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

Running title: Life stage-specific insect rhythm responses to ALAN

Jenna L. B. Kirk[1], Breanna Beard[1], Sophia C. Anner[1], Dae-Sung Hwangbo[1], Alycia C. R. Lackey[1]

[1]Department of Biology, University of Louisville, Louisville, KY

Corresponding author: Jenna L. B. Kirk

jenna.burns.1@louisville.edu

139 Life Sciences Building, University of Louisville, Louisville, KY 40292

Key words: Artificial light at night, biological rhythms, sleep

Word count: 4544

Number of figures: 4

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 seasonal insect: the walnut husk fly (*Rhagoletis juglandis*). We tested for life-stage specific effects of ALAN across four experimental groups: pupal ALAN exposure only, adult ALAN exposure only, pupal and adult ALAN exposure, and pupal and adult dark night exposure (control). Pupal exposure to ALAN delayed developmental timing; these flies eclosed later than dark-night controls. In adults, ALAN disrupted sleep-wake cycles regardless of pupal exposure, indicating an immediate behavioral response to light exposure. Notably, flies that were exposed to ALAN as pupae only slept more during the transition from day to night as adults compared to dark-night control flies. This reveals a lasting effect of ALAN on circadian regulation from pupal to adult stages. Despite these seasonal and circadian shifts, 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 track 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 longer timescales, like diapause, migration, and molting. Operating on shorter timescales, circadian (daily) rhythms are synchronized with the 24-hour light cycle and drive daily functions, like sleep-wake cycles, singing, and enzyme activity. Importantly, these functions are inextricably tied to fitness traits like reproduction and survival (Gaston 2017).

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). Disruption of biological timing via ALAN can have direct consequences for behavior, physiology, and fitness across taxa. For example, experimental exposure to ALAN has had negative effects on foraging behavior in mammals (Hoffmann et al 2022), physiology in plants (Wei et al 2023), and reproduction in insects and birds (Gomes et al 2024).

Not surprisingly, many of ALAN's impacts are mediated through disruption of biological rhythms, 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 initiation 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). Exposure to ALAN has been shown to reduce diapause initiation (Westby and Medley 2020; Merckx et al 2023), potentially because animals may perceive ALAN as an extension of day length. However, little is known about the impact of ALAN on diapause termination.

In addition to seasonal effects, 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). Yet, research on ALAN and sleep remains incomplete and sometimes contradictory (Aulsebrook et al 2018). Species responses vary widely, even among close relatives (Sun et al 2017), and with sexual dimorphism (Pranevicius et al 2025). Further, 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). Thus, 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).

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 potential 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* (Cresson). First, we tested whether ALAN impacts processes governed by circannual and circadian 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*, with photoperiod as a secondary cue; they eclose earlier when exposed to longer day lengths (Feder et al 2010). Thus, we hypothesized that if flies perceive ALAN as a longer day length, then flies under ALAN exposure would eclose earlier. We also hypothesized that ALAN would shift sleep-wake cycles. We predicted that flies under ALAN would sleep less at night and more during the day, and potentially less overall. We hypothesized that ALAN would decrease fitness via disrupted biological rhythms. We tested whether ALAN impacts three fitness-related traits: survival to sexual maturity, adult body size, and female fecundity.

Finally, we tested whether ALAN's effects on sleep-wake cycles and fitness were life-stage specific using exposure treatments at two distinct life stages: post-winter pupa

and adult. We hypothesized that ALAN exposure at only the pupal stage would alter adult phenotype, indicating carryover effects of early-life light exposure. Thus, we expected that flies exposed to ALAN only as pupae would eclose earlier, have altered sleep-wake cycles, and have lower fitness than pupae exposed to dark nights. Next, we hypothesized that ALAN exposure at only the adult stage would directly disrupt circadian-regulated behaviors and lead to reduced fitness compared to dark-night controls. Thus, we expected that flies exposed to ALAN only as adults would have altered sleep-wake cycles and declines in fitness-related traits. Next, we hypothesized that exposure to ALAN across both pupal and adult stages would produce additive or synergistic effects, so we expected these flies to have the strongest deviations in behavior and/or fitness traits relative to controls. 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* [Torrey]) infested with *Rhagoletis juglandis* (Cresson) 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). Thus, all pupae experienced dark nights at this stage. We then changed the incubator settings to 4°C 0:24 L:D cycle to simulate winter. In nature, pupae are buried in soil during winter, experiencing total darkness. 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 1400 lx, equivalent to an overcast day). During the night, retro-fitted LED lights (UTILITECH) lit the ALAN incubator (80 lx, equivalent to bright urban night lighting), while the dark night incubator was not lit (0 lx).

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 at the conclusion of the experiment (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 (LAMs; TriKinetics Inc, Waltham, MA, USA). The LAM system requires 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), caps for tubes (model # CAP25-BLK), and software (DAMSystem311 and DAMFileScan113). We used four activity monitors (one for each experimental group). Each monitor has 32 independent activity channels, which measure sleep and activity by proxy of infrared beam breaks and sensors.

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. We randomly selected age-matched

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.

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).

We processed the raw data collected with Counting Macro (Version 5.19.9) using the protocol described in 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. 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 within each experimental group.

We measured body size and fecundity in flies used in locomotor activity trials as well as all flies that survived to at least 21 days (range 21-43 days, median 31 days), excluding flies that were desiccated following freezing and therefore unsuitable for dissection. For body size, the total sample size was n = 549 (ALAN pupae: n = 283; dark pupae: n = 266). For fecundity, the total sample size was n = 262 (pupal ALAN only: n = 73 ; adult ALAN only: n = 63 ; pupal and adult ALAN: n = 63; control: n = 63). 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; Lackey et al 2025). Under 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).

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. We included sex because males are known to eclose sooner than females in *Rhagoletis* (Bush 1966). Thus, ALAN could affect eclosion timing in 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. Body size and age were initially included as covariates to assess potential confounding effects, and housing container ID was evaluated as a random effect to account for potential non-independence among group-housed individuals. However, including these terms did not improve model fit or qualitatively change the results, so we excluded these terms from the final analyses.

