Locomotor activity in field captured crepuscular four-striped field mice, *Rhabdomys dilectus* and nocturnal Namaqua rock mice, *Micaelamys namaquensis* during a simulated heat wave

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Highlights

- Heatwaves did not induce activity shifts in either of the study species.
- Day time activity increased in both species during the simulated heatwave.
- Water drinking increased during the simulated heatwave.
- In the absence of microclimates, access to water may alleviate heat stress.

Abstract

Activity of animals is influenced by ambient temperature and increasing temperatures brought about by climate change may impose a heat stress risk. Previous studies investigating the effect of heat waves on activity usually measure animals at different, but constant temperatures, however, rarely are they studied under a natural temperature cycle. General activity, behavioural flexibility and frequency of water drinking counts during a normal day, hot day and a simulated heat wave temperature cycle were studied in the crepuscular four-striped field mouse, *Rhabdomys dilectus*, and the nocturnal Namaqua rock mouse, *Micaelamys namaquensis*. Both *R. dilectus* and *M. namaquensis* showed typical daily locomotor activity under control conditions. During the heat wave, peak activity times changed for *R. dilectus*, but both species exhibited higher bouts of activity for the heat wave during the day compared to the control, which was accompanied by an increased amount of time spent drinking water. The increased activity during the heat wave is likely due to enhanced water requirements and potentially a form of behavioural thermoregulation as animals may be uncomfortable and try to move to cooler areas. Thus, in the absence of a typical microclimate, access to water may allow rodents to overcome heat stress from extreme temperatures without having to shift their temporal active times.

Keywords: *Rhabdomys dilectus*, *Micaelamys namaquensis*, activity, heat wave, behavioural flexibility, temperature cycle

List of Abbreviations:

12L:12D – 12 hours light: 12 hours dark
14L:10D – 14 hours light: 10 hours dark
TNZ – Thermal neutral zone

1. Introduction

Activity patterns of animals are a fundamental aspect of their biology and reflect important life history qualities such as foraging, predator avoidance and minimising environmental risks (Vieira et al., 2010). One such environmental risk is ambient temperature (Kronfeld-Schor and Dayan, 2008), and with animals occurring in a world where they are exposed to climate change with elevated temperatures, the environmental risk may result in a geographic shift, a change in activity patterns and/or abundance (IPCC, 2014). Environmental risks such as heat waves (lasting for several days) are occurring more rapidly due to the intensity and frequency of warming climatic events (IPCC, 2014). Thus, examining the influence of temperature on the activity patterns of a diurnal and a nocturnal species may deepen our understanding on how temperature may influence animals occupying different temporal niches.

Daily activity patterns, at least for small mammals, are commonly placed in three categories namely diurnal (day active), nocturnal (night active) and crepuscular (activity peaks at dusk and dawn). However, non-circadian and acyclic activity patterns also occur (Baumgardner et al., 1980; Vieira et al., 2010). Diurnal animals generally show higher variability in activity between and within species, whereas nocturnal animals show less variability (Kronfeld-Schor and Dayan, 2008). Activity is an important mechanism of behavioural thermoregulation (Huey et al., 2012; Huey and Tewksbury, 2009; Stevenson, 1985), especially when individuals can be behaviourally flexible in their activity times (Sassi et al., 2015). Species that are not flexible in their activity times (obligate diurnal species) face a trade-off between remaining in their safety zone as compared to exposing themselves to high heat loads and water stress to obtain resources (Huey et al., 2012; McCain and King, 2014; Murray and Smith, 2012). Switching of temporal activity in rodents is not common in light of environmental change (Kronfeld-Schor and Dayan, 2008; Vieira et al., 2010), as diurnal small mammals will generally just avoid extreme temperatures by minimising their exposure through the use of microclimates (Bozinovic et al., 2000; Murray and Smith, 2012; Vispo and Bakken, 1993). However, it has been speculated that diurnal rodents such as the mesic four-stripe field mouse *Rhabdomys dilectus* (de Winton, 1987) may be at risk of undergoing a geographic shift due to climate change (Du Toit, 2019; Rymer et al., 2013). The majority of small mammals utilize microclimates to escape thermal stress (Scheffers et al., 2014), but animals can still be thermally stressed as a result of metabolic heat production even within a microclimate (Huey et al., 2012). Thus, microclimates alone may not be sufficient to buffer a species from climate change and thermal stress.

Previously, simulated heat waves have mostly been measured by exposure to different, but constant temperatures (Adamo et al., 2012; Dittmar et al., 2014; Fischer et al., 2014), but rarely with a fluctuating temperature cycle (Stahlschmidt et al., 2017). This difference is important as an animal’s performance in a constant environment is not always similar to that in an ecologically relevant environment, which is constantly fluctuating (reviewed in Angilletta Jr and Angilletta (2009)). For example, constant temperatures during a heat wave simulation have negative effects on butterfly development (Fischer et al., 2014), whereas fluctuating heat wave temperatures do not (Klockmann et al., 2016), but food availability can alter these results (Adamo et al., 2012).

Since rodents do not sweat or pant, they have to resort to other methods of thermoregulation such as saliva spreading to increase evaporative water loss; rodents that do not use saliva spreading suffer exaggerated responses to heat stress (Hainsworth, 1967). Generally, rodents are likely to reduce behaviours that will increase thermogenic activity such as eating (Hammond et al., 2001; Sassi and Novillo, 2015), lowering activity rate (Murray and Smith, 2012; Zub et al., 2009) and potentially shifting activity times (Murray and Smith, 2012; Sassi et al., 2015), however this latter behaviour is not common in rodents (Kronfeld-Schor and Dayan, 2008; Vieira et al., 2010). The activity of animals changes when individuals are extremely heat stressed and are subject to dehydration from hyperthermia, accompanied by drinking more water and increased activity through escape behaviour (Hainsworth, 1967; Hainsworth et al., 1968). Hainsworth et al. (1968) proposed water drinking becomes more rapid during dehydration and starts to occur more frequently at the later stages of heat stress.

