


ORIGINAL RESEARCH

Fertility up in flames: Reduced fertility indices as a consequence of a simulated heatwave on small African mammals

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Introduction

As global temperatures rise, regions around the world are experiencing significant climatic shifts, characterized by fluctuating temperatures and more frequent, intense heatwaves (Conradie et al., 2019). These changes pose a heightened threat to small animals, which, due to their large surface area to volume ratio are particularly vulnerable to dehydration and overheating (Lovegrove, 2003; McKechnie & Wolf, 2019; van Jaarsveld et al., 2021; Wallace et al., 2021). While considerable attention has been paid to the impact of anthropogenic climate change on ectotherms (Paaijmans et al., 2013), and smaller endotherms such as birds (Dunn & Møller, 2019) and bats (Festa et al., 2023), there is growing concern regarding how small terrestrial mammals are coping with these shifts (Lovegrove et al., 2013; McCain & King, 2014; McKechnie & Wolf, 2019; Mitchell et al., 2018; van Jaarsveld et al., 2021; Wells et al., 2022).

Small mammals have a large surface area to volume ratio (Gardner et al., 2011). While beneficial for rapid heat loss in

Abstract

With the increasing frequency and intensity of heatwaves due to climate change, the survival and reproductive success of mammals could be under significant threat. However, the specific effects of these environmental stressors on mammalian reproductive fitness remain insufficiently explored. This study investigates the impact of a simulated heatwave on male fertility indices in two African rodent species: the mesic four-striped field mouse (*Rhabdomys dilectus*) and the Namaqua rock mouse (*Micaelamys namaquensis*) during the breeding season. We measured key indicators of male fertility, including testes mass, testes volume, seminiferous tubule diameter, the presence of sperm, and plasma testosterone levels. Our findings reveal that both species experienced significant effects on male fertility indices, with the smaller *R. dilectus* showing a decline in all fertility indices following a simulated heatwave. These results suggest that the projected increase in heatwave events may compromise the reproductive success of small mammals, potentially leading to population declines. Finally, this study highlights the need for focused studies on the effect of heatwaves on long-term reproductive success in both males and females.

cooler environments (Gardner et al., 2011), it can also lead to rapid heat gain in hotter conditions, potentially endangering their survival (Scheffers et al., 2014). This makes small mammals particularly sensitive to elevated ambient temperature (T_a), especially when microclimatic protection is unavailable (Bennett et al., 2024; Gardner et al., 2011; Scheffers et al., 2014; van Jaarsveld et al., 2021). Current evidence highlights the immediate threats of hyperthermia and dehydration stress to their survival (Jacobs et al., 2021; Jacobs, Bennett, et al., 2020; Jacobs, Oosthuizen, et al., 2020; van Jaarsveld et al., 2021), and also points to reproductive challenges such as reduced fertility, increased offspring mortality, and ecological mismatches (Dantas et al., 2021; Durant et al., 2007; Kucheravy et al., 2021; Prendergast et al., 2001).

In recent years, the role of T_a in shaping the fitness of small mammals has garnered increasing attention often looking beyond just survival and focusing on reproductive success (Cunningham et al., 2021; Oswald et al., 2011; Tabh & Nord, 2023). Many animals time their reproductive activities to

coincide with environmental conditions that maximize food availability, often linked to rainfall and T_a (Bronson, 1985, 2009; Skagen & Adams, 2012; Varpe, 2017). For example, breeding activity in zebra finches (*Taeniopygia guttata*) is strongly influenced by rainfall and T_a (Zann *et al.*, 1995), while the Mongolian gerbil (*Meriones unguiculatus*) also depends on food availability and T_a (Karakas *et al.*, 2005). In other species, T_a alone is the primary predictor of breeding activity, with cooler T_a fluctuations driving reproductive timing more than rainfall or food resources (Alagaili *et al.*, 2017; Hart *et al.*, 2019, 2020; Hart, Alharbi, *et al.*, 2021; Sarli *et al.*, 2015, 2016; van Heezik *et al.*, 2002). For instance, in the trumpeter finch (*Bucanetes githagineus*), T_a was found to be the sole factor influencing the timing of breeding, rather than rainfall (Barrientos *et al.*, 2007). However, direct evidence of how heatwaves affect fertility indices in mammals, and particularly small mammals, remains scarce.

Steroid hormones, such as testosterone, play a central role in regulating breeding activity by driving seasonal changes in the testes. In males, elevated testosterone levels in the blood promote the growth of seminiferous tubules, which in turn boosts sperm production and enlarges the testes, signaling reproductive readiness (Ngalamenoa *et al.*, 2024). However, exposure to high temperatures and hyperthermia can disrupt male fertility by impairing spermatogenesis and reproductive functions, largely through its negative effects on testosterone synthesis (Agarwal *et al.*, 2008; Setchell, 2018). Under heat stress, animals employ a range of thermoregulatory strategies to protect reproductive function, especially in temperature-sensitive tissues like the testes. These strategies include mechanisms specific to the testes, such as radiating heat through thin skin layers and using a counter-current heat exchange system to maintain an optimal temperature (Boni, 2019; Durairajanayagam *et al.*, 2014; Hansen, 2009; Kastelic & Rizzoto, 2021; Maloney *et al.*, 2003). Additional body-wide cooling processes, such as evaporative water loss, panting, and seeking out cooler environments, help regulate both body and testicular temperatures (Boni, 2019; Durairajanayagam *et al.*, 2014; Hansen, 2009; Kastelic & Rizzoto, 2021; Maloney *et al.*, 2003). Other adaptations to help regulate both body and testicular temperatures include reducing food intake to lower metabolic heat production (Hammond *et al.*, 2001), minimizing activity levels (Ackermann *et al.*, 2020), and shifting activity to cooler periods of the day (Grenfell *et al.*, 2024; Hart, Alharbi, *et al.*, 2021; Hart, van Jaarsveld, *et al.*, 2021; Murray & Smith, 2012; Sassi *et al.*, 2015; van Jaarsveld *et al.*, 2019). When these cooling mechanisms are insufficient, even mild heat stress can lead to decreased testosterone production, impairing spermatogenesis, causing germ cell loss, and potentially resulting in infertility (Durairajanayagam *et al.*, 2015; Paul *et al.*, 2009; Rizzoto *et al.*, 2020). The extent of testicular damage caused by heat stress depends on factors such as the duration, intensity, and frequency of heat exposure, as well as the body size of the animal (Durairajanayagam *et al.*, 2015; Qari *et al.*, 2021). Heat stress quickly reduces testicular size and fertility by interrupting spermatogenesis, causing germ cell apoptosis, and impairing Sertoli cells, which are essential for supporting germ cells. Additionally, suppression of Leydig

