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UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

Effects and consequences of natural and artificial light at night on small mammals in peri-urban Johannesburg, South Africa

Tasha Oosthuizen

Submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

in the Faculty of Science

at the University of Witwatersrand, Johannesburg

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Declaration

I, Tasha Oosthuizen, declare that this thesis submitted for the degree of Doctor of Philosophy at the University of Witwatersrand, Johannesburg, is my own work. It has not been submitted before for any degree or examination at any other university.



Tasha Oosthuizen

27/05/2024

Abstract

Studies investigating artificial light at night (ALAN) have increased over recent years. However, research examining the influence of ALAN on southern African small mammal species are lacking and even information on their basic biology is scarce. To close this knowledge gap, I investigated the effect of ALAN on different facets of animal behaviour in African small mammals. Firstly, I evaluated the impact of the natural (lunar cycle) and ALAN on the community composition and species abundance in two populations of small mammals. I chose two field sites: one facing Johannesburg (exposed to ALAN) and one facing away. I conducted mark-recapture trapping to ascertain the occurrence and abundance of small mammals. The Light site had both a higher species composition and a higher animal abundance when compared to the composition and abundance of the Dark site. The lunar cycle had an effect; on nights with a full moon, the species composition and animal abundance of both study sites declined, while on new moon nights, the opposite occurred, with an increase in both the species composition and abundance on the Light and Dark sites. The absence of a negative ALAN effect on the Light site can potentially be ascribed to the availability of microhabitats for small mammals to escape illumination, leaving them seemingly unaffected. Next, I assessed the locomotor activity of three species of commonly occurring rodents on the study area, one crepuscular (19 single-striped grass mice, *Lemniscomys rosalia*), one species with reportedly variable activity (19 angoni vlei rats, *Otomys angoniensis*) and one nocturnal (19 southern multimammate mice, *Mastomys coucha*). They were captured at a different location than the mark-recapture study sites and tested in captivity under natural (exposed to natural light and temperature changes), laboratory (standard laboratory conditions; 12h light:12h dark and constant temperature) and ALAN treatments. *Lemniscomys rosalia* exhibited crepuscular activity under all three experimental treatments, *Otomys angoniensis* was mostly nocturnal with some diurnal activity. The temporal activity profiles of the two species that showed some activity during the light hours were unaffected by ALAN. *Mastomys coucha* displayed strictly nocturnal activity during the natural and laboratory treatments, but during ALAN treatments the temporal activity profiles of some animals shifted so that they were active during the start of the day. *Lemniscomys rosalia* and *O. angoniensis* were more active under the natural treatment, whilst *M. coucha* was more active in the laboratory treatment. When exposed to 2 Lux ALAN presented remotely, there was no effect on the level of activity in *O. angoniensis*, *L. rosalia* showed a reduction of about 20% in its activity, whereas *M. coucha* reduced its activity by more than 50%. Finally, I studied how ALAN impacted the foraging behaviour of the three species under four treatments (during the day, at night, 2 Lux ALAN and 10 Lux ALAN). Foraging behaviour differed in the three species under different light conditions. *Lemniscomys rosalia* was risk-averse when feeding during the diurnal and nocturnal (no light at night) treatments. *Otomys angoniensis* showed irregular responses in their foraging behaviour under all foraging treatments. *Mastomys coucha* showed no differences when feeding under any of the nocturnal treatments, but it was inactive under the diurnal treatment. Overall, my study revealed that the effect of

ALAN is not similar for all small mammalian species and appear to depend on both the spatial and temporal niches that the different species occupy. Strictly nocturnal animals seem to be affected the most, whereas animals that are active during the day showed lesser responses. Given the rapid increase in urbanisation and anthropogenic disturbances, more and more species are exposed to ALAN. Species that prefer darker, more secluded habitats appear to be more vulnerable and at higher risk of local extinctions as a result of disturbances, such as ALAN and habitat transformation. My study highlights that ALAN affects both nocturnal and diurnal rodents to the extent that it can have fitness consequences, including changed active times, foraging efficiency, movement patterns and susceptibility to predation. Finally, the disruption of rodent behaviour can have cascading effects for ecosystems and my study also emphasises the importance of safeguarding our night skies to protect biodiversity.

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Chapter 1: General Introduction

1.1 Circadian biology

Most living organisms have evolved endogenous rhythms that coincide with external environmental cycles (Buijs et al., 2003; Dominoni et al., 2016). Although endogenous rhythms are self-sustaining, they are not exactly 24 hours long and must be synchronised or entrained to rhythmic external cycles daily (Buijs et al., 2003). The daily solar cycle is the most predictable environmental cycle and therefore, light is the primary environmental cue to which animals entrain their rhythms (Benstaali et al., 2001; Tapia-Osorio et al., 2013). Other environmental factors such as temperature, feeding times and in some cases locomotor activity can also affect the endogenous rhythms of animals. In the absence of light, these factors are to some extent able to entrain circadian rhythms, but when light is present, it overrides these effects and the secondary external cycles re-enforce light entrainment (Benstaali et al., 2001; Ikeno et al., 2014). By synchronising behavioural and physiological processes to cyclic environmental factors, animals gain extrinsic adaptive fitness, since it enables an organism to predict environmental conditions (Sharma, 2003). Animals can also obtain intrinsic fitness by synchronising their internal processes such that the entire organism is in harmony with its environment (Sharma, 2003).

Light influences the internal biological clock that is responsible for the generation of innate biological rhythms (Buijs et al., 2003; Dominoni et al., 2016). In mammals, the master biological clock is called the suprachiasmatic nucleus (SCN) and is located above the optic chiasm in the hypothalamus of the brain (Buijs et al., 2003; Tapia-Osorio et al., 2013). The SCN receives photic information from a subset of light-sensitive retinal ganglion cells (RGCs) located within the retina of the eye. This system enables animals to entrain to the environmental light/dark cycle, identify environmental changes, and elicit an appropriate response (Benstaali et al., 2001; Tapia-Osorio et al., 2013). Most animals have a specific temporal niche, such that approximately 26% of extant mammals are predominantly diurnal, while about 44% of mammals are nocturnal (Benstaali et al., 2001; Jones et al., 2009; Prugh and Golden, 2014). The remaining proportion of mammals are either crepuscular, showing activity bouts during dawn and dusk, or cathemeral, i.e., species that display irregular activity periods during both day and night (Benstaali et al., 2001; Jones et al., 2009; Prugh and Golden, 2014). Diurnality in mammals is thought to have evolved from nocturnality (Roll et al., 2006) and diurnal animals have preserved some of the nocturnal morphological features, such as retinae that are rod-dominated (Peichl, 2005). However, the majority of diurnal species have higher proportions of cones than nocturnal species (Peichl, 2005). Cones are important for colour detection; they are highly acute but not very sensitive to light (Peichl, 2005). Rods have lower acuity but are very sensitive to light and enhance vision in low

light conditions (Peichl, 2005). In the presence of light, diurnal species become more active, which is termed positive masking (Ikeno et al., 2014). Negative masking is the presence of light that causes a decrease in activity in nocturnal species (Ikeno et al., 2014). Thus, the presence of light awakens diurnal animals and promotes sleep in nocturnal animals (Yan et al., 2020).

Temporal niche partitioning in the timing of activity occurs in sympatric species that have specialised morphological adaptations of the eyes to be active within certain periods (Bennie et al., 2014; Lear et al., 2021). However, morphological adaptations alone do not determine the activity period, since factors, such as predation pressure, competition, food resources, weather conditions and human disturbances all play a role in defining the temporal niche of a particular species (Bennie et al., 2014; Lear et al., 2021).

1.2 Lunar cycle and animal behaviour

Rodents are important prey species and often have to weigh the costs and benefits of foraging against potential predation risks (Lima and Dill, 1990; Mandelik et al., 2003). Most rodents are nocturnal (Hawkins and Golledge, 2018) and can use the moonlight to navigate under low light levels. The lunar cycle creates different intensities of natural light depending on the phase of the moon (Weaver, 2011). A full lunar cycle is 29.5 days and the light intensity of a new moon is around 0.0001 Lux, whereas a full moon can reach intensities of up to 2 Lux (Weaver, 2011). Visibility to predators can lead to several behavioural changes in prey species, such as increased vigilance during full moon nights (Russart and Nelson, 2018). During moonlit nights, prey animals tend to avoid foraging in open areas because increased illumination creates greater predation risk (Mandelik et al., 2003). There are several notable examples. During full moon nights, fewer wood mice (*Apodemus sylvaticus*) were trapped compared with new moon nights (Perea et al., 2011). The higher illumination from a full moon resulted in increased foraging efficiency of short-eared owls (*Asio flammeus*), but their prey species, deer mice (*Peromyscus maniculatus*), decreased their activity and feeding (Clarke, 1983). Nocturnal common spiny mice (*Acomys cahirinus*) that foraged in an open habitat, were significantly influenced by moonlight, with mice visiting fewer artificial food trays to limit detection under moonlit nights (Mandelik et al., 2003). Allenby's gerbils (*Gerbillus andersoni allenbyi*) were more vigilant during the brightest full moon phase followed by the waning moon, waxing moon and lastly the new moon and they ceased foraging sooner during the waxing moon, followed by the full, new, and waning moon (Kotler et al., 2010). During the brighter part of the lunar cycle (full and waxing moon), these gerbils also increased vigilance and reduced foraging, resulting in a poorer body condition, but as the cycle progressed and starvation increased, the gerbils spent more time foraging despite exposure and risk

(Kotler et al., 2010). The response of animals to varying levels of light intensity over the lunar cycle can facilitate predictions of behavioural alterations during artificial light at night.

1.3 Artificial light at night and animal behaviour

Artificial light close to urbanised areas can be brighter than moonlight and is more constant, both throughout the night and over consecutive days. As a result, artificial light at night (ALAN) has the potential to affect the natural nocturnal behaviour of animals more severely than moonlight (Falchi et al., 2016; Russart and Nelson, 2018). The rapid growth rate of the human population increases the need for more structures and developments, and it encroaches on natural habitats, impacting wildlife. Most roads in urbanised areas are lit by streetlights and depending on the light bulbs used, the light intensities of streetlights range between 1 and 10 Lux at ground level (Preto and Gomes, 2019), which far exceeds the natural light intensities reflected by the moon.

Light pollution is a broad term, defined as the change in the natural low light levels at night as a result of the increased artificial light at night (Raap et al., 2015). The two main categories of light pollution are point source and skyglow. Point source is artificial light that is concentrated within a specific area and can be further separated into light trespass, glare, over-illumination, and clutter (Rajkhowa, 2012). Light trespass is the presence of unwanted light found in a person's property, which can result in insufficient sleep (Gaston et al., 2012; Rajkhowa, 2012). Glare, as a result of street and vehicle lights, is a common safety issue, since the eye is not always able to process the high light intensity (termed photostress) and the after-effects can persist for up to an hour after exposure (Gaston et al., 2012; Rajkhowa, 2012). Over-illumination is the excessive use of lights resulting from improper positioning of lights that provide light beyond the desired area (Gaston et al., 2012; Rajkhowa, 2012). The inappropriate arrangement of lights is termed light clutter and can lead to disorientation, especially along roads (Gaston et al., 2012; Rajkhowa, 2012). Sky glow, in contrast, is the product of all the extra light that is reflected into the sky and then reflected back to earth by the atmosphere, increasing the brightness of the sky (Gaston et al., 2012; Rajkhowa, 2012). Skyglow is measured in magnitude per square arc-second (mag/arcsec^2) and can range from 22.0 mag/arcsec^2 for the darkest areas and less than 17.5 mag/arcsec^2 in the brightest areas (https://lightpollutionmap.info/, Appendix Table 1). The artificial brightness is measured in µcd/m^2, and ALAN in most laboratory studies is measured in Lux. The different ways to measure light depend on the scientific field of study (Hänel et al., 2018), I used a handheld light meter, measuring in Lux, which is in accordance with past laboratory studies.

Artificial light at night can have several negative effects on people, including disrupted sleep patterns, melatonin suppression, depressive symptoms, and fatigue (reviewed in Cho et al., 2015). Several studies have shown negative impacts of ALAN on wildlife (Gaynor et al., 2018; Łopucki et al.,

2021; Sanders et al., 2021; Willems et al., 2021). Under ALAN, diurnal and crepuscular animals can extend their active hours, whereas nocturnal species often decrease their activity throughout the night (Russart and Nelson, 2018). Animals which experience ALAN can also alter their general behaviour in terms of their active period, home range size, and their interactions with conspecifics (Hoffmann et al., 2019). Species that show some diurnal activity, such as striped field mice (*Apodemus agrarius*) and bank voles (*Myodes glareolus*), decreased their activity throughout the daylight hours under ALAN (Hoffmann et al., 2019). In addition, these species showed no difference in diurnal and nocturnal home range sizes under ALAN, in contrast to the distinctly larger home range sizes during the day under natural conditions (Hoffmann et al., 2019). This was possibly because of activity asynchronisation under ALAN, since individual interactions decreased and conspecific home ranges did not overlap and as a result could lead to missed mating opportunities (Hoffmann et al., 2019). This can also have an indirect effect on predation risk, since animals can no longer rely on group safety. Likewise, by being active throughout the entire day, animals can now be predated on by a wider variety of predators (Hoffmann et al., 2019). Common spiny mice (*A. cahirinus*) reduced their general activity, as well as foraging behaviour (i.e., the number of visits and movements between food patches decreased significantly) during ALAN (Rotics et al., 2011). This change in risk perception reduces movement between patches and increases within-patch use (Rotics et al., 2011), which then decreases predation risk by birds of prey, such as owls, that hunt moving prey (Mandelik et al., 2003; Rotics et al., 2011). During continuous ALAN, animals will alter their spatial movement, impacting landscape connectivity by avoiding more exposed foraging patches (Bird et al., 2004). Pinyon mice (*Peromyscus truei*) were trapped less frequently under ALAN, possibly because they were avoiding illuminated areas (Willems et al., 2021). ALAN can also be beneficial to some species if they increase their activity during the night (Dominoni et al., 2016), allowing for better detection of predators and food (Prugh and Golden, 2014). Six wader bird species increased their nocturnal food intake by 78% when they foraged in areas with ALAN (Santos et al., 2010). Even though the increased nocturnal activity of diurnal species under ALAN is widely mentioned anecdotally, there are a limited number of studies with empirical evidence to test its effects. Thus, there is a need for more studies focussing on how diurnal species experience ALAN and their responses to light at night.

Living in urban environments could provide benefits that are absent in non-urban, more natural areas (Łopucki et al., 2021). Urban animals have greater and more constant access to food resources. Due to the limited suitable habitat and increased availability of food in an urban environment, one could expect increased aggressive interactions between individuals (Łopucki et al., 2021). However, the opposite was observed in the striped field mouse (*A. agrarius*), where individuals were more tolerant of each other when food was present, apparently to prevent physical injury and excess stress, or to avoid wasting time and energy on competition rather than foraging (Łopucki et al., 2021). An alternative explanation can be that individuals cannot defend large quantities of food, leading to a greater tolerance

of conspecifics (Łopucki et al., 2021; Thomas et al., 2018). Yet, this tolerance was not observed in common spiny mice (*A. cahirinus*) in a more natural setting with ALAN; at higher ambient light intensities, there were greater levels of intraspecific competition since most mice were competing to forage in the shaded areas, which offered safety from predators (Rotics et al., 2011). Competition was greater because the mice lowered their overall activity in the light, and focussed their foraging to a very limited period after the nocturnal illumination (Rotics et al., 2011).

1.4 Risk-sensitive foraging behaviour

All animals make foraging decisions daily, while taking different risks, such as ALAN, into account. These decisions include what to eat, where to eat, how long to spend eating, and how to obtain the food with the lowest estimated risk possible. The Optimal Foraging Theory (OFT) was developed in an attempt to understand and predict these behaviours (Pyke et al., 1977). The OFT assumes that the consumer will always forage optimally, with regard to the diet choice, choice of feeding patch, choice of when to leave a patch and the movement decisions between patches (Bartumeus and Catalan, 2009; Pyke et al., 1977). The Marginal Value Theorem and the OFT together maintain that the animal will constantly maximise its fitness by gaining the most energy while foraging in a patchily distributed resource environment (Bartumeus and Catalan, 2009; Charnov, 1976), but many scientists have challenged the OFT. One of the questions is whether there is optimal behaviour in nature since an animal would have to know and learn everything from their environment to make optimal choices, but this would require long periods of time, which is not always available to a foraging animal (Craft, 2016; Pierce and Ollason, 1987). There are also various risks that each consumer faces when foraging, which are not taken into account (Craft, 2016; Pierce and Ollason, 1987). Yet, OFT helped develop other foraging theories, for example, the Risk-Sensitive Foraging Theory (RSFT) (Barnard et al., 1985).

Foraging choices under risky situations are explained by the RSFT (Barnard et al., 1985; Craft, 2016), maintaining that a risk-sensitive or risk-averse individual will opt for the food choice with a fixed return, whereas a risk-prone individual will show bias towards the food choice with a variable return (Barnard et al., 1985; Craft, 2016). Bank voles (*M. glareolus*) showed risk-sensitive foraging in terms of microhabitat use, since individuals foraged under vegetation of a certain height that they perceived as safe, and each individual had its own range of preferred vegetation heights (Dammhahn et al., 2022). Australian rodents showed a similar response, where more food was consumed in microhabitats of dense and unburnt vegetation, compared with burnt and exposed microhabitats (Doherty et al., 2015).

The RSFT is important for understanding the influence of ALAN on different populations of small mammals and their perception of risk and their responses to foraging decisions. The Mongolian five-toed jerboa (*Allactaga sibirica*) decreased its food searching efforts under ALAN, yet increased its

vigilance and spent less time in each foraging patch, leaving more food uneaten (Zhang et al., 2020). Santa Rosa beach mice (*Peromyscus polionotus leucocephalu*s) limited their activity in illuminated patches and also consumed fewer seeds closer to a light source (Bird et al., 2004). Dwarf striped hamsters (*Cricetulus barabensis*) foraged faster in a food patch under ALAN, and reduced their active times and thus body mass in patches with both low vegetation and the presence of ALAN (Shuai et al., 2023). Overall, many small mammals perceive ALAN as high risk and adjust their foraging behaviour accordingly.

1.5 General biology of my study species

As the literature suggests, both the lunar cycle and ALAN influence the behaviour of multiple species with different temporal niches. Thus, I studied a variety of species, but three were the focus of multiple chapters in this thesis and they were selected based on their abundance and their temporal niche to represent a range of temporal preferences. These were the single-striped grass mouse (*Lemniscomys rosalia*), the Angoni vlei rat (*Otomys angoniensis*), and the southern multimammate mouse (*Mastomys coucha*).

The single-striped grass mouse (Family: Muridae, Figure 1) is terrestrial and has been described as diurnal with crepuscular activity, or crepuscular with diurnal bouts (Kingdon, 2013; Skinner and Chimimba, 2005). This species occurs within tall and dense vegetation and can be found singly, in pairs, or in small groups, and is thus tolerant of conspecifics (Skinner and Chimimba, 2005). However, some laboratory studies suggest aggression between conspecifics (Kingdon, 2013). This species is granivorous and breeds during the summer months (Monadjem et al., 2015; Skinner and Chimimba, 2005).



Figure 1. The single-striped grass mouse. Photo credit: MK Oosthuizen

The Angoni vlei rat (Family: Muridae, Figure 2) is terrestrial and has been described as diurnal, crepuscular or nocturnal (Kingdon, 2013; Skinner and Chimimba, 2005). It is found in grasslands and woodlands, often close to water sources (Kingdon, 2013; Skinner and Chimimba, 2005). This species has been observed singly, in pairs or in small groups and breeding can occur throughout the year, but peaks in the summer months (Skinner and Chimimba, 2005). It is strictly herbivorous (Skinner and Chimimba, 2005).



Figure 2. The Angoni vlei rat. Photo credit: MK Oosthuizen

The southern multimammate mouse (Family: Muridae, Figure 3) is terrestrial and strictly nocturnal (Kingdon, 2013; Skinner and Chimimba, 2005). This species can occur in a wide range of habitats, including human-dense areas (Kingdon, 2013; Skinner and Chimimba, 2005). It is omnivorous, but relies mostly on grass seeds and will opportunistically eat arthropods (Skinner and Chimimba, 2005). In resource-scarce times, it can be cannibalistic (Skinner and Chimimba, 2005). It breeds aseasonally, and under favourable conditions, it can experience population eruptions, with the potential to produce a maximum of 24 pups per litter (Monadjem et al., 2015; Skinner and Chimimba, 2005). Since individual home ranges show a high degree of overlap, it is thought to be tolerant towards conspecifics. It is also a post-burn pioneer that colonise previously disrupted (i.e., burnt) areas after which specialist species become the dominating species (Perrin et al., 2001; Skinner and Chimimba, 2005).



Figure 3. The southern multimammate mouse. Photo credit: MK Oosthuizen

1.6 Study area

I conducted my research at the Cradle Nature Reserve (-25.9214, 27.8503), Gauteng, South Africa, an approximately 9000-hectare property within the Magaliesberg Biosphere Reserve (MBR). The MBR consists of a grassland plateau and sub-Saharan savanna. There are also some Afromontane Forest fragments still present in the area (Mucina and Rutherford, 2006). This site is part of a world heritage site, namely the Cradle of Humankind World Heritage site, which aids in maintaining the integrity of the area and the biodiversity that falls within it (https://magaliesbergbiosphere.org.za/). This site includes cultural heritage along with many archaeological sites.

This reserve is uniquely suited for my research objectives, since it is about 38km from the centre of Johannesburg, the largest metropolitan area in southern Africa. Johannesburg is rapidly expanding and as a result has a high concentration of ALAN. The Cradle Nature Reserve has a skyglow measurement of between 20.4 and 19.4 magnitude/arc second^2, whereas urbanised areas of Johannesburg measure at approximately 18.5 magnitude/arc second^2 (Figure 4, https://lightpollutionmap.info/, Appendix Table 1). The area surrounding Johannesburg measures the highest artificial sky brightness compared with the rest of southern Africa (Figure 4). Within my study area, I chose three separate study sites. One site faced Johannesburg with a high concentration of ALAN reflected from the city (i.e., Light site) and one site faced the opposite direction and received less ALAN (i.e., Dark site) in comparison to the Light site. Lastly, I chose a site separate from the first two with a high abundance of the three study species to catch the laboratory animals without impacting the mark-recapture study.

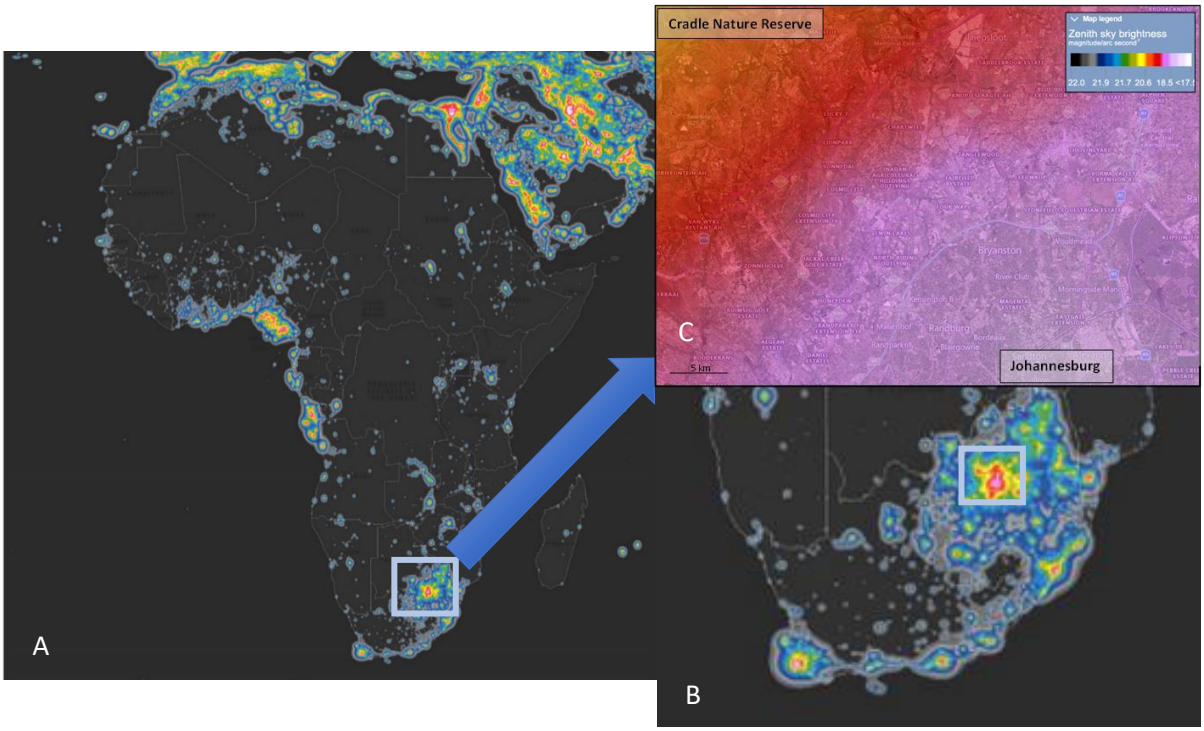


Figure 4. A & B - The artificial sky brightness of Africa, with the area of my study site indicated in the light blue square. Black indicates the lowest artificial brightness, measured at < 1.74 µcd/m^2, blue measures between 13.9 and 27.8 µcd/m^2, yellow between 223 and 445 µcd/m^2, red between 890 and 1780 µcd/m^2 and pink between 3560 and 7130 µcd/m^2. Image extracted from Falchi et al. 2016. C - The reported skyglow for the Cradle Nature Reserve and Johannesburg, the nearest and largest metropolitan area to the study site. This is measured using magnitude per arc second^2, areas with a measurement closer to black, or 22 magnitude per arc second^2, have very little exposure to skyglow and areas closer to white, or less than17.5 magnitude per arc second^2, have a relatively high exposure to skyglow. https://lightpollutionmap.info (Falchi et al., 2016).

1.7 Motivation for this study

As the human population grows over time, we encroach on the natural world at an ever-increasing rate. It is thus important for us to understand how this will influence the natural world and what we could potentially do to mitigate the negative consequences. With the human population increasing by approximately 1% per year (Roser et al., 2019), infrastructure development for human habitation also increases (Rotics et al., 2011). Aside from important consequences, such as habitat fragmentation and water pollution, a large concern of human expansion is ALAN (Gaynor et al., 2018; Rotics et al., 2011). A study investigating skyglow across the world in 2016, found that approximately 23% of the earth's land surface was exposed to ALAN (Falchi et al., 2016). Another study examining ALAN over four years found a 2.2% increase in the earth's area that was exposed to light (Kyba et al., 2017). Furthermore, some locations, including Africa, showed significant growth in artificial lighting over a limited study period (Kyba et al., 2017). These studies are some of the few available that considered all

countries, but they were conducted more than five years ago, making their findings important, but possibly underestimating the severity of ALAN in the present day.

Laboratory studies, which are the standard protocol for ALAN studies on rodents, frequently generate results that differ from experiments under natural conditions. Therefore, it is important to understand how animal responses to light compare in laboratory and field settings. Although more studies are being done on the effects and implications of ALAN on animal behaviour, very little has been done on wild animals in their natural habitat (Raap et al., 2015). The limited number of studies that are available are biased toward wild populations of birds. For example, great tits (*Parus major*) showed increased parental care when a light source was placed at the entrance of nesting boxes (Titulaer et al., 2012) and female blue tits (*Cyanistes caeruleus*) emerged earlier in the morning when they experienced artificial lighting (Schlicht et al., 2014). During the first study year of ALAN exposure, the great tit (*P. major*) and pied flycatcher (*Ficedula hypoleuca*) laid their eggs earlier (de Jong et al., 2015). One study on rodents showed that the nocturnal Cairo spiny mice (*A. cahirinus*) decreased their overall activity and foraging under ALAN in field conditions, whereas diurnal golden spiny mice (*A. russatus*) did not (Rotics et al., 2011).

Very few studies have been conducted on the impact of ALAN on rodents in southern Africa, a taxonomic group that comprises the majority of mammals in most ecosystems in the subregion. Presently, studies on urbanisation and its impact on animal behaviour are taxonomically biased towards species that can easily disperse once they experience unsuitable conditions, such as avian species (Mazza et al., 2020). Rodents are excellent models to study the effect of urbanisation and ALAN on animal behaviour since they are easy to track, capture, house and maintain. Rodents have limited dispersal abilities that force them to remain in an altered area (Mazza et al., 2020). Furthermore, rodents are key biological constituents of ecosystems, since they play a role in distributing plant seeds and they serve as prey for multiple predators, both aerial and terrestrial (Viljoen and Oosthuizen, 2023). Drastic changes in rodent behaviour will have knock-on effects in the wider ecosystem. By understanding how urbanisation and ALAN alter rodent behaviour, we gain insight into the responses of other animals and how functional trophic levels can be modified.

1.8 Aim and Objectives

The main aim of my study was to investigate the influence of artificial light at night on southern African small mammals, specifically rodents.

Study objectives, hypotheses, predictions:

- To assess whether and how the lunar cycle and ALAN influenced small mammal community assemblages by investigating the abundance and composition of two populations at a peri-urban field site, one facing the largest metropolitan area in southern Africa (Johannesburg) and one facing away. I predicted that the lunar cycle and ALAN would negatively affect species abundance and composition, specifically that high light levels would relate to a lower abundance and composition of species.
- To evaluate how the activities of a diurnal/crepuscular species, a species with variable activity and a nocturnal species differed between natural, laboratory and ALAN treatments. I expected all three rodent species selected for study would show higher overall activity during a natural treatment, which comprised of animals being confined to cages but kept in an outside enclosure where they experienced natural light and temperature fluctuations. I anticipated activity to be lower in a standard laboratory treatment (12L:12D at a constant 24°C). I predicted the lowest activity to occur under the ALAN treatment in the laboratory (12L:12D, constant 24°C and 2 Lux light during the D phase).
- To investigate how the foraging behaviour of three rodent species with different activity profiles, was influenced by different environmental risk levels using ALAN. I hypothesised that the diurnal/crepuscular rodent would experience the control treatments as high risk and the ALAN treatments as normal risk. I expected the two remaining rodent species would experience a "high risk environment" under ALAN treatments and subsequently alter their foraging behaviour and reduce movement compared with the control treatments.

1.9 Layout of thesis

My thesis consists of seven chapters: a general introduction (Chapter 1) followed by five data chapters (Chapters 2 – 6) and concluding with the general discussion (Chapter 7). Chapters 2 to 6 have been written as individual manuscripts intended for submission to different academic journals. Chapter 3 has been published in the Journal of Chronobiology International. There is some overlap in information presented throughout the thesis, but the overall editing of the thesis was maintained in each data chapter. The pages have been numbered sequentially across the thesis, but the figures and tables are numbered successively within each chapter and each chapter has its own reference list. All the data chapters were co-authored by both my supervisors who oversaw all aspects of the different chapters. However, I was the principal investigator who collected the data, conducted experiments, ran formal analyses, wrote up each chapter, and compiled the thesis.

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Chapter 2: The impact of artificial light at night and the lunar cycle on small mammal trapping near a large metropolitan area in South Africa

Abstract

Nocturnal light levels affect animal activity, for example, rodents lower their activity during full moon nights because of increased visibility and predation risk. The illumination of a full moon pales in comparison to the illumination provided by artificial light at night (ALAN) close to human habitation, which is rapidly increasing with the growing human population. I investigated how the abundance and composition of a small mammal community changes over the lunar cycle during different seasons and how this differs between a site (Light site) facing Johannesburg, the largest metropolitan area in southern Africa, and a control site (Dark site). Using baited live traps, I caught more animals on the Light site compared with the Dark site, contrary to expectations. I trapped the lowest abundance and composition during the full moon on both sites and this increased closer to the new moon when visibility decreased. Shannon and Simpson diversity indices and Pieloe's evenness score indicated a diverse community on both sites, which differed across seasons. The Light site had a higher small mammal diversity during winter and the Dark site during autumn. There is a paucity of African-based studies that investigate the influence of the lunar cycle and ALAN on the abundance and diversity of small mammal communities. My study shows trends opposite to those expected in communities exposed to ALAN and this could be because of the comparatively low light levels or the use of microhabitats that ameliorate the effects of ALAN.

Keywords: abundance, ALAN, anthropogenic disturbance, lunar cycle, small mammals, South Africa

2.1 Introduction

As the human population continues to grow exponentially, the demand for housing and development increases at a rapid pace. This causes the transformation and depletion of natural spaces that force wild animals to seek other suitable habitats (Sol et al., 2013). However, the remaining natural areas are impacted by another growing challenge, i.e., artificial light at night (ALAN) (Finch et al., 2020). Falchi et al. (2016) estimated that 23% of the earth's surface was already exposed to ALAN and this percentage has definitely increased since their study was published. Animals use light as a source of temporal information (Benstaali et al., 2001) and the increased levels of ALAN results in changes that were not

anticipated or cannot be mitigated currently. These changes include shifts in spatial use patterns of the affected individuals, altered interspecific interactions and foraging behaviours etc.

We can investigate the responses of prey animals to ALAN by comparing changes in their behaviour during dark and full moon. A full moon provides the highest illumination in the lunar cycle, which is approximately 2 Lux (Penteriani et al., 2013). In the few days around full moon, prey species decrease their activity to avoid predator detection because of the greater visibility (Kotler et al., 2010; Kronfeld-Schor et al., 2013; Prugh and Golden, 2014). For example, rabbits (*Oryctolagus cuniculus*) travelled longer distances and used simpler movement patterns as their main predator avoidance strategy during new moon and became less active and employed more complex moves to avoid predator detection during full moon nights (Penteriani et al., 2013). Prey animals thus modify behaviour to minimise the risk of exposure and detection by predators during nights with high visibility.

