in the UHI effect in our experiment by selecting temperatures that mimic differences between urban and rural areas within the biome where our mosquitoes originate, we did not account for temporal variation in the UHI effect. Second, urbanization has been shown to lead to rapid evolution and adaption (Diamond et al., 2017, 2018; Vorhees et al., 2013), and the diapause trait is particularly prone to quick evolutionary shifts such that the specific conditions needed to induce diapause can vary dramatically between laboratory and field populations (Batz et al., 2020; Kingsolver and Nagle, 2007). Future studies examining how UHIs affect more recently-established laboratory colonies and field-collected insects from highly urbanized locations could demonstrate the extent to which *Cx. pipiens* can adapt their diapause response to urban warming.

5. Conclusion

Temperature greatly impacts the physiology of insects (González-Tokman et al., 2020), including in the regulation of seasonal responses (Saunders, 2014). Anthropogenic changes to the environment, such as urbanization, can alter temperature patterns and thereby disrupt the seasonal physiology of insects and other animals (Marshall et al., 2020). Overall, we found that exposure to relatively small and consistent increases in temperature (+2 °C and +4 °C) in the presence of short days inhibits diapause initiation in females of *Cx. pipiens*. This corroborates prior studies showing that increased temperatures likely inhibit diapause in flesh flies and moths (Merckx et al., 2021; Mukai et al., 2021). Female mosquitoes reared at higher temperatures were reproductively active, as demonstrated by increased ovarian development, a higher blood-feeding proclivity, and the ability of UHI-exposed females to lay eggs and produce larvae at rates that were similar or higher than non-diapausing females that were reared in summer-like conditions. However, in contrast to our hypothesis, females reared at these higher temperatures accumulated the same levels of lipid reserves as diapausing females that were preparing to overwinter. Yet despite garnering high lipid reserves lipid reserves, females that were reared at increased temperatures were not able to survive without food as long as diapausing females even when both groups were overwintered in the same conditions. Our results suggest that urban warming has the potential to extend the time *Cx. pipiens* is active in the autumn in temperate environments, thereby increasing the risk of vector-borne disease. As temperatures increase with urbanization (UNDESC, 2019; Oke, 1973), it is imperative to consider how the UHI effect will impact the seasonal responses of disease vectors and other insects.

Author statement

Lydia Fyie: Conceptualization, Methodology, Investigation, Formal Analysis, Visualization, Writing – original draft, Writing- Review & Editing, and Funding acquisition. Hannah Tronetti: Investigation, Funding acquisition. Mary Gardiner: Conceptualization, Methodology, Writing – Review & Editing, Supervision. Megan Meuti: Conceptualization, Methodology, Resources, Writing – Review & Editing, Supervision, Funding acquisition.

Funding sources

This work was supported by OARDC SEEDS grants awarded to L.F and M.M.; the Undergraduate Research Apprenticeship Program at The Ohio State University awarded to H.T., NSF IOS grant 1944324 awarded to M.M, and NIH R21-AI144266 awarded to M.M.

Declaration of competing interest

The authors have no competing interests to declare.

Data availability

Data are available in the Figshare public repository (10.6084/m9.figshare.21774860).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2023.103594.

References

Acero, J.A., Arrizabalaga, J., Kupski, S., Katzschner, L., 2013. Urban heat island in a coastal urban area in northern Spain. Theor. Appl. Climatol. 113, 137–154. https://doi.org/10.1007/s00704-012-0774-z.

Alto, B.W., Juliano, S.A., 2001. Precipitation and temperature effects on populations of *Aedes albopictus* (Diptera: Culicidae): implications for range expansion. J. Med. Entomol. 38, 646–656.

Andreadis, T.G., 2012. The Contribution of *Culex pipiens* complex mosquitoes to transmission and persistence of West Nile virus in North America. J. Am. Mosq. Control Assoc. 28, 137–151. https://doi.org/10.2987/8756-971X-28.4s.137.

