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Light pollution disrupts seasonal reproductive phenotypes and reduces lifespan in the West Nile vector, *Culex pipiens*

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ABSTRACT

Females of the Northern House mosquito, *Culex pipiens*, are important disease vectors as they transmit pathogens including West Nile virus. These females survive the winter by entering diapause, a state of dormancy, characterized by the accumulation of lipids, cessation of blood-feeding, and reproductive arrest. Diapause is cued by photoperiod, so as days become short in late summer and early fall, female *Cx. pipiens* prepare to overwinter and disease transmission decreases. We previously demonstrated that Artificial light at night (ALAN) causes female *Cx. pipiens* to avert diapause and continue to blood-feed when reared under short-day conditions. Additionally, light pollution alters seasonal differences in mosquito activity and nutrient reserves. However, it is unclear how exposure to ALAN affects blood-feeding and fecundity in long-day reared females, as well as the survival of *Cx. pipiens* exposed under both short and long-day conditions. In this study, we hypothesized that females exposed to ALAN in long-day conditions would have a lower proclivity to blood-feed, reduced fecundity, and reduced survival. Results from our lab-based experiments demonstrate that females exposed to ALAN in long-day conditions were less likely to blood-feed but were more fecund than long-day reared females that were not exposed to ALAN, and that ALAN exposure did not affect lifespan of long-day reared females. Additionally, we hypothesized ALAN exposure under short-day conditions would reduce survival, and our data supports this hypothesis. Overall, our results demonstrate that ALAN is an important urban stressor that has the potential to affect reproduction and lifespan in mosquitoes, and therefore has the potential to create evolutionary tradeoffs.

1. Introduction

Light pollution caused by artificial light at night (ALAN) has been shown to negatively impact both plants and animals (reviewed by Falcón et al., 2020). ALAN disrupts natural light:dark cycles that many organisms use to coordinate daily and seasonal activities. Therefore, ALAN can adversely affect organismal behavior and physiology, reduce survival, and alter fecundity (Navara and Nelson, 2007; Sanders and Gaston, 2018; Sanders et al., 2023). For example, birds exposed to ALAN can become reproductively active up to one month earlier (Dominoni et al., 2013) and ALAN can also advance juvenile development in orb-web spiders (Willmott et al., 2018). ALAN also disrupts plant phenology, growth, and resource allocation (Bennie et al., 2016). Additionally, exposure to ALAN disrupts migrations in seabirds and sea turtles, inhibits diel vertical migrations in zooplankton, desynchronizes spawning in corals, and reduces long-term survival in coral reef fish (Marangoni et al., 2022, Schligler et al., 2021). ALAN exposure impacts the physiological processes associated with development and metabolism in insects (reviewed by Desouhant et al., 2019), and there is sufficient evidence to suggest that ALAN poses a risk to insect conservation worldwide (Stewart, 2021).

Mosquito abundance and vector-borne disease transmission is higher in urban areas (LaDeau et al., 2015). This is likely in part because urban areas contain more breeding habitats for container-breeding mosquitoes such as *Culex* and *Anopheles* species (Townroe and Callaghan, 2014). ALAN is also higher in urban areas (Chepesiuk, 2009) and has been shown to increase mosquito biting behavior in day-active, yellow fever mosquitoes (*Aedes aeygpti*; Rund et al., 2020). Therefore, humans and animals that live in urban areas likely face an increased risk of being infected with a mosquito-borne disease (reviewed by Kernbach et al., 2018).

Research suggests that ALAN exposure alters and impairs immune

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function in animals (Durrant et al., 2020; Malek and Haim, 2019; Ouyang et al., 2018). For example, ALAN exposure increased West Nile virus (WNV) titers in house sparrows (Kernbach et al., 2019) and songbirds exposed to ALAN were more likely to carry malaria infections (Becker et al., 2020). Therefore, urbanization can impact immunity in both insect vectors and their vertebrate hosts, and thereby increase disease transmission. However, it is currently unclear how ALAN disrupts the physiological and behavioral processes of insects. Better understanding these impacts will enhance our ability to predict and mitigate seasonal trends in vector-borne disease transmission in urban areas.