Here our focus is on the effect of extreme temperature regimes on animal activity. In this study, we investigated the locomotor activity of two rodent species namely the, *R. dilectus* and the Namaqua rock mouse, *Micaelamys namaquensis* (Smith, 1834). *Rhabdomys dilectus*, one of the few diurnal/crepuscular rodent species (Schumann et al., 2005), is a mesic species within the grassland and savannah biomes and relies strongly on ground vegetation cover and water. Grass cover provides a thermal buffer for striped mice in grasslands in order to avoid extreme temperature fluctuations (Rymer et al., 2013). This species can breed for seven months per year, representing opportunistic breeding (Jackson, 2000; Rymer et al., 2013). The temperature preferences of *R. dilectus* are not known, however, the thermoneutral zone (TNZ) of *Rhabdomys pumilio* (Sparrman 1784), the desert living counterpart is 32^{o}C (Haim and Fourie, 1980). Its diet includes seeds, fruits, green plant material, Acacia pods, *Protea* flower bracts, *Pinus* bark and arthropods (Fuller and Perrin, 2001; Monadjem et al., 2015; Skinner and Chimimba, 2005) with, depending on season, opportunistic omnivorous feeding (e.g. insects) (Perrin and Curtis, 1980).

Rhabdomys dilectus is mostly solitary compared to the more arid-adapted species which appears to be more communal (Rymer et al., 2013).

Micaelamys namaquensis inhabits a range of biomes, but is a rock crevice dweller, preferring rocky environments (Russo et al., 2010; Skinner and Chimimba, 2005). This is a strictly nocturnal species (van der Merwe et al., 2014). They are omnivorous and granivorous, having a wide diet range including seeds, grass, nuts, and fruits (Denys, 1994). This species is described as communal and/or social, but groups generally consist of family members (Nicolson and Fleming, 2004). *Micaelamys namaquensis* is a seasonal breeder with breeding confined to the rainy summer months, linked to food availability (Muteka et al., 2006). *Micaelamys namaquensis* has a TNZ between 26-34 ^{o}C (Buffenstein, 1984). Since the width of the TNZ of *R. dilectus* is unknown, the TNZ cannot be directly compared. Both species differ in their urine production where *M. namaquensis* has a daily rhythm of urine production, with *R. pumilio* lacking a 24 hour urine rhythm with urine release occurring primarily during the onset of activity (Van der Merwe et al., 2017) Morphologically, *M. namaquensis* is larger in size compared to *R. dilectus*.

Our aims were to examine locomotor activity during a long summer day with a temperature cycle and compare this to a transition day with intermediate temperatures and then subsequently a simulated heat wave, for both species. We predicted that *M. namaquensis* would not shift its activity times and will remain primarily nocturnal, with minimal diurnal activity during heat stress periods. As for *R. dilectus*, we predicted two potential outcomes namely that day time activity may be reduced during heat wave conditions, with increased night-time and/or twilight activity during heat stress conditions. Alternatively, we predicted that the activity will remain the same (no shift in activity time), but with overall reduced activity during heat stress conditions. Stress conditions are defined here as exposure to ambient temperatures which exceed the TNZ of animals for an extended time period, which may lead to increased heat absorption, causing heat stress. Lastly, we predicted that water drinking would increase during the simulated heat wave.

2. Methods

2.1. Animal maintenance and acclimation

Experimental animals were wild caught using metal Sherman traps (26 cm x 9 cm x 9 cm), baited with a mixture of oats and peanut butter. Six male *Rhabdomys dilectus* were caught in Rietvlei nature reserve (3800ha, Centurion, South Africa, -25° 53' 29.39" S, 28° 17' 22.80" E) between January 2019 and March 2019, whereas six male *M. namaquensis* were collected at Telperion nature reserve (7350ha, Gauteng, South Africa, 25° 43' 40.4" S, 28° 59' 56.4" E) in February 2019. Once captured, the rodents were maintained in field cages and transported to the Zoology and Entomology Department, at the University of Pretoria. All individuals were weighed to the closest ± 0.1 g. Before experimentation, *R. dilectus* had a mean body mass of 46±2g and *M. namaquensis* had a mean body mass of 67±6g. The body mass of the animals did not change significantly during the experiment. Animals were weighed weekly to assess body condition. The mice were housed in a climate-controlled room at the University of Pretoria and acclimated to laboratory conditions (12L:12D, 40% RH and 23^{o}C) for at least a month prior to the commencement of experimentation to minimise the stress influence of bringing wild animals into captivity. All animals were housed individually in 40 cm x 25 cm x 12 cm containers lined with wood shavings and a rock, toilet rolls, a small plastic container for a nest, with tissue paper for nesting material. Mice were provided with *ad libitum* water and food. The food was provided every second day in the form of sunflower seeds, corn, banana, carrot, apple shavings or sweet potato slices in a way to encourage movement around the cage as a source of enrichment. Cages were cleaned out weekly.

2.2. *Calculation of temperatures for simulated climate conditions*

In order to simulate the heat wave and obtain natural weather conditions, weather station data from each geographic region from the South African Weather Service where the rodents were captured was collected. Weather station data for simulating daily temperatures for *R. dilectus* and *M. namaquensis* were taken from 2015-2016 between September and March. For *R. dilectus*, the average monthly minimum was 13.1^{o}C and the maximum was 34.8^{o}C. The absolute maximum temperature was 38.2^{o}C. The average monthly minimum was 13.8^{o}C and monthly max was 27.9^{o}C. The maximum temperature was 35.8^{o}C. The temperature parameters were corrected using a temperature-humidity index as climate control rooms that were used had constant humidities. This index was calculated from the wet and dry bulb air temperatures for a particular day according to the following formula: Temperature humidity index = 0.72 (W + D) + 40.6, where W is wet bulb and D is dry bulb temperature in degree centigrade. The following website was used to convert climatic data values to heat index values (http://www.wpc.ncep.noaa.gov/html/heatindex.shtml)).