Animal activity patterns are regulated by circadian rhythms, which are primarily influenced by light (Benstaali et al., 2001). Artificial and unnatural light patterns can result in the desynchronisation of activities, meaning that animals could mistime activities due to altered light cues or miss potential mating encounters due to different individual temporal patterns (Gaston et al., 2012). Moreover, desynchronisation could alter the behaviour of competing species that utilise separate temporal niches and impact species interactions (Hoffmann et al., 2018), and ultimately coexistence. Bank voles (*Myodes glareolus*), which altered their activity and feeding times because of ALAN, showed temporal overlap in activity times with wood mice (*Apodemus sylvaticus*), increasing competition because of interspecific encounters (Hoffmann et al., 2018). Competition is not the only interspecific interaction that changes under ALAN. Since rodents are a prey source for both terrestrial and aerial predators (Gutman et al., 2011), ALAN can cause a permanent state of high predation risk, thus impacting their foraging and other activities (Kotler et al., 2010). For example, the Mongolian five-toed jerboa (*Allactaga sibirica*) spent less time in artificially illuminated patches and had an overall reduced food intake despite being more efficient at finding food under light (Zhang et al., 2020). Animals can therefore experience reduced fitness under ALAN, if they have to employ the behavioural adaptations they use under high intensity light in the lunar cycle (Zhang et al., 2020).

Within a small mammal community, there are a multitude of natural factors influencing the population dynamics of individual species. Biotic factors, such as intra- and interspecific competitors and predators, and abiotic factors, such as climatic factors and the lunar cycle, act in tandem to create the spatial and temporal environment of an animal (Pratas-Santiago et al., 2017). The combination of these factors creates a complex system and teasing apart the components of these systems is necessary to understand the larger processes and dependencies in an ecosystem (Radchuk et al., 2016). For example, the amount of available food is dependent on rainfall, and during periods of low rainfall, there could be low food availability, resulting in competition for resources (Shilereyo et al., 2023). Moreover, the

amount of rainfall influences the thickness of vegetation cover, which could impact how small mammals move on the ground. Foraging success is also affected by the lunar cycle. Predation risk is higher during full moon nights (Kotler et al., 2010). However, depending on levels of satiety, prey species could risk being exposed if they need to forage to meet their energy needs or reduce foraging if they are not energetically compromised (Bedoya-Perez et al., 2013).

My study aimed to investigate how the abundance and composition of small mammals on two field sites differed over the lunar cycle, seasonally and under the influence of ALAN. This study is important because little is known about how African small mammals respond to changes in moon illumination and to artificial light at night. I conducted small mammal trapping surveys in two areas at a peri-urban field site, outside Johannesburg, South Africa. One site faced the city of Johannesburg (Light site), and the other faced away from the city (Dark site). I had three predictions. 1) Since the Light site received a greater level of ALAN, I predicted that I would trap fewer species and lower abundance of animals on this site, as the predation risk is presumably higher because of the increased visibility. I did not necessarily expect that trapping fewer animals would be related to more animals being preyed upon, but that the animals would be more cautious and would not enter traps as readily as in areas with lower visibility. I expected to trap more animals and species on the Dark site, since there were fewer days with high illumination. 2) Population fluctuations differ seasonally based on the number of available resources. Thus, I established how the abundance and composition of small mammals varied seasonally. I predicted the highest small mammal abundance in spring and summer during the breeding season for most small mammals, and lower numbers during autumn and winter because of the declining number of resources. 3) Since the moon phase is associated with levels of predation risk, I also studied how the abundance and composition changed across the lunar cycle. I predicted that greater moon illumination (days around a full moon) would result in lower trapping success, whereas reduced moon illumination (days around a new moon) would show a higher trapping success. I expected this trend to be present on both trapping sites.

2.2 Materials and Methods

2.2.1 Trapping sites

I conducted my research on the Cradle Nature Reserve (-25.9214, 27.8503) located within the Magaliesberg Biosphere in South Africa. This area consists of both savanna and grassland biomes with some woody vegetation and herbaceous grasses (Mucina and Rutherford, 2006; Ramahlo et al., 2022). Two trapping sites were identified. The sites were visually inspected, and I established that they were of similar slope and elevation (Appendix Figures 1 and 2). The first site (Light site) faced Johannesburg, which is the largest metropolitan area in southern Africa with high levels of ALAN and the second site

(Dark site) faced away from Johannesburg (Figure 1). These two sites were approximately 1.7km apart and very few ALAN sources were present on the property itself with the majority of the sources being located at quite a distance and buffered with densely wooded areas.

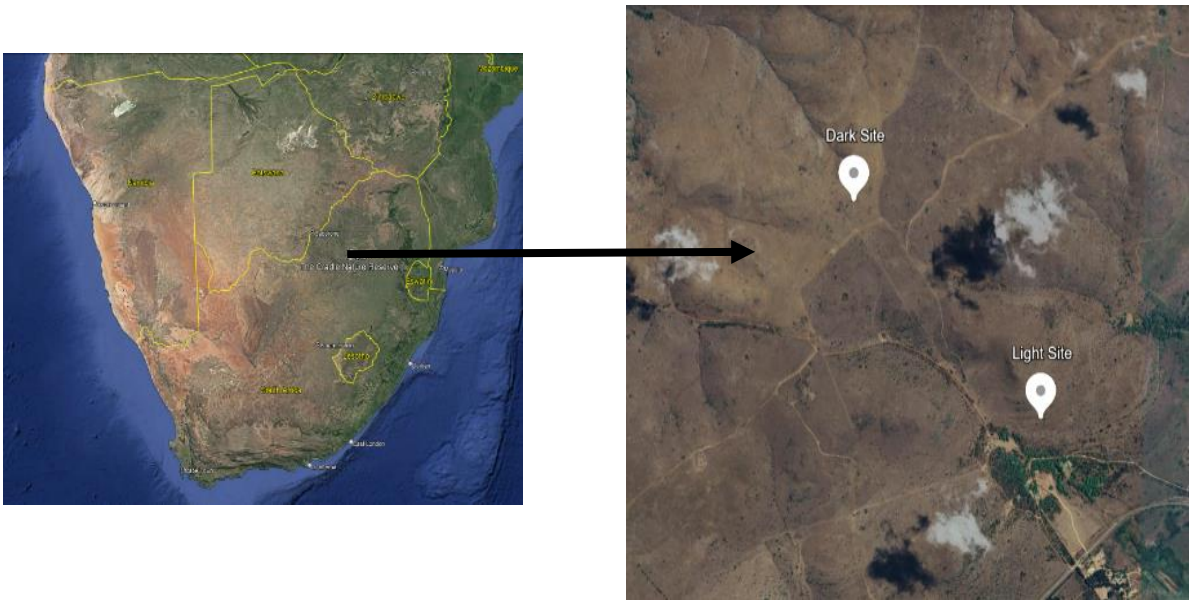


Figure 1. The locations of the Light and Dark sites on the Cradle Nature Reserve property. The Light site faced Johannesburg and the Dark site faced the opposite direction.

2.2.2 Animal trapping and measurements

I trapped small mammals in four seasons within one year (Table 1), using 75 PVC live animal traps per site (7.5 x 7.5 x 30cm). A permanent grid was set up on each site to ensure traps were placed in approximately the same position during each trapping session and the traps were covered with loose vegetation to aid in insulation and mimic a more natural look. All traps per site were separated by 10m and placed in five rows of 15 traps each. The traps were baited with a mixture of sunflower seeds, sunflower oil, rolled oats, granola and salt. Traps were opened at approximately 17h00 every day and checked the following morning at sunrise (between 05h00 and 06h30 depending on the season). Traps were closed during the day since I was interested in the activity of nocturnal rodents to assess the impact of light at night on this guild.

Table 1. The seasonal sampling period dates for the Dark and Light sites on the Cradle Nature Reserve.

Seasons	Start date	End date
Autumm	10/05/2022	10/06/2022
Winter	03/08/2022	03/09/2022
Spring	11/11/2022	12/12/2022
Summer	30/01/2023	02/03/2023

Trapping lasted for a total of 32 continuous days per season (Table 1) to account for the varying illumination levels throughout a full lunar cycle. During winter, cotton wool was placed in the traps to provide some warmth for the occupants during cold nights. When I confirmed that the trap contained a small mammal, I emptied the contents of the trap into a transparent plastic Ziploc® freezer bag to identify the animal to species level where possible. Three cryptic species that were not morphologically identifiable were later identified through mtDNA cyt *b* sequencing using tissue from trapped individuals, obtained by cutting a small piece of the animal's external ear. I weighed the animal using a hanging scale (Pescola®, Switzerland, 1g precision). I sexed each animal using their anogenital distance (longer in males than females). Thereafter, the reproductive status of each animal was recorded; males were classified as either scrotal or non-scrotal and females were classified as pregnant when the abdomen was swollen, lactating when milk could be expressed from the nipples and if neither was observed I considered them non-reproductive (White and Geluso, 2012). Each animal was fitted with a pair of unique ear tags (National Band & Tag Company, USA), which allowed me to identify re-captured individuals. The animal was then released at the site of capture. A trapping permit was approved by the Gauteng Department of Agriculture and Rural Development (CPF6-0231) and the University of Witwatersrand Animal Research Ethics committee gave ethical clearance for this study (2021/08/09B).

To assess the influence of the lunar cycle on the trapping, I downloaded the moon illumination data from the Time and Date website (https://www.timeanddate.com/moon/south-africa/johannesburg?month=3&year=2023, accessed April 2023). The moon illumination percentages (moon phase per day) used in the analyses were retrieved from this website, it was calculated at lunar noon and took refraction into account. In addition, weather data from Lanseria, Johannesburg was accessed through the VisualCrossing website (https://www.visualcrossing.com/weather/weather-data-services#, accessed April 2023). The downloaded weather variables included the minimum temperature, wind speed, cloud cover, humidity and precipitation per trapping day.

2.2.3 Data analyses

In winter, the Dark site was burnt down completely after only 10 days of trapping because of a runaway fire, thus I did not use winter data for the Dark site. Species richness was calculated within site and season, using the count of species per site and per season (Table 2). All further data analyses were done using the R software (R v4.2.1, Boston, United States). I calculated the diversity of the small mammals by site and season using the Shannon and Simpson diversity indices and Pielou's evenness index in the "vegan" package (Oksanen et al., 2022). To analyse whether the indices differed between sites and seasons, I ran a Kruskal-Wallis test for the Simpson index, since it was not normally distributed (Shapiro-Wilk test: $P < 0.05$) and t-tests for the Shannon and Pielou indices, as they were normally distributed (Shapiro-Wilk test: $P > 0.05$). In order to assess the homogeneity of populations across sites and seasons, I used the *betadisper* function to determine the Bray-Curtis dissimilarity score, using means per site per season (Oksanen et al., 2022).

I used linear models to analyse which factors influenced the abundance and composition of the populations. Both response variables (abundance of animals caught - including new and recaptured animals, and the composition - the number of different species caught per day) were tested for normality using the Shapiro-Wilk test and were non-parametric ($P < 0.05$). The abundance of animals and composition of species were considered daily to coincide with the changes in moon illumination. I analysed the data using generalized linear models (GLMs), with a Poisson distribution and log link function. Predictor variables included the site, season and moon illumination as percentage illumination.

To obtain the most parsimonious model per response variable, I used the *drop1* function to remove non-significant variables in a stepwise manner. All model versions were then compared using the "MuMIn" package (Barton, 2023), and the model with the highest weight and lowest AICc was used for all reported results per response variable (Appendix Table 2). Post-hoc comparisons were completed for all significant categorical variables using the "emmeans" package (Lenth et al., 2020). If moon illumination significantly influenced the response variables, it was further analysed using Spearman correlations. These correlations were run using the moon illumination percentage with the response variables, namely abundance of animals caught and composition of species. All tests were two-tailed, and model significance set at 0.05.

Weather variables including minimum temperature, wind speed, cloud cover, humidity and precipitation, were included in the preliminary analysis using the residuals obtained from a principal component analysis (PCA). The PCA was completed using the "FactoMineR" (Husson et al., 2023) and "factoextra" (Kassambara and Mundt, 2022) packages in R. Five different principal components (PCs) were extracted (Appendix Table 3) and the PC explaining more than 50% of the variance was considered in the GLMs. However, the *drop1* function excluded PC1 from all GLMs, and thus the PC values were excluded from all further analysis.

2.3 Results

The models for the statistical analyses considered all predictor variables and their interactions. The final models used depended on whether some variables were retained in the analyses (Appendix Table 2). For ease of explaining the statistical analyses, I presented the outcomes for predictor variables separately below.

2.3.1 Trapping success

During the 128 trapping days (38 400 trap nights), a total of 396 small mammals were caught of which 72.2% were recaptured individuals (Figure 2). The highest number of new captures was during autumn on the Light site and during spring and summer on the Dark site (Table 2). The highest number of recaptured animals was during winter on the Light site and during autumn on the Dark site (Table 2).

I caught significantly more individual small mammals on the Light site compared with the Dark site ($\chi^2 = 193.37$, df = 1, $P < 0.001$, Figure 2). Season influenced the number of small mammals caught ($\chi^2 = 11.72$, df = 3, $P = 0.008$, Figure 2). The post-hoc comparisons showed that the number of animals caught during winter was higher than both autumn and summer ($P \leq 0.041$). None of the other comparisons were significant ($P \geq 0.225$).

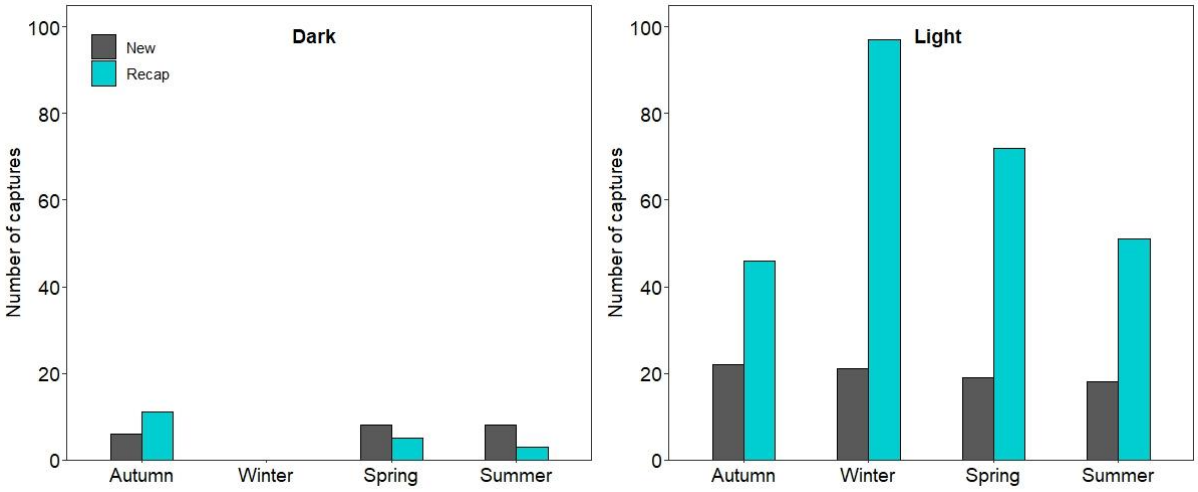


Figure 2. The number of small mammals captured by season on each trapping site at the Cradle Nature Reserve, Gauteng, South Africa. There are no data displayed for winter at the Dark site, since it burned down during the trapping period.

A variety of species were caught on both sites, including southern multimammate mice (Mastomys coucha), bushveld gerbils (Gerbillicus leucogaster), Angoni vlei rats (Otomys angoniensis), Musk shrews (Crocidura spp.) and Namaqua rock mice (Micaelamys namaquensis) (Table 2). The chestnut climbing mouse (Dendromys mystacalis), pygmy mice (Mus minutoides) and four-striped grass mice (Rhabdomys dilectus chakae) were caught only on the Light site. None of the species were unique to

the Dark site (Table 2). The identity of Otomys angoniensis, Rhabdomys dilectus chakae and Mastomys coucha were confirmed with genetic testing.

I caught significantly more species on the Light site compared with the Dark site ($\chi^2 = 129.93$, df = 1, $P < 0.001$). Season was not retained in the most parsimonious model (Appendix Table 2).

Table 2. The abundance of small mammals and species richness by season in Dark and Light trapping sites at the Cradle Nature Reserve. *M. coucha, O. angoniensis* and *R. d. chakae* were identified genetically. The NA's inserted under Dark site in winter, represents when trapping was stopped because the site burned down.

	Autumn		**Winter**		**Spring**		**Summer**	
	Dark	**Light**	**Dark**	**Light**	**Dark**	**Light**	**Dark**	**Light**
Chestnut climbing mouse (*Dendromys mystacalis*)	0	0	NA	1	0	0	0	0
Bushveld gerbil (*Gerbillicus leucogaster*)	2	3	NA	1	4	2	5	1
Southern multimammate mouse (*Mastomys coucha*)	0	11	NA	11	4	7	0	11
Namaqua rock mouse (*Micaelamys namaquensis*)	2	0	NA	1	0	0	2	0
Pygmy mouse (*Mus minutoides*)	0	0	NA	4	0	8	0	0
Angoni vlei rat (*Otomys angoniensis*)	1	7	NA	1	0	2	1	3
Four-striped grass mouse (*Rhabdomys dilectus chakae*)	0	0	NA	0	0	0	0	3
Musk shrew (*Crocidura spp.*)	1	1	NA	2	0	0	0	0
Total new captured animals	6	22	NA	21	8	19	8	18
Total recaptured animals	11	46	NA	97	5	72	3	51
Total captured animals	17	68	NA	118	13	91	11	69

Sex ratio (Males/Females)	0.89	1.00	NA	7.14	3.33	0.66	0.83	1.96
Species richness (N)	4	4	NA	7	2	4	3	4

2.3.2 Diversity indices

The Simpson diversity index was highest on the Dark site during autumn (Table 3). During summer, the Simpson diversity index was similar on both Light and Dark sites (Table 3). The lowest diversity was on the Dark site during spring (Table 3) and this low diversity was also supported by the Shannon index (Table 3). The Shannon index revealed the highest diversity during winter at the Light site (Table 3). The Pielou's evenness index indicated that the evenness was high for all sites in all seasons, with the lowest during winter on the Light site (Table 3).

The Bray-Curtis dissimilarity composite score showed that the Light site had a similar composition of species in all seasons. Following the dissimilarity score, the Dark site had similar species composition in spring and autumn, but a less diverse species composition during summer and autumn. Due to the fire, I did not consider these values in winter, since I was unable to trap for more than 10 days. The Bray-Curtis dissimilarity mean was 0.59 for the Dark site and 0.29 for the Light site (Table 3).

Table 3. Bray-Curtis dissimilarity and diversity indices by season and site for small mammal trapping done on Cradle Nature Reserve, Gauteng, South Africa.

Bray-Curtis	**Autumn**		**Winter**		**Spring**		**Summer**	
	Dark	**Light**	**Dark**	**Light**	**Dark**	**Light**	**Dark**	**Light**
Winter	NA	NA	-	-	-	-	-	-
Spring	0.67	0.25	1.00	0.27	-	-	-	-
Summer	0.14	0.25	0.50	0.46	0.60	0.25	-	-
Simpson index	0.72	0.63	NA	0.67	0.50	0.67	0.53	0.57
Shannon index	1.33	1.12	NA	1.46	0.69	1.21	0.90	1.06
Pielou's index	0.96	0.81	NA	0.75	1.00	0.87	0.82	0.76

2.3.3 Lunar cycle

Moon illumination, regardless of season and site (both were predictor variables described above), was a significant predictor of the number of small mammals caught ($\chi^2 = 26.97$, df = 1, $P < 0.001$; R = -0.25,

Figure 3). During brighter moonlight (i.e., full moon), fewer animals were captured compared with a darker moon (i.e., new moon). On days with a darker moon, I caught more species ($\chi^2 = 12.57$, df = 1, $P < 0.001$; R = -0.24, Figure 3). Both the abundance and composition of animals were negatively influenced by the moon illumination. The Light and Dark site showed a decrease in the number of animals caught when the moon illumination was high, and the opposite pattern was seen when the moon illumination decreased (Figure 3). During new moon nights, more animals were caught in autumn and winter than summer and spring (Figure 3).

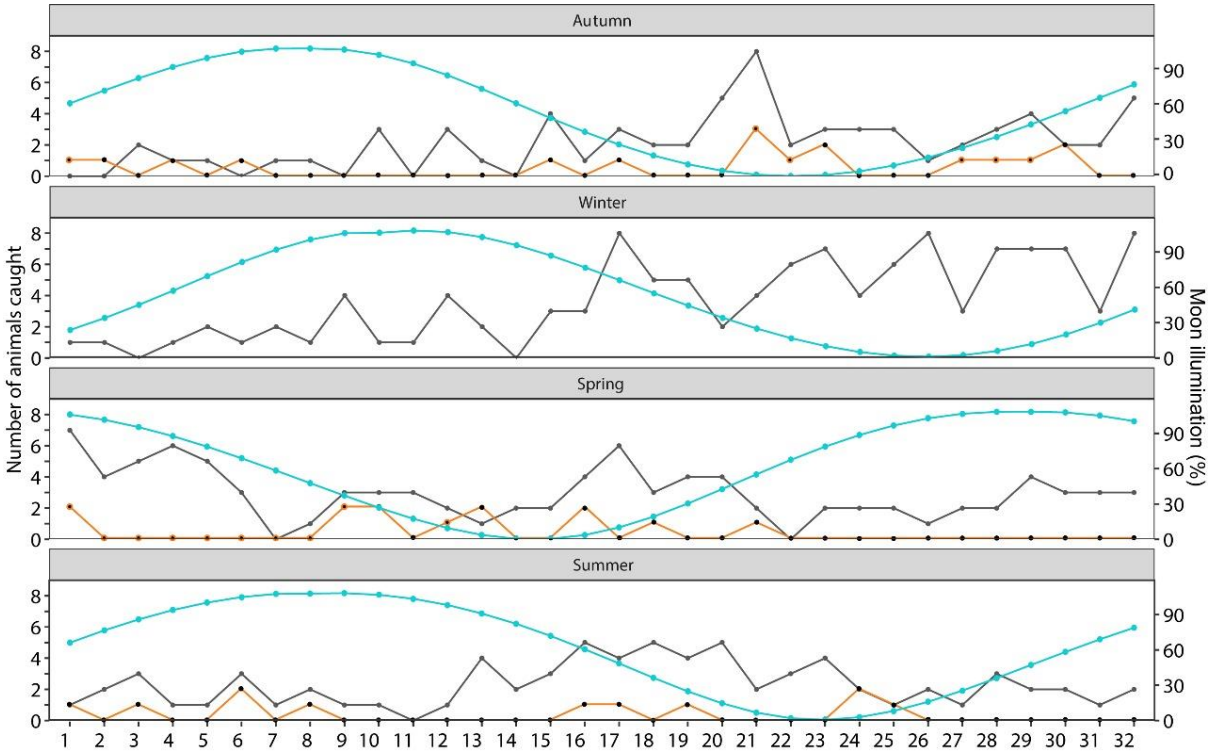


Figure 3. The total number of captures per day per season on the Dark (orange line) and Light (grey line) sites with the associated moon illumination percentage (blue line) of each day.

2.3.4 Weather variables

Although my model selection excluded weather variables from the analyses, this does not suggest that the weather variables considered had little to no influence on the population abundance and composition. There could have been more complex interactions that my analysis was not sensitive enough to detect for, or more variables I did not include in the analysis, such as mean daily temperature. I captured more animals on both sites on nights without rain compared with nights with rain (Table 4). With respect to the minimum nocturnal temperature, I caught the lowest number of animals when the minimum temperature was between 0 and 5°C (Table 4). The captures increased at temperatures of 5 - 10°C and 15 - 20°C but decreased at temperatures between 10 - 15°C (Table 4). There is no clear trend,

but temperature and rainfall seem to impact the capture numbers to a degree and could be working in unison with other weather variables.

Table 4. The number of captures based on whether it rained and minimum temperature during the night on both sites on the Cradle Nature Reserve.

Precipitation		
Did it rain during the night?	**Number of captures**	
	Light site	*Dark site*
Yes	74	7
No	286	36
Minimum night temperature		
Temperature classes (°C)	**Number of captures**	
	Light site	*Dark site*
0 - 5	6	0
5.1 - 10	134	17
10.1 – 15	86	5
15.1 - 20	127	21

2.4 Discussion

I investigated the influence of ALAN (site location) and the lunar cycle (moon phase) on African small mammal abundance, species composition and diversity near Johannesburg, the largest metropolitan area in southern Africa. I predicted that the Light site (facing Johannesburg) would have a lower abundance and diversity of small mammals than the Dark site (facing away from Johannesburg), since the more intense ALAN and associated increased visibility on the Light site would pose a significant predation risk for the small mammals. My prediction was not supported. The Light site had a significantly higher abundance of small mammals and an overall higher diversity than the Dark site. Three species were caught on the Light site but not on the Dark site, these were the chestnut climbing mouse, the four-striped grass mouse, and the pygmy mouse. This difference could be attributed to the vegetation density on the two sites, since the Dark site seemed to be barer than the Light site. Additionally, the Dark site faced away from the city and visually it looked darker than the Light site, but ALAN was not measurable with a handheld Lux meter that measured up to two decimals. Thus, I could have caught more individuals and species on the Light site because of a lower level of ALAN than anticipated and/or the denser vegetation providing microrefugia. The Light site seemed to have denser vegetation and more trees than the Dark site, and this could have been more attractive to a range of small mammal species, adding to the richer diversity on the Light site.

The lowest diversity on the Light site was recorded during summer, possibly because of it being the rainy season with an associated increase in available natural resources. Seasonal diversity indices were lowest on the Dark site during spring, followed by summer. Spring was the trapping season that followed the burning event on the Dark site. This could have resulted in the low diversity, as only some species (such as *M. coucha*) can tolerate the lack of resources and increased exposure. Controlled burning of some sections of the property occurs yearly, but runaway fires are irregular occurrences. I caught the same number of *G. leucogaster* as *M. coucha* in spring on the Dark site. *Gerbillicus leucogaster* is also a pioneer species but they are more abundant in areas with frequent burning compared with control areas (Kingdon, 2013; Korn, 1987). Diversity was higher for the other seasons for both sites, indicating the sites were not dominated by a single species. It is possible that during autumn and winter, the naturally occurring resources were low and multiple species preferred the bait and as a result I caught a higher number of species. However, the diversity index scores of the Dark site were affected by the small number of captures, and should be interpreted with caution (Bashalkhanov et al., 2009). The evenness scores remained similar in all seasons and between the Light and Dark sites, suggesting that the small mammal communities were close to even.

The small mammal communities were not dominated by a single species. For example, the shrew species occurred at both sites (although in low abundances). Shrews have been used as bioindicators to identify healthy environments (Pankakoski et al., 1994). Yet, the occurrence of the *M. coucha* on both sites is indicative of a disturbed area (Perrin et al., 2001). A similar pattern of high *Mastomys* captures and lower shrew captures was recorded in another ecosystem close to my study site (Ramahlo et al., 2022) and a forest reserve based in Tanzania (Michael et al., 2016). Southern multimammate mice were caught on the Dark site in spring only. This species is recognised as a pioneer species in post-burn environments (Rowe-Rowe, 1995; Skinner and Chimimba, 2005) and as this particular spring was preceded by fire in winter, it supports the species presence in spring.

Bushveld gerbils had the highest number of newly captured small mammals on the Dark site. Adult gerbils are tolerant of other adult gerbils in the same area and breed year-round, with peaks during certain times of year (Kingdon, 2013; Monadjem et al., 2015). Multimammate mice were the highest number of newly captured small mammals on the Light site. Similarly, two other studies reported high numbers of multimammate mice in sites exposed to high levels of disturbances (Michael et al., 2016; Ramahlo et al., 2022). However, these studies did not investigate ALAN directly, but rather human disturbances. Multimammate mice are aseasonal breeders, which can experience population eruptions under favourable environmental conditions since they are prolific breeders (maximum 24 offspring per litter; mean = 11 pups) (Monadjem, 1998; Skinner and Chimimba, 2005). This could explain the high rate of newly captured individuals on this site. Although I did recapture some *M. coucha* individuals, there were more newly captured individuals in comparison. Some of the other species caught either breed seasonally (explained below) or they are territorial and intolerant of strangers (specified below),

which potentially explains the higher recapture number of the individuals of these species, such as *O. angoniensis*. If only a limited number of individuals from the same species occurred within my study site and they were territorial, I would most likely only recapture these same individuals over time, which is a challenge of the mark-recapture trapping method (Reich and Gardner, 2014).

Angoni vlei rats (*O. angonienis*) were caught on both study sites. This species can breed throughout the year but breeding peaks during spring and summer (Skinner and Chimimba, 2005). *Otomys angoniensis* is described as anti-social and territorial (Packer, 1980), but some literature report it to occur in pairs or groups (Kingdon, 2013; Skinner and Chimimba, 2005), possibly during the breeding season. It is apparently diurnal with some instances of crepuscular and nocturnal activity, which could explain its low capture numbers in my study (Skinner and Chimimba, 2005). However, in the laboratory, it is mainly nocturnal (Chapter 4). Namaqua rock mice (*M. namaquensis*) were also caught on both study sites. Similar to *O. angoniensis, M. namaquensis* is a seasonal breeder during the wet summer months (Skinner and Chimimba, 2005). The literature indicates that it occurs in small colonies, although aggressive interactions between individuals have been observed in the laboratory (Heike Lutermann, pers. communication). During the breeding season, a male *M. namaquensis* territory overlaps with those of multiple females (Fleming and Nicolson, 2004). The aggressiveness of this species could be the reason I caught so few individuals on my study plot. If the species is aggressive, overlap of conspecific home ranges would be reduced, resulting in fewer individuals being caught across the trapping grid. Alternatively, the habitat from the two study sites could have been less ideal for this species, which prefer more rocky areas and possibly reduced the likelihood of being trapped (Skinner and Chimimba, 2005). A single chestnut climbing mouse (*D. mystacalis*) was caught on the Light site. Climbing mice are seasonal breeders. They spend most of time at the top of long vegetation (Skinner and Chimimba, 2005), potentially decreasing the probability of capturing them. Pygmy mice (*M. minutoides*) were only caught on the Light site and are presumed to be seasonal breeders (Skinner and Chimimba, 2005). They are aggressive towards conspecifics in the laboratory (Skinner and Chimimba, 2005) and their aggressiveness could be the reason for fewer captures of this species on my trapping sites. An alternative explanation could be that my traps were not sensitive enough to detect this small species upon entry into the trap. Finally, the four-striped grass mice (*Rhabdomys dilectus chakae*) were only captured on the Light site and have been described as diurnal and crepuscular (Monadjem et al., 2015). This species is seasonally territorial (Skinner and Chimimba, 2005), both its active time and their territoriality could be the cause for low capture success.

The abundance of small mammals was greater in winter than autumn, spring and summer, which was unexpected since most savanna small mammals are more abundant in the breeding season i.e., spring and summer (Meheretu et al., 2015; Skinner and Chimimba, 2005). There are several possible explanations for this outcome. 1) Capture success was based on whether the bait was preferred over the surrounding vegetation. These small mammals often breed during the hot and wet seasons in

spring and summer (Skinner and Chimimba, 2005). During these months, vegetation density is greater because of higher precipitation. The increased vegetation density is linked to increased resource abundance (Shilereyo et al., 2023), which could result in the capture of fewer small mammals, since they could have preferred their natural food over the bait in the traps (Adler and Lambert, 1997; Aplin et al., 2003). 2) In spring and summer, the small mammals could have expanded their home ranges in search of potential mates and possibly also experienced increased mortality as a result of competitive interactions, resulting in lower capture rates (Ramahlo et al., 2022; Rocha et al., 2016). During one of the dry seasons (autumn), I still trapped more animals on the Light site compared with the Dark site. Contrary to my findings, Ramahlo et al. (2022) found that study areas with a low level of anthropogenic disturbances, similar to my Dark site, resulted in higher densities of small mammals during the dry seasons. There could be other factors influencing this difference, such as the density and presence of predators (which was outside of the scope of my study). I caught fewer small mammals during spring on both sites, but it is important to note that the Dark site burned down in the preceding winter, and this most likely resulted in the lower diversity observed. This would have reduced the availability of resources and exposed the small mammals to greater predation risk (González et al., 2022). The exception would be the multimammate mouse, a post-burn pioneer species (Leirs et al., 1994). Although the weather variables were excluded from the analysis, it could still have played a role. Precipitation and temperature are directly related to the number of available resources and competition. Cloud cover could still impact the visibility and predation risk, by either concealing a full moon or reflecting ALAN from the city (Kyba et al., 2011), increasing the concentration of ALAN on the site, but this has to be investigated further.

As predicted, I caught significantly more individuals and species on nights of a new moon and capture rate declined across the cycle, with the lowest capture rate around full moon. Past studies indicated that rodents decrease their overall activity with increased visibility (Penteriani et al., 2013; Pratas-Santiago et al., 2016), as reported for wood mice (*A. sylvaticus*), especially in areas with limited vegetation cover (Perea et al., 2011). As my trapping sites were geographically close together, the overhead moon illumination was similar on the two sites. Thus, the same pattern of low capture rates under brighter moon illumination was apparent on both sites. From our current knowledge, it is more likely that the levels of ALAN that reached the Light site is not as bright and influential as I thought initially. Yet, it is vital to take into account that none of these factors act in isolation, and there could be multiple factors interacting which resulted in the patterns I observed here. Artificial light at night along with artificial noise, available resources, the lunar cycle, weather variables and more, are all playing a role on each organism and their underlying neuroendocrine systems, which facilitates their responses and behaviours (Falcón et al., 2020). Rodent captures appeared to be higher in new moon in both autumn and winter and slightly lower in spring and summer and is probably linked to the availability of resources, yet this was not tested for specifically. The available literature on the influence of lunar cycles

and ALAN on animals is still growing. Presently, the investigations are focused on the simpler questions surrounding the influence of light on animals, but there is not enough information to completely understand all the factors and interactions involved in animal responses, not to mention specific species.

