The impact of wildlife tourism on elephants, *Loxodonta africana*, in South Africa

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“Finally I saw that the very land itself had risen, that the sunbaked land had taken form as something vast and alive and was in motion. The land walked as multitudes, their strides so utterly of the earth that they seemed the source of the very dust. The cloud they raised engulfed us, seeped into every pore, coated our teeth, sifted into our minds. Both flesh and metaphor. That big.

And you could see their heads, like warriors‘ shields. Their great breaths, gushing in and out, resonant in the halls of their lungs. The skin as they moved, wrinkled with time and wear, batiked with the walk of ages, as if they lived within the creased maps of the lives they’d travelled. [...]

Their rumbles rolled through the air like distant thunder approaching, vibrating through the undulating ground and the roots of trees, rallying families and friends from the hills and rivers, sending among themselves greetings and recognitions and news of where they had been; sending to us a sign of something coming.“

Carl Safina, Beyond Words: What Animals Think and Feel

Abstract

Wildlife tourism may aid in the protection of species and habitat and contributes to countries' economies. However, wildlife tourism has also been identified as a potential stressor in many species, affecting individuals' survival, reproduction, welfare, and behaviour. We can investigate whether something elicits a stress response in an individual by studying its behaviour, movement, and faecal glucocorticoid concentrations. African elephants, *Loxodonta africana*, are mega-herbivores threatened by poaching and habitat loss. Simultaneously, they are iconic animals which tourists are keen to observe in their natural habitat. Nevertheless, studies assessing wildlife tourism impacts on African elephants are scarce. I studied a population of approximately 1200 free-ranging elephants in Madikwe Game Reserve, South Africa, over a period of 15 months from April 2016 to June 2017. I investigated whether wildlife tourism affected elephants' faecal glucocorticoid metabolite (fGCM) concentrations, vigilance or aggressive behaviour, or the direction of herd movement in relation to tourist vehicles present. I further investigated whether wildlife tourism impacted on elephant space use, in the form of home range size and journey length. Wildlife tourism was either defined as tourist pressure (the total number of tourists in Madikwe per month), or as immediate tourist presence (in form of number of game drive vehicles present during an observation). I used a mixed model approach, controlling for age, sex, habitat and herd type (such as lone males, bull, cow-calf or mixed groups), as well as a proxy of plant productivity, season, temperature, and time of day throughout those analyses. High tourist pressure was significantly related to increased fGCM concentrations. Elephants were more likely to display conspecific-directed aggression during times of high tourist pressure. During game drive vehicle presence, the likelihood of elephant herds moving away from vehicles increased with increasing numbers of vehicles present. Elephants' home range size and journey length were not affected by wildlife tourism. The results presented in this thesis suggest that wildlife tourism is a stressor for free-ranging elephants in fenced reserves. I present management recommendations to improve elephant welfare and increase tourist safety: to introduce a maximum speed of approach and minimum distance between vehicles and elephants, as well as establishment of an area with limited tourist activity.

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1.1 Wildlife tourism

Demand by tourists to observe or interact with wildlife has been one of the fastest growing form of tourism worldwide, especially in Africa (Gössling, 2000; Christie & Crompton, 2001; Orams, 2002; Tapper, 2006; World Tourism Organization, 2014; Balmford *et al.,* 2015). For example, visitors to protected areas in countries such as Kenya and South Africa bring revenue of up to USD$90 million per year (World Tourism Organization, 2014), and up to USD$5.2 and USD$5.9 million per year in the Serengeti National Park and Ngorongoro Conservation area in Tanzania (Thirgood *et al.,* 2006). Further, an estimated 86 million people observed wildlife in 2016 in the United States alone (U.S. Department of the Interior, 2016). These numbers indicate the large scale of wildlife tourism. There are various forms of wildlife tourism: tourists can interact directly with wild animals by feeding, petting, riding or walking with them, or they can observe animals in their natural habitat (Orams, 2002; Millspaugh *et al.*, 2007; Moorhouse *et al.*, 2015). In this thesis, wildlife tourism, unless specifically noted otherwise, refers to non-consumptive tourism occurring in animals' natural habitat, not to zoos or safari parks.

1.1.1 Benefits of wildlife tourism

In many cases, tourism can aid in funding and protecting habitats (e.g. through entrance fees) and the flora and fauna within, and ultimately promote biodiversity and natural ecological processes (Burger & Gochfeld, 1993; Reynolds & Braithwaite, 2001; Newsome *et al.*, 2005; Lindsey *et al*., 2007; Maciejewski & Kerley, 2014; Dinets & Hall, 2018). Financial benefits to conservation agencies and protected areas include, for example, funds for poaching patrol units, upkeep of infrastructure, or veterinary interventions (P.Nel, P.Hattingh, C.Catton, K.Potgieter, *pers.comm.;* Aveling & Aveling, 1989; McNeilage, 1996; Higginbottom *et al.*, 2001). Furthermore, tourists may spend significant amounts of money on accommodation, local enterprises, local guides and in local communities, all of which contributes towards a country's economy (Higginbottom *et al.*, 2001; Weaver 2001; Higginbottom 2004; Snyman, 2012; World Tourism Organization, 2014; U.S. Department of the Interior, 2016; Penteriani *et al.,* 2017) and can provide socio-economic incentives for conservation-oriented land-use and management (Hearne & Mackenzie, 2000; Higginbottom *et al.*, 2001). Especially in developing countries, wildlife tourism makes up a significant proportion of local and national economies (Naidoo *et al.,* 2016). Additionally, tourism can increase the motivation of tourists to become involved in biodiversity-oriented behaviours, financially support species' care, encourage and support habitat preservation, and to buy wildlife-friendly products (Skibins *et al.,* 2013). Higginbottom (2004) lists a wide range of positive impacts of wildlife tourism on economies and conservation (Box 1.3 and 1.4, p. 8-9 in Higginbottom, 2004).

Benefits of wildlife tourism for conservation are often indirect, for example by providing a sustainable source of income for locals which in turn promotes positive attitudes of locals towards preservation of species and habitats, and potentially high animal welfare standards by promoting performance of natural behaviours in species' natural habitats (Higginbottom, 2004; Snyman, 2012; Moorhouse *et al.*, 2015; Wardle *et al.*, 2018). Note, however, that conservation does not necessarily ensure good welfare for wild animals, especially when non-consumptive tourism is insufficient in covering financial costs of protecting natural habitats (see Sekar & Shiller (2020) for a review). Further, direct community involvement can be of benefit for conservation and wildlife tourism has also been reported to have positive effects on human mental health (Muloin, 1998; Penteriani *et al.,* 2017). For example, whale, *Cetacea spp.*, and sea turtle, *Chelonioidea spp.*, tourism has been shown to provide educational, economic and political benefits, in form of employment and educational opportunities to locals, and politicians becoming more supportive of conservation (Wilson & Tisdell, 2003; Pegas *et al.,* 2013). Mountain gorilla, *Gorilla beringei beringei*, tourism in Uganda contributes significantly towards monitoring animals daily and hence has reduced poaching (Aveling & Aveling, 1989; McNeilage, 1996). In Samburu, Kenya, tourism in the form of wildlife viewing provides income and employment for local people (Kuriyan, 2002). Where tourism has provided an incentive for conservation, community involvement has further been reported to increase species diversity, and poaching has decreased (Bajracharya *et al.,* 2005; Stronza & Gordillo, 2008).

There are, however, limitations to the role of ecotourism in wildlife conservation, as income from tourism often does not cover costs of protected areas, and successful community-run tourism projects are scarce (Walpole & Thouless, 2005). For example, in some areas with community-based conservation projects, poaching has remained unchanged (Gibson & Marks, 1995). This is often due to political instability, corruption, and benefits of tourism not being distributed evenly amongst local stakeholders and communities (Morgan, 1994; Kiss, 2004; Leader-Williams & Hutton, 2005; Spiteri & Nepal, 2006). Further, local communities may not be involved in the conservation of habitat, which can cause conflict between local people and those aiming to protect and conserve habitat (Das & Chatterjee, 2015). Therefore, although the concept of wildlife tourism could be a promising tool for conservation of species and habitats, the reality and implementation often fall short (Das & Chatterjee, 2015).

Lindsey and colleagues (2007) have provided a good review of the potential role of ecotourism in wildlife conservation and Snyman (2012) and Das and Chatterjee (2015) have assessed how tourism can affect local economies and community perceptions of conservation. Further, Stem and colleagues (2003) and Spiteri and Nepal (2006) discuss the potential benefits as well as shortcomings with regards to ecotourism (or so-called incentive-based conservation in Spiteri & Nepal, 2006) as a conservation tool in more detail. There has been little systematic research reporting positive or no effects of wildlife tourism on animal behaviour specifically, whilst much research has reported negative effects of tourism on animal behaviour (Higginbottom *et al.*, 2001; Higginbottom, 2004; Moorhouse *et al.*, 2015; Wardle *et al.*, 2018).

1.1.2. Disadvantages of wildlife tourism

Negative effects of wildlife tourism have been reported and it has been identified as a potential stressor in a wide range of species (Orams, 2002; Millspaugh *et al.*, 2007; Moorhouse *et al.*, 2015; Sarmah *et al.*, 2017). A stressor is an unpredictable or uncontrollable stimulus which elicits a physiological or behavioural response (McEwen & Wingfield, 2003; see below). Such negative effects related to wildlife tourism include alteration, destruction, or pollution of the natural habitat, reduced reproductive output and increased mortality of animals, decreased individual animal welfare, and negative effects on animal diet, for example by potentially reducing the time spent foraging (Liptrap, 1993; Gössling, 2000; McEwen & Wingfield, 2003; Higginbottom, 2004; Reynolds & Braithwaite, 2001; Bhattacharjee *et al.*, 2015; Moorhouse *et al.*, 2015; Wardle *et al.*, 2018). Wildlife tourism can further displace wildlife and lead to animals emigrating from their natural habitat and thereby alter population structures and enable exotic species to establish themselves within the habitat (Reynolds & Braithwaite, 2001; Muntifering *et al.,* 2018).

Negative responses by animals to tourism-related stressors can be physiological, behavioural, or related to space and habitat use (Green & Giese, 2004; Moorhouse *et al.*, 2015). In this thesis, I will focus on such negative responses, review current research regarding those types of responses across different animal species and then focus on my study species' behavioural ecology, known stressors, and known effects of anthropogenic disturbance. Most published literature on the negative responses of animals to tourists focuses on direct interactions between animals and tourists, or on increased competition and aggression between animals and potential spread of diseases with regards to supplementary feeding (Orams, 2002), and research on the potential effect of wildlife-watching is lacking. Negative responses can have consequences for animal welfare, tourist safety (Jachowski *et al.*, 2012), and even species survival, which is especially of concern for species affected by poaching, habitat loss and declining population numbers, either for locally or internationally threatened or endangered species (Chase *et al.*, 2016). Additionally, effects on animals from tourists observing them, without directly interacting with them, are not always well understood (Wardle *et al.*, 2018). Assessing short-term physiological and behavioural changes in animals has been suggested to present robust information on negative effects of stressors, such as wildlife tourism (Green & Giese, 2004). However, to reliably measure and assess changes in animal physiology and behaviour in response to a stressor, scientists need to understand the underlying mechanisms of the stress response.

1.2 The stress response and allostatic load

Stress is a difficult concept to define as it refers to several multidimensional, interrelated categories, such as stress stimuli, the processing systems which include the subjective perception of stress, the consequences of experiencing stress, the stress response (Levine, 2005), and the overstimulation of the stress response resulting in disease (Romero, 2004). According to Romero and colleagues (2009) part of the difficulty with the definition of stress can be addressed by distinguishing between "stimuli as stressors, the emergency responses as the stress response, and the over-stimulation of the emergency responses as chronic stress".

The stress response has evolved under positive selective pressures and is an adaptation to challenges in the environment of an individual (Selye 1936; McEwen & Wingfield, 2003; Koolhaas *et al.*, 2011). Throughout an animal's life, it will repeatedly encounter unpredictable events that have the potential to be stressors (McEwen & Wingfield, 2003). What an animal perceives as a stressor is difficult to define and can depend on personality traits, past experiences (Koolhaas *et al.*, 1999; Bradshaw *et al.*, 2005; Levine, 2005), on individual preferences, and on the perceived amount of control and predictability an individual has in a given situation (Levine, 2005; Nelson & Kriegsfeld, 2017). An immediate response to a perceived stressor, for example, is an increase in heart rate and respiration, whilst behaviours which are non-essential to immediate survival, such as digestion, are supressed (Gabrielsen & Smith, 1995; Ellenberg *et al.,* 2006; Nelson & Kriegsfeld, 2017). A stress response which successfully allows an individual to cope with a stressor and ensures the individual's survival, is adaptive. A simple example of a stress response being adaptive is if a zebra, *Equus spp*., encounters a lion, *Panthera leo,* in which case the lion is a stressor for the zebra. The zebra flees as an immediate response to the stressor and survives, hence making the stress response adaptive.

The basic concepts of stress which have been widely used include those of homeostasis, allostasis and the Reactive Scope model (McEwen & Wingfield, 2003; Romero, 2004; Romero *et al.,* 2009; Sterling, 2012; Palme, 2019). These will be introduced briefly below. For the purpose of this thesis, a stressor will be defined following McEwen and Wingfield (2003), where a stressor is an unpredictable and/ or uncontrollable event or stimulus which elicits physiological and behavioural responses in addition to those imposed by the normal life cycle. The physiological, hormonal, or behavioural responses, in turn, are the stress response (Romero, 2004).

An individual ideally should be in a stable physiological state, called homeostasis, during which physiological systems are maintained within an ideal range to sustain life (McEwen & Wingfield, 2003). Allostasis is the concept of maintaining stability through change and therefore serves to achieve homeostasis; in response to a stressor an organism activates neural and/or neuroendocrine systems, and/or responds behaviourally in order to cope (Palme, 2019). Usually, an external stressor disrupts homeostasis and triggers a stress response. Such responses are referred to as allostatic state (McEwen & Wingfield, 2003). The cumulative response of those changes in reaction to a stressor is referred to as allostatic load and, as mentioned before, is adaptive, as it enables an individual to cope with changes (McEwen & Wingfield, 2003). Allostatic load can hence result from a difference between the amount of resources, such as energy required to maintain an organism, and the amount of resources which are available (McEwen & Wingfield, 2003). An individual's available resources must be divided amongst needs such as growth, cell maintenance, immune function, reproduction, and, in homeotherms, thermogenesis (Nelson & Kriegsfeld, 2017). However, if resources are in short supply and normal adaptive responses become insufficient, non-essential needs, such as growth, digestion, and reproduction are supressed and immune function is compromised (Nelson & Kriegsfeld, 2017). Hence, if resources are not sufficient to cope with the stressor it results in allostatic overload and an individual's survival is compromised (Nelson & Kriegsfeld, 2017). There are two types of allostatic overload: Type I allostatic overload refers to states during which the demand for energy exceeds the amount of energy which is available, whilst Type II allostatic overload refers to states during which enough or even excess energy is available but other environmental conditions, such as social conflict, act as stressors for a prolonged period (McEwen & Wingfield, 2003).

There are criticisms of the model of allostasis and allostatic overload (Romero *et al.,* 2009). Energy gain and expenditure are not well understood and vary greatly, especially depending on the time frame during which they are measured (Romero *et al.,* 2009). Furthermore, energy budgets are hard to compare between different species and individuals may alter rates of energy consumption during different life-history stages (Walsberg, 2003). The concept of allostasis, as introduced by McEwen & Wingfield (2003), depends heavily on the concept of energy gain *vs.* energy demand. However, a response to a stressor may not be sustained for a prolonged period or may not be energetically demanding (such as a freeze response upon encountering a predator). Therefore, behavioural and cognitive responses to stressors may not use significant amounts of an individual's energy budget and may not be related to energy budgets (Romero *et al.,* 2009). The allostasis model would therefore only detect effects of stressors if they are extreme enough to affect the energy budget of an individual (McEwen & Wingfield, 2003), for example if an individual performs so many or frequent freeze responses that it no longer manages to forage for sufficient amounts of food. However, short term behavioural and cognitive responses provide substantial information about stressors (Romero *et al.,* 2009).

In addition to the allostatic model mentioned previously (McEwen & Wingfield, 2003), the Reactive Scope model has been introduced by Romero and colleagues (2009). This model describes four ranges of homeostatic states, depicted in Figure 1.1. Mediators in reaction to a stressor can include secretion of hormones, changes in heart rate or neurotransmitter concentrations, as well as changes in specific behaviours (see Table 1 in Romero *et al.*, 2009). To sustain life, basic homeostatic processes are required, such as a sufficient blood pressure. If those basic requirements are not met, it results in homeostatic failure and an organism cannot survive (Fig. 1.1; Romero *et al.,* 2009). Predictive homeostasis represents the range of functions an organism adapts to, and which it encounters predictably, but which vary depending on life-history stages, circadian function, and seasonality (Fig. 1.1; Romero *et al.,* 2009). Reactive homeostasis is an adaptive mediator response to random, unpredictable stressors which challenge homeostasis (Romero *et al.,* 2009). Changes in mediators within the predictive and reactive homeostatic range (Fig. 1.1) form the ‘normal reactive scope’ and are fluent and adaptive (Romero *et al.,* 2009). When an organism encounters many stressors within a short amount of time, increasing physiological mediators beyond the normal reactive scope of the organism, it can result in a pathological state called homeostatic overload (Fig. 1.1). Similarly, if a singular stressor cannot be avoided or the organism cannot cope, it results in homeostatic overload.

If an organism is in a constant state of homeostatic overload or enters homeostatic overload too frequently, the reactions related to coping with the stressor can become harmful and pathological themselves. Maintaining physiological systems within the reactive homeostatic range further accumulates a cost which is referred to as wear and tear (Romero *et al.,* 2009) and such wear and tear, over time, decreases the range of the reactive scope. Therefore, continued or frequent stress can result in the organism being unable to cope with additional occurring stressors (Romero *et al.,* 2009). Eventually, this may narrow the reactive scope through wear and tear so much that the individuals' threshold of homeostatic overload intersects the threshold of predictive homeostasis, ultimately collapsing into homeostatic failure (Romero *et al.,* 2009; see also Fig.4 in Romero *et al.,* 2009). Further, if an organism is in homeostatic overload for a prolonged period of time, it becomes chronic and can have negactive effects on an individual's health, affect behaviour and cognitive processes, as well as neuro-endocrine and autonomic functions (O'Connor *et al.,* 2000; Sapolsky, 2002; Möstl & Palme, 2002; McEwen & Wingfield, 2003; Romero *et al.,* 2009).

The magnitude of a response can be reduced dependent on the amount of control an individual has in removing itself from the stressor, whether the individual has outlets of frustration, and whether the individual can become habituated to the stressor (Nelson & Kriegsfeld, 2017). Whilst homeostatic failure is usually a constant threshold over time, how wide an individual's reactive homeostatic range is can vary throughout time and hence an individual may be able to react to a stressor within its normal reactive scope at one time, but the same stressor may push an individual into homeostatic overload at another time (Fig. 1.1C; Romero *et al.,* 2009). Further, the range of the normal reactive scope can also differ between individuals (Romero *et al.,* 2009).

The stress response, according to Romero and colleagues (2009) as well as following the definition of a stressor above, is the change in physiological mediators which occurs within the reactive homeostatic range. This means that, for example, changes in mediators associated with breeding, reproduction, changes in photoperiod, or hibernation fall within the predicitve homeostatic range (Romero *et al.,* 2009) and are not regarded as the stress response.

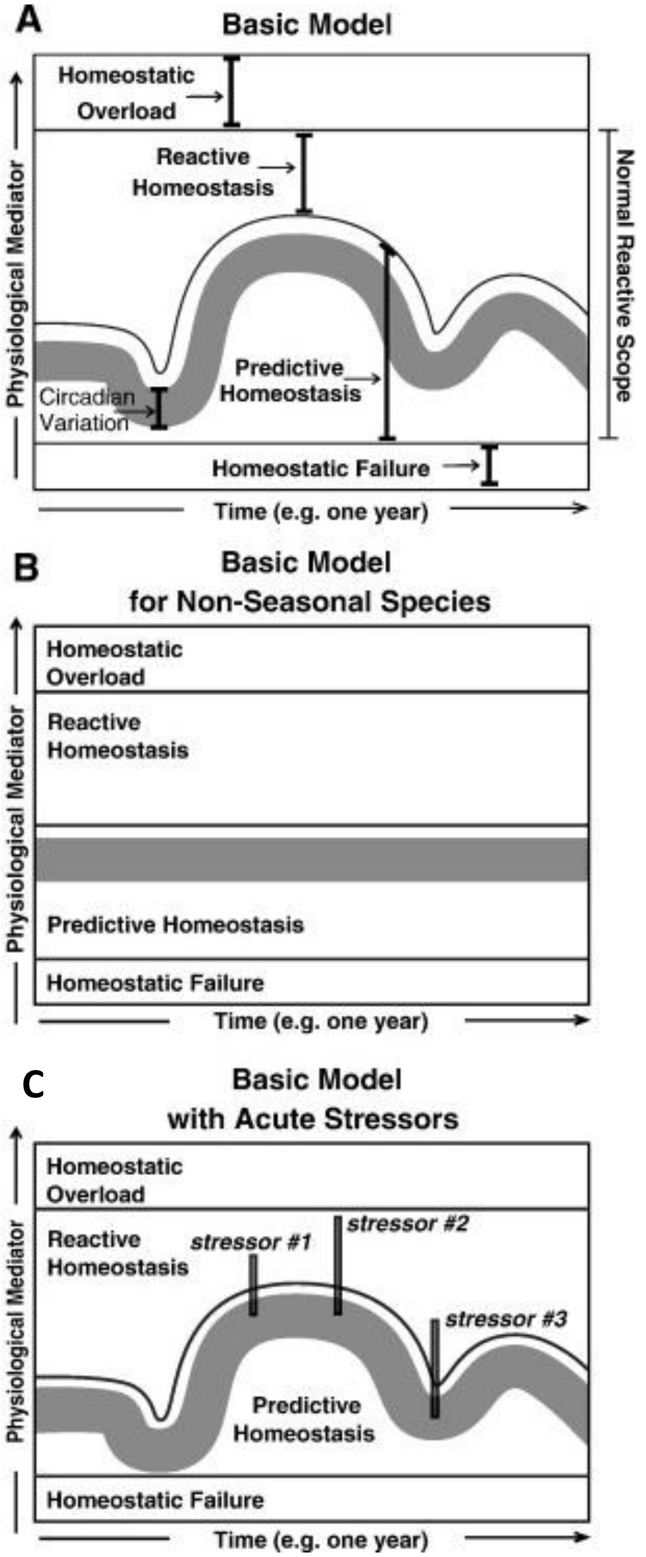

Figure 1.1

(A) Graphical model of concentrations of different physiological mediators on the *y*-axis vs. time. The lowest range depicts concentrations that are too low to maintain homeostasis, termed Homeostatic Failure. Above this threshold is the Predictive Homeostasis range that varies according to predictable life-history changes. The circadian variation in concentrations is depicted as a gray bar (bottom = circadian nadir, top = circadian peak). The Predictive Range extends slightly above the circadian peak to encompass predictable daily events such as foraging. Above the Predictive Homeostasis range is the Reactive Homeostasis range, which represents concentrations of the physiological mediator necessary to maintain homeostasis following an unpredictable event that threatens homeostasis. The Predictive and Reactive Homeostasis ranges form the normal reactive scope for that physiological mediator. Above the threshold of the Reactive Homeostasis range is the Homeostatic Overload range. (B) A simplified version of the graphical model presented in A in a nonseasonal species such as humans. (C) A graphical depiction of the response to stressors. Each vertical line represents both a rapid spike of the mediator into the Reactive Homeostasis range in order to maintain homeostasis and a rapid decrease in the mediator once the stressor has ended. Stressor #2 is a stronger stressor than #1 and thus requires a stronger response to maintain homeostasis. Stressors #2 and #3 are of equivalent strength, but occur at different times of year. Consequently, the mediator is at different concentrations in the Predictive Homeostasis range and stressor #3 is less likely to elicit a mediator response that extends into the Homeostatic Overload range. Reprinted from The reactive scope model – A new model integrating homeostasis, allostasis, and stress, Hormones and Behavior 55/3, Romero LM, Dickens MJ, Cyr NE, page 378, Copyright (2009), with permission from Elsevier. License number 4744230020699.

In response to a stressor, the neural and the endocrine system of individuals are activated (Nelson & Kriegsfeld, 2017) with the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic adrenomedullary system being the key components to the stress response (Koolhaas *et al.,* 2011). In order to monitor stress response in populations of wild animals, non-invasive methods are required. Whilst we cannot measure the neural response to stress non-invasively, the endocrine system's response can be measured non-invasively (Nelson & Kriegsfeld, 2017). Hormones are organic chemical messengers, released from endocrine cells, which travel through the blood system to interact with cells and cause biological responses (Nelson & Kriegsfeld, 2017). Hormones can change gene expression or the rate of cellular function in their target cells and can increase the probability of a behaviour occuring in the presence of a specific stimulus (Nelson & Kriegsfeld, 2017). Steroid hormones can be measured in substrates such as blood and faeces (see below) and therefore represent a great tool for the assessment of individuals' responses to perceived stressors (Nelson & Kriegsfeld, 2017).

1.3 The endocrine system and glucocorticoids

There may be a wide range of physiological responses to stressors, e.g. increased heart rate and respiration, and changes in blood flow (Gabrielsen & Smith, 1995; Ellenberg *et al.*, 2006). Here I focus on the response of a specific class of steroid hormones which has been well studied and can be measured relatively cheaply and non-invasively. Further, as a mediator of the physiological stress response (McEwen & Wingfield, 2003; Romero *et al.,* 2009; Palme, 2019) it is often considered the ‘stress hormone‘: glucocorticoids (GCs).

An integral part of the endocrine stress system is the HPA axis (Fig. 1.2) and the associated negative feedback loops (Smith & Vale, 2006; Sheriff *et al.*, 2011; Palme, 2019). In response to a perceived stressor, the hypothalamus releases corticotropin-releasing hormone (CRH) and vasopressin into the portal circulation, which triggers the release of adrenocorticotropic hormone (ACTH) from the pituitary gland into the general circulation, and this, in turn, acts on the adrenal cortex to regulate the synthesis and release of GCs (Handa *et al.*, 1994; Levine, 2005; Smith & Vale, 2006). GCs then activate receptors, hippocampus, hypothalamic, and preoptic areas, all in the central nervous system, which terminate the release of CRH and ACTH (Levine, 2005). The HPA axis is modulated and regulated by a variety of factors such as neurotransmitters, neuropeptides, and adrenal and gonadal steroid hormones (Handa *et al.*, 1994).

Cholesterol is the precursor to all vertrebrate steroid hormones (Nelson & Kriegsfeld, 2017), such as GCs. Steroids move easily through cell membranes and are not stored in cells but leave them immediately after production. Further, steroids are fat soluble but not very soluble in blood, which means they require carrier proteins to transport them through blood (Nelson & Kriegsfeld, 2017). Carrier receptors transport steroids through the blood, and release them so the steroids can enter a cell through its membrane or by binding to a steroid receptor in the cell membrane (Nelson & Kriegsfeld, 2017). Whilst steroids are bound to co-receptors they are inactive, but when the steroid binds to a steroid receptor, the co-receptor is released and the steroid-receptor complex is activated (Nelson & Kriegsfeld, 2017). Those steroid-receptor complexes then bind to DNA (deoxyribonucleic acid) in the cell nucleus which stimulates or inhibits the transcription of specific mitochondrial ribonucleic acid (mRNA; Nelson & Kriegsfeld, 2017). Such mRNA is translated to specific enzymes or proteins which produce a physiological response (Nelson & Kriegsfeld, 2017).

When GCs bind to glucocorticoid receptors, it leads to physiological and behavioural changes which allow an organism to cope with the stressor and re-establish predicitve homeostasis (Busch & Hayward, 2009; Romero *et al*., 2009). There are two types of those receptors, known as Type I and Type II. Type I receptors are associated with GC concentrations maintained during predictive homeostasis, and, as they have a high affinity to bind to GCs, they are the first receptor type to bind to GCs (Romero, 2004). Type II receptors have a lower affinity, only bind when GC concentrations are high, and are associated with reactive homeostasis (Romero, 2004). There are more Type II receptors, compared to Type I receptors, therefore having a higher capacity for GCs (Romero, 2004).

Steroid receptors are influenced in their numbers by levels of glucocorticoids. When GCs are elevated for a prolonged period of time, glucocorticoid receptors are saturated, resulting in a surplus of GCs and to compensate, less receptors are produced (Romero, 2004; Busch & Hayward, 2009). Due to the now reduced amount of available receptors, less GCs can bind and the biological effect is reduced as lower concentrations of secreted GCs saturate receptors (Romero, 2004). Therefore, if GCs are elevated for a long period of time or too often, it may alter the baseline of GCs or the duration of GC secretion (McEwen & Wingfield, 2003; Gobush *et al*., 2008). This means that, following prolonged or chronically perceived stress, secretion of GCs is, in fact, reduced and concentrations of GCs are low.

1.3.1 Glucocorticoids‘ effects on physiology and behaviour

Perceiving something as a stressor can result in a physiological and behavioural response, however, a hormonal or cardiovascular response itself does not always indicate that a stimulus is a stressor but can also be related to factors such as sexual activity (Koolhaas *et al.,* 2011). Physiological mediators such as glucocorticoids are unable to distinguish between a perceived stressor or arousal, such as sexual activity or excitement, and increase in response to both. As such, investigating only one physiological response in order to assess a stressor is insufficient (Koolhaas *et al.,* 2011) and instead should be done alongside investigations of behavioural responses which can be observed directly and non-invasively.

Figure 1.2 gives a simplistic overview of the HPA axis and the associated negative feedback loop, as well as other mechanisms in response to a perceived stressor which act in the short-term, as discussed in the following paragraphs. GCs promote the conversion of protein and lipids into usable carbohydrates and are therefore integral for energy regulation (McEwen & Wingfield, 2003; Palme, 2019). Alongside GCs, epinephrine and norepinephrine are released from the adrenal cortex, which act within seconds to increase respiratory rate and make energy available for muscles (Nelson & Kriegsfeld, 2017) which increases the ability for individuals to perform coping behaviours, such as the fight or flight response. The hormone effects in response to a stressor are discussed in detail in a range of literature (see for example Sapolsky *et al.,* 2000), but all serve to facilitate coping behaviour and responding to unpredictable stressors. Effects include replenishing glucose from energy stores and gluconeogenesis (generation of glucose), enhanced delivery of substrates to muscles, stimulation of immune function, inhibition of reproductive physiology and behaviour, increase of cerebral blood flow, increased cardiac tone, decreased feeding and appetite and sharpened cognition (Sapolsky *et al.,* 2000; Sapolsky, 2002).

