Artificial light at night has life stage-specific effects on biological rhythms in a specialist insect

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Abstract

Artificial Light at Night (ALAN) has infiltrated many once-dark nightscapes, introducing a novel cue on biological clocks. Recent research has uncovered effects of ALAN on behavior, physiology, and fitness across taxa. However, questions remain about ALAN's effects on key biological rhythm functions like diapause and sleep, and how disruptions to these rhythms can be linked to fitness declines. Further, most studies test effects of ALAN at the adult stage only, but little is known about the specific effects of ALAN across multiple life stages. We measured the outcomes of ALAN exposure on diapause termination (i.e., adult eclosion), sleep-wake cycles, and fitness-related traits (i.e., survival, body size, and fecundity) in a specialist insect: the walnut husk fly (*Rhagoletis juglandis*). We tested for life-stage specific effects of ALAN by implementing four experimental groups: pupal ALAN exposure only, adult ALAN exposure only, pupal and adult ALAN exposure, and pupal and adult dark night exposure (control). We found that pupal flies exposed to ALAN eclosed later than dark night pupae. Also, all adult flies exposed to ALAN had altered sleep-wake cycles compared to dark night adults. Further, flies that were exposed to ALAN as pupae only slept more during the transition from day to night compared to control flies. Finally, we found no fitness effects of ALAN. This work highlights the ability of ALAN to impact crucial functions like diapause and sleep, including through pupal exposure that has lasting effects into adulthood.

Keywords: artificial light at night, sleep, behavior, circadian rhythms, phytophagous insect

Introduction

Life on Earth has long experienced consistent rhythms of light intensity, with nights of virtually zero lux and days that average a few thousand lux (Rich and Longcore 2013). These daily oscillations, together with seasonal variations, have orchestrated the evolution of the biological clock (Helm et al. 2017). This internal system is regulated by external environmental cues, or *zeitgebers*—the most potent of which is light (Aschoff 1965). Importantly, biological clocks allow organisms across taxa to synchronize their biology with predictable changes to the light environment, resulting in biological rhythms that regulate crucial functions (Aschoff 1989). For example, circannual (seasonal) rhythms are synchronized with seasonal changes in photoperiod (i.e., the relative length of day and night) and drive functions on a broad timescale, like diapause, migration, and molting. Additionally, circadian (daily) rhythms are synchronized with the 24-hour light cycle and drive daily functions, like sleep-wake cycles, singing, and enzyme activity (Gaston et al. 2017). These functions are inextricably tied to fitness; for example, experimental manipulation of photoperiod can result in diminished reproductive fitness in birds (i.e., smaller hatching size) (Clark and Reed 2012).

In the face of urbanization, previously dark nightscapes have been infiltrated by up to 100 lux of artificial light at night (ALAN) (Rich and Longcore 2013), introducing a novel stimulus on biological clocks. ALAN encompasses streetlights, security lights, and other outdoor lighting employed by humans for safety and entertainment (Aulsebrook et al. 2018). Many environments in and around urban areas rarely, if ever, experience completely dark nights (Bennie et al. 2015), and ALAN prevalence continues to increase worldwide by ~3-5% annually (Hölker et al. 2010). Recent research has revealed many consequences of ALAN on behavior, physiology, and fitness across taxa. For example, experimental exposure to ALAN has had negative effects on mammalian foraging behavior (Hoffmann et al. 2022), plant physiology (Wei et al. 2023), and insect reproduction (Gomes et al. 2024).

Not surprisingly, ALAN is also known to disrupt biological rhythm functions, including seasonal processes like diapause. Diapause is a type of dormancy that allows organisms to halt development in order to survive adverse seasonal conditions (Tauber and Tauber 1976). In temperate zones, diapause entry is often cued by shortened days (i.e., autumn), while diapause termination is often cued by lengthened days (i.e., spring) (Meuti and Denlinger 2013). Animals may perceive ALAN as an extension of day length, and ALAN has been shown to reduce diapause initiation (Westby and Medley 2020) (Merckx et al. 2023). However, little is known about the impact of ALAN on diapause termination.

ALAN is also known to disrupt daily processes, such as sleep-wake cycles. For example, experiments in nocturnal rats show that they shift daytime sleep to nighttime in the presence of ALAN (Stenvers et al. 2016). However, ALAN's impact on sleep is a critical but understudied area given the central role of sleep in regulating physiology and behavior (Aulsebrook et al. 2018). Research on ALAN and sleep remains incomplete and sometimes contradictory (Aulsebrook et al. 2018). Species responses vary widely, even among close relatives[18], and little is known about how ALAN affects the sleep of diurnal animals—particularly invertebrates, whose sleep in general is poorly understood (Lesku et al. 2019).

Disruptions to biological rhythms can trigger cascading effects on fitness, making it essential to examine both immediate and downstream consequences. Although fitness costs of disrupted biological rhythms are often assumed (i.e., decreased sleep leading to decreased fitness), this relationship remains poorly understood, especially in the context of ALAN (Aulsebrook et al. 2018). There is an increasing focus on investigating ALAN's role in disrupting fitness-related traits, particularly in invertebrates, with declines in survival (McLay et al. 2017), body size (Fuse et al. 2024), and fecundity (Jiang et al. 2023). However, effects of ALAN are inconsistent, as ALAN has also been linked with increased survival (Gomes et al. 2024). Further research across diverse taxa is therefore needed to clarify the fitness consequences of ALAN and the links between biological rhythm disruptions and fitness costs.