2.5 Conclusions

My study is one of the first to investigate the influences of the lunar cycle and ALAN on African small mammals. Although the Light site faced Johannesburg and was exposed to a higher level of ALAN than the Dark site, I caught more animals and more species here than expected. The reason for this is unclear, as the abundance and composition decreased under a full moon night, which suggests a strong response to increased illumination. Thus, either the amount of ALAN that reached the Light site was not sufficient compared with a full moon, or the animals make use of microhabitats that provide sufficient cover in the presence of ALAN. There are possibly other contributing factors (e.g., vegetation, predators) that were undetected in my study. These findings should be investigated further since small mammals are food sources to a range of predators and changes in abundance and composition in their communities because of anthropogenic changes will have knock-on effects on ecosystems.

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Chapter 3: Wild mice in an urbanized world: effects of light at night under natural and laboratory conditions in the single-striped grass mouse (*Lemniscomys rosalia*)

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Abstract

Urbanization, and the accompanying artificial light at night (ALAN), can disrupt the activity of animals. Such disruptions at the base of a food web can ripple through the ecosystem. Most studies of ALAN are performed in the laboratory. Thus, we lack basic information about the circadian responses of animals under natural environmental conditions to fully evaluate the impact of ALAN. We studied the behaviour and activity of wild-caught, peri-urban single-striped grass mice (*Lemniscomys rosalia*) under a natural treatment and in a standard laboratory treatment, including dim light at night to mimic conditions that they could experience. The species exhibited predominantly crepuscular activity under all experimental treatments. It showed the highest level of activity under the natural treatment, whereas ALAN significantly suppressed its activity. Males were more active than females under all experimental treatments. The marked changes in activity under ALAN is of particular concern since global change in combination with urbanization can lead to a change in vegetation density and composition that will decrease the number of suitable microhabitats and expose small mammals to novel habitat changes. We suggest that the single-striped mice could become vulnerable because of urbanization, leading to impacts on its ecosystem broadly.

Keywords: activity, artificial light at night, laboratory, rodent, natural environmental conditions, urbanization

3.1 Introduction

Urbanization is increasing rapidly worldwide, and the accompanying environmental changes create new challenges for wildlife (Sanders et al., 2021). In addition to habitat loss, anthropogenic disturbances

such as artificial light at night (ALAN) poses a significant threat to the natural behaviours of animals (Ikeno et al., 2014; Mazza et al., 2019). In their natural habitats, animals synchronise their physiology and behaviour to cyclic environmental cues, with the light-dark cycle being the most prominent and reliable cue (Aschoff, 1960; Benstaali et al., 2001). ALAN can alter the perception of light levels, causing animals to shift or modify their temporal and/or spatial activity patterns (Dupke et al., 2017; Gaynor et al., 2018).

Temporal activity patterns of mammals evolved over millennia. Early mammals are believed to have been nocturnal, with diurnality emerging as a secondary evolutionary trait (Roll et al., 2006). Globally, about 70% of all mammals are nocturnal (Bennie et al., 2014), and many species show anatomical adaptations of their visual systems that match their temporal niches (Peichl, 2005). Nocturnal animals usually have a larger proportion of rods in their retinae that have low acuity but are very sensitive to light, whereas diurnal animals have a higher proportion of cones adapted for colour vision, with a high acuity but a lower sensitivity (Peichl, 2005; Rotics et al., 2011b). Nevertheless, both nocturnal and diurnal species are affected by ALAN. Light can affect animals in two ways, the first is masking, a direct and immediate effect that does not alter entrainment of the circadian rhythms, and the second is the disruption in the circadian responses by altering the length and expression of the rhythm (Hoffmann et al., 2018; Mrosovsky, 1999). In their natural habitat, diurnal and nocturnal animals experience light at different times of the temporal day, and also at different intensities, and can therefore have different responses to light at night. Diurnal animals can extend their active time into the night, thereby disrupting their sleep patterns (Aschoff, 1960; Fonken et al., 2010; Russart and Nelson, 2018). In contrast, the activity of nocturnal animals may be suppressed (Aschoff, 1960; Viljoen and Oosthuizen, 2023; Zhang et al., 2020).

Experiments to test the effect of ALAN are often conducted under controlled laboratory settings where environmental conditions such as ambient temperature and light are kept constant (Ackermann et al., 2020; Kronfeld-Schor et al., 2013). The laboratory environment eliminates variation in environmental variables, and results are more predictable and easier to interpret. However, it is not always clear whether laboratory outcomes are representative of behaviour in natural environments (Calisi and Bentley, 2009). Several studies highlight differences in the circadian behaviour of animals in the laboratory and the field (Blanchong et al., 1999; Levy et al., 2007; Rotics et al., 2011a; Rotics et al., 2011b; Yan et al., 2020).

To investigate how animals would react in a natural environment, a more realistic approach should be adopted (Ackermann et al., 2020; Kronfeld-Schor et al., 2013). A blend between a laboratory and a natural treatment on the same individuals would be an ideal approach since this would provide an opportunity to assess the response capacity of animals to changing environmental conditions. A natural environment has fluctuating temperature and lighting conditions and a natural soundscape. For example,

in response to a simulated dawn and dusk period in a laboratory setting, diurnal animals extended their activity into the increased light hours (Ackermann et al., 2020), such that these results could be more representative of animal behaviour in a natural environment. In another study, researchers compared the activity of four-striped mice (*Rhabdomys pumilio*) in a natural enclosure with that of the same species in the laboratory under a square wave light cycle (light switches on and off immediately without a dawn and dusk period). The results revealed similar activity patterns between the two groups, but the onsets and offsets of activity differed (Schumann et al., 2005).

The effect of anthropogenic disturbances on southern African small mammals is virtually unknown. The circadian biology of several rodents and other small mammalian species has been investigated in the laboratory (Ackermann et al., 2020; Hoole et al., 2017, 2012; Perrin, 1981; Ribble and Perrin, 2005; Schumann et al., 2005; van der Merwe et al., 2014, 2012, 2011), some more detailed than others. Only a single, very recent study considered the effects of ALAN on the behaviour of a southern African rodent (Viljoen and Oosthuizen, 2023). This illustrates a significant paucity in published literature on the effect of light at night on African small mammals.

We studied a small African rodent, the single-striped grass mouse (*Lemniscomys rosalia*), originating from a peri-urban area (Skinner and Chimimba, 2005). This species is terrestrial, mostly herbivorous and is found within grasslands with long and dense grasses that provide good cover (Monadjem and Perrin, 1997; Skinner and Chimimba, 2005). Very little is known about the single-striped grass mouse, although it has been reported to be active during the day with possible crepuscular activity (Skinner and Chimimba, 2005). Skinner and Chimimba (2005) also suggest that this species used burrows. However, several other sources indicate that they make untidy grass nests on the ground at the base of grass clumps (Chidumayo, 1977; Kingdon, 2013; Monadjem unpublished data; N Pillay personal observation). This species is solitary and have been observed to show aggressive interactions in the laboratory, yet males and females can share a nest in the presence of offspring (Kingdon, 2013).

Our study is the first to empirically test the temporal activity of *Lemniscomys rosalia.* We assessed the locomotor activity of wild-caught single-striped grass mice under controlled light and temperatures in the laboratory and under natural ambient conditions in an outside enclosure. In the outside enclosure, animals were exposed to similar ambient conditions and other disturbances in their natural environment. Therefore, we expected their activity to closely resemble their natural behaviour. We compared the activity of single-striped grass mice under the natural environmental treatment with that under a standard laboratory treatment, and predicted more variable activity under natural conditions, whereas in the laboratory, we expected more activity during the day. We also evaluated the activity when animals were subjected to artificial light at night (ALAN) in the laboratory and compared this to activity under the standard laboratory treatment and the natural environmental treatment. We expected animals under ALAN to increase their activity compared to when they are exposed to dark

nights in the laboratory, thus showing more activity compared to activity under natural conditions. We predicted that males would be more active than females since rodent males are normally the more explorative sex and more risk prone compared to females.

3.2 Materials and Methods

3.2.1 Animal capture

Trapping took place during the austral spring of 2022 at the Cradle Nature Reserve (-25.9214, 27.8503), Gauteng, South Africa. The study site is located in a peri-urban area close to Johannesburg, the largest metropolitan area in Africa, and is therefore exposed to a high concentration of ALAN. We trapped mice using PVC small mammal live traps, baited with a mix of sunflower seeds, sunflower oil, oats, granola, salt and peanut butter. Traps were placed in areas with signs of rodent presence, such as rodent runways and beneath bushes. When a single-striped grass mouse was captured, it was transported to a field laboratory for further processing. At the laboratory, the mouse was weighed, sexed and checked for pregnancy. Pregnant animals were excluded from the study and released at the capture site. We used 19 adult single-striped grass mice (10 males (57.44g ± 3.45) and 9 females (45.38g ± 1.91)) in this study.

3.2.2 Animal maintenance

Study animals were housed individually in transparent, plastic experimental cages (60 x 40 x 36cm). Mice were in their cages at least a month before the commencement of the experimentation. The cages had wire mesh lids and cage floors were layered with 2cm of sand (Kiddies play sand, EDCO Trading cc, RSA) to cover the entire surface. Each cage had a plastic shelter, hay and tissue paper for nesting material, a rock and a stick for enrichment, and *ad libitum* water. Animals were fed daily at random times, and their diet consisted of fresh food (apples, carrots or sweet potato) and sunflower and millet seeds. To monitor body condition, the mice were weighed after each experimental treatment. The experimental procedures were approved by the University of Witwatersrand Animal Research Ethics Committee (2021/08/09/B), and we obtained a collection permit from the Gauteng Department of Agriculture and Rural Development (CPF6-0231).

3.2.3 Experimental design

The activity of the mice was detected using infrared motion detectors (BMT Digital PIR Motion Sensor, Communica, RSA). The sensors were mounted on top of the lids of each cage without blocking external light and positioned to cover movement of the whole floor. Cardboard dividers were inserted between

cages to prevent neighbouring mice from influencing each other. Mice were allowed to acclimate to their surroundings for three days before experiments.

Mice were subjected to three experimental treatments in the following order: (1) laboratory (LAB), (2) artificial light at night (ALAN) and (3) natural ambient conditions (NAT). Each experimental treatment lasted 10 days during which locomotor activity counts were continuously recorded with VitalView software (VitalViewTM, Minimitter Co., Sunriver, OR, USA). The LAB treatment took place under standard laboratory conditions with 12h dark and 12h light (LED warm white light, Leroy Merlin, RSA; ± 50 Lux at ground level) and constant temperature set to 24°C. During the ALAN treatment, environmental conditions remained the same as during LAB, but a LED light connected to a dimmer (Light: LightWorx, 9W 3000K LED, LightWorx LED & Electric Supply LLC, New Jersey; Dimmer: 500W rotary dimmer, Shuttle, Cape Town) was added to provide 2 Lux of indirect light at night at the level of the cages. The dim light remained on permanently for the duration of the ALAN experimental treatment; it was dim enough that it did not add additional light during the day. For the last treatment (NAT), mice were moved to an outside enclosure attached to the field laboratory (Appendix Figure 3). The enclosure had an opaque roof, and the sides were covered with wire mesh to expose mice to natural light and temperature, but not rain. The NAT treatment included the natural soundscape and anthropogenic disturbances such as noise, intermittent car traffic and lights, but the animals were trapped within close proximity of the laboratory, therefore the disturbances were not deemed novel. Cages were cleaned after every treatment.

3.2.4 Data analyses

Activity data were visually assessed using double-plotted actograms generated with ActiView (ActiView^{TM}, Minimitter Co., Sunriver, OR, United States). Activity counts were summed and recorded per minute and, were converted to per hour counts per animal and we analysed it using R (R v4.2.1, Boston, United States). The dataset was not normally distributed (Anderson-Darling normality test: $P < 0.05$), thus we analysed the data using a generalized linear mixed model (GLMM) with a Gamma distribution and log link function, using the lmer package (Bates et al., 2015). The response variable was activity counts, the fixed variables sex (male/female), experimental treatment (LAB, ALAN or NAT) and the time of day (light/dark), and we considered all interactions. To account for individual variation, we included the animal ID as the random variable. We used Tukey HSD for our post-hoc comparisons. Two males that displayed stereotypical behaviour (abnormally high activity and repetitive movements concentrated in one part of the cage), were excluded from the analyses, resulting in a final sample of 17 mice. A Spearman correlation ($P < 0.05$) was conducted to assess the relationship between the activity counts and temperature during the NAT treatment. The correlation was not performed on the laboratory conditions since the ambient temperature was constant during these treatments. We

visually examined the actograms for each animal per experimental setting in order to determine the on and offsets of activity, however, the start and end of the activity were too variable over the days and between mice. This is also the reason the alpha (duration of activity) could not be determined accurately.

3.3 Results

The experimental treatment had a significant effect on the total activity counts ($\chi^2 = 104.395$, df = 2, P < 0.001, Figure 1). Mice were least active during ALAN and most active under the semi-natural environmental conditions (NAT) (NAT < LAB < ALAN, P < 0.001, Figure 1). Under NAT, the activity counts were weakly negatively correlated with the temperature (r = -0.10, P > 0.001, Figure 2).

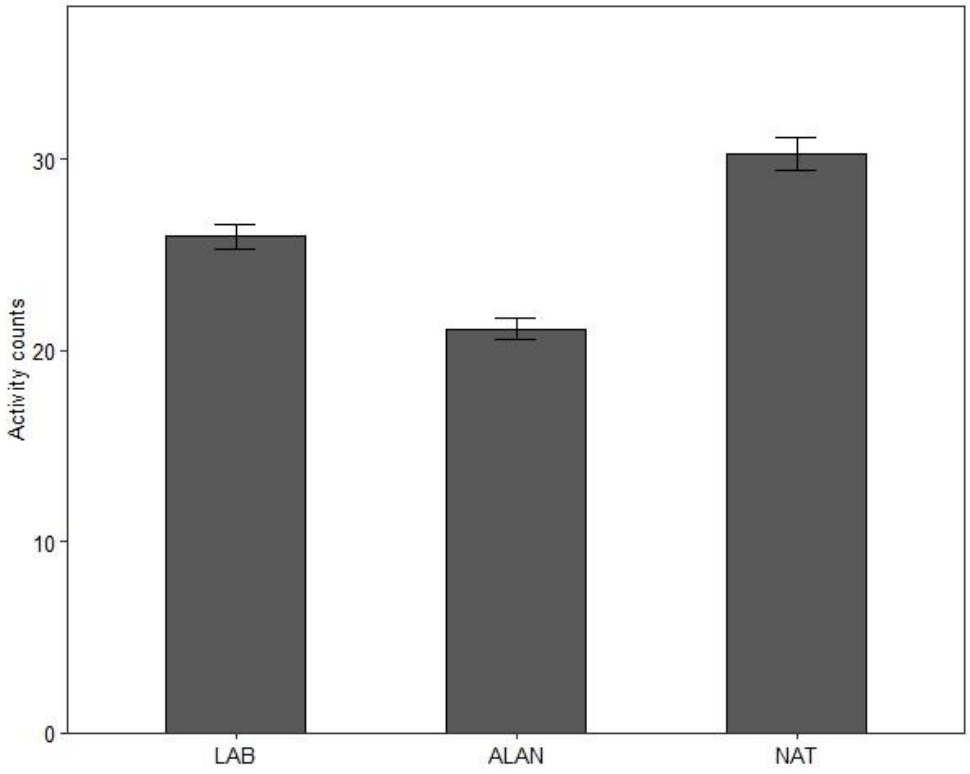


Figure 1. The total activity counts of all *Lemniscomys rosalia* (mean ± SE) during the three environmental treatments (LAB – Laboratory treatment, ALAN –2 Lux light at night, NAT – semi-natural environmental treatment). The lack of letters on the graph indicates no non-significant differences.

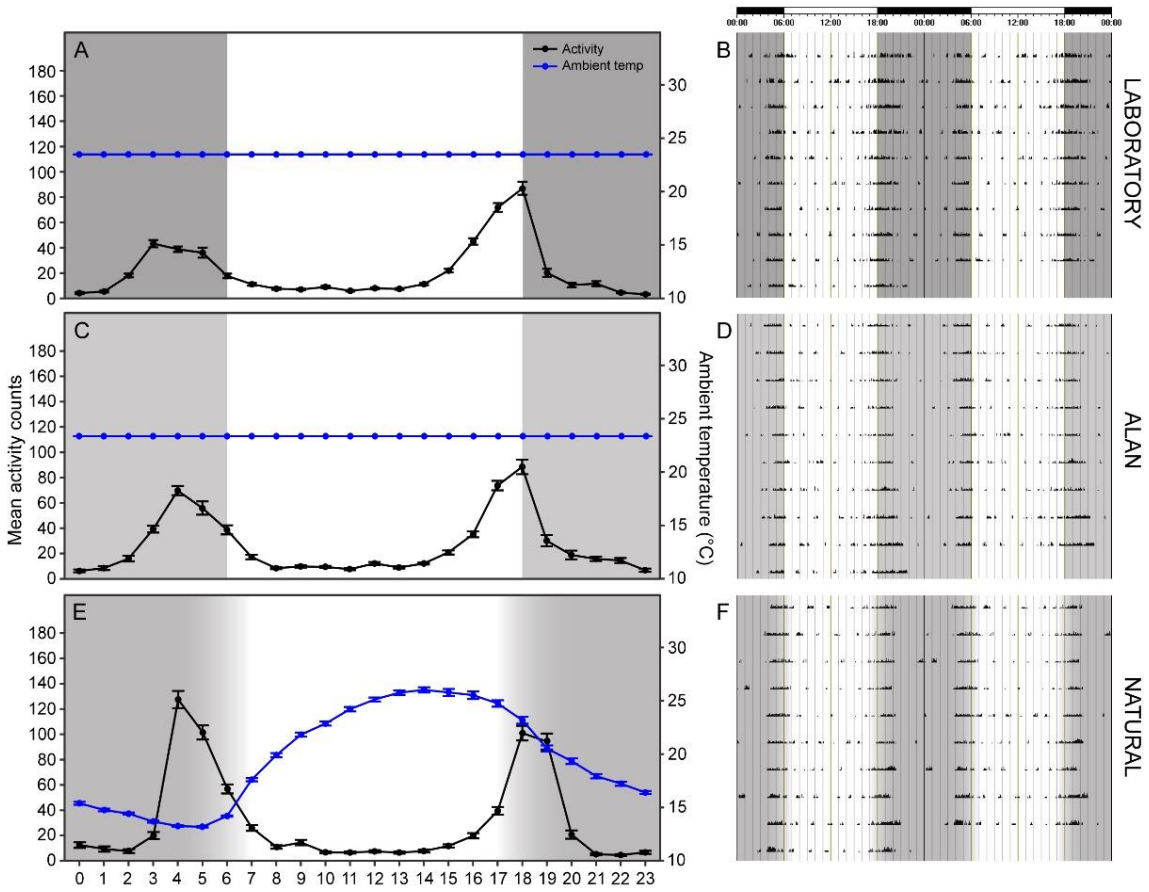


Figure 2. The activity of *Lemniscomys rosalia* over the 24-hour day for the three experimental treatments. A, C, E – Hourly activity counts (mean ± SE) of all individuals (black line) and the ambient temperatures (blue line) during the three different treatments. B, D, F - Actograms of a single representative individual corresponding to the treatment in the figure to the left of it. The LAB (A and B) and ALAN (C and D) experimental treatments were on a square wave regime and a constant temperature, whereas NAT (E and F) mice were exposed to the natural dawn and dusk (approximately 05:00-07:00 and 17:00-19:00) and naturally fluctuating temperatures. Shaded areas show periods of darkness, and the faded periods between 05h00 and 07h00, and 17h00 and 19h00 indicate dawn and dusk.

Overall, sex did not influence the activity counts ($\chi^2 = 3.394$, df = 1, P = 0.065), but mice showed more activity during the night compared to the day ($\chi^2 = 365.569$, df = 1, P < 0.001, Figure 2).

The interaction between sex and experimental treatment significantly affected the activity ($\chi^2 = 77.872$, df = 2, P < 0.001, Figure 3A). Females showed significantly higher activity in NAT compared to both ALAN and LAB (P < 0.001 for both, Figure 3A). Males were significantly less active during ALAN compared to LAB and NAT (P < 0.001 for both, Figure 3A). Across all three settings, males were more active than the females (P ≤ 0.014, Figure 3A). All other interactions between sex and experimental treatment were not significant (P ≥ 0.249, Figure 3A).

The interaction between sex and time of day significantly affected the level of activity (χ^2 = 52.823, df = 1, P < 0.001, Figure 3B). Both males and females were significantly less active during the light phase compared to the dark phase (P < 0.001, Figure 3B) and females were significantly less active during the dark period compared to males (P < 0.001, Figure 3B). None of the remaining comparisons involving sex and time of day affected the activity (P ≥ 0.5081, Figure 3B).

Activity was significantly influenced by the interaction between the experimental treatment and time of day (χ^2 = 140.3561, df = 2, P < 0.001, Figure 3C). During the dark phase, mice displayed reduced activity under ALAN compared to LAB and NAT and lower activity during LAB than NAT (NAT < LAB < ALAN; P < 0.001 for all interactions, Figure 3C). Under ALAN, the activity counts were lower during the light compared to the dark phase (P = 0.004, Figure 3C), and the same was observed for LAB and NAT (P < 0.001 for both, Figure 3C). No other comparisons between the experimental treatment and time of day were significant (P ≥ 0.160, Figure 3C).

The three-way interaction between sex, experimental treatment and time of day did not affect the activity counts (χ^2 = 1.434, df = 2, P = 0.488).

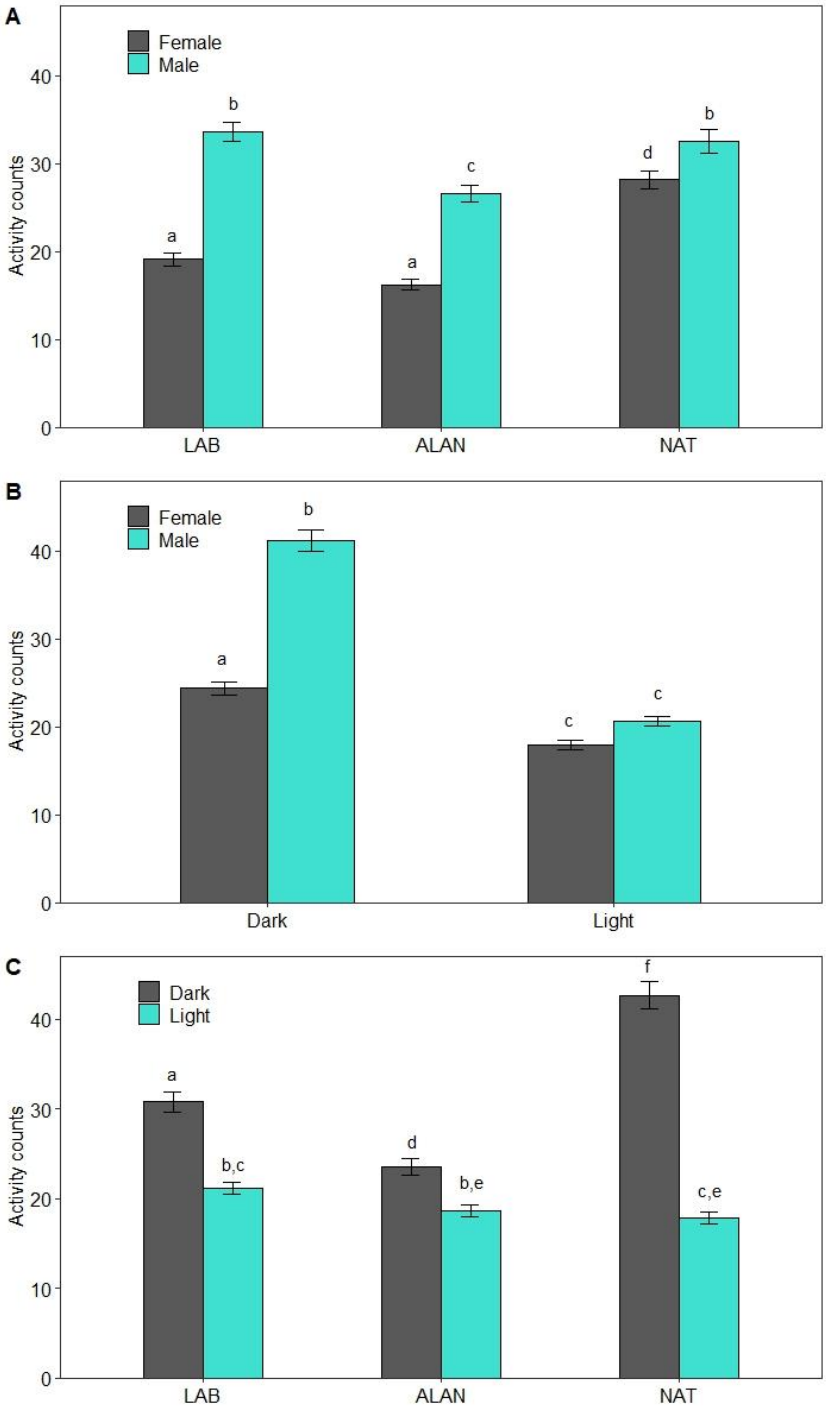


Figure 3: A - The total activity counts (mean ± SE) of male and female *Lemniscomys rosalia* during the three different experimental treatments. Bars with the same letters indicate insignificant differences. B - The total activity counts (mean ± SE) of male and female *Lemniscomys rosalia* during the light phase and dark phase during the three experimental treatments. Bars with the same letters indicate insignificant differences. C - The total activity counts (mean ± SE) of *Lemniscomys rosalia* during the dark and light phases of the three different experimental treatments. Bars with the same letters indicate insignificant differences.

3.4 Discussion

Urbanization and the accompanying anthropogenic changes transform both the spatial and temporal landscapes of wildlife. Large areas of natural habitat are being transformed and, in addition to habitat loss, wildlife faces other disruptions, such as ALAN and anthropogenic noise (Willems et al., 2021). Rodents are an important component of ecosystems. However, the biology of most of southern Africa's rodents are poorly studied (Viljoen and Oosthuizen, 2023). To predict the responses of rodents to anthropogenic changes and the broader implications for the ecosystem, it is important to investigate the effects of urbanization and its repercussions on wildlife. We investigated the locomotor activity of the single-striped mouse (*L. rosalia*) under standard and ALAN laboratory treatments and a natural environmental treatment to assess how they might be affected by anthropogenic pressures.

Single-striped grass mice displayed predominantly crepuscular activity with distinct peaks at dawn and dusk, but also some diurnal and nocturnal activity. The species was previously described as crepuscular (Monadjem et al., 2015), diurnal (Monadjem and Perrin, 1997), or diurnal with some crepuscular activity (Skinner and Chimimba, 2005). Nevertheless, our results showed that they were more active during dark hours compared to light hours during all three experimental treatments, although the level of recorded activity differed between treatments. We suggest that the transition period between light and dark was the significant driver behind their locomotor activity.

We assumed that the activity of the mice under the natural environmental treatment would be similar to the activity of the mice in their natural habitat. During the natural treatment, mice were exposed to both natural and anthropogenic sounds as well as natural light and indirect ALAN. Therefore, our setup is representative of an anthropogenic environment. As predicted, the mice displayed the highest level of activity under natural ambient conditions with the additional disturbances. The higher activity could be as a result of the natural temperature cycle. Ambient temperature cycles support and enhance locomotor activity rhythms, with animals displaying higher and more robust activity rhythms under ambient temperature cycles compared to constant ambient temperatures (van Jaarsveld et al., 2019). The decreased activity during daylight hours could be a result of competition with the diurnal four-striped grass mouse (*Rhabdomys* spp.) that occurs syntopically with *L. rosalia* where they were caught. Two *Acomys* species were found to display nocturnal activity when they occur separately, but when they occur together in the same space, temporal segregation was observed (Gutman et al., 2011).

In the laboratory, the single-striped mice were less active compared to the natural environmental conditions. They displayed a similar temporal activity profile but with a reduced activity peak at dawn. The reduced activity in the laboratory could result from wild-caught animals being housed in the laboratory. Laboratory conditions were darker and quieter and can lead to stress in experimental animals. The overall activity decreased further when ALAN was added under the standard laboratory

treatment. The mice were indirectly exposed to continuous ALAN in the laboratory for the duration of the night, which could have increased their anxious response over a longer period.

ALAN has variable effects on the behaviour and physiology of diurnal rodents, but little is known about its influence on crepuscular animals. Some species extend their active times into the night (reviewed in Gaynor et al., 2018), whereas others perceive the high illumination as high risk in terms of predation (Ditmer et al., 2021). In the diurnal Nile grass rat (*Arvicanthis niloticus*), ALAN has been linked to adverse behaviours such as depressive-like behaviours and reduced cognitive abilities (Fonken et al., 2009). Diurnal spiny mice (*A. russatus*), studied in a natural experimental setup under ALAN, showed increased stress levels that led to decreased reproductive output and survival (Vardi-Naim et al., 2022). Single-striped mice appear to fall in the latter group, showing more adverse effects under ALAN, acting more similar to nocturnal animals in response to ALAN by decreasing nocturnal activity. Caged animals are usually provided with reduced cover compared to animals in their natural environment, and ALAN in the laboratory is presented more continuously. Although mice in the outside enclosure in our study were also exposed to ALAN, the light was much further away, and animals were not exposed to it continuously for the duration of the night. The ALAN treatment in our study represented a more intense scenario of light at night that would likely not be experienced naturally in peri-urban populations of single-striped grass mice.

Several previous studies raised concern regarding the extrapolation of laboratory results to natural environments since laboratory experiments are highly controlled and the ambient conditions are quite different from natural conditions (Ackermann et al., 2020; Kronfeld-Schor et al., 2013). However, our results illustrate that single-striped grass mice behave similarly in the laboratory and under natural conditions, although the magnitude of activity is greater under natural conditions. The effect of ALAN appears to be amplified under controlled conditions, probably because the light source was placed closer to the animals and for a more continuous period without a real escape for the mice. Therefore, we believe that the temporal activity that this species displayed in the laboratory can be extrapolated to field scenarios with relative confidence, although at a lower amplitude, and it obviously would not reflect the natural general behaviours of the animals.

Our study animals were wild-caught from a peri-urban study site and were therefore already exposed to some level of ALAN in their natural habitat. However, these mice are mainly found in areas with tall grasses and good vegetation cover (Skinner and Chimimba, 2005) that provide shelter from both predators and ALAN, especially during the summer growing season. Densely vegetated areas are a critical resource for small mammals seeking refuge from predators and other unfavourable environmental conditions (Teckentrup et al., 2018). However, future climate change predictions forecast dryer and warmer climates in our study area that could drastically change the vegetation composition and density (Idris et al., 2022). The remaining dense vegetation could become highly attractive to a

variety of species seeking refuge, resulting in competition that could ultimately affect individual fitness and the community structure. Single-striped mice are one of two grassland species (the other being *Rhabdomys dilectus*) that show some diurnal activity at our study site. They have similar life history traits and will likely share the same fate in response to habitat changes. Both species, serve as prey for a variety of predators, and should they disappear because of changes in their habitat, there will be knock-on effects for predators that are dependent on them as a food source.

Finally, we investigated the influence sex had on the activity. Males were more active during the laboratory treatment and the females more active during the natural treatment. This could be as a result of males being more explorative of a novel environment and risk prone compared to the more risk-averse females (Jolles et al., 2015). Both males and females showed the lowest activity during ALAN, possibly suggesting an increased predation risk for both males and females over the experimental period.

3.5 Conclusions

Our study provides the first comparisons of activity between traditional laboratory and natural environmental treatments in a wild-caught peri-urban South African rodent. We confirmed that the single-striped grass mouse is primarily crepuscular, with varying levels of diurnal activity and even more varied nocturnal activity. They were adversely affected by ALAN in the laboratory, as indicated by the reduction in activity, responding similarly to nocturnal species under the influence of ALAN. They were most active in the natural environmental treatment when exposed to naturally fluctuating light and temperature cues. We propose that activity under the natural treatment corresponds to the natural activity of the species, and they were not adversely affected by the presence of anthropogenic factors in our experimental setup, perhaps because the disturbances were not experienced directly. In their natural habitat, dense vegetation would buffer the effects of ALAN. Our study contributes to the conservation of biodiversity by assessing the response capacity of a species occupying a basal trophic level and how changes in its behaviour and survival could impact the ecosystem it occupies. Changes in the activity and behaviour in our study and in other studies in response to ALAN appears to be exaggerated in the laboratory but could be used to predict the future pressures and the response of rodents to expanding urbanization and the accompanying ALAN.

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3.7 Declarations of interest

The authors declare no conflict of interest.

