Evaluation of anthropogenic disturbance on African clawless otter (*Aonyx capensis*) physiological stress, behaviour, and population density using non-invasive methods

by

Tshepiso Lesedi Majelantle

Submitted in partial fulfilment of the requirement for the degree Master of Science (Zoology)

in the

Department of Zoology and Entomology Faculty of Natural and Agricultural Sciences

University of Pretoria

2019

DECLARATION

I, Tshepiso Lesedi Majelantle, declare that this dissertation, which I hereby submit for the degree, Master of Science (Zoology), at the University of Pretoria, is my own work and has not been submitted by me for a degree at this or any other tertiary institution.

ETHICS STATEMENT

I, Tshepiso Lesedi Majelantle, declare that the research described in this work has observed the ethical standards required in terms of the University of Pretoria's Code of Ethics for Researchers and the Policy guidelines for responsible research and received approval from University of Pretoria Animal Ethics Committee

Signature: 

Tshepiso Lesedi Majelantle
Date: 22/11/2019

DISCLAIMER

Chapters 2 and 3 in this dissertation were structured, and formatted, according to the scientific journal submitted for publication. Therefore, there is an inevitable overlap and repetition in the introduction and methods sections between chapters.

SUMMARY

Evaluation of anthropogenic disturbance on African clawless otter (*Aonyx capensis*) physiological stress, behaviour, and population density using non-invasive methods

by

Tshepiso Lesedi Majelantle

Supervisor: Prof. Andre Ganswindt

Department: Mammal Research Institute, Department of Zoology and Entomology

University: University of Pretoria

Co-supervisor: Dr. Trevor McIntyre

Department: Life and Consumer Sciences

University: University of South Africa

Degree: Master of Science (Zoology)

Keywords: Faecal glucocorticoid metabolites, EIA validation, non-invasive hormone measurement, stress, camera traps, random encounter model, urbanization

Abstract

Land transformation for anthropogenic use is the leading cause of species declines globally, but some species are able to succeed in these environments. African clawless otters (*Aonyx capensis*) are the most widely distributed otter species in Africa and occur in a wide variety of habitats, including transformed landscapes. Thus, they are a good model species to investigate animal adaptions to anthropogenic environments. The aim of the study was to examine and compare the stress-related endocrine responses, population density, and behaviour of African clawless otters (ACOs) from a transformed area and natural areas using non-invasive techniques. An enzyme-immunoassay for measuring faecal glucocorticoid metabolite (fGCM)

concentrations as a measure of stress in ACOs was established by comparing fGCM concentrations from a captive otter before and after a stress event caused by translocation to a different site. Thereafter, fresh faeces from ACO latrines were collected, and camera traps were set up at a transformed area (Millstream Farm) and two natural areas (Verloren Vallei Nature Reserve and Cobham Nature Reserve). Camera trap arrays, consisting of between 18 and 24 cameras, were placed on all three sites, recording otter presence for a total of 2439 camera days. From the five different enzyme-immunoassays (EIAs) tested, the cortisol and oxoaetiocholanolone (measuring 11,17 dioxoandrostanes) EIAs showed the highest response (74% and 48% increase, respectively) 30-, 24- hours after the stress event. For both EIAs, a desiccation experiment illustrated that alterations in fGCM concentrations after defecation is acceptable (≤ 16.2 %) for samples collected up to 3 hours post-defecation. Using the cortisol EIA for subsequent analyses, fGCM concentrations of animals from the transformed area (n = 20; mean (± SD): 0.468 (± 0.539) µg/g dry weight (DW)) were significantly higher (p = 0.019) than those from otters in the natural areas (n = 17; 0.242 (± 0.226) µg/g DW), with an overall difference of 220%. Using a random encounter model approach, the transformed area was estimated to have the highest density of ACOs (8.2 ± 2.3 km^{-2}), whereas Verloren Vallei and Cobham Nature Reserve (natural areas) had estimated densities of 0.7 ± 0.2 km^{-2} and 2.1 ± 0.6 km^{-2}, respectively. There was a significant difference (p = 0.007) between group sizes in the transformed area (detections = 112; group size range = 1 – 5) and natural areas (detections = 29; group size range = 1 – 3) and in otter activity time (p = 0.033, activity overlap = 66.5 ± 8.33 %) between Verloren Vallei and Millstream farm. With the newly established non-invasive method, this study demonstrates that ACOs show increased adrenocortical activity in a transformed environment. Conversely, the otters exhibit substantial behavioural plasticity to exploit the anthropogenic landscape. Transformed areas such as Millstream farm provide ACOs with suitable habitat and abundant food resources, evidently supporting higher than average otter population densities. Such densities likely exacerbate conflict with trout farm managers due to their perceived depredation impact on trout stocks. Further studies evaluating the potential causes of elevated fGCM concentrations and investigate approaches to reduce human-otter conflict will contribute to African clawless otter conservation.

RESEARCH OUTPUTS

Journal articles

Majelantle TL, McIntyre T & Ganswindt A (2019). Monitoring the effects of land transformation on African clawless otters (*Aonyx capensis*) using faecal glucocorticoid metabolite concentrations as a measure of stress. *Integrative Zoology, In press*

Majelantle TL, Ganswindt A, Jordaan RK, Slip DJ, Harcourt RG & McIntyre T (2019). Increased population density and behavioural flexibility of African clawless otters (*Aonyx capensis*) in resource-rich anthropogenic environments. *Urban Ecosystems, Submitted*

McIntyre T, **Majelantle TL**, Slip DJ & Harcourt RG (2019). Quantifying imperfect camera trap detection probabilities: implications for density modelling. *Wildlife Research, In press*

Conference presentations

Oral Presentations

11th International Symposium of Integrative Zoology (2019)

Title: A comparison of African clawless otter (*Aonyx capensis*) population density, group size, and activity time in transformed and natural areas

7th International Society of Wildlife Endocrinology Conference (2019)

Title: Ecological Espionage: Application of non-invasive methods to evaluate anthropogenic disturbance in elusive African clawless otter (*Aonyx capensis*)

39th Zoological Societies of Southern Africa congress (2019)

Title: A comparison of African clawless otter *Aonyx capensis* behaviour and stress hormone levels in rural and peri-urban areas.

14th International Otter congress IUCN SSC Otter specialist group (2019)

Title: Poo Power: non-invasive monitoring of stress-related physiological responses in African clawless otter

LIST OF ABREVIATIONS

ACO	African clawless otter
ACTH	Adrenocorticotropic hormone
DW	Dry Weight
EIA	Enzyme-immunoassay
fGCM	Faecal glucocorticoid metabolites
GCs	Glucocorticoids
HPA	hypothalamic-pituitary-adrenocortical
REM	Random encounter model

ACKNOWLEDGEMENTS

This project would not be possible without the funding provided by the Department of science and technology through the National Research Foundation. I would also like to thank the University of Pretoria Animal Ethics Committee for providing me ethical clearance. Further, I would like to thank Mpumalanga Tourism and Parks Agency, Ezemvelo KZN Wildlife, Millstream Farm, Wild4life wildlife rehabilitation centre, and Millvale Golf Estate for permission to collect data and samples on site. Very few words can describe how grateful I am to my supervisors, Andre Ganswindt and Trevor McIntyre. Their invaluable knowledge, absolute brilliance, sacrificial time, kindness, and humility inspired me to continuously work as best as I can. I am grateful to them for allowing me the opportunity to explore and travel through this project. To Andre and Trevor, I genuinely look up to both of you, and you are a powerful inspiration to me and all your students. I would like to thank Nicole Duplaix for contributing towards my travel through the otter specialist group. I am indebted to Stefanie Ganswindt, Nicole Hagenah, and Abongile Ndzungu from the Endocrine Research Laboratory for all their hard work and for everything I have learnt from them for the past two years. I would like to thank Rowan Jordaan for all his instrumental help and contribution as well as Rob Harcourt and David Slip for all their vital contributions. I am grateful to Andrea, Marie, Dewald, Ruben, Wayde, Shannon, Damien, Nico, Ayesha, Aaliyah and Yasoda for all their help during field work. To my family, Anna, Neo, Mynie, Paul and so many others and my friends, Lindelani, Shannon, Tshego, Tumi, Rori, Marc and so many others, I blame all of you. The past two years have been a challenging journey and a test of endurance. Without your non-stop encouragement, incessant support, and unremitting love, I would have stopped this journey a long time ago. So thank you.

Finally, a special and eternal thanks to Moreri Khanie, my grandfather, I would not be here without his kindness, love and genuine belief in education and excellence.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: GENERAL INTRODUCTION

1.1 Background and Scope of Research Theme

1.1.1 Land Transformation

Archaeological evidence suggests that human induced land transformation for settlement and agricultural purposes dates back to the early Holocene era (~10 000 years ago) (Gupta 2004). In addition, the increase in carbon dioxide and methane since 8 000 years ago and 5 000 years ago respectively, suggest that human induced climate change also began during the Holocene era (Ruddiman 2003). Since then, the world human population has grown rapidly from 2.5 billion people in 1950 to 6.5 billion people in 2005, and is projected to reach 9.2 billion people worldwide by the year 2050 (Bongaarts 2009). The expansion of the human population also includes an increase in human induced land transformation for agricultural, industrial and settlement purposes (Meyer & Turner 1992; Ramankutty *et al.* 2002). Humans have consequently influenced about 83% of the land (Sanderson *et al.* 2002; Fischer & Lindenmayer 2007) and 41% of the ocean (Halpern *et al.* 2008).

Land transformation by humans entails: replacing natural vegetation with favoured vegetation (for example crops) and/or anthropogenic structures (for example roads and buildings), change of natural community structure, addition of artificial or provisioned food sources, and increased human disturbance (McKinney 2002, 2006). Subsequently, land transformation has led to factors such as habitat degradation, species over-exploitation, competition with introduced species, and pollution (Kerr & Currie 1995; Woodroffe 2000; Marzluff & Ewing 2001; McKinney 2001; Byers 2002; McKee *et al.* 2004). In addition, transformed environments promote biotic homogenization because they are constructed to meet the requirements of humans only (McKinney 2006). Some transformed habitats have isolated heterogeneous habitats (Honnay *et al.* 1999; McIntyre *et al.* 2001; Gibb & Hochuli 2002), where the surrounding matrix is inhospitable to some species living within these isolated pockets (Öckinger *et al.* 2012; Freeman *et al.* 2018). Thus, land transformation can be linked to the population decline and extinction of numerous species globally (Woodroffe 2000; McKinney 2001) and species richness tends to decrease along gradients of increasing transformation (McKinney 2001).

1.1.2 Urban Adapters

There are species which are able to exploit transformed areas or even urban areas, and these species are often referred to as "adapters" or "exploiters" (McKinney 2006). These species are faced with selective pressures such as increased human activity, noise, and toxin levels, but gain benefits such as increased food resources and, in some instances, decreased or altered predation risk (Bonier *et al.* 2006). A framework proposed by Evans *et al.* (2010) suggests that colonisation of urban areas by urban exploiters entails: (1) arrival into an urban area, (2) adjustment to urban habitats, and (3) spread within- and to other urban areas (Figure 1.1). Sol *et al*. (2013) suggested an additional phase, which is an increase of population size determining if the urban exploiter will persist and spread to other urban areas. The success of exploiters in urban areas depends on a combination between environmental factors (e.g. magnitude of change) and species traits (e.g. adaptive phenotypic plasticity and genetic adaptation) (Evans *et al.* 2010; Sol *et al.* 2013).

There is growing evidence that urban exploiters display behavioural modifications when compared to rural counterparts, and their ability to utilise these transformed habitats is therefore possibly linked to their behavioural and phenotypic plasticity (Lowry *et al.* 2013). For example, raccoons (*Procyon lotor*) adjust their movements, reduce their home range size, and attain high population densities in patches with rich artificial resources in urbanised sites as compared to natural areas (Prange *et al.* 2004). Bush babies (*Galago moholi*) in urban areas have higher body mass index values presumably due to access of rich artificial food resources, and show more frequent aggressive behaviour towards conspecifics, and higher stress hormone concentrations, when compared to their rural counterparts (Scheun *et al.* 2015). However, animal adjustment to urban environments is also possibly due to a sorting process or an evolutionary response (Sol *et al.* 2013). Although research on urban exploiters in and around cities began in the 1970s, most studies are based on "old" urban areas in the northern hemisphere (Magle *et al.* 2012). For example, a review by Santini *et al.* (2019) identified 182 mammalian urban dwellers and visitors globally, but only 26% of the species occur in Africa. Thus, there is a knowledge gap on urban exploiters in Africa, although the continent is rapidly urbanising (Magle *et al.* 2012).

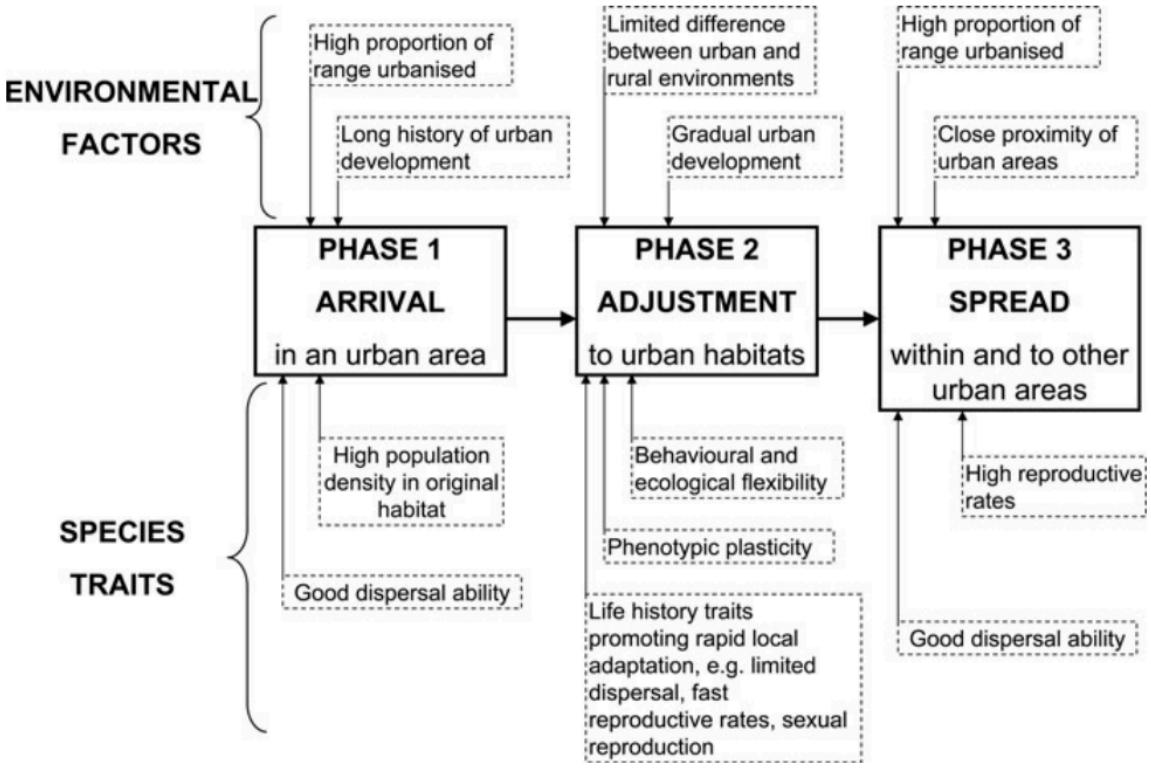

Figure 1.1: Schematic representation of the three stages of the urbanisation process proposed by Evans *et al.* (2010)

1.2 African Clawless Otters

Otters are in the *Lutrinae* subfamily which has 13 extant species (Koepfli & Wayne 1998). Of these, the African clawless otter (*Aonyx capensis*), common otter (*Lutra lutra*), Congo clawless otter (*Aoynx congicus*), and spotted-necked otter (*Hydrictis maculicollis*) occur in Africa (Koepfli & Wayne 1998; Somers & Nel 2013). The African clawless otter is the most widely distributed otter species in Africa (Figure 1.3). Their distribution range stretches from Senegal to Ethiopia then southwards throughout east Africa to South Africa; they are absent in the Congo basin where they are replaced by the Congo clawless otter (Somers & Nel 2013). In 2015, the IUCN changed the status of the African clawless otter from Least Concern to Near Threatened based on their population decline, habitat loss and degradation, and pollution (Jacques *et al.* 2015).

Figure 1.2: African clawless otter (© Rowan K Jordaan).

The broadened bunodont molars and manual dexterity of African clawless otters suggest that they possibly evolved from a lineage of crab specialists (Rowe-Rowe 1977; Davis & Pineda-Munoz 2016). Their diet also includes fish, frogs, insects, and sporadically, birds and small mammals (Somers & Nel 2013). African clawless otter respond to the abundance and behaviour of their prey (Somers & Nel 2004; Nel & Somers 2007). For example, they consume more fish during winter likely because fish swimming efficiency is reduced in colder waters (Rowe-Rowe 1977). Their diets remain consistent over time (years), but are not consistent between populations (Jordaan *et al.* 2015) . The differences in diet over a spatial scale may be attributed to differences in water temperature, climate, prey abundance, prey selection, and anthropogenic influence (Verwoerd 1987; Somers 2000; Jordaan *et al.* 2015).

