



# Artificial light at night alters diurnal and nocturnal behavior and physiology in green anole lizards

Laura A. Taylor<sup>a,b</sup>, Christopher J. Thawley<sup>c</sup>, Olive R. Pertuit<sup>a</sup>, Abigail J. Dennis<sup>a,d</sup>, Isabela R. Carson<sup>a</sup>, Chen Tang<sup>a,e,f</sup>, Michele A. Johnson<sup>a,\*</sup>

<sup>a</sup> Department of Biology, Trinity University, San Antonio, Texas, 78212 United States of America

<sup>b</sup> Department of Biological Sciences, University of North Texas, Denton, Texas 76203 United States of America

<sup>c</sup> Department of Biological Sciences, University of Rhode Island, Kingston, Rhode Island, 02881 United States of America

<sup>d</sup> Department of Biological Sciences, North Dakota State University, Fargo, North Dakota United States of America

<sup>e</sup> Environmental Science Programme, Beijing Normal University - Hong Kong Baptist University United International College, Zhuhai, China

<sup>f</sup> Johns Hopkins University, Baltimore, Maryland, 21218 United States of America

## ARTICLE INFO

### Keywords:

ALAN  
*Anolis carolinensis*  
Exploratory behavior  
Foraging  
Metabolism  
Social behavior

## ABSTRACT

Artificial light at night (ALAN) disrupts biological rhythms across widely diverse organisms. To determine how energy is allocated by animals in different light environments, we investigated the impacts of ALAN on behavior and physiology of diurnal green anole lizards (*Anolis carolinensis*). Two groups of 24 adult lizards (half males, half females) were maintained in a controlled lab setting for six weeks. One group was exposed to a simulated natural summer light-dark cycle; the other was exposed to ALAN that simulated urban, nocturnal light exposure. After an acclimation period, we conducted four behavioral trials. One trial examined behavioral time allocation over two 24 h periods, and three others were conducted during mid-day and mid-night: open field tests, to examine exploratory behavior; foraging trials, to examine prey consumption; and social interaction trials, to examine same-sex interactions. We then measured each lizard's snout-vent length and mass of its body, abdominal fat pads, liver, and, for males, testes. Lizards exposed to ALAN were more likely to be awake at night, using nocturnal light to explore, forage, and display to conspecifics. However, during the day, ALAN lizards were less likely to be awake, slower to move, and females displayed less frequently. ALAN lizards had heavier fat pads and testes, but ALAN did not impact body mass, liver mass, or snout-vent length. In sum, ALAN appears to cause a broad shift towards increased nocturnal activity and may alter metabolic and reproductive processes. Future work should examine the fitness consequences of these behavioral and physiological changes.

## 1. Introduction

Urbanization is dramatically altering the landscape of the Earth. Urban habitats differ from natural habitats due to a multitude of factors, including the urban island heat effect [72], anthropogenic noise [32], habitat alteration and fragmentation [3], and the alteration of the natural light-dark cycle via light pollution, such as artificial light at night (ALAN; [48]). ALAN is direct or indirect illumination from an anthropogenic light source at night, causing an overall increase in the amount of time an area is illuminated during a 24 h period. It is generally associated with urbanization, as it is commonly caused by streetlights, internal and external lighting of buildings, road vehicle headlights, and shipping and offshore infrastructure [26]. ALAN introduces light into

places and times, and at intensities and emission spectra, that do not naturally occur. Unlike other anthropogenic stressors (e.g., temperature, pollution, habitat fragmentation), there are no natural analogues of ALAN [26,27].

Due to ALAN's alteration of the natural light-dark cycle, ALAN disrupts the environmental cues that entrain natural biological rhythms in living organisms, therefore altering organisms' physiology and behaviors (reviewed in [27]). For example, ALAN suppresses the daily production of melatonin across a variety of vertebrate taxa, including perch (*Perca fluviatilis*, [7]), European blackbirds (*Turdus merula*, [18]), green anole lizards (*Anolis carolinensis*, [58]), and humans (*Homo sapiens*, [56]). Further, ALAN can cause dramatic disruptions to sleep-wake cycles [e.g., great tits (*Parus major*, [70,71]), Wistar rats (*Rattus norvegicus*

\* Corresponding author.

E-mail address: [mjohnso9@trinity.edu](mailto:mjohnso9@trinity.edu) (M.A. Johnson).

<https://doi.org/10.1016/j.physbeh.2022.113992>

Received 27 June 2022; Received in revised form 23 September 2022; Accepted 7 October 2022

Available online 9 October 2022

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*domestica*, [77]), humans [51]). In seasonal breeders, ALAN can cause an advancement in reproductive development (e.g., European blackbirds; [19]) or a delay in testis regression (Siberian hamsters, *Phodopus sungorus*; [34]). These changes can have dramatic effects on the ecology, behavior, and reproduction of animals in ALAN-illuminated environments, including cities. However, much of this existing research has primarily focused on mammals and birds (although this is starting to change), and our current knowledge of the impacts of ALAN on other taxa, including reptiles, is limited [27].

*Anolis carolinensis*, the green anole lizard, is an excellent model system in which to study the impacts of ALAN. Green anoles are common diurnal lizards that are found in disturbed and natural areas [57] and have been well-studied in the lab and field (reviewed in [49,50]). In urban environments, these lizards are impacted by habitat structure and increased presence of competitors, but little is known about how urbanization affects other aspects of their biology [44]. Green anoles are highly visual organisms that detect electromagnetic spectra from wavelengths of 358 nm (UV) to 625 nm (the border between orange and red visible light in the electromagnetic spectrum; [39,68]); this sensitivity overlaps with common emission spectra peaks of LEDs used in many ALAN sources [25]. Behavior in this species is well characterized and easily observed [28,37,38], including display behaviors (pushups, head-bobs, and extension of a throat fan called a dewlap) and sleep behavior [10]. There is also a robust body of literature on the impacts of photoperiod on green anole behavior and physiology (e.g., [23,47,58]), and a growing body of research on the impacts of ALAN on anole behavior and physiology (e.g., [42,52,78]).

In this study, we analyzed the impacts of ALAN on the behavior and physiology of green anoles to test the hypothesis that ALAN alters sleep-wake cycles, therefore resulting in altered behavioral allocation across the day, and altered energy allocation (using changes in organ mass as a proxy) across the breeding season. We thus measured a range of traits associated with behavioral and energy allocation, including activities and organs that are costly during the reproductive season. As observations of green anoles in the field have demonstrated that they are active and forage in the presence of ALAN [54], we first predicted that the lizards exposed to ALAN would be awake more frequently during the night than the lizards exposed to a natural light-dark cycle. However, we predicted the lizards exposed to ALAN would compensate for this decline in sleep at night with increased sleep during the day. Secondly, because anoles in the field (reviewed in [65]) and in the lab [59] have increased nocturnal activity when exposed to ALAN, we predicted that lizards exposed to ALAN would exhibit a higher rate of general activity at night, and correspondingly less activity during the day than lizards exposed to a natural light-dark cycle.

Third, we predicted that lizards exposed to ALAN would consume more prey than lizards exposed to a natural light-dark cycle because, in a previous experimental study, male green anoles exposed to an 18L:6D cycle consumed three times their standard metabolic requirement and five times as many mealworms than the control green anoles which were exposed to 9L:15D cycle [23]. Fourth, as both male and female anoles use visual social displays in aggressive conspecific interactions [38], we predicted that lizards exposed to ALAN would display more frequently at night, when they could see other lizards, than lizards exposed to a natural light-dark cycle.

