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

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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

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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 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 1500 *g* 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 $\mu g g^{-1}$ 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

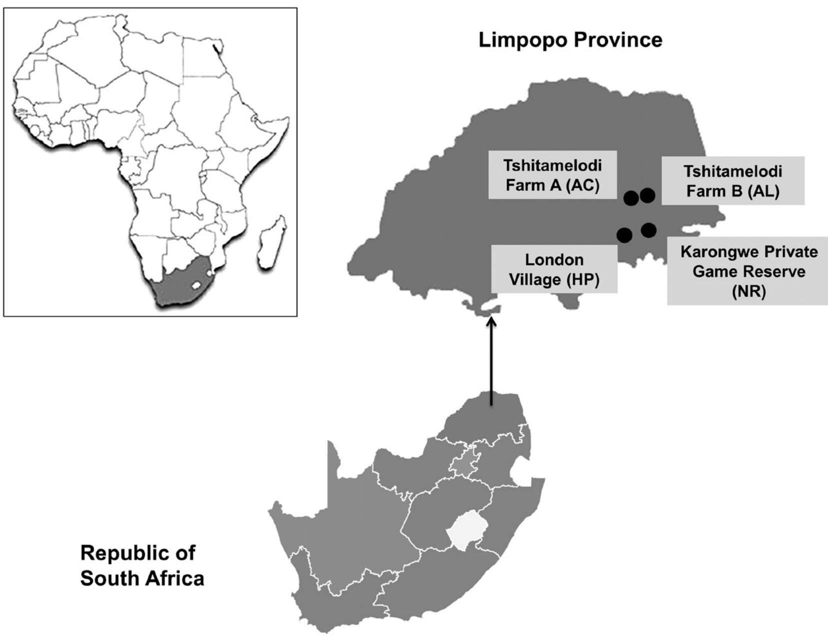

Figure 1. Sampling sites from which the Namaqua rock mouse (*M. namaquensis*) faecal samples were collected: Karongwe Private Game Reserve (NR), London village (HP), Tshitamelodi crop farm (AC) and Tshitamelodi livestock farm (AL), all in Limpopo Province, South Africa. See Table 1 for a description 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 μg g^{-1} DW (range 0.89–3.11 μg g^{-1} DW) during capture, compared to 2.75 μg g^{-1} DW (range 1.09–9.98 μg 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 μg g^{-1} DW (range 0.74–12.73 μg g^{-1} DW, n = 20) was recorded at the crop farm (AC), 2.40 μg g^{-1} DW (range 0.46–10.21 μg g^{-1} DW, n = 10) at the livestock farm (AL), and 3.26 μg g^{-1} DW (range 2.22–12.09 μg 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 μg 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 (3.59 μg g^{-1} DW, range 1.48–10.21 μg g^{-1} DW, n = 4), followed by winter (3.16 μg g^{-1} DW, range 1.01–12.73 μg g^{-1} DW, n = 10) and summer (2.86 μg g^{-1} DW, range 0.46–12.09 μg g^{-1} DW, n = 22). The lowest overall individual median fGCM concentration was found during spring (1.47 μg g^{-1} DW, range 0.74–4.73 μg 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).

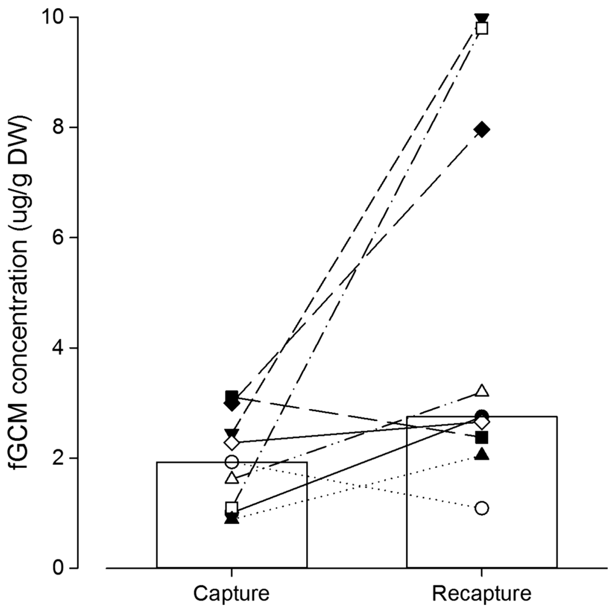

Figure 2. Faecal glucocorticoid metabolite (fGCM) concentrations of Namaqua rock mouse *Micaelamys namaquensis* individuals (n = 9) from Limpopo Province, South Africa, at capture and recapture (48 h later).

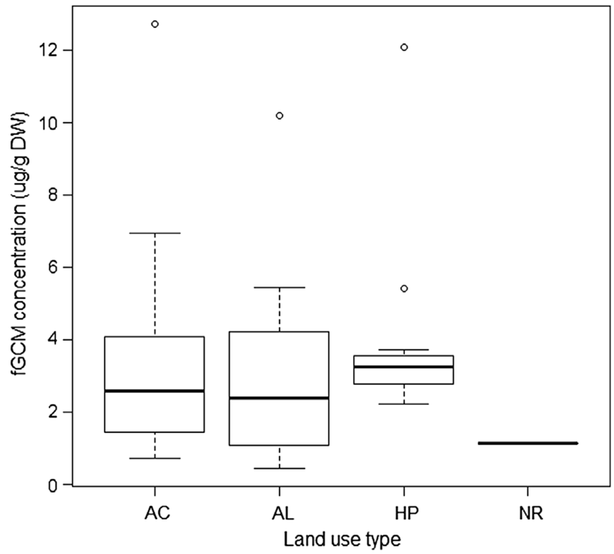

Figure 3. Individual means of faecal glucocorticoid metabolite (fGCM) concentrations of Namaqua rock mice *Micaelamys namaquensis* (n = 42) sampled in Limpopo Province, South Africa, across the following landscapes: 1) agricultural crop farm (AC) (n = 20); 2) agricultural livestock farm (AL) (n = 10);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

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.

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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).

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