# Poo's potential

## Linking land use practice to stress-related hormone levels in leopards (*Panthera pardus*)


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## Introduction

The leopard's versatility as a generalist predator facilitates occupation of a wide range of habitats [4]. Leopards may therefore be exposed to different environmental, physiological, and psycho-social stressors depending on where they happen to be at a particular point in time.

So far, we are not equipped with the tools to determine how leopards cope with different perceived stressors, what the potentially harmful effects of chronic stress may be, or how rapid changes caused by increasing urbanisation and changes in land use may affect this iconic African species. In terms of monitoring adrenocortical activity, a non-invasive approach would be beneficial as animals are not disturbed during sample collection and thus sampling in this manner is both safe and feedback free [1,2].

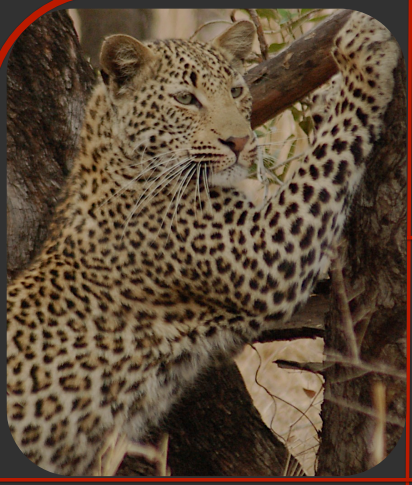
## Objectives

1. Establish a reliable test system to determine glucocorticoid metabolite levels from leopard faeces.
2. Comparing faecal glucocorticoid metabolite (fGCM) levels of free-ranging leopards occupying different land use types.

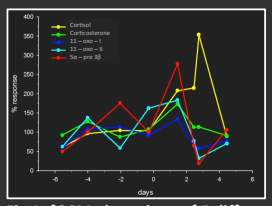
## Methods

i. Determine stress-related physiological responses in leopard faeces by conducting an ACTH (adrenocorticotropic hormone) challenge test on captive individuals (1 adult male & 1 adult female) housed at Predator World, Pilanesburg.
ii. Measure gastrointestinal transit time to link hormonal events in the blood to the time it takes the respective signals to appear in the faeces [5].
iii. Determine the stability of fGCMs post-defecation and monitor changes in sample morphology to develop indicators for ageing samples in the field [3].
iv. Collect samples from free-ranging leopard in a protected (Mala Mala Game Reserve, Mpumalanga) and unprotected peril-urban (Hoedspruit Wildlife Estate, Limpopo) areas.
v. All faecal material was collected according to accepted methods and frozen at -20°C [1,2,5]
vi. Extraction and analysis of faecal steroid hormones will take place at the Endocrine Research Laboratory, University of Pretoria according to Ganswindt et al. 2002 [1].

## Results



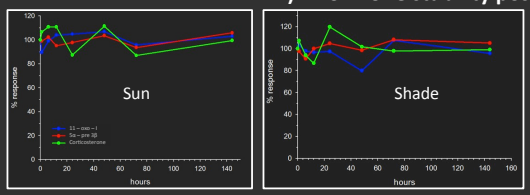
### i) - ACTH challenge test




- 5 different enzyme-immunoassays (EIA) were evaluated:
  a) cortisol (detecting fGCMs with a 11,17,21-trihydroxy-20-one structure)
  b) corticosterone (detecting fGCMs with a 11β,21-diol-3,20-dione)
  c) 11-oxoetiocholanolone I (detecting 11,17 dioxoandrostanes; 11,17-DOA)
  d) 11-oxoetiocholanalone II (detecting fGCMs with a 5β-3α-ol-11-one structure; 3α,11-oxo-CM),
  e) 5α-pregnane-3β,11β,21-triol-20-one (measuring 3β,11β-diol-CM),
- Three of the five EIA's, (b), (c) and (e) were identified as suitable

**Fig 1. fGCM alterations of 5 different enzyme-immunoassays following ACTH administration in one male leopard**

### iii) - Hormone stability post-defecation




- The 11,17-DOA, 5α-pregnane-3β,11β,21-triol-20-one, and corticosterone EIA seem suitable for determining fGCM concentrations in up to 45h old faecal material
- 5α-pregnane-3β,11β,21-triol-20-one EIA showing least variation

**Fig 2. Relative change in fGCM concentration post-defecation for sun (left) and shade (right) conditions**

### ii) - Gastrointestinal transit time

- Determined GIT ranged between 10h – 41h for the male and 8h – 45h for the female
- In both sexes availability, quality and quantity of food appears to influence the lag time

### iii) - Morphological characteristics


- Faeces exposed to shade and sun conditions show little change in colour over time
- Faeces kept in shade conditions did not retain their moisture content unless protected from wind

**Fig 3. Changes in visual appearance of leopard faeces exposed to full shade (top) and full sun (bottom) conditions**

## Conclusions

- ✓ **A reliable non-invasive method for assessing adrenocortical function in leopards based on fGCM analysis has been established**
- ✓ **GIT is highly variable in leopards and is influenced by food availability and quantity**
- ✓ **Post-defecation fGCM concentrations remain sufficiently stable for up to 6 days**

## Outlook

- With a solid foundation in place and sample collection from leopards in different areas in progress, a comparison of stress-related hormone levels from free-ranging leopards occupying an array of land use types will soon be possible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