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: *ggplot*2 (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

To evaluate potential shifts in circannual rhythms, we tested the effect of ALAN on eclosion timing. We found that pupae exposed to ALAN eclosed an average of 1.3 days later ($F = 30.15$; $df = 1, 1068$; $p = 5.0 \times 10^{-8}$; Fig. 1) than pupae exposed to dark nights. Eclosion timing did not differ between the sexes ($F = 2.93$, $df = 1, 1068$, $p = 0.087$; Supp. Fig. 1).

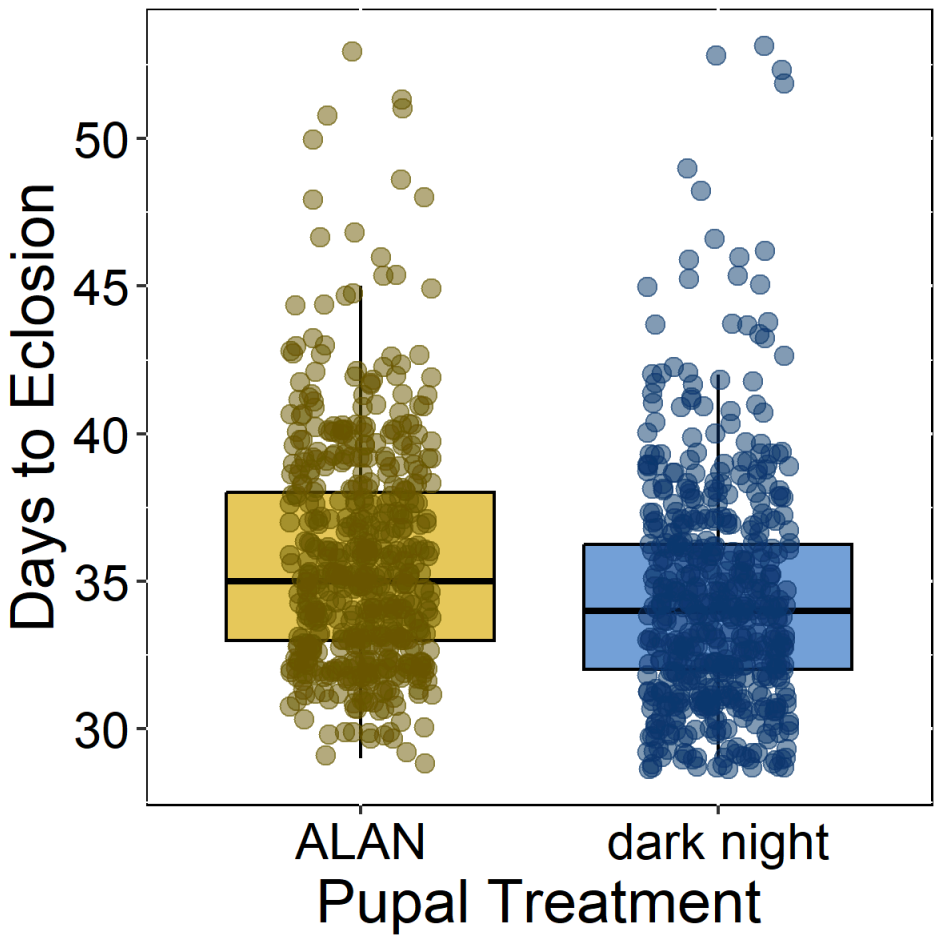


Figure 1: Effect of pupal treatment on days to eclosion. Boxplots show medians, and points are individuals.

Effect of ALAN on sleep-wake cycles

To evaluate shifts in circadian rhythms, we tested the effect of ALAN on sleep-wake cycles. First, we tested for sex differences in percent sleep. Females had higher total percent sleep than males ($F = 7.78$, $df = 1, 197$, $p = 0.0058$; Supp. Fig. 2). However, importantly, the pupal and adult treatment effects on all metrics of percent sleep did not differ between the sexes (all $F < 0.90$, $df = 1, 197$, $p > 0.34$). Thus, we combined data across the sexes in our analyses. In Figure 2, we visualized patterns of percent sleep per hour for the four experimental groups. In addition to total percent sleep in 24 hours, we also analyzed four periods that were likely to differ functionally or behaviorally: daytime, nighttime, day:night transition, and night:day transition. The dark blue line (control) provides a baseline where sleep is low during the day, lowest at the day:night transition, then quickly shifts to nearly 100% at night (Fig. 2). We found that regardless of pupal exposure, the percent sleep of flies exposed to ALAN as adults was lower during the nighttime ($F = 40.05$, $df = 1, 197$, $p = 1.6 \times 10^{-9}$; Fig. 3a), higher during the daytime ($F = 39.13$, $df = 1, 197$, $p = 2.4 \times 10^{-9}$; Fig. 3b), and higher overall ($F = 4.55$, $df = 1, 197$, $p = 0.034$; Fig. 3c) compared to flies not exposed to ALAN as adults. We found the same pattern during the night:day transition period; all flies exposed to ALAN as adults had lower percent sleep during this period than flies not exposed to ALAN as adults ($F = 26.74$, $df = 1, 197$, $p = 5.7 \times 10^{-7}$; Supp. Fig. 3). Notably, during the day:night transition period, there was a significant interaction of pupal treatment and adult treatment on percent sleep ($F = 5.85$, $df = 1, 197$, $p = 0.017$; Fig. 4). Post-hoc pairwise comparisons revealed that regardless of pupal treatment, all adults that received ALAN

slept more during this transition than adults that did not receive ALAN (for all pairwise comparisons: $t > 2.95$, $df = 197$, $p < 0.019$). Also, flies that received ALAN as pupae only slept more during this transition than control flies ($t = 2.78$, $df = 197$, $p = 0.030$).

