

Non-invasive monitoring of adrenocortical activity in free-ranging Namaqua rock mice *Micaelamys namaquensis* from South Africa in response to anthropogenic land use and season

Authors: Ramahlo, Mmatsawela, Chimimba, Christian, Pirk, Christian, and Ganswindt, André

Source: Wildlife Biology, 2019(1) : 1-6

Published By: Nordic Board for Wildlife Research

URL: <https://doi.org/10.2981/wlb.00544>

The BioOne Digital Library (<https://bioone.org/>) provides worldwide distribution for more than 580 journals and eBooks from BioOne's community of over 150 nonprofit societies, research institutions, and university presses in the biological, ecological, and environmental sciences. The BioOne Digital Library encompasses the flagship aggregation BioOne Complete (<https://bioone.org/subscribe>), the BioOne Complete Archive (<https://bioone.org/archive>), and the BioOne eBooks program offerings ESA eBook Collection (<https://bioone.org/esa-ebooks>) and CSIRO Publishing BioSelect Collection (<https://bioone.org/csiro-ebooks>).

Your use of this PDF, the BioOne Digital Library, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Digital Library content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne is an innovative nonprofit that sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Non-invasive monitoring of adrenocortical activity in free-ranging Namaqua rock mice *Micaelamys namaquensis* from South Africa in response to anthropogenic land use and season

Mmatsawela Ramahlo, Christian Chimimba, Christian Pirk and André Ganswindt

M. Ramahlo (<https://orcid.org/0000-0003-3433-6728>) ✉ (m.ramahlo@zoology.up.ac.za), C. Chimimba and A. Ganswindt, Mammal Research Inst. (MRI), Dept of Zoology and Entomology, Univ. of Pretoria, Private Bag X20, 0028 Hatfield, South Africa. MR and CC also at: DST-NRF Centre of Excellence for Invasion Biology (CIB), Dept of Zoology and Entomology, Univ. of Pretoria, Hatfield, South Africa. – C. Pirk, Social Insects Research Group (SIRG), Dept of Zoology and Entomology, Univ. of Pretoria, Hatfield, South Africa.

Stress in animals has been linked to behavioural and physiological changes in response to environmental, social and anthropogenic stimuli. Hence, stress-related responses in animals, especially in rodents, have been used as biological indicators of ecosystem health. This study aimed to establish an enzyme immunoassay (EIA) for monitoring adrenocortical activity in free-ranging Namaqua rock mice *Micaelamys namaquensis* (Rodentia: Muridae) using faeces as a prerequisite for assessing the effects of anthropogenic land use and season on faecal glucocorticoid metabolite (fGCM) concentration. Rodents were live-trapped seasonally across four land use types: an agricultural crop farm, an agricultural livestock farm, a human-populated site and a nature reserve; all situated in Limpopo Province, South Africa. Determined fGCM concentrations from capture and recapture events were used for biologically validating an EIA detecting steroids with a 5α - 3β - 11β -diol structure. Recapturing resulted in a significant overall 40% elevation of individual fGCM concentrations demonstrating the effectiveness of the chosen EIA to reliably detect glucocorticoid output in the study species. Neither land use type nor season affected fGCM concentrations in the species, suggesting that land use and season-related environmental changes do not necessarily act as stressors for *M. namaquensis*, presumably due to their adaptive and resilient nature. Such species can be used to identify ecosystems affected by human-mediated disturbances and allow insights into the management and restoration of these threatened ecosystems and their associated species.

Keywords: anthropogenic impact, ecosystem health, faecal glucocorticoid metabolite, land use types, *Micaelamys namaquensis*, non-invasive stress hormone monitoring, South Africa

Small mammal community structure and population dynamics can be used as indicators of the level of disturbance experienced by a habitat and thus ecosystem health (Ferreira and Van Aarde 1999, 2000, Umetsu and Pardini 2007). In this regard, the monitoring of stress-related physiological responses of animals to a particular habitat allow insights into human impacts on species and ecosystem health (Fair and Becker 2000, Morgan and Tromborg 2007, Brearley et al. 2012). Wild rodents experience many different stressors, including intra- and inter-specific competition, predator–prey interactions and seasonal variation resulting in fluctuations of food and water availability (Boonstra et al. 1998, Prevedello et al. 2013, Dantzer et al. 2016, Navarro-Castilla et al. 2017, Dantzer et al. 2018). Their

comparably small body size however, often affords them the ability to, a priori, escape stressful situations, which can lead to distinct shifts in community structure (Touma et al. 2004, Harper and Austad 2012). As a result of the constant nature of certain stressors within the natural environment, employing strategies such as avoidance might not offer long-term survival mechanisms (Tarlow and Blumstein 2007, Blumstein et al. 2016). Thus, increased proximity to humans and direct human interference as well as changes to natural habitats resulting from human practices, may cause stress in rodents (Monadjem 1999, Singleton et al. 2003, Ellenberg et al. 2007).

