Environmental effects on the circadian systems of a diurnal (*Rhabdomys dilectus*) and nocturnal (*Micaelamys namaquensis*) rodent species: with specific reference to light pollution.

by:

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Declaration

I, Simone Ackermann declare that the dissertation/thesis, which I hereby submit for the degree MSc Zoology at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature 

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Month Year September 2019

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Summary

Environmental effects on the circadian systems of a diurnal (*Rhabdomys dilectus*) and nocturnal (*Micaelamys namaquensis*) rodent species: with specific reference to light pollution.

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The presence of artificial light at night (ALAN) is one of many contributing factors to global change today. The spectral range of ALAN can also alter the potential effects of light pollution in certain contexts which creates an exceptionally complex cascade of impacts. The purpose of this thesis was to examine the interactions of various environmental factors including ALAN on biological variables, locomotor activity and corticosterone concentration, of two species of rodent. This was accomplished by manipulating the environmental factors; environmental enrichment, temperature and lighting in captivity. A pilot field study was also conducted in order to test the future feasibility of incorporating information garnered from the laboratory study into larger scale real world experiments. The two species were collected from the field and was subsequently subjected to various light cycles, during which locomotor activity was monitored and urinary corticosterone stress hormone was assessed. Results showed that *Micaelamys namaquensis*, a nocturnal species, reacted favourably to the addition of enrichment by increasing activity levels whereas *Rhabdomys dilectus*, a diurnal species decreased activity levels while improving the strength of entrainment. Both *M. namaquensis* and *R. dilectus* decreased activity during a light cycle which simulated natural dawn and dusk patterns of light. The two species reacted differently when a 24hr

ambient temperature cycle was introduced, with *M. namaquensis* increasing its locomotor activity and *R. dilectus* decreasing overall activity. *M. namaquensis* decreased its average activity in response to ALAN and did not show any difference in reaction towards different types of light at night. *R. dilectus* on the other hand increased its activity under ALAN but also showed no preference between different spectra of light at night. While corticosterone concentrations were monitored during all the environmental factor experiments, fluctuations in hormone concentrations were noted, however found to be statistically non-significant. Thus, only speculations could be made regarding the impacts of the various environmental factors on the stress physiology of *M. namaquensis* and *R. dilectus.* These results highlight the importance of considering species specific outcomes even under virtually identical circumstances. Understanding the impacts of environmental factors is crucial in order to extrapolate laboratory-based findings into real world experiments. This work can be used to further understand the impacts of different environmental factors on the circadian systems of nocturnal and diurnal rodent species as well as the potential implication of ALAN under various environmental conditions. In future, this can be combined into a large-scale field experiment in order to monitor the impacts of light pollution using the methodology elucidated during the pilot study. The results of this study show that the impacts of ALAN can be incredibly diverse and specific to the species in which they are examined

Chapter 1: General Introduction

The advent of artificial light, while having proven extremely beneficial for humankind, has come with a multitude of hidden costs to the surrounding environments and biodiversity (Plainis et al. 2006; Gaston and Bennie 2014; Gaston et al. 2015a). Many of these costs have become the focus of major research only in the last few decades. The presence of artificial light at night (ALAN) has pervaded the natural environment to such an extent that it has begun to alter the lengths of natural rhythms of day and night that animals and insects have evolved to for millennia (Gaston et al. 2012; Davies et al. 2013b). This intrusion is most obvious during night-time, eroding ecological darkness at levels far beyond the natural night-time light generated by celestial bodies. Over 30% of vertebrates and 60% of insects are nocturnal (Horváth et al. 2009) making the opportunities for ALAN to interfere with the natural cycles which govern their existence all the more likely.

Light pollution is generally accepted as the deleterious effects of light in spatial and temporal spaces where it has not historically occurred. ALAN arises from a multitude of sources; street lighting, advertising lighting, architectural lighting and security lighting to name a few (Gaston et al. 2013). Although most of these sources are found primarily within the urban landscapes of today's cities, and their direct effects are experienced most profoundly within urban areas, these sources have the ability to spread the effect of light pollution up to hundreds of kilometres outside the confines of cities (Kyba and Hölker 2013). This phenomenon is referred to as sky glow (Longcore and Rich 2004); it is caused by the reflection of direct illumination off surfaces commonly found in urban landscapes such as roads or glass windows into the atmosphere, were it is reflected back onto the surface of the Earth. This happens multiple times, and with each reflection the light is propagated through the environment further from its original source however, it will lose some energy with each reflection (Kyba et al. 2011).

The presence or absence of ALAN is not the only factor to consider when studying the potential impact of light pollution. Recent advancements in lighting technology has led to the creation of light emitting diodes (LED) as energy efficient alternatives to traditional lighting technologies (Schubert and Kim 2005; Pawson 2013). Traditional luminaires emitted light that is concentrated at the low energy and long wavelength end of the visible light spectrum (Elvidge et al. 2010), which is why they often appear yellow. However, with the

invention of LEDs the average spectrum of light emitted by luminaires in the environment showed a significant shift towards whiter, fuller spectrum light, which contains much larger proportions of shorter wavelengths blue and ultraviolet light (Spoelstra et al. 2015). This trend towards full spectrum white light has exacerbated many of the effects of light pollution (Davies et al. 2013a). Light produced by LEDs has much shorter wavelengths, which propagate through the environment much faster and with higher intensities than their long wavelength counter parts (Tan et al. 2012). This increases the radius and intensity of sky glow produced from the urban sources (Bierman 2012; Luginbuhl et al. 2014).

ALAN has been shown to have a multitude of impacts on behaviour, physiology and ecology of a host of different taxa, such as the European black bird (*Turdus turdus*) where reproductive processes are initiated a month earlier in the season in response to low level LAN exposure (Rotics et al. 2011; Dominoni et al. 2013c). The impacts most often studied in literature are at individual, or species level (Bird et al. 2004; Poot et al. 2008; Bedrosian et al. 2011). However, when considering the sum of various species level impacts, the scale of ALAN's impact becomes apparent (Perkin et al. 2011; Davies et al. 2013b). It has the power not only to influence the individual, but to disrupt the ecological processes of entire ecosystems (Bennie et al. 2015). In two species of sympatric but temporally portioned spiny mice, exposure of ALAN led to a contraction of activity in both the diurnal and nocturnal species, thus creating a relatively unexploited temporal niche open to invasive species when neither species are active (Rotics et al. 2011) A fair amount of research is based on terrestrial mammal and bird species with documented impacts on their foraging behaviour (Bird et al. 2004) , physiology (Dominoni et al. 2013a), daily circadian rhythms (Stone et al. 2009), and community compositions (Meyer and Sullivan 2013). Far less research has been conducted on the effects of light pollution on invertebrate, marine and aquatic species (Gaston et al. 2015b).

Studying light pollution is incredibly challenging given the sheer scale of the phenomenon. Remote sensing techniques and satellite imagery have given insights into the global extent of light pollution and sky glow (Cinzano et al. 2001; Cinzano and Falchi 2014; Falchi et al. 2016). The proliferation of light pollution globally is estimated at 6% per year however, this estimate is highly variable according to geographic location, with some regions estimated as high as 20% per year (Hölker et al. 2010). While satellite images highlight the regions likely to be affected most by ALAN, there is often a disconnect

between the conclusions made by remote sensing and the actual experience of organisms to ALAN on the ground (Gaston et al. 2015b). Field based observational studies and experimental studies have attempted to identify the local effects of ALAN on organisms, some with great success (Dominoni et al. 2013b; Spoelstra et al. 2015; de Jong et al. 2016; Ouyang et al. 2017). However, more often they produce data with large amounts of variation from which limited conclusions can be drawn. This is due to the lack of a well-developed understanding of how potential impacts of light pollution vary among factors such as individual organisms, life stages and sexes (Gaston et al. 2015b). This is where lab-based studies may represent a viable opportunity to rapidly assess some of the expected impacts of ALAN, as well as potential sources of intra-species variation found in field studies (Bedrosian et al. 2011, 2013). Lab studies are relatively inexpensive compared to field studies and can be done in shorter periods of time with greater numbers of replicates. Data gathered in lab-based studies can be successfully extrapolated for use in field studies however, only if conditions are carefully controlled and there is understanding of the inherent effect of laboratory conditions on the expected result of the experiment (Calisi and Bentley 2009).

Lab studies mostly require the maintenance of animals in captivity. The conditions they are housed in are often standardised to control factors that are potential sources of variation while manipulating a single factor to determine its effect (Eskola et al. 1999; Tsai et al. 2003). These include factors such as lighting, ambient temperature and the complexity of the environments they are maintained in (Morgan and Tromborg 2006), all of which contribute to the simulated environment in which experiments take place. Lighting in captivity is provided by luminaires which do not provide full spectrum light akin to that of natural sunlight, and at much lower intensities than natural light (Morgan and Tromborg 2006). Ambient temperatures are often chosen based on the comfort of the researcher instead of ideal operating temperature of the organism (Stoinski et al. 2001), and environmental enrichment is kept to a minimum as it is thought to increase variation in results (Olsson et al. 2003). All these factors influence the opportunities for an animal to engage in positive normal behaviour and increase the frequency at which stereotypic behaviour occurs (Mason 2010).

There are many studies highlighting the range of stereotypies these housing conditions cause and the impacts they have on the physiological and behavioural

parameters of individual species (Bashaw et al. 2001; Würbel 2001; Steenkamp 2003; Ellenberg et al. 2007). Regularly the development of stereotypic behaviours due to impoverished living conditions, directly influences the parameters being targeted by the experiment at hand, there by skewing the results obtained. There is also growing evidence to support the assumption that increasing environmental complexity in captivity does not cause as much variation in results as previously believed (Augustsson et al. 2003). Stereotypic behaviours are one cause of the difference in results often found when similar studies are conducted in a lab setting and in the field (Calisi and Bentley 2009). Ultimately it should be the goal of all lab-based studies to allow the animals being studied to exhibit as many natural species-specific behaviours as possible, in order for results to be relevant when extrapolated to field studies.

Advancements in modern lighting and temperature control technology can allow researchers to simulate increasingly complex environments in lab-based studies. This creates a golden opportunity to use lab-based inquiries to study a phenomenon like light pollution and the effects of ALAN. Lab based studies can be performed much more rapidly than field studies and often require a smaller sample size to reach the same statistical power of a field study (Calisi and Bentley 2009). Lab studies can provide base line information, which in turn could be used to explain the large amounts of variation found in field studies of ALAN. However, caution must be exercised in lab studies to remove the impacts of captivity itself from the results associated with conclusions drawn from the data (Tsai et al. 2003).

With this thesis, I will attempt to generate such base line data for two species of common South African rodent, one diurnal and the other nocturnal, which can be used to inform and aid interpretation of later field studies regarding the impacts of different spectra of ALAN. Several factors will be included that have often been highlighted in literature as being information deficient and likely to contribute to variation found in large-scale studies. Individuals of each species will be considered separately, and they will be separated by sex. Firstly the daily activity profiles of each species will be examined in relation to; environmental enrichment, different light and temperature cycles, which are common elements manipulated in laboratory studies, to elucidate each species reaction to these factors. This will also be used to eliminate any potential interferences from some laboratory elements with other outcomes in this study. Subsequently the effect of different spectra of

ALAN will be tested to examine potential impacts of light pollution on these two species under laboratory conditions. Furthermore, glucocorticoid stress hormone concentrations will be monitored in order to explore the effect of the above-mentioned factors on the stress physiologies of these two species, which could influence their behaviour in the wild.

This thesis includes a pilot field experiment, which aims at determining species behavioural responses towards different spectra of ALAN. The experiment was conducted as a pilot study in order to adapt the methodology of a similar experiment to fit local conditions and identify possible adjustments that must be made for such an experiment to be relevant in a South African context. Results obtained from the lab-based elements of this thesis can then be combined with the methodology proofed for the field study to conduct future research on the impacts of ALAN in South Africa. It is the hope that the information generated in the lab-based experiments can allow researchers to clarify if not reduce some of the variation in results found in future field studies there by strengthening the foundation of future research on the impacts of light pollution.

Chapter 2: Effects of enrichment, natural light cycles, temperature and artificial light at night on the locomotor activity of a captive nocturnal (*Micaelamys namaquensis*) and a diurnal (*Rhabdomys dilectus*) rodent.

Introduction

Animals of many species, especially rodents, are maintained in captivity across the world for the purpose of research. The most frequently kept rodents are laboratory bred white mice that have been artificially selected to assume the desirable traits needed for the study they are used for (Miller et al. 2002). However, in some cases wild-caught rodents are necessary to test principles that will later be extrapolated to a larger scale in field studies (Calisi and Bentley 2009). To obtain the most accurate results and repeatability, captive studies often standardise living conditions for the housing of animals (Baumans and Van Loo 2013a). This supposedly removes potential variation in the rodents' responses to the experimenter's manipulations. However, it has been shown that over simplified living conditions may lead to animals developing unnatural stereotypic behaviours, which are indicative of captivity induced stress and would cause animals to react abnormally to experimental manipulations (Mason 1991; Berkson et al. 1995).

Studies involving the physiological reactions of animals to factors manipulated by the experimenter are the most common types of research to involve captive rodents. This is mostly since such studies often require frequent monitoring and taking of biological samples such as blood. It is therefore convenient to keep the subject animals captive, rather than to attempt to recapture them for each sampling event (Baumans 2005). Obtaining data on the physiological reaction to a stimulus often leads to the understanding of the behavioural patterns in animals, as a physiological response can translate into a behavioural reaction (Killen et al. 2013). A physiological trait that has been studied in a wide variety of captive organisms is that of investigating circadian rhythms (Rajaratnam and Redman 1999; Tang et al. 1999; van der Merwe et al. 2014), which translates in to the activity patterns followed by an animal over a daily cycle.

The daily activities of almost all animals are governed by endogenously generated circadian rhythms (Reuss 1996). The suprachiasmatic nuclei (SCN) is a small paired structure located in the basal hypothalamus, that is responsible for keeping time and acts as the internal clock mechanism in mammals (Benstaali et al. 2001; Evans and Silver 2016). Circadian rhythms expressed by the SCN have periods that are usually slightly shorter or longer than 24 hours under constant conditions (Reppert and Weaver 2002). These rhythms are constantly synchronised or entrained to an external cue (zeitgeber) such as daily cycles of light and dark (Sharma 2003). Entrainment is achieved by altering the levels of clock genes and proteins in the SCN depending on photic input received via an animal's retina (Albrecht 2015). Although daily lighting regimes are the most reliable stimuli to synchronise the SCN, mammalian circadian rhythms may also entrain to non-photic cues such as temperature, rainfall and even social cues (DeCoursey 1986; Rajaratnam and Redman 1999; Slotten et al. 2005; Refinetti 2010). Male laboratory mice exposed to an ambient temperature cycle ranging between 24°C and 32°C successfully entrained their wheel running activity to the temperature cycles (Refinetti 2010). However the entrainment fidelity was only about 60 – 80% and took much longer to attain stability under the ambient temperature cycle compared to under the presence of a light – dark cycle (Refinetti 2010). The synchronisation of circadian rhythms to both photic and non-photic cues impart an evolutionary advantage to the animal, ensuring that both short term and longer term activities that are time sensitive, such as foraging times, migration or seasonal breeding occur at the correct times (Sharma 2003; Froy 2011).

Keeping study animals in captivity allows researchers to control any potential *zeitgebers* in climate-controlled rooms. Environmental factors such as light, temperature and environmental complexity of the cages are carefully manipulated in these rooms. Most studies investigating circadian rhythms use standard square wave light-dark (LD) cycles, where the illuminance level of light does not vary temporally (Schumann et al. 2005). Since it is known that some animals entrain their locomotor activity periods to changes in light at dawn and dusk, natural or twilight light cycles may have a profound impact on the onset and offset of activity in rodents (Boulos et al. 2002).

Circadian studies are often conducted at constant temperatures mostly around room temperature (between 20°C and 25°C) to prevent animals from entraining to temperature cycles. Even when temperature cycles are presented, it is usually at a constant higher

ambient temperature for a set period of time, followed by a constant lower ambient temperature for a set time (Ellis et al. 2009; Refinetti 2010). Very few circadian studies have been performed simulating a natural ambient temperature cycle in the laboratory to assess the effect on circadian rhythms (van Jaarsveld et al. 2019).

Although maintaining animals in captivity affords great opportunities for studying the aspects of an animal's physiology, little work has been done on the effects that standardised conditions may have on the subjects being studied. Animals are usually kept in captivity with limited environmental enrichment during such experiments, with an enrichment standardised along ethical guidelines pertaining to the species (Lewejohann et al. 2006). These standardised conditions allow factors to be studied independently without interaction between two or more at the same time. However, when animals are housed in impoverished conditions that do not allow them to express their natural repertoire of behaviours, they can develop stereotypic behaviours (Mason 1991). The development of these stereotypic behaviours is one of the largest sources of variation in lab based studies (Calisi and Bentley 2009) . Understanding how different environmental factors present in laboratories can potentially influence the experiment being conducted can greatly increase the accuracy of lab studies. This is particularly valuable when investigating the impacts of rapidly growing research areas such as light pollution. Light pollution is a phenomenon associated with global change and is rapidly expanding (Gaston et al 2015a). Being able to remove or account for the confounding effects of different environmental conditions in captivity will be a great asset when studying new phenomena such as light pollution.

In this study, I investigated two wild caught rodent species, *Rhabdomys dilectus* (Mesic four-striped grass mouse) and *Micaelamys namaquensis* (Namaqua rock mouse) that are both widely distributed throughout South Africa. *Rhabdomys dilectus* is a small solitary species that inhabits mesic and humid grasslands in the eastern part of southern Africa (Rambau et al. 2003; Meynard et al. 2012). It has been described as being primarily diurnal (Schradin and Pillay 2003; Rymer et al. 2013). On the other hand, *M. namaquensis* is a polygynous, communally nesting species, that prefers rocky habitats with tall grass (Russo et al. 2010). Previous studies have described it as being strictly nocturnal (Muteka et al. 2006; van der Merwe et al. 2014).

My aims were to examine the effect of three factors most commonly controlled for in captive studies. Firstly, the influence of cage enrichment on locomotor activity of two

rodent species were examined. Secondly, the effect of using square wave light-dark cycle was compared to a simulated dawn-dusk light cycle. This was one of the first studies to simulate a dawn-dusk cycle where illuminance increased and decreased along a temporal scale. Thirdly, the impact of using constant temperature was compared to using a natural temperature cycle simulating an average daily temperature cycle in South Africa. Lastly, the impacts of two types of ALAN on locomotor activity and entrainment of the rodent species were examined.

Materials and Methods

Animal Capture and Housing

Twelve (six males and six females) Namaqua Rock Mice (*M. namaquensis*) and twelve (six males and six females) mesic Four Striped Grass Mouse (*R. dilectus*) were captured from their native habitats. The *R. dilectus* were obtained from high rainfall grassland regions within Rietvlei Nature Reserve in Pretoria (-25.882125S, 28.263915E), South Africa whereas *M. namaquensis* were caught on rocky outcrops with sparse tufts of grass growing among rocks in Telperion Private Game Reserve on the border of the Gauteng province (-25.703790, 28.981446). Trapping permits were obtained from Gauteng Nature Conservation (CPF6-0126) and the respective nature reserves. Ethical approval was obtained for the experiments through the University of Pretoria's Animal Use and Care Committee (EC018-16).

Animals were caught using Sherman live traps, baited with a mixture of peanut butter and oats rolled into small balls. During the trapping period for *M. namaquensis,* traps were opened before dusk and closed again at dawn as *M. namaquensis* is known to be strictly nocturnal (van der Merwe et al. 2014). However, during the trapping effort for *R. dilectus,* traps remained open for 24-hour periods since *R. dilectus* is known to be crepuscular in some studies (Schumann et al. 2005). Entry into the game reserve was limited to between the hours of 07:00 and 17:00, which excludes most of the dawn and dusk periods, thus traps could not be closed. Traps were checked at 07:00, between 11:00 and 13:00, and at 16:30. Individuals captured were visually inspected to determine relative age, only adult males and adult, non-gravid females were retained and transported to the Small Animal Physiological Research Facility on the experimental farm at the University of

Pretoria, where they were maintained for the duration of the experiment. All other animals, including non-target species were released again.

Animals were housed individually in climate-controlled rooms where the light and temperature conditions could be manipulated. Animal cages measured 58 × 38 × 36 cm in size and where lined with fresh wood shavings at the start of every new light cycle. Newly caught animals were given a two-week habituation period to acclimatize to captive conditions. Animals were provided with empty plastic containers to use for shelter as standard items throughout all the experiments. Other enrichment items were added during the experiment. The mice were provided with *ad libitum* access to water from feeder bottles that were kept full and were maintained on a mixed diet of sliced fresh food (such as banana, carrot and apples), mixed seeds and specialised rodent pellets (Reggie Rat & Mimi Mouse, Supreme Pet Foods, Suffolk, United Kingdom) to fill all their dietary requirements. Cages were always cleaned at the end of a light cycle, before the next one was initiated.

Experimental Setup

To capture the locomotor activity of the mice, a passive infrared captor (Quest PIR internal passive infrared detector; Elite Security Products [ESP], Electronic Lines, London, United Kingdom) was fitted to the top of each cage. Sensors were placed into custom made holders that maintained the correct angle of the captor to cover the entire floor of each cage. The sensors require displacement of the animal to record activity, thus stationary activities such as grooming, and eating were not recorded. Activity counts were captured per minute by the program Vital View (Minimitter Co. Inc., Sunriver, Oregon; http://minimitter.com) on a computer system. Data were downloaded from the computer at the end of each light cycle and then imported into Microsoft Excel and prepared for data analysis.

