INFLUENCE OF RANGING BEHAVIOR AND VEGETATION QUALITY ON STRESS HORMONE LEVELS OF AFRICAN ELEPHANTS AT MPALA RANCH, LAIKIPIA COUNTY, KENYA

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REG. NO. N50/38843/2016

A RESEARCH THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ENVIRONMENTAL SCIENCE IN THE SCHOOL OF ENVIRONMENTAL STUDIES OF KENYATTA UNIVERSITY

June 2021

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University or for any other award.

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ACKNOWLEDGEMENT

I would like to give my sincere gratitude and thanks to the following people who have immensely contributed to my thesis from the initial stage which included applying for funds to undertake this study to its completion. Without their support, this study would not have been successful.

First and foremost, I would like to express my sincere gratitude and thanks to my mentor and advisor Dr. Janine L. Brown, a Reproductive Physiologist and head of the Endocrine laboratory at Smithsonian Conservation Biology Institute. Through her mentorship, support and guidance, I was successful in receiving grants from National Geographic Society (Early Career Grant) who have funded 95% of this work and from IDEA Wild which funded me the equipment that I used to undertake this study. Additionally, through her mentorship and in collaboration with her parent institution, Smithsonian Conservation Biology Institute (SCBI), I received training from Dr. Nicole Boisseau of Smithsonian Conservation Biology Institute on endocrine techniques for assessing physiological stress and reproductive status of wildlife species from 23rd of November to 15th of December 2019. I would also like to express my sincere gratitude to other supervisors Dr. Geoffrey Macharia and Dr. Thomas Obade for their guidance and support through constant reviews on my work. Without their corrections proofreading and insights to my work, this study would not have been a success.

I would also like to express my sincere gratitude and thanks to Dr. Dino J. Martins, the Executive Director of Mpala Research Center. Through his support, I received food and accommodation while I was undertaking my study at no cost. Additionally, through his support, I received several recommendation letters that accompanied my application to different grant organizations including National Geographic Society (NGS), Ruffords Small Grant (RSG), and Idea Wild all of which were successful and I received the grants. Through his support by providing me with accommodation at Mpala, I attended a training at Mpala on Spatial Analysis Training Workshop for Biodiversity Conservation and Monitoring that was held at Mpala Research Center from 18th-26th September 2018. The training equipped me with skills on using Google Earth Engine, a cloud based geospatial processing platform using JavaScript to process and generate sentinel images for my study area and for calculating Normalized Difference Vegetation Index in R Core Team

(2017) and answering my third objective in my study. The training also enhanced my skills on the use of R programming, a statistical computing software.

I would also like to express my sincere gratitude and thanks to other members of staff at Mpala Research Centre. I am grateful to Fardosa Hassan for her guidance on the procedures for acquiring my research permits from Kenya Wildlife Service (KWS) and from National Commission for Science, Technology and Innovation (NACOSTI). Through Fardosa's support, I was able to get all my supplies including cooler box, plastic vials, gloves and other stationery that were required in undertaking this study. I am also grateful to Joseph Irura, the Information Technologist (IT) expert at Mpala for his assistance on trouble shooting numerous error codes from my JavaScript in the process of extracting satellite image for Mpala boundary in the Google Earth Engine. Additionally, I am also grateful to all the security team who risked their lives to accompany me to the field and protected me from aggressive elephant families who would occasionally charge at my vehicle during my field work study. Without their support and protection, this study would not have been possible.

I would also like to express my sincere gratitude and thanks to Dr. Margaret F. Kinnaird, the Practice Leader Wildlife at World Wildlife Fund (WWF) International. During the period she was the Executive Director at Mpala Research Centre, she gave me an opportunity to study elephants at Mpala. Through this opportunity, I acquired skills on monitoring elephants using the catalog recognition files and I was able to catalog all the migrants and resident elephant families that were found at Mpala and has subsequently led me to undertaking this study based on personal observation on the variation in behavior for both resident and migrant elephant families as well as the ongoing levels of human elephant conflict in Laikipia.

Finally, I would wish to express my sincere gratitude and thanks to my loving mother Roselyne Nyawiri for her unwavering support both financially and emotionally and even when the journey seemed to be insurmountable. I will forever be indebted for your help and support.

DEDICATION

This thesis is dedicated to my family Mrs. Roselyne Nyawiri, Mr. Jimmy Joseph Owuor, Gerry Owuor, Fiona Kate, Diana Okatch, and Nancy Atieno for their financial and emotional support towards my education.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACTH	Adrenocorticotropic Hormone
AIC	Akaike's Information Criterion
BCS	Body Condition Score
CRH	Corticotrophin-releasing Hormone
CV	Coefficient of Variation
EIA	Enzyme Immunoassay
FGM	Fecal Glucocorticoid Metabolite
GEE	Google Earth Engine
GPS	Global Positioning System
HEC	Human Elephant Conflict
HPA	Hypothalamic-Pituitary Adrenal
HPA Axis	Hypothalamic Pituitary Adrenal Axis
Hrs	Hours
ID	Identification
IgG	Immunoglobulin-G
IT	Information Technology
IUCN	International Union for Conservation of Nature
KWS	Kenya Wildlife Service
LMM	Linear Mixed Model
MIKE	Monitoring Illegal Killing of Elephant
NACOSTI	National Commission for Science, Technology and Innovation
NDVI	Normalized Difference Vegetation Index
NGS	National Geographic Society

NIR	Near-Infrared
PIKE	Proportion of Illegally Killed Elephants
RSG	Ruffords Small Grant
SD	Standard Deviation
SEM	Standard Error Mean
SNS	Sympathetic Nervous System
SP	Spatial
Std. DEV	Standard deviation
Std. Error	Standard Error
TIFF	Tagged Image File Format
TMB	Tetramethylbenzidine
UNEP	United Nations Environmental Programme

ABSTRACT

Heterogeneous landscapes like those characteristic of Laikipia County, Kenya, consist of a mosaic of land-use types, which exert differential physiological stress on elephants that occupy and traverse them. Previous studies have showed elephants to be more stressed outside protected areas, however, little is known of whether ranging behavior and their persistence in private ranchlands offers a better alternative to communal ranchlands where lethal methods are used to control human-elephant conflicts. This study was therefore designed to determine: i) how stress hormone concentrations between resident and migrant elephant families that are found within Mpala Ranch vary; ii) the relationship between stress hormone concentrations and the sampling location within Mpala Ranch; and iii) determine whether a change in Normalized Difference Vegetation Index influences stress hormone concentrations in elephants at Mpala Ranch. Existing catalog recognition files for monitoring elephant families prepared during the period 2009 to 2014 was used to identify the resident and migrant families using their ears and tusks idiosyncrasies while a cross-sectional survey method was used to obtain data from the study area. Whenever a family herd or individual bull(s) of known elephant was encountered, their behavioral response to the observer was recorded and the vehicle's engine was turned off. The individuals were then monitored until defecation occurred. Dung samples were collected from successfully identified individuals within thirty minutes after elephants had dispersed from the area and immediately placed in a cooler box with ice packs until freezing at the laboratory. Their behavioral response to vehicle presence as a proxy for past negative human elephant interaction also was recorded. Sentinel 2 satellite images for the period April 2019 to August 2019 were processed using Google Earth Engine and exported to R programming for analysis of vegetation greenness. Stress hormones were analyzed using enzyme immunoassay validated for African elephants. The concentrations of fecal glucocorticoid metabolite was calculated from optical densities from Multiskan plate reader at 450 nm using sigmoidal dose response. Contrary to what was hypothesized, resident elephants had a higher stress response compared to the migrant elephants. Resident elephants had higher physiological stress response compared to the migrant elephants overall but further differences were found to be related to region. Migrant elephants had lower physiological stress response than resident elephants in the northern part of Mpala but no differences were found in the southern part of Mpala. Additionally, individuals that were terrified and running away had a higher physiological stress response compared to other behavioral reactions. Similarly, a higher stress response was observed during the dry season compared to the wet season. Findings from this study therefore reveal differing physiological and behavioral response to environmental and anthropogenic factors between resident and migrant elephants at Mpala ranch. Result of this study will benefit Laikipia Ranch Owners Association and communal ranches in identifying land use types that are compatible with conservation which will be critical in establishing wildlife corridors and connectivity for wildlife to minimize human-wildlife conflict and reduce overall physiological stress response in elephants that are traversing the area.

CHAPTER ONE: INTRODUCTION

1.1 Background of the study

Laikipia County is a non-protected savannah region (9,666 Km^2) located on the equator and is divided into a mosaic of privately, publicly and communally owned ranches. The area hosts the second largest population of elephants outside protected areas in Kenya (Omondi, Bitok, & Mayienda, 2002; Litoroh *et al.*, 2010; Ngene *et al.*, 2013). This is a recent phenomenon as historical records showed that elephants were not seen within Laikipia County until late 1960s (Neumann, 1966). The increase in elephant abundance was attributed to intense poaching that occurred between the 1970s and 1980s in the adjacent Samburu County and the creation of multiple artificial water sources for livestock in Laikipia.

Thouless (1993) categorized Laikipia elephants into four groups based on their ranging behaviours: 1) permanent residential family groups with small ranges; 2) semi-permanent family groups residing in several land holding with large ranges overlapping several private and community landholdings; 3) seasonal migrants that move outside of Laikipia to evergreen forests; and 4) long distance migrants that move between Laikipia and Samburu. A study by Goldenberg *et al.* (2016) however revealed evidence of spatial segregation between sub-population of elephants within Laikipia-Samburu ecosystem. Mpala Ranch, a 200 Km^2 private landholding in northwestern Laikipia is located within the corridor of an elephant migratory route and is visited by three of the four groups of elephants described above.

Between 2009 and 2014, an elephant monitoring program was carried out to understand how elephants use Mpala Ranch and the surrounding community ranches, as well as to determine how family groups aligned with Thouless (1993) classifications and if any family groups could be considered Mpala resident. Results from Goldenberg *et al.* (2016) identified photographic records of 573 breeding females (113 family groups); and only 15% (12 family groups) of the breeding females were considered to be residents at Mpala. A resident family is considered to be those families which spends more that 75% of their time per year at Mpala ranch.

International Union for Conservation of Nature (IUCN) classifies 25% of mammals as either extinct or threatened with extinction ((IUCN, 2019). This is attributed to factors such as poaching, human-wildlife conflict, habitat loss and fragmentation, as well as isolation of populations (Graham, Douglas-Hamilton, Adams, & Lee, 2009; UNEP & IUCN, 2013; Thouless *et al.*, 2016). This is true for African elephants, which experienced an upsurge in poaching across the continent between 2010 and 2012 that threatens their sustainability (Wittemyer *et al.*, 2014). Within the Laikipia-Samburu ecosystem, the Proportion of Illegally Killed Elephants (PIKE) increased in the past 11 years, peaking at 70% of all the deaths in 2012 (Ihwagi *et al.*, 2015). As a result, the population of elephants in Laikipia-Samburu ecosystem declined from 7,415 in 2008 (Litoroh *et al.*, 2010) to 6,454 in 2012 (Ngene *et al.*, 2013).

There has, however, been a disconnect between the declining numbers of wildlife with human disturbance owing to its reliance on the use of mathematical population models to predict the impact of human disturbance (McCormick & Romero, 2017). The use of mathematical population models to monitor wildlife population trends is disadvantageous due to its inability to explain the causes of changes in behavior, demography, reproduction, health and welfare by observation alone.

The emergence of conservation endocrinology as a research field has, however, enabled ecologist to quantify the impact of both anthropogenic and environmental disturbance on the physiological stress response and reproductive function, and how these disturbances affect the physiological state and reproductive output of wildlife species, including elephants at individual and population levels (Busch & Hayward, 2009; Ganswindt *et al.*, 2012). The use of conservation endocrinology as a research field also has a predictive capacity by quantifying the effects of disturbances to inform management decisions.

Dantzer, Fletcher, Boonstra, and Sheriff (2014) defined physiological stress as a state that occurs when animals have to make extreme and or prolonged physiological or behavioral adjustments to cope with adverse environments and maintain homeostasis. Stress may be short-term as from predator attacks or long-term as from drought. Acute stress is normal and usually results in production and discharge of glucocorticoids from the adrenal cortex, as well as a range of physiological behaviors to cope with acute environmental challenges (Wingfield *et al.*, 1998). Chronic stress, on the other hand, can negatively affect animal physiology in many ways, including suppressing immune function leading to increased disease susceptibility and decreased wound healing, inhibiting reproduction, and decreasing growth rates (Sapolsky, Romero, & Munck, 2000), all of which are detrimental to population fitness.

This study was motivated by the ongoing anthropogenic disturbances in Laikipia County, which has resulted in increased fear response from migrant elephants that in most cases are exposed to anthropogenic disturbances, such as poaching, human-elephant conflict and competition from different land use systems. The migrant elephants also often flee in response to vehicle presence, creating an unsafe environment for the young calves that may be abandoned or injured. As a result there was need to assess how ranging behavior, behavioral reaction to vehicle presence, and vegetation quality influenced the physiological state of African elephants at Mpala Ranch.

1.2 Problem Statement/Justification

There has been a continuous decline of wildlife species both inside and outside protected areas in sub-Saharan Africa (Craigie *et al.*, 2010; Chase *et al.*, 2016). Elephants, however, face numerous threats outside protected areas including illegal killing (Douglas-Hamilton, 1987; Wittemyer *et al.*, 2014; Chase *et al.*, 2016) as well as human-elephant conflict (Gadd, 2005; Graham *et al.*, 2010). Mpala Ranch lies in the heart of Laikipia at the cross road of known elephant migrations. The ranch is classified as a 'pro-wildlife property' and remains unfenced, but it is bounded by some ranches which are less tolerant of elephant presence, which increases Human Elephant Conflict (HEC) and has resulted in elephant deaths due to competition for access to resources. Migrant elephants appear to be particularly affected as they wander into hostile ranchlands, and suffer from injuries hence seek refuge within Mpala. Little is however known of whether vegetation quality, ranging behavior and their persistence in private ranchlands offer a better alternative to communal ranchlands where lethal methods are used to control human-elephant conflict.

Several studies have observed the effects of human activities on glucocorticoid hormone concentrations of African elephants outside protected areas (Foley, Papageorge, & Wasser, 2001; Millspaugh *et al.*, 2007; Gobush, Mutayoba, & Wasser, 2008; Jachowski, Slotow, & Millspaugh, 2012; Ahlering *et al.*, 2013; Tingvold *et al.*, 2013; Hunninck *et al.*, 2017). Additionally, Ihwagi *et al.* (2018) observed that elephants responded to anthropogenic disturbance by altering their speed of travel in areas perceived to be having higher poaching incidences and other anthropogenic perturbation. Little is however known on how anthropogenic activities and behavioral response to past or present anthropogenic activities manifest itself to affect the physiological state of African elephants.

This study will hence provide a quantitative assessment on how ranging behavior, vegetation quality and behavioral response to vehicle presence as a proxy to past or present anthropogenic disturbance affect the physiological state of African elephants in human occupied landscapes. This will be important to monitoring elephant aggression towards people and increased levels of human-elephant conflict in human occupied landscapes for management intervention and species conservation. The study therefore sought to compare how physiological stress response of resident and migrant elephants with different exposure to anthropogenic disturbance differ.

1.3 Research questions

1. How do stress hormone concentrations between the resident and migrant African elephants found within Mpala Ranch compares?
2. How does the concentration of faecal glucocorticoid metabolites (FGM) vary within different sampling locations at Mpala ranch?
3. How do changes in Normalized Difference Vegetation Index (NDVI) influence faecal glucocorticoid metabolites (FGM) concentrations of African savannah elephants in Mpala ranch?

1.4 Hypothesis

HO: Resident elephants whose home ranges are restricted to within Mpala Ranch do not experience lower physiological stress from anthropogenic perturbation compared to long distance migrant elephant families whose home ranges overlaps several ranches within Laikipia County.

HA: Resident elephants whose home ranges are restricted to within Mpala Ranch experiences lower physiological stress from anthropogenic perturbation compared to long distance migrant elephant families whose home ranges overlaps several ranches within Laikipia County.

1.5 Objectives

1.5.1 General objectives

To determine the influence of ranging behavior and vegetation quality on the stress hormone levels of African elephants at Mpala Ranch, Laikipia County.

1.5.2 Specific objectives

1. To assess how stress hormone concentrations between the resident and migrant elephants that are found within Mpala Ranch differ.
2. To assess the relationship between concentrations of faecal glucocorticoid metabolites (FGM) and the location of sampling within Mpala ranch.
3. To determine whether a change in normalized difference in vegetation index (NDVI) influences faecal glucocorticoid metabolites (FGM) concentrations in African savannah elephants in Mpala ranch.

1.6 Significance of the study

This study was important in understanding how elephants that reside outside of protected areas adjust their space use patterns in both private and communal ranches with different disturbance levels and vegetation quality. Information generated from this study would benefit private ranch owners and Northern Rangeland Trust (a membership owned community conservancy) in mitigating negative human elephant interaction. This study would also benefit Kenya Wildlife Service in understanding elephant risk-management behavior and reducing human-elephant conflict. Additionally, this study would benefit Laikipia County government on land use planning and policy formulation within wildlife dispersal areas in human occupied landscapes.

1.7 Conceptual Framework

The diagram on conceptual framework illustrates that the concentration of Faecal Glucocorticoid Metabolite which is the dependent variable, is influenced by Mpala location, ranging behavior, and vegetation quality i.e. normalized difference vegetation index (see Figure 1). Mpala location is divided into north and south of Mpala, ranging behavior is divided into resident and migrant elephant families, and vegetation quality is divided into dry and wet season. Both resident and migrant elephant families are further divided into age, sex, Body Condition Score (BCS), reproductive status (presence or absence of temporal gland secretion), and temperament (elephant's reaction index) which acts as an intervening variable.

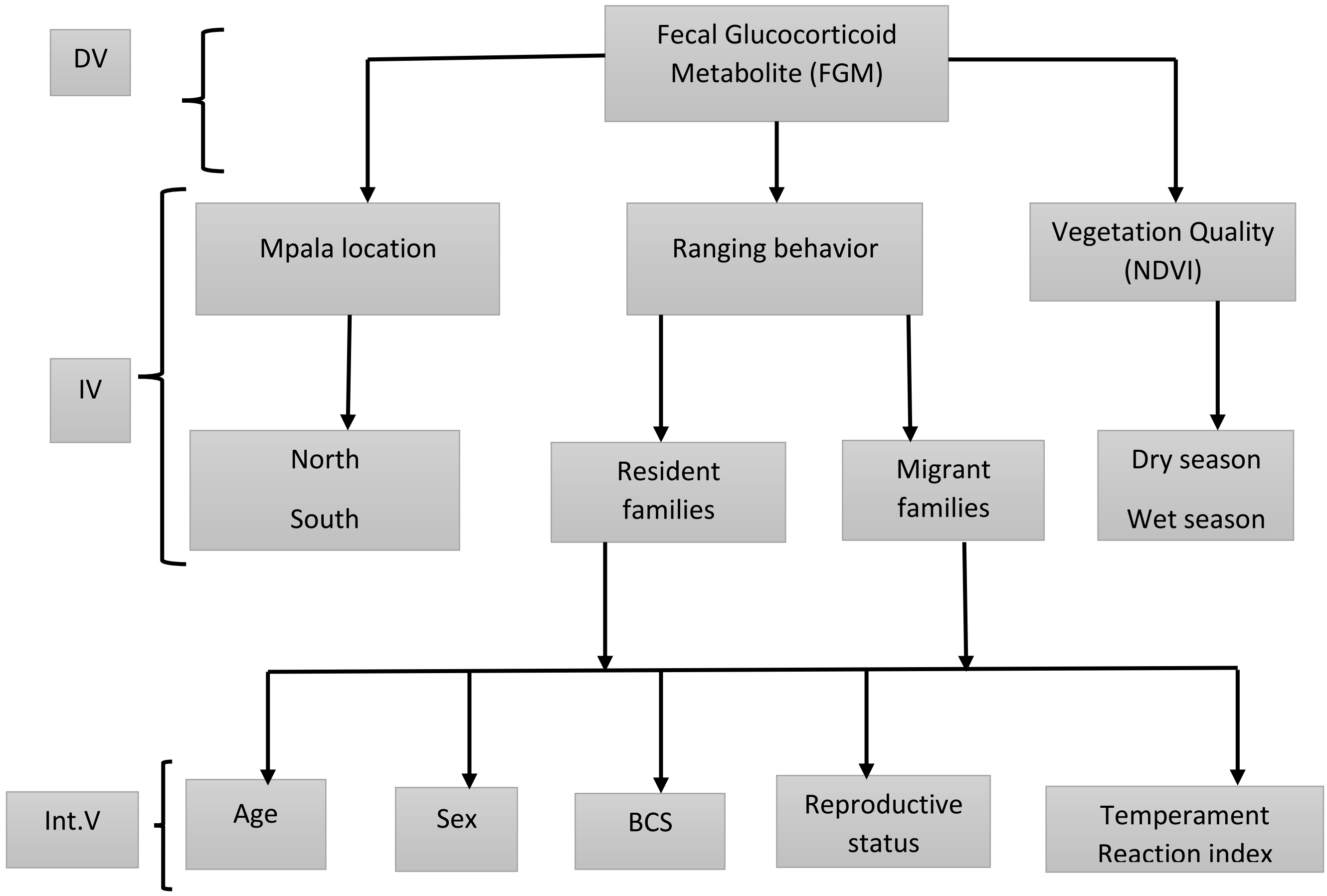


Figure 1. 1: Conceptual framework of the study

Key DV = Dependent variable, IV = Independent variable & Int.V = Intervening variable.