Stress can be defined as a stimulus that disrupts an animal's homeostasis, and can refer to any emotional or physical stimulus that continues for any given period of time or intensity (Rivier and Rivest 1991, Navarro-Castilla et al. 2017). In mammals, a perceived stressor activates the hypothalamo–pituitary–adrenal axis which results in the secretion of glucocorticoids (Rivier and Rivest 1991, Ganswindt et al. 2012). Consequently, the increased production of these

This work is licensed under the terms of a Creative Commons Attribution 4.0 International License (CC-BY) <<http://creativecommons.org/licenses/by/4.0/>>. The license permits use, distribution and reproduction in any medium, provided the original work is properly cited.

hormones can be used as a reliable indicator of stress (Touma and Palme 2005, Palme 2019). Currently, glucocorticoids and their metabolites are quantifiable by a variety of matrices including blood, saliva, urine and faeces (Sheriff et al. 2011). Using animal excreta often allows a non-invasive or minimally invasive approach as animals do not need to be present during sampling. In addition, animals will only experience mild discomfort if temporal restraint in movement is involved during sampling (Laver et al. 2012, Möstl 2014). Adequate techniques for a non-invasive quantification of respective hormones, however, need to be validated in terms of their suitability for the species-specific hormone matrix of interest to ensure a reliable quantification of respective steroid metabolites (Touma and Palme 2005, Hodges et al. 2010).

The Namaqua rock mouse *Micaelamys namaquensis* (A. Smith 1834) is a communal-living murid rodent that is well-adapted to high temperatures and dry conditions and is omnivorous, consuming grasses and seeds in natural environments (Skinner and Chimimba 2005, Muteka et al. 2006). The species often undergoes periodic population fluctuations due to environmental factors such as rainfall and temperature, resulting in high mortality during dry periods, followed by high natality during wet conditions (Dickman et al. 1999). This response to environmental factors makes it a suitable model for investigating the effects of anthropogenic stressors on small mammal communities as responses to changes in the immediate environment, whether natural or anthropogenic, can be observed within a short period of time (Muteka et al. 2006, Avenant 2011, Fagir and Ueckermann 2014). In addition, *M. namaquensis* is a highly resilient species that has been known to re-establish after environmental fluctuations, which can result in population eruptions in areas that have been previously disturbed (Russo et al. 2010). Thus, *M. namaquensis* could be used as an indicator species for determining the effects of human practices on ecosystem health and biodiversity (Avenant 2000, 2011, Avenant et al. 2008). By monitoring the response of this species to stressors, areas of high disturbance and thus in need of restoration could be identified (Ferreira and Van Aarde 2000, Martin 2003).

Therefore, with research on endocrine activity in rodents being based mainly on reproduction and stress in European and American rodents, our study aimed to investigate stress-related hormone activity specifically in African murid rodents. Since there has been no previous validation on an EIA for the Namaqua rock mouse, we aimed to evaluate the reliability of an EIA detecting steroids with a 5α - 3β - 11β -diol structure for monitoring faecal glucocorticoid metabolite (fGCM) concentrations in captured and recaptured *M. namaquensis*. We also aimed to assess the effects of anthropogenic land use and seasonal variation on fGCM alteration in this species.

Material and methods

Study area

Micaelamys namaquensis was sampled seasonally (Southern Hemisphere autumn, winter, spring and summer) between March 2016 and January 2017 at four sites (Plug 2000,

Table 1) with different land use types in the Hoedspruit region, in Limpopo Province, South Africa (Fig. 1).

Sampling design

Rodents were sampled at each of the four study sites once per season with a single survey consisting of five consecutive trap nights. Fifty Sherman live traps (H.B. Sherman Traps Inc. Florida, USA) were baited with a mixture of oats, peanut butter and tinned pilchards, and placed 10 m apart in a 5×10 grid following standard procedures (Avenant and Cavallini 2007). Traps were checked in the mornings from 05:30 (and baited before 09:00) and afternoons from 15:30 (and baited before 18:00) over a 24-h period, and captured rodents were removed and identified up to species level (Mills and Hes 1997, Skinner and Chimimba 2005), with *M. namaquensis* being weighed and sexed on site. All *M. namaquensis* individuals used in the study were captured between 18:00 and 06:00 and traps were checked for dried faecal pellets. All faecal pellets found in traps were collected for each individual to ensure that the required amount of dried faecal matter per sample was obtained. A single faecal sample was obtained from each individual at capture, and if applicable, at each recapture event as animals were not housed. Animals were marked at capture by unique toenail clipping, which consisted of numbered clipping of toenails to ensure identification of recaptured individuals (Monadjem and Perrin 2003, Meheretu et al. 2015). Toenail clipped individuals were treated with antiseptic Mercurochrome (Barrs Pharmaceutical Industries, Cape Town, South Africa) to prevent infection (Aplin et al. 2003). All sampled individuals were released at their capture site after processing.