Light Cycles

The experiment consisted of six consecutive light cycles that were presented to both species in the following order LD_{NE} (non-enriched), LD_{E} (enriched), LD_{N} (natural light), LD_{NT} (natural temperature), LD_{H} (halogen light) and LD_{LED} (LED light). Under the LD_{NE} cycle, animals were subjected to a 12L (Light):12D (Dark) (06:00-18:00 light) square wave light cycle (~330 lux), a constant ambient temperature (T_a) of 25˚C and no additional environmental enrichment apart an empty plastic shelter. During the LD_{E} cycle, mice were presented with similar environmental conditions as during the LD_{NE} cycle, but with the addition tissue paper as

nesting material, three toilet rolls and a rock as environmental enrichment. During the LD_N cycle, while maintaining enrichment elements, the square wave light cycle was replaced with a 14L:10D light cycle (05:00-19:00 light). This light cycle had light decreasing and increasing along a stepwise ramp at 10% intervals starting from 100% to a minimum of 20% for the first two and last two light hours of the day to simulate dawn and dusk. During LD_{NT}, the ambient temperature cycled between 16°C (at 04:00) and 30°C (at 15:00) to simulate temperatures of an average summer day. Cycles were presented for 21-23 days, with one or two days between the different cycles to allow for cleaning of the cages and resettlement of the animals. After the LD_{NT} cycle animals were exposed to two further cycles LD_H and LD_{LED} where light was presented during the dark phase. These cycles followed the same protocol as the LD_{NT} with the addition of 10 hours of artificial light at night meant to simulate the lowest levels of night-time light pollution experienced commonly in South Africa. A Eurolux 28W 2900K halogen (Eurolux, Milnerton, South Africa) light bulb was used in LD_H and a K Light Import 7W 3000K LED (K Light, Milnerton, South Africa) light bulb was employed in LD_{LED}. The light bulbs that were used to simulate light pollution were switched on at 18:00 and turned off at 06:00 in order to mimic the period of transition between streetlights and daylight. ALAN bulbs were installed in the centre of the experimental room, and the light intensity calibrated to achieve the smallest possible range of values (3.2 – 4.8 lux) measure on each animal's cage floor.

Data Analysis

The data collected from the animals during the different light cycles were downloaded from the computer at the research facility. Microsoft excel was used to clean and standardise data as only the last 15 days of each light cycle was included in the analysis. The program Actiview Biological Rhythm Analysis 1.2 software (Minimitter Co. Inc., Sunriver, Oregon, http://www.minimitter.com) was used to create double plotted actograms for each individual animal to visualise their locomotor activity. All statistical analyses were performed in IBM SPSS v25 (SPSS Inc., Chicago, Illinois). Hourly activity counts were compared between different light and temperature cycles, and different light phases (light / crepuscular / dark). Activity data were not normally distributed therefore non-parametric generalized linear mixed models were used. Light cycle, light phase and gender were used as fixed factors in the analysis. All two way and three-way interactions of factors were investigated, and least significant difference was used in the post hoc tests. The average onset and offset times of

activity as well as chi-square periodograms for each animal under each light cylce were calculated using Clocklab software (ClockLab™; Actimetrics, Evanston, Illinois, USA).

Results

12L: 12D without enrichment

In *M. namaquensis,* the average activity counts per hour during the dark phase of LD_{NE} was significantly higher than average light phase activity counts per hour ($F_{1, 23.31} = 674.84$, $P < 0.001$; Fig 1). There were no significant differences observed in activity counts between male and female activity during LD_{NE} ($F_{1, 23.31} = 2.13$, $P = 0.14$). *M. namaquensis* was strictly nocturnal (Fig 2) with 97.96% of its activity (Fig 3) occurring during the dark phase of LD_{NE}. The average onset time for *M. namaquensis* was 17:49 and offset occurred around 05:58, rendering the active time a total of 11 hours and 51 minutes (Table 1).

R. dilectus showed no significant differences in their average activity counts per day during the light and dark phases of LD_{NE} ($F_{1,22.70} = 0.50$, $P=0.50$). During LD_{NE} only three individuals showed a clear diurnal rhythm (Fig 4a), with the remaining nine individuals showing less organised activity patterns with large peaks of activity during the dark phase (Fig 4b). The activity in the light phase ranged from being diurnal in some individuals to showing slight crepuscular activity. Light phase activity of *R. dilectus* amounted to 55.86% of total activity and dark phase activity accounted for 44.14% (Fig 5). Females were significantly more active than males in $_{LDNE}$ ($F_{1,22.70} = 72.81$, $P< 0.001$). On and offsets could only be determined for a few individuals due to the weak entrainment. Average time of activity onset was 05:24 and offset of activity occurred around 19:22 (Table 1).

12L: 12D with environmental enrichment

M. namaquensis significantly increased its activity during LD_E after the addition of enrichment items when compared to LD_{NE} ($F_{5,23.32} = 91.08$, $P<0.001$). The average activity counts per hour during the dark phase of LD_E were significantly higher than during the light phase ($F_{1, 23.32} = 2447.53$, $P < 0.001$; Fig 1). Entrainment patterns for *M. namaquensis* did not change during LD_E with all the individuals still maintaining strictly nocturnal activity (Fig 2). The proportion of activity also did not change much with 97.16% of total activity taking place in the dark phase (Fig 3). Female *M. namaquensis* were significantly more active than

males during LD_E ($F_{1,23.32} = 25.72$, $P < 0.001$). The mean time of activity onset was 17:52 whereas offset occurred at 06:07, and alpha was 11 hours and 44 minutes (Table 1).

In contrast, *R. dilectus* significantly decreased its activity after the introduction of enrichment objects in its habitat ($F_{5,22.70} = 64.15$, $P < 0.001$). Three individuals remained less organised under LD_E whereas, four showed a clear transition toward diurnal activity and three became crepuscular (Fig 4b) with clear bimodal peaks of activity during the transition periods between light and dark. The average activity counts per hour during the light phase where significantly higher than during dark phase ($F_{1,22.70} = 90.76$, $P < 0.001$), with the proportion of daytime activity also increasing to 66.49% and night-time activity decreasing to 33.51% (Fig 5). Female *R. dilectus* were again significantly more active when compared to the males ($F_{1,22.70} = 22.92$, $P < 0.001$). Average onset of activity for *R. dilectus* was 04:12 and offset occurred at 19:10 rendering their active time as a total of 14 hours and 57 minutes (Table 1).

Table 1. The average times of activity onsets, when activity commenced; offset when activity completely ceased and alpha, the average total number of hours each species was active during each light cycle.

Species	Light cycle	Mean onset	±SE	Mean offset	±SE	Mean alpha	±SE
Micaelamys namaquensis	LD_{NE}	17:49	00:11:15	05:58	00:10:37	11:51	00:16:19
	LD_E	17:52	00:11:49	06:07	00:07:41	11:44	00:15:59
	LD_N	18:51	00:10:32	05:15	00:14:32	13:35	00:21:46
	LD_{NT}	18:56	00:08:48	05:14	00:09:46	13:41	00:14:37
	LD_H	18:59	00:14:57	05:33	00:57:45	13:25	00:54:51
	LD_{LED}	18:55	00:13:22	05:22	00:08:29	13:32	00:17:31
Rhabdomys dilectus	LD_{NE}	05:24	01:01:20	19:22	00:16:58	13:57	00:50:45
	LD_E	04:12	00:47:55	19:10	00:15:52	14:57	01:04:46
	LD_N	04:16	00:17:32	19:56	00:20:13	15:39	00:33:23
	LD_{NT}	04:32	00:11:33	19:43	00:16:19	15:10	00:18:01
	LD_H	04:00	00:31:56	20:08	00:24:58	16:08	00:40:55
	LD_{LED}	03:36	00:40:06	20:10	00:37:30	16:33	00:49:50

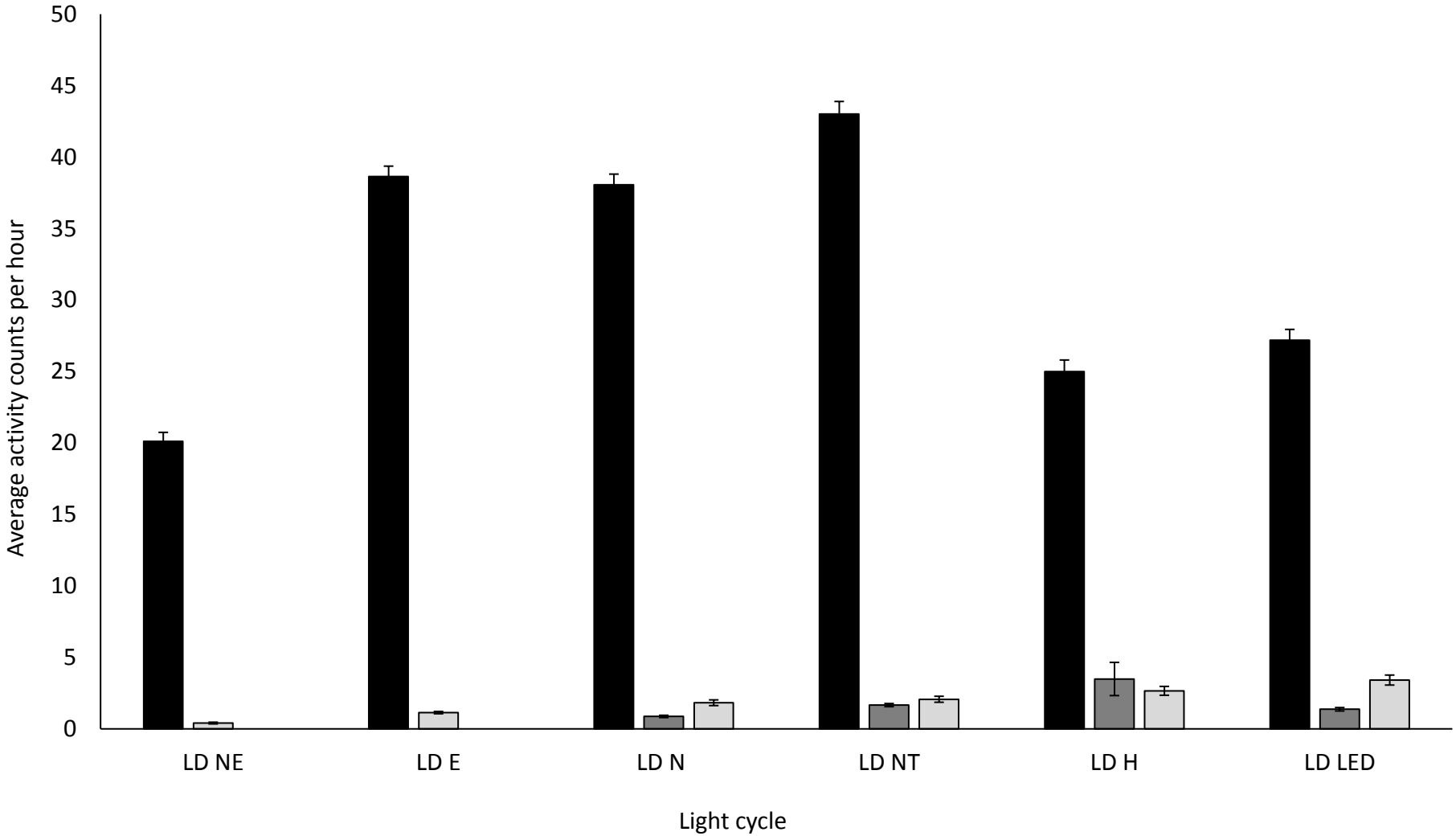

Figure 1. The average locomotor activity counts per hour of *Micaelamys namaquensis* during dark (black), twilight (dark grey) and light (light grey) hours during six different light cycles. LD_{NE} and LD_{E} were square wave cycles and did not have a simulated twilight period hence the dark grey is absent from the representation.

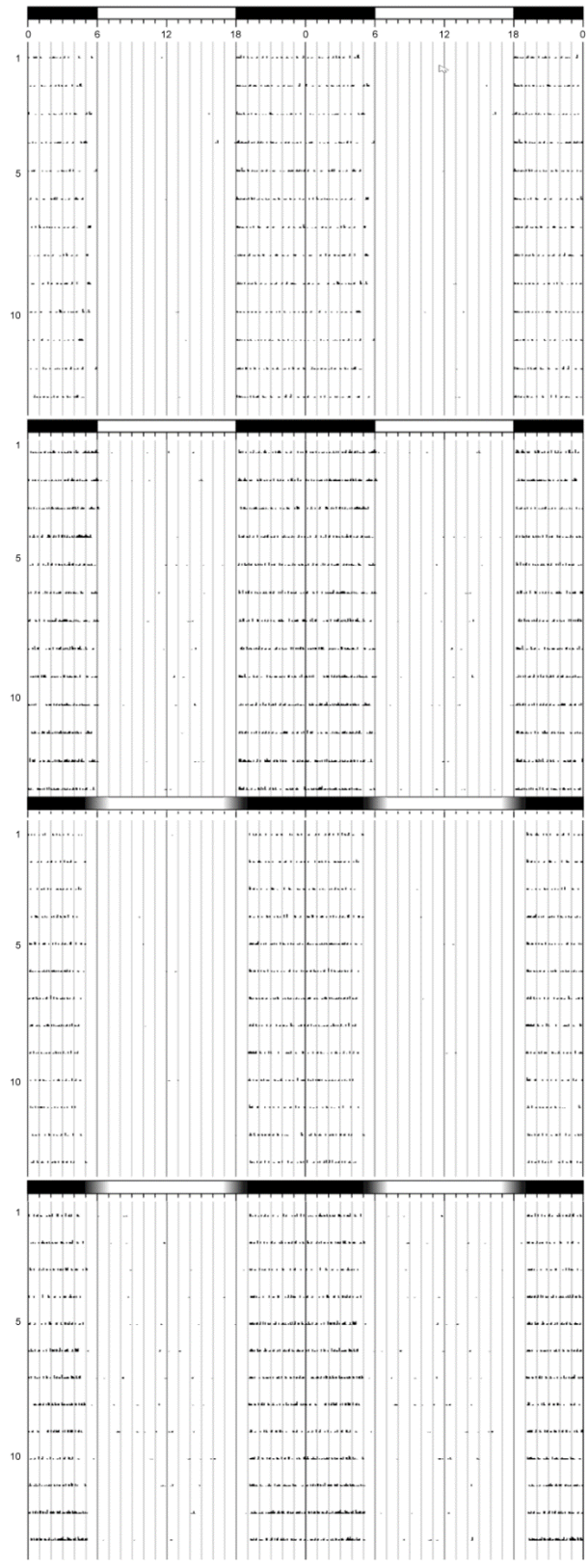

Figure 2. Double plotted actogram of *Micaelamys namaquensis* no. 10 showing robust nocturnal entrainment during square wave light cycles LD_{NE} and LD_{E} as well as during the natural dawn-dusk cycles LD_{N} and LD_{NT}. *M. namaquensis* was very responsive to changes in light cycles which can clearly be seen in the contraction of activity at the start of LD_{N}. White and black bars on top of the actograms indicate light and dark periods, with consecutive days on the y-axis.

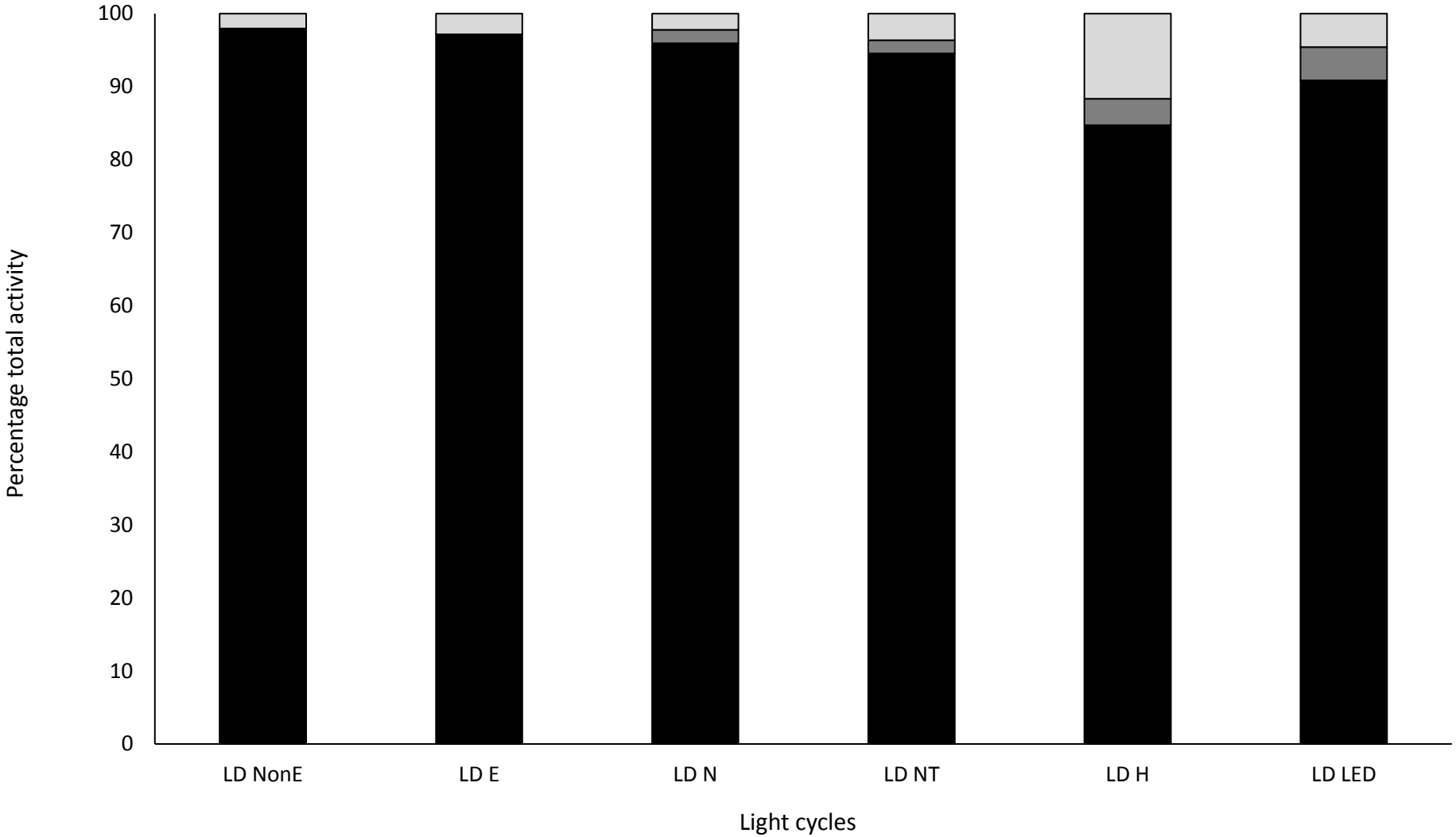

Figure 3. The percentage total activity expressed by *Micaelamys namaquensis* during dark (black), twilight (dark grey) and light (light grey) hours across six different light cycles. LD_{NE} and LD_{E} were square wave cycles and did not have a simulated twilight period hence is not represented in the figure.

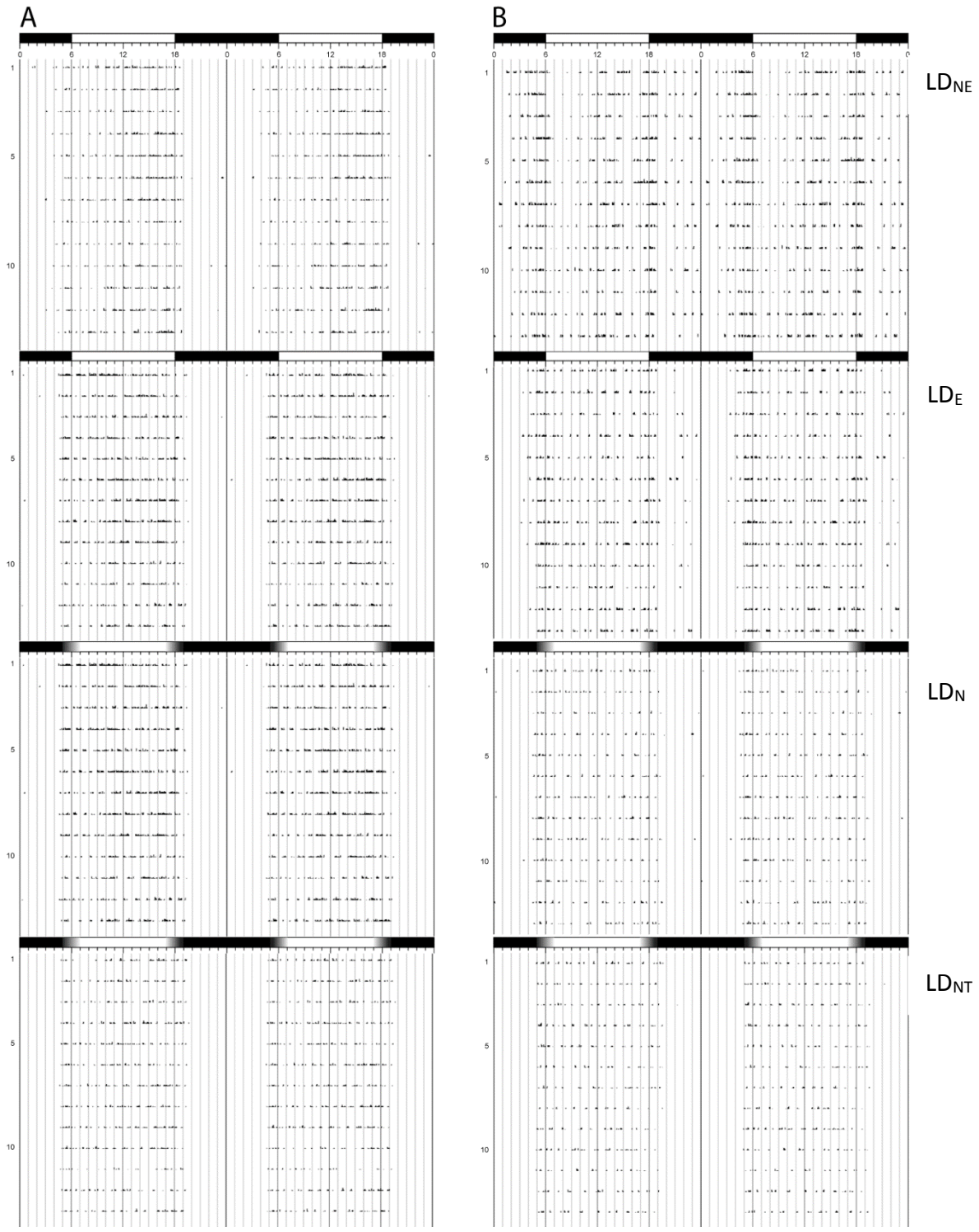

Figure 4. Double plotted actogram of *Rhabdomys dilectus* No.10 during LD_{NE}, LD_{E}, LD_{N} and LD_{NT} showing A) robust diurnal entrainment maintained throughout the four light cycles and B) disorganised activity pattern showing transitions after the addition of enrichment items to become more diurnally entrained (no. 6). White and black bars on top of the actograms indicate light and dark periods, with consecutive days on the y-axis.