Arnfield, A.J., 2003. Two decades of urban climate research: a review of turbulence, exchanges of energy and water, and the urban heat island. Int. J. Climatol. 23, 1–26. https://doi.org/10.1002/joc.859.

Backe, K., Rousselet, J., Bernard, A., Frank, S., Roques, A., 2021. Human health risks of invasive caterpillars increase with urban warming. Landsc. Ecol., 0123456789 https://doi.org/10.1007/s10980-021-01214-w.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Software 67. https://doi.org/10.18637/jss.v067.i01.

Batz, Z.A., Clemento, A.J., Fritzenwanker, J., Ring, T.J., Garza, J.C., Armbruster, P.A., 2020. Rapid adaptive evolution of the diapause program during range expansion of an invasive mosquito. Evolution 74, 1451–1465. https://doi.org/10.1111/evo.14029.

Brans, K.I., De Meester, L., 2018. City life on fast lanes: urbanization induces an evolutionary shift towards a faster lifestyle in the water flea *Daphnia*. Funct. Ecol. 32, 2225–2240. https://doi.org/10.1111/1365-2435.13184.

Buczek, A., Ciura, D., Bartosik, K., Zajac, Z., Kulisz, J., 2014. Threat of attacks of *Ixodes ricinus* ticks (Ixodida: Ixodidae) and Lyme borreliosis within urban heat islands in south-western Poland. Parasites Vectors 7, 1–9. https://doi.org/10.1186/s13071-014-0562-y.

Chang, V., Meuti, M.E., 2020. Circadian transcription factors differentially regulate features of the adult overwintering diapause in the Northern house mosquito, *Culex pipiens*. Insect Biochem. Mol. Biol. 121, 103365 https://doi.org/10.1016/j.ibmb.2020.103365.

Chick, L.D., Strickler, S.A., Perez, A., Martin, R.A., Diamond, S.E., 2019. Urban heat islands advance the timing of reproduction in a social insect. J. Therm. Biol. 80, 119–125. https://doi.org/10.1016/j.jtherbio.2019.01.004.

Ciota, A.T., Matacchiero, A.C., Kilpatrick, A.M., Kramer, L.D., 2014. The effect of temperature on life history traits of *Culex* mosquitoes. J. Med. Entomol. 51, 55–62. https://doi.org/10.1038/jid.2014.371.

Cordonnier, M., Bellec, A., Escarguel, G., Kaufmann, B., 2020. Effects of urbanization–climate interactions on range expansion in the invasive European pavement ant. Basic Appl. Ecol. 44, 46–54. https://doi.org/10.1016/j.baae.2020.02.003.

Dale, A.G., Frank, S.D., 2014a. Urban warming trumps natural enemy regulation of herbivorous pests. Ecol. Appl. 24, 1596–1607. https://doi.org/10.1890/13-1961.1.

Dale, A.G., Frank, S.D., 2014b. The effects of urban warming on herbivore abundance and street tree condition. PLoS One 9, e102996. https://doi.org/10.1371/journal.pone.0102996.

Denlinger, D.L., Armbruster, P.A., 2014. Mosquito diapause. Annu. Rev. Entomol. 59, 73–93. https://doi.org/10.1146/annurev-ento-011613-162023.

Denlinger, D.L., Armbruster, P.A., 2016. Molecular physiology of mosquito diapause. In: Advances in Insect Physiology. Elsevier Ltd., pp. 329–361. https://doi.org/10.1016/bs.aiip.2016.05.002

Detinova, T.S., 1962. Age-grouping Methods in Diptera of Medical Importance, with Special Reference to Some Vectors of Malaria. World Health Organization.

Diamond, S.E., Cayton, H., Wepprich, T., Jenkins, C.N., Dunn, R.R., Haddad, N.M., Ries, L., 2014. Unexpected phenological responses of butterflies to the interaction of urbanization and geographic temperature. Ecology 95, 2613–2621.