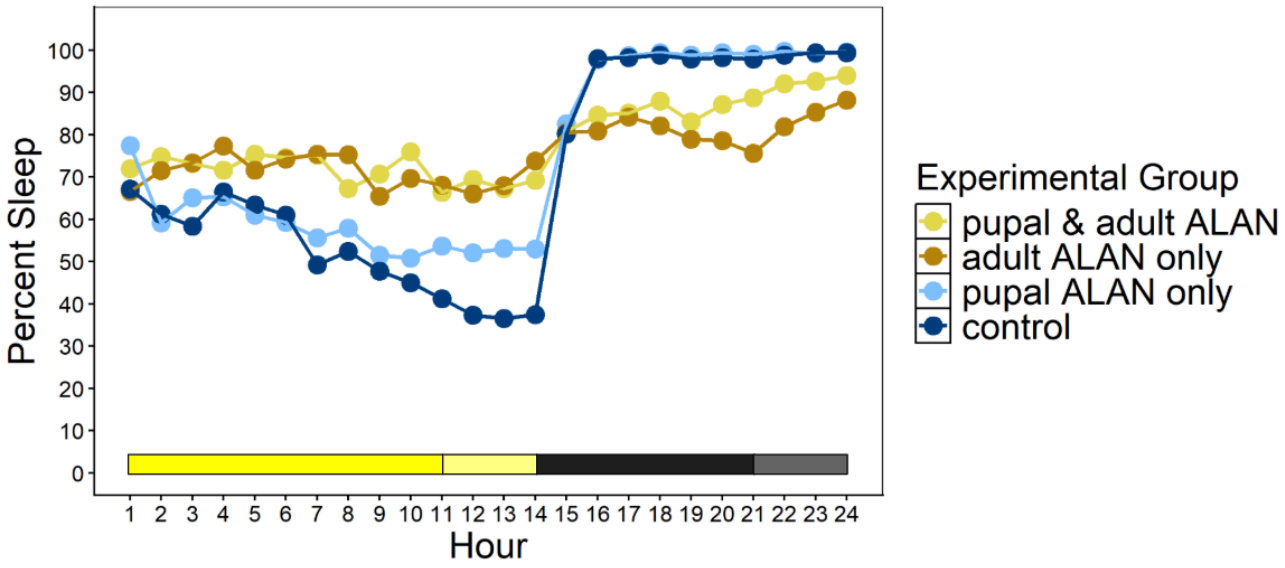


Figure 2: Percent sleep across 24 hours for all four experimental groups with data for the sexes combined. For each experimental group, points indicate average percent sleep per hour and lines connect hourly observations. The yellow bar indicates the 14 hour daytime period, and the black bar indicates 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.

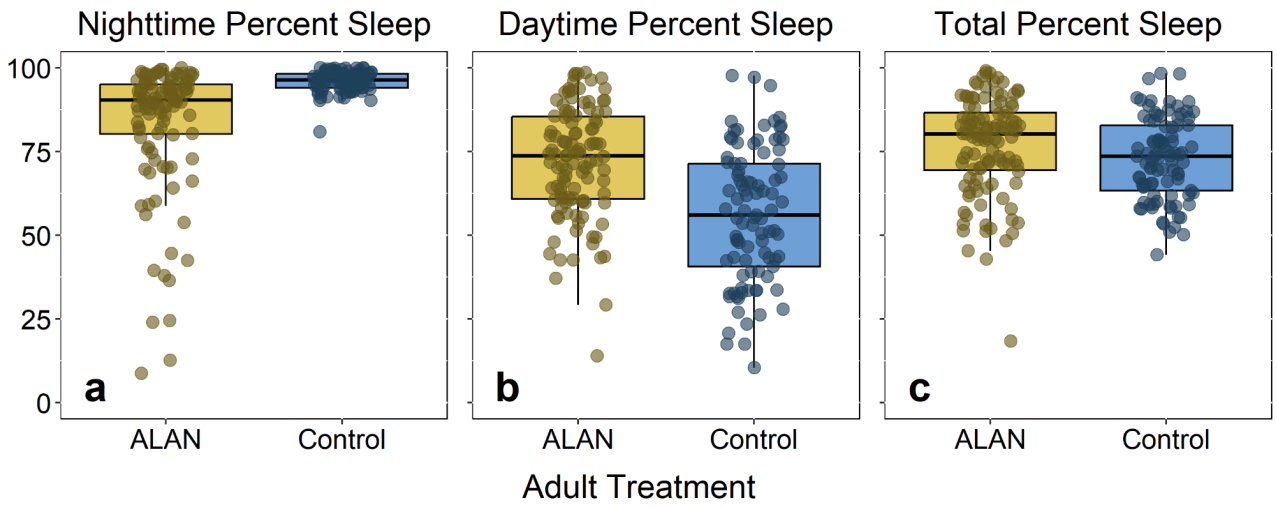


Figure 3: Effect of adult treatment on nighttime percent sleep (a), daytime percent sleep (b), and total percent sleep (c). Boxplots show medians, and points are individuals.