We also tested a series of predictions regarding the physiological impacts of ALAN exposure. We predicted that lizards exposed to ALAN would have heavier fat pads, livers (a primary storage location for lipids; [17]), and overall body mass, as well as increased snout-vent length (SVL), due to increased foraging over the course of the trials. ALAN has been found to increase fat storage and overall body mass in mammals, such as humans [74] and Siberian hamsters [34]. We also predicted that the male lizards exposed to ALAN would have heavier testes than those in a natural light-dark cycle, because male green anoles experimentally exposed to longer light periods maintained enlarged testes from the summer breeding season into the fall [47].

## 2. Methods

### 2.1. Study species and housing

We captured 48 free-living, adult green anole lizards (*Anolis carolinensis*; 24 males and 24 females) at Trinity University, an urban campus in San Antonio, Texas, during mid-June 2019 (the middle of the green anole breeding season). All lizards were presumably exposed to ALAN in this environment prior to capture. We captured all lizards with a dental floss loop and temporarily held each lizard in an individual cloth sack for 1–5 h before transporting them to the Trinity University Vivarium. On the day of capture, we measured each lizard's snout-vent length (SVL) to the nearest mm using a 6 in clear plastic ruler and mass to the nearest 0.1 g using a 10 g Pesola spring scale. We randomly assigned each lizard to one of the two treatment groups (i.e., ALAN or control), such that equal numbers of males and females were assigned to each group.

Across the duration of the study, the two treatment groups were housed in separate climate-controlled rooms, following the standard housing and care protocol for anole lizards [75]. In the control room, temperature ranged 25.6–28.2 °C and humidity ranged 60–68%, while the temperature in the ALAN treatment room ranged 26.4–29.2 °C and humidity ranged 55–67% (on average, the two rooms differed by 1% humidity, and 0.5 °C). Randomly assigned pairs of lizards (one male, one female) were housed together in large (37.5 × 21.0 × 28.0 cm) plastic cages (Kritter Keeper, Lee's Aquarium and Pet Products, San Marcos, CA), following standard breeding-season housing procedures. Lizards were housed in pairs to maintain breeding season behavior and physiology, which may be altered if they were housed in isolation. Each cage contained two small PVC pipe perches; a wire mesh hammock that stretched across the width of the cage; and a small plastic pot filled with moist sphagnum peat moss (Fertilome Bonham, TX), in which females could lay eggs (the "nest box"; see Supplementary Figure 1). The bottom of each cage was lined with R'zilla terrarium liner (Zilla, Franklin, WI). The cages were separated with plyboard to prevent visual contact between lizards in different cages. We fed each lizard 2–3 crickets or mealworms dusted with Fluker's calcium/phosphorus powder (Flukers, Port Allen, LA) three times a week, and misted the cages daily to provide drinking water.

All lizards in the control and ALAN groups were exposed to standard reptile lighting conditions. Directly over the cages in both rooms hung two T8 ReptiSun 5.0 UVB fluorescent bulbs (Zoo Med Laboratories, San Luis Obispo, CA) which were set to a 12.5L:11.5D cycle to mimic the natural light-dark cycle of a summer day in San Antonio, Texas ([61]). These bulbs simulate the full spectrum of sunlight, with emission peaks in the violet (410 and 440 nm), green (550 nm), and yellow (580 nm) spectra, as well as a broadband emission in the UVB and UVA spectrum (centered at 350 nm; [87]). Additionally, the ceiling lights (32 watt GE T8 Starcoat ECO bulbs, GE, Boston, MA; emission peak in the blue spectrum at 450 nm and a broadband emission centered at 600 nm across the green-red spectrum) in the rooms turned on 30 min before the cage lights turned on and turned off 30 min after the cage lights turned off, to mimic dawn and dusk, respectively. Therefore, all lizards were exposed to some level of light from 0600 to 1930 each day.

In addition, ALAN lizards were exposed to a nocturnal light source that mimicked the ALAN exposure that lizards experience on Trinity University's urban campus. We measured nocturnal light intensity on two clear nights at 2200 in May 2019 with a LI-250A Light Meter (LI-COR Biosciences, Lincoln, NE), and found the average intensity under campus lamppost lights was 1.33  $\mu\text{mol}/\text{m}^2/\text{second}$  (approx. 98.5 lux; SD = 0.16  $\mu\text{mol}/\text{m}^2/\text{second}$ ). To simulate this light exposure in the lab, we used a D802-LED 12" low-profile area light (Deco Lighting, Inc. Commerce, CA), identical to those used for nocturnal lighting on Trinity University's campus. The emission spectrum of this light, measured using a custom Ocean Optics USB2000+ spectrometer (Largo, FL) peaked in the blue spectrum at 450 nm, and a broadband emission centered at 600 nm across the green-red spectrum. The light was

covered with four layers of black mesh deer cloth to provide a light intensity of  $1.21 \mu\text{mol}/\text{m}^2/\text{second}$  (approx. 89.6 lux;  $\text{SD} = 0.14 \mu\text{mol}/\text{m}^2/\text{s}$ ;) at a distance of 180.0 cm from the lizard cages in the ALAN room. This cloth reduced the light's intensity to a similar intensity to the campus lamppost lights without changing its emission spectrum. To ensure each cage received an equivalent light exposure over the course of the experiment, we rotated the ALAN cages three times a week. Control cages were rotated on the same schedule.

All animal experiments were conducted in accordance with guidelines from the National Research Council's Guide for the Care and Use of Laboratory Animals.

## 2.2. Overview of behavioral trials

After a 12 d period during which lizards were acclimated to the facility in their treatment's lighting conditions (control or ALAN), over the following four weeks we performed a series of four behavioral trials, with at least 24 h separating each trial on an individual lizard. All trials were conducted in the room where each lizard was housed, and ALAN and control light treatments continued throughout the duration of the study. During nocturnal trials, in which ALAN lizards continued to be exposed to ALAN and control lizards were exposed only to darkness, we used a red-light flashlight (HQRP, Harrison, NJ; emission peak in the red spectrum at 650 nm) to observe lizards without disturbing them, as green anoles have extremely low detection of light with spectral emissions greater than 625 nm [68]. We covered the flashlight with eight layers of VWR Light-Duty Tissue Wipers (Radnor, PA) to minimize its light intensity ( $0.002 \mu\text{mol}/\text{m}^2/\text{second}$ ), as the spectral emissions for the red flashlight were close to the limit of green anole sensitivity to light.

Each type of trial was repeated twice for each lizard. For three of these trials – open field tests (OFTs), foraging trials, and social interaction trials – each lizard participated in one mid-day (1000–1400) and one mid-night (2200–0200) trial. The fourth trial consisted of two 24 h behavioral point observations. The schedule of these trials was as follows. We first conducted OFTs over a period of 9 d. These trials were followed 5 d later by the two 24 h behavioral point observations, separated by 1 d. The first half of the foraging trials were conducted 5 d after the behavioral point observations, followed by 6 d of social interaction trials, and the second half of the foraging trials (see Supplementary Figure 2). The order of mid-day and mid-night trials for the OFTs, social interaction trials, and foraging trials was not the same for all lizards (some experienced nocturnal trials first; others diurnal trials first). Lizards were randomly selected to participate in each trial session.

Mid-day and mid-night OFTs, foraging trials, and social interaction trials were conducted within a standard arena (a  $62.5 \times 34.5 \times 33.0$  cm mesh cage, which was not the lizard's home cage), and each lizard had a 10 min acclimation period under an opaque plastic container ( $14.5 \times 10.0 \times 7.5$  cm) before the 10 min trial began. After the acclimation period, the observer removed the opaque plastic container and moved behind a blind 1.5 m away. Once the OFT, foraging, or social interaction trial was completed, the lizard was placed back in its home cage. One lizard was observed during each OFT and foraging trial, while two lizards of the same sex were observed during each of the social interaction trials.

