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# Establishing enzyme immunoassays for quantifying fecal glucocorticoid metabolites in six southern African rodents using biological and physiological stimuli

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**Introduction:** Global landscape transformations and increased resource use are leading to an overall decline in ecosystem health and natural habitat quality. Due to their low trophic position, small body size, and close association with environmental conditions, rodents can serve as effective biological indicators of ecosystem health and anthropogenic disturbance by monitoring their behavior and physiology, specifically stress-related endocrine responses. This study aimed to establish minimally invasive methods for quantifying fecal glucocorticoid metabolites (fGCMs) in six southern African rodent species (*Aethomys ineptus*, *Lemniscomys rosalia*, *Mastomys coucha*, *Micaelamys namaquensis*, *Otomys angoniensis*, and *Steatomys pratensis*).

**Methods:** Where possible, one male and one female from each species were live trapped in the Magaliesberg Biosphere, North West province, South Africa, and individually housed on-site for enzyme immunoassay (EIA) validation experiments. Biological (handling and temporary removal from enclosure) and physiological (Synacthen-induced adrenocorticotrophic hormone or ACTH challenge test) stimuli were administered, and fGCM concentrations were determined from samples collected before, during, and after each intervention.

**Results and discussion:** A 5 $\alpha$ -pregnane-3 $\beta$ ,11 $\beta$ ,21-triol-20-one EIA (detecting fGCMs with a 5 $\alpha$ -3 $\beta$ , 11 $\beta$ -diol structure) was identified as the most suitable assay across all six species assessed. Two additional EIAs appear suitable for some of the species tested: Oxotiocholanolone II, measuring fGCMs with a 5 $\beta$ -3 $\alpha$ -ol-11-one structure (*A. ineptus*, *O. angoniensis*, and *S. pratensis*); and a Corticosterone EIA (*M. coucha*, *O. angoniensis*, and *S. pratensis*). Establishing and validating minimally invasive techniques for monitoring stress-related animal responses in rodents can be beneficial for evaluating the impact of anthropogenic disturbances on natural environments and their associated fauna.

## KEYWORDS

ACTH challenge, biological validation, fGCMs, non-invasive hormone monitoring, small mammals, stress-related biomarkers

## 1 Introduction

Assessing stress-related physiological responses and associated behavioral alterations of wildlife has become a widely used approach for evaluating animal welfare and advancing our understanding of ecological processes (Reeder and Kramer, 2005; Angelier and Wingfield, 2012). By quantifying physiological responses to anthropogenically induced environmental changes in indicator species, we can assess the impact of human-mediated disturbance on wildlife and, indirectly, on the various habitats they occupy (Hilty and Merenlender, 2000; Cadotte et al., 2015; Jepsen et al., 2021). A stress response, either acute or chronic, occurs when a perceived stressor threatens the homeostasis of an individual (Palme et al., 2005; Hulsman et al., 2011). When a stressor is perceived, the sympathetic-adrenomedullary axis and the hypothalamic-pituitary-adrenal (HPA) axis are stimulated, resulting in, among other processes, the secretion of glucocorticoids (GCs). These GCs are circulated through the blood and are subsequently metabolized and excreted through urine and feces (Reeder and Kramer, 2005; Touma and Palme, 2005). Short-term elevations of GCs can benefit the animal, for example, by supporting energy demands to evade predators. Long-term elevations, however, can lead to health issues such as suppression of the animal's immune response, reduced reproductive success, and impaired HPA regulation (Dobson and Smith, 2000; Hing et al., 2014). Therefore, quantifying stress-related biomarkers like GCs or their metabolites can be a useful tool for wildlife management and conservation strategy development.

Monitoring GC concentrations by using feces as a hormone matrix has become increasingly common as quantification of fecal glucocorticoid metabolites (fGCMs) is minimally invasive compared with other matrices like blood or saliva (Palme, 2005). Fecal collection typically does not require restraint or immobilized, and samples can often be obtained without direct interaction with the study animal (Palme et al., 2005). Minimally invasive approaches reduce pain, limit sampling-induced stress and allow for the release of wild-caught individuals, making them particularly suitable for long-term monitoring of free-ranging wildlife populations (Ganswindt et al., 2003; Laver et al., 2012). Due to species and sex-specific variations in fGCM composition and metabolism, respective test techniques must be validated to ensure reliable quantification of fGCMs in females and males of different indicator species.

Small mammals such as rodents are reliable biological indicator species of anthropogenic disturbance and, subsequently, ecosystem health in various global environments (Hilty and Merenlender, 2000; Horváth et al., 2001; Avenant et al., 2008). Human-mediated transformation of natural landscapes can ultimately lead to habitat degradation or destruction, and depletion of natural resources (Umetsu and Pardini, 2007; Irwin et al., 2010; Waters et al., 2016). Rodents are ideal biological sentinels of ecosystem health because their ecology places them among the first vertebrates to experience change (Dickman, 1999; Singleton et al., 2003; Avenant and Cavallini, 2007). As small-bodied, abundant mammals occupying low trophic levels, rodents are directly and rapidly

affected by shifts in climate, habitat structure, and food availability (Kerley et al., 1990; Weibull et al., 2003). These pressures act on the same energetic and survival pathways regulated by the HPA axis, making stress-related hormone quantification in rodents a sensitive and early indicator of ecosystem disturbance. Rodents are reliable short-lived model species that can be studied over several generations, and population and behavioral trends can be identified in response to environmental changes (Galetti et al., 2009; Merritt, 2010). Rodents can use microhabitats and have a diverse diet, and can thus persist across a wide range of landscapes (Canova and Fasola, 1993; Skinner and Chimimba, 2005). They are ecosystem drivers who cycle nutrients through the soil, disperse seeds to aid in plant growth, and provide food for predators, and their successful establishment in a landscape does not require interspecific interactions with other taxonomic groups (Joyce et al., 1997; Monadjem et al., 2015). Food resource quality and abundance are directly linked to litter size, weaning success, and nesting survival, indicating that species richness and abundance are correlated with food quality and quantity in natural landscapes (Gilbert and Krebs, 1991; Monadjem and Perrin, 2003).

As such, wildlife species population and behavior fluctuations can be linked with human population growth and anthropogenic activities such as agriculture, urbanization, and mining, among others (Marris, 2008; World Wildlife Fund, 2016; Christie et al., 2019). Identifying biological indicator species and monitoring their population dynamics, behavior, and physiology, specifically measuring their adrenocortical activity, can provide insights into the impact of anthropogenic disturbance and global climate change on wildlife in a rapidly changing world. Due to the resilient characteristics displayed by rodents, it is essential to monitor them in both natural and transformed landscapes to gauge the critical limit of their tolerance of environmental changes brought about by climate change and anthropogenic activities. Monitoring the behavioral responses of adaptive and resilient species can also inform on less adaptive species (McKee et al., 2004; Watson et al., 2004; Colles et al., 2009). Finding reliable methods for monitoring rodent adrenocortical activity in response to environmental stressors can improve our understanding of anthropogenic impacts on their habitat. However, most stress-related rodent research has been conducted on laboratory-reared animals, and the majority of the currently established enzyme immunoassays (EIAs) have been validated for species in the northern hemisphere, with limited research on free-ranging species in the southern hemisphere (Touma et al., 2004; Touma and Palme, 2005; Sheriff et al., 2011). As such, further validation of EIAs is necessary to support research in the global south.