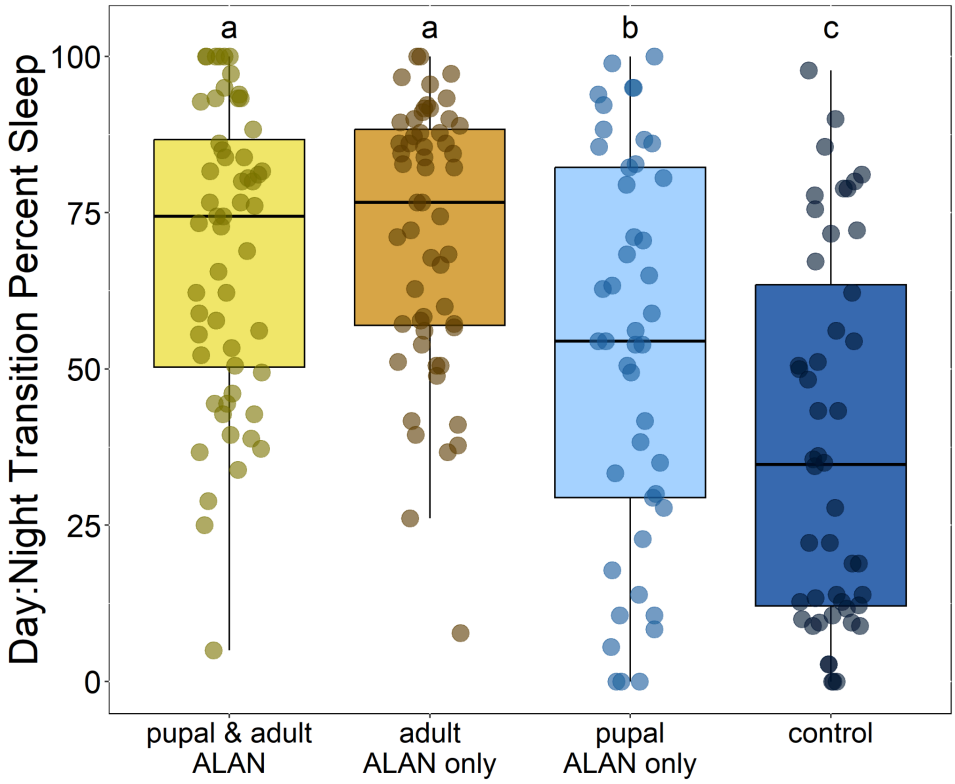


Figure 4: Effect of experimental group on day:night transition percent sleep. Boxplots show medians, and points are individuals. Different letters indicate post-hoc comparisons with significant differences.

Effect of ALAN on fitness

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; Supp. Fig. 4). For adult body size, we found no effect of pupal treatment (F = 1.33, df = 1, 545, p = 0.25; Supp. Fig. 5). Also, body size did not differ between the sexes (F = 0.04, df = 1, 545, p = 0.84; Supp. Fig. 6). For female egg load, we found no effect of pupal treatment (X^2 = 0.00, df = 1, p = 0.996; Supp. Fig. 7) or adult treatment (X^2 = 3.2741, df = 1, p = 0.07038; Supp. Fig. 7).

Discussion

Overall, our results indicate that ALAN disrupts biological rhythms 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 and to synchronize their life cycles with seasonal resource availability, including host plant fruiting in *Rhagoletis* (Tauber and Tauber 1976; Feder et al 2010; Inskeep et al 2022). Previous studies have shown that ALAN reduces diapause initiation in other insects (Westby and Medley 2020; Merckx et al 2023), but our findings provide new insight into ALAN's role in altering diapause termination (i.e., adult eclosion). Contrary to our hypothesis that flies would perceive ALAN as an extended day and eclose sooner, pupae exposed to ALAN actually eclosed slightly later than

those not exposed to ALAN. One possible explanation is that ALAN suppresses melatonin, a hormone that regulates diapause termination (Jones et al 2015). Our finding suggests that ALAN may influence diapause termination through a complex interplay of perceived photoperiod and hormonal regulation, warranting further investigation into the underlying mechanisms.

Next, our findings show that exposure to ALAN shifts sleep-wake cycles in this 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 possible mechanism is true disruption to the circadian rhythm (i.e., changes in clock development, mechanism, and/or gene expression). In this case, the altered sleep-wake cycle could 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 did not impact day, night, or total percent sleep, we discovered a striking effect of pupal exposure to ALAN during the day:night transition period. Flies exposed to ALAN only as pupae slept significantly more during this transition than flies that were never exposed to ALAN. This finding indicates that ALAN

exposure limited to the post-winter pupal period had lasting effects on adult fly sleep behavior, suggesting the presence of a critical development window for circadian regulation. Additionally, once again, melatonin suppression by ALAN may underlie this effect, 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 vary widely across species, with both positive and negative fitness outcomes under ALAN exposure (e.g., positive in lizards (Thawley and Kolbe 2020), negative in wallabies (Robert et al 2015)). 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). This variability highlights that ALAN's fitness effects can differ even among closely related organisms, and that species- or stage-specific factors may be important. In insects, for example, larval energy reserves strongly influence 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 and provide insights into how early-life light exposure shapes adult physiology, behavior, and reproductive outcomes.

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, flies in our experiment

exhibited *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.

Acknowledgements

We thank undergraduate research assistants Marlee Mincher and Brianna Neubacher for training JLBK in dissection techniques. We would also like to thank Shayna Earl for feedback on earlier versions of this manuscript. This research was supported by the National Institutes of Health Postbaccalaureate Research Education Program (PREP) under grant number 1R25GM148314 (awarded to JLBK). We received additional support from the University of Louisville PREP program, Department of Biology, and startup funds to ACRL. Finally, we received support from the National Institutes of Health award R15 ES028440-02A1.

Declarations

Funding:

This research was supported by the National Institutes of Health Postbaccalaureate Research Education Program (PREP) under grant number 1R25GM148314 (awarded to JLBK). We received additional support from the University of Louisville PREP program, Department of Biology, and startup funds to ACRL. Finally, we received support from the National Institutes of Health award R15 ES028440-02A1.

Conflicts of interest:

The authors have no relevant financial or non-financial interests to disclose.

Ethics approval:

Insects used in this study were collected with permission. Research was conducted in accordance with University of Louisville ethical guidelines.