Diamond, S.E., Chick, L., Perez, A., Strickler, S.A., Martin, R.A., 2017. Rapid evolution of ant thermal tolerance within an urban heat island. Biol. J. Linn. Soc. 121, 248–257. https://doi.org/10.1093/biolinnean/blw047.

Diamond, S.E., Chick, L.D., Perez, A., Strickler, S.A., Martin, R.A., 2018. Evolution of thermal tolerance and its fitness consequences: parallel and non-parallel responses to urban heat islands across three cities. Proc. R. Soc. B Biol. Sci. 285, 20180036 https://doi.org/10.1098/rspb.2018.0036.

Eldridge, B.F., 1968. The effect of temperature and photoperiod on blood-feeding and ovarian development in mosquitoes of the *Culex pipiens* complex. Am. J. Trop. Med. Hyg. 17, 133–140.

Eldridge, B.F., 1987. Diapause and related phenomena in Culex mosquitoes: their relation to arbovirus disease ecology. In: Current Topics in Vector Research, vol. 4. Springer, New York, NY, pp. 1–28. https://doi.org/10.1007/978-1-4612-4712-8_1.

Farajollahi, A., Fonseca, D.M., Kramer, L.D., Marm Kilpatrick, A., 2011. "Bird biting" mosquitoes and human disease: a review of the role of *Culex pipiens* complex mosquitoes in epidemiology. Infect. Genet. Evol. 11, 1577–1585. https://doi.org/10.1016/j.meegid.2011.08.013.

Fisman, D., 2012. Seasonality of viral infections: mechanisms and unknowns. Clin. Microbiol. Infect. 18, 946–954. https://doi.org/10.1111/j.1469-0691.2012.03968.x.

Fox, J., Weisberg, S., 2019. An *R* Companion to Applied Regression, Third. ed. Sage, Thousand Oaks, CA.

Fyie, L.R., Gardiner, M.M., Meuti, M.E., 2021. Artificial light at night alters the seasonal responses of biting mosquitoes. J. Insect Physiol. 129, 104194 https://doi.org/10.1016/j.jinsphys.2021.104194.

González-Tokman, D., Córdoba-Aguilar, A., Dáttilo, W., Lira-Noriega, A., Sánchez-Guillén, R.A., Villalobos, F., 2020. Insect responses to heat: physiological mechanisms, evolution and ecological implications in a warming world. Biol. Rev. 95, 802–821. https://doi.org/10.1111/brv.12588.

Gross, J., Ligges, U., 2015. Nortest: Tests for Normality.

Gutierrez, A.P., Ponti, L., 2014. Analysis of invasive insects: links to climate change. Invasive Species Glob. Clim. Chang. 45–61. https://doi.org/10.1079/9781780641645.0045.

Hahn, D.A., Denlinger, D.L., 2007. Meeting the energetic demands of insect diapause: nutrient storage and utilization. J. Insect Physiol. 53, 760–773. https://doi.org/10.1016/j.jinsphys.2007.03.018.

Hahn, D.A., Denlinger, D.L., 2011. Energetics of insect diapause. Annu. Rev. Entomol. 56, 103–121. https://doi.org/10.1146/annurev-ento-112408-085436.

Hajdasz, A.C., Otter, K.A., Baldwin, L.K., Reudink, M.W., 2019. Caterpillar phenology predicts differences in timing of mountain chickadee breeding in urban and rural habitats. Urban Ecosyst. 22, 1113–1122. https://doi.org/10.1007/s11252-019-00884-4.

Hamblin, A.L., Youngsteadt, E., López-Uribe, M.M., Frank, S.D., 2017. Physiological thermal limits predict differential responses of bees to urban heat-island effects. Biol. Lett. 13, 20170125 https://doi.org/10.1098/rsbl.2017.0125.