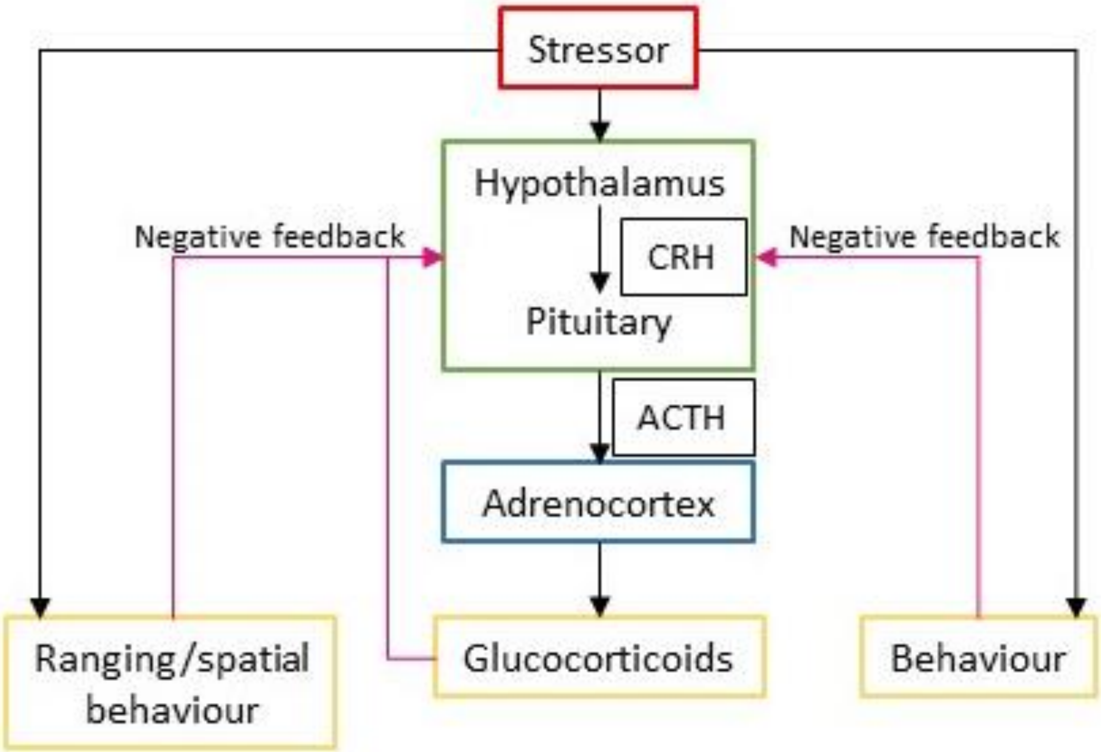

Figure 1.2

Simplistic overview of the hypothalamic-pituitary-adrenal (HPA) axis. A stimulus is perceived as a stressor by an individual, sending a signal to the hypothalamus, situated in the brain. The hypothalamus secretes corticotropin releasing hormone (CRH) which stimulates the pituitary (situated in the brain) to release adrenocorticotrophic hormone (ACTH). This stimulates the adrenocortex (situated just above the kidneys) to release glucocorticoids (GCs). GCs feed back to the hypothalamus and pituitary to inhibit further release of their respective hormones, until GCs are depleted, and the hypothalamus and pituitary are no longer inhibited. The perceived stressor can also lead to changes in, for example, an individual’s behaviour or ranging behaviour, which in turn serve as coping mechanisms that can reduce or inhibit further release of GCs. Additionally, GCs, behaviour, and ranging behaviour are affected by other intrinsic and extrinsic factors such as life history stages, temperature, or season. Further impacts of GCs, for example on the immune system, have been excluded from this figure as they are not investigated in this thesis. Figure partly adapted from Romero, 2004.

GCs can directly inhibit or enhance the hypothalamic-pituitary-gonadal (HPG) axis, which secretes reproductive hormones such as testosterone and oestrogen (Handa *et al.*, 1994). High GC concentrations impair reproduction by reducing sexual drive and performance (Nelson & Kriegsfeld, 2017). GCs are affected by reproductive status in males as well as females (Sheriff *et al*., 2011). For example, testosterone inhibits the HPA axis, whilst oestrogen enhances the response of the HPA axis (Handa *et al.*, 1994). In species which have pronounced mating seasons, males often experience peaks in testosterone during mating season, simultaneously inhibiting GCs. Females experience a peak in oestrogen, alongside an enhanced response of the HPA axis, at the end of the follicular phase, just before ovulation, and then oestrogen is elevated during the luteal phase alongside a peak of luteinizing hormone and follicle-stimulating hormone. Increased GC concentrations inhibit the synthesis and release of luteinizing hormone and follicle-stimulating hormone and hence suppress ovulation (Whirledge & Cidlowski, 2010). As reproduction is energetically demanding, in the short-term, suppression of reproduction is an adaptive response if individuals are within their reactive homeostatic range or experiencing homeostatic overload (Romero *et al.,* 2009). This allows for a wider scope of coping with the stressor, without the energy demands of reproduction.

Besides affecting the HPG axis, GCs also affect an individual’s behaviour (Sapolsky, 2002). Behaviour, in return, can feed back and affect hormone concentrations, where, for example, a behaviour which was performed to cope with the stressor will lower the amount of GCs which are released (Fig. 1.2; Nelson & Kriegsfeld, 2017). For example, increased GCs are related to potential coping behaviours such as increased movement and foraging behaviour, promote the ability of an individual to move into a new habitat or return to a previously disturbed habitat (Fig. 1.2; McEwen & Wingfield, 2003; Busch & Hayward, 2009). Further, vasopressin, released alongside GCs, enhances memory consolidation and retrieval (Sapolsky, 2002; Nelson & Kriegsfeld, 2017). This enables individuals to form memories of, for example, where stressors were encountered, which coping strategies were successful in removing the individual from the stressor and to retrieve this information the next time this specific stressor is encountered.

Another effect of GCs is that on immune function and susceptibility to disease. GCs have immunosuppressive and anti-inflammatory characteristics, for example by inhibiting the synthesis, release, and/ or efficiency of mediators such as cytokines which promote immune and inflammatory reactions (Sapolsky *et al.,* 2000). This may be adaptive, if immunosuppression serves to avoid an overshoot of autoimmune reactions or extreme concentrations of cytokines, which themselves can be toxic and therefore require controlled concentrations (Sapolsky *et al.,* 2000). However, long-term increases in GCs are related to apoptosis (cell death) of a wide range of other mediators such as macrophages, natural killer cells, and dendric cells (Bereshchenko *et al.,* 2018) and hence have negative effects on organisms' immune function and ability to fight disease.

Changes in physiology and behaviour, as discussed, are adaptive and enable individuals to cope with perceived stressors in the short-term (Busch & Hayward, 2009; Sheriff *et al.*, 2011). Prolonged secretion of GCs, however, can have negative effects on an individual's behaviour, reproduction, immune system, and health (Sapolsky, 2002). Effects can become long-lasting if a stressor is encountered repeatedly or if a coping mechanism fails to successfully remove the individual from the stressor. Further, effects can be long-lasting when a coping mechanism results in changes in behaviour, but the consequences of the new behaviour are perceived as a stressor. Long-term, if an individual is in homeostatic overload (Fig. 1.2), chronically elevated GCs suppress physiological processes and behaviours non-essential for immediate survival, such as reproduction, digestion, memory-related skills, and growth (McEwen & Wingfield, 2003; Busch & Hayward, 2009; Romero *et al.,* 2009; Sheriff *et al.*, 2011). For example, long-term elevated GCs can interrupt a female’s oestrus cycle, pregnancy, or lactation (Nelson & Kriegsfeld, 2017). Additionally, GCs supress storage of energy and promote energy use from adipose tissue and liver stores (Sapolsky *et al.*, 2000). Therefore, GC secretion over time leads to loss of muscle mass, reduces growth, and delays cutaneous wound healing (Nelson & Kriegsfeld, 2017). Further, long-term homeostatic overload reduces the birth of new neurons (neurogenesis) in the hippocampus and impairs memory (Nelson & Kriegsfeld, 2017). Long-term effects of perceived stressors can also increase aggression (Sapolsky, 2002; Nelson & Kriegsfeld, 2017) and individuals may either become more anxious and therefore more vigilant, or depressed and therefore lethargic (Fig. 1.2; McEwen & Wingfield, 2003; Busch & Hayward, 2009; Romero *et al.,* 2009).

The endocrine stress response can be measured in various substrates, allowing us to assess how animals respond to potential stressors. However, those various substrates represent GC concentrations over different periods of time and are more or less invasive to collect. Hence all those substrates have specific advantages and disadvantages associated with their measurements.

1.3.2 Measuring glucocorticoids

1.3.2.1 Substrates in which glucocorticoids can be measured

As GCs circulate in the plasma of an individual, they are metabolized predominantly by the liver as well as the kidney, where they become inactivated and more soluble in water (Taylor, 1971; Palme, 2019). The resulting metabolites are then excreted mostly as conjugates via bile, urine, hair, feathers, milk, saliva, or eggs (Fig. 1.3; Palme, 2019). Those steroids excreted in bile move through the intestines to be excreted in faeces and can undergo further metabolism by bacterial enzymes or can be partly reabsorbed in the intestine through enterohepatic circulation, where they re-enter the liver via the circulation (Palme, 2019).

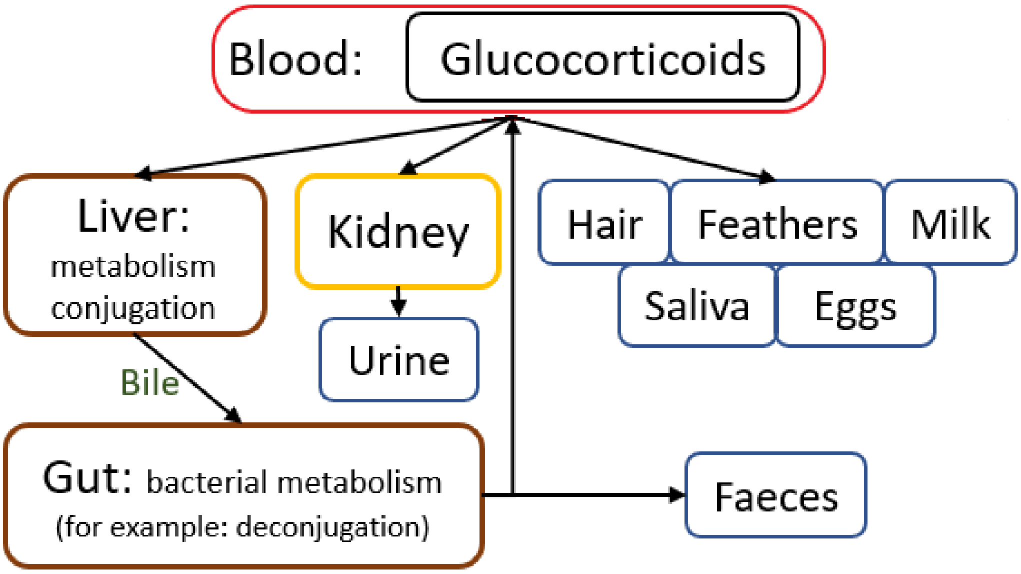

Figure 1.3

Scheme of the substrates in which glucocorticoids (GCs) can be measured. The hypothalamic-pituitary-adrenal (HPA) axis has released GCs into the blood stream, from where they can enter various other substrates. Figure adapted from Möstl & Palme, 2002.

When measuring GCs, values taken from the various substrates are representative of different time frames and feasibility of sampling varies. Further, some sampling techniques are more invasive than others. As this thesis is concerned with wild and free-ranging animals, invasive was here defined as a procedure which requires direct contact with the animal whilst the animal is fully conscious but restrained, sedated, or anaesthetised. Non-invasive was defined following Pauli *et al.*'s (2010) definition of unperceived, non-invasive sampling, where animals are unaware of sampling and, therefore, are unaffected by it.

Some substrates provide measurements of GC concentrations in reaction to a potential stressor within minutes following the stressor. For example, an individual's blood can be sampled as GC concentrations increase 2 – 5 mins following the stressor (Sheriff *et al.,* 2011). Further, collecting blood provides a chance to collect a range of other information such as genetic data (Sheriff *et al.,* 2011). However, restraint and taking of blood itself is a stressor for individuals and hence such concentrations are not representative of previous stressors alone. As such restraint/ capture and direct contact itself is likely to be stressful for wild animals, the measured concentration of GCs would be unlikely to be useful for someone aiming to assess a stress response to anything but the current intervention. Further, collecting this kind of sample is invasive, and for wild, potentially dangerous, difficult to handle, or protected species, collection requires experts such as veterinarians to handle or even sedate the animal (Sheriff *et al.,* 2011). Similar to blood, saliva concentrations of GCs represent measurements about 20 – 30 mins following a stressor (Sheriff *et al.,* 2011). Saliva is less invasive to collect than blood as it does not require the insertion of a needle, but it still requires either direct contact with the animal, or collection of saliva from something the animal licked or chewed on. This in turn increases the risk for contamination, for example with other fluids or saliva from other animals (Sheriff *et al.,* 2011). For repeated measurements, the sample would need to be collected from the same substrate for consistency. Therefore, collection of saliva and blood for repeated analysis of GCs from free-ranging animals would be invasive and impractical.

To collect GC concentrations representative of a period of weeks, months or even years, hair and feathers can be collected (Sheriff *et al.,* 2011). GCs in a specific part of the hair or feather are representative of the average concentration during the time of growth, for example, the part closest to the root is representative of the most recent GC concentrations, whilst the part furthest from the root represents concentrations from months or years ago, depending on the speed of growth. Note that hormones are only deposited in feathers during the time of growth and, once grown, feathers are gradually abraded and then shed. Feathers may grow faster than hair and, in theory, may even have sections which correspond to daily GC concentrations (Grubb, 1989), whilst hair can correspond to GC concentrations up to several years ago (Sheriff *et al.,* 2011). Samples should be washed to avoid contamination with GC by secretions from skin, for example, on the outside of the sample, however this potentially penetrates the sample itself and removes GCs (Sheriff *et al.,* 2011), making GCs in hair and feathers difficult to measure reliably. To gain insight into the effect of something occurring within a specific timeframe, a researcher needs to collect a whole sample (including, or close to the root) just before the onset of that timeframe as well as one just after, and from the same individual (Sheriff *et al.,* 2011). For free-ranging animals, taking hair or feathers would likely require capture and sedation, posing ethical concerns of such sampling techniques and making it invasive. Alternatively, when using hair traps, it may not be possible to assign a sample to a specific individual and other associated information such as sex, age, or ID.

Collecting milk or eggs to measure GCs limits sampling to females. Further, for mammals it limits sampling to the timeframe of pregnancy or lactation (Sheriff *et al.,* 2011). Eggs and milk require careful collection to avoid contamination with, for example, secreted GCs on skin or contamination from hands or water, and careful processing to control for varying GC concentrations depending on the embryonic development (von Engelhardt & Groothuis, 2005; Aerts, 2018). However, collection of those samples requires either the removal of the eggs or capture of the female to take a sample of her milk (Sheriff *et al*., 2011).

Concentrations of GCs can be taken from urine and faecal samples to assess a potential stressor. Following a stressor, GCs in urine are excreted within a few hours, in the first sample following the stressor or in one or two samples later, depending on the species and frequency of urination (Palme *et al.,* 1996; Ganswindt *et al.,* 2003). When collecting urine from free-ranging animals contamination with bacteria is possible where the urine mixes with substrates such as soil and faeces. Bacteria can potentially break down GC metabolites within the sample rendering results inaccurate (Sheriff *et al.*, 2011). Following a stressor, metabolized GC concentrations related to the stressor will be present in the faeces according to the species-specific gut-passage time (Palme *et al.*, 1996; Palme 2019), usually several hours to a few days. It should further be considered that diet can have an effect on gut-passage time and metabolite concentrations, as can sex, age, or health. Faecal samples can be collected from the inside of the faecal matter which avoids contamination with soil or bacteria but may have been contaminated with urine. However, temperature, rain, time a sample has been lying on the ground before collection, and degradation after defecation by bacteria or microbes can affect GCs in faeces (Palme, 2019). Before reaching urine or faeces, metabolites can be reabsorbed when passing through the liver (Fig. 1.2) or be affected by microflora in the intestines, hence reflect a fraction of the total GCs excreted in response to a stressor (Sheriff *et al.*, 2011). GCs in urine and faeces are species-, and sometimes sex specific and techniques to measure them require validation (see below). However, urine and faeces can be collected repeatedly and non-invasively from captive and free-ranging animals and their collection does not require restraint or capture, hence limits ethical concerns to those of human presence within the species' habitat (Sheriff *et al.*, 2011).

1.3.2.2 Measuring faecal glucocorticoid metabolites (fGCMs)

Different collection, storage, and extraction methods can affect the measured concentrations of GCs, and consistency in all those methods is integral for assessing GCs of a study species over time (Palme, 2019). As such, it is important to collect samples within a certain time frame of excretion, which can be species- and environment specific. Samples can be stored on ice before being frozen, which only imposes minor costs related to sampling vials and gloves, and a cooler box with ice packs. However, such storage requires a constant cold chain in order to keep bacteria from further breaking down metabolites. If, alternatively, samples are stored in ethanol, then ethanol, vials and gloves need to be bought. Storage in ethanol raises concerns as different percentages of ethanol have different polarities and the recovery of steroid metabolite concentrations can be considerably affected depending on the polarity of the mix of steroid metabolites in the faecal sample (Palme *et al.,* 2013). Further, ethanol may affect concentrations of metabolites during storage, resulting in the measured concentration being lower compared to the actually excreted concentration (L.Yon, *pers.comm.*). To measure fGCMs, there are two main methods: chromatographic methods, such as liquid chromatography (LC) used in combination with mass spectrometry (MS), or immunoassays (IA). LC-MS studies are often too costly, unsuited for large sample sizes, and the chemical make-up of the measured metabolites needs to be known (Palme, 2019). A range of reviews have previously compared IA methods (Wudy *et al.*, 2018) but I will give a brief overview of them.

Immunoassays, usually, are either radioimmunoassays (RIAs) or enzyme immunoassays (EIAs). They are based on the principle of competitive binding of an antibody to an antigen (Nelson & Kriegsfeld, 2017). An antibody produced in response to any antigen, such as a hormone, has a binding site specific for that antigen (Nelson & Kriegsfeld, 2017). This means that a specific quantity of antibody has a specific number of binding sites for its antigen. In RIAs, a radioactive label is added to an antigen and this competes with the antigens from the sample to bind at specific sites of an antibody (Möstl *et al.*, 2005; Sheriff *et al.*, 2011; Nelson & Kriegsfeld, 2017). For either IA a standard curve needs to be developed, by adding a known amount of labelled antigen as well as unlabelled antigen to a known amount of antibody. As the unlabelled and labelled antigen competes to bind to the antibody, the amount of labelled antigen can then be measured. This process is repeated for different concentrations of unlabelled antigen, which produces a standard curve, showing the proportion of bound labelled antigen versus the total amount of unlabelled antigen (Nelson & Kriegsfeld, 2017). In EIAs, no radioactive material is used, but an enzyme, which changes the colour of the substrate, is added to the antigen (Nelson & Kriegsfeld, 2017). The standard curve is produced using a spectrometer, which reads the colour of each sample and produces a colour gradient along which different colours represent different concentrations of GCs, and against which samples can be matched (Nelson & Kriegsfeld, 2017).

IA techniques require highly purified hormone for a specific antibody to be prepared, usually raised in an animal such as a rabbit, chicken, or goat (Nelson &

Kriegsfeld, 2017). There is potential for contamination of the raised antibody, for example with endotoxins in the antigen or diluents or through non-sterile working conditions (Saeed *et al.,* 2017). However, EIAs are cheaper in terms of equipment used and do not require radioactive materials at all, making them safer to people using those assays as well as the environment and hence are the most frequently used method (Sheriff *et al.*, 2011; Palme, 2019). In order to reliably assess fGCMs and because of complexities and variation of steroid metabolism and excretion between species, the assay used needs to be validated (Sheriff *et al.*, 2011; Palme, 2019). This can be done through physiological validation, such as an ACTH challenge test, or biological validation, such as measuring fGCMs before and after a presumed stressful event (see Palme, 2019, for more details and advantages and disadvantages of each method). In short, physiological validation by injecting ACTH causes the release of GCs and, based on species-specific gut passage time, a peak in fGCMs occurs when the released GCs have been metabolised and excreted. If this peak can be detected by the used assay, it has been successfully validated. However, physiological validation is invasive, and the amount of ACTH administered is often very high compared to concentrations occurring naturally (Sheriff *et al.*, 2011; Palme, 2019). Biological validation is done through measurement of fGCMs before and after a presumed stressful event such as capture, transportation, or other anthropogenic disturbances (Sheriff *et al.*, 2011; Palme, 2019). In each case, a baseline of fGCMs needs to be established before the presumed stressor occurs and all faeces after the presumed stressor should be collected for a prolonged period of time depending on the species-specific gut-passage time, until all GCs related to the presumed stressor have been excreted and GC concentrations return to baseline. Further disturbance following the initial presumed stressor can result in further fGCM increases and should be avoided in order to gain insight into how long it takes for GC concentrations to return to baseline following a presumed stressor. Validation should be carried out on several individuals of each sex in order to gain a detailed understanding and to understand a species- rather than individual-specific response.

It is necessary to know how long it takes for secreted steroids to be metabolised and excreted when measuring them in faeces. The species-specific gut passage time of GCs has traditionally been assessed through radiometabolism studies, during which an individual is injected with radiolabelled GCs and all faeces are collected afterward. The time lag between the injection and secretion of those radiolabelled GCs is then measured, further allowing one to assess the proportion of GCs excreted via faeces (Palme, 2019). This is important as some GCs are reabsorbed through the intestine and hence GC concentrations in faeces only represent a fraction of the released concentrations of GCs overall. Alternative methods exist, for example by mixing small artificial markers such as plastic beads into an individual's food and measuring time until those are excreted in faeces (Stringer *et al.,* 2020). Sound validation measures of plasma GC concentrations being reflected by the measured faecal metabolites are important for the use of fGCM measurements in studies of stressors (Palme, 2019). As IAs depend on specific antibodies which measure different metabolites in faeces, comparisons between different studies are often not possible unless the exact same extraction and assay method was used (Palme, 2019). Further references on radiometabolism studies and details on validation processes can be found in a recent review by Palme (2019) but go beyond the scope and aim of this thesis.

When assessing fGCMs, it is vital to consider factors which can affect and influence their concentrations. Those factors can be related to the diet and an individual's metabolism, individual differences, sex, reproductive state, and season (Palme, 2019). Knowing the individual, its sex and reproductive state, and the season in which a sample was secreted, is therefore necessary to account for variation due to such factors when assessing other potential stressors.

1.4 Wildlife tourism as a stressor

A variety of factors may affect specific aspects of hormone concentrations, behaviour or other physiological measures, and as one or all of them could be affected by a stressor at different times or simultaneously, it is therefore important to combine a variety of measures in order to study an animal‘s response to a potential stressor (Sevi, 2009; Fig. 1.2). For example, GCs can not only increase due to a perceived stressor, but also due to exertion and, further, they can also decrease with prolonged exposure to a stressor (Mason & Veasey, 2010). Therefore, by collecting information on physiological, behavioural and space use patterns, we are able to achieve a more informed result (Sevi, 2009). Further, such responses are often connected, where, for example, we can use behavioural indicators to predict the effect of a stressor on biological functions (Rushen, 2000; Fig. 1.2).

As mentioned at the beginning of this chapter, one factor which has been identified as a stressor in a range of taxa, is wildlife tourism. As discussed above, an individual may respond to stressors within its reactive scope or it may experience homeostatic overload (Fig. 1.1; Romero *et al.,* 2009). Further, responses may be short-term and adaptive, or long-term and maladaptive. In order to assess whether a specific stimulus presents a stressor to an individual, we can measure physiological mediators which occur in response to this stimulus. Palme (2012) noted that responses to stressors are not only dependent on the context but are also very complex, and therefore different measurements should be combined to assess stress responses. Besides the measurement of physiological indicators such as GCs, focal observations of animals can provide insight into disturbance and stress-induced changes. For example, running away has been used as a behaviour indicating perceived stressors in animals, which serves to remove the animal from the vicinity of a stressor, such as a predator (Burke *et al.,* 2008). Further, self-directed behaviours, such as self-scratching and self-grooming, have been used as indicators of perceived stressors in primates, goats and rats, and are directly related to physiological measures such as GC concentrations (Troisi, 2002; Fraser *et al*., 2008; Schino *et al*., 1996).

1.4.1 Wildlife tourism and glucocorticoids

Presence of tourists within an animal's habitat has been linked to increased GC concentrations in mammals and birds (Fowler, 1999; Müllner *et al.*, 2004; Behie *et al.*, 2010; Shutt *et al.,* 2014). In some studies, tourist activity has been defined as mere presence in the animal's habitat, whilst other tourism-related factors which have been assessed were close proximity of tourists to animals and human activity in the form of vehicle use within animals' habitats. Tourism intensity, in the form of total number of tourists, and direct interactions between animals and tourists have also been identified as stressors (see following paragraphs).

Short-term, adaptive responses have been observed in yellow-eyed penguins, *Megadyptes antipodes* (Ellenberg *et al.,* 2007). Penguins from two sites, one undisturbed from tourism and another which experiences high numbers of tourists each day, had similar baseline GC concentrations (Ellenberg *et al.,* 2007). However, in response to capture and handling, penguins at the disturbed site had a higher magnitude increase in

GCs compared to penguins at the undisturbed site (Ellenberg *et al.,* 2007). The similar baseline concentrations could indicate that the increase in GCs related to handling and capture of individuals at the tourism disturbed site was short-term and not chronic. However, results may have been biased because individuals which perceived tourism as a stressor may have left the disturbed site already (indicated by lower nest numbers in relation to available habitat as well as reduced fledgling survival at the disturbed site), and because samples were collected late in the breeding season and therefore were biased toward penguins still attending nests and potentially best able to cope with stress (Ellenberg *et al.,* 2007). Further, no long-term data were collected to assess whether responses associated with human proximity were occurring at high frequency (Ellenberg *et al.,* 2007). Barbary macaques, *Macaca sylvanus,* at a site frequented by tourists throughout the year, had significantly elevated fGCMs following high rates of aggressive interactions with tourists in the form of tourists physically striking or pushing macaques, or throwing items towards macaques (or pretending to do so; Maréchal *et al.*, 2011). Mean tourist number at the site as well as rates of neutral (where tourists did not interact directly with macaques such as talking to-, or photographing them), feeding (where tourists handed-, or threw food to macques), and aggressive (see above) interactions between tourists and macaques were related to self-scratching, a measure of anxiety in this species (Maréchal *et al.*, 2011). Feeding interactions occurred on average 5.5 times per hour, neutral interactions 2.4 times per hour, and aggressive interactions 1.7 times per hour, where interactions were classed as separate events if they were separated by at least ten seconds (Maréchal *et al.*, 2011). Further, the authors reported that monkeys were observed climbing and resting in trees when many tourists were present or very close, which authors interpreted as an escape response (Maréchal *et al.*, 2011). However, maximum or mean number of tourists and percentage of time tourists were present was not related to fGCMs during the study period (Maréchal *et al.*, 2011), indicating that the increase in fGCMs following aggressive interactions may have been a short-term response within individuals' reactive scope (Romero *et al.,* 2009).

Responses to stressors can include short- as well as long-term responses and can change over time. Magellanic penguins, *Spheniscus magellanicus,* exposed to tourists for one hour each day over a previous period of three years, showed an increased GC response to human presence from their baseline, whilst penguins which had been exposed to very high levels of tourism with human visits occurring, according to the author, most of the day during the majority of the breeding season over the past 20 years did not show significantly increased GC concentrations (Fowler, 1999). As baselines between those two populations were similar, this suggests that individuals at the tourist exposed site have habituated to tourist presence. Similarly, western lowland gorillas, *Gorilla gorilla,* undergoing habituation to humans for the past two years since 2008 before the study was carried out in 2010 to 2011, had significantly increased fGCM concentrations compared to gorillas which had undergone habituation for ten years, since 2000. The group undergoing habituation was encountered 130 times on 90 days, between one to three times within a single day between November 2010 and December 2011 by the habituation team (Shutt *et al.,* 2014). However, groups which have been habituated for over ten years and visited by tourist for over six years did not have increases in fGCMs related to daily tourist presence (Shutt *et al.,* 2014), but did have significant increases in fGCMs when tourists came closer than the permitted seven meters (Shutt *et al.*, 2014), indicating that groups no longer perceive tourist presence as a stressor but did perceive close approaches as stressful, with the potential to become chronic if this minimum distance is ignored frequently..

Long-term, chronic responses to wildlife tourism related stressors have been observed in black howler monkey, *Alouatta pigra,* groups, frequently visited by tourists

(Behie *et al.,* 2010). Monkeys had significantly elevated fGCMs compared to groups which were not visited by tourists over a period of several years (Behie *et al.*, 2010), which may suggest tourism is a long-term stressor to those groups. Similarly, in response to high use of vehicles, researchers reported significantly increased fGCMs in grey wolves, *Canis lupus,* over two winters and in red deer, *Cervus elaphus,* over a thirty-day period (Creel *et al.*, 2002), and tourism was suggested to be a long-term stressor (Millspaugh *et al.*, 2001). Similarly, European pine martens, *Martes martes,* had elevated faecal cortisol metabolites in areas with high tourist numbers and during periods of high tourism, indicating responses which lasted for the spring, as well as summer season (Barja *et al.*, 2007), indicating long-term stress. In ski tourism areas, where black grouse, *Tetrao tetrix,* and capercaillie, *Tetrao urogallus,* are regularly flushed out of their burrows, disturbance by tourists and tourism intensity was significantly related to increased concentrations on consecutive days (Arlettaz *et al.*, 2007), and during the approximately 4 – 5 months long ski season (Thiel *et al.*, 2008) suggesting a long-term stress response. In wildcats, *Felis silvestris,* high tourism intensity was linked to elevated fGCMs for a period of nearly four years, indicating a long-term stress response (Piñeiro *et al.*, 2013). Further Tatra chamois, *Rupicapra rupicapra tatrica,* had significantly elevated faecal cortisol metabolite concentrations with high tourist disturbance and larger numbers of visitors to the reserve lasting all summer (Zwijacz-Kozica *et al.*, 2013).