cells reduces testosterone production, further contributing to testicular shrinkage. Damage is compounded by processes such as vasoconstriction, ischemia, and oxidative stress, leading to cellular damage and a loss of tissue volume (Ayad *et al.*, 2022; Paul *et al.*, 2008; Setchell, 2018).

Most laboratory studies on testicular heat stress rely on constant T_a , which do not reflect the variable conditions found in nature (Banks *et al.*, 2005; Cao *et al.*, 2009; Paul *et al.*, 2009; Pérez-Crespo *et al.*, 2008; Yaeram *et al.*, 2006). This leaves the impacts of natural T_a fluctuations—more representative of wild conditions—less understood (Jacobs *et al.*, 2021; Jacobs, Bennett, *et al.*, 2020; Jacobs, Oosthuizen, *et al.*, 2020; Stahlschmidt *et al.*, 2017). To address this dearth of knowledge, we simulated a natural heatwave under controlled laboratory conditions to assess male fertility markers in two African rodents: the four-striped field mouse (*Rhabdomys dilectus*) and the Namaqua rock-rat (*Micaelamys namaquensis*) (see Data S1 for details). The small diurnal *R. dilectus*, is adapted to mesic environments like grasslands and savannas, where it depends on dense grasses for shelter as it does not burrow. Its short limbs and tail provide limited thermal windows to aid in temperature regulation (Coetzee, 1970; du Toit *et al.*, 2016; Ganem *et al.*, 2012; Rymer *et al.*, 2013). In contrast, the larger nocturnal *M. namaquensis* is adapted to both arid and mesic environments. Its longer tail serves as a thermal window, aiding in heat dissipation, and it can utilize various microclimates, such as rocky crevices or dense grass, based on the landscape, as it does not burrow (Kryštufek, Haberl & Baxter, 2008; van Jaarsveld *et al.*, 2021). Additional details on the ecology, physiology, and morphology of these two species are provided in the supplementary material. The behavioral, physiological, and morphological differences between the species suggest that *M. namaquensis* is more heat tolerant than *R. dilectus*.

By exposing these rodents to a T_a cycle mimicking an extreme heatwave, we examined potential impacts on testicular health and fertility during their summer breeding season, when heatwaves are most common. We hypothesized that heat stress would lower fertility indices in both species, with *R. dilectus* likely more vulnerable due to its smaller size, mesic adaptations, and limited thermoregulatory capacity in open grasslands. In contrast, *M. namaquensis*, being larger and more adapted to arid and mesic environments, may show greater resilience to heatwaves.

Materials and methods

Ethical statement

All experiments were conducted in full compliance with the current laws of South Africa, under ethical clearance from the Animal Ethics Committee at the University of Pretoria (clearance number: EC008-17). The animal collection was authorized by the Gauteng Nature Conservation Authority (permit numbers CPF6-0134 and CPB4-000011). All animals were handled and cared for according to the ASAB guidelines (ASAB Ethical Committee/ABS Animal Care Committee, 2024).

Animal maintenance and acclimation

Wild-caught experimental animals were captured using metal Sherman traps (26 × 9 × 9 cm), baited with a mixture of oats and peanut butter. Twelve male *R. dilectus* were captured in Rietvlei Nature Reserve (3800 ha, Centurion, South Africa, −25°53′ 29.39″ S, 28°17′22.80″ E) between January 2019 and March 2019, while 10 male *M. namaquensis* were collected at Telperion Nature Reserve (7350 ha, Gauteng, South Africa, 25°43′40.4″ S, 28°59′56.4″ E) in February 2019. Following capture, the rodents were housed in field cages and transported to the Department of Zoology and Entomology at the University of Pretoria. Aspects regarding their activity, sociality, habitat, food and microclimate use, breeding, thermoregulation, and appendage sizes are supplied as Data S1.

Upon arrival, the mice were acclimated to laboratory conditions in a climate-controlled room at the University of Pretoria. Before experimentation, all animals were maintained and acclimated in captivity for a minimum of 1 month. A lighting schedule of 14 Light:10 Dark was used to simulate a long summer day, both species have been observed to be fertile in the summer months (Jackson, 2000; Muteka *et al.*, 2006; Rymer *et al.*, 2013). This included 10 h of daylight (maximum light intensity), 10 h of nighttime (minimum light intensity) and 4 h of “twilight” with increasing and decreasing light intensities simulating dawn (2 h) and dusk (2 h), respectively and 40% relative humidity (RH), and a T_a of 23°C. Each animal was housed individually in containers measuring 40 × 25 × 12 cm, lined with wood shavings and containing enrichment items such as rocks, toilet rolls, and a small plastic container for nesting. Tissue paper was provided as nesting material. *Ad libitum* access to water (a likely condition in a mesic environment, considering there is no drought) and food was provided, with food consisting of sunflower seeds, corn, banana, carrot, apple shavings, and sweet potato slices, offered every other day. Cages were cleaned on a weekly basis.