Consent to participate:

Not applicable

Consent for publication:

Not applicable

Data and code availability:

Data and R code are publicly available in the Open Science Framework Repository and can be accessed with the following link:
https://osf.io/6b35h/overview?view_only=fecfd09440c24e30aebe74a15a0e0978

Authors' contributions:

JLBK: Conceptualization (lead), Data curation (lead), Formal analysis (lead), Funding acquisition (lead), Investigation (lead), Methodology (lead), Project administration (lead), Visualization (lead), Writing - original draft (lead), Writing - reviewing and editing (lead) **BB:** Conceptualization (supporting), Data curation (supporting), Formal analysis (supporting), Investigation (supporting), Methodology (supporting), Writing - reviewing and editing (supporting) **SCA:** Conceptualization (supporting), Formal analysis (supporting), Methodology (supporting), Resources (lead), Writing - reviewing and editing (supporting) **DSH:** Conceptualization (supporting), Methodology (supporting), Resources (lead) **ACRL:** Conceptualization (supporting), Formal analysis (supporting), Funding acquisition (lead), Methodology (supporting), Project administration (supporting), Resources (lead), Supervision (lead), Visualization (supporting), Writing - original draft (supporting), Writing - reviewing and editing (supporting)

References

Alonso-Pimentel H, Korer JB, Nufio C, Papaj DR (1998) Role of colour and shape stimuli in host-enhanced oogenesis in the walnut fly, Rhagoletis juglandis. Physiol Entomol 23(2):97–104

Aschoff J (1965) Circadian Rhythms in Man. Science 148(3676):1427-1432

Aschoff J (1989) Temporal orientation: circadian clocks in animals and humans. Animal Behaviour 37:881–896

Aulsebrook AE, Jones TM, Mulder RA, Lesku JA (2018) Impacts of artificial light at night on sleep: A review and prospectus. J Exp Zool Pt A 329(8–9):409–418

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. Stat Softw 67:1–48

Bennie J, Duffy JP, Davies TW, Correa-Cano ME, Gaston KJ (2015) Global Trends in Exposure to Light Pollution in Natural Terrestrial Ecosystems. Remote Sensing 7(3):2715–2730

Berger D, Walters R, Gotthard K (2008) What limits insect fecundity? Body size- and temperature-dependent egg maturation and oviposition in a butterfly. Funct Ecol 22(3):523–529

Berlocher SH, Enquist M (1993) Distribution and Host Plants of the Apple Maggot Fly, Rhagoletis pomonella (Diptera: Tephritidae) in Texas. Kans Entomol Soc

66(1):51–59

Bush GL (1966) The taxonomy, cytology, and evolution of the genus Rhagoletis in North America (Diptera, Tephritidae). BMCZ 134:431-562

Calvo D, Molina JM (2005) Fecundity–Body Size Relationship and Other Reproductive Aspects of Streblote panda (Lepidoptera: Lasiocampidae). Ann Entomol Soc Am 98(2):191–196

Chen S, Reichert S, Singh C, Oikonomou G, Rihel J, Prober DA (2017) Light-dependent regulation of sleep/wake states by prokineticin 2 in zebrafish. Neuron 95(1):153-168

Damulewicz M, Tyszka A, Pyza E (2022) Light exposure during development affects physiology of adults in Drosophila melanogaster. Front Physiol 13:1008154.

Dominoni DM, Borniger JC, Nelson RJ (2016) Light at night, clocks and health: from humans to wild organisms. Biol Lett 12(2):20160015

Feder JL, Powell THQ, Filchak K, Leung B (2010) The diapause response of Rhagoletis pomonella to varying environmental conditions and its significance for geographic and host plant-related adaptation. Entomol Exp Appl 136(1):31–44

Fox J, Weisberg S (2018) An R Companion to Applied Regression.

Fuse S, Yoshida T, Kobayashi Y (2024) Effects of artificial night lighting on a web-building spider species in urban green spaces. Acta Oecol 124:104017.

Gaston KJ, Davies TW, Nedelec SL, Holt LA (2017) Impacts of Artificial Light at Night

on Biological Timings. Ann Rev of Ecol Evol Syst 48:49–68

Gomes E, Lemaître J-F, Rodriguez-Rada V, Débias F, Desouhant E, Amat I (2024) Foraging at night under artificial light: impacts on senescence and lifetime reproductive success for a diurnal insect. Anim Behav 210:85–98.

Helm B, Visser ME, Schwartz W, Kronfeld-Schor N, Gerkema M, Piersma T, Bloch G (2017) Two sides of a coin: ecological and chronobiological perspectives of timing in the wild. Philos Trans R Soc Lond B Biol Sci 372: 20160246

Hendricks JC, Finn SM, Panckeri KA, Chavkin J, Williams JA, Sehgal A, Pack AI (2000) Rest in Drosophila Is a Sleep-like State. Neuron 25:129–138

Hoffmann J, Hölker F, Eccard JA (2022) Welcome to the Dark Side: Partial Nighttime Illumination Affects Night-and Daytime Foraging Behavior of a Small Mammal. Front Ecol Evol 9:779825

Hölker F, Moss T, Griefahn B, Kloas W, Voigt CC, Henckel D, Hänel A, Kappeler PM, Völker S, Schwope A, Franke S, Uhrlandt D, Fischer J, Klenke R, Wolter C, Tockner K (2010) The Dark Side of Light: A Transdisciplinary Research Agenda for Light Pollution Policy. Ecol Soc 15(4):13

Hopkins GR, Gaston KJ, Visser ME, Elgar MA, Jones TM (2018) Artificial light at night as a driver of evolution across urban–rural landscapes. Front Ecol Environ 16(8):472–479

Inskeep KA, Doellman MM, Powell THQ, Berlocher SH, Seifert NR, Hood GR, Ragland

GJ, Meyers PJ, Feder JL (2022) Divergent diapause life history timing drives both allochronic speciation and reticulate hybridization in an adaptive radiation of Rhagoletis flies. Mol Ecol 31(15):4031–4049

Jiang X, Ren Z, Hai X, Zhang L, Wang Z, Lyu F (2023) Exposure to artificial light at night mediates the locomotion activity and oviposition capacity of Dastarcus helophoroides (Fairmaire). Front Physiol 14:1063601.