2.3. *Control, transition and heat wave simulation*

The mice were transferred to individual cages 60 cm x 40 cm x 30 cm lined with wood shavings, a small plastic container for a nest and tissue paper as well as a toilet roll for nesting material. Animals were left for 24 hours prior to the onset of the recording of activity. Lighting was set on a 14L:10D long day schedule, which included 10 hours of daylight (maximum light intensity), 10 hours of night time (minimum light intensity) and 4 hours of 'twilight' with increasing and decreasing light intensities simulating dawn (2 hours) and dusk (2 hours) respectively. The maximum light intensity was 330 lux and the minimum was 0 lux. The long day photoperiod was accompanied by a typical temperature cycle, which was determined through climatic data obtained from the South African weather service for each species separately.

Each species was measured separately at their own temperature cycle and all individuals within a species were measured simultaneously. The mice were maintained at controlled temperatures (minimum of 19^{o}C and a maximum of 29^{o}C for *R. dilectus,* and minimum of 16^{o}C and a maximum of 26^{o}C for *M. namaquensis*) for five days, transition temperatures (minimum of 22^{o}C and a maximum of 34^{o}C for *R. dilectus,* and a minimum of 20^{o}C and a maximum of 32^{o}C for *M. namaquensis*) for one day, and heat wave temperatures (minimum of 24^{o}C and a maximum of 39^{o}C for *R. dilectus,* and a minimum of 24^{o}C and a maximum of 38^{o}C for *M. namaquensis*) for 3 days. During changes in treatment (control to transition, and transition to heat wave) temperatures were set to represent the new treatment condition starting at 9:00 am. During experimentation, experimenter influence was kept to a minimum with a 30 min interval (between 8:00-8:30 am) for feeding and checking the general welfare of the animals. Activity data were not corrected for the influence of feeding during this time. The mice were given a fixed amount of water and food (sunflower seeds every day, fresh fruit/vegetables every second day).

2.4. Video footage and activity measurements

Microsoft HD 3000 live cams were modified in order to record video footage throughout the day and night to determine animal activity and water drinking activity. The software program Open Broadcasting Software was used to record the videos. The cameras were placed above the cage in order to capture the area inside the cage for animal activity.

2.5. Water drinking analyses

An animal was considered to be drinking water when the mouse was clearly seen drinking, or the mouse's muzzle was over the tip of the water bottle. A count stopped when the mouse's muzzle moved away from the tip of the bottle and a new count would begin if an animal placed its muzzle over the tip of the bottle to drink again. Total drinking counts were recorded with timestamps and the amount of time spent drinking during each count was determined through frame-by-frame analysis using QuickTime Player (Apple. Inc. 2010-2016) and converted to seconds.

2.6. Statistical analyses

Video footage was analysed using Ethovision XT 11.5 (Noldus Information technology) and raw data extracted to Excel. Ethovision measures activity through centre-point detection of the mouse which is the only moving object inside the cage. Pixel changes from grayscale values from 0 (black) to white (255) are detected from one frame to the next. If several pixels change in their grayscale value the mouse is considered to be active by the program, whereas low rates of pixel changes result in the animal being inactive. Some days were lost due to corrupted video files. The raw data values were used to calculate the sum of activity counts per minute and per hour, which was repeated for all available video data. Total activity count and normalized activity counts per hour were calculated for each individual for each treatment and light phase. Furthermore, normalized drinking counts and time spent drinking (drinking duration) were also calculated. The onsets and offsets of activity and peak activity times were determined visually by generating actograms with Actiview Biological Rhythm Analysis 1.2 software (Minimitter Co. Inc., Sunriver, Oregon, http://www.minimitter.com). Periodograms were constructed with Clocklab software (ClockLab™; Actimetrics, Evanston, Illinois, USA). Statistical analyses were performed in SPSS (version 25) (IBM Corp. Armonk, NY). Since activity data were not normally distributed and are repeated measures, a generalised linear mixed model (GLMM) was used to determine statistical significance for normalized activity counts per hour as the response variable, with light phase and treatment as fixed factors, with individual identity x day interaction as a random factor. The *post hoc* Fishers least significant difference test was used where significant differences were detected. Degrees of freedom was calculated using the Satterthwaite's correction. Another GLMM was run for each species with water drinking count and duration as the dependant variables and light phase and treatment as fixed variables, with individual identity x day interaction as a random factor. One *R. dilectus* was removed from the analysis due to it being an extreme outlier with consistent high counts compared to all other individuals across all treatments during all light phases. Significance levels were maintained at $p<0.05$. Normalized activity, counts, duration spent drinking in seconds per hour represents the mean of each treatment in each light regime, with the overall mean expressed as means ± standard error (SEM).

3. Results

3.1. Daily locomotor activity rhythms

3.1.1. Rhabdomys dilectus

The overall normalized activity per hour of *R. dilectus* did not significantly change between different treatments (F=0.244, df=2, df=129, p=0.784) (Fig. 1; Table 1). During the control period, the time of day significantly influenced the normalized activity per hour counts (F=15.761, df1=2, df2=138, p=<0.001). *Rhabdomys dilectus* demonstrated peaks in activity around twilight, this phase had significantly higher normalized activity per hour compared to the day (p<0.001) and night (p=0.006) (Table 1). Night activity was significantly higher than day activity (p=0.001). *Rhabdomys dilectus* demonstrated a lot of intermittent activity resulting in variable onset and offset of activity.