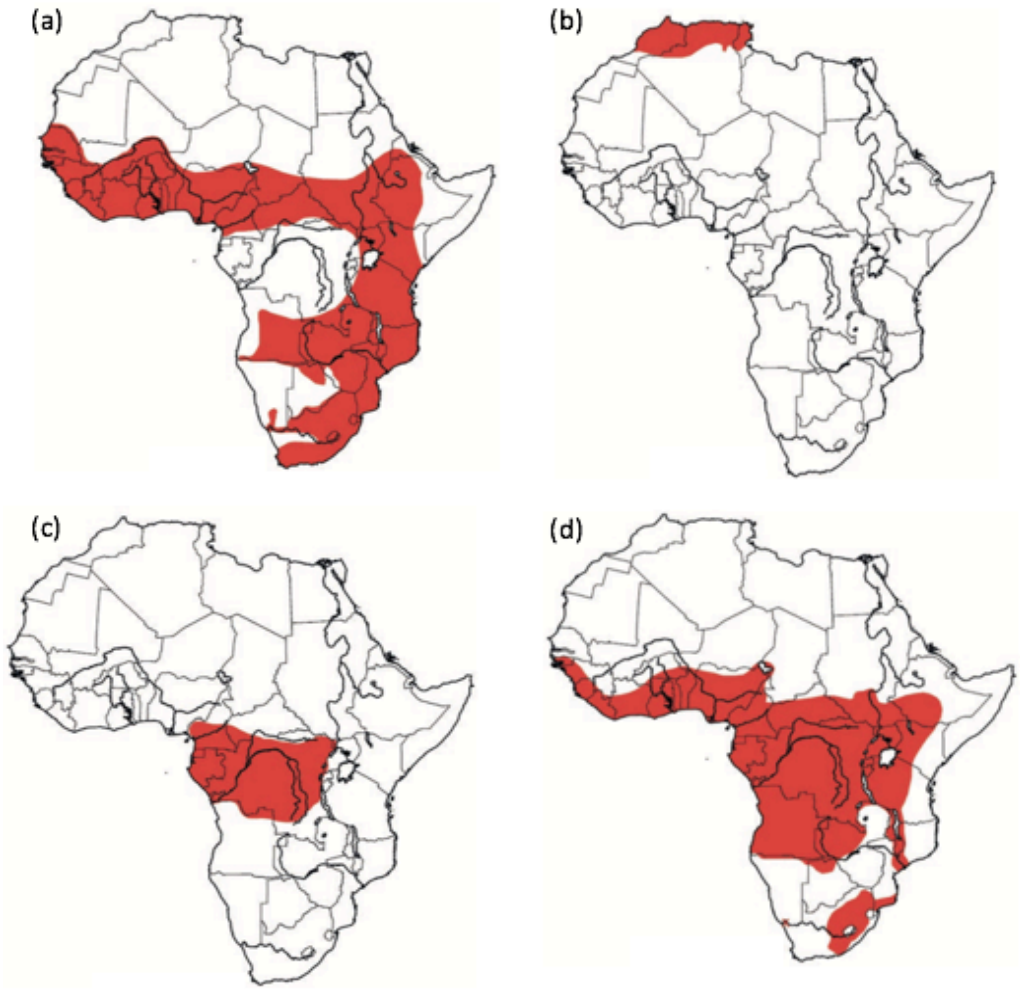

Figure 1.3: Maps from Somers & Nel (2013) showing the distribution (shaded) of the four African otters; (a) *Aonyx capensis*, (b) *Lutra lutra*, (c) *Aonyx congicus,* and (d) *Hydrictis maculicollis*

African clawless otters are mainly solitary but occasionally occur in pairs or small groups (max five individuals) (Arden-Clarke 1986; Somers & Nel 2004). Observations on African clawless otter reproductive biology suggests they are continuous breeders (can reproduce throughout the year), producing one to three cubs after gestation periods of about 60 - 64 days (Somers & Nel 2013). African clawless otters occur in a wide range of habitats such as riparian, impoundments, estuaries and mangroves, both inland and coastal (Nel & Somers 2007), whereby freshwater availability is the main habitat requirement (Van Niekerk *et al.* 1998). Within these habitats, the otters prefer areas with rocky outcrops, boulders and reedbeds (Van Niekerk *et al.* 1998; Somers & Nel 2004). African clawless otters rarely venture more than 15 m from fresh

water sources (Rowe-Rowe 1992; Larivière 2001), their core home range length varies between 0.2 to 9.8 km, and female otters exhibit territorial behaviour (Somers & Nel 2004). African clawless otters presence is not influenced by proximity to urban areas (Okes & O'Riain 2017) and genetic evidence suggests that their movement is not affected by physical barriers in urbanised areas (Ponsonby *et al.* 2019). Thus, they occur in transformed and urban areas, however in relatively low densities compared to natural areas (Ponsonby & Schwaibold 2019; Ponsonby *et al.* 2019). Since African clawless otters display such a broad distribution encompassing a variety of diets, habitats and climate regimes, they are a useful model species for studying phenotypic adaptability and potential fitness costs in the face of increasing anthropogenic pressure. However, since otters are shy and elusive, the application of non-invasive techniques and indirect measurements for a respective monitoring are required to gain such insights (Macdonald & Mason 1983).

1.3 Non-invasive monitoring of hormones as an indicator of stress

There is no formal agreement on the definition of "stress" since the term can be applied to a broad range of disciplines. In the context of this study, stress is defined as a general syndrome occurring in response to any stimulus (stressors) that threatens or appears to threaten the homeostasis of an individual (Selye 1936; Wielebnowski 2003). Vertebrates cope with stressors by initiating a behavioural- (e.g. change in social behaviour (Tonelli *et al.* 2008)), autonomic nervous- (e.g. change in heart rate (Von Borell *et al.* 2007)), and neuroendocrine- (e.g. release of adrenocorticotropin hormone) responses to restore homeostasis (Moberg & Mench 2000). The neuroendocrine stress response includes the activation of the hypothalamic-pituitary-adrenocortical (HPA) axis and the sympatho-adrenomedullary system (Palme 2019). In addition, the HPA axis is activated in response to factors such as diet, reproduction, circadian rhythms and ageing (Cavigelli *et al.* 2005).

The HPA axis activation results in an increase of secretion of glucocorticoids (GCs) by the adrenal glands (Palme 2019). Catecholamines are secreted rapidly in response to short term (acute) stressors, and a have a brief half-life in the blood (Palme *et al.* 2005). Glucocorticoids are transported to target tissues via the blood, thereafter, circulating hormones are metabolized by the liver and excreted often as conjugates

via the kidneys into the urine or via the bile into the gut (Palme *et al.* 2005; Touma & Palme 2005) (Figure 1.4). GCs or their metabolites are also present in hair/feathers, milk, saliva, and eggs (Palme 2019) (Figure 1.4). There are two primary GCs, specifically cortisol (4-pregnene-11ß,17α,21-triol-3,20-dione) which is primarily secreted by fish and most mammals, and corticosterone (4-pregnene-11ß,21-diol-3,20-dione) primarily secreted by birds, amphibians, reptiles and some rodents (Palme 2019). The secretion of these hormones results in a shift in energy balance to assist the animal with coping with the stressor, and the magnitude of the stressor perceived by the animal determines the amount of hormones secreted (Wielebnowski 2003). However, long-term exposure to these hormones leads to immunosuppression, atrophy of tissue, decreased reproductive capacity and impaired growth (Möstl & Palme 2002; von der Ohe & Servheen 2002). Thus, measurement of GCs or their metabolites in the matrices highlighted in Figure 1.4 can be used as an indication of the welfare status of an animal (Lane 2006).

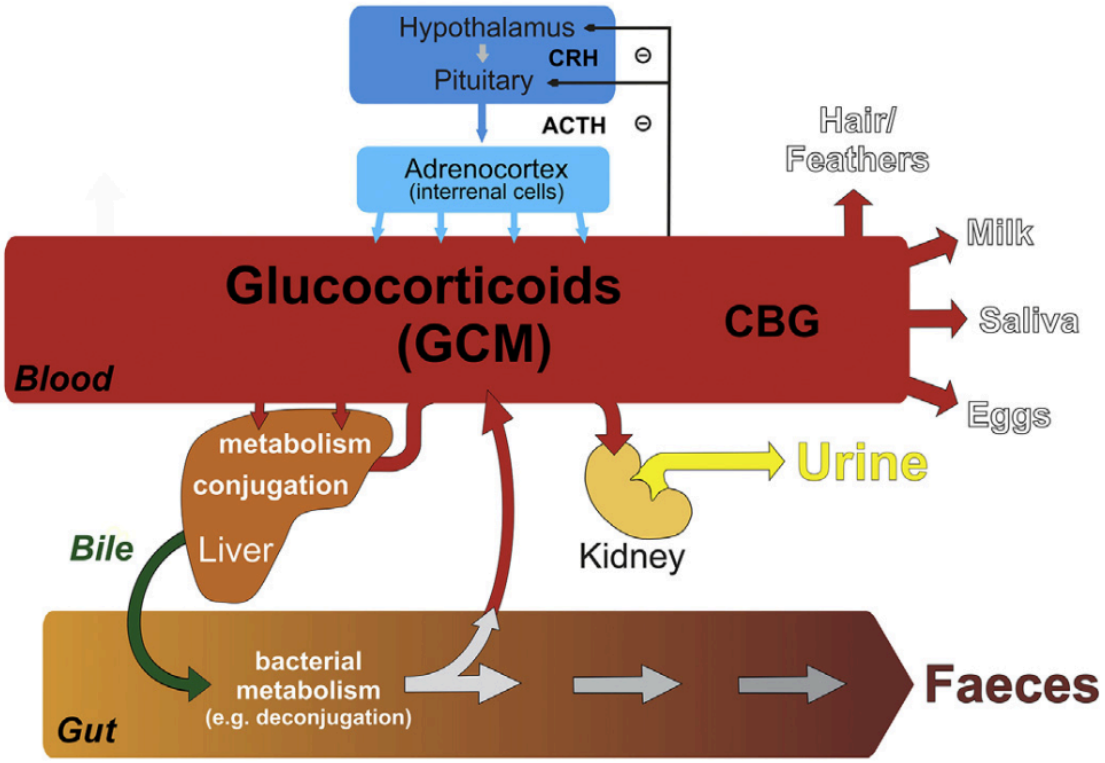

Figure 1.4: Representation of the secretion of glucocorticoids proposed by Palme (2019)

Measurement of glucocorticoid concentrations in matrices such as blood and urine usually require handling or confinement of animals which could be perceived as stressful and thus affect the reliability of the results (Möstl & Palme 2002). However, measuring GC concentration in alternative matrices such as faeces provide a non-invasive approach, because faeces can be collected easily, animals are usually not disturbed during sample collection, and thus sampling is feedback free (Touma & Palme 2005). However monitoring of faecal glucorticoid metabolite (fGCM) concentrations in a species studied for the first times must be reliably validated (Ganswindt *et al.* 2012), as species- and sex-specific differences in GC metabolism exists (Palme *et al.* 2005). Further species- and sex-specific time delay of fGCM concentrations post stressor have been reported (Webster *et al.* 2018), which is closely related to the animals' intestinal transit time (Palme *et al.* 2005) and food intake (Kalliokoski *et al.* 2012). Thus, the total amount of GC metabolites excreted via faeces varies between species (Wasser *et al.* 2000) and sex (Touma *et al.* 2003). Further, peak responses to a perceived stressor, in the form of highest glucocorticoid output, can vary between individuals of the same species (e.g. Ganswindt *et al.* 2012). In addition to assure the reliability of samples collected to quantify fGCM output, the time-dependent stability of GC metabolites post defecation (Crossey *et al.* 2018) and the effect of the circadian rhythm on circulating GCs (Ganswindt *et al.* 2012) should be investigated to warrant comparability between different faecal samples analysed.

1.4 Camera Trapping

Traditionally, African clawless otter density estimates are based on their characteristic sign and faeces at latrine sites (Rowe-Rowe 1992). However, these methods are labour intensive and limited by bias due to fieldworker experience and weather conditions (e.g. rain) (Wilson & Delahay 2001). In addition, the method may underestimate the number of individuals in areas with high otter density (Ruiz-Olmo *et al.* 2001). Camera trapping is a convenient tool in ecological studies because it is cost effective and less time consuming compared to traditional methods (Cutler & Swann 1999). The usefulness of camera traps extends in particular to elusive and cryptic species, including otters. Thus, over recent years, studies based on camera trap data have become increasingly popular (Burton *et al.* 2015; Caravaggi *et al.* 2017). One method for estimating animal densities from camera trap data is the Random

Encounter Model, which utilizes the ideal gas theory, as proposed by Rowcliffe *et al.* (2008). Random Encounter Models (REMs) are particularly useful because they do not require uniquely identifiable individuals (Rowcliffe *et al.* 2008). In addition, the REM model approach is a well validated method, which has performed better than traditional methods such as dung counts (Pfeffer *et al.* 2018) and line transects (Zero *et al.* 2013). However, REMs assume that: the movement of target species is random, detections represent independent contacts between cameras and animals, and the population is closed (Rowcliffe *et al.* 2008). Thus, the effective application of the model requires careful camera trap placement to allow for random encounter opportunities (Rowcliffe *et al.* 2013), as well as prior knowledge on the movement ecology of the study species. Camera traps also provide valuable information on facets such as behaviour (Caravaggi *et al.* 2017). For example, the application of camera traps provided information on African clawless otter scent marking behaviour (Jordaan *et al.* 2017), Giant otter (*Pteronura brasiliensis*) social behaviour (Leuchtenberger *et al.* 2014a), and Giant otter activity patterns (Leuchtenberger *et al.* 2014b).

1.5 Research Aim and Objectives

1.5.1 Aim

The aim was to examine and compare the stress-related endocrine response, population density, and behaviour of African clawless otters from a transformed area and natural areas using non-invasive techniques.

1.5.2 Objectives

The specific objectives were:

1 To examine the suitability of enzyme-immunoassays for monitoring stress-related physiological responses in African clawless otter faeces by using a translocation as a biological validation.

2 To investigate the effect of bacterial metabolism and environmental factors on the stability of faecal glucocorticoid metabolite (fGCM) levels post-defaecation.

3 To evaluate and compare fGCM concentrations as a measure of stress in African clawless otters occurring in natural and transformed areas.

4 To estimate and compare African clawless otter population densities in a transformed area and natural areas using a random encounter model approach, based on camera trap data.

5 To compare the group size and activity time for African clawless otters occurring in a transformed area and natural areas based on camera trap data.

1.6 Dissertation Structure

The dissertation is divided into four chapters of which two are based on data. The first chapter includes an introduction on land transformation, urban adapters, African clawless otters, and non-invasive monitoring of hormones as an indicator of stress and camera trapping. The aims and objectives are specified in this chapter. The second chapter addresses the first three objectives. Specifically, the chapter includes the validation of faecal glucocorticoid metabolites as a measure of stress in African clawless otters and application of the method to compare the fGCM levels of otters occurring in a transformed area and natural areas. The third chapter includes the application of camera trap data to quantify African clawless otter densities, group size and activity time and compared these between a transformed area and natural areas (objectives 4 and 5). Both the second and third chapter are in a peer-reviewed paper format and are submitted for publication to Integrative Zoology (*In press*) and Urban Ecosystems (*Submitted*), respectively. The final chapter highlights the major findings of the study and concludes with recommendations for management strategies and future studies.

CHAPTER 2: MONITORING THE EFFECTS OF LAND TRANSFORMATION ON AFRICAN CLAWLESS OTTERS (*AONYX CAPENSIS*) USING FAECAL GLUCOCORTICOID METABOLITE CONCENTRATIONS AS A MEASURE OF STRESS

2.1 Abstract

In a time of increasing environmental change caused by anthropogenic disturbance, the need for understanding animal adaptations to man-made environments increases. In this regard, the measurement of stress-related endocrine markers provides a useful tool to examine the impact of environmental challenges and its physiological consequences in wildlife occupying such space. The aims of this study were to validate faecal glucocorticoid metabolite (fGCM) concentrations as a measure of stress using samples from a male African clawless otter (Aonyx capensis; n= 1), and secondly to compare fGCM concentrations of otters occurring in a transformed and two natural areas in South Africa. From the five different enzyme-immunoassays (EIAs) tested, a cortisol and oxoaetiocholanolone (measuring 11,17 dioxoandrostanes) EIA revealed the highest response (74% and 48% increase, respectively) 30-, 24- hours after a stress event (translocation of a captive individual as part of its rehabilitation prior to release), respectively. For both EIAs, fGCM concentrations were comparable for samples collected up to 3 hours post-defecation. Using the cortisol EIA for subsequent analyses, fGCM concentrations of animals from the transformed area (n = 20; mean (± SD): 0.468 (± 0.539) µg/g dry weight (DW)) were significantly higher (p = 0.013) than those from otters in the natural areas (n = 17; 0.242 (± 0.226) µg/g DW). These preliminary results suggest that African clawless otters may have increased adrenocortical activity that could be due to conditions linked to living in a transformed environment.

This data chapter is accepted for publication in Integrative Zoology

2.2 Introduction

Land use change (land transformation) is the most important factor contributing to habitat degradation, alteration, and fragmentation globally (Marzluff & Ewing 2001). In addition, land transformation promotes biotic homogenization, because transformed habitats are constructed to meet the requirements of humans (McKinney 2006). There exists isolated heterogeneous habitats within transformed areas (Honnay *et al.* 1999; McIntyre *et al.* 2001; Gibb & Hochuli 2002), where the surrounding matrix is inhospitable to some species living within these isolated pockets (Öckinger *et al.* 2012; Freeman *et al.* 2018).

Food availability is a key factor contributing to population size and dynamics (Chapman *et al.* 2015) and transformed areas may offer benefits such as increased food resources, shelter and in some instances decreased or altered predation, which some species are able to exploit (Bonier *et al.* 2006; McKinney 2006).However, species living in urban environments are faced with selective pressures such as increased human activity, noise, and toxin levels (Bonier *et al.* 2006).As a result, species roaming in transformed areas tend to have elevated stress-related hormone levels compared to those living in more natural environments (Fokidis *et al.* 2009; Scheun *et al.* 2015; McLennan *et al.* 2019).

There are many definitions of stress. Herein, stress is defined as a general syndrome occurring in response to any stimulus (stressors) that threatens or appears to threaten homeostasis of an individual (Selye 1936; Wielebnowski 2003). When confronted with a stressor, an animal will rely on different biological systems (for example behavioural, autonomic nervous, neuroendocrine and immune) to prompt a stress response to cope with the stressor and restore homeostasis (Moberg & Mench 2000). The stress response includes the activation of the hypothalamic-pituitary-adrenocortical (HPA) axis and the sympatho-adrenomedullary system, which is sensitive to environmental stressors (Cavigelli *et al.* 2005). In addition, the HPA axis responds to factors such as alteration in diet (Kalliokoski *et al.* 2012) or reproductive status, circadian rhythms and ageing of an animal (Cavigelli *et al.* 2005). HPA axis activation results in an increase in the secretion of catecholamines and glucocorticoids by the adrenal glands (Palme 2019). Catecholamines are secreted rapidly in response to (short term) stressors

(Palme 2005), while glucocorticoids (GCs) are secreted in response to acute and long-term stressors. The secretion of these hormones in response to perceiving a stressor stimulate rapid changes in the physiology of the animal (e.g. increased heart rate and shift in energy balance) to allow it to respond and ideally cope with the stressor (e.g. initiate fight or flight response) (Wielebnowski 2003). However, if stressor(s) are perceived over a prolonged period, it may decrease individual fitness by immunosuppression, and atrophy of tissues (Möstl & Palme 2002). At population level, a prolonged stress response can decrease reproductive capacity, increased disease susceptibility and impaired growth (von der Ohe & Servheen 2002; Wielebnowski 2003).

Measuring concentrations of GC or its metabolites in matrices such as faeces instead of blood provides a non-invasive approach. Especially when working with free-roaming wildlife, faeces can often be collected more easily and safely, and animals are usually not disturbed during the procedure and thus sampling can be regarded as feedback free (Touma & Palme 2005). Therefore, measuring GC metabolites in faeces gives reliable information on the welfare status of animals (Lane 2006). There are species- and sex-specific differences in the types of GC metabolites found in faeces (Wasser *et al*. 2000; Touma *et al*. 2003; Palme *et al*. 2005) and multiple criteria should be taken into account when applying any non-invasive approach for glucocorticoid metabolite measurement from faeces in a species being investigated for the first time (Ganswindt *et al.* 2012). To account for these differences, an assay measuring faecal glucocorticoid metabolite (fGCM) concentrations must first be established through analytical, physiological and/or biological validation (Touma & Palme 2005). In short, assay reliability is examined by stimulating a stress response in an animal and measuring fGCM concentrations before and after the stress event, and suitable assays will show a related peak in fGCM concentrations (Palme 2019). GCs get metabolized prior excretion and resulting fGCMs can be even further metabolized by bacterial enzymes post-defecation (Palme 2005). Furthermore, activation of the HPA axis responds to the circadian rhythm in most vertebrates (Cavigelli *et al.* 2005) and consequently diurnal variations of fGCMs have been reported (Touma & Palme 2005; Ganswindt *et al.* 2012), Thus, the time-dependent stability of GC metabolites post defecation (Crossey *et al.* 2018) and the effect of the circadian rhythm of circulating

GCs (Ganswindt *et al.* 2012) must be investigated to warrant the comparability between different samples.