Insects present a valuable model for investigating how ALAN influences organisms across development and in different ecological contexts. The effects of ALAN may change across life stages due to differences in exposure or sensitivity. For instance, insects often have sedentary early life stages (i.e., pupae), followed by highly mobile later stages (i.e., adult) that offer them an opportunity to escape challenging conditions. Also, there are known critical periods in development at which certain light environments yield irreversible effects. For example, in *Drosophila melanogaster*, a lack of light during pre-adult development leads to smaller brain size and altered adult behavior (Damulewicz et al. 2022). Further, many ALAN studies are conducted on adults only, with little attention given to ALAN's distinct effects on other life stages. Thus, there is a large gap in our understanding of the life stage-specific effects of ALAN.

Here, we tested the effects of ALAN on biological rhythm functions and fitness across multiple life stages in the walnut husk fly, *Rhagoletis juglandis*. First, we tested whether ALAN impacts two functions that are dictated by biological rhythms: diapause termination (i.e., adult eclosion) and sleep-wake cycles. *R. juglandis* larvae emerge from infested walnuts in the fall, then they pupate and enter diapause in the winter. As temperatures and daylengths increase in the spring, diapause is terminated and pupae develop into adults and eclose. Temperature is the primary cue for diapause initiation and termination in *Rhagoletis*, but they are known to eclose earlier when exposed to longer day lengths (Feder et al. 2010). Thus, we hypothesized that flies perceive ALAN as a longer day length, so we expected that flies under ALAN would eclose earlier. We also hypothesized that ALAN shifts sleep-wake cycles, so we expected that flies under ALAN would sleep less at night and more during the day, and potentially less overall. We also tested whether ALAN impacts fitness by observing three fitness-related traits: survival to sexual maturity, adult body size, and female fecundity. We hypothesized that ALAN decreases fitness, so we expected that exposure to ALAN would result in declines in survival, body size, and fecundity.

Finally, we tested for ALAN's effects on sleep-wake cycles and fitness with exposure at two distinct life stages: post-winter pupa and adult. We hypothesized that ALAN exposure at only the pupal stage alters adult phenotype, indicating carryover effects of early-life light exposure. Thus, we expected that flies exposed to ALAN as pupae only would eclose earlier than those exposed to dark nights, as well as have altered sleep-wake cycles and declines in body size, survival, and fecundity. Next, we hypothesized that ALAN exposure at only the adult stage directly disrupts circadian-regulated behaviors and leads to reduced fitness compared to controls. Thus, we expected that flies exposed to ALAN as adults only would have altered sleep-wake cycles and declines in fitness-related traits. Next, we hypothesized that continuous exposure to ALAN across both pupal and adult stages produces additive or synergistic effects, so we expected these flies to have the strongest deviations in behavior and/or fitness traits relative to controls. Finally, we hypothesized that individuals reared entirely in dark night conditions maintain baseline biological rhythms and fitness measures, so these "no ALAN" flies acted as our control. Overall, our results will deepen understanding of ALAN's role in impacting key biological rhythm functions and fitness in the face of increasing urbanization. Our findings will especially contribute to our knowledge of ALAN's effects on organisms across life stages.

Materials and Methods

Insect collection and rearing

We collected fallen Arizona walnuts (*Juglans major*) infested with *Rhagoletis juglandis* larvae from Southwestern Research Station in Portal, Arizona (31.883577, -109.205775), on August 17-18, 2023. We brought the walnuts back to our lab at the University of Louisville to collect larvae as they naturally emerged from the fruit. We placed collected larvae in plastic containers with damp vermiculite to mimic natural soil conditions. To allow larvae to pupariate, we housed them in a Percival incubator (model # I36VLC9) for 28 days at standard rearing conditions of 22°C and a 14:10 L:D cycle (Berlocher and Enquist 1993). We then changed the incubator settings to 4°C 0:24 L:D cycle to simulate winter. We removed pupae from winter conditions after 11 months, and randomly assigned groups of 50 pupae into one of two Percival incubators (see *Light treatments* below). We checked pupae daily for eclosed adults. We housed same-sex groups of three to six flies that eclosed on the same day in 32 oz. plastic containers (11.43 x 13.97 cm). Flies had access to water and food (3:1 sucrose:hydrolyzed yeast) *ad libitum*.

Light treatments

We used two Percival incubators to create the light treatments. Both incubators were configured to the standard 14:10 L:D cycle at 22°C. During the day, both incubators were exposed to the same light conditions (approximately 1400lx, equivalent to an overcast day). During the night, retro-fitted LED lights (UTILITECH) lit the ALAN incubator (80lx, equivalent to bright urban night lighting), while the dark night incubator was not lit (0lx).