Females of the Northern house mosquito, *Culex pipiens*, transmit WNV between birds and mammals (Turell et al., 2005, Molaei et al., 2006). In the fall, females of this species typically enter a dormant overwintering period known as diapause. This period is marked by the cessation of blood-feeding (Bowen et al., 1988) and therefore reproductive inactivity, with an increase in sugar feeding and fat accumulation (Robich and Denlinger, 2005, Sim and Denlinger, 2009). Female mosquitoes preparing for diapause must accumulate sufficient fat reserves to survive winter conditions before resting in overwintering habitats such as cellars or culverts (Rozsypal et al., 2021, Koenraadt et al., 2019). Movement may deplete these energy reserves more rapidly; thus, fat accumulation can be a limiting factor in the length of time female mosquitoes can survive the winter (Rozsypal et al., 2021). The token stimuli to trigger diapause in *Cx. pipiens* are the short days or photoperiods in early fall, that cause female mosquitoes prepare for the winter by gorging on sugar (Robich and Denlinger, 2005), stop seeking vertebrate hosts and therefore no longer transmit mosquito-borne pathogens (Eldridge, 1987).

Our previous laboratory experiments demonstrate that ALAN exposure causes short-day reared females of *Cx. pipiens* to avert diapause (Fyie et al., 2021) and that ALAN disrupts the daily activity of long and short-day reared mosquitoes (Wolkoff et al., 2023). Specifically, female *Cx. pipiens* exposed to ALAN under short-day conditions exhibited large egg follicles and an increased proclivity to blood-feed, demonstrating that they had averted diapause (Fyie et al., 2021). Additionally, ALAN disrupts the daily and seasonal responses of *Cx. pipiens* and allows them to accumulate fat while remaining reproductively active (Wolkoff et al., 2023, Fyie et al., 2021). Our lab has also shown that the circadian clock regulates diapause entry and seasonal trends in fat accumulation (Meuti et al., 2015, Chang and Meuti, 2020), and that ALAN alters the abundance of several transcripts in the circadian clock (Fyie et al., 2024). Additionally, studies on a variety of organisms have shown that ALAN disrupts metabolism and effects lipid accumulation (Rumanova et al., 2022, Levy et al., 2020, Fonken and Nelson, 2014). Taken together, these studies suggest that ALAN disrupts the circadian clock and resource accumulation during diapause and may therefore affect the long-term survival of both diapausing and non-diapausing female *Cx. pipiens*.

Our goal was to determine if the presence of ALAN in long-day, summer-like conditions affects blood-feeding and fecundity of *Cx. pipiens* and to assess whether ALAN reduces the lifespan of both long-day and short-day reared mosquitoes. To do this we reared female mosquitoes under long and short-day conditions with and without ALAN and measured their reproductive capacity and/or survival. We hypothesized that female mosquitoes that were reared under long-day conditions and constantly exposed to ALAN would have a lower proclivity to blood-feed due to their crepuscular activity in absence of ALAN (Veronesi et al., 2012). We also hypothesized that because light pollution decreases reproductive development and survival in other animals (Li et al., 2022; O'Connor et al., 2019), ALAN-exposed females would be less fecund, and have a shorter lifespan than non-ALAN exposed, non-diapausing females. We also hypothesized that females constantly exposed to ALAN in short-day conditions would avert diapause, as evidenced by having large egg follicles even in the absence of a bloodmeal, and a shorter lifespan than non-ALAN exposed, diapausing females.

2. Materials and methods

2.1. Light treatment and insect rearing conditions

Mosquitoes were reared in long-day, diapause-averting (16 hrs light, 8 hrs dark, 18℃) or short-day, diapause-inducing (11.5 hrs light, 12.5 hrs dark, 18℃) conditions either in the presence or absence of ALAN. All growth chambers were fitted with identical lights that emitted ~19000 lx during the photophase, which is equivalent to a cloudy day (Gaston et al., 2013). Both ALAN treatment groups were exposed to "warm white" LEDs (3000 K, Lighting EVER, 1800016-DW-US; Fyie et al., 2024) resulting in a nighttime light exposure at night of ~4 lx as in our previous studies (Fyie et al., 2021, Wolkoff et al., 2023). Mosquitoes were maintained with *ad libitum* access to 10 % sucrose solution and water.

All mosquitoes originated from a laboratory colony of *Cx. pipiens* that was established in 2013 from field-collected egg rafts in Columbus, Ohio (Buckeye strain). In 2021, additional field-collected mosquitoes were added to the colony to increase its genetic diversity. Mosquitoes were reared in clear plastic containers and cages (~220 larvae per container) to allow for full exposure to light. Containers were regularly rotated in the incubator to ensure equal exposure to light across their lifespan (n = 6 containers per each of the 4 treatments resulting in ~1320 larvae/treatment). Three different cohorts of mosquitoes were established for the long-day treatment groups and two cohorts of mosquitoes for the short-day treatment groups. The blood-feeding proclivity and fecundity were measured in female mosquitoes from long-day rearing conditions with and without ALAN. The egg follicle size and diapause status of non-bloodfed females were measured in female mosquitoes from the short-day rearing conditions with and without ALAN. The survival of mosquitoes from all treatment groups (short-day and long-day, with and without ALAN) in the absence of food was also evaluated.