When stress is perceived long-term it can become chronic and have negative consequences for animals (Romero *et al.,* 2009). The reactive scope can narrow under such circumstances and individuals become less able to cope with additional stressors (Fig. 1.2; Romero *et al.,* 2009). Additionally, if individuals are within homeostatic overload for too long, ‘wear and tear’ (the concept of the cost in maintaining physiological mediators) accumulates, resulting in a gradual decrease in the ability of individuals to cope (Romero *et al.,* 2009).

Recently, high tourist pressure in the form of increased numbers of vehicles, has been linked to increased fGCM concentrations in tigers, *Panthera tigris,* in two protected areas in India (Tyagi *et al.,* 2019). The tourist season lasted nine months, indicating that tourist pressure could be a long-term stressor to tigers (Tyagi *et al.,* 2019). Further, juvenile hoatzins, *Opisthocomus hoazin,* frequently visited by tourists, had a stronger increase in corticosterone secretion during experimental capture and handling, whilst juveniles not visited by tourists secreted less corticosterone, suggesting that previous tourist visits were perceived as stressors by the juveniles (Müllner *et al.*, 2004). Although baseline corticosterone concentrations were similar in juveniles frequently visited to those not visited, each time juveniles were exposed to visits their corticosterone levels remained elevated for over one hour following the visit (Müllner *et al.*, 2004). As tourist visits can occur daily (Müllner *et al.*, 2004), this could indicate a frequently perceived long-term stressor (Romero *et al.,* 2009). Additionally, the same study reported that, for three consecutive years, juvenile survival of birds not visited by tourists was significantly higher, compared to surivial of fledglings in nests which were visited by tourists due to a higher mortality in weeks three to six after chicks had left the nest (Müllner *et al.*, 2004), indicating that juveniles may be experiencing negative fitness effects associated with long-term perception of stress (Romero *et al.,* 2009).

However, tourism may not always be related to increased GC concentrations. In their study, Romero and Wikelski (2002) found that Galapagos marine iguanas, *Amblyrhynchus cristatus*, at tourist-exposed sites had lower corticosterone concentrations than those from a known population which was chronically stressed from a prolonged drought related to an El Niño resulting in wide-spread mortality. The authors suggested that iguanas may have habituated to tourism but noted that this may not have been beneficial, as the long-term downregulation of corticosterone concentrations could compromise their ability to respond appropriately to another stressful stimulus. However, such downregulation would indicate chronic stress, rather than true habituation (Romero *et al.,* 2009). Additionally, even though tourist-exposed iguanas, compared to iguanas affected by drought, had lower corticosterone concentrations, they may still have had elevated corticosterone concentrations overall. Further, gorillas and penguins, following long periods of habituation or high and frequent exposure to tourists as discussed in the examples above, did not have significantly elevated GC concentrations in response to tourist presence anymore (Ellenberg *et al.,* 2007; Schutt *et al.,* 2014). Gorillas still had significant GC increases related to other stimuli such as very close proximity to tourists, indicating that, although the initial stress response was long-term, they became habituated to tourism (Schutt *et al.,* 2014) rather than having experienced a down-regulation of GCs as suggested in the study by Romero and Wikelski (2002).

Overall, results from different studies suggest that there are individual differences in how animals respond to tourism and a variety of factors may affect this, such as past experiences. Further, it can depend on the frequency, proximity, or duration of tourist visits and overall nature of tourism. Without prolonged sampling and consideration of various potential confounding factors as well as incorporating a variety of measures, such as behaviour and physiological mediators, conclusions as to whether effects of perceived stress related to tourism are long- or short-term are difficult to draw.

1.4.2 Wildlife tourism and behaviour

Wildlife tourism, however, not only results in the secretion of glucocorticoids as physiological mediators in various species but those GCs and the perception of a stressor can also affect animal behaviour (Fig. 1.2). Animals change their behaviour and their interactions with their environment and each other in order to adapt to living conditions and to react to stressors (Martin & Bateson, 2007). Changes in behaviour to unpredictable stressors are within the reactive scope (Romero *et al.,* 2009) and are often the initial response to human-altered conditions, improving animals' likelihood to cope and survive (Wong & Candolin, 2015). Therefore, changes in behaviour are useful to observe when trying to assess whether a specific factor, such as tourism, is a stressor. Further, a specific stressor can potentially invoke changes in a wide range of behaviours such as aggressive behaviours (Mellor *et al*., 2000), foraging, resting, vigilance, or any other species-specific behaviour. Large increases in conspecific-directed aggression have also been identified as a behaviour which may indicate a perceived stressor (Jennings & Prescott, 2009). Furthermore, an increased occurrence of any specific behaviour may be related to poor welfare in animals if they are related to an abnormal time budget such as over grooming or restlessness (Jennings & Prescott, 2009).

Stress-related behaviours are behaviours which are species- specific and performed in response to a perceived stressor. Often, stress-related behaviours are self-directed, such as self-scratching and nail biting, and some specific self-directed behaviours have been observed in a range of species such as primates (Maestripieri *et al*., 1992; Schino *et al.,* 1996; Das *et al.,* 1998; Bassett *et al.,* 2003), domestic goats, *Capra hircus* (Schino, 1998), or rats, *Rattus spp.* (Weiss *et al.,* 2004). Although some studies have linked performance of stress-related behaviour directly to other physiological indicators of stressors, such as increases in GCs, many specific species' behaviours have been identified to be related to perceived stressors through repeated and rigorous observational study, but without providing direct links to increases in GCs (Poole, 1999; Poole & Granli, 2009). Therefore, where a direct link between stress-related behaviour and increases in GCs in response to a stressor has not yet been established, other potential causes should be considered when interpreting stress-related behaviour. Further, without such validation it may be inappropriate to refer to those behaviours as stress-related. Additionally, aggressive behaviour, directed at human observers or conspecifics, has been suggested to be related to the causal link with anxiety, where the threat- or actual occurrence of aggression leads to animals being more anxious (Maréchal *et al*., 2011).

Provision of food by tourists to wild animals, for example, can increase conspecific-directed as well as human-directed aggression and stress-related behaviour in animals and can facilitate transmission of disease from humans to animals due to close contact or contamination of food with reverse zoonotic diseases (Orams, 2002; Moorhouse *et al.*, 2015). However, mere observation of wildlife by tourists has also been reported to cause changes in animals' behaviour, such as in aggression, foraging, resting, and parenting behaviours. Studies investigating how tourists viewing animals can affect animal behaviour have reported increased stress-related behaviour in animals (harp seals, *Phoca groenlandica* (Kovacs & Innes, 1990), Barbary macaques (Maréchal *et al.*, 2011), and Asian elephants, *Elephas maximus* (Ranaweerage *et al.*, 2015)), increased aggression towards tourists as well as conspecifics (Dusky damselfish, *Stegastes fuscus* (Benevides *et al.,* 2019), harp seals (Kovacs & Innes, 1990), sea lions, *Neophoca cinerea* (Osterrieder *et al.,* 2017), and Asian elephants (Ranaweerage *et al.*, 2015)), and increased vigilance of animals during tourist presence (pinnipeds (Kovacs & Innes, 1990; Osterrieder *et al.,* 2017), Indian rhinoceros, *Rhinoceros unicornis* (Lott & McCoy, 1995), black rhinoceros, *Diceros bicornis* (Muntifering *et al.,* 2018), bears, *Ursus spp.* (Dyck & Baydack, 2004; Penteriani *et al.,* 2017), and damselfish (Benevides *et al.,* 2019)).

Foraging and resting behaviour have also been shown to be affected by wildlife tourism, for example, animals spent less time foraging or resting, or were disturbed during hunts when tourists were around (dolphins, *Cetacea spp.* (Mann & Smuts, 1999; Lusseau & Higham, 2004), manta rays, *Mobula spp.* (Murray *et al.,* 2019), lions (Sindiyo &

Pertet 1984), and cheetah, *Acinonyx jubatus* (Roe *et al.*, 1997); (Orams, 2002)). Parental behaviour has been shown to be decreased during presence of tourists, in the form of nest or egg abandonment (birds (Green & Higginbottom, 2001), American alligators, *Alligator mississippiensis*, and Nile crocodiles, *Crocodylus niloticus* (Cott, 1969; Deitz & Hines, 1980)). Further, human activity has been shown to affect social bonds in animals, where high human activity, human infrastructure and traffic related to wildlife tourism was linked to weaker social bonds in spotted hyaenas, *Crocuta crocuta* (Belton *et al.,* 2018) and giraffes, *Giraffa camelopardalis* (Muller *et al.,* 2019).

Another specific type of behavioural response to wildlife tourism is movement and ranging behaviour. Spatial movement of animals can be in the form of immediate, short-term responses to tourist presence, which can be observed by researchers in the field. Alternatively, spatial movement can be related to long-term responses, where areas of habitat used change over months or even years, for example in African savannah elephants, *Loxodonta africana,* in Kenya (Ngene *et al.*, 2009), which can be difficult to study by direct observation. Short- and long-term changes in habitat use and spatial movement may be adaptive, as they may allow animals to remove themselves from a stressor or avoid it altogether. However, if they occur frequently, over prolonged periods of time, or pose a new stressor themselves (for example by pushing animals into less optimal habitats or by increasing the amount of time animals allocate to movement and thereby reducing feeding or resting time), those responses may become chronic (Fig. 1.2; Romero *et al.,* 2009).

Changes in habitat use by animals related to human presence have been reported in a range of species. Animals have been reported to increase travel speed and distance between themselves and tourists, or retreat in response to mere tourist presence (dolphins (Lusseau & Higham, 2004), mountain goats, *Oreamnos americanus* (Lott, 1992), boobies, *Sula spp.* (Burger & Gochfeld, 1993), Indian rhinoceros (Lott & McCoy, 1995), American bison, *Bison bison*, mule deer, *Odocoileus hemionus*, pronghorns, *Antilocapra americana* (Taylor & Knight, 2003), bears (Penteriani *et al.,* 2017), and various marine species (Burgin & Hardiman, 2015; Murray *et al.,* 2019; Benevides *et al.,* 2019)). Additionally, animals may identify areas within their home range where they can avoid the stressor and move into those areas either for short periods of time, or long-term (so-called refuge areas; Wingfield & Romenofsky, 1997).

Observations of common blackbirds, *Turdus merula*, showed that pedestrians in parks were related to flushing responses and movement away from pedestrians (Fernández-Juricic & Tellería, 2000). Human settlements or roads were avoided by African savannah and forest elephants, *L. cyclotis,* where researchers found that animals only visited areas during the night or avoided them completely, by only recording fresh elephant dung and signs of elephants foraging on vegetation in the morning, or where, by using tracking collars, researchers found that elephants avoided human settlements completely (Munshi-South *et al.*, 2008; Chase & Griffin, 2009; Orrick, 2018). Black howler monkeys have been shown to utilise habitat in higher canopy with increasing numbers of tourists present, assessed by researchers observing them (Treves & Brandon, 2005). Further, direct negative interactions between animals and humans, in the form of humans directing aggressive behaviour towards animals, such as chasing them or throwing rocks, have been shown to increase time spent moving in wild vervet monkeys, *Chlorocebus pygerythrus* (Thatcher *et al.*, 2019), which in turn may have negative consequences on time spent feeding or socialising.

Whether animals avoid humans, such as in the examples above, or are attracted to them can vary with other confounding factors. For example, bears, raccoons, *Procyon lotor*, skunks, *Mephitis*, and hyenas, *Hyaenidae spp.*, moved closer to human settlements, thought to be driven by access to food resources (Sindiyo & Pertet, 1984; Mattson, 1990; Gill, 2002).

1.5 Study species – elephants

There is a lack of research into effects of wildlife tourism on elephants. There are three species of elephants, Asian elephants, African savannah elephants, and African forest elephants (Blanc, 2008; Choudhury *et al.,* 2008; Roca *et al.,* 2015). Asian elephants occur in south- and southeast Asia, are decreasing in numbers, threatened with habitat loss, habitat fragmentation, and poaching and are classed as threatened (Choudhury *et al.,* 2008). They utilise shrub and grassland as well as forest habitats, with home ranges ranging from 30 km^2 to 600 km^2 and population estimates, although extremely crude, are around 40 000 - 50 000 individuals (Choudhury *et al.,* 2008). The African forest elephant occurs in forest areas in west and central Africa and is slightly smaller in size than Asian and African savannah elephants (Cardoso *et al.,* 2019). As African forest elephants have only recently been identified as a separate species and studies within their habitats are extremely difficult, exact population estimates do not exist, however, their population is thought to have declined by approximately 62% between 2002 – 2011 (Maisels *et al.,* 2013). African savannah elephants are the largest of all elephant species, occurring in east and southern Africa in a wide range of habitats, including wetland, forest, grassland, shrubland, savanna, marine and desert habitats (Blanc, 2008), with the most recent population estimate of approximately 352 271 individuals (Chase *et al.,* 2016) and home ranges varying widely (see section 1.5.7.1 below).

Average group sizes differ between the three species, with average numbers of 2.38 adult females in Asian elephants, 5.03 adult females in savannah elephants and between 1.48 and 2.7 adult females with dependants in forest elephants (see Table 2 in Nandini *et al.,* 2018). Further, some studies suggest that forest elephants have more fragmented social networks and fewer social associations compared to Asian or African savannah elephants (Schuttler *et al.,* 2014). The smaller group sizes have been suggested to be due to ecological constraints of forest habitats (Schuttler *et al.,* 2014), which is supported by relatively small group sizes in African savannah elephants occurring in the extreme desert environment of Namibia (Leggett *et al.,* 2003). Numerous studies have been published on Asian or African savannah elephants, and especially African savannah elephants have been studied continuously at long-term study sites, whilst only limited research has been conducted on the African forest elephant (Poulsen *et al.,* 2018).

Elephants are amongst the most popular species for wildlife viewing, particularly for international tourists, and have a high value for tourism (Lindsey *et al.*, 2007; Epps *et al.*, 2011). As discussed previously, wildlife tourism can be a widely used sustainable method to conserve natural habitats (see 1.1 in this Introduction) either through financial benefits towards anti-poaching efforts or upkeep of infrastructure, education of visitors, or socio-economic benefits. Given the large demand by tourists to observe wild elephants, it is surprising that effects of wildlife tourism on wild African elephant welfare and behaviour have not been studied (but see Ranaweerage *et al.,* 2015 for a study on wild Asian elephants). This thesis is focused on the African savannah elephant (henceforth elephant unless otherwise noted) and I will therefore present information most relevant to the study species in the following parts. As African savannah elephants have been well studied for more than 45 years, providing us with a detailed understanding about their behavioural ecology, they are a suitable species on which to investigate effects of wildlife tourism.

1.5.1 The African savannah elephant

1.5.1.1 Social system

African elephants live in complex social systems with close knit relationships that develop over long lifespans (McComb *et al.*, 2001; McComb *et al.*, 2011). In the wild, male elephants have lifespans of over 60 years (Hollister-Smith *et al.,* 2007) and females have lifespans of over 70 years (Moss, 2011; Lee *et al.,* 2012). First tier units are made up of mothers and daughters, and second tier units are made up of closely related individuals in core family groups (Moss, 2001). This is followed by third tier units, such as bond and kinship groups made up from separate core groups which have split apart from each other in the past but do associate with each other at times. Fourth tier units, such as clans, are several bond groups which associate with each other for short periods of time, fifth tier units are subpopulations, and ultimately sixth tier is the whole population (Moss & Poole, 1983; Wittemyer *et al.*, 2005). Elephants live in a fission-fusion society, as herds can fission when ecological constraints are high, or fuse when ecological conditions allow (Moss & Poole, 1983; Archie *et al.*, 2006a). However, second tier units are very robust and only fission under extreme circumstances and fission is further affected by the age of the herd‘s matriarch, where older matriarchs have been shown to lead larger herds (Wittemyer *et al.*, 2005). The fission-fusion societies of elephants are some of the most complex of all mammals (Wittemyer *et al.*, 2005) alongside dolphins*,* chimpanzees, *Pan spp.*, and humans, *Homo sapiens* (Vance *et al.*, 2009).

Female elephants live in herds with several related females and their immature offspring and display high levels of cooperation and affiliation with female kin, where, for example, calves are raised by their mother as well as other females in the herd (Moss &

Poole, 1983; Lee & Moss, 1986; Lee, 1987; McComb *et al.*, 2011). Herds are led by matriarchs, usually the oldest and largest female in the herd and, in populations undisturbed by poaching, females form a number of non-random, stable, and well-developed relationships with members of their herd (Wittemyer *et al.*, 2005). Although relationships between adult males have historically been thought to be relatively weak and random (Chiyo *et al.*, 2011; Moss & Poole, 1983), by taking into account varying sexual states, Goldenberg and colleagues (2014) were able to show that associations between bulls were far stronger than previously suggested. Elephants are not territorial (Douglas-Hamilton, 1972) and agonistic interactions occur over point resources such as waterholes and fruiting trees (Archie *et al.*, 2006a). The dominance relationships between different herds depend on the age of each herds‘ matriarch, making matriarch age an important factor (Wittemyer & Getz, 2007). This means that, for example, at a waterhole the herd of the oldest matriarch would be able to drink before another herd whose matriarch was younger.

1.5.1.2 Reproduction

Female elephants enter oestrus throughout the year, but oestrus only lasts for two to six days, a time during which males express high interest in females (Moss, 1983). The oestrous cycle of female African elephants lasts approximately 13.7 ± 2.3 weeks, with a luteal phase of 8.1 ± 2.3 weeks and a follicular phase of 5.6 ± 1.5 weeks (Fieβ *et al.*, 1999). On average, female elephants will conceive their first calf between the ages of 11 to 13 years (Moss, 2001). The gestation period of a female elephant is 22 months, followed by 12+ months of lactational anoestrus (Hodges *et al.*, 1994; Lee *et al.,* 2016), resulting in an inter-calving interval of approximately three to five years (Moss, 2001). Further, around week 30 of a pregnancy, females have a rapid increase in oestrogen levels for a duration of 30 – 35 weeks (Fieβ *et al*., 1999). In the wild, elephants breed throughout the year (Buss & Smith, 1966; Poole, 1994; Hufenus *et al.,* 2018), however, in areas in which primary productivity fluctuates strongly throughout the year, an increased number of conceptions and births have been observed to occur during the wet season (Rasmussen *et al.*, 2006; Wittemyer *et al*., 2007; Hufenus *et al.,* 2018). On average, female elephants will conceive their first calf between the ages of 11 to 13 years (Moss, 2001).

Adult males can weigh over six tonnes, twice the weight of an adult female (Owen-Smith, 1992). They come into musth, a state during which adult males are most sexually active, have elevated androgen levels, show increased aggression, and have increased mating success with females (Poole, 1989). In wild populations, males reach sexual maturity around 17 years but rarely start reproducing before the age of 30 partly because older, more mature bulls supress musth in younger bulls (Poole, 1994; Slotow & van Dyk*,* 2001). This means that a younger bull in musth may drop out of musth within minutes of encountering an older bull in musth (Slotow *et al.,* 2000; Y.Pretorius, *pers. comm.*) and thereby older and larger bulls dominate access to females in oestrus. Duration of musth increases with age, with younger males often only exhibiting signs (such as dribbling of urine and a strong scent) for a few days and with older males being in musth for up to several months (Poole, 1989). Although elephant dominance usually follows a clear pattern based on size, a bull in musth will temporarily outrank a larger male, if that male is not in musth (Poole, 1989).

1.5.1.3 Development

Calves nurse exclusively for the first three months of their lives, when they start to feed independently, but are dependent on milk for about two to three years and often continue to suckle for longer and remain in close proximity to their mothers until the age of six to eight years (Lee & Moss, 1986; Lee, 1987; Moss, 1996). Once calves are weaned, but before beginning to be reproductively active or, in the case of male elephants, leaving their natal herd, animals are considered juveniles (Moss, 1996). Upon reaching sexual maturity, around 15 – 17 years of age, males leave their natal herd and start associating with other adult bulls in bull groups, or roam solitary, whilst females remain in their natal herd (Moss & Poole, 1983; Poole, 1994; Moss, 2001). However, even though males may in theory be able to father young from such a young age, paternity is usually only observed from their mid-twenties, with a peak in paternity occurring between the ages 45-53 years (Hollister-Smith *et al.,* 2007). Young bulls have been shown to undergo an intense period of learning and development once they leave their natal herd and preferentially associate with older bulls, suggesting a so-called ‘shadowing effect‘ where young bulls learn from older ones (Evans & Harris, 2008; Goldenberg *et al.*, 2014). Social play is observed across all ages and sexes (Lee & Moss, 2014).

1.5.1.4 Communication

Elephants use physical, visual and auditory communication to maintain bonds (Charif *et al.*, 2005). They possess temporal glands (TG), situated between their ear and eye, from which they secrete when they are particularly distressed, aggressive, or excited (Buss *et al.*, 1976; Poole & Moss, 1981), during periods of high heat and, in case of bulls, during musth. Elephants also possess a vomeronasal organ in their mouth with which they can process chemical signals such as pheromones (Göbbel *et al.*, 2004; Ngwenya *et al.*, 2011).

Communication between individuals is highly vocal with so called rumbles, a low-frequency and harmonically rich vocalization, the majority of which are only in the infrasonic range, inaudible to humans, but some with frequencies in the infrasonic range and harmonics that extend well into the audible range of humans (Soltis, 2010). Vocalisations also come in form of snorts, trumpets and higher frequency calls, detectable by the human ear (Langbauer, 2000). The high amplitudes of elephant low-frequency rumbles travel along the earth‘s surface as well as through air (O'Connell-Rodwell *et al.* 2006; O'Connell-Rodwell, 2007; Gunther *et al.* 2004). Pacinian corpuscles, quick-adapting encapsulated primary mechanoreceptors which respond to mechanical deformation and vibratory stimuli, are found in elephant trunk tips, tongues, and feet (Bell *et al*., 1994; Rasmussen & Munger, 1996; Kubota, 2005; Bouley *et al*., 2007) and are thought to pick up vibrations of low-frequency rumbles. Long-distance contact calls are utilised to coordinate movements in time and space between individuals when they are out of sight (Poole *et al.,* 1988; Langbauer *et al*., 1991; McComb *et al.,* 2003).

Female elephants have been shown to be able to distinguish over 100 individuals by their contact rumbles (McComb *et al.,* 2000) and females in close social relationships are more likely to respond to rumble vocalisations of each other (Soltis *et al.,* 2005). Males utilize significantly less unique types of distinguishable vocalisations and vocalise less frequently compared to females (Poole, 1994) and are able to distinguish vocalizations of familiar females from unfamiliar ones, to which they will display longer attentive behaviour (Stoeger & Baotic, 2017). Both sexes have a variety of unique rumbles associated with specific circumstances such as making contact, maintaining group cohesion, or reproductive state.

1.5.1.5 Cognition

Having the largest cerebral cortex of all terestrial animals (Hart *et al.,* 2001), elephants are highly intelligent (Byrne *et al.,* 2009; Greco *et al*., 2013). They have been shown to be able to distinguish between human ethnic groups based on visual (colour of clothing) as well as acoustic (dialect) cues, and can distinguish human male and female voices as well as human adult and child voices (Bates *et al.,* 2007; McComb *et al*., 2014). They further exhibit social learning and possibly exhibit cultural transmission of knowledge between generations (McComb *et al.*, 2011), they have an excellent spatial memory (Polansky *et al.,* 2015), and are thought to have a concept understanding of death, investigating and carrying bones of conspecifics (Fayrer-Hosken *et al*., 1997; Goldenberg & Wittemyer, 2020). Further, there is evidence of self-recognition (Dale *et al.,* 2011) and recently, the potential for culture was suggested based on elephants sharing environmental components such as specific travel routes and exhibiting site fidelity over generations and thereby generating unique opportunities for exchange of information between different generations (Fishlock *et al.* 2016).

1.5.1.6 Distribution and population estimates

Historically, African elephants roamed vast areas, across country borders (Epps *et al.*, 2013). Today, elephants occur in 37 countries in sub-Saharan Africa, they are listed as vulnerable, and their habitat is becoming increasingly fragmented (Thouless *et al.*, 2016). Habitat encroachment by humans and poaching are fast decreasing their numbers (Bouche *et al*., 2011; Maisels *et al*., 2013). In 2003, over 80% of elephant habitat occurred outside of protected areas (Blanc *et al.*, 2003), whilst a continent-wide survey in 2016 (Chase *et al.,* 2016) suggested that 84% of elephants occurred inside protected areas. In 1979 the first Pan-Africa elephant survey reported approximately 1.3 million individuals (Douglas-Hamilton, 1979), in 1989, ten years later, this number had fallen to 600 000, and in 2016 a continent wide survey of savannah elephants provided an estimate of approximately 352 271 elephants (Chase *et al.,* 2016). A recent study found that, in a conservatively interpreted scenario where poaching was non-existent, approximately 967 000 individual African savannah elephants would exist (Robson *et al*., 2017).

In South Africa specifically, the most recent population estimate available lies at 26 168 individuals (Pretorius *et al.,* 2019). However, of the 78 discrete populations identified, 59% have been suggested not to be socially viable, meaning that there are fewer than 34 individuals, a number suggested by Pretorius and colleagues as the minimum requirement for elephants to have a normal social hierarchy to prevent maladaptive behaviour (Pretorius *et al.,* 2019). Further, they found that 77% of the populations were not genetically viable, with fewer than 100 individuals present, which is the minimum number of individuals required to keep inbreeding at 1% per generation (Pretorius *et al.,* 2019).

1.5.1.7 Home ranges

Elephants' seasonal ranges are influenced by energetic constraints, social factors and abundance and distribution of resources (Osborn, 2004; Cerling *et al.*, 2006; Wittemyer *et al.*, 2008). In unfenced areas with seasonal variation such as Kenya, Tanzania, Namibia, and Botswana, elephant home ranges are generally larger during the summer months, compared to the drier winter months, when they are restricted to areas close to water sources (Lindeque & Lindeque, 1991; Shannon *et al.*, 2006; Chase & Griffin, 2009; Purdon & van Aarde, 2017; Ngene *et al*., 2009). In fenced areas such as in South Africa, however, no consistent home range pattern based on season has been reported and home range size appears to depend on specific habitat characteristics (Shannon *et al.*, 2006; Orrick, 2018). It has been suggested that an abundance of water and forage is related to small home ranges in elephants. Additionally, elephant distribution is also thought to be influenced by human settlements (Chase & Griffin, 2009). In Kenya, elephants have shifted their home ranges away from areas of heavy poaching and towards areas with higher relative primary productivity (Goldenberg *et al.,* 2018).

Reported home range sizes, as well as methods to assess those, vary greatly (Osborn, 2004) and the details are provided with references for readers interested in how each study has calculated home range sizes specifically. Adult bulls roam either solitary or in bachelor herds, hence reported home ranges only apply to the specific bull, whilst female home ranges are assumed to apply to her core (first and second tier) breeding herd as breeding herds move as cohesive units. Osborn (2004) provides a list of studies which use 100% minimum convex polygons and report home range sizes for female elephants from various locations which range between 10 – 57 km^2 and 5 800 – 8 700 km^2, and male elephants which range between 32 – 60 km^2 and 1 300 – 2 981 km^2 (Table 2 in Osborn, 2004). Other, more recent studies report between 1 679 – 10 168 km^2 for individual male elephants in the Okavango Delta, Botswana (Evans *et al.*, 2013) and an average home range size of 1 537 km^2 (±446.6) for 13 individual elephants from separate herds in the Laikipia District, Kenya (Graham *et al.*, 2009). Using different methodologies, home range sizes of an average 1 690.5 km^2 (±2 660.9) for 17 female elephants from separate herds (95% autocorrelated kernel density estimation; Goldenberg *et al.,* 2018) have been reported in the Laikipa Plateau, Kenya, and a mean of 4 701 km^2 (±1 603) for nine individual elephants (five male and four female) from separate herds in the Gourma, Mali (localized convex hull; Wall *et al.*, 2013).

Home ranges of elephants in smaller, fenced areas appear to be significantly smaller as reported for Pongola Game Reserve, South Africa, where breeding herds and individual bulls ranged between 17.5 – 71.5 km^2 (95% kernel; Shannon *et al.*, 2006), and for Karongwe Private Game Reserve, South Africa, where breeding herds‘ and individual bull home ranges were approximately 27 and 19.5 km^2 on average, respectively (95%

isopleth; Orrick, 2018). Comparing home ranges of elephants in areas with annual rainfall ranges similar to those of South Africa, van Aarde and colleagues (2008) have shown that home ranges of elephants whose movement was not restricted were much larger (authors did not provide methodologies or locations). The range for cow-calf herds was approximately 300 – 2 100 km^2 in unfenced populations, compared to approximately 200 – 1 200 km^2 in fenced South African populations (Figure 2 in van Aarde *et al*., 2008).

1.6 Wildlife tourism impacts on African and Asian elephants

One area which has not been well studied across elephant species, is whether wildlife tourism represents a stressor to elephants. For conservation and population management to be succesful and effective, an understanding of the diverse and flexible interactions between humans and elephants is required (Mumby & Plotnik, 2018). In order to assess a factor as a potential stressor, a variety of measures can be assessed, including glucocorticoids and changes in behaviour (Fig. 1.2). As detailed research is available on how other factors (e.g. sex, season, age) affect GCs and behaviour in African elephants, this allows us to account for the effects of those factors, whilst investigating a factor on which information is lacking: wildlife tourism.

To my knowledge, no research has investigated the effect of non-consumptive wildlife tourism on African elephants. However, some direct tourist-elephant interactions, as well as the effects of other anthropogenic activities have been studied in African savannah, as well as in Asian elephants. As research is limited, I will continue to present information related to African as well as Asian elephants.