In a time of increasing environmental change caused by the increase in the human population, thus increasing demand in land use change, studies on the biology of taxa which exploit a wide range of habitats and how animals adapt to transformed areas become increasingly important (Bateman & Fleming 2012). African clawless otters (*Aonyx capensis*) display a broad distribution encompassing a variety of habitats and climate regimes, with a distribution range stretching from east Africa to South Africa (excluding the Congo basin) (Somers & Nel 2013). The species responds behaviourally to the abundance and behaviour of their prey (Somers & Nel 2004; Nel & Somers 2007), and are able to utilize resources provided by anthropogenic landscapes (Ponsonby & Schwaibold 2019). Therefore, African clawless otters are a useful model species for studying phenotypic adaptability and potential fitness costs in the face of increasing anthropogenic pressure.

The aim of this study was to evaluate and compare fGCM concentrations as a measure of stress in African clawless otters occurring in natural and transformed areas. As a prerequisite, the study also aimed to establish a non-invasive approach for measuring fGCM concentrations in a male African clawless otter. More specifically, this part of the study aimed to: a) determine stress-related physiological responses in African clawless otter faeces by monitoring a transport event as a form of a biological validation (n =1), b) investigate the potential influence of time of day (circadian rhythm) on fGCM excretion, and c) examine the changes in fGCM concentrations over time after defecation. The hypothesis was that the otters occurring in the transformed area will have higher fGCM concentrations compared to the natural areas due to factors linked to anthropogenic disturbance.

2.3 Materials and Methods

2.3.1 Study sites and sample collection

Faecal samples were collected from free-ranging otters at Cobham Nature Reserve (n = 13 collected between 8 October – 1 November 2018), at Verloren Vallei Nature

Reserve (n = 4 collected between 28 June – 19 August 2018), and at Millstream Farm (n = 20 collected between 26 June – 18 August 2018) (Figure 2.1).

Cobham Nature Reserve (29°41'58.8"S, 29°24'50.3"E) is situated in the Southern Maloti-Drakensberg Park, KwaZulu Natal, South Africa. The size of the reserve is 520 km^2 with two main vegetation zones, Alti-montane and Afro-montane grassland. Samples were collected along the Polela river. The area has an annual average rainfall of 800 mm and temperatures range between -15 °C and 35 °C (SAWS 2018). The 58.91 km^2 Verloren Vallei Nature Reserve (25°19'10.9"S, 30°07'38.8"E) in Mpumalanga, South Africa, is situated in the Highveld montane grassland and contains several permanent wetlands. The reserve has an annual mean rainfall of 664 mm and temperatures range between -13 ˚C and 21.9 ˚C (SAWS 2018). Both nature reserves, (Cobham and Verloren Vallei) have little anthropogenic transformation on the landscape and were therefore chosen as areas that are mostly ecologically intact and characterized by very low levels of direct anthropogenic disturbance.

The third study site, Millstream Farm (25°27'07.3"S 30°05'30.7"E), is situated in the Mpumalanga Highveld, South Africa. Millstream Farm offers accommodation on-site and recreational fly-fishing in eight dams and 13 weirs along the Witpoort River (all stocked with rainbow trout, *Oncorhynchus mykiss*). While offering suitable habitat for otters in terms of the vegetation cover, water- and food availability, this area is also characterized by increased human presence (permanent inhabitants and visiting anglers). The farm is located 21 km from Verloren Vallei Nature Reserve and is characterized as experiencing similar climatic conditions.

African clawless otter faeces were identified based on the shape, size and characteristic odour (Stuart & Stuart 2000). Samples were collected from comparatively large faecal material (diameter > 2 cm) since these samples were likely from adult otters (Stuart & Stuart 2000). The freshness of a sample was estimated based on moisture content, temperature and anal jelly texture. In addition, to avoid collecting older samples, samples were not collected during wet weather conditions (e.g. rain and fog). While wearing gloves, about half of the fresh faecal material was thoroughly mixed and placed in labelled small plastic containers and put on ice immediately. Samples were frozen within 1 hour of collection and kept frozen until

further processing and analysis at the Endocrine Research Laboratory, University of Pretoria, South Africa. The study was performed under the approval of the University of Pretoria Animal Use and Care Committee (Project number EC012-18) and the University of KwaZulu Natal Animal Research Ethics Committee (Project number AREC/033/018).

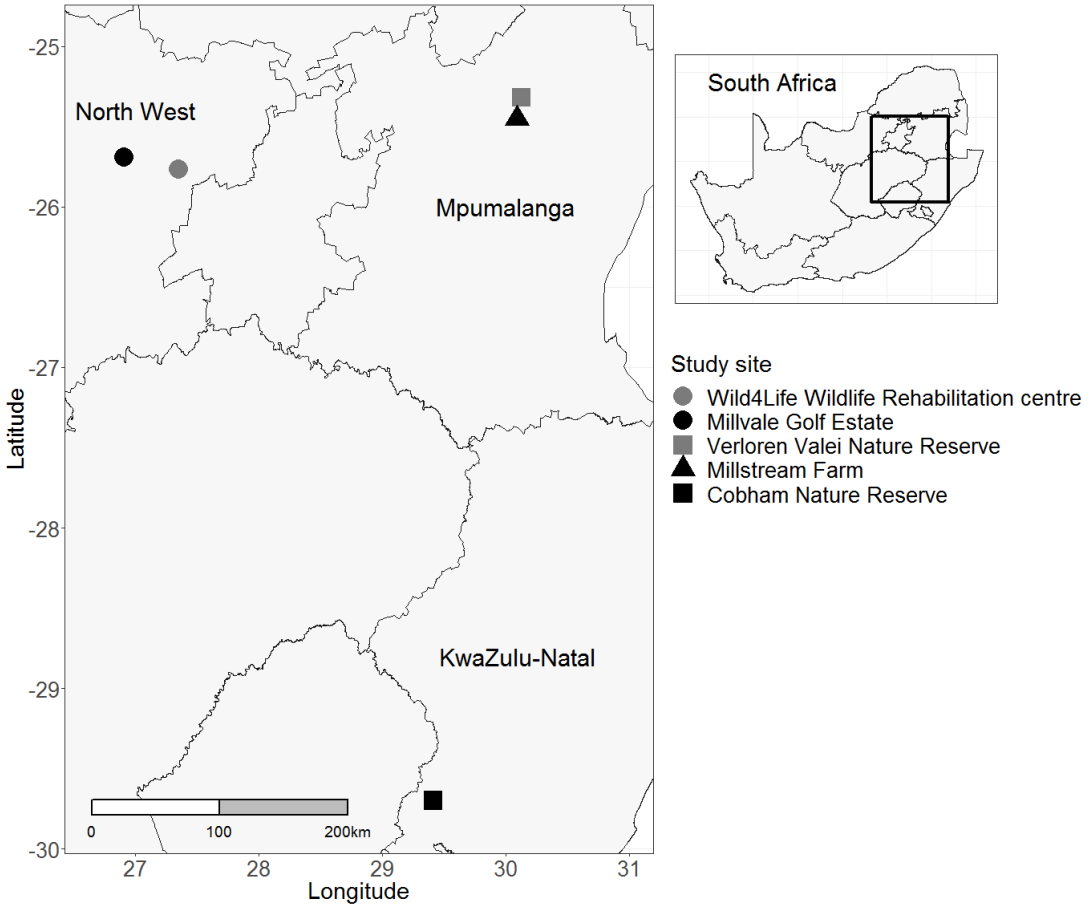

Figure 2.1:Representation of spatial locations where African clawless otter fresh faecal samples were collected from 8 June 2018 – 1 November 2018.

2.3.2 Sampling during animal translocation

Faecal samples were collected from one captive adult male otter between 8 June and 8 August 2018. A mobile cage (2m x 1m x 1m) within its enclosure was baited with fish and the otter voluntarily entered. The otter was translocated in the mobile cage (2m x 1m x 1m) within a vehicle from the Wild4Life wildlife rehabilitation centre to Millvale Golf Estate, both located in Rustenburg, South Africa (61 km apart) (Figure 2.1). In both areas, the otter was housed in an enclosure (5m x 3m x 3m), which included a pool and foraging pans and was fed fish or chicken once a day. In addition, the rehabilitation program at Millvale Golf Estate included a once a week foraging

event for the otter in the nearby lake. Samples which were voided during the day (07:00 – 18:00) were collected within 1 hour of defecation (n = 55), whereas samples voided at night (00:00 – 06:00) were collected in the morning (06:00) (n = 10). In total, 65 faecal samples were collected (28 before and 37 after-translocation).

2.3.3 *Time-dependent alteration in fGCM concentration post-defecation and influence of defecation time*

To evaluate alteration in fGCM concentration post-defecation in samples when frozen at different times, ten fresh samples collected before the translocation were thoroughly mixed and immediately divided into 27 subsamples. Subsamples were exposed to air and direct sunlight at room temperature (21.7°C – 34.5°C). Subsequently, three subsamples (triplicates) were frozen at -20°C after 0, 1, 3, 6, 12, 24, 48, 72 hours and 1 week of exposure.

Samples collected from the captive otter during the morning (07:00 – 09:00; n = 10 and evening (16:00 – 17:30; n = 10) were used to explore the influence of time of defecation on fGCM concentrations.

2.3.4 Faecal sample processing and steroid analysis

Faecal samples were lyophilized, pulverized and sieved through a mesh to remove existing indigestible material (Fieß et al. 1999). A weighed amount (0.10-0.11 g) of faecal powder was extracted by adding 3 ml of 80% ethanol in water and vortexed for 15 minutes. Thereafter, samples were centrifuged at 1500xg for 10 minutes. The supernatant formed was transferred into microcentrifuge tubes and stored at −20 °C for further analysis.

A subset of 14 samples from the translocation event (samples 72 hours prior (n = 3) and up to 5 days after; n = 11) were measured for faecal glucocorticoid metabolite (fGCM) concentrations using five enzyme immunoassays (EIAs): i) a 11β-hydroxyaetiocholanolone EIA (detecting fGCMs with a 5β,3α,11β-diol structure), ii) a 11-oxoaetiocholanolone I EIA (detecting 11,17-dioxoandrostanes), iii) a 11-oxoaetiocholanolone II EIA (detecting fGCMs with a 5β-3α-ol-11-one structure), iv) a 5α-pregnane-3β,11β,21-triol-20-one EIA (detecting fGCMs with a 5α-3β, 11β-diol

structure), and v) a Cortisol EIA. Detailed assay characteristics, including full descriptions of the assay components and cross-reactivities have been provided by Palme and Möstl (1997) for the 11-oxoaetiocholanolone I and Cortisol EIA, by Möstl *et al*. (2002) for the 11-oxoaetiocholanolone II EIA, by Touma *et al.* (2003) for the 5α-pregnane-3β,11β,21-triol-20-one EIA, and by Frigerio *et al.* (2004) for the 11β-hydroxyaetiocholanolone EIA. The sensitivities of the EIAs were 0.6 ng/g dry weight (DW) (Cortisol, 11-oxoaetiocholanolone I and 11-oxoaetiocholanolone II), 1.2 ng/g DW (11β-hydroxyaetiocholanolone), and 2.4 ng/g DW (5α-pregnane-3β,11β,21-triol-20-one), respectively. Intra-assay coefficients of variation (CV) of high- and low-concentration controls were 9.56 % and 12.56 % (Cortisol), 8.33 % and 8.65 % (11-oxoaetiocholanolone I), 6.41% and 10.30 % (11-oxoaetiocholanolone II), 6.67 % and 13.18 % (11β-hydroxyaetiocholanolone), 10.20 % and 10.42 % (5α-pregnane-3β,11β,21-triol-20-one), respectively. Subsequently, only the Cortisol and 11-oxoaetiocholanolone I EIAs were used for analysing the faecal extracts derived from the time-dependent alteration in fGCM concentration post-defecation experiment (see results section). Finally, the influence of defecation time and free-ranging otter sample fGCM concentrations were determined using the Cortisol EIA. The serial dilutions of faecal extracts gave displacement curves that were parallel to the respective standard curves, the relative variation of the slope of the trend lines, < 5% for the Cortisol EIA, < 3% for the 11-oxoaetiocholanolone I EIA. Inter-assay coefficients of variation of high- and low-concentration controls were 5.96 % and 6.90 % for the Cortisol, and 5.65 % and 6.11 % for the 11-oxoaetiocholanolone I EIA, respectively. All steroid concentrations are expressed per mass of faecal DW matter.

2.3.5 Data Analysis

All statistical analysis were conducted using the program R, with the use of the R Studio interface (R Core Team 2016). Suitable EIAs were identified based on the increase in fGCM concentration following translocation by calculating the percentage response based on the highest fGCM peak post translocation over the median baseline fGCM concentration calculated from samples collected over 72 h before the translocation (n = 3). To determine a time-dependent alteration in fGCM concentration post-defecation, fGCM concentrations for each subsample were expressed as percentages based on the triplicate mean value of subsamples exposed for 0 hours

(representing 100 %) for the respective EIA. The fGCM concentration percentages from 11-oxoaetiocholanolone I EIA were normally distributed. However, fGCM concentration percentages derived from the Cortisol EIA had a positive skewed distribution and large differences in variance of the data observed; and thus, were $\log_{10}$ transformed prior to statistical analysis. A repeated measures one-way analysis of variance (ANOVA) was used to test the difference between fGCM concentration at each time point in relation to t=0 for each EIA. Pair-wise t-tests, with a Bonferroni correction, were conducted post-hoc to determine which time treatments were significantly different from t=0. A student's t-test was also used to test the differences in fGCM concentrations between samples collected in the morning and evening, as well as for differences in fGCM concentrations between study areas (these data were also $\log_{10}$ transformed).

2.4 Results

2.4.1 Biological validation

From the five different EIAs tested, the Cortisol and 11-oxoaetiocholanolone I EIA revealed the highest transport-related response (74 % and 48 %, respectively). Peak fGCM levels were found 30 hours (second sample collected post-transport) and 24 hours (first sample collected post-transport) after transport for the two EIAs, respectively. The remaining three EIAs tested showed only a marginal to no elevation in fGCM concentrations a day after transport (Table 2.1, Figure 2.2).

Table 2.1:Summary of fGCM concentrations (μg/g DW) before (up to 72 h) and after (24 – 30 hours) translocation of a male African clawless otter determined with five different EIAs. Bold font indicates selected EIAs for subsequent analysis

EIA	**Cortisol**	**11-oxoaetiocholanolone I**	11-oxoaetiocholanolone II	11β-hydroxyaetiocholanolone	5α-pregnane-3β,11β,21-triol-20-one
Baseline median (μg/g DW)	**0.878**	**0.821**	0.357	0.595	0.178
Post translocation Peak (μg/g DW)	**1.536**	**1.216**	0.197	0.632	0.208
Response (%)	**174%**	**148%**	55%	106%	116%
Hours after translocation	**30**	**24**	30	24	24

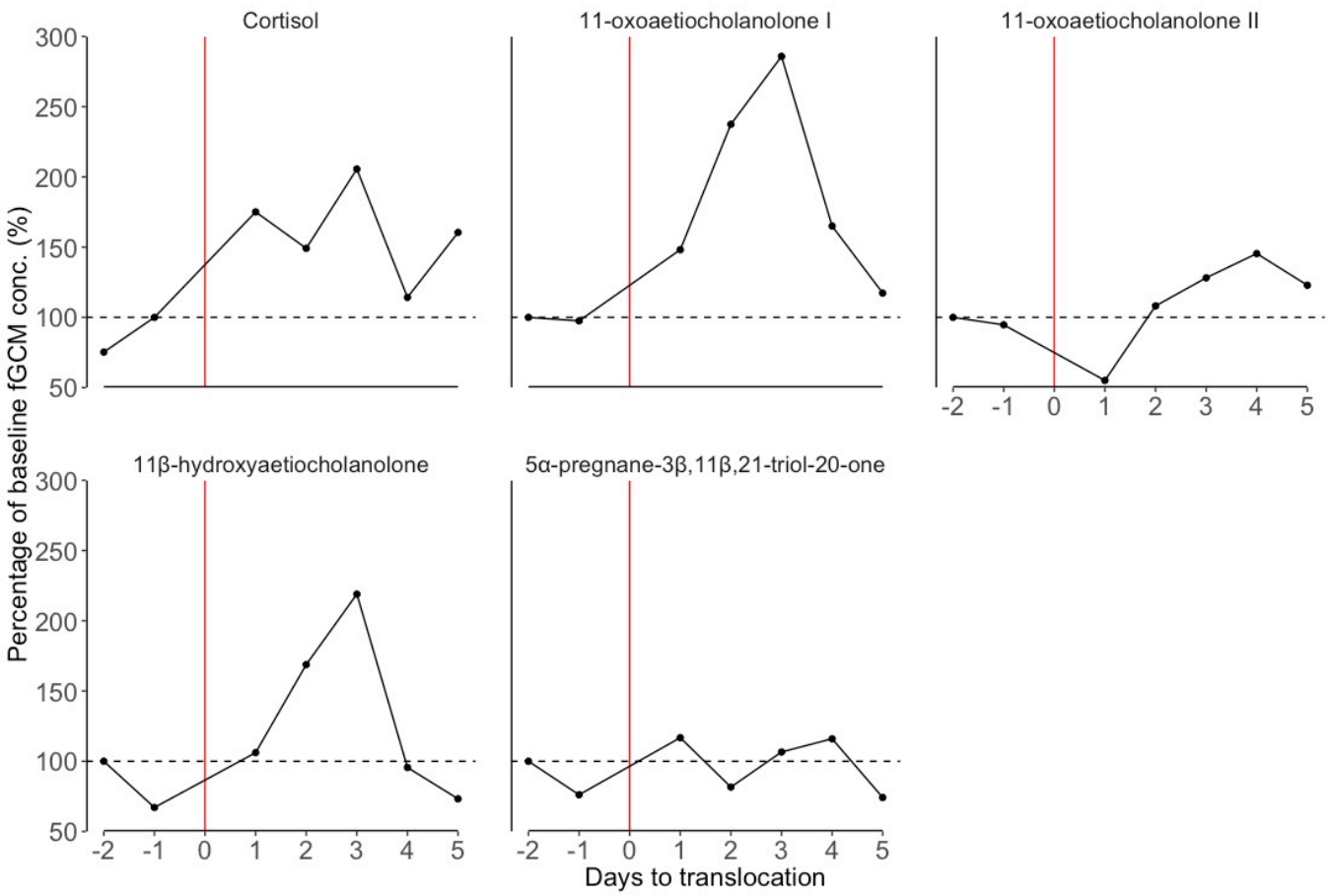

Figure 2.2: Median percentage change of African clawless otter fGCM concentrations (µg/g DW) in relation to baseline. Red line represents translocation event, dotted line indicates median baseline fGCM concentration before the translocation, and point represents maximum fGCM percentage change.