Simulated dawn-dusk light cycle

The introduction of a simulated natural dawn-dusk period in the light cycle effectively increased the light hours to 14 and reduced the completely dark hours to 10. After the addition of a simulated natural dawn-dusk cycle, *M. namaquensis* significantly decreased its overall activity counts per hour when compared to LD_E ($F_{5, 23.31} = 91.0.8$, $P < 0.001$). The average activity count per hour in the dark phase of LD_N was significantly higher than activity both in the light phase and twilight phase ($F_{2,23.31} = 1098.10$, $P < 0.001$), while there was no significant difference between the average activity count per hour of the twilight and light phases ($F_{2,23.31} = 1098.10$, $P = 0.39$). The animals exhibited 95.96% of their activity during the dark phase of LD_N and only 2.20% and 1.84% during light and twilight respectively (Fig 3). *M. namaquensis* showed clear responses to the addition of a natural dawn dusk cycle by delaying their average onset of activity by 59 minutes and advancing their offset of activity with one hour and 8 minutes (Table 1), thus compressing their nocturnal activity to fit into the shortened dark phase of LD_N (Fig 2). Although males were more active than females during LD_N there was no significant difference observed in their average activity counts per hour ($F_{1,23.31} = 0.13$, $P = 0.72$).

Locomotor activity for *R. dilectus* decreased significantly ($F_{5,22.70} = 64.15$, $P < 0.001$) during LD_N when compared to LD_E. Entrainment patterns for *R. dilectus* became much more defined during LD_N with all individuals now showing rhythmic activity patterns (Fig 4a). Two animals remained crepuscular with the remaining 10 showing distinct diurnal entrainment. Average activity counts per hour during the twilight phase were significantly higher than activity counts for both the light phase and the dark phase ($F_{2,22.70} = 186.89$, $P < 0.001$, Fig 6) with 52.81% of activity taking place during the light phase, 36.47% during the twilight phase and only 10.73% during darkness (Fig 5). *R. dilectus* females were more active than males, however, not significantly so ($F_{1,22.70} = 1.68$, $P = 0.12$). *R. dilectus* responded to the addition of the natural dawn dusk cycle by increasing their number of active hours (Table 1). They shifted their onset of activity to 04:16 delaying it by four minutes and offset of activity occurred at 19:56, 46 minutes later than during LD_E.

Dawn-dusk light cycle with natural temperature cycle

During the LD_{NT} cycle, where both a natural dawn-dusk cycle and a temperature cycle were present, *M. namaquensis* significantly increased its activity compared to LD_N ($F_{5,23.31} = 91.08$, $P < 0.05$). *M. namaquensis* spent 94.55% of its activity during the dark phase of the light

cycle and 3.63% and 1.82% in the light and twilight respectively (Fig 3). All animals showed strictly nocturnal activity for the duration of LD_{NT} (Fig 2) and nocturnal activity levels were significantly higher than activity levels during the twilight or light phase ($F_{2,23.31}$ = 1279.24, P < 0.001, Fig 1). There was no significant difference in the level of activity between the light and twilight phase ($F_{2,23.31}$ = 1279.24, P = 0.76). The average time of activity onset and offset were not substantially different from LD_N, 18:56 and 05:14 respectively (Table 1). During $LD_{NT,}$ females were significantly more active than males ($F_{1,23.31}$ = 34.45, P < 0.001).

R. dilectus further decreased level rate of activity when compared to the previous light cycle ($F_{5,22.70}$ = 64.15, P < 0.001; Fig 6). The mice showed significantly higher levels of activity during twilight hours when compared to light ($F_{2,22.70}$ = 161.66, P < 0.001) and dark hours ($F_{2,22.70}$ = 161.66, P < 0.001, Fig 6). All individuals showed rhythmic activity during LD_{NT}, four individuals showed crepuscular rhythms and the eight remaining individuals showing diurnal activity patterns (Fig 4a). *R. dilectus* displayed 52.81% of its activity during the light phase, 36.64% during the twilight hours and only 10.67% during the dark hours (Fig 5). Females were significantly more active than males ($F_{1,22.70}$ = 5.14, P < 0.05). There was little change in the average times of activity onset and offset of *R. dilectus*, with onset occurring at 04:32 and offset at 19:43 (Table 1).

Halogen light at night

M. namaquensis significantly decreased its level of activity after the addition of ALAN to the light cycle ($F_{5,23.31}$ = 91.08, P < 0.001). While activity levels were still significantly higher during the dark phase compared to both the twilight phase and the light phase ($F_{5,23.31}$ = 371.96, P < 0.001, Fig 1), the proportion of activity in the dark phase decreased to 84.74%, while twilight and light phase activity increased to 3.61% and 11.65% respectively (Fig 3). All the individuals remained strongly nocturnal during the presence of ALAN (Fig 7b). There was no significant difference between the levels of activity during light and twilight hours ($F_{2,23.31}$ = 371.93, P = 0.58). During LD_H there was no significant difference between the activity counts per hour of males and females ($F_{1,22.70}$ = 0.32, P = 0.57). The average times for onset and offset of activity for *M. namaquensis* remained very similar to LD_{NT} (Table 1).

R. dilectus increased its overall level of activity significantly during LD_H when compared to LD_{NT} ($F_{5,22.70}$ = 64.15, P < 0.01). Twilight activity levels were significantly higher than both light and dark phase activity ($F_{2,22.70}$ = 119.48, P < 0.001; Fig 6). The proportion of activity *R. dilectus* displayed during the light phase of the light cycles decreased to 45.56%

whereas dark phase increased to 18.37% and twilight phase remained similar to LD_{NT} at 36.04% (Fig 5). *R. dilectus* shifted its activity onset times almost half an hour earlier to 04:00 during LD_H compared to LD_{NT}, whereas their average activity offsets occurred approximately 20 minutes later than during LD_{NT} (Table 1). There was no significant difference between the levels of activity of males and females during LD_H ($F_{1, 22.70} = 0.32$, $P = 0.57$).

LED light at night

In *M. namaquensis*, the addition of white ALAN caused activity levels to increase slightly, however, this was not significant when compared to the LD_H difference ($F_{2,23.31} = 91.08$, $P = 0.44$). Activity counts per hour during the dark phase of LD_{LED} remained significantly higher than the activity rates during the light and twilight phase ($F_{2, 23.31} = 507.86$, $P < 0.001$, Fig 1). There was also no significant difference between the levels of activity of the light and twilight phase ($F_{2, 23.31} = 507.86$, $P = 0.06$). The amount of activity during the dark phase and twilight increased to 90.85% and 4.56% respectively whereas light phase activity decreased to 4.58% (Fig 3). The average onset of nocturnal activity was 18:55 and the offset of activity occurred at 05:22 on average (Table 1). Males where more active than females, but no significance difference was found ($F_{1, 23.31} = 0.096$, $P = 0.76$).

In *R. dilectus*, the average level of activity during LD_{LED} was lower than when compared to LD_H, but not significantly so ($F_{5,22.70} = 64.15$, $P = 0.07$). The average activity counts per hour was significantly higher during twilight hours than during both the light and dark phase of LD_{LED} ($F_{2,22.70} = 133.28$, $P < 0.001$, Fig 6). Light phase activity levels were also significantly higher than that during the dark phase ($F_{2,22.70} = 133.28$, $P < 0.001$). The highest proportion of *R. dilectus* activity was still in the light phase (42.20%), with twilight having the second highest amount of activity (39.04 %) and dark hours showing the lowest proportion (18.76%; Fig 5). The average onset of activity for *R. dilectus* was shifted approximately 30 min earlier shifting to 03:36 compared to LD_H but, average time of offset remained similar 20:10 (Table 1). During LD_{LED} males were significantly more active than females ($F_{1, 22.70} = 46.34$, $P < 0.001$).

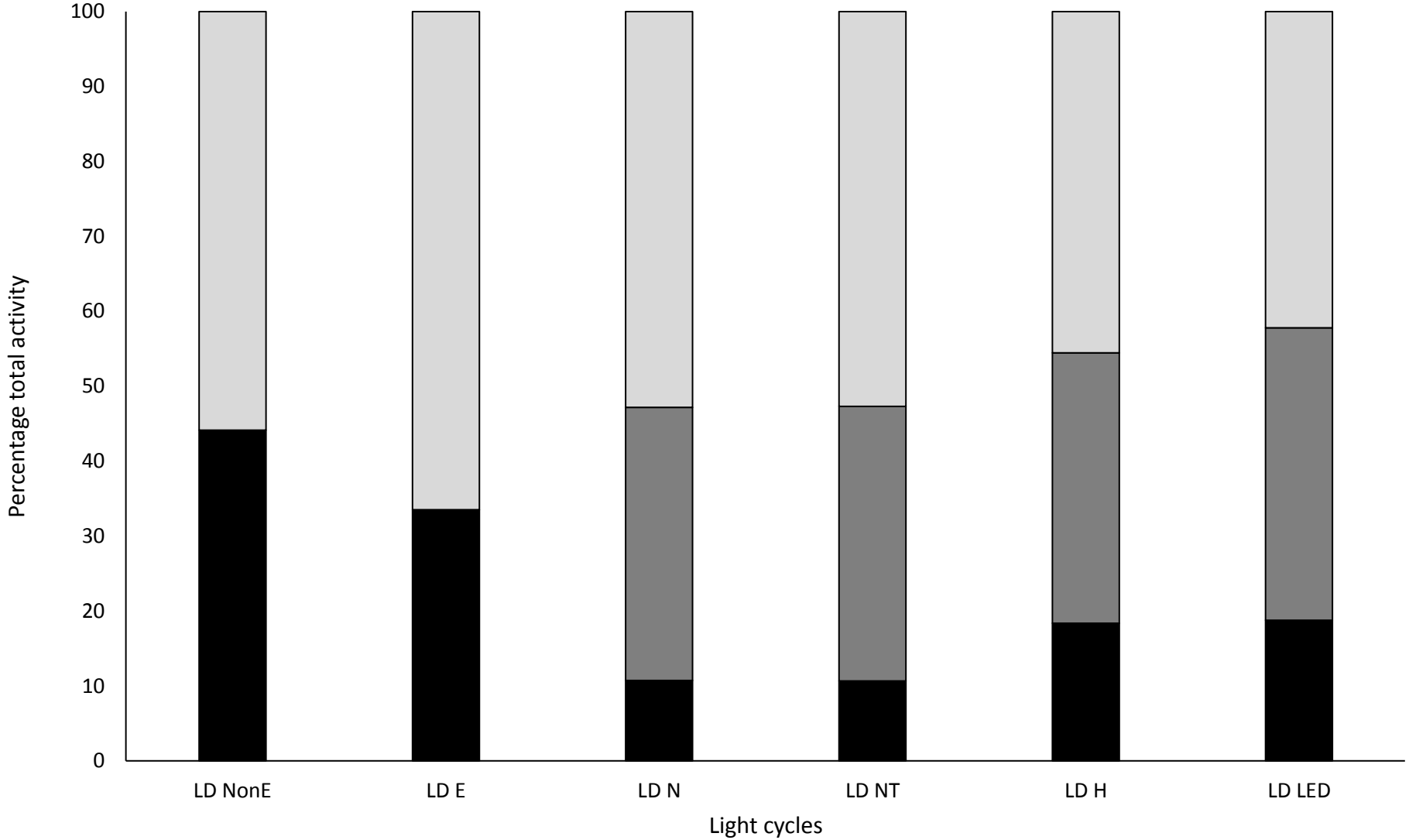

Figure 5. The percentage total activity expressed by *Rhabdomys dilectus* during dark (black), twilight (dark grey) and light (light grey) hours across six different light cycles. LD_{NE} and LD_{E} were square wave cycles they did not have a simulated twilight period hence is not represented in the figure.

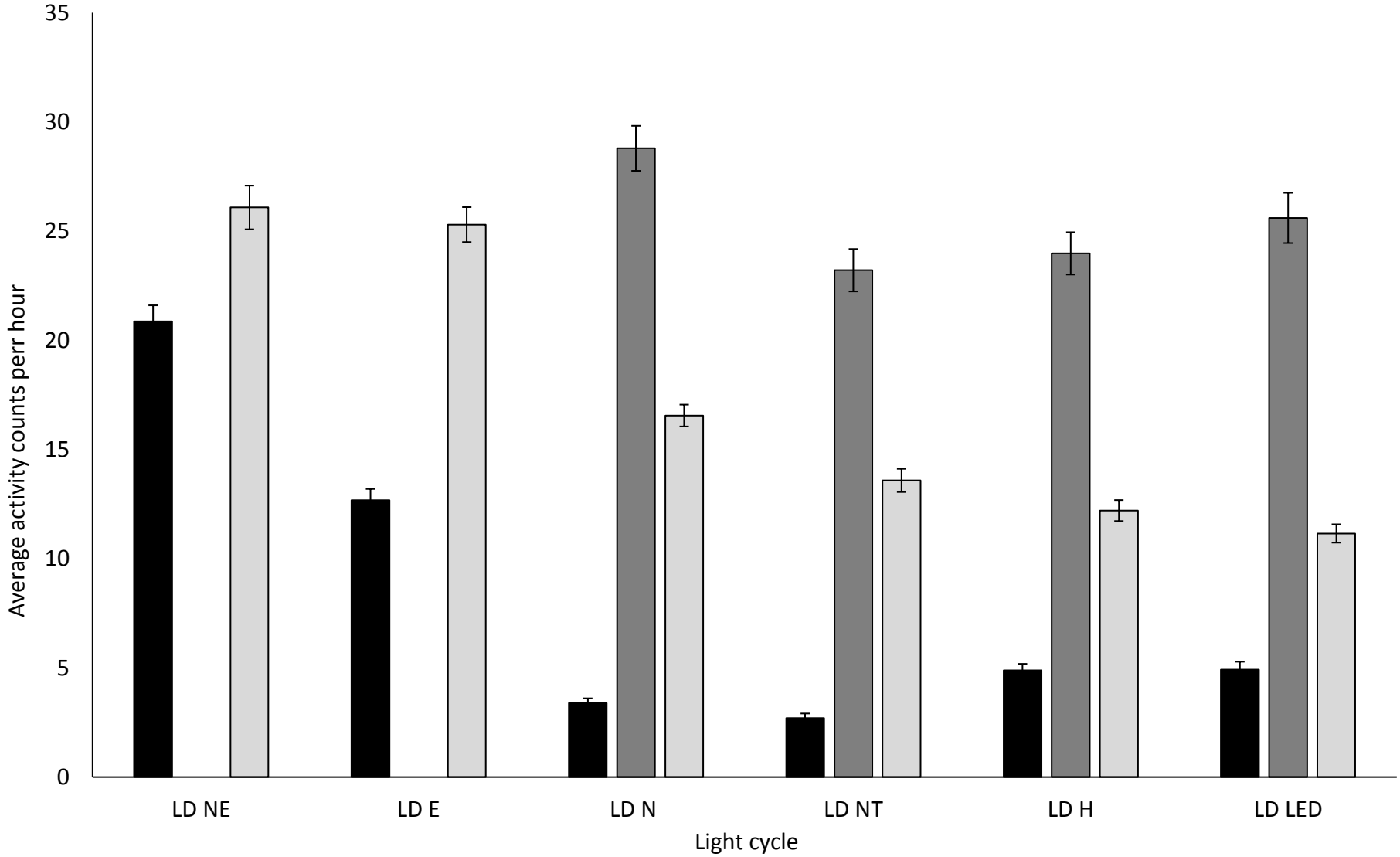

Figure 6. The mean locomotor activity counts per hour of *Rhabdomys dilectus* during dark (black), twilight (dark grey) and light (light grey) hours for all light cycles. LD_{NE} and LD_{E} were square wave cycles they did not have a simulated twilight period hence the dark grey is absent from the representation.

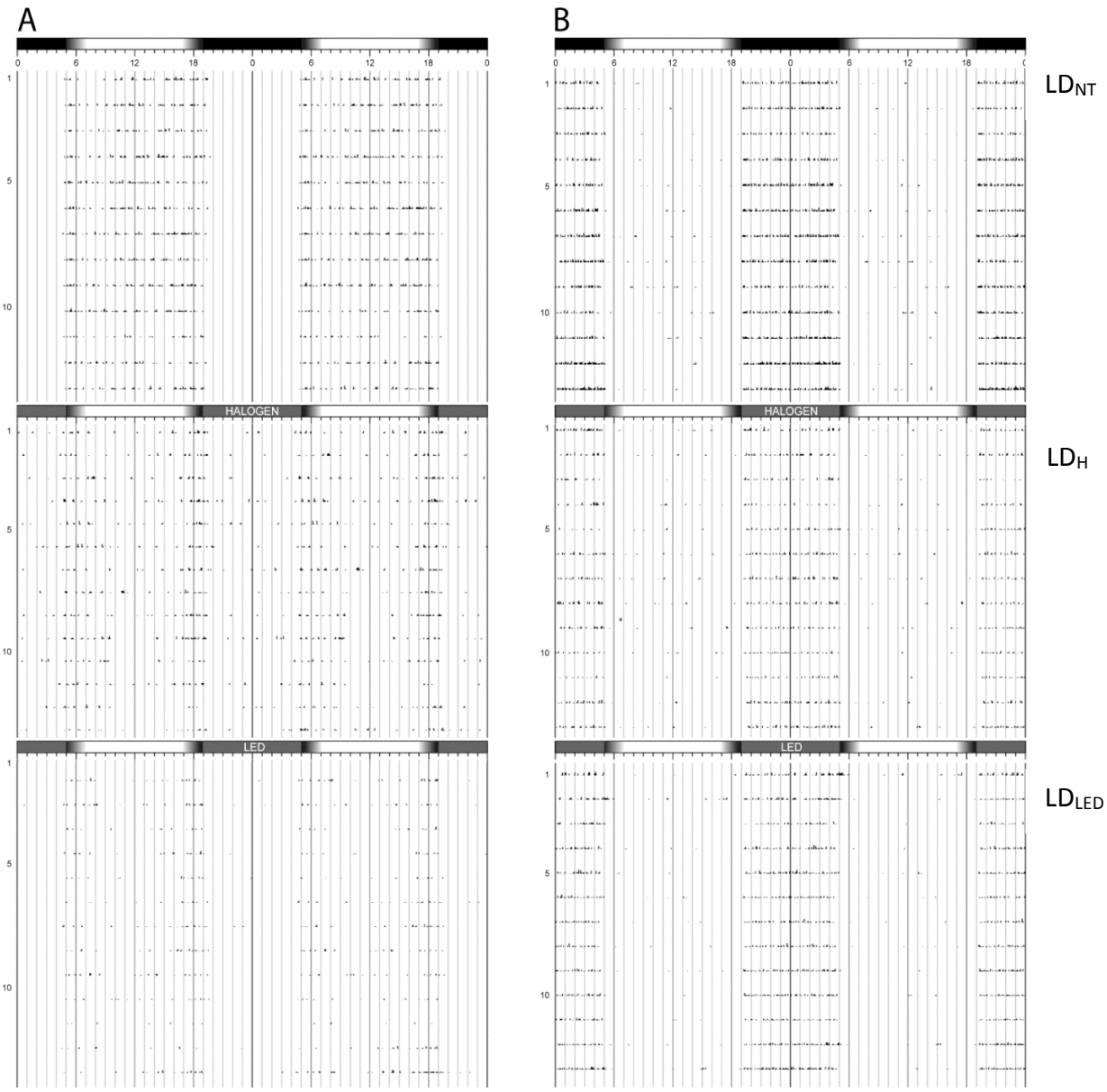

Figure 7. Double plotted actogram of A) *Rhabdomys dilectus* and B) *Micaelamys namaquensis* during LD_{NT}, LD_{H} and LD_{LED} showing the varying reactions to the spectral content of ALAN. White and black bars on top of the actograms indicate light and dark periods, with consecutive days on the y-axis.