2.2. Blood-feeding proclivity and fecundity

We measured the blood-feeding proclivity of seven-day old female mosquitoes that were reared under long-day conditions with or without ALAN. For cohort one, five cages, each containing fifteen one week-old females, were established for both the ALAN-exposed and unexposed treatments. For cohorts two and three, three cages, each containing fifteen one week-old females, were established for the ALAN exposed and unexposed treatments (n = 11 cages/light treatment total). To promote higher levels of blood-feeding, one day before the blood meals were offered, the 10 % sucrose solution was removed from the cage. Approximately 12 mL of chicken blood (Pel-Freeze Biologicals, Rogers, AR, USA) were mixed with 200 μL 0.1 M ATP, and approximately 3 mL of this blood solution was heated using a Hemotek artificial membrane feeding system (Blackburn, UK) and then offered to mosquitoes. Bloodmeals were offered during the first two hours of scotophase (lights-off), such that ALAN-exposed females were subjected to low levels of light pollution whereas the non-ALAN treatment fed in the dark. Following blood-feeding, the abdomens of females were visually assessed to determine whether they took a full or partial blood-meal (Fyie et al., 2021), and the 10 % sucrose solutions were added to the cages.

Oviposition water was placed in each cage four days after blood-feeding, and females were allowed to oviposit over a course of three days. Each day, egg rafts were collected from both ALAN-exposed and unexposed treatment groups, placed in individual condiment cups (1 oz.), and were allowed to hatch. Photos of each egg raft were taken using the Leica LAS V4.13 microscope program. The number of eggs in each egg raft were counted using the Cell Counter plugin in ImageJ (Mallard et al., 2013). Then, larvae that hatched were preserved in ethanol and later counted to determine the total number of offspring each female produced, as well as the hatch rate (number of larvae/number of eggs, Huck et al., 2021; Fyie et al., 2023). The total number of larvae produced by all the females in each cage (n = 15 females/cage), whether they had blood-fed or not was compared to determine the impact of ALAN on the fecundity of long day-reared mosquitoes. Generally, each female lays a single egg raft, which allows us to determine how many females laid eggs and the number of offspring each female produced.

2.3. *Egg follicle measurements*

We assessed the diapause status of our short-day reared mosquitoes that were not offered a bloodmeal. Notably, the egg follicles of non-blood-fed, non-diapausing females rapidly grow to be ~90 um in length three days after adult emergence whereas the egg follicles of short-day reared, non-blood-fed diapausing females do not grow larger than ~75 um in length (Meuti et al., 2018). Therefore, we collected a subset of females that had been reared in the presence or absence of ALAN. Female mosquitoes were collected seven days post-adult eclosion and euthanized by freezing (n = 10 female mosquitoes from each treatment). Ovaries were dissected in 0.9 % NaCl solution. Egg follicles were separated using a dissecting needle and were measured at 200-fold magnification with an inverted microscope (Nikon). The average egg follicle length (n = 10 egg follicles) was calculated for each female. The diapause status of each female was determined based on the average egg follicle length, such that females were categorized as “diapause” if their average egg follicle length <75 μm, “nondiapause” if their average egg follicle length >90 μm, and “intermediate” if their average egg follicle length was between 75–90 μm (Fyie et al., 2021, Meuti et al., 2015).

2.4. *Survival*

The survival of female mosquitoes from each cohort (n = 2 cohorts for long-day females; n = 2 cohorts for short-day females), and all four treatment groups were analyzed to determine how ALAN impacts the lifespan of mosquitoes in the absence of food. Long-day reared females that were not used for blood-feeding proclivity experiments and short-day reared females that were not used for egg follicle length were placed in cages (n = 147 females for SD ALAN, n = 148 females for LD ALAN, n = 144 females for LD Dark, and n = 94 females for SD Dark). Females were eight days post-adult eclosion at the start of this experiment and were only provided a water source. Dead females (i.e., those that no longer responded to stimuli) were counted and removed every other day, allowing us to measure the median lifespan of each treatment group.