Extreme climate events (ECEs)

In order to define our methodology to be in line with ECEs, our data represent a climatological extreme as the selected temperatures are at the extreme tail of the T_a s obtained from the climate data (van de Pol *et al.*, 2017). A biological impact (an event that would influence the behaviour, ecology, and evolution of an organism) of these selected T_a s could not be determined as research on these parameters has not previously been measured. However, laboratory studies suggest T_a s above 36°C would have a biological impact on rodent reproduction (Yaeram, 2002; Yaeram *et al.*, 2006). We selected maximum T_a to represent extreme conditions and set a 3-day exposure duration to simulate a natural heatwave. This duration reflects typical heatwave length, which often spans a few days (Meehl & Tebaldi, 2004). Since this was a one-time, short-term exposure without any prior detrimental conditions known to affect these animals, we define our ECE as a brief, single occurrence of an extreme climate condition.

Climate data analyses

Climate data spanning 1994–2023 from the South African Weather Stations situated in Irene (25°54′36.00″ S, 28°12′36.00″ E) and Witbank (25°49′55.20″ S, 29°11′31.20″ E), Gauteng, South Africa were collected, which were the two closest weather stations to where *R. dilectus* (Irene) and *M. namaquensis* (Witbank) were caught.

Ambient temperature and RH data from the weather station data were converted to a heat index (HI) (Equations in Data S1) based on the formula by Rothfus (Blazejczyk *et al.*, 2012; Rothfus, 1990; Steadman, 1984). The heat index is a measure of how hot it feels to the animal when RH is factored in with the actual T_a . The daytime (5:00–19:00) HI averages are presented in the supplementary material for Irene (Fig. S1a–c) and Witbank (Fig. S2a–c) along with the daily nighttime averages (19:00–05:00) for Irene (Fig. S3a–c) and Witbank (Fig. S4a–c) for December, January and February, respectively. The daily HI maximums are presented in Irene (Fig. 1) and in Witbank (Fig. 2) for December, January and February respectively. All 3 months show a trend toward a greater frequency of hotter temperatures over time.

The hourly T_a s within these periods (Figs. 1 and 2) were used to simulate the extreme heat wave temperatures for *R. dilectus* and *M. namaquensis* respectively.

Experimental design

Animals were weighed to the nearest 0.1 g and transferred to new individual cages (60 × 40 × 30 cm) lined with wood shavings, a small plastic container for a nest and tissue paper, and a toilet roll for nesting material. *Rabdomys dilectus* were significantly lighter than *M. namaquensis* at the start of the experiment (*R. dilectus*: 42.2 ± 1.77 g; *M. namaquensis*: 53.8 ± 3.51 g; Mann–Whitney test: $U = 2.0$, $P < 0.0001$).

Animals were left for 24 h before the onset of the experiment to give the animals time to acclimate. The animals were separated into a control group and an experimental group. The climate control rooms could only be maintained at a constant RH of 40%, as such room temperatures were adjusted, such that each species was measured at an apparent T_a profile corresponding to their natural habitat. Additionally, all individuals of a species were measured simultaneously under the climatic scenarios provided.

Control groups were kept at typical daily T_a s for the full duration of the experiment (9 days) on a cycle that oscillated from a minimum of 19°C and 16°C and a maximum of 29°C and 26°C for *R. dilectus* (Fig. 3a) and *M. namaquensis* (Fig. 3b), respectively. In contrast, for the heatwave simulation experimental group, all animals were first maintained at control T_a for 5 days then transitioned to a single “hotter” day to T_a that oscillated between a minimum of 22°C and 20°C and a maximum of 34°C and 32°C for *R. dilectus* and *M. namaquensis* respectively (Fig. 3a,b). A 3-day simulated heatwave followed this hotter day with T_a oscillating between