Jones TM, Durrant J, Michaelides EB, Green MP (2015) Melatonin: a possible link between the presence of artificial light at night and reductions in biological fitness. Philos Trans R Soc Lond B Biol Sci 370(1667):20140122

Kuo T-H, Williams JA (2014) Increased Sleep Promotes Survival during a Bacterial Infection in Drosophila. Sleep 37(6):1077–1086

Lachmann AD, Papaj DR (2001) Effect of host stimuli on ovariole development in the walnut fly, *Rhagoletis juglandis* (Diptera, Tephritidae). Physiol Entomol 26(1):38–48

Lackey, ACR, Damron K, Gebhardt A, Anner SC, Alizadeh M (2025) Species differences in reproductive timing and egg load in two fly species adapted to different host plants. Physiol Entomol 50(3):245–252

Lenth RV (2017) emmeans: Estimated Marginal Means, aka Least-Squares Means

Lesku JA, Aulsebrook AE, Kelly ML, Tisdale RK (2019) Chapter 20 - Evolution of Sleep and Adaptive Sleeplessness. In: Dringenberg HC (ed) Handbook of Behavioral

Neuroscience. Elsevier, pp 299–316

Lesku JA, Rattenborg NC, Valcu M, Vyssotski AL, Kuhn S, Kuemmeth F, Heidrich W, Kempenaers B (2012) Adaptive Sleep Loss in Polygynous Pectoral Sandpipers. Science 337(6102):1654–1658

McLay LK, Green MP, Jones TM (2017) Chronic exposure to dim artificial light at night decreases fecundity and adult survival in Drosophila melanogaster. Insect Physiol 100:15–20

Merckx T, Nielsen ME, Kankaanpää T, Kadlec T, Yazdanian M, Kivelä SM (2023) Dim light pollution prevents diapause induction in urban and rural moths 60(6):1022-1031

Meuti ME, Denlinger DL (2013) Evolutionary Links Between Circadian Clocks and Photoperiodic Diapause in Insects. Integr Comp Biol 53(1):131–143

Neilson WTA, McAllan JW (1965) Effects of Mating on Fecundity of the Apple Maggot, *Rhagoletis pomonella* (Walsh). Can Entomol 97(3):276–279

Pfeiffenberger C, Lear BC, Keegan KP, Allada R (2010) Processing Sleep Data Created with the Drosophila Activity Monitoring (DAM) System. Cold Spring Harb Protoc

Pranevicius A, Biondi G, Iyer AR, Seiffe A, Depino AM, de la Paz Fernandez M (2025) Sexually dimorphic and clock genespecific effects of artificial light at night on Drosophila behavioural rhythms. Philos Trans R Soc Lond B Biol Sci 292: 20251170

Rich C, Longcore T (2013) Ecological Consequences of Artificial Night Lighting. Island Press

Robert KA, Lesku JA, Partecke J, Chambers B (2015) Artificial light at night desynchronizes strictly seasonal reproduction in a wild mammal. Philos Trans R Soc Lond B Biol Sci 282:20151745

Sasmita HI, Tu W-C, Bong L-J, Neoh K-B (2019) Effects of larval diets and temperature regimes on life history traits, energy reserves and temperature tolerance of male Aedes aegypti (Diptera: Culicidae): optimizing rearing techniques for the sterile insect programmes. Parasites Vectors 12(1):578

Skocbat T, Haimov I, Lavie P (1998) Melatonin - the key to the gate of sleep. Ann Med 30(1):109–114

Stenvers DJ, van Dorp R, Foppen E, Mendoza J, Opperhuizen A-L, Fliers E, Bisschop PH, Meijer JH, Kalsbeek A, Deboer T (2016) Dim light at night disturbs the daily sleep-wake cycle in the rat. Sci Rep 6(1):35662

Sun J, Raap T, Pinxten R, Eens M (2017) Artificial light at night affects sleep behaviour differently in two closely related songbird species. Environ Pollut 231:882–889

Swaddle JP, Francis CD, Barber JR, Cooper CB, Kyba CCM, Dominoni DM, Shannon G, Aschehoug E, Goodwin SE, Kawahara AY, Luther D, Spoelstra K, Voss M, Longcore T (2015) A framework to assess evolutionary responses to anthropogenic light and sound. Trends Ecol Evol 30(9):550–560

Tauber M, Tauber C (1976) Insect Seasonality: Diapause Maintenance, Termination, and Postdiapause Development. Annu Rev Entomol 21:81–107

Thawley CJ, Kolbe JJ (2020) Artificial light at night increases growth and reproductive output in Anolis lizards. Philos Trans R Soc Lond B Biol Sci 287(1919):20191682

Van de Schoot E, Wesselingh RA, Van Dyck H (2025) Artificial light at night reduces larval survival and constrains female body mass in a capital breeding moth. Basic Appl Ecol 85:38–44

Wei Y, Li Z, Zhang J, Hu D (2023) Effects of artificial light at night and drought on the photosynthesis and physiological traits of two urban plants. Front Plant Sci 14:1263795

Westby KM, Medley KA (2020) Cold Nights, City Lights: Artificial Light at Night Reduces Photoperiodically Induced Diapause in Urban and Rural Populations of Aedes albopictus (Diptera: Culicidae). J Med Entomol 57(6):1694–1699

Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis

Wickham H, François R, Henry L, Müller K, Vaughan (2025) dplyr: A Grammar of Data Manipulation

Yee WL, Chapman PS, Sheets HD, Unruh TR (2009) Analysis of Body Measurements and Wing Shape to Discriminate Rhagoletis pomonella and Rhagoletis zephyria (Diptera: Tephritidae) in Washington State. Ann Entomol Soc Am 102(6):1013–1028

Figure Legends

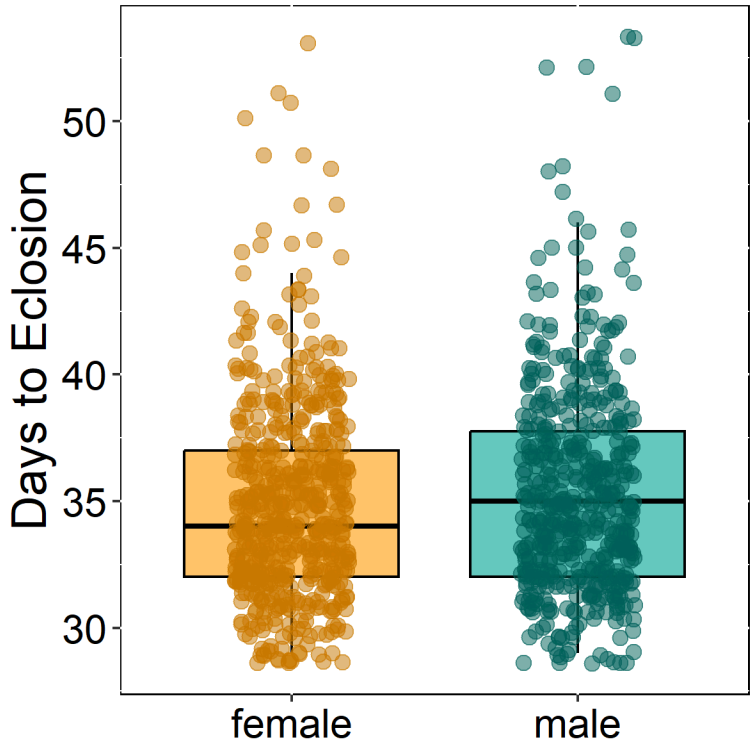
Figure 1: Effect of pupal treatment on days to eclosion. Boxplots show medians, and points are individuals.

