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# Developing a non-invasive field method for monitoring thyroid hormone concentrations in black rhinoceros, Grevy's zebra and African elephant

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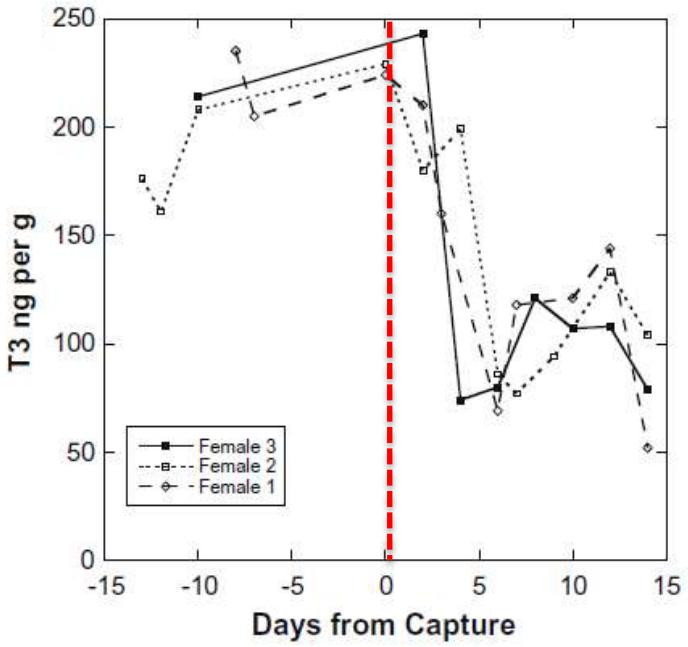
# Introduction

## Background

- Thyroid hormones (T3 and T4) are involved in a number of important physiological processes
- Thyroid hormones have been shown to decrease in response to diminished food intake[1,2]
- May explain why some populations in situ perform better than others



1. Eales, J.G. (1988) *American Zoologist* 28, 351-362
2. Wasser, S.K. et al. (2010) *General and Comparative Endocrinology* 168, 1-7


Hawkins, E. et al (2015). *Journal of Wildlife Diseases* 51

# Introduction

## Why use poo?

- Non-invasive
- Easy to collect
- Broad time frame

## Poo and thyroid hormones

- In order to get the thyroid hormones out of the faeces it must first be extracted
- The concentration of hormone is measured using a total T3 enzyme immunoassay (EIA)

# Extraction process

- Faecal samples are collected as part of standard husbandry routine
- Stored at -20$^{o}$C

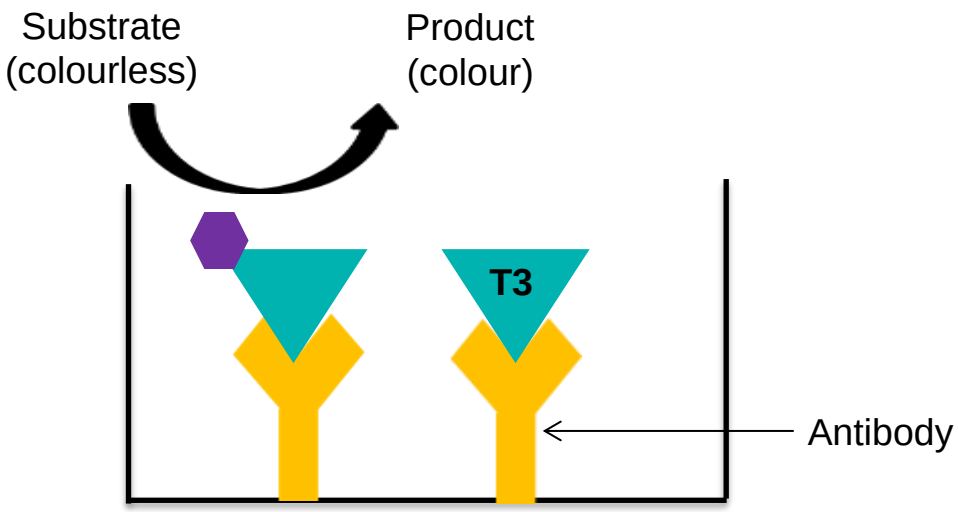
Laboratory method:

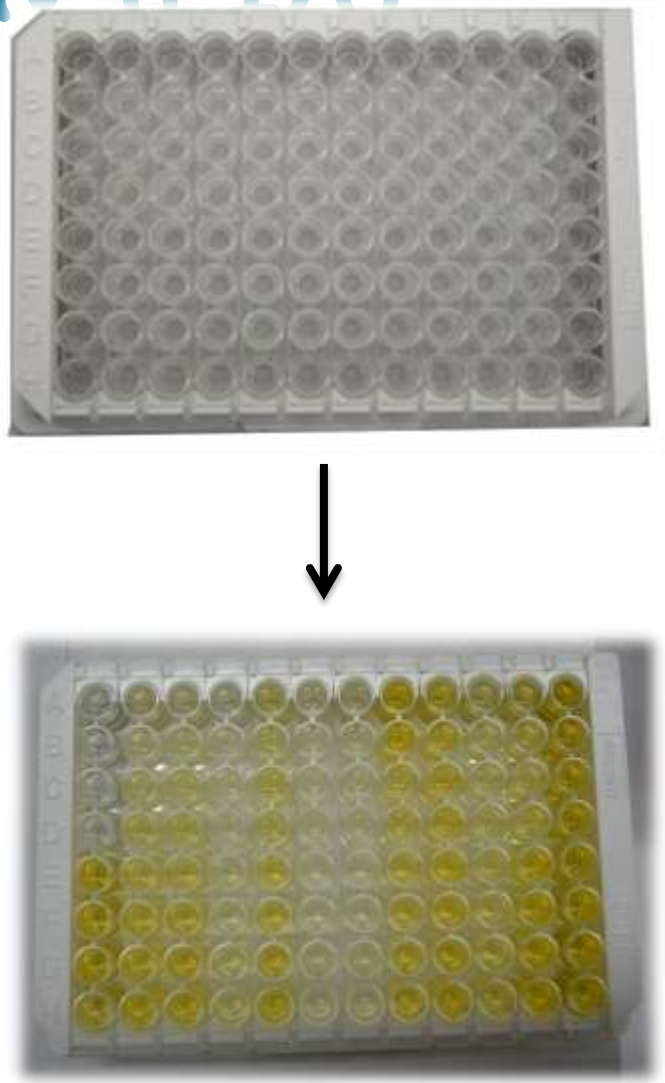
**0.5g faeces + 5ml 90% methanol → spun → dried → re-suspended 1ml 100% methanol**

# Introduction

## Enzyme Immunoassay (EIA)

- Used to determine the concentration of T3 hormone in our samples



# Introduction

## The problems with poo

- The current lab method requires samples to be stored at -20$^{o}$C
- BUT may not have access to electricity/equipment in the field
- Cartridges have successfully been used to store faecal samples for analysis of reproductive and stress hormones[1]

Field extraction method:

**0.5g faeces + 90% methanol → diluted and loaded onto cartridge → eluted with 5ml 100% methanol → dried and re-suspended**

1. Edwards, K.L. et al. (2014). *Conservation Physiology* 2

# Aims & Hypotheses

The main aims of the study were:

- to develop a non-invasive field method for extracting and storing faecal samples for analysis of thyroid (T3) hormone
- to validate the use of a total T3 EIA for measuring T3 in the faeces of megaherbivores (black rhinoceros, Grevy's zebra, African elephant)
- to produce results that are comparable with the standardised laboratory method

It was hypothesised that:

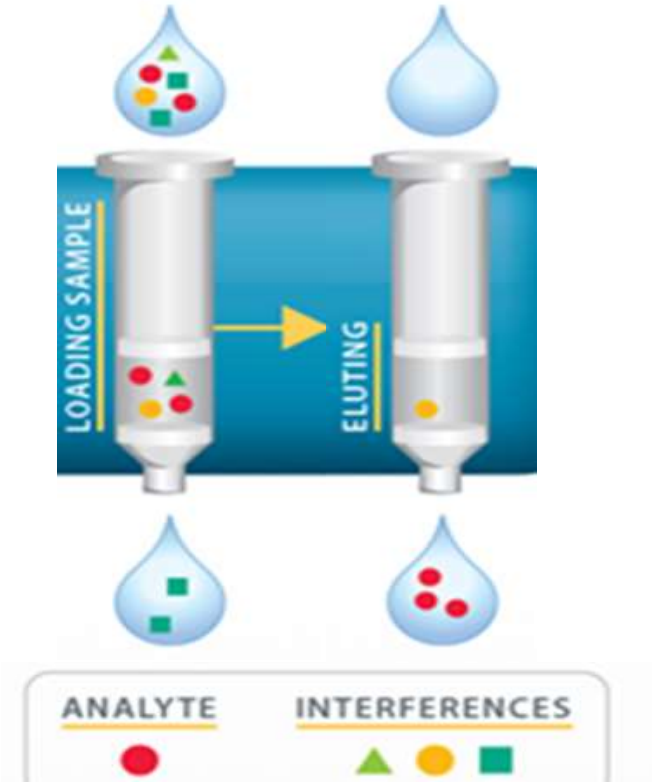
1. T3 recovery will be observed at >40% methanol solution
2. T3 recovery would be improved by using acid to elute and/or plastic tubes to dry samples
3. Lab extracted and field extracted samples will be comparable