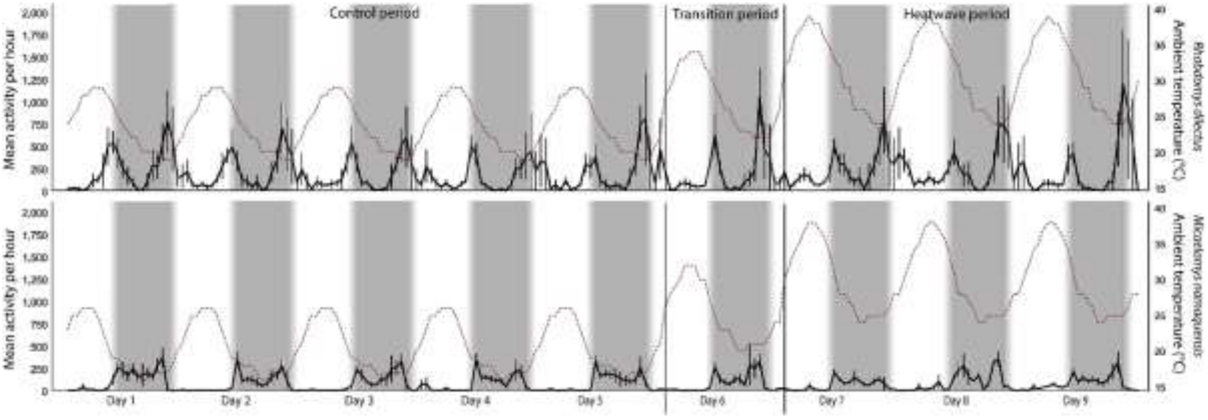

Fig. 1. Mean activity per hour for the four-striped field mouse *Rhabdomys dilectus* and the Namaqua rock mouse *Micaelamys namaquensis* across treatments (separated by black vertical lines) over a 14L:10D long day. Red dotted line indicates the temperature cycles in ^{o}C. White areas represent day, shaded areas represent night and shaded transitions indicate twilight conditions. Vertical lines above counts represent SEM.

Table 1. Normalized mean activity count per hour for each individual during the control, transition and heat wave treatment for each light phase (light, twilight and dark) for the four-striped field mouse *Rhabdomys dilectus* and the Namaqua rock mouse *Micaelamys namaquensis*.

Rhabdomys ID	1	2	3	4	5	6	Mean
Control: Light	32,116	83,11067	49,46156	86,84067	52,08822	387,8538	115,25 ± 55,19
Control: Twilight	73,72556	435,24	213,835	669,2844	306,405	1301,37	499,98 ± 180,38
Control: Dark	69,01267	274,8193	108,8611	228,0056	141,5584	52,068	145,72 ± 36,27
Transition: Light	39,64	65,4	13,53	112,91	89,38	488,7044	134,93 ± 72,20
Transition: Twilight	86,975	159,05	122,075	470,9	109,15	1128,45	346,10 ± 166,94
Transition: Dark	123,4944	445,2689	54,32	346,51	279,1878	76,08444	220,81 ± 65,25
Heat wave: Light	89,14704	79,18667	77,5643	93,14667	36,52	488,5181	144,01 ± 69,39
Heat wave: Twilight	69,2375	370,3	93,225	526,0333	30,225	1282,058	395,1798 ± 194,50
Heat wave: Dark	113,7894	580,9494	39,21296	365,35	159,15	136,2185	232,45 ± 82,70
Micaelamys ID	1	2	3	4	5	6	
Control: Light	17,74	1,73	1,9	14,57	7,58	5,32	8,14 ± 2,72
Control: Twilight	9,94	6,53	8,11	40,48	129,65	8,23	33,82 ± 19,88
Control: Dark	61,49	144,6	180,81	229,02	255,83	176,56	174,72 ± 27,87
Transition: Light	1,92	4,32	1,4	11,6	11,44	7,97	6,44 ± 1,86
Transition: Twilight	3,38	11,65	5,38	12,22	56,33	18	17,83 ± 7,99
Transition: Dark	35,32	100,85	137,6	312,78	299,7	158,27	174,09 ± 45,17
Heat wave: Light	15,08	19,53	11,33	41,31	10,92	8,66	17,81 ± 4,95
Heat wave: Twilight	37,34	35,68	18,37	84,32	34,39	8,04	36,36 ± 10,69
Heat wave: Dark	49,47	111,59	173,63	147,14	232,14	124,83	139,80 ± 25,10

Normalized activity per hour for *R. dilectus* during the transition period did not differ significantly from the control during the day (p=0.637), twilight (p=0.163) or night (p=0.400) (Fig. 1; Table 1). Activity peaks did not differ from that during the control period.

During the heat wave, normalized activity per hour for *R. dilectus* did not differ significantly from the control during the day (p=0.097), twilight (p=0.913) or night (p=0.510) (Fig. 1; Table 1). Peaks in activity shifted an hour later after dusk and an hour earlier before dawn during the heat wave. Some individuals increased their intermittent activity during the heat wave with less rest periods between activity bouts.

All animals under control conditions demonstrated 24-hour activity cycles. These cycles drastically changed for 2 individuals with rhythms becoming drastically longer, with the 3 other individuals deviating slightly from the 24-hour activity cycle by lowering activity to 23-23.5-hour cycles. From this it is clear that heat wave disrupted the 24 activity rhythms of the animals.

3.1.2. Micaelamys namaquensis

The overall normalized activity per hour of *M. namaquensis* did not significantly change between different treatments (F=104, df1=2, df2=12, p=0.902) (Fig. 1; Table 1).