3.8 Funding sources:

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Chapter 4: Temporal activity patterns under natural and laboratory conditions in the Angoni vlei rat, *Otomys angoniensis*

Abstract

Urbanisation and its associated ecological disruptions, such as artificial light at night (ALAN), is of growing concern for wildlife. Very little is known about the general behaviour of African rodents and, consequently, also about their responses to environmental alterations. A basic foundation of animal behaviour is required to determine how comparable behaviour under natural conditions is to that of animals in the laboratory, to assess to what extent laboratory experiments can be extrapolated to the field. Furthermore, light at night applied in the laboratory will give an indication of the sensitivity of the species to anthropogenic light at night. In this study, the locomotor activity of 19 Angoni vlei rats (*Otomys angoniensis*) was investigated to assess their activity under a standard laboratory environment, remote light at night (rLAN) and a more natural experimental design. This species acts as prey to multiple predators and its possible flexible temporal preferences could be beneficial to adapting and surviving the changing nightscape. Although this species has been reported to have variable activity patterns, my results suggest that it is more nocturnal in captivity. *Otomys angoniensis* showed higher activity under natural conditions compared with the constant environmental conditions in the laboratory. Males and females showed a similar amount of activity throughout the treatments. This suggests that this species is sensitive to environmental changes. The animals did not show a reduction in activity in response to light at night, as would be expected from strictly nocturnal rodents. This indicates that the active time of vlei rats may be flexible, as can be seen from previous reports of variable activity, and that the temporal niche of these animals may depend on prevailing environmental conditions.

Keywords: artificial light at night, laboratory, locomotor activity, natural environmental conditions, *Otomys angoniensis*, temporal activity

4.1 Introduction

The human population is growing at an estimated rate of 1% per year (Roser et al., 2019) and results in increasing urbanisation and the expansion of infrastructure. This contributes to habitat fragmentation, water and air pollution and artificial light at night (ALAN) (Gaynor et al., 2018). ALAN was estimated to affect approximately 23% of the earth's surface in 2016 (Falchi et al., 2016) and it is most likely more widespread now and will continue to increase over time and further impact the behaviour of animals.

Light is one of the most reliable environmental cues used by animals to synchronise their innate biological rhythms to the external environment (Benstaali et al., 2001; Ikeno et al., 2014). Changes in environmental light are captured by the retina and the animal reacts accordingly (Benstaali et al., 2001; Tapia-Osorio et al., 2013). These light changes could be subtle, such as cyclic changes in moonlight or more dramatic like artificial lights that provides persistent illumination for the duration of the night at a consistently high intensity. For example, several mammalian studies have reported significant changes in behaviour depending on the phase of the lunar cycle (Griffin et al., 2005; Kotler et al., 2010; Mandelik et al., 2003). The illumination of a full moon can reach up to 2 Lux at ground level depending on the surrounding vegetation cover (Weaver, 2011). ALAN could potentially have a larger effect as it can reach intensities of 10 Lux and higher, and it is more persistent (Preto and Gomes, 2019). Moreover, ALAN could change the natural activity patterns of diurnal and nocturnal species because ALAN is brighter than the natural available light at night.

Animals are adapted to their specific temporal niches, primarily linked to their visual acuity. Diurnal species typically have a higher proportion of cones than nocturnal species, affording them acute, vision in colour (Peichl, 2005). Nocturnal species usually have very few cones since they are obsolete in the low light conditions that prevail at night, whereas the rods are sensitive enough to be used at night (Peichl, 2005). More than 40% of extant mammals are nocturnal, which increase the need for more studies on mammal responses to ALAN (Jones et al., 2009; Prugh and Golden, 2014).

Mammals that are exposed to ALAN can show a range of behavioural alterations, including suppression of activity, changes in the time of activity, the areas they utilise and conspecific and predator interactions (Hoffmann et al., 2019). Strictly nocturnal species, such as common spiny mice (*Acomys cahirinus*) (Rotics et al., 2011a,b) and African pygmy mice (*Mus minutoides*) (Viljoen and Oosthuizen, 2023), decreased their overall activity when subjected to direct light at night (dLAN). Moreover, when exposed to ALAN, nocturnal Swiss-Webster mice altered the time of food intake from night-time to the daytime, which lead to weight gain and possibly decreased survival (Fonken et al., 2010). Diurnal species can use ALAN as an extension of daylight hours, resulting in extended activity into the night. This additional active time can be utilised for foraging or it can cause an overlap in temporal niches between inter- or intraspecific competitors (Fonken et al., 2010). Asynchronous behaviour between conspecifics has been observed in the predominantly diurnal striped field mice (*Apodemus agrarius*) and polyphasic bank voles (*Myodes glareolus*) when they were exposed to ALAN, but there were no changes between heterospecifics (Hoffmann et al., 2019). This can cause missed mating opportunities, missed opportunities to establish and defend territories and thus impacting fitness, since prey species can no longer derive the benefits of safety in numbers (Hoffmann et al., 2019). Increased illumination at night results in an increase in predation risk for prey species, and ALAN can result in mammals altering their movement patterns to avoid well-lit areas (Rotics, et al., 2011a). For example, nocturnal pinyon mice (*Peromyscus truei*) were trapped less often under ALAN (Willems et al., 2021).

The impacts of ALAN on animal behaviour have been the focus of an increasing number of studies over the last few years. These studies have been biased toward species in the Northern hemisphere and were mainly restricted to a laboratory setting (Spoelstra et al., 2015). Limited studies have been conducted on wild-caught rodent species, and laboratory studies are often focussed on a single variable (Ackermann et al., 2020; Kronfeld-Schor et al., 2013; Viljoen and Oosthuizen, 2023), such as the influence of a running wheel on rodent activity. However, the behaviour observed under laboratory conditions is not always comparable to that of animals in a natural environment (Ackermann et al., 2020; Kronfeld-Schor et al., 2013). We require a more representative experimental setting to test the natural behaviour of animals, which can then be used to more accurately predict animal responses to the variable in question (Viljoen and Oosthuizen, 2023).

In this study, I aimed to investigate the activity of the Angoni vlei rat (*Otomys angoniensis*) under natural conditions, controlled laboratory conditions and the influence of light at night. The literature has been inconsistent with regards to its temporal niche, since it has been reported as diurnal, crepuscular, and nocturnal in different studies (Kingdon, 2013; Skinner and Chimimba, 2005), thus I first had to ascertain its active period in captivity. It is strictly herbivorous, living in dense vegetation in mesic regions of southern Africa and is often found in close proximity to water sources (Kingdon, 2013; Skinner and Chimimba, 2005). This species is a medium-sized rodent and is preyed upon by multiple predators, such as various owl species, felids and jackal (Kingdon, 2013; Skinner and Chimimba, 2005). Its absence will thus have cascading effects on the ecosystem. It facilitates movement patterns of other small mammal species since vlei rats construct rodent highways in the dense vegetation (Kingdon, 2013; Skinner and Chimimba, 2005).

I investigated how light at night affected the temporal activity patterns of *O. angoniensis* by subjecting it to different environmental and lighting treatments, including a natural light and temperature regime (NAT), a standard laboratory treatment (LAB), and a laboratory treatment with a 2 Lux remote light during the dark phase of the light cycle (rLAN). I expected 1) that this species will display diurnal activity, regardless of treatments, because I trapped them at different times of the day. Moreover, 2) I anticipated the species to show the highest activity levels under the NAT treatment because although experiments were in captivity, individuals were exposed to natural fluctuations in light and temperature. I expected higher activity under a dark night than when dim light at night was present. I tested both sexes and anticipated 3) that males would be more active than females since this species is polygynous (Skinner and Chimimba, 2005) with males needing to travel more to find potential mates.

4.2 Materials and Methods

4.2.1 Animal capture and maintenance

During the summer of 2023, I trapped 19 vlei rats (10 females and 9 males) at a peri-urban study site, the Cradle Nature Reserve, Gauteng, South Africa (-25.9214, 27.8503). I trapped the rodents in an area that faced Johannesburg but was separate from the Light and Dark trapping sites to avoid influencing the small mammal populations on site. I baited 45 PVC live traps with a combination of sunflower seeds, oil, oats, granola, salt and peanut butter. As this species is known to make rodent highways (Skinner and Chimimba, 2005), I strategically placed the traps within these high-use areas. After capture, the rats were weighed (Pescola® hanging scale, Switzerland, 1g precision), and sexed (using anogenital distance, males have a larger distance than females) and females were checked for signs of pregnancy (swollen abdomen). If a female was pregnant, she was released into the area where she was caught.

Subsequently, the vlei rats were introduced to individual plastic, transparent rodent cages (60 x 40 x 36cm), lined with 2cm of sand (Kiddies play sand, EDCO Trading cc, RSA). The cages were stationed at a field laboratory on the Cradle Nature Reserve property (Appendix Figure 3). The lids of the cages were modified by removing the middle section and replacing it with expanded mesh. This allowed an infrared detector to detect animal movement (BMT Digital PIR Motion Sensor, Communica, RSA) while preventing the escape of the animals. Cardboard dividers were added between all cages to prevent the IR sensors from capturing the activity of neighbouring animals. Each rat was provided with an empty plastic 1L yogurt container, tissue paper and hay to be used as nesting material. Enrichment materials included a rock and stick for each cage. The vlei rats had *ad libitum* access to water and were fed a combination of dry food (sunflower seeds, yellow maize and Burgess Excel Guinea Pig Nuggets, Burgess Group Inc., United Kingdom) and fresh food (carrot, apple, and sweet potato). I also provided them with fresh grass and hay (Tiny Friends Farm Russel Rabbit Tasty Hay, Marltons Pets and Products, United Kingdom) on alternate days since this species is herbivorous (Skinner and Chimimba, 2005). Animals were acclimated to the laboratory after capture for a minimum of five days before the experiments commenced. The experiments were approved by the University of Witwatersrand Animal Research Ethics Committee (2021/08/09/B) and the animals were trapped under a trapping permit from the Gauteng Department of Agriculture and Rural Development (CPF6-0231).

4.2.2 Experimental design

I assessed the activity of *O. angoniensis* over three consecutive experimental treatments (this was not counterbalanced, since the experiment required all animals to experience the same natural conditions to prevent additional confounding factors). The first was under standard laboratory conditions (LAB,

Appendix Figure 4), the second under laboratory conditions in the presence of remote light at night (rLAN – a light positioned on the opposite side of the room, providing indirect light), and in treatment three, the rats were exposed to natural environmental conditions (NAT). Each treatment was preceded by three acclimation days, followed by 10 experimental days. The LAB treatment consisted of a room without external cues and the temperature was kept constant at 24°C ± 1°C, with the lighting set to 12h light and 12h dark (overhead lights used during the light phase measured at ± 40 Lux). After the 13 days of the LAB treatment, I kept the rats in the same room, and added a light (Light: LightWorx, 9W 3000K LED, LightWorx LED & Electric Supply LLC, New Jersey; Dimmer: 500W rotary dimmer, Shuttle, Cape Town) dimmed to 2 Lux. This light remained switched on for 24h each day for the duration of the treatment. After the rLAN treatment, I moved the rats to an ambient laboratory, which consisted of an outdoor enclosure that was fenced off and had a roof. The fence prevented other animals from entering the space, whilst allowing test rats to experience natural temperature and light fluctuations (Appendix Figure 5). The average photoperiod for the duration of the NAT treatment was 11:39:42. The activity of the animals was detected by motion detectors and captured as activity counts per minute per animal using VitalView software (VitalViewTM, Minimitter Co., Sunriver, USA). To ensure the animals' wellbeing (i.e., to check for any drastic change in weight possibly caused by extreme stress), I weighed them between treatments while their cages were cleaned.

The identity of *Otomys angoniensis* was confirmed genetically and the GenBank sequence reference number was AM408343.1.

4.2.3 Data analyses

To visually inspect my activity data, I produced actograms for each individual using ActiView software (ActiView™, Minimitter Co., Sunriver, United States). Data produced by VitalView consisted of the number of times animal movement was detected over a minute per animal. Then I added the counts per minute to produce counts per hour per animal. This resulted in 240 data points per animal per treatment. To analyse these data, I used R (v4.2.1, Boston, United States) and tested for the normality of the counts (all 240 data points per animal per treatment) using the Anderson-Darling normality test and found that the distribution was not normal ($P < 0.05$). I analysed the data using the *lmer* function through the "lme4" package to run a generalized linear mixed model (GLMM, Bates et al., 2015). I used the hourly counts as the response variable in the model with a Gamma distribution and log link function. The predictor variables included the experimental treatments (i.e., LAB, rLAN, or NAT), the time of day (i.e., light or dark hours of the day), the sex (male or female), and all interactions. The random variable included in the model was the identity of all animals in the experiment. Post-hoc analysis was completed for all significant variables, using the Tukey HSD method.

4.3 Results

The activity counts of *O. angoniensis* were significantly influenced by the experimental treatment (χ^2 = 3431.72, df = 2, P < 0.001, Figure 1). During the NAT treatment, the activity levels were significantly higher than the two laboratory treatments (P < 0.001 for both, Figure 1). The activity levels did not differ between the LAB and rLAN treatments (P = 0.192, Figure 1). Animals displayed more nocturnal activity than diurnal activity (χ^2 = 3411.63, df = 1, P < 0.001) and sex did not affect the activity levels (χ^2 = 0.13, df = 1, P = 0.715).

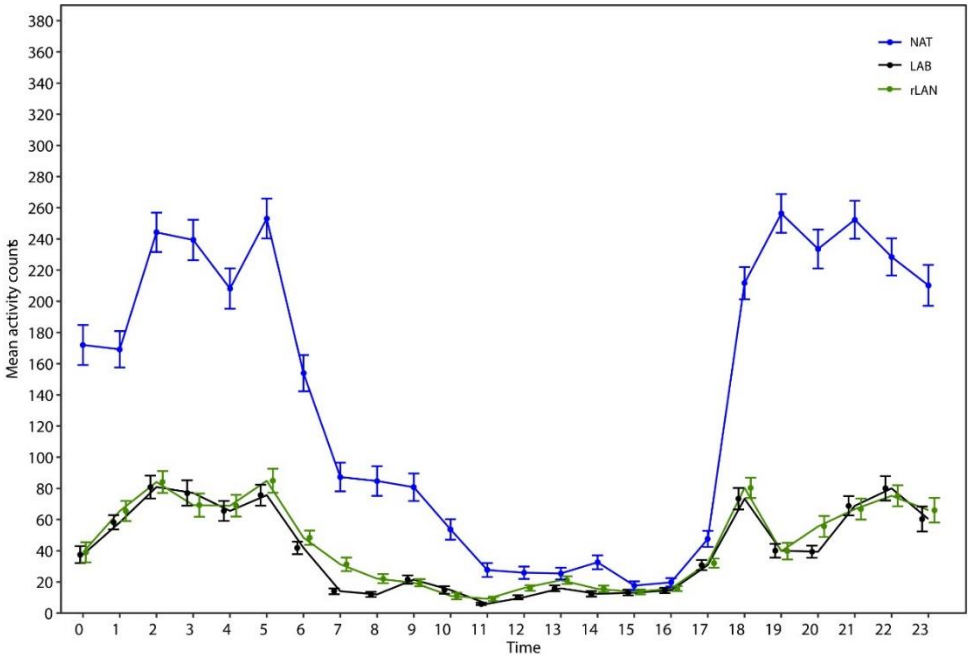


Figure 1. The hourly activity counts (mean ± SE) of *O. angoniensis* during each experimental treatment illustrated over the 24h day.

The activity counts were influenced by the interaction between the experimental treatment and the time of day (χ^2 = 87.82, df = 2, P < 0.001, Figure 2). The rats showed more nocturnal activity during all three experimental treatments (P < 0.001 for all, Figure 2). During both light and dark hours, the activity was higher under the NAT treatment than the LAB and rLAN treatments (P < 0.001 for all, Figure 2). There was no difference between the LAB and rLAN treatments and none of the other comparisons for this variable were significant (P ≥ 0.054).

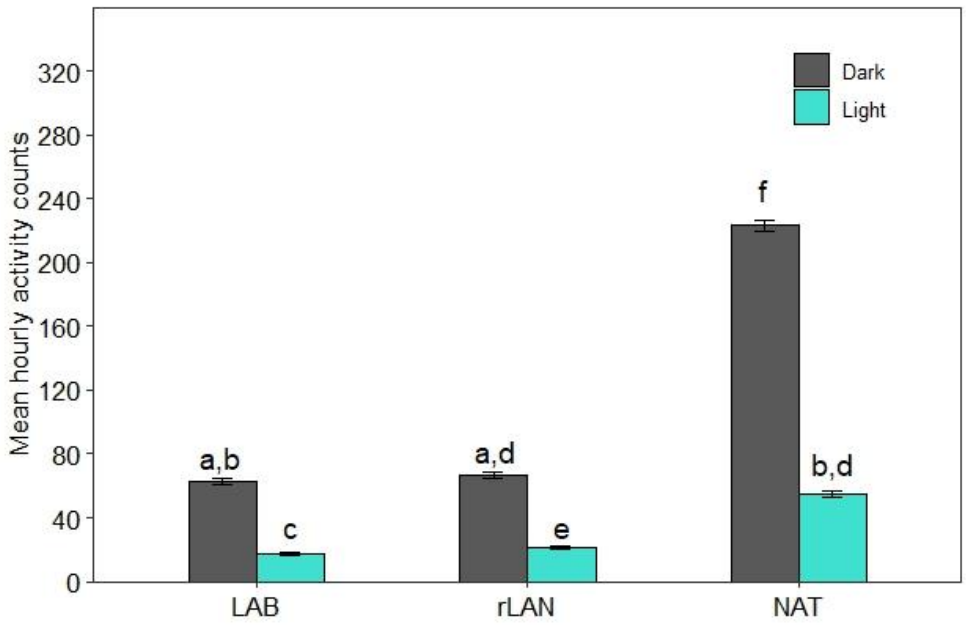


Figure 2. The mean hourly activity counts (mean ± SE) recorded for O. angoniensis individuals during the dark and light hours across experimental treatments. The same letters on the plots indicate non-significant differences between treatments.

The interaction between experimental treatments and sex significantly impacted activity counts ($\chi^2 = 15.60$, df = 2, $P < 0.001$, Figure 3). Both males and females showed higher activity during the NAT treatment compared with both the LAB and rLAN treatments ($P < 0.001$ for all, Figure 3). None of the remaining comparisons between sex and treatment were significant ($P \geq 0.634$).

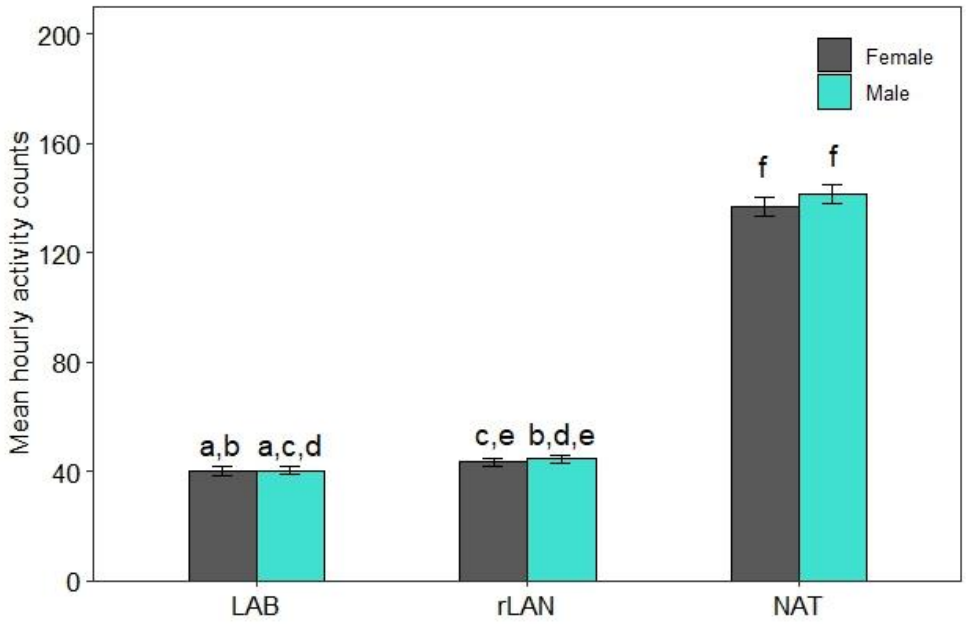


Figure 3. The mean hourly activity counts (mean ± SE) of O. angoniensis individuals across the day per experimental treatment with females and males illustrated separately. The same letters on the plots indicate non-significant differences between treatments.

The interaction between time of day and sex significantly affected activity ($\chi^2 = 111.50$, df = 1, $P < 0.001$). Both males and females were more active during the dark hours than the light hours ($P < 0.001$ for both). During the light hours, males were more active than females ($P < 0.001$). No significant differences were seen between males and females during the dark hours ($P \geq 0.086$).

The three-way interaction between sex, experimental treatment and time of day influenced the activity counts significantly ($\chi2 = 89.97$, df = 2, $P < 0.001$, Figure 4). Activity counts of females during the dark hours in the NAT treatment were higher than both the LAB and rLAN treatments and the same pattern was seen for males in the dark hours ($P < 0.001$ for all). During the light hours, both males and females showed higher activity counts in the NAT treatment compared with both the LAB and rLAN treatments ($P < 0.001$ for all). Both males and females were more active during the dark hours across all treatments ($P < 0.001$ for all) compared with the light hours. None of the other comparisons for the three-way interaction were significant ($P \geq 0.105$).

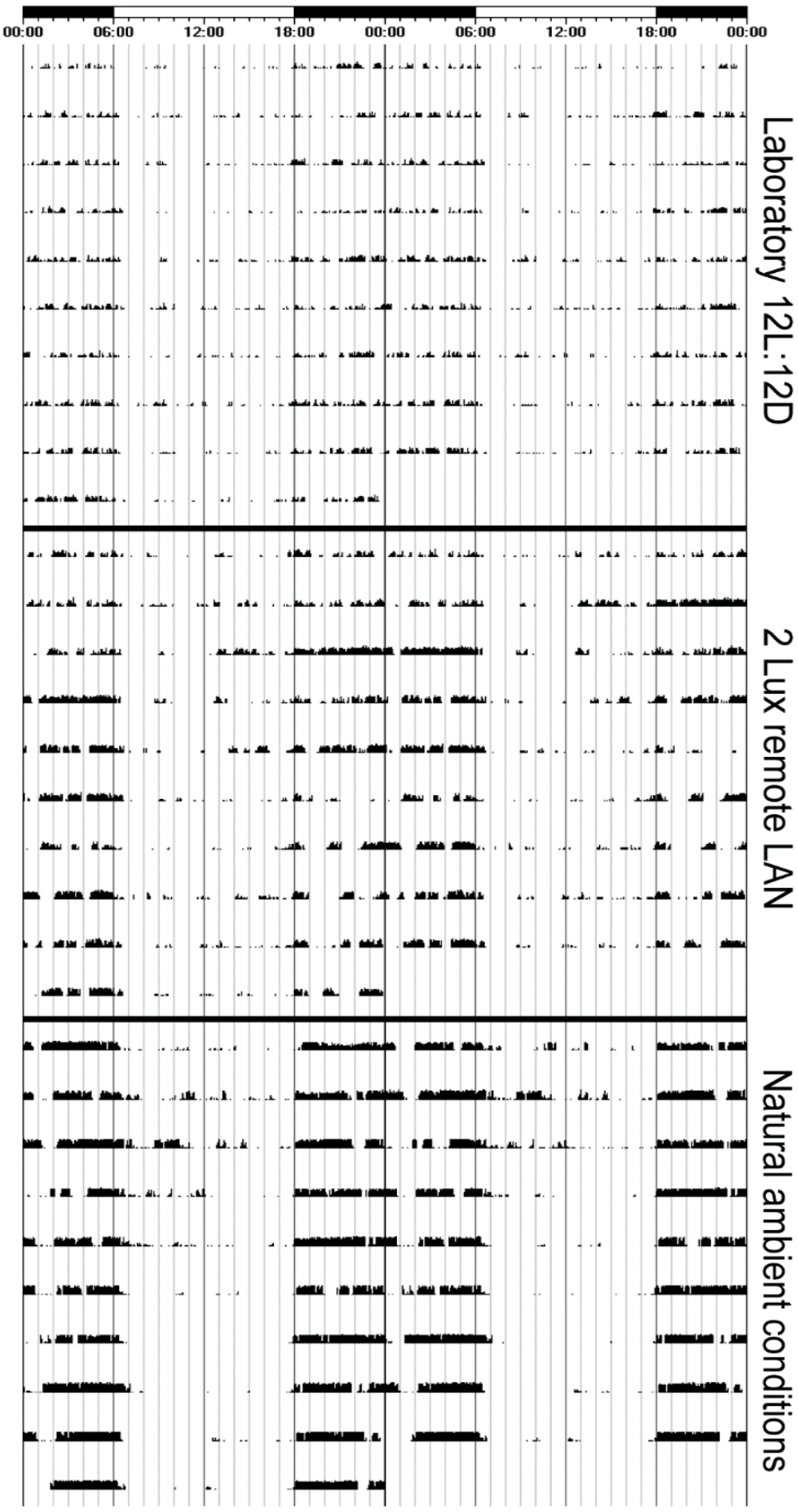

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Laboratory 12L:12D
2 Lux remote LAN
Natural ambient conditions

Figure 4. A representative double-plotted actogram of an *O. angoniensis* female to illustrate activity during the different experimental treatments. LAB – Laboratory 12L:12D, rLAN – 2 Lux remote LAN and NAT – Natural ambient conditions. The top bar indicates the light and dark hours of each day, with two days represented next to each other on each line. The black spikes illustrate activity counts per minute.

4.4 Discussion

Here, I investigated how *O. angoniensis* responded to natural light and different lighting conditions in the laboratory. I captured *O. angoniensis* in the traps throughout the day, but with the highest frequencies in the mornings (75% at 06:00 with first check and 90% before midday). However, in contrast to my expectation, *O. angoniensis* showed low diurnal activity and higher nocturnal activity levels throughout the treatments. The literature reports different active times for *O. angoniensis* including diurnal, crepuscular and nocturnal activity (Kingdon, 2013; Skinner and Chimimba, 2005), but no studies indicate how this conclusion was reached. Similar to *O. angoniensis, O. irroratus* has also been reported with variable activity in the literature (Skinner and Chimimba, 2005). However, these reports are substantiated with past studies, including the conclusion that *O. irroratus* is nocturnal based on the presence of its remains in owl pellets and crepuscular based on trapping studies and laboratory experiments (Davis, 1972 and references herein). My findings on the activity profile of *O. angoniensis* seem to suggest a more flexible temporal niche compared with other rodents and appear to have the ability to change their temporal activity according to prevailing conditions. Alternatively, other contributing factors, such as predation pressure could have an influence. Norway rats (*Rattus norvegicus*) are nocturnal under laboratory conditions, but in the presence of nocturnal red foxes (*Vulpes vulpes*), wild Norway rats were diurnal and predation pressure was suggested as the cause of this difference (Fenn and Macdonald, 1995). A similar conclusion was reached when a wild population of nocturnal laboratory golden hamsters (*Mesocricetus auratus*) were observed to be mainly diurnal (Gattermann et al., 2008).

I predicted *O. angoniensis* would be the most active under the NAT treatment and it showed the highest activity when exposed to natural environmental conditions. The co-occurring, crepuscular rodent *Lemniscomys rosalia,* also had higher activity when exposed to natural environmental conditions compared with standard laboratory conditions (refer to Chapter 3). Under the NAT treatment, *O. angoniensis* was exposed to natural ambient environmental light and temperature fluctuations, as well as natural sounds, all of which were absent from the LAB and rLAN treatments. Both light and temperature fluctuations influence the intensity of activity in other southern African rodents, such as four-striped mice (*Rhabdomys dilectus*) and Namaqua rock mice (*Micaelamys namaquensis*) (Ackermann et al., 2020). Ackermann et al. (2020) simulated natural temperature and light cycles in a

laboratory setting and illustrated the importance of these factors in the accurate representation of circadian rhythms in these species.

I further predicted that *O. angoniensis* would be the least active under rLAN compared with LAB and NAT, but there was no difference in activity between LAB and rLAN. These experimental treatments were representative of standard laboratory conditions under which rodents are routinely studied, and the constant overhead lights simulated an anthropogenic environment. However, the response of *O. angoniensis* was dissimilar to what one would expect from a nocturnal species, since it did not drastically lower its activity between a dark night and the introduction of rLAN during the night. The syntopic *M. minutoides* showed a significant reduction in their activity when it was exposed to LAN in a laboratory setting (Viljoen and Oosthuizen, 2023). Similarly, nocturnal Siberian hamsters (*Phodopus sungorus*) showed a considerable reduction in total locomotor activity and night time activity when exposed to LAN in the laboratory (Bedrosian et al., 2013). Nocturnal wood mice (*A. sylvaticus*) were observed less frequently on camera traps when LAN was introduced to a forest edge habitat (Spoelstra et al., 2015). European hedgehogs (*Erinaceus europaeus*) showed a similar response to *O. angoniensis* in that ALAN had no significant influence on their activity (Finch et al., 2020). However, Finch et al. (2020) did not mention possible reasons for the lack of influence seen in their results, aside from individual differences. The response of *O. angoniensis* could be that it is adaptable to changing surroundings which could indicate that this species has behavioural flexibility or morphological adaptations to be active in day- or night-time. These adaptations could include the proportion of rods and cones present in their eyes, but this still has to be investigated. An alternative explanation could be that this species was less active in the laboratory setting because of the lack of cover and being extremely exposed, in contrast to the dense vegetation they normally occupy. Furthermore, the overhead lights in the laboratory were also dimmer than the sunlight *O. angoniensis* was exposed to in the natural environmental conditions.

Overall, males and females displayed a similar amount of activity. I expected males to be more active since polygynous rodent males are often the more explorative sex in search of mates, whereas females remain in familiar areas where food resources are known (Jolles et al., 2015).

4.5 Conclusions

Urbanisation and ALAN are predicted to substantially change the natural world and how animals perceive it. *Otomys angoniensis* has mainly been observed in the field and past literature concluded the temporal niche of this species on the time of observation or trapping. My study is the first to empirically test the active period of this species and it was shown to be more nocturnal. My conclusions emanate from testing the responses of the rats to changing environments, it is beneficial to design more realistic

experiments and to not test rodents solely under the constant conditions of a laboratory setting (Viljoen and Oosthuizen, 2023). By using a more representative design, we can gain more information on behaviour. *Otomys angoniensis* significantly lowered its activity under both a laboratory setting and the presence of rLAN. This suggests that it is sensitive towards environmental changes. The lack of differences between a dark night and LAN in the laboratory, implies that light is not the primary driver of the behavioural changes from the natural to laboratory environment. However, this implies that *O. angoniensis,* even with flexibility in temporal preference, could experience difficulty to adapt to a more urbanised world and a combination of ALAN and habitat loss can possibly lead to local population declines.

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Chapter 5: A pioneer rodent species faces negative consequences under expanding urbanisation: insight into the activity profiles of the nocturnal multimammate mice (*Mastomys coucha*)

Abstract

Studies investigating urbanisation and the associated artificial light at night (ALAN) have increased over the last decade, examining an array of influences on animal behaviour. Many of the studies on the influence of ALAN on rodent behaviour have been confined to a laboratory setting, but this is not necessarily representative of natural populations. In this study, I evaluated the activity of a wild-caught nocturnal rodent species, the southern multimammate mouse (*Mastomys coucha*), collected from a peri-urban site, under four different treatments. This included a treatment in an outdoor enclosure (natural temperature and light fluctuations) and three treatments in a laboratory setting. In the laboratory, animals were tested under a standard 12L:12D light regime, and two 2 Lux ALAN regimes, remote LAN (light source on the opposite side of the experimental room) and direct LAN (light suspended directly above the cages). This species was more active under laboratory conditions than the outdoor treatment. Animals reduced their activity under ALAN, and this reduction was intensity dependent. Activity levels decreased by approximately 50% under remote LAN and 75% under direct LAN. The onset of activity was later during the two LAN treatments and resulted in more activity during the light phase of the day. Overall, males were more active than females, except during direct LAN, when the sexes showed similar levels of activity. My results suggest that this species would be negatively impacted by an increase in ALAN. Under such conditions, it could escape the direct influences of light by using burrows more frequently and for longer durations in their exposed habitats. This could possibly have negative consequences, since longer times in burrows will lead to a reduction in foraging time and have an influence on their overall survival.

Keywords: activity profile, artificial light at night, circadian rhythms, *Mastomys,* nocturnal rodent, urbanisation

5.1 Introduction

Nocturnal species are typically exposed to low levels of environmental light at night. Natural levels of light at night can be as high as 2 Lux during a full moon and as low as 0.001 Lux during a new moon (Alaasam et al., 2021; Emmer et al., 2018). Night-dwelling mammals show several adaptations to their

temporal niche, which can include enlarged eye sizes to increase the amount of light entering the eye or a larger proportion of rods to assist with low light levels at night (Peichl, 2005). Nocturnal prey animals have also evolved behavioural adaptations to reduce predation risk during full moon nights, when the visibility is better for predators (Hoffmann et al., 2022). This includes reducing activity (Griffin et al., 2005), and concentrating their activity to the darkest parts of the night (Daly et al., 1992; Pratas-Santiago et al., 2017). Prey species also occupy thick vegetation that provides cover (Mandelik et al., 2003) and they perform complex movement patterns that make their capture challenging (Prugh and Golden, 2014).

There are several examples of rodents changing their behaviour on full moon nights. Wood mice (*Apodemus sylvaticus*) showed decreased food consumption when they detected predator cues and their anti-predator responses increased when the predator cues were identified in combination with high visibility during full moon nights (Navarro-Castilla and Barja, 2014). Crested porcupines (*Hystrix cristata*) avoided moonlight and reduced their activity on full moon nights, regardless of the visibility of the moon and the type of habitat, i.e., exposed or covered (Mori et al., 2014). Nocturnal common spiny mice (*Acomys cahirinus*) reduced their foraging under a full moon and in the few days following a full moon night, although no changes were observed in the days before the full moon (Gutman et al., 2011). Behavioural adaptations in response to full moon illumination levels have associated costs, such as decreased food consumption and increased competition for refugia (Finch et al., 2020; Navarro-Castilla and Barja, 2014; Perea et al., 2011). Thus, there is a trade-off between remaining safe and consuming enough food to meet energy requirements (Kotler et al., 2010; Viljoen and Oosthuizen, 2023).