Faecal sample processing and extraction

Faecal samples from *M. namaquensis* were obtained from Sherman traps using gloves, and subsequently stored in labelled 5 ml cryotubes and frozen immediately at -20°C until further processing. Samples were freeze-dried, weighed and pulverised with a pestle and mortar (Fraňková et al. 2012). Dried samples weighing between 0.040 and 0.060 g were extracted with 1.5 ml of 80% ethanol (Touma et al. 2003). The suspensions were vortexed for 15 min and centrifuged at $1500g$ for 10 min, and the supernatants were then decanted and stored in 1.5 ml Eppendorf tubes at -20°C until analysis.

Hormone analysis

Immunoreactive faecal glucocorticoid metabolite (fGCM) concentrations were determined using three different EIAs: 1) a 5α -pregnane- 3β , 11β , 21 -triol- 20 -one EIA (detecting fGCMs with a 5α - 3β - 11β -diol structure) that has formerly been used to reliably monitor adrenocortical activity in rodents such as mice, 2) a cortisol EIA and 3) a corticosterone EIA. Detailed assay characteristics, including full descriptions of the assay components and cross-reactivities are described by Touma et al. (2003) for the 5α -pregnane- 3β , 11β , 21 -triol- 20 -one EIA, and by Palme and Möstl (1997) for the cortisol and corticosterone EIAs. As only the 5α -pregnane- 3β , 11β , 21 -triol- 20 -one EIA reliably depicted

Table 1. Sampling sites and their geographic coordinates for *Micaelamys namaquensis* from four land use types with their associated characteristic vegetation (Plug 2000) in the Hoedspruit region, Limpopo Province, South Africa.

| Study site | Land use type (abbreviation) | Location | Characteristic vegetation |
|-------------------------------|----------------------------------|----------------------|--|
| Karongwe Private Game Reserve | nature reserve (NR) | 24.1957°S, 30.5677°E | mixed lowveld bushveld vegetation |
| London Village | human-populated site (HP) | 24.2997°S, 30.5823°E | mixed lowveld bushveld, maize crops |
| Tshitamelodi Farm A | agricultural crop farm (AC) | 24.1666°S, 30.3978°E | maize, tomatoes, peppers and butternut |
| Tshitamelodi Farm B | agricultural livestock farm (AL) | 24.1666°S, 30.3978°E | planted fruit trees |

capture/recapture-related fGCM alterations and also revealed highest quantities (about 100× higher than the cortisol and 5× higher than the corticosterone assay), fGCM concentrations are only reported for this EIA. Sensitivity, determined at 90% binding was 2.4 µg g⁻¹ faecal dry weight (DW) for the 5α-pregnane-3β,11β,21-triol-20-one assay, 0.6 ng g⁻¹ faecal DW for the cortisol assay and 1.8 ng g⁻¹ faecal DW for the corticosterone EIA. Inter-assay coefficients of variation, determined by repeated measurement of high- and low-value quality controls, were 6.37% and 10.09%, respectively, for the 5α-pregnane-3β,11β,21-triol-20-one EIA, 6.05% and 9.44%, respectively, for the cortisol EIA, and 5.52% and 12.45%, respectively, for the corticosterone assay. Intra-assay coefficients of variation, determined by repeated measurement of high- and low-value quality controls, were 6.62% and 6.70%, respectively, for the 5α-pregnane-3β,11β,21-triol-20-one EIA, 4.64% and 5.96%, respectively, for the cortisol EIA, and 5.75% and 4.84%, respectively, for the corticosterone assay. Hormone analyses were performed at the Endocrine Research Laboratory, University of Pretoria, Pretoria, South Africa, as described previously (Ganswindt et al. 2002).

Statistical analyses

Assay reliability was examined in the form of a biological validation by comparing fGCM concentrations of captured

and recaptured (up to 48 h later) *M. namaquensis* individuals using a pairwise t-test (n=9; three females and six males). To deduce baseline fGCM concentrations for the biological validation group during original capture, an iterative process was followed where concentrations of individuals exceeding the mean ± 1.5 SD were excluded, the average successively recalculated, and the elimination process repeated until no values exceeded the mean ± 1.5 SD (Brown et al. 1999, de Bruin et al. 2014). This process excluded three of the initially 12 captured/recaptured individuals, and thus statistical tests were conducted on individual fGCM values of the remaining nine animals. Differences in fGCM concentrations across land use types, season as well as the interactive effects of both variables, were analysed using generalised linear models (package lm) (Bolker et al. 2009). Alpha level for all tests was set at α=0.05. All statistical analyses were based on algorithms in R ver. 3.2.1 (<www.r-project.org>) and were performed using the MASS package (Venables and Ripley 2002) in the R Studio (<www.r-project.org>) interface.