Established EIAs can aid in quantifying fGCM concentrations of focal species and provide a summary of the perceived stressors that have activated the HPA axis of an individual over a period dependent on the gut passage time of the animal (Touma and Palme, 2005). It is not possible to attribute specific perceived stress events to measured hormone concentration values, and since we are unable to distinguish between an animal's response to anthropogenic versus other extrinsic and intrinsic stimuli, it is

important to standardize research protocols to ensure that observed differences between individuals are primarily from specific experimental variables (Vitousek et al., 2019). While it is not possible to link potential stressors to their hormone signals, we can compare average population hormone concentrations of focal species across different landscapes to identify overall variability (Dantzer et al., 2014). To reduce ecological noise, we can design experimental protocols that control for the season, time of day, the focal species, their sex and age, and individual immune responses, among others (Touma et al., 2003; Touma and Palme, 2005).

This study examined the suitability of five different EIAs for quantifying fGCMs in six southern African rodent species (*Aethomys ineptus*, *Lemniscomys rosalia*, *Mastomys coucha*, *Micaelamys namaquensis*, *Otomys angoniensis*, and *Steatomys pratensis*) by a) inducing a potential biological stress event through direct handling, temporary removal from the enclosure, and alteration of the immediate environment, and b) conducting an ACTH challenge test as a form of physiological challenge.

## 2 Materials and methods

### 2.1 Study area and data collection

The study animals were captured around the Magaliesberg mountain range (25°41'00.1"S 27°57'51.8"E), which extends through the Gauteng and North West provinces of South Africa. The Magaliesberg Biosphere is characterized by the Savannah Biome, which covers the Gold Reef Mountain Bushveld vegetation type at higher altitudes and the Marikana Thornveld and Moot Plains Bushveld in lower lying areas (Rutherford and Mucina, 2006). The study sites, located in the De Wildt (25°40'05.5"S 27°55'23.1"E) and Zilkaatsnek (25°39'25.8"S 27°56'34.9"E) regions of Magaliesberg, were characterized by hot, wet summers (November to March) and cold, dry winters (April to October) (Boonzaaier and Hough, 2011). The average seasonal temperatures in the region were 20.5 °C (min – max: 15.13 °C – 25.47 °C) in the wet season and 11.56 °C (5.2 °C – 18.3 °C) in the dry season (Magaliesburg climate (South Africa), 2021).

The animals were captured using 40 Enviro-Care live traps over a four-week period between December 2020 and January 2021. The traps (length x height x width: 255 x 78 x 80 mm) were constructed out of galvanized sheet metal and were placed 10 m apart in a 5 x 8 trapping grid following standard procedures (Aplin et al., 2003; 2011). Each trap was baited with a mixture of oats and peanut butter. The traps remained open throughout the day and night to ensure diurnal and nocturnal species trapping and were checked in the morning between 05:30 and 09:00 and again between 15:00 and 17:00 daily. Captured animals were sexed and identified up to species level (Joyce et al., 1997; Newbery, 1999; Linzey et al., 2003; Skinner and Chimimba, 2005). Selected adult individuals from each species were weighed using a spring scale and transported to an on-site animal housing research facility. Traps were rebaited and returned to their original positions in the trapping grid daily (Aplin et al., 2003).

### 2.2 Focal species

Six study species were selected based on their dominant abundance and trappability in the region: the Tete veld rat (*Aethomys ineptus*, Thomas & Wroughton 1908), the single-striped grass mouse (*Lemniscomys rosalia*, Thomas 1904), the southern multimammate mouse (*Mastomys coucha*, Smith 1834), the Namaqua rock mouse (*Micaelamys namaquensis*, Smith 1834), the Angoni vlei rat (*Otomys angoniensis*, Wroughton 1906), and the fat mouse (*Steatomys pratensis*, Peters 1846). Two individuals from each species were used in the study, resulting in 12 focal animals. Where possible, one female and one male from each species were used for biological and physiological validation experiments. Two species, *M. namaquensis* (two females) and *M. coucha* (two males), deviated from the standard protocol because only individuals from one sex were captured. Studies with small sample sizes, while limited in statistical power, can improve our understanding of animal ecology and provide valuable insight into future research methodology and interpretation of results (Bissonette, 1999; Bayazit, 2009; Kastelic et al., 2023).

Focal animals transported to the on-site research facility were temporarily housed in a separate room (approximately 8 m x 15 m), and the experiment was conducted approximately three days after capture. The experimental room was equipped with an air conditioning unit that was used daily at intermittent times to allow air circulation and prevent rodents from overheating during summer. The room was kept closed to minimize the risk of infiltration by pets of on-site staff and wildlife from the surrounding fields. Animals were housed individually in enclosures of 380 mm *l* x 110 mm *h* x 284 mm *b*. Fresh grass was collected daily and placed in the enclosures with wood shavings for feeding or nesting. For species between 6 g and 60 g, tins (73 mm *l* x 62 mm *h* x 62 mm *b*) were used for nesting, whereas larger species (> 60 g) were given buckets (120 mm *l* x 130 mm *h* x 130 mm *b*). In addition, depending on the individual's body size, enrichment objects (cardboard rolls and different-sized boxes) were placed in enclosures. All animals were additionally fed once a day on mixed feed grains, a vegetable (carrot, cucumber, bell pepper), and a fruit (apple, pear) option, and water was provided ad libitum.

### 2.3 Experimental design

Two days prior to the biological validation experiment, fecal samples were collected from all captive individuals at 0–8 h post-defecation three times a day (04:00, 12:00, and 20:00) to establish a pre-stressor baseline. At the start of the biological validation, animals were removed from their enclosures for about 30 min using a non-transparent handling bag, weighed using a scale and body mass recorded, then placed in a clear 110-l storage container (*l* x *b* x *h*: 800 x 400 x 430 mm) for observation while enclosures were cleaned. Following cleaning, animals were returned to their enclosures, which had different olfactory and visual cues from the cleaning agent used (70% ethanol) and the newly replaced enrichment objects. Animal handling, temporary removal from the enclosure and return to an altered environment were identified as potential stressors for individuals, and it was

TABLE 1 Assay-specific intra- and inter-assay variances (determined through repeated measurements of high and low quality controls generated from the respective standard stock solutions), sensitivities, and assay parallelism (determined using pooled samples for assays proven reliable to quantify fGCMs in one or more focal species, with respective serial dilutions of the fecal extracts giving displacement curves parallel to the standard curves) for the six Rodentia species (*Aethomys ineptus*, *Lemniscomys rosalia*, *Micaelamys namaquensis*, *Otomys angoniensis*, and *Steatomys pratensis*) captured in the Magaliesberg Biosphere, North West province, South Africa.

EIA	Intra-assay variance (%)	Inter-assay variance (%)	Assay sensitivity (ug)	Parallelism					
				<i>Aethomys ineptus</i>	<i>Lemniscomys rosalia</i>	<i>Mastomys coucha</i>	<i>Micaelamys namaquensis</i>	<i>Otomys angoniensis</i>	<i>Steatomys pratensis</i>
5 $\alpha$ -pregnane-3 $\beta$ ,11 $\beta$ ,21-triol-20-one (5 $\alpha$ )	3.15 and 3.61	10.27 and 10.40	2.4	<3%	<3%	<5%	<4%	<1%	N/A
Oxoetiocholanolone I (Oxo I)	4.09 and 4.81	13.02 and 14.16	1.2	<3%	<5%			<5%	No parallelism
Oxoetiocholanolone II (Oxo II)	4.94 and 6.84	8.71 and 13.46	0.75	<1%			<5%	<5%	<5%
Corticosterone (CC)	4.57 and 5.74	8.34 and 11.72	2.4						
Cortisol (C)	4.42 and 6.24	11.46 and 13.29	0.75		<5%				<1%

Associated relative variation of slopes of the respective parallelism trendlines are also presented (%).

assumed that these actions might elicit a quantifiable stress response in affected animals (Ramahlo et al., 2019). Following this, fecal samples were once again collected from all captive individuals at 0–8 h post-defecation three times a day (04:00, 12:00, and 20:00) to monitor any elevations in fGCM concentrations relative to the prestressor baseline.