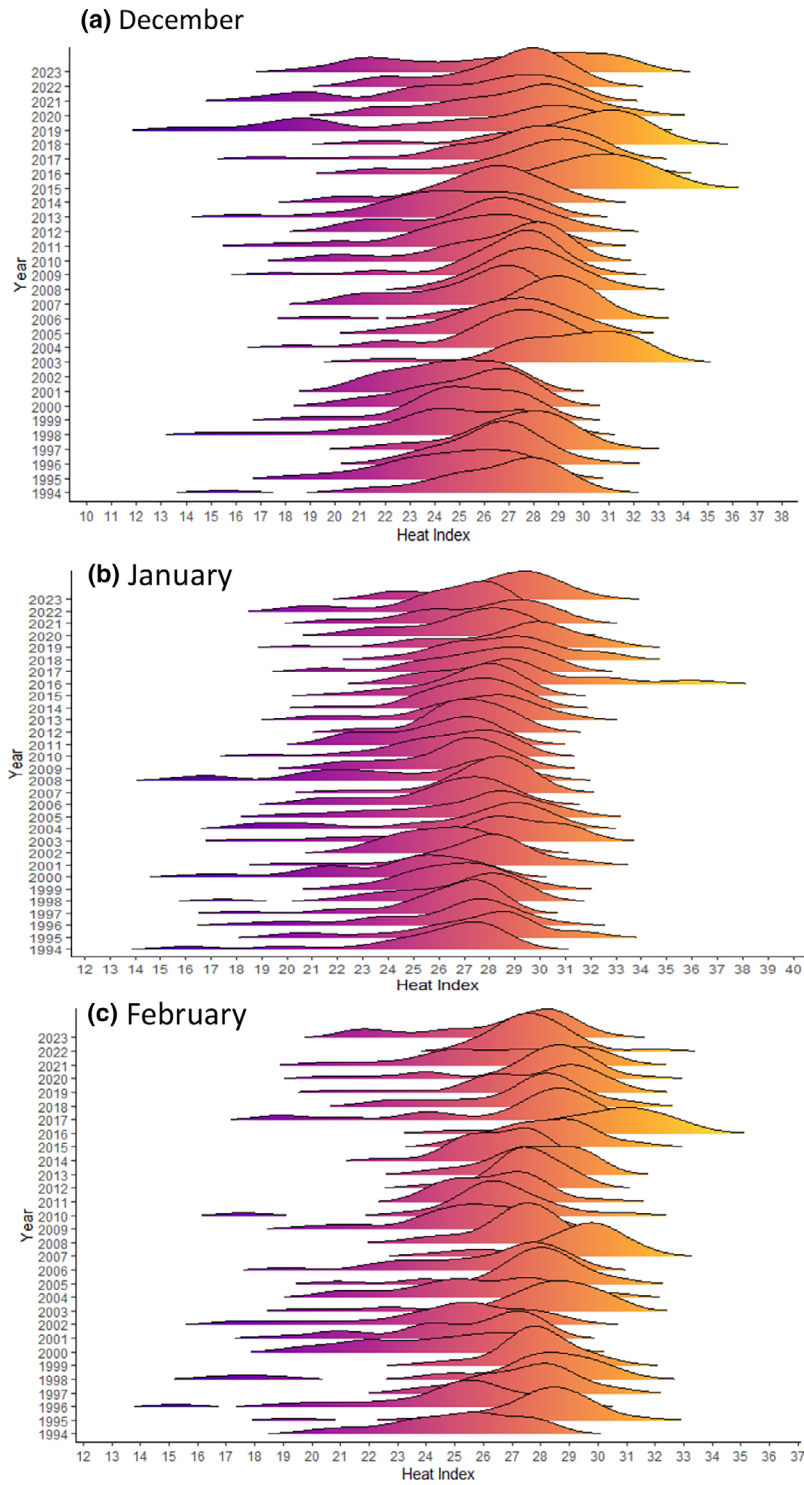


Figure 1 The apparent maximum temperatures (heat index) at Irene Gauteng, South Africa (25°54'36.00" S, 28°12'36.00" E) for the summer months of (a) December, (b) January, and (c) February for the years of 1994–2023 where *Rhabdomys dilectus* inhabits. All 3 months show a trend towards a greater frequency of hotter temperatures over time.

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a minimum of 24°C and 24°C with a maximum of 39°C and 38°C *R. dilectus* and *M. namaquaensis* respectively (Fig. 3a,b). All animals were provided with *ad libitum* water and food. All animals were given a container to make a nest, but this container did not act as a microclimate as it would not offer any buffering effect from T_a .

Variables like radiant heat and wind speed were excluded due to the complexity of replicating these conditions in a laboratory setting (Kántor & Unger, 2011). While laboratory environments cannot fully replicate nature, they allow precise control over specific factors, enabling clearer insights into direct physiological responses to heat stress (Altwegg *et al.*, 2017; van de Pol *et al.*, 2017).

Dissection and testes processing

All animals were subsequently euthanized with an overdose of isoflurane within 1 day after the end of the experimental cycle. Once the animal had expired, whole blood was obtained from the heart using a 3.0 cc syringe with a 25 g × 5/8" needle. Immediately after collection, the blood was transferred into a vacutainer lithium heparin blood collection tube and centrifuged at 4500g for 15 min. Plasma was collected and stored at -20°C for plasma testosterone analysis. Subsequently, testes were removed from the body cavity, and excess fat and connective tissue were removed from the testes, the latter of which was weighed using a balance (Adam Equipment PW 254 PW Series Analytical Balance, 250 g capacity, with 0.0001 g readability). The maximum length and width of the testes were recorded using digital vernier calipers (Mitutoyo America Corporation, Aurora, IL, USA). Testicular volume was determined using Equation 1 for the volume (V - mm³) of an ellipsoid, where a is one-half the maximum length, and b is one-half the maximum width (Hart, Alharbi, *et al.*, 2021; Hart, van Jaarsveld, *et al.*, 2021).

$$V = \frac{4}{3} * a * b^2 \quad (1)$$

The testicular measurements (mass and volume) for each testis were recorded and then summed and averaged for each individual. The average testes mass was converted to milligrams (mg).

Histology procedure

Small blocks of testicular tissue were fixed after euthanasia and testicular measurements in 4% phosphate-buffered glutaraldehyde, pH 7.4 and routinely prepared for microscopy (Du Plessis & Soley, 2012). Sections of approximately 300 nm thickness were cut with a Leica EM UC7 ultramicrotome, collected on glass slides, stained with 1% toluidine blue and mounted with a coverslip before viewing with an Olympus BX63 light microscope. Images of the seminiferous tubules were captured with an Olympus DP72 camera and the diameter of the tubules was measured using Cell Sense imaging software. Two measurements, perpendicular to each other, were made for each tubule. If the two measurements differed by

more than ten percent, the tubule and its measurements were discarded; thus, only true cross-sections were used for measurements (Botha *et al.*, 2022). The diameter of 50 seminiferous tubules (µm) per individual male was used for the statistical evaluations. The presence or absence of fully formed sperm cells was recorded.