1.8 Definition of terms

Acute stress: This is a condition in which an animal takes a short time period before returning back to basal levels (within a few days to a week) after exposure to stressor factor.

Chronic stress: This is a condition in which an animal takes a longer time period before returning back to basal level (usually several weeks or months depending on a species) after exposure to stressor factor.

Corticosteroids: These are a class of steroid hormones which are produced in the adrenal cortex of vertebrates

Delay time: This is the difference between the time of defaecation and the time before the samples are collected for freezing.

Ecosystem services: Ecosystem services are conditions and processes through which natural ecosystems, and the species that make them up, sustain and fulfill human life.

Glucocorticoids: These are steroid hormones that bind the glucocorticoid receptors which are present in all vertebrate animal cell and play an important role in physiological process e.g. stress responses, metabolism and immune responses.

Gut-transit time: This is the time taken for the steroids to be metabolized in the liver, excreted through the bile fluid and into the rectum. For elephants, this usually takes 48 hrs.

Home range: This is the area traversed by an individual in its normal activities of food gathering, mating, and caring for the young.

Human-elephant conflict: This is an interaction between humans and elephants (often negative) which results in loss of life or socio-economic losses.

Migrant families: These are families who spend less than six months of their time at Mpala Ranch or those who come only during the dry or wet season lasting no more than six months at Mpala

Pro-wildlife properties: These are private or communal properties that are tolerant of wildlife presences by practicing wildlife conservation, ecotourism and ranching.

Resident families: These are elephant families who spend more than 75% of their time at Mpala ranch.

1.9 Scope and limitations

This study was limited to sampling the migrant families who were found within Mpala Ranch by the time this study was being undertaken. This study was also limited to accessibility of bad weather roads particularly “black cotton” vertisol soils during the rainy season.

CHAPTER TWO: LITERATURE REVIEW

2.1 Assessing stress in wildlife

Environmental perturbation has been observed to be the major selective force in natural populations of wildlife (Reeder & Kramer, 2005). Such activities may elicit physiological stress in animals and affect the behavior of populations as well as their demographic rates (Hockin *et al.*, 1992; Frid & Dill, 2002). Different techniques have been employed to assess health and welfare of wildlife, both in captivity and in the wild, such as visual body condition scoring; laboratory analyses of reproductive cycle, stress, nutritional and immune biomarkers; behavioral monitoring, and fitness evaluations. Stress occurs when animals have to make extreme and or prolonged physiological or behavioral adjustments to cope with adverse environmental conditions and maintain homeostasis (Dantzer *et al.*, 2014).

Stress can be short-term and helps an animal to escape from life threatening events such as predator attack (Wingfield *et al.*, 1998) or long-term which can have a negative effect on an animal's fitness (Sheriff, Bosson, Krebs, & Boonstra, 2009). Prolonged exposure to stress results in the release of glucocorticoid. This can negatively affect the physiology of an animal by suppressing their immune function which results in increased susceptibility to diseases, decreased wound healing, inhibition of reproduction and decreased growth rate (Sapolsky *et al.*, 2000), all of which are detrimental to an individual fitness.

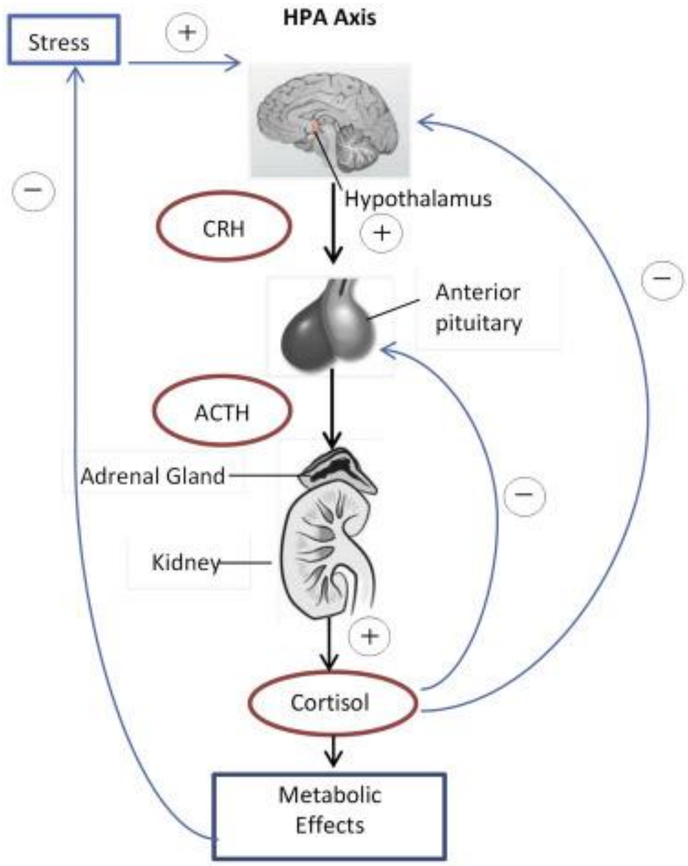


Figure 2. 1: A schematic diagram of the negative and positive feedback mechanism of the hypothalamic-pituitary-adrenal axis (HPA Axis). (Source: Kulkarni et al., 2016).

Stress responses are mediated by two neuroanatomical systems: i.e. sympathetic nervous system (SNS) which results in the release of catecholamine; and activation of hypothalamic-pituitary-adrenal (HPA) axis which in turn reestablishes a state of homeostasis (Sapolsky *et al.*, 2000). Hypothalamic-pituitary-adrenal is a major neuroendocrine system responsible for regulating stress and other body systems, such as the immune system. Any environmental perturbation will trigger the release of corticotrophin-releasing hormone (CRH) from the hypothalamus which in turn travels to the pituitary gland (anterior lobe) to trigger the release of adrenocorticotropic hormone (ACTH), which in turn stimulates the release of glucocorticoids (cortisol or corticosterone or a combination both) from the adrenal cortex (Selye, 1976). A schematic diagram of the HPA axis is shown in Figure 2.1.

Individuals experiencing chronic stress have higher levels of glucocorticoids (Sapolsky, 2005), which circulate in blood (plasma or serum), and are subsequently excreted in urine, feaces, and saliva, and deposited in feathers and hair (Sheriff *et al.*, 2011). Assessing stress in wildlife typically involves noninvasive monitoring through the use of faecal glucocorticoid metabolites analyses. This method is advantageous owing to the ease at which samples can be collected non-invasively, thereby reducing capture induced bias on the animal (Millspaugh & Washburn, 2004). Additionally, this method represent hormonal state of an animal over the hours preceding collection of the samples (Palme, 2005) and is more preferred when carrying out longitudinal studies on individuals (Touma & Palme, 2005).

Although excreted steroids are prone to bacterial and environmental degradation (Wasser, Risler, & Steiner, 1988; Beehner & Whitten, 2004), faecal sample collection can still be used as a proxy for measuring the physiological state of an animal (Palme, 2005; Touma & Palme, 2005) as long as the samples are collected within 8 hours after defecation. Freezing the samples after defecation usually halts degradation and hence preserve the hormone samples for long period before extraction processes can be carried out (Whitten, Brockman, & Stavisky, 1998; Palme, 2005; Ziegler & Wittwer, 2005).

Endocrine monitoring has predictive value for quantifying anthropogenic impacts on physiological responses, how they may affect behavior, and understanding how these responses interact synergistically to affect the population viability (Wikelski & Cooke, 2006; Cooke *et al.*, 2013). However, there are other intervening factors that must be taken into consideration when interpreting glucocorticoid data in animals. These include seasonal changes (Romero, 2002; Sheriff *et al.*, 2012) reproductive status (Rasmussen, Ganswindt, Douglas-Hamilton, & Vollrath, 2008), sex (Huber, Palme, & Arnold, 2003; Touma, Sachser, Möstl, & Palme, 2003) and changes in diet (Goymann, Trappschuh, Jensen, & Schwabl, 2006), all of which can affect glucocorticoid production.

2.2 Ranging behavior of African elephants

Spatial and temporal movement of wildlife within a landscape has been widely documented in a variety of taxa and it is caused by a complex interplay between extrinsic and intrinsic factors which operate at multiple spatio-temporal scales (Martin *et al.*, 2013). Extrinsic factors include predictable variations in the environment such as ability of an animal to obtain food, to find mate and to avoid predation which could subsequently affect their fitness (Lima & Zollner, 1996; Hopcraft *et al.*, 2014). Intrinsic factors on the other hand include individual physiological attributes (Wingfield, 2005; Nathan *et al.*, 2008).

Wildlife movement within a landscape consists of two major components: the migrant populations moving over long distances and having wider home ranges and the resident populations with small home ranges (Thouless, 1996; Dingle & Drake, 2007; Purdon, Mole, Chase, & Van Aarde, 2018). Patterns of spatial movement of wildlife in heterogeneous landscapes has a consequence on their foraging behavior, their reproductive success, and their fitness across spatio-temporal gradients (Dingle & Drake, 2007; Owen-Smith, Fryxell, & Merrill, 2010; Rolandsen *et al.*, 2017).

There has been a popular belief that conservation of wildlife in private ranches offers an integral role to the persistence of large mammals (Hunt, 1997; Figgis, 2004). This is due to their role in providing payment for ecosystem services, offering wildlife tourism and game ranching and offering conservation easements and leases. This is in contrast to communal ranches where livestock stocking levels are high, lethal methods are used to control wildlife and human populations keep on increasing (Kinnaird & O'brien, 2012).

Private land use systems would therefore be beneficial to elephant's internal physiological state with majority of the populations facing threats from different anthropogenic disturbances such as poaching, human-elephant conflict, land use changes, and habitat fragmentation, (Wittemyer *et al.*, 2014; Chase *et al.*, 2016; Thouless *et al.*, 2016). These anthropogenic disturbances have forced large mammals including elephants to alter their movement patterns and or flee away in response to human presence, which creates an unsafe environment for the calves and juveniles (Haidt, Kamiński, Borowik, & Kowalczyk, 2018). Anthropogenic disturbances therefore can lead to decreased energetic gains (Williams, Lusseau, & Hammond, 2006) and ultimately reduced reproductive success and fitness. There is therefore need to assess how elephants that reside outside of protected areas adjust their space use patterns in both private and communal ranches with different disturbance levels and vegetation quality and how anthropogenic disturbances manifest themselves on the physiological state of elephants.

Laikipia County has been witnessing extreme and unpredictable weather patterns including prolonged drought conditions. This resulted in numerous cattle invasion into private ranches and thereby affecting wildlife. In 2017 for instance, 18 elephants were reported to be killed as a result of the livestock invasion. In Mugie Ranch, elephants, giraffes, zebras and other wildlife were killed as a result of the conflict. The population of elephants in Sosian and Mugie ranches were hardest hit as a result of the conflict. Such anthropogenic activities may have likely elicited negative behavioral response to elephants. This study hence explored how the changes in behavior (i.e. negative reaction to vehicle presence) could have elicited higher physiological stress response in African elephants within Laikipia County.

Mpala Ranch which lies in the heart of Laikipia at the cross road of known elephant migratory routes, has been receiving migrant elephant families from ranches that were hardest hit from the livestock invasion and poaching. These anthropogenic disturbances have resulted in increased fear responses from migrant families who flee in response to vehicles, creating an unsafe environment for young calves and resulting in potential deleterious physiological changes associated with stress. With wildlife conservation placing greater emphasis on multiple use areas like Laikipia, there is a need to understand how these disturbances affect the survival and physiology of African elephants.

2.3 Effects of human activities on the physiological stress response of elephants

Previous studies have shown that elephants appear to be more stressed outside than within protected areas (Tingvold *et al.*, 2013; Hunninck *et al.*, 2017). A study by Thouless (1996) indicated that, elephant's home range size varied within Laikipia-Samburu ecosystem with resident families being restricted to only one ranch, while the migrants have home ranges overlapping several ranches. Additionally Ihwagi *et al.* (2018) observed that elephants responded to anthropogenic disturbances by altering their speed of travel at night in areas perceived to be posing higher level of risks. However, studies by Tingvold *et al.* (2013) and Hunninck *et al.* (2017) have failed to take into account the spatial variation that exists outside protected areas and subsequently different levels of exposures to anthropogenic disturbance outside protected areas.

A study by Kinnaird and O'brien (2012) indicated that Laikipia county has a high number of livestock that is currently influencing the distribution of wildlife negatively. Space For Giants (2017) observed that in 2015, about 250,000 livestock that were being led by 6,000 herders invaded wildlife ranches in Laikipia, an act that resulted in an upsurge of conflict over grazing in Laikipia. Besides cattle invasion into wildlife ranches, Laikipia County has been grappling with numerous incidences of human wildlife conflict with incidences of Human-Elephant Conflict (HEC) in the county being the worst in Kenya and among the worst in East Africa. Such anthropogenic activities could result in changes in behavior, demography, reproduction, as well as their health and welfare. Hence the need to assess and compare the physiological stress response of different migratory groups of African elephants exposed to these anthropogenic disturbances.

In addition to the anthropogenic disturbance, Laikipia County has been witnessing prolonged drought conditions and extreme and unpredictable weather patterns which are expected to amplify in the coming years due to global warming. Of particular concern is the land use change in Laikipia County which has been experienced over the past three decades due to climate change (M'mboroki, Wandiga, & Oriaso, 2018) which may influence forage availability for African elephants and subsequently increased crop raiding incidences. Heightened conflict and inadequate financial base are likely to be witnessed (Khalid, 2011) and hence the need to assess how anthropogenic disturbance affectes the health and welfare of wildlfie particularly the long ranging African elepphants which resides in human occupied landscapes. There is therefore need for conservationist to examine the intensity at which anthropogenic activities begins to alter physiology and how these activities may be detrimental to the species (Wikelski & Cooke, 2006).

2.4 Using conservation physiology tools to evaluate habitat quality

Remote sensing has been used as a powerful tool to predict the rangeland productivity in savannah ecosystem and other habitat ecosystems (Richardson & Everitt, 1992). Analysis of Normalized Difference Vegetation Index has been used as an indices of vegetative health and is calculated by the difference in the intensity of the Near-Infrared (NIR) radiance and the visible red wavelength (Huete, Justice, & Van Leeuwen, 1999). As a result, Normalized Difference Vegetation Index quantifies the photosynthetic capacity of each pixel within the image. Normalized Difference Vegetation Index values ranges between -1.0 to +1.0 with values closer to -1.0 associated with no vegetation while values closer to +1.0 being associated with dense vegetation.

Normalized Difference Vegetation Index can also be used to measure vegetation quality for herbivores as greening can be associated with lower food quality i.e. low nutrient content, low digestibility, higher crude fiber content and low crude protein content (Griffith *et al.*, 2002). On the other hand, browning can be associated with lower food quality i.e. higher nutrient content, higher digestibility, lower crude fiber content and higher crude protein content. An assessment of habitat quality therefore, is being used by conservation physiologist to measure its resulting impact on the physiological state of animals and the overall body score index. Pokharel, Seshagiri, and Sukumar (2017) observed the influence of body condition scores on Asian elephants with dry season associated with lower body condition scores while the wet season being associated with higher body condition scores. This subsequently affected their physiological stress response. Similarly, Foley *et al.* (2001) observed that dry seasons are usually associated with higher levels of stress. Unpredictable weather patterns has made it difficult to determine when the wet season or the dry season is likely to commence.

Although the study of stress within different taxonomic group in wildlife has been increasing over the years, there is sparse knowledge on how migration and behavior influences the physiological state of wildlife particularly elephants which are known to move widely. This study therefore sought to fill this knowledge gap by assessing the physiological stress response of two ranging behavior (i.e. resident elephants and migrant elephants) and their behavioral reaction to vehicle presence affect their physiological state. The study also sought to assess how vegetation quality via assessment of normalized difference in vegetation quality affect the physiological state of African elephants.

CHAPTER THREE: METHODOLOGY

3.1 Introduction

This section provides a detailed description of the study area, the experimental design used and how samples were collected from individuals using a catalogue recognition file prepared for monitoring different elephant individuals. This section also gives a detailed description of how samples were processed and analyzed in the endocrine laboratory at Mpala Research Centre and the processing of the sentinel 2 satellite images from April 2019 to August 2019 when this study was carried out.

3.2 Study area

This study was carried out at Mpala Ranch, which lies north of the equator on the Laikipia plateau (latitude $0^{0}17'32''$N, longitude $36^{0}53'52''$E). The ranch covers an area of approximately 200 km^2 of semi-arid savanna. A map of Mpala ranch is shown below.

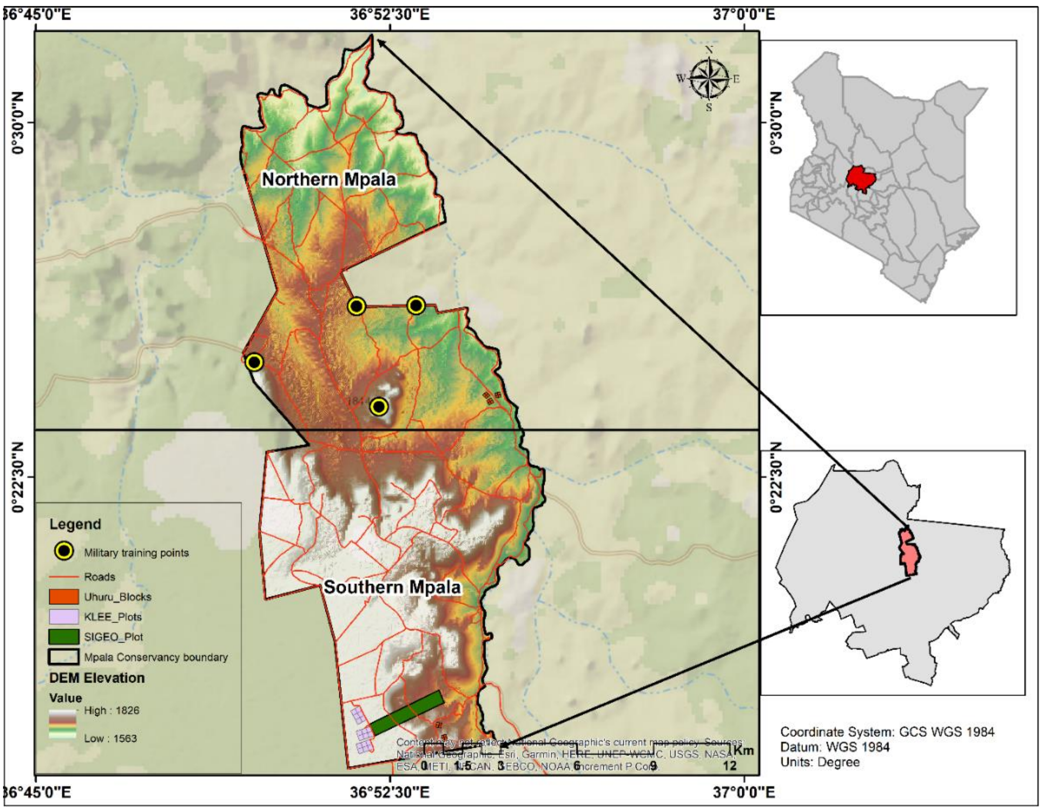


Figure 3. 1: A map showing the location of Mpala Ranch located in Laikipia County in Kenya.

3.2.1 Climate

The climate consists of weak tri-modal annual patterns with a north-south rainfall gradient. Rainfall usually peaks during the month of April and May for the long rains and

August and October for the short rains while the dry season occurs from January to March (Augustine & McNaughton, 2004; Pringle, 2008; Goheen *et al.*, 2013).

3.2.2 Vegetation and wildlife

The study area is characterized by woody vegetation with *Acacia drepanolobium* accounting for 98% of the overstory vegetation (Young, Okello, Kinyua, & Palmer, 1997) in the “black cotton” vertisol soils while the “red” vertisol soil is dominated by *Acacia brevispica*, *Acacia mellifera*, and *Acacia etbaica*. Other woody species includes *Croton dichogamous*, *Rhus vulgaris* and *Grewia* species (Young, Patridge, & Macrae, 1995). Elephant numbers in this area ranges from 300 – 900 individuals depending on the seasons and difference in movement patterns (MRC, 2014), with some being resident, while others are migrants. Apart from African elephants (*Loxodonta africana*), Mpala ranch is also home to threatened species such as lions (*Panthera leo*), leopards (*Panthera pardus*), cheetahs (*Acinonyx jubatus*), and African wild dogs (*Lycaon pictus*). The area also supports large herbivores including the endangered Grevy’s zebras (*Equus grevyi*), and reticulated giraffe *(Giraffa camelopardalis*), among other large herbivores such as African Buffaloes (*Syncerus caffer*).