Two days after the biological validation experiment was concluded, the same individuals were subjected to a physiological validation experiment (ACTH stimulation test). Each animal received a single dose (2.5 IU) of a synthetically prepared Synacthen-saline solution (Synacthens/Synacthen Depots; Novartis, Basel, Switzerland) administered intramuscularly, after which they were returned to their enclosures for monitoring over 24 h. During this 24-h period, enclosures were checked hourly for fecal samples, which were collected and frozen immediately to monitor for any elevations in fGCM concentrations relative to the prestressor baseline. Following the 24-h period, the sampling protocol reverted to fecal collection three times a day for the following seven days. At the end of the physiological validation experiment, all animals were released back into the environment at their capture sites. All frozen fecal material was transported to the Endocrine Research Laboratory of the University of Pretoria, South Africa, for further processing.

## 2.4 Steroid extraction and fGCM quantification

Frozen fecal material was freeze-dried and pulverized following Fraňková et al. (2012). Subsequently, dried fecal powder was extracted with 80% ethanol in water (0.016 – 0.024 g of fecal powder in 0.5 ml solvent, 0.025 – 0.037 g in 1 ml solvent, and 0.038 – 0.055 g in 1.5 ml solvent) (Touma et al., 2003). The suspensions were vortexed for 15 min and then centrifuged at 1500 g for 10 min. Supernatants were decanted into 1.5 ml Eppendorf tubes and stored at -20 °C until analysis.

Steroid extracts were quantified for immunoreactive fGCMs using five different enzyme immunoassays (EIAs): (i) a 5 $\alpha$ -pregnane-3 $\beta$ ,11 $\beta$ ,21-triol-20-one EIA (detecting fGCMs with a 5 $\alpha$ -3 $\beta$ , 11 $\beta$ -diol structure); (ii) an 11-oxoetiocholanolone (detecting 11,17-dioxoandrostanes; hereon referred to as Oxoetiocholanolone I); (iii) an 11-oxoetiocholanolone (detecting fGCMs with a 5 $\beta$ -3 $\alpha$ -ol-11-one structure; hereafter referred to as Oxoetiocholanolone II); (iv) a Corticosterone EIA; (v) and a Cortisol EIA. The selection of these five EIAs was based on their successful application in hormone quantification in laboratory-reared rodents, and detailed assay characteristics, including a full description of assay components and cross-reactivities, have been provided by Touma et al. (2003) for the 5 $\alpha$ -pregnane-3 $\beta$ ,11 $\beta$ ,21-triol-20-one EIA, by Palme and Möstl, (1997) for the 11-oxoetiocholanolone I, Corticosterone, and Cortisol EIAs, and by Möstl et al. (2002) for the 11-oxoetiocholanolone II EIA. The assay sensitivity and respective intra- and inter-assay coefficients of variances, determined by high- and low- concentration controls, are provided in Table 1. Parallelism tests were conducted for EIAs proven reliable to quantify fGCMs in one or more focal species, with respective serial dilutions of fecal extracts giving displacement

curves parallel to the standard curves. All values obtained are reported as  $\mu\text{g}\cdot\text{g}^{-1}$  DW.

## 2.5 Data analysis

For each individual, three samples were collected prior to biological validation, and three samples post biological validation. Following physiological validation, each individual was checked hourly and all voided samples were collected 0–24 h post-ACTH injection: *A. ineptus* ( $\varnothing = 5$ ,  $\sigma = 7$ ); *L. rosalia* ( $\varnothing = 8$ ,  $\sigma = 13$ ); *M. coucha* ( $\sigma = 7$ ,  $\sigma = 3$ ); *M. namaquensis* ( $\varnothing = 5$ ,  $\varnothing = 5$ ); *O. angoniensis* ( $\varnothing = 4$ ,  $\sigma = 12$ ); and *S. pratensis* ( $\varnothing = 1$ ,  $\sigma = 5$ ). Three additional samples were collected 24–72 h after physiological validation. All samples obtained during the experimental period were analyzed for a total of nine samples combined with the sample size obtained up to 24 h post-ACTH injection for each individual: (*A. ineptus* ( $\varnothing = 14$ ,  $\sigma = 16$ ); *L. rosalia* ( $\varnothing = 17$ ,  $\sigma = 22$ ); *M. coucha* ( $\sigma = 16$ ,  $\sigma = 12$ ); *M. namaquensis* ( $\varnothing = 14$ ,  $\varnothing = 14$ ); *O. angoniensis* ( $\varnothing = 13$ ,  $\sigma = 21$ ); and *S. pratensis* ( $\varnothing = 10$ ,  $\sigma = 14$ ). No samples were excluded from analysis.

Presumed baseline fGCM concentrations of three samples obtained 24 h before and after biological validation, and three samples obtained 24–72 h after the Synacthen injection were used to calculate individual median baseline fGCM concentrations. For each experimental individual, the biological peak value was the highest fGCM concentration obtained from three samples collected up to 24 h post-handling (biological validation), and the physiological peak was the highest fGCM concentration obtained from hourly sample collection up to 24 h post ACTH injection. For low sample sizes, the comparison of differences between baseline and peak fGCM concentrations is a common method (Brown et al., 1999, 2001). This allows each animal to serve as its own control, enabling absolute differences and percentage increases to be calculated, thereby reducing issues associated with individual variation in basal and peak fGCM concentrations (Romero, 2004; Palme et al., 2005). The percentage increases in fGCMs for each experiment were calculated using the median (baseline) values described above against the highest (peak) fGCM concentration obtained up to 24 h after the biological stressor and the physiological challenge for each individual and species.

A minimum 100% increase between baseline and peak fGCM concentrations was classified as a significant difference because a 2-fold increase is more likely measuring a physiological adrenocortical response and is not only attributed to metabolic variation (Romero, 2004; Touma and Palme, 2005). An assay was classified as able to reliably quantify fGCMs in a species if a 100% increase in fGCM concentrations was detected from both biological and physiological validation experiments in the female and male (Heistermann et al., 2006; Sheriff et al., 2009; Dantzer et al., 2014). An EIA was classified as potentially reliable when a respective increase in fGCM concentrations was found from either biological or physiological validation in both the female and male. When such an increase was only detected in one sex, the EIA was said to be potentially reliable for the particular sex of that species.

## 2.6 Ethics statement

This study was approved by the Natural and Agricultural Sciences Faculty Animal Ethics Committee of the University of Pretoria, Pretoria, South Africa (Ethics clearance number EC044-18) and complied with Section 20 of the Animal Diseases Act of South Africa, 1984 (SDAH-Epi-18111309000).