To assess the impacts of ALAN at two distinct life stages—post-winter pupa (hereafter, “pupa”) and adult—we used four experimental groups: pupal ALAN exposure only, adult ALAN exposure only, pupal and adult ALAN exposure, and pupal and adult dark night exposure (control). Pupae were randomly assigned to ALAN or dark night conditions, and as adults eclosed, they were randomly assigned to ALAN or dark night conditions.

Effect of ALAN on eclosion timing

To measure ALAN’s impact on eclosion timing, we recorded the number of days it took for flies to eclose as adults after being moved into spring temperatures (n = 1072). Specifically, we checked pupal flies daily and recorded the day that each individual eclosed. We discarded pupae that did not eclose (n = 278).

Effect of ALAN on sleep-wake cycles

To quantify ALAN’s impact on sleep-wake cycles, we used a Locomotor Activity Monitoring System (TriKinetics Inc, Waltham, MA, USA) to track percent sleep over time. The system contains the following components: data collection computer, power supply interface unit (model # PSIU9), activity monitors (model # LAM29), 25 mm diameter × 125 mm long Pyrex glass tubes (model # PGT25x125), and caps for tubes (model # CAP25-BLK). We used four activity monitors (one for each experimental group). Each monitor has 32 independent activity channels, which measure activity by using infrared beams and sensors.

We randomly selected flies that had reached sexual maturity (21 days, Neilson and McAllan 1965) and had eclosed within a few days of each other. Flies used in activity trials were 23.5 ± 2.8 days old. We loaded one fly into each tube, and we positioned one tube horizontally into each of the 32 channels. We then placed the monitors into their respective adult light treatment incubator. We provided a gelatinous food (3:1 sucrose:hydrolyzed yeast by mass + 1% agar in water) *ad libitum* in the cap of each sampling tube.

The DAMSystem3 (Waltham, MA, USA) data collection program collected data from each activity monitor as beam breaks in 1-minute bins. We processed the raw data using CountingMacro (Pfeiffenberger et al. 2010). The standard definition of sleep is five contiguous minutes of no beam breaks (Hendricks et al. 2000). We obtained percent sleep per hour data for each individual fly.

For each trial, we loaded flies during the 14 h light period. The first 24 h were used for acclimation and excluded from analysis. We quantified sleep for the subsequent 24 h (days 2–3). We considered flies with less than six total beam breakings in the last 12 hours of the trial to be dead, and removed them from the analysis (n = 47). Between both sexes, this yielded a total sample size of n = 209 (pupal ALAN only: n = 56; adult ALAN only: n = 46; pupal and adult ALAN: n = 58; control: n = 49). From these samples, we obtained measurements of daytime percent sleep (14 h of day), nighttime percent sleep (10 h of night), and total percent sleep (24 h). We also quantified sleep during 3 h transition periods surrounding light changes (“day:night transition” and “night:day transition”) to assess whether flies behaviorally anticipated the change in light or simply responded after it occurred.

Effect of ALAN on fitness

To test ALAN’s impact on fitness, we measured survival to sexual maturity (n = 359), body size (n = 549), and female fecundity (n = 263). To measure survival to sexual maturity, we recorded natural deaths daily throughout the entire experiment. When flies were found dead, we froze them for later analysis. We excluded flies that did not die naturally (i.e., stuck in food/water dishes). We also excluded flies that were used in Locomotor Activity Monitoring System trials, which were frozen directly after each trial. We calculated the proportion of flies that survived to sexual maturity by experimental group.

To measure body size and fecundity, we immediately froze flies after locomotor activity trials. Additionally, throughout the rearing of all experimental flies, we froze flies that were potentially sexually mature (at least 21 days) after they experienced a natural death. Finally, at the conclusion of the experiment, we froze all remaining flies. We pinned each frozen fly on a Petri dish filled with Sylgard 184 clear elastomer. We used a Leica S9i stereomicroscope (Leica Microsystems) with camera, coded zoom, and LAS X software to measure the right hind leg tibia length, which is a common proxy for body size (Yee et al. 2009). Next, we measured egg load in adult females as a proxy for fecundity (Calvo and Molina 2005, Berger et al. 2008). Using the microscope, we dissected the ovaries in Ringer's solution and removed oocytes. We spread oocytes into a single layer and counted only fully mature oocytes, which are elongated, tapered at one end, and opaque (Alonso-Pimentel et al. 1998, Lachmann and Papaj 2001). We discarded flies that were desiccated or otherwise unsuited for dissection, and excluded flies that were less than 21 days of age at death.

Statistical Analysis

To test whether pupal treatment (ALAN or dark), sex, or their interaction predicted days to eclosion, we used a linear model with days to eclosion as the response variable and pupal treatment and sex as interacting predictor variables. Within *Rhagoletis*, males are known to eclose sooner than females (Bush 1966), and ALAN could affect the sexes differently.

To test whether pupal treatment, adult treatment, sex, or their two-way interactions predicted percent sleep at different times of day, we used five separate linear models. In each model, pupal treatment, adult treatment, sex, and the two-way interactions were predictor variables, and one of the following five metrics of percent sleep was a response variable: daytime percent sleep, nighttime percent sleep, total percent sleep, day:night transition percent sleep, night:day transition percent sleep. Additionally, we used linear mixed effect models to check for a random effect of housing container ID, and found no effect. We also found no effects of body size or age when included as predictor variables in the linear models, so we removed these two variables from the final models.

