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Artificial Light at Night (ALAN): An Anthropogenic Challenge for Urban Lizard Behavior and Physiology

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ARTIFICIAL LIGHT AT NIGHT (ALAN): AN ANTHROPOGENIC CHALLENGE FOR URBAN LIZARD BEHAVIOR AND PHYSIOLOGY

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Abstract

Artificial light at night (ALAN) is a byproduct of anthropogenic illumination that disrupts the behaviors and physiologies of organisms as diverse as mammals, birds, non-avian reptiles, fishes, and insects. In a time of increasing urbanization, discovering the impacts of ALAN on urban organisms is crucial to conservation efforts. In this study, we investigated the impacts of ALAN on the behaviors and physiology of the green anole lizard (*Anolis carolinensis*). Two groups of 24 urban wild-caught adult green anoles (12 males, 12 females per group) were exposed to two different light-dark cycles in a controlled lab setting for six weeks. One group was exposed to a light-dark cycle that simulated the natural light-dark cycle of a summer day in San Antonio, Texas, and the other group was exposed, in addition to the natural light-dark cycle, to an ALAN source that simulated the light intensity of the streetlights on an urban university campus. After an acclimation period, we conducted a series of behavioral trials. Three trials were repeated during mid-day and mid-night: open field tests, to examine exploratory behavior; foraging trials, to examine prey consumption; and conspecific trials, to examine same-sex interactions. The fourth trial examined behavioral time allocation over two 24 h periods. At the conclusion of behavioral trials, we measured each lizard's body mass and snout-vent length (SVL) and the mass of its abdominal fat pads, liver, and reproductive tissues. Our data demonstrate that lizards exposed to ALAN were more likely to be awake at night. While they were awake, lizards exposed to ALAN used the light to explore, forage, and display to conspecifics. However, during the day, lizards exposed to ALAN were more likely to be asleep, were slower to move and forage, and females displayed less frequently than females not exposed

to ALAN. Lizards exposed to ALAN had heavier fat pads and males had heavier testes, but ALAN did not impact liver mass, overall body mass, or female reproductive tissue mass. In sum, ALAN appears to cause behavioral trade-offs between diurnal and nocturnal activity and alters metabolic and reproductive processes within green anoles. These behavioral and physiological changes could cause the lizards to be exposed to novel situations and impact higher-level organization within the urban environment.

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Table of Contents

Abstract	3
Acknowledgements	5
Table of Contents	7
Introduction	8
Methods	26
Results	39
Discussion	59
References	71

Introduction

Biological rhythms

Biological rhythms are an organism's internal clocks of life events over a set time period (Bradshaw and Holzapfel 2010). Biological rhythms are synchronized to the natural environment by environmental cues, known as zeitgebers (Aschoff 1960, Bradshaw and Holzapfel 2010). While the abiotic world is principally organized by the effects of temperature, the biotic world is principally organized by the effects of light (Aschoff 1989, Bradshaw and Holzapfel 2010). The properties of light that influence biological rhythms include daily variation in the timing of light and dark over a 24 h period (or, the light-dark cycle), and seasonal variation in day length (or, photoperiod) (Bradshaw and Holzapfel 2010, Aulsebrook et al. 2018). Changes of the light-dark cycle and photoperiod correlate to daily and seasonal patterns, respectively, because patterns of light are determined by the rotation, tilt, and orbital motion of the Earth in relation to the sun, which, in turn, influences temperature patterns and the distribution of life (Gaston et al. 2014).

Biological rhythms can be organized into tidal (13 h), daily (24 h), lunar (29.5 d), and seasonal (365 d) cycles (Foster and Roenneberg 2008). The most well-known biological rhythms are circadian rhythms, which are the life events of an organism organized over a roughly 24 h period (Bradshaw and Holzapfel 2010). Mammalian circadian rhythms are primarily controlled by the activity of a central pacemaker: the suprachiasmatic nuclei of the hypothalamus (SCN; reviewed in Dibner et al. 2010). Light information is received by photosensitive retinal ganglion cells in the retinas, which transmit the information to the SCN. The SCN's gene expression is altered due to this information, which is in turn relayed to other parts of the brain and the body through neurochemicals, endocrine signals, body temperature rhythms, and feeding cycles. Circadian rhythms in birds are controlled by multiple pacemakers, including the pineal gland, the

retinae, and the medial SCN and visual SCN, the avian homologs to the SCN (reviewed in Cassone and Westneat 2012). These four elements work in tandem to regulate the daily cycles of melatonin, a hormone that is crucial to regulating sleep-wake cycles in all vertebrates (Jones et al. 2015). In amphibians, reptiles, and fishes, the pineal gland alone is the central pacemaker of circadian rhythms, regulating rhythms primarily through the production of melatonin (Underwood 1989/1990).

The internal life events of biological rhythms set by light can impact biological phenomena from the level of an individual to an entire ecosystem. At the level of the individual, biological rhythms entrained by light include daily cycles of hormones, such as melatonin in goldfish (*Carassius auratus*, Iigo et al. 1991), testosterone in male humans (*Homo sapiens*, Plymate et al. 1989), and corticosterone and adrenocorticotropin in fancy rats (*Rattus norvegicus domestica*, Atkinson and Waddell 1997), as well as daily cycles of behaviors, such as foraging in stingless bees (*Scaptotrigona* aff *depilis*, Bellusci and Marques 2001) and movements of brown shrimp (*Crangon crangon*, Adhub-Al and Naylor 1975). Seasonal biological rhythms entrained by light include reproductive and migration cycles, such as the ovarian cycle of pony mares (*Equus ferus caballus*, Freedman et al. 1979), flowering in thale cress (*Arabidopsis thaliana*, Gregory and Hussey 1953), and reproduction, molt, and migration cycles in a variety of bird species (reviewed in Dawson et al. 2001, Gwinner 2003). At the level of a population, light entrained biological rhythms have impacted the diversity and abundance of individual species, such as Asian tiger mosquitoes (*Aedes albopictus*, Urbanski et al. 2012); community organization, such as the bacterial composition in the rumen of Soay sheep (*Ovis aries*, McEwan et al. 2005); and even the distribution of broad taxa, such as biogeographic patterns of terrestrial mammals (reviewed in Bennie et al. 2014).

ALAN: A disruptor of biological rhythms

While biological rhythms can persist in artificial environments, without zeitgebers biological rhythms become desynchronized from the natural environment (Bradshaw and Holzapfel 2010). Additionally, zeitgebers might not accurately reflect the natural environment. For example, anthropogenic ecological light pollution does not accurately reflect the natural light-dark cycle (Longcore and Rich 2004). A specific type of ecological light pollution is artificial light at night (ALAN). In ALAN, the lighting source is illuminated during the night, causing an overall increase in the amount of time an area is illuminated during a given 24 h period. ALAN is a byproduct of outdoor illumination, such as street lights, internal and external lighting of buildings, road vehicle headlights, and shipping and offshore infrastructure (Gaston et al. 2014). ALAN is unique, as it introduces light into places and times, and at intensities and emission spectra, that do not naturally occur (Gaston et al. 2014).

While ALAN refers specifically to direct illumination that extends to hundreds of meters, the scattering of ALAN can also create a phenomenon known as skyglow, which can extend to thousands of kilometers (Gaston et al. 2014, Gaston et al. 2015). Skyglow is the scattering of ALAN over a large area by small particles or droplets suspended in the atmosphere, known as aerosols (Kyba et al. 2011). Skyglow is an issue on a global scale, as it is documented in nearly every country in the world (Cinzano et al. 2001). The biological effects of skyglow are mostly unknown due to challenges in study design (Gaston et al. 2014); however, the phenomenon of skyglow demonstrates the effects of ALAN can reach far from the original light source.

There are multiple different types of artificial lighting sources, including liquid fuel, pressurized fuel, incandescent, fluorescent, mercury vapor, metal halide, high pressure sodium,

low pressure sodium, and light emitting diode lamps (Elvidge et al. 2010). Recent trends in developed nations have moved away from incandescent and fluorescent lighting and towards LED lighting, due to LED lighting's high efficiency, quality, and increasing affordability (Haitz and Tsao 2011). All LED emission spectra approximating white light contain an emission peak in the blue spectrum at 450 nm and a broadband emission centered at 600 nm across the green-red spectrum (Elvidge et al. 2010). The emission spectrum of each ALAN source differs from each other, but all ALAN sources have emission spectra that differ from the emission spectra of natural electromagnetic radiation sources, such as the sun, moon, and stars (Gaston et al. 2015). Natural electromagnetic radiation sources emit radiation from 200 nm to over 4000 nm, with a single peak in the visible light spectrum, from 400 nm to 700 nm (Mecherikunnel and Richmond 1980). However, most electromagnetic radiation below 290 nm is absorbed by the atmosphere and therefore does not reach the Earth's surface (Mecherikunnel and Richmond 1980).

Due to ALAN's disruption of the natural light-dark cycle, ALAN alters organisms' physiologies and behaviors (reviewed in Gaston et al. 2015). The physiological and behavioral changes can have neutral, beneficial, and detrimental effects, depending on characteristics of the individual organism and the species as a whole (reviewed in Gaston et al. 2015).

ALAN's impacts on hormones

Physiologically, ALAN alters hormone concentrations, which can lead to the disruption of metabolic, reproductive, and immunological functions regulated by hormones (reviewed in Ouyang et al. 2018). ALAN suppresses the daily production of melatonin across a variety of taxa, including humans (McIntyre et al. 1989), European blackbirds (*Turdus merula*, Dominoni et al. 2013a), European perch (*Perca fluviatilis*, Brüning et al. 2015), and green anoles (*Anolis*

carolinensis, Moore and Menaker 2011). In general, shorter wavelengths (blue light) suppress melatonin production more than longer wavelengths (orange-red light) (e.g., Senegalese soles, *Solea senegalensis*, Oliveira et al. 2007; humans, West et al. 2011; social voles, *Microtus socialis*, Zubidat et al. 2011). Melatonin suppression due to ALAN suppressed immune functioning in Siberian hamsters (*Phodopus sungorus*, Bedrosian et al. 2011) and when given melatonin supplements while exposed to ALAN, black field crickets (*Teleogryllus commodus*) showed increased immune functioning (Jones et al. 2015). In fact, melatonin has been implicated to be the mechanistic link between the presence of ALAN and reductions in biological fitness across multiple taxa (Jones et al. 2015).

Additionally, ALAN has mixed effects on daily cycles and baseline levels of glucocorticoids, depending on the species and ALAN source. In great tits (*Parus major*), exposure to white ALAN, but not green ALAN, caused increased baseline corticosterone (CORT) concentrations and the increase in baseline CORT was dose-dependent (Ouyang et al. 2015). Birds that nested closer to the ALAN source had higher baseline CORT concentrations than birds that nested further away (Ouyang et al. 2015). In Siberian hamsters, exposure to ALAN dampened daily fluctuations of cortisol (Bedrosian et al. 2013). As glucocorticoids aid in maintaining an organism's energy balance, dysregulation could have severe impacts on the fitness of an organism (Ouyang et al. 2018). However, in European perch (Brüning et al. 2015) and brown anoles (*Anolis sagrei*, Thawley and Kolbe 2020), ALAN did not impact glucocorticoid production. Therefore, the impacts of ALAN on the daily cycles and baseline levels of glucocorticoids are not universal.

ALAN's impacts on reproductive physiology

The impacts of ALAN on hormones can lead to changes in reproductive development; however, not all studies make the connection between altered hormone concentrations and reproductive changes. ALAN alters the timing of seasonal reproductive processes differently in short-day breeders (fertile in the fall where daylength is decreasing) than in long-day breeders (fertile in the spring where daylength is increasing; reviewed in Ouyang et al. 2018).

In short-day breeders, ALAN delays or inhibits reproductive events, such as the suppression of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) production in female European perch (Brüning et al. 2016) and delayed birth dates in tammar wallabies (*Macropus eugenii*, Robert et al. 2015). In long-day breeders, ALAN advances and prolongs the timing of reproductive maturation and breeding, such as an advancement in reproductive development of European blackbirds (Dominoni et al. 2013b) and a delay in testis regression in the fall for Siberian hamsters (Ikeno et al. 2014). However, de Jong et al. (2015) found ALAN did not affect the lay date of great tits and pied flycatchers (*Ficedula hypoleuca*) consistently from year to year, indicating that the timing of reproductive system development might not impact the timing of birth.

ALAN's impacts on metabolism

Physiologically, ALAN's impacts extend beyond reproduction and can cause alterations to metabolic processes. Studies of the impacts of ALAN on metabolic processes have focused primarily on humans and rodent models. In humans, ALAN is linked to obesity (e.g., McFadden et al. 2014, Rybnikova et al. 2016). In rodent models, such as Swiss-Webster mice (*Mus musculus*, Fonken et al. 2010) and Siberian hamsters (Ikeno et al. 2014), ALAN caused increased body mass. Most of these studies provided food *ad libitum* or were correlational and therefore

did not monitor the organisms' food intake. Fonken et al. (2010) did however monitor the Swiss-Webster mice food intake and found ALAN shifted the nocturnal mice food intake to the daytime, when they are typically inactive. In addition, Fonken et al. (2010) found that ALAN caused reduced glucose processing in Swiss-Webster mice.

Other organisms in which ALAN's effects on metabolism have been studied include domesticated chickens (*Gallus gallus domesticus*, Pan et al. 2014), where ALAN caused increased body mass as well. In free-living great tits, ALAN caused reductions in daily energy expenditure, which was linked to increased food availability in the presence of ALAN (Welbers et al. 2017). However, to my knowledge, Welbers et al. (2017) is the only study examining ALAN's impacts on metabolism on free-living wild organisms.

ALAN's impacts on sleep-wake cycles

Physiological alterations due to ALAN could be the mechanism for behavioral changes, such as disturbed sleep-wake cycles. Few studies have been conducted on sleep-wake cycles, outside humans (Gaston et al. 2017, but see Raap et al. 2015, 2016, Stenvers et al. 2016). The exact purpose of sleep is debated, but it is generally thought to be important for energy conservation or restoration (Berger and Phillips 1995), brain function and memory consolidation (Tononi and Cirelli 2006), and brain and neuromuscular development (Roffwarg et al. 1966). Sleep deprivation has been shown to impact multiple organisms across a variety of taxa with effects such as reduced immune functioning (e.g., humans, Irwin et al. 1996; Wistar-Hannover rats, *Rattus norvegicus domestica*, Zager et al. 2007), impaired social signaling (e.g., European honey bees, *Apis mellifera*, Klein et al. 2010), and impaired memory consolidation (e.g., chickens, Jackson et al. 2008; flies, *Drosophila* spp., Le Glou et al. 2012). It is crucial to

determine which species' sleep-wake cycles are disrupted by ALAN in order to mitigate the potential negative effects.

In humans, ALAN is found to disrupt sleep-wake cycles, leading to poor sleep quality and sleep fatigue (Martin et al. 2012). Specifically, ALAN causes increased waking after sleep onset, as well as altered sleep stage lengths (Cho et al. 2016). Mammalian sleep is divided into two sections: non-REM sleep, which, in humans, is divided into Stages N1, N2, and N3, and REM sleep. ALAN increased time spent in Stage N1, decreased time spent in Stage N2, and increased time spent in REM sleep, which is associated with melatonin deficiencies (Kocher et al. 2006, Cho et al. 2016). ALAN is linked to insomnia in elderly individuals (Obayashi et al. 2014) and delayed sleep phase disorder in adolescents (Auger et al. 2011).

In the few non-human studies on sleep-wake cycles, ALAN causes similar disruptions to sleep-wake cycles. ALAN exposure in the winter months causes great tits to wake up earlier, take longer to fall asleep, and overall sleep less (Raap et al. 2015). However, there was no effect on sleep bout length or frequency (Raap et al. 2015). In the spring months, ALAN exposure of great tits caused the same effects as in the winter, but they had a reduced frequency of sleep bouts (Raap et al. 2016). In Wistar rats (*Rattus norvegicus domestica*), ALAN disrupted the sleep-wake cycle, with the nocturnal rats sleeping more during the night and less during the day (Stenvers et al. 2016). Additionally, ALAN dampened the rhythm of non-REM sleep in Wistar rats (Stenvers et al. 2016). Overall, disruptions to sleep-wake cycles by ALAN may lead to other behavioral adjustments, as explored below.