## 3 Results

In terms of absolute concentrations, the overall peak values of fGCMs varied considerably, with the highest and lowest being measured by the  $5\alpha$ -pregnane- $3\beta$ , $11\beta$ , $21$ -triol- $20$ -one (peak value range: 0.5 – 13.5  $\mu\text{g}\cdot\text{g}^{-1}$  DW) and Cortisol (peak value range: 0.02 – 0.29  $\mu\text{g}\cdot\text{g}^{-1}$  DW) EIAs, respectively (Table 2). The remaining assays also showed variable peak value ranges: Oxoetiocholanolone I (0.02 – 0.34  $\mu\text{g}\cdot\text{g}^{-1}$  DW); Oxoetiocholanolone II (0.26 – 2.92  $\mu\text{g}\cdot\text{g}^{-1}$  DW); and Corticosterone (0.42 – 2.73  $\mu\text{g}\cdot\text{g}^{-1}$  DW). The timing of peak values obtained for each assay also differed between species and sexes across the two validation methods: ranging from 7–24 h post-biological stimulation and 1–23 h post-physiological stimulation.

The small sample size used in the study prevented any statistical analyses, but we noted an increase in fGCM concentrations across all animals following biological or physiological stimulation and a decrease after habituation from the stimulant. Assays that were able to detect a 100% or greater increase between baseline and peak fGCM concentrations for a particular species and sex were seen as reliable for that group (Heistermann et al., 2006).

The five investigated assays detected a greater than 100% increase in the six species with varying levels of success (Table 2). The  $5\alpha$ -pregnane- $3\beta$ , $11\beta$ , $21$ -triol- $20$ -one EIA was able to reliably detect fGCM concentrations in three murid species (*A. ineptus* females, *M. namaquensis* females, *O. angoniensis* females and males) and *S. pratensis* (females and males). The Oxoetiocholanolone II EIA detected fGCM concentrations in female and male *O. angoniensis*, *M. namaquensis* females, and *S. pratensis* females. While the Cortisol EIA was able to detect reliable increases in fGCM concentrations of *L. rosalia* females, *M. namaquensis* females, and *S. pratensis* males, those fGCMs were detected in trace concentrations across species. The Oxoetiocholanolone I and Corticosterone EIAs were only able to reliably detect fGCM increases in *S. pratensis* males. Longitudinal profiles of immunoreactive baseline and peak fGCM concentrations are shown in Supplementary Figure 1 for each species.

## 4 Discussion

The study compared the ability of five EIAs to reliably detect variation in fGCM concentrations of six southern African rodent species, five from the Muridae family (*A. ineptus*, *L. rosalia*, *M.*

TABLE 2 Summary of biological and physiological validation baseline and peak fGCM concentrations, percentage increases, and the hours post-injection that a physiological peak was detected per enzyme immunoassay denoting suitability for six Rodentia species in the Magaliesberg Biosphere, North West province, South Africa after experiencing a potential biological stressor (handling and temporary removal from enclosure), and a physiological stressor (ACTH administration) for each of the five enzyme immunoassays tested: 5 $\alpha$ -pregnane-3 $\beta$ ,11 $\beta$ ,21-triol-20-one (5 $\alpha$ ); Oxoetiocholanolone I (Oxo I); Oxoetiocholanolone II (Oxo II); Corticosterone (CC); Cortisol (C).

Species	Sex	EIA	Baseline ( $\mu\text{g}\cdot\text{g}^{-1}$ DW)	Biological validation			Physiological validation		
				Peak ( $\mu\text{g}\cdot\text{g}^{-1}$ DW)	% increase	Hours post-handling	Peak ( $\mu\text{g}\cdot\text{g}^{-1}$ DW)	% increase	Hours post-injection
<i>Aethomys ineptus</i>	♀	5 $\alpha$	4.61	9.81	<b>113</b>	11	10.90	<b>136</b>	5
		Oxo I	0.05	0.16	<b>202</b>	24	0.11	99	5
		Oxo II	0.76	1.06	40	19	1.26	65	5
		CC	1.49	2.73	83	24	2.29	54	5
		C	0.11	0.29	<b>158</b>	24	0.11	98	5
	♂	5 $\alpha$	3.31	5.23	58	11	8.52	<b>158</b>	17
		Oxo I	0.13	0.17	33	24	0.34	<b>168</b>	17
		Oxo II	0.89	1.41	58	17	2.60	<b>193</b>	17
		CC	0.85	0.98	15	17	1.54	81	17
		C	0.05	0.07	29	17	0.08	63	17
<i>Lemniscomys rosalia</i>	♀	5 $\alpha$	1.12	1.68	50	24	1.52	35	20
		Oxo I	0.01	0.04	<b>350</b>	24	0.04	<b>312</b>	20
		Oxo II	0.30	0.50	68	24	0.51	69	20
		CC	0.38	0.68	78	17	0.66	71	8
		C	0.04	0.12	<b>186</b>	24	0.09	<b>113</b>	8
	♂	5 $\alpha$	0.49	1.34	<b>174</b>	8	0.51	5	4
		Oxo I	0.02	0.04	<b>113</b>	8	0.03	74	23
		Oxo II	0.23	0.36	59	11	0.26	13	8
		CC	0.28	0.50	77	8	0.42	48	4
		C	0.01	0.02	28	11	0.10	<b>727</b>	7
<i>Mastomys coucha</i>	♂	5 $\alpha$	3.43	13.49	<b>293</b>	11	6.14	79	10
		Oxo I	0.02	0.03	88	8	0.02	51	10
		Oxo II	0.44	0.90	<b>105</b>	8	0.73	66	10
		CC	0.80	2.51	<b>216</b>	11	1.49	87	10
		C	0.05	0.13	<b>143</b>	11	0.09	63	10
	♂	5 $\alpha$	3.54	5.09	44	11	4.96	40	5
		Oxo I	0.03	0.05	44	8	0.04	10	5

(Continued)

TABLE 2 Continued

Species	Sex	EIA	Baseline ( $\mu\text{g}\cdot\text{g}^{-1}$ DW)	Biological validation			Physiological validation			
				Peak ( $\mu\text{g}\cdot\text{g}^{-1}$ DW)	% increase	Hours post-handling	Peak ( $\mu\text{g}\cdot\text{g}^{-1}$ DW)	% increase	Hours post-injection	
<i>Micaelamys namaquensis</i>		Oxo II	0.39	0.70	80	11	0.72	85	5	
		CC	1.15	1.50	30	11	1.66	44	5	
		C	0.07	0.08	17	8	0.09	36	5	
	♀	5 $\alpha$	1.91	5.05	<b>164</b>	8	4.84	<b>153</b>	5	
		Oxo I	0.01	0.02	64	8	0.02	98	5	
		Oxo II	0.49	0.84	71	8	0.60	22	5	
		CC	1.21	1.84	51	8	1.93	59	5	
		C	0.07	0.11	54	8	0.13	84	5	
		♀	5 $\alpha$	2.18	4.70	<b>115</b>	24	5.22	<b>139</b>	3
			Oxo I	0.01	0.02	22	8	0.02	19	5
			Oxo II	0.40	1.08	<b>173</b>	24	0.79	<b>100</b>	3
			CC	1.38	2.42	76	8	1.55	12	8
			C	0.07	0.20	<b>194</b>	24	0.16	<b>131</b>	7
	<i>Otomys angoniensis</i>	♀	5 $\alpha$	2.46	5.30	<b>115</b>	24	5.21	<b>112</b>	1
			Oxo I	0.05	0.32	<b>533</b>	8	0.08	151	1
Oxo II			0.76	2.40	<b>217</b>	24	1.88	<b>149</b>	1	
CC			0.69	1.41	<b>103</b>	24	1.03	49	1	
C			0.07	0.24	<b>262</b>	8	0.11	62	1	
♂		5 $\alpha$	1.55	6.16	<b>298</b>	11	3.38	<b>118</b>	5	
		Oxo I	0.03	0.09	<b>147</b>	8	0.03	93	3	
		Oxo II	0.89	2.42	<b>172</b>	11	1.94	<b>118</b>	2	
		CC	0.97	1.58	63	11	1.09	12	3	
		C	0.07	0.29	<b>309</b>	24	0.14	96	3	
<i>Steatomys pratensis</i>	♀	5 $\alpha$	2.06	4.92	<b>139</b>	11	4.98	<b>142</b>	9	
		Oxo I	0.06	0.06	99	8	0.06	3	9	
		Oxo II	0.98	2.07	<b>111</b>	11	2.92	<b>197</b>	9	
		CC	0.51	0.72	42	8	1.10	<b>116</b>	9	
		C	0.06	0.09	36	11	0.13	<b>106</b>	9	
	♂	5 $\alpha$	1.73	5.16	<b>197</b>	8	3.89	<b>124</b>	12	