Plasma testosterone analysis

Plasma testosterone concentrations were determined using a commercially available coated tube assay kit (Coat-a-Count MG12191 testosterone kit, IBL International GmbH, Hamburg, Germany). The assay was able to determine plasma testosterone concentrations of 6–1200 ng/dL. Cross-reactivity of the Coat-a-Count testosterone antibody was 1.8% with 19-Nortestosterone, 0.31% with dihydrotestosterone, and <0.01% with other steroids tested. We validated the assay by testing for parallelism between serial dilutions of both species' plasma (obtained from an individual with high testosterone concentrations) and the standard curve (Chard, 1978). The curves were parallel and not significantly different from the reference preparation (*R. dilectus*: $F = 2.4$, $P = 0.3$; *M. namaquaensis*: $F = 1.2$, $P = 0.1$). The intra-assay coefficient of variation was 7.9%. The sensitivity of the assay (90% binding) was 5 ng/dL or 0.175 nmols/L.

Data analysis

The normality of model residuals for male fertility indices (testicular mass (mg) and volume (mm³), seminiferous tubule diameter (µm), and plasma testosterone (ng/dL)) was assessed using Shapiro–Wilk tests. To meet this assumption, log transformations were applied to any variables that did not initially display normality. Levene's test was used to confirm homogeneity of variances for the model. Following these transformations, the residuals of the model were confirmed to meet the normality assumption. Species were analyzed separately using general linear models with treatment (control vs. heatwave) as the fixed factor and body mass as a covariate with the various male fertility indices as the response variables. Body mass of each species before and after experimentation was assessed using a paired *t*-test (*R. dilectus*) and Wilcoxon matched-pairs signed rank test (*M. namaquaensis*).

All statistical analyses were performed in R 4.0.5, and statistical significance was assumed at $P \leq 0.05$. All data are presented as mean ± standard error (SE). All data presented in the Supplementary material (Table S2).

Results

All fertility indices decreased in male *R. dilectus* subjected to heatwave conditions compared to the controls. This included a reduction in plasma testosterone levels, seminiferous tubule diameter, and testicular mass and volume (Fig. 4, Table 1). While sperm cells were observed in the seminiferous tubules of male *R. dilectus* under control conditions, they were notably absent in those exposed to heatwave conditions.

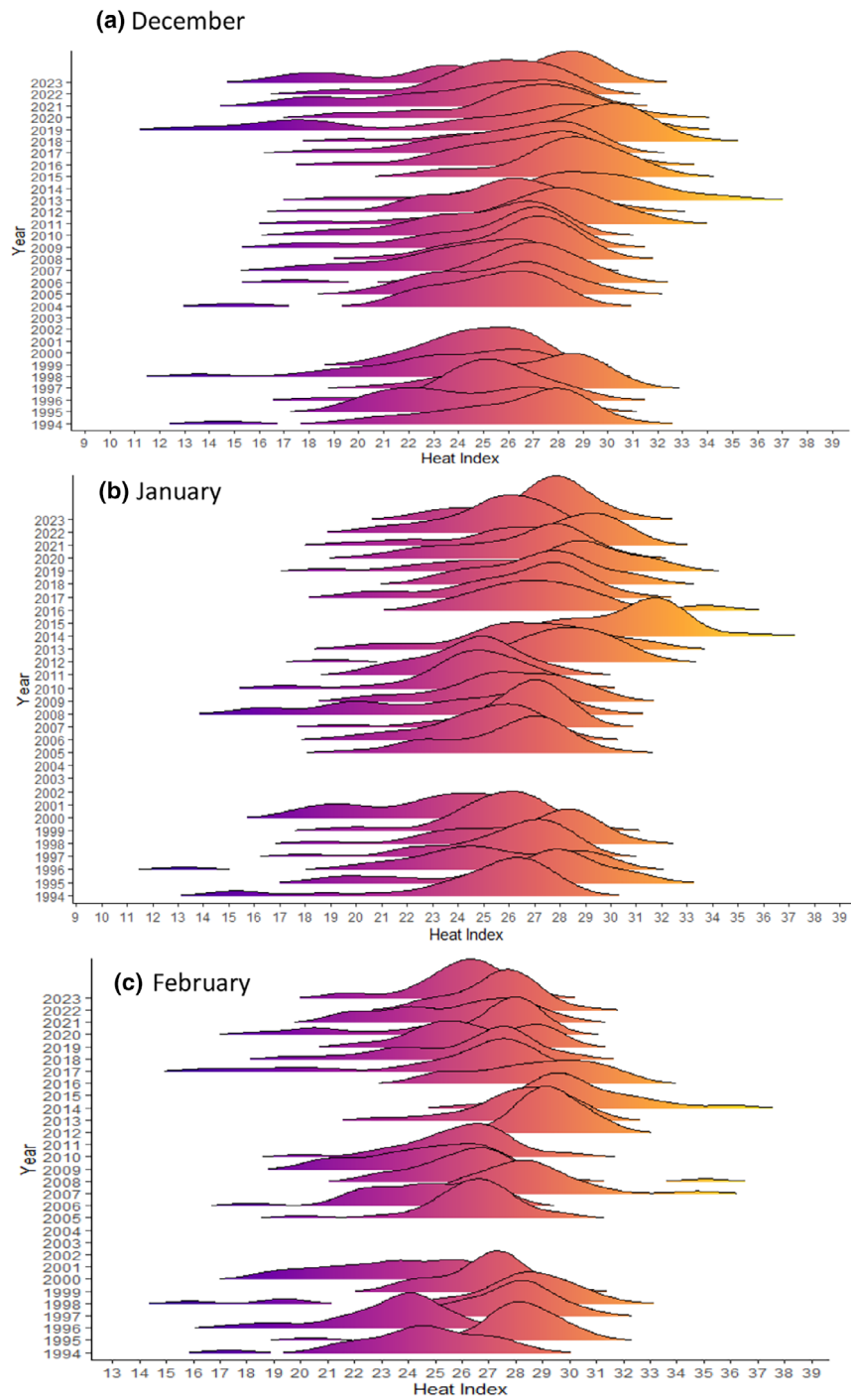


Figure 2 The apparent maximum temperatures (heat index) for weather station data from Witbank, Gauteng, South Africa (25°49'55.20" S, 29°11'31.20" E) for the summer months of (a) December, (b) January, and (c) February for the years of 1994–2023 where *Micaelamys namaquensis* inhabits. All 3 months show a trend towards a greater frequency of hotter temperatures over time.