To evaluate the fitness consequences of ALAN exposure, we analyzed survival, body size, and egg load across pupal and adult treatment groups. We tested whether proportions of survival to 21 days differed between the four experimental groups using a Pearson's Chi-squared test. To test for pupal treatment or sex effects on body size, we used a linear model with pupal treatment, sex, and their interaction as predictor variables and adult tibia length as the response variable. To test whether pupal treatment, adult treatment, or their interaction predicted egg load, we used a linear model with egg load as the response variable and pupal treatment and adult treatment as interacting predictor variables. We ran all statistics in R version 4.4.2 (R Core Team 2025) and used the following packages: ggplot2 (Wickham 2016), car (Fox and Weisberg 2018), dplyr (Wickham et al. 2025), lme4 (Bates et al. 2015), emmeans (Lenth 2017).

Results

Effect of ALAN on eclosion timing

First, we tested the effect of ALAN on eclosion timing. We found that pupae exposed to ALAN eclosed an average of 1.3 days later ($F = 30.15$, $df = 1$, $p = 5.0\times10^{-8}$; Fig. 1) than pupae exposed to dark nights. Eclosion timing did not differ between the sexes (F = 2.93, df = 1, p = 0.087; Appendix Fig. 1).

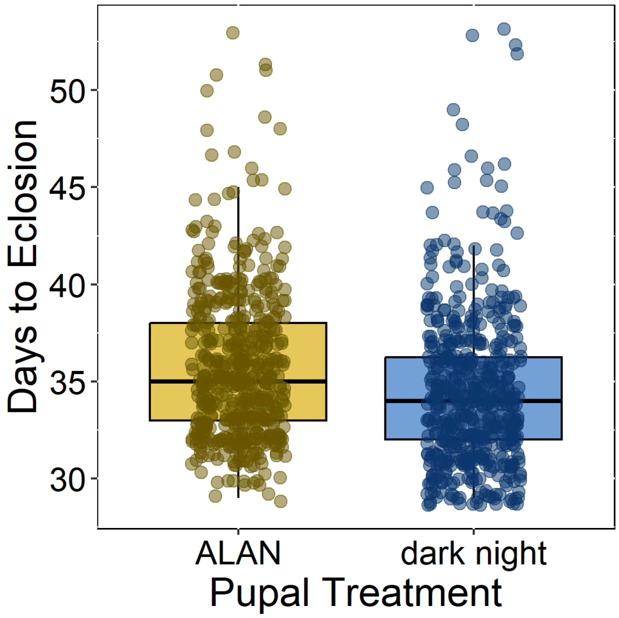

Figure 1: Effect of pupal treatment on days to eclosion. Boxplots show medians, and points are individuals.

Effect of ALAN on sleep-wake cycles

Next, we tested the effect of ALAN on sleep-wake cycles. We found that regardless of pupal exposure, the percent sleep of flies exposed to ALAN as adults was lower during the nighttime (F = 40.05, df = 1, $p = 1.6\times10^{-9}$; Fig. 3A), higher during the daytime (F = 39.13, df = 1, $p = 2.4\times10^{-9}$; Fig. 3B), and higher overall (F = 4.55, df = 1, p = 0.034; Fig. 3C) than the percent sleep of flies not exposed to ALAN as adults. Further, during the day:night transition period, all flies exposed to ALAN as adults had higher percent sleep than those not exposed to ALAN as adults (F = 4.87, df = 1, p = 0.028; Fig. 4). Additionally, during this day:night transition, flies exposed to ALAN as pupae only had higher percent sleep than control flies (*t* = 2.78, df = 197, p = 0.030; Fig. 4). Finally, during the night:day transition period, all flies exposed to ALAN as adults had lower percent sleep than flies not exposed to ALAN as adults (F = 26.74, df = 1, p = 5.7×10^{-7}; Appendix Fig. 2). Finally, females had higher percent sleep than males (*total percent sleep*: F = 7.78, df = 1, p = 0.0058; Appendix Fig. 3).