ALAN's impacts on awake behaviors

Behaviorally, ALAN is typically thought to reduce daily movements and foraging time of nocturnal or light-shy organisms during the night, but extend the daily movements and foraging time of diurnal organisms into the night. For example, when exposed to ALAN, nocturnal common spiny mice (*Acomys cahirinus*) decreased their nocturnal activity and foraging (Rotics et al. 2011), light-shy aquatic invertebrates communities reduced their drift (Perkin et al. 2014), and lesser horseshoe bats (*Rhinolophus hipposideros*) changed their flight routes to avoid areas with ALAN (Stone et al. 2009). Sea turtle hatchlings born on beaches with ALAN either became disoriented and wandered in circuitous paths for hours, or became misoriented and crawled towards ALAN instead of out to sea (Salmon 2006). Diurnal organisms such as jumping spiders (*Platycryptus undatus*, Frank 2009) and brown anoles (Brown and Arrivillaga 2017) use ALAN to forage at night. However, there are also some nocturnal species that use ALAN to forage, such as common pipistrelle bats (*Pipistrellus pipistrellus*, Spoelstra et al. 2015), Australian garden orb-web spiders (*Eriophora biapicata*, Willmott et al. 2019), and common redshanks (*Tringa tetanus*, Dwyer et al. 2013). Therefore, ALAN can have negative or positive effects on the daily movements and foraging, depending on the ecology of the species.

ALAN can also cause changes in daily and seasonal social communication. The most well-studied model for ALAN's impacts on social communication is mating calls in birds. Bird song in multiple species, including Eurasian blue tits (*Cyanistes caeruleus*), great tits, European blackbirds, and European robins (*Erithacus rubecula*), began earlier in the day (Kempenaers et al. 2010) and earlier in the season (Da Silva et al. 2015) in the presence of ALAN. Additionally, ALAN reduced flashing behavior of two species of firefly, *Photuris versicolor* and *Photinus pyralis*, in mating contexts (Firebaugh and Haynes 2016). Male common glow-worms (*Lampyris noctiluca*) were unable to find bioluminescent females with exposure to very low levels of

ALAN (0.1 lux; Bird and Parker 2014). However, in Trinidadian guppies (*Poecilia reticulata*), exposure to ALAN did not impact the amount of time the guppies spent with two male and two female companions during the day (Kurvers et al. 2018). To my knowledge, no study has looked specifically at the impact of ALAN on intrasexual communication, which is important in establishing and maintaining a territory, locating and identifying related individuals, conveying information to conspecifics, and facilitating group unity (Gillam 2011).

Reptiles and ALAN

Within the literature on ALAN, there is a general lack of information about the effects of ALAN on reptiles, with the exception of sea turtle hatchlings and females (Perry and Fisher 2006, Salmon 2006). Reptiles are a diverse group of organisms that are central to the maintenance of ecosystem functions and services critical to our survival, including supporting services, such as in nutrient cycling (DéVault and Krochmal 2002); regulating services, such as in seed dispersal (Valido and Olesen 2007); provisioning services, such as a protein source (Peres 2000), a raw material (Prestridge et al. 2011), and in medicine (Alves and Santana 2008); and cultural services, such as in ritualistic use (Neto et al. 2009). Unfortunately, there is a global decline in reptile species diversity due to habitat loss and degradation, introduced invasive species, environmental pollution, diseases, unsustainable use, and climate change (Gibbons et al. 2000, Sinervo et al. 2010). Climate change in particular disproportionately harms reptiles compared to birds and mammals (Rolland et al. 2018). Lizards in particular are expected to experience species extinction levels of 20% and local extinction levels of 39% by 2080 (Sinervo et al. 2010). With the current extinction threat due to climate change, it is imperative to understand potential synergistic factors causing reptile declines, such as ALAN.

One species of lizard, *Anolis carolinensis*, the green anole lizard, is an excellent model system to study the impacts of ALAN. Green anoles are common diurnal lizards that are found in disturbed and natural areas (McMillan and Irschick 2010) and have been well-studied in both the lab and field (reviewed in Lovern et al. 2004, Losos 2009). Green anoles are highly visual organisms that detect electromagnetic spectra from wavelengths of 358 nm (UV) to 625 nm (the border between the orange and red spectrum), which overlaps with the peaks of the emission spectra of LEDs (Provencio et al. 1992, Kawamura and Yokoyama 1998). Their general behavior is well characterized and easy to observe (Greenberg and Noble 1944, Jenssen et al. 1995, Jenssen et al. 2000), and their sleep behavior is readily identifiable (Clark and Gillingham 1990).

Green anoles are seasonal breeders, with a breeding season from April to mid-September. Many behavioral (e.g., Jenssen et al. 1995, Nunez et al. 1997) and physiological traits (e.g., Dessauer 1955a; Licht 1971, 1973) are influenced by this seasonal reproductive cycle.

Seasonal changes in green anole reproductive physiology

In female green anoles, ovaries start to enlarge at the beginning of spring and the yolk is developed by late March (Dessauer 1955a). Ovarian enlargement and development is stimulated by warmer temperatures in the spring, but not by photoperiod length (Licht 1973). Humidity and social cues also affect the ovarian cycle, as low humidity (Summers 1988), as well as low social status and crowding (Summers et al. 1995), inhibit ovarian development. In April, females begin egg production (Dessauer 1955a). Females lay one egg at a time about every two weeks by alternating egg production between their two ovaries (Licht 1973). By mid-August, most adult females stop producing eggs, however, some individuals continue to produce eggs until mid-September (Licht 1973). In mid-September, the females' ovaries have regressed, with small

follicles lacking yolk and small oviducts (Licht 1973). The regression of the ovaries appears to be controlled by an endogenous rhythmicity, as they do not cease or postpone the regression with changes in temperature or photoperiod length (Licht 1973). No reproductive events occur from mid-September until late March (Dessauer 1955a).

For male green anoles, the low temperature of the first few months of the year promotes testis growth (Licht 1969). In early March, increasing temperatures stimulate spermatogenesis (Licht 1969). Male anoles have seven stages of spermatogenesis: stage 1 is fully regressed, inactive testes; stages 2-5 are the progression of recrudescence (testes development); stage 6 is the peak of spermatogenic activity and is marked by the development of accessory sexual structures; and stage 7 is regression of the testes (Licht 1967a). Testes are heaviest in April and May (Dessauer 1955a), and stage 6 of spermatogenesis coincides with peak testis mass in April (Licht 1971). Testis mass plateaus between May and mid-July (Licht 1971) and then declines over the rest of the summer months (Dessauer 1955a). Testes cease spermatogenesis by the end of August (Licht 1971) and are fully regressed (at their lowest mass) in September and October (Dessauer 1955a). Testis regression depends primarily on the photoperiodic cue of decreasing daylength (Licht 1971). Males' testes begin to increase in mass during November and December (Dessauer 1955a), and this process is controlled primarily by temperature cues (Licht 1967b).

Other physiological attributes vary in tandem with the reproductive cycle, including metabolic processes. Green anoles store the majority of their lipids in three or four places: abdominal fat pads, the liver, eggs (for females in the breeding season), and the "carcass" (i.e., intramuscular lipids and diffuse adipose deposits; Dessauer 1955a). Anoles typically do not have dissectible fat pads before mid-July (Licht 1971), but the fat pads increase in mass over the late summer and are the largest in October (Dessauer 1955a). Over the course of the winter, fat pads

decline in size and are undetectable in the spring months (Dessauer 1955a). The liver shows a similar pattern of seasonal variation, with the highest mass in the fall and lowest mass in the spring, and this seasonal variation is more pronounced for males than females (Dessauer 1955a). Liver mass starts increasing, due to synthesis of liver protein, with the increasing appetite of anoles in the late spring (Dessauer 1955b).

Seasonal changes in green anole reproductive behaviors

In March, male and female anoles emerge from their winter refugia at the same time and disperse before they begin breeding behaviors, fully develop their sexual organs, and initiate territorial responses (Jenssen et al. 2001). Male territorial behaviors gradually develop over March and April, and courtship during this time is infrequent and brief (Jenssen et al. 2001). Males and females establish overlapping territories that they behaviorally defend, and male territories are significantly larger than female territories (Jenssen and Nunez 1998, Bush et al. 2016, Kamath and Losos 2017).

From May to July, male anoles are considered socially oriented organisms (Jenssen et al. 1995). Males spend two-thirds of their waking time on defense (patrolling and displaying), courtship, and copulation from May to July, with the majority of the time spent on social display (Jenssen et al. 1995). Males move an average of 27 m within an hour and display an average of 100 times per hour (Jenssen et al. 1995). Males proportionally spend less time foraging during this time of intense social display as compared to August through September and frequently opportunistically forage while on defense (Jenssen et al. 1995).

In contrast to males, females from May to July spend about 80% of their time inactive (Nunez et al. 1997). Females rarely display outside courtship, copulation, and intrasexual

encounters (Nunez et al. 1997). Females engage in similar levels of courtship and copulation to males from May to July, but they rarely have intrasexual encounters that result in aggression (0.3% observed time; Jenssen et al. 1995, Nunez et al. 1997). Additionally, females engage in similar levels of foraging as males, but primarily forage from a stationary position (Nunez et al. 1997).

In August to September, males are less engaged in social activities as they move less (8 m/h), display infrequently (6 displays per hour), and do not engage in courtship displays (Jenssen et al. 1995). They have even been found to cohabitate and congregate together during this time (Jenssen et al. 1996). In turn, males spend triple their time on foraging, as compared to foraging rates from May to July, and primarily forage from a stationary position (Jenssen et al. 1995). Additionally, males spend more time on higher (> 2 m) and thinner (> 8 cm) perches from August to September, which may be due to increased foraging in the canopy (Jenssen et al. 1995). While there is little behavioral data on females from August to September, based upon their increased resting metabolic rates, females also increase feeding rates during this time period (Orrell et al. 2004). Females also likely do not engage in courtship displays and copulation during this time period, in parallel to male behavior (Jenssen et al. 1995).

From December to March, male and female green anoles are inactive over 90% of the time (Jenssen et al. 1996). Males only move at an average rate of 2.5 cm/h, while females move at an average of 1.5 cm/h (Jenssen et al. 1996). The remainder of time is spent close to their nighttime refugia, foraging, interacting socially, or responding to predator threats (Jenssen et al. 1996).

Study design

In this thesis, I will analyze the impacts of ALAN on the behavior and physiology of green anole lizards, including the lizards' sleep-wake cycle. Altered sleep-wake cycles indicate disturbed circadian rhythms, which can lead to deviations in behavioral patterns, as well as altered metabolic and reproductive processes. There is a robust body of literature on the impacts of different photoperiod length on green anole behavior and physiology (e.g., Fox and Dessauer 1957, Licht 1971, Moore and Menaker 2011), and yet, few studies have analyzed the effects of changes in photoperiod length in the context of anthropogenic stressors on green anoles. This gap provides an opportunity to investigate the intersection of ecology, behavior, and conservation in the context of ALAN's impacts on sleep cycles and energy allocation, particularly in the context of reptiles.

I tested the hypothesis that because ALAN changes the natural light-dark cycle, it modifies the green anole lizards' sleep-wake cycle, thereby influencing behavioral patterns and the physiology of the lizards. Using urban wild-caught green anole lizards exposed to either a natural light-dark cycle or a natural light-dark cycle and ALAN in the laboratory, we conducted four types of behavioral trials in the laboratory, including open field tests (OFTs), undisturbed behavioral observations, foraging trials, and social interaction trials. For the OFTs, foraging trials, and social interaction trials, we conducted one diurnal and one nocturnal trial. After the behavioral trials, we measured a series of morphological traits to evaluate potential physiological effects of ALAN.

The first round of trials we conducted were OFTs. OFTs are standardized trials used in many animal taxa to determine an animal's general activity levels (Walsh and Cummins 1976). In the field, shifts to increased nocturnal activity with exposure to ALAN have been documented in multiple species of diurnal lizards (reviewed in Perry and Fisher 2006), including brown

anoles (Brown and Arrivillaga 2017) and green anoles (McCoid and Hensley 1993). In the lab, four species of *Anolis* lizards had increased locomotor activity at night with exposure to pulses of ALAN (Moore et al. 2012). In the OFTs, I predicted that lizards exposed to an ALAN treatment would exhibit a higher rate of general activity at night, and correspondingly less activity during the day, than control lizards.

In the undisturbed behavioral observations, every hour we recorded a snapshot of each lizard's activity. This allowed us to gain insight into their 24 h sleep-wake cycle, as well as potential differences in behavioral allocation. As observations of green anoles have shown that they are active and foraging in the presence of ALAN (McCoid and Hensley 1993), I predicted that lizards exposed to an ALAN treatment would be awake and change perch location more frequently during the night than control lizards. To my knowledge, no studies have examined the diurnal behaviors of lizards exposed to ALAN, therefore I do not have a basis to make predictions of diurnal behaviors. However, I believed that lizards exposed to ALAN would be more likely to be asleep and less active than control lizards during the day, because the exposure to ALAN would decrease their sleep at night.

The third round of trials we conducted were foraging trials, where we provided a lizard with two crickets. In a previous experimental study, juvenile and adult 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 who were exposed to 9L:15D cycle (Fox and Dessauer 1957). Fox and Dessauer (1957) postulated that because the 18L:6D lizards were awake more frequently due to the exposure to an elongated photoperiod, they had higher energy demands than the 9L:15D lizards and subsequently required more food. Therefore, I

predicted that lizards exposed to an ALAN treatment would consume more prey than control lizards because ALAN mimics an elongated photoperiod.

Concurrent with the foraging trials, we conducted same-sex conspecific social interaction trials where we recorded the interactions of two lizards. Both male and female green anoles more frequently display when they view individuals of the same sex, but females display less frequently in intrasexual encounters than male green anoles (Jenssen et al. 2000). Male green anoles will also perform nondirected displays, where they display to unspecified and/or undetected conspecifics (Jenssen et al. 2012), but female green anoles do not perform nondirected displays (Jenssen et al. 2000). Because at night, lizards exposed to ALAN can see their respective conspecific and lizards not exposed to ALAN cannot, I predicted that lizards exposed to ALAN would display more frequently at night than lizards in a normal light-dark cycle. Additionally, I predicted that females would display less frequently than males, regardless of treatment.

At the conclusion of the behavioral trials, we performed morphological measurements to determine the potential physiological effects of ALAN, including effects on reproduction and metabolism. Male green anoles exposed to 13.5L:10.5D and longer light periods have been shown to maintain enlarged testes from the summer breeding season into the fall (Licht 1971), while females do not maintain their ovaries with the increased photoperiod length (Licht 1973). I could not make a prediction of how females reproductive tissue mass would differ, because I collected the total reproductive tissue mass of female lizards (including all developing eggs), without separately massing the ovaries. However, the two groups of females were all in breeding condition and were likely in the same ovarian stage. I predicted males exposed to ALAN would have heavier testes than males not exposed to ALAN.

We addressed metabolic processes by recording overall body mass and snout-vent length (SVL), as well as fat pad and liver mass. Abdominal fat pads are the primary storage organs of lipids in reptiles (Derickson 1976), but the liver acts as an intermediate between lipid storage and utilization primarily for reproduction (Hahn 1967). Therefore, both are crucial to determining potential effects of ALAN on metabolic and reproductive processes. Previous studies of anoles do not provide a clear extrapolation to my study. Brown anoles exposed to ALAN in the early spring months showed increased growth over a period of eight weeks, but no difference in body condition, as measured by SVL and body mass (Thawley and Kolbe 2020). When exposed to a 18L:6D cycle, adult male green anoles grew more (as measured in SVL) in the fall than anoles exposed to a 9L:15D cycle, but both treatment groups had a similar liver, fat pad, and overall body mass in the fall and winter (Fox and Dessauer 1957). Many lizards starved to death in this study, and Fox and Dessauer (1957) stated that this may have been the cause of their nonsignificant results. In mammals, such as humans (Rybnikova et al. 2016) and Siberian hamsters (Ikeno et al. 2014), ALAN has been found to cause increases in fat storage and overall body mass. Based upon mammalian literature and Fox and Dessauer (1957), I predicted lizards exposed to ALAN would have larger fat pads, livers, and overall body mass, as well as increased SVL.

Methods

Study species and housing

We captured 48 free-living adult green anole lizards (24 males and 24 females) at Trinity University in San Antonio, Texas during mid-June 2019. 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 clear plastic ruler and mass to the nearest 0.1 g using a 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.

The two treatment groups were housed in separate climate-controlled rooms in the Trinity University Vivarium, following the standard housing and care protocol for anole lizards (Sanger et al. 2008). Across the duration of the study, 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%. Two lizards (one male, one female) were housed together in a large plastic cage (Kritter Keeper; 37.5 x 21.0 x 28.0 cm^3). Each cage contained two small PVC pipe perches; a wire mesh hammock that stretched across the width of the cage, offering an additional perch; and a small plastic plant pot filled with moist sphagnum peat moss (Fertilome Bonham, TX), in which females could lay eggs (the "nest box"). The bottom of each cage was lined with R'zilla terrarium liner. The cages were separated with plyboard sheets 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 three times a week. We also misted the lizard cages daily to provide drinking water.