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.

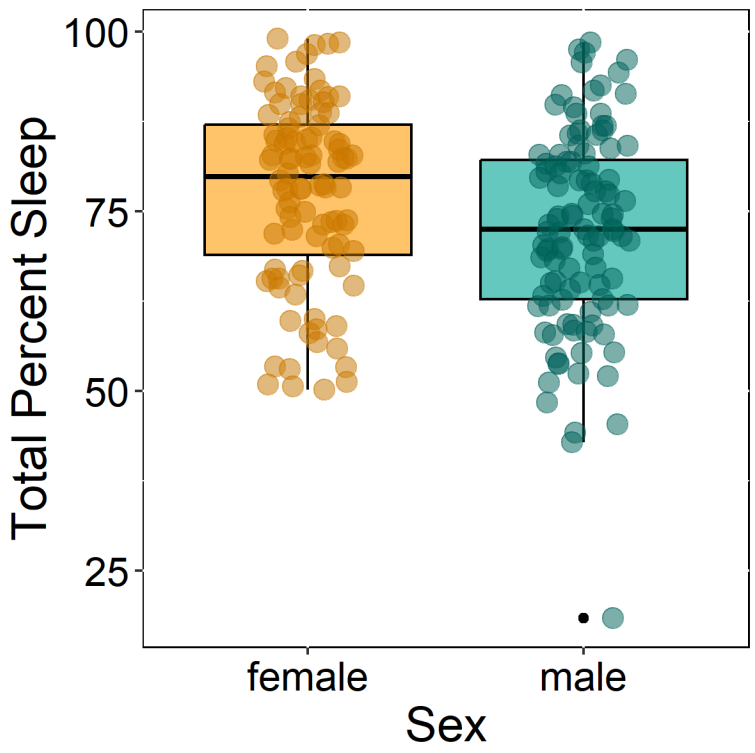
Figure 3: Effect of adult treatment on nighttime percent sleep (a), daytime percent sleep (b), and total percent sleep (c). Boxplots show medians, and points are individuals.

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.

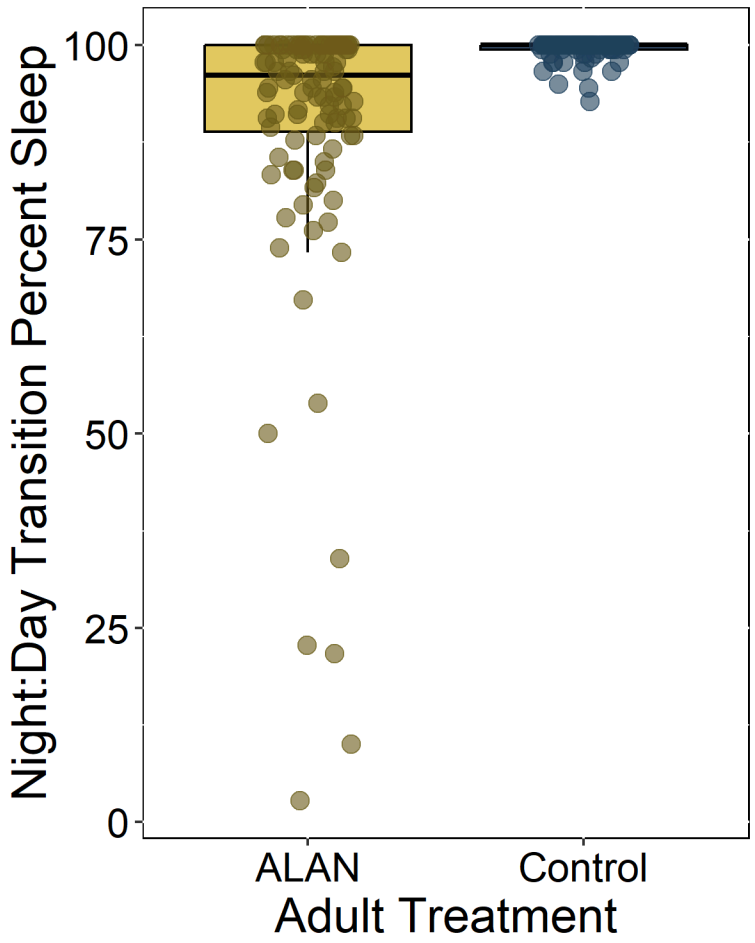
Supplementary Material



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

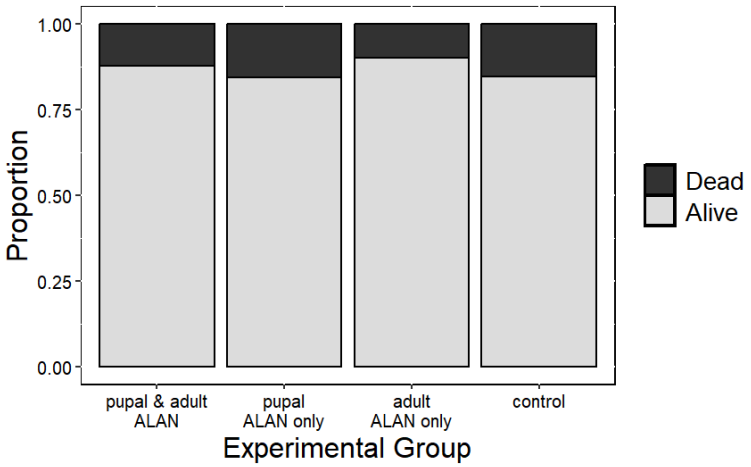


Supplementary Figure 2: Effect of sex on total percent sleep. Boxplots show medians, and points are individuals.