2.4.2 *A time-dependent alteration of fGCM concentrations post-defecation*

There was a significant change in the alteration of fGCM concentrations post-defecation over time for the Cortisol EIA ($F_{8,18}$ = 21.089, n = 27, p < 0.001) (Figure 2.3). The Cortisol EIA showed a significant (p = 0.009) 269% increase in fGCM concentrations after 12 hours (3.101 ± 0.057 µg/g DW). Concentrations peaked at a 505% increase and had high variability after 24 hours (p = 0.001, 4.251 ± 1.491 µg/g DW). Although not statistically significant (p = 1.000), there was already a 38% increase in fGCM concentration after 6 hours post-defecation (1.159 ± 0.136 µg/g DW).

There was also a significant change in the alteration of fGCM concentrations post-defecation over time for the 11-oxoaetiocholanolone I EIA ($F_{8,18}$ = 8.567, n = 27, p < 0.001) (Figure 2.3). At 48 hours, there was an 82% decrease in fGCM concentrations measured by 11-oxoaetiocholanolone I EIA, which was statistically significant (p = 0.018, 0.213 ± 0.124 µg/g DW). Although not statistically significant (p = 1.000), fGCM concentrations already decreased by 38% after 12 hours post-defecation (1.150 ± 0.545 µg/g DW), with higher variability in fGCM concentrations between triplicates already at 6 hours (SD = 48%).

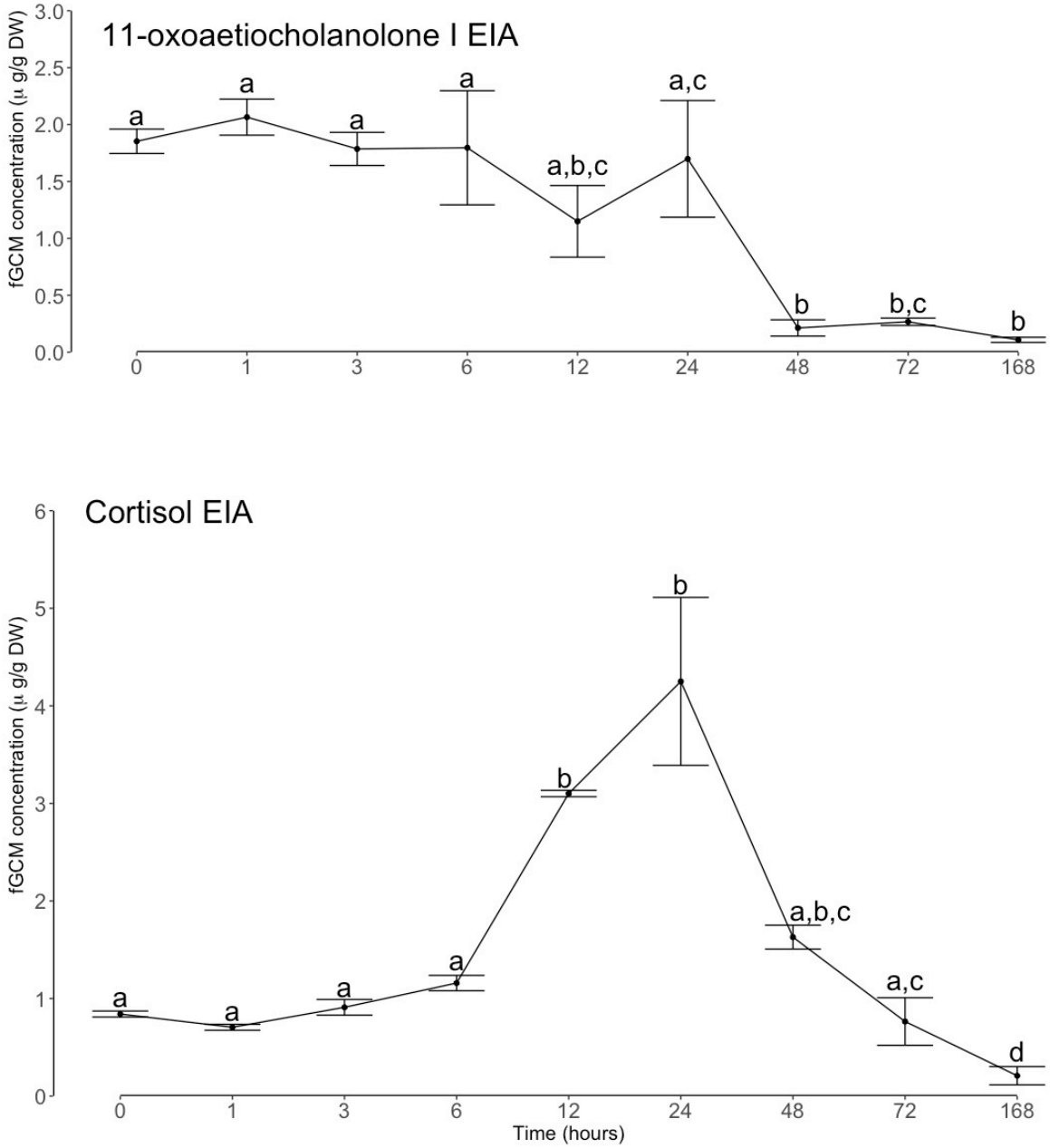

Figure 2.3: Time dependent change in African clawless otter fGCM concentrations (µg/g DW) post-defecation (0–168 hours). Point represents mean fGCM concentrations (µg/g DW), and lines above and below show mean ± SD. Different superscripts indicate statistically significant differences ($p < 0.05$) between time points.

2.4.3 Influence of defecation times

There was no significant difference ($F_{1,18} = 0.059$, $n = 20$, $p = 0.811$) in fGCM concentrations between fresh samples collected during the morning (mean ± SD = 0.704 ± 0.133 µg/g DW) and evening (mean ± SD = 0.660 ± 0.125 µg/g DW).

2.4.4 fGCM concentrations of African clawless otters in natural and transformed areas

There was no significant difference ($F_{1,15}$ = 1.433, n = 17, p = 0.250) between fGCM concentrations of otters from the two nature reserves; Cobham (n = 13, mean ± SD = 0.211 ± 0.202 µg/g DW) and Verloren Vallei (n = 4, mean ± SD = 0.346 ± 0.301 µg/g DW). Thus, the data from the two study sites were combined (here on referred to as "natural areas") for subsequent data analysis. Faecal samples collected from otters at Millstream ("transformed area", n = 20, mean ± SD = 0.468 ± 0.539 µg/g DW) had significantly higher ($F_{1,35}$ = 6.809, n = 37, p = 0.013) fGCM concentrations compared to those collected from animals at the natural areas (n = 17, mean ± SD = 0.242 ± 0.226 µg/g DW) (Figure 2.4).

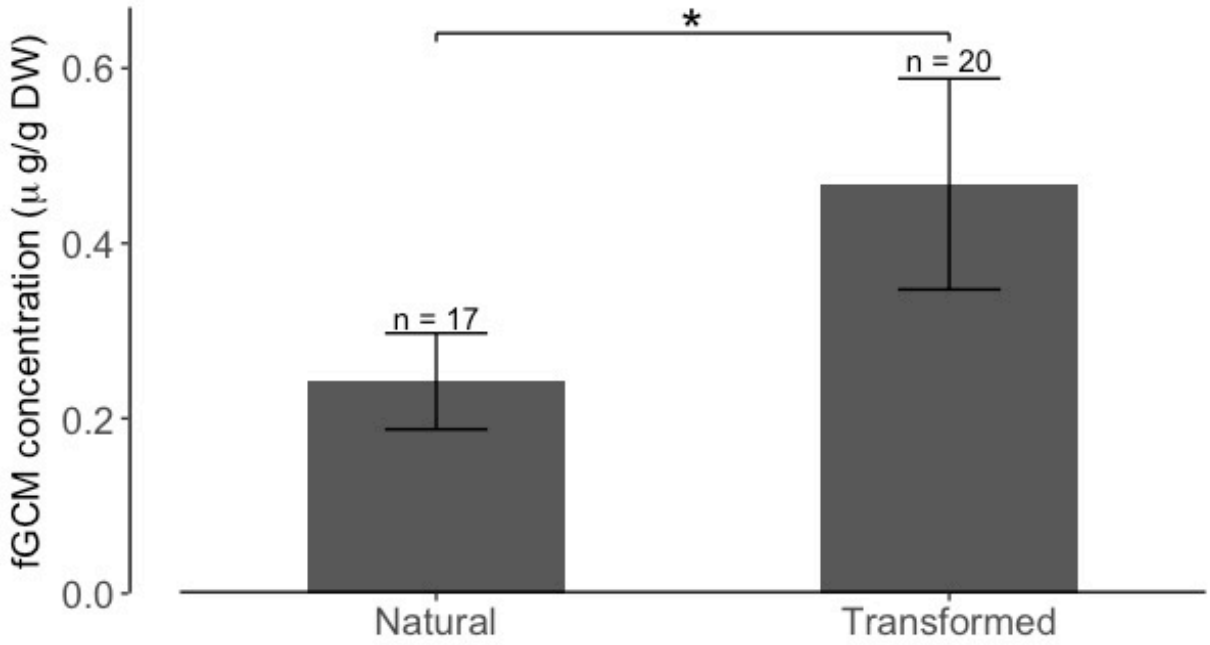

Figure 2.4: Comparison of fGCM concentrations of otters from Natural areas and the Transformed area. Box represents mean fGCM concentrations (µg/g DW), and lines above and below indicate the mean ± SD. Statistically significant difference ($p < 0.05$) represented by * and n denotes the number of samples.

2.5 Discussion

The results reported here firstly illustrate how a translocation event was successfully used as a biological stressor for examining the suitability of enzyme-immunoassays for measuring fGCM concentrations in African clawless otters. The study further shows

that an EIA utilizing an antibody against cortisol-3-CMO can be used to reliably measure fGCM concentrations in a male African clawless otter, and that the time of day for faecal sample collection may not affect fGCM concentrations. Further, limited changes in fGCM concentrations were evident in samples collected within a 3-hour post-defecation window, but became significantly different at time periods exceeding 6 hours post-defecation. When interpreted conservatively, this result suggests that samples should be collected within 3 hours of defecation to ensure reliable measurement of fGCMs. Lastly, fGCM concentrations of African clawless otters were significantly higher in a transformed area (Millstream farm) characterized by anthropogenic disturbance, when compared to natural areas (Verloren Vallei Nature Reserve and Cobham Nature Reserve) with minimal human presence. Thus, indicating that factors related to human dominated landscapes could inflict stress on African clawless otters reflected by an increase in their adrenocortical activity. Alternatively, differences in fCGM concentrations could be due to metabolic or dietary differences among habitats which might change concentrations in fGCMs but not circulating cortisol concentrations (Rangel-Negrin *et al.* 2009).

The validation of EIAs for reliably monitoring adrenocortical activity in mammals using alterations in fGCM concentrations is often achieved by performing an adrenocorticotropic hormone stimulation test (ACTH challenge) (Touma & Palme 2005; Ganswindt *et al.* 2012; Scheun *et al.* 2015; Webster *et al.* 2018; Jepsen *et al.* 2019). In addition to pharmacologically stimulating GC production, studies increasingly include biological validations such as individual separation (Ganswindt *et al.* 2012; Jepsen *et al.* 2019), reproductive suppression (Laver *et al.* 2012), or translocations (Goymann *et al.* 1999; Franceschini *et al.* 2008). Such presumed stressors, specifically translocation, are highly likely to induce a stress response (Dickens *et al.* 2010) and are therefore good opportunistic events for biologically validating a monitoring system like EIAs (Goymann *et al.* 1999; Rothschild *et al.* 2008; Dickens *et al.* 2010). Ideally, both a pharmacological and biological validation should be conducted. However, as highlighted by Palme (2019), ACTH challenge requires permission to conduct animal experimentation, which often are not easily granted due to endangered species regulations and/or lack of access to captive individuals. The results of this study affirm the usefulness of translocations as an alternative to an ACTH challenge for EIA validation. Application of high-pressure liquid chromatography

would be a helpful addition to assay validation to assess possible sex-related differences in fGCM composition excreted as well as potential co-measurement of androgen metabolites with fGCM assays tested.

The tested cortisol and 11-oxoaetiocholanolone I EIAs showed the highest transport related response 30 hours and 24 hours after the translocation, respectively. Similarly, Zalewski (2011) used an EIA with an antibody against cortisol for monitoring stress responses of North American river otter (*Lontra canadensis*) to ecological factors such as habitat quality. In other African mustelids, EIAs measuring 5β,3α,11β-diol structures or 11,17-dioxoandrostanes were used to determine changes in fGCM concentrations in meerkats, *Suricata suricatta*, (Goncalves *et al.* 2016) or banded mongooses, *Mungos mungo*, (Laver *et al.* 2012). These reports highlight the variation in the suitability of EIAs for monitoring alterations in fGCM concentration within the Mustelidae family, and underlines the importance of validating EIAs when applying fGCM monitoring to a species for the first time.

Using the Cortisol EIA, the alterations in fGCM concentrations seen post-defecation were characterized by a distinct temporary increase in fGCM concentrations. This resembled the pattern reported for African wild dogs, *Lycaon pictus*, (Crossey *et al.* 2018) and African buffalo, *Syncerus caffer,* using the 11,17 DOA EIA (Ganswindt *et al.* 2012). In contrast, the 11-oxoaetiocholanolone I EIA alteration showed a decrease in fGCM concentration similar to that of banded mongoose (Laver *et al.* 2012) and African elephants, *Loxodonta africana,* (Webber *et al.* 2018). The observed differences in the alteration in African clawless otter fGCM concentration post-defecation are possibly due to bacterial conversion. Bacterial conversion alters the composition of immunoreactive compounds within a sample, which then could cross-react differently with the antibodies utilized in the respective applied EIA(s) (Washburn & Millspaugh 2002; Lexen *et al.* 2008). However, further research similar to Lexen *et al.* (2008), including samples from more individuals, are necessary to better understand the effects of potential alteration in immunoreactive compound composition for fGCM monitoring in African clawless otter.

Apart from the differences in the alteration of fGCM concentrations seen over time when using the Cortisol and 11-oxoaetiocholanolone I EIA, there was comparatively

higher variation between triplicates at 6 – 72 hours post defecation compared to respective steroid levels of sample triplicates taken at the other sampling points. There are at least two possible reasons for the variation within triplicates: (1) sample material was not sufficiently mixed when conducting the experiment; or (2) there were different rates of bacterial conversion between triplicates as a consequence of varying environmental conditions due to sample placement (Webber *et al.* 2018; Webster *et al.* 2018). The first reason is a more likely cause for especially the 24 hour triplicates since high variance in fGCM concentrations can be seen when analysing respective samples with both, the Cortisol and 11-oxoaetiocholanolone I EIA. For triplicates taken at 6 and 12 hours post-defecation substantial variation was only evident when samples were analysed with the 11-oxoaetiocholanolone I EIA, whereas triplicates taken at 48 and 72 hours post-defecation revealed substantial variation only when using the Cortisol EIA for analysis. These EIA specific differences might be a result of different rates of bacterial conversion between triplicate samples, as variation in environmental conditions such as sunlight, temperature, and humidity are likely to affect the duration of bacterial enzyme activity, and consequently might affect immunoreactive compound composition within a sample (Webber *et al.* 2018; Webster *et al.* 2018). Since the exposure to certain environmental variables like sunlight might not have been fully standardized in this study, the higher variation found in some triplicates could be indicative of varying bacterial enzyme sensitivity between these samples, resulting in quantitative differences in respective immunoreactive compound compositions relevant only for the one or the other EIA. However, additional investigations exploring the effect of environmental conditions and time on fGCM concentrations in African clawless otters are necessary.

Although fGCM concentrations were stable (as indicated by no evidence for statistically significant differences) for up to 6 hours post defecation when measured with the Cortisol EIA, an overall difference in fGCM concentrations of 38% was recorded when comparing respective steroid levels between 3 and 6 hours. It is important to note a possible biological significance associated with such a change - for example, McLennan et al. (2019) found a 32% difference in fGCM concentrations between subadult and adult chimpanzees. Thus, taking potential biological meaningful alterations in fGCM concentrations into account, respective hormone metabolite levels of African clawless otters can be regarded as comparable when faeces as hormone

matrix has been collected within 3 hours post-defecation and fGCM concentrations were then determined with the described Cortisol EIA. Due to the predominant nocturnal behaviour of free-ranging African clawless otters, collecting samples that are fewer than 3 hours old (fresh), requires collecting from latrines as early as possible. To identify those samples, collectors should look out for anal jelly and water vapor when collecting material (T Majelantle, 2018, pers. obv.). Alternatively, since the results suggest that time of day potentially does not affect fGCM concentration, all faecal material present at a known latrine could be marked (e.g. with glitter as performed by Guertin *et al.* (2010)). Thereafter, the site should be checked every 3 hours, thereby ensuring all newly deposited samples (without glitter) are sufficiently fresh.

A notable limitation of our study is that our samples were limited to one male captive otter. Thus, confounding factors abound such as sex (Webster *et al.* 2018), age (McLennan *et al.* 2019), reproductive state (Wolf *et al.* 2018), and diet (Kalliokoski *et al.* 2012) could not be accounted for. Moreover, the results on potential influence of circadian rhythm on fGCM concentrations are further limited by possible differences in captive and wild African clawless otter daily activity patterns. Monitoring of additional captive otters would be needed to validate the fGCM assay more comprehensively.

African clawless otters in the transformed (anthropogenically disturbed) area were characterized by higher stress-related biomarkers compared to the otters monitored in natural areas. The difference in fGCM concentrations found is unlikely due to study design, chosen study sites, or season. Verloren Vallei Reserve and Millstream samples were collected within the same time within the same area, and thus the otters encountered the same broad environmental conditions. While Cobham samples were collected at a different location and time, the similarity between overall fGCM concentrations of these samples and those from Verloren Vallei support the approach to include data from Cobham in the study and combining the two data sets under the label ‘Natural areas’. However, since field samples were collected from unknown adult individuals, sex and reproductive status could not be accounted for (a limitation applicable to all study sites). Thus, the possibility of some bias due to the small sample sizes cannot be excluded, particularly at Verloren Vallei. Unfortunately, small sample sizes could be expected, due to the shy and elusive behaviour of African clawless

otters and the fresh sample requirement. Yet, samples from Millstream Farm had considerably higher fGCM concentrations than both natural sites (analysed separately as well as combined). Thus, it is likely that the disturbances associated with the transformed environment are responsible for elevated fGCM levels in African clawless otters. The significantly higher fGCM concentrations in transformed landscapes compared to natural areas is consistent with results reported for other species inhabiting both types of habitats. Examples of similar responses include studies on chimpanzees (McLennan *et al.* 2019), African lesser bushbabies, *Galago moholi*, (Scheun *et al.* 2015), European blackbirds, *Turdus merula*, and Northern mockingbirds, *Minus polyglottos*, (Fokidis *et al.* 2009).