Artificial light at night (ALAN) is the introduction of unnatural light sources to brighten areas not previously illuminated at night (Hölker et al., 2010). ALAN is a consequence of human technological development that has increased illumination at night, which has a negative impact on wildlife populations. ALAN presents more constant and intense levels of illuminance than full moon nights and can have similar if not larger cost implications for wildlife (Raap et al., 2015). For example, both Santa Rosa beach mice (*Peromyscus polionotus leucocephalus*) and bank voles (*Myodes glareolus*) visited a lower number of foraging patches under ALAN in the field, presumably to avoid predator detection (Bird et al., 2004; Hoffmann et al., 2022). This effect is also apparent in the laboratory. African pygmy mice (*Mus minutoides*) showed an 80% reduction in their general activity and a delay in the onset of their activity when they were exposed to 0.5 Lux light at night (Viljoen and Oosthuizen, 2023).

The effects of environmental light on activity are mediated via a subset of photoreceptive retinal ganglion cells (RGCs) in the eye (Bonmati-Carrion et al., 2017). These cells convey information about the time of day to the circadian pacemaker, located in the suprachiasmatic nucleus (SCN) in the anterior hypothalamus (Bonmati-Carrion et al., 2017). Altered signals, produced by ALAN for example, can

result in indirect changes in activity rhythms in mammals or direct changes through masking (positive masking is when activity increases with the addition of light and negative masking results in decreased activity under additional light) (Mrosovsky, 1999). Nocturnal mammals can shift their activity towards the day time as a result of obscured photic cues or attempt to increase foraging opportunities lost during the night (Hoffmann et al., 2022; Ikeno et al., 2014). This alteration in temporal niche can increase the competition between species in different temporal niches (Hoffmann et al., 2022). Diurnal and crepuscular species can also become more active at night because of the increased visibility to exploit additional resources (Longcore and Rich, 2004).

My study focussed on a nocturnal opportunistic granivore, the southern multimammate mouse (*Mastomys coucha*), which is widely distributed throughout southern Africa (Skinner and Chimimba, 2005). It is a pioneer species that can dominate recently disturbed areas and is prone to population explosions under favourable conditions (Skinner and Chimimba, 2005). This species is often found within human-dominated landscapes and is an important vector for diseases and a frequent pest of crops (Kennis et al., 2008). I investigated how the activity of *M. coucha* differed when it was housed in an outdoor enclosure with fluctuating light and temperature cues or a laboratory treatment with constant temperature and 12L:12D. I further assessed the response of *M. coucha* under 2 Lux light at night, both when the light was presented directly above the cages (dLAN) and from an indirect or remote light source (rLAN), presented from approximately 2m away. I used the two different ALAN treatments to investigate how the distance to light with intervening microhabitat barriers could influence the responses of the nocturnal *M. coucha*. I predicted that 1) the activity of *M. coucha* would be higher under the natural environmental conditions since it is more similar to conditions in the natural habitat of the species with light and temperature fluctuations. I expected decreased activity under laboratory conditions, since it had constant temperature, and the laboratory lights were dimmer than the sunlight experienced under natural conditions. Nocturnal rodents decrease their activity when exposed to light at night and use microhabitats that shield them from direct light, such as areas with dense cover. However, when the light source is situated in close proximity to rodents, it penetrates the microhabitat and disrupts the rodents' activity (Dickerson et al., 2023; Mandelik et al., 2003). I therefore expected 2) *M. coucha* will decrease its activity under the 2 Lux ALAN treatments. Direct ALAN (light originating from above the cage) was expected to elicit a more acute response compared with remote ALAN (light source further away), since direct ALAN could prevent the use of cage contents from acting as temporary microrefugia. In nature, *M. coucha* uses burrows, where it is presumably more concealed from the light and there are more refuge areas compared with their cage in this experiment. The outside enclosure and laboratory treatments provided cues for the animals to determine time of day, and thus I hypothesized 3) that *M. coucha* would remain strictly nocturnal under these conditions. The addition of dim light in the two LAN treatments, which was on for 24h a day, would make the change from day and night less apparent. The change between day and night would be indicated by a change in light

intensity instead of the complete presence or absence of light. The laboratory overhead lights were still on a 12L:12D schedule and at a higher intensity compared with the dim lights used for the LAN treatments. Therefore, I predicted 4) that *M. coucha* would shift its activity towards the day and delay the onset and offset of activity. Finally, I assessed whether activity would differ between the sexes. I expected 5) that this promiscuous species (Kennis et al., 2008) would not show any differences in activity between the sexes, since both sexes would experience similar risks when in search for potential mates.

5.2 Materials and methods

5.2.1 Study animals

I trapped 19 southern multimammate mice in the Cradle Nature Reserve, Gauteng, South Africa (-25.9214, 27.8503) during the summer of 2023. Species identity was confirmed through DNA analysis against GenBank sequence reference number: KY754025.1.

The study site has a dry winter and wet summer and falls within the Magaliesberg Biosphere Reserve South Africa, which is primarily part of the Savanna biome (Mucina and Rutherford, 2006). The mice were captured using PVC live small mammal traps baited with a mixture of oats, peanut butter, salt, sunflower oil, and sunflower seeds. Since *M. coucha* is nocturnal, traps remained open during the night and were checked before sunrise each morning. I obtained permission to trap small mammals from the Gauteng Department of Agriculture and Rural Development (permit CPF6-0231).

Trapped mice were taken to a field laboratory approximately 500m from the trapping site (Appendix Figure 3). In the laboratory, they were weighed (Pescola® hanging scale, Switzerland, to the nearest gram). I sexed the mice using the anogenital distance (larger distance present in males) and housed them individually in transparent cages (60 x 40 x 36cm) for the duration of the experiment. Each cage contained approximately 2cm of sand (Kiddies play sand, EDCO Trading cc, RSA), a shelter, and a rock, stick and empty toilet roll for enrichment. Mice had *ad libitum* access to water and were fed a combination of seeds and fresh food (millet and sunflower seeds with either a piece of apple, carrot, or sweet potato) once per day to avoid excessive disturbance. Each cage was equipped with an infrared motion sensor (BMT Digital PIR Motion Sensor, Communica, RSA) placed approximately three quarters to the back of the lid to capture any movement of a mouse using VitalView software (VitalViewTM, Minimitter Co., Sunriver, USA). All cages were separated with cardboard dividers to prevent visual signals between neighbouring mice and IR motion sensors from capturing activity from neighbouring animals. To further avoid disruptions and added stress, mice were weighed, and cages were cleaned after each experimental treatment (every 14 days).

5.2.2 Experimental design

Mice were subjected to four treatments in sequence. I first exposed the mice to the open enclosure treatment (NAT, Figure 1), where they experienced natural temperature and light fluctuations (including a dawn and dusk period, average photo period was 10:46:12). This enclosure had a roof to prevent direct sunlight or rain from entering the cages, and it was fenced off to prevent direct contact with predators (Appendix Figure 5). The mice were acclimated to these conditions for three days, followed by 10 experimental days and this protocol was repeated for each of the remaining treatments. In the second treatment (LAB, Figure 1), the mice were transferred to an enclosed standard laboratory room (Appendix Figure 4) with controlled environmental conditions at 24℃ ± 1℃ and a 12h light 12h dark light cycle. The overhead LAB lights measured at approximately 40 Lux and automatically switched on at 06:00 and switched off at 18:00. During the third treatment, rLAN (remote light at night, Figure 1), the original laboratory conditions were retained, and in addition, a lightbulb (LightWorx, 9W 3000K LED, LightWorx LED & Electric Supply LLC, USA; Dimmer: 500W rotary dimmer, Shuttle, RSA) was placed on the opposite side of the laboratory room, dimmed to 2 Lux at cage level. This illuminated the experimental room during the night. The night light penetrated the transparent cages, which resulted in a light intensity of 1.5 Lux inside the cage, measured with a hand-held Lux meter (Major Tech, RSA, to the nearest 0.01 Lux). The final experimental treatment, dLAN (direct light at night, Figure 1) also retained the original laboratory conditions and consisted of warm white LED strip lights (12 V/DC 3528) fixed above the cages that were dimmed to 2 Lux at the bottom of the cages. Following the results obtained from the rLAN component of the experiment, and since I expected that dLAN could have an increased response compared with rLAN, I reduced the number of experimental days to seven. Only 14 mice formed part of dLAN, since I had a limited number of LED strip lights that I could place over the cages. For the rLAN and dLAN treatments, the additional lights remained on during both the night and day. The temperature remained constant. All procedures were approved by the University of Witwatersrand Animal Ethics Committee (2021/08/09/B).

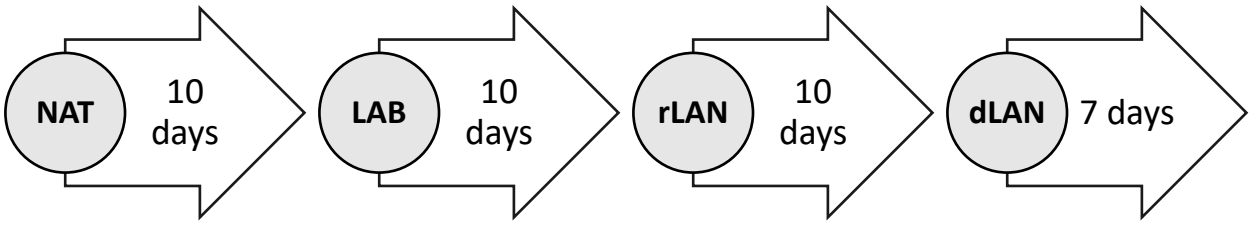


Figure 1. An experimental timeline representing each treatment: NAT – natural environmental treatment, LAB – laboratory conditions, rLAN – remote light at night and dLAN – direct light at night.

5.2.3 Data analyses

Activity data, recorded using VitalView software, was visually assessed using ActiView (ActiView™, Minimitter Co., Sunriver, United States). I recorded data as counts per minute per mouse, and I summed the minute data to counts per hour per mouse for data analysis. The data set was tested for normality using the Anderson-Darling normality test and was not normally distributed ($P < 0.05$). I thus analysed the data using a generalized linear mixed model (GLMM) with a Gamma distribution and log link function in R software (R v4.2.1, Boston, United States). Activity counts per hour were used as the response variable and the mouse identity was the random variable. The fixed variables included experimental treatment (NAT, LAB, rLAN and dLAN), time of day (light or dark), sex and all interaction terms. For the analysis, I did not include the dawn and dusk period of NAT but rather assigned all treatments 12h light and 12h dark to be able to compare activity between treatments. I ran post-hoc comparisons using the Tukey HSD method for all significant variables. I determined the onset of activity of each mouse by recording the mean time the mouse became active during each treatment. A similar approach was followed to determine the offset of activity, i.e., the mean time the mouse stopped activity during each treatment. To analyse whether the onset and offset of activity differed between experimental treatments, I conducted a Kruskal-Wallis test, since the onset and offset were not normally distributed ($P < 0.05$). I then compared the treatments using the Dunn test with the Bonferroni method. I computed the duration of the biological rhythm (alpha; time activity stopped – activity start time) in ClockLab (ClockLab™, Actimetrics, USA) and ran a Kruskal Wallis test with the Dunn test and Bonferroni method to determine whether the duration of activity differed between treatments. I also assessed the period the mice were active (tau) for the LAB, rLAN and dLAN treatments using ClockLab and analysed it in the same way than the onset and offset data, since the active period of the mice was also not normally distributed ($P < 0.05$).

5.3 Results

The activity of the mice during the four treatments differed significantly ($\chi^2 = 470.13$, $df = 3$, $P < 0.001$, Figure 2). Activity levels were significantly higher in the LAB treatment than all other treatments ($P < 0.001$ for all, Figure 2). The activity during the NAT treatment was 11.1% lower than the LAB treatment. Activity was reduced by 55.7% in the rLAN treatment compared with the LAB treatment, and activity in the dLAN treatment decreased by 75.5% compared with the LAB treatment. The activity levels in the NAT treatment were higher than both the rLAN and dLAN treatments ($P < 0.001$ for both, Figure 2). The mice were more active during the rLAN treatment compared with the dLAN treatment ($P < 0.001$, Figure 2). The time (or phase) of day influenced the activity significantly ($\chi^2 = 7219.07$, $df = 1$, $P < 0.001$); mice were significantly more active during the dark phase than the light phase. Activity was

influenced by the sex of the mice with males being significantly more active than females ($\chi^2 = 81.99$, df = 1, $P < 0.001$).

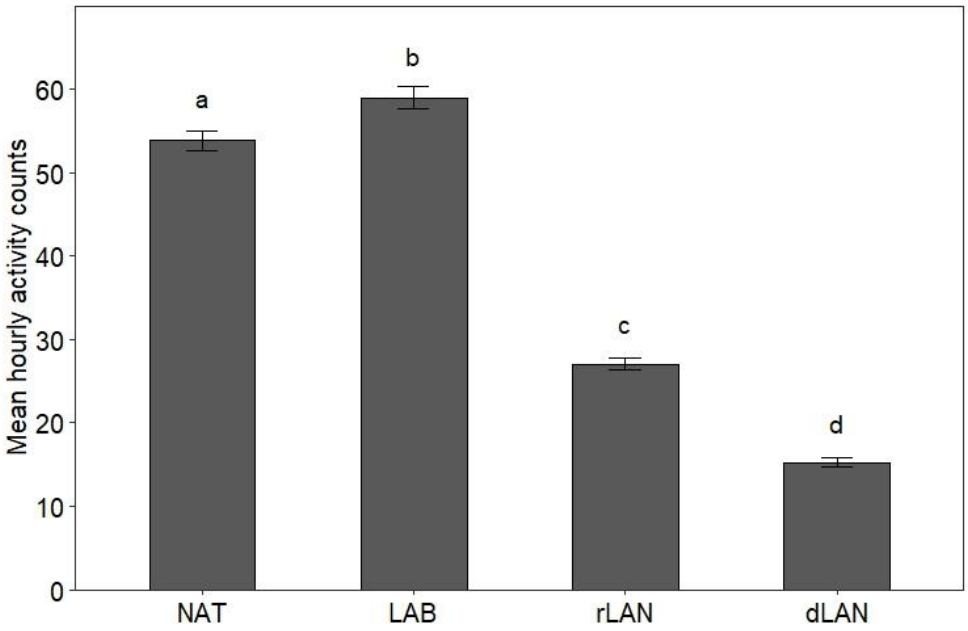


Figure 2. The mean hourly activity counts (mean ± SE) of all M. coucha individuals in four experimental treatments. The same letters on the plots indicate non-significant differences between treatments. NAT – natural environmental conditions, LAB – laboratory conditions, rLAN – light at night on the opposite side of the room and dLAN – light at night suspended directly above the cages.

The activity levels of the mice were significantly influenced by the interaction between treatment and time of day ($\chi^2 = 515.78$, df = 3, $P < 0.001$, Figure 3). Activity for all treatments was higher during the dark phase than the light phase ($P < 0.001$ for all, Figure 3). Activity in the dark phase was the highest in the LAB treatment, followed by the NAT treatment and then the rLAN treatment, with the lowest nocturnal activity during the dLAN treatment ($P < 0.001$ for all, Figure 3). None of the remaining comparisons were significant ($P \geq 0.620$, Figure 3).

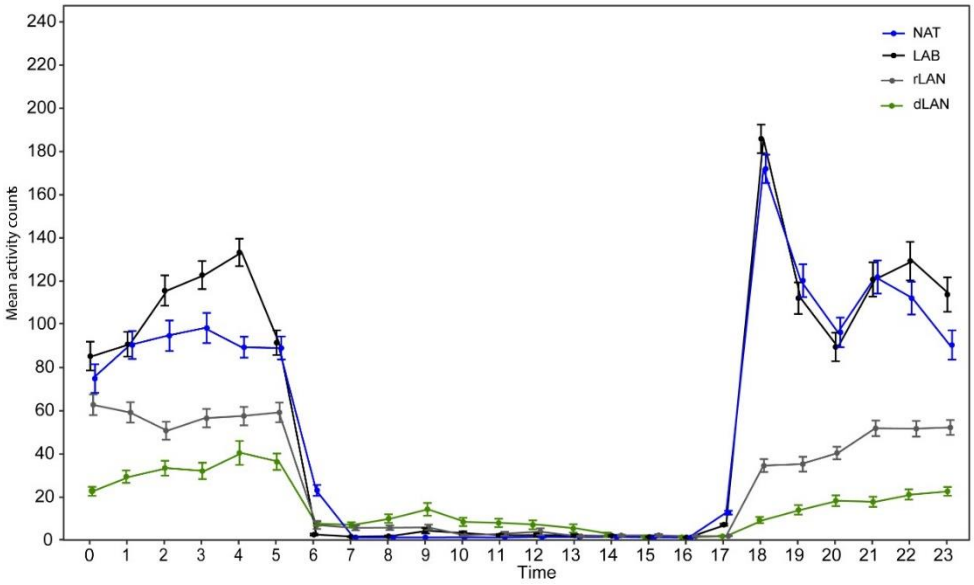


Figure 3. The hourly activity counts (mean ± SE) for all individuals during the 24h of the day in the different experimental treatments. NAT – natural environmental conditions, LAB – laboratory conditions, rLAN – light at night on the opposite side of the room and dLAN – light at night suspended directly above the cages.

The interaction between treatment and sex influenced the activity levels of the mice ($\chi^2 = 7.27$, df = 3, $P < 0.001$, Figure 4). Both males and females showed the highest activity scores in the LAB treatment compared with all other treatments, followed by the NAT and rLAN treatments and they showed the lowest activity under the dLAN treatment ($P < 0.001$ for all, Figure 4). Males showed significantly higher activity during the NAT, LAB and rLAN treatments than females ($P \leq 0.002$, Figure 4), but there was no significant difference in the sexes during the dLAN treatment ($P = 0.398$, Figure 4).

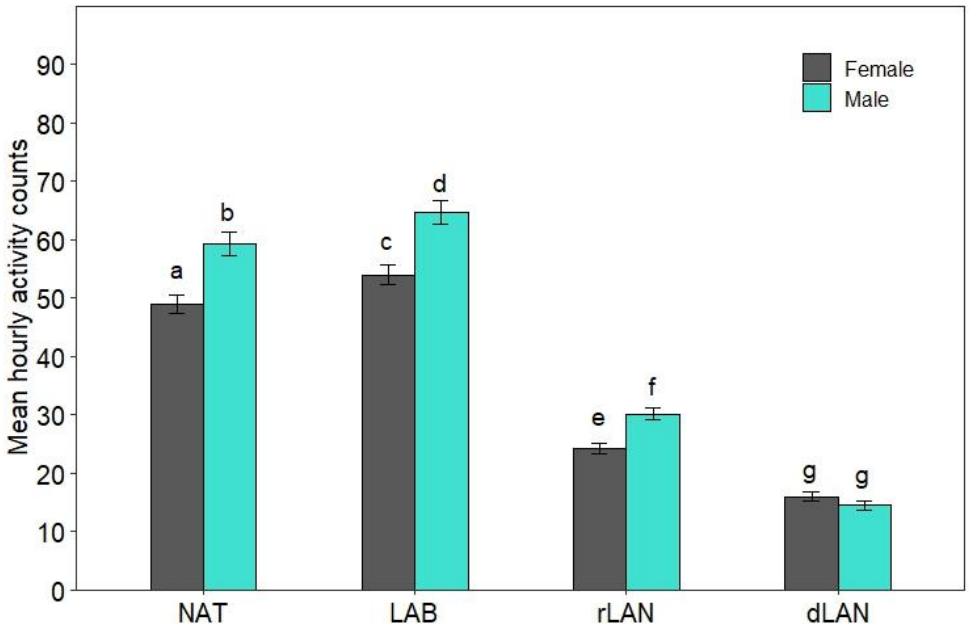


Figure 4. The mean hourly activity counts (mean ± SE) of all the female and male *M. coucha* in four different experimental treatments. The same letters on the plots indicate non-significant differences between treatments. NAT – natural environmental conditions, LAB – laboratory conditions, rLAN – light at night on the opposite side of the room and dLAN – light at night suspended directly above the cages.

The interaction between time of day and sex was a significant predictor of activity ($\chi^2 = 67.06$, df = 1, $P < 0.001$, Figure 5). During the dark phase, males were more active than females ($P < 0.001$, Figure 5), but the sexes did not differ in their activity during the light phase ($P = 0.922$, Figure 5). Both males and females were more active during the dark phase compared with the light phase ($P < 0.001$, Figure 5).

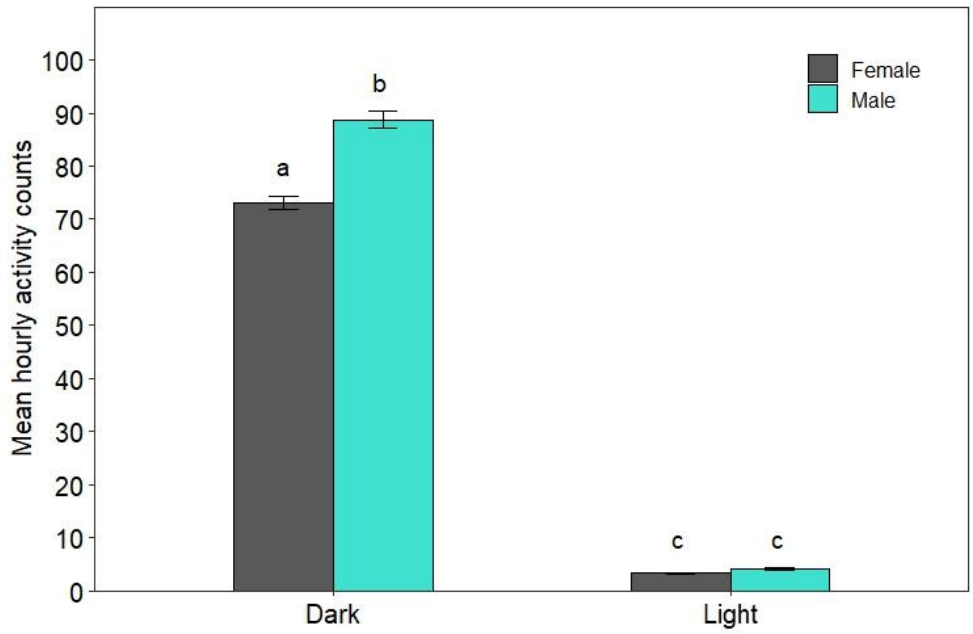


Figure 5. The mean hourly activity counts (mean ± SE) of all the female and male M. coucha in the dark and light phases of the day. The same letters on the plots indicate non-significant differences between phases. NAT – natural environmental conditions, LAB – laboratory conditions, rLAN – light at night on the opposite side of the room and dLAN – light at night suspended directly above the cages.

The three-way interaction of treatment, time of day and sex, showed a significant influence on the activity levels ($\chi^2 = 4.59$, df = 3, P = 0.003). Both sexes showed higher activity during the dark phase for all treatments compared with the light phase ($P < 0.001$ for all). Males showed higher activity than females during the dark phase for all treatments ($P < 0.001$ for all), excluding the dLAN treatment ($P = 0.968$). Both males and females showed the highest activity during the dark phase in the LAB treatment, followed by the NAT, then rLAN and finally dLAN treatments ($P < 0.001$). None of the remaining comparisons were significant ($P \geq 0.982$).

The start time of activity of *M. coucha* differed significantly between treatments (Kruskal-Wallis test: $\chi^2 = 46.35$, df = 3, $P < 0.001$). Mice became active significantly later during the dLAN treatment (20:26 ± 0.77, Figure 5) compared with the activity onset in the NAT treatment (17:54 ± 0.20), the LAB treatment (17:58 ± 0.09) and the rLAN treatment (18:56 ± 0.36; $P \leq 0.001$, Figure 5). The mean onset of activity under the rLAN treatment was also significantly later compared with both the NAT and LAB treatments ($P \leq 0.001$), but the mean onset of activity did not differ between the NAT and LAB treatments ($P = 0.998$, Figure 6).

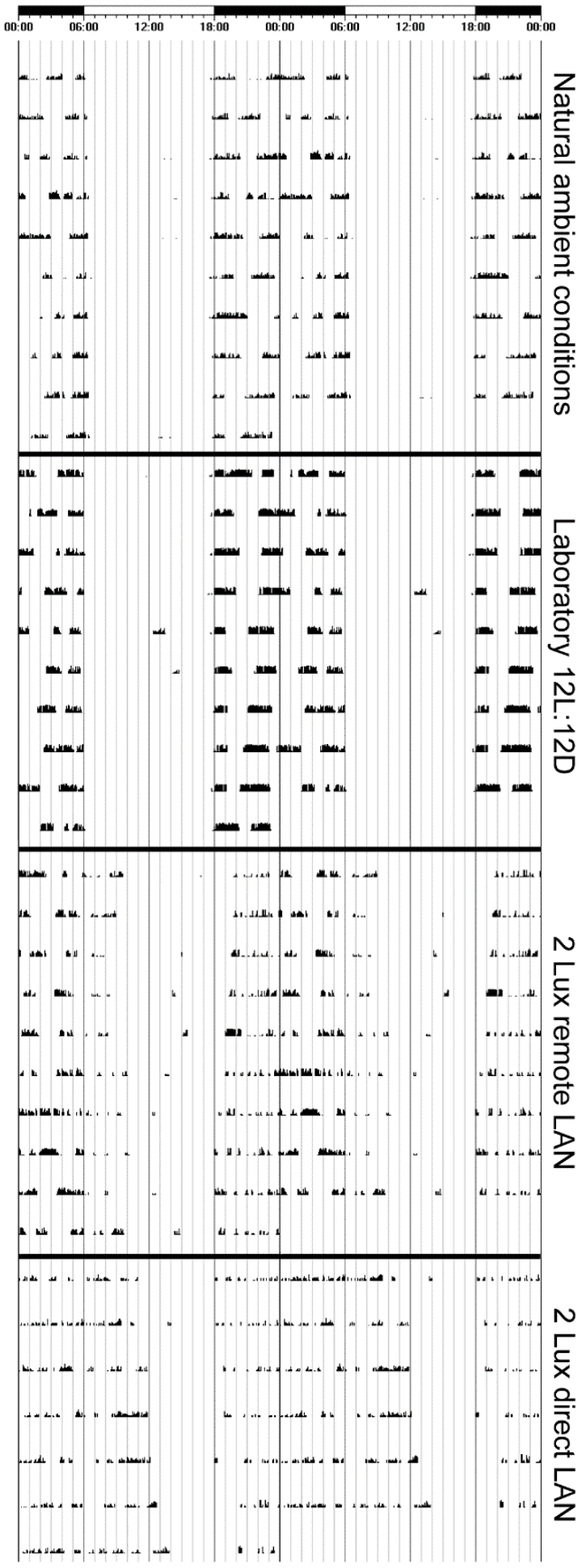

00:00
06:00
12:00
18:00
00:00
06:00
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00:00
Natural ambient conditions
Laboratory 12L:12D
2 Lux remote LAN
2 Lux direct LAN

Figure 6. A double-plotted actogram of a single *M. coucha* to illustrate the changes in onset (start of activity) and offset (end of activity) during the different experimental treatments. NAT – Natural ambient conditions, LAB – Laboratory 12L:12D, rLAN – 2 Lux remote LAN, dLAN – 2 Lux direct LAN. The top bar indicates the light and dark hours of each day, with two days represented next to each other. The black spikes illustrate activity counts per minute.

Like the onset of activity, treatment significantly influenced the offset of activity (Kruskal-Wallis test: $\chi^2 = 14.75$, df = 3, P = 0.002). The mice showed a significantly later offset of activity during the dLAN treatment (08:04 ± 0.79) than the NAT treatment (05:34 ± 0.29, P = 0.011) and the LAB treatment (05:29 ± 0.29, P = 0.002, Figure 6), but not the rLAN treatment (05:59 ± 0.88; P = 0.052, Figure 6). The mean offset of activity of the NAT, LAB and rLAN treatments were not significantly different (P = 1.000).

Treatment was not a significant predictor of the duration of activity (Kruskal-Wallis test: $\chi^2 = 4.92$, df = 3, P = 0.178). The duration was 11:29:24 ± 00:00:15 in the NAT treatment, 11:39:12 ± 00:00:20 in the LAB treatment, 11:02:24 ± 00:00:41 in the rLAN treatment, and 11:39:10 ± 00:01:14 in the dLAN treatment. There was no significant difference in the mean period of activity between the dLAN and the LAB or rLAN treatments (Kruskal-Wallis test: $\chi^2 = 3.86$, df = 2, P = 0.145).

5.4 Discussion

I investigated aspects of the general circadian biology of *M. coucha,* a pioneer species in disturbed areas, under different environmental conditions. I compared the activity of *M. coucha* under natural environmental conditions and standard laboratory conditions. I subsequently assessed how, and to what extent, ALAN could influence the general activity of *M. coucha*. I predicted that activity of *M. coucha* would be highest under conditions that were the closest to what they experience in their natural habitat and that they would reduce their activity under the artificial laboratory treatment. However, *M. coucha* was more active under the controlled LAB treatment. These findings contradict those of previous studies, since the syntopic diurnal four-striped field mouse (*Rhabdomys pumilio*) showed higher mean activity counts in a natural setting than those from the constant laboratory setting (Schumann et al., 2005). Similarly, wild-caught crepuscular single-striped grass mice (*Lemniscomys rosalia*) and nocturnal vlei rats (*Otomys angoniensis*) were more active during a natural treatment than a controlled laboratory treatment (Chapters 3 & 4). I suggested that the observed decrease in activity in *L. rosalia* and *O. angoniensis* under laboratory conditions was because of the novel setup and the lack of natural cues and fluctuations (Chapters 3 & 4). Additionally, the difference in light levels between the natural conditions and the laboratory overhead lights could also have been the driving factor behind the decrease in activity in *L. rosalia* and *O. angoniensis* and could have led to an increase in activity in the nocturnal *M. coucha.*

As expected, *M. coucha* reduced its activity under both LAN treatments compared with the dark night (LAB) treatment. Past studies showed that nocturnal laboratory rodents under LAN conditions decreased activity levels (Spoelstra et al., 2015; Viljoen and Oosthuizen, 2023), showed signs of depressive behaviours, reduced learning and memory (Fonken et al., 2012) and also altered the time of food consumption (Fonken et al., 2010). Some of these findings can have costs in free-living animals because a small modification in the time of food consumption can lead to weight gain (Fonken et al., 2010).

Artificial light at night is constant and can be more intense than natural night light levels. Nocturnal animals appear to respond to ALAN in an intensity-dependent manner, where activity decreases as light intensity increases (Viljoen and Oosthuizen, 2023). Similarly, the intensity of the light source and the level of cover available can affect the activity of an animal. Activity reduction under dLAN was observed in a syntopic African rodent, the African pygmy mouse (*M. minutoides*) that showed an 88% decrease in activity (Viljoen and Oosthuizen, 2023). The Patagonian leaf-eared mice (*Phyllotis xanthopygus*) showed a similar response to night light by decreasing their overall activity (Kramer and Birney, 2001). Under remote light at night (rLAN), *M. coucha* reduced its overall activity by more than 50% than when exposed to a dark night in the LAB. It reduced its activity by 75% under the direct light (dLAN) treatment, where it did not have the opportunity to escape the light by hiding behind features in the cages. This could be because the mice responded to the continuous light cues and mistiming the normal period of activity.

Overall, *M. coucha* was more active during the dark hours than the light hours, which was expected since they are nocturnal (Skinner and Chimimba, 2005). However, my results revealed that the onset and offset of activity were less defined under LAN treatments. During rLAN the onset of activity drifted to later in the evening compared with the onsets in the NAT and LAB treatments. However, the onset returned to around 18:00 later in the rLAN treatment, suggesting that the mice became used to the presence of the rLAN. A similar delay in activity onset was observed in the strictly nocturnal *M. minutoides* (Viljoen and Oosthuizen, 2023). The dLAN intensity was probably brighter than preferred, resulting in a delayed onset in *M. coucha* activity. It is possible that the mice waited for the light to be switched off to exit their shelter and after some time they had no other choice but to leave the shelter to feed. The mice also increased their diurnal activity under dLAN with a later offset of activity.

Presently, we lack information on the eye morphology and proportions of rods and cones in many species, including *M. coucha,* and the research into how different morphological eye types perceive and adapt to high intensity light is still ongoing. We know that the pupil of a nocturnal mouse will be completely open on nights with only starlight and the pupil closes steadily as the intensity of the light increases (Bushnell et al., 2016). This suggests *M. coucha* would experience more challenges with

the high intensity of ALAN and difficulty distinguishing between low and high intensity light cues (Stöckl and Foster, 2022). ALAN sources can be much brighter than the natural light sources at night, such as the moon and stars (Stöckl and Foster, 2022). Thus, if a rodent runs from within thick vegetation into the open where an ALAN source is present, it will experience a significant increase in light intensity, requiring time for the eyes and visual neurons to respond to the increased illumination (Stöckl and Foster, 2022). If the eyes and neurons take too long to adjust to the higher intensity light this could have negative consequences for the rodent (Stöckl and Foster, 2022), such as being vulnerable to predators they are not able to detect during this period.

My results revealed no significant differences in the duration of activity in *M. coucha,* suggesting that *M. coucha* did not expand or contract its activity significantly under the experimental treatments. The period of activity also did not differ between treatments.