## 2.3. Behavioral point observations

To quantify differences in behavioral allocation during the night and day, we conducted behavioral point observations of lizards in their home cages for two nonconsecutive 24 h periods, from 1800 to 1800 of the following day. Each hour, we observed each lizard and recorded its location in the cage (see Supplementary Figure 1) and its behavior at the time of observation.

Lizards were “asleep” when they exhibited closed eyes, immobile body, head resting on substrate, and all limbs positioned against the substrate [10,21]. If lizards were awake, they were assigned one of three

behavioral categories, which encompassed all observed behaviors during the trials. Lizards were “alert” if eyes were open and body not moving, whether or not head or body were in contact with the substrate. “Locomotor behavior” included any movements around the cage, and “display” included dewlap extensions, pushups, and head-bobs. Finally, lizards were “non-observable” if the observer could not see the lizard's head (e.g., the lizard was fully under the cage carpet or buried within the nest box) and so was unable to determine if the lizard was awake or asleep. As a proxy for activity between observation periods, we determined the proportion of intervals between observations during which lizards moved from one substrate to another for the day and night separately (see Supplementary Figure 1).

## 2.4. Open field tests

OFTs are standardized trials used in many animal taxa to determine an animal's level of general activity [84]. We recorded the latency to each lizard's first movement (i.e., a locomotor movement that resulted in a change of location in the cage) in s and its total number of movements during the trial.

## 2.5. Foraging trials

To determine if foraging frequency and efficiency differed between day and night, we performed foraging trials. Lizards were not fed within 24 h prior to trials. After the pre-trial acclimation period described above, we placed two live crickets in the arena while removing the opaque plastic container. We recorded the time to each lizard's first movement, time to first cricket consumption, and time to second cricket consumption. If the lizard ate a cricket before the observer was positioned behind the blind, we recorded the time to first movement and time to cricket consumption as 1 s.

## 2.6. Social interaction trials

To assess differences in the frequency or intensity of aggressive interactions between lizards that were or were not exposed to ALAN, we conducted social interaction trials. Lizards were randomly paired with a lizard of the same sex from the same treatment group such that they interacted in the same lighting conditions to which they were acclimated. We paired different lizards in the nocturnal and diurnal trials. The two lizards were placed on opposite sides of the standard arena, under separate opaque containers. We placed a small branch equidistant between the two containers, to provide a vertical perch over which the anoles might compete (e.g., [8,31]). During each trial, two observers (one for each lizard) recorded the total number of head-bobs and pushups (here combined into one measure called “push-bobs”), dewlap extensions, and locomotor movements performed, along with the latency to first movement in s. If one lizard bit its partner or the lizards locked jaws, we immediately stopped the trial and separated the lizards. This occurred in two diurnal trials between ALAN males.

## 2.7. Morphological measurements

After the conclusion of all trials in late July 2019, we measured each lizard's SVL and body mass as described above. Each lizard was then euthanized using a two-step protocol. We first injected a lizard with a 2% MS-222 solution, and once it was unresponsive to a firm toe pinch, delivered an injection of 50% MS-222 solution [11]. The lizard was then rapidly decapitated. We subsequently harvested and massed each lizard's abdominal fat pads, liver, and, for males, testes to the nearest 0.0001 g.

## 2.8. Statistical analysis

### 2.8.1. Overview of behavioral trials

Our analytical approach for behavioral data used generalized linear mixed models (GLMMs) to test whether anoles differed in behaviors based on the interaction between treatment (ALAN vs. control) and time (day vs. night), unless otherwise noted. We used mixed models with random effects for individual identity to account for multiple observations from each lizard. For logistic, multinomial, and Poisson regressions, in initial models we included the main effects of sex, treatment, time, and two- and three-way interactions. In some cases, we found that including all interactions with sex in initial models caused issues with model fitting and performance. Due to this complication, we used simpler initial models which only included sex as a main effect in all cox regression models and the analysis of movements during social interaction trials. For the non mixed-effect cox regression models of foraging time data that did not include interactions (see Supplementary Table 1), we initially included treatment and sex as fixed factors.

To select final models, we *a priori* decided to drop terms including sex and interactions from initial models if likelihood ratio tests (LRTs) determined that they did not significantly contribute to the model. As a result, none of our final GLMMs and cox regressions included terms for sex, and we report initial and final models in Supplementary Table 1. Multinomial mixed models were fit using *xtnlogit* and estimated marginal probabilities calculated using *margins* in Stata (Version 17; [76]). We fit logistic and Poisson mixed models using the *lme4* package [1] and cox regression models using the *coxme* package [79], both in R (Version 4.0.3; [69]). Models were evaluated via visual inspection of residual plots and functions in the *DHARMA* package in R where appropriate [30].

To perform *post hoc* tests on interaction terms in models, we used custom contrasts in the *emmeans* package in R [46] to focus on four comparisons of *a priori* interest (e.g., ALAN day vs. night, control day vs. night, ALAN day vs. control day, ALAN night vs. control night).

### 2.8.2. Behavioral point observations

We examined whether ALAN treatment and time of day influenced activity patterns of lizards by modeling the probability of lizards being awake, non-observable, or asleep using a multinomial mixed-effects model. After fitting the model, we calculated estimated marginal probabilities with 95% confidence intervals of each lizard state and compared them to assess differences in activity. The final model included treatment, time, and their interaction as fixed effects, with individual identity as a random effect. While the lizards were awake, we calculated the percent of observations in which the lizards were alert, displaying, and performing locomotor behaviors (see Supplementary Table 2).

To assess the impact of ALAN on locomotor movements, we compared whether lizards differed in the probability of moving between observations, as defined by a change in position from one substrate to another within the cage, using a GLMM with a logistic distribution. The final model included treatment, time, and their interaction as fixed effects, with individual identity as a random effect.

### 2.8.3. Open field tests

We compared lizards in the two treatments in time to first movement in the OFTs using a mixed-effects cox regression model. Additionally, we compared number of movements during the trials using a GLMM with a Poisson distribution. In both models, we included treatment, time, and their interaction as fixed effects and individual identity as a random effect.

### 2.8.4. Foraging trials

To understand ALAN's impacts on locomotor behavior during foraging, we compared time to first movement during the trials with the same mixed-effects cox regression structure as in the OFTs (above). To

assess ALAN's impacts on prey consumption, we compared lizards in the two treatments in their times to consume the first and second crickets during the trials. Due to the lack of variation in the control lizards in the nocturnal trials (i.e., none of the control lizards consumed crickets at night), we performed two sets of pairwise analyses. In the first set, for the diurnal trials, we compared the lizards' time to consuming their first and second crickets using a separate cox regression model for each cricket. Within these models, we included treatment as a fixed effect. In the second set, we compared the ALAN lizards' time to consuming their first and second cricket during the diurnal and nocturnal trials using a mixed-effects cox regression model for each cricket. Within these models, we included time as a fixed effect and individual identity as a random effect.

### 2.8.5. Social interaction trials

To assess the impact of ALAN on same-sex social interactions, we compared the lizards in the two treatment groups in the number of push-bobs and dewlap displays using a series of permutation tests in the *coin* package in R [33]. We used permutation tests to examine same-sex social interactions because the data violated assumptions of generalized linear models and contain a high number of ties. We conducted separate permutation tests on each sex, due to differences in display behavior by sex [37,63]. Additionally, the control lizards did not perform social displays at night, except for a single push-bob recorded for one male. Due to this lack of variation in the control lizards in the nocturnal trials, we performed a series of pairwise analyses. We compared between treatment groups the number of push-bobs or dewlap displays performed by lizards of the same sex within the diurnal or nocturnal trials. Within the ALAN treatment group, we compared the number of push-bobs or dewlaps displays made by lizards of the same sex between the diurnal and nocturnal trials.