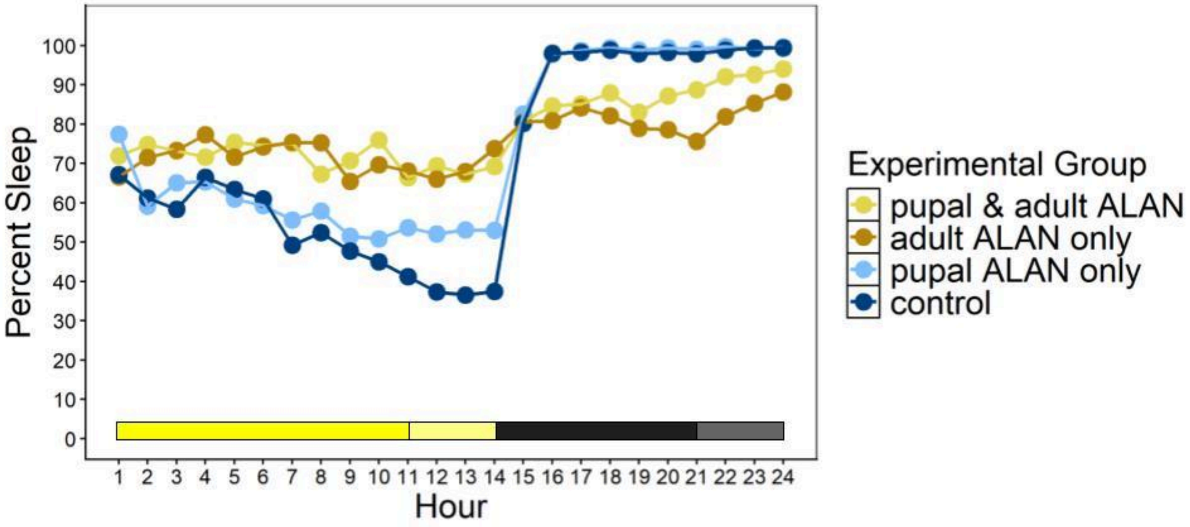

Figure 2: Overview of percent sleep across 24 hours for all four experimental groups. The yellow bar indicates the 14 hour daytime period, and the black bar indicates the 10 hour nighttime period. The pale yellow bar indicates the 3 hour day:night transition period, and the gray bar indicates the 3 hour night:day transition period.

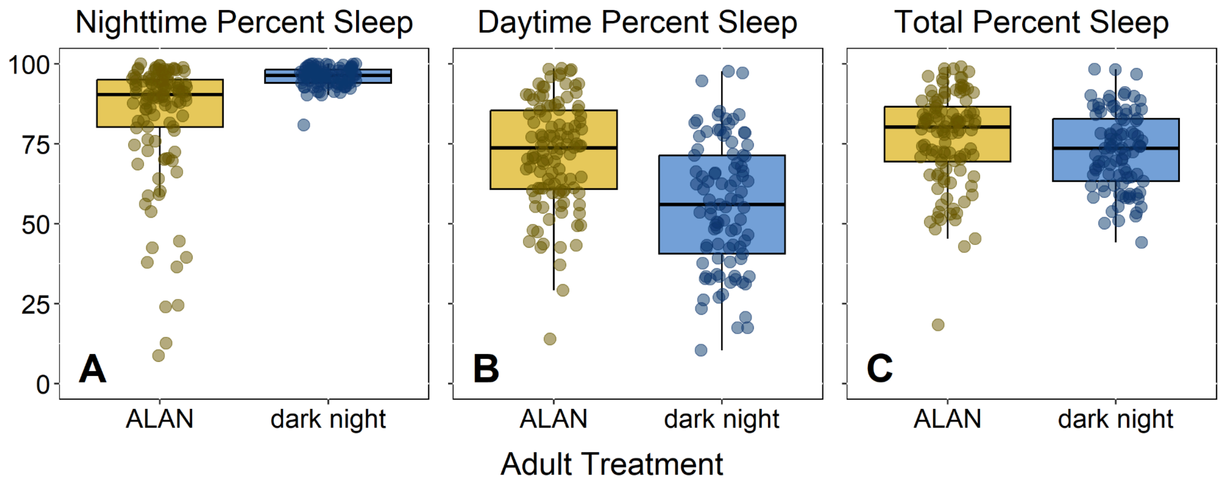

Figure 3: Effect of adult treatment on nighttime percent sleep (A), daytime percent sleep (B), and total percent sleep (C). Boxplots show medians, and points are individuals.

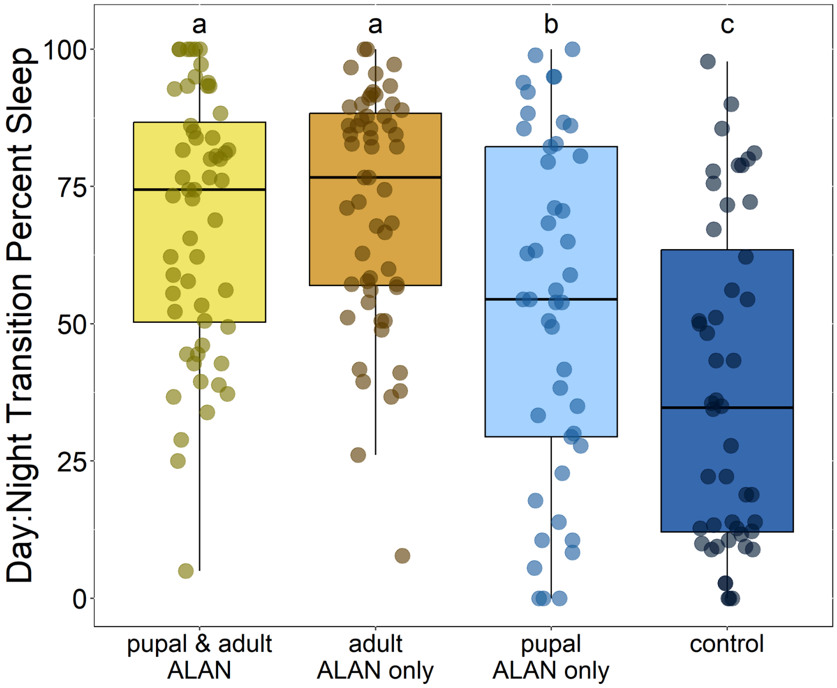

Figure 4: Effect of experimental group on day:night transition percent sleep. Boxplots show medians, and points are individuals. Different letters indicate post-hoc comparisons with significant differences.