(Continued)

TABLE 2 Continued

Species	Sex	EIA	Baseline ( $\mu\text{g}\cdot\text{g}^{-1}$ DW)			Biological validation			Physiological validation		
			Peak ( $\mu\text{g}\cdot\text{g}^{-1}$ DW)	% increase	Hours post-handling	Peak ( $\mu\text{g}\cdot\text{g}^{-1}$ DW)	% increase	Hours post-injection	Peak ( $\mu\text{g}\cdot\text{g}^{-1}$ DW)	% increase	Hours post-injection
		Oxo I	0.05	<b>616</b>	8	0.34	616	8	0.11	139	12
		Oxo II	0.85	42	8	1.21	42	8	1.58	86	12
		CC	0.44	<b>206</b>	8	1.33	206	8	0.98	124	9
		C	0.06	<b>121</b>	8	0.13	121	8	0.26	343	12

Bold values indicate a 100% increase between fGCM concentrations pre- and post- stressor for biological and physiological validation.

*coucha*, *M. namaquensis*, *O. angoniensis*) and one from the Nesomyidae family (*S. pratensis*), in response to adrenocortical stimulation through biological and physiological means. Based on the limited sample size, three EIAs were able to reliably detect greater than 100% increases in fGCM concentrations in more than one species (the  $5\alpha$ -pregnane- $3\beta,11\beta,21$ -triol-20-one, the Oxoetiocholanolone II, and Corticosterone).

In each of the six species studied, elevated fGCM concentrations were detected in each of the 12 individuals by at least two of the five assays to varying extents within a concentration range and response time comparable to other studies on vertebrates (Palme et al., 2005; Touma and Palme, 2005; Heistermann et al., 2006). There was, however, no single EIA identified that was applicable to all species in the study, but rather assay reliability for quantifying adrenocortical activity varied among species. The EIAs were able to quantify a 100% increase between baseline and peak values obtained from biological and physiological validation of certain species: the  $5\alpha$ -pregnane- $3\beta,11\beta,21$ -triol-20-one EIA (female *A. ineptus*, female *M. namaquensis*, female and male *O. angoniensis*, and female and male *S. pratensis*); the Oxoetiocholanolone II EIA (female and male *O. angoniensis*, female *M. namaquensis*, and female *S. pratensis*); the Oxoetiocholanolone I and Corticosterone EIAs (male *S. pratensis*); and the Cortisol EIA (female *L. rosalia*, female *M. namaquensis*, and male *S. pratensis*). Several studies have shown that some vertebrates including mammals and birds excrete Cortisol in relatively trace amounts in their feces (Palme, 2005; Touma and Palme, 2005; Heistermann et al., 2006). Our study supports these findings as the Oxoetiocholanolone I and Cortisol EIAs were only able to detect fGCM concentrations in trace amounts ( $0.02 - 0.34 \mu\text{g}\cdot\text{g}^{-1}$  DW and  $0.02 - 0.29 \mu\text{g}\cdot\text{g}^{-1}$  DW, respectively). Despite the detection of overall trace amounts, some differences were observed across species and sexes, with the female *A. ineptus* and male *S. pratensis* showing measurable peak fGCM concentrations following biological and physiological validation, respectively. Overall, the Oxoetiocholanolone I and Cortisol EIA appear to be unsuitable assays for fGCM quantification in the southern African rodents investigated, as concentrations were below detectable limits (Palme and Möstl, 1997; Goncalves et al., 2016; Fanson et al., 2017). Heistermann et al. (2006) reported that the variability in assay reliability within closely related taxonomic groups is a vital reminder that assay effectiveness should not be inferred, but rather tested in case of inter- and intra-specific variation in measuring differences in adrenocortical activity (Brown et al., 1994; Touma and Palme, 2005).

Mammalian GCs excreted from feces and urine can differ between species and between sexes (Touma et al., 2003; Palme et al., 2005; Sheriff et al., 2011). In terms of intraspecific sex-related variation in assay suitability, Hill et al. (2015) reported that rodents exhibit alternative reproductive tactics (ARTs) influenced by their varied living strategies (communal, returning, and solitary, among others). Some are communal breeders who give birth in shared nests and care for their young, some individuals leave the nest to give birth and return, while others leave the nest to give birth and never return (Merritt, 2010; Schradin et al., 2013; Hill et al., 2015). These tactics can affect endocrine control and steroid hormone concentrations in adult individuals.

The relatively high fGCM concentrations seen in the females of the communal-living species compared to males may be explained by the stress associated with group-living social dynamics (Hill et al., 2015). Van Kesteren et al. (2012) and Schradin et al. (2013) showed that communal living leads to physiological stress in reproductive individuals. Hill et al. (2015) reported that females of solitary-living species have comparatively lower GC levels, as living singly does not require as much cooperative behavior, which can be perceived as stressful. Although individuals were housed singly, enclosures were grouped by species, and animals could see and smell females and males of the different species. Overall, communal living species (*A. ineptus*, *L. rosalia*, and *M. coucha*) showed lower percentage increases between baseline and peak fGCM concentrations, as they may be used to large group interactions or may have already been impacted by the experimental isolation. Exceptions were the females of *A. ineptus* and *M. namaquensis*, possibly due to the presence of gestating females and pups of their species at the research facility (Van Kesteren et al., 2012; Schradin et al., 2013).

The single living species, *O. angoniensis* and *S. pratensis* (> 100% increase detection by all five EIAs), had higher percentage-increase detectability across the different EIAs, likely due to their solitary ecology, and the uncharacteristic group living at the research facility. Conversely, the two species with the lowest percentage-increase detection rates were *L. rosalia* and *M. coucha*, who are communal, synanthropic and reported to persist in transformed landscapes (Skinner and Chimimba, 2005). These species may have adapted to anthropogenic landscapes and increased human encroachment by maintaining elevated fGCM concentrations to aid with predator evasion and overall survival, as seen in red-backed voles (*Clethrionomys gapperi*) with consistent fGCM concentrations post-stressor (Harper and Austad, 2001; 2012). As the variability within and between species and the physiological and behavioral hormone-linked differences between related taxonomic groups were seen in a small sample size, it is necessary to investigate larger sample sizes and further validate the results.