3.3 Research Design

3.3.1 Experimental design

A cross-sectional research design method was used to obtain data from the study area; i.e. Mpala Ranch. A cross-sectional research design is a type of observational study design where the investigator measures the outcome or exposure in the populations at the same time. The study area was first divided into North and South sections along rainfall gradient as described by Goheen *et al.* (2013). The two locations also varied in levels of anthropogenic disturbance arising from the number of cattle “bomas”, military ballistic training sites, and frequency of illegal cattle grazing. The two sections of Mpala (North and South) were sampled by driving random routes as well as communicating with different security outposts located in different parts of Mpala about elephant presence.

3.3.2 Identification of elephants

A pictorial database of the catalog recognition file developed by the Mpala Elephant Research Project which contained all the resident and migrant elephant families was loaded into a Samsung Galaxy Tablet A T580 10.1" and used in the field to identify different elephant herds as well as the bull(s).

Plate 3. 1: Identifying a lone bull using the database on the tablet.

Both male and female adult individuals were identified using their ears and tusks idiosyncrasies. In the plate 3.1 above, the tusk appeared to be curved upwards and is a unique feature which can be used to identify the individuals. Once the individual(s) were successfully identified in the pictorial database, the individual(s) were monitored from a distance as shown in plate 3.2 below until defecation was observed from any member of the family group.

Plate 3. 2: Monitoring family herd from a distance around the dam for faecal sample collection.

In the event that a new family was not found within the pictorial database, photos of the adult females in the group were taken and the age of each of their calves estimated and used in preparing a catalogue recognition file based on the method described by Moss (1996). A sample of a catalogue recognition file for monitoring elephants and prepared during the Mpala Elephant Research project is shown in plate 3.3. The AI1 shown below has got inverted “V-shaped” cuttings on both sides of the ears with wrinkled ear lobe which can be used as a unique feature to identify this female elephant. Several holes are also visible on both sides of the ears.

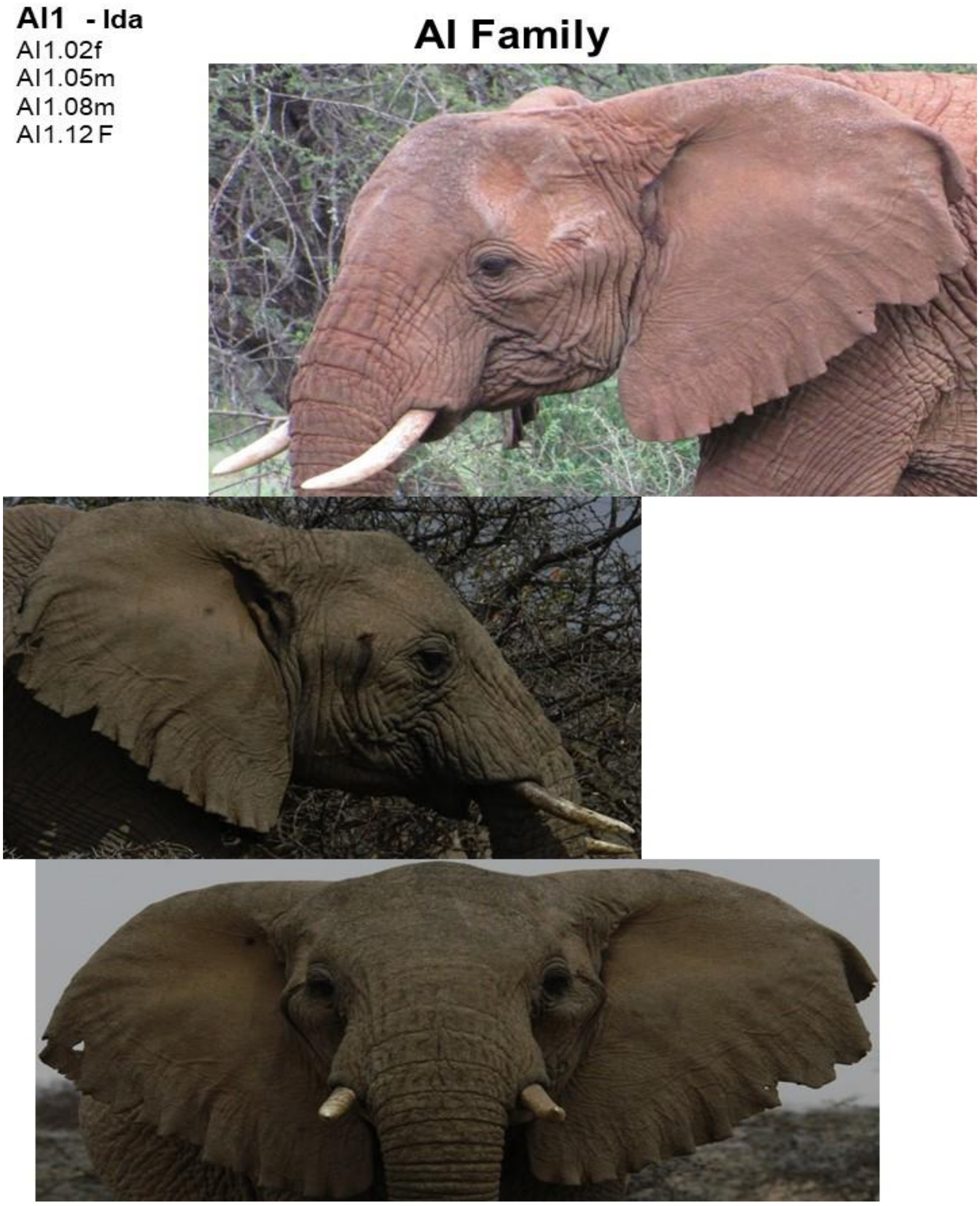


Plate 3. 3: A sample of a catalogue recognition file for AI family (matriarch) with four calves AI1.02F (female calf born in 2002), AI1.05M (Male calf born in 2005), AI1.08M (Male calf born in 2008) and AI1.12F (Female calf born in 2012)

3.4 Sample size and sampling procedure

Elephants that were monitored at Mpala Ranch between 2009 and 2014 and included photographic records of breeding females N_{Mpala}=573 and dispersed males N_{Mpala}=139 (Goldenberg *et al.*, 2016) were targeted for faecal sample collection. The sample size (n) was obtained using the formula developed by Cochran (1963) due to the heterogeneity of the population.

$$n = \frac{Z^2 pq}{e2}$$

Where: n= sample size

Z^2= the abscissa of the normal curve that cuts off an area α at the tails (1 - α equals the desired confidence level is 95%),

e= the acceptable sampling error

p= the estimated proportion of an attribute that is present in the population

q= 1-p

Based on the formula described above, a sample size of n = 100 individuals was targeted for fecal sample collection.

3.5 Data collection

3.5.1 Measuring behavioral reaction to vehicle presence in African elephants as a proxy to human disturbance

Data was collected by the principle investigator accompanied by a security guard. For behavioral data, the principle investigator in accompany of the security guard, aimed at approaching an elephant to a distance of about 20 m before the vehicle engine was turned off. Their behavior to vehicle approach was categorized as: 1) calm within 20 m of vehicle approach; 2) skittish on vehicle approach; 3) frightened and avoiding the vehicle; 4) terrified and running away. This was used as an indicator of whether the family group had experienced poaching or other forms of human disturbance such as human elephant conflict. Since it was difficult to distinguish between 'frightened and avoiding vehicle' and 'terrified and running away' in dense vegetation types, the two categories were collapsed into one defined as 'showing fear and avoiding vehicle' during analysis.

Plate 3. 4: A family herd calm within 20 m of the research vehicle

An individual or family group was considered to be calm within 20m if the activity of the family group or individual was not interrupted by the researcher's approach on a vehicle to within 20m of vehicle approach. In the plate 3.4, the family group was resting under a tree and the researcher's approach to vehicle did not interrupt their activity i.e. resting under a tree.

Plate 3. 5: A skittish female elephant upon vehicle approach.

An individual or family group was considered to be skittish upon vehicle approach if the activity of the family group or individual was slightly interrupted by the researcher's approach on a vehicle but the family group or individual thereafter remained calm after a few minutes. This is shown by plate 3.5.

Plate 3. 6: A family herd frightened and avoiding an approaching vehicle

An individual or family group was considered to be frightened and avoiding vehicle if the activity of the individual or family group was interrupted and the individual(s) started to walk away from an approaching vehicle as shown in plate 3.6.

Plate 3. 7: A terrified female elephant running away from the vehicle.

An individual or family group was considered to be terrified and running away from the vehicle if the activity of the individual or family group was interrupted and the individual(s) started running away from the approaching vehicle as shown in plate 3.7.

3.5.2 Collecting fecal samples for hormonal analysis

While the vehicle engine was turned off, each individual was monitored at a distance of at least 20 m depending on how they first reacted to vehicle response until defecation was observed. If the investigator was not sure of the identity of any individual, then the sample was not collected. If the individual was successfully identified in the catalog, using pictorial database of the individuals in the Samsung Galaxy Tablet A T580 10.1", then all information was recorded. Information recorded from sampled individual included the GPS coordinate of the area, time of defecation, time of collection, Mpala location, group type, age group, ranging behavior, and reaction index (1. Calm within 20m, 2. Skittish on vehicle approach, 3. Frightened on vehicle approach, 4. Terrified and running away), sex, reproductive condition in males (presence or absence of musth), body condition scoring, and the number of individuals in the family herd, Mpala location based along rainfall gradient (Goldenberg *et al.*, 2016).

The group type was classified as cow/calf, lone bull, lone female, lone calf, or mixed (both cow/calf and adult bull). The age group was classified as 1) juvenile (0-8yrs.), 2) Sub-adult (9-17 yrs.) 3) Adult (≥18 yrs.) using the method described by (Moss, 1996). Body condition was assessed through visual observation of the body fat deposits around the ribs, back bone, pelvic and the depression of the pelvic and lumbar regions using the method described by Morfeld *et al.* (2014). The body condition scoring chart was printed, laminated and used in the field as a guide for scoring the body condition of different individuals whose samples were collected. An individual was given a Body Condition Score (BCS) of 1, 2, 3, 4 or 5 with 1 representing very thin and 5 representing very fat. A detailed description for BSC-1, BCS-2, BSC-3, BSC-4, and BSC-5 is provided in the plate 3.8.

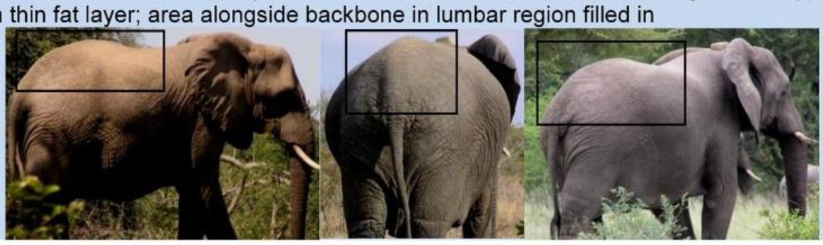
BCS	Description and example photographs
1	**Ribs:** Clearly visible **Pelvic Bone:** Protrudes, deep depression in front and depression or flattened area behind pelvic bone **Backbone:** Prominent from tail head to shoulders, deep depression alongside backbone in lumbar region 
2	**Ribs:** Not visible and appear to be covered by a very thin fat layer **Pelvic Bone:** Clearly visible, gradual sunken area in front and flattened area behind pelvic bone **Backbone:** Clearly visible from tail head to mid-back, depression alongside backbone in lumbar region 
3	**Ribs:** Not visible **Pelvic Bone:** Visible as a ridge, entire pelvic bone may not be visible, slight sunken or flattened area in front and/or behind pelvic bone **Backbone:** Visible from tail head to mid-back, sloping alongside backbone in lumbar region 
4	**Ribs:** Not visible **Pelvic Bone:** Not visible **Backbone:** Visible as ridge from tail head to mid-back, no obvious depression and fat beginning to accumulate alongside backbone in lumbar region 
5	**Ribs:** Not visible **Pelvic Bone:** Not visible **Backbone:** Difficult to differentiate, may be visible from tail head to pelvic bone region and appears to be covered with a thin fat layer; area alongside backbone in lumbar region filled in 

Plate 3. 8: Scoring chart for body condition (source: Morfeld et al., 2014).

To account for variation in the concentration of faecal glucocorticoid metabolites in different parts of the dung boli, each dung bolus was divided in the middle and samples from each dung boli collected (Ganswindt, Rasmussen, Heistermann, & Hodges, 2005). The dung samples were placed into 60ml fecal plastic tubes and stored in a cold box with ice packs in the field and transferred to a -80^{0}C freezer within 6 hours to limit bacterial metabolism of the hormone (Möstl & Palme, 2002).

The principle investigator aimed at collecting the dung samples within an hour of defecation and that no sample was exposed to rain as this could influence the concentration of the fecal glucocorticoid metabolite (Palme, 2005).

Plate 3. 9: Collecting dung samples by splitting each dung boli and sampling the inner section of the dung.

Plate 3. 10: Faecal sample being collected after family group has moved to a safer distance from the researcher’s vehicle.

3.5.3 Assessment of vegetation quality via analysis of NDVI

Sentinel 2 images for Mpala Ranch for the month of April, May, June, July, and August 2019 were extracted using Google Earth Engine (GEE), a cloud based geospatial processing platform. The area of interest i.e. Mpala Ranch was first defined using the geometric coordinate polygon for Mpala in JavaScript programming language. The satellite images were then filtered by the dates of acquisition (April, May, June, July and August) when this study was undertaken, and masking the clouds to only 5% using JavaScript programming code language. The images generated from each month when this study took place were sent to google drive and loaded to R programming, a statistical computing language R Development Core Team (2018) in form of Tagged Image File Format (TIFF) for further analysis.

Processing and generation of Normalized Difference Vegetation Index (NDVI) maps were done in R Core R Development Core Team (2018) using SP package for spatial analysis (Pebesma & Bivand, 2005), Rgdal package for geospatial data abstraction (Keitt, 2010), raster package for geographic data analysis and modelling (Hijmans & Van Etten, 2016), Viridis package for aesthetically pleasing color palette, (Garnier, Ross, & Rudis, 2018), and ggplot2 package for elegant graphical analysis (Wickham, 2016). NDVI for each month was calculated using the following equation

NDVI = (Band 8-NIR – Band 4-Red)/ (Band 8-NIR + Band 4-Red).

The mean NDVI values for every cells were then generated and NDVI graph plotted.

3.6 Processing faecal samples

3.6.1 Extraction of faecal samples

Faecal samples were thawed to room temperature and 0.5g (±0.02) of well-mixed sample was weighed in a 16x100 mm glass tubes. Five ml of 90% of absolute methanol (4.5 ml of 100% methanol and 0.5 ml of distilled water) was added to each weighed sample and vortexed using multi-pulse vortex at a speed of 70 with 1 pulse/second for 30 minutes. The samples were then centrifuged at 2700 rpm for 20 minutes. The subsequent extract in each glass tube was poured into an identically labeled 16x100 mm glass tube and dried under air in a warm water bath.

Plate 3. 11: A well-mixed faecal sample being weighed into a 16X100mm glass tube.

The tubes were then reconstituted with 1 ml of assay buffer and sonicated until the dried materials were removed from the sides of the tube. A parallelism check was conducted by serially diluting the extract with assay buffer to determine the dilution factor needed, which was determined to be 1:4; i.e. 100 µL of the neat concentrate to 300 µL of buffer solution.

3.6.2 Enzyme immunoassay

A pre-coated 96 well plate of donkey anti-sheep Immunoglobulin-G (IgG) [Catalog Number X061-EIA Arbor Assays (www.arborassays.com)] was used. Before analysis, several reagents were prepared. An Assay Buffer Concentrate, Catalog Number X065-55ml Arbor Assays was first prepared by diluting the concentrate to 1:5 i.e. adding one part of the concentrate to four parts of deionized water. Wash Buffer Concentrate, (Catalog Number X007 125ml, Arbor Assays) was prepared by diluting the concentrate to 1:20 i.e. adding one part of the concentrate to nineteen parts of deionized water. Corticosterone standard (100,000 picograms per milliliter i.e. pg/ml, Catalog Number C151-625UL, Arbor Assays) was serially diluted 2-fold to 10000, 5000, 2500, 1250, 625, 312.5, 156.25, and 78.125 pg./ml by using 50µl of corticosterone stock solution to 250µl assay buffer and mixed well. Control 1 (representing 30% binding) and Control 2 (representing 70 % binding) were set at 200 pg/ml and 3000pg/ml.

All these reagents were loaded into the 96 well plate in a systematic order in duplicate. 50µl of assay buffer, standards, controls and fecal extracts (diluted 1:4) were loaded into the plate. A corticosterone conjugate solution (25 µl) was added to all 96 wells followed by the corticosterone antibody solution (except in the first two wells, non-specific binding).

Plate 3. 12: Sonicating the extracts in an ultrasonic bath in the sonicator.

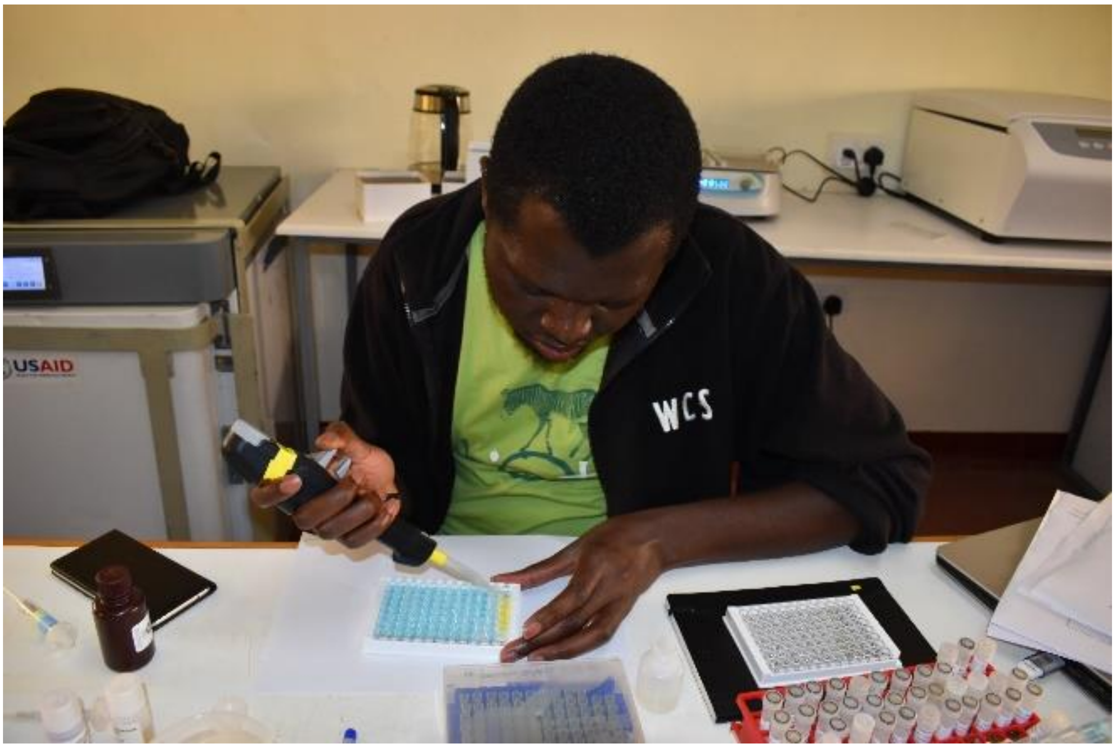


Plate 3. 13: Loading the 96 well plates using a repeater pipette.

The plate was left to incubate for 1 hour on a plate shaker. The plate was thereafter washed with the wash solution and 100µl of Tetramethylbenzidine (TMB) solution added into all wells. 25µl of stop solution was then added to the wells and the optical density read on a plate reader (Thermo Scientific Multiskan FC) at 450 nm using the sigmoidal dose response from www.myassays.com for interpretation of the results and the curve.