Lizards in the control and ALAN groups were exposed to standard reptile lighting conditions, while the ALAN lizards were also exposed to an additional nocturnal light source. Directly over the cages in both rooms hung two T8 ReptiSun 5.0 UVB fluorescent bulbs, which were set to a 13.5L:10.5D cycle to mimic the natural light-dark cycle of a summer day in San Antonio, Texas (National Oceanic and Atmospheric Administration n.d.). 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; Zoo Med Laboratories, Inc. n.d.). Additionally, the ceiling lights (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, the lizards were exposed to some level of light from 0600 to 1930 each day.

To mimic the ALAN exposure that lizards experience on Trinity University's urban campus, I measured the light intensity and emission spectrum of the lamppost lights on Trinity University's campus and created a light treatment for the ALAN group that matched these conditions. I measured the light intensity directly under 3 lights, as well as 1 m, 2 m, 3 m, 4 m, and 5 m away from the 3 lights at 2200 in May 2019 with a LI-250A Light Meter (LI-COR Biosciences, Lincoln, NE). There was a consistent gradual decline in light intensity from directly under the light to 3 m away from the light, but beyond 3 m the pools of light from different lights started to overlap, causing the decrease in intensity to level off. Directly under the lamppost lights, the average intensity was 1.33 μmol/m^2/second (s = 0.16 μmol/m^2/second). To simulate this light exposure in the lab, I used a D802-LED 12" low-profile area light (Deco Lighting, Inc. Commerce, CA), identical to those lights used for nocturnal lighting on Trinity University's

campus, and I measured its emission spectrum using an Ocean Optics USB2000+ (Custom) spectrometer (Largo, FL) inside the Trinity University vivarium with all standard lights turned off. The emissions spectrum had an emission peak in the blue spectrum at 450 nm, and a broadband emission centered at 600 nm across the green-red spectrum.

I then installed the D802-LED 12" low-profile area light at a distance of 180.0 cm, directly parallel to the lizard cages in the ALAN room. I fitted four layers of black mesh deer cloth over the light to provide a light intensity of 1.21 μmol/m^2/second (s = 0.14 μmol/m^2/s), in order to roughly mimic the light intensity that I measured directly below the lampposts on Trinity University's campus. This cloth reduced the light's intensity 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. We also rotated the control cages three times a week to ensure that each cage was handled in the same way.

Overview of behavioral trials

After a 12-day acclimation period, we performed a series of four behavioral trials. We conducted these trials over the course of four weeks in late June through mid-July, with at least 24 h separating each trial on an individual lizard. All behavioral trials were conducted in the room where each lizard was housed. For three of the trials, open field tests (OFTs), foraging trials, and social interaction trials, each lizard underwent one diurnal and one nocturnal trial. The diurnal trials occurred between the hours of 1000 and 1400, while the nocturnal trials occurred between the hours of 2200 and 0200 of the following day. In the nocturnal trials, we used an HQRP Red Flashlight (emission peak in the red spectrum at 650 nm) to observe the lizards without disturbing them, as green anoles have extremely low detection of light with spectral

emissions greater than 625 nm (Provencio et al. 1992). We covered the flashlight with eight layers of VWR Light-Duty Tissue Wipers to minimize its light intensity (0.002 μmol/m^2/second), as the spectral emissions for the red flashlight were close to the limit of green anole sensitivity to light.

The first trials conducted were open field tests (OFTs), performed over the course of 9 d. The second round of trials occurred 5 d after the conclusion of the OFTs and consisted of two 25 h behavioral observation trials separated by 1 d. The third and fourth rounds of trials were foraging trials and social interaction trials. The first half of the foraging trials was conducted 5 d after the conclusion of the behavioral observation trials. The social trials were conducted over the course of 6 d between the two foraging trials. The second half of the foraging trials was conducted the day after the conclusion of the conspecific trials.

Open field tests

To measure differences in the general activity levels of the lizards, we performed a series of OFTs. Each lizard was placed in the OFT arena (a 62.5 x 34.5 x 33.0 cm^3 mesh cage) under a 14.5 x 10.0 x 7.5 cm^3 opaque plastic container, placed in the middle of the arena. After a 10 min acclimation period under the opaque container, we removed the container and the observer moved behind a blind that was 1.5 m away. We recorded the latency to each lizard's first movement in s and its total number of movements over a 10 min trial period. If the lizard did not move, we recorded the latency to movement as 600 s. At the conclusion of the 10 min trial, the lizard was returned to its home cage.

Behavioral observation trials

To determine differences in behaviors and sleep/wake allocation during the night and day, we conducted behavioral observations of the lizards in their home cages for two nonconsecutive periods of 24 h from 1800 to 1800 of the following day. At 1 h intervals, we observed each lizard and recorded its location in the cage (Fig. 1) and its behavior at the time of observation (Table 1). If the lizard was designated to be non-observable, it was presumably asleep under the cage carpet, or fully buried within the nest box. However, as I cannot confirm the sleep status of a lizard I could not observe, I created a category specifically for this outcome.

I defined sleep based upon a behavioral definition of closed eyes, head resting on substrate, and all limbs positioned against substrate (Flanigan 1973, Clark and Gillingham 1990). A behavioral definition is most commonly utilized to define sleep in reptiles because there is a lack of consensus of an electrophysiological definition of sleep in reptiles. While in mammals and birds, sleep is defined based on behavioral and electrophysiological evidence (Aserinsky and Kleitman 1953, Ookawa and Gotoh 1964), electrophysiological evidence of reptilian sleep has only recently emerged in two lizard species: bearded dragons (*Pogona vitticeps*, Shein-Idelson et al. 2016) and tegu (*Salvator merianae*, Libourel et al. 2018). Through deep brain readings, the authors found these lizards exhibit sleep states homologous to those in mammals and birds, but the lizards' sleep state periodicity is different (Shein-Idelson et al. 2016, Libourel et al. 2018).

After the trials, we used an ethogram I created prior to the trials to assign each behavioral observation to a defined behavior. I next converted the behavioral data into count data, where I recorded the number of times each lizard conducted each recorded behavior in each 24 h trial for the day and night separately, as well as the full 24 h period. I defined day as the period from 0600 to 1900 and night as 2000 to 0500 of the next day. I next averaged the numbers for each recorded behavior across the two 24 h trials and then converted the count data into proportional

data. The proportional data allowed me to directly compare the behavioral observations between night and day, as the day included 15 time points and the night included ten.

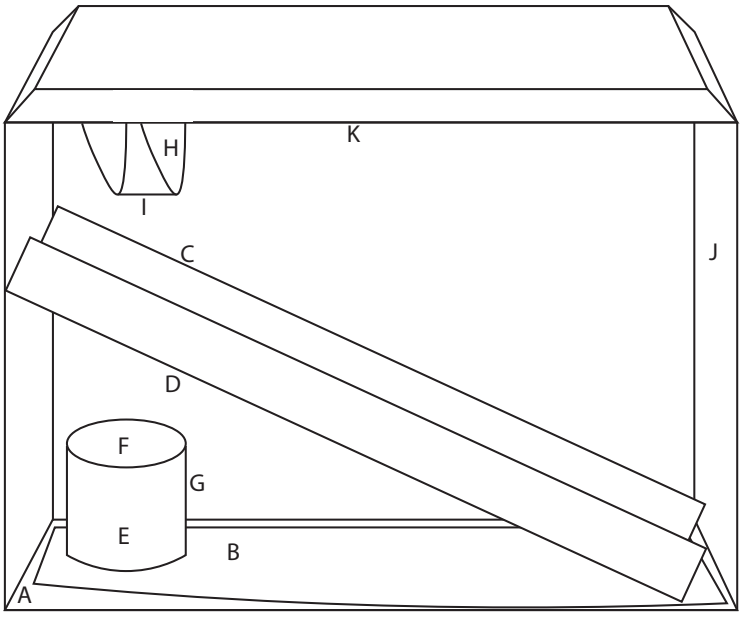

Figure 1. Potential locations of each green anole within its home cage during the behavioral observation trials. A) Under the cage carpet, B) On top of the cage carpet, C) On top of the PVC pipes, D) Hanging underneath the PVC pipes, E) Buried within the nest box, F) On top of the nest box, G) On the sides of the nest box, H) On top of the hammock, I) Hanging underneath the hammock, J) On the sides of the cage, and K) Hanging from the ceiling of the cage.

Table 1. Recorded behaviors of green anoles during behavioral observations.

Behavior	Definition
Alert	Eyes open, body not moving, body and head may or may not be in contact with substrate
Displays	Raising and lowering the front half of its body by extending and contracting its forelimbs (sometimes all limbs) in a rhythmic fashion and/or moving its head up and down in a rhythmic fashion with all four limbs in contact with ground (head-bobs and pushups)
Locomotor movements	Moving from one location to another (climbing, jumping, running, walking)
Non-observable	Observer cannot see animal (likely under the cage carpet) or, because of the animal's head position, unable to determine if awake or asleep
Asleep	Eyes closed, body not moving, head and body in contact with substrate

Foraging trials

To determine if foraging may occur during the night and to quantify the efficiency of foraging at night and during the day, we conducted foraging trials. Lizards were last fed a minimum of 24 h prior to each trial. During the trial, each lizard was placed in the foraging chamber (a 62.5 x 34.5 x 33.0 cm^3 mesh cage) under a 14.5 x 10.0 x 7.5 cm^3 opaque plastic container, placed in the middle of the chamber. After a 10 min acclimation period under the opaque container, we placed two live crickets in the foraging chamber, while removing the container. The observer subsequently moved behind a blind 1.5 m away from the arena. We recorded the time to each lizard's first head movement, time to each lizard's first body movement, time to first cricket consumption, and time to second cricket consumption over a 10 min trial period. If the lizard ate a cricket before the observer was positioned behind the blind, we recorded the time to first head movement, body movement, and cricket consumption as 1 s. If the lizard did not move or consume a cricket, we recorded the time as 600 s. At the conclusion of the 10 min trial, the lizard was returned to its home cage.

Social interaction trials

To determine potential differences in same sex interactions between the ALAN and control groups, we conducted social interaction trials. Each lizard was placed in an arena (a 62.5 x 34.5 x 33.0 cm^3 mesh cage), under a 14.5 x 10.0 x 7.5 cm^3 opaque plastic container, placed on one side of the chamber. Once we placed one lizard under one opaque container, we placed a second lizard under another opaque container on the other side of the arena. The lizards were randomly paired (without respect to body size) with another 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 placed a small branch equidistant between the two opaque chambers. Because green anoles are arboreal, vertical perches are commonly used in anole competition trials to provide a standardized resource over which they might compete (e.g., Henningsen and Irschick 2012, Bush et al. 2016). After a 10 min acclimation period under their respective opaque containers, we removed the containers, and two observers (one observer for each lizard in the trial) moved behind a blind 1.5 m away. We recorded the number of behavioral events, including head-bobs and push-ups (here combined into one measure called "push-bobs"), dewlap extensions, and locomotor movements, along with the latency to first movement in s, over each 10 min trial. If one lizard bit another lizard, or the lizards locked jaws, we stopped the trial and separated the lizards. This occurred in two diurnal trials between males in the ALAN room. At the conclusion of the 10 min trial, or when we stopped the trials before 10 min, each lizard was returned to its home cage.

Morphological measurements

After four weeks of trials, we performed a series of morphological measures on each lizard in late July 2019. We measured each lizard's SVL (mm) using a clear plastic ruler and its body mass (g) using a Pesola spring scale, and each lizard was then euthanized using a two-step protocol. We first injected a lizard with a 2% MS-222 solution, followed by an injection of a 50% MS-222 solution, once it was unresponsive to a firm toe pinch (Conroy et al. 2009). The lizard was then rapidly decapitated. We subsequently harvested and massed (g) each lizard's abdominal fat pads, liver, and reproductive tissues (ovaries and developing eggs of females, testes of males) to the nearest 0.0001 g.

Statistical Analysis

Open Field Tests: To understand ALAN's impacts on exploratory behaviors, I compared lizards in the two treatments (ALAN or normal light-dark cycle) in time to first movement in the OFTs during the diurnal and nocturnal trials, separately, using Mann-Whitney U tests. Mann-Whitney U tests were used due to the non-normal distribution of the residuals that could not be corrected with transformations. Additionally, I compared lizards in the two treatments in number of movements during the diurnal and nocturnal trials, separately, using two-way ANOVAs. For both ANOVAs, sex and treatment (ALAN or normal light-dark cycle) were included as fixed factors. The interaction between sex and treatment was included in initial analyses, but this interaction did not significantly explain the variation in the data as determined by a likelihood ratio test and was omitted from the final model.

Behavioral Observation: To assess ALAN's impacts on behavioral allocation, I compared lizards in the two treatments in proportion of time spent awake, asleep, and non-observable during the day and at night separately, as well as across the full 24 h period using Mann-Whitney U tests. While the green anoles were awake, I calculated the relative frequency (%) of time the lizards spent alert, displaying, and performing locomotor movements.

Foraging Trials: To assess ALAN's impacts on prey consumption, I compared lizards in the two treatments in time to first movement, time to first cricket consumed, and time to second cricket consumed during the diurnal and nocturnal trials, separately, using Mann-Whitney U tests. Mann-Whitney U tests were used due to the non-normal distribution of the residuals that could not be corrected with transformations.

Social Interaction Trials: To assess the impact of ALAN on same-sex social interactions, I compared the lizards in the two treatments in number of push-bobs and dewlap displays using

Mann-Whitney U tests, split by sex and by time of trial. I used Mann-Whitney U tests to examine same-sex social interactions because of the low variation in the control group's behavior during the nocturnal trial (i.e., control lizards in the dark did not perform social displays). Additionally, I compared lizards in the two treatments in number of movements during diurnal and nocturnal trials, separately, using two two-way ANOVAs. For both ANOVAs, sex and treatment were included as fixed factors. Additionally, the interaction between sex and treatment was included for the diurnal ANOVA. However, in the nocturnal ANOVA, the interaction between sex and treatment did not significantly explain the variation in the data as determined by a likelihood ratio test and was omitted from the final model.

Morphological Measurements: To determine ALAN's impacts on the physiology of green anoles, I compared lizards in the two treatments in SVL, body mass, fat pad mass, liver mass, and reproductive organ mass using a series of linear models.

To compare the difference between pre- and post-treatment SVL, as well as body mass, I compared lizards of the two treatment groups using two-way linear models with the initial models including the treatment, sex, and the interaction between treatment and sex as fixed factors and initial SVL (or body mass) as a covariate. The interaction between treatment and sex did not explain the variation in the data better than simpler models without the interaction as determined by a likelihood ratio test and, therefore, the interaction was omitted from the final models. The final models included treatment and sex as fixed factors and initial SVL (or body mass) as a covariate.

To determine the impact of ALAN on fat pad and liver mass, I compared lizards of the two treatments using two-way linear models with the initial models including treatment, sex, and the interaction between treatment and sex as fixed factors, as well as final mass as a covariate.

The interaction between treatment and sex did not explain the variation in the data better than simpler models without the interaction as determined by a likelihood ratio test and, therefore, the interaction was omitted from the final models. The final models included treatment and sex as predictors and final mass as a covariate. Models were also run with the data log-transformed, to meet assumptions of normality. However, analyses with untransformed and transformed data generated similar results; therefore, only the results of untransformed data are shown here.

To determine the impact of ALAN on testis mass, I compared lizards of the two treatments using a linear model with the initial model including treatment as a fixed factor and final mass as a covariate. However, final mass did not significantly explain the variation in the data as determined by a linear model with only final mass and was omitted from the final model. To determine the impact of ALAN on female reproductive organ mass, I compared lizards of the two treatment groups using a Mann-Whitney U test, because of the non-normal distribution of the residuals that could not be corrected with transformations.

Linear models were conducted in R (Version 3.5.2; R Core Team 2018), and Mann-Whitney U tests were conducted using IBM SPSS Statistics (Version 26).

Results

Open field tests

During the day, lizards exposed to ALAN were slower to make their first movement (median = 261.5 s, s = 227.26), as compared to lizards exposed to a normal-light dark cycle (median = 135.0 s, s = 167.27; U_{48} = 168.0, P = 0.013, r = 0.36; Fig. 2). However, at night, lizards exposed to ALAN were quicker to make their first movement (median = 63.5 s, s = 164.83), as compared to lizards exposed to a normal light-dark cycle (median = 217.0 s, s = 231.43; U_{48} = 167.0, P = 0.012, r = 0.36; Fig. 2).