We compared number of movements during the trials using a GLMM with a Poisson distribution. The model included treatment and time as fixed effects and individual identity as a random effect. The interaction between treatment and time was dropped during model selection, because it did not significantly explain the variation in the data, as determined by an LRT (see Supplementary Table 1).

### 2.8.6. Morphological measurements

To determine ALAN's impacts on the physiology of green anoles, we compared lizards in the two treatments in SVL, body mass, fat pad mass, liver mass, and, for males, testis mass using a series of linear models conducted in R [69]. We *a priori* decided to include sex as a fixed factor in all morphology analyses (except for testis mass) due to the sexual dimorphism of green anoles. We included sex\*treatment interaction terms in these initial models. However, we dropped these interaction terms from all final models as they did not significantly contribute to the models, as determined by LRTs (see Supplementary Table 1).

To compare the difference between pre- and post-treatment SVL, we compared lizards of the two treatment groups using linear models that included treatment and sex as fixed factors and initial SVL as a covariate. We performed parallel analyses with pre- and post-treatment body mass. We also compared fat pad and liver mass of lizards in the two treatments using linear models that included treatment and sex as fixed factors and final body mass as a covariate. We compared testis mass of male lizards in the two treatments using a linear model including treatment as a fixed factor and final body mass as a covariate.

## 3. Results

### 3.1. Behavioral point observations

The interaction between treatment and time influenced activity patterns of lizards and the probability of them being awake, non-observable, or asleep during the behavioral point observations ( $\beta_{\text{Awake}} = 3.27$ ,  $Z = 4.24$ ,  $P < 0.001$ ). For both treatments, lizards were more



likely to be awake during the day and asleep at night (Fig. 1). During the day, control lizards were more likely to be awake than ALAN lizards, and ALAN lizards were more likely to be unobserved than control lizards (Fig. 1). At night, while ALAN and control lizards did not differ in their probabilities of being unobserved, ALAN lizards were more likely to be awake and less likely to be asleep than control lizards (Fig. 1).

The interaction between treatment and time influenced the probability that a lizard changed their position among substrates between behavioral point observations ( $Z = 4.41, P < 0.001$ ; see Supplementary Figure 3). During the day, there was no difference in the probability of an ALAN or control lizard making a substrate change ( $P = 0.493$ ), indicating similar rates of movement around their home cage (see Supplementary Figure 3). However, at night, ALAN lizards were more likely to change their substrate than control lizards ( $P = 0.032$ ; see Supplementary Figure 3). Both ALAN and control lizards changed their substrate less frequently at night than during the day (ALAN:  $P < 0.001$ , control:  $P < 0.001$ ).

### 3.2. Open field tests

During the OFTs, the interaction between treatment and time influenced lizards' time to first movement ( $Z = 4.11, P < 0.001$ ). During the day, ALAN lizards were slower to make their first movement than control lizards ( $P = 0.041$ ; Fig. 2a). However, at night, ALAN lizards were quicker to make their first movement than control lizards ( $P = 0.014$ ; Fig. 2a). Within treatment groups, ALAN lizards were quicker to make their first movement at night than during the day ( $P = 0.002$ ; Fig. 2a), while control lizards did not differ in their time to first movement between day and night ( $P = 0.069$ ; Fig. 2a).

The interaction between treatment and time also influenced the number of movements made by lizards during the OFTs ( $Z = 10.55, P < 0.001$ ; Fig. 2b). ALAN lizards moved more frequently at night than control lizards ( $P < 0.001$ ; Fig. 2b). In fact, ALAN lizards moved more

frequently at night than during the day ( $P < 0.001$ ; Fig. 2b), while, conversely, control lizards moved more frequently during the day than at night ( $P < 0.001$ ; Fig. 2b). During the day, ALAN and control lizards did not differ in their number of movements ( $P = 0.113$ ; Fig. 2b).

### 3.3. Foraging trials

Just as in the OFTs, the interaction between treatment and time influenced the lizards' time to first movement during the foraging trials ( $Z = 4.08, P < 0.001$ ; see Supplementary Figure 4). While ALAN and control lizards did not differ in their time to first movement during the day ( $P = 0.119$ ), at night ALAN lizards were quicker to move than control lizards ( $P = 0.009$ ; see Supplementary Figure 4). Within treatment groups, ALAN lizards did not differ in their time to first movement during the day and at night ( $P = 0.556$ ), but control lizards were quicker to move during the day than at night ( $P < 0.001$ ; see Supplementary Figure 4).

During the day, ALAN and control lizards did not differ in the time to consume their first ( $Z = -1.05, P = 0.296$ ; Fig. 3a) or second cricket ( $Z = -1.91, P = 0.056$ ; Fig. 3b). In contrast, none of the control lizards consumed a cricket at night, while 19 of the 24 ALAN lizards consumed at least one cricket, and 9 of 24 consumed both. In fact, more ALAN lizards consumed crickets at night than ALAN or control lizards did during the day. ALAN lizards did not differ in the time to consume their first ( $Z = 1.69, P = 0.091$ ) or second cricket ( $Z = 1.20, P = 0.230$ ) between night and day.

### 3.4. Social interaction trials

During social interaction trials, treatment did not influence the number of movements made by the lizards ( $Z = -0.76, P = 0.450$ ). However, timing of the trial influenced the number of movements ( $Z = -4.47, P < 0.001$ ), as lizards in both treatments moved more frequently during the day than at night.

ALAN and control males did not differ in their number of push-bobs ( $Z = -1.14, P = 0.269$ ; Fig. 4a) or dewlap displays ( $Z = -0.74, P = 0.477$ ; Fig. 4b) during the day. However, at night, ALAN males performed more frequent push-bobs ( $Z = -2.01, P = 0.037$ ; Fig. 4a) and dewlap displays ( $Z = -2.44, P = 0.037$ ; Fig. 4b) than control males. Five ALAN males performed push-bobs and dewlap displays at night while no control males performed any stereotyped displays at night. ALAN males performed push-bobs more frequently during the day than at night ( $Z = 2.10, P = 0.038$ ; Fig. 4a), but did not differ in their number of dewlap displays performed during the day and at night ( $Z = 1.86, P = 0.064$ ; Fig. 4b).

ALAN and control females also did not differ in diurnal push-bobs ( $Z = 1.13, P = 0.271$ ; Fig. 5a) or nocturnal dewlap displays ( $Z = -1.00, P = 1.000$ ; Fig. 5b). However, at night, ALAN females performed push-bobs more frequently than control females ( $Z = -2.44, P = 0.037$ ; Fig. 5a). Five ALAN females performed push-bobs at night while no control females performed any displays at night. Yet during the day, ALAN females performed dewlap displays less frequently than control females ( $Z = 3.03, P = 0.005$ ; Fig. 5b). ALAN females did not differ in their number of push-bobs ( $Z = 0.54, P = 0.611$ ; Fig. 5a) or dewlap displays ( $Z = -1.00, P = 1.000$ ; Fig. 5b) performed during the day and at night.