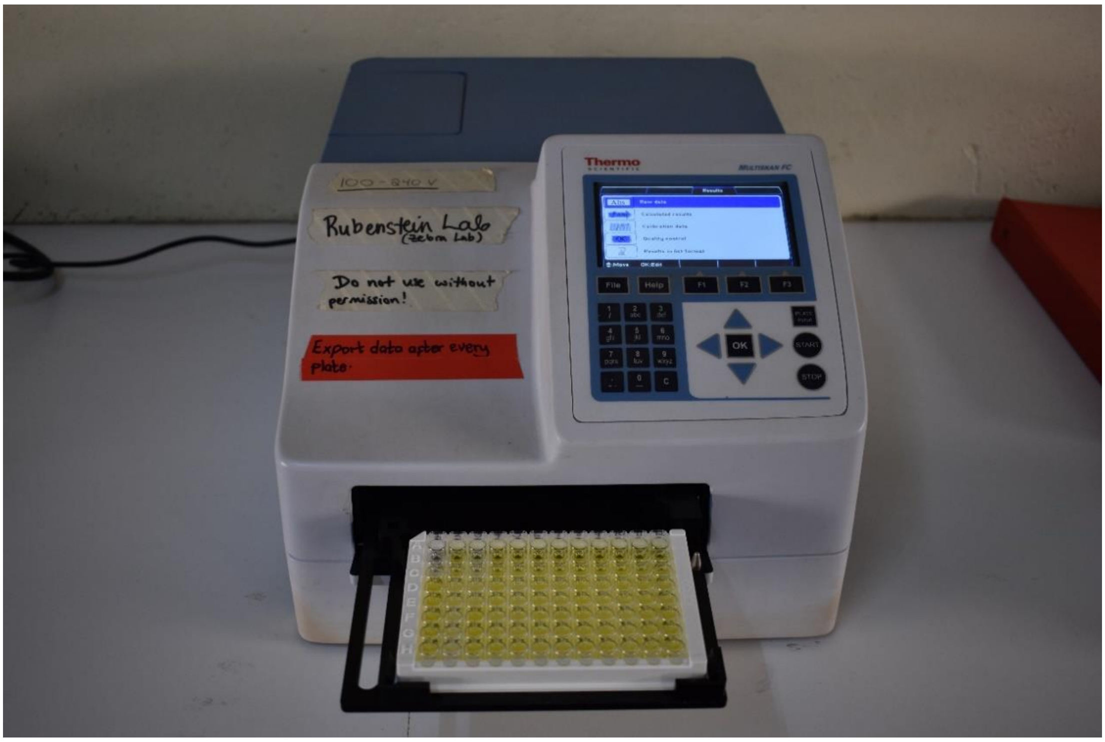


Plate 3. 14: A Thermo Scientific Multiskan FC plate reader with loaded plate wells and used in obtaining the readings on the concentrations of the hormones.

FGM measured in ng/g of wet weight was obtained by multiplying the concentration by the dilution factor (which was determined to be 4 based on the parallelism check), divided by each measured weight of the sample and dividing it again by 1000 to convert it from picogram (pg) to nanogram (ng). The inter-assay coefficient of variation (CV) was <10% between sample duplicates and the intra-assay CVs for two internal controls analyzed on each assay were 5.7% and 8.2% (N = 7 plate wells).

3.6.3 Analysis of factors influencing stress

Different factors including age, sex, Mpala location, were incorporated and used in modelling the factors that could be influencing stress in elephants at Mpala Ranch, Laikipia County, Kenya. Table 3. 1 shows different factor variables that were observed during fieldwork data collection.

Table 3. 1: A summary of factors and levels recorded and used in modelling variation of stress between resident and migrant elephant families.

Factor	Level	Description
Delay Time	Time of defecation Time of collection	The difference between the time of collection and the time of defecation.
Mpala Location	North South	Based on rainfall gradient described by Goheen *et al*., (2013) and different degrees of anthropogenic disturbances occurring in North & south of Mpala.
Group type	Cow/calf Mixed group Bull(s)	The group type category is based on the method described by Moss, 1996.
Age group	Juvenile Sub-Adult Adult	The age category is based on the method described by Moss, 1996.
Ranging behavior	Resident families Migrant families	Based on Wittemyer 2001.
Reaction Index	Calm to within 20m Skittish on vehicle approach Frightened & avoiding vehicle Terrified & running away	Based on categorization described by Goldenberg et al., 2017.
Sex	Male Female	Based on observing the shape of the head and their reproductive systems as described by Moss 1996.
Temporal gland secretion	Yes No	In males, the secretion of temporal gland is associated with musth period i.e. heightened sexual activity (Poole& Moss, 1981). In females, the secretion of temporal gland is associated with excitement e.g. when separated groups re-unite (Moss, 1988) or during distress (Douglas-Hamilton *et al*., 2006).
Body Condition Index	1-Very thin 2-Thin 3-Normal 4-Fat 5-Very fat	Based on categorization developed by Morfeld *et al*., 2014.
Family ID	Alpha-numeric system of naming	As developed in the catalog files.
Total counts	Numerical total	Based on total number of individuals in the herd or found to be associating at the time of observation.

3.6.4 Statistical analysis

Data was analyzed using R version 3.5.1 (R Development Core Team (2018). Wilcoxon Rank Sum Test was used to test the difference between predictor variables that were not normally distributed with two categories such as Mpala location (North or South) and Sex. Kruskal-Wallis test was used to test the difference between predictor variables that were not normally distributed with three or more categories e.g. age. Pearson's chi-squared test (χ^2) was used to test how likely that an observed difference between different categorical variables arose by chance. Linear Mixed Model (LMM) developed by Bates, Mächler, Bolker, and Walker (2014) was used to test the hypothesis that resident elephant families whose home ranges are restricted to within Mpala Ranch experienced lower physiological stress from anthropogenic perturbation compared to long distance migrant elephant families whose home ranges overlaps several ranches within Laikipia County. Akaike's Information Criterion (AIC) developed by Mazerolle (2013) was used to select the most suitable model that explains most of the variation in the data. Sjplot package developed by Lüdecke (2018) was used to test whether the selected model obeyed the assumptions of LMM. Result summaries were presented in tables and graphs as mean FGM concentrations ± Standard Error Mean (SEM).

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Introduction

This chapter reports on the findings emanating from this study which has been divided into three sections. The first section (4.2) reports on the findings related to how stress hormone concentrations between the resident and migrant elephants that are found within Mpala Ranch differ. The second section (4.3) reports on the findings between the concentration of fecal glucocorticoid metabolite and the sampling location within Mpala Ranch. The third section (4.4) reports on the findings on whether a change in NDVI influences glucocorticoid concentrations in African elephants at Mpala Ranch.

4.2 Physiological stress response between resident and migrant elephants

Data collection from this study was carried out from 23rd April 2019 to 30th August 2019. A total of 179 samples were successfully collected from 156 unique individuals with individual mean range between 2.58 to 15.55 ng/ml. The highest number of repeated samples collected from the same individual was 3 samples.

Plate 4. 1: Pots containing dung sampled from different elephants.

Demographic summary of the 156 unique individual elephants observed during the 4 month study period is shown in Table 4.1.

Table 4. 1: Demographic summary of elephants in the study monitored for behavior and FGM analyses from April to August 2019 at Mpala Ranch, Laikipia County, Kenya.

Demographic factors	**No. of individuals**	**Percentage (%)**
Age group		
Juvenile	44	28.21
Sub-Adult	32	20.51
Adult	80	51.28
Total	156	100.00
Group type		
Lone bull	3	1.92
Bulls in groups	12	7.69
Mother/calf pairs	86	55.13
Mixed groups (M/F)	55	35.26
Total	156	100.00
Sex		
Males	76	48.72
Females	80	51.28
Total	156	100.00
Ranging behavior		
Resident	57	36.54
Migrant	99	63.46
Total	156	100.00

The mean ± standard deviation (SD) observation days per month was 15 ± 4 days. Elephants were monitored until defecation was observed. The mean (SD) time between observed defecation to sample collection and placement in the cool box i.e. time delay between defecation and sample collection on the concentration of FGM was 10.75±5.06 minutes.

Majority of the samples were collected between 6-10 minutes after the elephant had defecated while few samples were collected between 26-30 minutes after the elephant had defecated (Table 4.2). Samples that were collected between 21-25 minutes had the highest FGM concentrations while samples that were collected between 26-30 minutes after the elephant had defecated had the lowest FGM concentrations.

Table 4. 2: Time delay in minutes before freezing the samples, their frequencies and the mean faecal glucocorticoid metabolites concentrations.

Time delay (minutes)	Frequency	Mean FGM
0-5	17	6.45
6-10	83	5.88
11-15	54	6.05
16-20	15	5.51
21-25	6	6.87
26-30	4	5.28

This study found no correlation between the time delay (i.e. the difference between the time that an elephant was observed to be defecating and the time the samples were collected and placed in a cooler box with ice packs) and the concentration of FGM (Spearman, R = -0.011, p = 0.88). This is indicated in Figure 4.1 below.

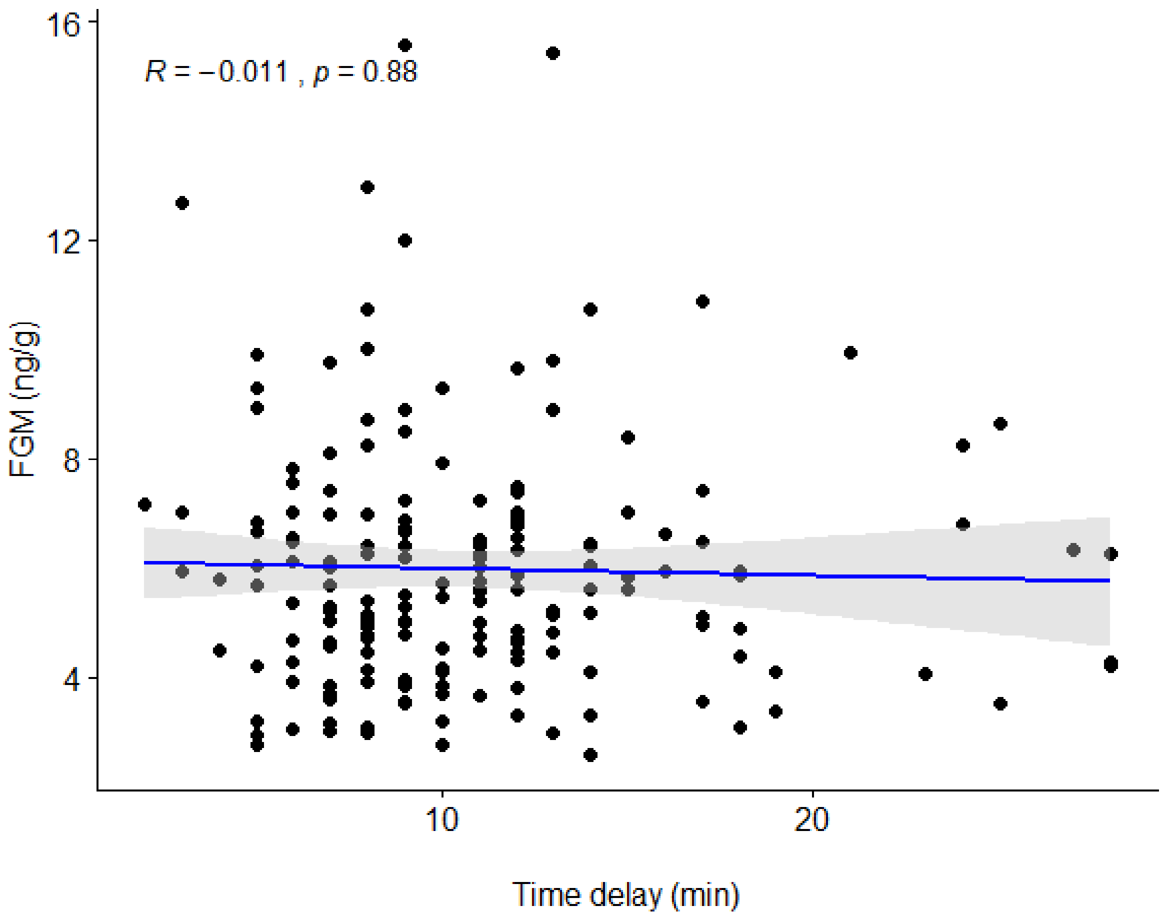


Figure 4. 1: A linear regression plot with 95% confidence interval (shaded areas) showing the relationship between time delay before samples were collected and frozen (in minutes) and FGM concentrations.

Results from this study indicates that the glucocorticoid hormones did not undergo bacterial enzyme degradation due to the delay in time between when an elephant had defecated and the time that the samples were collected and frozen into the cooler box with ice packs. Previous studies have observed the influence of time delay on measurements of faecal hormone metabolites which can compromise the reliability of hormonal monitoring. Webber *et al.* (2018) observed that faecal bacterial enzyme degradation starts to take place after 8 hrs of defecation in African elephants. Based on the fact that all the samples were collected within thirty minutes after defecation, the samples had not undergone any biological degradation by the time the samples were being collected. This explain why the regression line in the graph was fairly constant from the results and hence there was no relationship between the time delay (in minutes) and the concentration of FGM. Findings from this study corroborate previous studies which have observed similar findings of no relationship between time delay and FGM concentrations when samples are collected less than eight hours after defecation (Hunninck *et al.*, 2017).

There were no significant difference between the number of samples collected from males and females that were sampled during this study ($N_{females} = 92$, $N_{males} = 87$; $\chi^2 = 0.1397$, df = 1, p = 0.7086). There was a significant difference in FGM concentrations between females and males (Wilcoxon Rank Sum Test: W = 4908, p = 0.009) with females (6.38 ± 0.26 ng/g) having higher FGM concentrations compared to the males (5.54 ± 0.21 ng/g) as shown in figure 4.2.

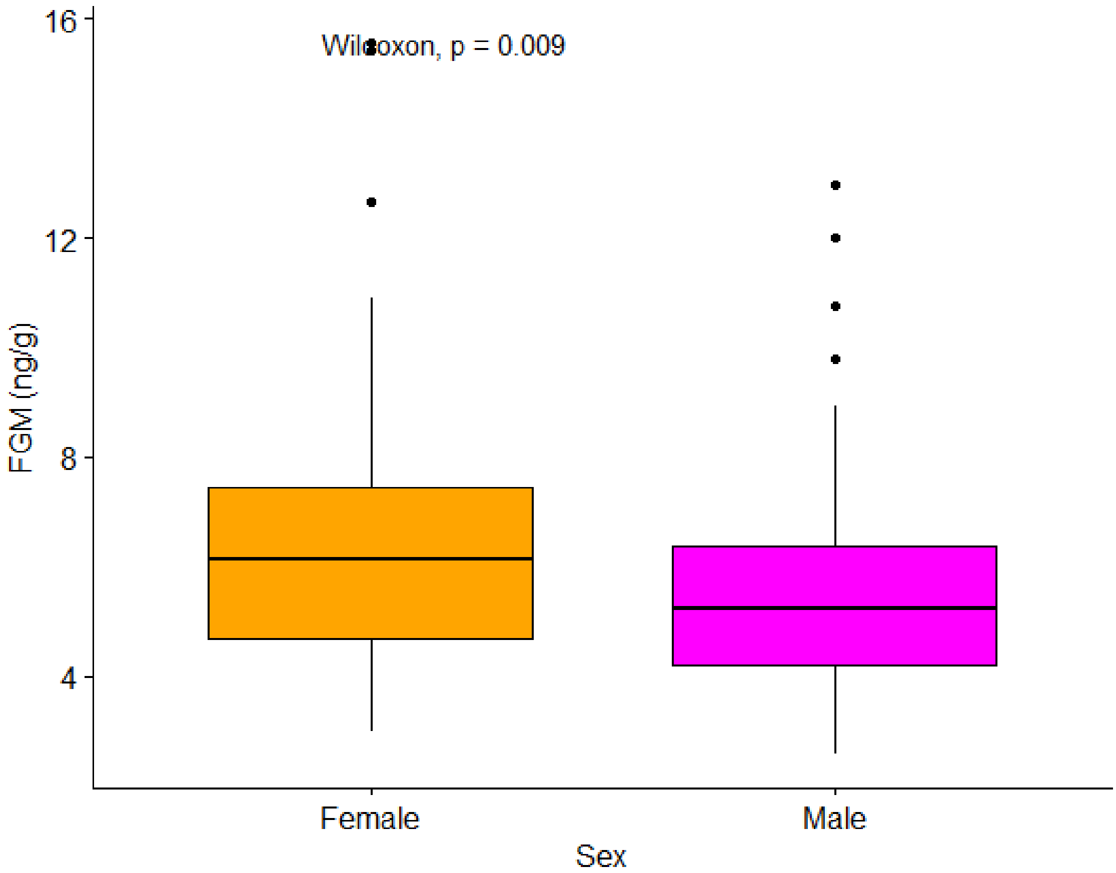


Figure 4. 2: Faecal glucocorticoid metabolites (FGM) concentrations for females and males.

The difference in FGM concentrations between the males and females been attributed to the social and physiological differences between the two sexes (Creel, Dantzer, Goymann, & Rubenstein, 2013), where the juveniles and adolescent females assisted in taking care of the calves (allomothering) as well as the adult females in the family herd (Lee, 1987). Findings from the study corroborate other authors who have found similar results where females have higher FGM concentrations than the males (Hunninck *et al.*, 2017), some studies have found the reverse (Ahlering *et al.*, 2013) while other studies have found no significant difference between males and females (Tingvold *et al.*, 2013). As a result it still remains to be determined whether the difference between these two sexes are due to their social and physiological differences or other intrinsic factors.

Majority of the samples were from the adults (N = 97) compared to juveniles (N = 48) or sub-adults (N = 34). Results from this study indicates that FGM concentrations did not significantly differ among different age groups (Kruskal-Wallis; $\chi^2 = 5.93$, df = 2, p = 0.052) as shown in Figure 4.3.

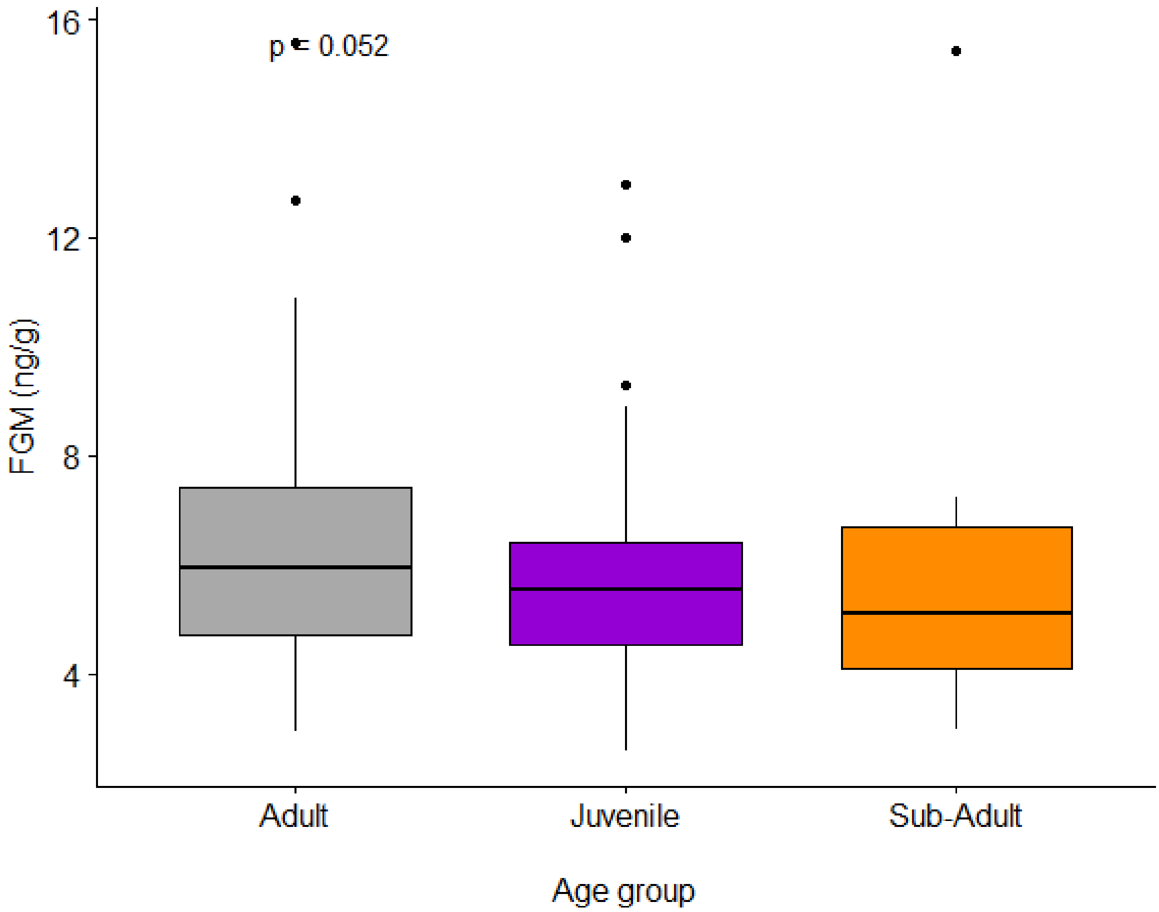


Figure 4. 3: Faecal glucocorticoid metabolites (FGM) concentrations for juveniles, sub-adults, and adults.