Several studies have assessed how factors related to other types of tourism or anthropogenic stressors may affect wild and captive African and Asian elephants' faecal

GC metabolite concentrations. Millspaugh and colleagues (2007) analysed fGCM concentrations of captive and wild African elephants in South Africa. By comparing fGCM concentrations of captive elephants to concentrations of free-ranging elephants sampled in the nearby Pilanesberg National Park they concluded that, within one to two months following transportation of captive elephants (where animals are transported between sites using large trucks), fGCMs of working elephants became indistinguishable from those of wild elephants. However, they only collected 10 – 15 samples per month between September of 2002 and September 2003 of wild elephants from unknown individuals and made this comparison based on assessment of data presented in their figures, rather than statistical assessment (Millspaugh *et al.*, 2007). Additionally, the authors collected samples of the five captive individuals over six 24-hour periods to compare days during which elephants interacted with humans in form of rides, to days without human interaction (Millspaugh *et al.,* 2007). They reported a statistically significant 1.2-fold increase in fGCMs of elephants on days of human interaction (fGCMs x=27.8 ng/g) compared to days without interaction (fGCMs x=22.9 ng/g; Millspaugh *et al.,* 2007). This suggests that direct interaction with humans may be a stressor to elephants. However, authors reported much higher fGCM concentrations associated with transport (170.8 ng/g and 103.5 ng/g) and loud noises such as thunderstorms, a concert and fireworks (93.6 ng/g, 77.3 ng/g, 76.4 ng/g; MIllspaugh *et al.,* 2007), indicating that human interaction was only a mild stressor. By contrast, recent research on captive Asian elephants has identified that individuals with more intense human contact, such as elephant-back riding, had, in fact, lower fGCM concentrations compared to individuals which were merely observed and fed by tourists (Norkaew *et al.,* 2019). The authors suggested that the high-calorie diets, fed by tourists, and a lack of movement contributed to the increased concentrations of fGCMs in Asian elephants, whilst increased activity in form of daily rides would decrease fGCMs of the individuals with more intense human contact (Norkaew *et al.,* 2019). Additionally, high calorie diets and low exercise were further related to other negative health implications such as high body condition scores (obesity; Norkaew *et al.,* 2019). In fact, exercise has been shown to decrease stress, anxiety, and depression (Coulter *et al.,* 2009). However, comparison of individuals between different sites is difficult and individual baselines for each elephant were not known in this study. Further, GC concentrations can be down-regulated following prolonged or chronic stress (see earlier in this chapter), and the lower concentrations of GCs in elephants used for rides could be the result of chronically perceived stress. Hence, no full conclusion can be drawn from these results. Nonetheless, overall, fGCMs were higher during the high tourist season in all Asian elephants studied (Norkaew *et al.,* 2019). Furthermore, the study did not compare the fGCM concentrations of Asian elephants that had no direct contact with humans at all (Norkaew *et al.,* 2019) and, therefore, it is possible that less disturbed Asian elephants exhibit lower fGCM concentrations compared to all individuals used in direct contact.

A study on elephants in Namibia found that fGCMs in 35 samples collected in unprotected areas were significantly higher compared to fGCMs in 56 samples collected within protected areas and suggested this to be due to human disturbance within the unprotected area (Hunninck *et al.*, 2017). Human disturbance occurred in form of high human presence, hunting of elephants, poaching and less controlled high-impact tourism, amongst others, compared to the protected national park (Hunninck *et al.*, 2017).

Aside from GCs, research has also assessed how anthropogenic stressors affect African and Asian elephants' behaviour. A behaviour used to identify stressors which has been linked to increases in GCs in wild African savannah elephants is swift movement away from a perceived threat such as tourist vehicles (Burke, 2005) or the location where a conspecific has been shot (Burke *et al.,* 2008). Further, increased GC concentrations have been linked to elephants restricting their space use to so-called refuge areas, with no- or limited human disturbance within their available habitat (Jachowski *et al.,* 2012).

Established elephant researchers have identified behaviours which indicate that elephants are alarmed or apprehensive. Those behaviours include elephants touching their own face, lips, or temporal glands with their trunks, swinging their foot, as well as elephants curling their trunk (Poole, 1999; Burke, 2005; Poole & Granli, 2009) and are here referred to as stress-related behaviours. However, no studies, to my knowledge, have validated those behaviours as indicating a perceived stressor by linking them to additional evidence of perceived stress, such as increased heart rate or GC concentrations. Despite this, researchers refer to these behaviours as stress-related and therefore, they have been included here.

To my knowledge, only one published study has assessed how wildlife tourism affects the behaviour of wild Asian elephants (Ranaweerage *et al.,* 2015). A study on 87 Asian elephants in the Udawalawe National Park, Sri Lanka, assessed the effect of tourism on elephant behaviour by comparing elephant behaviour during tourist presence and absence (Ranaweerage *et al.*, 2015). They recorded the frequency and duration of several elephant behaviours: alert (gaze fixed at tourist or guarded body posture), fear (running away), stress (ears flapping fast, tossing of soil, swaying of head, shoulders or whole body, circling), and aggressive behaviours (running towards or attacking tourist vehicles; Ranaweerage *et al.,* 2015). Tourist behaviour was recorded as being calm (no talking), loud (talking) or extreme (talking, clapping, waving, attempting to feed elephants or playing music). Loud or extreme tourist behaviour, close distance of game drive vehicles to elephants, loud vehicle noise, and time of day all increased fear, alert, and aggressive behaviours of elephants (Ranaweerage *et al.*, 2015). Furthermore, by comparing different group composition and sexes, the authors determined that male elephants performed more stress and aggressive behaviours in comparison to females, and solitary males were less affected by tourists compared to bull or breeding herds (Ranaweerage *et al.*, 2015).

Further behavioural effects in elephants related to tourists viewing them in Pilanesberg National Park, South Africa, were noted in an unpublished Masters thesis (Burke, 2005). Burke (2005) found that elephants in Pilanesberg National Park increased their stress-related and vigilance behaviours as distance between elephants and tourist vehicles decreased and with increasing numbers of vehicles present. Further, elephants became more responsive to tourist vehicles during periods of high tourist activity in the reserve, indicated by behaviours such as twisting their trunks, moving away or towards tourist vehicles, or displaying signs of aggression towards vehicles (Burke, 2005). Similarly, Pretorius (2004) in her Masters thesis, reported that tourist presence increased stress behaviours of elephants classed as avoidance, aggressive displays and increased mobility in form of faster and more frequent movement.

As research of wildlife tourism effects on wild elephant behaviour is scarce, assessing how other anthropogenic factors affect elephants may give some insight into possible effects of wildlife tourism. For example, McComb and colleagues (2014) found that herds of free-ranging elephants in Amboseli National Park performed investigative smelling, extending their trunks to gather olfactory information in response to human voices (McComb *et al.*, 2014). Tourists may elicit similar responses in elephants in areas where they accumulate to observe wildlife, such as at waterholes.

Where humans do occur within elephant habitats, human settlements and human activity causes elephants to alter drinking behaviour by utilising water sources away from humans during the wet season, increase speed of movement when traveling through unprotected areas, avoid main roads, or increasingly utilise areas closer to humans at night rather than during the day (Lewis, 1986; Hoare & Du Toit, 1999; Wittemyer *et al.*, 2005; Jackson *et al.*, 2008; Graham *et al.*, 2009; Roever *et al.,* 2013; Gaynor *et al.,* 2019). Those studies suggest that an increase in tourist presence in the form of accommodation (associated with wildlife tourism) or immediate presence of tourists may induce changes in elephants‘ spatial behaviour, even at a very fine scale by altering spatial behaviour within a 24 hour period.

1.7 Aims and objectives

I aimed to investigate the impact of wildlife tourism on African savannah elephants. If elephants perceived wildlife tourism as a stressor, elephants should develop coping mechanisms (Fig. 1.1; Fig. 1.4). Wildlife tourism could thereby affect elephant GC concentrations and/ or behaviour (Fig. 1.4). Perceiving wildlife tourism as a stressor could also affect elephant spatial behaviour, where elephants move away from tourists or alter ranging behaviour during times of high tourist pressure (Fig. 1.4).

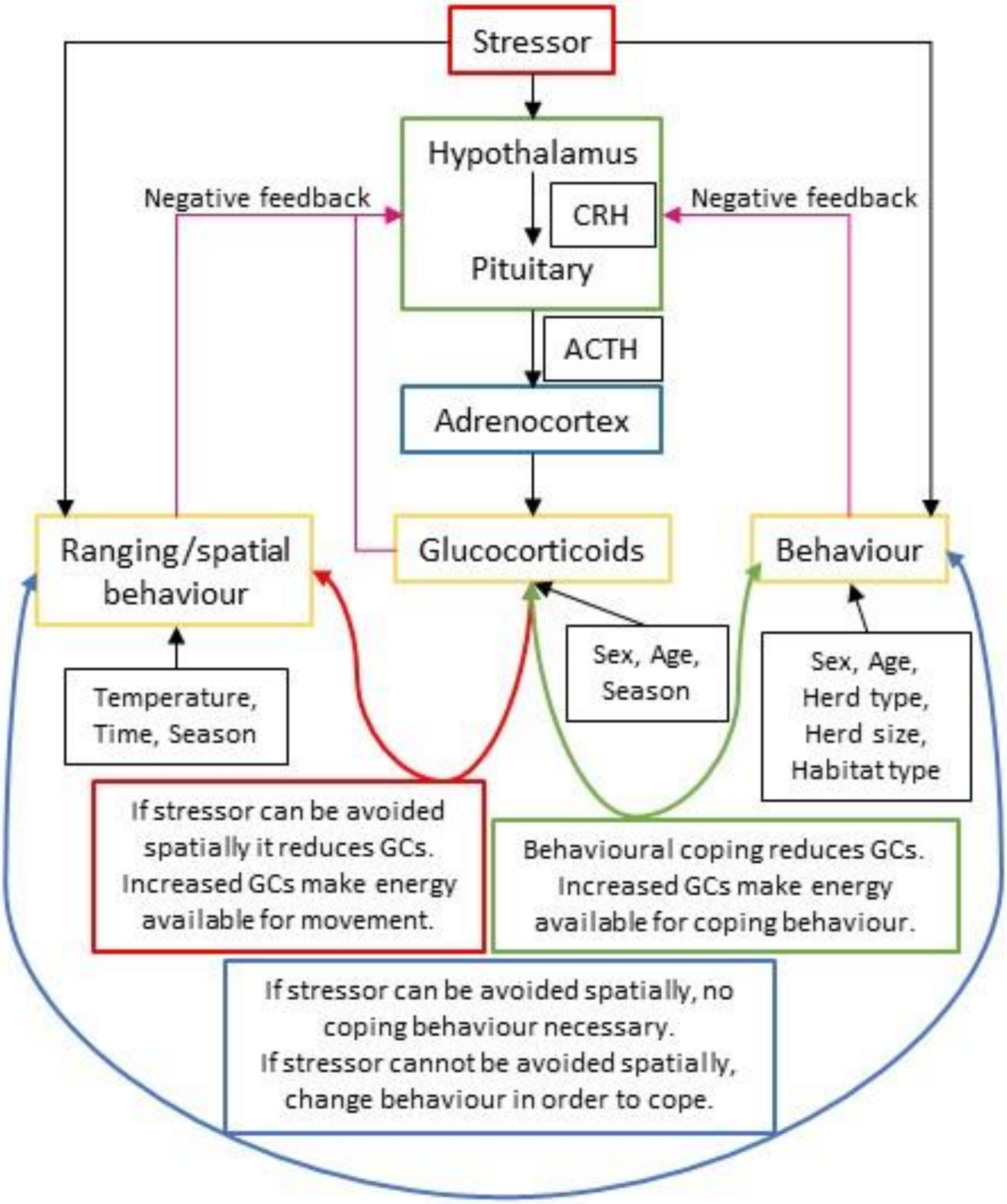

Figure 1.4

A schematic based on Figure 1.2 in this chapter of short-term changes in animals' physiology and behaviour in response to a stressor. The stressor's disruption of predictive homeostasis leads to increases in physiological mediators in order to allow an individual to cope. Increased concentrations of glucocorticoids (GCs) make energy available for additional coping behaviours or movement. In return, such coping behaviours and movement re-establish predictive homeostasis. For example, if an individual is able to avoid the stressor spatially, no coping behaviour or increase in GCs may be necessary, however, this may require increased vigilance behaviour in order to avoid the stressor (which in turn may reduce time available for other essential behaviours, potentially acting as a stressor itself). If the individual was unable to avoid the stressor spatially, it may result in changes in other behaviours (such as aggressive behaviour) as coping mechanisms. Further, the individual may move away from the stressor, when encountering it. Additionally, other extrinsic and intrinsic factors such as age, sex, season, and temperature can affect behaviour, GC concentrations, and ranging behaviour. If the stressor is encountered and coping mechanisms (release of GCs or behaviour) result in the individual being removed from the perceived stressor, the effect is adaptive and serves to re-establish predictive homeostasis. Figure partly adapted from Romero, 2004.

Because an increase in physiological mediators or changes in behaviour can indicate whether something is perceived as a stressor or not, I formed the following three main objectives.

1: To investigate the effect of wildlife tourism on faecal glucocorticoid metabolites in elephants.

If elephants perceived high tourist pressure (total number of tourists within the reserve each month) as a stressor, it would lead to an increase in their glucocorticoid concentrations as a physiological mediator response in order to cope with the stressor.

2: To investigate the effect of wildlife tourism on elephant behaviour.

If elephants perceived high tourist pressure (total number of tourists within the reserve each month) as a stressor it would lead to changes in behaviour in order to cope. This may result in increased vigilance behaviour to avoid the stressor, and/ or increased stress-related and conspecific-directed aggressive behaviour as a coping mechanism. This may be an indirect effect of perceived pressure of tourists in the reserve. Further, if elephants perceived tourists as a stressor, it may result in an increased likelihood of moving away from tourists observing them as a coping mechanism.

3: To investigate the effect of wildlife tourism on the ranging behaviour of elephants.

If tourists were a stressor, elephants may aim to avoid them in order to cope. Therefore, elephants may alter their ranging behaviour during high tourist pressure (total number of tourists within the reserve each month) throughout the year or throughout the day. Elephants could alter how far they move during months with higher tourist numbers, compared to months with lower tourist numbers in the reserve, and change fine-scale movement during the day in order to reduce the risk of encountering tourists.

1.8 Thesis structure

This first chapter presented a broad overview into how a stressor can be identified, wildlife tourism as a potential stressor to wild animals, and why this may be a problem. Furthermore, I identified a gap in our knowledge about how wildlife tourism may affect African elephants. In Chapter 2, I provide information about the study site and data collection methods. The following data chapters (Chapter 3 – 5) investigate the effect of total number of tourists within the reserve each month (henceforth tourist pressure), on fGCMs, behaviour, and movement of elephants whilst controlling for a range of other factors in their analyses (see Fig. 1.4).

Studying different levels of organisation allows us to understand ‘the bigger picture’ and assess whether a factor is, in fact, a stressor (Koolhaas *et al.,* 2011). The underlying physiology does not predict how an individual will behave but knowledge of the mechanism can inform our understanding of behaviour and vice versa (Martin & Bateson, 2007). As seen in the scheme presented in Figure 1.4, these measures are interconnected as hormones can affect behaviour, and behaviour can affect hormones. In order to study a stressor, multiple measures are required (Sevi, 2009). Further, each measure is affected by various things such as environmental (e.g. temperature, season) or life history (e.g. age, sex) factors, which will be discussed in each respective chapter.

As mentioned in Romero *et al.*'s paper (2009, Table 1, p. 379), the scheme in Fig. 1.4 incorporates different physiological systems in the form of hormones as well as behaviour. The hormone measure included in this thesis is GCs, which, as discussed in this chapter, make energy available for movement and changes in behaviour (Romero *et al.*, 2009). The behaviour measures included in this thesis relate to locomotion, vigilance, and aggressive behaviour, as well as behaviours that have been previously used to indicate perceived stressors, here referred to as stress-related behaviours (Poole, 1999; Poole & Granli, 2009).

In Chapter 3, I investigated the effect of tourist pressure on fGCMs from repeated samples collected from a set of individually identified elephants. Following this, I examined whether another set of individually identified elephants (mostly different to those in Chapter 3) repeatedly performed stress-related, vigilance, and conspecific-directed aggressive behaviour when tourist pressure increased, whilst controlling for a variety of factors known to potentially affect those behaviours (see Chapter 4). Further, Chapter 4 details findings of elephant herds' movement in relation to the immediate presence of game drive vehicles used by tourists to observe elephants. As I found increased fGCM concentrations and increased conspecific-directed aggressive behaviour in response to wildlife tourism in Chapters 3 and 4, in Chapter 5 I analysed factors affecting spatial use in three adult female elephants fitted with satellite collars, using monthly 95% isopleth home range sizes (areas containing 95% of all locations recorded by the collars) and mean hourly journey length, to analyse spatial patterns. As opportunistic sampling led to different samples of individuals in Chapters 3, 4, and 5, a comparative analysis of fGCMs, behaviour, and spatial patterns was not possible.

Lastly, in Chapter 6, I discuss wildlife tourism effects on elephants, placing these findings into a broader context for elephants across Africa and make recommendations for future research, as well as for local management. The four data chapters (Chapter 3 – 5) are formatted as journal articles, either published or in preparation, and so there will be some minor repetition across their introductions and methodologies. However, I have taken great care in re-phrasing parts of those chapters which are repeated to avoid plagiarism between chapters. Further, those chapters, because they are in the format of scientific publications, contain less background information or detail in their introductions and only discuss results related to each chapter. Readers should refer to this introduction or later discussion (see Chapter 6) for more detailed information and an overarching discussion of results. I have added links to chapters in the text to facilitate cross-referencing.

Chapter 2 - General Methodology

This chapter provides some background information on the study site and describes some general methods by which data were collected and analysed. Detailed statistical analysis will not be described in this chapter as this is specific to each chapter and therefore presented there, however, I present basic information on statistical methods that is relevant to all data chapters.

2.1 Study site

I collected data in the approximately 680 km^2 large Madikwe Game Reserve (hereafter also referred to as Madikwe), North West Province, South Africa (24°47’45.5712’’ south, 26°18’4.1688’’ east; Fig. 2.1). Madikwe was founded in 1991 in order to bring economic growth to the area and is run by a state/private/communal partnership. At the point of study, there were 33 lodges within Madikwe. Lodges conducted tourist activities in the form of game drives and guided walks and some lodges held concessions within the reserve to which only they had access (orange areas, Fig. 2.2) or could control access (pink areas, Fig. 2.2). The whole reserve, with the exception for the orange concessions was available for game drives and walks for all lodges. Only three game drive vehicles were permitted to conduct game drives within pink concessions at a time unless permission was granted otherwise (Fig. 2.2). This meant that orange concessions were almost exclusively utilised by the lodges within them, whilst any game drive vehicle had access to the pink concession following permission from the lodge controlling that concession. The number of tourists in Madikwe was limited as only guests staying at one of the lodges were allowed to enter the reserve.

Figure 2.1

Map of the southern end of Africa, showing South Africa and the location of Madikwe Game Reserve, indicated by a large red dot. Map data ©2019 AfriGIS (Pty) Ltd, Google.

The first elephants introduced to Madikwe in 1992, were 25 orphaned elephants from Kruger National Park, between 8 – 12 years old (Hofmeyr *et al.*, 2003), following culling operations (management operations during which adults in a herd were killed). In 1993, Madikwe then introduced 194 elephants from Zimbabwe containing entire breeding herds including calves as well as individuals ≥ 50 years of age (Hofmeyr *et al.*, 2003). No further information on sex, age, or numbers of herds concerning those 194 elephants was available. These animals had experienced a severe drought, two bush wars, and heavy poaching in Zimbabwe. In 1998 six adult bulls and in 1999 two adult bulls from Kruger National Park were introduced (all standing ≥ 3.2 m tall and therefore classed as adults; Hofmeyr *et al.*, 2003). The only elephants translocated out of Madikwe were 16 individuals to Quicama Park in Angola in 2000, and 29 individuals to private game reserves in South Africa in 2001 (12 to Sandhurst, Tosca, eight to Bayete, Eastern Cape, nine to Kwandwe, Eastern Cape; Scholes & Mennell, 2008; Hofmeyr *et al.*, 2003). Again, no further information on age, sex, or numbers of herds containing these individuals was available. In July 2017, Madikwe contained an estimated 1348 ± 128 elephants (July 2017, North West Parks Board, P. Nel, *pers.comm*.), representing one of the highest population densities (1.9 elephants per km^2) in South Africa. For comparison, the nearby Pilanesberg National Park reported 240 elephants in 2016 (Clark, 2016) bringing it to a density of 0.42 elephants per km^2, whilst Addo Elephant National Park has an estimated density of 0.37 elephants per km^2 (*Mammals,* 2020). Further population densities reported in 2008 can be found in Scholes and Mennell (2008), Chapter 2, Table 4 (pp. 111-112).

Madikwe was split into three main areas: east, west and south (Fig. 2.2), defined as such by field guides and staff in Madikwe. Private concessions (see Fig. 2.2) were not included in this study although all animals were free to roam between concessions and the main reserve. Although Figure 2.2 shows most roads, a few additional roads did exist whilst a few others shown were inaccessible (marked as crossed out pink lines in Fig. 2.2).

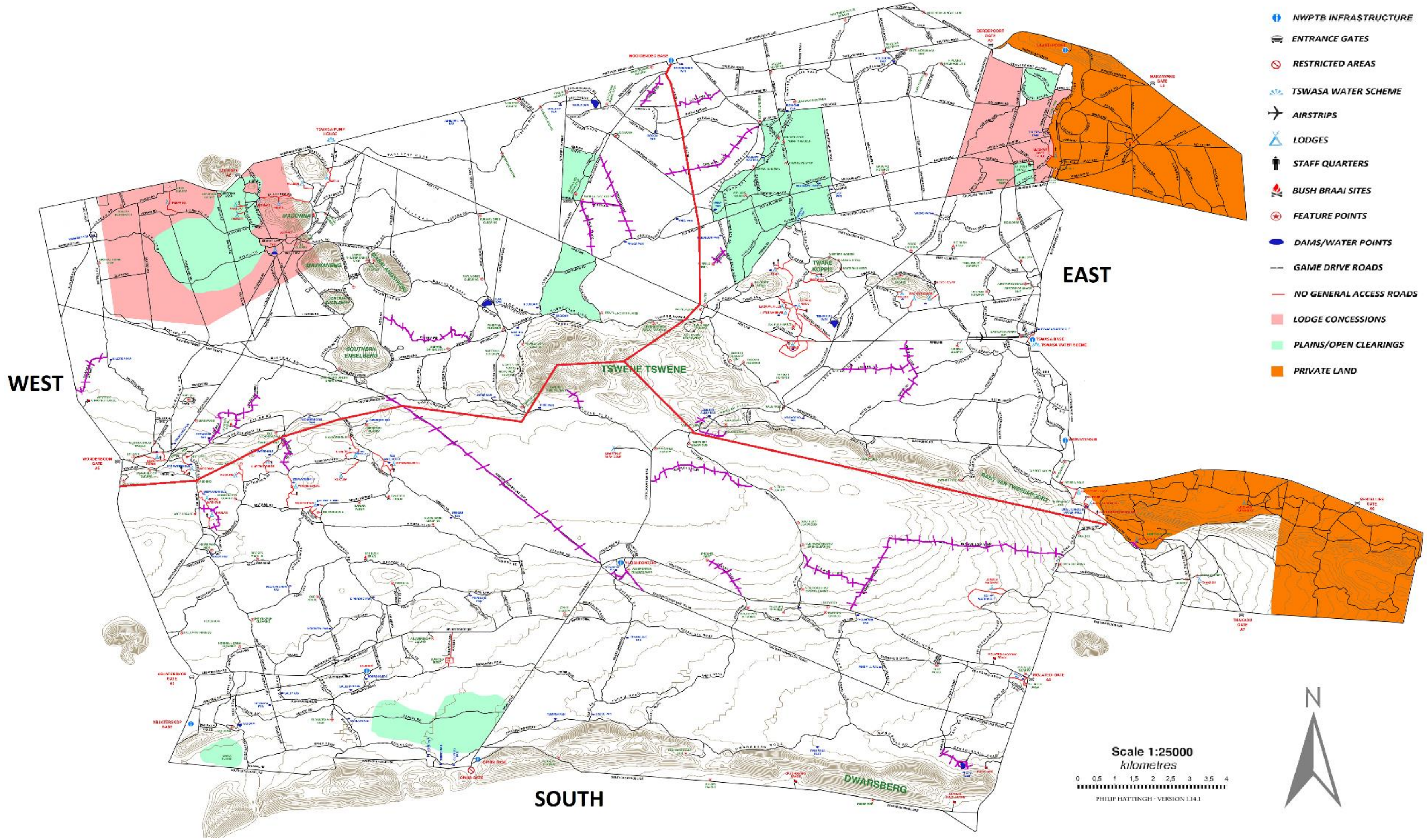

Figure 2.2 Map of Madikwe Game Reserve, South Africa, in 2014. Orange areas are private concessions, pink areas are private concessions used with lodge permission, and green areas are open plains where off-roading was prohibited. Black and red lines are roads. Game drives and research vehicles could only use black lines. Crossed out pink lines are roads no longer accessible with a research vehicle. East, west and south areas are indicated with the thicker red line. Map courtesy of P. Hattingh (2014) and reproduced with his permission.

2.2 Game drive protocols and regulations

Lodges situated in the reserve conducted game drives in the morning, from sunrise until approximately 10/11 am, and in the afternoon, approximately from 3:30/4 pm until 7/8 pm. However, depending on demand, lodges occasionally conducted game drives during the day, between 10/11 am and 3:30/4 pm. Each game drive included a drinks stop at a randomly chosen location anywhere in the reserve, during which guests disembarked from the vehicle for any duration but usually lasting approximately 20 minutes. A game drive vehicle (GD) was a large open vehicle, seating up to ten people as well as a spotter at the front of the vehicle. Guests were briefed not to stand up at any time, not to make loud noises and not to use camera or torch flashlight. Furthermore, eating, drinking (other than water), and smoking were strictly forbidden during game drives with the exception of drinks stops. Offroading was only permitted to visit high-value sightings such as big cats, buffalo, *Syncerus caffer*, or rare sightings such as smaller felines or honeybadgers, *Melivora capensis*. Offroading was only allowed up to 300 m off the road, had to be cleared with a park official, and was not utilised for the sighting of elephants.

The Code of Conduct of Madikwe Game Reserve set out rules for guides and researchers. It was forbidden to call to attract animals‘ attention, and all animals were regarded as potentially dangerous and had to be approached with caution. At a sighting, no more than three GDs were allowed and at a sighting along the fenceline, no more than two. The research vehicle was excluded from the count of GD vehicles. Any vehicle at a sighting had to be positioned with clear access to an escape route, before the engine was turned off. Only one engine was allowed to be running at a time (unless exceptional circumstances called for all vehicles to move at the same time). Further, vehicles were not supposed to position themselves between individuals of an elephant herd. When driving in Madikwe, the speed limit was 40 km/h on all main roads and 30 km/h on smaller roads.

2.3 Research vehicle protocol

As Madikwe's lodges offer high-end safari tourism with a high level of exclusive offers such as private game drives, as well as game drives often occurring within the surrounding area of a specific lodge, I could not join guests on those game drives. Further, this would have highly restricted my ability to collect behavioural observations of elephants, as GDs would not specifically locate elephant herds and often spent less than ten minutes with elephants (I. Szott, *pers.obs*.). Instead, research was carried out from a white Isuzu single-cab 4x4 pick-up from the 18th April 2016 until the 28th June 2017.

Upon spotting an elephant from the road, I scanned the surrounding area for additional animals and stopped the vehicle at a minimum distance of 30 m from the closest animal. If I only spotted an elephant once it was already closer than 30 m to the vehicle, I reversed the vehicle slowly to a safe distance of 30 m. In all cases I ensured that there were no additional animals immediately behind the research vehicle. If animals remained in proximity or approached the vehicle without displaying obvious signs of distress (stress-related or aggressive behaviour, see Table 2.3 below for details), the research vehicle remained in its current position. I allowed animals to approach the research vehicle as close as they chose to, if I did not detect signs of stress-related or aggressive behaviour. However, I did not allow any animal to touch the research vehicle and either raised my voice in order to deter them, or moved the research vehicle. If an animal directed his or her attention towards the research vehicle and displayed signs of stress-related or aggressive behaviour, the research vehicle was moved to increase the distance from the animal to 50 m.

The research vehicle engine was switched off as much as possible, for example when elephants did not display signs of distress upon first approach, after I had reversed to a distance of 30 m, or whilst they approached me. If a herd moved away from the research vehicle, but remained in vicinity of the road, I would follow at a distance that did not lead to behavioural signs of disturbance (e.g. animals turning around to face the research vehicle; Moss, 1996) and then switched off the engine again. Due to animals occasionally approaching the vehicle from behind vegetation from where I had been observing them with the engine switched off, I would speak in a calm voice in order to make my presence and location known to them in case they did not see me. Before starting the engine with animals in close vicinity, I would speak to increase the noise level more gradually and make animals aware of my presence rather than just starting the research vehicle engine. Speaking to elephants as they approached me from behind vegetation or before starting my engine was recommended to me by Dr Y. Pretorius.

2.4 Data collection schedule and selection of study area

Observations took place in the morning (sunrise – 11 am), during the day (11 am – 4 pm), or the afternoon (4 pm – sunset). Data collection was carried out in the three above mentioned areas (east, south, west). No data were collected at night. I collected data six days a week (rotating days off) for three weeks per month and rotated areas and time slots as above in order to collect data in all areas during all time slots and all days. For example, I would collect data during one week follwing a schedule of: day one (east/morning, west/afternon), day two (south/day), day three (west/morning, south/afteroon), day four (east/day), day five (south/morning, east/afternoon), day six (west/day). This would result in a total of approximately 39 hours of data collection in total (13 hours in each area) across those days, depending on time of sunset and sunrise. An area was sampled from the roads by driving unplanned routes (meaning I did not plan routes before but decided where to go once I was within an area based on prioritising areas not sampled for a prolonged period and reports of elephant sightings from guides), as well as communicating with field guides about elephant presence. As I had no other means of sampling an area, such as on foot accompanied by a ranger for safety, this was the only way of collecting data and limited my sampling to elephants encountered in the vicinity of roads. If areas were inaccessible due to bad road conditions (e.g. following heavy rain) or management operations, another area was sampled. Additionally, if elephants were not encountered within three hours spent in an area, I moved to another. In the fourth week of each month I transported dung samples for storage at the University of Pretoria.