Results

A total of 122 *Micaelamys namaquensis* individuals (47 females and 75 males) were captured during the study. From the 122 individuals captured, faecal samples were obtained and analysed from 42 individuals (17 females and

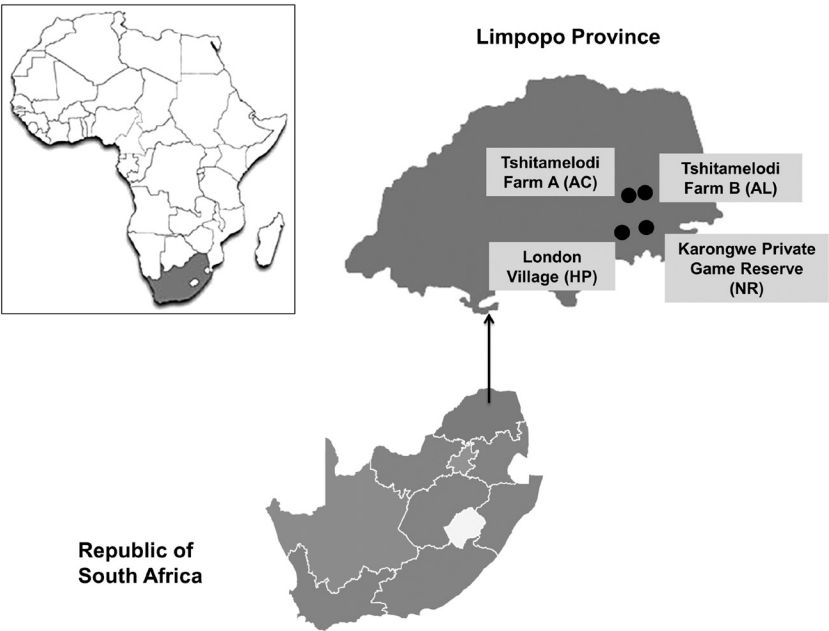


Figure 1. Sampling sites from which the Namaqua rock mouse (*M. namaquensis*) faecal samples were collected: Karongwe Private Game Reserve (NR), London village (HP), Tshitamelodi crop farm (AC) and Tshitamelodi livestock farm (AL), all in Limpopo Province, South Africa. See Table 1 for a description of these sample sites.

25 males). There were no significant differences between fGCM concentrations of females and males and thus subsequent statistical analyses were conducted on a pooled dataset of both sexes.

Individual fGCM concentrations during capture/recapture

The nine captured/recaptured individuals ($n=9$; three females and six males) showed a statistically significant increase in fGCM concentrations when recaptured up to 48 h later ($t_8 = -2.32$; $n=9$; $p=0.05$; Fig. 2). Overall individual median fGCM concentrations were $1.93 \mu\text{g g}^{-1}$ DW (range $0.89\text{--}3.11 \mu\text{g g}^{-1}$ DW) during capture, compared to $2.75 \mu\text{g g}^{-1}$ DW (range $1.09\text{--}9.98 \mu\text{g g}^{-1}$ DW) during recapture; demonstrating an overall 40% increase in respective fGCM concentrations during the second event (Fig. 2).

fGCM concentrations in relation to land use type and season

There were no statistically significant differences in fGCM concentrations of *M. namaquensis* across land use types ($F_{2,38}=0.35$; $n=41$; $p=0.71$; Fig. 3). An overall individual median fGCM concentration of $2.59 \mu\text{g g}^{-1}$ DW (range $0.74\text{--}12.73 \mu\text{g g}^{-1}$ DW, $n=20$) was recorded at the crop farm (AC), $2.40 \mu\text{g g}^{-1}$ DW (range $0.46\text{--}10.21 \mu\text{g g}^{-1}$ DW, $n=10$) at the livestock farm (AL), and $3.26 \mu\text{g g}^{-1}$ DW (range $2.22\text{--}12.09 \mu\text{g g}^{-1}$ DW, $n=11$) at the human-populated site (HP). Only one animal was sampled at the nature reserve (NR) showing an fGCM concentration of $1.15 \mu\text{g g}^{-1}$ DW.

There were no statistically significant differences in fGCM concentrations of *M. namaquensis* between seasons ($F_{3,37}=1.07$; $n=41$; $p=0.40$). The highest individual median fGCM concentration was found during autumn