Findings from this study corroborate other studies which have found no significant difference in FGM concentrations among different age groups (Ganswindt *et al.*, 2005). Some studies however, have found significant differences between different age categories in Asian elephants (Vijayakrishnan *et al.*, 2018) and has been attributed to the differences in metabolic rates.

Majority of the samples were collected from mother with calf groups (N = 118) compared to mixed groups (N = 45) or bulls (N = 16). There was a significant difference in FGM concentrations among different group types (Kruskal-Wallis; χ^2 = 6.46, df = 2, p = 0.04) with mothers with calf (6.83 ± 0.34 ng/g) having higher FGM concentrations compared to bull groups (5.57 ± 0.61 ng/g) and mixed male/female groups (5.77 ± 0.33 ng/g) as shown in Figure 4.4.

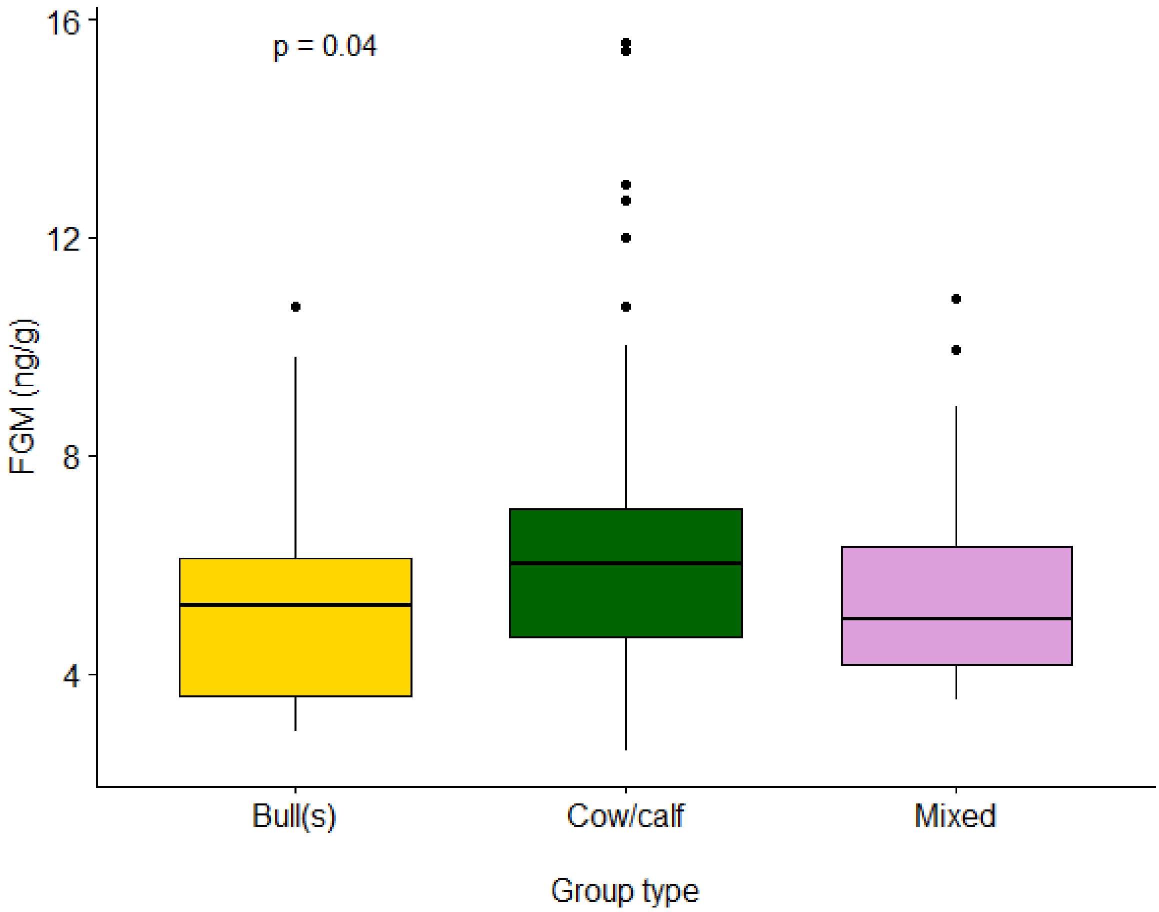


Figure 4. 4: Faecal glucocorticoid metabolites (FGM) concentrations for bulls, mother with calf and mixed group type.

This study found a relationship between group type and FGM concentrations with mothers with calf having a higher FGM concentrations than bulls and mixed group (i.e. both mother with calf and adult bulls). The high FGM concentrations in mother with calf could have been attributed to increased level of 'awareness' in a land use mosaic which posed a higher poaching risk to their calves (Boettiger *et al.*, 2011) and the role other adult females play in caring for the young ones i.e. allomothering (Lee, 1987). The higher responsibility roles played by the adult females within the family could have resulted in higher FGM concentrations.

Majority of the individuals were observed to be skittish on vehicle approach (N = 86) compared to individuals that were calm within 20 m of vehicle approach (N = 50) or terrified on vehicle approach (N = 43). There was a significant difference in FGM concentrations among different behavioral response to vehicle presence (Kruskal-Wallis; $\chi^2 = 15.78$, df = 2, p = 3.74e^{-04}) with individuals that were terrified and running away having higher FGM concentrations (6.95 ± 0.40 ng/g) than individuals that were calm within 20 m of the research vehicle (6.08 ± 0.29 ng/g) or individuals that were skittish upon vehicle approach (5.43 ± 0.22 ng/g) as shown in Figure 4.5.

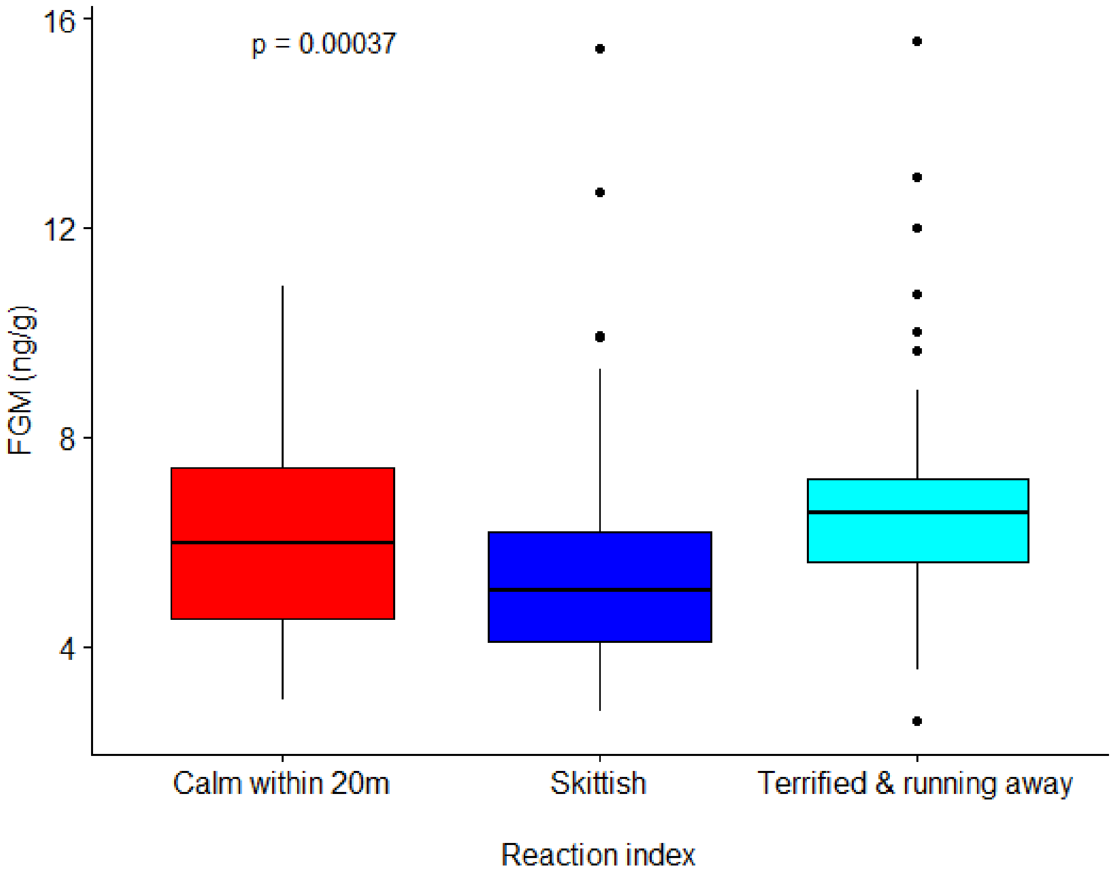


Figure 4. 5: Faecal glucocorticoid metabolites (FGM) concentrations for elephants that were calm within 20 m, skittish, and terrified and running away.

This study revealed the influence of past and or present negative interactions with humans on the physiological stress response of African elephants with individuals that were terrified and running away having higher FGM concentrations than individuals that were either calm within 20 m of vehicle approach or skittish on vehicle approach. Results from this study corroborate other studies which have shown wildlife ungulates to be increasing their flight initiation distance in response to hunting and human harassment (Stankowich, 2008; Tarakini, Crosmary, Fritz, & Mundy, 2014; Szott, Pretorius, & Koyama, 2019). This could manifest itself on the physiological state of wildlife and have been observed to influence behavioral changes and increased physiological stress in elephants (Szott, Pretorius, Ganswindt, & Koyama, 2020). In addition to this, Mpala also supports various activities including higher vehicular traffic from researchers which could results in higher physiological stress response among elephants that were terrified and running away.

Higher number of the samples were collected from males and females that were skittish on vehicle approach (N = 43) while fewer samples were collected from males that were terrified and running away (N = 18) as shown in Table 4.3. Additionally, females that were terrified and running away from an approaching vehicle (N = 25) had the highest FGM concentrations (7.22 ± 0.46 ng/g) while males that were skittish on vehicle approach had the lowest FGM concentration (5.08 ± 0.20 ng/g). Results from this study indicated that FGM concentration and elephant's reaction index differed between males and females ($p < 0.05$, post hoc pairwise comparison). Females that were calm within 20 m had a higher FGM concentrations than males that were calm within 20 m ($p < 0.05$, post hoc pairwise comparison). Females that were terrified and running away had higher FGM concentrations than males that were calm within 20 m of vehicle approach ($p < 0.05$, post hoc pairwise comparison). Additionally, Females that were terrified and running away from vehicle presence had a higher FGM concentrations than males that were skittish on vehicle approach ($p < 0.05$, post hoc pairwise comparison).

Table 4. 3: Samples collected from males and females that were calm within 20 m, skittish on vehicle and terrified and running away and their mean FGM concentrations, standard deviation and standard error.

Reaction Index	**Sex**	**Sample size (N)**	**Mean FGM**	**Std. Dev**	**Std. Error**
Calm within 20 m	Female	24	6.7	2.21	0.45
Calm within 20 m	Male	26	5.51	1.67	0.33
Skittish	Female	43	5.77	2.58	0.39
Skittish	Male	43	5.08	1.31	0.2
Terrified & running away	Female	25	7.22	2.29	0.46
Terrified & running away	Male	18	6.58	2.99	0.7

Results from this study revealed a possible past and or present anthropogenic disturbance differed between males and females with females being more susceptible to anthropogenic disturbance than males. Gobush *et al.* (2008) showed that poaching of matriarchs had a long term impact on the physiological stress response and reproductive output (assessed though measurement of the progesterone hormones) of other members within the family. Although this study did not analyze the progesterone levels of females, their behavioral reaction together with the higher FGM concentrations could be an indication of different levels of susceptibility between the two sexes. This could have a major impact on the reproductive output of the elephants resulting in population declines.

Higher number of samples were collected from migrant elephants that were skittish on an approaching vehicle (N = 59) compared to the resident that were skittish on an approaching vehicle (N = 27). On the contrary, fewer resident elephants were terrified and running away on an approaching vehicle (N = 6) compared to the migrant elephants that were terrified and running away (N = 37) as shown in Table 4.4. The physiological response of elephants to vehicle presence, based on FGM concentrations differed between ranging behavior ($p < 0.005$, post hoc pairwise comparison) with resident elephants that were calm within 20 m (6.33 ± 0.35 ng/g) having higher FGM concentrations than migrants that were skittish (5.06 ± 0.21 ng/g) ($p < 0.05$, post hoc pairwise comparison). Migrants that were skittish (5.06 ± 0.21 ng/g) also had lower FGM concentrations than migrants that were terrified and running away (6.59 ± 0.34 ng/g) ($p < 0.05$, post hoc pairwise comparison) as well as the residents that were terrified and running away (9.18 ± 1.77 ng/g) ($p < 0.05$, post hoc pairwise comparison).

Table 4. 4: Samples collected from resident and migrant elephants, their response to vehicle presence and their mean FGM concentrations, standard deviation and standard error.

Reaction index	**Ranging behavior**	**Sample size**	**Mean FGM**	**Std. Dev**	**Std. Error**
Calm within 20 m	Migrant	20	5.7	2.18	0.49
Calm within 20 m	Resident	30	6.33	1.9	0.35
Skittish	Migrant	59	5.06	1.58	0.21
Skittish	Resident	27	6.22	2.72	0.52
Terrified & running away	Migrant	37	6.59	2.06	0.34
Terrified & running away	Resident	6	9.18	4.35	1.78

Although the physiological demands of ranging over long distance exposes animals to stressful environments, making them more susceptible to disturbance (Wilcove & Wikelski, 2008), this study observed differences in behavioral and physiological stress response of African elephants with resident elephants having higher FGM concentrations than migrant elephants. The difference could have been attributed to the human activities that the resident elephants are exposed to in the northern part of Mpala including military ballistic training. Findings from this study are consistent with other study which have observed differences in behavioral response to vehicle presence between resident and migrants in Samburu and Buffalo Spring National Reserve (Goldenberg, Douglas-Hamilton, Daballen, & Wittemyer, 2017). The change in behavior i.e. responding negatively to an approaching vehicle could be an indication of anthropogenic disturbance which could be manifesting itself on the physiological stress response of African elephants.

Majority of the families consisted of small groups consisting of between 1 to 10 individuals while only one family had between 61 to 70 individuals. The mean number of individuals observed for every sampling session was 15±11 elephants as shown in Table 4.5.

Table 4. 5: Number of elephants observed in a herd, the frequency of the observations and the mean FGM concentrations.

Number of elephants	**Frequency**	**Mean FGM**
1-10	80	6.25
11-20	69	5.83
21-30	15	5.27
31-40	1	6.79
41-50	11	6.01
51-60	2	4.59
61-70	1	5.88

From the results, it was evident that the number of individuals within a family did not influence the concentration of FGM. Results showed no correlation between the number of individuals in the group and the concentration of FGM (Spearman $R = -0.088$, $p = 0.24$). This is shown in Figure 4.6.

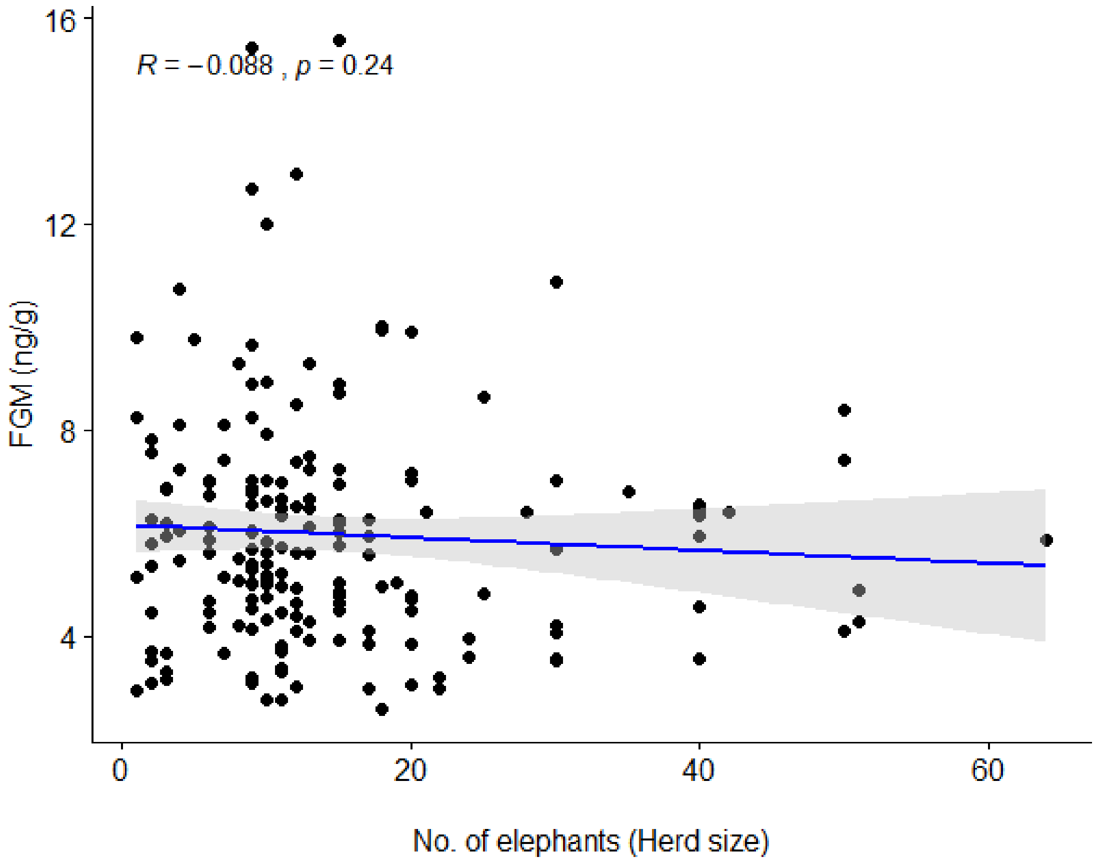


Figure 4. 6: A linear regression plot with 95% confidence interval (shaded areas) showing the relationship between number of individuals in a herd and FGM concentrations.

Although the study did not observe any relationship between the number of individuals in a herd and the physiological stress response of African elephants, previous studies have shown a positive relationship between the group size and the physiological stress response in African elephants particularly during the dry season (Foley *et al.*, 2001), due in part to the competition for resources within the herd during the dry season. Due to the limited time period that this study was carried out (4 months), it was not possible to determine the influence of season on the herd size and their FGM concentrations.

There were more migrant elephant families compared to the resident elephant families ($N_{Migrant}$ = 116, $N_{Resident}$ = 63; χ^2 = 15.693, df = 1, p = 7.451e^{-05}). The study found significant difference in FGM concentrations between resident and migrant elephants (Wilcoxon Rank Sum Test: W = 2918, p = 0.026) with resident elephants (6.55 ± 0.33 ng/g) having higher FGM concentrations than migrant elephants (5.65 ± 0.18 ng/g) as shown in Figure 4.7.

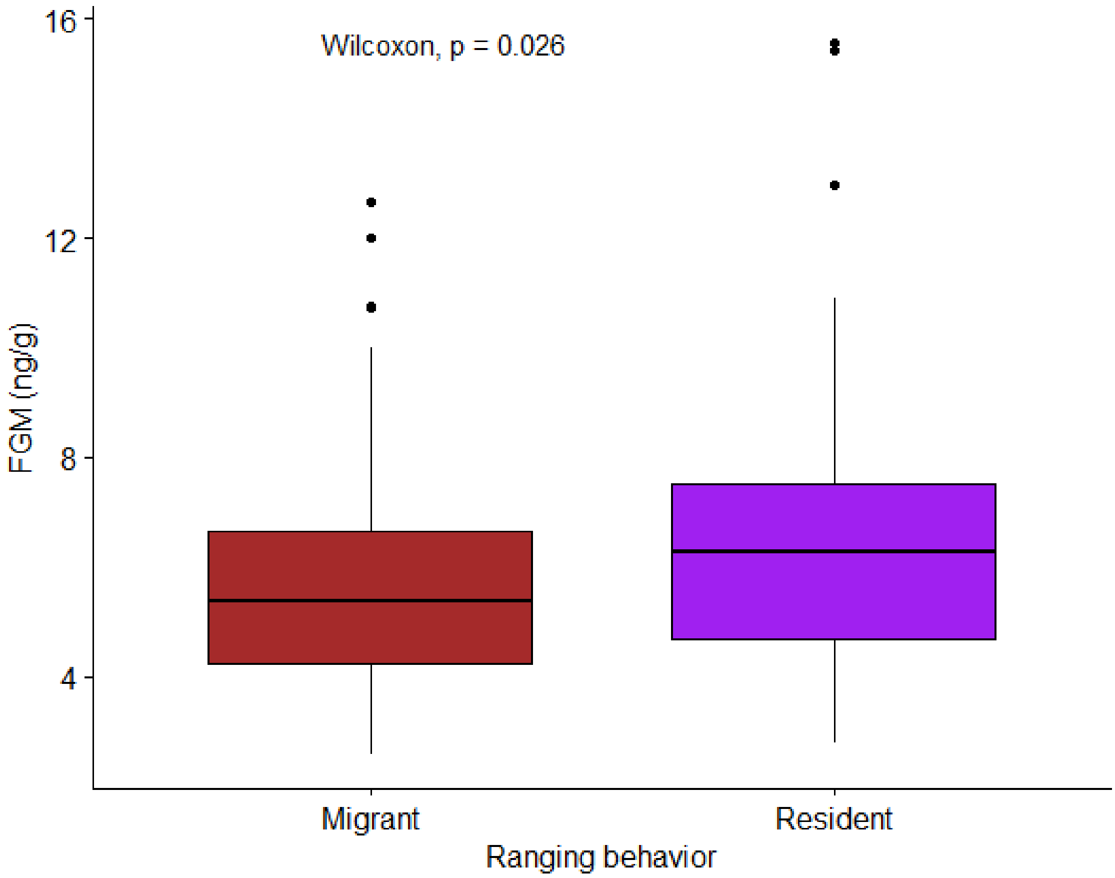


Figure 4. 7: Faecal glucocorticoid metabolites (FGM) concentrations for migrant and resident elephants.