### 3.5. Morphological measurements

Neither treatment ( $\beta_{\text{ALAN}} = -0.033, F_{1,44} = 0.002, P = 0.965$ ), sex ( $\beta_{\text{Female}} = -1.090, F_{1,44} = 0.599, P = 0.443$ ), nor initial SVL ( $\beta = -0.111, F_{1,44} = 1.264, P = 0.267$ ) was associated with final SVL. When controlling for differences in initial body mass, females lost more body mass on average over the course of the trials than males ( $\beta_{\text{Female}} = -0.487, F_{1,44} = 7.804, P = 0.008$ ) and lizards that had a higher body mass at the beginning of the study tended to lose less body mass over the course of the study than lizards that had a lower body mass at the beginning of the

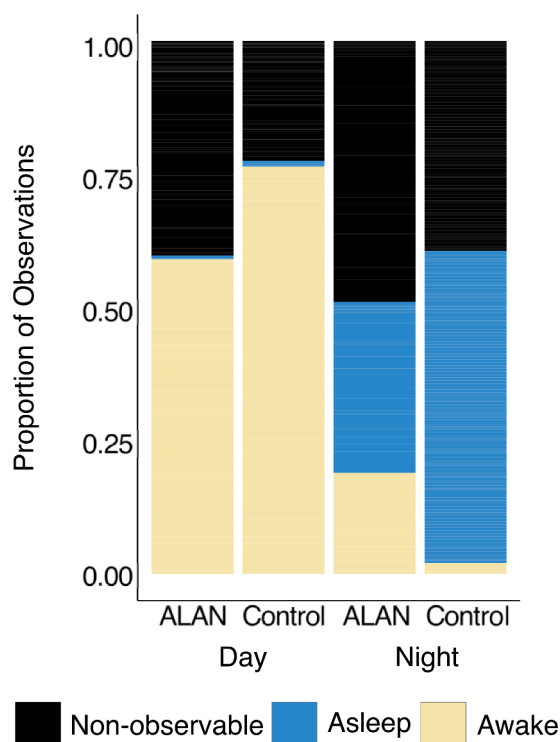
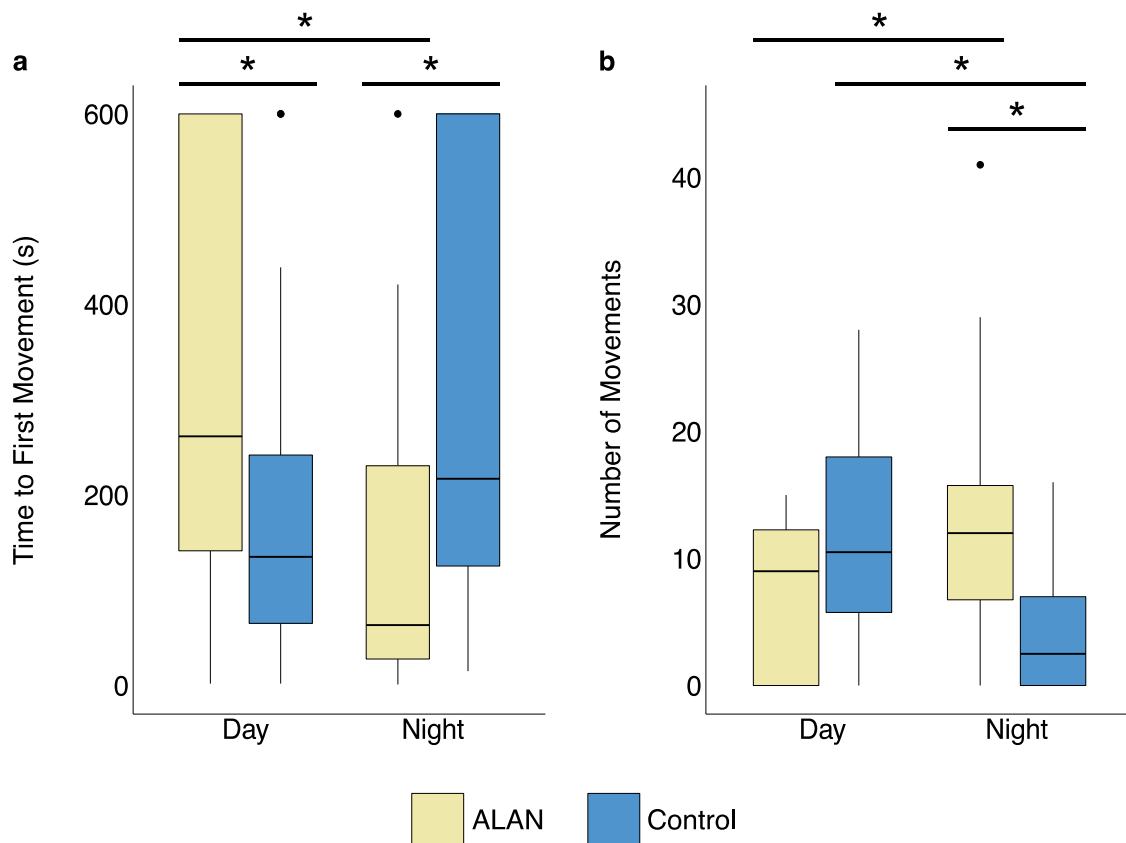


Fig. 1. Green anoles exposed to ALAN ( $N = 24$ ) were more likely to be awake and less likely to be asleep than control lizards exposed to a natural light cycle ( $N = 24$ ) at night. During the day, ALAN lizards were more likely to be non-observable than control lizards, and control lizards were more likely to be awake than ALAN lizards.



**Fig. 2.** Green anoles exposed to ALAN ( $N = 24$ ) differed from control lizards exposed to a natural light cycle ( $N = 24$ ) in a) their time to first movement and b) number of movements performed, in 10 min open field tests during the day and at night. The horizontal lines in the boxplots represent the median. Asterisks indicate the comparison between groups is significant ( $P < 0.05$ ).

study ( $\beta = -0.289$ ,  $F_{1,44} = 21.565$ ,  $P < 0.001$ ). However, treatment did not affect change in body mass over the course of the study ( $\beta_{\text{ALAN}} = 0.186$ ,  $F_{1,44} = 2.980$ ,  $P = 0.091$ ).

ALAN lizards had heavier abdominal fat pads than control lizards ( $\beta_{\text{ALAN}} = 0.018$ ,  $F_{1,43} = 5.664$ ,  $P = 0.022$ ; Fig 6a). Lizard sex was not associated with fat pad mass ( $\beta_{\text{Female}} = 0.021$ ,  $F_{1,43} = 2.081$ ,  $P = 0.156$ ). Additionally, lizards that had a higher final body mass had larger fat pads than lizards that had a lower final body mass ( $\beta = 0.016$ ,  $F_{1,43} = 7.339$ ,  $P = 0.010$ ). Yet, treatment did not affect liver mass ( $\beta_{\text{ALAN}} = 0.008$ ,  $F_{1,44} = 2.109$ ,  $P = 0.154$ ). Females had heavier livers than males when controlling for body mass ( $\beta_{\text{Female}} = 0.039$ ,  $F_{1,44} = 14.215$ ,  $P < 0.001$ ). Lizards that had a higher body mass at the end of the study also had heavier livers than lizards with a lower body mass at the end of the study ( $\beta = 0.031$ ,  $F_{1,44} = 56.833$ ,  $P < 0.001$ ). Finally, ALAN males had heavier testes than control males ( $\beta_{\text{ALAN}} = 0.026$ ,  $F_{1,22} = 28.981$ ,  $P < 0.001$ ; Fig. 6b).