# Methods – Hypothesis 1

## At what solvent concentration does T3 elute from the column?

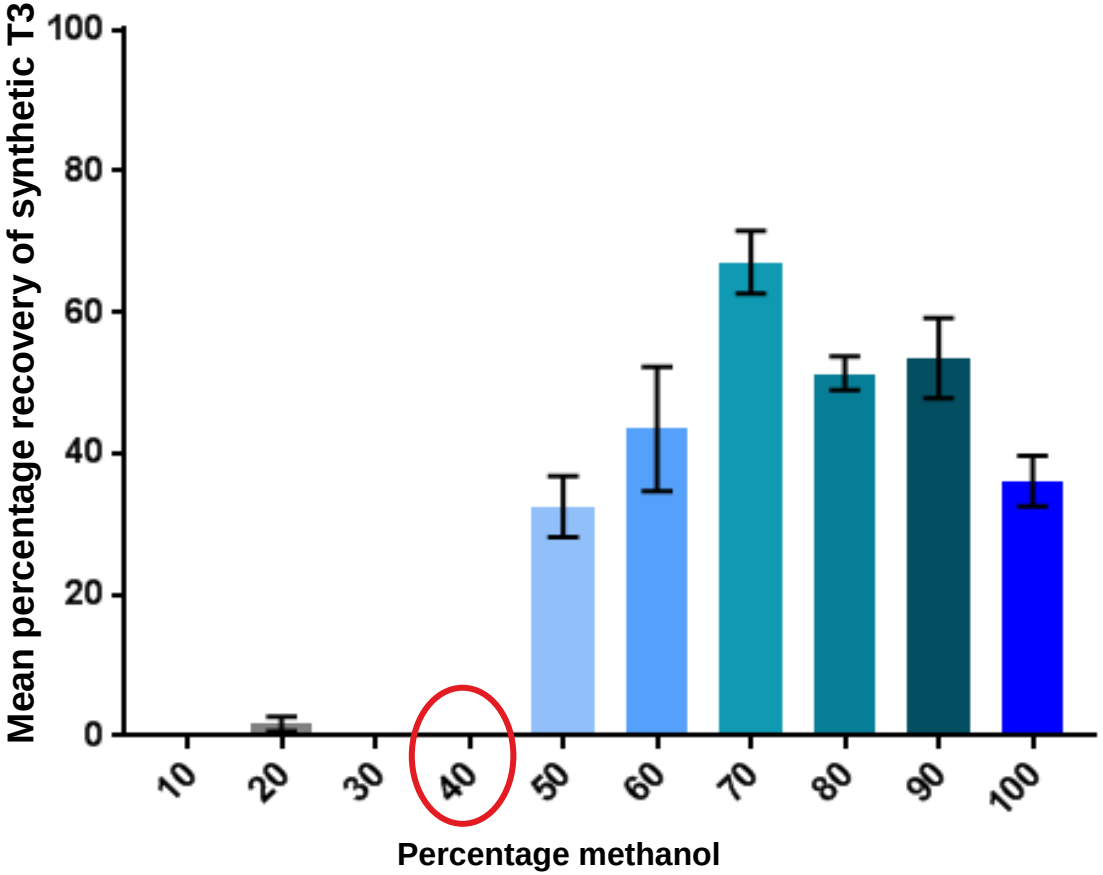
- Need hormone to stick to cartridge

1. A known concentration of synthetic T3 hormone was loaded onto cartridges
2. Cartridges were eluted with increasing concentrations of methanol (10%-100%)
3. Percentage recovery of hormone was measured using a total T3 enzyme immunoassay



# Results - Hypothesis 1

## Optimal loading concentration of solven+

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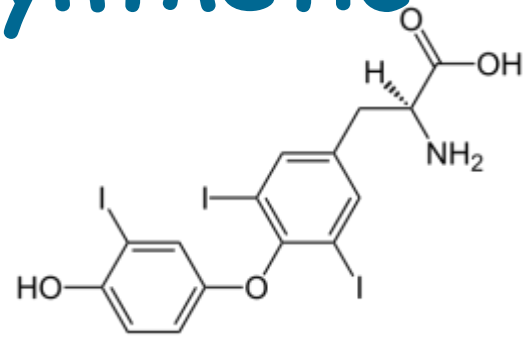
# Methods – Hypothesis 2

## Improving recovery of synthetic T3 hormone




- Low recovery of synthetic T3 was observed (~65%)
- Possibly due to T3 molecules interacting with silanol groups in the glassware used and/or in the cartridges

- A known concentration of synthetic T3 was loaded onto cartridges
- Cartridges were eluted with increasing concentrations of methanol (10%-100%)
- Liquid was evaporated off in either glass tubes or plastic tubes