Effect of ALAN on fitness

Finally, we tested for effects of ALAN on three fitness-related traits: survival to sexual maturity, adult body size, and female fecundity. We found no effect of experimental group on the proportion of flies that survived to sexual maturity ($X^2 = 2.32$, df = 3, p = 0.51; Appendix Fig. 4). For adult body size, we found no effect of pupal treatment (F = 1.33, df = 1, p = 0.25; Appendix Fig. 5). Also, body size did not differ between the sexes (F = 0.04, df = 1, p = 0.84; Appendix Fig. 6). For female egg load, we found no effect of pupal treatment ($X^2 = 0.00$, df = 1, p = 0.996; Appendix Fig. 7) or adult treatment ($X^2 = 3.2741$, df = 1, p = 0.07038; Appendix Fig. 7).

Discussion

Overall, our results indicate that ALAN disrupts biological rhythm functions in the walnut husk fly, *Rhagoletis juglandis*, with distinct effects at pupal and adult stages. First, we considered the effect of ALAN on diapause—a crucial phase that allows insects to survive unfavorable conditions (Tauber and Tauber 1976). Additionally, within *Rhagoletis*, diapause allows flies to synchronize their life cycle with host plant fruiting (Feder et al. 2010, Inskeep et al. 2022). Previous studies have elucidated effects of ALAN on diapause initiation[19], but our findings provide new insight into ALAN's role in altering diapause termination (i.e., adult eclosion). We found that pupae exposed to ALAN eclosed slightly later than pupae not exposed to ALAN. This does not support our hypothesis that flies perceive ALAN as an extended day. It is possible that the observed shift could be linked to melatonin, which regulates diapause termination and is suppressed by ALAN (Jones et al. 2015). Therefore, it is possible that ALAN could play a complex role in diapause termination via perceived photoperiod cues and hormonal regulation, warranting further investigation.

Next, our findings support ALAN's ability to shift sleep-wake cycles in a diurnal insect system. We found that regardless of pupal exposure, adult flies exposed to ALAN slept more during the day and less at night. However, the precise mechanism through which ALAN disrupts this cycle is challenging to disentangle (Aulsebrook et al. 2018). One such possible mechanism is true disruption to the circadian rhythm, in which case the altered sleep-wake cycle would persist when all light cues are removed (Dominoni et al. 2016). Alternatively, ALAN may elicit immediate behavioral responses that alter sleep and wakefulness without necessarily affecting the circadian rhythm itself (Chen et al. 2017). Our finding that ALAN adults slept more overall may support further investigation into the latter mechanism, as it suggests a behavioral "overcorrection" for lost sleep under ALAN nighttime conditions. Finally, although pupal treatment played no role in the overall day versus night view of the sleep-wake cycle, we discovered a striking effect of pupal exposure to ALAN during the day:night transition period. Flies exposed to ALAN as pupae only slept more during this transition than flies that were never exposed to ALAN. This suggests that ALAN exposure during only the post-winter pupal period has lasting effects on adult fly sleep behavior, pointing to a potential critical period. Additionally, once again, melatonin suppression by ALAN may be at work here, as melatonin is crucial for regulating transitions between sleep and wakefulness (Skocbat et al. 1998).

Contrary to our prediction, we found no link between biological rhythm function disruption and fitness consequences for survival to sexual maturity, body size, or fecundity. Notably, the fitness effects of ALAN are known to differ greatly between species, with both positive and negative fitness outcomes under ALAN exposure. For example, anolis lizards displayed increased growth and reproductive output under ALAN (Thawley and Kolbe 2020). Even within insects, ALAN exposure decreased survival rates in a moth (Van de Schoot et al. 2025) and a fly (McLay et al. 2017), but led to longer lifespans in a wasp (Gomes et al. 2024). Further study of ALAN's effects across a wider variety of organisms will allow us to begin to understand this variation. Additionally, larval energy reserves play a large role in determining adult fitness-related traits like body size and egg production (Sasmita et al. 2019). Therefore, a future experiment that exposes larvae to ALAN may reveal fitness effects not uncovered in the present study.

The lack of fitness effects reported here could also be explained by the fact that ALAN exposure did not lead to a decrease in total sleep. In fact, *R. juglandis* exhibits elevated total sleep under chronic ALAN exposure. As flies exposed to ALAN did not suffer sleep deprivation, we would not expect to see immediate, strong fitness costs, such as premature death from overexertion. Instead, increased sleep observed under ALAN could increase survival (Kuo and Williams 2014). However, future studies should look beyond sleep duration and also consider ALAN's role on sleep quality and composition (Aulsebrook et al. 2018). This may begin to provide insight into the assumed—but presently hidden—downstream costs of the dramatic shifts to the sleep-wake cycle that our study revealed. Future work could also reveal when changes to sleep-wake cycles do not lead to detrimental fitness outcomes. For instance, reduced sleep can increase reproductive opportunities; in the polygynous pectoral sandpiper, males that sleep the least sire the most offspring, pointing to a strong sexually selective force for tolerating low sleep (Lesku et al. 2012).