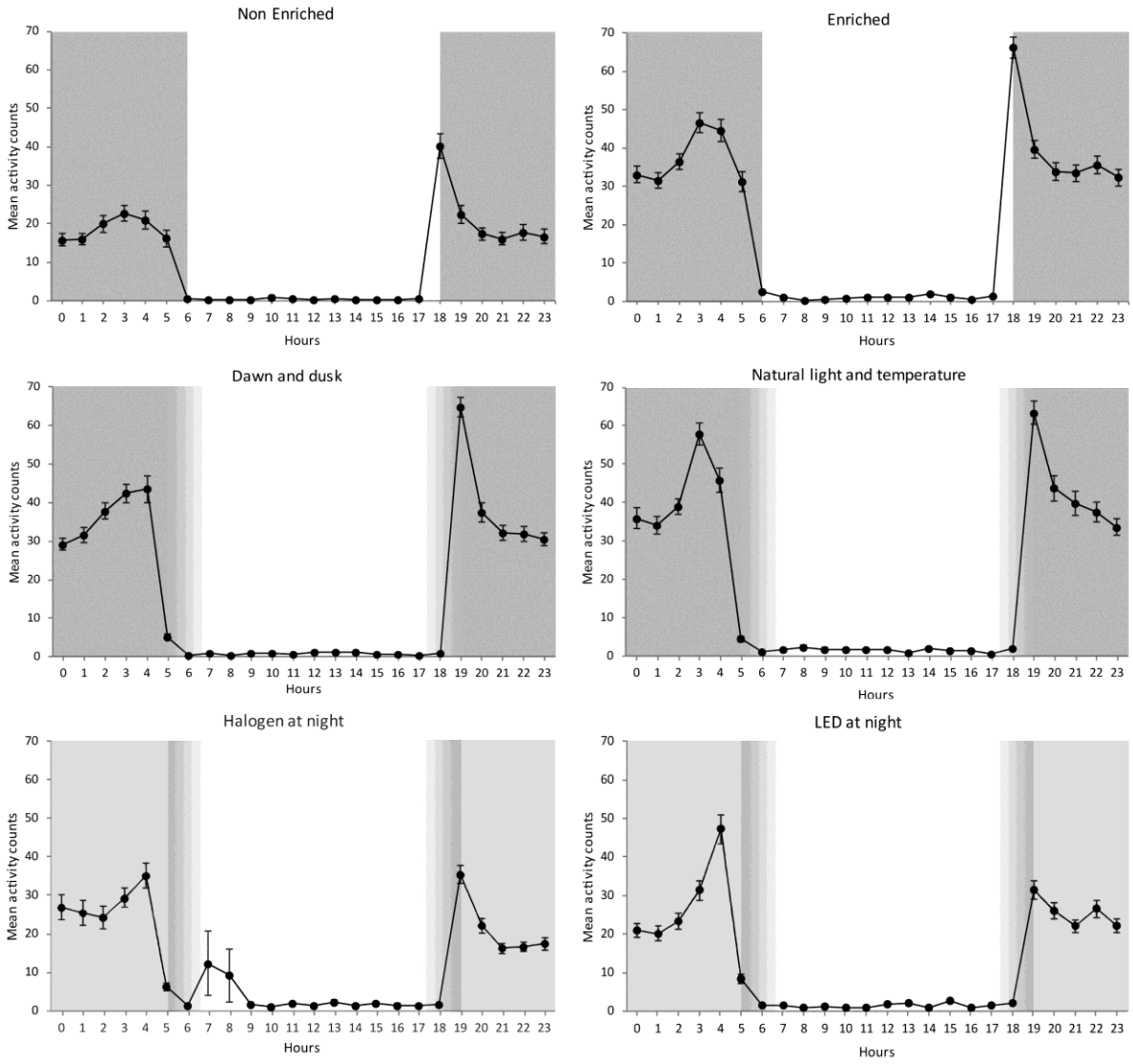

Figure 8 Periodograms of all *Micaelamys namaquensis* individuals, calculated as an average activity per hour across a 24hour period during six different light cycles.

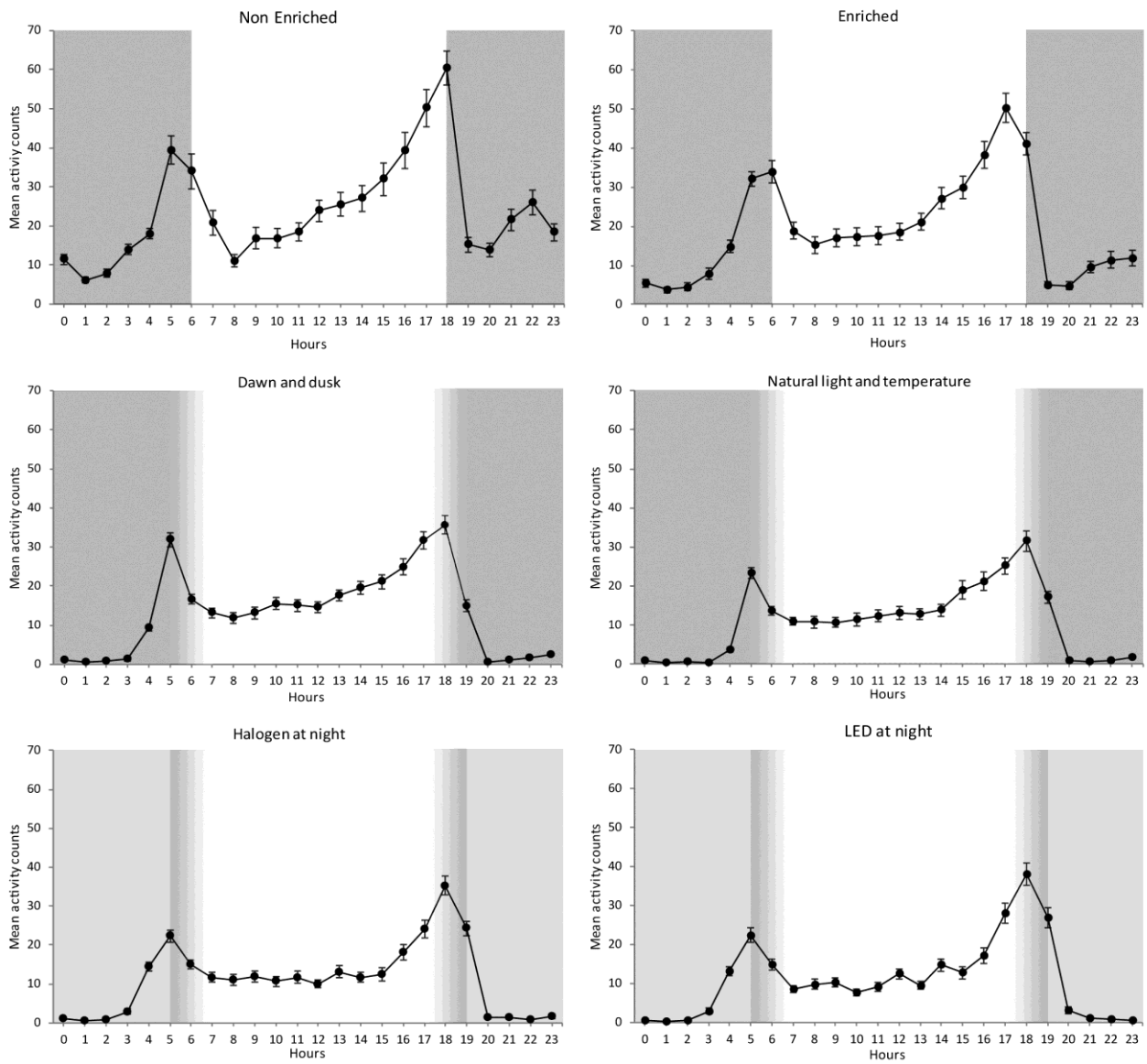

Figure 9. Periodograms of all *Rhabdomys dilectus* activity, calculated as average activity per hour across a 24hour period during six different light cycles.

Discussion

Several environmental factors that are commonly controlled for in laboratory studies investigating the circadian rhythms of animals were examined in this chapter. All these factors are known to affect the entrainment of circadian rhythms in animals in captivity (Oosthuizen et al. 2003; Refinetti 2010; Ackermann et al. 2016) and are therefore mostly standardised in laboratory studies i.e. constant temperature, constant illuminance levels and very little environmental complexity in an effort to elucidate the effect each factor has on circadian rhythms independently.

General locomotor activity

Locomotor activity is frequently used as a proxy for the entrainment of circadian rhythms (Ishii et al. 1996; Benstaali et al. 2001; Ackermann et al. 2016). In this study, *M. namaquensis* showed a robust and consistent nocturnal activity rhythm throughout all experimental conditions, with little individual variation occurring. This result is consistent with previous research which found *M. namaquensis* to be strictly nocturnal (van der Merwe et al., 2014; van der Merwe et al., 2017). In contrast, *R. dilectus* showed substantial inter-individual variation, not only in patterns of locomotor activity but also in their levels of activity, with some individuals being highly active and others being relatively inactive throughout the experiment. Similarly, the study by van der Merwe et al. (2017) similarly found an intraspecific variation in *R. pumilio*, a species closely related to *R. dilectus.* The genus *Rhabdomys* has been the subject of relatively recent reclassification and previously only one species was recognised within the genus (Castiglia et al. 2012), but more recently, the arid and mesic populations of *Rhabdomys* were split into separate species. The variable activity exhibited by *R. dilectus* could be an adaptation to inhabiting highly variable environments ranging from mesic grasslands to relatively arid savanna habitats. In these highly variable environments being able to quickly alter activity in response to day to day changes may be beneficial for resource acquisition and predator avoidance.

Effect of environmental enrichment

Although environmental enrichment is generally viewed as beneficial, it is a complex phenomenon. It is known to influence the brains and behaviour of rodents (Würbel 2001) with effects that can be interpreted as positive, negative or neutral to the well-being of an animal, depending on the study (Toth et al. 2011). Environmental enrichment has the

potential to increase variability in data, such that a larger sample size may be needed, it can also affect the reproducibility of the experiments and affect how data are interpreted (Hutchinson et al. 2005). It is extremely difficult to replicate the exact same cage conditions among laboratories around the world, which may explain the variation in results in an experiment duplicated in other locations.

In this study, *M. namaquensis* consistently displayed nocturnal activity patterns, but the addition of environmental enrichment caused a marked increase in the level of locomotor activity. An increase in activity may indicate the reduction of captivity induced stress as some research suggests that the suppression of activity in captive rodents is an indicator of physiological stress (Joshi and Pillay 2016b). In contrast, *R. dilectus* showed disorganised and disturbed activity patterns during the non-enriched cycle. However, with environmental enrichment the locomotor activity rhythms of six individuals became more structured and identifiable as either diurnal or crepuscular, while their average overall activity levels decreased. Previous research shows that *R. pumilio* responds to stressful situations in captivity by reducing its activity (Scantlebury et al. 2006). Another study suggests that captive *R. pumilio* are bolder and spend more time investigating novel items reflecting lower stress levels than their mesic counterpart (Rymer et al. 2008; Rymer and Pillay 2012). When interpreting the results in light of these findings, the environmental enrichment may have increased the stress levels of *R. dilectus* while in captivity, thus having a masking effect on their true circadian rhythms. In contrast, *M. namaquensis* could potentially have been experiencing less stress and therefore be encouraged to show the higher levels of activity in the presence of environmental enrichment.

Exposure to dawn and dusk

The gradual transition between light and dark that occur during the twilight hours of the day may provide different temporal cues to animals compared to abrupt changes in lighting (Tang et al. 1999). Both the colour and intensity of light changes dramatically during dawn and dusk indeed, species of cichlid have been shown to entrain their activity to the colour of light, and not its intensity (Pauers et al. 2012) whereas rodents seem to be able to entrain to the intensity of the light (Boulos et al. 2002).

In this study, only the intensity of the light was altered to simulate dawn and dusk with the spectral quality of the light remaining constant. *M. namaquensis* showed a further increase in their mean activity level, were extremely responsive to the change in

photophase, and immediately compressed their activity periods to be contained within the absolute darkness period. Both the onsets and offsets were shifted supporting the finding that this species is strictly nocturnal. In captive, nocturnal flying squirrels (*Glaucomys volans*), it was uncovered that arousal of the animals did not coincide with the onset of activity. Upon arousal from sleep, animals would sample the light conditions outside the nest box, but when it was still too light, they would return to sleep for another 30 min. Subsequently, another light sampling event would occur and activity would only commence once it was completely dark (DeCoursey 1986). *M. namaquensis* have been shown to be particularly sensitive to light stimuli, with the amplitude of their activity increasing with increasing intensity of external light exposure during the light phase (van der Merwe et al. 2017). It is possible that a light sampling procedure and ensuing phase shifting behaviour, similar to that of the flying squirrel, is employed by *M. namaquensis*. Light sampling behaviour in *M. namaquensis* may further be supported by the slightly but non-significant increase in the level of movement during twilight hours, as the passive IR motion detectors used in this study would capture their activity of emerging from the shelters.

Overall average locomotor activity was further decreased in *R. dilectus;* however, it did become more robustly entrained to the light cycle with all individuals now exhibiting clear rhythmic activity patterns. Although the proportion of activity exhibited in the light phase was higher than during twilight phase, the level of activity was significantly higher than during the twilight phase. During the natural light cycle, *R. dilectus* shifted the onsets and offsets of activity to increase the number of active hours. A similar result was found by Schumann et al. (2005), where the activity period of *R. pumilio* was increased by almost an hour when subjected to natural light cycles. In that study, most animals displayed clear bimodal activity during the square wave cycle and the natural cycle, however, these animals had access to running wheels (Schumann et al. 2005). Running wheels are known to affect locomotor activity levels and behaviour of animals (Vaanholt et al. 2007). These findings support the notion that *R. pumilio* is crepuscular rather than diurnal thus favouring higher levels of activity during the twilight hours.

Natural ambient temperature cycle

Studies investigating the effects of ambient temperature on the circadian biology of animals frequently use 12:12h cycles of high and low ambient temperatures to determine whether animals can entrain to such cycles (Francis and Coleman 1988; Ellis et al. 2009;

Refinetti 2010). Here I simulated a natural temperature cycle that gradually increased and decreased over a 24-hour period to simulate temperatures of an average summer day in the Highveld region of South Africa (South African Weather Service 2019). *M. namaquensis* showed the highest level of activity during this cycle. Rodents appear to show variable responses to lower ambient temperatures, many species show a reduction in locomotor activity at cooler temperatures (Vaanholt et al. 2007; Sears et al. 2009; Wróbel and Bogdziewicz 2015), whereas others increase their activity with lower ambient temperatures (Ishii et al. 1996). A nocturnal species such as *M. namaquensis* is by default active during the cooler parts of a 24-hour day as temperatures can drop considerably at night. The increase in activity levels exhibited by *M. namaquensis* may be a behavioural thermoregulatory response to maintain their body temperatures during cooler conditions. Alternatively, *ad libitum* food may be responsible for the increased activity. *M. namaquensis* had significantly reduced variation in internal body temperatures when exposed to lower ambient temperatures in captivity which was attributed to the provisioning of *ad libitum* food (Boyles et al. 2012). This suggest that if *M. namaquensis* were to be restricted in the amount of food they receive they may instead down regulate their activity levels instead of increasing them, however further investigation would be necessary to determine if this was the case.

R. dilectus displayed the lowest activity levels during this cycle, and in this case, higher day temperatures may suppress locomotor activity. *R. dilectus* is described as a diurnal species, thus the majority of its active time would fall in the warmest part of the ambient temperature cycle. The largest proportion of its activity was exhibited during the light phase of the light cycle however, activity rates were significantly lower than that of the twilight hours. The levels of activity for *R. dilectus* during twilight hours were reduced during LD_{NT} when compared to LD_{N}, this may be because gradual reduced light hours overlap with the hottest and coldest parts of the light cycles. Several other rodent species also show reduced locomotor activity at temperatures that approaches their thermoneutral zones (Finger 1976; Gordon 1993; Pálková et al. 1999; Gaskill et al. 2009; Oosthuizen and Bennett 2015). The ambient temperature cycle appears to reinforce the entrainment to the light cycle without causing a phase shift in this species, since the activity patterns of all individuals showed well-defined diurnal or crepuscular rhythms, but no changes were observed in the onsets and offsets of activity.

Halogen light at night

After the introduction of the first type of ALAN *M. namaquensis* showed a significant suppression in overall activity levels after the addition of ALAN. Their activity levels were severely reduced during the subjective dark hours of LD_H. The proportion of their activity exhibited in the light hours of the cycle also increased slightly which could be indicative of a circadian desynchronising effect of ALAN for *M. namaquensis*. However, *M. namaquensis* did remain nocturnally entrained during LD_H with no shift in their activity onset and offset times. Da Silva et al. (2015) found that the presence of ALAN advanced the onset of dawn song in some European songbirds (*Cyanistes caeruleus*) causing them to start singing earlier in areas affected by ALAN. *M. namaquensis* did not seem to adjust their onset times in response to ALAN, but their physiology may have been affected, indicated by a decrease in their activity levels. Exposure to ALAN masked seasonal light cues, causing a suppression of melatonin and delayed birth of offspring in tammar wallabies (*Macropus eugenii*) (Robert et al. 2015). This effect could be devastating for a seasonally breeding organism such as the tammar wallaby, or indeed *M. namaquensis* which has to time the birth of its young with the seasonal increase in availably of food to ensure maximal survival of offspring. Dominoni et al. (2013a) showed that not only did black birds (*Turdus merula*) advance the onset of their reproductive season by almost a month in the presence of ALAN but individuals captured in urban areas responded differently to ALAN treatments than individuals caught in relatively dark forests. The *M. namaquensis* used in this study were captured from a relatively ecologically dark area; *M. namaquensis* caught from areas of varying levels of light pollution may respond differently to the ALAN treatments in this study.

The addition of the halogen light proved to have an impact on *R. dilectus*; not only did they increase the level of their activity during the dark phase of LD_H but they also increased the total percentage of their activity expressed during dark hours, which were now contaminated by ALAN. Their activity levels during the light phase increased slightly and twilight activity levels remained the same. In *R. dilectus* the most profound effect of the ALAN seemed to be on onset and offset of activity times. They became active almost half an hour earlier than when compared to the previous cycle and ceased activity approximately 25 minutes later, which implies they expanded their active time to 16 hours in total. This expansion of activity may allow *R. dilectus* to expand into temporal niches that it has never occupied before. *R. dilectus,* is a generalist species (Skinner and Chimimba 2005) as well as

being a prolific breeder when conditions are favourable (Taylor and Green 1976), and its potential to be competitive with other species is quite high. It could possibly become a new competitor to some other nocturnal species of rodents. This temporal niche expansion could then eventually lead to physical range expansions and changes in distributional range for *R dilectus* which in turn will alter interspecific community dynamics of the ecosystems it occupies (Rotics et al. 2011). Altering the community dynamics of an ecosystem can have far reaching effects on ecosystem services and species abundances (Longcore and Rich 2004; Minnaar et al. 2015a). If the species does indeed expand toward lighted areas, they have the potential to become commensal in human settlement. The movement of a new organism into close proximity with humans poses a potential for zoonotic diseases to be transferred from these wild vectors to livestock and humans, especially if the organism is host to known vectors of diseases (Matthee et al. 2007).

LED light at night

The last light cycle saw the introduction of a LED light bulb which simulates the light emitted by newer technologies of street lighting (Davies et al. 2013b). These new LED technologies come in two main varieties; a neutral or warm white light which emits light in the 3000K to 4000K colour range and a cool white variety which emits light at the 5700K colour temperature (Aubé et al. 2013). In comparison the halogen light bulbs colour temperature is very low at 2900K. These differences in spectral qualities of light have different effects on the physiology and behaviour of animals (Longcore and Rich 2004). *M. namaquensis* was seemingly not affected by the shift in the spectral quality of the LED light at night. They increased their levels of activity after the addition of the LED light, but it was not significantly higher than under the exposure of the halogen light. The main contribution to this increase was during the dark phase where the *M. namaquensis* increased their level of activity slightly. They also maintained their robust nocturnal entrainment with virtually no shift in their onset and offset of activity times. The slight increase in the activity level of *M. namaquensis* could be attributed to habituation, as the presence of ALAN remained a constant factor for about six weeks. This may lead to the conclusion that the impact of ALAN is short lived in *M. namaquensis* and that given a long enough period under ALAN, the *M. namaquensis* would return to baseline activity levels seen in LD_{NT}, however, a longer period of exposure would be required to determine if this were true. Although allowing for some

increase in activity rate, LED light did have a continued suppression effect on activity rates keeping them much lower than those levels observed in LD_{NT}.

R. dilectus did not react to the change to LED light at night with regards to their average activity levels, nor the proportion of their activity exhibited in the various light phases, but their activity onset time did show a significant shift. They became active half an hour earlier, arousing from sleep at 03:36 continuing the trend observed in LD_H. They did not delay their offset times any further in response to LED light. Whereas *M. namaquensis* experienced short-term effects for light at night *R. dilectus* appears to experience more long-term effects from the presences of light at night, which are more pronounced under LED light than under halogen light. This would mean that as older lighting technologies are replaced with new LED luminaires, *R. dilectus* could expand its range of activity and eventually its distributional range even further. A possible explanation for this may be the unique visual capabilities of *R. dilectus*. Although the visual capabilities of *R. dilectus* have not been assessed, its closely related sister species *R. pumilio* has been found to have exactly 50% rod and 50% cones allowing them to see equally well in both dim and bright light (Schumann et al. 2005). This would allow *R. dilectus* to exploit the extended twilight period created by ALAN in human settlements. This would also bring it into direct competition with nocturnal species that emerge at twilight.

Chapter 3: The impact of environmental enrichment, natural light cycles, temperature and artificial light at night on the stress responses of nocturnal (*Micaelamys namaquensis*) and diurnal (*Rhabdomys dilectus*) rodent.