During the control period, *M. namaquensis* differed significantly in their normalized activity counts per hour in relation to time of day (F=19.051, df1=2, df2=10, p<0.001). *Micaelamys namaquensis* demonstrated clear onsets and offsets of activity associated with night time, with night time having a significantly higher normalized activity counts compared to the day (p=0.001) and twilight (p=0.006). Twilight activity was significantly higher than day time activity (p=0.002). Peaks in activity occurred during the night which was quite variable throughout the night.

Normalized activity per hour for *M. namaquensis* during the transition period did not differ significantly from the control during the day (p=0.789), twilight (p=0.057) or night (p=0.967) (Fig. 1; Table 1). Onsets and offsets of activity during the transition period was similar to that during the control period.

For *M. namaquensis,* normalized activity per hour during the heat wave significantly increased from the control only during the day (p=0.038), but did not change significantly during twilight (p=0.520) or night time (p=0.607) (Fig. 1; Table 1). Low levels of intermittent activity occurred in most *M. namaquensis* during the heat wave during the day starting between 11:00 and 13:00. Peak activity, offset and onset were similar between the control and transition treatment.

Five animals under control conditions demonstrated 24-hour activity cycles (with one individual demonstrating a 23.5-hour activity cycle), three of these six individuals dramatically shortened their activity periods, one individual drastically lengthening the period of its activity rhythm. The other 2 animals deviated slightly from the 24-hour rhythm by shortening the periods of their activity rhythms. Overall, rhythms were disrupted during the heat wave.

3.2. *Water drinking counts and durations*

3.2.1. Rhabdomys dilectus

Overall normalized water drinking counts per hour differed significantly between treatments for *R. dilectus* (F=4.353, df1=2, df2=12, p=0.015) (Fig. 2; Fig. 3; Table 2). Drinking counts did not change during the transition (p=0.165), but increased significantly during the heat wave (p=0.030) (Fig. 2; Fig. 3; Table 2). Drinking counts were not significantly different from control levels for the transition period during the day (p=0.905), twilight (p=0.067) or night time (p=0.472) (Fig. 2; Fig. 3; Table 2). Drinking counts were not significantly different from control levels for the heat wave during the day (p=0.103), twilight (p=0.206) or night time (p=0.181) (Fig. 2; Fig. 3; Table 2).

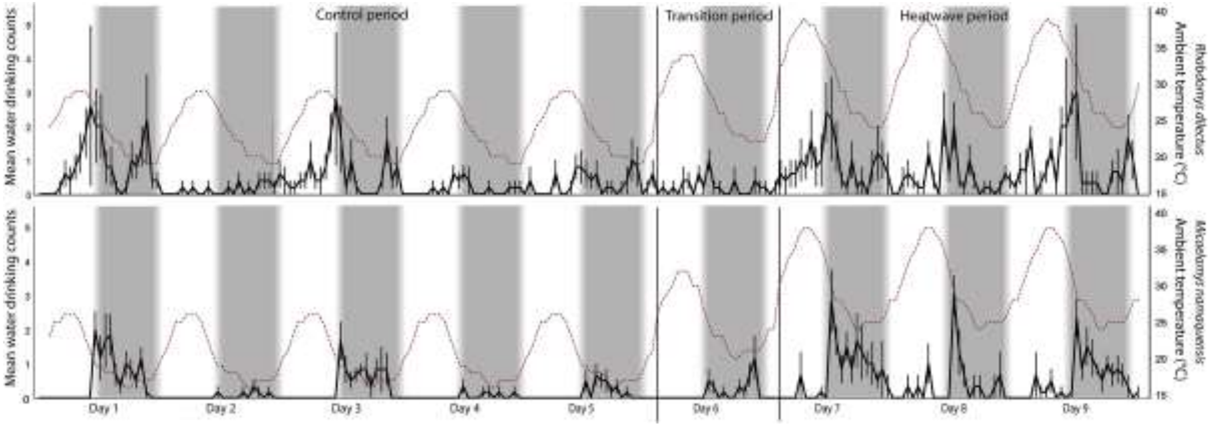

Fig. 2. Mean water drinking counts per hour for the four-striped field mouse *Rhabdomys dilectus* and the Namaqua rock mouse *Micaelamys namaquensis* across treatments (separated by black vertical lines) over a 14L:10D long day. Red dotted line indicates the temperature cycles in °C. White areas represent day, shaded areas represent night and shaded transitions indicate twilight conditions. Vertical lines above counts represent SEM.