Finally, for the first time to our knowledge, we characterized the sleep-wake patterns of *Rhagoletis* flies in standard rearing conditions. This baseline not only enabled us to compare sleep-wake rhythms under ALAN but also provides a framework for studying insect sleep across diverse contexts. Our findings that ALAN has life stage-specific effects on *Rhagoletis* fly biological rhythms highlights new opportunities for investigation. Notably, ALAN has been proposed as a driver of evolutionary change (Swaddle et al. 2015, Hopkins et al. 2018). Because biological rhythms evolved under natural cycles of light and dark, understanding how they respond to anthropogenic pressures like ALAN will deepen insights into organisms' abilities to cope with a changing world.

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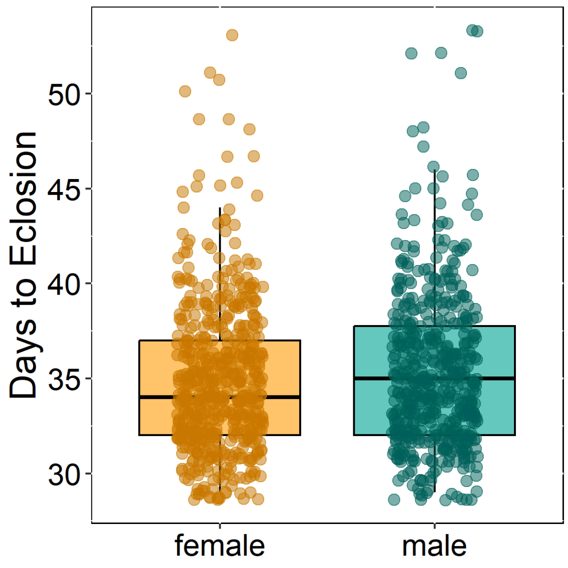
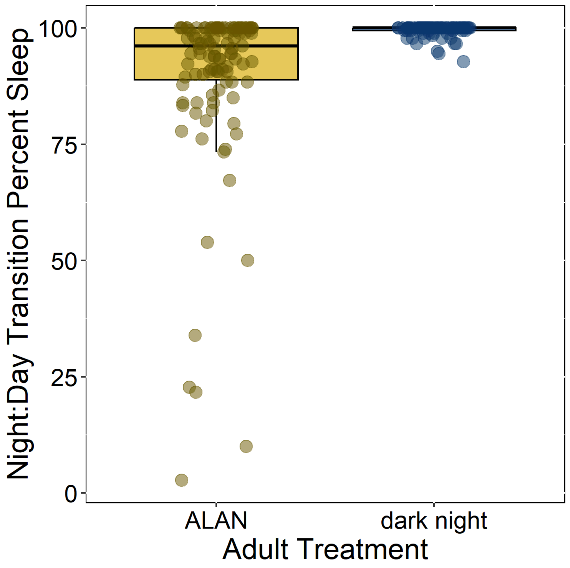
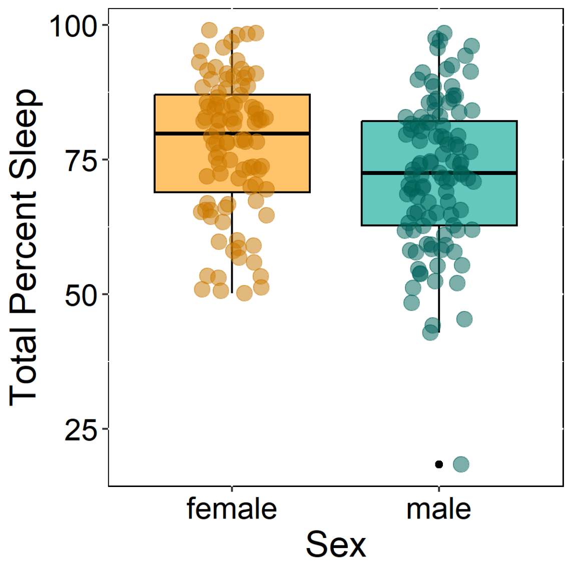
Appendix



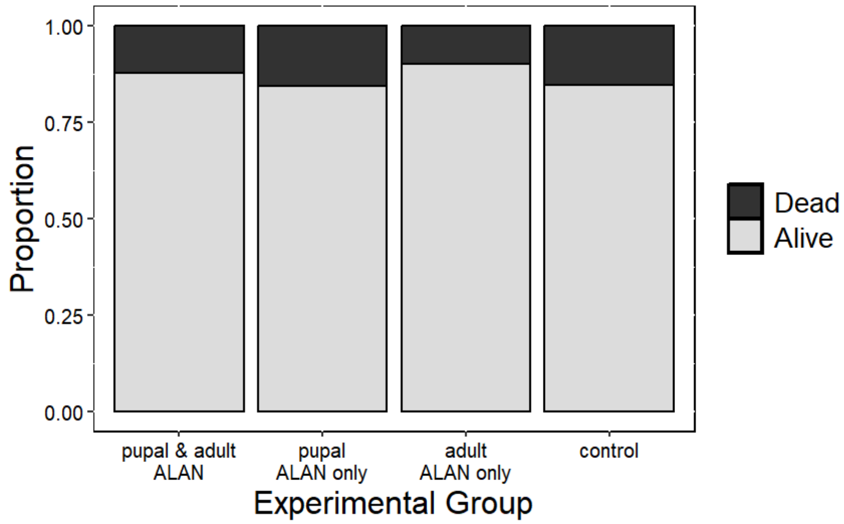
Appendix Figure 1: Effect of sex on days to eclosion. Boxplots show medians, and points are individuals.