4.2.1 Modeling the influence of ranging behavior, and different predictor variables on FGM concentrations of African elephants at Mpala.

A set of seven linear mixed effect models with different combinations of predictor variables were tested to assess the influence of ranging behavior, Mpala location with varying degree of anthropogenic disturbance and NDVI quality (as a measure of vegetation quality) on FGM concentrations of African elephants at Mpala Ranch. AICcmodavg developed by Mazerolle (2013) was used to compare the seven sets of models to evaluate the model that best explained most of the variation in the data. A summary of seven models selected based on AIC is shown in the Table 4.6.

Table 4. 6: Models on the relationship between stress levels of African elephants measured in FGM and predictor variables.

Model	Model description
1	Log(FGM) = β_o + β_1(Age group) + β_2(Group type) + β3(Ranging behavior) + β4(Mpala location) + β5(Reaction index) + β6(Body condition score) + β7(NDVI) + (1 | Family ID), data = Stress
2	Log(FGM) = βo + β1(Age group) + β2(Ranging behavior) + β3(Sex) + β4(Body condition score) + β5(NDVI) + (1 |Family ID), data = Stress
3	Log(FGM) = β0 + β1(Age group) + β2(Ranging behavior*Mpala location) + β3(Sex) + β4(Body condition score) + β5(Sex*Reaction index) + β8(NDVI) + (1 | Family ID) data = Stress
4	Log(FGM) = β0 + β1(Age group) +β2(Ranging behavior) + β3(Mpala location) + β4(Ranging behavior*Mpala location) + β5(Sex) + β6(NDVI) + (1 | Family ID/Family group) data = Stress
5	Log(FGM) = β0 + β1(Ranging behavior) + β2(Mpala location) + β3(NDVI) + (1 | Family ID) , data = Stress
6	**Log(FGM) = β0 + β1(Age group) + β2(Ranging behavior * Mpala location) + β3(Reaction index) + β5(NDVI) + (1 | Family ID), data = Stress**
7	Log(FGM) = β0 + β1(Age group) + β2(Ranging behavior) + β3(Mpala location) + β4(Ranging behavior*Reaction index) + β5(NDVI) + (1 | Family ID/Family group), data = Stress

The asterisk (*) between different predictor variables shows the interaction between two predictor factors. The best model (in bold) explained most of the variation in the data.

Results from the AIC values run on the models shown in Table 4.11 revealed that age, ranging behavior in interaction with Mpala location, reaction index, and NDVI (shown in bold) were the main predictor variables influencing the physiological stress in African elephants at Mpala Ranch. The results from the model-averaged coefficients showing parameter estimates relating to age, ranging behavior, Mpala location, reaction index and NDVI is shown in Table 4.7. Findings from this study revealed that ranging behavior was interacting with Mpala location to influence FGM concentrations of elephants with migrant elephants in the northern part of Mpala having lower FGM concentration than resident elephants in the southern part of Mpala. The model also showed that individuals that were terrified and running away on an approaching vehicle had a higher FGM concentrations than those that were either calm within 20 m or skittish on vehicle approach with NDVI also influencing FGM concentrations in African elephants at Mpala.

Table 4. 7: Model-averaged coefficients and parameter estimates related to ranging behavior, age, reaction index, Mpala location, NDVI and their interactions for elephants at Mpala Ranch. Asterisks (*) indicate interactions between two predictor variables.

Predictors	*Estimates*	*std. Error*	*CI*	*df*	*T Statistic*	*p*
(Intercept)	2.03	0.14	1.75 – 2.31	164.51	14.4	**<0.001**
Age group [Juvenile]	-0.14	0.05	-0.24 – -0.03	126.54	-2.62	**0.009**
Age group [Sub-Adult]	-0.19	0.06	-0.30 – -0.07	119.62	-3.18	**0.001**
NDVI	-0.92	0.28	-1.47 – -0.36	163.64	-3.24	**0.001**
Reaction Index [Skittish]	-0.05	0.05	-0.15 – 0.05	149.68	-0.91	0.364
Mpala location [South]	0.2	0.06	0.09 – 0.31	155.04	3.57	**<0.001**
Reaction Index [Terrified & running away]	0.23	0.06	0.10 – 0.36	163.46	3.55	**<0.001**
Ranging behavior [Resident]	0.32	0.07	0.19 – 0.45	133.88	4.7	**<0.001**
Ranging behavior [Resident] * Mpala location [South]	-0.34	0.09	-0.52 – -0.16	144.5	-3.69	**<0.001**
Random Effects						
σ^2	0.07					
τ_{00} Family ID	0.01					
ICC	0.11					
N Family ID	153					
Observations	174					
Marginal R^2 / Conditional R^2	0.320 / 0.393					

LMM (formula = Log(FGM) = Age group + Ranging behavior * Mpala location + Group type*Reaction index + NDVI + (1 | Family ID), data = Stress

Although this study had hypothesized that resident elephants whose home ranges are restricted to within Mpala Ranch experiences lower physiological stress from anthropogenic perturbation compared to long distance migrant elephant families whose home ranges overlaps several ranches within Laikipia County, findings from this study did not support the hypothesis. Contrary to what was hypothesized, resident elephants whose home ranges are restricted to within Mpala Ranch had higher physiological stress response compared to migrant elephant families whose home ranges overlaps several ranches within Laikipia County. One reason for the lower FGM concentrations in migrant elephants is due to the fact that elephants are known to track peak forage quality (Loarie, van Aarde, & Pimm, 2009) to improve their overall energy balance and this could have resulted in lower FGM concentrations among the migrant elephants. Results from this study corroborate other findings who have linked elephant's physiological states with space use patterns; i.e., the spatial refuge hypothesis, which predicts higher physiological stress in animals with more restricted space compared to those with (Jachowski *et al.*, 2012; Jachowski, Montgomery, Slotow, & Millspaugh, 2013).

Further differences in ranging behavior were found to be related to regions whereby in the northern part of Mpala, the resident elephants had higher FGM concentrations than the migrant elephants while no differences were found to exist between the migrant elephants and resident elephants in the south. The difference in FGM concentrations between resident and migrant elephants in the northern part of Mpala could have been attributed to the human activities which usually occurs in the northern part of Mpala including British military ballistic training which is held quarterly (Awuor, 2015) and harassment of elephants by herders around the water points. The training which is usually carried out in the northern part of Mpala ranch and involves drills, use of fire arms and explosives could have subsequently resulted in the higher FGM concentrations of the resident elephant family groups in the northern part of the ranch.

4.3 Influence of sampling location on the FGM concentration of African elephants at Mpala Ranch.

There were more elephants found in the northern part of Mpala compared to the southern part of the ranch (N_{North} = 103, N_{South} = 76; χ^2 = 4.0726, df = 1, p = 0.044). Results from this study revealed no differences in the concentrations of FGM between elephants that were sampled in the northern and southern part of the ranch (Wilcoxon Rank Sum Test: W = 952, p = 0.14) as shown in Figure 4.8.

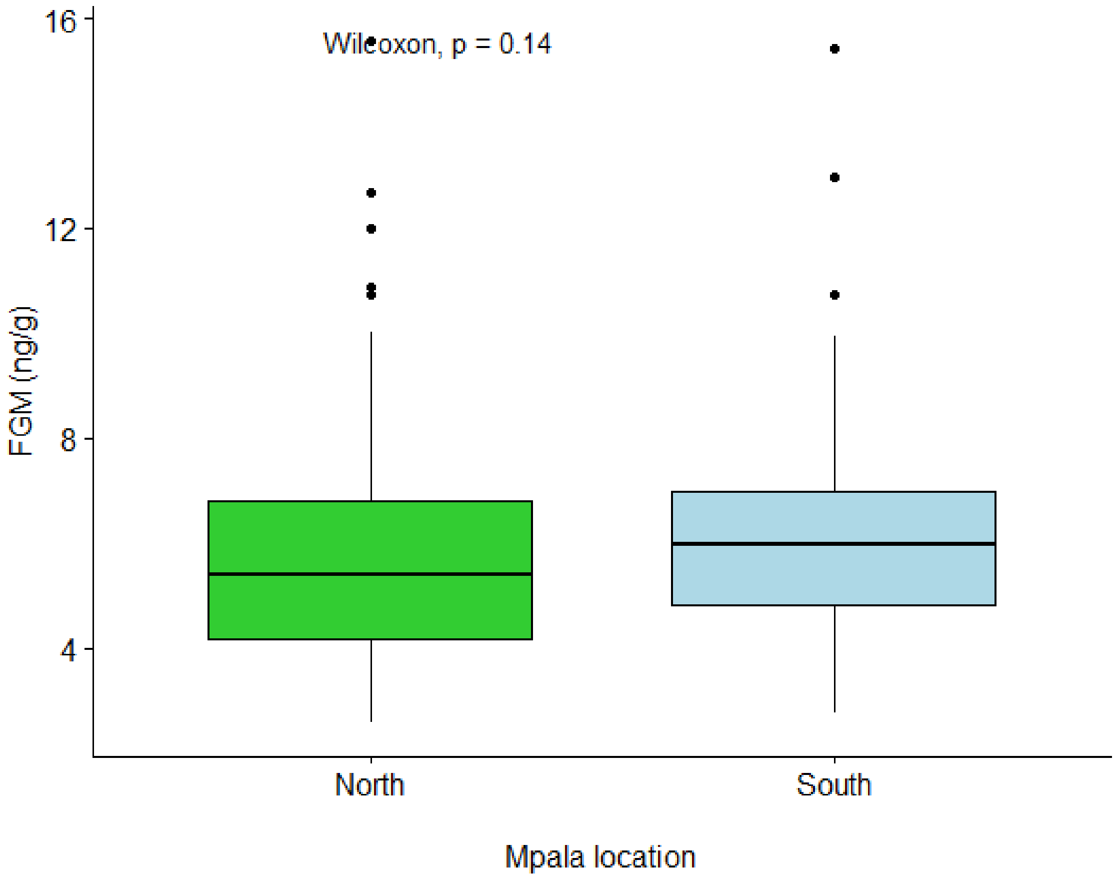


Figure 4. 8: Faecal glucocorticoid metabolites (FGM) concentrations for elephants found in the North and South of Mpala.

Resource fluctuation across habitat heterogeneity influences the movement patterns and foraging behavior of herbivore within a landscape (Fryxell *et al.*, 2008). The non-significant difference between individual elephants located in the northern and southern part of Mpala could have been attributed to low nutritional stress across Mpala ranch. As has been observed by Ditchkoff, Saalfeld, & Gibson (2006), landscape chages arising from habitat loss and environmental degradation, as well as land fragmentation are major factors influencing the distribution, conservation status, foraging behavior and nutritional status of wildlife.

There were more migrant elephant families in the northern part of Mpala (N = 73) while more resident elephant families were found in the southern part of Mpala (N = 33). Additionally, migrant elephants in the northern part of Mpala had the lowest FGM concentrations (5.39 ± 0.24 ng/g) while resident elephants in the northern part of Mpala had the highest FGM concentrations (6.93 ± 0.48 ng/g) as shown in Table 4.8.

Table 4. 8: Samples collected from migrant and resident elephants in the north and south of Mpala together with their mean FGM, standard deviation and standard error.

Ranging behavior	**Mpala location**	**Sample size (N)**	**Mean FGM**	**Std. Dev**	**Std. Error**
Migrant	North	73	5.39	2.06	0.24
Migrant	South	43	6.11	1.69	0.26
Resident	North	30	6.93	2.65	0.48
Resident	South	33	6.22	2.66	0.46

Further analysis between these two Mpala locations however, revealed that FGM concentration and Mpala location differed between resident and migrant elephants ($p < 0.05$, post hoc pairwise comparison). In the northern part of Mpala, resident elephants (6.93 ± 0.48 ng/g) had higher FGM concentrations than migrants (5.39 ± 0.24 ng/g) ($W = 663$, $p = 0.002$), whereas no such differences were observed in the south ($W = 772$, $p = 0.518$) as shown in Figure 4.9.

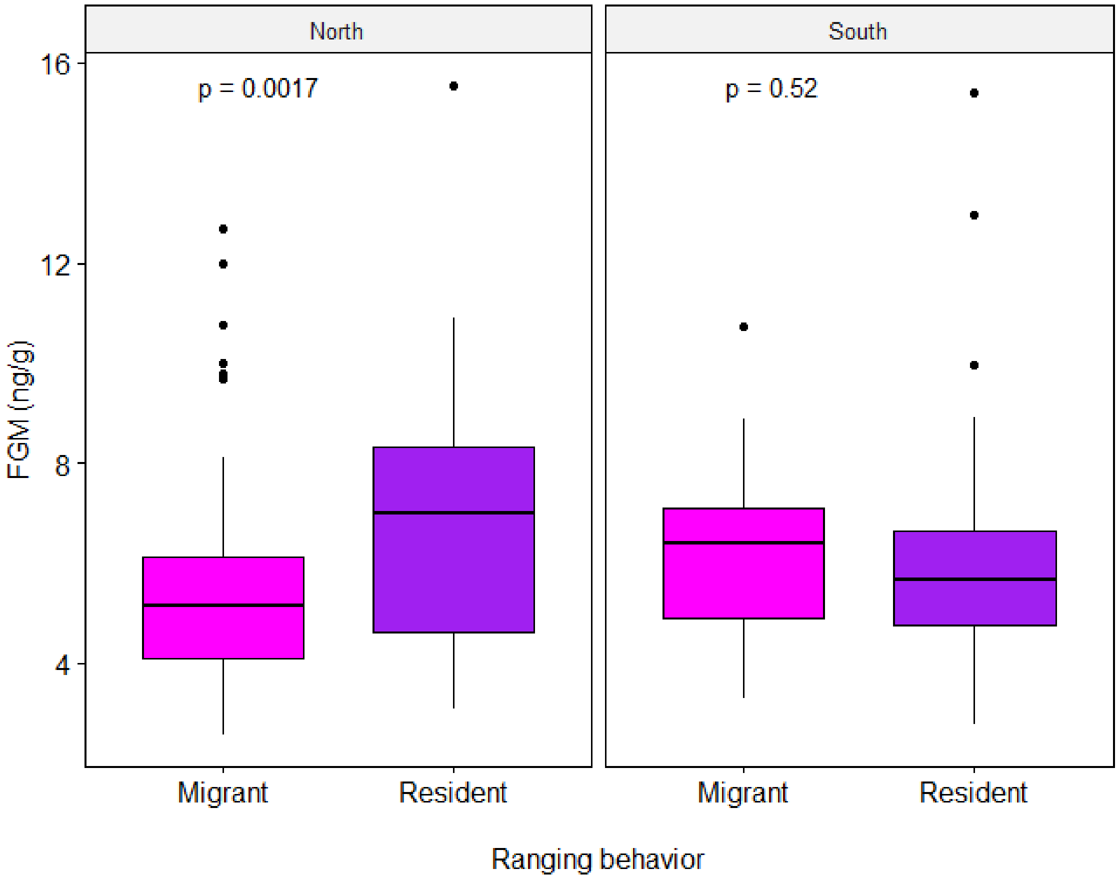


Figure 4. 9: Faecal glucocorticoid metabolite (FGM) concentrations in the north and south of Mpala between migrant and resident elephants.

A comparison of FGM concentration between ranging behavior by region revealed that there was a higher FGM concentrations in migrant elephants in the southern part of Mpala (6.11 ± 0.26 ng/g) compared to the northern part of Mpala (5.39 ± 0.24 ng/g) (W = 1097, p = 0.007), but no differences between regions for resident elephants (W = 600, p = 0.15) as shown in Figure 4.10.

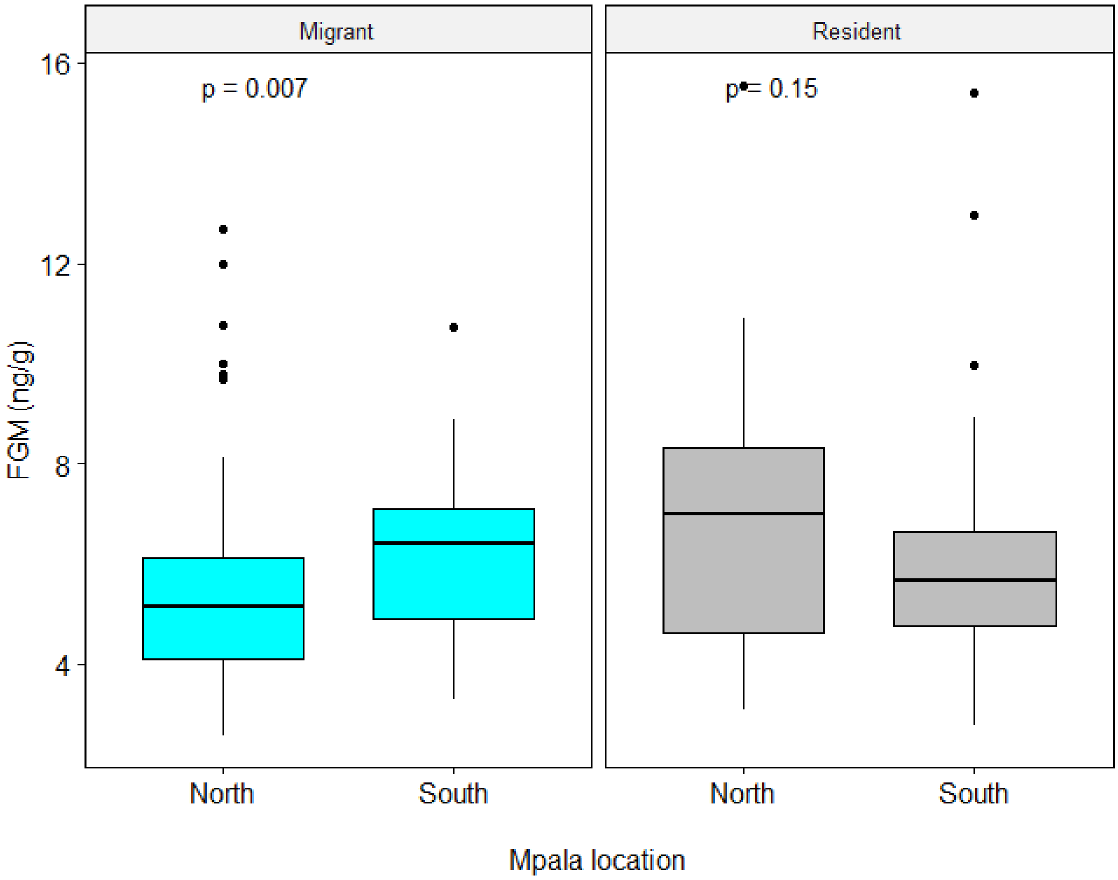


Figure 4. 10: Faecal glucocorticoid metabolite (FGM) concentrations for migrant and resident elephants in the north and south of Mpala Ranch.

Findings from this study indicated that it doesn't matter whether an elephant was found in the northern or southern part of Mpala but rather whether the elephant was a migrant or a resident. The difference in ranging behavior (i.e. migrants vs. residents) exposed the elephants to different anthropogenic disturbance particularly in the north where military ballistic training was conducted and affected the resident elephants that were traversing the northern part of Mpala. The FGM concentrations varied by ranging behavior in the north with resident elephants in the north of Mpala having higher concentrations than migrant elephants. Additionally, FGM concentrations varied between ranging behavior with migrants in the south having higher concentrations than migrants in the north of Mpala. The non-significant difference between resident elephants in the north and resident elephants in the south could have been attributed to the crossover of some resident elephants in the south to north of Mpala in search of forage resources. Other studies have however found the influence of habitat disturbance on the physiological stress of Asian elephants (Tang *et al.*, 2020) with elephants found within expanded tea plantations sites having higher FGM concentrations than elephants found in undisturbed sites.