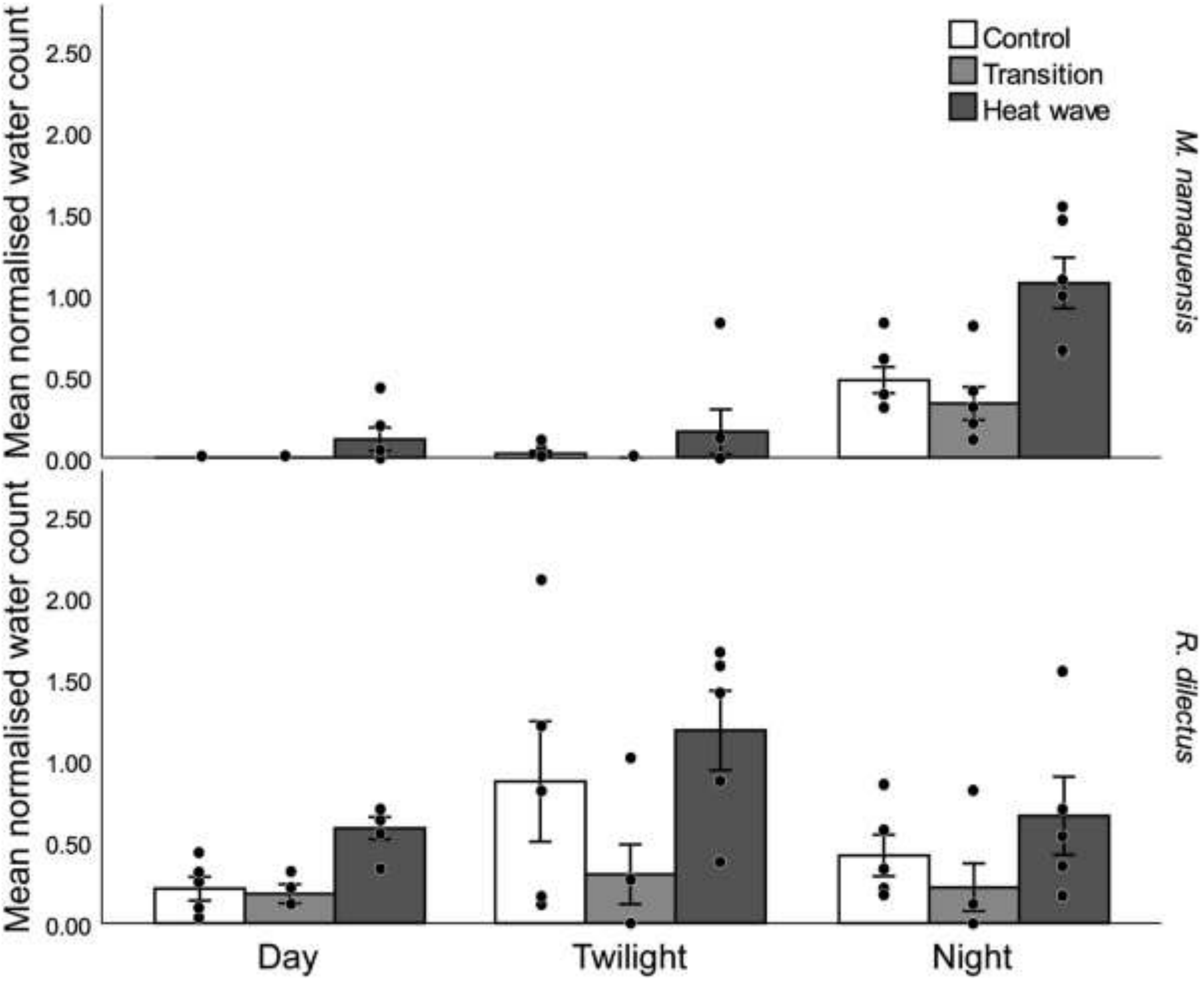

Fig. 3. Mean normalized water drinking count per hour for (A) the Namaqua rock mouse *Micaelamys namaquensis* and (B) four-striped field mouse *Rhabdomys dilectus* during the day, twilight and night of each treatment. Scatter plots represent individual data points.

Table 2. Normalized mean drinking count and duration (in sec) spent drinking water ratio with activity per hour during the control, transition and heat wave treatment for each light phase (light, twilight and dark) for the four-striped field mouse *Rhabdomys dilectus* and the Namaqua rock mouse *Micaelamys namaquensis*.

Rhabdomys	Normalized mean drinking count per hour/activity per hour	Normalized hourly mean drinking duration (s) /activity per hour
Control: Light	0,21/121,67	2,44/121,67
Control: Twilight	0,87/512,92	6,62/512,92
Control: Dark	0,42/119,90	3,37/119,90
Transition: Light	0,18/148,83	1,09/148,83
Transition: Twilight	0,30/175,92	2,06/175,92
Transition: Dark	0,22/175,92	2,36/175,92
Heat wave: Light	0,58/156,98	7,94/156,98
Heat wave: Twilight	1,18/400,16	14,47/400,16
Heat wave: Dark	0,66/162,74	8,27/162,74
Micaelamys		
Control: Light	0/8,14	0/8,14
Control: Twilight	0,03/33,82	0,26/33,82
Control: Dark	0,48/174,72	4,78/174,72
Transition: Light	0/6,44	0/6,44
Transition: Twilight	0/17,82	0/17,82
Transition: Dark	0,33/17,09	2,66/17,09
Heat wave: Light	0,11/17,81	8,17/17,81
Heat wave: Twilight	0,16/36,35	2,54/36,35
Heat wave: Dark	1,07/139,80	15,86/139,80