Hamblin, A.L., Youngsteadt, E., Frank, S.D., 2018. Wild bee abundance declines with urban warming, regardless of floral density. Urban Ecosyst. 21, 419–428. https://doi.org/10.1136/bmj.1.5645.714-d.

Harvey, W.R., 1961. Metabolic aspects of insect diapause. Annu. Rev. Entomol. 7, 57–80.

Hongoh, V., Berrang-Ford, L., Scott, M.E., Lindsay, L.R., 2012. Expanding geographical distribution of the mosquito, *Culex pipiens*, in Canada under climate change. Appl. Geogr. 33, 53–62. https://doi.org/10.1016/j.apgeog.2011.05.015.

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biom. J. 50, 346–363. https://doi.org/10.1002/bimj.200810425.

Huang, L., Li, J., Zhao, D., Zhu, J., 2008. A fieldwork study on the diurnal changes of urban microclimate in four types of ground cover and urban heat island of Nanjing, China. Build. Environ. Times 43, 7–17. https://doi.org/10.1016/j.buildenv.2006.11.025.

Huck, D.T., Klein, M.S., Meuti, M.E., 2021. Determining the effects of nutrition on the reproductive physiology of male mosquitoes. J. Insect Physiol. 129, 104191 https://doi.org/10.1016/j.jinsphys.2021.104191.

Imhoff, M.L., Zhang, P., Wolfe, R.E., Bounoua, L., 2010. Remote sensing of the urban heat island effect across biomes in the continental USA. Remote Sens. Environ. 114, 504–513. https://doi.org/10.1109/IGARSS.2010.5653907.

Jochner, S., Menzel, A., 2015. Urban phenological studies – past, present, future. Environ. Pollut. 203, 250–261. https://doi.org/10.1016/j.envpol.2015.01.003.

Johnson, J.C., Urcuyo, J., Moen, C., Stevens, D.R., 2019. Urban heat island conditions experienced by the Western black widow spider (*Latrodectus hesperus*): extreme heat slows development but results in behavioral accommodations. PLoS One 14, e0220153. https://doi.org/10.1371/journal.pone.0220153.

Jönsson, A.M., Harding, S., Krokene, P., Lange, H., Lindelöw, Å., Økland, B., Ravn, H.P., Schroeder, L.M., 2011. Modelling the potential impact of global warming on *Ips typographus* voltinism and reproductive diapause. Clim. Change 109, 695–718. https://doi.org/10.1007/s10584-011-0038-4.

Kerr, N.Z., Wepprich, T., Grevstad, F.S., Dopman, E.B., Chew, F.S., Crone, E.E., 2020. Developmental trap or demographic bonanza? Opposing consequences of earlier phenology in a changing climate for a multivoltine butterfly. Global Change Biol. 26, 2014–2027. https://doi.org/10.1111/gcb.14959.

Kilpatrick, A.M., Kramer, L.D., Jones, M.J., Marra, P.P., Daszak, P., 2006. West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. PLoS Biol. 4, 606–610. https://doi.org/10.1371/journal.pbio.0040082.

Kingsolver, J.G., Nagle, A., 2007. Evolutionary divergence in thermal sensitivity and diapause of field and laboratory populations of *Manduca sexta*. Physiol. Biochem. Zool. 80, 473–479. https://doi.org/10.1086/519962.

Koštál, V., 2006. Eco-physiological phases of insect diapause. J. Insect Physiol. 52, 113–127. https://doi.org/10.1016/j.jinsphys.2005.09.008.

Kuttler, W., 2008. The urban climate – basic and applied aspects. In: Urban Ecology. Springer US, Boston, MA, pp. 233–248. https://doi.org/10.1007/978-0-387-73412-5_13.

Lenth, R.V., 2021. Emmeans: Estimated Marginal Means, Aka Least-Squares Means.

MacRae, T.H., 2010. Gene expression, metabolic regulation and stress tolerance during diapause. Cell. Mol. Life Sci. 67, 2405–2424. https://doi.org/10.1007/s00018-010-0311-0.