Majority of the migrant elephant families that were sampled were skittish on vehicle approach (N = 38), while a few of the resident elephant families that were sampled were terrified and running away from the vehicle presence (N = 2). Resident in the southern part of Mpala that were terrified and running away from vehicle presence had the highest FGM concentrations (10.94 ± 2.02 ng/g), while migrant elephants that were calm within 20m of vehicle presence in the north had the lowest FGM concentrations (4.54 ± 0.73 ng/g). This is shown in Table 4.9.

Table 4. 9: Samples collected from reaction index of elephants in the north and south of Mpala for both migrant and resident elephants together with their mean FGM, standard deviation and standard error.

Reaction index	Mpala location	Ranging behavior	Sample size (N)	Mean FGM	Std. Dev	Std. Error
Calm within 20 m	North	Migrant	9	4.54	2.2	0.73
Calm within 20 m	North	Resident	14	6.98	2.23	0.6
Calm within 20 m	South	Migrant	11	6.66	1.71	0.51
Calm within 20 m	South	Resident	16	5.77	1.4	0.35
Skittish	North	Migrant	38	4.94	1.67	0.27
Skittish	North	Resident	12	6.41	2.15	0.62
Skittish	South	Migrant	21	5.29	1.41	0.31
Skittish	South	Resident	15	6.04	3.17	0.82
Terrified & running away	North	Migrant	26	6.36	2.24	0.44
Terrified & running away	North	Resident	4	8.31	5.07	2.54
Terrified & running away	South	Migrant	11	7.15	1.48	0.45
Terrified & running away	South	Resident	2	10.94	2.86	2.02

Findings from this study revealed that it doesn't matter whether an elephant was located in the south or north but rather the past and or current exposure to anthropogenic disturbance. This could explain why individuals that were terrified both in the north and in the south had significantly higher FGM concentrations than individuals that were either calm within 20 m or skittish on vehicle approach in the north or south as shown in Figure 4.11. The study did not however find comparable studies assessing FGM concentration and behavioral response of ungulates to variation in other habitat types or different locations.

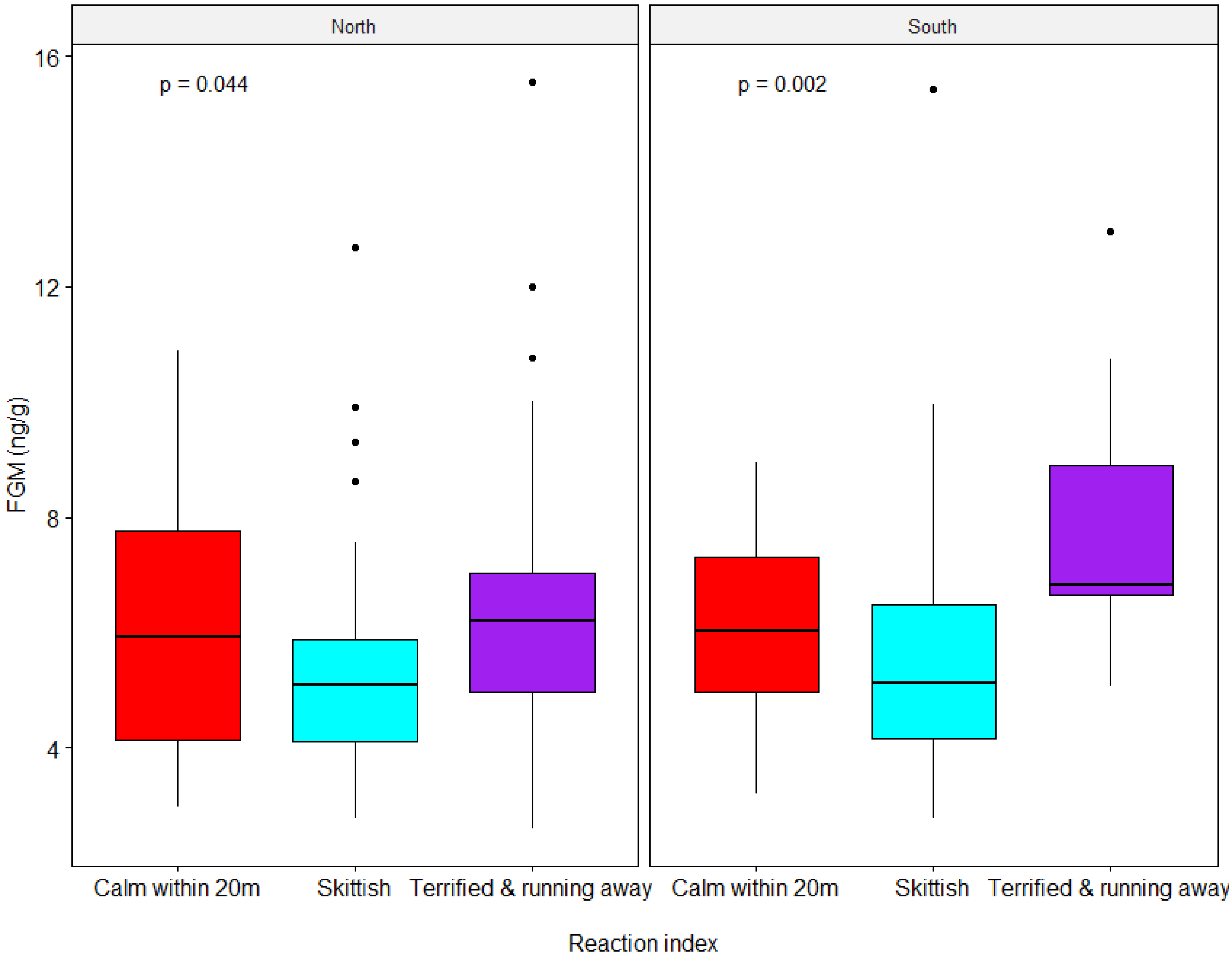


Figure 4. 11: Faecal glucocorticoid metabolite (FGM) concentrations for migrant and resident elephants in the north and south of Mpala Ranch.

4.4 Influence of NDVI on the FGM concentrations of African elephants at Mpala Ranch

The study was undertaken just before the onset of the long awaited rains towards the end of April 2019 to the end of August 2019. The mean NDVI for every month increased from 0.1451 in April to 0.2250 in May. The month of June, July, and August had a relatively higher NDVI value increasing from 0.4415 in June to 0.4431 in July before marginally dropping to 0.4105 in August. Values closer to -1 are usually related to dry or dead vegetation while values approaching +1 are related to green vegetation and therefore better forage quality.

Sentinel satellite images for the month of April was first processed in Google Earth Engine using JavaScript programming code language. The resulting TIFF image was then exported to R programming for analysis of NDVI. NDVI was obtained by the formula: NDVI = (Band 8-NIR – Band 4-Red)/ (Band 8-NIR + Band 4-Red). The mean NDVI for the month of April 2019 was found to be 0.1451. The mean rainfall received for the month of April 2019 was 6.86 mm of rainfall. This was the driest month during data collection period as shown in Figure 4.12.

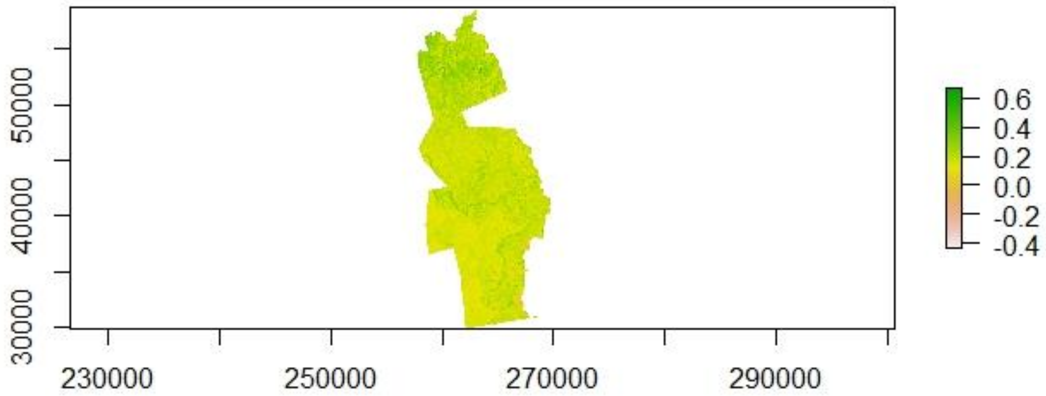


Figure 4. 12: Mean NDVI for Mpala Ranch in the month of April 2019.

Sentinel satellite images for the month of May was first processed in Google Earth Engine using JavaScript programming code language. The resulting TIFF image was then exported to R programming for analysis of NDVI. NDVI was obtained by the formula: NDVI = (Band 8-NIR – Band 4-Red)/ (Band 8-NIR + Band 4-Red). The mean NDVI for the month of May 2019 was found to be 0.2250. The mean amount of rainfall received for the month of May 2019 was 36.58 mm of rainfall. This is shown in Figure 4.13.

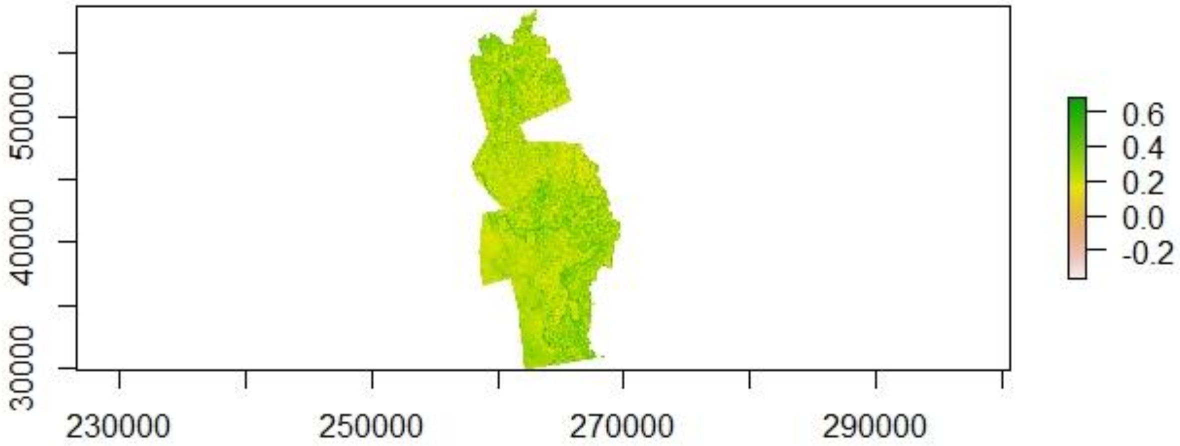


Figure 4. 13: Mean NDVI for Mpala Ranch in the month of May 2019.

Sentinel satellite images for the month of June was first processed in Google Earth Engine using JavaScript programming code language. The resulting TIFF image was then exported to R programming for analysis of NDVI. NDVI was obtained by the formula: NDVI = (Band 8-NIR – Band 4-Red)/ (Band 8-NIR + Band 4-Red). The mean NDVI for the month of June 2019 was found to be 0.4415. The mean rainfall received for the month of June 2019 was 125.22 mm of rainfall. This is shown in Figure 4.14.

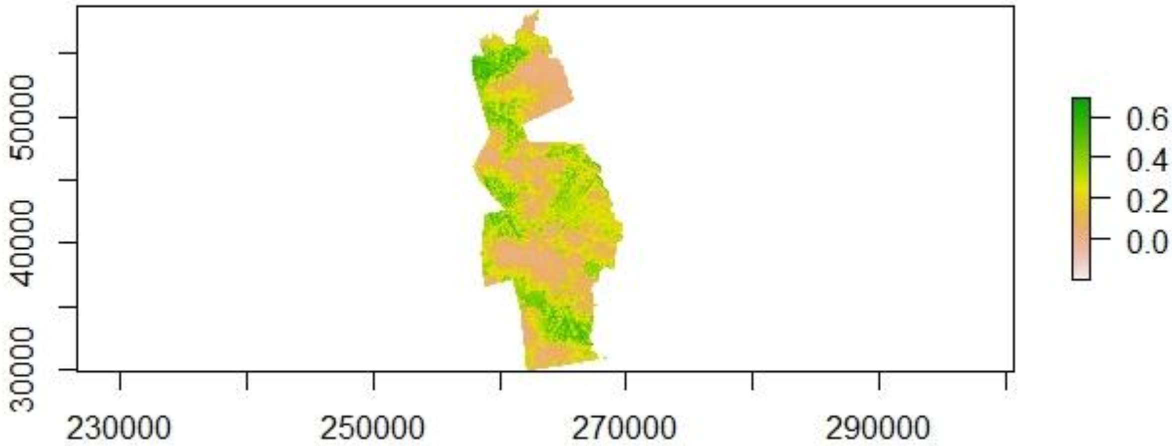


Figure 4. 14: Mean NDVI for Mpala Ranch in the month of June 2019.

Sentinel satellite images for the month of July was first processed in Google Earth Engine using JavaScript programming code language. The resulting TIFF image was then exported to R programming for analysis of NDVI. NDVI was obtained by the formula: NDVI = (Band 8-NIR – Band 4-Red)/ (Band 8-NIR + Band 4-Red). The mean NDVI for the month of July 2019 was found to be 0.4431. The mean rainfall received for the month of July 2019 was 37.34 mm of rainfall. This is shown in Figure 4.15.

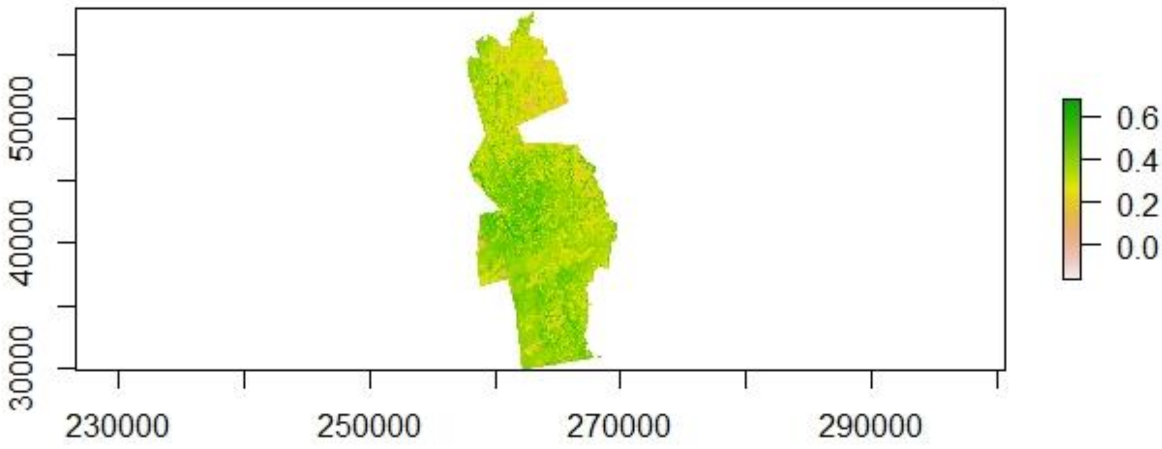


Figure 4. 15: Mean NDVI for Mpala Ranch in the month of July 2019.

Sentinel satellite images for the month of August was first processed in Google Earth Engine using JavaScript programming code language. The resulting TIFF image was then exported to R programming for analysis of NDVI. NDVI was obtained by the formula: NDVI = (Band 8-NIR – Band 4-Red)/ (Band 8-NIR + Band 4-Red). The mean NDVI for the month of August 2019 was found to be 0.4105. The mean rainfall received for the month of August 2019 was 81.08 mm of rainfall. This is shown in Figure 4.16.

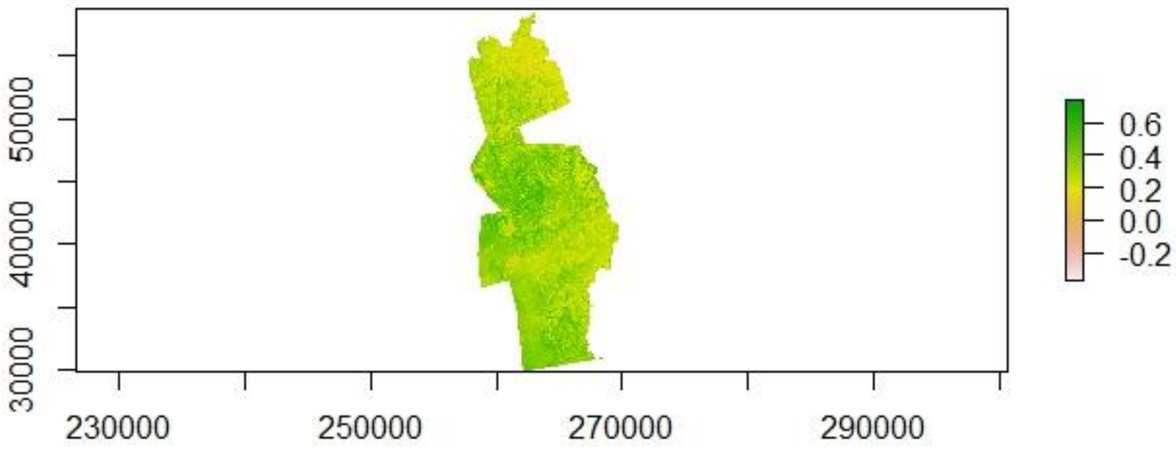


Figure 4. 16: Mean NDVI for Mpala Ranch in the month of August 2019.

Majority of the individuals had a BCS of 3 (N = 113) compared to individuals with a BCS of 4 (N = 47) or individuals with a BCS of 2 (N = 19). FGM concentrations varied by BCSs (Kruskal-Wallis; $\chi^2 = 7.626$, df = 2, p = 0.022) with BCS = 2 exhibiting higher FGM concentrations (7.18 ± 0.67 ng/g) than those with BCS = 3 (6.04 ± 0.22 ng/g) or BCS = 4 (5.34 ± 0.23 ng/g) as shown in Figure 4.17.

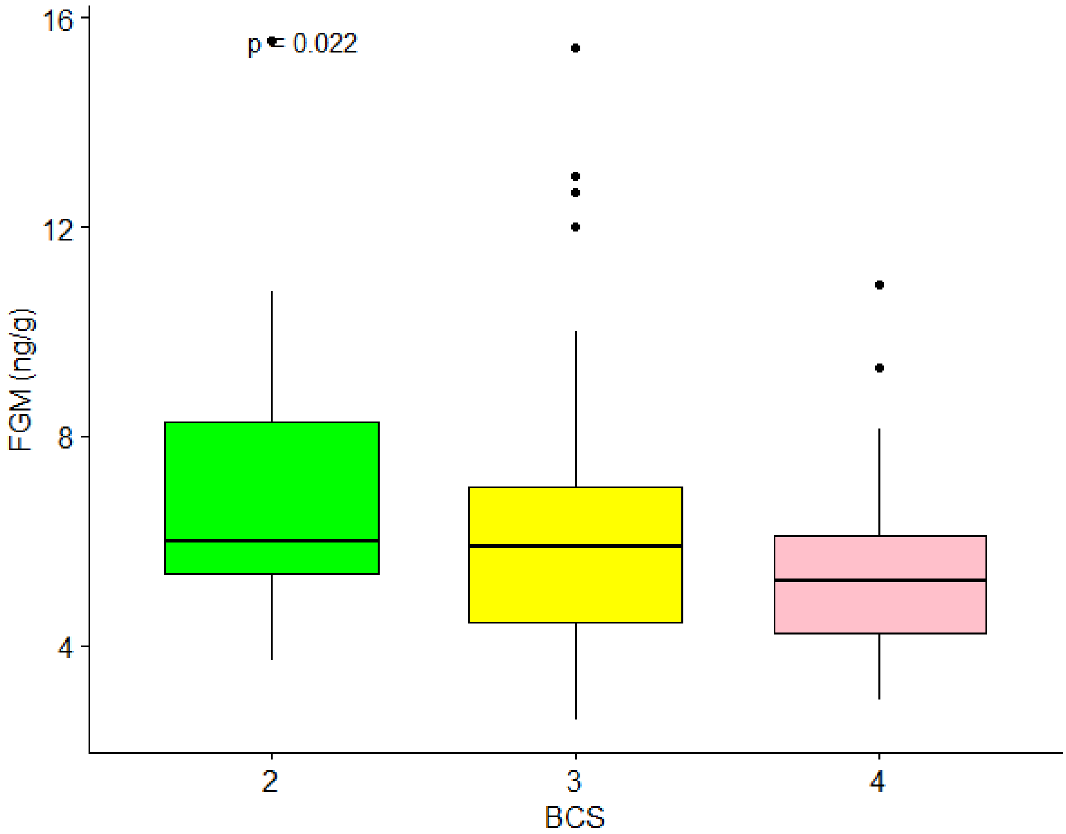


Figure 4. 17: Faecal glucocorticoid metabolite (FGM) concentrations in relation to body condition score (BCS). BSC 2 = thin, 3 = normal and 4 = fat.