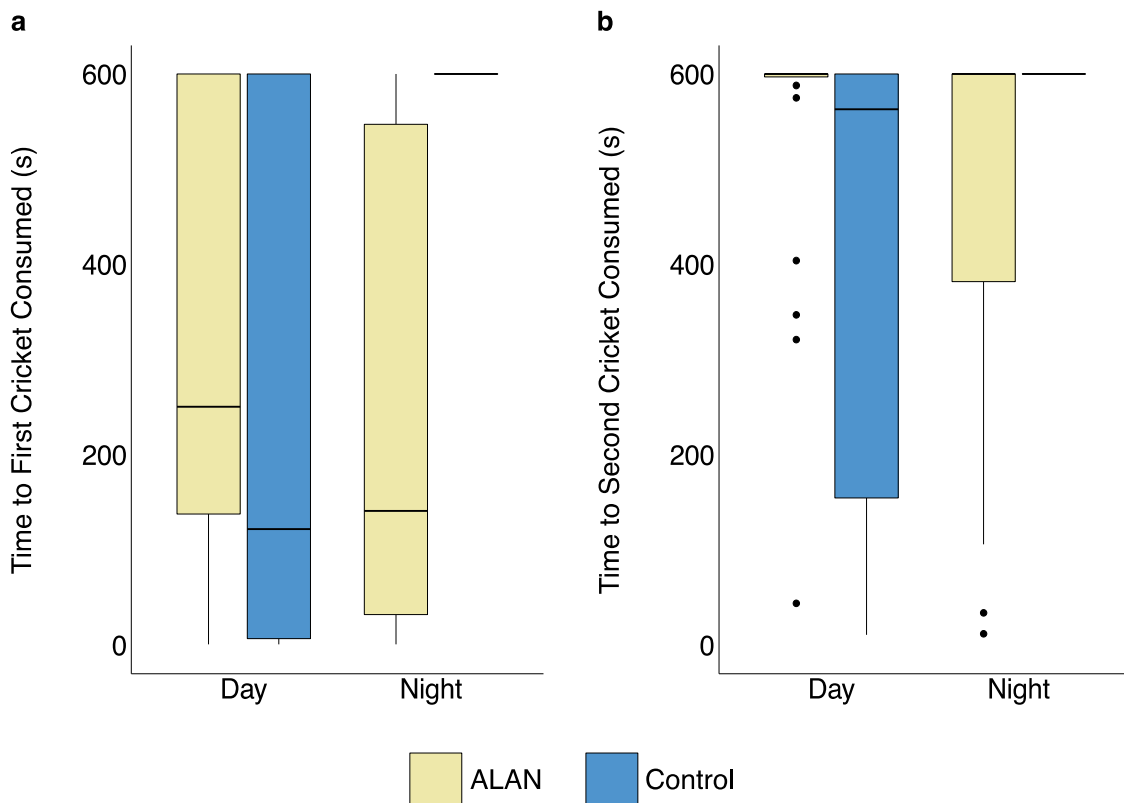
#### 4. Discussion

In this study, we found clear evidence that exposure to ALAN is associated with behavioral trade-offs and altered physiological processes in green anole lizards. When ALAN lizards were awake at night, these visually oriented animals appeared to use ALAN to move, forage, and display. However, during the day, ALAN lizards spent more time in refuge from light (unobserved) and were slower to move than control lizards that were exposed to a natural light-dark cycle. ALAN lizards also had increased fat accumulation, and ALAN males had heavier testes. We discuss the wide-ranging ecological and reproductive implications of these effects below.

##### 4.1. Altered sleep-wake and activity cycles

At night, when green anoles are generally asleep, ALAN lizards spent less time asleep and more time awake, consistent with studies of brown anoles (*Anolis sagrei*, [6]), humans [9], and nocturnal Wistar rats [77]. During the day, ALAN lizards were more likely to be non-observable, presumably sheltering from light to sleep. Sleep is important for energy conservation or restoration [5], brain function and memory consolidation [80], and brain and neuromuscular development [73]. Across species, a lack of sleep has been linked to multiple negative effects, such as reduced immune functioning (e.g., humans: [35]; Wistar-Hannover rats: [86]), impaired social signaling (e.g., European honey bees: [41]), impaired memory consolidation [e.g., chickens (*Gallus gallus domesticus*): [36]; flies (*Drosophila* spp.): [45]], and reduced endurance (brown anoles, [42]). Green anoles experiencing sleep deprivation as the result of ALAN may face similar costs.

In the field, diurnal sleeping may limit foraging and mating opportunities, yet these costs might be alleviated by increased activity during the night. Indeed, our OFTs demonstrated that ALAN lizards exhibited a relative decrease in general locomotor activity during the day, but an increase at night. However, previous studies have shown that ALAN results in different patterns of daily locomotor activity within and across species, and across seasons (e.g., [2,18,22]). This study was conducted during the green anole breeding season, during which trade-offs in activity cycles related to mating may be critical. Thus, future studies of ALAN using longitudinal focal observations, conducted across seasons, would allow a more complete perspective on individual differences in behavioral allocation and whether these differences persist outside of breeding periods.



**Fig. 3.** Green anoles exposed to ALAN ( $N = 24$ ) and control lizards exposed to a natural light cycle ( $N = 24$ ) did not differ in a) time to consume first cricket during diurnal 10 min foraging trials. At night, none of the control lizards consumed a cricket, while 19 ALAN lizards consumed their first cricket. b) During diurnal trials, control and ALAN lizards also did not differ in their time to consume their second cricket. At night, no control lizards consumed a second cricket, while nine ALAN lizards did. The horizontal lines in the boxplots represent the median .

#### 4.2. Foraging

Our results found that ALAN facilitates nocturnal feeding in lizards, similar to other visual predators, such as jumping spiders (*Platycryptus undatus*, [24]), common redshanks (*Tringa tetanus*, [20]), and brown anoles [6]. In fact, ALAN lizards fed at night more frequently than any other group, including ALAN lizards during the day. Nocturnal foraging may result in a cascade of altered ecological relationships. While ALAN provides nocturnal illumination, its intensity rarely mimics the intensity of sunlight over large areas. Therefore, green anoles foraging at night would potentially be clustered in limited areas, causing higher competition. Green anoles may also be exposed to novel predators during nocturnal foraging. Further, if diurnal predators of green anoles also expand their activity into the night, then there is the potential for food webs to shift, putting ecological pressure on nocturnal organisms who cannot expand their activity into the day. For example, a diurnal colubrid snake (*Borikenophis portoricensis anegadae*) has been documented using ALAN to hunt for *Anolis* lizards at night [66]. As community dynamics continue to shift over time, the costs of foraging under ALAN may increase.

#### 4.3. Social communication

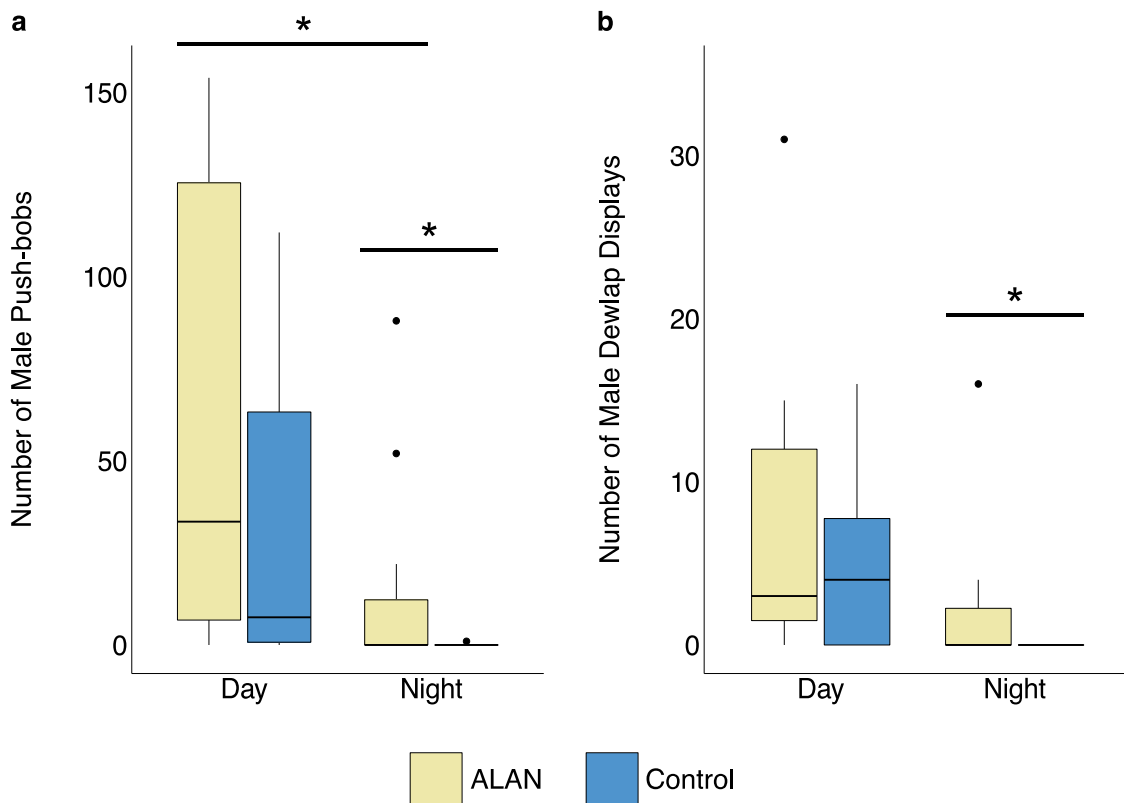
While the overall rates of display during the day did not differ between green anoles exposed to ALAN and those exposed to a natural light-dark cycle, only ALAN male lizards displayed at night. Green anoles of both sexes display more frequently when they can visually interact with conspecifics than when alone [38]; therefore, ALAN lizards likely displayed at night because the nocturnal light enabled them to see the other lizard in their trial. Similarly, several male diurnal bird species [e.g., European robins (*Erithacus rubecula*), European blackbirds, great