The effect of treatment on the number of movements during the day was marginally nonsignificant but trended towards lizards exposed to ALAN moving less frequently than lizards exposed to a normal light-dark cycle (β_{ALAN} = -3.958, $F_{1,45}$ = 3.935, P = 0.053). At night, lizards exposed to ALAN moved more frequently than lizards exposed to a normal light-dark cycle (β_{ALAN} = 8.833, $F_{1,45}$ = 16.790, P < 0.001; Fig. 3). Sex was not associated with the number of movements the lizards made during the day (β_{Female} = 1.292, $F_{1,45}$ = 0.419, P = 0.521) or at night (β_{Female} = -1.667, $F_{1,45}$ = 0.598, P = 0.443).

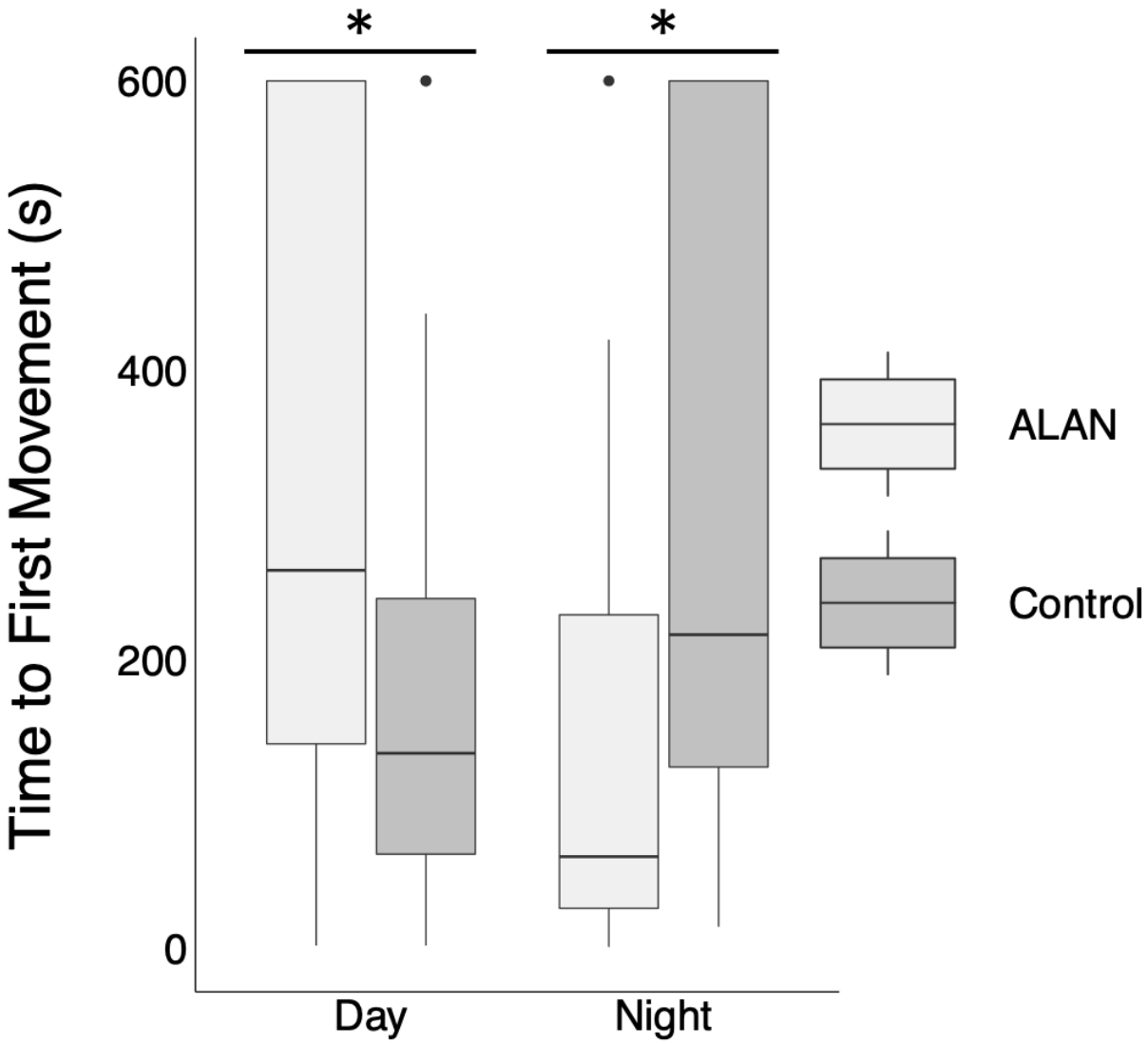

Figure 2. During the 10 min open field tests, green anoles exposed to ALAN (N = 24) were slower to make their first movement during the day than green anoles exposed to a normal light-dark cycle. However, at night, green anoles exposed to ALAN were quicker to make their first movement than green anoles exposed to a normal light-dark cycle. The horizontal line in the boxplot represents the median time to first movement. Asterisks indicate the comparison between groups is significant ($P < 0.05$).

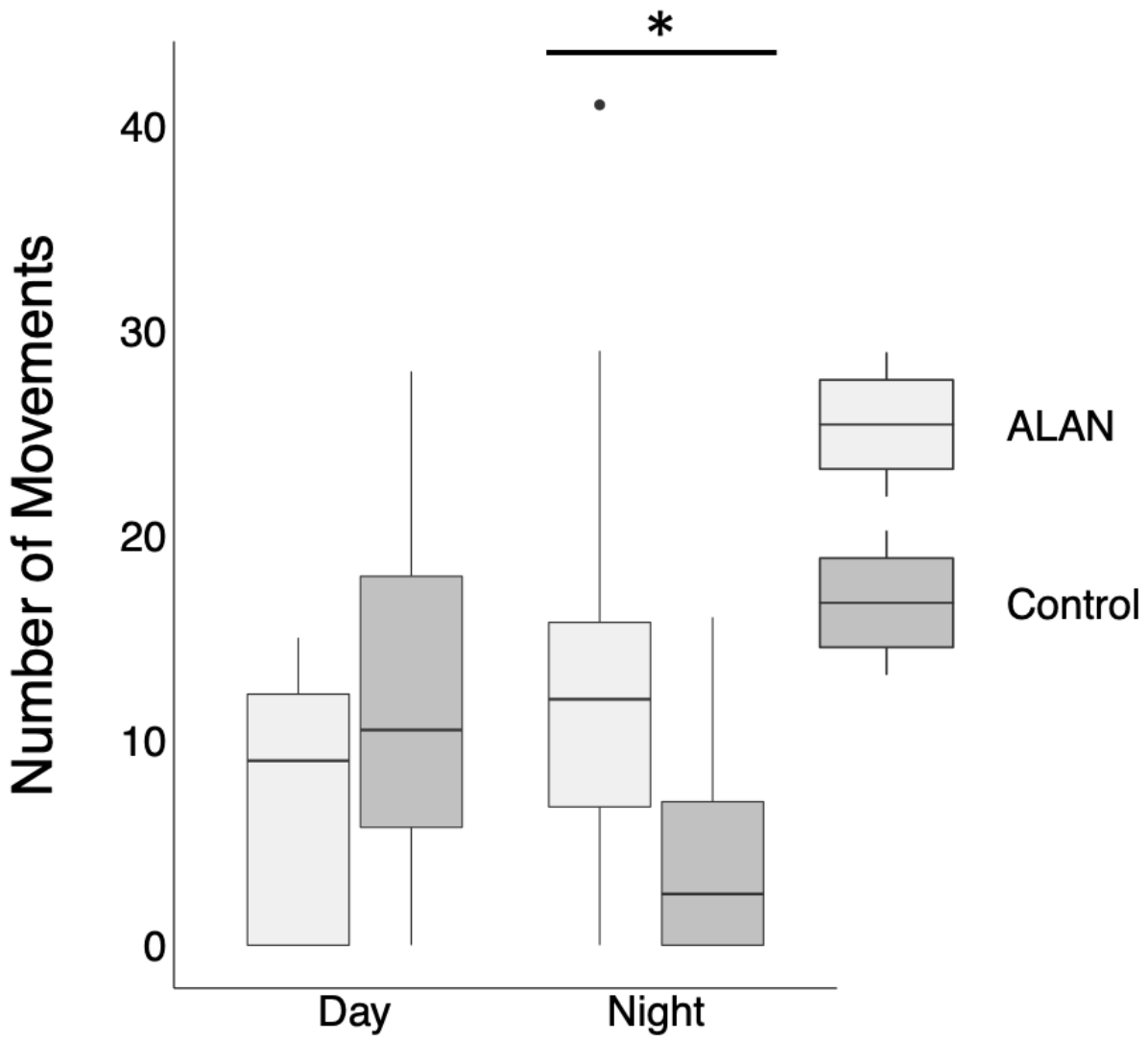

Figure 3. There was a marginal difference between the green anoles exposed to ALAN (N = 24) and exposed to a normal light-dark cycle (N = 24) in their total number of movements during the 10 min open field tests during the day, with green anoles exposed to ALAN moving less frequently than green anoles exposed to a normal light-dark cycle. However, at night, green anoles exposed to ALAN moved more frequently than green anoles exposed to a normal light-dark cycle. The horizontal line in the boxplot represents the median number of movements. Asterisk indicates the comparison between groups is significant ($P < 0.05$).

Behavioral observation trials

During the behavioral observation trials, each lizard was determined to either be awake, asleep, or non-observable (e.g., taking refuge from the light). If the lizard was awake, the lizard was recorded as either alert, performing displays, or performing locomotor movements. Across the full 24 h period, lizards exposed to ALAN or a normal light-dark cycle did not differ in the total proportion of time they spent awake (U_{48} = 264.0, P = 0.620, r = 0.07) or taking refuge (i.e., non-observable; U_{48} = 223.0, P = 0.178, r = 0.19). However, lizards exposed to ALAN were less likely to be asleep throughout a 24 h period (median = 0.15, s = 0.11) than lizards exposed to a normal light-dark cycle (median = 0.34, s = 0.17; U_{48} = 172.0, P = 0.016, r = 0.35; Fig. 4). When the lizards were awake, lizards exposed to ALAN spent 95.8% of the time alert, 0.2% of the time displaying, and 4.0% of the time moving, while lizards exposed to a normal light-dark cycle spent 96.1% of the time alert, 0.5% of the time displaying, and 3.5% of the time moving over a 24 h period.

During the night, lizards exposed to ALAN were more likely to be awake (median = 0.18, s = 0.18) than lizards exposed to a normal light-dark cycle (median = 0.00, s = 0.04; U_{48} = 93.0, P < 0.001, r = 0.62; Fig. 5). Additionally, lizards exposed to ALAN were less likely to be asleep (median = 0.33, s = 0.28) than lizards exposed to a normal light-dark cycle at night (median = 0.85, s = 0.42; U_{48} = 174.0, P = 0.017, r = 0.34; Fig. 6). There was not a difference between the two treatment groups in the time they spent taking refuge (i.e., non-observable; U_{48} = 261.0, P = 0.569, r = 0.08) at night. When the lizards were awake, lizards exposed to ALAN spent 97.7% of the time alert, 0.5% of the time displaying, and 1.8% of the time moving, while lizards exposed to a normal light-dark cycle spent 100.0% of the time alert, 0.0% of the time displaying, and 0.0% of the time moving at night.

During the day, lizards in the two treatment groups did not differ in their proportion of time spent asleep (U_{48} = 264.0, P = 0.445, r = 0.11). The difference in the proportion of time the two groups spent awake during the day was a marginally nonsignificant but trended towards lizards exposed to ALAN spending less time awake than lizards exposed to a normal light-dark cycle (U_{48} = 194.5, P = 0.052, r = 0.28). Additionally, lizards exposed to ALAN were more likely to be taking refuge (i.e., non-observable) during the day (median = 0.38, s = 0.32) than lizards exposed to a normal light-dark cycle (median = 0.12, s = 0.26; U_{48} = 192.0, P = 0.045, r = 0.29; Fig. 7). When the lizards were awake, lizards exposed to ALAN spent 95.7% of the time alert, 0.1% of the time displaying, and 4.2% of the time moving, while lizards exposed to a normal light-dark cycle spent 96.1% of the time alert, 0.5% of the time displaying, and 3.5% of the time moving during the day.

Across the entire 24 h daily period, there was no difference in the proportion of substrate changes between lizards exposed to ALAN or a normal light-dark cycle (U_{48} = 271.5, P = 0.733, r = 0.05). When considering only diurnal activity, there was also no difference in the proportion of substrate changes by lizards exposed to ALAN and to a normal light-dark cycle (U_{48} = 224.5, P = 0.190, r = 0.19). However, at night, lizards exposed to ALAN were more likely to change substrate (median = 0.13, s = 0.11) than lizards exposed to a normal light-dark cycle (median = 0.03, s = 0.05; U_{48} = 165.0, P = 0.008, r = 0.38; Fig. 8).

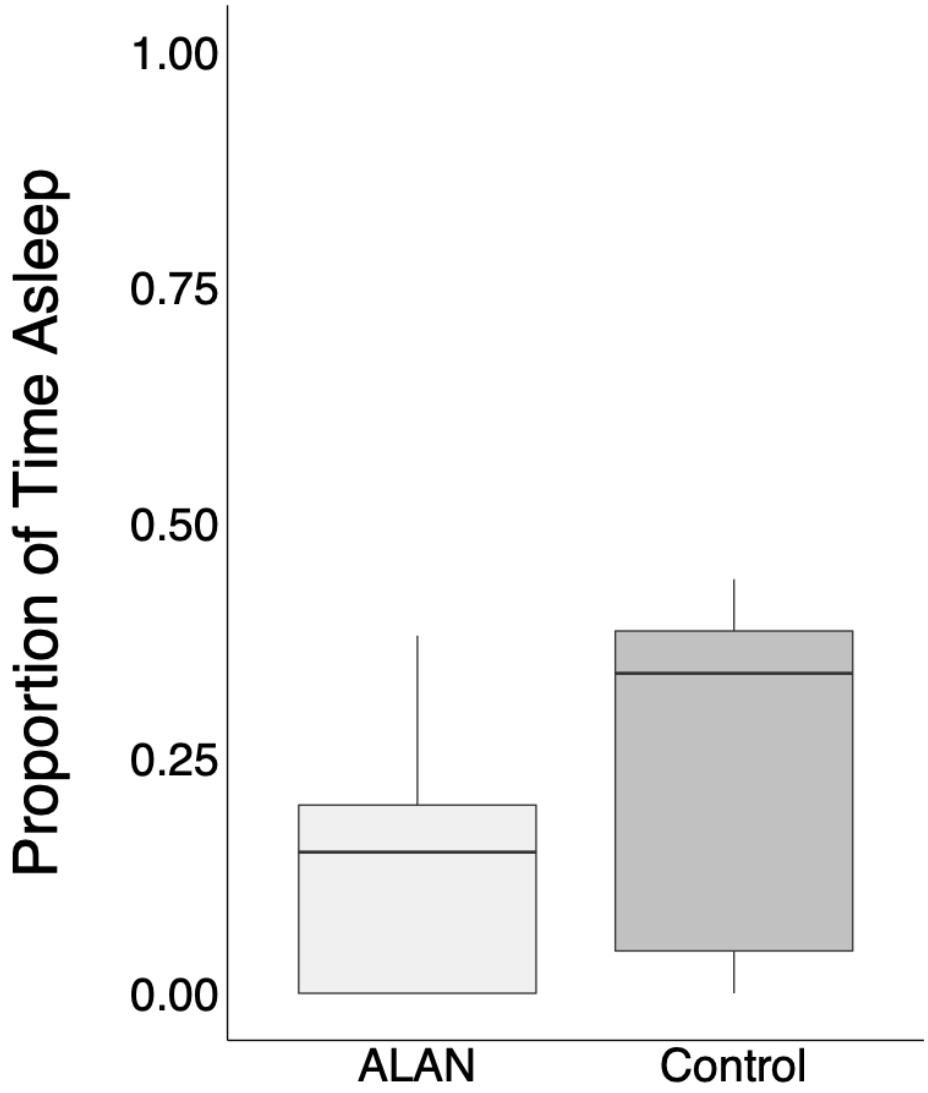

Figure 4. Green anoles exposed to ALAN (N = 24) spent proportionately less time asleep over a 24 h period than green anoles exposed to a normal light-dark cycle (N = 24) during the behavioral observational trials. The horizontal line in the boxplot represents the median proportion of time spent asleep.