2.5 Dung sample collection

I collected faecal samples of elephants upon watching an elephant defacate (mean ± SD between defecation and collection= 16 ± 12mins). Due to safety issues, I could only collect samples close to the road or in easily accessible areas where I could drive closer to the sample. The sample was collected wearing gloves by breaking up a minimum of three boluses and taking matter from the inside as to avoid contamination with the ground or urine. Upon sample collection, I recorded date, age, and sex, as well as time of defecation and collection, and longitude and latitude (using a Lenovo TAB 2 A8-50F). Additionally, I measured the length and diameter (in centimetres) of the bolus and assigned each sample a unique reference number. The matter was stored in commercial zip-lock bags, in which it was further homogenised by breaking and mixing it manually (Fig. 2.3). Each sample was transported in a cooler box containing ice blocks for no longer than four hours before storage in a freezer (-18 °C). Before storage in the freezer, samples were transfered to small vials holding approximately 50 g of faecal matter (Fig. 2.3). Those vials were transfered to the Endocrine Research Laboratory, University of Pretoria, South Africa, in a cooler box with several ice blocks.

Figure 2.3
Collection of African elephant, *Loxodonta africana*, faecal samples in the field in Madikwe Game Reserve. Faecal matter from a minimum of three boluses was homogenised in zip-lock bags before storage in a cooler box. Samples were then transferred into vials before being stored at -18 °Celsius.

2.6 Steroid extraction

All hormone extraction was carried out by experienced endocrine scientists at the Endocrine Research Laboratory, University of Pretoria, South Africa. Vials containing faecal matter were lyophilized at -50 °C using a laboratory freeze dryer (Alpha 1-2 LD plus, Christ) and then pulverized. The matter was sieved through mesh to remove any undigested faecal matter. Approximately 0.050 - 0.055 g of the remaining powder was extracted with 3 ml 80% ethanol in water, and the mixture was vortexed for 15 minutes, centrifuged for 10 minutes at 1500 g, and transferred to a microcentifuge tube for hormone analysis.

All of the following was carried out using a regular refridgerator (approximately 4 °C) for incubation, a Titramax 100 (Heidolph) plate shaker and an Elx800 reader (Biotek). Immunoreactive glucocorticoid metabolite (GCM) in diluted extracts (1:10 or 1:50 in aqueous buffer) was analysed using an 11-oxoetiocholanolone enzyme immunoassay (EIA; Möstl *et al.*, 2002). This EIA detects GCM with a 3α-hydroxy-11-oxo-structure, has been previously validated and has been repeatedly used to monitor adrenocortical activity in elephants (Ganswindt *et al.*, 2003; Ganswindt *et al*., 2005). 50 µL aliquots of standards (range = 0.98 - 250 pg/ml), quality control, and diluted faecal extracts were pipetted, in duplicate, into microtiterplate wells. To that, 50 µL of biotinylated 3α,11-oxo-CM label and antiserum (raised in a rabbit against 5β-Androstane-3α-ol-11-one-17-CMO) was added, and the plates were incubated in the dark in a refridgerator at 4 °C over night. The following day, plates were washed four times with a phosphate buffered solution made up of the following stock solution: 0.399 g NaCI (molecular weight (MW) = 58.44 g/mol), 5.752 g Na2HPO4 (MW = 141.96 g/mol), 1.005 g KCI (MW = 74.56 g/mol), 1.025 g KH2PO4 (MW = 136.09 g/mol), 1L H2O (RO) added to the final volume and the pH adjusted to 7.2. The washing solution was then made up from 1.92 L H_2O, 80 ml of the stock solution, and 1 ml Tween 20. Then, 150 µL (20 ng) of streptavidin-peroxidase was added to each well. Plates were incubated in the dark for 30 minutes, washed again using the same washing solution as before and, after adding 150 µL TMB (3,3‘,5,‘-Tetramethylbenzidine) substrate solution, incubated for a further 30 – 60 minutes. By adding 50 µL of 4N H_2SO_4 the reaction was terminated and the absorbance was measured at 450 nm. Serial dilutions of faecal extracts gave displacement curves that were parallel to the respective standard curve. Sensitivity of the assay at 90% binding was 1.2 ng/g dry faecal mass. Intra-assay variance, determined by repeated measurements of high and low-value controls ranged from 3.3% - 5.6% and inter-assay variance, determined by repeated measurements of high and low-value controls ranged from 9.5% - 12.3%.

2.7 Behaviour observations

Data were collected on a Lenovo TAB 2 A8-50F tablet using the Prim8 app (McDonald & Johnson, 2014). I noted herd composition, sex, and age of the focal individual. Herd composition was either a lone male (no other individuals visible within 500 m), a bull group (only adult males within 200 m of each other), a cow-calf group (adult females, juveniles and calves within 100 m of each other and no adult bulls within 500 m), or a mixed group (adult males within 500 m of a cow-calf group). Elephants were classed as calf (0 – 3 years of age), juvenile (4 – 12 years of age) or adult (13 years or older) and further a male was classed as an adult if he was encountered in a bull group or solitary, whilst a female was classed as an adult if she was seen with a dependent calf (Moss, 1983; Archie *et al.,* 2007; Y. Pretorius *pers.comm*.). Sexing calves was not always possible, therefore I did not always record calf sex. Females were identified based on presence of mammary glands, as well as an angled forehead, whilst males have a rounded forehead, no mamary glands, and wider skulls (Moss, 1996). I received two weeks of training in ageing and sexing elephants at the beginning of my study by Dr Y. Pretorius. However, due to a lack of previous experience I was unable to reliably distinguish between juvenile and sub-adult elephants. Therefore, I classed elephants in one of three age categories mentioned before, resulting in the most reliable method of aging elephants. Further factors to aid classification are listed in Table 2.1, taken from Moss (1996), Stoeger *et al.*, (2014), and Poole & Granli (2009). Previous research has shown that sex-specific size characteristics do not show large variation between geographical regions such as South Africa and Kenya, and can therefore be applied to African elephants in general (Shrader *et al.,* 2006; Trimble *et al.,* 2011).

Table 2.1. Factors used to aid identification of age and sex of African elephants, *Loxodonta africana*, in Madikwe Game Reserve.

Sex	Age	Indicators
Male	Adult	Twice the size of adult females A 17 year old male is as large as a 50 year old female Larger head, thicker tusks than juveniles and line from eyes to tusks begins to curve to hourglass contour
	Juvenile	Tusks longer than 10 cm Line from eyes to tusks is straight
Female	Adult	Large, more angled forehead Thicker tusks than juveniles Longer, more elongated body compared to males or juveniles Mammary glands
	Juvenile	Tusks longer than 10 cm Smaller size than adult female
Calf		Under one year of age: no tusks, fits under belly of large adult female, regularly suckling Under 18 months: no tusks, does not fit under large female 18 months to 2 years: tusks appear just beyond lip 2 – 3 years: tusks ~8 cm beyond lip 4 years: tusks ~10 cm

Each time I collected data, I chose a focal elephant based on visibility. If several individuals were equally visible, I observed the one closest to me first. Once a focal observation had finished, the next focal observation was carried out on the next best visible elephant. The focal elephant was observed using five-minute continuous sampling (Altmann, 1974). Simultaneously, an instantaneous group scan sample of the whole (or visible) herd was carried out every 30 seconds. The prim8 app was used for focal observations and scan behaviour was noted verbally on an Olympus Digital Voice Recorder VN-510. This scan sample noted the behaviour performed by the majority (75% or more) of all individuals in the herd at that time and was easy to assess and collect simultanously. Further, as the focal animal observation was collected on the tablet whilst I made note of scan samples verbally, this did not require me to change the equipment I was using. However, in the rare event that a focal animal observation prevented me from observing the herd behaviour, priority was given to collecting reliable focal observations. If a lone bull was observed, his behaviour was also the scan behaviour. I chose five minutes based on preliminary data collection in Madikwe, as sometimes individuals would move away within a short time of approximately 10 – 15 minutes, making longer observations more difficult. Only observations where the elephant or herd was visible for at least 50% of the five minute observation were retained for later analysis. For each five-minute focal, I collected a range of other factors such as date, herd size or whether game drive vehicles were present (Table 2.2). In the case where a GD, car, or other species arrived/left during an observation, it was coded on the app, producing an exact timestamp for time of the arrival/departure and duration of its presence.

Table 2.2. Factors recorded for five-minute focal observation of African elephants, *Loxodonta africana*, in Madikwe Game Reserve, South Africa.

Factor	Measurement	Description
Date	Day/ Month/ Year	Date of observation
Time	Hour/ Minute/ Second	Start time of observation
Location	Latitude/ Longitude	Location of observer
ID (if known) or age and sex	ID or calf/ juvenile/ adult and female/ male	ID of individual if known (see section 2.9) or age and sex as described table 2.1
Travel direction	Towards, crossing, away or parallel	Direction in which the focal herd moved during the observation in relation to the observer or, if present, game drive vehicles as assessed from the location of the herd at the beginning- compared to the end of the observation. If herds moved ten meters or more away from the observer or any present game drive vehicle (without approaching another vehicle present), it was classed as 'away', all other categories were later classed as 'stay'
Herd size	Count	Number of elephants in the herd in which the focal animal was observed
Habitat type	Open grassland, dense shrub, shrub, waterhole	Type of habitat in which the focal animal was observed. Open grassland: area vastly open with only occasional bushes or trees; dense shrub: shrub and trees in observed area, growing so densely that observation was only possible at close distance and dense enough to obstruct view of large areas of the body of the focal animal; shrub: various bushes and trees in area but not obscuring observation noticeably; waterhole: water accumulated either naturally or pumped artificially with enough water for one or more elephants to drink
Game drive vehicle presence	Count	Number of game drive vehicles and number of persons on those vehicles in total

The ethogram used for focal observations (Table 2.3) was designed using previously published ethograms (Langbauer, 2000; Horback *et al.*, 2012; McComb *et al.*, 2014; Doughty *et al.*, 2014; Hasenjager & Bergl, 2015; Asher *et al.*, 2015), and refined during an initial trial period in Madikwe between February and April 2016 and following personal communication with Dr Y. Pretorius. Scan sampling included any of the behaviours noted with an asterisk in Table 2.3. Behaviours were recorded as either state behaviours or events. During behavioural observations note of any errors during data entry or other factors that may have been relevant to the behavioural observation were noted on the dictaphone.

Table 2.3. Behavioural ethogram used in this study for five-minute focal observations of African elephants, *Loxodonta africana*, in Madikwe Game Reserve, South Africa. Abbreviation coding for the behaviour, description and whether it was recorded as a state or event. Behaviours which were recorded as scan behaviours of the entire elephant herd parallel to the five minute focal, are noted by an asterisk. Behaviours were classed as stress-related, aggressive or vigilance for later analysis based on relevant literature and feedback from elephant experts (provided below the table; Poole, 1999; Burke, 2005; Poole & Granli, 2009; Y. Pretorius, *pers.comm.*).

Behaviour	**Code**	**Description**	**State/Event**	**Class**
Bunch*[2,6]	bu	Animals stand close together with younger individuals closer to the core, facing outwards. Often this occurs suddenly, with a large number of animals moving together at once, decreasing the herd diameter, whilst scanning surroundings.	State	Stress
Charge[3,4,5,6] animal*	ca	Fast walk, often with ears out and head held high, towards an animal. Can be accompanied by a trumpet or an abrupt head shake.	State	Aggression
Charge[3,4,5,6] human*	ch	Fast walk, often with ears out and head held high, towards a human (usually in a vehicle). Can be accompanied by a trumpet or an abrupt head shake.	State	Aggression
Displace given[4,5,6]	dg	Focal animal is approaching a conspecific which leaves the currently occupied spot either just upon the approach or after being pushed out of its position. Focal animal then takes the occupied spot.	Event	Aggression
Ears flapping[6]	ef	Focal animal is moving ears in and out resulting in a loud noise when the ears hit the body. Can happen during locomotion or as part of a charge (then classed as charge rather than ears flapping).	Event	Aggression
Ears out*[3,4,5,6]	eo	Focal animal is spreading its ears outwards, away from the body, making it appear larger. Often head held high and jaw tucked in. Can also include the bottom part of the ears being folded back, forming a horizontal line. Can happen during locomotion.	State	Aggression
Head shake[1,3,4,5] human	hsh	Focal animal rapidly moves the head in a flowing motion tilting it from the right to the left, resulting in the ears flapping against the body and making a loud sound. Often, this is done whilst turning towards the human at which the head shake is directed.	Event	Aggression

Head shake[1,3,4,5] animal	hsa	Focal animal rapidly moves the head in a flowing motion tilting it from the right to the left, resulting in the ears flapping against the body and making a loud sound. Often, this is done whilst turning towards the animal at which the head shake is directed.	Event	Aggression
Head shake[1,3,4,5] random	hsr	Focal animal rapidly moves the head in a flowing motion tilting it from the right to the left, resulting in the ears flapping against the body and making a loud sound. Often, this is done whilst turning. No clear receiver of the head shake can be identified.	Event	Aggression
Pushing tree	pt	Focal animal is pushing a tree over with its body or head.	State	Aggression
Redirected aggression[6]	ra	Focal animal often will have received aggression by another individual or was the loser of a play or aggressive sparring interaction. Often redirected aggression can be throwing around leaves or sticks or turning rapidly from the dominant individual and push a tree over or uproot a bush.	State	Aggression
Run*[6]	run	Focal animal is moving fast without feeding and often within the whole herd moving away from a specific stimulus such as a predator. More than one foot is lifted off the ground at once. Can also be in context of excitement, when running toward a conspecific or waterhole or can be observed during social play. In those cases, note of this was taken and it was not classed as stress related.	State	Stress
Slap[5,6] given	slg	Focal animal is using its trunk, tail or head to strike a conspecific.	Event	Aggression
Smell down[2,3]	sd	Focal animal extends the trunk downwards, close to the ground, with the trunk tip curled horizontally. Often the trunk is rotated into several directions to pick up scent. This does not include extending the trunk towards a conspecific, other animal, object, or water at close proximity to pick up its scent. Can happen during locomotion.	State	Vigilance
Smell up*[2,3]	su	Focal animal extends the trunk up, over its head, often rotating the tip into several directions. This does not include extending the trunk towards a conspecific, other animal, object or water at close proximity to pick up its scent. Can happen during locomotion.	State	Vigilance
Sparring aggressive[1,4]	spa	Focal animal is pushing with conspecific head to head often with their trunks entwined and tusks clashing against each other. Use of force is visible.	State	Aggression
Standing tall*[1,4]	st	Focal animal is standing with its head held high up and glancing forwards over the trunk.	State	Aggression
Throw[1,3]	th	Focal animal throws something such as a branch or grass, using its trunk. Can happen during locomotion.	Event	Aggression

Trunk swing[3,4,6]	ts	Focal animal is swinging trunk backwards and forwards between the front legs, often whilst exhaling or stepping forward and facing a conspecific, animal or human. Can happen during locomotion.	Event	Aggression
Trunk to body	ttb	Focal animal is touching own body with trunk. Different from scratching. Can happen during locomotion.	State	Stress
Trunk to face[3]	ttf	Focal animals’ trunk is touching its face fleetingly. Can happen during locomotion.	Event	Stress
Trunk to mouth[3]	ttm	Focal animal puts the tip of its trunk into its own mouth without ingesting any food or water. Can happen during locomotion.	State	Vigilance
Trunk twirl[3]	tt	Focal animal is curling its trunk in a swift motion. Can happen during locomotion.	Event	Stress
Tusk[1,4,5,6] **given**	tg	Focal animal is pushing its tusks into conspecifics body. Can happen during locomotion.	Event	Aggression
Vigilance*[1,2]	v	Focal animals’ head is held high and ears are spread out at a 45-degree angle. Often the head is moved from one side to another such as to listen to the surrounding. Can be accompanied by smelling up/down.	State	Vigilance

1: (Horback *et al.*, 2012); 2: (McComb *et al.*, 2014); 3: (Langbauer, 2000); 4: (Doughty *et al.*, 2014); 5: (Hasenjager & Bergl, 2015); 6: (Asher *et al*., 2015)

2.8 Satellite collars

Three satellite collars, built by African Wildlife Tracking (www.awt.co.za), Pretoria, South Africa, were fitted and removed by the North West Parks Board during this study for management activities. The collars were made of a thick polyester belt with the sealed functional satellite unit in the middle (Fig. 2.4). The two straps were closed with a counterweight, which ensured that the satellite unit remained on top of the elephant's neck. The total weight of each collar was less than 10% of an adult elephants' body weight, approximately 15 kg.

Figure 2.4

Adult female African elephant, *Loxodonta africana*, in Madikwe Game Reserve, South Africa, with a fitted satellite collar and I. Szott holding the collar before fitting, showing the unit and rolled-up belt. Collar consisted of one belt with the unit at the top, which was closed using a counterweight to hold it in place.

A helicopter flew over an area within the reserve (communicated beforehand with the team on the ground to allow for cohesive and timely operating conditions) until it had located an elephant herd. The veterinarian on board the helicopter then selected a large adult female within the spotted herd which he sedated to be collared. None of the collared females were part of the same second-tier herd. The collars transmitted location data on an hourly basis and emitted a VHF (very high frequency) signal that allowed locating the individual using a VHF receiver. All collars were fitted by qualified TOPS (Threatened Or Protected Species) permitted veterinarians (TOPS standing permit #S21200, unique registration M132/6502195751085). The veterinarian would dart an adult female from a helicopter with 1.2ml of Etorphine (M99) in combination with Azaperone. Once the animal was unconscious, the collar was fitted on the ground, and the drugs were reversed with Naltrexone. All three collars were fitted without any complications and the procedure took no longer than 20 minutes for each collar. Each collared female was located in the field and visual confirmation was obtained that, in all cases, the female was reunited with (what was presumed to be) her second-tier herd. Collars were fitted in July, August and September 2016. One female repeatedly twisted the unit upside-down and the decision was made to remove the collar in April 2017. The remaining two collars were removed at the end of September 2018. Removal procedures followed the same protocols as fittings and no complications occured.

2.9 Individual identification of elephants

For behavioural observation and faecal sampling, I collected as many repeated samples from known elephants as possible. In order to fully ID an elephant, I photographed both ears and the front of the head for wrinkles, scars, and direction of tusk growth. I further identified whether the individual was left- or right ‘trunked‘, a feature synonymus to human left- or right handedness where an individual will wrap its trunk either the left or right way around an object or forage to grasp it (Racine, 1980; Dr Y. Pretorius, *pers.comm.*). Only if I was able to obtain all these details, the elephant was added into a database. When carrying out observations of elephants, I took photographs of both ears (when conditions in the field allowed) and aimed to match those against the database (see Appendix *I*) at a later stage in order to increase the total number of ID'd elephants observed. If I was unable to identify an individual, the data were retained under a non-ID code (number) alongside the age and sex profile of the individual.

2.10 Rainfall, temperature and tourist data

The South African Weather Service recorded rainfall data at four sites in Madikwe (Fig. 2.2) and provided these data in retrospect. These data were used to define wet and dry season. Total average rainfall in Madikwe was 189.69 mm. Wet season was defined as the period during which 95% of precipitation of the study year fell (Loarie *et al.,* 2009a, b). Mean (± SD) monthly rainfall during the dry season (April 2016 – September 2016 and March 2017 – June 2017) was 6.79 ± 7.79 mm. Mean (± SD) monthly rainfall during the wet season (October 2016 – February 2017) was 118.89 ± 63.51 mm.

The monthly average temperature (in ^{o}Celsius) from May 2016 – June 2017 was extracted from historical records online in hindsight (https://www.worldweatheronline.com/madikwe-weather-history/north-west/za.aspx). The temperatures were calculated from readings every three hours, with the first reading taken at 12 am. Monthly average temperature ranged from 16 ^{o}C to 30 ^{o}C (Fig. 2.5).

The total number of tourists visiting Madikwe each month was provided by the South African North West Parks Board, as visitors must sign in at the gate upon entering the reserve. Figure 2.6 shows the total number of tourists each month during the wet and dry season in Madikwe throughout the study period.

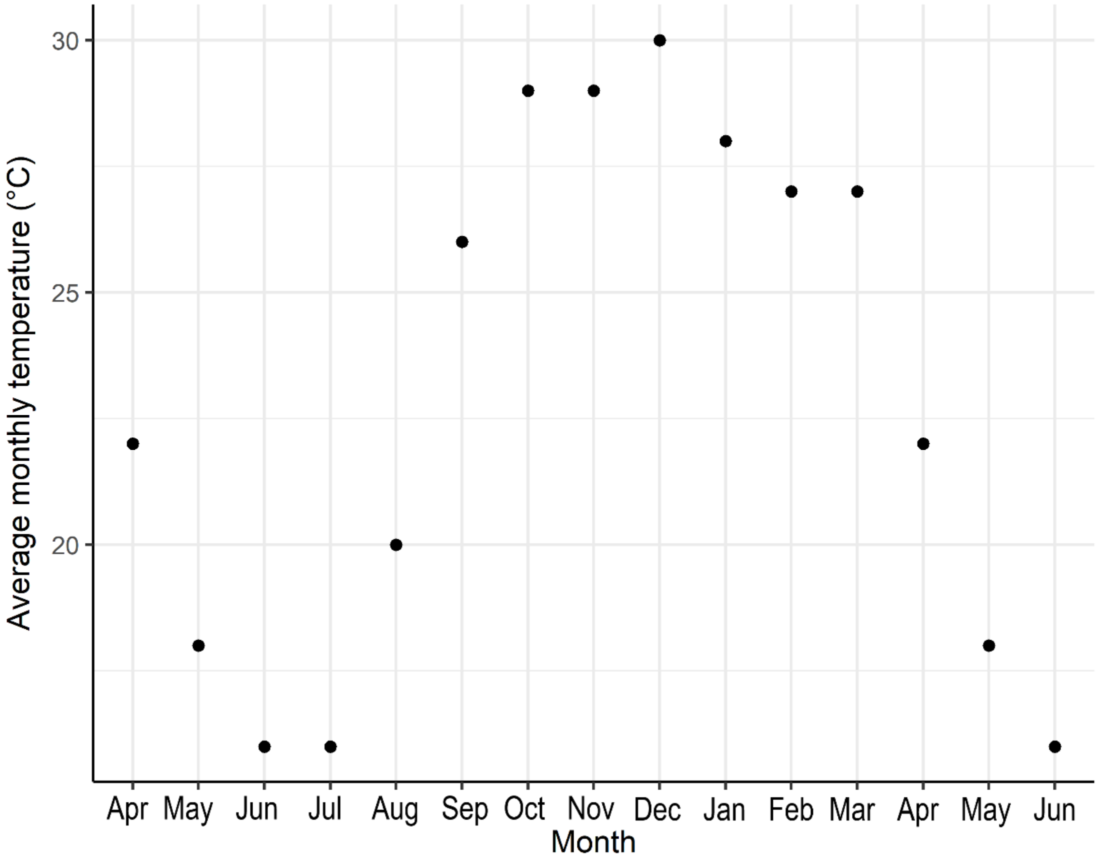

Figure 2.5

Average monthly temperature in Madikwe Game Reserve, South Africa, between May 2016 and June 2017.

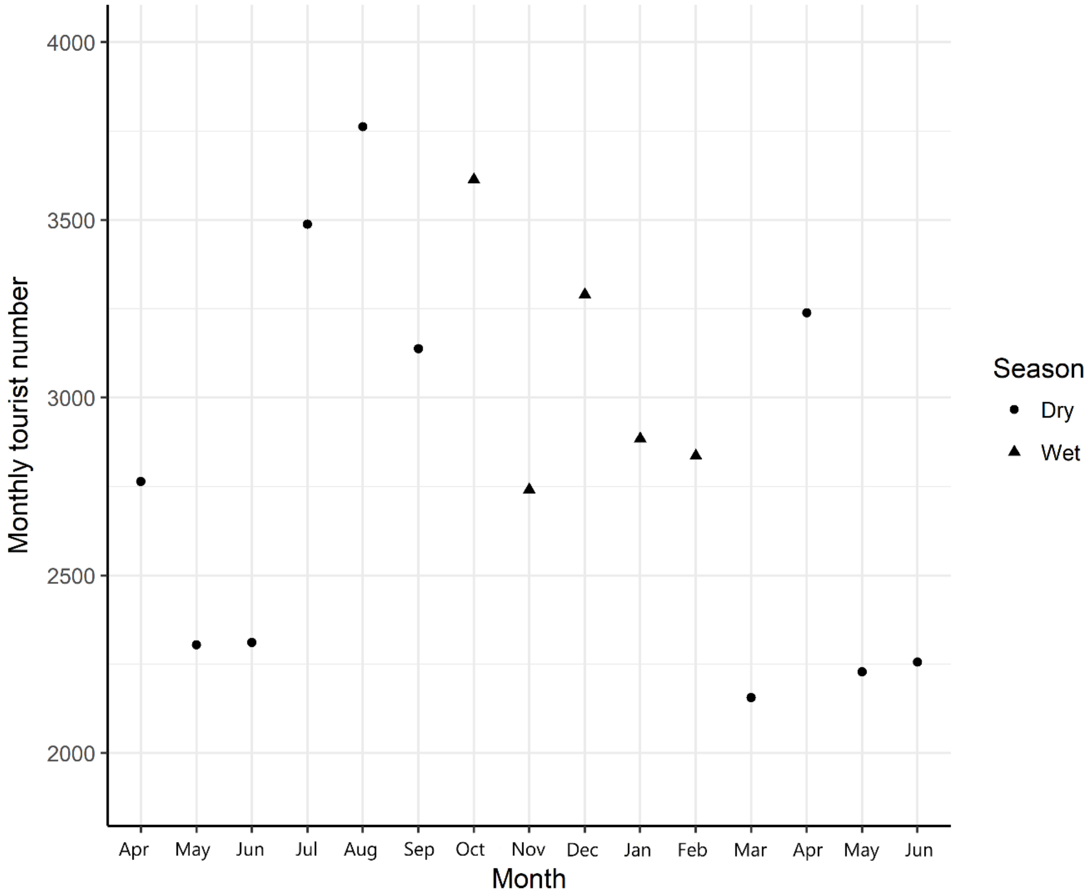

Figure 2.6

Total number of tourists per month in Madikwe Game Reserve, South Africa, between April 2016 and June 2017. Dry season (circles) lasted from April 2016 to September 2016 and from March 2017 to June 2017. Wet season (triangles) lasted from October 2016 to February 2017.

2.11 Ethical clearance

Ethical clearance for the research carried out for this PhD was received from Liverpool John Moores University (NK_IS/2016-6) as well as permission from the South African North West Parks Board. This research adhered to the Association for the Study of Animal Behaviour guidelines for the ethical treatment of animals.

2.12 Statistical analyses

All statsistical analyses were carried out using R Statistical Software, version 3.4.1 (R Core Team, 2000). In order to analyse a variety of factors affecting elephant fGCM concentrations, behaviour, or movement, I applied logistic regression models throughout the following chapters. As a first step, factors potentially included in models were assessed for collinearity using the variance of inflation factor (VIF; Fox & Monette, 1992). Instead of assessing collinearity between two factors in a model, VIF allows us to assess the correlation between any two factors whilst adjusting for all other factors in the data (Fox & Monette, 1992). Classic correlation coefficients assess numerical consequences whilst VIF assesses factors for their impact on the variability of estimates, and therefore aids in variable selection for predictions (Fox & Monette, 1992). Previous studies have suggested the use of cut-off values between five to ten, but as higher cut-off values can be too lenient, I used a VIF cut-off value of four throughout all data chapters (Hair *et al.*, 1995; Craney & Surles, 2002). Addtionally, if VIF values were above four, I inspected the *r*-coefficients to confirm which factors were highly correlated (indicated by values larger than ± 0.3) and in order to decide which factor to remove (Taylor, 1990).

Numerical variables in models were scaled and centred which rectifies issues associated with convergence warnings or large eigenvalues and improves model fit. Scale means that the mean and standard deviation of the entire vector is calculated and then each element is scaled by subtracting the mean before dividing it by the vector's standard deviation. Centre means that a variable is centred around zero. This method does not affect statistical interference in regression models and eases interpretation of effects where parameters have different scales (such as a large range of tourist numbers compared to values of a factor that ranges only between -1 to 1; Schielzeth, 2010).

Models in each chapter were assessed using an information-theoretic approach in order to identify the most parsimonious set of independent factors. For this, I used the Akaike information criterion (AIC) or the conditional AIC (AICc) for models with small sample sizes, to rank models (Burnham & Anderson, 2002). The MuMIn package (Barton, 2018) provides the function ‘dredge‘ which compared all possible candidate models based on a specified global model. It produced an output table with all those candidate models and their associated AIC/AICc values, from which the top model was chosen. The lowest AIC/AICc value indicated the best model and models within ≤ 2 AIC/AICc points were also thought to provide an equally good fit (Burnham & Anderson, 2002). However, when assessing models differing by ≤ 2 AIC/AICc, I applied further criteria as outlined in Leroux (2019), to identify the best model within the set of models. This followed the following three steps, building on each previous step (Leroux, 2019):

1: If model 2 has one additional parameter compared to model 1, but model 2 is ranked below model 1 (and therefore has a larger AIC/AICc value than model 1), then model 2 does not have a better fit compared to the simpler model 1.

2: If a model has a virtually identical value of log likelihood to another model, then it suggests that this more complex model is not a better fit. Whether log likelihood is virtually identical is subjective and investigators should be drawing inference from all available information. Note that Type I errors can be avoided by being cautious rather than misinterpreting uninformative parameters as useful.

3: If the additional parameter identified from the previous steps has a parameter estimate near zero and its confidence intervals overlap 0, then it is uninformative.

Linear models should contain a minimum number of 10 – 20 samples for each treatment level or experimental unit included in the model (Bolker *et al.,* 2009). Specifically, when including a factor as a treatment which has two levels (e.g. factor: sex, levels: male, female), a minimum of 20 observations are neccessary in order to assess this factor alone. Therefore, small sample sizes restrict the number of treatment levels which can be included in a linear model. The ranking of models based on AIC/AICc does not consider model fit and therefore, top models were only considered valid if they did not result in extreme violations of the minimum number of observations neccessary as described above. Further, the top model was assessed for model stability by visually inspecting histograms of residuals.