Based on the findings of the monitored translocation, an acute stress response seems to reflect in otter faeces about 30 - 48 hours after the stress event. Since samples were collected from each study site for a minimum of 25 days, the conducted sampling period reduces the likelihood of collecting samples primarily after an acute stress response. Possible stressors identified on this site include human activity, predators (including encounters with humans and their pets), food availability, and intra- and inter-specific competition. Since there is very limited direct contact with humans in Millstream Farm (T Majelantle, 2018, pers. obv.) due to African clawless otter nocturnal behaviour, human presence is considered an unlikely stressor. Limited food availability and resulting low food intake is associated with increased glucocorticoid levels; e.g. in ring-tailed lemurs (*Lemur catta*), Pride (2005) found a direct negative relationship between food intake and glucocorticoid levels. Otters on Millstream Farm are known to forage on the stocked rainbow trout and therefore food is considered likely to be relatively abundant here. Conversely, increased food availability and differential food intake could result in higher fGCM concentrations due to higher levels of circulating GCs linked to differential metabolism (Kalliokoski *et al.* 2012). In addition there is evidence that excessive body mass can lead to the prolonged activation of the HPA axis and glucocorticoid secretion in humans (Björntorp & Rosmond 2000). Similar results were reported for African lesser bushbabies, where animals living in transformed landscapes have higher BMI and concomitant increased levels of glucocorticoids, compared to animals living in natural areas (Scheun *et al.* 2015). Further research is thus absolutely required to find out more about the possible

stressors, including behaviour, food intake and related factors such as weight, body mass index and agility rate in the different landscapes.

2.6 Conclusion

African clawless otters occurring in a transformed area appear to show relatively higher stress-related biomarkers within the study period possibly due to anthropogenic disturbance. The ability to reliably validate adrenocortical function via fGCM monitoring in a male African clawless otter now provides a promising non-invasive tool to further investigate endocrine responses to putative stressful circumstances in this species in both captive and free-ranging settings.

CHAPTER 3: INCREASED POPULATION DENSITY AND BEHAVIOURAL FLEXIBILITY OF AFRICAN CLAWLESS OTTERS (*AONYX CAPENSIS*) IN RESOURCE-RICH ANTHROPOGENIC ENVIRONMENTS

3.1 Abstract

Land transformation for anthropogenic use is the leading cause of species declines globally. However, few species are able to succeed in anthropogenically disturbed environments and this ability is strongly linked to their behavioural plasticity. African clawless otters (*Aonyx capensis*) occur in a wide variety of habitats, and thus are good model species to investigate animal adaptions to anthropogenic environments. Therefore, we aimed to estimate and compare population densities, group size, and activity time of African clawless otters occurring in a transformed area, Millstream Farm, and natural areas, Verloren Vallei Nature Reserve and Cobham Nature Reserve. Camera trap arrays, consisting of between 18 and 24 cameras, were placed on all three sites, recording otter presence for a total of 2439 camera days. Using a random encounter model approach, the transformed area was estimated to have the highest density of African clawless otters (8.2 ± 2.3 km^{-2}), whereas otter densities at Verloren Vallei and Cobham Nature Reserve (natural areas) were estimated at 0.7 ± 0.2 km^{-2} and 2.1 ± 0.6 km^{-2}, respectively. There was a significant difference ($p = 0.007$) between group sizes in the transformed area (detections = 112; group size range = 1 – 5) and natural areas (detections = 29; group size range = 1 – 3). Furthermore, there was a significant difference in otter activity time ($p = 0.033$, activity overlap = 66.5 ± 8.33 %) between Verloren Vallei Nature Reserve and Millstream Farm. This study illustrates how African Clawless exhibit behavioural plasticity, occurring in greater densities, forming larger groups, and concentrating activity times in order to exploit a resource-rich anthropogenic environment. The results have implications for the understanding of human-otter conflict scenarios as well as potential otter conservation measures.

This data chapter has been submitted as a full article for publication in Animal Behaviour

3.2 Introduction

Good estimates of animal population density are important in ecology and conservation studies because they are essential to understanding population dynamics and factors such as probability of survival, density dependent population growth, probability of local extinction, or sensitivity to stochastic processes (Wright & Hubbell 1983). Estimates of animal population density can also be linked to habitat quality, trophic level occupied, and even individual body mass (Connor *et al.* 2000). Development of effective conservation and management strategies hinge upon knowledge of population size and change. As human-induced alterations to habitat increases, improved knowledge of animal population densities and how these may respond to anthropogenic pressure is increasingly important (Everatt *et al.* 2014). Urban and other human settled areas are becoming increasingly important for some wildlife, but often result in marked behavioural and/or life-history adaptations (Ditchkoff *et al.* 2006). Individual African clawless otters (*Aonyx capensis*) have significantly higher faecal glucocorticoid metabolite concentrations (applied as a measure of stress) in transformed areas characterized by anthropogenic disturbance, compared to those in natural areas (Majelantle *et al*. under review). However, since food availability seems the most important factor determining African clawless otter presence in a given habitat (Somers & Nel 2004; Nel & Somers 2007), they are also expected to exploit urbanized areas with rich resource patches (Ponsonby & Schwaibold 2019).

Previously, density estimates of African clawless otter populations were based on their characteristic signs (holts, rolling places and tracks) and deposited faeces at latrines (Rowe-Rowe 1992). Using this method, reported African clawless otter population density estimates in coastal habitats were of the order of one otter per 2 km of coastline (Arden-Clarke 1986) and estimates of density in freshwater habitats varied between one otter per 1.25 and one otter per 2.5 km of river (Perrin & Carugati 2000, 2006). The IUCN up-listed the status of African clawless otters to near threatened due to reported population declines by Somers & Nel (2013) and Kubheka *et al.* (2013), as well as general continent-wide declines in river water quality (Jacques *et al.* 2015; Okes *et al.* 2016). However, the precision and accuracy of the aforementioned population density estimates approaches have not been verified for African clawless

otters and may underestimate the number of individuals in areas with high otter density (Ruiz-Olmo *et al.* 2001). Using sign for surveying populations has potential variability and bias due to field workers' experience, season, age and sex related differences in behaviour, as well as differences in habitat and landscape (Wilson & Delahay 2001). This is also true for otters, although surveys of otter sign and spraints are generally useful in the broad identification of population status of otters, after the seasonal cycle of sprainting activity and periods of heavy rain have been accounted for (Mason & Macdonald 1987).

There are alternative non-invasive methods for estimating population density in otters. These include population genetics which use species specific sets of DNA primers to identify the number of unique genotypes within a sample area and thus measures relative population abundance (Deiner *et al.* 2017). DNA samples are extracted from material left behind by the animals, such as faeces and hair (Hájková *et al.* 2009), or by hair sampling techniques such as hair snares (Depue & Ben-David 2007). Recently, genetic techniques differentiating between African clawless otters and spotted-necked otters (*Hydrictis maculicollis*) faecal samples were developed by Madisha *et al.* (2015). In addition, 10 microsatellite primers developed for Eurasian otter (*Lutra lutra*) was successfully applied to African clawless otter population genetics (Ponsonby *et al.* 2019). Although this method gives more accurate population density estimates (Hájková *et al.* 2009), the method is labour intensive and comparatively expensive. Remote photography offers an alternative, rapid and relatively cost effective tool to estimate animal population density (Cutler & Swann 1999). Moreover, remote photography can provide additional data such as activity time, in addition to density estimates (Rowcliffe *et al.* 2014).

Established methods for estimating animal population density from remote photography data typically require additional information. For example, capture-mark-recapture models, or capture-resight models as applied to camera-trap data, require each individual to be uniquely marked and easily recognisable (Karanth 1995), while distance models require estimates on how far individuals are from the detection devices (Barlow & Taylor 2005). Random encounter models (REM) are a modification of the ideal gas theory and do not require uniquely identifiable individuals (Rowcliffe *et al.* 2008). However, REMs assume that: the movement of target species is random,

detections represent independent contacts between cameras and animals, and the population is closed (Rowcliffe *et al.* 2008). While target species generally do not move in a random fashion, by carefully placing camera trapping stations to allow for random encounter opportunities (e.g. by placing them in suitable habitat, but avoiding areas likely to be used preferentially such as food – or water sources) can be used to approximate the random movement assumption (Rowcliffe *et al.* 2013). This model approach is a well validated method which has performed better than dung counts (Pfeffer *et al.* 2018) and line transects (Zero *et al.* 2013) with ungulate species, for example the Lesser oriental chevrotain (*Tragulus javanicus*) (Gray 2018), and small carnivores, for example European pine marten (*Martes martes*) (Manzo *et al.* 2012).

Here, the aim was to estimate and compare African clawless otter population densities in a transformed and two natural areas. Since African clawless otters are not individually identifiable from images, population densities were estimated by applying a random encounter model approach. In addition, group size and activity time were determined and compared between the two land use types.

3.3 Materials and Methods

3.3.1 Ethical Note

The study was performed under the approval of the University of Pretoria Animal Use and Care Committee (Project number EC012-18) and the University of KwaZulu Natal Animal Research Ethics Committee (Project number AREC/033/018).

3.3.2 Study Areas

Cobham Nature Reserve (29°41'58.8"S, 29°24'50.3"E) and Verloren Vallei Nature Reserve (25°19'10.9"S, 30°07'38.8"E) are natural areas with low direct anthropogenic disturbance (Figure 3.1). Cobham Nature Reserve, in the Southern Maloti-Drakensberg Park, KwaZulu Natal, South Africa, has two main vegetation zones, namely Alti-montane and Afro-montane grassland and is 520 km^2 in area. Camera traps were set up along the Polela River. This area has an annual average rainfall of 800 mm and temperatures range between -15 °C and 35 °C. The Verloren Vallei Nature Reserve (Verloren Vallei) in Mpumalanga, South Africa, is in the Highveld montane grassland and has several permanent wetlands. Camera traps were placed

along multiple drainage lines and streams. This area has an annual mean rainfall of 664 mm and temperatures range between -13 ˚C and 21.9 ˚C. Millstream Farm (25°27'07.3"S 30°05'30.7"E), situated in the Mpumalanga Highveld, South Africa, is a transformed area, with substantial anthropogenic disturbance. The site has both accommodation and recreational fly-fishing, and is thus characterized by year-round, persistent human presence (permanent inhabitants and visiting anglers) (Figure 3.1). The Farm has eight dams and 13 weirs along the Witpoort River (all stocked with rainbow trout, *Oncorhynchus mykiss*) where camera traps were placed. The Farm is 21 km from Verloren Vallei, and the sites have similar climatic conditions.

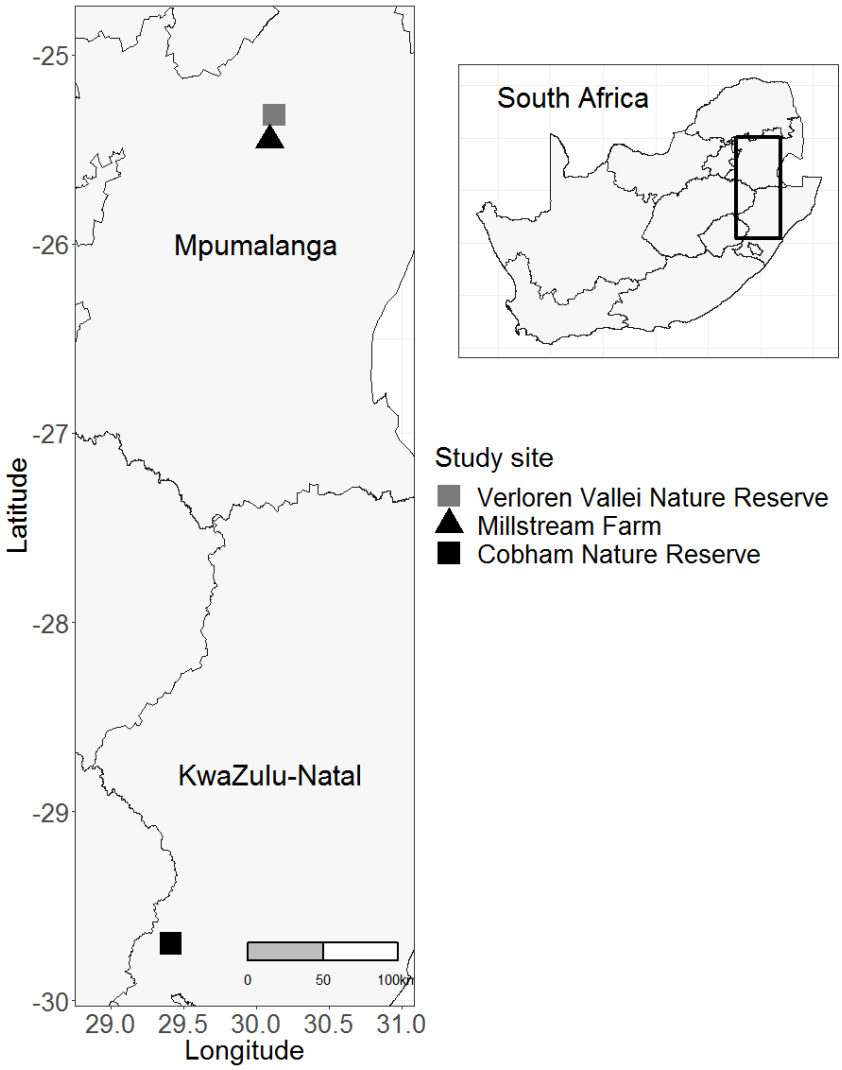

Figure 3.1: Locations where camera traps were placed from 8 June – 1 November 2018. Square points = natural areas, Triangle point = transformed area.

3.3.3 Camera trap detectability trails

To determine the optimum ranges and angles at which camera traps (PRIMOS ProofCam 3) would detect animals, *ex-situ* field trials were conducted. Further, these trials provided data that were used to calculate the probability of detection at a range of different heights and distances. Camera traps were set on poles at different heights (20, 40, 60, 80 and 100 cm) from the ground surface. The camera traps at heights 80 cm and 100 cm were tilted slightly downwards to capture as much of a predefined, demarcated area as possible. Distances from the camera traps were marked out with tennis balls placed 2m, 4m, 6m, 8m and 10m from the camera traps and at angles of 0°, 90˚, 45˚, 22.5˚ and 11.25˚ from the camera traps. For each trial, a mobile object (remote controlled car with a stuffed toy animal covered with a hot water bottle mounted atop) of similar size to an African clawless otter (length = 105 cm, height = 35 cm) was manoeuvred in an arc of known distance and angle marked by the tennis balls (McIntyre *et al. in press*). One photographic image with or without the mobile object represented "detection" during a trial, while inclusion of at least half of the mobile object represented "capture". The first and last capture of the mobile object was used to calculate the camera angle (θ) in each trial.

3.3.4 Field Camera Traps

Camera traps were set at Cobham (n = 24), Verloren Vallei (n = 22), and Millstream Farm (n = 18) between 26 June and 18 August 2018, and between 9 October and 27 November 2018 for a total of 2439.5 camera days (Table 3.1). As African clawless otters rarely venture more than 15 m from fresh water sources (Rowe-Rowe 1992; Larivière 2001), cameras were positioned within 15 m of, and pointed towards, water sources at each site. In addition, locations where the slope of the riverbank was accessible to otters were used. Camera traps were deployed between 200 m and 300 m apart and mounted on wooden stakes at heights of between 20 cm and 100 cm. The camera traps recorded bursts of three images when triggered, with a 30 second delay between trigger events. Each camera trap placement represented a camera station. At each camera station, the presence and absence of tracks and otter latrines within a 5 m radius of the camera station were noted along with camera trap placement height. Distances within the field of view of each camera were calibrated and marked by displaying distance labels (1 m intervals between 1 m and 6 m).

Table 3.1: Summary of camera trap deployments for each study site: n = number of camera traps, p = number of camera trap placements, Time = total number of camera days, Duration = camera trap deployment period.

Study Area	n	p	Duration	Time (Days)
Millstream	18	18	26 June – 18 August 2018	654.1
Verloren Vallei	22	24	28 June – 19 August 2018	950.8
Cobham	24	24	9 October – 27 November 2018	834.7

3.3.5 Data Analyses

All camera trap images were processed manually. When an African clawless otter was identified in an image, the study site, camera station, date, time and group size were recorded. Based on the African clawless otter average movement speed (Appendix A) multiple records of the same species at the same camera station were considered independent when images were taken at least 30 min apart.

Under field conditions, the probability of detecting an African clawless otter (when one is present) is not 1 due to variation between camera traps, distance, speed, and temperature (McIntyre *et al. in press*). In this study, otter detection probabilities were likely further influenced by differences in physical and environmental variables among study sites. The influence of latrine and track presence, camera trap height and study area on the probability of any individual camera trap station recording otter presence over the course of a survey was quantified by applying a binomial linear mixed effects model with the total time each camera trap was active as random effect. The model included variables such that:

Detection ~ latrine + track + camera placement height + study area + (1 | time active)

where; Detection = whether an individual camera trap recorded otter presence in a study area with confirmed otters present (0 or 1), latrine = presence/absence of an otter latrine within a 5 m radius of the camera station, track = presence/absence of

animal track within a 5 m radius of the camera station, time active = camera station total time active in days.

African clawless otter density (D km^{-2}) was calculated using a random effects model (REM) that considers density as a function of trapping rate, animal speed and the dimensions of the camera detection zone (Rowcliffe *et al*. 2008) such that:

$$D = \frac{y}{t} \times \frac{\pi}{Vr\,(2 + \theta)}$$

where; y = the number of independent photographs of African clawless otters, t = the total number of camera days (survey effort), V = the average speed of animal movement (calculated as distance, in km, travelled per day), r = the detection distance (radius) of the camera trap (km), and θ = the angle of the camera trap detection zone (radians). The average speed of otter movement was estimated from movement data on African clawless otters (Somers & Nel 2004) and from unpublished telemetry data of otters on Millstream Farm, as 8.278 km/day (Appendix A). The detection distance was the estimated maximum distance that African clawless otters were detected from camera traps, relative to the marked distances in the camera trap fields of view. Non-parametric bootstrapping with 10 000 iterations was used to obtain 95% confidence limits and standard deviation. Thereafter, the respective probabilities associated with each study site was accounted for by dividing the density estimate by the respective probability of detection to account for REM assumption of equal detection probability within a camera detection range and random movement of species (Rowcliffe *et al.* 2008).