2.5. *Data analysis*

All statistical analyses were performed using R 4.4.0 (R Foundation for Statistical Computing, Vienna, Austria). We used a binomial logistic regression to analyze differences in blood-feeding proclivity and the proportion of a blood-fed females that laid eggs in long-day reared females that were exposed or unexposed to ALAN; both light treatment and cohort were used as explanatory variables (n = 11 replicate cages/treatment). Cages where we collected a greater number of egg rafts than the number of blood-fed females (2/20 cages; likely occurring because the egg rafts had broken apart) were removed from the analysis of the proportion of blood-fed females that laid eggs. To determine if exposure to ALAN affected the number of eggs produced by individual females, we used a negative binomial mixed model with cage as a random effect (*lme4* package, Bates et al., 2015). Next, we determined if there were differences in the number of larvae produced by individual blood-fed females from both light treatments using a zero-inflated negative binomial model (*pscl* package, Zeileis et al., 2008). Due to high variability and overdispersion, we analyzed differences in the hatch rate using a generalized linear model with a quasibinomial distribution.

For all the above analyses, the model residuals were assessed for uniformity and overdispersion using the *DHARMa* package (Hartig, 2022). A type II ANOVA was performed on each model to generate Wald statistics (*car* package, Fox and Weisberg, 2018) and post-hoc multiple comparisons were examined using general linear hypotheses with Tukey’s contrasts (*glht* function, *multcomp* packages, Hothorn et al., 2008).

Finally, because ALAN-exposed females had a lower blood-feeding proclivity but higher fecundity than ALAN-unexposed females, we also compared the reproductive output (total number of larvae produced by all females within a cage) from each of the 11 cages of the ALAN-exposed and unexposed treatments using a Welch’s T-test.

A simple linear model was used to analyze the difference in egg follicle size of short-day reared, ALAN exposed and unexposed females. The model residuals were tested for normal distribution, uniformity, and outliers. A type II ANOVA was performed to test statistical differences between egg follicle size in ALAN exposed versus non-exposed mosquitoes.

The effects of photoperiod and ALAN exposure on female mosquito survival were analyzed using a Cox Proportional Hazards Model (*survival* package, Therneau, 2023; Therneau and Grambsch, 2000), using photoperiod, light treatment, their interaction, and cohort (n = 2–3) as explanatory variables clustered by cage. Post-hoc multiple comparisons of the interaction term were performed using estimated marginal means pairwise contrasts with a Tukey p-value adjustment (*emmeans* package, Lenth, 2023).

3. Results

3.1. *Blood-feeding proclivity and fecundity*

We found that exposure to ALAN impacted blood-feeding behaviors and associated reproduction in long-day reared, female *Cx. pipiens* (Fig. 1). Long day-reared females that were exposed to ALAN had a lower blood-feeding proclivity (24.8 ± 5.2 %; Mean ± SE, n = 11 cages) than the unexposed control group (49.7 ± 5 %; n = 11 cages), and this difference was statistically significant (Fig. 1a; $\chi^2 = 20.18$, $p < 0.001$). Additionally, blood-feeding significantly varied among cohorts ($\chi^2 = 10.2$, $p < 0.01$) such that females from cohort one were significantly more likely to blood-feed than females from cohort three (z = -3.136, $p < 0.01$).

Contrary to our hypothesis, ALAN-exposed females that took a blood-meal were more likely to lay eggs than those that were not exposed to ALAN (Fig. 1b; $\chi^2 = 7.2$, $p < 0.001$). Specifically, 60 ± 13 % of ALAN-exposed females laid egg rafts. In contrast only 39 ± 6.3 % of control females laid eggs following a blood-meal. The proportion of blood-fed females that laid eggs did not significantly vary among cohorts ($\chi^2 = 0.356$, $p > 0.05$). Additionally, females exposed to ALAN had more eggs per raft from those females that were not exposed to ALAN (Fig. S1a: $\chi^2 = 157.7$, $p < 0.001$).

Females exposed to ALAN produced more larvae per blood-fed female than their dark counterparts (Fig S1b: $\chi^2 = 6.8$, $p < 0.01$) such that ALAN-exposed females produced 118 ± 14 larvae compared to control females who produced 65 ± 12 larvae. Additionally, the hatch rate of eggs produced by ALAN-exposed females was significantly higher (70.2 ± 6.6 %), than the hatch rate of eggs produced by females in the dark control group (48 ± 6.9 %, $\chi^2 = 6.09$, $p < 0.05$). Hatch rate significantly varied among cohorts ($\chi^2 = 8.83$, $p < 0.05$) such that the eggs produced by females from cohort three had a significantly higher hatch rate than the eggs produced by females from cohort one (z = 2.43, $p < 0.05$).