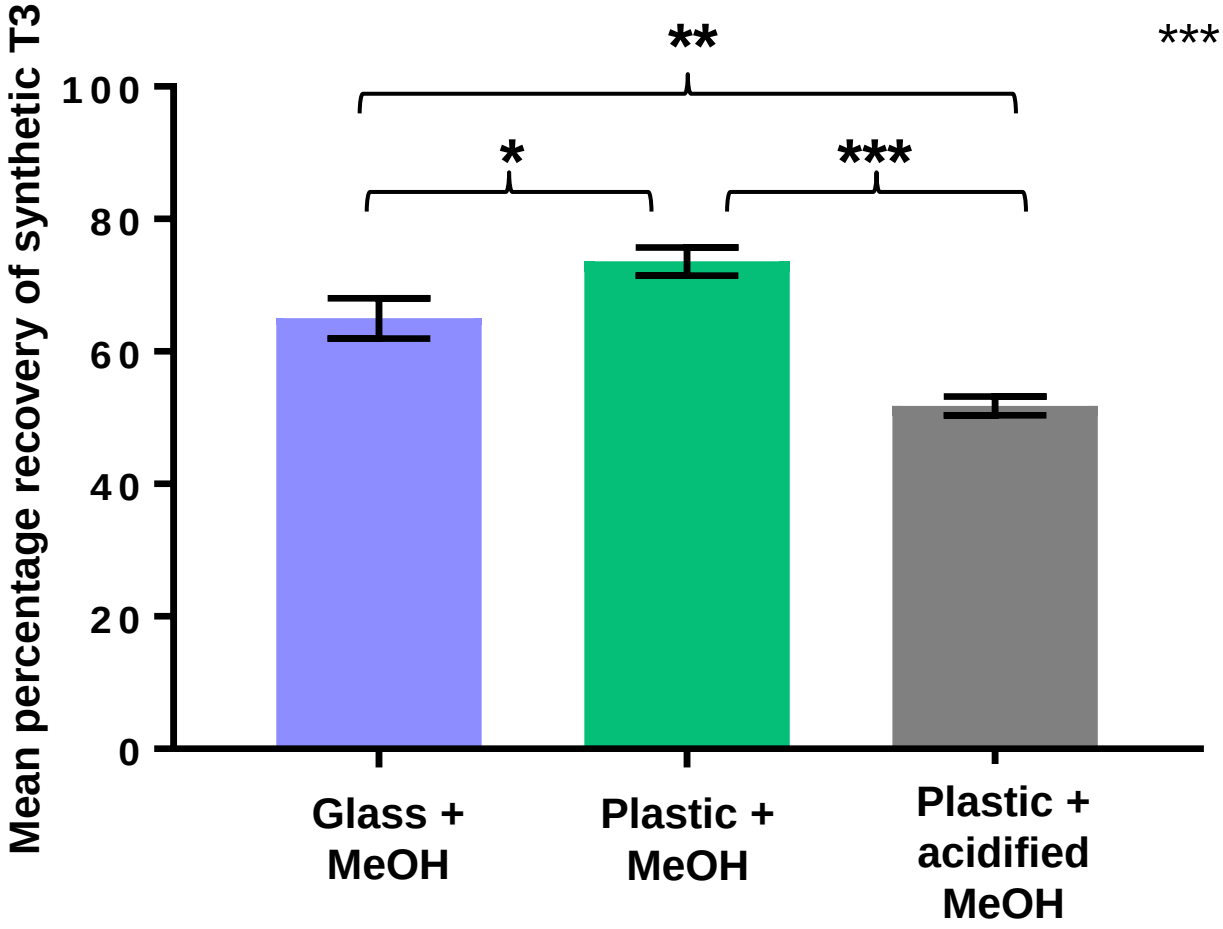
- A known concentration of synthetic T3 was loaded onto cartridges
- Cartridges were eluted with acidified methanol
- Liquid evaporated in plastic tubes

# Results - Hypothesis 2

## Optimising synthetic T3 recovery

- One-way ANOVA, Turkey's multiple comparisons test

* P=0.0406
** P=0.0007
*** P<0.0001

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# Methods – Hypothesis 3

## Chemical validation of three species

Parallelism

- A pooled faecal sample was created
- Run in parallel with standards of known concentrations
- Determines whether the assay is measuring T3 in the samples

Recovery

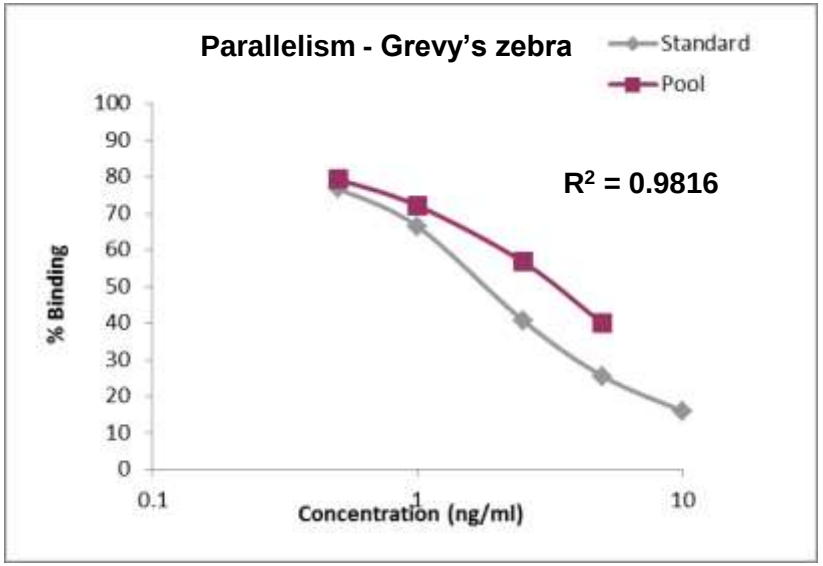
- Standards were spiked with the pooled sample
- Checks that the observed concentration of T3 corresponds to the true concentration
- Tests for interferences from other compounds