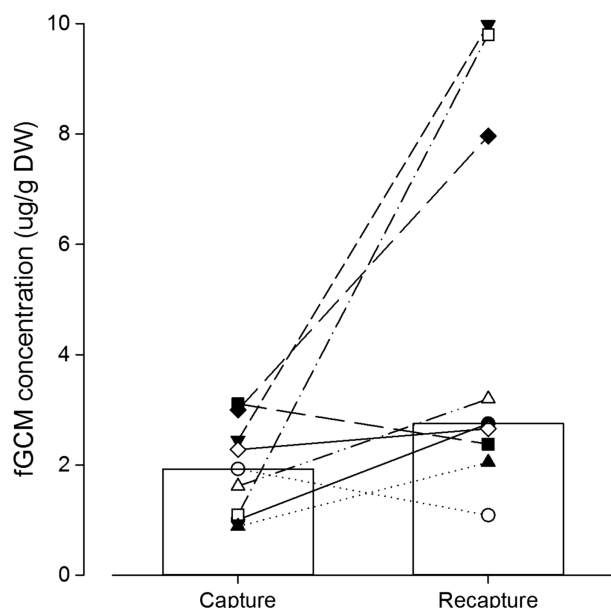


Figure 2. Faecal glucocorticoid metabolite (fGCM) concentrations of Namaqua rock mouse *Micaelamys namaquensis* individuals ($n=9$) from Limpopo Province, South Africa, at capture and recapture (48 h later).

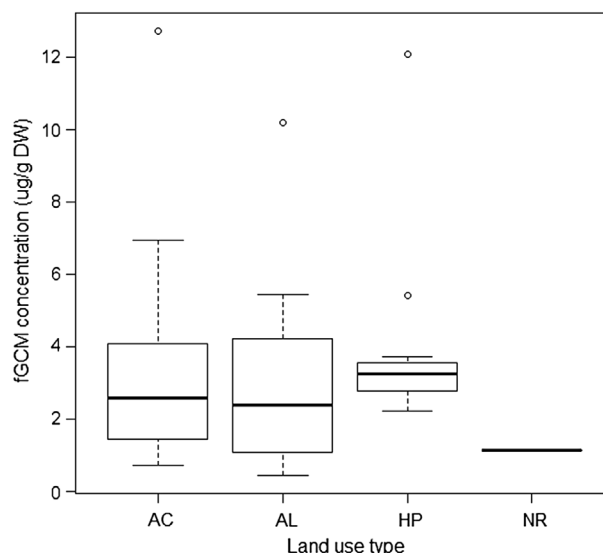


Figure 3. Individual means of faecal glucocorticoid metabolite (fGCM) concentrations of Namaqua rock mice *Micaelamys namaquensis* ($n=42$) sampled in Limpopo Province, South Africa, across the following landscapes: 1) agricultural crop farm (AC) ($n=20$); 2) agricultural livestock farm (AL) ($n=10$); 3) human-populated site (HP) ($n=11$); and 4) nature reserve (NR) ($n=1$). See Table 1 for a description of these sample sites.

($3.59 \mu\text{g g}^{-1}$ DW, range $1.48\text{--}10.21 \mu\text{g g}^{-1}$ DW, $n=4$), followed by winter ($3.16 \mu\text{g g}^{-1}$ DW, range $1.01\text{--}12.73 \mu\text{g g}^{-1}$ DW, $n=10$) and summer ($2.86 \mu\text{g g}^{-1}$ DW, range $0.46\text{--}12.09 \mu\text{g g}^{-1}$ DW, $n=22$). The lowest overall individual median fGCM concentration was found during spring ($1.47 \mu\text{g g}^{-1}$ DW, range $0.74\text{--}4.73 \mu\text{g g}^{-1}$ DW, $n=6$). There were no statistically significant effects from the interaction between land use and season ($F_{5,35}=1.17$; $n=41$; $p=0.34$).

Discussion

As demonstrated in a study on house *Mus musculus* and deer *Peromyscus maniculatus* mice, as well as Eurasian red squirrels *Sciurus vulgaris*, capture and restraint of free-ranging animals is usually perceived as a stressor and thus can be used to demonstrate the reliability of an EIA to determine biologically meaningful fGCM alterations in a species (Harper and Austad 2012, Dantzer et al. 2016, Santicchia et al. 2018). The significant elevation in fGCM concentrations in recaptured *Micaelamys namaquensis* in our study thus demonstrates the suitability of the 5α -pregnane- 3β , 11β , 21 -triol- 20 -one EIA to reliably monitor adrenocortical activity in the species using faeces as a hormone matrix. This finding also underlines the impact of presumed small-scale human-mediated disturbances such as animal handling on small mammals, as is also the case with the African lesser bush baby *Galago moholi* (Scheun et al. 2015). Łopucki et al. (2019) also found that small mammals such as the striped field mouse *Apodemus agrarius* often experience elevated hormone concentrations in response to human-mediated disturbances, followed by hormonal adjustments and a loss of the initial fear of humans. Although the chosen EIA determined changes in fGCM concentrations following a stressful

event, further validation in the form of an ACTH challenge could underline the suitability of the EIA to monitor adrenocortical activity in *M. namaquensis* (Laver et al. 2012). Such a combined approach of physiological and biological validation has, for example, been used in studies on banded mongooses and spiny mice (Nováková et al. 2008, Laver et al. 2012). Thus, conducting an additional ACTH challenge test in a future study would be recommended for reassuring that the selected EIA is in fact suitable to determine differences in fGCM concentrations in *M. namaquensis*.