Because ALAN reduced females’ proclivity to blood-feed but increased the likelihood that they would lay eggs and that those eggs would hatch, we also compared the total reproductive output of females from each treatment. ALAN-exposed females on average produced 322 ± 100 larvae per cage, whereas the control group on average produced 196 ± 60 larvae per cage. These differences were not statistically significant (T = 1.07, $p = 0.30$), demonstrating that the higher fecundity of ALAN-exposed females compensated for their lower rates of blood-feeding.

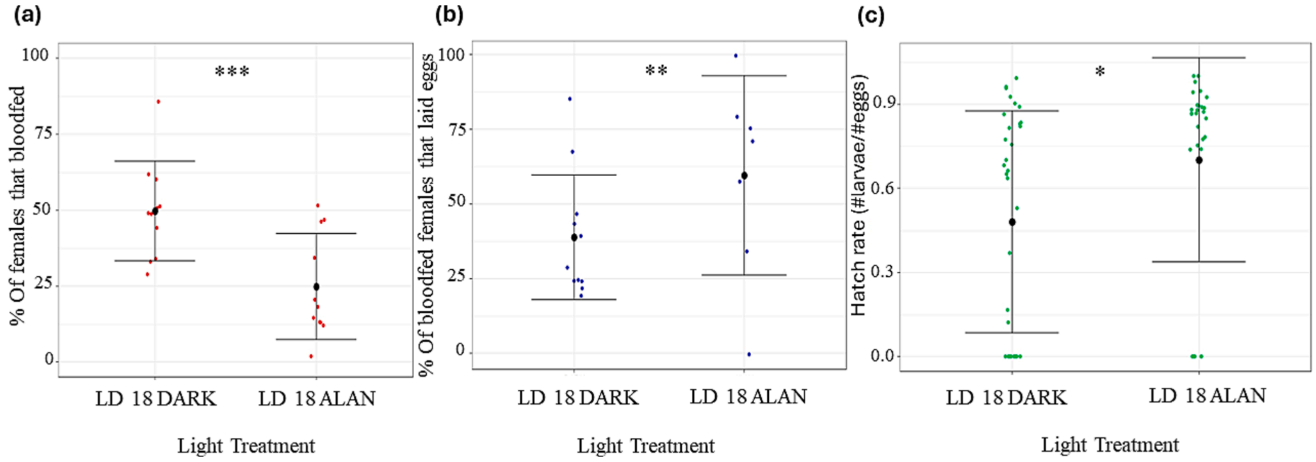

Fig. 1. Exposure to light pollution (ALAN) decreases blood-feeding proclivity (a), increases proportion of females that laid eggs (b), and increases the hatch rate of eggs produced (c) in female *Cx. pipiens*. Mosquitoes were reared under long day conditions (LD; 16 hrs of light 8, hrs of dark) at 18C. For panels (a) and (b), colored dots represent individual cages, each containing 15 females, while the mean ± standard error is represented in black. For panel (c) colored dots represent the number of larvae produced from egg rafts laid by individual females while the mean ± standard error is shown in black. All panels have asterisks indicating significant differences (Tukey's contrasts; *** indicates $p < 0.001$, ** indicates $p < 0.01$, * indicates $p < 0.05$).

3.2. Egg follicle length

Egg follicle measurements in short-day reared, non-blood-fed females were significantly larger in females that had been exposed to ALAN (91.65 μm ± 1.5; n = 10) compared to short-day reared, diapausing females (45.45 ± 2.8; n = 10, Fig. S2, F = 210.92, $p < 0.001$). While all short-day reared mosquitoes that were not exposed to ALAN had small enough egg follicles to be considered in diapause, none of the short-day reared females that had been exposed to ALAN had egg follicles small enough to be considered diapausing (<75 μm), demonstrating that our ALAN treatment caused these females to avert diapause.

3.3. Survival

ALAN exposure increased mosquito mortality as an interaction with photoperiod (Fig. 2, Table 1, z = 7.09, $p < 0.001$), such that ALAN-exposed females in short-day conditions had a higher mortality relative to ALAN-unexposed, diapausing counterparts (Fig. 2, EMMEANS: z ratio = −8.44, $p < 0.001$). However, ALAN exposure in long-day, non-diapause-inducing conditions did not impact mortality rate relative to the long-day reared, dark control (Fig. 2, EMMEANS: z ratio = 0.20, $p = 0.99$). Short-day reared females that were exposed to ALAN had higher mortality rates than both long-day reared mosquitoes with ALAN (EMMEANS: z ratio = −2.93, p = 0.018) and without ALAN (EMMEANS: z ratio = −7.24, p < 0.001). Female mosquitoes reared in short day-conditions without ALAN were less likely to die than mosquitoes reared in long day conditions without ALAN (Fig. 2, z ratio = 5.579, $p <$