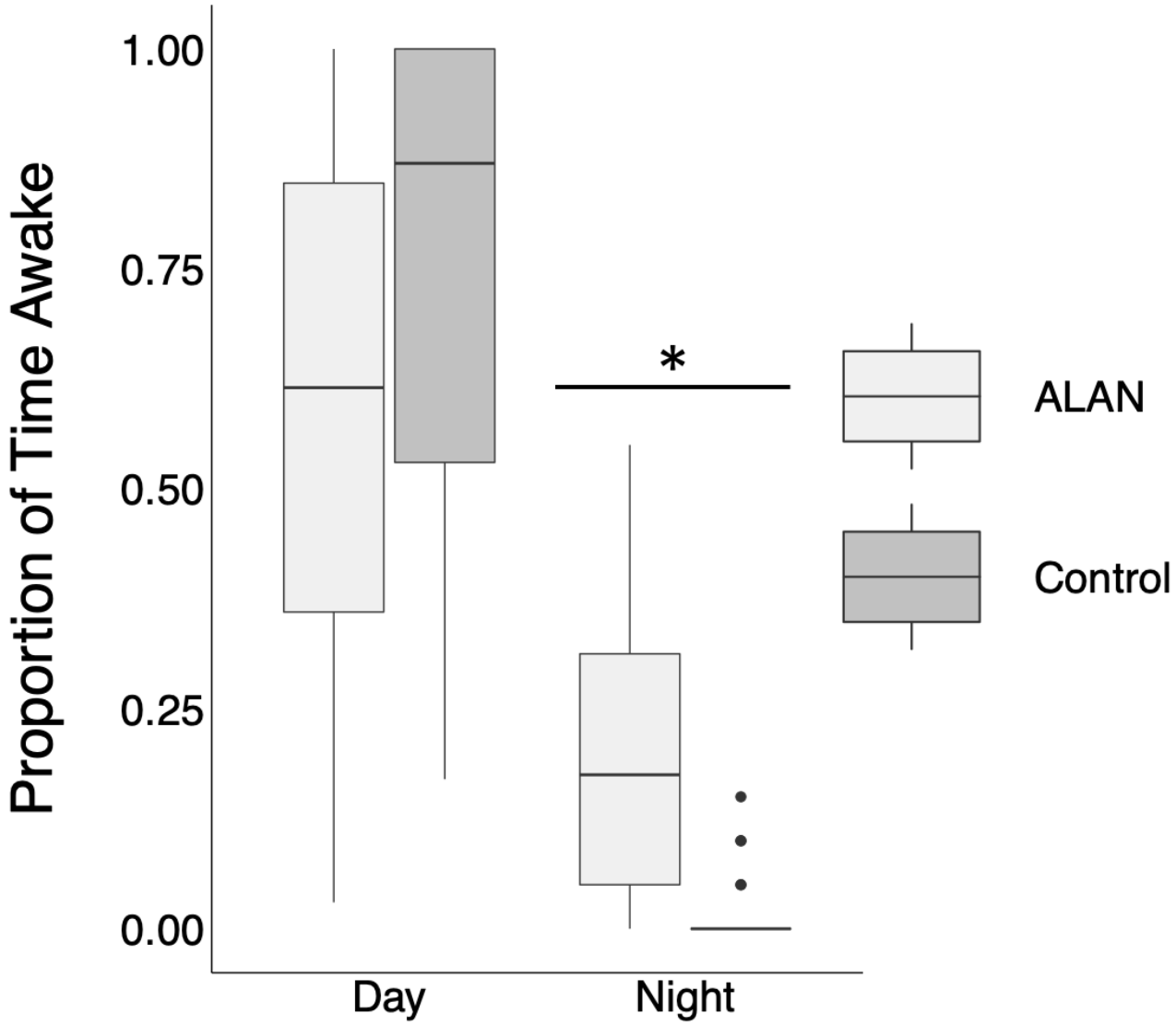

Figure 5. There was a marginal difference between the green anoles exposed to ALAN (N = 24) and exposed to a normal light-dark cycle (N = 24) in their proportion of time spent awake during the behavioral observation trials during the day, with green anoles exposed to ALAN spending proportionately less time awake than green anoles exposed to a normal light-dark cycle. However, at night, green anoles exposed to ALAN spent proportionately more time awake than green anoles exposed to a normal light-dark cycle. The horizontal line in the boxplot represents the median proportion of time spent awake. Asterisk indicates the comparison between groups is significant ($P < 0.05$).

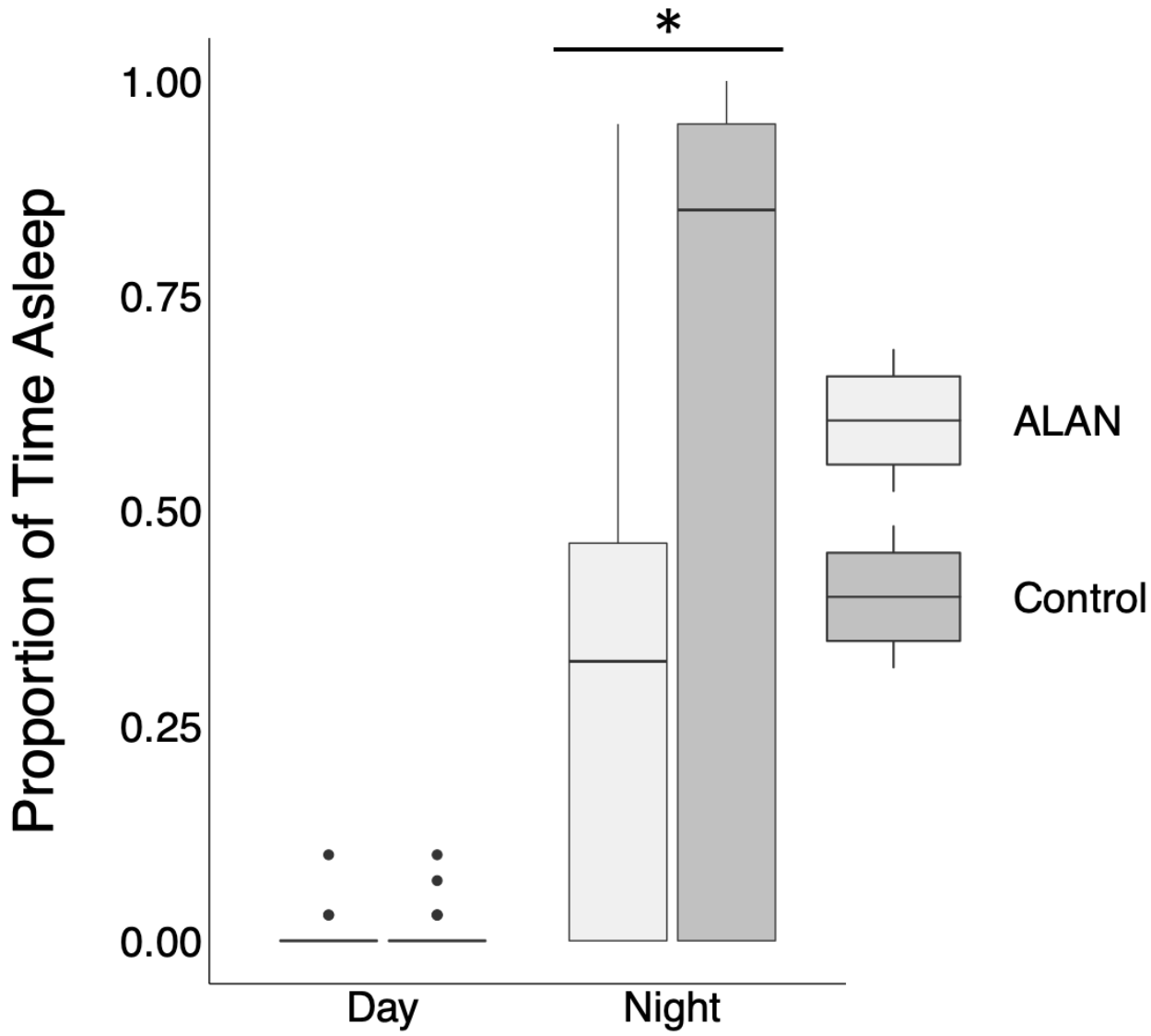

Figure 6. Green anoles exposed to ALAN (N = 24) and a normal light-dark cycle (N = 24) did not differ in their proportion of time spent asleep during the behavioral observational trials during the day. However, at night, green anoles exposed to ALAN spent proportionately less time asleep than green anoles exposed to a normal light-dark cycle. The horizontal line in the boxplot represents the median proportion of time spent asleep. Asterisk indicates the comparison between groups is significant ($P < 0.05$).

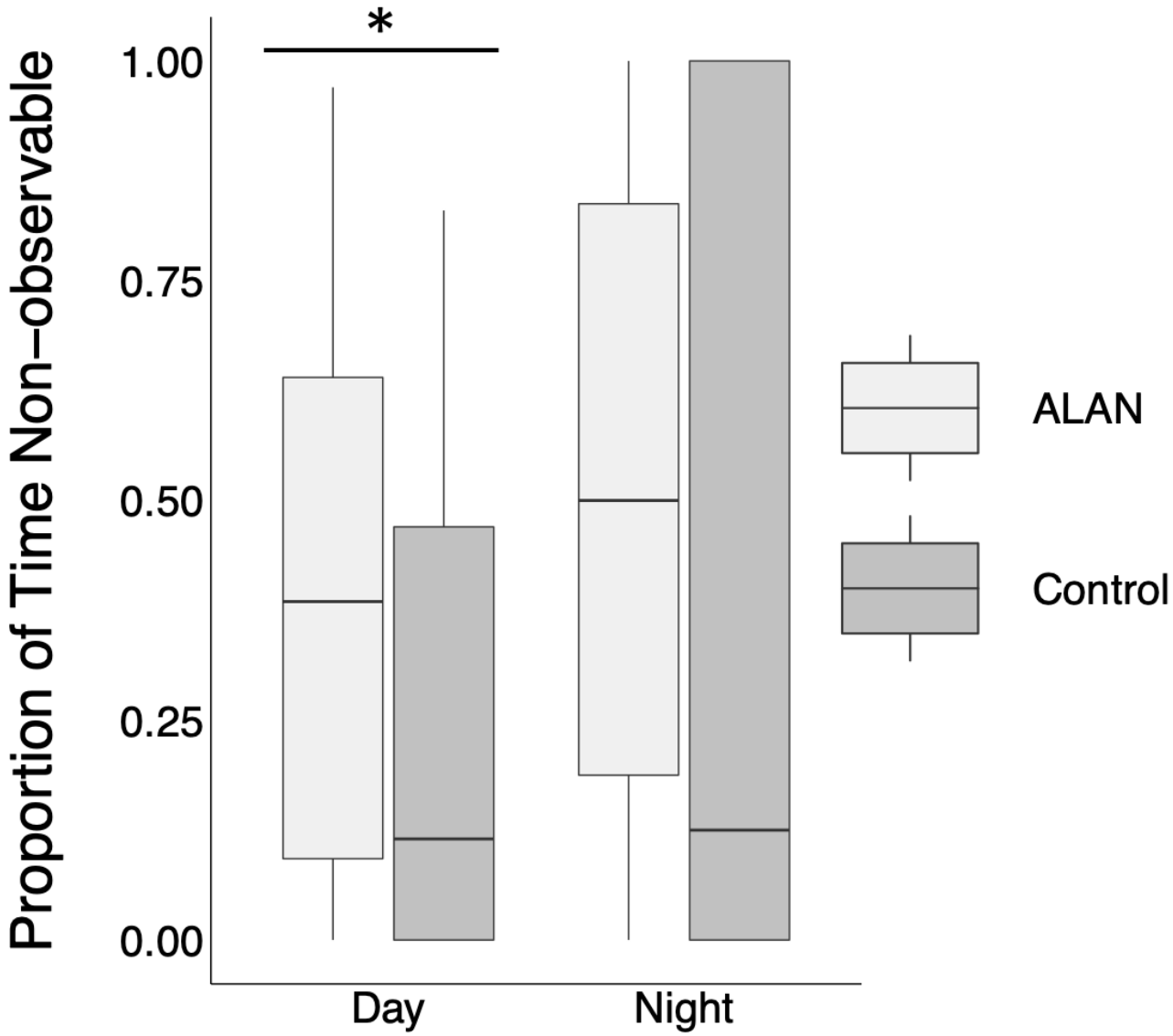

Figure 7. Green anoles exposed to ALAN (N = 24) spent proportionately more time taking refuge (i.e., non-observable) than green anoles exposed to a normal light-dark cycle (N = 24) during the behavioral observational trials during the day. However, green anoles exposed to ALAN and a normal light-dark cycle did not differ in their proportion of time spent taking refuge during the behavioral observational trials at night. The horizontal line in the boxplot represents the median proportion of time spent taking refuge. Asterisk indicates the comparison between groups is significant ($P < 0.05$).

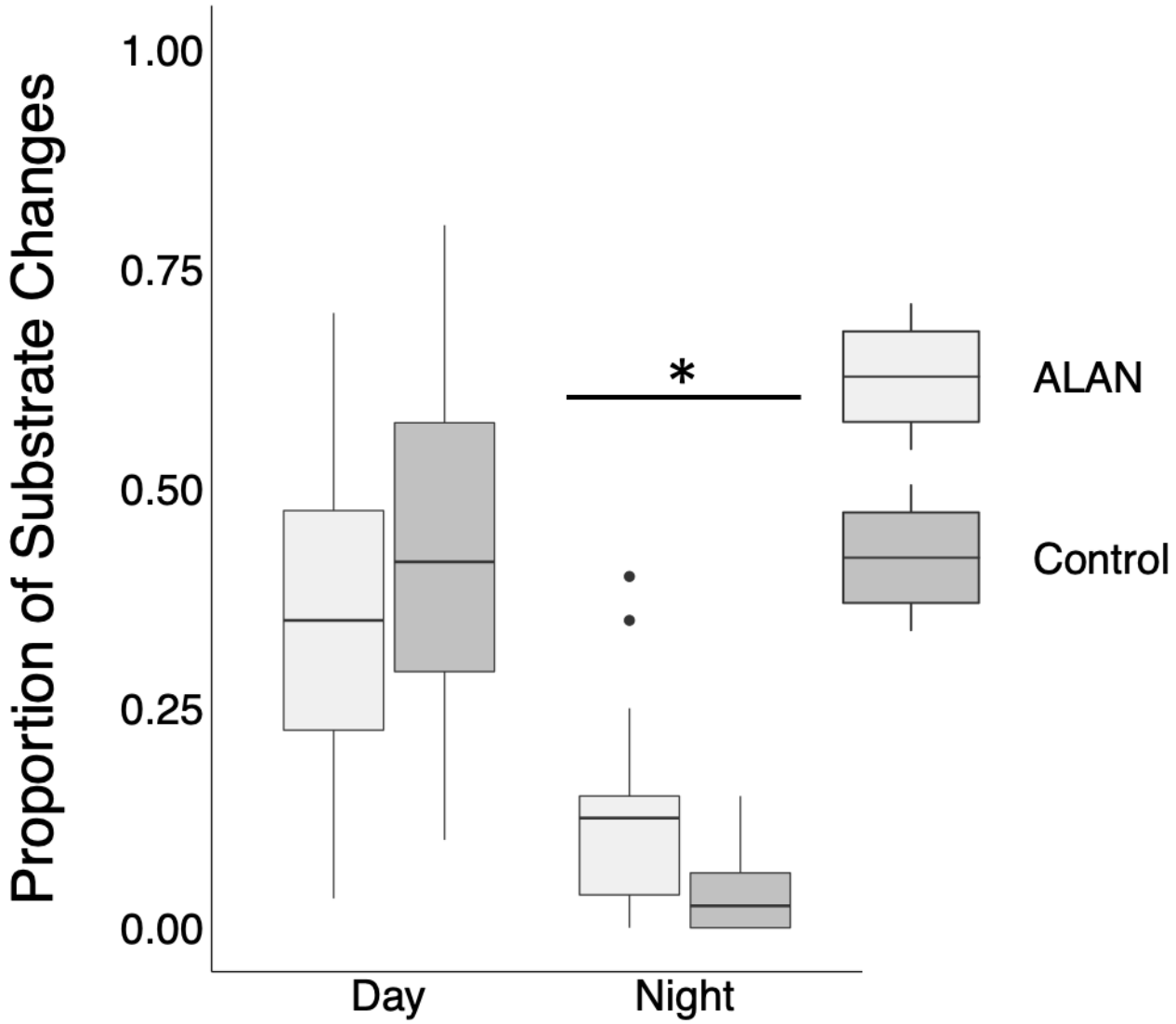

Figure 8. Green anoles exposed to ALAN (N = 24) and a normal light-dark cycle (N = 24) did not differ in their proportions of substrate changes during the behavioral observational trials during the day. However, at night, green anoles exposed to ALAN changed their substrate more frequently than green anoles exposed to a normal light-dark cycle. The horizontal line in the boxplot represents the median proportion of substrate changes. Asterisk indicates the comparison between groups is significant ($P < 0.05$).