Marshall, K.E., Gotthard, K., Williams, C.M., 2020. Evolutionary impacts of winter climate change on insects. Curr. Opin. Insect Sci. 41, 54–62. https://doi.org/10.1016/j.cois.2020.06.003.

Martínez-de la Puente, J., Ruiz, S., Soriguer, R., Figuerola, J., 2013. Effect of blood meal digestion and DNA extraction protocol on the success of blood meal source determination in the malaria vector *Anopheles atroparvus*. Malar. J. 12, 109. https://doi.org/10.1186/1475-2875-12-109.

Melaas, E.K., Wang, J.A., Miller, D.L., Friedl, M.A., 2016. Interactions between urban vegetation and surface urban heat islands: a case study in the Boston metropolitan region. Environ. Res. Lett. 11, 054020 https://doi.org/10.1088/1748-9326/11/5/054020.

Meng, L., Mao, J., Zhou, Y., Richardson, A.D., Lee, X., Thornton, P.E., Ricciuto, D.M., Li, X., Dai, Y., Shi, X., Jia, G., 2020. Urban warming advances spring phenology but reduces the response of phenology to temperature in the conterminous United States. Proc. Natl. Acad. Sci. USA 117, 4228–4233. https://doi.org/10.1073/pnas.1911117117.

Merckx, T., Nielsen, M.E., Heliölä, J., Kuussaari, M., Pettersson, L.B., Pöyry, J., Tiainen, J., Gotthard, K., Kivelä, S.M., 2021. Urbanization extends flight phenology and leads to local adaptation of seasonal plasticity in Lepidoptera. Proc. Natl. Acad. Sci. USA 118, e2106006118. https://doi.org/10.1073/pnas.2106006118.

Meuti, M.E., Short, C.A., Denlinger, D.L., 2015. Mom matters: diapause characteristics of *Culex pipiens-Culex quinquefasciatus* (Diptera: Culicidae) hybrid mosquitoes. J. Med. Entomol. 52, 131–137. https://doi.org/10.1093/jme/tju016.

Mimet, A., Pellissier, V., Quénol, H., Aguejdad, R., Dubreuil, V., Rozé, F., 2009. Urbanization induces early flowering: evidence from *Platanus acerifolia* and *Prunus cerasus*. Int. J. Biometeorol.

Misslin, R., Telle, O., Daudé, E., Vaguet, A., Paul, R.E., 2016. Urban climate versus global climate change-what makes the difference for dengue? Ann. N. Y. Acad. Sci. 1382, 56–72. https://doi.org/10.1111/nyas.13084.

Mitchell, C.J., 1983. Differentiation of host-seeking behavior from blood-feeding behavior in overwintering *Culex pipiens* (Diptera: Culicidae) and observations on gonotrophic dissociation. J. Med. Entomol. 20, 157–163. https://doi.org/10.1093/jmedent/20.2.157.

Mitchell, C.J., Briegel, H., 1989. Fate of the blood meal in force-fed, diapausing *Culex pipiens* (Diptera: Culicidae). J. Med. Entomol. 26, 332–341. https://doi.org/10.1093/jmedent/26.4.332.

Mitchell, C.J., Monath, T.P., Sabattini, M.S., 1980. Transmission of St. Louis encephalitis virus from Argentina by mosquitoes of the *Culex pipiens* (Diptera: Culicidae) complex. J. Med. Entomol. 17, 282–285. https://doi.org/10.1093/jmedent/17.3.282.

Mpho, M., Holloway, G.J., Callaghan, A., 2000. Fluctuating wing asymmetry and larval density stress in *Culex quinquefasciatus* (Diptera: Culicidae). Bull. Entomol. Res. 90, 279–283. https://doi.org/10.1017/S0007485300000390.