Group sizes of African clawless otters among study sites data was quantitative discrete and thus was compared using a non-parametric Mann-Whitney U test. To compare activity time between sites, previously published definitions (Gómez *et al*. 2005) to classify otters as diurnal (<10% of records at night), nocturnal (≥ 90% of records at night), mostly diurnal (10 – 29% of records at night), mostly nocturnal (70% - 89% of records at night), or cathemeral (30 -69% of records at night) were followed. As the activity time data had a circular distribution, small sample size and was non-parametric, the Wheeler-Watson test was used to test for significant differences

between sites. Otter daily activity pattern was determined using a non-parametric circular kernel-density function (see Ridout & Linkie 2009), and measured the extent of a coefficient of overlap (Δ) ranging between 0 and 1 was used to measure the extend overlap between two kernel-density estimates (Ridout & Linkie 2009; Meredith & Ridout 2014). The overlap was assumed as the area lying under both density curves. Since sample sizes were small (<75),the smoothing constant Δ_4 was used (Ridout & Linkie 2009; Meredith & Ridout 2014). Three Verloren Vallei African clawless otter detections were discarded for activity period analysis due to incorrect date stamps caused by camera trap failure. All statistical analysis were conducted using the program R, with the use of the R Studio interface (R Core Team 2016).

3.4 Results

There was no significant relationship between camera height and the probability of any individual camera trap recording African clawless otter presence ($\chi^2 = 0.544$, df = 1, p = 0.461). This probability was also not significantly different between locations where tracks where present or not ($\chi^2 = 0.023$, df = 1, p = 0.881) or where otter latrines were present or not ($\chi^2 = 0.619$, df = +1, p = 0.432). However, the probability of recording African clawless otter presence differed significantly among study areas ($\chi^2 = 7.151$, df = 2, p = 0.028; Table 3.2).

Table 3.2: The probability of individual camera traps detecting African clawless otter presence during the course of a survey in each study area. P = probability of detection, CI = 95 % confidence interval, SD = Standard deviation.

Study Area	P	CI	SD
Cobham	0.442	0.226 – 0.682	0.544
Verloren Vallei	0.423	0.202 - 0.681	0.510
Millstream Farm	0.856	0.511 - 0.971	0.887

3.4.1 Density

There were 10, 19, and 112 African clawless otter detections from Cobham, Verloren Vallei, and Millstream Farm, respectively. Millstream Farm had the highest density of African clawless otters, which was 12- and four-fold higher than Cobham and Verloren Vallei, respectively (Table 3.3).

Table 3.3: Random encounter model estimation of African clawless otter densities in each study site, SD = Standard deviation

Study Area	Density (km^{-2})	SD
Cobham	0.729	0.235
Verloren Vallei	2.067	0.824
Millstream Farm	8.248	2.368

3.4.2 Group Size

Group sizes for African clawless otters at Cobham (1.1 ± 0.3; group size range: 1 – 2; detections = 10) did not differ significantly from Verloren Vallei (1.1 ± 0.5; group size range = 1 – 3; detections = 19) (W = 99, df = 1, p = 0.715). Group size data from these two sites were therefore combined into “natural areas”. However, group sizes on Millstream (“transformed area”) (1.6 ± 0.9; group size range = 1 – 5; detections = 112) were significantly larger than the natural areas (1.1 ± 0.4; group size range = 1 – 3; detections = 29) (W = 1045, df = 1, p = 0.007) (Figure 3.2).

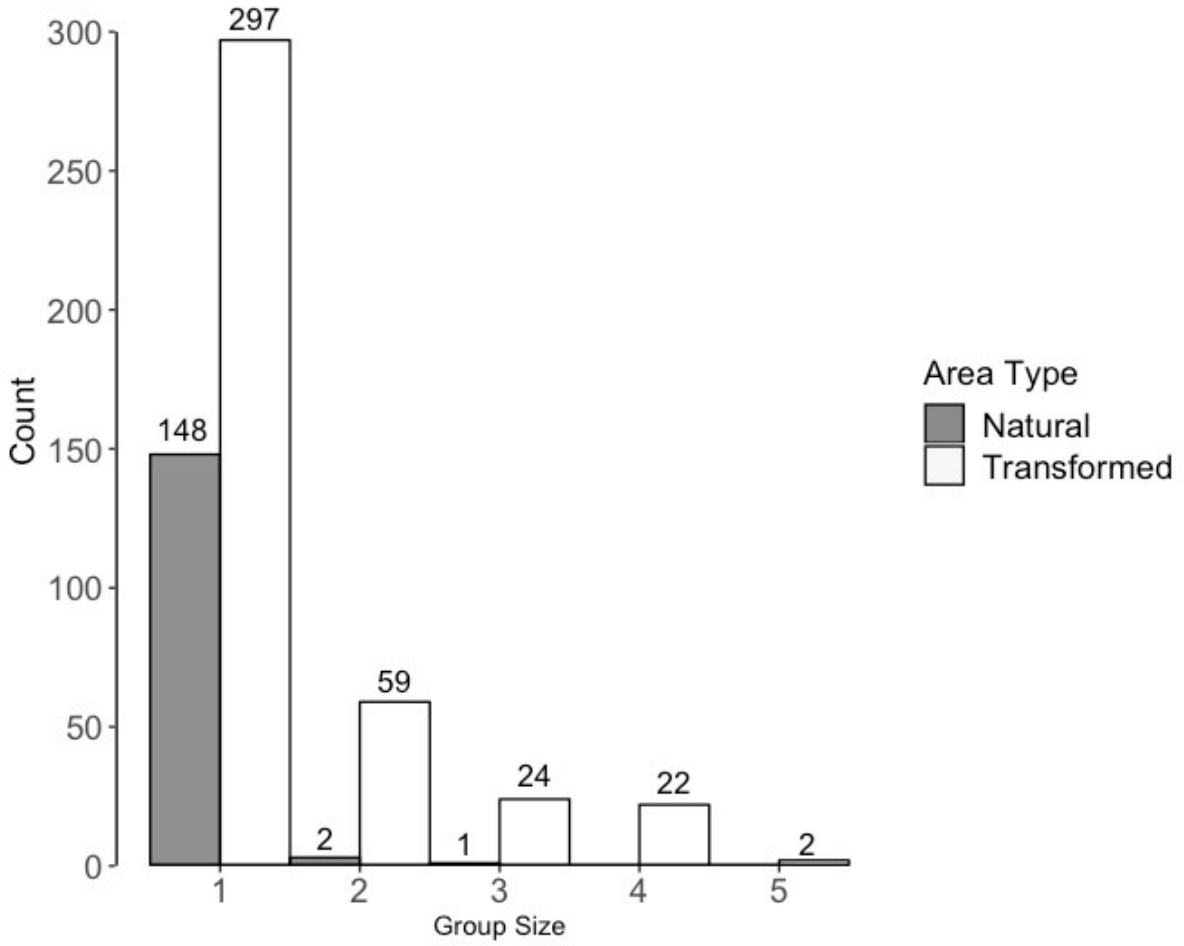

Figure 3.2: Histogram for African clawless otter group sizes recorded with camera traps at the transformed area (Millstream Farm) and the natural areas (Cobham and Verloren Vallei).

3.4.3 Time of activity

Seventy-seven percent of the Verloren Vallei otter records were obtained at night (between 18h00 and 05h00), and this population was therefore classified as "mostly nocturnal". In contrast, 90% of the Cobham and 91% of the Millstream otter records were obtained at night and these populations were therefore classified as "nocturnal" (Figure 3.3). There was a significant difference in otter activity time ($p = 0.038$, activity overlap = 67.2 ± 8.3 %) between Verloren Vallei and Millstream Farm. There was no significant difference in African clawless otter activity time between Cobham and Verloren Vallei ($p = 0.533$, activity overlap = 54.2 ± 12.1 %), and Cobham and Millstream Farm ($p = 0.088$, activity overlap = 55.8 ± 11.2 %).

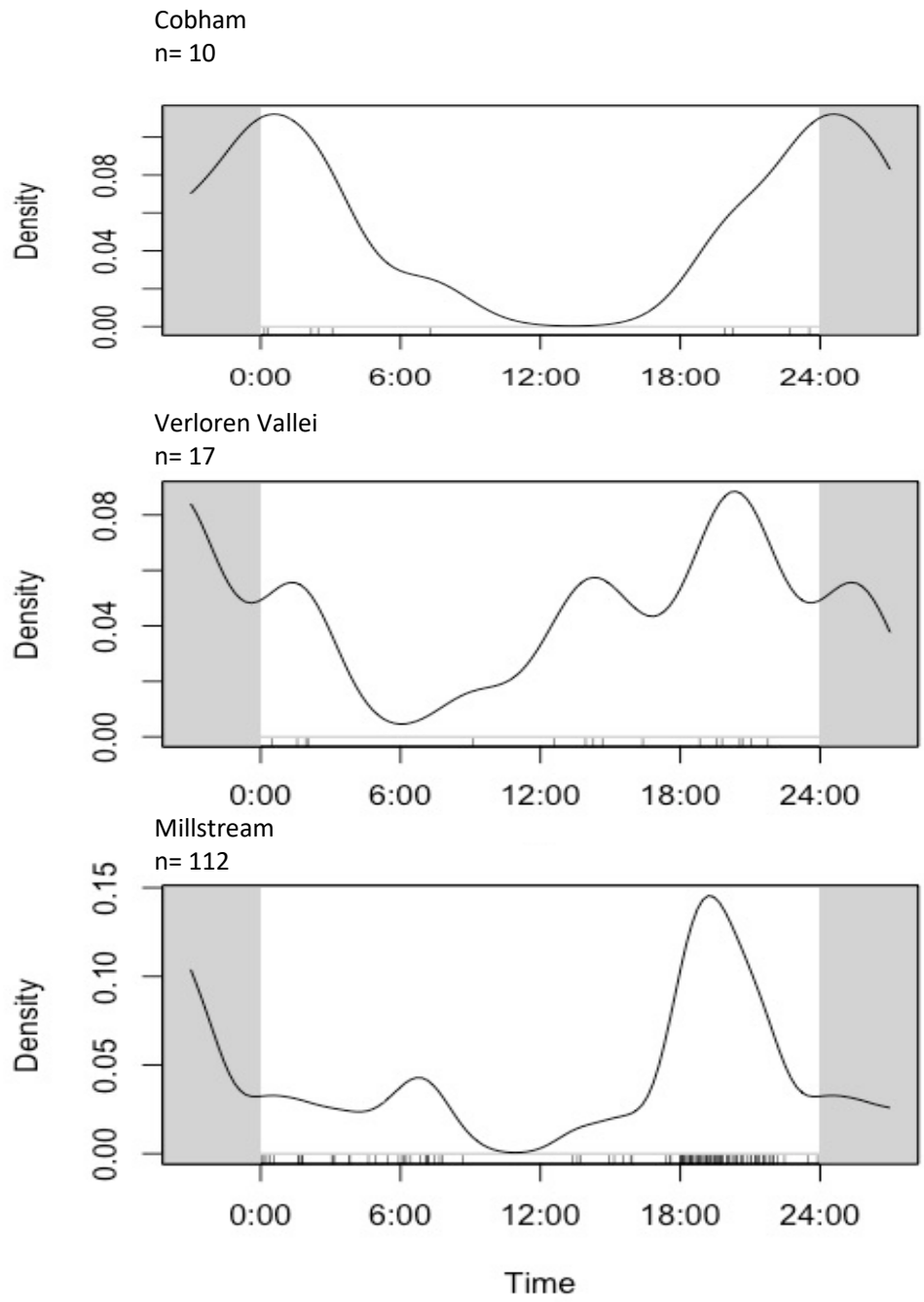

Figure 3.3: Fitted kernel density curve for African clawless otter activity recorded with camera traps at three different sites. n = number of records.

3.5 Discussion

The study reports density estimates for African clawless otters determined from random encounter models applied to camera trapping surveys. The density estimates indicated that African clawless otters occur in considerably higher densities at the more disturbed site of Millstream Farm, compared to natural areas with minimal anthropogenic disturbance at two other sites (Verloren Vallei and Cobham). Moreover, otter group sizes differed significantly between the land use types, with otters typically moving in larger groups more frequently at Millstream Farm. Activity time only differed between otters at Millstream Farm and those at Verloren Vallei.

Based on otter sign surveys, previous estimates of African clawless otter population density in freshwater habitats such as the Drakensburg have been estimated at 1 otter / 2.5 km (Perrin & Carugati 2006). When converting the data to the same metric as Perrin & Carugati (2006), the otter density estimates at Verloren Vallei of 3.6 otters / 2.5 km, and Cobham estimates of 2.1 otters / 2.5 km. While an increase in population density between the study periods (between 2006 and 2018) cannot be excluded, it is likely that the sign-based surveys result in a general underestimation of otter densities in this general study area (Ruiz-Olmo *et al.* 2001). The results were potentially influenced by some REM model assumptions not being met. For example, it is not certain that the populations of African clawless otters are closed. However, there was no evidence, at least in the case of Millstream Farm where otters were instrumented, to suggest that resident otters regularly disperse to areas outside of the general study areas. Furthermore, camera trap placements were focused on areas considered to be accessible to otters, potentially violating the REM model assumption of camera trap placements that are random with respect to animal movement (Cusack *et al.* 2015). This bias was also considered likely to be minimal, since otter detections were independent of any signs of activity in the current study.

Collectively, results suggest that African clawless otter occurrence in transformed areas is not dependent on human activity alone, but a combination of factors such as resource availability and other anthropogenic disturbance factors such as pollution. African clawless otters occur in natural areas in the Nama Karoo ecosystem of South Africa's Western Cape, but were not found in adjacent areas transformed for

agricultural purposes because the otters were probably limited by fresh water sources and this farmland was characterised by dry riverbeds (Drouilly & O'Riain 2019). Similarly, in the Gauteng Province African clawless otters were present in areas transformed for settlement purposes (urban and peri-urban areas), but spraint site density estimates in these areas were lower than where human activity was minimal (Ponsonby & Schwaibold 2019). In the Cape Peninsula region of the Western Cape, African clawless otter presence was not influenced by proximity to urban areas at the landscape scale, but otters preferred non-canalized sections of river with low pollution (Okes & O'Riain 2017). This is consistent with the findings that reported that river otter (*Lontra canadensis*) occupancy is not determined by land use type but the availability of vegetative cover and freshwater sources (Hanrahan *et al.* 2019). In this study, African clawless otters occur in much higher densities on Millstream Farm, compared to the natural areas surveyed here likely due to the availability of vegetative cover and resource rich anthropogenic food resources.

The high densities of otters found on Millstream Farm were most likely due to rich patches of food associated with anthropogenic disturbance (e.g. stocked trout), rather than other land transformation types. Similar results have been reported elsewhere for smooth coated otter (*Lutra perspicillata*) and Eurasian otter (*Lutra lutra*) displaying high population densities and visitation rates near fish farms despite frequent anthropogenic disturbance, and presumably due to the rich patches of artificial resources (Anoop & Hussain 2004; Freitas *et al.* 2007). Neotropical otters (*Lontra longicaudis*) occur more frequently in the vicinity of fishing gear and boats, where they are reported to exploit fish trapped by fishing gear (Andrade *et al.* 2019). Recent evidence suggests that African clawless otters show substantial dietary-linked behavioural plasticity (Jordaan *et al.* 2019). This likely allows them to exploit anthropogenically disturbed environments where high densities of prey are available, like that reported here.

Resource availability may also influence African clawless otter social organisation, as is evidenced by the larger group sizes reported here at Millstream Farm compared to the natural areas. Similar results have been reported for river otters adjusting their social organisation to improve foraging efficiency (Hanrahan *et al.* 2019). These results accord with group size alterations predicted by the resource dispersion

hypothesis proposed by Macdonald (1983) and Carr & Macdonald (1986). Accordingly, animals independently adjust their territory and group sizes, based on the frequency distribution (in both space and time) of the food sources available. For example, African striped mouse (*Rhabdomys pumilio)* female home ranges decrease when provisioned food resources increase (Schoepf *et al.* 2015) and African lion (*Panthera leo*) maximum group size, in turn, is determined by patch richness (Valeix *et al.* 2012).

African clawless otters are considered mainly crepuscular (active during dawn and dusk) (Somers & Nel 2004). However, the current results show that otters on Millstream Farm were nocturnal, as most detections occurred between 18h00 and midnight. Otters at Verloren Vallei were mostly nocturnal in their activity patterns, with 76% of detections at night, and the rest of activity spread throughout the day. The difference in activity time on Millstream is possibly due to the otters avoiding direct encounters with humans who were generally not active at night there, or due to differences in prey activity and accessibility. The peak activity time between 18h00 and midnight may suggest that the otters at Millstream achieved their required daily energy intake faster than otters in the natural areas due to the artificially high prey base. American black bears (*Ursus americanus*), in urban areas also shift their activity times from crepuscular to nocturnal and are active for shorter durations to avoid direct contact with humans and take advantage of anthropogenic food sources (Beckmann & Berger 2003).

3.6 Conclusion

Behavioural plasticity is strongly linked to an animals' ability to succeed in anthropogenically disturbed environments (Lowry *et al.* 2013; Sol *et al.* 2013). This study highlights how African clawless otters adjust their population density, group size, and activity times, suggesting behavioural plasticity allows them to exploit an anthropogenic landscape providing new food sources. The ability of otters to exploit such environments provides new possibilities for otter conservation, given the increasing concern about global population declines (Jacques *et al.* 2015; Okes *et al.* 2016). However, the African clawless otter ability to exploit resource rich anthropogenic enviroments such as trout farms is also linked to human-otter conflict

(de Vos 2018), and so ethical mitigation measures may also be required for successful conservation and management.

CHAPTER 4: GENERAL CONCLUSION

The current expansion of the human population increases the demand for land transformation (Meyer & Turner 1992; Ramankutty *et al.* 2002), especially in rapidly urbanising regions such as Africa and Asia (McDonald *et al.* 2008). While continuing urbanisation increases the risk of survival of some vertebrates species (Woodroffe 2000; McKinney 2001), information on related physiological and behavioural alterations, and population status of animals that are able to exploit transformed landscapes is lacking in Africa (Magle *et al.* 2012). This is an important knowledge gap because the continent has different biomes and diversity when compared to the relatively extensively studied areas in the northern hemisphere (Magle *et al.* 2012). In addition, studies on the biology of urban exploiters and how they adapt to transformed areas will allow prediction of further changes in wildlife in anticipation for future expansion (Bateman & Fleming 2012).

African clawless otters (*Aonyx capensis*) were selected as a model species for studying phenotypic adaptability and potential fitness costs associated with living in an anthropogenic environment. The availability of artificial anthropogenic food sources (Chapman *et al.* 2015) and behavioural plasticity (Sol *et al.* 2013) is linked to an urban adapter's success in a transformed landscape and these otters display a broad distribution encompassing a variety of habitats (both inland and coastal) and climate regimes (Somers & Nel 2013). In addition, recent studies suggest that human presence and activity does not affect the probability of their occupancy (Okes *et al.* 2016), thus they are able to utilize resources provided by anthropogenic landscapes (Ponsonby *et al.* 2019). Finally, African clawless otters show differences in diet over a spatial and temporal scale possibly to exploit the abundance and behaviour of their prey (Verwoerd 1987; Somers 2000; Jordaan *et al.* 2015).