There is interspecific variation in fGCM concentrations and responses to experimental protocols such as handling and injection during ACTH tests, which further emphasizes the need for assay validation before fGCM quantification. To understand this variation and potentially improve our ability to extrapolate assay reliability in large taxonomic groups, future research should also investigate GC composition and metabolism as a function of phylogenetic relatedness (Goncalves et al., 2016). Such research should include the characterization of GC metabolite compositions associated with the study species and the determination of gut passage time in said species. This information may improve the accuracy of *a priori* hormone assay selection because some assays can be eliminated from validation experiments if their associated hormone structures are not found in a species. Determining the gut passage time of different species may also improve our understanding of GC metabolism and fecal excretion. A longer gut passage time may indicate prolonged digestion and, potentially, increased metabolic activity of some hormones and not others. This

may explain the trace amounts of hormones detected by some EIAs in this study, whereas others were present in greater concentrations. Additionally, spatial and temporal niches should be considered when conducting future research on adrenocortical activity in communal versus solitary, or diurnal versus nocturnal species because circadian rhythms and activity patterns may affect hormone activity in different species (MacDougall-Shackleton et al., 2019).

It is important to note that variability in fGCM concentrations across species may also be linked to capture and handling stress (Hing et al., 2014). Initial capture from study sites and transportation to the research site, capture and handling during biological and physiological experiments, and daily collection of fecal samples may influence HPA axis activity and potentially mask the responses from the validation experiments. As we are unable to isolate and identify the effects of specific stressors in southern African rodents, further research with larger sample sizes is required to better understand their responses to different stressors and improve our experimental protocol, minimizing researcher impact on hormone activity data collection in future.

Based on the research question, future studies that use EIAs to quantify average population hormone concentrations in free-ranging southern African rodents should standardize data collection methodology and incorporate environmental controls for seasonality, land-use type, proximity to humans, species ecology and life history traits, among others. Our study relied on experimental perceived stressors, whereas field-based ecological studies are likely to include different variables that influence rodent physiology and hormone responses.

Established EIAs can aid in quantifying fGCM concentrations of focal species and provide a summary of the perceived stressors that have activated the HPA axis of an individual over a period that is dependent on the gut passage time of the animal (Touma and Palme, 2005). It is not possible to attribute specific perceived stress events to measured hormone concentration values, and since we are unable to distinguish between an animal's response to anthropogenic versus other extrinsic and intrinsic stimuli, it is important to standardize research protocols to ensure that observed differences between individuals are primarily from specific experimental variables (Vitousek et al., 2019). While it is not possible to link potential stressors to their hormone signals, we can compare average population hormone concentrations of focal species across different landscapes to identify overall variability (Dantzer et al., 2014). To reduce ecological noise, we can design experimental protocols that control for the season, time of day, the focal species, their sex and age, and individual immune responses, among others (Touma et al., 2003; Touma and Palme, 2005).

Conclusions drawn from the results are based on small sample sizes and should be interpreted as such (Bissonette, 1999). This species-specific variation in assay suitability within seemingly closely related rodent species highlights the importance of EIA validation before adrenocortical activity can be quantified in a species (Ganswindt et al., 2003; Millsaugh and Washburn, 2004). The 5 $\alpha$ -pregnane-3 $\beta$ ,11 $\beta$ ,21-triol-20-one EIA can be used to monitor stress-related fGCM concentrations in selected southern

African rodent species, with the potential for use in other as yet untested species. A tested and reliable common EIA can standardize future research so that intraspecific comparisons can be made across different studies of the same species. This can have various implications for small mammal research because researchers conducting captive studies can reliably quantify the impact of their experiments on their study animals and, where necessary, adjust their protocols to improve their ethical compliance. Importantly, these results also indicate that the number of EIAs to be tested for a specific species can be limited, which is particularly valuable in the Global South, where research funding is often scarce.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The animal study was approved by the Natural and Agricultural Sciences Faculty Animal Ethics Committee of the University of Pretoria, Pretoria, South Africa. The study was conducted in accordance with the local legislation and institutional requirements.

## Author contributions

MR: Methodology, Writing – original draft, Conceptualization, Formal Analysis, Data curation, Writing – review & editing, Investigation. MS: Investigation, Writing – review & editing, Funding acquisition, Resources, Supervision. DH: Methodology, Investigation, Writing – review & editing, Conceptualization. AG: Resources, Writing – review & editing, Investigation, Formal Analysis, Methodology, Supervision, Conceptualization.

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## References

Angelier, F., and Wingfield, J. C. (2012). Importance of the glucocorticoid stress response in a changing world: theory, hypotheses and perspectives. *Gen. Comp. Endocrinol.* 190, 118–128. doi: 10.1016/j.ygcen.2013.05.022

Aplin, K. P., Brown, P. R., Jacob, J., Krebs, C. J., and Singleton, G. R. (2003). *Field methods for rodent studies in Asia and the Indo-Pacific. 1st ed* (Canberra, Australia: CSIRO Publishing).

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## Conflict of interest

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcosc.2026.1738537/full#supplementary-material>

Aplin, K. P., Suzuki, H., Chinen, A. A., Chesser, R. T., ten Have, J., Donnellan, S. C., et al. (2011). Multiple geographic origins of commensalism and complex dispersal history of black rats. *PLoS One* 6, e26357. doi: 10.1371/journal.pone.0026357

(2021). Magaliesburg climate (South Africa). Available online at: <https://en.climate-data.org/africa/south-africa/gauteng/magaliesburg-29792/> (Accessed February 2, 2026).