Mpho, M., Callaghan, A., Holloway, G.J., 2002. Temperature and genotypic effects on life history and fluctuating asymmetry in a field strain of *Culex pipiens*. Heredity 88, 307–312. https://doi.org/10.1038/sj/hdy/6800045.

Mukai, A., Yamaguchi, K., Goto, S.G., 2021. Urban warming and artificial light alter dormancy in the flesh fly. R. Soc. Open Sci. 8, 210866 https://doi.org/10.1098/rsos.210866.

Murdock, C.C., Evans, M.V., Mcclanahan, T.D., Miazgowicz, L., Tesla, B., 2017. Fine-scale variation in microclimate across an urban landscape shapes variation in mosquito population dynamics and the potential of *Aedes albopictus* to transmit arboviral disease. PLoS Neglected Trop. Dis. 11, e0005640 https://doi.org/10.5061/dryad.s3953.

Musolin, D.L., 2012. Surviving winter: diapause syndrome in the southern green stink bug *Nezara viridula* in the laboratory, in the field, and under climate change conditions. Physiol. Entomol. 37, 309–322. https://doi.org/10.1111/j.1365-3032.2012.00846.x.

Neil, K., Wu, J., 2006. Effects of urbanization on plant flowering phenology: a review. Urban Ecosyst. 9, 243–257. https://doi.org/10.1007/s11252-006-9354-2.

Nguyen, H.Q., Andersen, D.K., Kim, Y., Jang, Y., 2018. Urban heat island effect on cicada densities in metropolitan Seoul. PeerJ 6, e4238. https://doi.org/10.7717/peerj.4238.

Nguyen, H.Q., Serret, H., Bae, Y., Ji, S., Chae, S., Kim, Y.I., Ha, J., Jang, Y., 2020. Not all cicadas increase thermal tolerance in response to a temperature gradient in metropolitan Seoul. Sci. Rep. 10, 1343. https://doi.org/10.1038/s41598-020-58276-0.

Oke, T.R., 1973. City size and the urban heat island. Atmos. Environ. 7, 769–779. https://doi.org/10.1016/0004-6981(73)90140-6.

Oke, T.R., 1982. The energetic basis of the urban heat island. Q. J. R. Meterological Soc. 108, 1–24.

Olson, D.M., Dinerstein, E., Wikramanayake, E.D., Burgess, N.D., Powell, G.V.N., Underwood, E.C., D'Amico, J.A., Itoua, I., Strand, H.E., Morrison, J.C., Wettengel, W.W., Hedao, P., Kassem, K.R., 2001. Terrestrial ecoregions of the world: a new map of life on earth. Bioscience 51, 933–938. https://doi.org/10.1641/0006-3568(2001)051.

Onyeka, J.O.A., Boreham, P.F.L., 1987. Population studies, physiological state and mortality factors of overwintering adult populations of females of *Culex pipiens* L. (Diptera: Culicidae). Bull. Entomol. Res. 77, 99–111. https://doi.org/10.1017/S0007485300011585.

Parris, K.M., Hazell, D.L., 2005. Biotic effects of climate change in urban environments : the case of the grey-headed flying-fox (*Pteropus poliocephalus*) in Melbourne. Aust. Now. 124, 267–276. https://doi.org/10.1016/j.biocon.2005.01.035.

Peffers, C.S., Pomeroy, L.W., Meuti, M.E., 2021. Critical photoperiod and its potential to predict mosquito distributions and control Mmedically important pests. J. Med. Entomol. 58, 1610–1618. https://doi.org/10.1093/jme/tjab049.

Pickett, S.T.A., Cadenasso, M.L., Grove, J.M., Nilon, C.H., Pouyat, R.V., Zipperer, W.C., Costanza, R., 2001. Urban ecological systems: linking terrestrial ecological, physical, and socioeconomic components of metropolitan areas. Annu. Rev. Ecol. Systemat. 32, 127–157. https://doi.org/10.1146/annurev.ecolsys.32.081501.114012.