# Methods - Hypothesis 3

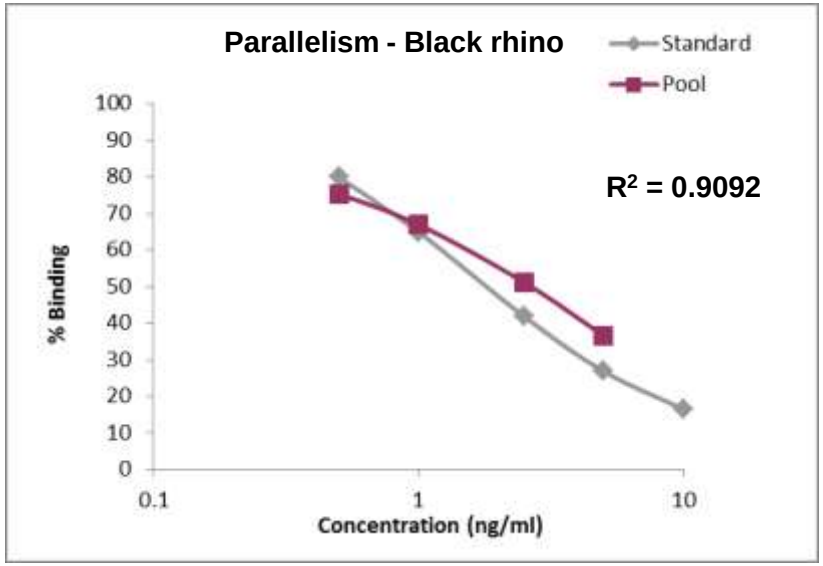
## Study animals

- 120 samples were collected from 13 adult female pregnant and non-pregnant megaherbivores, consisting of:
  - 4 black rhinoceros (*Diceros bicornis*)
  - 3 Grevy's zebra (*Equus greyvi*)
  - 6 African elephants (*Loxodonta africana)*

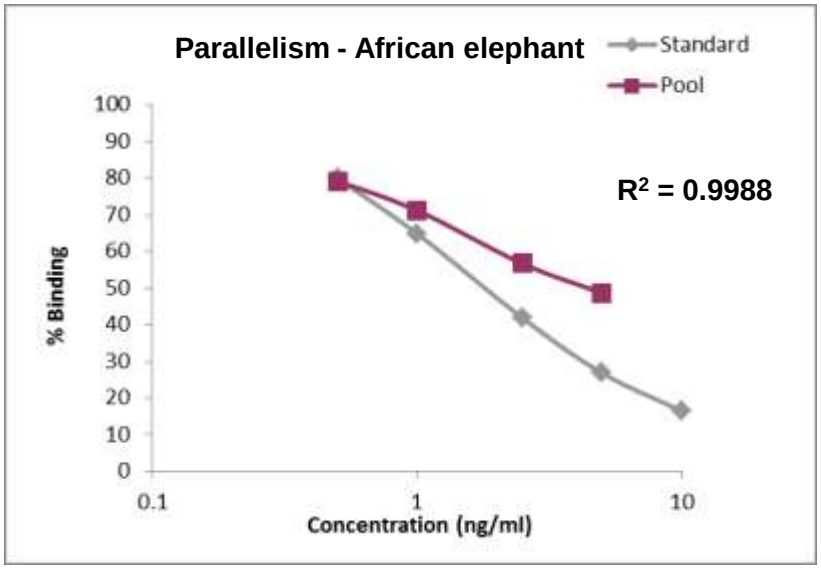
# Results - chemical validation




Recovery: $R^2$ = 0.9985




Recovery: $R^2$ = 0.9780




Recovery: $R^2$ = 0.9968

For parallelism to be significant:

- $R^2$ >0.9
- X variable (slope) >0.5
- Intercept is approximately 0

# Methods – Hypothesis 3

## Comparison of lab and field method

- Faecal samples were split into two sub-samples
- Extracted in parallel by either the lab method or the optimal field method
- T3 concentration were quantified using a T3 enzyme immunoassay

Laboratory method:

**0.5g faeces + 5ml 90% methanol → spun + dried → re-suspended in 100% methanol**

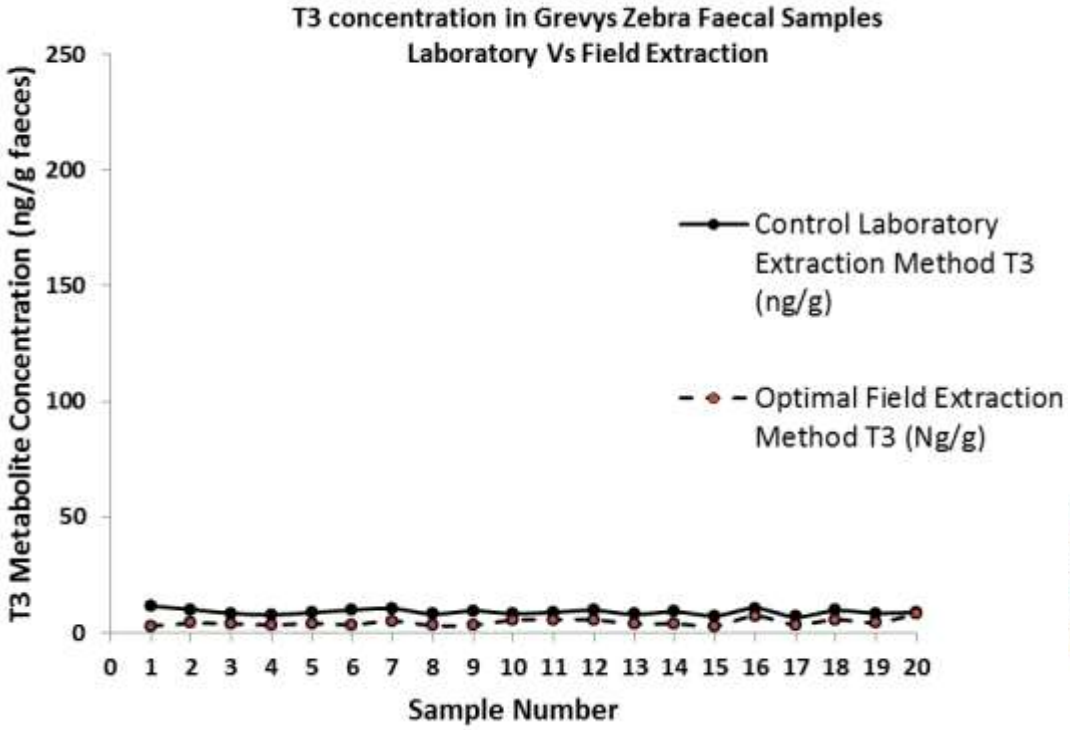
Field method:

**0.5g faeces + 5ml 90% methanol → diluted and loaded onto cartridge → eluted with 5ml 100% methanol → dried and re-suspended in 100% methanol**