Once the top model had been identified using the information theoretic approach and given that it was supported by a minimum number of observations for the number of treatment levels included, I analysed significance of factors in form of *p*-values or 95% confidence intervals using appropriate methods, presented in each respective chapter. Information theoretic methods assesses models as relative model probabilities but provide no information about the importance of each individual predictor (fixed effect) in a given model and further fail to distinguish between variables with weak or strong effects (Harrison *et al.,* 2018). As this thesis was focused on the effect of tourism on elephants, it was reasonable to assess this specific effect in more detail in order to understand how strongly it affected the response variable (Mundry, 2011; Symonds & Moussalli, 2011).

In order to visualise results, I used packages which plot effects as calculated in the regression models, as presenting fitted values or predicted probabilities is the standard approach when presenting results from mixed models (Zuur *et al.,* 2009). Presenting raw data of single factors over a figure which represents the effects assessed by mixed models is inappropriate as it would misrepresent relationships (Zuur *et al.,* 2009). Further, I aimed to provide tables with descriptive statistics to show trends in the raw data in each respective chapter.

Chapter 3 – Effect of wildlife tourism on glucocorticoid concentrations of elephants

Szott I., Pretorius Y., Ganswindt A., Koyama N.F. (2019) Physiological stress response of African elephants to wildlife tourism in Madikwe Game Reserve, South Africa. Wildlife Research, 47:34-43. doi: 10.1071/WR19045. See Appendix II

This chapter has been adapted from a paper published in Wildlife Research. Wildlife tourism aids in funding and protecting habitats and wildlife (Reynolds & Braithwaite, 2001; Newsome et al., 2005; Maciejewski & Kerley, 2014), however, it has also been shown to be related to increased glucocorticoid concentrations in a wide range of species (e.g. Creel et al., 2002; Behie et al., 2010; Maréchal et al., 2011). In this chapter, I investigated whether tourist pressure was positively related to faecal glucocorticoid metabolite concentrations in free-ranging African elephants.

Abstract

Wildlife tourism has been shown to be a perceived stressor to a variety of species and can negatively affect survival, reproduction, welfare, and behaviour of individuals. In African elephants, *Loxodonta africana*, increased glucocorticoid (GC) concentrations have been linked to use of refugia, rapid movement through corridors, and heightened aggression towards humans. However, we are unaware of any studies assessing the impact of tourism pressure (tourist numbers) on GC concentrations in elephants and this study is the first to do so. We used faecal GC metabolite (fGCM) concentrations to investigate whether tourist numbers in Madikwe Game Reserve, South Africa, were related to changes in a physiological measure of stress in elephants. We repeatedly collected dung samples (n=43) from 13 individually identified elephants over 15 months. Using an information theoretic approach based on Akaike Information Criterion, we ran Generalised Linear Mixed Models and a Kenward-Roger approximation to assess the impact of monthly tourist numbers, season, age, and sex on elephant fGCM concentrations. The best model included season and tourist number. High tourist numbers were significantly related to elevated fGCM concentrations. Overall, fGCM concentrations increased by 112% (from 0.26 to 0.55 µg/g dry weight) in the months with the highest tourist pressure, compared to months with the lowest tourist pressure. Managers of fenced reserves should consider providing potential alleviation measures for elephants during high tourist pressure, for example, by ensuring that refuge areas (areas within animals' home ranges, where perceived stressors can be avoided) are available. This may be of even higher importance if elephant populations have had stressful experiences with humans in the past, such as poaching or translocation. Such management action will improve elephant welfare and increase tourist safety. Although tourism can generate substantial revenue to support conservation action, careful monitoring of its impact on wildlife is required to manage potential negative effects.

3.1 Introduction

Wildlife conservationists can use stress-related hormone measurements to assess welfare, translocation success, and the ability to cope with injury, disease, and environmental challenges (Millspaugh & Washburn, 2004; Teixeira *et al.*, 2007; Ganswindt *et al.*, 2010a). Perceiving something as a stressor is a normal process and may even be adaptive in the short term (McEwen & Wingfield, 2003; see Chapter 1). However, prolonged, or chronic perception of stressors can lead to changes in an individuals' behaviour, health, and cognition which might detrimentally affect welfare, reproduction, and survival (Sapolsky, 2002; McEwen & Wingfield, 2003; Bhattacharjee *et al.*, 2015; see Chapter 1).

What an individual perceives as a stressor depends on past experiences, personality traits, and the amount of control an individual perceives to have in a given situation (Koolhaas *et al.,* 1999; Bradshaw *et al*., 2005; Nelson & Kriegsfeld, 2017). When something is perceived as a stressor, the neural and/ or neuroendocrine system and/ or behavioural responses are activated to cope with the stressor (McEwen & Wingfield, 2003; Romero *et al.,* 2009; Palme, 2019). The neuroendocrine response involves activation of the hypothalamic-pituitary-adrenal axis, resulting in increased secretion of hormones referred to as glucocorticoids (GCs; Nelson & Kriegsfeld, 2017). In the short term, increased concentrations of GCs are linked to adaptive responses (see Chapter 1; Romero *et al.,* 2009) such as an increase in movement and foraging behaviour, as well as vigilance, and a decrease in behaviours non-essential to immediate survival such as reproduction and resting (McEwen & Wingfield, 2003; Busch & Hayward, 2009; Sheriff *et al.,* 2011). However, increased GC concentrations over longer periods of time are related to suppression of reproductive hormones and the immune system, as well as muscle loss and reduced growth (Nelson & Kriegsfeld, 2017; see Chapter 1). If a stressor becomes chronic, individuals may therefore become more susceptible to predation, starvation and disease, as well as exhibit lasting changes of behaviour and reduced reproductive output (see Chapter 1; Reynolds & Braithwaite, 2001; McEwen & Wingfield, 2003; Teixeira *et al.,* 2007). Therefore, changes in GC concentrations are often interpreted to indicate a physiological response to stressors (Möstl & Palme 2002; Sapolsky 2002; Touma & Palme 2005) and used as a welfare indicator alongside other measures such as observations of behaviour, body condition, or health.

GCs can be measured using faecal glucocorticoid metabolite (fGCM) concentrations excreted in dung. This approach is advantageous because it does not require restraint or capture of animals and thus does not interfere with an animal's natural behaviour (Sheriff *et al.,* 2011; Goymann, 2012). Further, especially in free-ranging animals, restraint itself would likely be a stressor and change GC concentrations and hence pose ethical concerns and make GC concentrations related to the stressor of interest undistinguishable from GC concentrations related to the stress caused by restraint (Palme, 2019). Monitoring fGCMs, amongst a suite of measures, therefore allows us to assess animal welfare non-invasively, for example by assessing effects of environmental conditions such as season, temperature and rainfall, or by assessing potential anthropogenic stressors such as human presence or hunting (Millspaugh & Washburn, 2004; Millspaugh *et al.*, 2007; Palme, 2012; Scheun *et al.,* 2015). One potential stressor that has been studied in various wildlife species, is tourism. Wildlife tourism can take several forms, such as tourists watching, feeding, or petting animals, or animals being transported to tourism sites (Orams, 2002; Millspaugh *et al.*, 2007; Sarmah *et al.*, 2017). Wildlife tourism has been linked to elevated glucocorticoid concentrations in a range of species (e.g. African elephants (Millspaugh *et al.*, 2007); western capercaillie (Thiel *et al*., 2008); mountain hare, *Lepus timidus* (Rehnus *et al*., 2014); black howler monkeys (Behie *et al.*, 2010); western lowland gorillas (Shutt *et al.*, 2014); wildcats (Piñeiro *et al.*, 2013); Tatra chamois (Zwijacz-Kozica *et al.*, 2013); grey wolves and red deer (Creel *et al.*, 2002)).

Funding from wildlife tourism can aid in the protection of habitat, biodiversity, and ecological processes (see Chapter 1; Reynolds & Braithwaite 2001) and has become increasingly common over the past few years (Orams, 2002). For example, entrance fees aid in funding anti-poaching units, upkeep of park infrastructure, and maintenance (P. Nel, *pers.comm.*). However, assessing how wildlife tourism affects the behaviour and welfare of the wildlife being viewed is difficult and studies doing so are relatively scarce (Ranaweerage *et al*., 2015). African elephants are one of the most popular species for tourists to observe across Africa (Lindsey *et al.*, 2007), and are threatened with a drastic decline in numbers due to habitat loss and poaching (Chase *et al.*, 2016).

To conduct wildlife tourism in a sustainable and welfare-focused manner, it is important to understand whether overall tourist pressure, in form of number of tourists within an elephants' habitat, increases elephant GC concentrations. Further, it has been suggested that increased perceived stress underlies aggressive behaviour in elephants (Slotow & Van Dyk, 2001; Bradshaw *et al*., 2005; Bradshaw & Schore, 2007). In line with this, Jachowski and colleagues (2012) as well as another elephant expert (Y. Pretorius, *pers.comm*.) suggest that long-term and chronic perception of stressors is linked to elephants becoming hyper-aggressive towards humans and causing human fatalities. It is therefore important that managers monitor potential stressors in their elephant population to increase tourist safety. Even so, the effect of tourism on elephants has only been investigated in a few studies, all of which indicated a negative effect of tourism. Asian elephants have been reported to show signs of disturbance (in form of aggression such as attacking tourist vehicles, and stress-related behaviour such as swaying their body or tossing soil) in response to wildlife viewing (Ranaweerage *et al.*, 2015). In working African elephants, fGCM concentrations were slightly higher on days during which elephants and humans interacted directly (in form of elephant back rides) compared to days without interaction (Millspaugh *et al.*, 2007). In contrast, Norkaew and colleagues (2019) found that captive Asian elephants used for elephant back rides had, in fact, lower fGCM concentrations compared to captive Asian elephants which were only observed and fed by tourists. So far, no study has assessed how wildlife viewing affects fGCM concentrations of elephants.

Concentrations of fGCMs provide estimates of circulating steroid levels for an estimated two to three days prior to when the sample was collected; this roughly corresponds with the gut passage time of an elephant (Ganswindt *et al.*, 2003; Laws *et al.*, 2007). Further, fGCM concentrations in African elephant dung have been shown to be stable for up to twenty hours before collection in samples collected in South Africa (Webber *et al.*, 2018). Yet, elephant fGCMs must be interpreted with care, as elephants secrete GCs in response to many factors. For example, an elephant's GC secretion may shift according to ecological changes, increasing during low availability of key nutrients, during the dry season, and following large fires within elephants' habitat (Foley *et al.*, 2001; Viljoen *et al.*, 2008a; Woolley *et al.*, 2008). Social and environmental stressors are related to increases in elephant fGCMs, such as injury (Ganswindt *et al.*, 2010a), or the trophy hunting of conspecifics (Burke *et al.*, 2008), living outside of protected areas (Hunninck *et al.*, 2017), living in areas of high poaching risk, in herds with weak social bonds, or in herds lacking older matriarchs (Gobush *et al.*, 2008). Further, increased intra-group competition for forage and water, especially during the dry season and in larger herds, has been linked to elevated fGCM concentrations (Foley *et al.*, 2001). Reintroduced or translocated herds have increased fGCM concentrations for six to ten years following translocation (Jachowski *et al.*, 2012) and, at a population level, an even longer-term stress response for over ten years has been suggested (Jachowski *et al.*, 2013a). Additionally, non-stressful stimuli such as reproductive activity and physical activity can increase GC concentrations (Nelson & Kriegsfeld, 2017), whilst long-term, chronic stress can cause a downregulation of GC concentrations and hence individuals under chronic stress may have lower concentrations than non-stressed individuals (Dickens & Romero, 2013; see Chapter 1).

Given the lack of research on the impact of wildlife tourism on fGCM concentrations in elephants, here we investigated the effect of monthly tourist numbers on fGCM concentrations in a large population of elephants in Madikwe Game Reserve, South Africa. We hypothesised that high tourist presence would be an unpredictable stressor for elephants and, therefore, predicted that fGCM concentrations in elephants would be elevated during times of high tourist pressure. We included age and sex of the sampled individual as potential influencing factors in our analysis, although some studies have found no effect of sex (Pinter-Wollman *et al.*, 2009) or age (Viljoen *et al.*, 2008a). We further included season as a potential covariate, as it has been shown that fGCM concentrations are elevated during the dry season (Viljoen *et al.*, 2008a; Jachowski *et al.*, 2012). However, because water is artificially pumped at Madikwe and available throughout the year, we expected season to have a minimal effect. No hunting of elephants took place in Madikwe or other potential impacting sporadic events such as large fires, and no elephants with visible injuries were sampled. However, the founding population originates from traumatic backgrounds which included bush wars, poaching and culling (see further detail below under 3.2.1), which may have led to those elephants associating humans with negative experiences and hence a perceived stressor. Madikwe has strict driving regulations in place, with a maximum of three game drive vehicles at an elephant sighting at a time and private vehicles are restricted to main roads. Given these restrictions, we expected tourism to have a minimal effect on elephant fGCM concentrations.

3.2 Materials and Methods

3.2.1 Study site

Madikwe is a fenced reserve, managed by a state/private/communal partnership and is approximately 680 km^2 in size (Fig. 3.1). In total, 228 elephants were introduced to

Madikwe between 1992 and 1999 from various traumatic backgrounds such as poaching and bush wars (see Chapter 2; Bradshaw *et al.,* 2005). First, 25 orphaned elephants between 8 – 12 years of age were introduced following culling operations in Kruger National Park (Davis & Brett, 2003). This was followed by 194 individuals in entire herds from Zimbabwe, aged from a few months to over 50 years, from an area experiencing extreme drought and heavy poaching (Davis & Brett, 2003; P. Nel *pers.comm*.). Today, this founding population has grown to 1348 ± 128 elephants (July 2017, North West Parks Board, P. Nel, *pers.comm*.), representing one of the highest population densities (1.9 elephants per km^2) in South Africa.

Wildlife viewing in Madikwe is conducted from game drive vehicles: large, open vehicles driven by qualified field guides, seating up to ten people. Game drives are mainly carried out in the morning (from sunrise to 11 am) or afternoon (from 3:30 pm to sunset). No more than three vehicles were permitted at a sighting at a time, and guests were briefed on appropriate behaviour, such as no shouting or eating, which guides enforced. A higher number of tourists in Madikwe directly relates to higher numbers of game drive vehicles on the roads (P. Hattingh, C. Catton, K. Potgieter, *pers.comm.*; I. Szott, *pers.obs.*). The current Code of Conduct in Madikwe does not stipulate a minimum distance between elephants and game drive vehicles. There is no limitation to the total number of game drive vehicles conducting game drives within Madikwe. Off-roading in Madikwe occurred when viewing certain animals such as leopard, *Panthera pardus*, lion, buffalo, or cheetah. Although off-roading did not occur to specifically view elephants, off-roading for other species meant that elephants could encounter vehicles off-road. Madikwe is accessible for tourists throughout and contains no restricted areas.

Each of the 33 lodges at Madikwe has their own waterhole, providing water all year round (Fig. 3.1). The reserve is also bordered by the Marico River on the eastern side and contains large artificial dams that pump water throughout the year. According to Mucina and Rutherford (2006), Madikwe contains three main vegetation types. Dwaalboom thornveld contains ultramafic clay plains with a nearly continuous herbaceous layer dominated by grass species, deciduous microphyllous trees and shrubs and a few broadleaf species. Madikwe dolomite bushfeld contains a continuous herbaceous layer dominated by grass species and a woody layer dominated by deciduous trees. The Dwaarsberg-Swartruggens mountain bushveld has various combinations of tree and shrub layers as well as dense grass layers (Mucina & Rutherford, 2006). Elephants have access to the whole reserve and can be encountered across all of the previously mentioned vegetation types.

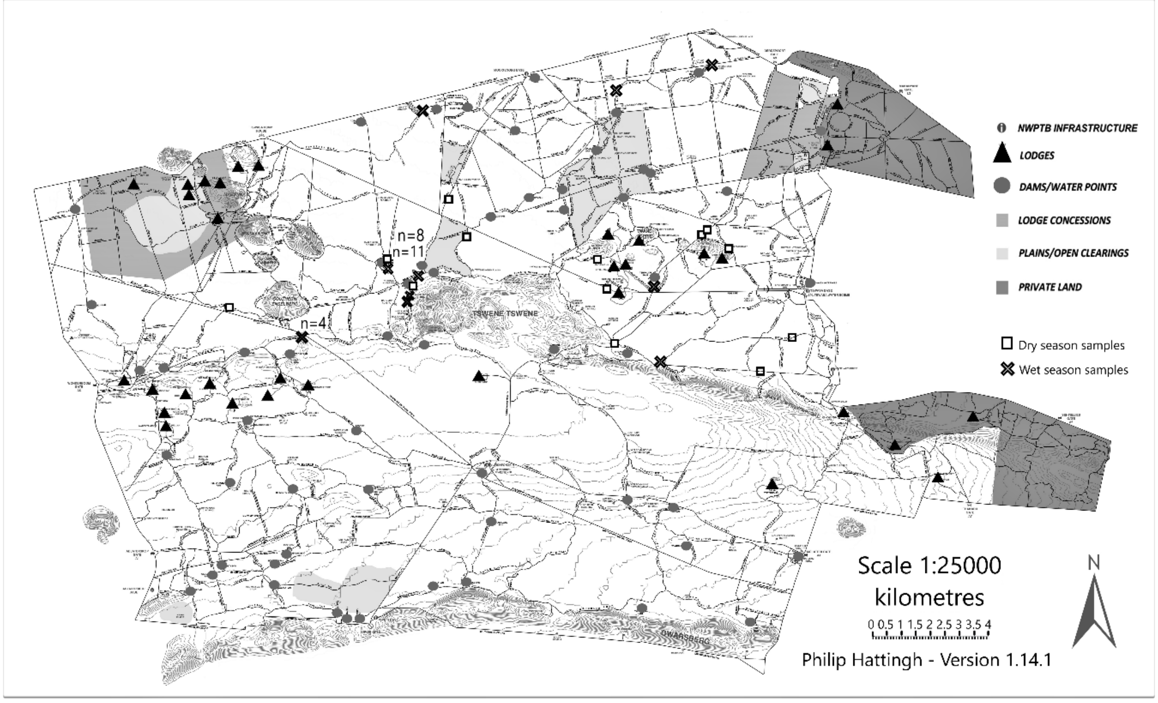

Figure 3.1

Map of Madikwe Game Reserve, South Africa, as of 2014. Game drives take place throughout the whole reserve. Dark grey areas are private concessions, used for game drives only by their respective lodge, grey areas are private concessions used for game drives by any lodge with prior permission but usually restricted to three vehicles within the area at any time. Light grey areas are open plains in which off-roading is prohibited. Lines are roads, triangles are lodges, and circles are waterholes (year-round or during wet season). Crosses and squares are locations at which dung samples of African elephants, *Loxodonta africana,* were collected during the dry season (squares) and wet season (crosses). Where several dung samples were collected at the same location, the number of samples (n) is given. Map courtesy of P. Hattingh (2014) and reproduced with his permission.

3.2.2 Data and sample collection

The principal investigator collected the faecal samples between April 2016 and June 2017 throughout Madikwe, spending similar amounts of time in the different areas of the reserve searching for individuals that could be observed defaecating (Fig. 3.1). As no previous information on Madikwe's elephant population was available, the number of sampled elephants was limited to the individuals we were able to identify reliably, so we could collect repeated faecal samples from each. We identified elephants on the basis of distinguishing characteristics such as holes and notches in their ears, wrinkles across the face and orientation of tusk growth (Poole & Granli, 2009), resulting in 12 known individuals of four different cow-calf groups, as well as one solitary adult male. The cow-calf individuals included five adult females, three juvenile males, three juvenile females, and one male calf. Sampling for this study was restricted to elephants encountered near roads, which led to a low rate of sightings of known elephants and, consequently, a low number of faecal samples collected. In total, 43 faecal samples were collected (mean ± SD per individual = 3.31 ± 1.9, Table 3.1) with a mean ± SD of 3 ± 3 samples per month.

Samples were collected with gloves following previously published protocols (Ganswindt *et al.*, 2010a, b; see Chapter 2). In short, matter was taken from the inside of two to five boluses and homogenised by hand. We stored approximately 50 g of faecal matter in a cooler box on ice and transferred it to a freezer at -18 °C, in sterile vials, no longer than four hours after collection. For each sample, we recorded the sex, age class (adult (13 years or older), juvenile (4 – 12 years), calf (0 – 3 years); Moss, 1996; Poole & Granli, 2009), and ID of the defaecating individual, the time, and the longitude and latitude on a Lenovo TAB 2 A8-50F tablet. The average time between observing an elephant defaecating and sample collection was 16 min (± 12min).

We defined wet and dry season based on average monthly rainfall measured at four stations in Madikwe by the South African Weather Service (www.weathersa.co.za). Average total rainfall in Madikwe during the study period was 189.69 mm. We classed wet season as the period in which 95% of precipitation for the study year fell (Loarie *et al.*, 2009a, b). During the dry season (April 2016 to September 2016 and March 2017 to June 2017), the mean (± SD) monthly rainfall was 6.79 ± 7.79 mm, and during the wet season (October 2016 to February 2017), the mean monthly rainfall was 118.89 ± 63.51 mm. South African North West Parks Board provided the total number of tourists visiting Madikwe each month. The number of tourists was assessed as the number of guests counted at the gate to the reserve, and the total number of tourists per month, within each season, is shown in Figure 2.5 in Chapter 2.

3.2.3 Steroid extraction and faecal glucocorticoid metabolite analysis

Steroid extraction and analysis was performed by the Endocrine Research Laboratory, University of Pretoria, South Africa, and followed previously published protocols (Fieß *et al.*, 1999; Ganswindt *et al.*, 2003, 2010b; see Chapter 2). In short, faecal matter was lyophilised and pulverised before being sieved through a mesh to remove any undigested faecal matter. Between 0.050 and 0.055 g of the remaining powder was extracted with 3 mL 80% ethanol in water. The suspension was vortexed for 15 minutes and then centrifuged for 10 minutes at 1500 g at room temperature and the supernatant then transferred to a microcentrifuge tube. An 11-oxoetiocholanolone enzyme immunoassay (EIA; detecting fGCMs with a 5β-3α-ol-11-one structure (Möstl *et al.*, 2002)) was used to measure immunoreactive fGCMs in diluted extracts (1:10 or 1:50 in aqueous buffer). This EIA has been validated and repeatedly used to monitor adrenocortical activity in elephants (Ganswindt *et al.*, 2003, 2005, 2010a). Sensitivity of the assay at 90% binding was 1.2 ng/g dry faecal mass. Repeated measurements of high- and low-value controls determined intra-assay variance of 3.3% and 5.6% (15 and 16 plates used for high- and low-quality control, respectively) and inter-assay variance of 9.5% and 12.3% (13 plates used).

3.2.4 Data analysis

We analysed data in R v.3.4.1 (R Core Team, 2000) and assessed factors to rule out collinearity using variance of inflation factor (VIF) analysis (Fox & Monette, 1992; see Chapter 2) in the *car* package (Fox & Weisberg, 2011), using a cut-off value of four. All VIF values were below two. Tourist number was scaled and centred. We analysed the samples with a Generalized Linear Mixed Effects Model with a gamma error structure and log link because data were non-parametric and resembled a normal distribution with a log10 transformation. Using the ‘glmer’ command (*lme4* package (Bates *et al.*, 2014)) we ran the following global model with all possible two-way interactions:

$$glmer\ (formula \\ = fGCMs \sim Tourist * Season\ +\ Tourist * Age\ +\ Tourist \\ * Sex\ +\ Season * Age\ +\ Season * Sex\ +\ Age * Sex \\ +\ (1|ID), data\ =\ Data, family\ =\ Gamma\ (link\ =\ \text{“}log\text{”}))$$

We then used the ‘dredge’ function in the MuMIn package (Barton, 2018) in order to compare all possible models based on their conditional Akaike Information Criterion (AICc; Burnham & Anderson, 2002; see Chapter 2). The ‘dredge’ function produced a table with all possible candidate models and their associated AICc values, from which the top model was chosen. However, a linear model should contain a minimum of 10 – 20 samples per treatment level or experimental unit (Bolker *et al.,* 2009). This means that, for example, to include the treatment level of season with the experimental units of wet and dry season, a minimum of 20 observations would be necessary. Therefore, our sample size restricted the number of factors which could reliably be assessed in a model, and we would only consider the top model if this did not result in an overly complex model. Where several models were within 2 AICc of each other, we further followed the criteria detailed in Leroux (2019; see Chapter 2) to identify one single best model.

As we were interested in the specific effect of tourism, we aimed to gain further understanding on how strongly each fixed effect identified in the AICc top model affected the response variable (Mundry, 2011; Symonds & Moussalli, 2011; Harrison *et al.,* 2018). Therefore, we further assessed this top model to obtain *p*- values for our fixed effects and, to control for the small sample size of our study, we used a Kenward-Roger approximation fitted with restricted maximum likelihood estimation (Kenward & Roger, 1997; Luke, 2017), with the *afex* package (Singmann *et al.*, 2018). Significance was assigned at $p \leq 0.05$.

Although our sample size (n=43) was slightly lower than previously recommended for a Kenward-Roger approximation, it was close to n=45, which has been suggested to provide robust results (Arnau *et al.,* 2013). Further, Arnau and colleagues (2013) showed that data with small to moderately skewed response variables (indicated by values of 0.8 and 1.6 respectively) are best assessed with a Kenward-Roger approximation. An approximate ratio of 1:2 in kurtosis between the largest and smallest group (in our case wet and dry season, respectively) indicates a robustness of 60% or higher for the Kenward-Roger approximation (Arnau *et al.,* 2013). In our case, skewness of GC concentrations was 0.72, and wet season kurtosis of tourist pressure was 3.73, whereas dry season kurtosis was 1.69.

We plotted graphs using the packages *effects* (Fox, 2003) and *ggplot2* (Wickham, 2016), by using the unscaled data for ease of interpretation (see Chapter 2).

3.3 Results

Overall, fGCM concentrations ranged from 0.05 to 1.02 µg/g dry weight (DW) with an overall mean (± SD) of 0.39 (± 0.22) µg/g DW (Table 3.1). Tourist numbers ranged from 2156 to 3762 tourists per month, with an increase of 74.5% from the lowest to the highest tourist numbers and with an average (± SD) of 2831 (± 563) throughout the study period. During the dry season, tourist numbers ranged from 2156 to 3762 tourists per month, and during the wet season, they ranged from 2741 to 3614 tourists per month.

Table 3.1 Faecal glucocorticoid metabolite (fGCM) concentrations of 13 individually identified African elephants, *Loxodonta africana*, in Madikwe Game Reserve, South Africa. Concentrations are in µg/g dry weight (DW). ID number of individuals, their age, and sex are presented (with overall mean (± SD) fGCM concentrations) and a breakdown of number (n) of samples collected during the dry and wet season.

Sex	Age class	ID	fGCM concentration µg/g DW dry season	fGCM concentration µg/g DW wet season	n samples/ individual
Female 0.38 ± 0.2	Adult 0.40 ± 0.21	1	0.46 0.58	-	2
		2	0.56	0.91	2
		3	0.2 0.22 0.64 0.23	0.17 0.34 0.4 0.19	8
		4	0.47 0.6	0.16 0.59	4
		5	0.16 0.42 0.24	-	3
	Juvenile 0.35 ± 0.23	6	0.37	0.39 0.31 0.19 0.55	5
		7	-	0.26 0.6	2
		8	-	0.09 0.38	2
Male 0.48 ± 0.28	Adult 0.10 ± 0.06	9	0.14	0. 05	2
	Juvenile 0.48 ± 0.26	10	0.53	0.57 1.02	3
		11	0.27 0.53 0.12 0.74	0.26 0.24	6
		12	0.55	0.49	2
	Calf 0.21 ± 0.12	13	-	0.29 0.12	2
n samples/ season			20	23	43

The top model included only tourist pressure and season according to the AICc model selection table (Table 3.2). As this model was ranked above the more parsimonious model, the additional parameter of season was considered informative (Leroux, 2019; see Chapter 2).

Table 3.2 Fixed effects included in the top models for the effect of various factors on African elephant, *Loxodonta africana*, faecal glucocorticoid metabolite (fGCM) concentrations of individually identified elephants in Madikwe Game Reserve, South Africa, as ranked by conditional Akaike information criterion (AICc). Degrees of freedom (df), log likelihood, delta value and weight for each model are also reported.

Fixed effects included	**df**	**Log Likelihood**	**AICc**	**delta**	**weight**
Tourist + Season	5	13.744	-15.9	0.00	0.192
Tourist	4	11.668	-14.3	1.58	0.087
Tourist + Season + Sex	6	14.185	-14.0	1.83	0.077
Null model	3	10.134	-13.7	2.22	0.063

The Kenward-Roger approximation on this model showed that high monthly tourist numbers in Madikwe were significantly related to elevated fGCM concentrations (Table 3.3, Fig. 3.2a, b). Season did not have an effect on fGCM concentrations but fGCMs were higher during the dry, compared to the wet season (Table 3.3, Fig. 3.3). Removing the adult male and calf from the data set or nesting ID in social group did not change these results. Removing six individuals (n=14 samples) that did not have samples in both high and low tourist numbers (above and below the mean tourist number) did not change the effect of tourist numbers on fGCM concentrations either.

Table 3.3 GLMM results of the fixed effects on faecal glucocorticoid metabolites of African elephants, *Loxodonta africana*, in Madikwe Game Reserve, assessed with a Kenward-Roger approximation. Significant effects are shown in bold.