Although anthropogenic land use might affect the structure of small mammal communities (Vieira et al. 2009), it does not seem to alter related fGCM concentrations in *M. namaquensis*. These findings concur with a study on captive crossbred rabbits, where Buijs et al. (2011) found that the immediate habitat of an individual has no significant effect on its glucocorticoid metabolite concentration. Due to the availability of food year-round in disturbed areas such as farms and towns, small mammal communities are effectively able to co-exist by occupying a variety of niches, thus somewhat nullifying the effects of land use differences (Loman 1991, Weibull et al. 2003, Codron et al. 2015). Pedersen and Greives (2008) showed that fGCM levels are influenced by food availability in small mammal populations in the wild. Scheun et al. (2015) found that *G. moholi* living in areas of high anthropogenic disturbance show higher fGCM concentrations than those in rural areas, indicating that human activities such as urbanisation and agriculture may pose a threat to natural ecosystems and species. In addition, Brearley et al. (2012) demonstrated that squirrel gliders *Petaurus norfolcensis* living in fragmented habitats and habitats near urban developments in southeast Queensland, Australia have higher glucocorticoid levels than those living away from urban edges. As shown in *A. agrarius*, however, hormonal adjustments to anthropogenic activities such as land use can be observed in small mammals, thus showing little to no elevation in the hormone concentrations of rodents living in disturbed areas (Łopucki et al. 2019). Thus, there is a need for further research to investigate in more detail the different types of potential stressors affecting *M. namaquensis*.

In our study, season did not have a significant effect on fGCM concentrations in the Namaqua rock mouse. This may be due to the availability of food during dry months of the year as crops are planted year-round. The ability of *M. namaquensis* to persist across various land use types, and its associated lack of related changes in fGCM concentrations of individuals across sites, suggests that the species has the ability to adapt to landscape disturbances and increased human population density (Abu Baker and Brown 2012, Makundi et al. 2016). This adaptive resilience suggests that *M. namaquensis* may be able to recover more effectively from disturbance than many other species as it is prone to fluctuating population cycles as a result of human-mediated disturbances (Russo et al. 2010). The use of such resilient and adaptive species in research can allow insights into the likelihood of wildlife populations and ecosystems recovering from human-induced disturbances as well as how to better manage threatened species in disturbed landscapes.

Our study represents one of the few conducted on southern African murid rodents and the monitoring of hormone activity in the species. In addition, our study

represents one of the few examples of non-invasive monitoring of stress-related endocrine activity in free-ranging southern African murid rodent species and could thus be used as baseline research data in future studies.

Acknowledgements – We thank the Centre of Excellence for Invasion Biology (CIB) for project funding. We also thank the landowners at Karongwe Private Game Reserve and Tshitamelodi Farms, and Ntate Gibson Mahlo from London Village, Hoedspruit for kindly giving their permission to work on their land. We are grateful to the staff and interns at GVI Limpopo for logistical support during fieldwork. We also thank Stefanie Ganswindt and Lezaan Prinsloo for expert help with laboratory techniques.

Permits – This study was performed with the approval of the Animal Ethics Committee of the University of Pretoria, Pretoria, South Africa (Ethics clearance number EC019-16).

References

- Abu Baker, M. A. and Brown, J. S. 2012. Patch use behaviour of *Elephantulus myurus* and *Micaelamys namaquensis*: the role of diet, foraging substrates and escape substrates. – Afr. J. Ecol. 50: 167–175.
- Aplin, K. P. et al. 2003. Field methods for rodent studies in Asia and the Indo-Pacific. – CSIRO Publishing.
- Avenant, N. L. 2000. Small mammal community characteristics as indicators of ecological disturbance in the Willem Pretorius Nature Reserve, Free State, South Africa. – S. Afr. J. Wildl. Res. 30: 26–33.
- Avenant, N. L. 2011. The potential utility of rodents and other small mammals as indicators of ecosystem ‘integrity’ of South African grasslands. – Wildl. Res. 38: 626–639.
- Avenant, N. L. and Cavallini, P. 2007. Correlating rodent community structure with ecological integrity, Tussen-die-Riviere Nature Reserve, Free State Province, South Africa. – Integr. Zool. 2: 212–219.
- Avenant, N. L. et al. 2008. Correlating small mammal community characteristics and habitat integrity in the Caledon Nature Reserve, South Africa. – Mammalia 72: 186–191.
- Blumstein, D. T. et al. 2016. Fitness and hormonal correlates of social and ecological stressors of female yellow-bellied marmots. – Anim. Behav. 112: 1–11.
- Bolker, B. M. et al. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. – Trends Ecol. Evol. 24: 127–135.
- Boonstra, R. et al. 1998. The impact of predator-induced stress on the snowshoe hare cycle. – Ecol. Monogr. 68: 371–394.
- Brearley, G. et al. 2012. Influence of urban edges on stress in an arboreal mammal: a case study of squirrel gliders in southeast Queensland, Australia. – Landsc. Ecol. 27: 1407–1419.
- Brown, J. L. et al. 1999. Hormone secretion in the Asian elephant (*Elephas maximus*): characterization of ovulatory and anovulatory luteinizing hormone surges. – Biol. Reprod. 61: 1294–99.
- Buijs, S. et al. 2011. Glucocorticoid metabolites in rabbit faeces – influence of environmental enrichment and cage size. – Physiol. Behav. 104: 469–473.
- Codron, J. et al. 2015. Stable isotope evidence for trophic niche partitioning in a South African savanna rodent community. – Curr. Zool. 61: 397–411.
- Dantzer, B. et al. 2016. Measurement of fecal glucocorticoid metabolite levels in Eurasian red squirrels (*Sciurus vulgaris*): effects of captivity, sex, reproductive condition and season. – J. Mamm. 97: 1385–1398.
- Dickman, C. R. et al. 1999. Long-term dynamics of rodent populations in arid Australia: the influence of rainfall. – Wildl. Res. 26: 389–403.