- Avenant, N. L., and Cavallini, (2007). Correlating rodent community structure with ecological integrity, Tussen-die-Riviere Nature Reserve, Free State Province, South Africa. *Integr. Zoology* 2, 212–219. doi: 10.1111/j.1749-4877.2007.00064.x
- Avenant, N. L., Watson, J., and Schulze, E. (2008). Correlating small mammal community characteristics and habitat integrity in the Caledon Nature Reserve, South Africa. *Mammalia* 72, 186–191. doi: 10.1515/MAMM.2008.023
- Bayazit, V. (2009). Evaluation of cortisol and stress in captive animals. *Aust. J. Basic Appl. Sci.* 3, 1022–1031. Available online at: <http://www.ajbasweb.com/old/ajbas/2009/1022-1031.pdf>
- Bissonette, J. A. (1999). Small sample size problems in wildlife ecology: a contingent analytical approach. *Wildlife Biol.* 5, 65–71. doi: 10.2981/wlb.1999.010
- Boonzaaier, W., and Hough, I. (2011). Magaliesberg Biosphere proposed management plan. *Brits.* 1–119.
- Brown, J. L., Bellem, A. C., Fouraker, M., Wildt, D. E., and Roth, T. L. (2001). Comparative analysis of gonadal and adrenal activity in the black and white rhinoceros in North America by noninvasive endocrine monitoring. *Zoo Biol.* 20, 463–486. doi: 10.1002/zoo.10028
- Brown, J. L., Schmitt, D. L., Bellem, A., Graham, L. H., and Lehnhardt, J. (1999). Hormone secretion in the asian elephant (*Elephas maximus*): characterization of ovulatory and anovulatory luteinizing hormone surges. *Biol. Reprod.* 61, 1294–1299. doi: 10.1095/biolreprod61.5.1294
- Brown, J., Wasser, S., Wildt, D., and Graham, L. (1994). Comparative aspects of steroid hormone metabolism and ovarian activity in felids, measured noninvasively in feces. *Biol. Reprod.* 51, 776–786. doi: 10.1095/biolreprod51.4.776
- Cadotte, M. W., Arnillas, C. A., Livingstone, S. W., and Yasui, S. L. E. (2015). Predicting communities from functional traits. *Trends Ecol. Evol.* 30, 510–511. doi: 10.1016/j.tree.2015.07.001
- Canova, L., and Fasola, M. (1993). Food habits and trophic relationships of small mammals in six habitats of the northern po plain (Italy). *Mammalia* 57, 189–200. doi: 10.1515/mamm.1993.57.2.189
- Christie, A. P., Amano, T., Martin, P. A., Shackelford, G. E., Simmons, B. I., and Sutherland, W. J. (2019). Simple study designs in ecology produce inaccurate estimates of biodiversity responses. *J. Appl. Ecol.* 56, 2742–2754. doi: 10.1111/1365-2664.13499
- Colles, A., Liow, L. H., and Prinzing, A. (2009). Are specialists at risk under environmental change? Neocological, paleoecological and phylogenetic approaches. *Ecol. Lett.* 12, 849–863. doi: 10.1111/j.1461-0248.2009.01336.x
- Dantzer, B., Fletcher, Q. E., Boonstra, R., and Sheriff, M. J. (2014). Stress in vertebrates measures of physiological stress: a transparent or opaque window into the status, management and conservation of species? *Conserv. Physiol.* 2, 1–18. doi: 10.1093/conphys/cou023
- Dickman, C. R. (1999). "Rodent-ecosystem relationships: a review," in *Ecologically-based rodent management*. Eds. G. R. Singleton, L. A. Hinds, H. Leirs and Z. Zhang (Australian Centre for International Agricultural Research, Canberra, Australia), 113–133. doi: 10.1016/S0140-6736(00)88507-4
- Dobson, H., and Smith, R. F. (2000). What is stress, and how does it affect reproduction? *Anim. Reprod. Sci.* 60, 743–752. doi: 10.1016/S0378-4320(00)00080-4
- Fanson, K. V., Best, E. C., Bunce, A., Fanson, B. G., Hogan, L. A., Keeley, T., et al. (2017). One size does not fit all: monitoring faecal glucocorticoid metabolites in marsupials. *Gen. Comp. Endocrinol.* 244, 146–156. doi: 10.1016/j.ygcen.2015.10.011
- Fraňková, M., Palme, R., and Frynta, D. (2012). Family affairs and experimental male replacement affect fecal glucocorticoid metabolites levels in the Egyptian spiny mouse *Acomys cahirinus*. *Zoological Stud.* 51, 277–287.
- Galetti, M., Giacomini, H. C., Bueno, R. S., Bernardo, C. S.S., Marques, R. M., Bovendorp, R. S., et al. (2009). Priority areas for the conservation of Atlantic forest large mammals. *Biol. Conserv.* 142, 1229–1241. doi: 10.1016/j.biocon.2009.01.023
- Ganswindt, A., Palme, R., Heistermann, M., Borragan, S., and Hodges, J. K. (2003). Non-invasive assessment of adrenocortical function in the male African elephant (*Loxodonta africana*) and its relation to musth. *Gen. Comp. Endocrinol.* 134, 156–166. doi: 10.1016/S0016-6480(03)00251-X
- Gilbert, S. B., and Krebs, C. J. (1991). Population dynamics of *Clethrionomys* and *Peromyscus* in Southwestern Yukon 1973–1989. *Holarctic Ecol.* 14, 250–259. doi: 10.1111/j.1600-0587.1991.tb00659.x
- Goncalves, I. B., Heistermann, M., Santema, P., Dantzer, B., Mausbach, J., Ganswindt, A., et al. (2016). Validation of a fecal glucocorticoid assay to assess adrenocortical activity in meerkats using physiological and biological stimuli. *Conserv. Physiol.* 11, 1–22. doi: 10.1371/journal.pone.0153161
- Harper, J. M., and Austad, S. N. (2001). Effect of capture and season on fecal glucocorticoid levels in deer mice (*Peromyscus maniculatus*) and red-backed voles (*Clethrionomys gapperi*). *Gen. Comp. Endocrinol.* 123, 337–344. doi: 10.1006/gen.2001.7682
- Harper, J. M., and Austad, S. N. (2012). Fecal glucocorticoids: a noninvasive method of measuring adrenal activity in wild and captive rodents. *Physiol. Biochem. Zoology* 73, 12–22. doi: 10.1086/316721
- Heistermann, M., Palme, R., and Ganswindt, A. (2006). Comparison of different enzymeimmunoassays for assessment of adrenocortical activity in primates based on fecal analysis. *Am. J. Primatology* 68, 257–273. doi: 10.1002/ajp
- Hill, D. L., Pillay, N., and Schradin, C. (2015). Alternative reproductive tactics in female striped mice: solitary breeders have lower corticosterone levels than communal breeders. *Hormones Behav.* 71, 1–9. doi: 10.1016/j.yhbeh.2015.03.004
- Hilty, J., and Merenlender, A. (2000). Faunal indicator taxa selection for monitoring ecosystem health. *Biol. Conserv.* 92, 185–197. doi: 10.1016/S0006-3207(99)00052-X
- Hing, S., Narayan, E., Thompson, R. C. A., and Godfrey, S. (2014). A review of factors influencing the stress response in Australian marsupials. *Conserv. Physiol.* 2, 1–17. doi: 10.1093/CONPHYS/COU027
- Horváth, A., March, I. J., and Wolf, J. H. D. (2001). Rodent diversity and land use in Montebello, Chiapas, Mexico. *Stud. Neotropical Fauna Environ.* 36, 169–176. doi: 10.1076/snfe.36.3.169.2130
- Hulsman, A., Dalerum, F., Ganswindt, A., Muenscher, S., Bertschinger, H. J., and Paris, M. (2011). Non-invasive monitoring of glucocorticoid metabolites in brown hyaena (*Hyaena brunnea*) feces. *Zoo Biol.* 30, 451–458. doi: 10.1002/zoo.20325
- Irwin, M. T., Wright, P. C., Birkinshaw, C., Fisher, B. L., Gardner, C. J., Glos, J., et al. (2010). Patterns of species change in anthropogenically disturbed forests of Madagascar. *Biol. Conserv.* 143, 2351–2362. doi: 10.1016/j.biocon.2010.01.023
- Jepsen, E. M., Scheun, J., Dehnhard, M., Kumar, V., Umapathy, G., and Ganswindt, A. (2021). Non-invasive monitoring of glucocorticoid metabolite concentrations in native Indian, as well as captive and re-wilded tigers in South Africa. *Gen. Comp. Endocrinol.* 308, 113783. doi: 10.1016/J.YGCEN.2021.113783
- Joyce, P., Reid, H., Barrett, J., and Lubbe, E. (1997). "Rodents," in *The complete book of southern African mammals*. Eds. M. G. L. Mills and L. Hes (Struik Publishers, Cape Town, South Africa), 120–164.
- Kastelic, M., Gregurić Gračner, G., Tomažič, I., Kvapil, P., Harej, M., and Dovč, A. (2023). Comparison of cortisol concentrations in different matrices in alpine ibex (*Capra ibex*) at the zoo. *Animals* 13, 1–15. doi: 10.3390/ani13152491
- Kerley, G. I. H., Knight, M. H., and Erasmus, T. (1990). Small mammal microhabitat use and diet in the southern Kalahari, South Africa. *South Afr. J. Wildlife Res.* 20, 123–126. doi: 10.10520/AJA03794369\_2615
- Laver, P. N., Ganswindt, A., Ganswindt, S. B., and Alexander, K. A. (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. doi: 10.1016/j.ygcen.2012.08.011
- Linzey, A. V., Kesner, M. H., Chimimba, C. T., and Newbery, C. (2003). Distribution of veld rat sibling species *Aethomys chrysophilus* and *Aethomys ineptus* (Rodentia: Muridae) in southern Africa. *Afr. Zoology* 38, 169–174. doi: 10.1080/15627020.2003.11657205
- MacDougall-Shackleton, S. A., Bonier, F., Romero, L. M., and Moore, I. T. (2019). Glucocorticoids and "stress" are not synonymous. *Integr. Organismal Biol.* 13, 1–8. doi: 10.1093/iob/obz017
- Marris, E. (2008). Pre-emptive strike: outwitting extinction. *Natural Climate Change* 1, 140–141. doi: 10.1038/climate.2008.114
- McKee, J. K., Sciuilli, P. W., David Foose, C., and Waite, T. A. (2004). Forecasting global biodiversity threats associated with human population growth. *Biol. Conserv.* 115, 161–164. doi: 10.1016/S0006-3207(03)00099-5
- Merritt, J. F. (2010). *The biology of small mammals* (Maryland, United States: Johns Hopkins University Press).
- Millspaugh, J. J., and Washburn, B. E. (2004). Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. *Gen. Comp. Endocrinol.* 138, 189–199. doi: 10.1016/j.ygcen.2004.07.002
- Monadjem, A., and Perrin, M. (2003). Population fluctuations and community structure of small mammals in a Swaziland grassland over a three-year period. *Afr. Zoology* 38, 127–137. doi: 10.1080/15627020.2003.11657200
- Monadjem, A., Taylor, P. J., Denys, C., and Cotterill, F. P. D. (2015). *Rodents of Sub-Saharan Africa: a biogeographic and taxonomic synthesis* (Berlin, Germany: De Gruyter).
- Möstl, E., Maggs, J. L., Schrötter, G., Besenfelder, U., and Palme, R. (2002). Measurement of cortisol metabolites in faeces of ruminants. *Veterinary Res. Commun.* 26, 127–139. doi: 10.1023/A:1014095618125
- Newbery, C. H. (1999). A key to the Soricidae, Macroscelididae, Gliridae and Muridae of Gauteng, North West Province, Mpumalanga and the Northern Province, South Africa. *Koedoe* 42, 51–55. doi: 10.4102/koedoe.v42i1.221
- Palme, R. (2005). Measuring fecal steroids: guidelines for practical application. *Ann. New York Acad. Sci.* 1046, 75–80. doi: 10.1196/annals.1343.007
- Palme, R., and Möstl, E. (1997). Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. *Int. J. Mamm. Biol.* 62, 192–197.
- Palme, R., Rettenbacher, S., Touma, C., El-Bahr, S. M., and Möstl, E. (2005). Stress hormones in mammals and birds: comparative aspects regarding metabolism,