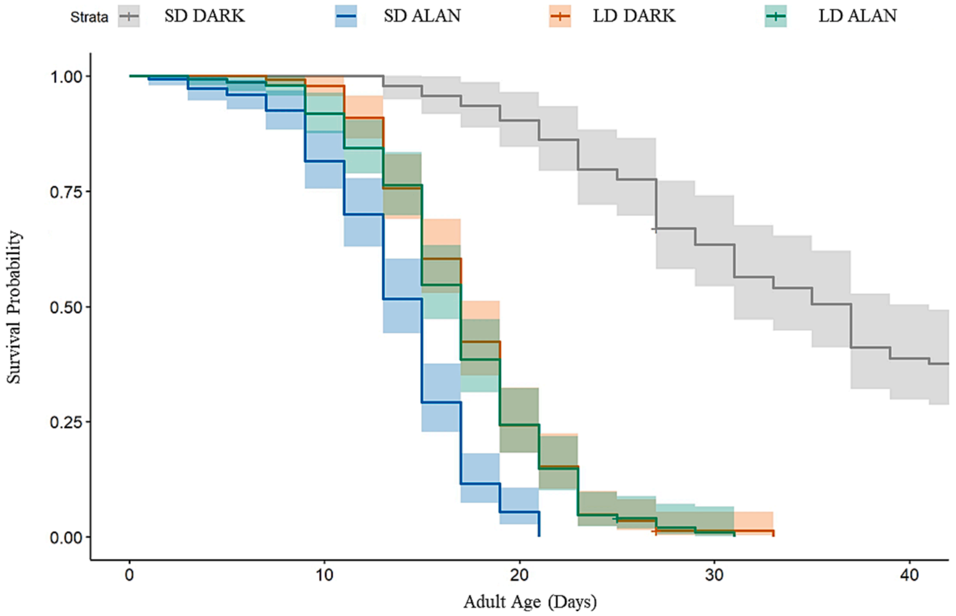

Fig. 2. ALAN reduces mosquito survival under short-day conditions but does not affect survival under long-day conditions. Kaplan Meier survival curves for female mosquitoes reared under short-day (SD; 11.5 hrs light, 12.5 hrs dark, 18℃) and long-day conditions (LD; 16 hrs light, 8 hrs dark, 18℃) either in the presence or absence of light pollution (ALAN and DARK respectively; (n = 147 females for SD ALAN, n = 148 females for LD ALAN, n = 144 females for LD Dark, and n = 94 females for SD Dark). 95 % Confidence Intervals are shown shaded around each curve.

Table 1
Summary for Cox Proportional Hazards Model.

Parameter	Coefficient	Robust SE	Hazard Ratio (HR) (95 % CI)	Test Statistic	p-value
ALAN	−0.036	0.180	0.96 (0.68–1.37)	−0.202	0.840
Photoperiod	−2.180	0.314	0.11 (0.06–0.21)	−6.932	<0.001
ALAN x Photoperiod	2.829	0.400	16.93 (7.74–37.02)	7.087	<0.001
Cohort	0.349	0.152	1.42 (1.05–1.91)	2.295	0.022
Omnibus Wald Test				100.5 (4 df)	<0.001

0.001). There were significant differences between the cohorts of mosquitoes used for the survival analyses, such that females from cohort two had significantly higher mortality rate compared to females from cohort one ($z = 2.295$, $p < 0.05$).

4. Discussion

Urban landscapes and ALAN significantly alter seasonal behaviors associated with diapause in *Cx. pipiens*. Studies from our lab demonstrate that exposure to ALAN in short-day, diapause-inducing conditions causes female *Cx. pipiens* to avert diapause and leads to changes in seasonal blood-feeding behavior, daily activity levels, nutrient composition and the abundance of transcripts encoding circadian clock proteins (Fyie et al., 2021, 2024; Wolkoff et al., 2023). Additionally, previous work in our lab has shown that the circadian clock is linked to the insulin signaling pathway to regulate seasonal trends in lipid accumulation (Meuti et al., 2015, Chang and Meuti, 2020). In long-day conditions, ALAN suppressed carbohydrate and glycogen levels in non-diapausing females, while ALAN exposure under short-day conditions caused diapausing females to have an increase in the variability of lipid content (Wolkoff et al., 2023).