- de Bruin, R. et al. 2014. The pattern of ovulation in the southern African spiny mouse (*Acomys spinosissimus*). – Mamm. Biol. 79: 318–324.
- Ellenberg, U. et al. 2007. Elevated hormonal stress response and reduced reproductive output in yellow-eyed penguins exposed to unregulated tourism. – Gen. Comp. Endocrinol. 152: 54–63.
- Fair, P. A. and Becker, P. R. 2000. Review of stress in marine mammals. – J. Aquat. Ecosyst. Stress Recover. 7: 335–354.
- Fagir, D. and Ueckermann, E. 2014. The Namaqua rock mouse (*Micaelamys namaquensis*) as a potential reservoir and host of arthropod vectors of diseases of medical and veterinary importance. – Parasit. Vectors 7: 1–11.
- Ferreira, S. and Van Aarde, R. 1999. Habitat associations and competition in *Mastomys–Saccostomus–Aethomys* assemblages on coastal dune forests. – Afr. J. Ecol. 37: 121–136.
- Ferreira, S. and Van Aarde, R. 2000. Maintaining diversity through intermediate disturbances: evidence from rodents colonizing rehabilitating coastal dunes. – Afr. J. Ecol. 38: 286–294.
- Fraňková, M. et al. 2012. Family affairs and experimental male replacement affect fecal glucocorticoid metabolites levels in the Egyptian spiny mouse *Acomys cahirinus*. – Zool. Stud. 51: 277–287.
- Ganswindt, A. et al. 2002. Assessment of testicular endocrine function in captive African elephants by measurement of urinary and fecal androgens. – Zoo Biol. 21: 27–36.
- Ganswindt, A. et al. 2012. Determining adrenocortical activity as a measure of stress in African buffalo (*Syncerus caffer*) based on faecal analysis. – Afr. Zool. 47: 261–269.
- Harper, J. M. and Austad, S. N. 2012. Fecal glucocorticoids: a noninvasive method of measuring adrenal activity in wild and captive rodents. – Physiol. Biochem. Zool. 73: 12–22.
- Hodges, J. K. et al. 2010. Endocrine monitoring of reproduction and stress. Kleiman, D. G. et al. (eds), Wild mammals in captivity: principles and techniques for zoo management. Univ. of Chicago Press, 447–467.
- Laver, P. N. et al. 2012. Non-invasive monitoring of glucocorticoid metabolites in banded mongooses (*Mungos mungo*) in response to physiological and biological challenges. – Gen. Comp. Endocrinol. 179: 178–183.
- Loman, J. 1991. The small mammal fauna in an agricultural landscape in southern Sweden, with special reference to the wood mouse *Apodemus sylvaticus*. – Mammalia 55: 91–96.
- Łopucki, R. et al. 2019. Hormonal adjustments to urban conditions: stress hormone levels in urban and rural populations of *Apodemus agrarius*. – Urban Ecosyst. 22: 435–442.
- Makundi, R. et al. 2016. Farmer's perceptions of rodents as crop pests: knowledge, attitudes and practices in rodent pest management in Tanzania and Ethiopia. – J. Agric. Ext. Rural Dev. 8: 39–46.
- Martin, G. 2003. The role of small ground-foraging mammals in topsoil health and biodiversity: implications to management and restoration. – Ecol. Manage. Restor. 4: 114–119.
- Meheretu, Y. et al. 2015. Reproduction and survival of rodents in crop fields: the effects of rainfall, crop stage and stone-bund density. – Wildl. Res. 42: 158.
- Mills, M. G. L. and Hes, L. 1997. Rodents. – In: Joyce, P. et al. (eds), The complete book of southern African mammals. Struik Publishers, pp. 120–164.
- Monadjem, A. 1999. Geographic distribution patterns of small mammals in Swaziland in relation to abiotic factors and human land-use activity. – Biodivers. Conserv. 8: 223–237.
- Monadjem, A. and Perrin, M. 2003. Population fluctuations and community structure of small mammals in a Swaziland grassland over a three-year period. – Afr. Zool. 38: 127–137.
- Morgan, K. N. and Tromborg, C. T. 2007. Sources of stress in captivity. – Appl. Anim. Behav. Sci. 102: 262–302.