Males were more active than the females at night, contrary to my expectation. In polygamous rodents, females often remain in their territories close to known resource locations, whereas the males expand their territories in search of mates (Jolles et al., 2015). Thus, males are usually more risk prone. However, promiscuous species would not show a difference between the sexes in terms of their roaming and the risks they experienced. The results could indicate sex-specific differences, which are not yet apparent. Males were more active than females in all treatments except for the dLAN treatment, where both sexes of *M. coucha* showed no difference in activity. This seems to suggest that the males are possibly more explorative, but both sexes experienced dLAN similarly, as too high risk.

5.5 Conclusions

My study involved the investigation of activity patterns of *M. coucha* under different light-dark treatments. The mice were more active under constant temperature conditions in the LAB treatment than the more natural environment in the NAT treatment. Yet, *M. coucha* showed a significant decrease in activity under rLAN and its activity decreased even more under dLAN. Sex differences were apparent under the first three treatments namely NAT, LAB and rLAN, with males being more active, but both sexes responded similarly under dLAN. *Mastomys coucha* is a pioneer species, often colonising burned areas before other rodents and appeared to be accustomed to open and exposed areas. In these exposed areas, *M. coucha* could use its burrow systems to escape predator detection and would most likely use the burrows more often and for longer durations throughout the night under the threat of increased illumination. The potential disappearance of this species will have severe consequences as they act as a post-burn pioneer and can have population eruptions under favourable conditions, providing crucial food sources for predators. This study is vital in providing a baseline of activity information on a pioneer African rodent species. Rodents are critical to the ecosystem, but if we do not understand how our urban

expansion will affect their existence, we will not be able to predict the wider impact on an ecosystem because of the changes in prey species composition.

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Chapter 6: Risk-sensitive foraging under different ALAN treatments in three African rodents

Abstract

Foraging decisions are made daily by all animals to fulfil their energy requirements and are influenced by a range of factors, including the risk of foraging. Predation risk has an influence during foraging in prey species, especially in open spaces. Animals have evolved a range of behaviours to avoid detection, such as reducing activity or remaining in microrefugia. Artificial light at night (ALAN) represents an unnatural environment for nocturnal animals in particular and could be perceived as an increased predation risk. I investigated the foraging behaviour of three southern African rodents *Lemniscomys rosalia, Otomys angoniensis* and *Mastomys coucha* with different temporal niche preferences, under different light conditions. Individuals of each species were tested in four treatments: Diurnal (tested in daylight), Nocturnal (tested in the dark), Low intensity LAN (2 Lux) and High intensity LAN (10 Lux). I recorded a range of behaviours under the different treatments to establish how the species responded to different lighting conditions. Risk-averse behaviour was observed in *L. rosalia* (crepuscular) during the Diurnal treatment, and they appeared to be more cautious in the Nocturnal treatment. This species showed normal foraging behaviour under the two LAN conditions, possibly because of the similarity between the LAN illumination and the dawn and dusk illumination levels. Surprisingly, *O. angoniensis* (nocturnal) showed a tendency for risk-aversive behaviour in all treatments. These treatments were conducted in a very exposed environment and this species is accustomed to living and foraging under dense vegetation and could have perceived the bare arena as too risky. During the Diurnal treatment, *M. coucha* (nocturnal) was inactive and it did not vary in its foraging behaviour between the three nocturnal treatments. Overall, the foraging responses of the study species were in line with their temporal niches, but it is not necessarily congruent with the behaviour observed in other species with similar temporal niches. The level of perceived risk in the treatments could have been different for each species and the habitat preferences of these species could have had an influence on their responses.

Keywords: artificial light at night, crepuscular, nocturnal, predation risk, risk-sensitive foraging, rodent

6.1 Introduction

Animals make a variety of choices while searching for food, and this is largely dependent on the present state of the animal. This can include health status and the level of satiety (Kotler et al., 2010), as well

as the energetic payoff, the handling and searching times for food and the level of real or perceived risk (Pyke et al., 1977). Perceived predation risk can include the level of exposure of an area based on surrounding vegetation or the nocturnal illumination of the area (Mazza et al., 2018). The optimal foraging theory (OFT) proposes that an animal will act in a specific way to gain the highest amount of energy possible for a given length of time spent foraging (Pyke et al., 1977). However, the assumptions of this theory are not always applicable to free-living animals that experience risks and have to modify their decisions accordingly (Craft, 2016). As a result, the risk-sensitive foraging theory (RSFT) was developed as an extension of the OFT, to take into account the risk and level of uncertainty animals experience while foraging (Craft, 2016). The RSFT attempts to explain the type of foraging strategy an animal will employ, depending on its surrounding conditions, as well as its ability to meet its energy requirements (Barnard et al., 1985; Craft, 2016). An animal is considered as risk-averse when it chooses the option with the known content and risk-prone if it chooses a variable option (Craft, 2016). Sprague-Dawley rats adopted a more risk-prone strategy when little foraging effort was needed, regardless of the energy contents of the food reward (Kirshenbaum et al., 2000), but the authors did not describe the behaviour forming risk-prone or risk-averse strategies. Nevertheless, when the food reward was high in energy and the associated effort to obtain it was higher, they were more risk-averse (Kirshenbaum et al., 2000).

One of the most significant influences on a prey animal's foraging behaviour is the risk of predation, which can be detected directly by assessing predator attacks, or indirectly through olfactory cues of predators or increased visibility (Kelleher et al., 2021). Species can adopt different anti-predator behaviours while foraging. Some species prefer foraging during the darker periods of the night (Daly et al., 1992; Pratas-Santiago et al., 2017), while others forage under dense cover (Mandelik et al., 2003). The anti-predator strategy often depends on predator type. On full moon nights, when the visibility was better and detection by predators was greater, deer mice (*Peromyscus manuculatus*) confined their foraging to densely covered areas to avoid aerial predators (Orrock and Fletcher, 2014). However, when the terrestrial island fox (*Urocyon littoralis*) population increased, the mice no longer showed increased foraging under cover, since this posed a greater risk of being attacked by foxes (Orrock and Fletcher, 2014). Generally, mammals decrease foraging during full moon nights to avoid predator detection (Kelleher et al., 2021; Prugh and Golden, 2014). Vigilance seems to increase while foraging around full moon nights compared with new moon nights (Kotler et al., 2010). A full moon can provide approximately 2 Lux light, whereas light illumination at new moon is lower at 0.001 Lux (Alaasam et al., 2021; Emmer et al., 2018). Allenby's gerbils (*Gerbillus andersoni allenbyi*) showed the highest vigilance on full moon nights and gave up foraging earlier during the waxing and full moon nights, applying a risk-averse approach with high illumination (Kotler et al., 2010).

Artificial light at night (ALAN), the presence of unnatural anthropogenic light sources in the landscape, is much more prevalent lately (Sanders et al., 2021). Prey species have to account for this

additional light source while foraging. In contrast to the lunar cycle, ALAN is usually constant and the brightness does not change during the night and is not cyclical (Gaston et al., 2013). ALAN, such as streetlights, can reach illumination levels between 10 and 40 Lux at ground-level, and some ALAN sources are well above 100 Lux (Gaston et al., 2014). Prey species have adapted to the lunar cycle and its associated risks through behavioural adaptations over evolutionary time but might not be able to employ these behaviours constantly under ALAN, since it is not feasible to, for example, stop foraging under ALAN. The Mongolian five-toed jerboa (*Allactaga sibirica*) was able to better detect food under the increased ALAN, but changed its foraging behaviour by showing increased foraging speed and decreased food selectivity (Zhang et al., 2020).

Most animals are specialised for a particular temporal niche. For example, nocturnal species have larger eyes and retinas equipped with a higher proportion of rods than diurnal animals (Peichl, 2005). Nocturnal species are also less active at night under ALAN and behave risk-prone to acquire food (Fonken et al., 2010; Russart and Nelson, 2018). Diurnal animals can extend their active time into the night, because artificial illumination essentially extends their day and allows for additional foraging opportunities (Russart and Nelson, 2018). This can also lead to more competitive interactions with nocturnal species (Seymoure et al., 2023).

I studied three syntopic southern African rodent species to assess their risk-sensitive foraging under ALAN. I selected these three species because of their difference in habitat and diet, but also because they had different temporal niches, one crepuscular, one variable activity and one nocturnal. 1) The single-striped grass mouse, *Lemniscomys rosalia,* has been reported as both diurnal and crepuscular (Skinner and Chimimba, 2005), although it was active around twilight in captivity (Chapter 3). This species is granivorous, and occur in dense vegetation (Skinner and Chimimba, 2005). 2) The Angoni vlei rat, *Otomys angoniensis*, has variable activity, having been described as diurnal, crepuscular and/or nocturnal (Kingdon, 2013; Skinner and Chimimba, 2005). In the laboratory, this species showed primarily nocturnal activity (Chapter 4). It is strictly herbivorous and occurs in grasslands close to water sources and it makes runways that are often used by smaller rodents (Kingdon, 2013; Skinner and Chimimba, 2005). 3) The southern multimammate mouse, *Mastomys coucha,* is strictly nocturnal (Skinner and Chimimba, 2005) both in the field (Kingdon, 2013; Skinner and Chimimba, 2005) and in captivity (Chapter 5). It occurs in a range of habitats and is an omnivore (Skinner and Chimimba, 2005). *Mastomys coucha* is a pioneer species that populates disturbed and post-burn areas (Skinner and Chimimba, 2005).

I assessed whether the species traded off reduced activity against foraging during the day and night and under ALAN. Individuals were subjected to four different treatments in captivity, namely Diurnal, which was conducted during daytime (natural daylight with overhead laboratory lights, light of ± 40 Lux combined) and three treatments were conducted at night - Nocturnal (complete darkness

for the duration of the experiment), LLAN (low intensity light at night, 2 Lux light) and HLAN (high intensity light at night, 10 Lux light). I hypothesised that the crepuscular species (*L. rosalia*) would be more risk-averse in the Diurnal and Nocturnal treatments, but not under the LLAN and HLAN treatments. I expected the nocturnal species (*O. angoniensis* & *M. coucha*) would be more risk-averse under all the treatments besides the Nocturnal treatment. Even though *O. angoniensis* has been reported to have variable activity (Skinner and Chimimba, 2005), I found it to be more nocturnal in a concurrent study in captivity (Chapter 4). I made six predictions regarding the experimental treatments (Table 1). Additionally, I investigated the influence of sex on the foraging behaviour for each species, but since both sexes have to feed, I did not make *a priori* predictions of a sex difference.

Table 1. Predictions for the influence of treatment on six response variables on the foraging behaviour of *L. rosalia* (L. r.)*, O. angoniensis* (O. a.) and *M. coucha* (M. c.). For each prediction, I indicated differences (>) or similarities (=) by treatment per species.

Response variable	**Species**	**Treatment**
Latency to move: Time elapsed to start moving	L. r.	Diurnal = Nocturnal > LLAN = HLAN Since this species is crepuscular, I expected that it would move the fastest in the LAN treatments, as it is used to low light conditions (LLAN would be more representative of the earlier mornings and evenings whereas HLAN would represent later morning and afternoons). I predicted that it would show the longest latency before moving under the Diurnal and Nocturnal treatments because of a higher risk during its inactive periods.
	O. a	LLAN = HLAN > Diurnal=Nocturnal I expected this species to move faster in the Diurnal and Nocturnal treatments since it is capable of being active in either of these. However, I predicted it to show a longer latency under the ALAN treatments because of the increased illumination and level of exposure.
	M. c	Diurnal > HLAN > LLAN > Nocturnal Since the Nocturnal treatment is the safest of the treatments for this nocturnal species, I expected it to move the fastest in the Nocturnal treatment, followed by the riskier LLAN treatment, but show the longest latency under the Diurnal and HLAN treatments which poses the highest visibility and thus highest risk.

Latency to approach seeds: Time elapsed before moving towards seeds	L. r.	Diurnal = Nocturnal > LLAN = HLAN I expected this species to approach seeds in a similar pattern to the latency to move based on level of exposure.
	O. a.	LLAN = HLAN > Diurnal=Nocturnal I expected a similar response to approach seeds than the latency to move based on level of exposure.
	M. c.	Diurnal > HLAN > LLAN > Nocturnal I expected the same pattern in the latency to approach seeds as in the latency to move based on the amount of risk experienced.
Duration of feeding: Amount of time spent eating seeds	L. r.	LLAN = HLAN > Nocturnal > Diurnal I anticipated this species would feed for longer during the LLAN and HLAN treatments, under low light levels compared with the complete darkness in the Nocturnal treatment and the very bright surroundings of the Diurnal treatment, since it posed more risk. I expected this species to show a significant increase in risk-sensitive foraging during the Diurnal and Nocturnal treatments compared with the other treatments.
	O. a.	Nocturnal > Diurnal > LLAN = HLAN Since this species showed mostly nocturnal behaviour in the laboratory, I predicted that it would spend the most time feeding in the Nocturnal treatment followed by the Diurnal treatment. I expected it to spend the least amount of time foraging under ALAN. I anticipated this species to be more risk-sensitive in the ALAN treatments.
	M. c.	Nocturnal > LLAN > HLAN = Diurnal I expected this species to spend less time feeding in the Diurnal treatment, since it is outside of its active period, and in the HLAN treatment, since it is too risky to remain exposed for long periods of time. Under the Nocturnal treatment, I expected it to eat for the longest time, since it represents its natural foraging time. I expected this species to show an increased risk-sensitive foraging under the Diurnal, LLAN and HLAN treatments compared with the Nocturnal treatments.

Number of feeding bouts: Number of times going to eat seeds	L. r.	LLAN = HLAN > Nocturnal > Diurnal I expected this species to conduct fewer feeding bouts under more risky conditions (i.e., complete darkness in the Nocturnal treatment and bright Diurnal treatment) and move more in the LLAN and HLAN treatments, since that represented its natural lighting circumstances. Similar to duration foraging, I expected this species to show increased risk-sensitive foraging under the Diurnal and Nocturnal treatments.
	O. a.	Nocturnal > Diurnal > LLAN = HLAN I predicted that this species would experience the ALAN treatments as very risky and make fewer foraging bouts in these treatments. Since it is known to be active during the day and night, but would be more satiated during the Diurnal treatment, I anticipated the most frequent foraging bouts in the Nocturnal treatment. I expected this species to show increased risk-sensitive foraging in the ALAN treatments.
	M. c.	Nocturnal > LLAN > HLAN = Diurnal I expected this species to experience elevated risk under the HLAN and Diurnal treatments and thus predicted that it would decrease feeding bouts. I expected it to show the highest frequency under the Nocturnal treatment, since the visibility is low, decreasing under the LLAN treatment since the visibility increased. Thus, I expected this species to show more risk-sensitive foraging under the Diurnal, LLAN and HLAN treatments.
Duration spent in a shelter: Time spent inside the shelter	L. r.	Nocturnal = Diurnal > HLAN > LLAN Under the riskiest treatments, Diurnal and Nocturnal, I predicted that the species would spend more time in the shelter. Under the HLAN, I expected it to spend slightly less time out in the open than during the LLAN treatment, since it is a little riskier than LLAN.
	O. a.	Diurnal > LLAN = HLAN > Nocturnal Since this species was mainly active at night in captivity, I expected it to spend the most in the shelter during the Diurnal treatment, followed by the ALAN treatments. I anticipated it to spend more time out of the shelter in its active period in the Nocturnal treatment.

	M. c.	Diurnal > HLAN > LLAN > Nocturnal I expected that this species would spend the longest time in the shelter during the Diurnal treatment. During the HLAN treatment, the risk would be the highest of all the night treatments and I expected it to remain under shelter for a prolonged period. I expected it to risk being more exposed and spend less time in the shelter under the Nocturnal treatment.
Mass of seeds consumed: quantified by the mass of eaten seeds	L. r.	LLAN = HLAN > Nocturnal > Diurnal I expected the same trend to Duration of feeding because of the level of risks experienced.
	O. a.	Nocturnal > Diurnal > LLAN = HLAN Similar to the Duration of feeding prediction, I anticipated this species to eat more in the Nocturnal, followed by the Diurnal treatment. I predicted that it would consume the least amount of seeds in the ALAN treatments.
	M. c.	Nocturnal > LLAN > HLAN > Diurnal I expected a similar reason for the mass of seeds consumed than the Duration of feeding. Since this species is not active during the day, I expected the lowest mass of seeds consumed, followed by HLAN that posed high risk. During the Nocturnal treatment, which is its main active period, I predicted the highest mass of seeds to be consumed.

6.2 Materials and methods

6.2.1 Study site

I conducted my study on the Cradle Nature Reserve, Gauteng, South Africa (-25.9214, 27.8503) between October 2022 and July 2023. My study site was exposed to a relatively high concentration of ALAN, since it was situated close to Johannesburg, the largest metropolitan area in southern Africa. This site is dominated by savanna and grassland biomes and falls within the Magaliesberg Biosphere region.

6.2.2 Test species

I selected three wild-caught rodent species for my study. I trapped the rodents using 40 PVC live small mammal traps placed in areas with signs of rodent presence (rodent runways or faeces). Traps were baited with a combination of peanut butter, oats, sunflower oil, salt, granola and sunflower seeds. I checked the traps every three hours during the day, to avoid hyperthermia or dehydration of any trapped animals, and before sunset and sunrise for nocturnal species. After a rodent was captured, it was identified to genus level and transported to the field laboratory for further processing. The captured animals were later identified to species level (Chapter 4 & 5). I weighed individuals (Pescola® hanging scale, Switzerland, 1g precision) and sexed them using the anogenital distance (shorter distance in females than males). I captured 19 *L. rosalia* individuals, 16 *O. angoniensis* and 17 *M. coucha* individuals. Mice were housed individually in cages (60 x 40 x 36cm) of which the bottom was covered in sand (Kiddies play sand, EDCO Trading cc, RSA) and equipped with a shelter, with nesting material and enrichment materials, such as a rock and a stick. Individuals were given *ad libitum* access to water and fed once daily with a combination of fresh food, such as apple or sweet potato, and seeds, including sunflower seeds and millet seeds. *Otomys angoniensis* received grass and hay on alternate days since it is herbivorous and prefers grass (Kingdon, 2013; Skinner and Chimimba, 2005). Prior to experiments, the study species were maintained in the laboratory for a minimum of a month to allow for acclimatisation to the novel surroundings.

6.2.3 Experimental design

6.2.3.1 Seed preference testing for all three species

Prior to the start of the experiment, I ascertained the seed preference of each individual to tailor the experimental design to each individual's preference, following Rymer et al. (2021). Two days prior to testing for preference, I placed the individuals on a restricted diet consisting of a quarter of their daily rations to motivate participation in the experiment. In the arena, I placed a shelter on one side and three food containers on the opposite side (Figure 1). These containers (2.5cm in diameter and 1cm in height) were placed equidistant from each other and from the sides of the arena. The testing arena floor was covered in sand (the same brand used in their housing cages). I removed the rodent from its cage and placed it in a testing arena with opaque sides, close to the shelter. The arena had a mesh lid to prevent the occupant jumping out and allowing real-time video monitoring of behaviour from above. A camera (Microsoft LifeCam HD-3000, Microsoft, USA) was mounted above the arena to capture the behaviour and not disrupt the normal behaviour of the test animals.

The rodents were housed in an animal room adjacent to the room where the seed preference and foraging experiments were conducted. Before the start of the trial, I weighed seeds to approximately

2g per seed type and placed them in the containers in a random order on the opposite side of the arena. The seed types included millet, shelled sunflower seeds and whole maize kernels. For *O. angoniensis,* I used rolled oats instead of millet since it did not eat millet prior to the experiments (pers. obs.). I monitored the behaviour of individuals through a video feed for 30 minutes to ascertain their preferred seed type (i.e., the seed type they spent the longest time eating) (Table 2). If the test rodent did not participate, it was retested on the following day and given more time to feed on the seeds (maximum time 2h). After each experiment, the test rodent was returned to its home cage and the sand was removed from the arena and the arena was cleaned with F10 (F10 Products, Health and Hygiene (Pty) Ltd, RSA) and water (1:10 dilution) and wiped down with tissue. Each test rodent had new sand in their arena, to avoid remaining olfactory cues from previous test rodents.

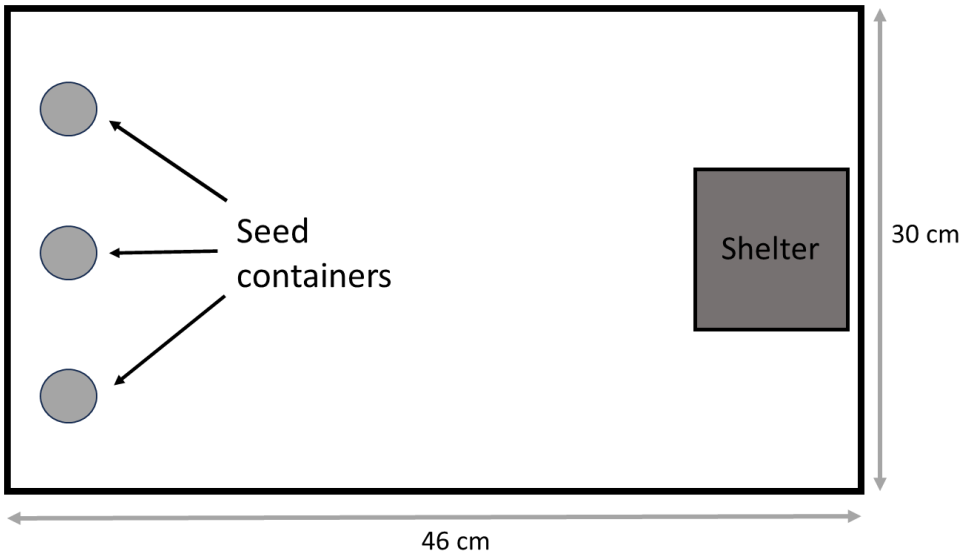


Figure 1. Top view of the seed preference test arena, with the shelter on one side and the seeds in the seed containers on the opposite side. A mesh lid was placed on top of the arena and the camera was suspended over the arena.

Table 2. The number of individuals of *L. rosalia, M. coucha* and *O. angoniensis* that preferred different seed types. *Otomys angoniensis* was tested with oats instead of millet (see text).

Species	Corn	Millet	Oats	Sunflower seeds
L. rosalia (n = 19)	2	9	NA	8
O. angoniensis (n = 16)	4	NA	10	4
M. coucha (n = 17)	2	13	NA	2

6.2.3.2 Foraging experiment: L. rosalia and O. angoniensis

Individuals were tested in arenas similar to the seed preference arena, and their behaviour was video recorded from above using EthoVision XT v11.5 (Noldus Information Technology Inc., The Netherlands) for analysis. To allow for simultaneous recording of the behaviour of more than one individual, each mouse was placed in a single arena which was part of a larger area with three other similar arenas, all with opaque sides to prevent visual communication with neighbouring mice. I placed the test rodents on a restricted diet of approximately 1g of carrot and sunflower seeds (since they had the highest fat content of all the seeds) in their home cage 24h before the experiment. To start the experiment, test individuals were placed into the experimental arena and approximately 2g of preferred seeds were distributed over the surface of the sand opposite the shelter immediately after the introduction of the animal. The behaviour of the test rodent was recorded for 30 minutes. Individuals were returned to their home cages after the experiment and all remaining seeds were collected by sifting the sand and weighed to calculate the amount of seeds consumed. The sand was removed, and the arena and shelter were cleaned using an F10 dilution and tissues.

Each individual was subjected to four treatments in sequence in a standard laboratory room at a constant 24°C temperature and a 12h light 12h dark regime. The treatments were conducted in the same order for all the individuals: 1) Diurnal – conducted during the light part of the light-dark cycle with ± 40 Lux combined overhead lights and natural light through the windows, 2) Nocturnal – completed during the dark part of the cycle. 3) LLAN – a light dimmed to 2 Lux was suspended above the arena during the dark part of the light-dark cycle. 4) HLAN – a light dimmed to 10 Lux was suspended above the arena during the dark part of the light-dark cycle. I chose 2 Lux as the low intensity setting since the light intensity at a full moon can reach up to 2 Lux and this is the brightest natural nocturnal light intensity. I chose 10 Lux as the high intensity setting since some streetlights measure around 10 Lux at ground level. During the Nocturnal treatment, the camera overlooking the arena was equipped with infrared lights to be able to record the behaviour of test rodents with no external lights. I used the same light in the LLAN and HLAN treatments together with a dimmer (Light: LightWorx, 9W 3000K LED, LightWorx LED & Electric Supply LLC, New Jersey; Dimmer: 500W rotary dimmer, Shuttle, RSA), to set the light either to 2 or 10 Lux depending on the treatment. Each individual was tested once per treatment and had two rest days between treatments. During the inter-treatment period, the rodents were fed their usual daily diet on the first day and put on a restricted diet on the following day to prepare for the next treatment. This inter-treatment period was necessary to allow me to assess all test rodents within a similar time period each day.

6.2.3.3 Preference and foraging experiment: M. coucha

For *M. coucha,* I had to adjust the experimental design since it was impacted more severely by changes to its environment and food. I did not place this species on a restricted diet since it was stressed by the food restriction. Individuals were presented with three seed choices in the same seed preference arena used with the first two species (Figure 1). If they had not eaten anything after 30 minutes, the first seed they ate after any period of time longer than 30 minutes was selected as the preferred seed type. The seed options included millet, shelled sunflower seeds and maize.

Individuals were also not tested in a separate arena, but rather in their own home cages. I removed all sand and old food from their cages before the start of the experiment. Similar to the other species, the behaviour of each individual was recorded with a camera suspended over the cage. The shelter, rock and stick remained in the cages during the treatments and the room temperature was maintained at 24°C temperature, with a 12h light 12h dark regime. They were subjected to the same four treatments (as described above), but these were run daily (i.e., no inter-treatment period) to reduce stress. For the Diurnal treatment, I added 2g of the preferred seed to each individual's cage before 06:00 (when the lights in the laboratory turned on to indicate the light cycle), also placing it opposite to where the shelter was located in the cage. The seeds remained in the cage until 18:00 (the laboratory lights turned off to indicate the dark part of the cycle) and then I collected all the remaining seeds. On the following night, the Nocturnal treatment commenced using the same procedure. I added the seeds just before 18:00 and I removed them the following morning at 06:00. I followed the same design on the third night for the LLAN treatment by adding a light dimmed to 2 Lux. On the last night for the HLAN treatment, I dimmed the same light as before to 10 Lux. I supplied these rodents with their normal daily diet as soon as the experimental seeds were collected and weighed. I cleaned any remaining food from the cage before the next experimental seeds were placed in the cage.

For each species and experimental treatment, I recorded five behaviours (Table 3) after the animal left the shelter for the first time. These were recorded for 30 minutes.

Table 3. The foraging variables recorded from the videos of each treatment with associated descriptions.

Recorded variable	**Description**
Latency to move (s)	The time elapsed from the start of the treatment until the rodent started to move from any position it was in at the start of the video, with more than half of their body length.
Latency to approach seeds (s)	The time elapsed from the start of the treatment until the rodent moved past half of their experimental section towards the seeds.
Duration of feeding (s)	The time spent feeding on the seeds.

Frequency of eating	The number of times the test rodent fed on seeds, provided that each bout occurred 5 seconds apart.
Duration spent in shelter (s)	The time spent inside of the shelter.

6.2.4 Data analysis

I analysed the behaviours of the species separately because of the different protocols used. Analyses were run in R (R v4.2.1, Boston, United States) and I tested each response variable in Table 3 for departure from normality using Shapiro-Wilk test. The mass of seeds consumed in each experiment was calculated by the initial mass of weighed seeds – final mass of seeds collected after the experiment. All variables were not normally distributed ($P > 0.05$) except for the duration of feeding for *L. rosalia* ($P < 0.05$). Predictor variables included treatment (Diurnal, Nocturnal, LLAN and HLAN), sex and the interaction between these variables. I included the animal identity (repeated measure analyses) as a random variable in each model. All non-normally distributed response variables were analysed using generalized linear mixed models (GLMMs) with a Gamma distribution and a log link function, except for the frequency of feeding, for which a Poisson distribution and log link function were used. The duration of feeding for *L. rosalia*, was analysed using a general linear mixed model with a Gaussian distribution and identity link function. I ran all models using the *lme4* package (Bates et al., 2015). All significant variables were further analysed using post-hoc pairwise comparisons with the *emmeans* package (Lenth et al., 2020).

6.3 Results

6.3.1 Latency to start moving

The latency to start moving in *L. rosalia* was influenced by the treatment ($\chi^2 = 20.03$, df = 3, $P < 0.001$). The latency was longer in the Diurnal treatment than the LLAN ($P = 0.035$) and HLAN treatments ($P = 0.048$, Figure 2). The latency was longer in the Nocturnal treatment than the LLAN ($P = 0.004$) and the HLAN treatments ($P = 0.006$, Figure 2). None of the remaining treatment comparisons were significant ($P \geq 0.805$). The latency to move was not significantly influenced by sex ($\chi^2 = 3.56$, df = 1, $P = 0.059$) and the interaction between treatment and sex ($\chi^2 = 5.73$, df = 3, $P = 0.126$).

The latency to move in *O. angoniensis* was not significantly influenced by treatment ($\chi^2 = 4.93$, df = 3, $P = 0.177$), sex ($\chi^2 = 2.13$, df = 1, $P = 0.145$) or the interaction between treatment or sex ($\chi^2 = 2.30$, df = 3, $P = 0.513$).

Treatment influenced the latency to move ($\chi^2 = 55.09$, df = 3, $P < 0.001$) in *M. coucha* and latency was longer in the Diurnal treatment than the Nocturnal, LLAN and HLAN treatments ($P < 0.001$ for all,

Figure 2). None of the remaining comparisons were significant ($P \geq 0.583$). The latency to move was also not significantly influenced by sex ($\chi^2 < 0.01$, df = 1, P = 0.988) or the interaction between treatment and sex ($\chi^2 = 0.44$, df = 3, P = 0.932).

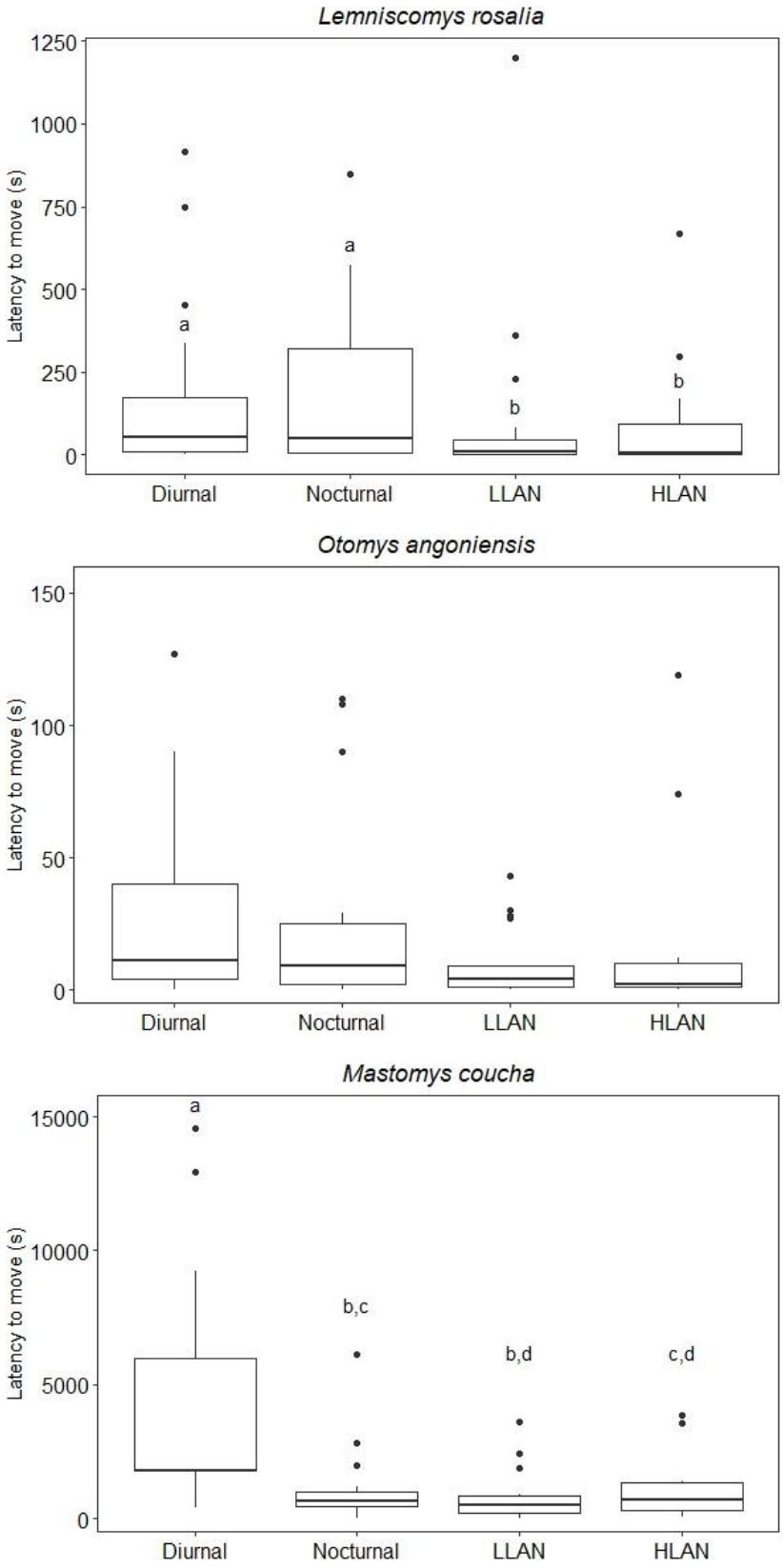


Figure 2. The difference in latency to move (s) between treatments for all species. Horizontal lines are medians, boxes are 1st and 3rd interquartiles, whiskers are minimum and maximum values and dots are outliers. The same letters on the plots indicate non-significant differences between treatments. Plots without alphabets indicate no overall model statistical significance.