As we hypothesized, the results from our blood-feeding experiments indicated that ALAN suppressed blood-feeding in females that were reared in long-day, diapause-averting conditions (Fig. 1a). This suggests that daily transitions from light to dark are important for blood-feeding (reviewed by Duffield, 2024) and that ALAN disrupts these daily cues. Insect feeding behavior is regulated by the circadian clock (Beck, 1980) and research has shown that *Culex* species primarily feed under dark conditions in the early scotophase (Yee and Foster, 1992). Furthermore, exposure to ALAN under long-day conditions reduced locomotor activity (Wolkoff et al., 2023). The insect circadian clock regulates not only feeding activity, but also locomotor activity, which has been demonstrated in *Drosphila melanogaster* (Konopka and Benzer, 1971) and *Aedes aegypti* (Shetty et al., 2022). Therefore, it is possible that ALAN exposure under long-day conditions lowers the proclivity to blood-feed by preventing the circadian clock to properly track natural light:dark cycles, thereby changing daily locomotor activity and natural feeding rhythms.

Interestingly, our results showed that while the proclivity to blood-feed was lower in ALAN-exposed mosquitoes, females that took a blood-meal were more likely to lay eggs and that those eggs were more likely to hatch (Fig. 1b, c). We also found that the higher fecundity of ALAN-exposed females compensated for their lower blood-feeding rates, such that the total reproductive output of ALAN-exposed and ALAN-unexposed cages of mosquitoes was statistically similar. This is somewhat surprising because *D. melanogaster* exposed to dim constant light had a reduced rate of oviposition and lower overall fecundity (McLay et al., 2017). Therefore, it is possible that light exposure has different effects on reproduction in insect species.

Additional work is needed to better understand why exposure to ALAN reduced blood-feeding but increased the fecundity of blood-fed females. As previously mentioned, our lab showed that ALAN-exposure under long-day conditions reduced carbohydrate and glycogen stores (Wolkoff et al., 2023), and it is therefore surprising that these females produced more offspring as we would expect them to have lower energetic reserves. It is possible that over the course of a female mosquito's lifetime and after several gonotrophic cycles, ALAN-exposed, long-day mosquitoes may indeed produce fewer offspring, but future experiments would be necessary to test this. It is also possible that exposure to ALAN increased the size of the bloodmeal of female mosquitoes, allowing them to produce more eggs. As we did not measure or assess the size of the bloodmeal in our experiments, it is difficult to say if this is the reason why ALAN-exposed females were able to produce more offspring. While the impact of ALAN on blood-meal size has not been examined before, previous studies found that parasitoid wasps, *Venturia canescens*, and several species of moths engage in less feeding behavior or begin feeding later when exposed to nighttime illumination (van Langevelde et al., 2017, Gomes et al., 2021).

Additionally, it is possible that ALAN increased the mating success and/or amount of sperm that male mosquitoes transferred to female mosquitoes. As we did not measure the proportion of females that had sperm in their spermathecae in our experiments, future work is necessary to determine how light exposure affects mating behavior. The effects of ALAN on mating success have largely been examined in nocturnal insects (reviewed in Owens and Lewis 2018, Desouhant et al., 2019). For example, in fireflies and glow-worms attraction to females and both male and female courtship flashing is reduced under ALAN (Firebaugh and Haynes 2016, Owens and Lewis 2022, Kivelä et al., 2023) such that female common glow-worms appear to take longer to successfully mate in the field in light-polluted areas (Van den Broeck et al., 2021). Geometrid moths, *Operophtera brumata*, that are caught near illuminated trees are less likely to have been mated (Van Geffen et al., 2015a), perhaps due to changes in sex pheromone production (Van Geffen et al., 2015b). However, the effects of ALAN on mating success in other arthropods have been more mixed. Female variable field crickets, *Gryllus lineaticeps*, that were exposed to ALAN were more likely to have a spermatophore or sperm-filled spermatheca after copulation (Stahlschmidt et al., 2022). Interestingly, *Drosophila melanogaster* take longer to commence copulation when exposed to ALAN but have no changes to the duration of copulation (McLay et al., 2018), and field crickets, *Teleogryllus commodus*, that are exposed to light pollution take longer to respond to mating calls but had no difference in contacting the source of the call (Thompson et al., 2019). Similarly, ALAN had no effect on the likelihood of mating in the Australian garden orb-web spider (Willmott et al., 2018). Therefore, future studies should examine how ALAN specifically impacts mating success in *Cx. pipiens*.