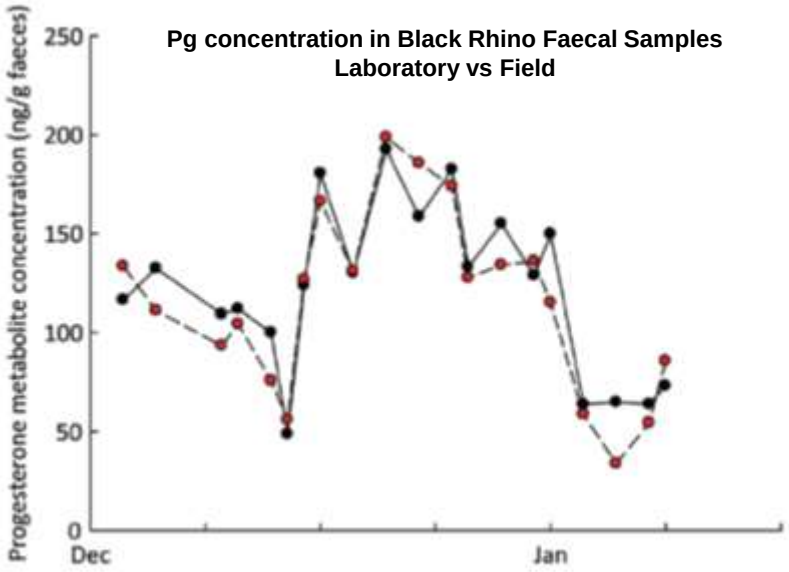
# Results – Hypothesis 3

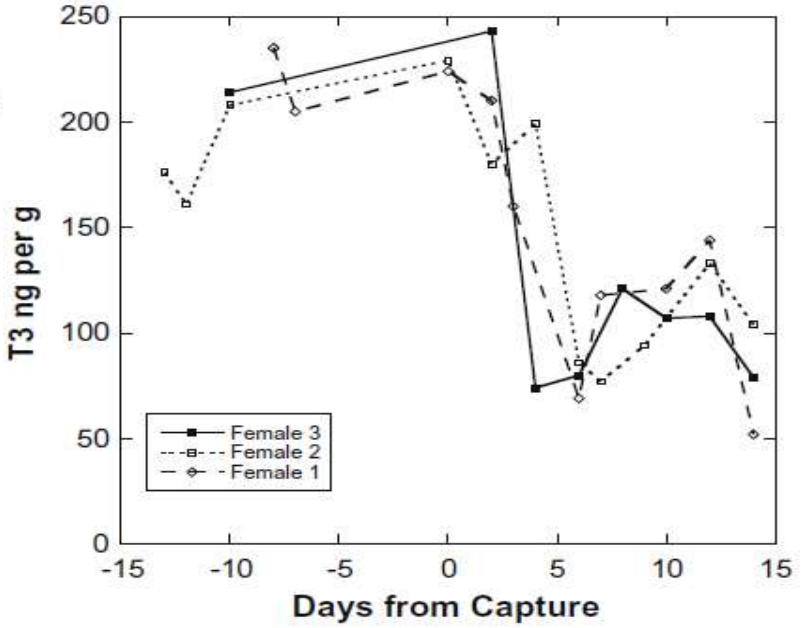
## Lab vs Field – Grevy's Zebra

Linear regression, Pearson's correlation




r = 0.455, P = 0.044, n = 20





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Wasser, S.K. et al. (2010) *General and Comparative Endocrinology* 168, 1-7

# Conclusions

- T3 hormone recovery was observed at >40% methanol
- Use of plastic tubes significantly improved recovery of synthetic T3 ($p<0.05$) but acid significantly reduced recovery of synthetic T3 ($p<0.05$)
- The use of a total T3 EIA was chemically validated to measure faecal T3 in three megaherbivore species

- With further development this method could be used to:
  - Monitor T3 concentrations in a variety of megaherbivores
  - Determine how nutritional status of different areas is impacting on the reproductive success of populations in situ
  - Inform conservation efforts and population management decisions on a continental scale

# Acknowledgements


- Thank you to the rhino team at Chester Zoo for help with sample collection

- Thank you to Rebecca Mogey and Sue Walker at Chester Zoo for guidance and assistance with this project

THANK
YOU!