Introduction

Animals often experience adverse conditions during their day to day activities, these stressors stem from a range of causes such as behavioural, environmental and demographic events (Creel 2001). Stressors such as these create a hormone-based stress response within an organism. The hormones released cause a number of physiological changes designed to limit energy consuming physiological processes that are not essential for the immediate survival of the organism (Buchanan 2000), thereby ensuring its survival. Resources are rather directed toward essential survival mechanisms during stressful events. Endocrine stress responses are generated by the hypothalamic – pituitary – adrenocortical (HPA) axis (Romero et al. 2009). Stress responses are initiated when the hypothalamus receives stimulus caused by external stressors. The hypothalamus then releases corticotropin releasing hormone (CRH) which stimulates the anterior pituitary gland which causes it to release adrenocorticotropic hormone (ACTH). ACTH acts principally on the adrenal cortex situated near the kidneys, which in response produces glucocorticoid (GC) hormones (Sapolsky et al. 2000). The release of GC hormones can be considered either acute or chronic depending on the duration of release.

Acute stress responses are short lived and generally have an adaptive value (Morgan and Tromborg 2006) to the organism facilitating its survival during stressful events (Buchanan 2000). An increase in circulating GC hormones redirects resources within the organism and mobilizes glucose resources that enables the immediate survival of an organism during taxing events (Buchanan 2000; Sapolsky et al. 2000). The primary glucocorticoid hormones secreted during a stress response is corticosterone (dominant in most birds and mammals) and cortisol (dominant in humans) (Wasser et al. 2000). The concentration of stress hormones generated during acute stress responses return to baseline levels relatively quickly. On the other hand, chronic stress is the result of prolonged periods of regular exposure to stressors which result in constant secretion of GC, leading to

consistently high concentrations of stress hormones in an organism. Chronic stress is generally considered to be maladaptive and has a wide range of negative cascading effects. Some effects of chronic stress include reduced activation of the HPA in response to acute stressors (Goliszek 1996), suppression of reproductive hormones (Tilbrook 2000) and the suppression of cell-mediated immune responses (Dhabhar 2002).

A major source of both acute and chronic stress in animals is being kept in captivity, which is often necessary for either short periods or extended periods of time in order to facilitate research goals. There are a multitude of causes of stress in captivity including; capture (Gregory et al. 1996; Moorhouse et al. 2007; Dickens et al. 2009), housing conditions (Waiblinger and König 2004; Clubb and Mason 2007), lighting conditions (van der Meer et al. 2004) and ambient temperature (Marai and Rashwan 2004). Chronic stress in captivity can lead to maladaptive behaviours and physiological conditions, which in turn can skew experimental results, thus scientists try to mimic as natural an environment as possible. The traditional convention for maintaining captive animals is to subject them to standard 12h light and 12h dark cycles, with no variation in light intensity during the light portion of the cycles. This does not simulate the temporal changes in light as a natural cycle of sunrise and sunset does and this can lead to increase anxiety in captive animals due to the sharp contrasts between light and dark. Furthermore, captive animals, especially rodents, are kept in small simple cages due to a restriction of housing space (Baumans and van Loo 2013). When these animals are not provided with stimulation which allows them to exhibit natural behaviours such as burrowing and nesting, they develop behavioural abnormalities which may influence physiological data collected during the experiments (Lewejohann et al. 2006; Mason 2010). In captivity an animal's ability to behaviourally regulate its preferred ambient temperature is greatly reduced and in order to standardise studies, animals are frequently maintained at constant temperature. Animals will once again develop abnormal behaviours in response to temperatures that are not considered optimal for them (Morgan and Tromborg 2006).

Light pollution is an emerging factor to which much of the observed global change can be attributed (Gaston et al. 2012). Over recent years many studies have attributed physiological changes discovered in organisms to light pollution (Fonken et al. 2010), one such change being the potential cause of chronic stress. Studies have found that the presence of ALAN impairs the cell mediated immune responses in some species such as

Siberian hamsters (Bedrosian et al. 2011, 2013), Japanese quail (Moore and Siopes 2000), and the rat (Oishi et al. 2006), which could have a negative impact on the survival. The onset of avian seasonal reproduction has experienced a temporal shift in the presence of ALAN, with the development of testes in European blackbirds being offset 3 months earlier in the presence of ALAN (Dominoni et al. 2013c). European blackbirds have also been shown to adapt to urban environments, where anthropogenic disturbance is frequent, by showing reduced stress responses to anthropogenic stimuli compared to individuals living in the wild (Partecke et al. 2006). The spectrum of light emitted by sources of artificial light also plays a role on the impacts of light pollution of the physiologies of animals. Blue fluorescent light (510 - 550nm) was found to be most effective at supressing pineal melatonin concentrations compared to red light (652 – 668nm), which had virtually no effect of pineal melatonin (Brainard et al. 1984). Ouyang et al., (2017) found that nesting great tits under a white light treatment showed decreased concentrations of oxalic acid, this decrease in oxalic acid is indicative of sleep deprivation. Great tits nesting under, green and red lights and dark conditions did not show any change to oxalic acid levels (Ouyang et al. 2017). Many of the deleterious effects found to be caused by the presence of ALAN, are similar to those caused when chronic stress is experienced.

Two species were selected for this study, a diurnal rodent (*R. dilectus)* and a nocturnal rodent (*M. namaquensis).* These two species are widely spread across South Africa and are likely to have been impacted by light pollution at both an ecological and physiological level. These rodent species have highly responsive physiologies to light input. Both species have been highlighted as possible bio-indicators to assess ecosystem health (Avenant 2011). Stress responses for both species have previously been investigated, and radioimmune assays have been validated using the ACTH challenge method. *Rhabdomys dilectus* has higher levels of corticosterone concentrations overall in captive conditions than its arid counterpart *R. pumilio* (Rymer et al. 2013). *M. namaquensis* showed significant responses in corticosterone concentrations and urine production when subjected to three different monochromatic light sources (van der Merwe et al. unpublished)

This chapter set out to determine the effects of different environmental conditions on the physiological stress levels of *R. dilectus* and *M. namaquensis* in captivity. Parameters investigated were the effect of cage environment enrichment, gradual light changes, a daily temperature cycle and the impact of ALAN. Once a baseline was established, the effect of

each of these environmental factors were investigated. I wanted to determine the impact that different spectra of light, commonly used in sources that contribute to light pollution globally, would have on the corticosterone concentrations of these animals. Given the literature on these two species, I posited that stress levels would be lower with the successive addition of enrichment, natural light and temperature. On the other hand, existing literature concerning the physiological effects of light pollution suggest that both species would show increased levels of stress under the presence of ALAN and that the effect would be greatest under the LED light used in this experiment.

Methods and Materials

Animal Capture and Housing

The animals used in this section of the study were the same individuals used in Chapter 2 of this thesis. Thus, the methodology followed in order to capture the animals and maintain them under laboratory conditions are the same as those described in the animals capture and housing section on page 15.

Urine Collection

Urine collections were done between 7:00 am and 11:00am in the morning on the last day of each of the light cycles described in the Light Cycles section of this thesis on page 16. The infrared captors were disabled prior to capture. Each individual was captured as quickly and gently possible to minimise the impact of handling stress and placed inside a urine trap. The urine trap was then placed on a shelf in the experimental room and monitored every 10 minutes for the presence of urine. Once urine was detected in the urine trap it was collected using a Pasteur pipette and transferred to an Eppendorf tube. The tube was then labelled, placed in a plastic Ziploc bag and frozen as soon as the sample was recorded. Samples were kept frozen until hormone analysis was performed.

Urine traps consisted of a hollow PVC pipe, closed at one end with galvanised wire mesh and with a removable lid on the other end. The lid was partially covered with the same galvanised wire mesh to allow for natural light to penetrate and air circulation while animals were enclosed. Each urine trap had its own collection tray which was washed and sterilized before each use. The wire mesh bottom of the urine trap allowed urine and faeces

to fall through onto the collection tray and prevented animals from stepping in their urine while urine traps were being monitored.

During urine collection while animals were not contained in their home cages, their cages were cleaned, and bedding replaced. Animals were returned to their home cages no more than 4 hours after being placed in the urine traps.

Hormone Extraction and Creatinine Determination

Corticosterone analysis was performed by the endocrine laboratory at the Onderstepoort campus, University of Pretoria. Urinary glucocorticoid metabolite concentrations were measured for immunoreactive CCs by a 96 –well microtiter enzyme immunoassay plate coated with IgG, following previously established methods published by Ganswindt et al. (2002). For hormone determination, 50µl aliquots of standards (range from 0.98-250pg for CCs), quality controls and diluted urinary extracts were pipetted in duplicate into a 96 well microtitre plate coated with IgG. Subsequently 50µl of biotinylated corticosterone label and antiserum were added, the plates were then covered and incubated overnight at 4°C. Following the incubation, the plates were washed four times and patted dry on a clean towel and immediately 150µl (20ng) of streptavidin-peroxidase was added to each well. The plates were then covered again and incubated at 4°C on a plate shaker for 45 minutes. Plates were washed again before 150µl peroxidase substrate solution was immediately added to each well and then plates were covered and further incubated for between 30-60 minutes. Incubation of the plate is considered complete when optical density of the zero wells reaches 0.8 to 1.0. The reaction was terminated by adding 50µl of 4N H_2SO_4 and the absorbance was measured at 450nm using the Gen5 software provided. The assays used in the analysis of urine samples from *R. dilectus* had an intra assay variance of 4.15% - 5.14%, and an inter assay variance 6.29% - 10.05% with a sensitivity 0.06ng/ml. The assay used to analyse urine samples from *M. namaquensis* had an intra assay variance 4.15% - 5.41%, an inter assay variance 5.58% - 6.68% and a sensitivity of 1.5 ng/ml.

Creatinine concentrations were determined to correct corticosterone concentration values according to the concentration of urine. All solutions used in the creatinine determination were freshly prepared. Picric reagent was prepared by adding one-part saturated picric acid solution and one-part alkaline triton solution, which consisted of 4.2ml triton and 12.5ml NaOH in 66ml distilled water, in 10 parts distilled water. 210µl of Picric reagent was pipetted into micro-wells, along with 7µl of each urine sample and allowed to

incubate at room temperature in darkness for two hours. Plates were then read on a plate reader at an absorbance of 492nm at 25°C.

Data Analysis

The corticosterone concentrations were corrected according to the creatinine concentration in the urine sample in order to compensate for the difference in water content of each individual sample. A generalised linear mixed model was used to analyse the data since the data contains repeated measures and the sample size was relatively small. IBM SPSS was used for the statistical analysis.

Results

Rhabdomys dilectus

No urine samples were collected for *R. dilectus* during LD_{NE} therefore this group was not included in the analysis. Mean corticosterone concentration during LD_E was 418.40 ± 204.98ng/mg (Fig 10). Males had higher concentrations of corticosterone than females although not significantly so ($F_{1, 43} = 0.247$, P = 0.622, Fig 11). Corticosterone levels after LD_N (480.89 ± 236.92 ng/mg) were not significantly different from that after LD_E ($F_{4, 43} = 0.339$, P = 0.843). Males again had higher corticosterone concentrations than females. (Fig 11), this difference was not significant ($F_{1,43} = 0.514$, P = 0.477). Mean corticosterone levels were lower during LD_{NT} (239.84 ± 119.11 ng/mg) when compared to LD_N, however not significantly so ($F_{4,43} = 0.339$, P = 0.368, Fig 10), and males and females had similar stress hormone concentrations during LD_{NT}. ($F_{1,43} = 0.015$, P = 0.903). After the addition of halogen LAN the average corticosterone concentration of the animals rose to 384.70 ± 191.11 ng/mg ($F_{4,43} = 0.339$, P = 0.523). Male animals once again had higher average corticosterone levels than females ($F_{1,43} = 1.206$, P = 0.278). The addition of LED LAN caused the average stress hormone concentration to increase (401.15 ± 199.28 ng/mg, Fig 10) however once again this difference was not significant ($F_{4,43} = 0.339$, P = 0.953) when compared to LD_H. Finally, males once again retained a higher average level of corticosterone than females however, the difference was non-significant ($F_{1,43} = 0.894$, P = 0.350).

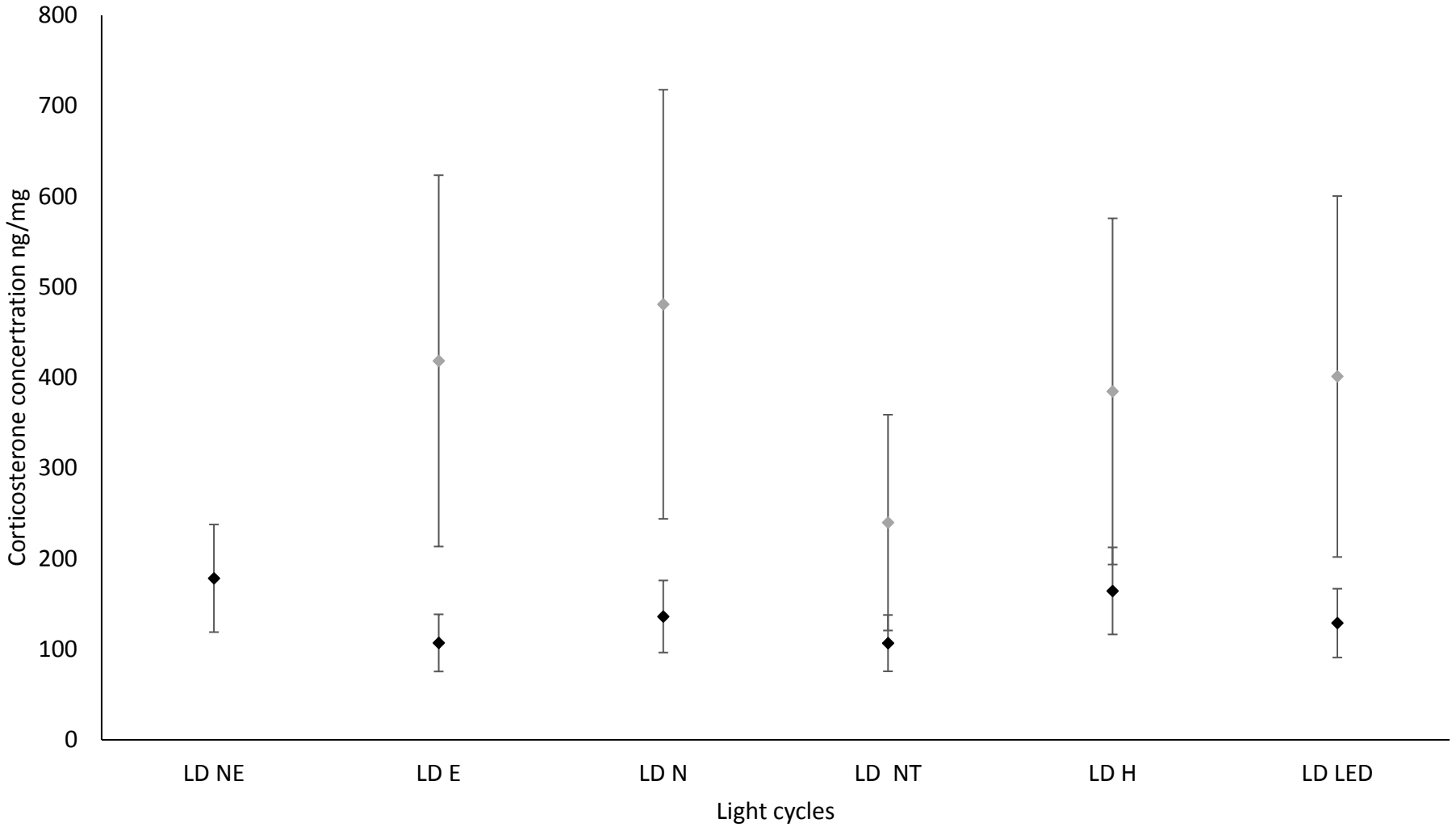

Figure 10. Average concentration of corticosterone stress hormone in urinary samples taken from *R. dilectus* (grey dots) and *M. namaquensis* (black dots) analysed after each of the six different light cycles (x-axis).

Micaelamys namaquensis

The average corticosterone hormone level during LD_{NE} for *Micaelamys namaquensis* was 178.32 ± 59.40 ng/mg (Fig 12). Females maintained higher concentrations of corticosterone in their urine than males (Table 2) however, this difference was not significant ($F_{1,46} = 3.521$, $P = 0.067$, Fig 10). Mean corticosterone concentrations decreased after the introduction of enrichment items during LD_E to a level of 107.02 ± 31.53 ng/mg, but this decrease was not statistically significant ($F_{5, 46} = 0.446$, $P = 0.295$). Female animals had significantly higher ($F_{1, 46} = 7.482$, $P < 0.01$) average stress hormone levels during LD_E than males (Fig 12). After LD_E natural light regimes were introduced and the light cycle called LD_N, the average corticosterone concentrations increased again to 136.17 ± 39.81 ng/mg, but this difference was not significant ($F_{5,46} = 0.446$, $P = 0.569$), females had higher stress hormone concentrations during LD_N than males ($F_{1, 46} = 7.807$, $P < 0.01$). During LD_{NT} the average stress hormone concentrations decreased once again to 106.75 ± 31.09 ng/mg and females still had significantly higher corticosterone levels when compared to males ($F_{1, 46} = 7.857$, $P < 0.01$). After the addition of ALAN in LD_H the average corticosterone concentrations increased once more to a level of 164.42 ± 48.07 ng/mg (Fig 12). Females had significantly higher corticosterone concentrations in their urine than males ($F_{1, 46} = 8.224$, $P < 0.01$). The addition of LED light at night reflected as a decrease in average urinary corticosterone levels in *M. namaquensis* to 128.87 ± 37.97 ng/mg, however this decrease was not significant ($F_{5, 46} = 0.446$, $P = 0.565$). Females had significantly higher corticosterone levels during LD_{LED} - than males ($F_{1, 46} = 7.215$, $P < 0.01$, Fig 10).

Table 2. Average corticosterone values (ng/mg) for male and female *Rhabdomys dilectus* and *Micaelamys namaquensis* during several different light cycles.

Species	Light Cycle	Female	±SE	Male	±SE
Rhabdomys dilectus	LD_E	384.478	198.723	455.324	235.341
	LD_N	420.232	217.203	550.294	290.15
	LD_{NT}	245.272	126.772	234.518	127.224
	LD_H	296.953	153.484	498.363	270.357
	LD_{LED}	325.767	168.377	493.976	267.977
Micaelamys namaquensis	LD_{NE}	337.527	143.71	94.21	31.383
	LD_E	171.517	52.414	66.771	21.343
	LD_N	222.816	68.09	83.219	25.905
	LD_{NT}	168.552	50.81	67.604	21.044
	LD_H	282.363	86.287	95.736	29.802
	LD_{LED}	201.679	61.631	82.367	26.328

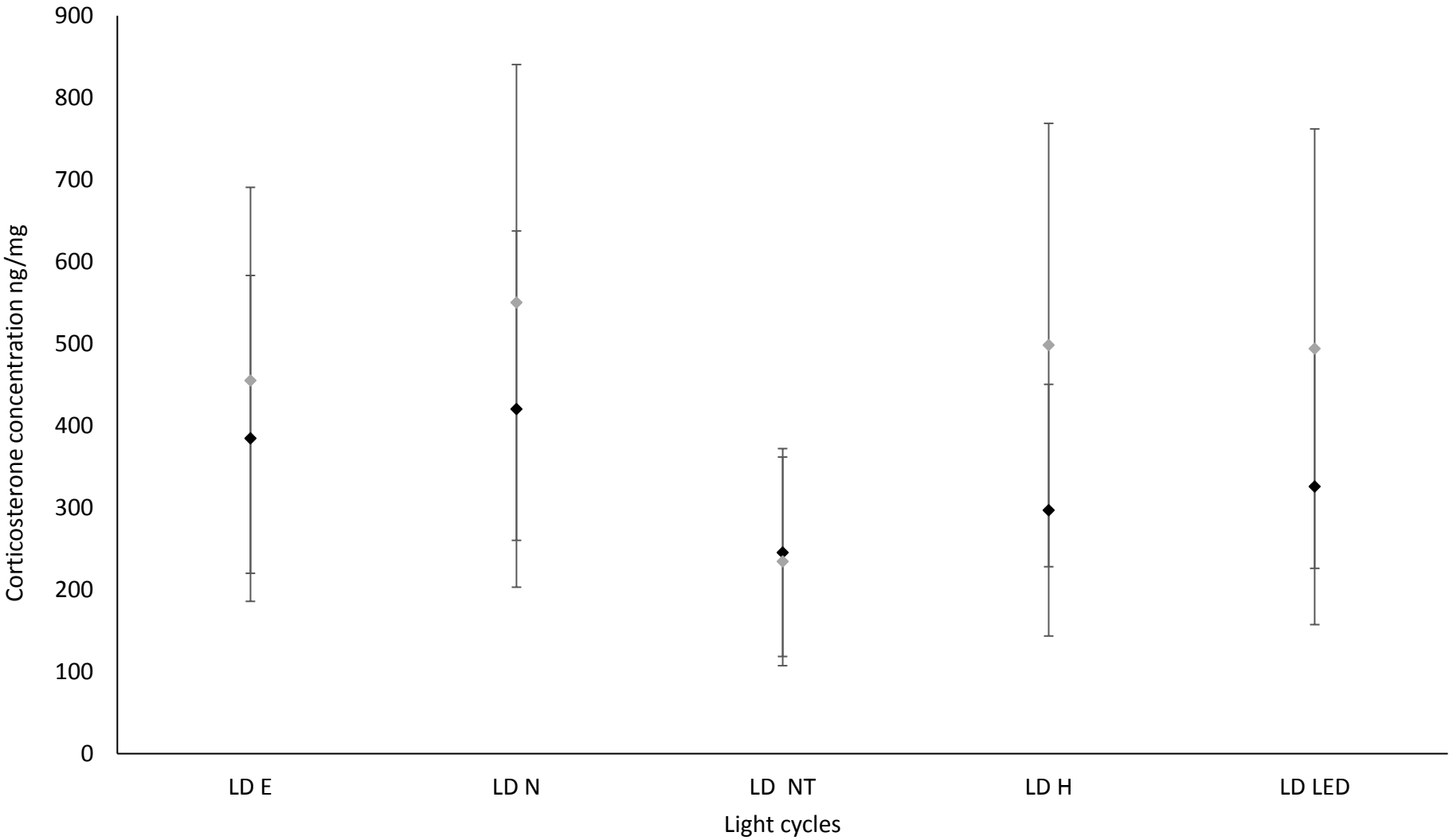

Figure 11. Mean corticosterone concentration values for male (grey dots) and female (black dots) *R. dilectus* analysed from urinary samples at the end of six light cycles (x-axis).