Pickett, S.T.A., Cadenasso, M.L., Grove, J.M., Boone, C.G., Groffman, P.M., Irwin, E., Kaushal, S.S., Marshall, V., McGrath, B.P., Nilon, C.H., Pouyat, R.V., Szlavecz, K., Troy, A., Warren, P., 2011. Urban ecological systems: scientific foundations and a decade of progress. J. Environ. Manag. 92, 331–362. https://doi.org/10.1016/j.jenvman.2010.08.022.

Ravasi, D., Guidi, V., Flacio, E., Lüthy, P., Perron, K., Lüdin, S., Tonolla, M., 2018. Investigation of temperature conditions in Swiss urban and suburban microhabitats for the overwintering suitability of diapausing *Aedes albopictus* eggs. Parasites Vectors 11, 212. https://doi.org/10.1186/s13071-018-2803-y.

Reisen, W.K., Thiemann, T., Barker, C.M., Lu, H., Fang, Y., Lothrop, H.D., 2010. Effects of warm winter temperature on the abundance and gonotrophic activity of Culex (Diptera: Culicidae) in California. J. Med. Entomol. 47, 230–237.

Robich, R.M., Denlinger, D.L., 2005. Diapause in the mosquito *Culex pipiens* evokes a metabolic switch from blood feeding to sugar gluttony. Proc. Natl. Acad. Sci. USA 102, 15912–15917. https://doi.org/10.1073/pnas.0507958102.

Rozsypal, J., Moos, M., Rudolf, I., Košťál, V., 2021. Do energy reserves and cold hardiness limit winter survival of *Culex pipiens*? Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 255, 110912 https://doi.org/10.1016/j.cbpa.2021.110912.

Saaroni, H., Ben-Dor, E., Bitan, A., Potchter, O., 2000. Spatial distribution and microscale characteristics of the urban heat island in Tel-Aviv. Israel. Landsc. Urban Plan. 48, 1–18. https://doi.org/10.1016/S0169-2046(99)00075-4.

Sanburg, L.L., Larsen, J.R., 1973. Effect of photoperiod and temperature on ovarian development in *Culex pipiens pipiens*. J. Insect Physiol. 19, 1173–1190. https://doi.org/10.1016/0022-1910(73)90202-3.

Saunders, D.S., 2014. Insect photoperiodism: effects of temperature on the induction of insect diapause and diverse roles for the circadian system in the photoperiodic response. Entomol. Sci. 17, 25–40. https://doi.org/10.1111/ens.12059.

Schatz, J., Kucharik, C.J., 2014. Seasonality of the urban heat island effect in madison, Wisconsin. J. Appl. Meteorol. Climatol. 53, 2371–2386. https://doi.org/10.1175/JAMC-D-14-0107.1.

Schmidlin, T., 1989. The urban heat island at Toledo, Ohio. Ohio J. Sci. 89, 38–41.

Seress, G., Hammer, T., Bókony, V., Vincze, E., Preiszner, B., Pipoly, I., Sinkovics, C., Evans, K.L., Liker, A., 2018. Impact of urbanization on abundance and phenology of caterpillars and consequences for breeding in an insectivorous bird. Ecol. Appl. 28, 1143–1156. https://doi.org/10.1002/eap.1730.

Sim, C., Denlinger, D.L., 2008. Insulin signaling and FOXO regulate the overwintering diapause of the mosquito *Culex pipiens*. Proc. Natl. Acad. Sci. USA 105, 6777–6781. https://doi.org/10.1073/pnas.0802067105.

Sim, C., Denlinger, D.L., 2009. Transcription profiling and regulation of fat metabolism genes in diapausing adults of the mosquito *Culex pipiens*. Physiol. Genom. 39, 202–209. https://doi.org/10.1152/physiolgenomics.00095.2009.

Sim, C., Kang, D.S., Kim, S., Bai, X., Denlinger, D.L., 2015. Identification of FOXO targets that generate diverse features of the diapause phenotype in the mosquito *Culex pipiens*. Proc. Natl. Acad. Sci. USA 112, 201502751. https://doi.org/10.1073/pnas.1502751112.