6.3.2 Latency to approach seeds

The latency to approach seeds was influenced by the treatment ($\chi^2 = 44.98$, df = 3, $P < 0.001$) in *L. rosalia*. This species was slower to approach seeds during the Diurnal treatment than the LLAN and HLAN treatments ($P < 0.001$ for both, Figure 3) and approached it slower in the Nocturnal treatment compared with both the LLAN ($P = 0.003$) and HLAN treatments ($P < 0.001$, Figure 3). None of the remaining treatment comparisons were significant ($P \geq 0.596$). Sex ($\chi^2 = 1.48$, df = 1, $P = 0.223$) and the interaction between treatment and sex ($\chi^2 = 2.89$, df = 3, $P = 0.409$) did not significantly influence the latency to approach seeds.

Treatment influenced the latency to approach the seeds ($\chi^2 = 27.64$, df = 3, $P < 0.001$) in *O. angoniensis*. During the Diurnal treatment, the latency was longer compared with the LLAN ($P = 0.010$, Figure 3) and HLAN treatments ($P < 0.001$, Figure 3). The latency was also significantly longer in the Nocturnal treatment than the HLAN treatment ($P = 0.006$, Figure 3) but none of the remaining treatment comparisons were significant ($P \geq 0.229$). Sex did not influence the latency ($\chi^2 = 2.95$, df = 1, $P = 0.086$), yet the interaction between treatment and sex was significant ($\chi^2 = 12.94$, df = 3, $P = 0.005$, Figure 4). Females in the Diurnal treatment had a longer latency to approach seeds than the HLAN treatment ($P = 0.022$, Figure 4) and they showed a longer latency to approach seeds in the Nocturnal treatment compared with LLAN ($P = 0.023$) and HLAN treatments ($P = 0.005$). In the Diurnal treatment, males had a long latency to approach seeds compared with the Nocturnal ($P = 0.018$) and HLAN treatments ($P = 0.002$, Figure 4). During the Nocturnal treatment, females were slower to approach seeds than males ($P = 0.013$, Figure 4). None of the remaining comparisons were significant ($P \geq 0.093$, Figure 5).

For *M. coucha,* treatment influenced the latency to approach the seeds ($\chi^2 = 170.57$, df = 3, $P < 0.001$), with the latency in the Diurnal treatment being significantly longer than all other treatments ($P < 0.001$ for all, Figure 3). None of the remaining treatment comparisons were significant ($P \geq 0.708$). Sex did not significantly influence the latency ($\chi^2 = 0.01$, df = 1, $P = 0.917$) and neither did the interaction between treatment and sex ($\chi^2 = 0.70$, df = 3, $P = 0.873$).

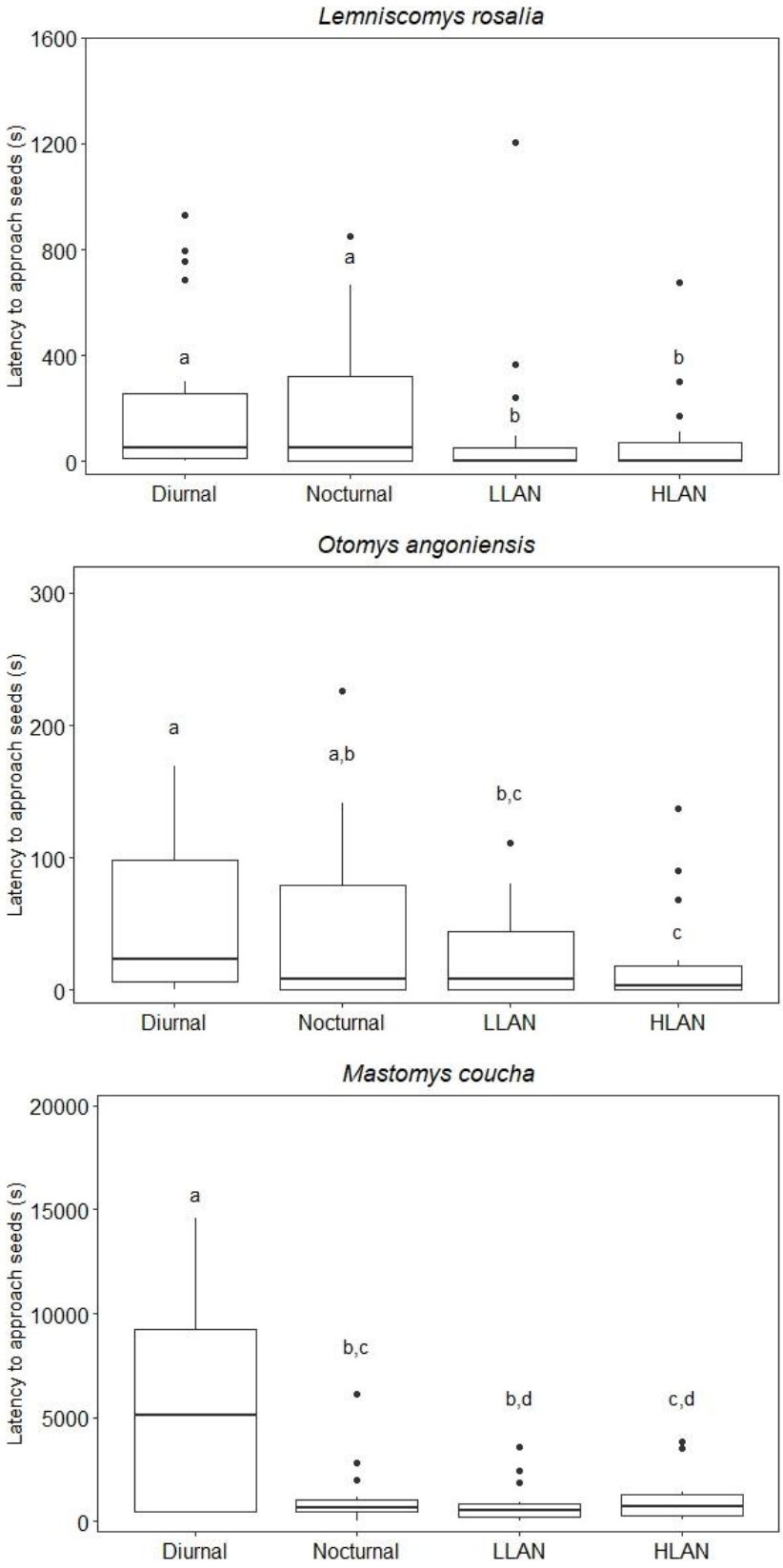


Figure 3. The latency to approach seeds (s) during each treatment for each species. Horizontal lines are medians, boxes are 1st and 3rd interquartiles, whiskers are minimum and maximum values and dots are outliers. The same letters on the plots indicate non-significant differences between treatments.

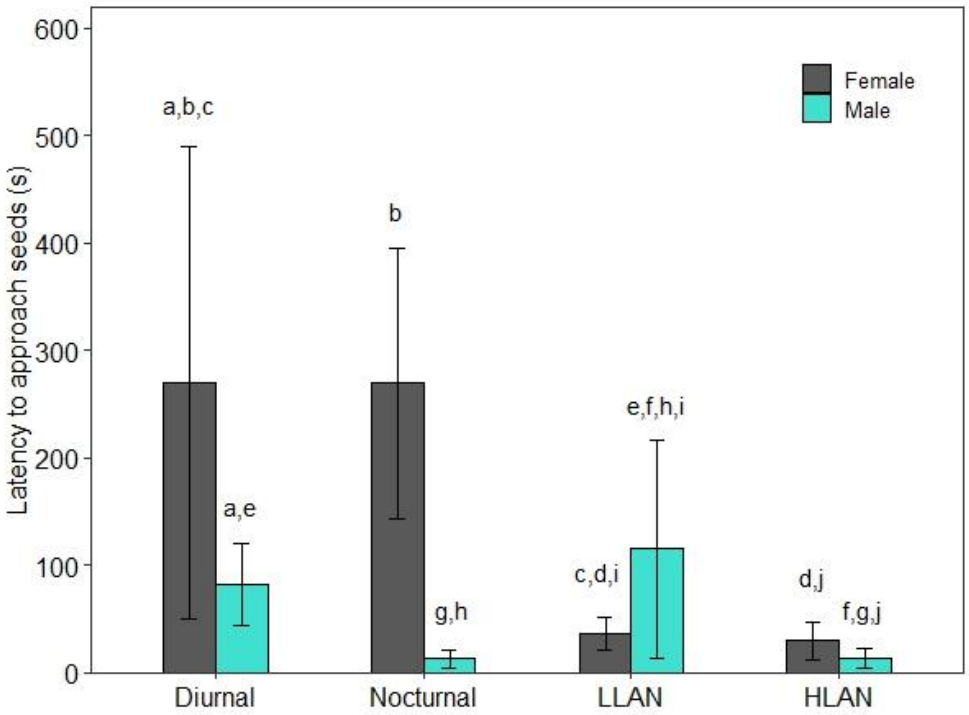


Figure 4. The latency to approach the seeds (mean ± SE) for *O. angoniensis* females and males for all treatments. The same letters on the plots indicate non-significant differences between treatments.

6.3.3 Duration of feeding

Treatment influenced the duration of feeding (χ^2 = 16.64, df = 3, P < 0.001) for *L. rosalia*, with the duration spent feeding in the Diurnal treatment being shorter compared with the Nocturnal (P = 0.029, Figure 5) and LLAN treatments (P = 0.002, Figure 5). None of the other treatments differed significantly (P ≥ 0.524). The feeding duration was not influenced by sex (χ^2 = 1.20, df = 1, P = 0.273) or the interaction between treatment and sex (χ^2 = 1.02, df = 3, P = 0.795).

For *O. angoniensis,* the duration of feeding was not significantly influenced by treatment (χ^2 = 2.52, df = 3, P = 0.471), sex (χ^2 = 0.18, df = 1, P = 0.672) and the interaction between treatment and sex (χ^2 = 0.85, df = 3, P = 0.839).

The duration of feeding was influenced by the treatment (χ^2 = 50.12, df = 3, P < 0.001) for *M. coucha*. The duration was shorter in the Diurnal treatment compared with the Nocturnal, LLAN and HLAN treatments (P < 0.001 for all, Figure 5). None of the other treatment comparisons were significant (P ≥ 0.339). The duration of feeding was not influenced by sex (χ^2 = 0.66, df = 1, P = 0.417) or the interaction between treatment and sex (χ^2 = 0.07, df = 3, P = 0.995).

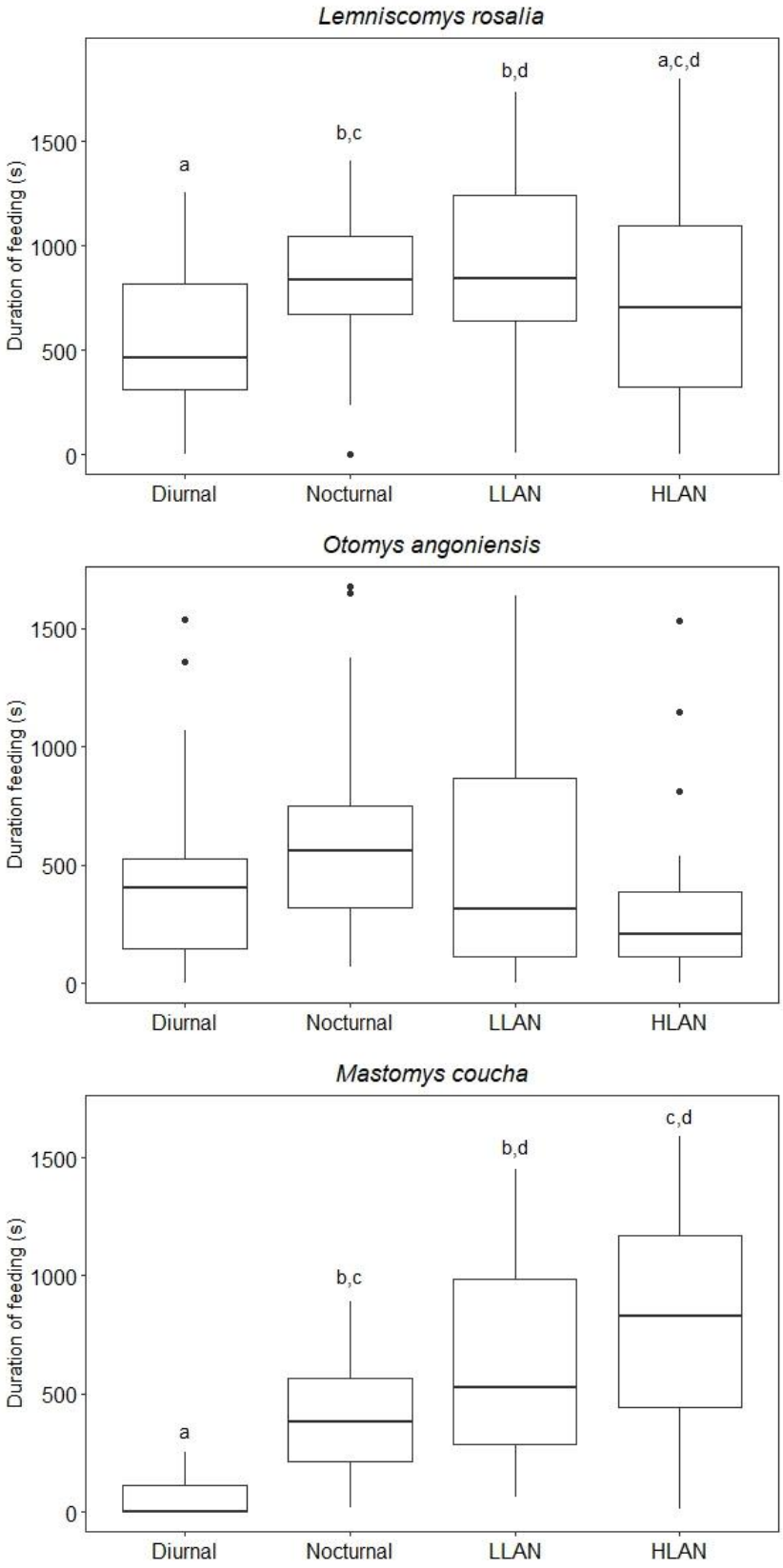


Figure 5. The duration of feeding (s) in each treatment for all the species. Horizontal lines are medians, boxes are 1st and 3rd interquartiles, whiskers are minimum and maximum values and dots are outliers. The same letters on the plots indicate non-significant differences between treatments. Plots without alphabets indicate no overall model statistical significance.

6.3.4 Frequency of feeding

For *L. rosalia,* the feeding frequency was influenced by the treatment ($\chi^2 = 12.76$, df = 3, P = 0.005). The frequency was higher in the Diurnal treatment compared with the HLAN treatment (P = 0.006, Figure 6) but none of the remaining comparisons were significant (P ≥ 0.063). Sex did not influence the frequency ($\chi^2 = 0.29$, df = 3, P = 0.590) and neither did the interaction between treatment and sex ($\chi^2 = 0.68$, df = 3, P = 0.878).

The frequency feeding was influenced by treatment ($\chi^2 = 9.73$, df = 3, P = 0.021) in *O. angoniensis,* but no differences were found in the posthoc comparisons (P ≥ 0.079). Both sex ($\chi^2 = 0.09$, df = 1, P = 0.765) and the interaction ($\chi^2 = 0.40$, df = 3, P = 0.941) did not influence the foraging frequency.

Treatment influenced the frequency of feeding ($\chi^2 = 33.88$, df = 3, P < 0.001) for *M. coucha*. Feeding frequency during the Diurnal treatment was significantly lower than the Nocturnal, LLAN and HLAN treatments (P < 0.001, Figure 6). None of the remaining treatment comparisons were significant (P ≤ 0.323). The frequency was not influenced by sex ($\chi^2 = 3.51$, df = 1, P = 0.061) or the interaction term ($\chi^2 = 1.02$, df = 3, P = 0.797).

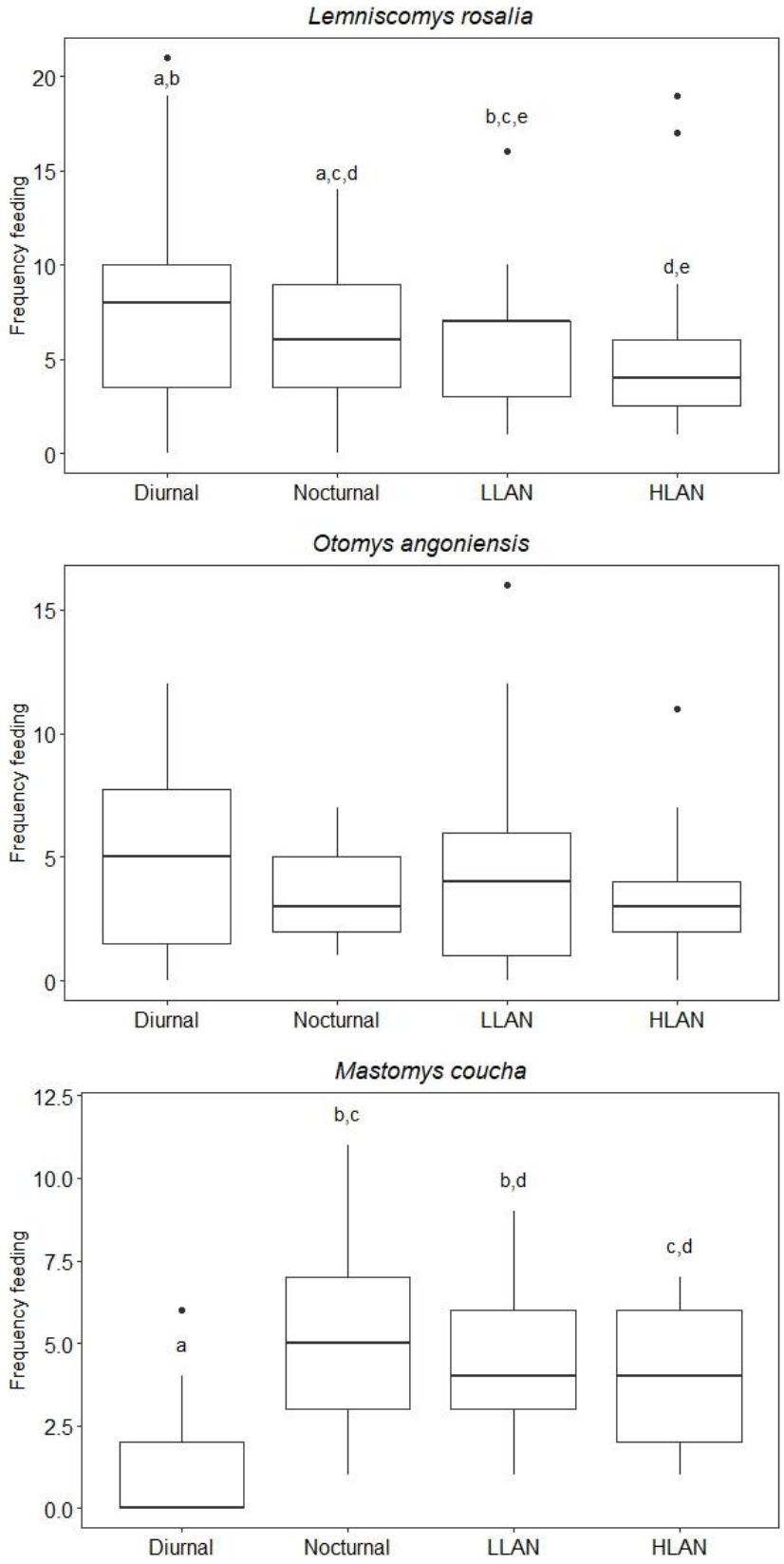


Figure 6. The frequency feeding during each treatment for all species. Horizontal lines are medians, boxes are 1st and 3rd interquartiles, whiskers are minimum and maximum values and dots are outliers. The same letters on the plots indicate non-significant differences between treatments. Plots without alphabets indicate no overall model statistical significance.

6.3.5 Risk-sensitive foraging

Under the Diurnal treatment, *L. rosalia* showed risk-sensitive foraging with regard to the duration of feeding. Foraging bouts were shorter during the Diurnal treatment compared with the Nocturnal and LLAN treatments, indicating risk aversive behaviour. Similarly, *L. rosalia* showed a risk-averse foraging strategy in the Diurnal treatment since it made multiple feeding bouts, possibly to reduce the time exposed while eating.

No risk-sensitive foraging was observed in *O. angoniensis* between treatments with the duration of feeding. Similarly, *O. angoniensis* did not show risk-sensitive foraging with regard to the frequency of feeding.

During the Diurnal treatment, *M. coucha* spent the shortest duration feeding, indicating risk-averse behaviour compared with the rest of the treatments. Likewise, *M. coucha* showed significant risk-sensitive foraging between treatments, employing a risk-averse strategy by making fewer feeding bouts in the Diurnal treatment compared with the three remaining treatments.

6.3.6 Duration spent in the shelter

Treatment influenced the duration spent in the shelter ($\chi^2 = 10.08$, df = 3, P = 0.018) for *L. rosalia*, which was longer in the Diurnal treatment than the Nocturnal treatment (P = 0.009, Figure 7). Time spent in the shelter did not differ significantly between the other treatments ($P \geq 0.232$). The duration was not influenced by sex ($\chi^2 = 0.67$, df = 1, P = 0.413) or the interaction between treatment and sex ($\chi^2 = 0.65$, df = 3, P = 0.884).

For *O. angoniensis,* the duration spent in the shelter was influenced by the treatment ($\chi^2 = 35.83$, df = 3, $P < 0.001$). Individuals spent more time in the shelter during the Diurnal treatment compared with the Nocturnal (P = 0.039), LLAN and HLAN treatments ($P < 0.001$ for both, Figure 7) and more time was spent in the shelter during the Nocturnal treatment than the HLAN treatment (P = 0.042, Figure 7). None of the remaining treatment comparisons were significant ($P \geq 0.066$). Sex did not influence the duration in the shelter ($\chi^2 = 2.56$, df = 1, P = 0.110). The interaction between treatment and sex significantly influenced the duration in the shelter ($\chi^2 = 9.82$, df = 3, P = 0.020, Figure 8). In the Nocturnal treatment, females spent more time in the shelter than males (P = 0.020, Figure 8). Females spent more time in the shelter during the Nocturnal treatment compared with the LLAN (P = 0.045) and HLAN treatments (P = 0.006, Figure 8), also during the Diurnal treatment compared with the LLAN (P = 0.006) and HLAN treatments (P = 0.001, Figure 8). Males spent more time in the shelter during the Diurnal treatment compared with the Nocturnal (P = 0.008), LLAN (P = 0.004) and HLAN treatments (P = 0.013, Figure 8). None of the remaining comparisons were significant ($P \geq 0.998$).

The duration spent in the shelter was not influenced by treatment (χ^2= 2.38, df = 3, P = 0.497) in *M. coucha*, sex (χ^2 = 0.05, df = 1, P = 0.822) nor the interaction between treatment and sex (χ^2 = 0.42, df = 3, P = 0.937).

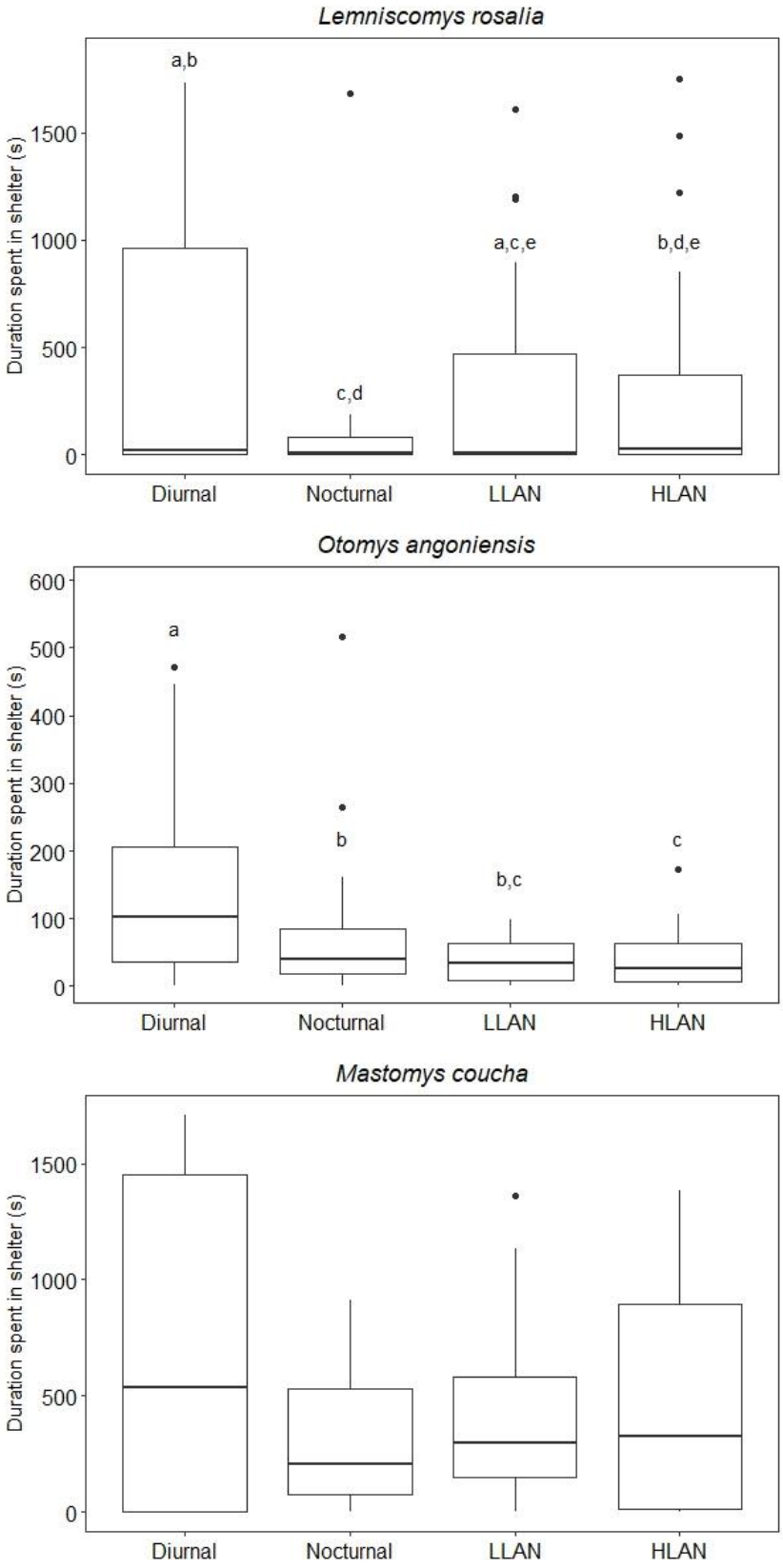


Figure 7. The duration spent in the shelter (s) in each treatment for all species. Horizontal lines are medians, boxes are 1st and 3rd interquartiles, whiskers are minimum and maximum values and dots are outliers. The same letters on the plots indicate non-significant differences between treatments. Plots without alphabets indicate no overall model statistical significance.

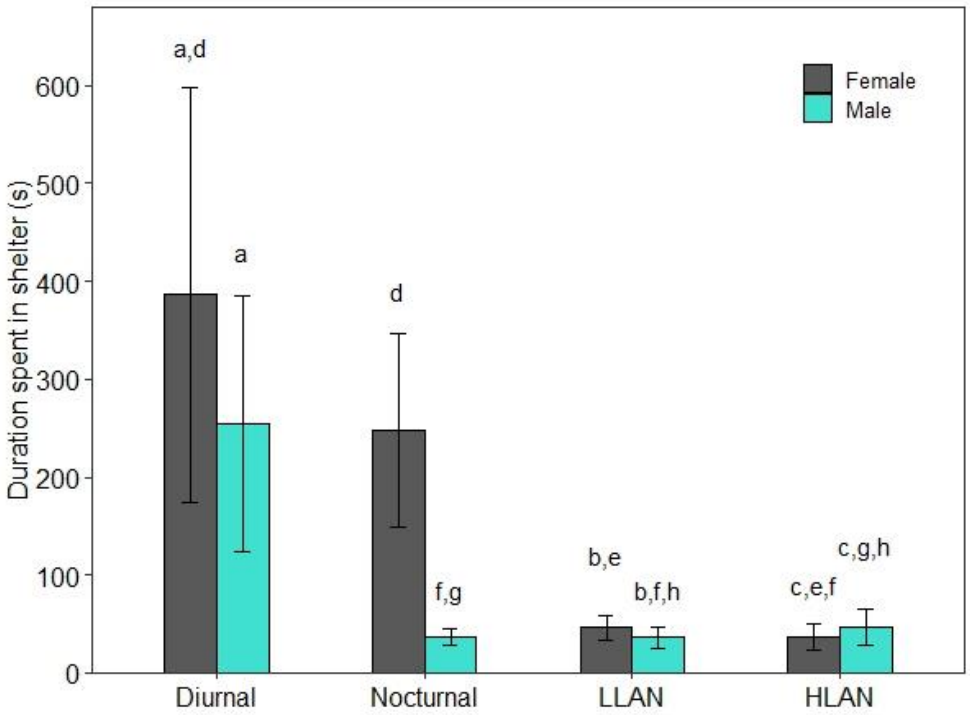


Figure 8. The duration spent in the shelter (s; mean ± SE) in the different treatments for *O. angoniensis* females and males. The same letters on the plots indicate non-significant differences between treatments.

6.3.7 Mass of seeds consumed

For *L. rosalia,* treatment influenced the mass of seeds consumed (χ^2 = 12.13, df = 3, P = 0.007). More seeds were consumed in the Nocturnal (P = 0.036) and the LLAN treatments (P = 0.22) than the Diurnal treatment (Figure 9). None of the other treatments differed significantly from each other (P ≥ 0.106). The mass of seeds consumed was not influenced by sex (χ^2 = 0.62, df = 1, P = 0.430), nor the interaction between treatment and sex (χ^2 = 0.70, df = 3, P = 0.874, Figure 9).

The mass of seeds consumed were influenced by treatment (χ^2 = 26.11, df = 3, P < 0.001) in *O. angoniensis*. There were less seeds consumed in the Diurnal treatment compared with the Nocturnal (P = 0.004), LLAN (P < 0.001) and HLAN treatments (P = 0.014, Figure 9). None of the remaining treatment comparisons were significant (P ≥ 0.203). The mass of seeds consumed was not influenced by sex (χ^2 = 1.57, df = 1, P = 0.211) or the interaction term (χ^2 = 0.12, df = 3, P = 0.990).

Treatment influenced the mass of seeds consumed (χ^2 = 862.78, df = 3, P < 0.001) for *M. coucha*, a lower mass of seeds was consumed in the Diurnal treatment compared with the Nocturnal, LLAN and HLAN treatments (P < 0.001 for all, Figure 9). None of the remaining treatment comparisons were significant (P ≥ 0.999). The mass was not influenced by sex (χ^2 = 0.06, df = 1, P = 0.807) or the interaction between treatment and sex (χ^2 = 0.75, df = 3, P = 0.862).

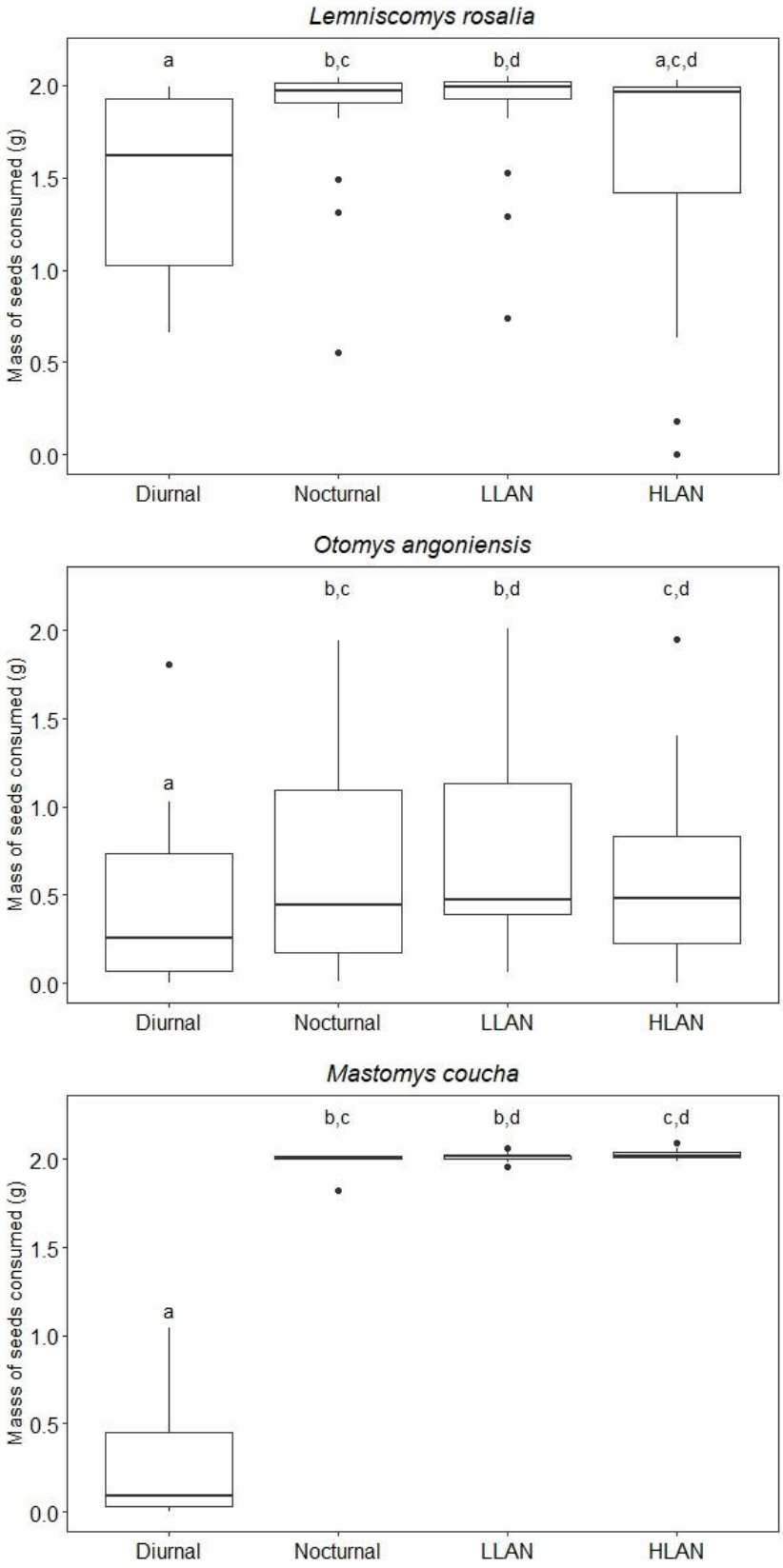


Figure 9. The mass of seeds that were consumed over after each treatment (g) for each species. Horizontal lines are medians, boxes are 1st and 3rd interquartiles, whiskers are minimum and maximum values and dots are outliers. The same letters on the plots indicate non-significant differences between treatments.