[a]SE=Standard error, [b]df=Degrees of Freedom

Fixed effect (reference level)	Level	Estimate (± SE[a])	df[b]	F	*p*-value
Intercept		0.400 ± 0.05			
Tourist		**0.090 ± 0.04**	**36.93**	**6.08**	**0.02**
Season (Dry)	Wet	0.057 ± 0.03	34.09	2.74	0.11

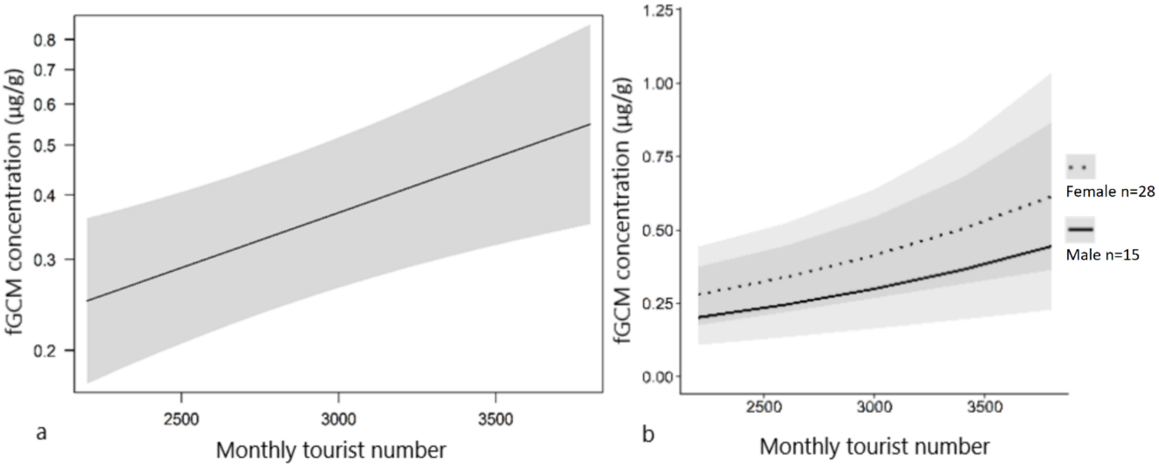

Figure 3.2

Effect of total tourist numbers per month (*p*=0.02), as assessed by a Generalised Linear Mixed Effects Model and Kenward-Roger approximation, on faecal glucocorticoid metabolite (fGCM) concentration (µg/g dry weight) of free-ranging African elephants, *Loxodonta africana*, in Madikwe Game Reserve, South Africa. Fig. 3.2a presents the overall effect of tourist pressure, whilst Fig. 3.2b presents the effect of tourist pressure on female and male elephants. Grey areas represent 95% confidence intervals.

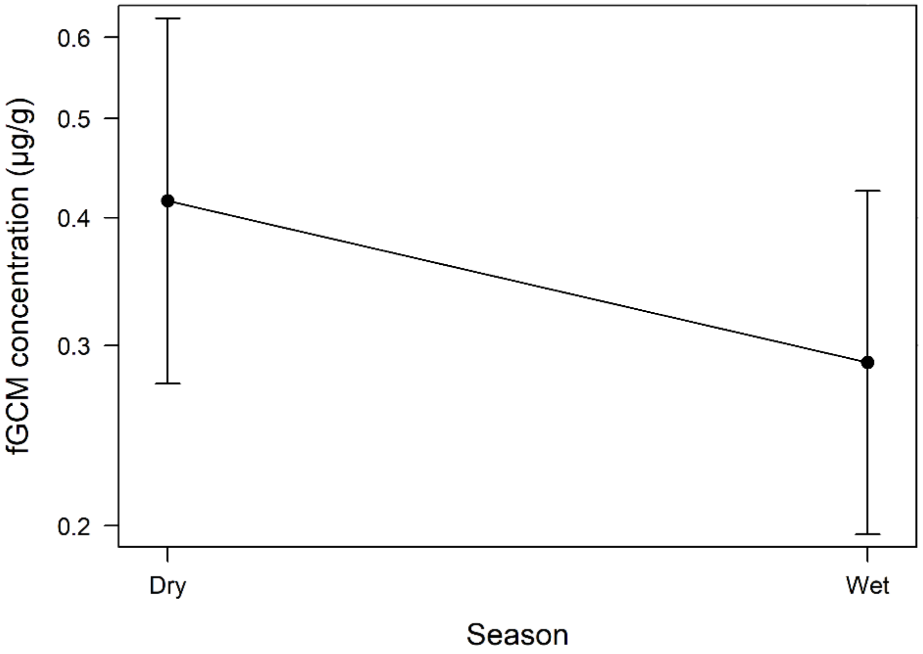

Figure 3.3

Effect of season (*p*=0.11), as assessed by a Generalised Linear Mixed Effects Model and a Kenward-Roger approximation, on faecal glucocorticoid metabolite (fGCM) concentration (µg/g dry weight) of free-ranging African elephants, *Loxodonta africana*, in Madikwe Game Reserve, South Africa. Dry season lasted from April 2016 to September 2016 and from March 2017 to June 2017. Wet season lasted from October 2016 to February 2017. Grey areas represent 95% confidence intervals.

3.4 Discussion

Our aim was to investigate the response of African elephants to tourist pressure as a potential stressor, by using fGCM concentrations of elephants and the number of visitors per month in Madikwe Game Reserve. We found that increasing tourist pressure was related to increasing fGCM concentrations, indicating that elephants reacted to an unpredictable stressor (Romero *et al.,* 2009). Season was included in the top model but was not statistically significant at the $p \leq 0.05$ level. We did not find any effect of age and sex on fGCM concentrations, in line with some previous studies (Viljoen *et al.*, 2008a; Pinter-Wollman *et al.*, 2009). Our results indicate that wildlife tourism is a stressor to elephants and are consistent with the results of previous behavioural studies linking elevated fGCM concentrations to heightened aggression of elephants towards humans (Slotow *et al.,* 2008; Jachowski *et al.,* 2012), use of refuge areas with limited human disturbance (Jachowski *et al*., 2013b, c), and human interactions (Millspaugh *et al*., 2007). Our study has thus contributed to a growing body of evidence that tourist pressure is related to an increase in physiological mediators (Romero *et al.,* 2009), in the form of increased fGCM concentrations, in elephants. Further, our study adds to a small body of literature on the effects of non-consumptive wildlife tourism as a stressor in a range of other species such as mountain hares, capercaillie, chamois, primates, and wildcats (Thiel *et al*., 2008; Behie *et al.*, 2010; Piñeiro *et al.*, 2013; Zwijacz-Kozica *et al.*, 2013; Shutt *et al.*, 2014; Rehnus *et al*., 2014).

Research on widlife tourism as a stressor highlights the need to monitor the potential for perceived stressors in wildlife populations exposed to tourism. If animals perceive wildlife tourism as a stressor and GC concentrations are elevated as a consequence of that for a prolonged period of time or frequently, then the negative effects of such chronically elevated GCs (see Chapter 1) could have wide-reaching negative effects on animal welfare, reproduction, growth, immune and inflammatory reactions, memory, and tourist safety (Sapolsky *et al.,* 2000; Sapolsky, 2002; Nelson & Kriegsfeld, 2017). In order to establish whether perceived stress is chronic, fGCMs need to be monitored for a prolonged period of time and in a larger number of individuals, in order to investigate whether they are chronically elevated in relation to high tourist pressure.

Madikwe's strict regulations of only three vehicles in any sighting could have potentially limited the effect of tourist activity on perceived stress in elephants and we had expected only subtle effects of tourism on fGCM concentrations. Further, elephants could have habituated to tourist presence throughout the years, in which case we would not see an effect of tourist pressure on fGCM concentrations. However, we found that fGCM concentrations increased from the lowest estimate of 0.26 µg/g DW when tourist pressure was low, to 0.55 µg/g DW during times of high tourist pressure, an increase of 112% (Fig. 3.2a). It is unknown which stimuli related to tourism may have caused an increase in elephant‘s GC concentrations, but possibilities include increased air traffic, vehicle noise, or vehicle encounter rate.

This study has further provided the first published record of fGCM concentrations of the Madikwe elephant population. The mean (± SD) fGCM concentration from samples collected for this study was 0.39 (± 0.22) µg/g DW. No data of female African elephant fGCM concentrations have been published, with which a comparison of absolute values would be possible. This is due to, for example, differences among studies in methodologies such as sampling protocol, steroid extractions, and steroid assays used (Palme, 2019), as well as differences between different laboratories using the same assay. However, previous studies from Kruger National Park, South Africa, using the same collection procedure, as well as steroid extraction and assay protocols, have provided an estimated fGCM concentration of 0.29 and 0.30 µg/g DW for two adult bulls (Ganswindt *et al.*, 2010a) and a median of approximately 0.32 µg/g DW for six adult bulls (Ganswindt *et al.*, 2010b), which are similar to average concentrations from Madikwe. The two adult bulls from Kruger National Park were also observed to exhibit an increase of 169% and 23% in fGCM concentrations, respectively, to values of 0.78 and 0.37 µg/g DW, during a stressful period of injury (Ganswindt *et al.*, 2010a). The increase of 23% and 169% related to injury in those Kruger bulls fall above and below the increase of 112% related to tourism presented in the current study, indicating that an increase in fGCM concentrations related to tourism is comparable to an increase in fGCM concentrations related to injury. However, the small sample size of only two bulls in Ganswindt *et al.'s* (2003) study should be taken into consideration when interpreting this result.

During the time that the study presented in this thesis was carried out, 38 samples from elephants in Pilanesberg National Park, South Africa, were collected (April – July 2016). Those samples were collected, stored, and analysed using the same methodology/ laboratory as in this thesis and values are therefore comparable on the basis of assay methodology, sample collection, storage and preparation (Palme, 2019). Samples were from one juvenile female, two adult females, 16 adult males and 19 unknown individuals. Pilanesberg samples had a mean (± SD) fGCM concentration of 0.41 ± 0.22 µg/g DW (0.43 ± 0.14 µg/g DW for two adult and one juvenile female, 0.45 ± 0.19 µg/g DW for 16 adult males). The mean (± SD) fGCM concentration of six samples collected in Madikwe during those same months from three adult females and one adult male was 0.45 ± 0.24 µg/g DW. The average values in Pilanesberg and Madikwe were therefore very similar for that period. In Madikwe, tourist numbers during those months were relatively low, especially in May and June. As no further data were collected in Pilanesberg, it was not possible to analyse what factors could have affected those fGCM concentrations. As Pilanesberg has self-driven tourism operating during the daytime, tourist pressure may be a factor affecting elephants there, too. Further, total number of tourists within Pilanesberg each day or month is likely higher, due to the nature of day visits. Future research should aim to assess fluctuations in tourism across seasons in Pilanesberg, in order to gain further insight and should further record details such as behaviour, life history traits, and environmental factors. However, direct comparisons between Madikwe and Pilanseberg based on these data are not possible, due to the many differences between the reserves, such as type of tourism, vegetation, water availability, and elephant population density and history. Additionally, whether high tourist pressure affects elephants in Pilanesberg in a similar manner to that in Madikwe remains speculative.

Fences have been shown to force elephants to revisit foraging patches more frequently, restrict elephant movement, and increase frequency of interactions with unrelated family herds (Munshi-South *et al.* 2008; Loarie *et al.* 2009b), adding to perceived stress of elephants. High densities of elephants, such as at the study sity, could increase competition and frequency of interactions even more, presenting additional stressors. However, if increased competition as a result of Madikwe's high density was a stressor, this would coincide with the dry season and the associated limitation in available resources rather than tourist pressure. Nevertheless, the average fGCM concentration of Madikwe's elephants was similar to baseline concentrations of Kruger bulls (Ganswindt *et al.,* 2010b) and values from Pilanesberg (see above). As season did not significantly affect GC concentrations, this may suggest that the Madikwe population is, in terms of fGCM concentrations, unaffected by its high density at this stage. However, further comparable fGCM concentrations from other populations are required in order to confirm this.

Given the traumatic background of the originally translocated elephants in Madikwe, those individuals may be more prone to perceive humans as a negative stressor. Any sampled individual younger than 22 years at the point of this study would have been born in Madikwe, and hence at least seven of the 13 individuals included in this study could not have been part of this founding population. It is unlikely that those six remaining individuals were the same who were originally introduced. Additionally, so called 'problem animals' are usually shot after attacking humans, with several such cases occuring before 2000 in Madikwe (Slotow *et al.,* 2008). We did not observe elephants to be extremely aggressive towards tourists, unless game drive vehicles approached individuals at a very close distance (<10 meters; I. Szott, Y. Pretorius, *pers.obs*.). Increases in aggression in elephants are a concern for human safety and elephant welfare. Further research is needed in order to identify which stimuli are perceived stressors to elephants in order to inform management of reserves, especially during times of high tourist pressure.

If individuals perceive stressors repeatedly or long-term, physiological mediator responses can become chronic. This would be a concern for elephant health and welfare, as chronic perception of stressors has been linked to an increased risk for disease and loss of body condition, reduced reproductive output, and impaired cognitive function (McEwen & Wingfield, 2003; Millspaugh & Washburn, 2004; Palme, 2012; Scheun *et al.*, 2015; see Chapter 1). Further, chronically perceived stress has been linked to elephants becoming hyperaggressive toward other species and aggressive towards humans (Bradshaw *et al*., 2005; Slotow *et al.,* 2008), which would likely lead to elephants being euthanized.

To assess whether a stress response is chronic, it is necessary to know what duration of time, as well as what magnitude of increase in GC concentrations constitutes as a biologically meaningful response (Romero *et al.,* 2009). It is impossible to assess whether perception of stress is chronic, without knowledge of baseline values (Millspaugh & Washburn, 2004). Furthermore, there is no existing consensus endocrine profile to identify chronically perceived stress across species (Dickens & Romero, 2013). Busch and Hayward (2009) suggest that perceived stress becomes chronic when GCs are elevated for days or weeks, but also state that sometimes GCs drop with exposure to severe stressors. Other studies have classed stress responses as chronic, when the associated physiological mediator response lasted for weeks or months (*e.g.* elk (Millspaugh *et al*., 2001)), or was repeated over successive days (*e.g.* black grouse (Arlettaz *et al.,* 2007)).

In their 2007 paper, Millspaugh and colleagues assessed fGCM concentrations of elephants in relation to human interaction, transport of elephants, as well as during thunderstorms and fireworks. Although their methodologies differed and, as discussed previously, direct comparisons cannot be drawn (Palme, 2019), it is possible to assess the percentage increase of fGCM concentrations during those events. During days on which no elephant-human interactions took place, compared to those where elephants interacted with humans, researchers observed an increase of 21% in fGCM concentrations (Millspaugh *et al.,* 2007). On human interaction days, average fGCM concentrations (± SD) was 27.8 ng/g (± 10.7) whilst on non-interaction days it was 22.9 ng/g (± 8.3; Millspaugh *et al.,* 2007). Although standard deviation of those averages overlapped, their statistical analysis indicated that those were significantly different, nonetheless (Millspaugh *et al.,* 2007). Authors further reported that transporting individual elephants was associated with average fGCM concentrations of 65 ng/g, but up to higher values of 170.8 ng/g and 103.5 ng/g, using the same assay and methodologies (Millspaugh *et al.,* 2007). Further, previous research using the same assay and methodology as Millspaugh and colleagues (2007) has reported that fGCM concentrations of 90 ng/g would indicate a stressed elephant, as this was the recorded peak following an ACTH challenge (Wasser *et al.,* 2000). The presumed baseline of elephants in Millspaugh *et al.*'s (2007) study was approximately 25-35 ng/g (as assessed from Figure 1 in Millspaugh *et al.,* 2007) and the authors suggested that an increase of 20 ng/g from baseline would be biologically meaningful, which would be an increase between 86 – 160%. Therefore, although statistically significant, the increase of 21% in fGCM concentrations related to human interaction days, was not biologically significant.

Tingvold and colleagues (2013) sampled elephant fGCMs for three months around Serengeti National Park, Tanzania, and reported that elephants in areas with higher human disturbance had chronically elevated fGCM concentrations. The difference in fGCM concentrations between the two sampled areas, calculated from their reported mean concentrations inside and outside of the protected area, was 85% and standard deviation did not appear to overlap, as assessed from concentrations presented in their figure (Figure 2 in Tingvold *et al.*, 2013). Additionally, elephants at iSimangaliso Wetland Park, South Africa, sampled in 2001, 2002, 2005, and 2006, were reported to have chronically elevated fGCM concentrations compared to two other parks in South Africa (Pilanesberg and Phinda) sampled from 2003 – 2005 (Jachowski *et al*., 2012). In this study, the increase in magnitude from reported baseline values at Pilanesberg and Phinda (approximately 30 ng/g) to chronically elevated values at iSimangaliso (approximately 50 ng/g), was 67%, and 95% confidence intervals of average concentrations at iSimangaliso did not overlap with those from Phinda or Pilanesberg (Jachowski *et al.,* 2012).

Chronic stress could be related to high tourist pressure in Madikwe. Previous work assessed perceived stress as chronic following an 85% increase of fGCMs from baseline for three months (Tingvold *et al.,* 2013) and following a 67% increase from fGCM concentrations in comparable populations for several years (Jachowski *et al.,* 2012). In Madikwe, an increase of 90% from fGCMs associated with the lowest tourist number (approximately 0.25ng/g and 2150 tourists; Fig. 3.2) related to 0.475 ng/g and approximately 3400 tourists per month. If such an increase was sustained for several months, it could fall within the range of a biologically meaningful and chronic response (see above, Millspaugh *et al.,* 2007; Jachowski *et al.,* 2012; Tingvold *et al.,* 2013). During July, August, and October 2016, tourist number was above 3400. The fGCM concentrations of 0.475 ng/g associated with a tourist pressure of 3400 tourists (Fig. 3.2) over two consecutive months (July-August) in Madikwe could therefore present a chronically elevated stress response. Further, even an elevated stress response over successive days (Arlettaz *et al.,* 2007) or weeks (Millspaugh *et al.,* 2001) has been suggested to be chronic and hence, October 2016, as well as July and August 2016, could also have presented a period of chronic stress to elephants in Madikwe. However, this conclusion remains speculative until a baseline has been established for the Madikwe elephant population.

Madikwe presents one of the highest population densities of elephants in South Africa, where potential effects of chronically increased GCs on suppression of reproduction and hence population growth were not perceived as a problem by local staff and stakeholders due to the large number of elephants in the reserve (I. Szott, *pers.obs*.). Additionally, the increase in population from originally introduced elephants (*n*=228) to when this study was carried out (~1300) does not indicate a negative effect of GCs on reproduction in the Madikwe population. However, if tourist pressure affects fGCMs in elephant populations elsewhere, the related effect of increased GCs suppressing reproduction as well as other negative consequences of chronic stress (see Chapter 1) could be adding to the worldwide decline in elephant numbers (Chase *et al.*, 2016).

There were some limitations to our study. Firstly, we only sampled one adult bull and overall had a small sample size with only few repeated samples collected for some elephants and so results should be interpreted with caution. Larger sample sizes and more samples collected may have detected an effect of sex or age. Some studies have found an effect of age, where adults had higher concentrations compared to juveniles (Pinter-Wollman *et al*., 2009, *n*=38 samples) or sex, where females had lower fGCM concentrations compared to males (Ahlering *et al.,* 2013, *n*=272 samples). However, other studies have not found effects of age (Viljoen *et al*., 2008a, *n*=74 samples) or sex (Pinter-Wollman *et al*., 2009, *n*=39 samples) on fGCMs in elephants, in line with our study.

Our sampling period of 15 months covered only one wet season and one-and-a-half dry seasons. As expected, from the year-round supply of artificially pumped water at Madikwe, we found that fGCM concentrations did not increase significantly during the dry season. However, season was included in the top model, even if not statistically significant and fGCM concentrations were higher in the dry, compared to the wet season (Fig. 3.3). Season not being significant was unusual, as most studies do find an effect of season on elephant fGCMs (*cf.* Foley *et al.*, 2001; Viljoen *et al.*, 2008a). Prolonged sampling across more seasons with a larger sample size may indicate an effect of season.

Oestrogen and testosterone may affect GC concentrations (Handa *et al*., 1994) and reproductive state in form of pregnancy or parturition can therefore affect fGCM concentrations in animals (Palme, 2019). Unfortunately, we were not able to collect information on reproductive state of our sampled females. We are therefore unable to investigate, or control for the potential effect of this on fGCMs. However, at least three of the adult females had suckling calves and were lactating throughout the study period, thus the increase in fGCM concentrations was unlikely to be due to a shift from non-lactation to lactation. Additionally, the effect of tourist pressure followed the same trend in male and female elephants (Fig. 3.2b), suggesting that reproductive state did not affect how females were affected by increasing tourist pressure. Musth itself has previously been investigated for its effect on fGCMs of free-ranging African and Asian elephants and was shown to be related to decreased concentrations of GCs, in line with the suppressive effect of testosterone on the HPA axis (Handa *et al*., 1994; Ganswindt *et al*., 2010b; Ghosal *et al.,* 2013). We did, however, not include males showing signs of musth (urine dribbling, swollen temporal glands, strong smell) in our study.

The small sample size limited the complexity of factors and interactions we could include in our model for statistically appropriate analysis. However, the top model only included two factors and we then used a repeated-measures study design, included ID of each animal to control for individual variation, and applied a Kenward-Roger correction to adjust the *p*-values. Given that we found such a distinct significant effect for tourist pressure with a limited number of samples, even with adjusted *p*-values, the effect of tourist pressure on fGCM concentrations reported here appears to be robust. However, further research is needed, including additional and varied measures to assess stress, in order to identify which exact stimuli are perceived stressors to elephants, so as to inform management of fenced reserves, especially during times of high tourist pressure and to assess whether perceived stress in elephants is chronic.

In order to come to a meaningful conclusion about baseline concentrations of fGCMs and whether they may be chronically elevated, comparisons need to be made with other populations. This requires assessment of fGCM concentrations in other parks and populations. However, such assessment needs to carefully consider differences between parks, such as types of tourism (self-driven or guided), water availability, and population density. Further, prolonged sampling is required to establish stable baseline values.

With regard to direct management implications in Madikwe, the authors encourage the establishment of a refuge area for elephants, as well as other wildlife. Available refuge areas and corridors with limited human disturbance are vital for elephants with increased concentrations of fGCMs (Jachowski *et al.*, 2012, 2013b, c; see Chapter 1). Access to such an area could add to elephants' sense of control, which can reduce perceived stress (Nelson & Kriegsfeld, 2017). As prolonged high fGCM concentrations have been linked to aggression of elephants towards humans (Bradshaw *et al*., 2005; Jachowski *et al.,* 2012), such refuge areas would potentially allow elephants to avoid human contact, and thereby decrease perceived stress and aggression in elephants and increase human safety. However, we are unable to advise on a specific size of such a refuge area as more research would be required to do so. Further, we are not able to link any specific aspect of high tourist pressure to the increase in fGCMs, such as increased traffic, more frequent encounters between elephants and tourists, various smells or any other stimulus. Nonetheless, a large designated area should be established in which no guided walks are conducted, where off-roading of vehicles is strictly forbidden and vehicles are restricted to roads, in order to establish a true refuge area where the probability of encountering humans is reduced. A subsequent assessment of fGCMs and behaviour of elephants should be made to determine if this type of intervention had the desired effect. Due to the southern area of Madikwe having fewer roads in place already, this area may present the best opportunity to establish such a refuge area. A strictly enforced refuge area would likely not only be of benefit for elephants but also for other animals in Madikwe during times of high tourist pressure. Further this would allow lodges in Madikwe to add information on this as advertisement that they prioritise animal welfare.

The present study has added to a growing body of literature investigating the impacts of wildlife tourism on wildlife. Increased tourist pressure led to higher fGCM concentrations in Madikwe elephants. A refuge area, in which tourist access is restricted, would likely add to elephants' sense of control, and may aid in reducing perceived stress related to high tourist pressure. This will increase animal welfare standards as well as human safety during such times.

Author contributions

I.S. carried out data collection, statistical analysis, conceptualized the project and wrote the paper.

Y.P. co-supervised the project and edited the final draft of the paper.

A.G. carried out hormone sample analysis and edited drafts of the paper.

N.K. supervised and conceptualized the project and edited drafts of the paper.

Summary of Chapter 3

In this chapter, I investigated whether wildlife tourism had an effect on fGCM concentrations of free-ranging African elephants. The results showed that high tourist pressure was related to higher levels of fGCMs in the individually identified Madikwe *elephants. Managers should provide wild populations with areas to which tourist access is restricted in order to allow animals to seek refuge during times of increased perceived stress as determined by increased fGCMs. Further, by providing elephants with refuge areas, human safety could be increased during such times. In the next chapter, I will extend this analysis to elephant behaviour and assess whether high tourist pressure was further related to changes in stress-related, aggressive or vigilance behaviour of elephants.*

Chapter 4 – Impact of wildlife tourism on elephant behaviour

Szott, I.D., Pretorius, Y., Koyama, N.F. (2019) Behavioural changes in African elephants in response to wildlife tourism. Journal of Zoology, 308: 164-174. doi: 10.1111/jzo.12661. See Appendix III

A version of this chapter has been published in the Journal of Zoology. Wildlife tourism has been linked to stress-related, aggressive, and vigilance behaviour, as well as animals retreating from tourists in a range of species (eg. Lott & McCoy, 1995; Taylor & Knight, 2003; Dyck & Baydack, 2004; Maréchal et al., 2011; Ranaweerage et al., 2015). In Chapter 3, I presented and discussed evidence that high tourist pressure, in form of total numbers of tourists in the reserve each month, was related to increased faecal glucocorticoid concentrations in individually identified, free-ranging African elephants in Madikwe Game Reserve. Based on this, I expected to observe increased stress-related behaviour, increased vigilance, and increased conspecific-directed aggression in individually identified elephants in Madikwe during times of high tourist pressure. I further expected that elephants would become increasingly likely to move away from tourists who were observing them from game drive vehicles, with increasing numbers of vehicles present.

Abstract

Eco-tourism and human-wildlife interaction can lead to increases in vigilance and aggression as coping mechanisms in many species when animals perceive tourism as a stressor. However, studies investigating effects of wildlife viewing on animals are scarce. We present the first study investigating the impact of wildlife tourism on African elephant, *Loxodonta africana,* behaviour. Over 15 months, we studied the effect of monthly tourist pressure (tourist numbers) on the occurrence of stress-related, vigilance, and conspecific-directed aggressive behaviour in 26 individually identified elephants, using five-minute continuous focal observations. Further, we studied the effect of presence of up to three game drive vehicles on the direction of travel of non-identified elephant herds using scan sampling of herds' movement during a five-minute observation. We analysed the effect of tourist pressure and vehicle presence using and information theoretic method with the Akaike Information Criterion. We ran Generalised Linear Mixed Models, including the following additional factors: habitat type, herd type, herd size, and season in the direction of travel model, as well as sex and age in the behaviour models. As tourist pressure increased, conspecific-directed aggression in elephants increased. Further, we found that elephant herds became increasingly likely to move away with increasing numbers of vehicles present. Results suggest that reserves should monitor elephant behaviour to identify when tourist pressure has potential effects on elephant welfare, and train guides to monitor behaviour and adjust minimum distances of their vehicles to elephants to encourage high animal welfare standards and tourist safety. This study further contributes to a small but growing body of literature on non-consumptive wildlife tourism effects on wild animals.

4.1 Introduction

Observing wildlife as a non-consumptive tourist attraction for recreational purposes has become increasingly popular (Orams, 2002) and can play a key role in global wildlife conservation (Burger & Gochfeld, 1993; Newsome *et al.*, 2005; see Chapter 1). Wildlife viewing, where carried out sustainably, facilitates protection of wildlife habitats, biodiversity, and natural ecological processes worldwide (see Chapter 1; Reynolds & Braithwaite, 2001; Maciejewski & Kerley, 2014). In terms of the management of such protected wildlife habitats, tourist satisfaction is usually a driving goal (Novellie, 1991). Nevertheless, negative impacts on animal welfare, caused by wildlife tourism, have been reported (Moorhouse *et al.*, 2015). Where negative impacts persist long-term, they can potentially lead to decreased reproduction, increased risk of predation, starvation, susceptibility to diseases, altered spatial habitat use (see Chapter 1; Reynolds & Braithwaite, 2001; Teixeira *et al.*, 2007; Bhattacharjee *et al.*, 2015), and lasting changes of behaviour (McEwen & Wingfield, 2003). However, impacts of wildlife tourism on animals are not well understood (Wardle *et al.*, 2018). The few studies that have assessed viewing impact on animals found increases in fearful, alert, aggressive, vigilant, and stress-related behaviour (Asian elephants (Ranaweerage *et al.*, 2015), Indian rhinoceros (Lott & McCoy, 1995), harp seals (Kovacs & Innes, 1990), polar bears, *Ursus maritimus* males (Dyck & Baydack, 2004), boobies (Burger & Gochfeld, 1993)), reduced reproductive fitness (Adelie penguins, *Pygoscelis adeliae* (Giese, 1996), bald eagle, *Haliaeetus leucocephalus* (Grubb & King, 1991)), increased probability of retreat (American bison, mule deer, pronghorns, (Taylor & Knight, 2003), mountain goats (Lott, 1992)), and increased physiological stress responses (African elephants (see Chapter 3), Magellanic penguin (Fowler, 1999)).

Although tourism impacts can be measured in various ways, by observing behaviour, we can detect immediate responses of animals to environmental factors (Taylor & Knight, 2003; see Chapter 1). Mega-fauna, such as African elephants, are among the most popular species for wildlife viewing, particularly for international tourists (Lindsey *et al.*, 2007), yet, research assessing the impact of tourist pressure or tourist presence on elephant behaviour is scarce. Elephants in unfenced areas have been reported to avoid human roads and settlements by altering their behaviour and movement (Hoare & Du Toit, 1999; Douglas-Hamilton *et al.*, 2005; Jackson *et al.*, 2008; Graham *et al.*, 2009; Roever *et al.*, 2013), suggesting active avoidance of human contact by some herds. Only one study has investigated viewing-induced disturbance in elephant behaviour (Asian elephants (Ranaweerage *et al.*, 2015)) in a relatively large population of over 1000 individuals in a fenced national park. Tourist behaviour and vehicle presence increased the likelihood of elephants switching their behaviour from feeding to fear, alert, stress-related or aggressive behaviour. Fear was defined as elephants running away from tourists, alert was defined as elephants adopting a guarding position or fixing their gaze at tourists, stress was defined as elephants tossing soil, swaying repetitively, circling or flapping their ears fast, and aggression was defined as elephants running towards tourists or attacking tourists (Ranaweerage *et al.,* 2015). Additionally, increasing tourist pressure has been shown to be related to increased GC concentrations of individuals in our study population of African elephants (see Chapter 3).