- excretion, and noninvasive measurement in fecal samples. *Ann. New York Acad. Sci.* 1040, 162–171. doi: 10.1196/annals.1327.021
- Ramahlo, M., Chimimba, C., Pirk, C., and Ganswindt, A. (2019). Non-invasive monitoring of adrenocortical activity in free-ranging Namaqua rock mice *Micaelmys namaquensis* from South Africa in response to anthropogenic land use and season. *Wildlife Biol.* 2019, 1–6. doi: 10.2981/wlb.00544
- Reeder, D. M., and Kramer, K. M. (2005). Stress in free-ranging mammals: integrating physiology, ecology, and natural history. *J. Mammalogy* 86, 225–235. doi: 10.1644/BHE-003.1
- Romero, L. M. (2004). Physiological stress in ecology: lessons from biomedical research. *Trends Ecol. Evol.* 19, 249–255. doi: 10.1016/j.tree.2004.03.008
- Rutherford, M. C., and Mucina, L. (2006). Biomes and bioregions of southern Africa: the vegetation of South Africa, Lesotho and Swaziland. *Strelitzia* 19, 31–51.
- Schradin, C., Kenkel, W., Krackow, S., and Carter, C. S. (2013). Staying put or leaving home: endocrine, neuroendocrine and behavioral consequences in male African striped mice. *Hormones Behav.* 63, 136–143. doi: 10.1016/j.yhbeh.2012.10.004
- Sheriff, M. J., Bosson, C. O., Krebs, C. J., and Boonstra, R. (2009). A non-invasive technique for analyzing fecal cortisol metabolites in snowshoe hares (*Lepus americanus*). *J. Comp. Physiol. B: Biochemical Systemic Environ. Physiol.* 179, 305–313. doi: 10.1007/s00360-008-0314-4
- Sheriff, M. J., Dantzer, B., Delehanty, B., Palme, R., and Boonstra, R. (2011). Measuring stress in wildlife: techniques for quantifying glucocorticoids. *Oecologia* 166, 869–887. doi: 10.1007/s00442-011-1943-y
- Singleton, G. R., Hinds, L. A., Krebs, C. J., and Spratt, D. M. (2003). *Rats, mice and people: rodent biology and management*. Eds. G. R. Singleton, L. A. Hinds, C. J. Krebs and D. M. Spratt (Canberra: Australian Centre for International Agricultural Research).
- Skinner, J., and Chimimba, C. (2005). “Order Rodentia,” in *The Mammals of the Southern African Sub-region, 3rd ed.* Ed. D. van der Horst (Cambridge University Press, Cape Town, South Africa), 77–209. doi: 10.1017/CBO9781107340992
- Touma, C., and Palme, R. (2005). Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. *Ann. New York Acad. Sci.* 1046, 54–74. doi: 10.1196/annals.1343.006
- Touma, C., Palme, R., and Sachser, N. (2004). Analyzing corticosterone metabolites in fecal samples of mice: a noninvasive technique to monitor stress hormones. *Hormones Behav.* 45, 10–22. doi: 10.1016/j.yhbeh.2003.07.002
- Touma, C., Sachser, N., Möstl, E., and Palme, R. (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. doi: 10.1016/S0016-6480(02)00620-2
- Umetsu, F., and Pardini, A. R. (2007). Small mammals in a mosaic of forest remnants and anthropogenic habitats - evaluating matrix quality in an Atlantic forest landscape. *Landscape Urban Plann.* 22, 517–530. doi: 10.1007/s10980-006-9041-y
- Van Kesteren, F., Sillero-Zubiri, C., Millar, R., Argaw, K., Macdonald, D. W., and Paris, M. (2012). Sex, stress and social status: patterns in fecal testosterone and glucocorticoid metabolites in male Ethiopian wolves. *Gen. Comp. Endocrinol.* 179, 30–37. doi: 10.1016/j.yggen.2012.07.016
- Vitousek, M. N., Johnson, M. A., Downs, C. J., Miller, E. T., Martin, L. B., Francis, C. D., et al. (2019). Macroevolutionary patterning in glucocorticoids suggests different selective pressures shape baseline and stress-induced levels. *Am. Nat.* 193, 866–880. doi: 10.1086/703112
- Waters, C. N., Zalasiewicz, J., Summerhayes, C., Barnosky, A. D., Poirier, C., Galuszka, A., et al. (2016). The Anthropocene is functionally and stratigraphically distinct from the Holocene. *Science* 351, 137–148. doi: 10.1126/science.aad2622
- Watson, J. E. M., Whittaker, R. J., and Dawson, T. (2004). Habitat structure and proximity to forest edge affect the abundance and distribution of forest-dependent birds in tropical coastal forests of southeastern Madagascar. *Biol. Conserv.* 120, 315–331. doi: 10.1016/j.biocon.2004.03.004
- Weibull, A.-C., Östman, Ö., and Granqvist, A. (2003). Species richness in agroecosystems: the effect of landscape, habitat and farm management. *Biodiversity Conserv.* 12, 1335–1355. doi: 10.1023/A:1023617117780
- World Wildlife Fund (2016). *Living planet report 2016: risk and resilience in a new earth* (Switzerland: Gland).