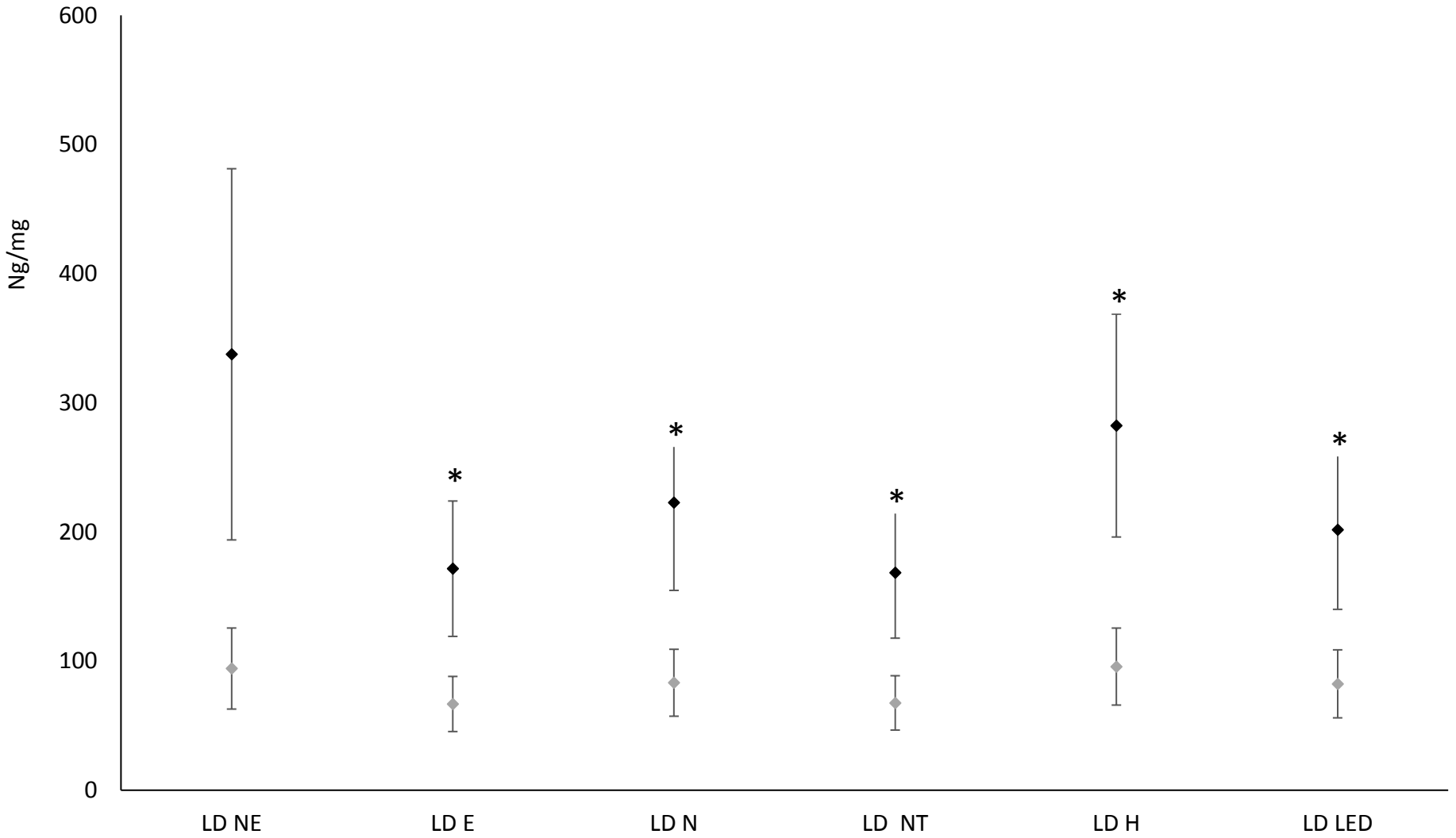

Figure 12. Average corticosterone concentration values for male (grey dots) and female (black dots) *M. namaquensis* analysed from urine samples at the end of six light cycles (x-axis).

Discussion

I investigated the effects of three environmental factors on the stress hormone concentrations of two species of rodent kept in captivity. After the effects of enrichment, natural light cycles and temperature was established, ALAN was introduced using two of the most common types of light attributed to light pollution, in order to determine the possible impacts of ALAN on the stress physiologies of *R. dilectus* and *M. namaquensis*.

The effect of environmental enrichment

Environmental enrichment has been shown to have both positive and negative effects on animals. Belz et al. (2003) reported a reduction of glucocorticoid hormones in animals housed in environmentally enriched conditions, while Moncek et al. (2004) found rats to show lower resting stress hormones concentrations if housed in unenriched environments. This suggests that the effects of enrichment on the wellbeing of animals can be species and context specific, and this idea has indeed been highlighted in literature (Mason 2010). Ideally the type of enrichment provided to a species should allow it to express at least a partial range of the behaviours that it would exhibit in its native habitat (Hutchinson et al. 2005). For example, burrowing animals should be provided with opportunities to burrow instead of climb, as providing enrichment that requires climbing may increase stress instead of alleviating it.

The effect of enrichment on the stress levels of *R. dilectus* could not be assessed in this study due to the lack of urine samples. No samples could be collected due to extremely low urine production rates. The low rate of urine production for *R. dilectus* could possibly be explained by the type of lighting used in the facility where they were housed. The lighting consists of fluorescent tubes lights which produces a proportion of short wavelength light. Van der Merwe et al (unpublished) found that, a very closely related species *R pumilio*, showed decreased volume of urine production under the influence of short wavelength lights in captivity. It may be likely that stress hormone concentration for *R. dilectus* would have been slightly higher under non-enriched conditions than during LD_{EN}. This could be due to captive stress, and if corticosterone concentration decreased after enrichment this may have purely been as a result of habituation. Certain personality groups in *R. dilectus* reacts favourably to enrichment by increasing their activity in an enriched environment (Joshi and Pillay 2016b). In the case of *R. dilectus* , which has been suggested to be a less bold species

than *R. pumilio* (Rymer and Pillay 2012), the increased activity in captivity can be seen as a proxy for reduced anxiety levels (Ijichi et al. 2013). However, this cannot be compared in our study and are therefore speculative and requires a dedicated study to confirm. Furthermore, according to literature, the lack of enrichment provided during LD_{NE} would also likely have exacerbated the stress experienced by *R. dilectus* , as there was no provision of "safe" spaces the animals could retreat to (Ottesen et al. 2014).

M. namaquensis showed a decrease in average corticosterone concentration after the introduction of environmental enrichment although this difference was not significant, suggesting that for this species at least, the type of enrichment provided alleviated the stress caused by prolonged periods of captivity. Among the enrichment items given to *M. namaquensis*, the opportunity to engage in nest building most likely had the most pronounced effect on reducing physiological stress. Nest-building behaviour is common in many rodent species and has been highlighted as a key enrichment strategy for those kept in captivity (Manser et al. 1998; Hutchinson et al. 2005). Female *M. namaquensis* displayed significantly higher stress than their males counter parts in spite of the addition of enrichment, although both sexes stress levels were lower than those recorded during LD_{NE}, suggesting that enrichment reduced stress in both male and female *M. namaquensis*.

Effect of dawn-dusk simulation

After animals entrained to a square wave light cycle, with abrupt transitions between light and dark, they were introduced to LD_N which simulated a gradual change in light intensity similar to dawn and dusk in nature. Light acts as the most prominent cue to animals in their natural habitats, signalling to an organism via their circadian rhythms when to time important events such as reproduction or foraging (Sharma 2003; Froy 2011). Lighting conditions in captivity are often maintained at constant intensities with very little variation which does not reflect natural light conditions (Morgan and Tromborg 2006). Incorrect maintenance of light conditions in captivity can have deleterious effects ranging from disruption in circadian activity (Ikeda et al. 2000), increased anxiety behaviours in animals (Pollard and Littlejohn 1994) and increase in the incidences of injury due to aggressive behaviours (Moinard et al. 2001).

The addition of a natural light cycle was expected to decrease physiological stress as it is closer to natural conditions the animals experience in the wild (Baumans 2005). However, the average stress levels for both species increased and *R. dilectus* males

continued to show higher concentration of stress hormone than females. Females of *M. namaquensis* also continued to exhibit higher levels of stress than males, even though both sexes experienced an increase in stress with exposure to the dawn-dusk cycle. There is very little literature on the use of simulated dawn dusk cycles in captive animal studies. Tang et al. (1999) investigated the effects of a square wave and a natural dawn dusk light cycle on the circadian rhythms of Syrian hamster, which exhibited different circadian characteristics under each light cycle.

The unexpected increase in corticosterone concentrations after the addition of natural light wave could possibly be attributed to the lights used in the housing facility to generate the dawn-dusk cycles. Fluorescent tubes emit light discontinuously which leads to a phenomenon called flicker which many species are sensitive to (Morgan and Tromborg 2006). This flicker is imperceptible to human vision however may be detectable by some species (Greenwood et al. 2004) and becomes more pronounced when the frequency of the light is attenuated to create the slow dimming effect of the sun setting. European starlings (*Sturnus vulgaris*) exhibit markers of chronic stress when kept under low frequency fluorescent lights in captivity (Evans et al. 2012) and it has been suggested that the flicker produced by fluorescent light could also induce stress responses in rats (Brandão and Mayer 2011).

The types of luminaires and the quality of light they produced can be critical not only to the wellbeing of animals in captivity, but also the results obtained from captive studies (Morgan and Tromborg 2006). Light is an ever-present stimulus, constantly providing input to organisms about the state of their environment, this makes its potential to act as a stressor even greater. Moreover, it means that lighting conditions should be carefully considered according to the species of captive animals and a source chosen based on what will minimise the physiological stress the animal experiences and not the source that is most convenient for the researcher.

Temporal temperature cycle

Temperature is another factor often given little or no thought to for animals in the laboratory, unless temperature is actively manipulated as part of experimental procedure. Choices concerning the temperatures animals are maintained under are often arbitrary or made based on convenience for the researchers working with them. Animals thus often end

up in situations where, for example tropical animals are maintained at temperate conditions, which could be considered stressful.

In this study, animals were initially maintained at 25°C since this was the value most commonly found in literature concerning these two species and would facilitate comparisons between this study and others (Schumann et al. 2005; van der Merwe et al. 2014; van der Merwe et al. 2017). Upon the introduction of a simulated temperature cycle *R. dilectus* and *M. namaquensis* both showed a reduction in their average stress hormone concentrations although the difference was not significant. This would suggest that constant temperatures are perceived as unnatural and can act as a stressor for both species in this study, and that allowing temperature to vary temporally as it would in natural daily rhythms reduces the stress these animals undergo in captivity. Ambient temperature cycles can reinforce light as a zeitgeber for activity and other physiological rhythms (Buhr et al. 2010) and the removal thereof may be stressful to the animals.

This effect seemed especially pronounced for *R. dilectus* as male corticosterone concentrations decreased to become almost equal with that of the females during LD_{NT}, suggesting that temperature could be of particular importance in the stress physiology of male *R. dilectus*. The reaction of male *R. dilectus* suggests that they may be particularly sensitive to temperature in captivity. *R. dilectus* has been highlighted as a species that experiences more stress in captivity (Rymer et al. 2008) than other closely related species and this will be important to consider in future captive studies on the species. Female *M. namaquensis* however were still maintaining higher stress levels than males although they did decrease in response to the natural temperature gradient.

R. dilectus and *M. namaquensis* occur sympatrically in much of their distributional ranges and as such the temperature cycle used in this experiment was tailored to reproduce the average temperature, they would encounter in the wild during an average summer day in South Africa. This approach has one major drawback in that it makes standardisation among laboratories even more difficult, as another group of researchers would have to recreate the exact conditions of this experiment in order to compare their own findings with those of this study. However, if standardisation protocol were species specific it could eliminate some of the replication issues (Latham and Mason 2004). A set of guidelines such as those proposed here are already in place for common strains of white mice bred specifically for laboratory studies. Species specific guidelines can ensure not only wellbeing

of wild caught animals in captivity but can also vastly increase the scientific rigor of laboratory studies by eliminating confounding influences caused by the stress of captivity and making the results more applicable to real world problems.

Artificial light at night

The impacts of light pollution stem from far more than just the presence or absence of ALAN. The type of light contributing to light pollution also has a major effect. The prevailing belief is that where the presence of ALAN is unavoidable, longer, slower wavelengths of light will minimise the impacts of ALAN on the ecological darkness (Longcore et al. 2015). New technologies such as LED lighting, which produce shorter, faster wavelengths of light are said to increase the harmful effect of light at night. This theory was tested during the LD_H and LD_{LED} light cycles. LD_H introduced ALAN produced by a halogen light bulb, this simulated the type of light many older technologies of lighting would produce and has the characteristic yellow/orange hue associated with older streetlights. On the other hand, LD_{LED} introduced shorter wavelengths of light rendering light a whiter appearance and many new streetlights would appear similar once they are replaced with LEDs.

As expected, both *R. dilectus* and *M. namaquensis* showed increased concentrations of corticosterone in response to the presence of light at night, with male *R. dilectus* displaying the greatest increase and exhibiting greater stress levels than females. Female *M. namaquensis* also showed a greater increase than their male counterparts to the introduction of ALAN. Thereafter it was expected to see a further increase in corticosterone concentration among the two species in response to the addition of LED light. However, the anticipated response was not shown, *R. dilectus* did not experience any reaction to the addition of LED light and *M. namaquensis* even showed decreases concentrations of corticosterone under the influence of LED light.

While both species are clearly perturbed by the presence of light at night, the fuller spectrum of light did not seem to make as large an impact as expected. It has been suggested multiple times in the literature that impacts of light pollution are species specific (Gaston et al. 2015b; Grubisic et al. 2018) which seems to be true in this experiment. In the case of these two rodent species white light source can be expected to have the same, if not less impact than yellow light sources. *M. namaquensis* is a nocturnal rodent and as such is rightly assumed to be a species that would experience the greatest impact from ALAN, however its nocturnal adaptation may actually work in its favour. *M. namaquensis* eyes are

adapted to maximise vision in low light conditions and has a low density of short wavelength receptors in its retina which reduces its capacity to perceive the short wavelength blue light produced by the LED. This may explain why *M. namaquensis* became less stressed after the addition of LED light but was still more stressed by halogen light, which it is fully capable of perceiving. However, care should be exercised when interpreting the results of this study as only one sample of stress hormone was taken from each species. In future, a series of samples may be more suited to elucidating the finer scale of effects ALAN may incur. Birds on the other hand whose eyes are adapted to see much farther into the ultraviolet range of the light spectrum would likely be much more susceptible to the impacts of white LAN (Church et al. 1998; Cuthill et al. 2000).

Extended exposure to ALAN may affect long term physiological responses of animals dramatically. Dominoni et al (2013b) found that ALAN advanced the reproductive physiology of European birds. Raap et al (2016) established that it may be possible for early life exposure to ALAN may have long term adverse fitness consequences of blue tit chicks. Although a large body of work has been done on long term effects of ALAN on bird species (Kirby and Froman 1991; Moor and Siopes 2000) there is virtually no evidence to suggest that the same impacts observed in birds are not possible in mammal species. A study by Bedrosian et al. (2011) showed that elevated stress hormone levels as a result of chronic exposure to dim LAN can lead to suppression in immune responses in hamsters. It is therefore feasible to assume a host of similar consequences could potentially also occur in *R. dilectus* and *M. namaquensis* should ALAN continue to increase at its current rate and they become chronically exposed in their natural environments.

Throughout the study female *M. namaquensis* maintained significantly higher concentrations of corticosterone than males. Individual isolation could explain the consistently higher stress levels displayed by the females compared to male individuals. In their native habitat these rodents are considered communal with females often grouping together in shared nests. Social isolation has been shown to cause physiological stress responses in social animals, especially in laboratory mice (Calisi and Bentley 2009). While individual isolation is often necessary for the purposes of research it is important to consider as a source of stress and possible variation in the data of the experiments. Overall *R. dilectus* was much more stressed than *M. namaquensis*, for the duration of all the light

cycles. However, comparison cannot be made for the LD_{NE} cycle as no urine samples could be collected for *R. dilectus* during that light cycle.

Chapter 4: The impact of different spectral compositions of artificial light at night on the foraging behaviour of naïve nocturnal rodent communities.

Introduction

One of the great advances of modern technology is the invention of artificial light sources. This has allowed humans to extend their activity beyond the confines of daylight hours. Artificial light at night (ALAN) has become a wide spread and almost unavoidable phenomenon on Earth (Longcore and Rich 2004). The prevalence of artificial lighting has transformed the night time landscape over the past years (Gaston et al. 2014) causing what we know today as ecological light pollution (ELP). ELP disrupts the natural cycles of light and darkness present in the environment (Davies et al., 2013a). As a consequence of this disturbance many processes that have evolved over millions of years in conjunction with natural light cycles such as the circadian rhythms by which animals determine their activities may be disrupted. These activities include migrations, reproductive behaviours and foraging behaviours among others. The disruptions caused to these activities can trigger changes ranging from individual levels through to community levels (Bird et al. 2004; Davies et al. 2012; Dominoni et al. 2013c), thereby irreversibly altering the way in which an ecosystem functions.

Ecological light pollution has many sources, but street lighting is considered one of the largest contributing factors to light pollution worldwide (Cinzano et al. 2001; Gaston et al. 2012). The effects of street lighting are mainly grouped into two types, namely direct or indirect. Direct effects are caused by direct illumination of surfaces from the luminaires and the reflection of light from the surrounding surfaces (Gaston et al. 2013). Direct effects are experienced in close vicinity to the light source and usually at high intensities of light. On the other hand indirect effects are categorised as relatively low intensities of diffuse light experienced over much larger areas caused by the contribution of light from luminaires scattered in the atmosphere (Gaston et al. 2013) often referred to as sky glow. Not only does the domestic and commercial use of artificial light contribute to ELP, the spectral composition of the light used can also alter the impact the light may have on biodiversity.

A recently emerging trend in urban lighting has older forms of lighting being systematically replaced with better, more energy efficient alternatives in order to reduce the environmental impact of artificial lighting in line with international agreements such as the Kyoto protocol (Horváth et al. 2009; Bouslama and Schapaugh 2010). This drive toward energy efficiency in artificial lighting is also changing the spectral composition of ecological light pollution (Gaston et al., 2014; Davies et al., 2013b). At the forefront of this push towards energy efficiency is street lighting.

Common types of conventional street lightning used are high pressure sodium (HPS) lamps, mercury vapour (MV) and metal halide (MH). These lamps produce light in narrow discontinuous bands over a broad range of wavelengths. HPS lamps produce primarily yellow light, whereas high intensity discharge lamps such as MH and MV emit whiter light with significant peaks in the blue and ultraviolet wavelengths (Elvidge et al. 2010; Gaston et al. 2013). Light emitting diodes or LED lamps are the most common energy efficient replacements for the older types of lighting. LED lamps produce white light over a broad range of wavelengths with a continuous symmetrical emission curve that peaks in the very narrow wavelength region of ultraviolet light (Gaston et al. 2013). The changing spectral emission of sources that contribute to light pollution may serve to extend the harmful effects of ELP. The narrow wavelengths of light produced in LED lamps permeate through the environment much faster, but tend to dissipate more quickly, reducing the radius of effect of a single light source there by increasing the intensities of light pollution. Calculations done by Bierman, (2012) showed that LED lights could contribute between 10 and 20 percent more light scatter to skyglow, changing not only its colour but also increasing its intensity as well.

The presence of ALAN has effects on species ranging from insects, to reptiles, birds and mammals at varying ecosystems levels, starting at the individual and reaching up to the community level (Rotics et al. 2011; Dominoni et al. 2013c; Somers-Yeates et al. 2013). One of the most common effects of ALAN is the disorientation of species dependant on natural sources of light or ecological darkness. Sea turtle hatchlings (*Caretta caretta*) are disoriented and attracted toward ALAN inland on coastal roads and settlements and as a result do not find their way to the ocean during hatching periods thereby reducing their fitness when they do eventually orient towards the ocean (Lorne and Salmon 2007; Bourgeois et al. 2009). Birds on migratory paths are dependent on natural illumination of celestial bodies in order

to navigate their routes (Poot et al. 2008). Van Doren et al. (2017) monitored an annual tribute event, where artificial illumination increases significantly for seven days each year for seven years and found that approximately 1.1 million birds were affected by the resulting increased illumination. Birds were attracted to the areas in high densities, slowed their flight speeds and continued to follow circular flight paths around the event (van Doren et al. 2017). Many bats species commute daily between roosting sites and feeding sites, Stone et al., (2009) showed that street lighting along roads formed an effective barrier to the flight path of bats commuting to and from their roost site. The study also concluded that bats showed no sign of habituation and continually refused to cross the light barrier.

Communication systems may also be influenced by the presence and spectral content of ALAN. The presence of green light at night showed a marked increase in the number of call sequences recorded for common pipistrelle bats around the areas the lights were erected (Spoelstra et al. 2015), they theorised that this was due to an increased attraction of moths to green light compared to other spectrums of light tested. In the same study conducted by Spoelstra et al., (2015) they also found that irrespective of ALAN's colour the activity of deer mice (*Peromyscus maniculatus*) was suppressed. Nordt & Klenke, (2013) found that European blackbirds (*Turdus merula*) started their dawn song rituals, which males use to defend territories and attract suitable females, up to five hours earlier than normal in city centres compared to semi urban environments.