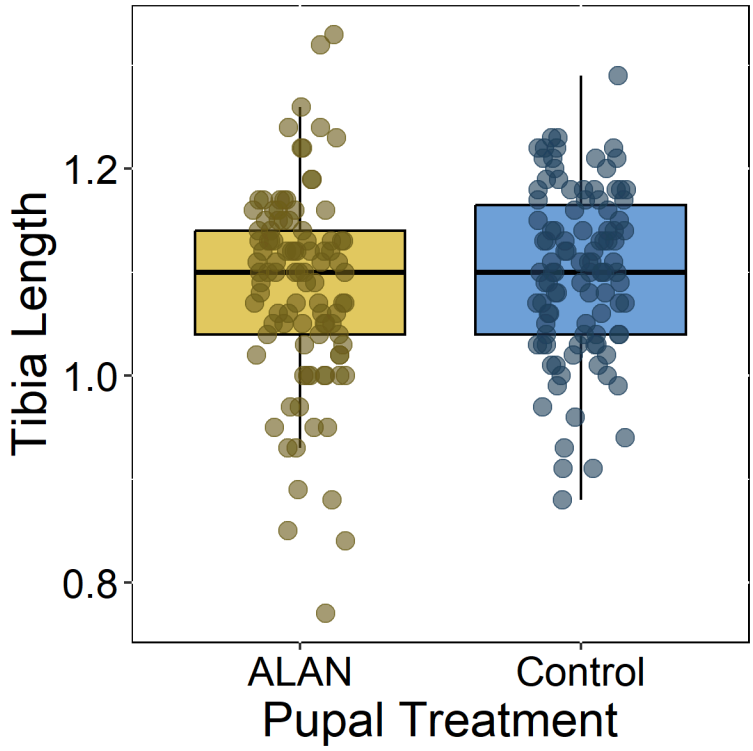


Supplementary Figure 3: Effect of adult treatment on night:day transition percent sleep.

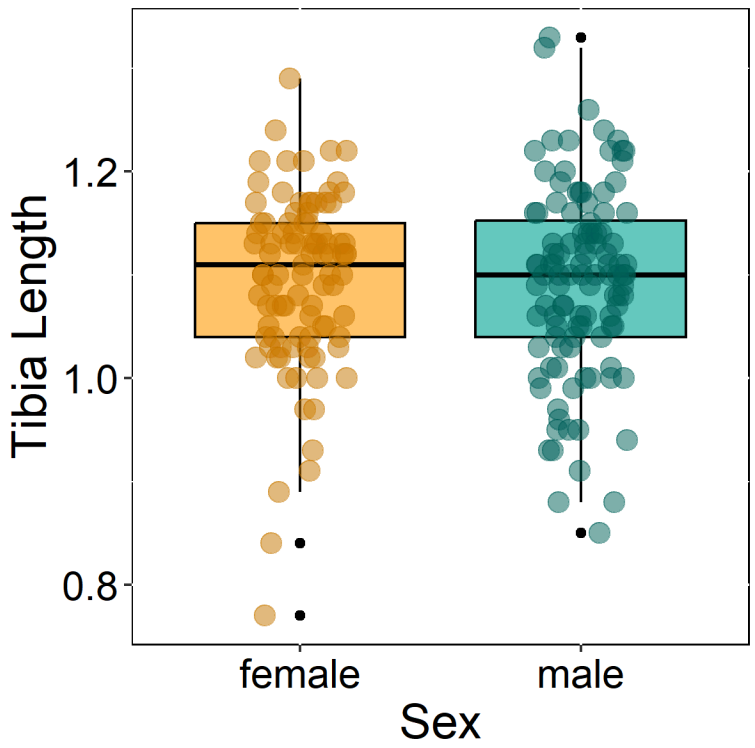
Boxplots show medians, and points are individuals.



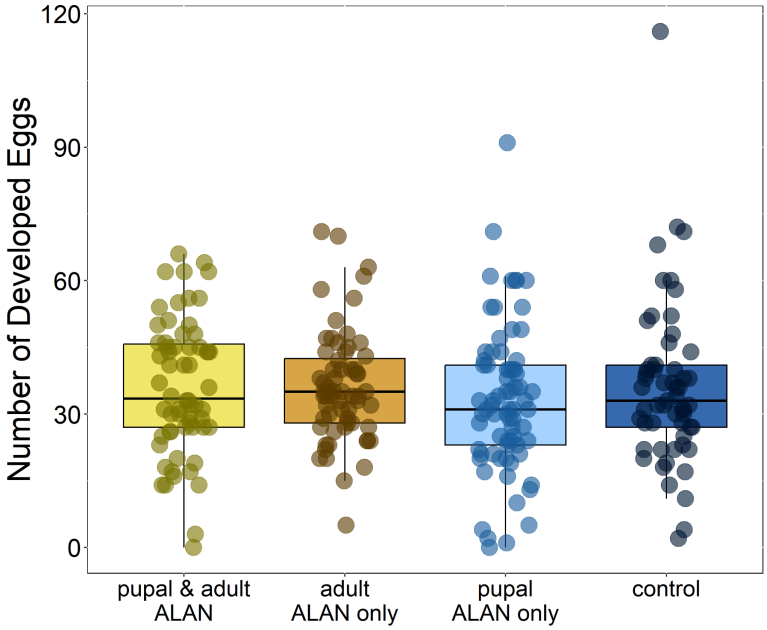
Supplementary Figure 4: Effect of experimental group on proportion of flies that survived to sexual maturity (21 days).



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



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



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