tits, and Eurasian blue tits (*Cyanistes caeruleus*)] exposed to ALAN advance their daily auditory mating signals into the night [12,13,40]. Interestingly, this pattern of performing typically diurnal signals with exposure to ALAN is consistent between the visual signals of green anoles and the auditory signals of birds, even though bird song can still be fully conveyed in the absence of light, while the information in visual signals cannot be conveyed without light.

Yet, exposure to ALAN may result in decreased display behaviors during the day. Our results showed that ALAN-exposed females displayed less frequently than control females during the day, but were able to perform nocturnal displays. ALAN females might have experienced a similar effect to that found by Kurvers et al. [43], who suggested that ALAN may reduce the motivation of Trinidadian guppies (*Poecilia reticulata*) to socialize. However, exposure to ALAN did not decrease male lizard displays, suggesting that the territorial social context of these trials may provoke male diurnal display behaviors regardless of ALAN exposure.

#### 4.4. Morphology and physiology

Green anoles exposed to ALAN did not grow in body length (SVL) during this study. This lack of growth is inconsistent with previous findings that adult brown anoles grew with exposure to ALAN in early spring (February through April; [78]), and adult green anoles grew with exposure to an extended photoperiod in fall and winter (September through January; [23]). However, this study was conducted during the late breeding season (late June through July), reflecting a period in which the effects of ALAN have not previously been evaluated in anoles. Therefore, exposure to ALAN may have varying effects across seasons. In addition, green anoles exposed to a natural light-dark cycle also did not grow in SVL over this study, suggesting that green anoles might not be



**Fig. 4.** Male green anoles exposed to ALAN ( $N = 12$ ) did not differ from control male lizards exposed to a natural light cycle ( $N = 12$ ) in their number of a) push-bobs or b) dewlap extensions during diurnal 10 min social interaction trials. However, at night, ALAN male lizards performed push-bobs and dewlap displays more frequently than controls. The horizontal line in the boxplots represents the median. Asterisks indicate the comparison between groups is significant ( $P < 0.05$ ).

growing during the late breeding season in general, or perhaps they were unintentionally food-limited in this study (although the presence of abdominal fat pads in the lizards suggests that excess energy was available).

Exposure to ALAN also did not impact green anole body mass. Adults of many species increase in body mass when exposed to ALAN [e.g., Siberian hamsters: [34]; mice (*Mus musculus*): [22]; humans: [55]; chickens: [64]; but see European blackbirds: [19] and common toads (*Bufo bufo*): [81]]. Fonken et al. [22] speculated that the increased body mass for Swiss-Webster mice in their study was due to increased feeding during the time the mice are typically inactive (daytime), because when feeding was restricted to only the time the mice are typically active (nighttime), the mice's body mass did not increase [22]. In the present study, all lizards received the same number of crickets at the same times, and generally all of the crickets were consumed within the day. This feeding regime may have precluded the development of any differences in body mass between treatments.

Unlike body size and mass, it appears that lipid storage was altered by exposure to ALAN. In green anoles, lipids are primarily stored in the abdominal fat pads, liver, eggs (for females in the breeding season), and "carcass" (i.e., intramuscular lipids and diffuse adipose deposits; [17]). While liver mass did not differ between ALAN and control lizards, even though the liver acts as the primary site of energy mobilization of lipids for reproduction in reptiles [29] and across vertebrate taxa (reviewed by [62]), ALAN lizards did have heavier abdominal fat pads than the control lizards. The increase in fat pad mass is similar to results in mice [22] but in contrast to European blackbirds, whose fat storage did not change with exposure to ALAN [19]. In our study, because there was no overall change in body mass, exposure to ALAN may have mobilized fat storage from the "carcass" to the abdominal fat pads within the lizards. Lipids stored in muscle are generally used for short-term energy demands (reviewed in [67]); therefore, a transfer of lipids from the "carcass" to

the fat pads would suggest that the ALAN lizards were storing lipids for long-term usage and utilizing less energy in their daily activity than control lizards. Consistent with this possibility, exposure to ALAN in free-living great tits caused a decrease in the great tits' daily energy expenditure [85]. A more direct physiological examination of energy allocation and metabolic rate under ALAN would be a valuable contribution to our understanding of this phenomenon.

Finally, ALAN was associated with an increase in male anole testis mass. The annual testis regression of green anoles is controlled by photoperiod length and begins after mid-July [17,47]. Previous research has shown that manipulations of light cycle in combination with pinealectomy in male green anoles altered testicular growth and development in season-specific ways [82,83], and that green anoles exposed to extended photoperiods in the summer-to-fall period had larger testes mass than male green anoles exposed to a normal light-dark cycle [47]. ALAN possibly acts similarly to an extended photoperiod; therefore, the males may have maintained their testis mass as the photoperiod cues, indicating that the breeding season was still occurring. Males of other species, such as Siberian hamsters [34] and European blackbirds [19] have a similar response to ALAN in their reproductive physiology. This pattern raises the possibility that light-responsive clock genes may be seasonally expressed in lizard testes (e.g., [16]), although clock gene expression in mouse testes is insensitive to photoperiod (e.g., [53,60]).