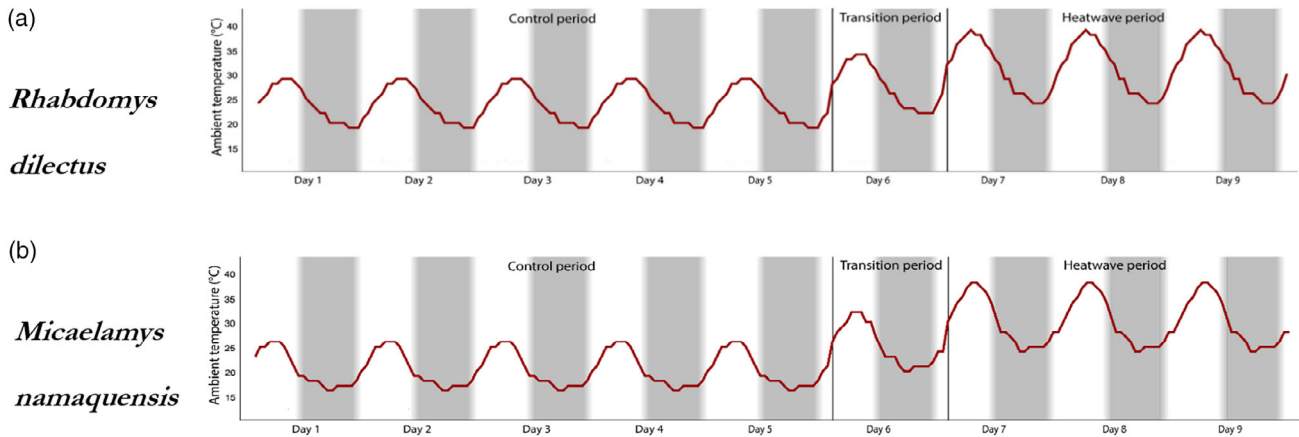


Figure 3 Photoperiod and ambient temperature ($^{\circ}\text{C}$) profile used to simulate control, transition, and heat wave temperatures for (a) the four-striped field mouse (*Rhabdomys dilectus*) and (b) the Namaqua rock rat (*Micaelamys namaquensis*). The white areas represent daytime, and the shaded areas represent nighttime. The black vertical lines represent changes in the experimental condition, with the control temperatures lasting 5 days, transition temperatures lasting 1 day and the simulated heat wave lasting 3 days. The red line represents the temperature cycle that animals were exposed to during the 9-day experiment (Figure adapted from Jacobs *et al.* (2020) and Jacobs *et al.* (2021)).

In male *M. namaquensis*, the impact of the simulated heatwave on fertility indices varied. Specifically, plasma testosterone levels and seminiferous tubule diameter exhibited a significant decrease when compared to the control (see Fig. 5, Table 1). However, testicular mass and volume were not significantly different between the control and heatwave conditions (see Fig. 5, Table 1). Unlike *R. dilectus*, sperm cells were observed in the seminiferous tubule of male *M. namaquensis* under both control and heatwave conditions.

Body mass did not affect any of the male fertility indices of *R. dilectus* ($t \leq 0.50$, $P \geq 0.63$ for all). Similarly, body mass did not affect any male fertility parameters of *M. namaquensis* ($t \leq 0.51$, $P \geq 0.12$ for all three) except for testicular mass ($t = 11.6$, $P < 0.0001$), which increased with body mass. We saw no change in body mass from before (*R. dilectus*: 42.1 ± 1.77 g; *M. namaquensis*: 64.3 ± 3.51 g), and after (*R. dilectus*: 43.9 ± 1.54 g; *M. namaquensis*: 64.9 ± 3.68 g) the stimulated heatwave (*R. dilectus*: $t = 1.92$, $P = 0.08$; *M. namaquensis*: $W = 9.0$, $P = 0.68$).

Discussion

This study set out to examine the impact of a simulated heatwave on male fertility indices in two small African rodent species, *R. dilectus* and *M. namaquensis*. The results demonstrate that exposure to a heatwave had a detrimental effect on fertility in both species, with *R. dilectus* experiencing more severe impairments. This finding suggests that acute heat stress can have significant implications for the reproductive success of small mammals.

Under controlled laboratory conditions with a long-day photoperiod simulating the natural light regime of the breeding season, both species displayed clear signs of reproductive activation. Key fertility indicators—testosterone levels, seminiferous tubule diameters, the presence of sperm, and testicular