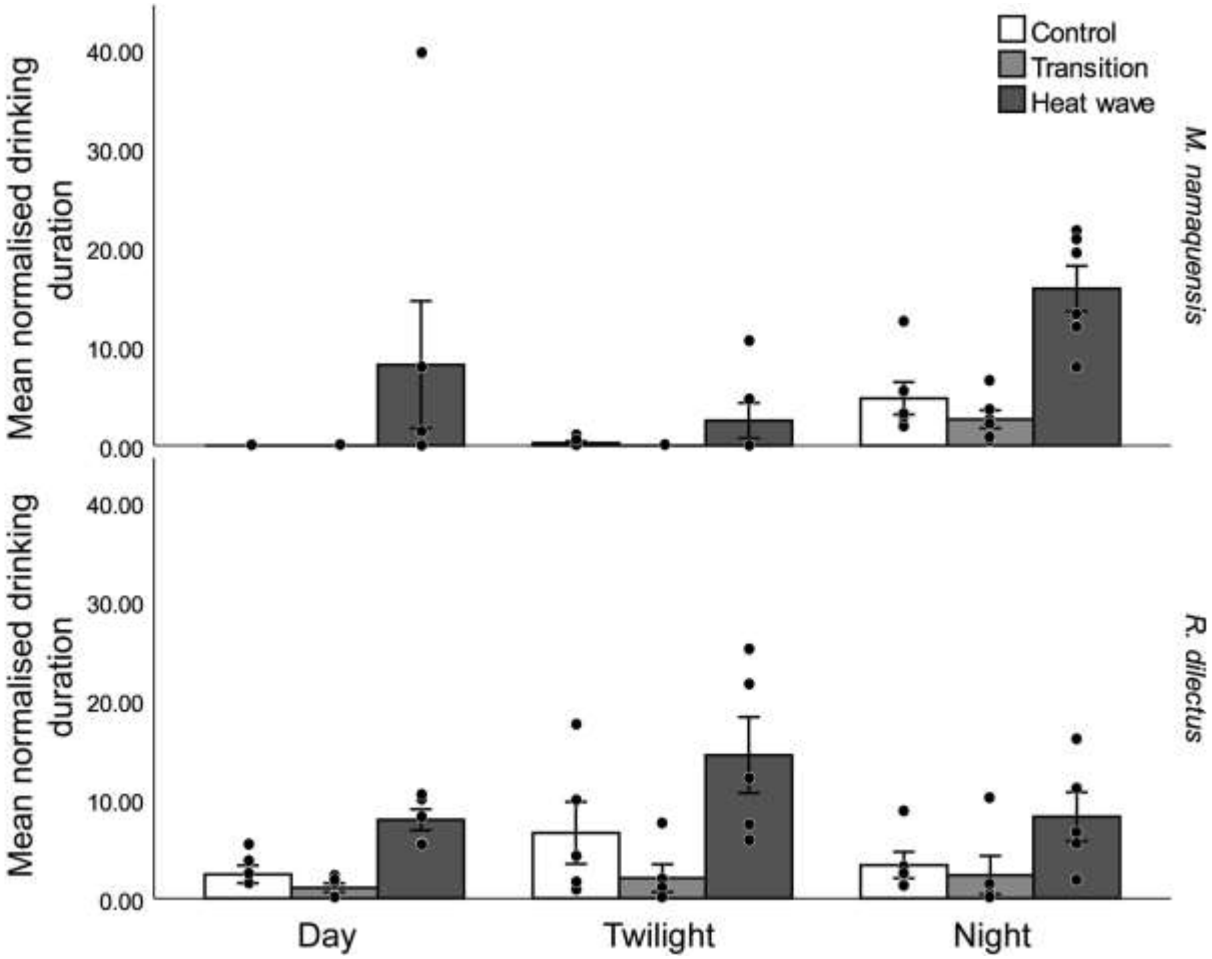

Fig. 4. Mean normalized water drinking duration (in sec) per hour for the Namaqua rock mouse *Micaelamys namaquensis* and four-striped field mouse *Rhabdomys dilectus* during the day, twilight and night of each treatment. Scatter plots represent individual data points.

Overall normalized water drinking duration in seconds per hour significantly differed for *R. dilectus* between treatments (F=5.052, df1=2, df2=120, p=0.008) (Fig. 4; Table 2). The time spent drinking water did not change from the control during the transition period (p=0.089), but significantly increased during the heat wave (p=0.024) (Fig. 4; Table 2). The time spent drinking water was significantly decreased from the control to the transition treatment only during twilight (p=0.025), but no significant changes during the day (p=0.089) or night time (p=0.653) (Fig. 4; Table 2). The time spent drinking water did not significantly increase from the control for the heat wave treatment during the day (p=0.104), twilight (p=0.279) or night time (p=0.094) (Fig. 4; Table 2).

3.2.2. Micaelamys namaquensis

Overall normalized water drinking counts per hour significantly differed for *M. namaquensis* between treatments (F=11.611, df1=2, df2=120, p=<0.001) (Fig. 2; Fig. 3; Table 2). Drinking counts did not significantly change during the transition period (p=0.469), but significantly increased during the heat wave (p<0.001) (Fig. 2; Fig. 3; Table 2). *Micaelamys namaquensis* did not drink water during the day for the control period, with no significant change during the transition period during the day (p=0.905), twilight (p=0.067) or night time (p=0.472) (Fig. 2; Fig. 3; Table 2). Drinking counts were not different from control levels for the heat wave during the day (p=0.103), twilight (p=0.206) or night time (p=0.181) (Fig. 2; Fig. 3; Table 2).

Overall normalized water drinking duration in seconds per hour significantly differed for *M. namaquensis* between treatments (F=7.508, df1=2, df2=144, p=0.001) (Fig. 4; Table 2). The time spent drinking did not change during the transition period (p=0.303), but significantly increased during the heat wave (p<0.001) (Fig. 4; Table 2). The time spent drinking water did not change during the transition period for the day (p=1.000), twilight (p=0.639) or night time (p=0.331) (Fig. 4; Table 2). During the heat wave, the time spent drinking water significantly increased during the day (p<0.001) and night (p=0.047), but not during twilight (p=0.052) (Fig. 4; Table 2).

4. Discussion

General daily activity of both study animals during control conditions were similar to that described in the literature, with *M. namaquensis* being strictly nocturnal

(Lovegrove and Heldmaier, 1994; Lovegrove et al., 1991; van der Merwe et al., 2014) and *R. dilectus* displaying diurnal and crepuscular activity (S. Ackermann, submitted). During the heatwave, *R. dilectus* demonstrated some higher activity during the night. Nocturnal activity has also been observed in *R. pumilio* (Schumann et al., 2005). It is generally proposed that the retinal structure correlates with the activity patterns of animals, for example, diurnal animals such as ground squirrels have a much higher percentage of cones compared to rods (Ahnelt, 1985; Kryger et al., 1998; Schumann et al., 2005), whereas nocturnal animals such as hamsters have rod dominated retinas (Calderone and Jacobs, 1999; Schumann et al., 2005). *R. pumilio* has been shown to have almost a 1:1.23 cone to rod ratio (van der Merwe et al., 2018). The photoreceptor composition of these animals would still allow them to see reasonably well during increased night time activity, as shown during the heat wave (peaks started at the onset of night time), should they have to drink water and forage, thus avoiding the high day time temperatures and possible dehydration. *Micaelamys namaquensis*, being nocturnal, have a higher cone to rod ratio (1:12.4) (van der Merwe et al., 2018), thus their vision may be impaired during the day since rods are more sensitive to light (Fain and Dowling, 1973), despite this, some individuals were still drinking water during the day.