Body condition score is increasingly being used as a conservation physiology tool to assess the nutritional stress related to poor quality vegetation (Madliger, Love, Hultine, & Cooke, 2018). Low body condition score has been associated with higher FGM concentrations due to nutrient-deficient diets and resource limitations resulting in increased protein catabolism and muscle deterioration, and subsequently elevated stress levels (Harvey, Phillips, Rees, & Hall, 1984). Findings from this study aligns with those of Pokharel *et al.* (2017), who observed a negative association between body condition score and FGM concentrations in wild Asian elephants.

Due to the changes in normalized difference in vegetation index during the month of May, June, July and August, an analysis was carried out to compare how body condition score had been changing over the month of May, June, July and August. Results from this study however, showed no significant difference between FGM concentrations and body condition score for the month of May June July and August as shown in Figure 4.18. Other studies, have observed the influence of seasonal fluctuations on the overall body condition score of elephants (Mumby *et al.*, 2015).

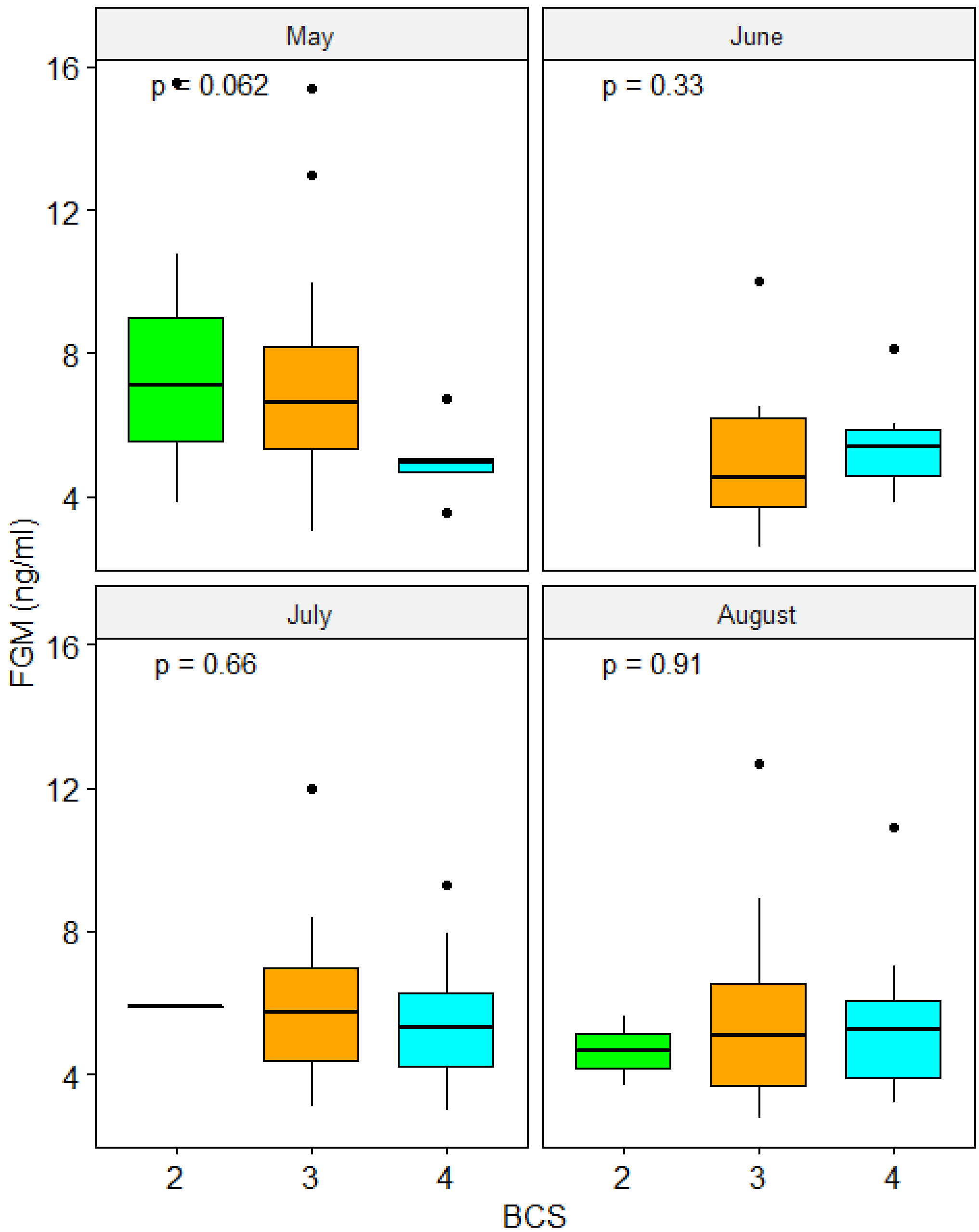


Figure 4. 18: Faecal glucocorticoid metabolite (FGM) concentrations in relation to body condition score (BCS) for the month of May, June July and August. BSC 2 = thin, 3 = normal and 4 = fat.

Majority of the samples were collected from males with a BCS of 3 (N = 58), while few samples were collected from males with a BCS of 2 (N = 4). Males with a BCS of 2 had the highest FGM concentration (7.74 ± 1.16 ng/g), while females with a BCS of 2 had the highest FGM concentrations (7.03 ± 0.80 ng/g). Additionally, males with a BCS of 4 had the lowest FGM concentrations (5.03 ± 0.22 ng/g), while females with a BCS of 4 had the lowest FGM concentrations (5.69 ± 0.43 ng/g) as shown in table 4.10.

Table 4. 10: Samples collected from BCS of 2, 3, and 4 from both males and females together with their mean FGM, standard deviation and standard error.

BCS	Sex	Sample size (N)	Mean FGM	Std. Dev	Std. Error
2	Female	15	7.03	3.11	0.8
2	Male	4	7.74	2.32	1.16
3	Female	55	6.52	2.42	0.33
3	Male	58	5.58	2.09	0.28
4	Female	22	5.69	2	0.43
4	Male	25	5.03	1.12	0.22

Analysis of FGM concentrations and body condition score for both males and females reveled that body condition score and FGM concentrations did not differ between males and females ($p > 0.05$, post hoc pairwise comparison). Other authors have however, found an association between FGM concentrations and BCS between different sexes with females having a lower BCS and higher FGM concentrations (Pokharel *et al.*, 2017). The differences has been attributed to the fact that males tend to crop raid to meet their higher energy demands compared to the female elephants with calves who tend to avoid the areas due to increased risks posed to the young ones.

Data was also compared on how body condition score varied between ranging behavior with majority of the migrants having a BCS of 3 (N = 58) while few resident elephants having a BCS of 2 (N = 7). Resident elephants with a BCS of 2 had the highest FGM concentrations (7.93 ± 1.40 ng/g) while migrants with a BCS of 2 had the highest FGM concentrations (6.74 ± 0.70 ng/g). Additionally, resident with a BCS of 3 had the lowest FGM concentrations (6.19 ± 0.32 ng/g) and this was in contrast to the migrant elephants where individuals with a BCS of 4 were the ones with the lowest FGM concentrations (5.10 ± 0.20 ng/g). This is shown in Table 4.11.

Table 4. 11: Samples collected from BCS of 2, 3, and 4 from both migrants and resident elephants together with their mean FGM, standard deviation and standard error.

BCS	Ranging behavior	Sample size (N)	Mean FGM	Std. Dev	Std. Error
2	Migrant	12	6.74	2.42	0.7
2	Resident	7	7.93	3.71	1.4
3	Migrant	58	5.89	2.22	0.29
3	Resident	55	6.19	2.39	0.32
4	Migrant	37	5.1	1.24	0.2
4	Resident	10	6.23	2.44	0.77

Analysis of FGM concentrations and body condition score for both migrants and resident elephants revealed that body condition score and FGM concentrations did not significantly differ between migrants and resident elephants ($p > 0.05$, post hoc pairwise comparison). Other authors have however, observed migrant individuals to be having better BCS due to their access to better quality vegetation during the dry season in areas that had previously received more rainfall compared to residents (Gaidet & Lecomte, 2013). The difference in the results could have been attributed to low nutritional stress for resident Mpala elephants and migrant elephants who track better vegetation quality to meet their nutritional demands.

Majority of elephants sampled in the northern part of Mpala had a BCS of 3 (N = 64) while few individuals sampled in the northern part of Mpala had a BCS of 2 (N = 8). Individuals in the southern part of Mpala with a BCS of 2 had the highest FGM concentrations (7.93 ± 0.90 ng/g) while individuals with a BCS of 4 in the southern part of Mpala had the lowest FGM concentrations (5.34 ± 0.41 ng/g). Additionally, individuals with a BCS of 2 in the northern part of Mpala had the highest FGM concentrations (6.15 ± 0.94 ng/g) while individuals with a BCS of 4 in the north had the lowest FGM concentrations (5.34 ± 0.28 ng/g) as shown in Table 4.12.

Table 4. 12: Samples collected from BCS of 2, 3, and 4 in the north and south of Mpala together with their mean FGM, standard deviation and standard error.

BCS	Mpala location	Sample size (N)	Mean FGM	Std. Dev.	Std. Error
2	North	8	6.15	2.67	0.94
2	South	11	7.93	2.98	0.9
3	North	64	5.84	2.67	0.28
3	South	49	6.3	2.34	0.33
4	North	37	5.34	1.7	0.28
4	South	10	5.34	1.28	0.41

Analysis of FGM concentrations and body condition score for elephants found in the north and south of Mpala revealed that body condition score and FGM concentrations did not significantly differ between north and south of Mpala ($p > 0.05$, post hoc pairwise comparison).

The monthly stress levels of African elephants was highest during the month of April i.e. peak of the dry season when there was a shortage of forage quality and lowest during the month of June i.e. wet season when there was relatively higher forage quality. This also coincided with the mean NDVI values which was highest during the month of April, an indication of an inverse proportion between FGM concentrations and fluctuations in environmental conditions. Monthly changes of FGM concentrations is shown in Table 4.13.

Table 4. 13: Samples collected during the month of April, May, June, July, and August 2019 together with the mean FGM concentrations and mean NDVI value for each month.

Month of the year	No. of samples	Mean FGM	Mean NDVI
Apr-19	4	9.59	0.1451
May-19	64	6.75	0.225
Jun-19	28	5.02	0.4415
Jul-19	47	5.59	0.4431
Aug-19	36	5.43	0.4105

From the results it was evident that during the month of April and May when the vegetation quality was low due to constrains in forage quality. The mean FGM concentrations were relatively higher compared to the month of June, July and August when the vegetation quality had improved due to availability of forage quality. This is a clear indication of the role that forage quality plays on the overall glucocorticoid outputs of wildlife, particularly African elephants.

4.4.1 Modeling the influence of FGM concentration on vegetation quality

The model that best explained the influence of FGM concentration on vegetation quality included sex and NDVI as the predictor variable and family ID as the random effect as shown in table 4.21 below. Findings from the model revealed that females had significantly higher FGM concentrations than males. Additionally, there was an inverse relationship between NDVI and FGM concentrations with FGM concentrations being high during the dry season and reducing when the vegetation quality (measured by NDVI) had improved.

Table 4. 14: Model-averaged coefficients and parameter estimates related to sex and NDVI for elephants at Mpala ranch.

Predictors	*Estimates*	*CI*	*P*
(Intercept)	2.36	2.09 – 2.62	**<0.001**
Sex [Male]	-0.14	-0.23 – -0.04	**0.003**
NDVI	-1.3	-1.87 – -0.72	**<0.001**
Random Effects			
σ^2	0.09		
τ_{00} Family ID	0		
N Family ID	153		
Observations	175		
Marginal R^2 / Conditional R^2	0.139 / NA		

The physiological stress level of elephants have been observed to increase with poor forage availability (Busch & Hayward, 2009). NDVI which is a measure of vegetation productivity (Carroll, DiMiceli, Sohlberg, & Townshend, 2004) and included in the model to assess its effects on FGM concentrations of elephants at Mpala. The NDVI had a significantly strong effect on the overall FGM concentrations of elephants at Mpala Ranch. Findings from this study corroborate that of other authors who have found similar results an indication of the influence of nutritious foraging resource on the physiological stress of elephants (Stabach, Boone, Worden, & Florant, 2015). The model also showed that females were more affected from low vegetation quality compared to the males. This has been attributed to social behavior differences between the two sexes whereby females live in groups and as a result are faced with resource competition within the family especially during the dry season. This is in contrast to the males who are generally solitary and are able to seek for high quality forage to enhance their reproductive competitiveness (Sukumar, 2003; Chiyo *et al.*, 2011).

CHAPTER FIVE: SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary

This study sought to assess how stress hormone concentrations between the resident elephant families and the migrant elephant families that are found within Mpala Ranch differ, assess the relationship between concentrations of faecal glucocorticoid metabolites (FGM) and the location of sampling within Mpala ranch and determine whether a change in normalized difference in vegetation index (NDVI) influences FGM concentrations in African savannah elephants in Mpala ranch. Finding from this study revealed that resident elephant families had higher Fecal Glucocorticoid Metabolite concentrations compared to the migrant elephant families. Although this study found no relationship between Fecal Glucocorticoid Metabolite concentrations and the location of sampling within Mpala ranch, the study found an inverse relationship between normalized difference in vegetation index (i.e. a measure of vegetation quality) and Fecal Glucocorticoid Metabolite concentrations. The study had hypothesized that resident elephant families whose home ranges are restricted to within Mpala Ranch experiences lower physiological stress from anthropogenic perturbation compared to long distance migrant elephant families whose home ranges overlaps several ranches within Laikipia County. The opposite was however true, resident elephant families whose home ranges are restricted to within Mpala Ranch had higher physiological stress response (via analysis of Fecal Glucocorticoid Metabolite concentrations) compared to the migrant elephant families. Results from the study resulted in the rejection of our hypothesis.

5.2 Conclusion

Findings from the study revealed that resident elephant families had higher Fecal Glucocorticoid Metabolite concentrations compared to the migrant elephant families. Results from the study can help conservation physiologist to understand how long ranging animals such as African elephants adjust their space use patterns in both private and communal ranches with differing human disturbance levels and improve their overall glucocorticoid outputs.

This study also found the influence of vegetation quality (i.e. via assessment of normalized difference vegetation index) on the Fecal Glucocorticoid Metabolite concentrations with dry season associated with higher Fecal Glucocorticoid Metabolite concentrations compared to the wet season where the Fecal Glucocorticoid Metabolite concentrations were generally low. Findings from this study reveals the influence of the environment on the overall glucocorticoid outputs. The study also found the influence of behavioral reaction to vehicle presence (used as a proxy for past negative human elephant interaction), age, sex, body condition scores, and social grouping (group type).

Results reveal the role that glucocorticoids play in different physiological function. It is therefore important to take into consideration the influence of glucocorticoids on age, sex, body condition score, and social groupings when relying on glucocorticoids to evaluate stress and welfare in wildlife and in particular, African elephants. Findings from the study also reveals the influence of behavioral reaction to vehicle presence as a proxy for past of present negative human elephant interaction on the physiological state of African elephant. This is important in monitoring elephant aggression towards people and increased levels of conflict in human occupied landscapes such as Laikipia for management intervention and mitigating negative human-elephant interactions.

5.3 Recommendations

This study recommend that there is need to minimize different anthropogenic activities such as military ballistic training which is usually carried out in the northern part of Mpala ranch and involves drills, use of fire arms and explosives and subsequently could have led to higher Fecal Glucocorticoid Metabolite concentrations among the resident elephant families. This study also recommend the need for peaceful coexistence with wildlife, particularly African elephants to minimize negative elephant's behavioral reaction to vehicle presence and has been attributed to poaching and human elephant conflict. This study also recommend the need to undertake a study on the nutritional stressors for both resident and migrant elephants to determine whether access to vegetation quality outside of Mpala could be acting as a 'pacifier' to the migrants which were observed to have lower Fecal Glucocorticoid Metabolite concentrations. This study was however, conducted from April 2019 when the vegetation quality was low to August 2019 when Mpala ranch had received significant amount of rainfall. As a results, data for wet season were oversampled. There is therefore a need to undertake a yearlong study to understand the physiological stress response of both the resident and the migrant elephant in different seasons.

CHAPTER SIX: REFERENCES

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CHAPTER SEVEN: APPENDICES

I. Data sheet

Date: Observer Name:

Serial No.				
	Group	Group	Group	Group
GPS				
Time dropped				
Time collected				
Mpala location				
Group type				
Age group				
Ranging behavior				
Sex				
Temporal gland secretion				
Body condition score index				
Family ID				
Total count				
Comments				

Key:

Group type: c/c-cow calf; M- Mixed; B- Bulls; LF- Lone female; LB- Lone bull; LC- Lone calf **Reaction Index** 1) Totally calm when within 20m, 2) Initially skittish on vehicle approach, 3) Frightened & avoiding vehicle, 4) Terrified & running**, Mpala location**: 1) South; 2) Central; 3) North **Age group**: 1) Juvenile (0-8 yrs.); 2) Sub-adult (9-17 yrs.); 3) Adult (>18 yrs), **Ranging behavior** 1) Resident family, 2) Migrant family; **Temporal gland secretion** 1) Nill, 2) Wet. Urine dribbling for bulls 1) Nill 2) wet

II. Budget

Itemized Expenses (US$)	National Geographic Society (NGS) grant (Ksh.)	Grant from other sources (Ksh.)	Total Budget (Ksh.)
Project Preparation			
Communications (Telephone)	0.0	0.0	0.0
Permits	1,000	6,000	7,000
Equipment			
GPS	0.0	17,000	17,000
Camera	0.0	54,300	54,300
Binoculars	3,000	12,000	15,000
Tablet	0.0	30,000	30,000
Dung sample tubes	6,000	0.0	6,000
Cooler box	6,000	0.0	6,000
Permanent pen	600	0.0	600
Labeling stickers	100	0.0	100
Project Implementation			
Vehicle hire + Fuel	456,700	0.0	456,700
Food & Accommodation	100,000	100,000	200,000
Laboratory costs	100,000	0.0	100,000
Compensation for Team members	100,000	0.0	100,000
Post-Project Expenses			
Printing services	3,300	2,000	5,300
Total	**776,700**	**221,300**	**998,000**

III Kenya Wildlife Service (KWS) Permit approval

KENYA WILDLIFE SERVICE

ISO 9001:2008 Certified

KWS/BRM/5001

4 June 2018

Mr. Sandy Oduor
P.O.Box 397-40404
RONGO
e-mail: oduorsandy@gmail.com
mobile:0727453288

Dear Mr. Oduor,

PERMISSION TO CONDUCT RESEARCH ON ELEPHANTS IN LAIKIPIA COUNTY, KENYA

We acknowledge receipt of your application requesting for permission to conduct research on a project titled: '**Influence of human activities on the stress hormone levels of African Elephants in Mpala Ranch, Laikipia County, Kenya'**. The study will generate vital data and information to enhance conservation and management of the species within its ranges.

You have been granted permission to conduct the study from **June 2018 – June 2019** upon payment to KWS academic research fees of **Ksh.6000** (Masters Study) However you will abide by the set KWS regulations and guidelines regarding the carrying out of research in and outside protected areas. You will also be required to work closely with our Senior Scientist in-charge of Mountain Conservation Area (MCA), whom you will give the progress report on the study.

You will submit a bound copy of your MSc thesis to the KWS Deputy Director, Biodiversity Research and Monitoring on completion of the study.

Yours Sincerely.

SAMUEL M. KASIKI, PhD, OGW
DEPUTY DIRECTOR
BIODIVERSITY RESEARCH AND MONITORING

Copy to:

- AD, Species Conservation and Management
- Senior Scientist, MCA
- Senior Warden, Laikipia County

P.O Box 40241-00100, Nairobi, Kenya.
Tel: +254-020-2379407/8/9-15. Mobile: +254-735 663 421, +254-726 610 508/9.
Email: kws@kws.go.ke Website:www.kws.go.ke

IV National Commission for Science, Technology and Innovation (NACOSTI) approval permit

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

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Off Waiyaki Way
P.O. Box 30623-00100
NAIROBI-KENYA

Ref. No. **NACOSTI/P/19/45794/28283**

Date: **13th March, 2019**

Sandy Oduor
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Effects of human activities on the stress hormone levels of African elephants in Mpala Ranch, Laikipia County"*** I am pleased to inform you that you have been authorized to undertake research in **Laikipia County** for the period ending **12th March, 2020.**

You are advised to report to **the County Commissioner and the County Director of Education, Laikipia County** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

GODFREY P. KALERWA MSc., MBA, MKIM
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Laikipia County.

The County Director of Education
Laikipia County.