6.4 Discussion

ALAN continues to spread into previously non-urban areas, exposing wildlife to novel cues that could negatively affect them and ultimately influence their fitness. Here, I investigated the foraging behaviour of one crepuscular rodent, one rodent with reportedly variable activity and one nocturnal rodent, under different active time and ALAN treatments. Light at night can increase both the actual and perceived risk of predation by rodents (Lima and Dill, 1990; Rotics et al., 2011), which adds to the importance of understanding prey responses under ALAN.

6.4.1 Lemniscomys rosalia

Lemniscomys rosalia showed risk aversive behaviour during the Diurnal treatment compared with the other treatments. One explanation for this could have been the level of satiety, since *L. rosalia* could have been more satiated in the Diurnal treatment, not needing to act risk-prone, compared with the other three treatments that occurred at night. Hunger is a strong motivator to feed but acts in a complex manner together with many other variables, such as risk and fear (Burnett et al., 2016). Thus, *L. rosalia* could have been more hunger-motivated during the three nocturnal treatments compared with the Diurnal treatment. Similarly, laboratory rats also showed a lack of motivation to forage in risky environments when they had a sufficient satiation level (Meng et al., 2024).

I also observed some risk-aversive behaviours during the Nocturnal treatment, mainly regarding the time it took *L. rosalia* to start moving and approach the seeds. The level of exposure to risks could have played a role, since my experimental setup had a single refuge, and the mice were forced to forage in the open. This species has been reported to prefer feeding under shrubbery with cover and its foraging effort decreased along with decreased vegetation cover (Loggins et al., 2019).

An alternative explanation could be that the experimental setup was novel (Zambetti et al., 2019). In the Diurnal treatment, *L. rosalia* was exposed to the experimental arena for the first time, since it was tested for seed preference in a separate arena. Thus, there could have been some hesitation in the Diurnal treatment because of the unfamiliar environment, which became more familiar with repetition (i.e., across treatments). A laboratory-based study on C57bl/6 mice showed that their knowledge gained from previous tests made it faster for them to locate the source in subsequent tests (Gire et al., 2016). Thus, repeated exposure to an arena can assist with the novelty and uncertainty of the test area over time. *Lemniscomys rosalia* could also have been more sensitive to the novelty of the experiment compared with the two nocturnal species who did not follow a similar pattern. Additionally, free-living *L. rosalia* is active during the day, and thus it is possible that its risk-averse behaviour was driven by an alternative factor other than risk from level of light experienced in the Diurnal treatment. For example, individual differences could have played a role, since the test animals were wild-caught

and underwent different risks and experiences which formed part of their foraging decisions in this experiment (Finch et al., 2020; Eccard et al., 2020).

6.4.2 Otomys angoniensis

Overall, *O. angoniensis* displayed risk aversive responses for half of the recorded behaviours (the latency to approach seeds, duration in the shelter and mass of consumed seeds) during the Diurnal and Nocturnal treatments and risk-prone responses in the LAN treatments. However, the lack of differences between treatments for the remaining half of recorded behaviours (latency to move, duration of feeding and frequency of feeding) could suggest a propensity towards it being risk averse. The lack of differences between treatments for these recorded behaviours could indicate that *O. angoniensis* perceived all treatments as similar in terms of risk level. Thus, behaving risk-averse and taking a similar amount of time before moving in each treatment and spending a comparable amount of time feeding in all treatments in order to remain safe.

Strictly nocturnal European hedgehogs (*Erinaceus europaeus*) also did not change their general activity or feeding activities at supplemental food trays under ALAN, apparently because of individual differences (Finch et al., 2020). *Otomys angoniensis* occupies habitats with dense vegetation (Skinner and Chimimba, 2005), which offers protection from predators. In captivity, this species may have perceived the reduced level of cover as a risk. The level of illumination affects perceived risk and resulting foraging behaviour, but the level of vegetation cover (i.e., the level of exposure in the habitat) has three times the influence on rodent foraging behaviour compared with moon illumination (Prugh and Golden, 2014). Other studies have shown that perceived threats influence prey behaviour even more than actual predation threats (Loggins et al., 2019; Verdolin, 2006). For example, reduced vegetation height increased the perceived predation risk in fat mice (*Steatomys pratensis*) and a similar pattern was seen in African pygmy mice (*Mus minutoides*) with reduced ground vegetation cover (Loggins et al., 2019). Likewise, Oldfield mice (*Peromyscus polionotus*) left more food in exposed foraging trays compared with covered foraging trays, because of the higher perceived predation risk (Orrock, 2004). Foraging behaviour in common voles (*Microtus arvalis)* differed under covered and open foraging patches, but there was high individual variation in behaviour and the individual responses resulted in dissimilar food rewards (Eccard et al., 2020). Some of these individual differences were attributed to the use of wild-caught *M. arvalis,* since each individual could have had different experiences with both actual and perceived predation risks that informed their foraging choices in the experiment (Eccard et al., 2020). Similarly, *O. angoniensis* was wild-caught and all the individuals possibly had their own experiences with predation, and the observed behaviour in this study could have been attributed to past experiences. The behaviour of *O. angoniensis* could be multifactorial and include its adaptive flexibility, the level of exposure when feeding, the hunting strategies of its predators, and individual experiences.

It is also possible that some of the rats chose not to make certain foraging decisions, resulting in a lack of statistical significance in some of the behaviours observed.

6.4.3 Mastomys coucha

At first glance, *M. coucha* appears to show risk-aversive behaviour in the Diurnal treatment, but when considering the active period of *M. coucha* (nocturnal), it was mostly just inactive rather than making an active foraging choice. I also found no differences among the nocturnal treatments, with different light intensities, suggesting that ALAN did not pose as big a risk as expected. *Mastomys coucha* occupies burrows and is a post-burn pioneer that has been described as one of the most adaptable African rodents (Kingdon, 2013). Thus, they are accustomed to being in open and exposed areas (Monadjem and Perrin, 1998) and have managed to adapt to increased visibility in these post-burn environments, most likely using burrows (Skinner and Chimimba, 2005). Similar to *M. coucha,* the closely-related *M. natalensis,* also increases its activity with decreasing vegetation height (Loggins et al., 2019); less cover correlates to increased predation risk (Mandelik et al., 2003). Likewise, *M. natalensis* assessed the direct predation risk and made foraging decisions regarding where to feed; it did not show a difference when foraging in an open or covered patch under control conditions, but in areas with avian predators, it showed increased foraging activity under covered patches (Mohr et al., 2003).

Contrary to my results, strictly nocturnal species decrease their overall activity, along with their foraging behaviours, under both full moon (measuring approximately 2 Lux) (Kotler et al., 2010; Orrock, 2004; Prugh and Golden, 2014) and ALAN (higher than 2 Lux) (Shier et al., 2020; Shuai et al., 2023; Zhang et al., 2020). The foraging behaviour of a Northern hemisphere pioneer species, the wood mouse (*Apodemus sylvaticus*), was not significantly influenced by indirect risks, such as moonlight (Díaz et al., 2005). Similar to *M. coucha,* Diaz et al. (2005) found that *A. sylvaticus* was more abundant in postfire areas, and their predators, small-spotted genets (*Genetta genetta*), were confined to the woodlands; such reduced overlap between the species is apparently a consequence of mice responding to direct predation risk. Thus, *M. coucha* could have used the combination of the available shelter and lack of direct predation risk to their advantage and showed little difference in foraging between the Nocturnal and ALAN treatments. *Mastomys coucha* could be better equipped to assess direct predation risks, similar to the closely-related *M. natalensis* (Mohr et al., 2003), which could be less advanced in *L. rosalia* and *O. angoniensis.*

6.4.4 Sex differences

Since both males and females have to gain energy by foraging, I predicted no sex differences in any of the species in this study. I found few instances of sex-specific foraging behaviour. Only *O. angoniensis* showed some sex differences. Overall, females seemed to be more hesitant in high risk open areas where the food was located, similar to laboratory rats (Zambetti et al., 2019).

6.5 Conclusions

Artificial light at night influences humans and wildlife, but we require more information on a wide range of animal groups and how they respond to ALAN. I investigated the foraging behaviour of three understudied African rodent species with different activity periods. *Lemniscomys rosalia* showed normal foraging behaviour in the ALAN treatments, as expected, but its possible risk-averse behaviour in the Diurnal treatment could have been because of satiety, novelty or individual differences instead of level of perceived risk. The lack of significantly different responses across treatments of *O. angoniensis* was contrary to other nocturnal animals, but it could have been related to its preference towards dense vegetation or because of individual differences based on experience. Similarly, *M. coucha* showed behaviour different from what was expected, which could have been a result of it naturally occurring in bare environments. Foraging decisions are influenced by a range of variables other than the level of satiation and level of exposure or risk and it is important to consider competing and synergistic factors. A rapidly changing environment will test the limits of adaptation of species. My study indicated the three species responded differently, possibly linked to their biology and perception of risk. ALAN is likely to expose them to novel cues and challenges.

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Chapter 7: General Discussion

Urbanisation and the associated artificial light at night (ALAN) have been thoroughly studied in some respects, although there are still many species in different ecosystems that have received less attention. Studies on ALAN and African species are scarce and presently we have to extrapolate the effect of illuminated nights from information on Northern hemisphere species that may have different biologies and environments. Additionally, ALAN studies on wild-caught species are lacking. The majority of rodent studies assessing the effect of ALAN are laboratory biased, and past research indicates that laboratory-based results cannot always be extrapolated to wild populations (Ackermann et al., 2020; Kronfeld-Schor et al., 2013). Free-living animals are exposed to a multitude of biotic and abiotic factors that can interact in complex ways, which can never accurately be simulated in the laboratory. These include biological factors, such as conspecific and predator interactions combined with environmental factors, such as resource availability, weather, moon phase, and vegetation composition and density. The responses derived from laboratory animals are based on a limited and controlled set of environmental variables and can potentially reveal patterns and responses different to a natural environment (Kronfeld-Schor et al., 2013). I attempted to close this knowledge gap by investigating different aspects, including activity and foraging behaviour, of several southern African rodents under natural conditions and in the laboratory to better understand their behaviour and responses to environmental changes, such as ALAN.

My research focussed on southern African rodent species with a range of different biologies. Overall, African rodent species are understudied (Viljoen and Oosthuizen, 2023), with most of the information gained from anecdotal field observations. Furthermore, several African rodents, such as *Otomys* and *Mastomys spp*., are morphologically indistinguishable from congenerics, and for many species, identification still requires genetic confirmation (Monadjem et al., 2015). Therefore, some of the anecdotal field observations could have been assigned to a specific species, but we know that some species are cryptic and require genetic confirmation. Thus, the observations could have been assigned to the wrong species based on external morphological characteristics. For example, a previous study on the activity patterns of vlei rats which were identified morphologically as *O. irroratus,* indicated that it was crepuscular in the laboratory but was both diurnal and nocturnal in nature (Davis, 1972). However, more recently *O. irroratus* was split into *O. irroratus* and *O. auratus* with differences in ecology, chromosomal number and molecular grounds (Engelbrecht et al., 2011; Taylor et al., 2009) and also possibly different temporal niches which is still to be confirmed.

One of my study species, *Lemniscomys rosalia,* is particularly understudied apart from some mark-recapture studies (Monadjem and Perrin, 1997; Saanya et al., 2022) and laboratory studies (Scott and Meester, 1988). A number of general field observations have also been recorded, yet these studies

are mostly biased towards *L. griselda* (Kern, 1981; Swanepoel, 1972). Very little is known about the behaviour of *L. rosalia,* and the lack of knowledge makes it difficult to relate experimental findings to its biology, as have been done in other studies. Linking experimental results to what is known about species biology and behaviour is common in Northern hemisphere studies, which include studies on bank voles (*Myodes glareolus*) (Hoffmann et al., 2018), field voles (*Microtus agrestis*) (Ergon et al., 2011) and striped hamsters (*Cricetulus barabensis*) (Wen et al., 2018).

7.1 Objectives and key findings of my study

I aimed to ascertain how ALAN impacted African rodents, following three broad areas. 1) I assessed whether natural light at night (i.e., lunar cycle) and ALAN influenced the abundance and composition of small mammals at two locations with different levels of exposure to ALAN (Chapter 2). 2) I investigated how different natural, laboratory and ALAN experimental treatments affected the activity of *L. rosalia* (reportedly diurnal and crepuscular; Chapter 3), *O. angoniensis* (apparently active during all times of the day; Chapter 4) and *M. coucha* (nocturnal; Chapter 5). 3) I studied the risk-sensitive foraging behaviour of the three rodent species by investigating their foraging behaviour under a range of natural and artificial lighting treatments (Chapter 6).

In Chapter 2, I investigated whether the abundance and composition of small mammal populations differed over the lunar cycle across seasons and the concurrent influence of geographic location of the trapping sites (facing toward or away from Johannesburg). My results revealed a higher species composition and abundance on the Light site (facing Johannesburg) compared with the Dark site (faced away from Johannesburg). Interestingly, I captured more small mammals on the Light site. This was unexpected since previous research has shown less activity in brighter areas because of increased visibility and thus a greater predation risk (Kotler et al., 2010; Prugh and Golden, 2014). The Light site was more densely vegetated and had a higher abundance of trees than the Dark site. The dense vegetation on the Light site could provide microrefugia, which could reduce or block the effects of ALAN. As I anticipated, the abundance and composition on both sites decreased around full moon and increased around new moon. This is congruent with past studies that reported that the predation risk was much higher with the improved visibility for predators under a full moon and the prey species reduce their activity (Clarke, 1983; Kotler et al., 2010; Perea et al., 2011). I was unable to measure the brightness of ALAN on either of the sites with a handheld light meter, so it is possible that ALAN was less intense than the 2 Lux of a full moon.

Although both the sites contained a diverse community (diversity indices data), there were seasonal differences. The Light site had a higher small mammal diversity in winter, while the Dark site had a higher diversity in autumn (animal abundance and species composition could not be assessed on the

Dark site during winter because it burned down). This was contrary to expectation, since small mammals are more often higher in abundance during summer and spring breeding season. Breeding results in greater recruitment of new individuals into the populations (Meheretu et al., 2015). The higher capture rate in winter could have been attributed to the reduced resource availability in the dry season attracting rodents to baited traps. In the resource abundant summer and spring (Shilereyo et al., 2023), the small mammals on the study sites were possibly not as dependent on the bait provided by the traps in preference to the available natural food (Adler and Lambert, 1997; Aplin et al., 2003). Overall, this study illustrated the importance of mark-recapture studies in a variety of locations and under different conditions, since it is still unclear to what extent ALAN can influence natural African small mammal species individually and at a population level.

In Chapter 3, I confirmed the active period of *L. rosalia.* My results revealed that this species displays more nocturnal activity under laboratory conditions, but the active hours were clustered around twilight times, suggesting crepuscular activity. Subsequently, I compared the activity of this species in the laboratory, under natural conditions and ALAN treatments and found that its highest activity was when it was exposed to natural conditions. The difference in light intensity between the natural environmental conditions and the overhead laboratory lights could have influenced this difference in activity levels. Alternatively, exposure to natural temperature cycles tend to reinforce activity rhythms, as was the case in Mahali mole-rats (*Cryptomys hottentotus mahali*) (van Jaarsveld et al., 2019). Van Jaarsveld et al. (2019) found more robust activity patterns (i.e., more precise rhythms) under simulated natural temperature fluctuations in the laboratory. The changes in natural sounds (for example bird songs and anthropogenic noises vary throughout the day and night) in the natural treatment could also have contributed to the higher activity, since these sounds were completely absent in the laboratory and ALAN treatments. As anticipated, *L. rosalia* reduced its activity under ALAN, possibly because of increased perceived risk of predation (Ditmer et al., 2021) or because of interrupted sleep, which could have resulted in depressive-like behaviours (Fonken et al., 2009). I found that males were more active than females. This is similar to the known pattern of polygamous rodent males, which explore more to find mates and are thus more frequently exposed to threats than females, who mostly remain in their territories (Jolles et al., 2015). My results showed that both sexes decreased their activity under ALAN, suggesting that when exposed to continuous ALAN, it is too risky for both sexes.

Using the same design as for *L. rosalia*, I studied how *O. angoniensis* responded to different experimental treatments, including natural, laboratory and ALAN treatments (Chapter 4). I ascertained that *O. angoniensis* showed nocturnal activity in captivity, and this was further supported by the timing of their capture, mostly confined to early dawn. This species also showed the highest activity in the natural treatment, indicating the importance of both light and temperature fluctuations to the circadian rhythm of this species. Similarly, Namaqua rock mice (*Micaelamys namaquensis*) and four-striped mice (*Rhabdomys dilectus*) were influenced by light and temperature changes (Ackermann et al., 2020).

Otomys angoniensis did not show a significant change in activity levels between the laboratory and ALAN treatments, which was puzzling. Normally, nocturnal rodents decrease their activity levels significantly under ALAN, both in the laboratory and in nature (Bedrosian et al., 2013; Spoelstra et al., 2015; Viljoen and Oosthuizen, 2023). My findings could be as a result of species-specific differences, such as their flexibility in active times, since their activity seems to depend on prevailing conditions (Davis, 1972), which could mean that dim light is not as influential to vlei rats. Another possibility could be that the cage layout was bare compared with their natural environment. *Otomys angoniensis* is found in densely vegetated areas, where it is often preyed upon by aerial predators, such as owls (Kingdon, 2013; Skinner and Chimimba, 2005), which detect moving prey (Mandelik et al., 2003). Bare surroundings could indicate high risk environments for species often preyed upon by predators with specialised hunting strategies, thus the bare cage layout could have been too risky to be more active than necessary. I found no sex differences. My results seem to indicate that this species could experience difficulty to adapt to increased use and intensity of light at night in combination with a barer environment and could show population declines closer to urbanised areas.

In Chapter 5, I investigated the influence of different experimental treatments on the activity of *M. coucha.* My results confirmed the nocturnality of this species, but it disproved my prediction of the mice being more active under natural conditions, as seen in the other two study species. *Mastomys coucha* showed the highest activity under the laboratory conditions. ALAN influenced the activity of *M. coucha* significantly, decreasing it by more than 50% from the laboratory activity to the activity under ALAN. This was in line with previous literature of nocturnal rodents under ALAN, such as Patagonian leaf-eared mice (*Phyllotis xanthopygus*) (Kramer and Birney, 2001), wood mice (*Apodemus sylvaticus*) (Spoelstra et al., 2015) and African pygmy mice (*Mus minutoides*) (Viljoen and Oosthuizen, 2023). My results also confirmed that the intensity of ALAN influenced the activity of *M. coucha*, with a further 25% decrease in activity under a closer light source. I also found that males were more active than females, similar to the pattern seen in *L. rosalia.* However, where both sexes of *L. rosalia* showed a similar response under remote ALAN, *M. coucha* males were still more active under remote ALAN but the results suggest that direct ALAN became too risky for them. This could indicate species-specific sex differences, yet we lack information on rodent sex differences in most research areas (Beery, 2018). Due to the adaptability of this species and the high reproductive output, *M. coucha* plays a key role in the recovery of post-burn environments. My results show that this species cannot respond predictably to all circumstances. However, if habitat transformation and ALAN continue to expand, this species could face serious fitness consequences which can impact its survival.

My last data chapter focussed on how ALAN influenced the risk-taking during foraging in *L. rosalia, O. angoniensis* and *M. coucha,* giving insight into how they perceive risky environments (Chapter 6). I investigated the risk-sensitive foraging behaviour under four treatments. A Diurnal treatment was included, since it was outside of the normal activity range of all three species in captivity.

In addition, a completely Nocturnal (i.e., dark) treatment represented the normal foraging time for two species and a riskier time for the crepuscular species. To assess the influence of ALAN, I included a low and high intensity treatment, which would represent the normal light intensity of *L. rosalia* (low intensity corresponded to early morning and evening whereas the high intensity treatment was representative of the later morning and afternoons) and represent artificial light sources to the nocturnal species. I found that the Diurnal treatment was the riskiest for *L. rosalia* and that its behaviour could have been driven by the level of hunger (Burnett et al., 2016; Meng et al., 2024) when the testing occurred or the level of exposure, since there was limited refuges. Alternatively, the novelty of the experiment could have had an influence (Zambetti et al., 2019), if this is the case, novelty is more influential to *L. rosalia* compared with the other two species. The results for *O. angoniensis* were complex and I suggested that the main reason for the risk-averse behaviour is the design of the experiment and the biology of this species. My design, for purposes of filming and observing the test subject, was very bare and exposed and this species seem to depend on the cover of vegetation immensely (Skinner and Chimimba, 2005). Similar to *O. angoniensis,* the dwarf-striped hamster (*Cricetulus barabensis*), which is also found within dense vegetation, showed a significant reduction in activity and experienced weight loss when exposed to ALAN and short vegetation (Shuai et al., 2023). The reduction in activity in *O. angoniensis* in both experiments (Chapters 4 and 6) suggest that this species will face difficulty under ALAN if they lose their preferred habitat. Lastly, I found that *M. coucha* was inactive during the daytime, but it unexpectedly was not influenced by the ALAN treatments. This was surprising after recording how their activity decreased under the ALAN treatments (Chapter 5). It is possible that this species had the enhanced ability to detect direct predator threats (which were absent from this experiment) similar to the closely related *M. natalensis* (Mohr et al. 2003), which could have been less developed in the other test species. I anticipated no sex differences, since both sexes have to feed, but I found sex differences for *O. angoniensis* only. This finding was surprising since it did not show any sex differences in activity (Chapter 4). This chapter revealed that not all animals follow the expected trend of behaviour under risky conditions, and we should investigate further to have a better understanding of the driving forces behind this behaviour and what the most important influencing factor is to each species.

7.2 ALAN and African rodents

Despite the high biodiversity of African species and the accelerated urbanisation of the growing cities in Africa, very little has been done in terms of how African species interact with urbanisation related

consequences, such as ALAN. Both community level and species-specific studies are required to investigate ALAN (Sanders et al., 2023), and both are scarce in African-based studies.

The abundance and composition of the nocturnal small mammals resulted in unexpected findings with regards to ALAN (Chapter 2). Generally, nocturnal rodents avoid ALAN by reducing their activity to remain safe from predation (Hoffmann et al., 2019; Rotics et al., 2011; Shuai et al., 2023), but there could be confounding factors involved in my study that I did not consider in my analysis. The abundance and composition changed across the lunar cycle which was predictable (Chapter 2) and similar to past studies, suggesting that different species of rodents react predictably to the lunar cycle globally, since both Northern (Clarke, 1983; Kotler et al., 2010; Mandelik et al., 2003; Perea et al., 2011) and Southern hemisphere (Chapter 2) species showed similar responses across the lunar cycle. The small mammals in the two study populations could indicate some flexibility to their response towards low light levels, but this could change if there are higher levels of ALAN or increased habitat transformation.

Limited studies have been done on crepuscular species and their response to ALAN, but crepuscular species are thought to extend their activity into the night under ALAN (Russart and Nelson, 2018; Seymoure et al., 2023). However, *L. rosalia* was more nocturnal and decreased activity under ALAN (Chapter 3). Thus, *L. rosalia* could perceive the illuminated areas as risky and experience an increase in predation risk similar to strictly nocturnal species. The behaviour of *L. rosalia* seems to suggest that they are flexible to an extent, they reduce activity under remote ALAN (Chapter 3) but continue to forage under dim ALAN sources (Chapter 6). This species could become selective in the areas it utilises, by preferring less illuminated and more covered spaces and it can also experience an increase in competition with strictly nocturnal species (Sanders et al., 2023; Seymoure et al., 2023).

Unexpectedly, the nocturnal *O. angoniensis* did not reduce its activity under ALAN (Chapter 4), as found in other nocturnal species, such as African pygmy mice (*M. minutoides*) (Viljoen and Oosthuizen, 2023) and Siberian hamsters (*Phodopus sungorus*) (Bedrosian et al., 2013). The foraging behaviour of *O. angoniensis* (Chapter 6), was also unlike past responses from nocturnal species (Shier et al., 2020; Shuai et al., 2023), possibly because it has more flexible temporal niches. However, ALAN did not have an overall impact on the activity and foraging behaviour of nocturnal European hedgehogs (*Erinaceus europaeus*), owing some of the alternate responses to individual differences (Finch et al., 2020), which could be an explanation for the *O. angoniensis* results. Thus, *O. angoniensis* could show these unpredictable behaviours because of differences in habitats where the different nocturnal species occur. Alternatively, this species could show fixed responses to the changing environment, resulting in maladaptive behaviour and possible survival consequences.

Predictably, the nocturnal *M. coucha* reduced its activity under ALAN (Chapter 5), similar to past nocturnal rodent species, both from the Northern hemisphere (Bedrosian et al., 2013; Hoffmann et

al., 2018) and from the Southern hemisphere (Kramer and Birney, 2001; Viljoen and Oosthuizen, 2023). However, this species did not show differences in foraging under completely unlit or lit conditions (Chapter 6), unlike other nocturnal species (Shier et al., 2020; Shuai et al., 2023). This suggests some flexibility in the behaviour of *M. coucha* under ALAN, which is to be expected of a generalist and a pioneer species (Skinner and Chimimba, 2005).

7.3 Implications of my research and future study areas

My study has now confirmed the circadian biology of three understudied southern African rodent species, and this made preliminary predictions to an illuminated nightscape possible. There are a limited number of the diverse group of southern African rodents that have been investigated with regards to their circadian biology under controlled laboratory conditions, including African pygmy mice (*M. minutoides*) (Hoole et al., 2017; Viljoen and Oosthuizen, 2023), four-striped field mice (*R. dilectus*) and Namaqua rock mice (*M. namaquensis*) (Ackermann et al., 2020), spiny mice (*Acomys spinosissimus*) (Hoole et al., 2012) and vlei rats (*O. irroratus*) (Davis, 1972). In one study, six *R. pumilio* individuals were in an outside enclosure under natural conditions (Schumann et al., 2005). The lack of studies investigating behaviour in an outdoor enclosure is not limited to southern Africa but is a global issue.

My research adds to the body of knowledge on African rodents. My study was the first to investigate lunar and ALAN effects on population composition and abundance in South Africa. Similar to Northern hemisphere studies, I found a significant difference across the lunar cycle, but I could not detect a significant influence of ALAN. My results indicate that African small mammals are sensitive to differences in light levels and surrounding conditions, such as available cover plays an important role in the interpretation of light as an indication of predation risk. Additional mark-recapture studies are required to focus on the influence of ALAN on small mammal populations. By conducting more of these studies, we could gain clarity on the factors acting with ALAN to influence small mammal populations. For example, the distance to the ALAN source, vegetation composition and density closest to the source and use of burrow systems and rodent highways as ways to escape increased visibility. Furthermore, we require more studies investigating how African species react to urbanisation and how microhabitats can play a role to counteract the effects of ALAN. Specifically, further investigation is needed into which microhabitats are the most effective against ALAN and whether these will remain available with a changing landscape.

I have also genetically confirmed the geographic location of two rodent species which adds to the existing knowledge of the distributional range of the different species. Most of the available literature on African rodents is based on general field observations conducted on sister species that are morphologically indistinguishable and could be the reason for unclear behaviours and trends observed.

Limited data is available on the geographical distribution of genetically confirmed species, to which my results can add, and this will also continue to grow in future to provide a more robust distribution range for different species.

In addition, I have now validated the temporal niches of three species using both a traditional laboratory approach and a more realistic natural approach. This will form an important basis for understanding the behaviours of these species in future studies and be able to predict their responses to environmental change more accurately. Future studies could also improve the traditional experimental design for activity monitoring to possibly include more cover around the outside of the cage, which would make the rodents feel less exposed and allow them to display more natural responses to increased ALAN. Designing a more realistic design could prove beneficial, comparable to the addition of enriching materials to a cage, the resulting responses and recorded behaviours could be more natural. Alternatively, a choice test could be designed to give rodents a choice between light with a shelter providing cover and light without any form of cover.

As with any field-based study or study on wild-trapped animals, there were some challenges and limitations to the experiments. Unfortunately, the age of the live animals is hard to determine and could have had an influence on the behavioural responses due to the natural experiences they have lived through, and this is not easily quantified. Capturing and introducing small mammals to a novel environment can be stressful and this can have an influence on the results. However, I attempt to minimise stress by incorporating a period of acclimation and including enrichment materials to the cages, along with using a more natural experimental environment.

7.4 Conclusions

My study was the first to investigate many facets of ALAN in wild southern African rodents, which laid groundwork for future studies. All five of my data chapters showed the implications of light, both natural and artificial, on the behaviour of potential prey species. ALAN is a significant avenue for further study, since it is predicted to impact more than 50% of the terrestrial areas around the world by 2052 (Kyba et al., 2017; Seymoure et al., 2023). This means we have less than 30 years to understand how species perceive and react to ALAN and which of these species will be able to adapt to it and survive. Evolution occurs over long time scales and the rate at which ALAN is expanding is too fast for many animals to adapt. Thus, more effort is needed to gain more knowledge on our African species before we lose our rich diversity. We still lack basic knowledge on many of the African species including their space use patterns and tolerance toward hetero- and conspecifics, for example. However, as a result of my research, there is additional information on three understudied southern African rodents to

provide a foundation for future research into rodent behaviour in general and rodent responses to altered environments.

7.5 References

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Appendix

Appendix Table 1. The colour and its associated artificial brightness scores measured in µcd/m^2 which corresponds to the world maps. This table was adapted from Table 1 in Falchi et al. (2016) and the sky brightness measures were accessed at https://lightpollutionmap.info/.

Colour on the map	Artificial brightness (µcd/m^2)	Sky brightness (mag./arc sec2)
Black	< 1.74	22.0 - 21.8
Dark grey	1.74 - 3.48	
Grey	3.48 - 6.96	
Dark blue	6.96 - 13.9	21.8 - 21.5
Blue	13.9 - 27.8	
Light blue	27.8 - 55.7	
Dark green	55.7 - 111	21.5 - 21.2
Green	111 - 223	21.2 - 20.9
Yellow	223 - 445	20.9 - 20.4
Orange	445 - 890	20.4 - 19.4
Red	890 - 1780	
Magenta	1780 - 3560	
Pink	3560 - 7130	
White	> 7130	< 17.5

Appendix Table 2. The models considered for the number of animals and species caught. The parsimonious models are indicated with an asterisk and were chosen as the models with the lowest AICc and highest weight values.

Response variable: Density – i.e., Number of animals caught				
Model number	**Variable dropped**	**DF**	**AICc**	**Weight**
Model 1*	Saturated (Site + season + moon illumination)	6	673.40	1.00
Response variable: Composition – i.e., Number of species caught				
Model number	**Variable dropped**	**DF**	**AICc**	**Weight**
Model 1	Saturated (Site + season + moon illumination)	6	554.30	0.47
Model 2*	Season	3	554.10	0.53

Appendix Table 3. The contributions of each weather variable to five separate principal components (PCs). The eigenvalue, variance percentage and cumulative variance percentage of each PC is included, with the most representative PC (explaining over 50% of the variance and an eigenvalue larger than 1) indicated with an asterisk.

	PC1*	**PC2**	**PC3**	**PC4**	**PC5**
Minimum temperature	0.490	-0.035	-0.460	0.715	-0.190
Wind speed	0.142	0.949	-0.103	-0.170	-0.197
Cloud cover	0.561	0.006	-0.158	-0.283	0.762
Humidity	0.523	-0.298	-0.017	-0.540	-0.587
Precipitation	0.389	0.090	0.867	0.297	0.003
Eigenvalue	2.700	1.021	0.732	0.371	0.177
Variance %	54.006	20.413	14.638	7.413	3.530
Cumulative variance %	54.006	74.418	89.057	96.470	100.000

Appendix figures



Appendix Figure 1. The Light site facing Johannesburg on the Cradle Nature Reserve.



Appendix Figure 2. The Dark site facing the opposite direction from the Light site on the Cradle Nature Reserve.



Appendix Figure 3. The field laboratory on the Cradle Nature Reserve property.



Appendix Figure 4. The standard laboratory room with no external windows in which the laboratory and ALAN treatments were conducted.



Appendix Figure 5. The area where the study animals were kept during the natural environmental treatment.