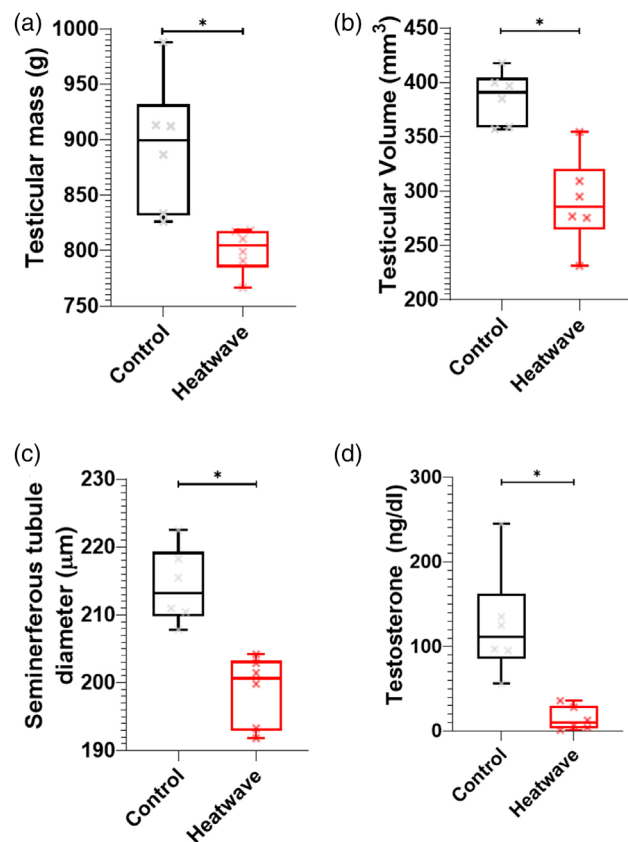
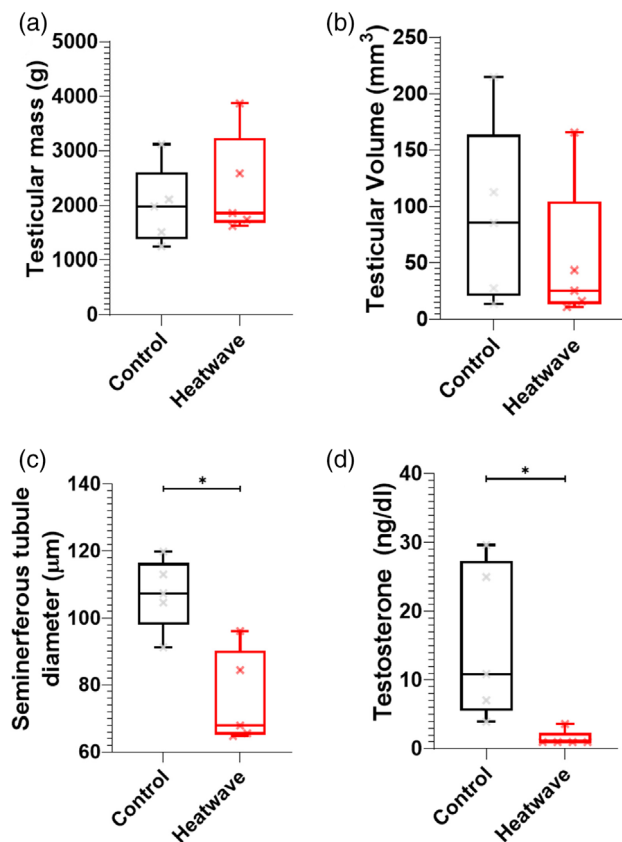


Figure 4 The reproductive parameters of (a) testicular mass (mg), (b) testicular volume (mm^3), (c) seminiferous tubule diameter (μm) and (d) plasma testosterone concentration (ng/dL) of four striped field mouse (*Rhabdomys dilectus*) captured from South Africa under control and stimulated heat wave conditions. *Indicates significance at $P \leq 0.05$. Data are shown as a maximum-minimum box plot.

Table 1 Statistical outputs of male fertility parameters between control and treatment conditions

Male fertility parameters	Species			
	<i>Rhabdomys dilectus</i>		<i>Micaelamys namaquensis</i>	
	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>
Testicular mass	-2.98	0.02*	-1.33	0.23
Testicular volume	-4.18	0.002*	-1.49	0.18
Seminiferous tubule diameter	-4.92	0.001*	-3.52	0.01*
Plasma testosterone	-4.11	0.003*	-2.59	0.04*

*Indicates significance at $P \leq 0.05$.**Figure 5** The reproductive parameters of (a) testicular mass (mg), (b) testicular volume (mm^3), (c) seminiferous tubule diameter (μm), and (d) plasma testosterone concentration (ng/dL) of the Namaqua rock rat (*Micaelamys namaquensis*) captured from South Africa under control and stimulated heat wave conditions. *Indicates significance at $P \leq 0.05$. Data are shown as a maximum–minimum box plot.

mass and volume—showed that these animals were physiologically prepared for reproduction (Krug, 2007; Muteka *et al.*, 2006; Rymer *et al.*, 2013). However, when exposed to simulated heatwave conditions, both species experienced a marked reduction in testosterone levels and seminiferous tubule diameters, suggesting a notable decline in fertility markers,

despite the photoperiod remaining consistent with the breeding season.

A key factor in reducing the impact of heat stress on small mammals is the availability of microclimates, which serve as critical refuges, protecting animals from extreme temperatures (Maclean, 2018; van Jaarsveld *et al.*, 2021; Varner & Dearing, 2014). Microclimates of small mammals can buffer temperatures by remaining 10–15°C cooler than daily maximum temperatures (Jackso *et al.*, 2002). In our study, we removed this temperature buffer to simulate a heatwave's direct effects without the relief offered by microclimatic refuges. The findings showed that, although both rodent species survived, their reproductive functions were significantly affected. Notably, *R. dilectus*, a smaller, more heat-sensitive species with typically greater access to microclimatic habitats, displayed more pronounced reductions in fertility, including a marked decrease in testicular mass and absence of sperm cells, indicating substantial reproductive stress. By contrast, *M. namaquensis* retained both testicular mass and sperm, suggesting greater resilience to heat stress. These results imply that without microclimate access, certain physical and behavioural traits—such as body and appendage size—may provide some buffering against heat stress impacts on reproduction.