One of the most often affected aspects of behaviour under ALAN is foraging patterns in species. A study conducted on Cape serotine bats (*Neoromicia capensis*), showed that when compared to ecological dark condition, bats ate more eared moths despite the density of moths decreasing around an experimental illumination treatment (Minnaar et al. 2015). Titulaer et al. (2012) showed that female great tits (*Parus major*) increased rates of food provisioning to their chicks when ALAN was present outside the nest, which could possibly have a negative fitness effect of the female parent because of excessive energy expenditure. ALAN caused several species of diurnal birds to aggregate around the source of artificial light, extending their activity period well into the night in order to forage on insects that were attracted by the artificial light (Lebbin et al. 2007). A study conducted on coastal assemblages of nocturnal waders found that visual foraging waders were drawn to artificially lit areas, and that their prey intake rate improved by 83% (Santos et al. 2010). Although the waders benefit positively from the increase in foraging success, foraging in

illuminated areas may draw them into degraded urban habitats where they can face increased pressure from predators (Santos et al. 2010).

The study conducted by Bird et al in (2004) showed that not only do beach mice (*Peromyscus polionotus leucocephalus*) forage less under lighted conditions at night, but that the spectral composition of the light also influences the amount of food beach mice are willing to forage. The beach mice foraged from artificially created foraging patches stocked with commercial seed, and the giving-up density (GUD) was used to determine the extent to which they foraged under the different experimental conditions (Bird et al. 2004). GUD is defined as the density of resource contained in a specified location at which an organism will stop feeding (Brown 1988). The willingness to forage or not, under a set of conditions is related to the benefit an organism perceives it will gain while weighing it up against the possible costs incurred while foraging. When the organism believes that the costs of foraging in a resource patch has begun to outweigh the benefits gained it will stop foraging and move on to a more beneficial resource area, this is when the GUD of the initial patch has been achieved (Shaner et al. 2007).

Factors that may affect the decision of an animal to forage in any particular resource patch includes, perceived, predation risk, surrounding vegetation cover and the quality of the resource in a patch. Animals will prefer higher quality food patches over poorer quality even if a patch is of high quality but under high risk of predation the organism may compromise and utilise a lower quality patch instead. Utilising a poorer quality patch with no threat of predation will mean an organism will forage in that patch for longer, resulting in a lower GUD. Higher risk resource patches will reflect much higher GUD, with more resource remaining after an organism has moved on.

This chapter was initiated as a result of the findings from a study performed by Bird et al. (2004). The main aim of the experiment was to investigate whether it would be possible to adapt the same methods used by Bird et al (2004) to assess how native South African rodent communities will response to the introduction of ALAN of different spectral signatures. The three types of luminaires chosen were selected to represent the most common types of street lighting erected in residential areas of South Africa. The study focussed on naïve rodents who have not encountered ecological light pollution to any extent, as an example of the possible implications of urban expansion into unpolluted habitat.

Methods and Materials

Site Description

Illumination experiments and rodent trapping were done in Tswalu Private Nature Reserve (E27.296414, S22.394355). Tswalu is located in the Northern Cape province of South Africa, in the Eastern Kalahari Bushveld. This location was selected as it represents one of the most naturally dark locations in South Africa and is free of almost any direct light pollutant sources. A light array and feeding trays were placed in a part of the reserve with as much homogenous grass cover as possible. The nearest source of any potential light pollution was a human settlement approximately 50km away. In order to standardise natural light levels, the experiment was undertaken during the 3 days prior to and 4 days after the new moon phase of the lunar cycle when ecological sources of light were as dim as possible.

Animal Trapping

Rodents were trapped with Sherman traps to establish a species composition estimate for the area where the illumination trials would take place. Sherman traps were placed approximately 500m away from the area where illumination trials took place to avoid interference. A total of 90 traps were placed out for 5 consecutive nights. Traps were laid out in a grid transect consisting of nine rows of 10 traps each spaced 10m apart. Rows were also spaced 10m apart. Traps were baited with a mixture of peanut butter and oats. Traps were set during early evening and checked for captures at dawn. Any animal caught in a trap was recorded to species level subsequently photographed and released. After animals were released all of traps were closed so that animals could not enter during the day and opened again the next evening at dusk after the light array had been activated.

Illumination Setup

Three luminaires were used in the light array: high pressure sodium (HPS) (BekaLux 70W HPS, Beka (PTY) LTD, South Africa), mercury vapour (MV) (BekaLux 80W MV, Beka (PTY) LTD, South Africa) and LED (LED Lume-mini 26W, Beka (PTY) LTD, South Africa). The lights were mounted on top of a telescopic pole (Teklite, TF 100 P-Series, Belgium), which could be extended to a maximum height of 4m above the ground. The pole was then raised to its maximum height and secured in place. The lights mounted on the pole were powered using two large batteries and an inverter (Power Star, Peak Energy, South Africa), that were mounted in a trailer parked 30m away from the light array.

The experiment was conducted for nine consecutive nights. The first two nights were control nights with the light array set up but not switched on. For the next six nights had the light array was switched on with two nights dedicated to the HPS, LED and MV lights respectively. A final control night was conducted on the last night. The streetlights were turned on at 7:00pm every night and turned off again at 6:00am each morning. The batteries and inverter were then disconnected from the luminaires and the trailer was hitched to a car in order to transport it back to the research facility where the batteries were recharged for the next night's experiments.

Artificial Foraging Patches

In order to compare the activity of rodents using foraging patches, 13 artificial foraging patches were constructed from plastic trays with a hole (approximately 10cm x 10cm) cut into each side. A 14th tray was made with holes in the sides that are small enough to exclude any animals including rodents but allow for airflow through the tray. This tray was placed 50m away from the light array each night to control for any weight gain or loss due to moisture in the atmosphere. Foraging trays were baited with 120g ± 1g sunflower seeds. When in use the foraging trays were placed at seven metre intervals starting directly under the streetlight and ending at 84 metres. The last feeding patch in the transect (No. 13) was placed so that it fell outside the direct illumination range of the streetlights. Each morning the trays were picked up, the remaining amount of seed separated from any debris and weighed, and the weight was recorded. Trays were cleaned out and rebaited for use during the following night.

Data Analysis

The amount of seed eaten at each foraging patch each night was determined and an average at each distance from the light source was calculated for each light treatment. The weights of seeds were corrected using a correction factor calculated using the control feeding patch. The weight gained or lost from the seeds during each night due to atmospheric water loss or gain was then subsequently added or subtracted from the averages calculated from each tray. Overall average GUDs of the four light treatments were compared using Kruskal- Wallis test for significance, as data were not normally distributed and to compensate for the small sample sizes. Mean GUDs at each distance under each light

treatment were displayed on a line graph. Cumulative species richness was calculated for the Sherman trapping effort as well as the average species composition per trap night.

Results

Animal Trapping

Sherman trapping effort consisted of 450 effective trap nights. In total six species were recorded namely *Gerbillurus vallinus, Gerbilliscus brandssii, Gerbilliscus leucogaster, Desmodillus auricularis, Mus indutus and Saccostomys campestris.* However due to difficulty distinguishing between *Gerbilliscus brandsii and Gerbilliscus leucogaster* the results for these two species were lumped together. These species occur sympatrically and are very similar in appearance thus positive identification could not be achieved for all occurrences of the two species. Effective species richness count was five with species saturation occurring after trap night three (Fig 13). The species caught in the highest proportions across all five trapping nights was *Gerbillurus vallinus,* with them comprising an average of 55.60% of the animals caught per night (Fig 14). The second most common species caught was *Gerbilliscus brandsii and Gerbilliscus leucogaster*, which when combined made up an average of 22.99% of the animals caught per night (Fig 14). *Desmodillus auricularis, Mus indutus* and *Saccostomys campestris* all had similar average capture proportions per night constituting 6.86%, 7.35 % and 7.21% respectively of animals trapped per night (Fig 14).

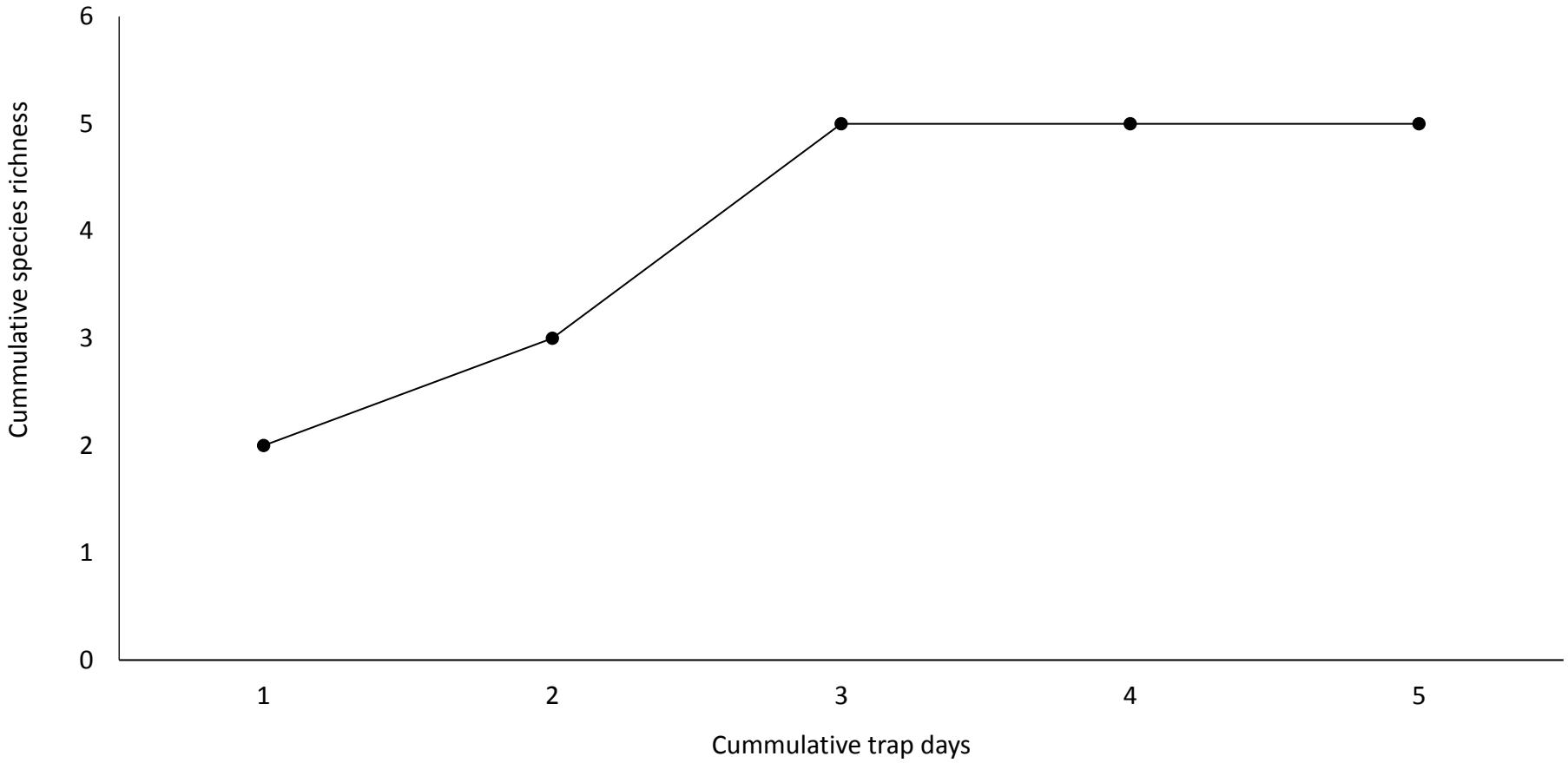

Figure 13. The cumulative species richness over five consecutive traps days (450 trap nights). Species saturation occurred the three nights with no new species being trapped after three nights.

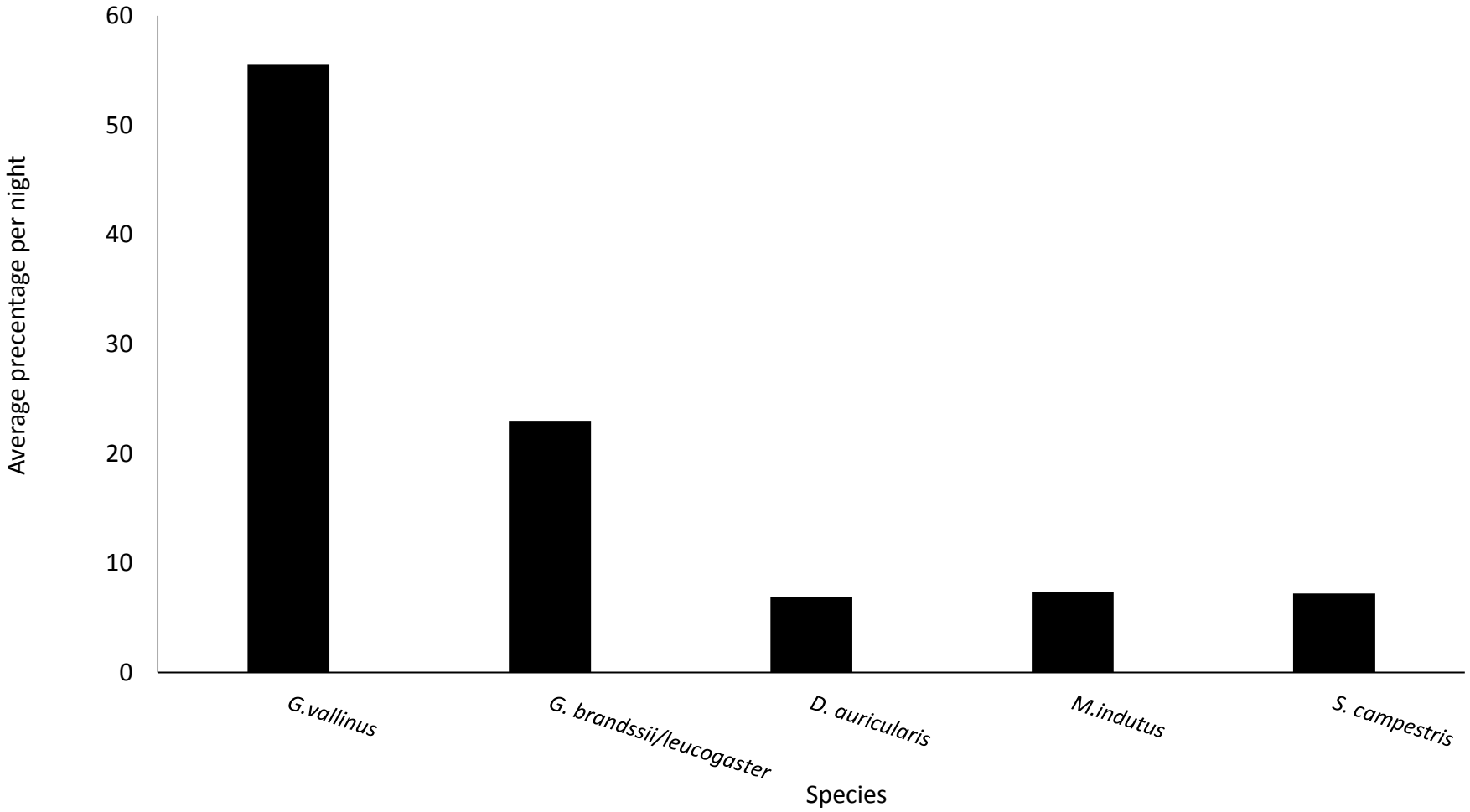

Figure 14. The average percentages of species that made up total individuals caught each night (n=5). *Gerbilliscus brandsii* and *Gerbilliscus leucogaster* were grouped into one column to reflect the percentage of both species captured each night.

Effect of Light on Foraging Activities

The average GUD (mean ± SE) across all distances during the control set up was 95.76 ± 3.14g. The foraging tray 63m away from the street light pole had the lowest GUD with 73.76 ± 10.69g and highest GUD was recorded at 35m as 116.25 ± 0.29 (Fig 15). The addition of HPS light lowered the overall foraging activity with GUD increasing to 99.13 ± 2.69 however this effect did not test as significant ($H = 4.876$, $P = 0.181$). Once again, the lowest mean GUD was recorded at 63m from the light source at 83.22 ± 18.32g and the highest GUD was 114.99 ± 0.80g at 35m (Fig 15). Although the addition of LED light lowered the average GUD to 88.63 ± 3.74g this effect was not significant ($H = 4.876$, $P = 0.181$). The highest GUD was recorded at 70m (113.04 ± 1.66g) and the lowest giving up density was recorded at 77m (64.30 ± 17.47g, Fig 15g). The GUD increased to 91.71 ± 3.33g under the influence MV light but again this impact was not significant ($H = 4.876$, $P = 0.181$). The highest GUD recorded was at 0m directly under the light array (118.66 ± 0.39g) and the lowest GUD was recorded at the furthest feeding tray (71.41 ± 7.31g).

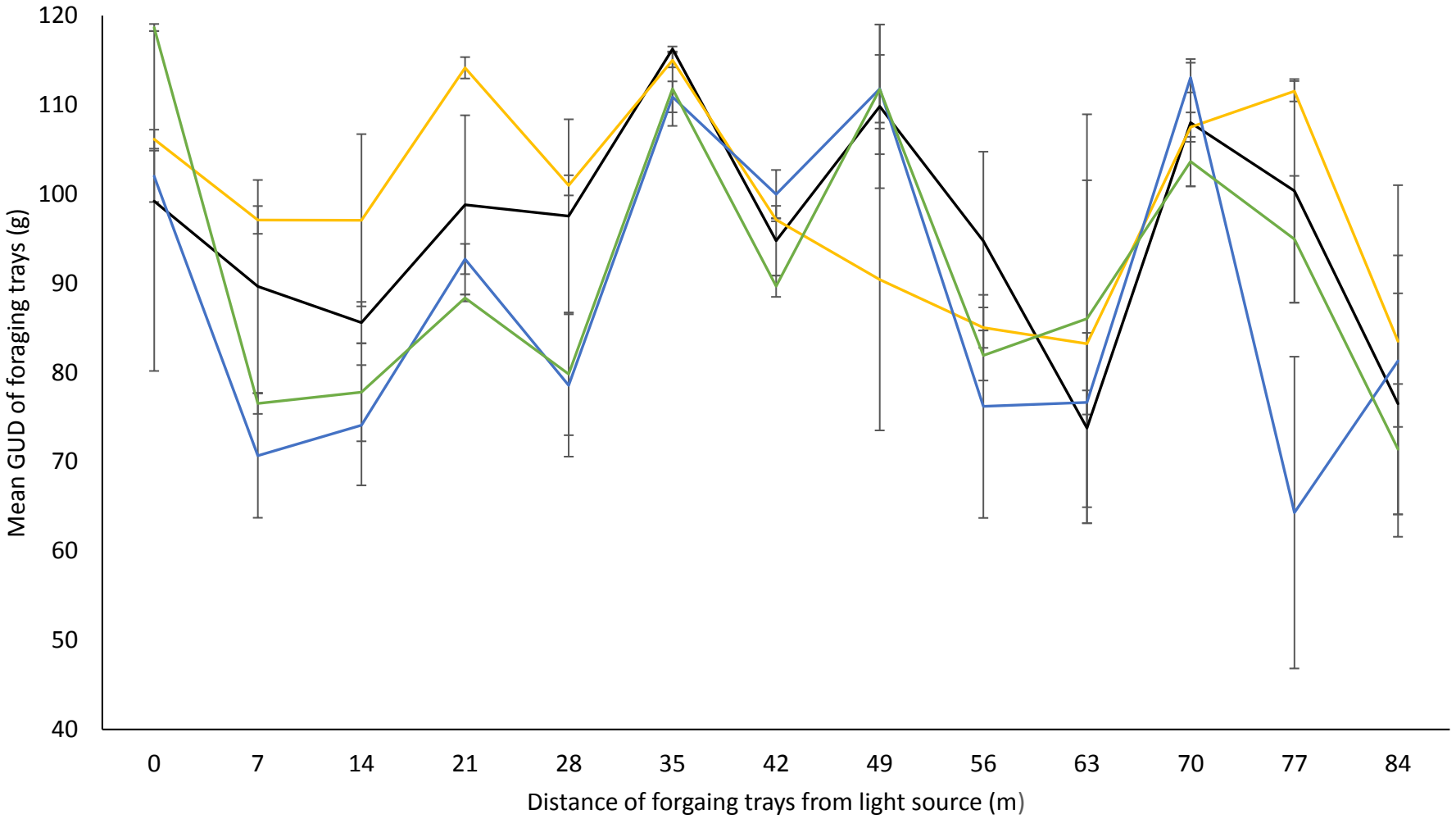

Figure 15. The average (GUD) ±SE at artificial foraging patches placed at each distance from the luminaire (x-axis) during each illumination trial; namely control (black line), HPS (orange line), LED (blue line) and MV (green line).

Discussion

This study was a pilot project to test whether the methods employed by Bird et al., (2004) could be adapted to investigate the influences of ALAN of different spectral signatures on the foraging activity of rodents in a South African context. The GUD of nocturnal rodent species in artificial foraging patches was recorded under four different lighting conditions. Trapping efforts yielded five species of rodent, all of which were expected to occur in the environment considering what is known about their distribution (Blaum et al., 2007,Skinner & Chimimba 2005). The data collected for *Gerbilliscus brantsii* and *Gerbilliscus leucogaster* had to be collated since positive species identification could not be discerned for all cases of the individuals caught. These two species are often considered cryptic and occur sympatrically, making identification extremely difficult (Skinner & Chimimba 2005). All the species caught during this study are known to consume seeds in their diets (Kerley 1989; Hurst et al. 2014). While their diet may shift as resource availability changes seasonally in the arid conditions of the Kalahari, they can be expected to make use of the grain-based bait provided in the artificial foraging patches.