Herein, the aim was to examine and compare the stress-related endocrine response, population density, and behaviour (daily activity pattern and group size) of African clawless otters from a transformed area (Millstream Farm) and natural areas (Cobham nature reserve and Verloren Vallei nature reserve). The shy and elusive behaviour of African clawless otters (Macdonald & Mason 1983) made the application of non-invasive techniques and indirect measurements for their monitoring imperative. Thus,

faecal glucocorticoid metabolites (fGCM) concentrations were used as an indicator of stress and animal welfare (Chapter 2). In addition, using camera trap data, random encounter models were used to calculate African clawless otter density (Chapter 3). Finally, otter activity patterns and group sizes were compared between the two land use types (Chapter 3).

A method for measuring faecal glucocorticoid metabolite (fGCM) concentrations as a measure of stress in African clawless otters was successfully established (chapter 2). Of the five test systems evaluated, the results suggest that an enzyme-immunoassay (EIA) utilizing an antibody against cortisol-3-CMO (Palme & Mostl 1997) is the most suitable EIA for measuring fGCM concentrations in African clawless otters. Faecal samples have to be collected up to 3 hours post-defecation to avoid biased results due to time dependent alteration of fGCM concentrations. However, the biological validation approach used for testing EIA reliability was limited and only included one translocated individual and no physiological validation. This limitation was due to lack of access to captive otters and restrictions in performing a physiological validation – such limitations being relatively common in similar studies (Palme 2019). Although the results gave important information on the suitability of the EIAs tested, additional studies including more individuals, preferably incorporating different sexes, as well as a physiological validation, is advisable to confirm general suitability of the identified EIA for monitoring faecal glucocorticoid metabolites as a measure of stress.

The results of this study show that African clawless otters utilizing a transformed area characterized by anthropogenic disturbance (Millstream farm) have higher overall fGCM concentrations when compared to otters in more natural areas. The comparatively higher fGCM concentrations determined for animals at Millstream Farm are possibly due to human activity (e.g. noise), predators (including encounters with humans and their pets), food availability, and intra- and inter-specific competition. Encounters with humans and their pets is considered an unlikely a high impact stressor due to African clawless otter nocturnal behaviour. The otters at Millstream Farm exploit the resource rich anthropogenic environment (e.g. stocked rainbow trout) (Jordaan *et al.* 2019). Increased food availability, differential food intake, and excessive body mass could result in higher fGCM concentrations due to higher levels of circulating GCs linked to differential metabolism and prolonged activation of the

HPA axis (Kalliokoski *et al.* 2012). Faecal samples were collected from unknown individuals, thus sex was not controlled for. While sex-related differences in fGCM concentrations cannot be excluded (Webster *et al.* 2018), this limitation applies across all sites and thus should not impact the overall difference in fGCM concentrations found between sites.

Random encounter models (REMs) were successfully applied to obtain density estimates of African clawless otters (chapter 3). The REM density estimate at Cobham is higher than previous African clawless otter estimates based on characteristic otter signs (holts, rolling places and tracks) at this study site (Rowe-Rowe 1992). While this is potentially because of an increase in the African clawless otter population (the previous estimates are based on data from 13 years ago). However, the differences may also be due to general underestimation associated with sign-based surveys (Ruiz-Olmo *et al.* 2001) or overestimation by the REM approach (Cusack *et al.* 2015). The current results are possibly influenced by violation of the REM; (i) closed population and (ii) random movement assumptions (Rowcliffe *et al.* 2008). However, there is no evidence which suggests that the otters regularly disperse to areas outside the study area and otter detections were independent of any signs of otter activity (movement was relatively random). Thus, the REM assumptions not being met is unlikely to have made a major difference to the overall density estimates.

African clawless otters occur in substantially higher densities on Millstream Farm compared to the natural areas. Moreover, otters alter their activity time and group sizes thus displaying substantial behavioural plasticity (chapter 3), presumably to optimally exploit resource-rich anthropogenic environments (Jordaan *et al.* 2019). The Cobham study site had a sample size of 10 detections, which, while sufficient for the random encounter model (Rowcliffe *et al.* 2008), was inadequate to determine activity time. Consequently, Cobham activity time results could not be compared to the two other sites. Limitations of this type can be avoided by increasing the camera trap deployment period; however, this was not possible due to the time constraints for the degree-related study.

The results suggest that African clawless otter behavioural plasticity and diet contribute to their success in transformed areas. The results also contribute to the

growing evidence that a species colonisation of transformed areas is dependent on the behavioural plasticity, physiology and the environmental conditions of the area. Thus, the suitable habitat and resource rich water sources in transformed areas such as Millstream Farm suggest potential for otter conservation. However, from a management perspective, relying on transformed areas such as Millstream Farm for otter conservation is probably not feasible due to otter-human conflict on such trout estates (de Vos 2018).

4. 1 Future Research

This dissertation reports on the application of methods to quantify faecal glucocorticoid metabolites in African clawless otters, and the use of camera traps to estimate African clawless otter population densities and activity times. The study highlighted the usefulness of using a translocation event as a biological validation to examine the reliability of enzyme immune assays to measure stress-related HPA axis activation, similar to work by Goymann *et al.* (1999) and Franceschini *et al.* (2008). However, future studies should perform a replication study which incorporates a physiological validation and more individuals. In addition, the study showed that African clawless otters have higher fGCM levels in a transformed area than their counterparts in the natural areas, although the absolute cause of this is unclear. Future research should investigate the possible cause of HPA activation by comparing fGCM concentrations of African clawless otters occurring in transformed areas characterized by low food availability and low otter densities. Although current studies comparing behavioural and physiological adaptations of urban adapters give useful insights (Santini *et al.* 2019), future studies should take it further by comparing these behavioural and physiological adaptations across continents and biomes. Such studies will identify if there are consistent and specialised adaptations across the different biomes. As there is potential for the animals to physiologically adapt to human dominated landscapes (Łopucki *et al.* 2019), long-term studies monitoring fGCM concentrations of urban adapters, including African clawless otters, should be carried out to examine if elevated fGCM concentrations will reduce over extended time periods. Finally, with the newly developed method for measuring fGCM concentrations as a measure of African clawless otter welfare, future studies should aim to investigate the usefulness of monitoring adrenocortical activity in African clawless otters as a proxy for

environmental health (e.g. freshwater system polluted by persistent inorganic pollutants.

CHAPTER 5: REFERENCES

Andrade AM, Arcoverde DL & Albernaz AL (2019). Relationship of Neotropical otter vestiges with environmental and anthropogenic factors. *Acta Amazonica* **49,** 183–192.

Anoop KR & Hussain SA (2004). Factors affecting habitat selection by smooth-coated otters (*Lutra perspicillata*) in Kerala, India. *Journal of Zoology* **263,** 417–423.

Arden-Clarke CHG (1986). Population density, home range size and spatial organization of the Cape clawless otter, *Aonyx capensis*, in a marine habitat. *Journal of Zoology* **209,** 201–211.

Barlow J & Taylor BL (2005). Estimates of sperm whale abundance in the northeastern temperate Pacific from a combined acoustic and visual survey. *Marine Mammal Science* **21**, 429–445.

Bateman PW & Fleming PA (2012). Big city life: carnivores in urban environments. *Journal of Zoology* **287,** 1–23.

Beckmann JP & Berger J (2003). Rapid ecological and behavioural changes in carnivores: the responses of black bears (*Ursus americanus*) to altered food. Journal of Zoology **261**, 207–212.

Björntorp P & Rosmond R (2000). Obesity and cortisol. *Nutrition* **16,** 924–936.

Bongaarts J (2009). Human population growth and the demographic transition. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **364**, 2985–2990.

Bonier F, Martin PR, Sheldon KS, Jensen JP, Foltz SL & Wingfield JC (2006). Sex-specific consequences of life in the city. *Behavioral Ecology* **18,** 121–129.

von Borell E, Langbein J, Després G, *et al.* (2007). Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals—A review. *Physiology & Behavior* **92,** 293–316.

Burton AC, Neilson E, Moreira D, *et al.* (2015). Wildlife camera trapping: a review and recommendations for linking surveys to ecological processes. *Journal of Applied Ecology* **52,** 675–685.

Byers JE (2002). Impact of non-indigenous species on natives enhanced by anthropogenic alteration of selection regimes. *Oikos* **97,** 449–458.

Caravaggi A, Banks PB, Burton AC, *et al.* (2017). A review of camera trapping for conservation behaviour research. *Remote Sensing in Ecology and Conservation*

3, 109–122.

Carr GM & Macdonald DW (1986). The sociality of solitary foragers: a model based on resource dispersion. *Animal Behaviour* **34,** 1540–1549.

Cavigelli SA, Monfort SL, Whitney TK, Mechref YS, Novotny M & McClintock MK (2005). Frequent serial fecal corticoid measures from rats reflect circadian and ovarian corticosterone rhythms. *Journal of Endocrinology* **184,** 153–163.

Chapman CA, Schoof VAM, Bonnell TR, Gogarten JF & Calmé S (2015). Competing pressures on populations: long-term dynamics of food availability, food quality, disease, stress and animal abundance. *Philosophical Transactions of the Royal Society B: Biological Sciences* **370,** 20140112.

Connor EF, Courtney AC & Yoder JM (2000). Individuals--area relationships: the relationship between animal population density and area. *Ecology* **81,** 734–748.

Crossey B, Ganswindt A & Chimimba C (2018). Faecal glucocorticoid metabolite concentrations and their alteration post-defaecation in African wild dogs *Lycaon pictus* from South Africa. *Wildlife Biology* **2018**.

Cusack JJ, Swanson A, Coulson T, *et al.* (2015). Applying a random encounter model to estimate lion density from camera traps in Serengeti National Park, Tanzania. *The Journal of Wildlife Management* **79,** 1014–1021.

Cutler TL & Swann DE (1999). Using remote photography in wildlife ecology: a review. *Wildlife Society Bulletin* **27,** 571–581.

Davis M & Pineda-Munoz S (2016). The temporal scale of diet and dietary proxies. *Ecology and Evolution* **6,** 1883–1897.

Deiner K, Bik HM, Mächler E, *et al.* (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular Ecology* **26,** 5872–5895.

Depue JE & Ben-David M (2007). Hair sampling techniques for river otters. *Journal of Wildlife Management* **71,** 671–674.

Dickens MJ, Delehanty DJ & Romero LM (2010). Stress: an inevitable component of animal translocation. *Biological Conservation* **143,** 1329–1341.

Ditchkoff SS, Saalfeld ST & Gibson CJ (2006). Animal behavior in urban ecosystems: modifications due to human-induced stress. *Urban Ecosystems* **9,** 5–12.

Drouilly M & O'Riain MJ (2019). Wildlife winners and losers of extensive small-livestock farming: a case study in the South African Karoo. *Biodiversity and Conservation* **28,** 1–19.

Evans KL, Hatchwell BJ, Parnell M & Gaston KJ (2010). A conceptual framework for the colonisation of urban areas: the blackbird *Turdus merula* as a case study. *Biological Reviews* **85,** 643–667.

Everatt KT, Andresen L & Somers MJ (2014). Trophic scaling and occupancy analysis reveals a lion population limited by top-down anthropogenic pressure in the Limpopo National Park, Mozambique. *PloS One* **9,** e99389.

Fieß M, Heistermann M & Hodges JK (1999). Patterns of urinary and fecal steroid excretion during the ovarian cycle and pregnancy in the African elephant (*Loxodonta africana*). *General and Comparative Endocrinology* **115,** 76–89.

Fischer J & Lindenmayer DB (2007). Landscape modification and habitat fragmentation: a synthesis. *Global Ecology and Biogeography* **16,** 265–280.

Fokidis HB, Orchinik M & Deviche P (2009). Corticosterone and corticosteroid binding globulin in birds: relation to urbanization in a desert city. *General and Comparative Endocrinology* **160,** 259–270.

Franceschini MD, Rubenstein DI, Low B & Romero LM (2008). Fecal glucocorticoid metabolite analysis as an indicator of stress during translocation and acclimation in an endangered large mammal, the Grevy's zebra. *Animal Conservation* **11,** 263–269.

Freeman MT, Olivier PI & van Aarde RJ (2018). Matrix transformation alters species-area relationships in fragmented coastal forests. *Landscape Ecology* **33,** 307–322.

Freitas D, Gomes J, Luis TS, *et al.* (2007). Otters and fish farms in the Sado estuary: ecological and socio-economic basis of a conflict. *Hydrobiologia* **587,** 51–62.

Frigerio D, Dittami J, Möstl E & Kotrschal K (2004). Excreted corticosterone metabolites co-vary with ambient temperature and air pressure in male Greylag geese (*Anser anser*). *General and Comparative Endocrinology* **137,** 29–36.

Ganswindt A, Tordiffe ASW, Stam E, Howitt MJ & Jori F (2012). Determining adrenocortical activity as a measure of stress in African buffalo (*Syncerus caffer*) based on faecal analysis. *African Zoology* **47,** 261–269.

Gibb H & Hochuli DF (2002). Habitat fragmentation in an urban environment: large and small fragments support different arthropod assemblages. *Biological Conservation* **106,** 91–100.

Gómez H, Wallace RB, Ayala G & Tejada R (2005). Dry season activity periods of some Amazonian mammals. *Studies on Neotropical Fauna and Environment* **40,**

91–95.

Goncalves IB, Heistermann M, Santema P, Dantzer B, Mausbach J, Ganswindt A & Manser MB (2016). Validation of a fecal glucocorticoid assay to assess adrenocortical activity in meerkats using physiological and biological stimuli. *PloS One* **11,** e0153161.

Goymann W, Möstl E, Van't Hof T, East ML & Hofer H (1999). Noninvasive fecal monitoring of glucocorticoids in spotted hyenas, *Crocuta crocuta*. *General and Comparative Endocrinology* **114,** 340–348.

Gray TNE (2018). Monitoring tropical forest ungulates using camera-trap data. *Journal of Zoology* **305,** 173–179.

Guertin DA, Harestad AS, Ben-David M, Drouillard KG & Elliott JE (2010). Fecal genotyping and contaminant analyses reveal variation in individual river otter exposure to localized persistent contaminants. *Environmental Toxicology and Chemistry* **29,** 275–284.

Gupta AK (2004). Origin of agriculture and domestication of plants and animals linked to early Holocene climate amelioration. *Current Science-Bangalore* **87**, 54–59.

Hájková P, Zemanová B, Roche K & Hájek B (2009). An evaluation of field and noninvasive genetic methods for estimating Eurasian otter population size. *Conservation Genetics* **10,** 1667–1681.

Halpern BS, Walbridge S, Selkoe KA, *et al.* (2008). A global map of human impact on marine ecosystems. *Science* **319,** 948–952.

Hanrahan AT, Rutter AU, Nielsen CK & Schauber EM (2019). Spatial ecology of river otters in a human-modified landscape. *Journal of Mammalogy* **100,** 1327–1339.

Honnay O, Endels P, Vereecken H & Hermy M (1999). The role of patch area and habitat diversity in explaining native plant species richness in disturbed suburban forest patches in northern Belgium. *Diversity and Distributions* **5,** 129–141.

Jacques H, Reed-Smith J & Somers MJ (2015) *Aonyx capensis*. *The IUCN Red List of Threatened Species* **2015**, e. T1793A21938767.

Jepsen EM, Ganswindt A, Ngcamphalala CA, Bourne AR, Ridley AR & McKechnie AE (2019). Non-invasive monitoring of physiological stress in an Afrotropical arid-zone passerine bird, the southern pied babbler. *General and Comparative Endocrinology* **276,** 60–68.

Jordaan RK, McIntyre T, Somers MJ & Bester MN (2015). An assessment of spatial and temporal variation in the diet of Cape clawless otters (*Aonyx capensis*) in

marine environments. *South African Journal of Wildlife Research* **45,** 342–353.

Jordaan RK, Somers MJ & McIntyre T (2017). Dancing to the message: African clawless otter scent marking behaviour. *The Italian Journal of Mammalogy* **28,** 277–279.

Jordaan RK, Somers MJ, Hall G & McIntyre T (2019). Plasticity and specialisation in the isotopic niche of African clawless otters foraging in marine and freshwater habitats. *Mammalian Biology* **98,** 61–72.

Kalliokoski O, Jacobsen KR, Teilmann AC, Hau J & Abelson KSP (2012). Quantitative effects of diet on fecal corticosterone metabolites in two strains of laboratory mice. *In Vivo* **26,** 213–221.

Karanth KU (1995). Estimating tiger Panthera tigris populations from camera-trap data using capture—recapture models. *Biological Conservation* **71,** 333–338.

Kerr JT & Currie DJ (1995) Effects of human activity on global extinction risk. *Conservation Biology* **9,** 1528–1538.

Koepfli K-P & Wayne RK (1998). Phylogenetic relationships of otters (Carnivora: Mustelidae) based on mitochondrial cytochrome b sequences. *Journal of Zoology* **246,** 401–416.

Kubheka SP, Rowe-Rowe DT, Alletson JD & Perrin MR (2013). Possible influence of increased riparian activity (stream modification and agricultural intensification) on abundance of South African otters. *African Journal of Ecology* **51,** 288–294.

Lane J (2006). Can non-invasive glucocorticoid measures be used as reliable indicators of stress in animals? *Animal Welfare* **15,** 331–342.

Larivière S (2001). *Aonyx capensis*. *Mammalian Species* **671,** 1–6.

Laver PN, Ganswindt A, Ganswindt SB & Alexander KA (2012). Non-invasive monitoring of glucocorticoid metabolites in banded mongooses (*Mungos mungo*) in response to physiological and biological challenges. *General and Comparative Endocrinology* **179,** 178–183.

Leuchtenberger C, Sousa-Lima R, Duplaix N, Magnusson WE & Mourão G (2014a). Vocal repertoire of the social giant otter. *The Journal of the Acoustical Society of America* **136,** 2861–2875.

Leuchtenberger C, Zucco CA, Ribas C, Magnusson W & Mourão G (2014b). Activity patterns of giant otters recorded by telemetry and camera traps. *Ethology Ecology & Evolution* **26,** 19–28.

Lexen E, El-Bahr SM, Sommerfeld-Stur I, Palme R & Mostl E (2008). Monitoring the

adrenocortical response to disturbances in sheep by measuring glucocorticoid metabolites in the faeces. *Wiener Tierarztliche Monatsschrift* **95,** 64.

Łopucki Rafałand Klich D, Ścibior A & Gołkebiowska D (2019). Hormonal adjustments to urban conditions: stress hormone levels in urban and rural populations of Apodemus agrarius. *Urban Ecosystems* **22,** 1–8.

Lowry H, Lill A & Wong BBM (2013). Behavioural responses of wildlife to urban environments. *Biological Reviews* **88,** 537–549.

Macdonald DW (1983). The ecology of carnivore social behaviour. *Nature* **301,** 379.