Foraging trials

Just as in the OFTs, lizards exposed to ALAN in the foraging trials were slower to make their first movement (median = 83.0 s, s = 146.20) than lizards exposed to a normal-light dark cycle during the day (median = 23.0 s, s = 82.48; U_{48} = 164.0, P = 0.010, r = 0.37; Fig. 9). At night, lizards exposed to ALAN were quicker to make their first movement (median = 59.0 s, s = 75.32) than lizards exposed to a normal light-dark cycle (median = 144.5 s, s = 236.09; U_{48} = 185.0, P = 0.034, r = 0.31; Fig. 9).

During the day, lizards exposed to ALAN and exposed to a normal light-dark cycle did not differ in the time it took them to consume their first cricket (U_{48} = 219.5, P = 0.149, r = 0.21; Fig. 10). However, lizards exposed to ALAN were slower to consume their second cricket during the day (median = 600.0 s, s = 126.72) than lizards exposed to a normal light-dark cycle (median = 563.0 s, s = 233.99; U_{48} = 183.0, P = 0.013, r = 0.36; Fig. 11).

At night, none of the lizards exposed to a normal light-dark cycle consumed a cricket (median = 600.0 s, s = 0.00), while 19 lizards exposed to ALAN consumed their first cricket (median = 141.0 s, s = 241.31; U_{48} = 60.0, P < 0.001, r = 0.77; Fig. 10) and nine lizards exposed to ALAN consumed their second cricket as well (median = 600.0 s, s = 208.16; U_{48} = 180.0, P = 0.001, r = 0.47; Fig. 11).

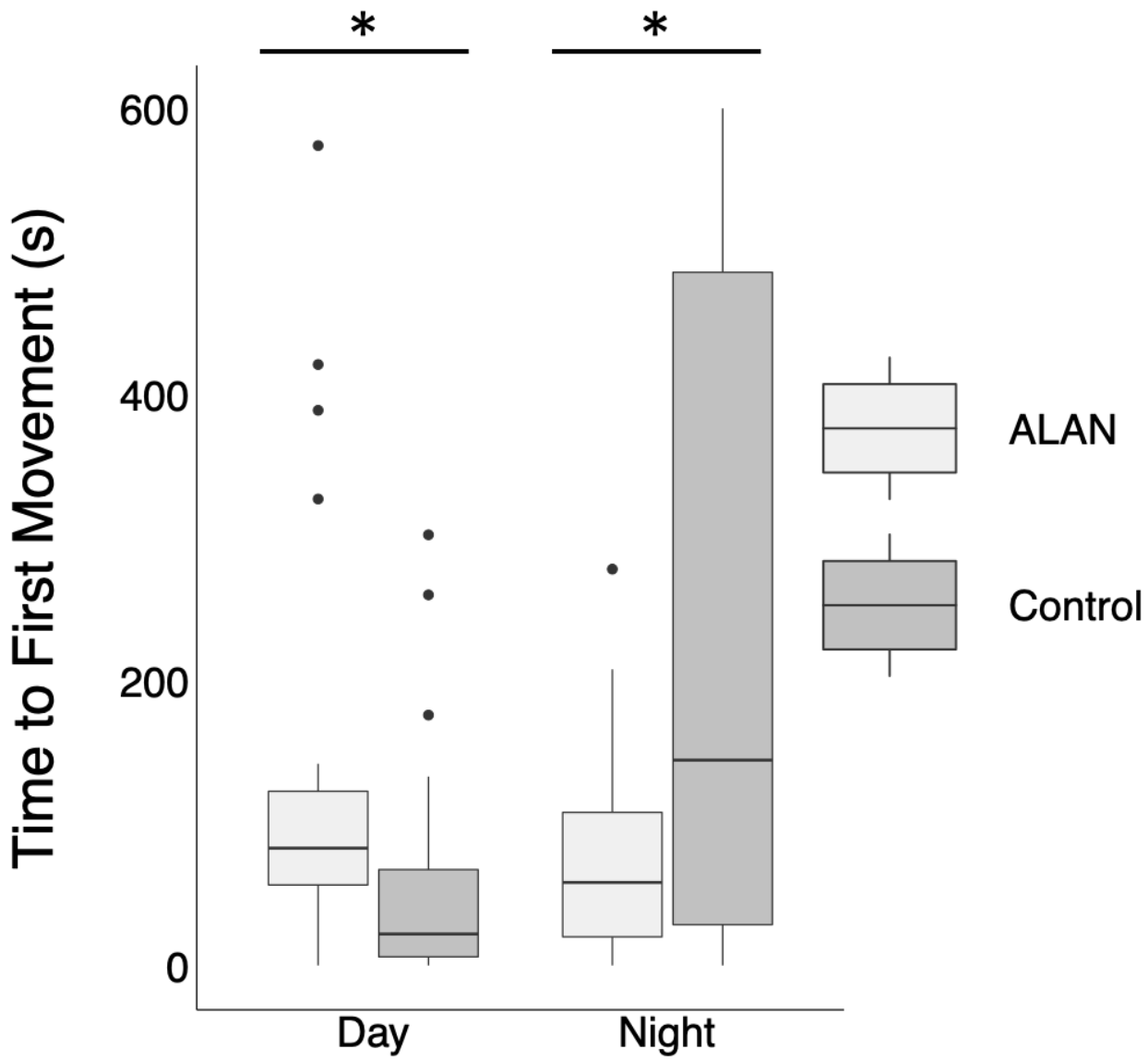

Figure 9. During the daytime 10 min foraging trials, green anoles exposed to ALAN (N = 24) were slower to make their first movement than green anoles exposed to a normal light-dark cycle. However, at night, green anoles exposed to ALAN were quicker to make their first movement than green anoles exposed to a normal light-dark cycle. The horizontal line in the boxplot represents the median time to first movement. Asterisk indicates the comparison between groups is significant ($P < 0.05$).

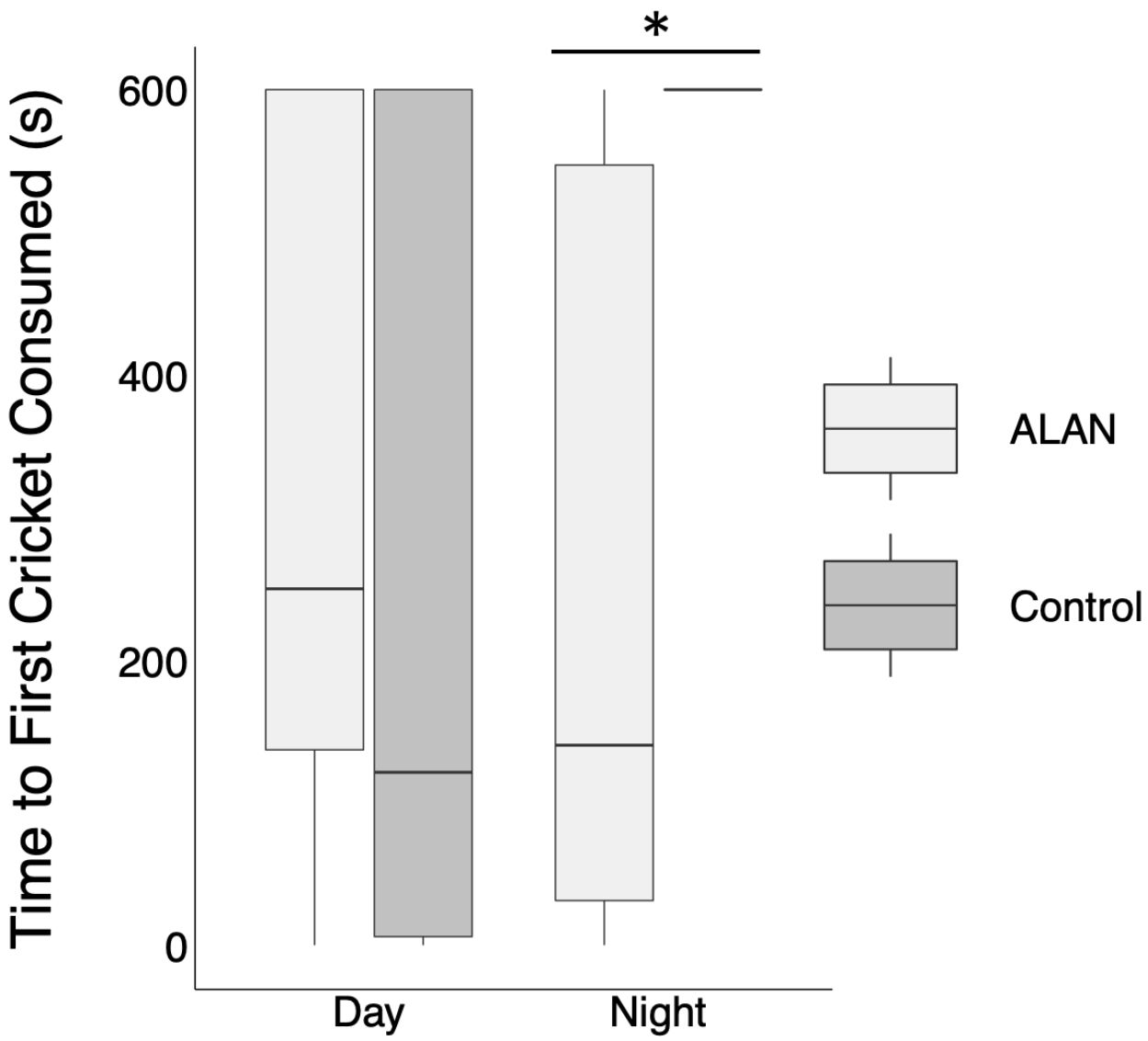

Figure 10. Green anoles exposed to ALAN (N = 24) and a normal light-dark cycle (N = 24) did not differ in the time it took them to consume their first cricket during the daytime 10 min foraging trials. At night, none of the green anoles exposed to a normal light-dark cycle consumed a cricket, while 10 green anoles exposed to ALAN consumed their first cricket. The horizontal line in the boxplot represents the median time to first cricket consumed. Asterisk indicates the comparison between groups is significant ($P < 0.05$).

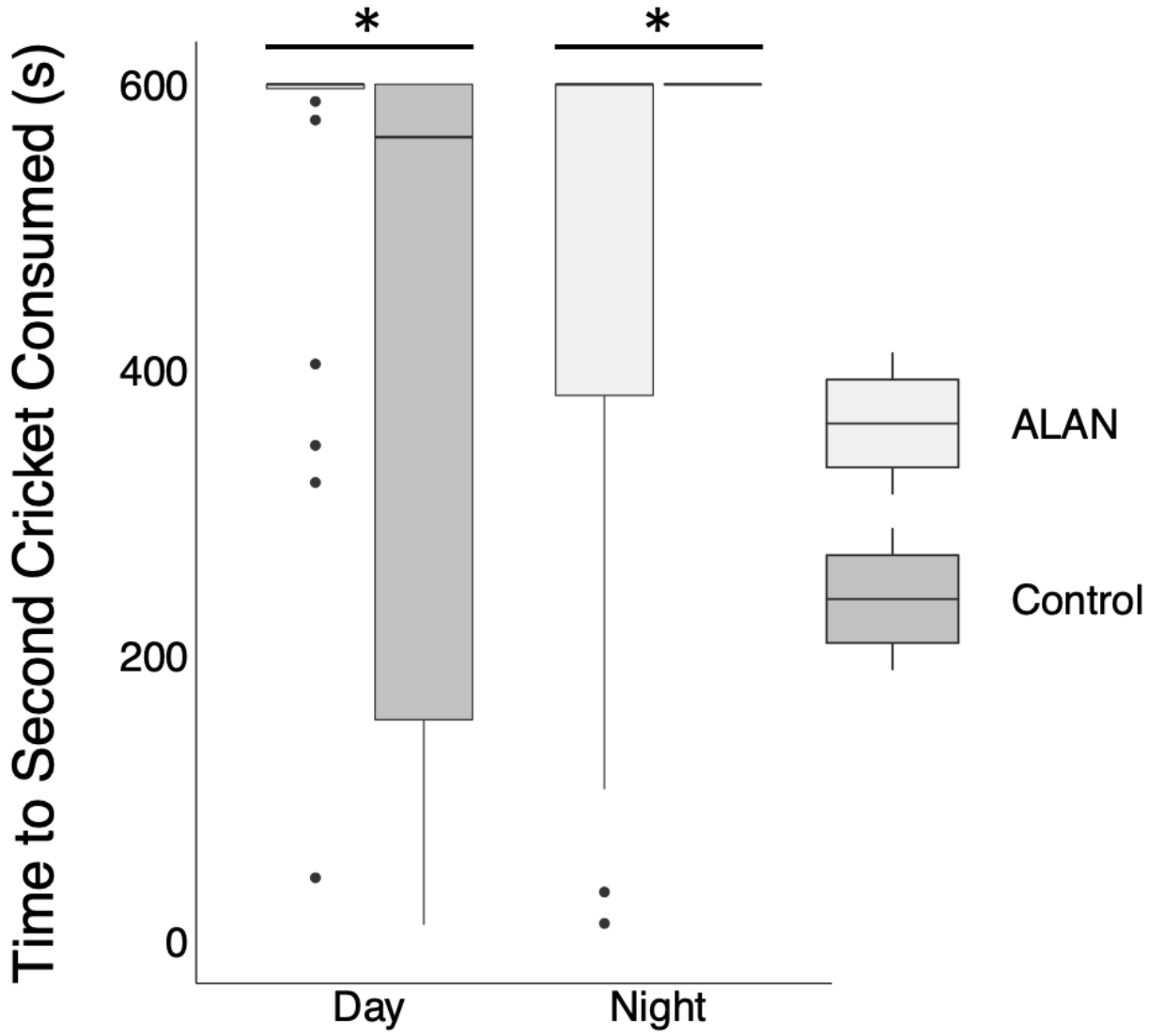

Figure 11. Green anoles exposed to ALAN (N = 24) were slower to consume their second cricket during the daytime 10 min foraging trials than green anoles exposed to a normal light-dark cycle. At night, none of the green anoles exposed to a normal light-dark cycle consumed a cricket, while nine green anoles exposed to ALAN consumed their second cricket. The horizontal line in the boxplot represents the median time to second cricket consumed. Asterisk indicates the comparison between groups is significant ($P < 0.05$).

Social interaction trials

During the social interaction trials, the interaction between treatment and sex influenced the number of movements during the day ($\beta_{Female*ALAN}$ = -8.500, $F_{1,44}$ = 4.359, P = 0.043; Fig. 12). Females exposed to ALAN moved less frequently than females exposed to a normal light-dark cycle, but males exposed to ALAN moved more frequently than males exposed to a normal light-dark cycle (Fig. 12). At night, neither treatment (β_{ALAN} = 1.811, $F_{1,45}$ = 0.305, P = 0.584), nor sex had a significant main effect on the number of movements (β_{Female} = 1.811, $F_{1,45}$ = 0.305, P = 0.584).

Males exposed to ALAN and to a normal light-dark cycle did not differ in their average number of push-bobs (night: U_{24} = 45.5, P = 0.128, r = 0.41; day: U_{24} = 52.5, P = 0.266, r = 0.23) or dewlap displays (night: U_{24} = 42.0, P = 0.089, r = 0.50; day: U_{24} = 59.5, P = 0.478, r = 0.15). However, none of the males exposed to a normal light-dark cycle performed stereotyped displays at night, while five males exposed to ALAN performed push-bobs and dewlap displays. Females also did not differ during their average number of push-bobs at night (U_{24} = 42.0, P = 0.089, r = 0.50) or during the day (U_{24} = 53.0, P = 0.291, r = 0.23) and their average number of dewlap displays at night (U_{24} = 66.0, P = 0.755, r = 0.20). However, none of the females exposed to a normal light-dark cycle performed stereotyped displays at night, while five females exposed to ALAN performed push-bobs and one female performed dewlap displays. During the day, females exposed to ALAN performed fewer dewlap displays (median = 0.0, s = 0.00) than females exposed to a normal light-dark cycle (median = 1.5, s = 2.27; U_{24} = 30.0, P = 0.014, r = 0.62; Fig. 13).