Our study also analyzed the survival of females exposed to ALAN in both short and long-day conditions. Non-blood-fed, ALAN-exposed females reared in short-day conditions had significantly larger egg follicles than their diapausing counterparts (Supplementary Figure S1), indicating these females averted diapause as we have previously demonstrated (Fyie et al., 2021). As hypothesized, the survival rate of the females in ALAN-exposed short-day conditions that had averted diapause was significantly lower than short-day reared diapausing females that were not exposed to ALAN (Fig. 2). What is somewhat surprising is that ALAN exposure reduced the lifespan of short-day reared females beyond that observed in long-day females that were reared with and without ALAN. Studies have shown that ALAN reduces lifespan in insects and other organisms relative to control groups that were exposed to normal daily light:dark cycles (McLay et al., 2017, Sheeba et al., 2000, Schligler et al., 2021). Notably, however, we found that female *Cx. pipiens* exposed to ALAN under long-day conditions lived just as long as females in the long-day, ALAN-unexposed control treatment. This demonstrates that ALAN exposure differentially affects mosquito lifespan depending on the seasonal context.

There are several possible explanations for why ALAN exposure in short-day conditions markedly reduced female survival. First, previous work in our lab showed that ALAN-exposed, short-day reared females not only averted diapause, but also had lower or more variable levels of lipid reserves as well as increased locomotor activity (Fyie et al., 2021, Wolkoff et al., 2023). Both a lack of energetic resources and behaviors that cause mosquitoes to burn through their reserves more quickly would be expected to reduce lifespan, especially when the females were denied access to food as in this laboratory-based study. Additionally, short-day reared, ALAN-exposed females may have perished quickly because they became reproductively active. The effects of ALAN and urbanization are associated with evolutionary tradeoffs between reproduction and survival in overwintering insects, such that for some insect's urbanization reduces these tradeoffs whereas in others it increases them (reviewed by Meuti et al., 2024). Our data suggest that the continued reproductive activity of short-day reared *Cx, pipiens* that are exposed to ALAN likely comes at a high cost to their survival.

Furthermore, we found that ALAN did not significantly impact the survival of long-day reared females, even though our data suggest that ALAN-exposed females likely devoted more energetic reserves to reproduction as they were more likely to lay eggs after blood-feeding and those eggs hatched at a higher rate. Although Paranjpe and Kumar Sharma (2005) found that exposure to constant light accelerated developmental timing of *D. melanogaster* without affecting lifespan, Kouser et al. (2014) found that exposure to constant light reduced both lifespan and fecundity in *D. melnaogaster*. It is therefore unclear precisely what effects constant light exposure can have on insects, and especially the low levels of light that we used in our study. Future studies are necessary to determine why ALAN exposure affects lifespan differently depending on the seasonal context and why it has such a significant and negative impact on the lifespan of short-day reared mosquitoes.

Additionally, future studies should address the effects of ALAN on fecundity and survival under long and short days in the field. In this laboratory-based study, mosquitoes were reared and kept under constant exposure to ALAN. However, it is unlikely in the field that mosquitoes would remain continuously exposed to ALAN after emerging as adults. Diapausing mosquitoes overwinter in caves and culverts, therefore, the constant and unescapable exposure to light that they were subjected to in our laboratory study may have been particularly detrimental to their survival. An additional potential weakness is that blood meal size was not accounted for when measuring reproductive outputs. Since blood meal size is a critical factor for female fecundity, future studies should address how blood meal size impacts reproductive output in ALAN exposed mosquitoes. Future studies should also address if mating status and insemination rates are the same between ALAN exposed and unexposed females to determine if this plays a role in the hatching rate differences observed.

5. Conclusions

Overall, this work characterized how ALAN exposure impacts blood-feeding proclivity, fecundity of female *Cx. pipiens* reared under long-day conditions, as well as the survival of long and short-day reared females. These findings add to the growing body of research on how ALAN disrupts organismal behaviors, physiology, and long-term evolutionary fitness. Our results show that ALAN significantly disrupts daily and seasonal aspects of behavior and physiology in the West Nile vector, *Cx. pipiens*. Additionally, our results show that ALAN may not have negative effects on mosquito reproduction. If mosquito populations are just as abundant in light-polluted areas, but levels of viruses are higher in birds and other reservoirs, this may contribute to the perfect conditions for the transmission of mosquito-borne illnesses in cities. Future work should examine the mechanisms by which ALAN increases the fecundity of blood-fed, long-day reared females while reducing the survival of short-day reared females. Better understanding how ALAN impacts the evolutionary fitness of *Cx. pipiens* in the urban landscape could inform mosquito surveillance and better direct control measures where and when needed most to limit vector-borne disease transmission.

CRediT authorship contribution statement

Maria A. Fiorta: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Lydia R. Fyie:** Writing – review & editing, Visualization, Formal analysis, Conceptualization. **Megan E. Meuti:** Writing – review & editing, Resources, Project administration, Funding acquisition, 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 data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jinsphys.2024.104725.

Data availability

Data will be made available on request.

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