Macdonald SM & Mason CF (1983). Some factors influencing the distribution of otters (*Lutra lutra*). *Mammal Review* **13,** 1–10.

Madisha MT, Ponsonby D, Schwaibold U, *et al.* (2015). Differentiation of two South African otter species (*Aonyx capensis* and *Lutra maculicollis*) from spraint based on partial CytB primer sets. *Global Ecology and Conservation* **4,** 8–13.

Magle SB, Hunt VM, Vernon M & Crooks KR (2012). Urban wildlife research: past, present, and future. *Biological Conservation* **155,** 23–32.

Majelantle TL, McIntyre T & Ganswindt A (2019). Monitoring the effects of land transformation on African clawless otters (*Aonyx capensis*) using fecal glucocorticoid metabolite concentrations as a measure of stress. *Integrative Zoology, In Review*

Manzo E, Bartolommei P, Rowcliffe JM & Cozzolino R (2012). Estimation of population density of European pine marten in central Italy using camera trapping. *Acta Theriologica* **57,** 165–172.

Marzluff JM & Ewing K (2001). Restoration of fragmented landscapes for the conservation of birds: a general framework and specific recommendations for urbanizing landscapes. *Restoration Ecology* **9,** 280–292.

Mason CF & Macdonald SM (1987). The use of spraints for surveying otter *Lutra lutra* populations: an evaluation. *Biological Conservation* **41,** 167–177.

McDonald RI, Kareiva P & Forman RTT (2008). The implications of current and future urbanization for global protected areas and biodiversity conservation. *Biological Conservation* **141,** 1695–1703.

McIntyre NE, Rango J, Fagan WF & Faeth SH (2001). Ground arthropod community structure in a heterogeneous urban environment. *Landscape and Urban Planning* **52,** 257–274.

McIntyre T, Majelantle TL, Slip DJ & Harcourt RG (2019). Quantifying imperfect

camera trap detection probabilities: implications for density modelling. *Wildlife Research, In press*

McKee JK, Sciulli PW, Fooce CD & Waite TA (2004). Forecasting global biodiversity threats associated with human population growth. *Biological Conservation* **115,** 161–164.

McKinney ML (2001). Role of human population size in raising bird and mammal threat among nations. In *Animal Conservation Forum*, pp 45–57. Cambridge University Press, England.

McKinney ML (2002). Urbanization, Biodiversity, and ConservationThe impacts of urbanization on native species are poorly studied, but educating a highly urbanized human population about these impacts can greatly improve species conservation in all ecosystems. *Bioscience* **52,** 883–890.

McKinney ML (2006). Urbanization as a major cause of biotic homogenization. *Biological Conservation* **127,** 247–260.

McLennan MR, Howell CP, Bardi M & Heistermann M (2019). Are human-dominated landscapes stressful for wild chimpanzees (*Pan troglodytes*)? *Biological Conservation* **233,** 73–82.

Meredith M & Ridout M (2014). Overview of the overlap package. *R. Proj.* 1–9.

Meyer WB & Turner BL (1992). Human population growth and global land-use/cover change. *Annual Review of Ecology and Systematics* **23**, 39–61.

Moberg GP & Mench JA (2000). *The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare*. CABI, Engaland.

Möstl E & Palme R (2002). Hormones as indicators of stress. *Domestic Animal Endocrinology* **23,** 67–74.

Möstl E, Maggs JL, Schrötter G, Besenfelder U & Palme R (2002). Measurement of cortisol metabolites in faeces of ruminants. *Veterinary Research Communications* **26,** 127–139.

Nel JAJ & Somers MJ (2007). Distribution and habitat choice of Cape clawless otters, in South Africa. *South African Journal of Wildlife Research* **37,** 61–70.

van Niekerk CH, Somers MJ & Nel JAJ (1998). Freshwater availability and distribution of Cape clawless otter spraints and resting places along the south-west coast of South Africa. *South African Journal of Wildlife Research* **28,** 68–72.

Öckinger E, Lindborg R, Sjödin NE & Bommarco R (2012). Landscape matrix modifies richness of plants and insects in grassland fragments. *Ecography* **35,** 259–267.

von der Ohe CG & Servheen C (2002). Measuring stress in mammals using fecal glucocorticoids: opportunities and challenges. *Wildlife Society Bulletin* **30,** 1215–1225.

Okes NC & O'Riain MJ (2017). Otter occupancy in the Cape Peninsula: Estimating the probability of river habitat use by Cape clawless otters, *Aonyx capensis*, across a gradient of human influence. *Aquatic Conservation: Marine and Freshwater Ecosystems* **27,** 706–716.

Okes N, Ponsonby DW, Rowe-Rowe D, Avenant NL & Somers MJ (2016). A conservation assessment of *Aoynx capensis*. In *The Red List of Mammals of South Africa, Swaziland and Lesotho*. South African National Biodiversity Institute and Endangered Wildlife Trust, South Africa.

Palme R (2005). Measuring fecal steroids: guidelines for practical application. *Annals of the New York Academy of Sciences* **1046,** 75–80.

Palme R (2019). Non-invasive measurement of glucocorticoids: Advances and problems. *Physiology & Behavior* **199,** 229–243.

Palme R & Mostl E (1997). Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. *Mammal Biology* **II,** 192–197.

Palme R, Rettenbacher S, Touma C, El-Bahr SM & Möstl E (2005). Stress hormones in mammals and birds: comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. *Annals of the New York Academy of Sciences* **1040,** 162–171.

Perrin MR & Carugati C (2000). Habitat use by the Cape clawless otter and the spotted-necked otter in the KwaZulu-Natal Drakensberg, South Africa. *South African Journal of Wildlife Research* **30,** 103–113.

Perrin MR & Carugati C (2006). Abundance estimates of the Cape clawless otter *Aonyx capensis* (Schinz 1821) and the spotted-necked otter *Lutra maculicollis* (Lichtenstein 1835) in the KwaZulu-Natal Drakensberg, South Africa. *Tropical Zoology* **19,** 9–19.

Pfeffer SE, Spitzer R, Allen AM, *et al.* (2018). Pictures or pellets? Comparing camera trapping and dung counts as methods for estimating population densities of ungulates. *Remote Sensing in Ecology and Conservation* **4,** 173–183.

Ponsonby DW & Schwaibold U (2019). Country otter, city otter: The distribution patterns of two otter species in an urbanized area of Gauteng, South Africa. *African Journal of Ecology* **57,** 148–154.

Ponsonby DW, Madisha MT, Schwaibold U & Dalton DL (2019). Genetic diversity of African clawless otters (*Aonyx capensis*) occurring in urbanised areas of Gauteng, South Africa. *South African Journal of Science* **115,** 1–8.

Prange S, Gehrt SD & Wiggers EP (2004). Influences of anthropogenic resources on raccoon (*Procyon lotor*) movements and spatial distribution. *Journal of Mammalogy* **85,** 483–490.

Pride RE (2005). Foraging success, agonism, and predator alarms: behavioral predictors of cortisol in *Lemur catta*. *International Journal of Primatology* **26,** 295–319.

R Core Team (2016). R: A language and environment for statistical computing [Computer software manual]. Vienna, Austria.

Ramankutty N, Foley JA & Olejniczak NJ (2002). People on the land: Changes in global population and croplands during the 20th century. *AMBIO: A Journal of the Human Environment* **31**, 251–257.

Rangel-Negrin A, Alfaro JL, Valdez RA, Romano MC & Serio-Silva JC (2009). Stress in Yucatan spider monkeys: effects of environmental conditions on fecal cortisol levels in wild and captive populations. *Animal Conservation* **12,** 496–502.

Ridout MS & Linkie M (2009). Estimating overlap of daily activity patterns from camera trap data. *Journal of Agricultural, Biological, and Environmental Statistics* **14,** 322–337.

Rothschild DM, Serfass TL, Seddon WL, Hegde L & Fritz RS (2008). Using fecal glucocorticoids to assess stress levels in captive river otters. *The Journal of Wildlife Management* **72,** 138–142.

Rowcliffe JM, Field J, Turvey ST & Carbone C (2008). Estimating animal density using camera traps without the need for individual recognition. *Journal of Applied Ecology* **45,** 1228–1236.

Rowcliffe JM, Kays R, Carbone C & Jansen PA (2013). Clarifying assumptions behind the estimation of animal density from camera trap rates. *Journal of Wildlife Management*.

Rowcliffe JM, Kays R, Kranstauber B, Carbone C & Jansen PA (2014). Quantifying levels of animal activity using camera trap data. *Methods in Ecology and Evolution* **5,** 1170–1179.

Rowe-Rowe DT (1977). Food ecology of otters in Natal, South Africa. *Oikos* **1977,** 210–219.

Rowe-Rowe DT (1992). Survey of South African otters in a freshwater habitat, using sign. *South African Journal of Wildlife Research* **22,** 49–55.

Ruddiman WF (2003). The anthropogenic greenhouse era began thousands of years ago. *Climatic Change* **61**, 261–293.

Ruiz-Olmo J, Saavedra D & Jiménez J (2001). Testing the surveys and visual and track censuses of Eurasian otters (*Lutra lutra*). *Journal of Zoology* **253,** 359–369.

Sanderson EW, Jaiteh M, Levy MA, Redford KH, Wannebo AV & Woolmer G (2002). The human footprint and the last of the wild: the human footprint is a global map of human influence on the land surface, which suggests that human beings are stewards of nature, whether we like it or not. *BioScience* **52,** 891–904.

Santini L, González-Suárez M, Russo D, Gonzalez-Voyer A, von Hardenberg A & Ancillotto L (2019). One strategy does not fit all: determinants of urban adaptation in mammals. *Ecology Letters* **22,** 365–376.

SAWS. *South African Weather Service.* [Cited 2 November 2018.] Available from URL: http://www.weathersa.co.za.

Scheun J, Bennett NC, Ganswindt A & Nowack J (2015). The hustle and bustle of city life: monitoring the effects of urbanisation in the African lesser bushbaby. *The Science of Nature* **102,** 57.

Schoepf I, Schmohl G, König B, Pillay N & Schradin C (2015). Manipulation of population density and food availability affects home range sizes of African striped mouse females. *Animal Behaviour* **99,** 53–60.

Selye H (1936) A syndrome produced by diverse nocuous agents. *Nature* **138,** 32.

Sol D, Lapiedra O & González-Lagos C (2013). Behavioural adjustments for a life in the city. *Animal Behaviour* **85,** 1101–1112.

Somers MJ (2000). Foraging behaviour of Cape clawless otters (*Aonyx capensis*) in a marine habitat. *Journal of Zoology* **252,** 473–480.

Somers MJ & Nel JAJ (2004). Movement patterns and home range of Cape clawless otters (*Aonyx capensis*), affected by high food density patches. *Journal of Zoology* **262,** 91–98.

Somers MJ & Nel JAJ (2013). *Aoynx capensis*. In *Mammals of Africa*, pp 103–108. Bloomsbury Publishing, England.

Stuart C & Stuart T (2000). *A Field Guide to the Tracks and Signs of Southern and East African Wildlife*. Struik Nature, South Africa.

Tonelli LH, Hoshino A, Katz M & Postolache TT (2008). Acute stress promotes

aggressive-like behavior in rats made allergic to tree pollen. *International Journal of Child Health and Human Development* **1,** 305.

Touma C & Palme R (2005). Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. *Annals of the New York Academy of Sciences* **1046,** 54–74.

Touma C, Sachser N, Möstl E & Palme R (2003). Effects of sex and time of day on metabolism and excretion of corticosterone in urine and faeces of mice. *General and Comparative Endocrinology* **130,** 267–278.

Valeix M, Loveridge AJ & Macdonald DW (2012). Influence of prey dispersion on territory and group size of African lions: a test of the resource dispersion hypothesis. *Ecology* **93,** 2490–2496.

Verwoerd DJ (1987). Observations on the food and status of the Cape clawless otter *Aonyx capensis* at Betty's Bay, South Africa. *African Zoology* **22,** 33–39.

de Vos M (2018). Human-predator conflict in the South African fly-fishing industry: fish survival probabilities and stakeholder perceptions. University of Pretoria.

Washburn BE & Millspaugh JJ (2002). Effects of simulated environmental conditions on glucocorticoid metabolite measurements in white-tailed deer faeces. *General and Comparative Endocrinology* **127,** 217–222.

Wasser SK, Hunt KE, Brown JL, Cooper K, Crockett CM, Bechert U, Millspaugh JJ, Larson S & Monfort SL (2000). A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. *General and Comparative Endocrinology* **120,** 260–275.

Webber JT, Henley MD, Pretorius Y, Somers MJ & Ganswindt A (2018). Changes in African Elephant (*Loxodonta africana*) faecal steroid concentrations post-defaecation. *Bothalia-African Biodiversity & Conservation* **48,** 1–8.

Webster AB, Burroughs REJ, Laver P & Ganswindt A (2018). Non-invasive assessment of adrenocortical activity as a measure of stress in leopards *Panthera pardus*. *African Zoology* **53,** 53–60.

Wielebnowski N (2003). Stress and distress: evaluating their impact for the well-being of zoo animals. *Journal of the American Veterinary Medical Association* **223,** 973–977.

Wilson GJ & Delahay RJ (2001). A review of methods to estimate the abundance of terrestrial carnivores using field signs and observation. *Wildlife Research* **28,** 151–164.

Wolf TE, Bennett NC, Burroughs R & Ganswindt A (2018). The impact of age-class and social context on fecal glucocorticoid metabolite levels in free-ranging male giraffes. *General and Comparative Endocrinology* **255,** 26–31.

Woodroffe R (2000). Predators and people: using human densities to interpret declines of large carnivores. *Animal Conservation Forum*, pp 165–173. Cambridge University Press, England.

Wright SJ & Hubbell SP (1983). Stochastic extinction and reserve size: a focal species approach. *Oikos* **41,** 466–476.

Zalewski JT (2011). Ecological factors influencing stress in northern river otters (*Lontra canadensis*). Humboldt State University.

Zero VH, Sundaresan SR, O'Brien TG & Kinnaird MF (2013). Monitoring an Endangered savannah ungulate, Grevy's zebra *Equus grevyi*: choosing a method for estimating population densities. *Oryx* **47,** 410–419

APPENDIX A

A.1 Capture, immobilisation, instrumentation and tracking

At suitable locations at Millstream Farm (e.g. latrine sites, holts, well used pathways, etc.) carnivore cage traps baited with fresh spraints from nearby sites and/or trout were set overnight and checked at sunrise. Once captured, the otter was examined and thereafter suitable otters (adult males or females over ~12 kg and non-pregnant or lactating females) were transported in the trap (covered to minimise disturbance) to Sterkspruit veterinary facility in Lydenburg. The otters were chemically immobilised upon arrival using a pre-calculated dosage (dependant on the estimated weight of the animal) of medetomidine and ketamine administered via a dart syringe fired through a mechanically pressurised blow-gun. Once immobilised, two devices (a VHF transmitter and TDR) were implanted into the abdominal cavity and tethered to the abdominal wall of the otter. Standard body size measurements and samples of vibrissae, fur and blood were also obtained. Antipamizole was administered to reverse the effects of the medetomidine, and the otter was provided with water and allowed to recover inside the trap. Otters were transported back to Millstream Farm and released at the site of capture around sunset when deemed to have fully recovered.

In the following days/weeks, otters (n = 2) were tracked with the use of a VHF Receiver, antenna, and non-invasive visual observations were attempted. When located, the otter's position was recorded and updated when the otter was mobile. When observed, behaviour was recorded with the use of a video camera (Panasonic HX-WA2, Panasonic Corporation, Osaka, Japan). Active tracking and following predominantly took place in the early evening following a period of diurnal inactivity by the otters. Active tracking was terminated when the observer detected changes in otter behaviour due to observer presence, due to inclement weather (thunderstorms) and when the otter moved off Millstream Farm. Attempts to relocate and observe the otter were made the following morning before sunrise. Additional position and movement data were obtained with the use of strategically placed camera traps (Bushnell Trophy Cam HD, USA) (n = 11). Ethical clearance was provided by the Animal Ethics Committee (AEC) of the University of Pretoria (Project Number EC087-14).

A.2 Data analysis and results

Movement data included otters travelling and foraging on land and in water. A general linear model was used to find the relationship between distance and time (both normally distributed). There was a significant relationship between distance and time ($F_{1,17} = 6.23$, n = 19, p = 0.023). The model slope (speed) was 0.096 m/s (Figure A.1) and was converted to 8.278 km/day.

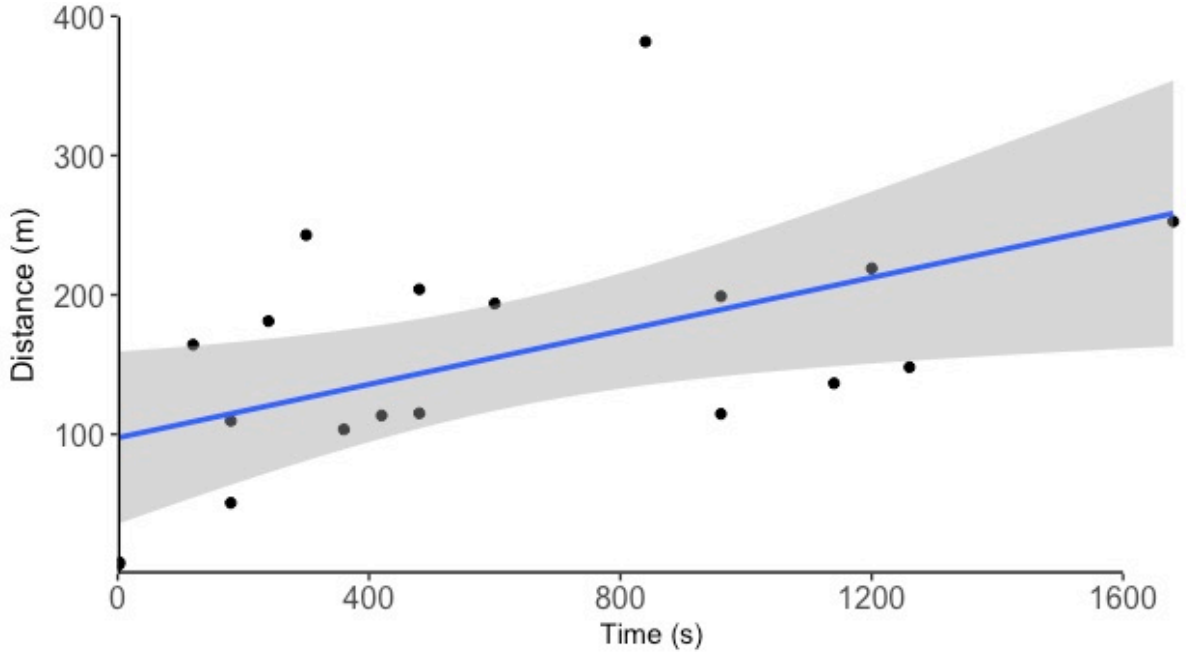

Figure 0.1: Relationship between African clawless otter distance travelled, and time taken. Blue line = regression line predicted by model, grey shade = 95 % confidence interval.