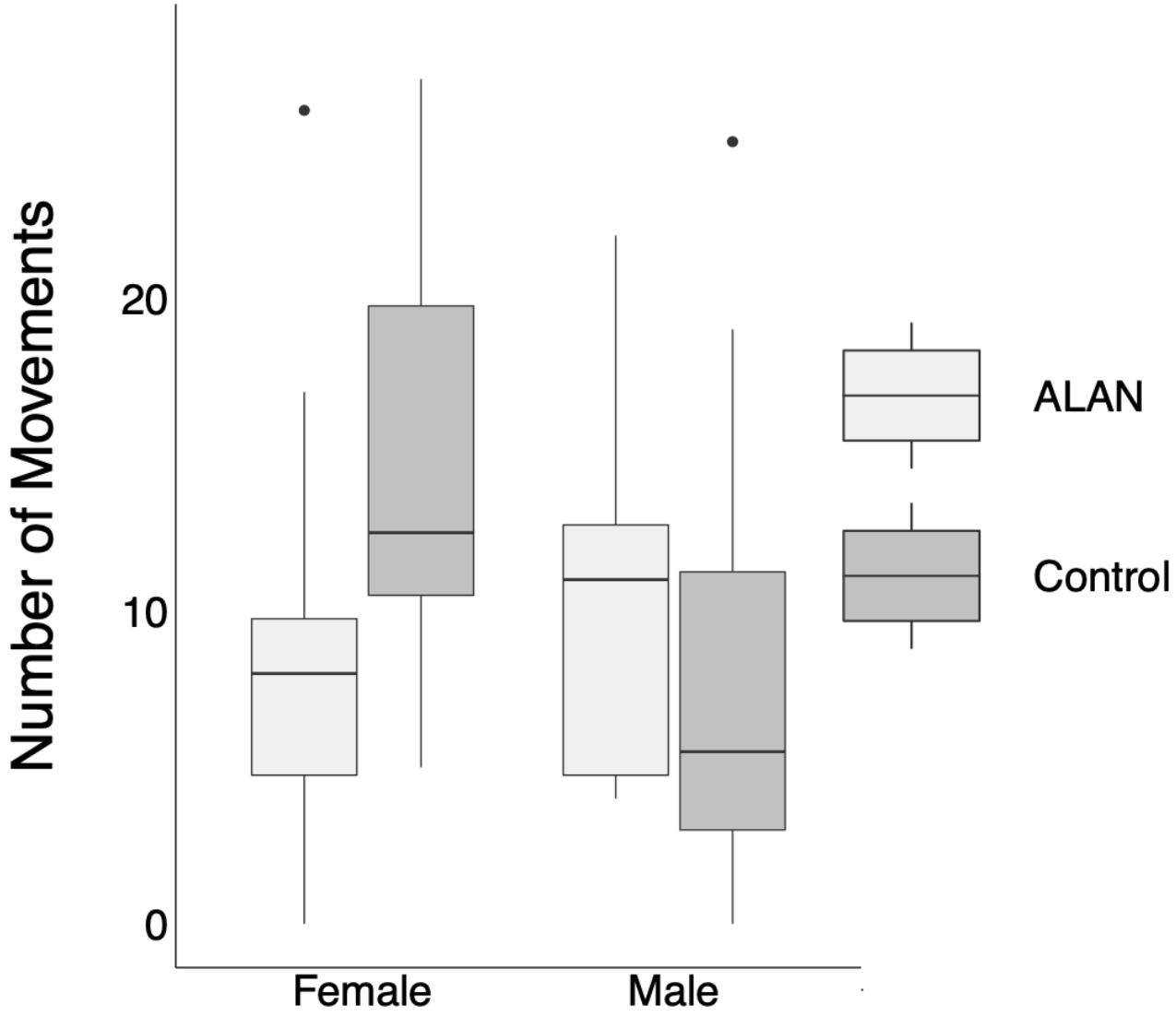

Figure 12. During the daytime 10 min social interaction trials, the interaction between sex and treatment (ALAN or control) had a significant effect on the number of movements the green anoles made. Females exposed to ALAN (N = 12) moved less frequently than females exposed to a normal light-dark cycle (N = 12), but males exposed to ALAN (N = 12) moved more frequently than males exposed to a normal light-dark cycle (N = 12). The horizontal line in the boxplot represents the median number of movements.

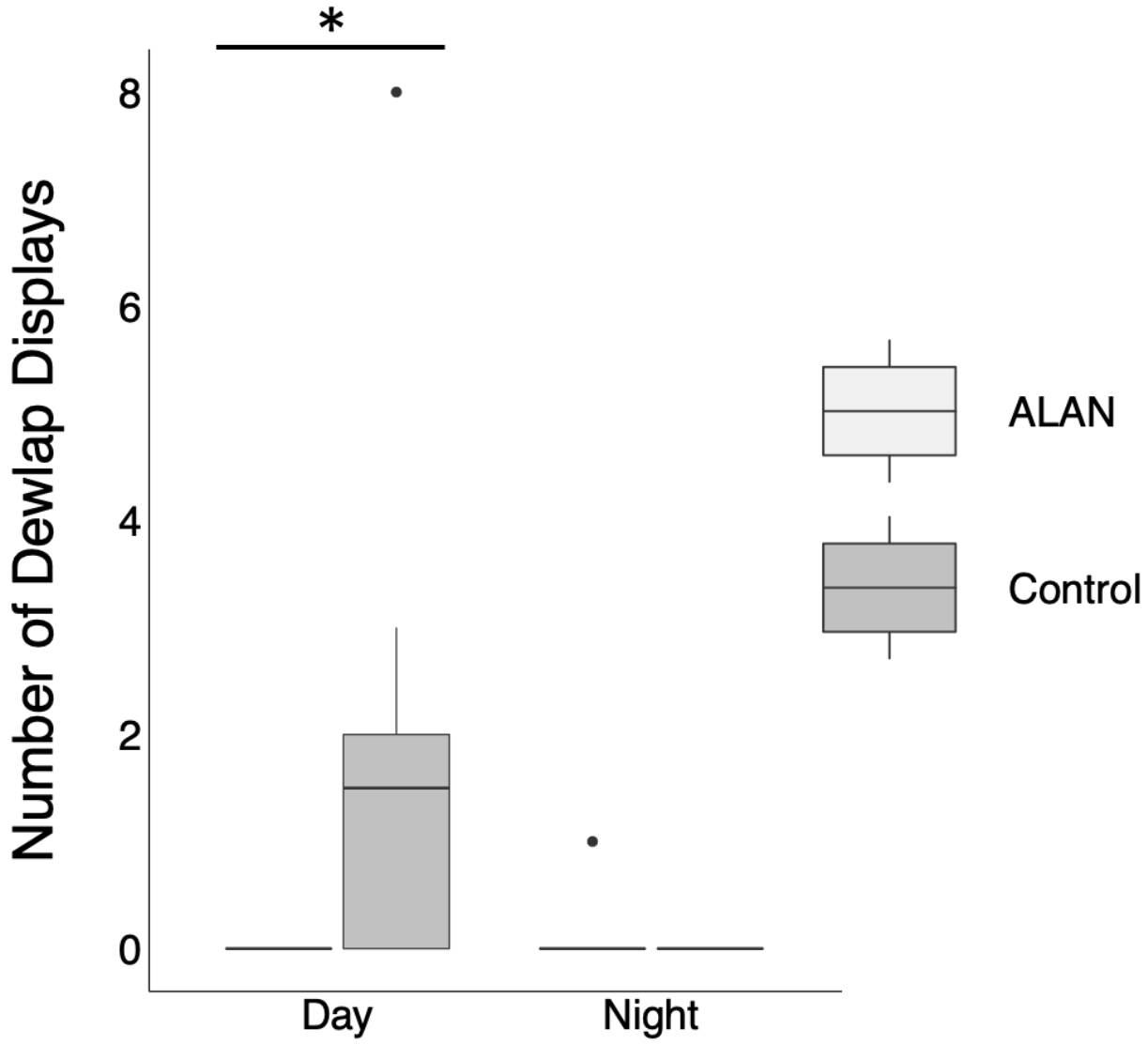

Figure 13. While female green anoles exposed to ALAN (N = 12) conducted fewer dewlap displays than female green anoles exposed to a normal light-dark cycle (N = 12) during the day in 10 min social interaction trials, female green anoles exposed to ALAN and exposed to a normal light-dark cycle did not differ in their number of dewlap displays performed at night. The horizontal line in the boxplot represents the median number of dewlap displays. Asterisk indicates the comparison between groups is significant ($P < 0.05$).

Physiology

Neither treatment (β_{ALAN} = -0.033, $F_{1,44}$ = 0.002, P = 0.965), sex (β_{Female} = -1.090, $F_{1,44}$ = 0.599, P = 0.443), nor initial SVL (β = -0.111, $F_{1,44}$ = 1.264, P = 0.267) was associated with final SVL.

Treatment did not have an effect on changes in body mass over the course of the study (β_{ALAN} = 0.186, $F_{1,44}$ = 2.980, P = 0.091). Males lost more body mass on average over the course of the trials than females (β_{Female} = -0.186, $F_{1,44}$ = 7.804, P = 0.008). 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 study (β = -0.289, $F_{1,44}$ = 21.565, P < 0.001).

Lizards exposed to ALAN had heavier abdominal fat pads than lizards exposed to a normal light-dark cycle (β_{ALAN} = 0.018, $F_{1,43}$ = 5.664, P = 0.022; Fig 14). Sex of the lizard was not associated with fat pad mass (β_{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 (β = 0.016, $F_{1,43}$ = 7.339, P = 0.010).

Treatment did not have an effect on liver mass (β_{ALAN} = 0.008, $F_{1,44}$ = 2.109, P = 0.154). Females had heavier livers than males (β_{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 (β = 0.031, $F_{1,44}$ = 56.833, P < 0.001).

Males exposed to ALAN had heavier testes than males in the normal light-dark cycle (β_{Male} = 0.026, $F_{1,22}$ = 28.108, P < 0.001; Fig. 15). Treatment did not impact female reproductive tissue mass (U_{24} = 60.5, P = 0.514, r = 0.14).

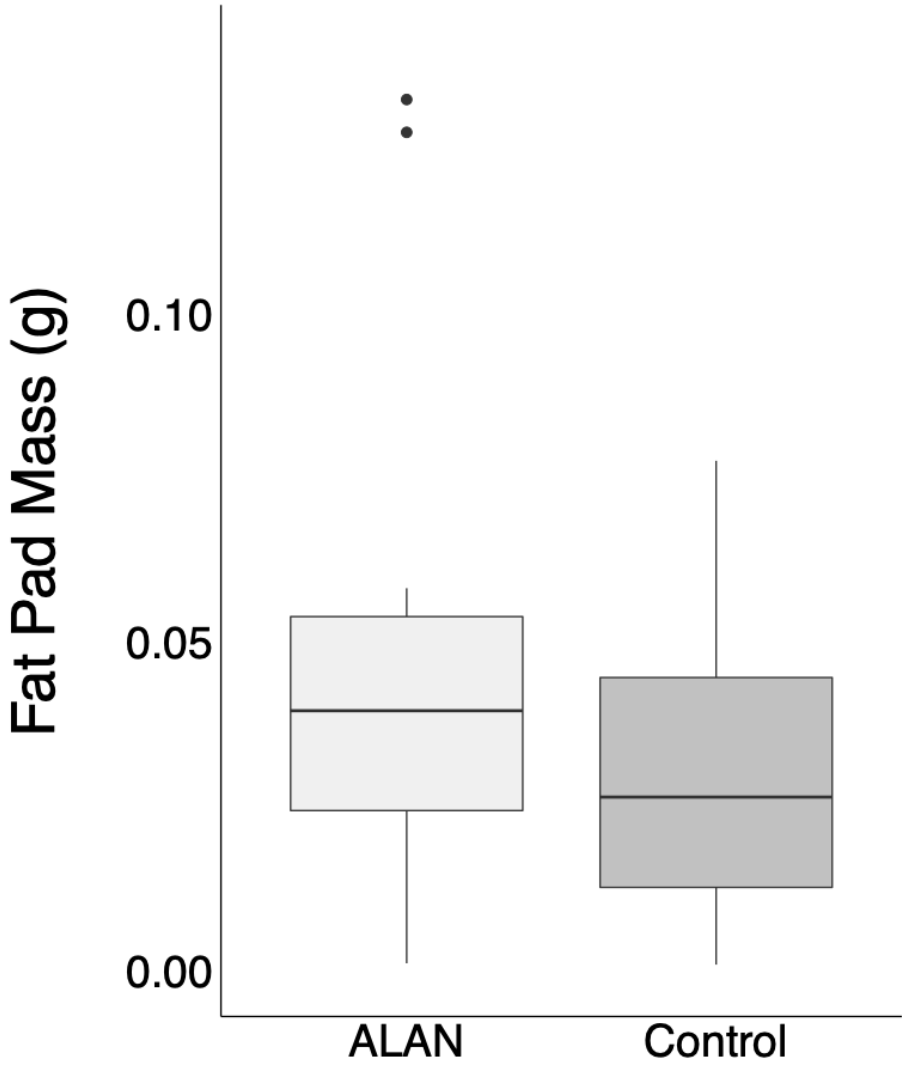

Figure 14. Green anoles exposed to ALAN (N = 24) had higher abdominal fat pad masses (g) than green anoles exposed to a normal light-dark cycle (N = 24). The horizontal line in the boxplot represents the median fat pad mass.

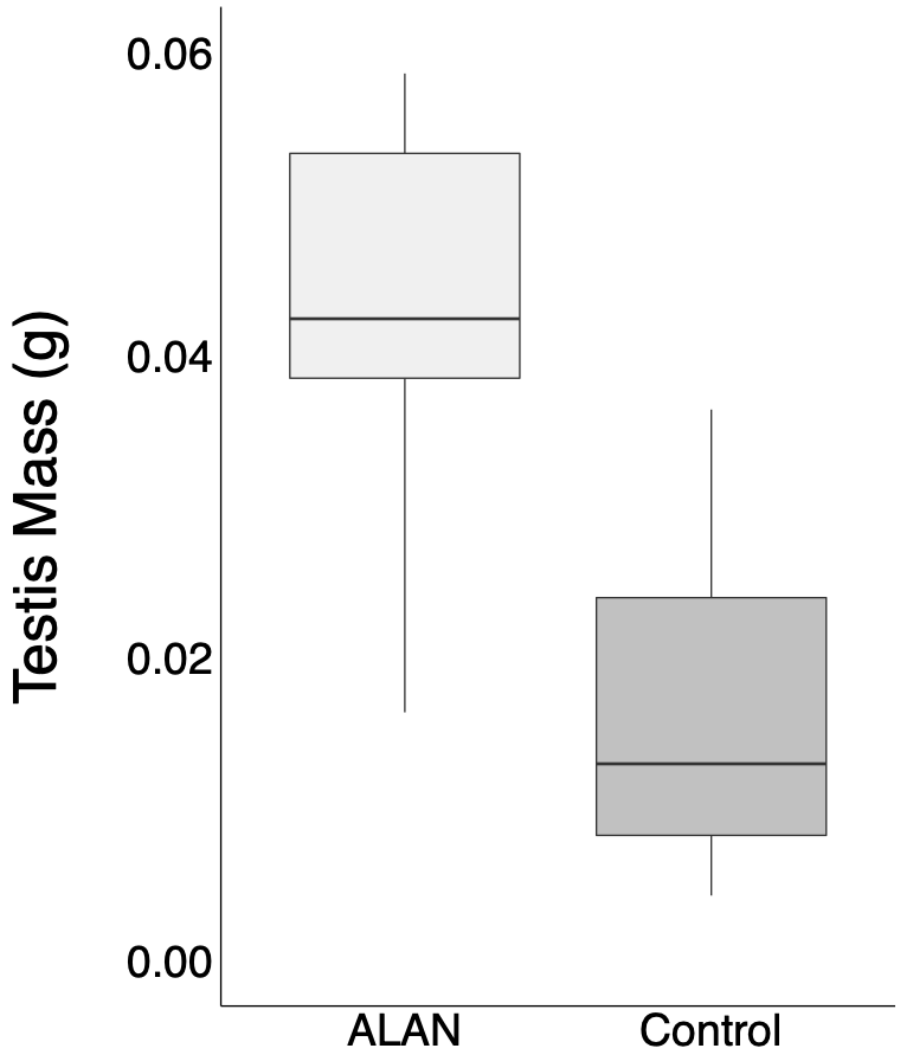

Figure 15. Male green anoles exposed to ALAN (N = 12) had higher testis masses (g) than green anoles exposed to a normal light-dark cycle (N = 12). The horizontal line in the boxplot represents the median testis mass.

Discussion

Overall, I found evidence that ALAN is associated with a behavioral trade-off and altered physiological processes within green anole lizards over a six-week exposure period. When the lizards exposed to ALAN were awake at night, they appeared to use ALAN to move, forage, and display. However, during the day, the lizards exposed to ALAN spent more time taking refuge from the light and were slower to move and forage than the lizards exposed to a normal light-dark cycle. Additionally, the females displayed less frequently during the day with exposure to ALAN. Physiologically, the lizards exposed to ALAN had heavier abdominal fat pads and the males exposed to ALAN had heavier testes than those exposed to a normal light-dark cycle. I consider these results in more detail below.