A decline in male fertility could lead to reduced offspring production, ultimately threatening the viability of populations (Bowler, 1972; Hurley *et al.*, 2018; McCowan & Griffith, 2021; Schou *et al.*, 2021; Setchell *et al.*, 2001; Yaeram *et al.*, 2006). Given the critical role that small rodents play in ecosystems, their decline could trigger cascading effects on food webs and ecosystem functions (Grimm *et al.*, 2013; Heleno *et al.*, 2020; Hernandez *et al.*, 2023; Zhang *et al.*, 2017). This underscores the importance of conserving these species as part of broader efforts to maintain ecosystem stability, particularly in the face of climate change. Protecting habitats that provide favourable microclimates—such as the shade and cooler temperatures offered by bushes, hedges, or tree cover in grassland ecosystems—is crucial for shielding these species from heat stress and helping them survive as temperatures rise (Graham *et al.*, 2018).

The long-term implications of these findings are concerning, particularly with respect to the recovery of male fertility following heat stress. Previous studies have shown that the recovery of testicular physiology in rodents can take several weeks to months, after only 30–60 min of exposure to temperatures above 39°C (Reid *et al.*, 1981; Setchell, 1998; Yaeram *et al.*, 2006). For instance, Kanter and Aktas (2009) found that Leydig cell recovery only began 40 days after heat exposure, with full recovery taking up to 140 days. Although the T_a extreme in our study was less severe, potentially allowing for a faster recovery, the short-term reproductive impairment observed could still have significant implications for population dynamics, especially if heatwaves become more frequent or prolonged. Further studies are needed to uncover the testicular recovery period these rodents may need to regain testicular function after a heatwave.

Our experimental design excluded factors such as radiant heat exchange and wind speed, highlighting that in natural settings, additional mechanisms for heat dissipation—such as

convective heat loss—might alleviate some of the thermal stress these animals experience (Mitchell *et al.*, 2018). Moreover, since female reproductive responses were not examined, it remains uncertain how reductions in male fertility may impact broader population dynamics (Kucheravy *et al.*, 2021). Therefore, further research exploring female fertility under similar heat-stress conditions would be essential for a fuller understanding of these dynamics.

Conclusion

This study highlights the acute impact of heatwaves on male fertility in small mammals, especially in smaller-bodied species. Our findings suggest that, without access to microclimatic refuges, even a single extreme heat event can significantly compromise reproductive function, with potential long-term implications for population viability. The vulnerability observed here raises important concerns for small mammals from non-arid regions or moderate climates, which may increasingly face similar reproductive challenges as global temperatures rise. While large mammals may benefit from a lower surface area-to-volume ratio that offers some protection against acute thermal stress, they are not immune; prolonged high temperatures could still impact their fertility, particularly in species with less efficient thermoregulatory adaptations or in habitats with limited cooling microclimates.

As climate change drives more frequent and intense heatwaves, understanding species-specific vulnerabilities to thermal stress is critical. This research underscores the importance of conservation strategies that address the thermal ecology of small mammals and emphasize the preservation of microclimates as natural buffers against climate extremes.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

Conceptualization PJJ, DWH, NCB; Methodology PJJ, DWH, LdP, NCB; Formal Analysis PJJ, DWH, LdP; Investigation PJJ, DWH, LdP, NCB; Resources NCB; Writing – Original

Draft Preparation PJJ, DWH; Writing – Review & Editing PJJ, DWH, NCB; Visualization; PJJ, DWH; Project Administration NCB, DWH; Funding Acquisition NCB.

Data availability statement

All data are presented in the manuscript and Data S1.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Life history of the mesic four-striped field mouse (*Rhabdomys dilectus*) and Namaqua rock mouse (*Micaelamys namaquensis*) from South Africa.

Table S2. Animal details and body mass (g) before and after experimentation, average testicular mass (mg), testicular volume (mm³) and seminiferous tubules diameter (µm) and plasma testosterone (ng/dL) for the mesic four-striped field mouse (*Rhabdomys dilectus*) and Namaqua rock mouse (*Micaelamys namaquensis*) from South Africa exposed to either control or heatwave conditions.

Figure S1. The day (5:00–19:00) apparent temperatures (heat index) for Irene Gauteng, South Africa (25°54'36.00" S, 28°12'36.00" E) for the summer months of (a) December, (b) January and (c) February for the years of 1994–2023 where *Rhabdomys dilectus* inhabits. All 3 months show a rightward trend to hotter temperatures and a reduction of colder temperatures.

Figure S2. The day (5:00–19:00) apparent temperatures (heat index) for weather station data from Witbank, Gauteng, South Africa (25°49'55.20" S, 29°11'31.20" E) for the summer months of (a) December, (b) January and (c) February for the years of 1994–2023 where *Micaelamys namaquensis* inhabits. All 3 months show a rightward trend to hotter temperatures and a reduction of colder temperatures.

Figure S3. The night (19:00–5:00) apparent temperatures (heat index) for Irene Gauteng, South Africa (25°54'36.00" S, 28°12'36.00" E) for the summer months of (a) December, (b) January and (c) February for the years of 1994–2023 where *Rhabdomys dilectus* inhabits. All 3 months show a rightward trend to hotter temperatures and a reduction of colder temperatures.

Figure S4. The night (19:00–5:00) apparent temperatures (heat index) for weather station data from Witbank, Gauteng, South Africa (25°49'55.20" S, 29°11'31.20" E) for the summer months of (a) December, (b) January and (c) February for the years of 1994–2023 where *Micaelamys namaquensis* inhabits. All 3 months show a rightward trend to hotter temperatures and a reduction of colder temperatures.