4.1. Fluctuating temperature and comparison to constant conditions

In a previous study, Mackay (2011) investigated *R. dilectus* under a 14D:10L light cycle (without twilight) with a constant ambient temperature for 3 days. Under these conditions, *R. dilectus* displayed activity peaks at 04h00 and again at 18h00. Activity was at its lowest during 22h00-02h00 with activity slightly increasing after 02h00 (Mackay, 2011). The activity peaks were of similar size at 04h00 and 18h00. In the present study, animals were subjected to a simulated dawn and dusk, and fluctuating ambient temperatures. Activity peaks occurred later in the morning, at 05h00 and at the same time in the evening (18h00), with a larger peak at 05h00 compared to the peak at 18h00. In both studies, animals displayed night time inactivity, with the lowest activity around midnight.

Differences in activity patterns could be attributed to either the fluctuating ambient temperatures, or the presence of a simulated dawn and dusk, neither of which was present in the study by Mackay (2011). Moreover, these animals were kept for much longer periods on a 14L:10D cycle compared to animals in the present study.

Micaelamys namaquensis was subjected to constant temperatures and a square wave light cycle in a study by van der Merwe et al. (2014). In our study, animals were subjected to a dawn and dusk and a natural temperature cycle, and rendered a similar activity profile with the same onset and offset of activity associated with night time.

4.2. Treatment on activity and water drinking count and duration

The normalized activity counts per hour for *Rhabdomys dilectus* increased with warmer temperatures suggesting that temperature directly contributed to increased activity. During the transition treatment, drinking counts and the time spent drinking water decreased. This, however, changed during the heat wave where overall drinking counts and overall drinking duration increased, but drinking count and duration during each specific light phase did not significantly increase from the control.

Micaelamys namaquensis non-significantly increased overall activity counts during the transition, but non-significantly decreased activity counts during the heat wave from control levels. Overall, activity levels were not significant due to their very low activity counts. Activity counts, however, significantly increased during the day for the heat wave. This is further exemplified by the increase in water drinking counts and duration during the day. Furthermore, drinking counts and duration further increased during the night suggesting some animals may have drank water during the night while trying to keep day time activity to a minimum during the heat wave.

Hainsworth (1967) explained that activity is likely to increase only when heat stress becomes severe, as it is well known that rodents will reduce activity during heat stress, and animals likely reduce their activity to compensate for the heat gain (Mitchell et al., 2018; Sassi et al., 2015). Furthermore, animals are likely to drink water only when dehydrated (Hainsworth et al., 1968). The provisioning of fresh fruit/vegetables may have alleviated some of the water stress in the animals. This was observed in Fig. 1B where every second day, when fresh fruit/vegetables were provided, water drinking and the duration of water drinking decreased, however, this was more evident during the control period. As can be seen in Table 2, in both species the drinking counts and duration per unit activity increased during the heatwave compared to the control during the day and at night. Furthermore, these counts of drinking per unit activity was much higher in *M. namaquensis*. Overall, both species appeared to have been stressed by the heat wave, but *M. namaquensis* relied much more on drinking water to cool down.

Furthermore, the increased activity during the day from the control in both species may imply that animals employed behavioural thermoregulation by moving around to try and find a place to cool down. Neither species shifted their primary activity period. However, *R. dilectus* did demonstrate some shift in peak activity such that the morning peak commenced about an hour earlier and the evening peak an hour later. This may have been sufficient for the species to compensate for the heat stress and did not require a complete shift from diurnal and crepuscular to nocturnal activity as is observed in the common degu (*Octodon degu*) (Vivanco et al. 2010). Both species, however, increased their activity during the day for the heat wave. This is consistent with the increased activity observed when animals are severely heat stressed (Hainsworth, 1967; Hainsworth et al., 1968). This also explains why during the heat of the day *R. pumilio* tried to avoid extreme temperatures to avoid heat stress (Schumann et al., 2005). Since these rodents did not have access to a microclimate that allows them to escape from heat wave temperatures, animals had to resort to other means of heat loss. Since heat stress was the highest during the day, the need for heat loss was greatest during this time, hence the increased activity during the day to either find a place to cool down or to reach the water bottle to drink. A similar finding was made by Murray and Smith (2012), where water availability was a potential driving force for continued day activity during extreme temperatures in the desert woodrat *Neotoma lepida* (Thomas, 1893).

In conclusion, the activity patterns of the diurnal species did not change with warmer ambient tempeartures, whereas the nocturnal species showed higher day time activity. The higher day time activity of *M. namaquensis* is surprising as activity in this species is typically strongly masked by light (Van der Merwe et al., 2014). This may indicate that this species may be more flexible in its activity than previously thought, and that the usual nocturnal activity may be anxiety related rather than a physical inability (such as photoreceptor composition) to be active during the day.

Reduced activity during mild heat stress might be sufficient for thermoregulation during a long day, however, if heat stress is severe and there is no access to a microclimate, then access to water will allow rodents to overcome the possible heat stress from heat waves. One important factor that was not considered in this experiment was the surface temperatures, as it may physically restrict rodents by preventing them from reaching water sources (Moses et al., 2012).

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Ethical standards

All experiments complied with the current laws of the country in which they were performed with ethics clearance number EC008-17. The authors declare that they have no conflict of interest.

Competing interests

No competing interests are declared.

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