Sleep-wake cycles

The green anoles experienced an overall decline in sleep throughout a 24 h period with exposure to ALAN, just as in female great tits (Raap et al. 2015, 2016). As previously mentioned, while the exact purpose of sleep is debated, sleep is important for energy conservation or restoration (Berger and Phillips 1995), brain function and memory consolidation (Tononi and Cirelli 2006), and brain and neuromuscular development (Roffwarg et al. 1966). A lack of sleep has been linked to multiple negative effects, such as reduced immune functioning (e.g., humans: Irwin et al. 1996, Wistar-Hannover rats: Zager et al. 2007), impaired social signaling (e.g., European honey bees: Klein et al. 2010), and impaired memory consolidation (e.g., chickens: Jackson et al. 2008, flies: Le Glou et al. 2012). The lack of sleep experienced by the lizards exposed to ALAN might have contributed to the declines in diurnal activity explained in subsequent sections.

The green anoles also experienced a behavioral trade-off with their sleep-wake cycle with exposure to ALAN, which is similar to the results Stenvers et al. (2016) found for nocturnal Wistar rats. The rats were more frequently awake in their normal inactive period (daytime) and less frequently awake in their normal active period (nighttime; Stenvers et al. 2016). During the night, lizards exposed to ALAN spent less time asleep and more time awake, which has also been observationally shown in brown anoles (Brown and Arrivillaga 2017) and experimentally shown in humans (Cho et al. 2016). During the day, lizards exposed to ALAN were more likely to be taking refuge from the light (e.g., non-observable). The lizards were likely sheltering from the light during the day in order to sleep. In the field, lizards would lose potential foraging and mating opportunities, leading to fitness declines, by sheltering from the light during the day. However, these costs might be alleviated by the increased time spent awake during the night.

Regardless of treatment, green anoles spent the majority of time alert when they were awake. However, during the night, the lizards in a normal-light dark cycle were only alert when they were awake at night, while lizards exposed to ALAN were observed displaying and moving as well. Yet, due to the experimental design of this study, in which hourly scans of behavior were performed, lizards were rarely observed moving or displaying at the moment of the scans. In the future, observational studies using longitudinal, focal observations would be able to better gauge specific differences in behavioral allocation, as focal observational studies continuously monitor individual study organisms.

General activity

The green anoles exposed to ALAN showed a trade-off in their general activity levels, with a relative decrease in general activity during the day, but an increase at night. This is similar

to the results found by Fonken et al. (2010) with nocturnal Swiss-Webster mice. Mice exposed to ALAN had an increase in locomotor activity during the day and a decrease at night (Fonken et al. 2010). Other species also have altered general activity due to ALAN, but the direction is not consistent among species or even within the same species. For example, nocturnal male Siberian hamsters had an overall reduction in locomotor activity across a 24 h period with exposure to ALAN (Bedrosian et al. 2011), but female Siberian hamsters had suppressed locomotor activity only at night with exposure to ALAN (Bedrosian et al. 2013). In contrast, diurnal male European blackbirds exposed to ALAN had increased locomotor activity across a 24 h period in the winter months (Dominoni et al. 2013a). However, in the summer, male European blackbirds had increased locomotor activity only during the night and in the morning with exposure to ALAN (Dominoni et al. 2013a). These seasonal changes in locomotor activity may be linked to reproduction, as European blackbirds breed during the summer and males sing early in the morning to attract females and warn off male rivals (Dabelsteen 1984). As green anoles are long-day breeders, just like European blackbirds, they may also experience this general activity trade-off only during their breeding season. Future studies should compare the impacts of ALAN exposure across a full year in order to determine if these changes in general activity are generalizable or hold true only during the breeding season.

Foraging

While green anoles were slower to forage during the day with exposure to ALAN, ALAN allowed them to forage at night. Common redshanks are also found to exploit ALAN for foraging using sight-based techniques (Dwyer et al. 2013). Therefore, it is logical that exposure to ALAN allows green anoles to hunt, as they are also visual predators. In other diurnal species,

such as jumping spiders (Frank 2009) and brown anoles (Brown and Arrivillaga 2017), ALAN allows for exploitation of the night-light niche. No study of which I am aware has explored the latency to forage during an organism's active time, but the green anoles' latency to forage during the day might be explained by the lack of sleep experienced by the lizards. As sleep is important for energy conservation and restoration (Berger and Phillips 1995), lizards exposed to ALAN might not have had the energy to rapidly react to a food stimulus during the day. A lack of energy would limit potential foraging opportunities during the day, but ALAN would allow them to explore new foraging opportunities at night.

While ALAN may provide illumination for the green anoles to hunt, ALAN's intensity rarely mimics the intensity of sunlight over large areas. Therefore, lizards hunting at night would potentially be clustered in limited areas, causing there to be higher competition during foraging at night. Additionally, lizards might be while exposed to novel predators while they forage at night. If diurnal predators of lizards also expand their activity into the night, then entire food webs could be shifted into the night, putting ecological pressure on nocturnal organisms who cannot expand their activity into the day. For example, a species diurnal colubrid snake (*Borikenophis portoricensis anegadae*) has been documented using ALAN to hunt for *Anolis* lizards at night (Perry and Lazell 2000). Therefore, lizards might not be able to escape from their diurnal predators through increased foraging and general activity at night.

Social communication

While the rate of display did not differ between the green anoles exposed to ALAN and exposed to a normal light-dark cycle, only lizards exposed to ALAN displayed at night. Additionally, females exposed to ALAN during the day displayed and moved less frequently

than females exposed to a normal-light dark cycle. Because both male and female green anoles display more frequently when they can see an individual of the same sex than when alone (Jenssen et al. 2000), I hypothesized that lizards exposed to ALAN would display more frequently at night than those exposed to a light-dark cycle. It is commonly thought that ALAN would facilitate nocturnal interactions of diurnal organisms, but there are few studies that examine the impact of ALAN on social communication (reviewed by Kurvers and Hölker 2015).

The few studies examining the effect of ALAN on social communication have found mixed results. Kurvers et al. (2018) found that exposure to ALAN did not impact the amount of time mixed-sex groups of Trinidadian guppies spent schooling during the day, but the sociability of the guppies exposed to ALAN declined over the course of the study. The authors ascribed the decline in sociability to a lack of motivation to socialize (Kurvers et al. 2018). Potentially, the female green anoles exposed to ALAN had a lack of motivation to socialize, due to a lack of sleep. As previously mentioned, sleep is important for energy conservation and restoration (Berger and Phillips 1995); therefore, a lack of sleep might have decreased the energy female lizards had to perform social displays and move in response to a female conspecific. These behavioral changes in the lab indicate that female lizards exposed to ALAN in the field might have declines in intrasexual territorial defense behaviors. Declines in territorial defense behaviors could lead to increased overlap between multiple females' territories, leading to increased competition for resources within an area typically designated for a smaller number of individuals.

While the Trinidadian guppies had a decline in sociability, diurnal bird species exposed to ALAN advance their daily auditory mating signals into the night. When exposed to ALAN, males of multiple bird species (e.g., European robins, European blackbirds, great tits, and

Eurasian blue tits) sang earlier in the day than when exposed to a normal-light dark cycle (Kempenaers et al. 2010, Da Silva et al. 2014, 2015). However, these auditory signals are not directly comparable with the visual signals of green anoles, as auditory signals can still be fully conveyed in the absence of light, while the information in visual signals cannot be conveyed without light.

Additionally, as the studies on bird auditory signals were conducted in the field, the authors could not separate the effects of bird song functioning dually as a deterrent of male competition and as an attractor for mates. My study explicitly evaluated intrasexual competitive interactions by eliminating the presence of the opposite sex. I wanted to evaluate the interactions that lead to aggressive defense of spaces for food, refuges, and potential mates. Even with the elimination of the opposite sex, male green anoles exposed to ALAN had the same display rates as male green anoles exposed to a normal light-dark cycle and even moved around more frequently within a territorial social context during the day. Potentially, the males maintained their display rates and increased their number of movements during the day within a territorial social context with exposure to ALAN because of strong sexually selected pressures during the breeding season to display (Jenssen et al. 1995). Once again, future studies should evaluate the impact of ALAN exposure on green anoles across a full year in order to determine if display rates change depending upon the season.

Physiology

The green anoles exposed to ALAN did not grow in body length (SVL) over the duration of the study. This lack of growth is surprising, given previous findings that adult brown anoles grew with exposure to ALAN (Thawley and Kolbe 2020) and adult green anoles grew with

exposure to an extended photoperiod (Fox and Dessauer 1957). However, Thawley and Kolbe (2020) conducted their study in early spring (February through April) and Fox and Dessauer (1957) conducted their study in fall through winter (September through January). My study therefore reflects a period of time that the effects of ALAN has not previously been evaluated in *Anolis* species. While my study was conducted over a similar amount of time to Thawley and Kolbe's (2020) study on adult brown anoles (six weeks vs. eight weeks, respectively), my study was conducted over a shorter period of time than Fox and Dessauer's (1957) study on adult green anoles (six weeks vs. five months, respectively). Therefore, I might not have exposed the green anoles to ALAN long enough to see similar effects on their SVL as in Fox and Dessauer (1957). In other species, ALAN's impact on body size is similarly mixed. Meyer and Sullivan (2013) found aquatic emergent insects had a decrease in body size, while terrestrial arthropods had an increase in body size with exposure to ALAN over the course of six weeks. In multiple bat species (greater horseshoe bat, *Rhinolophus ferrumequinum*; Geoffroy's bat, *Myotis emarginatus*; and lesser mouse-eared bat, *Myotis blythii*), juveniles exposed to ALAN over the course of several months had smaller forearm lengths, a common metric of body size in bats (Boldogh et al. 2007).

Exposure to ALAN did not impact green anole body mass, similar to results in European blackbirds (Dominoni et al. 2013b). Adults of most species have an increase in body mass when exposed to ALAN (e.g., hamsters: Ikeno et al. 2014, mice: Fonken et al. 2010, humans: McFadden et al. 2014, chickens: Pan et al. 2014). Only Fonken et al. (2010) explicitly tracked the timing and amount of food consumed by their study organism. The authors 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, the mice's body mass did not increase (Fonken et al. 2010). While I did not explicitly track the number of crickets consumed by the green anoles, the two treatment groups were fed the same number of crickets, and most, if not all, of the crickets were consumed during the day, with the exception of crickets provided during the nocturnal foraging trials. Therefore, future studies should explicitly track the amount of food the study organism consumed and when they feed in order to determine if the organisms are consuming a different amount of food, or consuming food at atypical times of the day.

Similar to body mass, ALAN did not appear to impact the liver mass of green anoles. Changes in liver mass can indicate either changes in lipid usage or storage, because the liver acts as the primary site of energy mobilization of lipids for reproduction in reptiles (Hahn 1967). However, the liver is crucial to energy mobilization and storage of lipids across multiple taxa, including birds, reptiles, and some mammals, such as humans (reviewed by Nguyen et al. 2008). Little research has focused on the impact of ALAN on energy mobilization and storage. I am only aware of one study that evaluated changes in energy expenditure, and the authors found that exposure to ALAN in free-living great tits caused a decrease in the great tits' daily energy expenditure (Welbers et al. 2017). However, Welbers et al. (2017) did not evaluate liver mass, even though, in bird species, the liver is the primary site of *de novo* lipogenesis, a complex metabolic pathway to create and store lipids (reviewed by Nguyen et al. 2008). Within green anoles, lipids are primarily stored in the abdominal fat pads, liver, eggs (for females in the breeding season), and "carcass" (Dessauer 1955a). Because lipid storage and mobilization in lizards is primarily for reproduction (Hahn 1967), it is logical that the liver mass of the females exposed to ALAN was similar to the females exposed to normal light-dark cycle group, because their reproductive tissue mass was also similar. While males had heavier testes with exposure to

ALAN, male green anoles store a very small proportion of lipids in their testes as compared to the amount of lipids female green anoles store in their developing eggs (Dessauer 1955a). Therefore, the maintenance of the testis mass might not involve lipid mobilization in the liver.

The green anoles exposed to ALAN had heavier abdominal fat pads than the green anoles exposed to a normal light-dark cycle, which is similar to Swiss-Webster mice (Fonken et al. 2010). However, the mice had a corresponding increase in body mass (Fonken et al. 2010), which the green anoles did not experience, even though they were exposed to ALAN for a similar amount of time (eight weeks vs. six weeks, respectively). In contrast to the green anoles, European blackbirds exposed to ALAN did not have a change in their fat storage, but they also did not have a change in their body mass, like the green anoles (Dominoni et al. 2013b). Potentially, the exposure to ALAN 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 Price 2016); therefore, a transfer of lipids from the "carcass" to the fat pads would indicate the lizards exposed to ALAN were storing the lipids for long-term usage and not utilizing as much energy in their daily activity as lizards exposed to a normal light-dark cycle. An increase in fat pad mass is beneficial for overwinter survival, as green anoles depend primarily on their fat stores for energy and spend most of their time inactive during the winter months (Dessauer 1955a, Jenssen et al. 1996). In future studies, the carcass should be massed at the end of the exposure period to ALAN along with the liver and fat pads, in order to get a more complete picture of lipid metabolism.

ALAN appeared to cause an increase in male green anoles' testis mass. The annual testis regression of green anoles is controlled by photoperiod length and begins after mid-July (Dessauer 1955a, Licht 1971). Male green anoles exposed to extended photoperiods

(13.5L:10.5D) in the summer to fall period had larger testes mass than male green anoles exposed to a normal light-dark cycle (Licht 1971). ALAN possibly acts similarly to an extended photoperiod; therefore, the males maintained their testis mass as the photoperiod cues indicated that the breeding season was still occurring. Other male long-day breeders, such as Siberian hamsters (Ikeno et al. 2014) and European blackbirds (Dominoni et al. 2013b) have a similar response to ALAN as the green anoles in their reproductive physiology. In male European blackbirds, exposure to ALAN is found to extend the time their testes are functional by nine to 12 days (Dominoni et al. 2013b).

However, the elongation of the male green anole reproductive tissue viability did not appear to have been replicated in the female green anole reproductive tissue mass. As most female green anoles produce eggs until mid-August and ovarian regression is controlled by an endogenous rhythmicity (Licht 1973), it is not surprising that female reproductive tissue mass was not different between the two treatment groups. However, to my knowledge, there is no literature on the impact of ALAN on female reproductive tissue mass. Because the females likely did not elongate their reproductive capacity past the end of the breeding season, as the reproductive tissue mass was the same between the two groups, the expense of the males maintaining their testis mass is likely not compensated through increased fitness gains. Therefore, the maintenance of testis mass a potential detriment for the males, as they are using energy to maintain their testes without the benefit of increased reproductive output. Instead, the males could have devoted this energy into other physiological processes, such as immune functioning and fat storage for the non-breeding season.

Limitations

One potential limitation in my study is that I did not randomize the order of trials. Most green anoles (with the exception of 12) experienced the day OFT prior to the night OFT, while all the lizards experienced the night social interaction trial prior to the day social interaction trial. Half of the lizards experienced the day foraging trial prior to the night foraging trial. In each treatment, the same number of lizards experienced the day (or night) trial prior to the night (or day trial), therefore the order of the trials should not affect the overall results found. However, randomization of the order of the day and night trials would ensure the results were not potentially affected by habituation to the trial conditions.

Conclusions

Taken together, I found that ecologically relevant levels of ALAN are linked to a wide variety of behavioral and physiological alterations in green anoles. The lizards exposed to ALAN slept less overall. As sleep is important for energy conservation and restoration, the lizards might not have had the energy to move and forage as quickly, as well as display as frequently during the day. Declines in these behaviors could cause the lizards to be less likely to survive and find a mate to pass on their genes. However, these costs appear to be compensated through increased general activity and foraging at night. Additionally, while the difference in the lizards' display rates between the two group at night was not different, only lizards exposed to ALAN displayed at night. Therefore, even competitive interactions might be compensated through increased nocturnal activity. Lizards exposed to ALAN appeared to maintain heavier fat stores, which is important for overwinter survival, and the males were able to maintain their testis mass, which indicates increased fitness by maintaining this expensive tissue. Potentially, these changes on an individual level could have cascading effects throughout the ecological community, leading to

new environmental challenges and altered ecological relationships. Other studies have demonstrated that exposure to ALAN in one, or multiple, species can lead to alterations in the behaviors of other species and even up to the community structure (e.g., Davies et al. 2012, Bennie et al. 2015, Davies et al. 2015). 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.

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