Differences in foraging activity were observed with each of the light treatments but, due to large variation, these differences were not significant. LED street lighting seems to have the greatest effect on foraging activity showing the largest decrease in overall GUD. A general decrease in GUD can be seen up to the foraging tray at 35m from the light source after which the trend dissolves and becomes erratic. The same holds true for the MV light trial, but the decrease in GUD is not as pronounced. On the other hand, the opposite trend is seen in the GUD's recorded under HPS light, with an increase in GUD when compared to the dark trial. The greatest effect of ALAN appeared to be confined to a radius of approximately 35m around the light source.

While the methodology of Bird et al. (2004) was employed, the results obtained were very different. Bird et al. (2004) tested the effect of two different lights and found that mice decreased their foraging activity systematically as distance increased from both light sources, however to a lesser extent under the influence of low-pressure sodium (LPS) light compared to a bug light. The main difference between these two lights was that LPS emits a monochromatic yellow light, whereas the bug light emits a wider spectrum of light (Bird et al. 2004). The HPS light used in this study increased the GUD of foraging rodents, similar to

the findings of the study by Bird et al. (2004), but both the lights with broader wavelengths (LED and MV) showed the opposite effect of the broad wavelength light used in Bird et al. (2004). However, despite our best efforts the study could not be replicated precisely. The study site of Bird et al. (2004) contained only one species, *Peromyscus polionotus leucocephalus,* the Santa Rosa beach mouse, and the environment comprised of mainly primary coastal sand dune with very sparsely distributed short grasses. On the other hand, in our study site in the Kalahari had far more vegetation cover from grasses, and small to medium shrubs despite choosing a homogenous open area. Thus, there was a much larger chance of microhabitat influences on the experiment. This study was also performed at the functional community level, with five species contributing to the foraging activity observed.

The increase in foraging activity observed under the influence of LED and MV contrasts other studies conducted on rodents in the presence of ALAN. A study conducted on wood mice under three colour lights found that their activity decreased under the presence of light regardless of its colour (Spoelstra et al. 2015), with white LED light, which is comparable to the LED light used in this study, having the greatest decreasing effect on wood mice activity. Other studies directed towards longer wavelength, narrow spectrum lights such as HPS light also found that the presence of light decreased the foraging activity of nocturnally active rodents (Rotics et al., 2011). Both the locomotor activity and the foraging activity of nocturnal spiny mice decreased after experiencing just three hours of ALAN exposure (Rotics et al. 2011). Anecdotal observations during the LED and MV light trials in this study showed that the light attracted large amounts of insects that would swarm around the light at night. Many of these insects would suffer fatal collision with the light and end up in high concentrations at the base of the light array. This could possibility have interfered with the foraging patches used in the experiment by adding a resource to the patch that was not accounted for in the data collection. The rodent species that were present during the illumination trials, although known to consume grains have been found to consume large quantities of insect matter in order to supplement their dietary requirements as seasonal availability of food changes.

In this study, the foraging activity was highly variable as the distance from the light source increased, with GUD both increasing and decreasing over distance, again this result was surprising, as GUD was expected to decrease as distance from the light source increased and light intensity waned. This result could be explained by the landscape of

microhabitats that influence an animal's perceived cost or benefit of utilising the resource patch. In order to maintain the integrity of the transect line the foraging trays were placed at locations that were at varying distances from vegetation cover that may serve as refugia from predation and as such influence the foraging activity that took place in each resource patch. Abu & Brown, (2012) found that *M. namaquensis* showed a preference for foraging in artificial resource patches located close to thick bush or rock crevices, significantly lowering their GUD when these escape substrates were nearby. Kotler et al., (1991) found that two sympatric gerbil species reduced foraging under illuminated conditions as well as preferring covered bush microhabitats to open habitat when light was present. The variability seen in the GUDs recorded across the distance transect could be explained by the confounding factor of a learning effect taking place during the study. Good quality foraging patches were provided for 9 consecutive nights. The provision of excellent quality of food in patches may skew the cost-benefit analysis of rodents so severely that they choose to forage in artificial patches regardless of perceived risk.

Predation risk and small mammal activity have been linked to the natural illumination levels of the moon (Perea et al. 2011). It is assumed that predation risk for rodents will increase with higher natural illumination around periods of full moon, especially when their predators are visually orienting species. Most studies have shown that during periods full moon rodents will decrease their foraging efforts, return from foraging trips with lower yields, and use less open high-risk habitats closer to their core areas or avenues of escape (Hughes and Ward 1993; Kotler et al. 1994; Orrock et al. 2004; Morris and Davidson 2008). Other species such as *Cynopterus sphinx,* a megachiropteran bat exhibits higher average feeding bouts during new moon conditions than during any other phases of the moon cycle (Elangovan and Marimuthu 2001). Tropical nightjar species, which are visually hunting aerial predators increase foraging intensity in twilight periods during new moon in order to compensate for the lack of light by which to identify prey (Jetz et al. 2003). Our result seems even more anomalous given that feeding activity only decreased under one light treatment, while increasing during what was considered the two most high-risk light treatments. This experiment was performed under new moon conditions which should yield the highest foraging rate of rodents, and the spectral content of the light used is different from the spectrum of light given off by moonlight.

Light is an integral part of human expansion, as cities grow to accommodate more inhabitants often the first action to follow the clearing of land is the setup of street lighting. The addition of street lighting not only extends the effect of direct lighting but also adds to the amount of sky glow produced by a single city extending its potential impacts further beyond the limits of the city itself. Together with the addition of more and more lighting, there is a trend towards more energy efficient solid-state lighting such as LED. These lights have a very different spectral composition compared to traditional HPS lighting. This suggests that there is an increasing need to understand not only the potential responses of organisms to the presence of ALAN, but also the impact new LED full spectrum lighting may have as it continues to increase.

Criticisms and Suggested Improvements

In order to refine and improve the methodology of Bird et al., (2004) a pilot study was conducted using the basic structure of their experiment. However, conditions under which their experiment was conducted varied dramatically from those during the pilot study. One of the aims of the pilot study was to identify features of the study that would need to be altered for it to be relevant in a South African context across a wide range of conditions.

Firstly, it is suggested that an identical control experiment is run simultaneously alongside the different light treatments instead of running a control before and after the light treatments. Climactic variables such as; relative humidity, ambient temperature and rainfall should also be recorded in order to correlate their impacts on feeding activity observed throughout the experiment. Natural illumination is known to affect the nocturnal activity of rodents and should also be considered, therefore it is recommended that the experiment be run across a full 28-day lunar cycle, to factor in the effect of natural illumination on foraging activity.

Secondly, secondary environmental effects which are not the intended target of the study should be recorded such as the attraction of insects seen in the pilot study, as they have the potential to alter the resource dynamics around which the whole experiment is based. Foraging trays in the pilot study were possibly over stocked with seed making the trend in foraging in relation to distance from the light source difficult to identify. In future, foraging trays should contain much less seed mixture, as well as being combined with a

foraging substrate to increase the cost of foraging at trays. The substrate should be easily separated from the seed mixture to facilitate accurate weight recording. Doing this should balance out the perceived risks and benefits of foraging at each tray leading to more accurate GUD measurements.

Lastly, the use of remote sensing techniques such as video recording or camera trapping could be employed to allow direct monitoring of each foraging tray. Parameters such as individual species, visitation rates, predation events, species interactions and more could then be included in the experiment to further support the foraging data and assess the impact of ALAN of rodent communities. Using the data gathered from direct monitoring potential impacts like changes in species communities and competitive exclusion can be identified as part of the experiment aims. Along with remote sensing, telemetry systems could be employed to garner unique individual data about foraging, counting the number of times a particular individual visits a foraging patch. Telemetric systems could also be used to monitor the movement of animals in the microhabitat surrounding the foraging patches and provide insights into the role the surrounding matrix plays in the foraging decisions of rodents.

Chapter 5: General Discussion

Light pollution is a global research challenge (Longcore and Rich 2004; Davies et al. 2013b; Gaston et al. 2015b). Many of the intricacies contributing to the impacts of the phenomenon are not known or understood yet. However, almost every piece of published literature has documented some effect of ALAN, suggesting that the impact of light pollution is incredibly widely spread (Gaston et al. 2015b). The purpose of this thesis was to use a lab-based study to examine effects of different spectra of ALAN on the circadian entrainment and physiological stress of two rodent species. In order to determine the impact of ALAN on these two physiological parameters in a captive setting, the effect of different abiotic factors in captivity were also investigated. Three environmental factors were considered, namely lighting, ambient temperature and environmental enrichment in order to determine their influence on the physiological stress and circadian rhythms of locomotor activity of these two species.

Environmental conditions of laboratory animals can have a large effect on their behaviour as well as their physiological states (Olsson et al. 2002; Latham and Mason 2004; Garner 2005). In this study, an increase in environmental complexity of the cage conditions significantly elevated the locomotor activity of the nocturnal species (*M. namaquensis*) and reduced their urinary stress hormone concentrations. This decrease was especially pronounced in females of *M. namaquensis* which were also significantly more active than males after enrichment was introduced. Enrichment also increased the robustness of entrainment of both species, strengthening the circadian entrainment to external cues, especially for *R. dilectus*. *R. dilectus* average locomotor activity decreased after enrichment was introduced however, no inference could be made regarding the effect of enrichment on the physiological stress of *R. dilectus* because no urine samples could be obtained for the preceding light cycle.

Stereotypic behaviours can cause desynchronization of the circadian rhythms of locomotor activity by detracting animals from normal behaviour through the performance of stereotypies (Garner and Mason 2002). This could potentially lead to an increase in their physiological stress as glucocorticoids are closely linked to the circadian rhythms of an organism (Irvine and Alexander 1994; Barriga et al. 2002; Otsuka et al. 2012). Providing enrichment in captivity can alleviate the negative effects of stereotypic behaviour as well as

prevent their development in the first place (Latham and Mason 2004). A study using Norwegian Brown rats (*Rattus norvegicus*) found that introducing environmental enrichment improved the frequency and quality of sleep patterns different age groups of rats (van Gool and Mirmiran 1986). Furthermore enriched BALB/c mice increased their activity and reduced of their corticosterone levels compared to non-enriched groups of mice (Roy et al. 2001). The reduction in stress hormones in this study may be an indication of lower captivity induced anxiety levels (Roy et al. 2001). It would seem given the results of this study that this is holds true for *M. namaquensis*, therefore environmental enrichment such as was provided here can be recommended for *M. namaquensis* to reduce the side effects of captivity induced stress. However, following the results of other studies and the fact that *R. dilectus* showed to decreased activity with increased stress, the results of this study suggested that *R. dilectus* may experience increased stress from the introduction of enrichment.

It is important to note that enrichment cannot be standardised between species (Latham and Mason 2004), as the aim of enrichment is to provide opportunities for an organism to display natural species-specific behaviour. In order to provide the correct enrichment, a good understanding of species natural behaviour and activities is essential (Olsson et al. 2003; Mason 2004). Traditionally enrichment was thought to increase variation in captive studies and reduce the replicability of an experiment, however increasing evidence to indicate the contrary is being discovered. Enrichment may actually enhance validity, reliability and replicability by reducing the amount of abnormally behaving animals in studies (Garner 2005).

External light and temperature act as *zeitgebers* in rodents (Refinetti 2015; Ackermann et al. 2016) and in most studies, light has been found to be the primary *zeitgeber* (van der Merwe et al. 2012; Katandukila et al. 2013) bringing about changes to circadian activity, for the entrainment of circadian rhythms while temperature has a relatively weak secondary effect on entrainment (Refinetti 2015). Simulated dawn-dusk cycles and temperature cycles that mimic average days in the habitats of both experimental species rendered mixed results. *M. namaquensis* activity levels decreased during LD_N while their corticosterone concentrations increased, which could mean that the introduction of natural light cycles led to a suppression of normal activity in these animals. It can be theorised that the source of increased stress observed was the unwitting introduction of

fluorescent flicker into the light cycle. When fluorescent luminaries are dimmed the frequency of flicker in the light emitted also decreases. This has the potential to bring the flicker rates within range of the critical flicker fusion frequency of an organism (Gilmour et al. 2008). Thus, the natural dimming meant to alleviate captivity induced stress, could have had quite the opposite effect, increasing physiological stress and resulting in a suppression of locomotor activity. In order to prove this theory, the critical flicker frequency for *M. namaquensis* would have to be established through further investigation and the flicker rate of the fluorescent lights used would have to be known.

M. namaquensis did increase their activity after the addition of an ambient temperature cycle in LD_{NT} and their stress hormone concentrations showed a decline although this change was not significant. Rodents have been shown to increase their locomotor activity as a behavioural thermoregulatory response to low ambient temperatures (Ackermann et al. 2016). Considering that *M. namaquensis* is a communally living species (Skinner et al. 2013) it is reasonable to assume that it will make use of huddling behaviour in order to reduce variation in body temperature at lower ambient temperature (Boyles et al. 2012).However, animals used in this study were housed individually and since *M. namaquensis* is strictly nocturnal, they did not have the opportunity to use huddling to regulate body temperature during the cool dark phase, thus making an upregulation of locomotor activity to maintain body temperature even more likely during the dark phase.

R. dilectus showed a somewhat anomalous combination of their locomotor activity in relation to stress during the LD_N and LD_{NT} cycles. In response to the natural light cycle their locomotor activity decreased and the amount of activity exhibited during the dark phase dropped dramatically. Their stress hormone concentrations increased marginally however not significantly. *R. dilectus* entrainment to the light cycle strengthened further with all animals maintaining either crepuscular or diurnal rhythm. This may suggest that a natural progression in the intensity of light is a stronger zeitgeber for the circadian rhythms of *R. dilectus* than the presence or absence of light alone. The inclusion of twilight parameters in the analysis showed that *R. dilectus* showed the highest activity during the simulated twilight period of the light cycle. A study conducted by Boulos & Macchi, (2005) found that a simulated twilight period decreased the variability of activity onset times for Syrian hamsters (*Mesocricetus auratus*) when compared to traditional square wave light

cycles. Another study on Syrian hamsters also concluded that twilight increases the strength of light as a zeitgeber as opposed to square wave light cycles (Boulos et al. 2002). However, research conducted on house mice found that under twilight periods there was large variation in the phase angle of entrainment, suggesting that for house mice twilight does not act as a stronger zeitgeber (Comas and Hut 2009).

However, after the introduction of an ambient temperature component, both average activity rate and stress hormone concentration decreased. Temperature had notable though not significant impact on the concentration of *R. dilectus* stress hormones. The ambient temperature cycle was considerably warmer during the light phase than the constant temperature of 25°C provided in the previous cycles and matched the ambient conditions in which *R. dilectus* is found naturally (Meynard et al. 2012). Their natural habitats regularly exceed 30°C (South African Weather Service 2019), and *R. dilectus* are very well adapted to heat stress (PJ Jacobs, University of Pretoria pers. comm). Thus, the more natural temperature conditions may have reduced the perceived temperature stress that *R. dilectus* was experiencing. The thermoneutral point for *R. pumilio*, which is closely related too *R. dilectus* , was determined to be around 32°C (Haim and Fourie 1980) thus the increase in temperature may have removed the need to behaviourally thermoregulate using increased activity.

This thesis demonstrates that simple housing conditions can have profound and varying, species-specific effects on measurements of locomotor activity when studying circadian rhythms. The circadian rhythms of both species entrained as expected according to previous literature however levels of activity and individual activity patterns differed somewhat. Laboratory studies are often used to test methods that can be implemented in larger field studies or in large scale conservation management decisions. Thus, improving our understanding of the effects that individual factors have on the outcomes of experiments, may prevent results obtained with interference from confounding factors from being implemented in important decisions. Further investigations are warranted to study the effects of factors in captivity not only on the activity of animals, but also on their behaviour and physiological traits to determine whether factors such as enrichment, light and temperature are truly affecting physiological traits and in which manner they are being affected.

After baseline information was established, and the effects of the simulated environmental factors were identified the effect of ALAN could be tested. The same two species, one diurnal and one nocturnal, were used for this part of the study. This part of the study aimed to elucidate the impacts of ALAN of the physiological stress and circadian rhythms of these two species. It is not only the presence or absence of LAN that has an impact on organisms, but also the type of LAN specifically the spectrum of light it is exposed too (Davies et al. 2013b; Stone et al. 2015). This study used two types of luminaries that have very different spectral emissions, intended to simulate the two most common types of street lighting used in South Africa.

M. namaquensis experienced an increase in corticosterone concentration as well as a suppression of their nocturnal activity after the first LAN was introduced in LD_H. However, while activity remained mostly suppressed during LD_{LED} stress hormone concentrations did decrease slightly. This may indicate a lesser impact of white LED light compared to yellow halogen light on the stress physiology of *M. namaquensis*, however from what is known from literature this is highly unlikely as most studies has found greater impact of LED light compared to other types (Gaston et al. 2014; Minnaar et al. 2015). This result was far more likely caused by habituation to the presence of light at night, thus the decrease in stress hormone concentrations but the remaining suppression of the circadian activity. Continued suppression of circadian rhythms may also lead to a decrease in corticosterone concentrations (Rohatagi et al. 1996).

Upon introduction of ALAN in the form of halogen light *R. dilectus* experienced an increase in the rates and proportion of activity expressed during the dark phase of LD_H accompanied by an increase in urinary corticosterone concentration. This would suggest that LAN is indeed a stressor for *R. dilectus*. Being a diurnal species LAN has the potential to disrupt several circadian rhythms including sleeping patterns and even extend activity periods well into the night-time depending on the intensity of LAN. This study has shown that even very low intensities of ALAN (~ 5 lux) can potentially disrupt the circadian activity of an organism and lead to increased stress. *R. dilectus* sustained increased nocturnal activity and elevated stress levels similar to LD_H after the addition of LED light at night. Prolonged exposure to ALAN on daily basis may lead to chronic stress in *R. dilectus*. The impact of chronic stress will not only impact on an individual level but also on a species population level.

It is clear from the results of this study that not only does the presence of ALAN contribute to the results but that the spectral qualities of the light also interact with the physiology of the two species investigated (Brainard et al. 1984). Both types of light tested gave very different results for *R. dilectus* and *M. namaquensis*. Although these results were obtained from a lab study, with care they can be extrapolated and used to predict possible impacts of ALAN in real world scenarios. The natural light regime and temperature cycle these two species would encounter throughout their natural range were simulated so any reaction they exhibited should remain relevant to scenarios in the field.

One aspect of species that may be affected by ALAN throughout their natural distributions is foraging behaviour. The results obtained from Chapter 4 of this thesis, hints at possible implications of the presence and spectral quality of ALAN of foraging behaviour of rodents. Although the experimental methodology used requires refinement in order to produce viable results in a South African context, ALAN had some interaction with the foraging patterns of nocturnal rodents. *M. namaquensis* may experience too great a perceived risk in the context of the field study conducted, given that under the influence of ALAN it suppressed locomotor activity and had higher concentrations of stress hormone. This could provide a temporal niche for *R. dilectus*, which occurs sympatrically with *M. namaquensis*, to expand into and competitively exclude *M. namaquensis* and other species. This is even more likely considering *R. dilectus* had increased nocturnal activity in response to ALAN and extended their active time by almost an hour.

Should a scenario like the one described above occur in nature the consequences may be far reaching beyond simply the individuals of the species involved (Kotler et al. 1994; Gaston et al. 2014; Abu and Joel 2018). Rodent populations are not constant such as those of larger mammals and vary in stochastic fashion with season. They are highly dependent on the environmental variables that traditionally influence population dynamics (David and Jarvis 1985). They are particularly sensitive to resource availability which tend to vary seasonally, many rodents only reproduce during periods that food is readily available. This makes their populations vulnerable to stochastic events, which have the potential to eradicate a population entirely if it occurs during the naturally low numbers of the population.

Disturbances in population dynamics of a species have the potential to impact upon entire community structures in ecosystems if they are severe enough (Gaston et al. 2014;

Hoffmann et al. 2018). One study has highlighted the importance of rodent communities, as they can be used as biological indicator organisms in South African grasslands to quantify biological degradation of ecosystems (Avenant 2011). Some studies have already started noting changes in species interactions which they attribute to light pollution (Davies et al. 2012, 2017). The findings in Minnaar et al. (2015) found changes in species interaction attributed to light pollution which may have long term impacts on the evolutionary histories of the species involved. According to published data these changes seem to be exacerbated by LED light in general (Davies et al. 2013b), even though the results of this thesis may suggest the halogen and LED have approximately the same magnitude of effect.

Research efforts have found an impact of light pollution in almost every taxon that has been investigated, not just in rodents (Hölker et al. 2010). While most literature focusses on impacts on mammal and bird species, amphibians, reptiles and fish are not exempt from being affected by light pollution (van Langevelde et al. 2011; Hölker et al. 2015; Brei et al. 2016). With these far-reaching effects at almost every functional level of physiology (Dominoni et al. 2013c; Robert et al. 2015)and ecology (Lyytimäki 2013) it comes as no surprise that there is increasing interest in the field of light pollution. There are however gaps in the literature concerning how the impact of light will vary spatially among different individuals (Gaston et al. 2014).There is need for methodical exploration of how environmental factors, inter- and intraindividual species variation will cause effects of ALAN to vary, in order to be able to apply research in global context.

This thesis has shown that ALAN has complex interactions with other environmental factors in species specific context on two aspects of rodent physiology. It also provides evidence that lab-based studies can be used to quickly and with minimal cost identify some of the individual variation seen in field studies on ALAN. This thesis will contribute useful information for further research to consider when studying the complex phenomenon of light pollution. Light pollution is a rapidly expanding, global problem, as such trying to minimise the impact has a very time sensitive agenda.

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