Sinclair, B.J., 2015. Linking energetics and overwintering in temperate insects. J. Therm. Biol. 54, 5–11. https://doi.org/10.1016/j.jtherbio.2014.07.007.

Stoeckli, S., Hirschi, M., Spirig, C., Calanca, P., Rotach, M.W., Samietz, J., 2012. Impact of climate change on coltinism and prospective diapause induction of a global pest insect – *Cydia pomonella* (L.). PLoS One 7, e35723. https://doi.org/10.1371/journal.pone.0035723.

Tauber, M.J., Tauber, C.A., Masaki, S., 1986. Seasonal Adaptations of Insects. Oxford University Press, New York, NY.

Therneau, T.M., 2020. A Package for Survival Analysis in R.

Therneau, T.M., Grambsch, P.M., 2000. Modeling Survival Data: Extending the Cox Model. Springer, New York, NY.

Townroe, S., Callaghan, A., 2014. British container breeding mosquitoes: the impact of urbanisation and climate change on community composition and phenology. PLoS One 9, e95325. https://doi.org/10.1371/journal.pone.0095325.

Tryjanowski, P., Sparks, T.H., Kuźniak, S., Czechowski, P., Jerzak, L., 2013. Bird migration advances more strongly in urban environments. PLoS One 8, e63482. https://doi.org/10.1371/journal.pone.0063482.

United Nations Department of Economic and Social Affairs, 2019. World Urbanization Prospects: the 2018 Revision.

Van Dyck, H., Bonte, D., Puls, R., Gotthard, K., Maes, D., 2015. The lost generation hypothesis: could climate change drive ectotherms into a developmental trap? Oikos 124, 54–61. https://doi.org/10.1111/oik.02066.

Van Handel, E., 1985. Rapid determination of total lipids in mosquitoes. J. Am. Mosq. Control Assoc. 1, 302–304. https://doi.org/10.1249/01.mss.0000229105.16274.4b.

Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics with S, Fourth. Springer, New York, NY.

Villalobos-Jiménez, G., Hassall, C., 2017. Effects of the urban heat island on the phenology of Odonata in London, UK. Int. J. Biometeorol. 61, 1337–1346. https://doi.org/10.1007/s00484-017-1311-7.

Vorhees, A.S., Gray, E.M., Bradley, T.J., 2013. Thermal resistance and performance correlate with climate in populations of a widespread mosquito. Physiol. Biochem. Zool. 86, 73–81. https://doi.org/10.1086/668851.

Youngsteadt, E., Ernst, A.F., Dunn, R.R., Frank, S.D., 2017. Responses of arthropod populations to warming depend on latitude: evidence from urban heat islands. Global Change Biol. 23, 1436–1447. https://doi.org/10.1111/gcb.13550.

Zeileis, A., Kleiber, C., Jackman, S., 2008. Regression models for Count data in R. J. Stat. Software 27.

Zhang, Z., Reinikainen, J., Adeleke, K.A., Pieterse, M.E., Groothuis-Oudshoorn, C.G.M., 2018. Time-varying covariates and coefficients in Cox regression models. Ann. Transl. Med. 6 https://doi.org/10.21037/atm.2018.02.12, 121-121.

Zhou, G., Miesfeld, R.L., 2009. Energy metabolism during diapause in *Culex pipiens* mosquitoes. J. Insect Physiol. 55, 40–46. https://doi.org/10.1016/j.jinsphys.2008.10.002.

Zipper, S.C., Schatz, J., Singh, A., Kucharik, C.J., Townsend, P.A., Loheide, S.P., 2016. Urban heat island impacts on plant phenology: intra-urban variability and response to land cover. Environ. Res. Lett. 11, 054023 https://doi.org/10.1088/1748-9326/11/5/054023.