- Möstl, E. 2014. Glucocorticoids, their metabolites and their measurement in various animal species. – Med. Weter. 70: 524–529.
- Muteka, S. P. et al. 2006. Reproductive seasonality in *Aethomys namaquensis* (Rodentia: Muridae) from southern Africa. – J. Mammal. 87: 67–74.
- Navarro-Castilla, A. et al. 2017. Does ungulate disturbance mediate behavioural and physiological stress responses in Algerian mice (*Mus spretus*)? A wild enclosure experiment. – Hystrix Ital. J. Mamm. 28: 165–172.
- Nováková, M. et al. 2008. The effects of sex, age and commensal way of life on levels of fecal glucocorticoid metabolites in spiny mice (*Acomys cahirinus*). – Physiol. Behav. 95: 187–193.
- Palme, R. 2019. Non-invasive measurement of glucocorticoids: advances and problems. – Physiol. Behav. 199: 229–243.
- Palme, R. and Möstl, E., 1997. Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. – Int. J. Mammal. Biol. 62: 192–197.
- Pedersen, A. B. and Greives, T. J. 2008. The interaction of parasites and resources cause crashes in a wild mouse population. – J. Anim. Ecol. 77: 370–377.
- Plug, I. 2000. Overview of Iron Age fauna from the Limpopo Valley. – S. Afr. Archaeol. Soc. Goodwin Ser. 8: 117–126.
- Prevedello, J. et al. 2013. Population responses of small mammals to food supply and predators: a global meta-analysis. – J. Anim. Ecol. 82: 927–936.
- Rivier, C. and Rivest, S. 1991. Effect of stress on the activity of the hypothalamic–pituitary–gonadal axis: peripheral and central mechanisms. – Biol. Reprod. 45: 523–532.
- Russo, I.-R. M. et al. 2010. Bioregion heterogeneity correlates with extensive mitochondrial DNA diversity in the Namaqua rock mouse, *Micaelamys namaquensis* (Rodentia: Muridae) from southern Africa – evidence for a species complex. – BMC Evol. Biol. 10: 307.
- Santicchia, F. et al. 2018. Stress in biological invasions: introduced invasive grey squirrels increase physiological stress in native Eurasian red squirrels. – J. Anim. Ecol. 87: 1342–1352.
- Scheun, J. et al. 2015. The hustle and bustle of city life: monitoring the effects of urbanisation in the African lesser bushbaby. – Naturwissenschaften 102: 57–67.
- Sheriff, M. J. et al. 2011. Measuring stress in wildlife: techniques for quantifying glucocorticoids. – Oecologia 166: 869–887.
- Singleton, G. R. et al. 2003. Rats, mice and people: rodent biology and management. – Canberra Publishing.
- Skinner, J. and Chimimba, C. 2005. Order Rodentia. – In: Van der Horst, D. (ed.), The mammals of the southern African subregion. Cambridge Univ. Press, pp. 77–209.
- Tarlow, E. M. and Blumstein, D. T. 2007. Evaluating methods to quantify anthropogenic stressors on wild animals. – Appl. Anim. Behav. Sci. 102: 429–451.
- Touma, C. and Palme, R. 2005. Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. – Ann. N. Y. Acad. Sci. 1046: 54–74.
- Touma, C. et al. 2003. Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. – Gen. Comp. Endocrinol. 130: 267–278.
- Touma, C. et al. 2004. Analyzing corticosterone metabolites in fecal samples of mice: a noninvasive technique to monitor stress hormones. – Horm. Behav. 45: 10–22.
- Umetsu, F. and Pardini, A. E. R. 2007. Small mammals in a mosaic of forest remnants and anthropogenic habitats – evaluating matrix quality in an Atlantic forest landscape. – Landscape Urban Plan. 22: 517–530.
- Venables, W. N. and Ripley, B. D. 2002. Modern applied statistics with S. Springer.
- Vieira, M. V. et al. 2009. Land use vs. fragment size and isolation as determinants of small mammal composition and richness in Atlantic Forest remnants. – Biol. Conserv. 142: 1191–1200.
- Weibull, A.-C. et al. 2003. Species richness in agroecosystems: the effect of landscape, habitat and farm management. – Biodiv. Conserv. 12: 1335–1355.