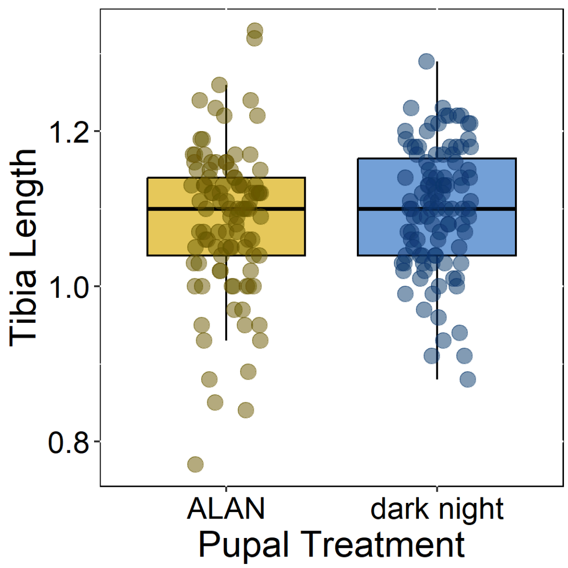
Appendix Figure 2: Effect of adult treatment on night:day transition percent sleep. Boxplots show medians, and points are individuals.



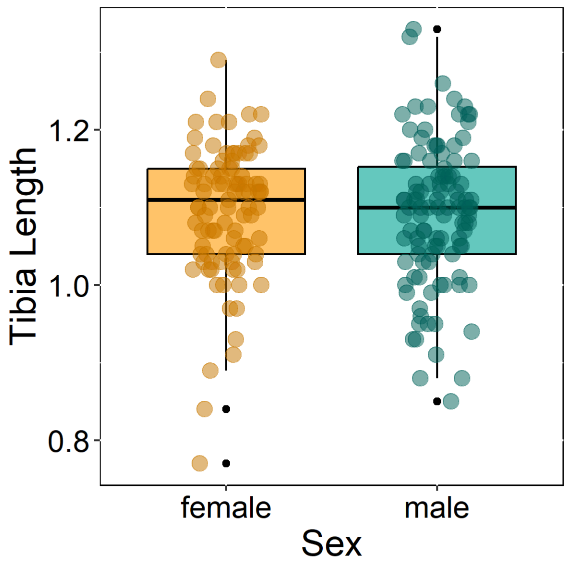
Appendix Figure 3: Effect of sex on total percent sleep. Boxplots show medians, and points are individuals.



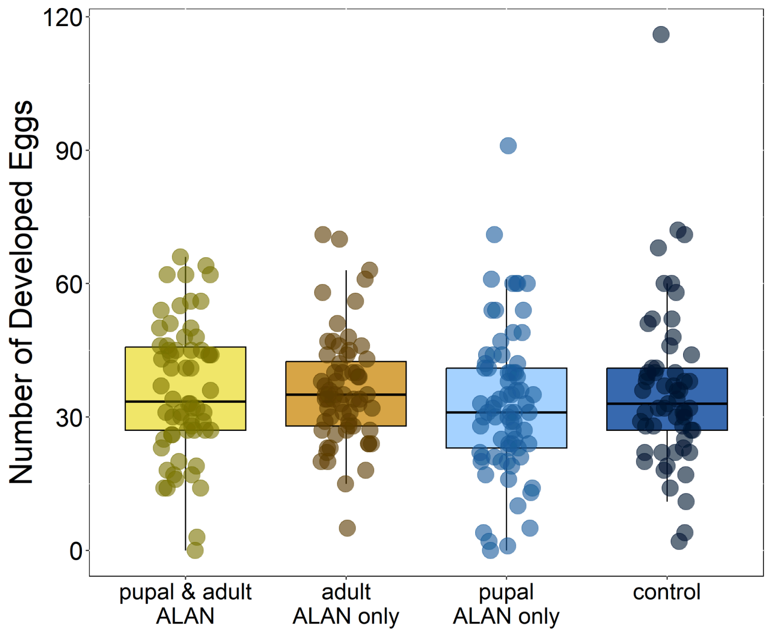
Appendix Figure 4: Effect of experimental group on proportion of flies that survived to sexual maturity (21 days).



Appendix Figure 5: Effect of pupal treatment on body size. Boxplots show medians, and points are individual measurements of tibia length.



Appendix Figure 6: Effect of sex on body size. Boxplots show medians, and points are individual measurements of tibia length.



Appendix Figure 7: Effect of experimental group on egg load. Points are the number of developed eggs of individual females.