## 5. Conclusions

In this study, we have shown that exposure to ALAN alters green anole foraging and display behavior, sleep patterns, energy allocation, and reproductive physiology. The behavioral and physiological effects of ALAN on individual animals could have cascading effects throughout the ecological community, leading to new environmental challenges and altered ecological relationships. Other studies have demonstrated that



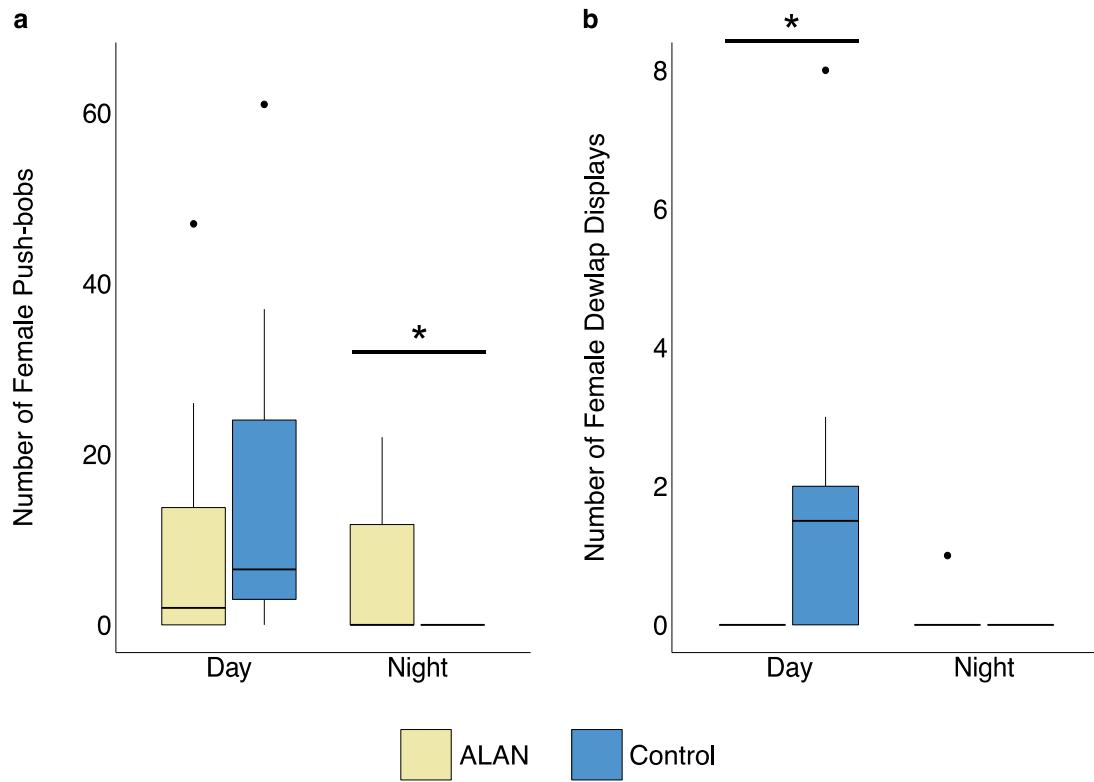


Fig. 5. In the 10 min social interaction trials, female green anoles exposed to ALAN (N = 12) and control female lizards exposed to a natural light cycle (N = 12) did not differ in a) their number of push-bobs during the day. ALAN females performed push-bobs more frequently than controls during the night. b) ALAN females performed dewlap displays less frequently than control females during the day, and female ALAN and control females did not differ in dewlap displays at night. The horizontal line in the boxplots represents the median. Asterisks indicate the comparison between groups is significant ( $P < 0.05$ ).

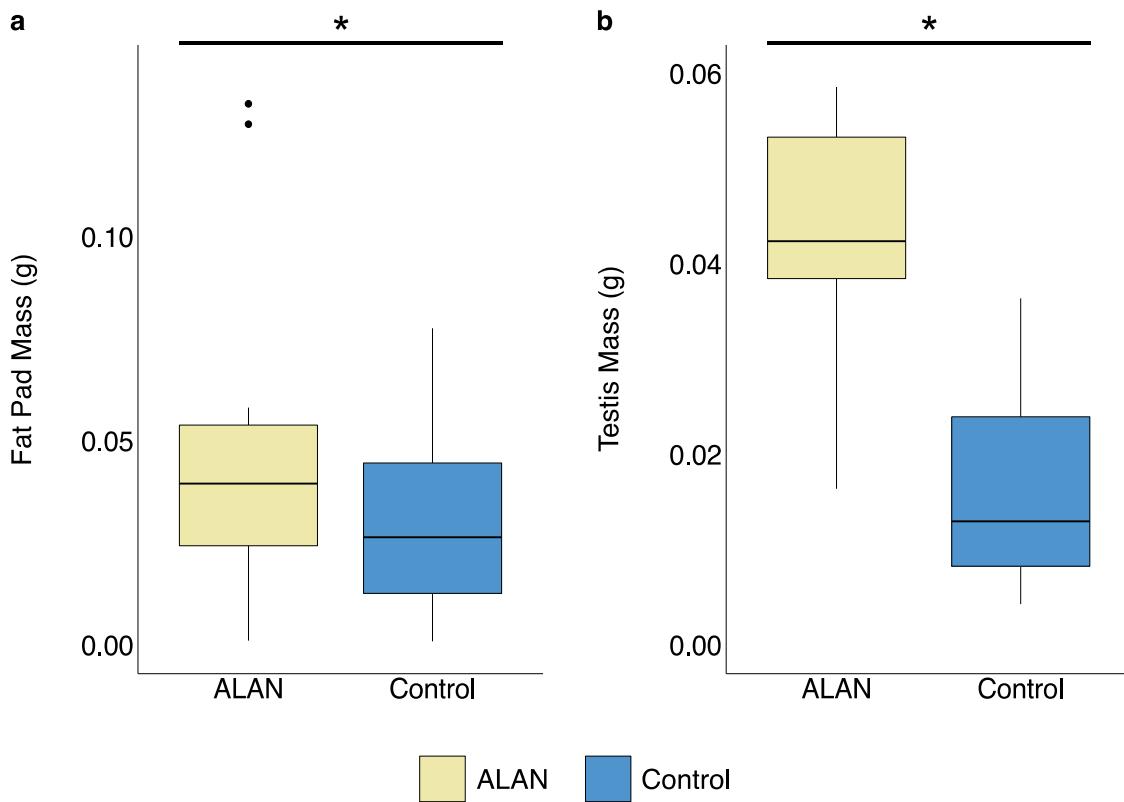


Fig. 6. Green anoles exposed to ALAN (N = 24) had a) higher abdominal fat pad mass than control lizards (N = 24). b) ALAN males (N = 12) had higher testis mass than control males (N = 12). The horizontal line in the boxplot represents the median. Asterisks indicate the comparison between groups is significant ( $P < 0.05$ ).

exposure to ALAN in one or more species can lead to changes in the behaviors of other species, and may even alter the community structure (e.g., [4,14,15]). Determining how physiological and behavioral changes in a focal species impact the wider community is important for informing future conservation efforts and management regarding ALAN.

### CRedit authorship contribution statement

The study was conceived and designed by Laura Taylor, Michele Johnson, and Christopher Thawley. Data were collected and interpreted by Laura Taylor, Olive Pertuit, Abigail Dennis, Isabela Carson, Chen Tang, and Michele Johnson, and data analyses were performed by Laura Taylor and Christopher Thawley. The first draft of the manuscript was written by Laura Taylor and Michele Johnson, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

### Declarations of Competing Interest

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

### Data availability

All data and R scripts for analyses presented here are available on figshare at: [https://figshare.com/collections/Supplementary\\_Materials\\_for\\_Taylor\\_et\\_al\\_-\\_Artificial\\_Light\\_at\\_Night\\_Alters\\_Diurnal\\_and\\_Nocturnal\\_Behavior\\_and\\_Physiology\\_in\\_Green\\_Anole\\_Lizards/6061181](https://figshare.com/collections/Supplementary_Materials_for_Taylor_et_al_-_Artificial_Light_at_Night_Alters_Diurnal_and_Nocturnal_Behavior_and_Physiology_in_Green_Anole_Lizards/6061181)

### Acknowledgements

The authors are grateful to Dale Cochran, Jim Shinkle, Troy Murphy, and David Lopez for technical assistance, and to the Trinity University Physical Plant for the loan of the streetlight. Funding: This work was supported by the Trinity University Department of Biology, Trinity University's Murchison Fellowship (to L. Taylor), Trinity's Biology Summer Undergraduate Research Fellowship (to A. Dennis), Trinity's Office of Academic Affairs (to O. Pertuit), and Texas Ecolab.

### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.physbeh.2022.113992](https://doi.org/10.1016/j.physbeh.2022.113992).

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