A widely used sustainable method to conserve elephant habitat is to allow wildlife tourism to take place in the form of viewing animals from vehicles, either self-driven or guided (Gössling, 2000; Lindsey *et al.,* 2007; World Tourism Organization, 2014). Elephant populations in South Africa are increasing and tourist demand to view elephants is high (Chase *et al.*, 2016; Arbieu *et al.*, 2017). Given the increasing numbers of elephants that are being viewed by tourists in South Africa, it is important to investigate the impact of tourist pressure on elephant welfare. To our knowledge, no published research has assessed the effect of tourist pressure or game drive vehicle presence on the behaviour of free-ranging African elephants.

In order to assess the effect of tourism, we need to consider other factors which may affect the behaviour of elephants. In elephants, physiological measures of stress responses have previously been shown to be affected by season, where low availability of water and key nutrients during the dry season increased elephants’ faecal glucocorticoid metabolite (fGCM) concentrations (Foley *et al.*, 2001; Viljoen*et al.*, 2008a). In fenced areas, elephants are forced to revisit foraging patches more frequently (Loarie *et al.*, 2009a) and an increased frequency of interactions with unrelated individuals is thought to present a consistent social stressor for elephants (Munshi-South *et al.*, 2008). Elephants compete over access to resources, and agonistic interactions have been reported to occur at point resources such as fruiting trees, waterholes (Archie *et al.*, 2006b), or mineral rich soil (I. Szott, *pers. obs*.). Further, adult bulls can regularly come into musth, a state during which testosterone levels are heightened, aggressive behaviour rises sharply, and bulls become more reproductively active (Poole, 1987; Ganswindt *et al.,* 2005; Hollister-Smith *et al.*, 2007; see Chapter 1). The occurrence and duration of musth is positively correlated with a bull's age (Poole, 1987). Even when not in musth, males have been shown to be the more aggressive sex (Ganswindt *et al.*, 2005; Hollister-Smith *et al.*, 2007), often engaging in dominance interactions with each other (Goldenberg *et al.*, 2014) or bullying younger males (Buss & Smith, 1966). Stress-related, vigilance or aggressive behaviour in elephants may therefore be caused by a variety of factors other than tourism, such as season, sex, age, herd size, herd type (lone male, bull group, cow-calf group or mixed group), and habitat type (open grassland, shrub, dense shrub or waterhole).

Immediate responses to a stimulus such as tourism can give insight into whether the stimulus is perceived as stressor by individuals, to which they respond by displaying coping mechanisms, such as specific behaviours (see Chapter 1; Romero *et al.,* 2009). Our aim was to investigate the effect of wildlife tourism on displays of aggressive, stress-related, and vigilance behaviours by elephants, as well as direction of elephant herd movement in relation to tourists viewing them. Madikwe Game Reserve in South Africa provided a suitable population to study the effects of tourist pressure on elephant behaviour. The founding population was introduced from various backgrounds, such as culling and poaching and, as the effects of such events can be long-lasting (Bradshaw *et al*., 2005; Gobush *et al.*, 2009; Jachowski *et al.*, 2013a), these elephants may be particularly sensitive to the presence of vehicles. Additionally, such traumatic experiences are not an exception for elephant populations across Africa (Chase *et al.*, 2016). Given that previous research found effects of wildlife tourism on stress-related, vigilance, and aggressive behaviour in viewed animals, we predicted that tourists would be a stressor for elephants and that elephants would increase vigilance during times of high tourist pressure in order to avoid encounters with tourists. If avoidance was not possible, we expected increased stress-related behaviour, such as running, touching their own face, or trunk twists (Poole, 1999; Burke, 2008; Poole & Granli, 2009) and aggressive behaviour, such as charging at each other or spreading their ears (see Chapter 2) in order to cope with the stressor. Because point resources, season, and sex are known to influence perceived stress and aggression in elephants, we included these factors in our analyses as control factors, alongside age and herd type (see Chapter 1). Lastly, as we hypothesized that tourists were a stressor, we predicted that elephant herds would be more likely to immediately retreat from tourists observing them from vehicles with increasing numbers of vehicles present representing a flight response to the stressor. Performing such behaviours would indicate a coping mechanism within the reactive scope of individuals, and could, if performed frequently or over prolonged periods of time, indicate the potential of homeostatic overload (see Chapter 1; Romero *et al.,* 2009).

4.2 Materials and Methods

4.2.1 Study site and driving regulations

Madikwe Game Reserve is a reserve managed by a state/private/communal partnership. The reserve, approximately 680 km^2 in size, was fenced and held an estimated 1348 ± 128 elephants (July 2017, P. Nel, *pers. comm.*) equivalent to 1.9

elephants per km^2, representing one of the highest population densities of elephants in South Africa. Elephants were first introduced to Madikwe in 1992 when 25 orphaned juvenile elephants from Kruger National Park culls (operations where herds of adult individuals were culled, and youngsters translocated to other reserves as a measure of population control) were introduced. In 1994, entire herds (194 individuals) from Zimbabwe were introduced having experienced a severe drought, two bush wars, and heavy poaching. In 1998 and 1999, six and two adult bulls (measured by a minimum 3.2 m shoulder height), respectively, were introduced from Kruger National Park.

Private vehicles are restricted to a few roads to travel between lodges and gates in Madikwe. Hence, elephant viewing occurs almost exclusively from game drive vehicles (GDs) where they encounter elephants on roads. A GD is a large open vehicle, driven by a qualified field guide, that seats up to ten people as well as a ‘spotter’ at the front of the vehicle. No more than three GDs were allowed at an elephant sighting. The researchers’ vehicle was not included in this number (see Chapter 2). All vehicles were obliged to park by leaving an unobstructed exit for the vehicle before switching the engine off but were not limited in how close they could approach elephants. Vehicles were not permitted to position themselves between individuals of a herd and had to remain on roads. All guests were briefed on appropriate behaviour. Standing up, loud noise or use of camera flash was not permitted. Eating, drinking, and smoking were strictly forbidden during game drives.

4.2.2 Data collection

Data were collected from the 18th of April 2016 until the 28th of June 2017 (see Chapter 2). The mean (± SD) number of observation days per month was 14 ± 5. An area was sampled by driving randomly selected routes, based on accessibility due to weather and previous reports of elephant sightings by guides, and by communicating with field guides about elephant presence and then searching for those elephants (see Chapter 2). Thirty-three lodges were spread across the reserve and conducted game drives in the morning from sunrise (approximately from 5:30 am) until approximately 11 am, and in the afternoon and evening between approximately 3:30 – 8 pm.

For the behaviour analysis, 26 individuals (14 males, 12 females) were identified based on distinguishing features. Herds included in travel direction analyses were herds which were encountered throughout data collection but were unknown and not individually identified. Upon spotting elephants, the researcher aimed to keep 30 m distance from the nearest elephant. If the animal was spotted at <30 m distance, the vehicle was slowly reversed to 30 m from the nearest elephant before the engine was switched off. When animal/s moved parallel to the road used by the researcher without displaying signs of distress (such as vigilance, body posture changes such as ‘ears out’ threats, or moving away whilst repeatedly looking back at the vehicle), the researcher followed at a distance before switching the engine off again. A bull group was defined as several bulls within a 500 m radius of each other, whilst a mixed group was defined as an adult bull within 200 m of a cow-calf group. All distances were approximated visually.

Data were collected on a Lenovo TAB 2 A8-50F tablet using the Prim8 app (McDonald & Johnson, 2014). The researcher classed elephants as juvenile or adult based on size (Moss, 1996; Poole & Granli, 2009). Adult females had mammary glands and an angled forehead, whilst adult males had a rounded forehead, wider skulls, and could be twice the size of adult females. Juveniles were smaller than adult females, moving and foraging independently, and had tusks of approximately ten centimetres in length. Once a sighting was made from the road, the researcher randomly selected an elephant of any age, which was in full view and close to the research vehicle, as the focal elephant to observe, using continuous focal animal sampling (Altmann, 1974) for five minutes. The researcher noted identity, if known, along with additional factors such as sex, age, and season (Table 4.1). If identity was not known at the time the observation was carried out, photographs of the individual were taken and later compared against a database for identification.

Table 4.1. Factors recorded for five-minute continuous behavioural observations of African elephants, *Loxodonta africana*, carried out in Madikwe Game Reserve, South Africa.

Factor	**Levels**	**Description**
Sex	Female, male	Sex of focal individual
Age	Adult, juvenile	Age of focal individual
Herd type	Lone male, bull group, cow-calf group, mixed group	Type of herd in which focal individual was observed
Herd size	1 – 100	Number of animals in the herd
Habitat type	Shrub, dense shrub, open grassland, waterhole[a]	Type of habitat the focal individual was observed in
Season	Dry, wet	Season during which observation took place
Vehicle	0 – 3	Number of GD vehicles present during the focal observation

[a] *shrub*= various bushes and trees in observed area but not obscuring observation noticeably; *dense shrub*= shrub and trees in observed area, growing so densely that observation only possible at close distance and dense enough to cover view of large areas of the body of the focal animal; *open grassland*= observation area vastly open with only occasional bushes or trees; *waterhole*= water accumulated either naturally or pumped artificially with enough water for one or more elephants to drink

The researcher noted the direction of travel of the whole herd by visually comparing herd location at the start and end of the focal observation and inferring direction of travel. If the centre of the herd (assessed visually through assessment of all visible elephants within the surroundings) increased its’ distance from the observer or, if present, the closest GD ≥10 m (without simultaneously approaching another GD), we classed it as ‘retreat’, otherwise, when herds did not increase their distance by more than 10 m or approached one GD whilst retreating from another we classed it as ‘stay’. Five-minute focal animal observations (Altmann, 1974) were conducted for as long as a herd stayed within the vicinity of the observer, or until all individuals in a herd had been observed once. We only recorded one herd movement observation per encounter, during the first five minutes after a herd was encountered or after a GD(s) arrived, as a measure of immediate reaction of herd movement to the potential stressor. If a herd had already been observed before a GD arrived, the herd movement observation used for analysis was the one during which the GD arrived.

According to previously published ethograms as well as following communication with an elephant expert, Dr Y. Pretorius, behaviours were categorised as stress-related (running, trunk touching own body or face, trunk twirling), vigilance (smelling, touching trunk to mouth, observing surroundings) or aggressive (charging, displacing another, redirecting aggression, having ears out or flapping, slapping, tusking, head shaking, pushing an object, standing tall, trunk swinging or aggressive sparring; see Chapter 1 and 2 for more detail). Because several aggressive behaviours could be directed at either humans or conspecifics, we made note of the direction of the recipient of the threat, and excluded all aggression explicitly directed at the researcher (*n*=5 occasions, where *n*=3 were ‘ears out’ and *n*=2 were ‘head shake’) in the analysis of conspecific-directed aggression.

Season was defined as wet or dry based on mean monthly rainfall measured at four stations within Madikwe by the South African Weather Service. Mean total rainfall during the study period was 189.69 mm. Wet season was defined as the period in which 95% of precipitation for the study year fell (Loarie *et al.*, 2009a, b) and therefore wet season lasted from October 2016 – February 2017 and dry season lasted from April 2016 – September 2016 and March 2017 – June 2017. North West Parks Board, South Africa, provided the total number of tourists visiting Madikwe each month. Tourist number across seasons can be seen in Figure 2.3 in Chapter 2. As each GD carries a maximum of ten tourists, higher tourist number overall generally leads to more GDs being used to accommodate all tourists during GD times and therefore directly relates to a higher number of vehicles on the road (unless tourists chose to stay at the lodges instead of conducting game drives, which is very unusual and would only represent a small number of guests (P. Hatting, C. Cotton, K. Potgieter, *pers. comm*.)).

4.2.3 Data analysis

Only focal observations where the animal was visible for $\geq$4 minutes 30 seconds (s) were retained for analysis. In order to assess the effect of monthly tourist numbers on elephant behaviour in the reserve, we analysed only those observations of individuals in herds with no vehicles present besides the research vehicle. This was to avoid potentially confounding effects on behaviour related to immediate presence of tourists and because the sample size of behavioural observations on individually identified elephants with and without game drive vehicles present was insufficient for analysis. We included individuals that had a minimum number of n=2 observations over the complete time period of the study.

For herd movement direction analysis, we included observations of unidentified herds with and without GDs present. Where a single GD arrived or left within the five-minute observation but was present for less than 60s, the observation was excluded from analysis. If GDs were present for more than 60s, the herd movement was considered to be in response to the number of GDs present for that time. This means that if one GD was present from the beginning, but a second GD arrived and stayed for over 60s, herd movement was assumed to be in response to two GDs present. If a second GD arrived but left in under 60s, the whole observation was considered to be in response to one GD.

Although continuous focal animal observations were carried out, data were zero inflated and therefore we decided to score specific behaviours as occurring or not within each five-minute observation. This presents a conservative estimate, as even observations during which several instances of one specific behaviour occurred, were still only recorded as occurring, rather than adding weight to it. We analysed data using R v.3.4.1 (R Core Team, 2000). We scored each behaviour as occurring or not, and elephant herd travel as away or stay, forming binary response variables. First, we assessed factors to rule out collinearity using variance of inflation factor analysis (see Chapter 2; Fox & Monette, 1992), using a cut-off value of four, where all values were below two. We specified General Linear Mixed Effects Models (package *lme4*, Bates *et al.*, 2014; Bolker, 2009) including all possible two-way interactions to analyse the effect of tourist pressure on stress-related and vigilance behaviour:

$$\begin{aligned} & glmer\ (formula \\ & \qquad = Behavioural\ category \sim Tourist * Herd\ type\ +\ Tourist * Sex \\ & \qquad +\ Tourist * Age\ +\ Tourist * Habitat\ type\ +\ Tourist * Season \\ & \qquad +\ Herd\ type * Habitat\ type\ +\ Sex * Age\ +\ Sex * Habitat\ type \\ & \qquad +\ Sex * Herd\ size\ +\ Sex * Season\ +\ Age * Season\ +\ Habitat\ type \\ & \qquad * Herd\ size\ +\ Habitat\ type * Season\ +\ Herd\ size * Season \\ & \qquad +\ (1|ID), family\ =\ binomial, data\ =\ Data) \end{aligned}$$

Due to insufficient data to support a more complex model, we ran the following global model to assess all possible models including two-way interactions for conspecific-directed aggression:

$$\begin{aligned} glmer\ (formula \\ &= Conspecific\ directed\ aggression \sim Tourist * Sex \\ &+ Tourist * Age\ +\ Tourist * Season\ +\ Sex * Age\ +\ Sex \\ &* Herd\ size\ +\ Sex * Season\ +\ Herd\ size * Season \\ &+ Herd\ type\ +\ Habitat\ type\ +\ (1|ID), family \\ &= binomial, data\ =\ Data) \end{aligned}$$

We scaled and centred the tourist pressure and herd size variables (see Chapter 2). Using the 'dredge' command in the MuMIn package (Barton, 2018), we compared the conditional Akaike Information Criterion (AICc) values (Burnham & Anderson, 2002; see Chapter 2). This function produced a table with all possible candidate models and their associated AICc values from which we identified the top model. Where several models were within 2 AICc of each other, we further followed the criteria detailed in Leroux (2019; see Chapter 2). However, as the top model gives no indication on how weak or strong each individual effect in this model affects the response variable (Mundry, 2011; Symonds & Moussalli, 2011; Harrison *et al.,* 2018), we further analysed significance of each fixed effect in the top model with a type II ANOVA (Langsrud, 2003). Where categorical fixed effects were significant, we assessed differences between the levels using a Tukey post-hoc test in the *multcomp* package (Hothorn *et al.*, 2008), checking that 95% confidence intervals did not cross zero.

For the direction of travel dataset, we excluded the open grassland habitat type from analysis as only *n*=5 observations had one GD present, resulting in poor model fit. The following *a priori* Generalised Linear Model was used:

$$glm\ (formula = Travel \sim Herd\ type\ *\ GD\ number\ +\ Habitat\ type\ *\ GD\ number\ +\ Season\ *\ GD\ number\ +\ Herd\ size, family = binomial, data\ =\ Data)$$

We scaled and centred GD number and herd size. To account for non-independence in the data due to potential pseudoreplication, we performed 1000 iterations of bootstrapping, using the package *boot* (Canty & Ripley, 2018) to obtain bootstrapped 95% confidence intervals. We considered fixed effects significant if confidence intervals did not cross zero. We plotted all graphs using the *effects*- (Fox, 2003) and *ggplot2* (Wickham, 2016) packages (see Chapter 2).

4.3 Results

A total of 156 observations of known individuals were collected (mean ± SD = 6 ± 6 per individual, Table 4.2). These observations were from ten adult males (18 observations as lone males, eight in bull groups, three in mixed groups), nine adult females (56 observations in cow-calf groups, 37 in mixed groups), three juvenile females (eight observations in cow-calf groups, two in mixed groups), and four juvenile males (16 observations in cow-calf groups, eight in mixed groups). We did not observe the following behaviours during our behavioural observations of individuals: slap, pushing object, standing tall, aggressive sparring (see Chapter 2). Removal of individuals with a small sample size or nesting ID in social group did not change the effect of tourist pressure reported below. We recorded travel direction of herds during 479 observations (81 bull groups, 141 cow-calf groups, 100 mixed groups, and 157 lone males).

Table 4.2. Information on number of observations collected of individually identified African elephants, *Loxodonta africana*, in Madikwe Game Reserve, South Africa. Sex, age, ID of the individual, as well as which herd it belonged to is presented alongside number of observations collected during the wet and dry season and the range of herd sizes the individual was observed in.

Sex	**Age**	**ID**	**Herd ID**	**Dry season observations**	**Wet season observations**	**Range of herd size**	**Total**
Female	Adult (*n*=9)	1	A	14	7	4-75	
		2	B	15	10	5-100	
		3	B	10	4	15-40	
		4	C	6	4	10-100	
		5	D	3	/	20-30	
		6	E	3	/	3-14	
		7	F	3	2	15-50	
		8	F	1	1	29-50	
		9	D	7	3	9-30	
	Total observations			62	31		93
	Juvenile (*n*=3)	10	A	3	3	19-40	
		11	C	/	2	14-25	
		12	D	1	1	27-30	
	Total observations			4	6		10
Male	Adult (*n*=10)	13		2	/	2-4	
		14		6	1	1-2	
		15		2	1	1-20	
		16		2	/	1	
		17		1	1	1	
		18		/	2	1-10	
		19		4	1	1-5	
		20		2	/	1-2	
		21		1	1	1	
		22		2	/	6-60	
	Total observations			22	7		29
	Juvenile (*n*=4)	23	A	4	2	5-75	
		24	B	8	6	15-40	
		25	F	1	1	20-29	
		26	D	/	2	20-30	
	Total observations			13	11		24

4.3.1 Stress-related behaviour

The following stress-related behaviours were observed: run (*n*=1 occasion), trunk to body (*n*=4 occasions), trunk to face (*n*=25 occasions), and trunk twirl (*n*=9 occasions). Based on the AICc values reported in table 4.3, the top model for factors explaining stress-related behaviour was the null model (see Chapter 2) and therefore none of the fixed effects had an effect on the likelihood of stress-related behaviour occurring. Other models within 2 AICc only differed by one parameter from this model and therefore were thought to be non-informative.

Table 4.3. Fixed effects included in the top models for the occurrence of stress-related behaviour in identified African elephants, *Loxodonta africana*, in Madikwe Game Reserve, South Africa, as assessed by conditional Akaike information criterion (AICc). Degrees of freedom, log likelihood, delta value and weight for each model are also reported.

Fixed effects included	df	Log Likelihood	AICc	delta	weight
Null model	2	-76.370	156.8	0.00	0.116
Tourist	3	-76.128	158.4	1.60	0.052
Season	3	-76.141	158.4	1.62	0.051
Age	3	-76.345	158.8	2.03	0.042

4.3.2 Vigilance behaviour

We observed smell up (*n*=9 occasions), smell down (*n*=97 occasions), trunk to mouth (*n*=38 occasions), and vigilance (*n*=169 occasions). Based on the AICc values reported in table 4.4, the top model for factors explaining vigilance behaviour included only habitat type (see Chapter 2). Other models within 2 AICc of this model did not differ by more than one additional parameter and were therefore non-informative.

Additionally, the model including the additional parameter of season*tourist was assessed and this parameter was close to zero and 95% confidence intervals crossed zero.

Table 4.4. Fixed effects included in the top models for the occurrence of vigilance behaviour in identified African elephants, *Loxodonta africana*, in Madikwe Game Reserve, South Africa, as assessed by conditional Akaike information criterion (AICc). Degrees of freedom, log likelihood, delta value and weight for each model are also reported.

Fixed effects included	df	Log Likelihood	AICc	delta	weight
Habitat type	5	-86.073	182.5	0.00	0.066
Habitat type + Age	6	-85.616	183.8	1.25	0.035
Habitat type + Sex	6	-85.640	183.8	1.30	0.034
Habitat type + Season	6	-85.747	184.1	1.51	0.031
Habitat type + Season*Tourist	8	-83.651	184.3	1.73	0.028
Habitat type + Herd size	6	-86.028	184.6	2.07	0.023

Vigilance behaviour was significantly more likely to occur at waterholes, compared to all other habitat types as assessed by a type II ANOVA (Table 4.5) and confirmed by a Tukey post-hoc test.

Table 4.5. Results of a type II ANOVA on a GLMM for the occurrence of vigilance behaviour in identified African elephants, *Loxodonta africana*, in Madikwe Game Reserve, South Africa. Fixed effects' estimates and standard errors (SE) are from the model summary and X^2 values, degrees of freedom (df) and *p*-values are from a type II ANOVA. Significant effects in bold, where significance was assigned at $p \leq 0.05$.

Fixed effect (reference level)	Levels	Estimate (± SE)	X^2	df	*p*-value
Intercept		-0.827(± 0.45)			
Habitat (Dense shrub)	Open grassland	0.827(± 0.78)	**32.296**	**3**	**<0.001**
	Shrub	0.112(± 0.41)			
	Waterhole	2.709(± 0.61)			

4.3.3 Conspecific-directed aggression

We observed charge (*n*=1 occasion), displace given (*n*=9 occasions), redirected aggression (in form of throwing of branches, *n*=2 occasions), ears out (*n*=10 occasions), ears flapping (*n*=4 occasions), tusking (*n*=4 occasions), head shake (*n*=8 occasions), and trunk swing (*n*=3 occasions). Based on the AICc values reported in table 4.6, the top model for factors explaining conspecific-directed aggression included habitat type, herd size, sex, and tourist (see Chapter 2). Other models within 2 AICc of this model did not differ by more than one parameter and were therefore not assumed to present a better fit. Further, the model with the additional parameter of sex*season was assessed and this parameter was close to zero and its 95% confidence intervals crossed zero, indicating that this was indeed a non-informative parameter.

Table 4.6. Fixed effects included in the top models for the occurrence of conspecific-directed aggression in identified African elephants, *Loxodonta africana*, in Madikwe Game Reserve, South Africa, as assessed by conditional Akaike information criterion (AICc). Degrees of freedom, log likelihood, delta value and weight for each model are also reported.

Fixed effects included	**df**	**Log Likelihood**	**AICc**	**delta**	**weight**
Habitat + Herd size + Sex + Tourist	8	-52.127	121.2	0.00	0.056
Habitat + Herd size + Sex*Tourist	9	-52.682	122.6	1.36	0.029
Habitat + Herd size + Tourist + Sex*Season	10	-50.722	123.0	1.73	0.024
Habitat + Herd size + Sex + Tourist + Age	9	-51.993	123.2	1.99	0.021

A type II ANOVA on the top model showed that increasing tourist pressure was significantly related to increased conspecific-directed aggression (Table 4.7, Fig. 4.1).

Male elephants were significantly more likely to perform conspecific-directed aggression compared to female elephants, and conspecific-directed aggression became increasingly likely to occur with increasing herd size (Table 4.7). Although conspecific-directed aggression appeared to be marginally affected by habitat type (Table 4.7), Tukey post-hoc tests between habitat types revealed that the confidence intervals crossed zero and were therefore not significant.

Table 4.7. Results of a type II ANOVA on a GLMM for the occurrence of conspecific-directed aggressive behaviour in identified African elephants, *Loxodonta africana*, in Madikwe Game Reserve, South Africa. Fixed effects' estimates and standard errors (SE) are from the model summary and X^2 values, degrees of freedom (df) and *p*-values are from a type II ANOVA. Significant effects in bold, where significance was assigned at $p \leq 0.05$.

Fixed effect (reference level)	**Levels**	**Estimate (± SE)**	**X^2**	**df**	***p*-value**
Intercept		-3.963(± 1.15)			
Tourist pressure		0.663(± 0.27)	**5.940**	**1**	**0.015**
Sex (Female)	Male	1.481(± 0.63)	**5.477**	**1**	**0.019**
Habitat (Dense shrub)	Open grassland	0.333(± 1.55)	7.693	3	0.053
	Shrub	0.887(± 1.45)			
	Waterhole	2.287(± 1.14)			
Herd size		**0.644(± 0.26)**	**6.107**	**1**	**0.014**

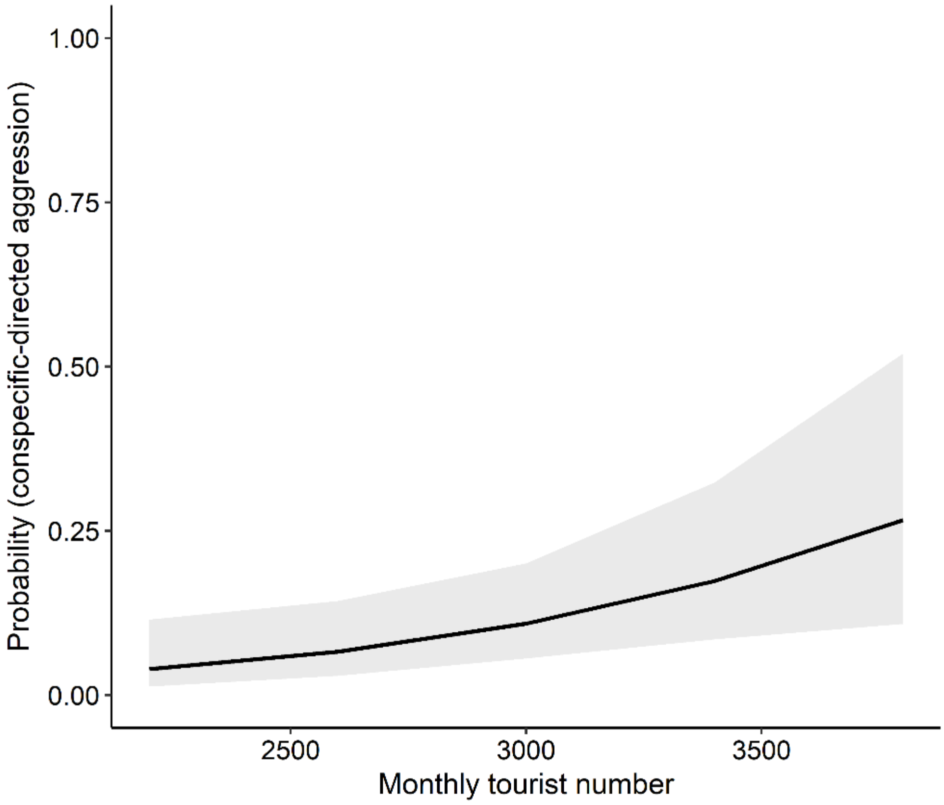

Figure 4.1.

Predicted mean probability of a Generalized Linear Mixed Model analysing the effect of tourist pressure on the probability of identified African elephants, *Loxodonta africana*, in Madikwe Game Reserve displaying conspecific-directed aggressive behaviour. Grey areas represent 95% confidence intervals.

4.3.4 Herd movement

Increasing number of GDs present was related to an increased likelihood of elephant herds moving away depending on the herd type (Table 4.8, Fig. 4.2). None of the other variables affected herd movement (Table 4.8).

Table 4.8. Results of a nonparametric bootstrap (1000 iterations) of a GLM for the impact of several fixed effects on the probability of African elephant herds, *Loxodonta africana*, in Madikwe Game Reserve, South Africa, moving away from game drive vehicles. Fixed effects' estimates and standard errors are from the model summary, and level comparisons and 95% Confidence Intervals are from bootstrapped confidence intervals. Significant effects are shown in bold.

Fixed effect (reference level)	**Levels**	**Estimate (± SE)**	**Levels (reference level vs. comparison level)**	**95% Confidence Intervals**
Intercept		-0.500(±0.39)		
Herd type (Bull group)	Cow-calf group	0.394(±0.35)	Bull group: Cow-calf group	-0.068 to 0.233
	Lone male	0.215(±0.32)	Bull group: Lone male	-0.095 to 0.169
	Mixed group	0.457(±0.40)	Bull group: Mixed group	-0.069 to 0.269
			Cow-calf group: Lone male	-0.187 to 0.093
			Cow-calf group: Mixed group	-0.112 to 0.159
			Lone male: Mixed group	-0.118 to 0.225
Vehicle		0.540(±0.66)		-0.230 to 0.481
Habitat (Dense shrub)	Shrub	-0.318(±0.27)	Dense shrub: Shrub	-0.221 to 0.060
	Waterhole	-0.340(±0.33)	Dense shrub: Waterhole	-0.241 to 0.086
			Shrub: Waterhole	-0.117 to 0.101
Season (Wet)	Dry	-0.128(±0.21)	Dry: Wet	-0.130 to 0.063
Herd size		0.005(±0.13)		-0.055 to 0.065
Herd type* GD (Bull group* GD)	Cow-calf group*GD	-0.485(±0.47)	Bull group*GD: Cow-calf group*GD	-0.360 to 0.038
	Lone male*GD	-0.283(±0.44)	Bull group*GD: Lone male*GD	-0.304 to 0.079
	Mixed group*GD	-0.515(±0.41)	**Bull group*GD: Mixed group*GD**	**-0.340 to -0.001**
			Cow-calf group*GD: Lone male*GD	-0.111 to 0.236
			Cow-calf group*GD: Mixed group*GD	-0.147 to 0.197
			Lone male*GD: Mixed group*GD	-0.174 to 0.063
Habitat type* GD (Dense shrub*GD)	Shrub*GD	0.034(±0.50)	Dense shrub*GD: Shrub*GD	-0.268 to 0.308
	Waterhole *GD	0.103(±0.52)	Dense shrub*GD: Waterhole*GD	-0.271 to 0.349
			Shrub*GD: Waterhole*GD	-0.092 to 0.138
Season*GD (Dry*GD)	Wet* GD	-0.044(±0.22)	Dry*GD: Wet*GD	-0.108 to 0.118

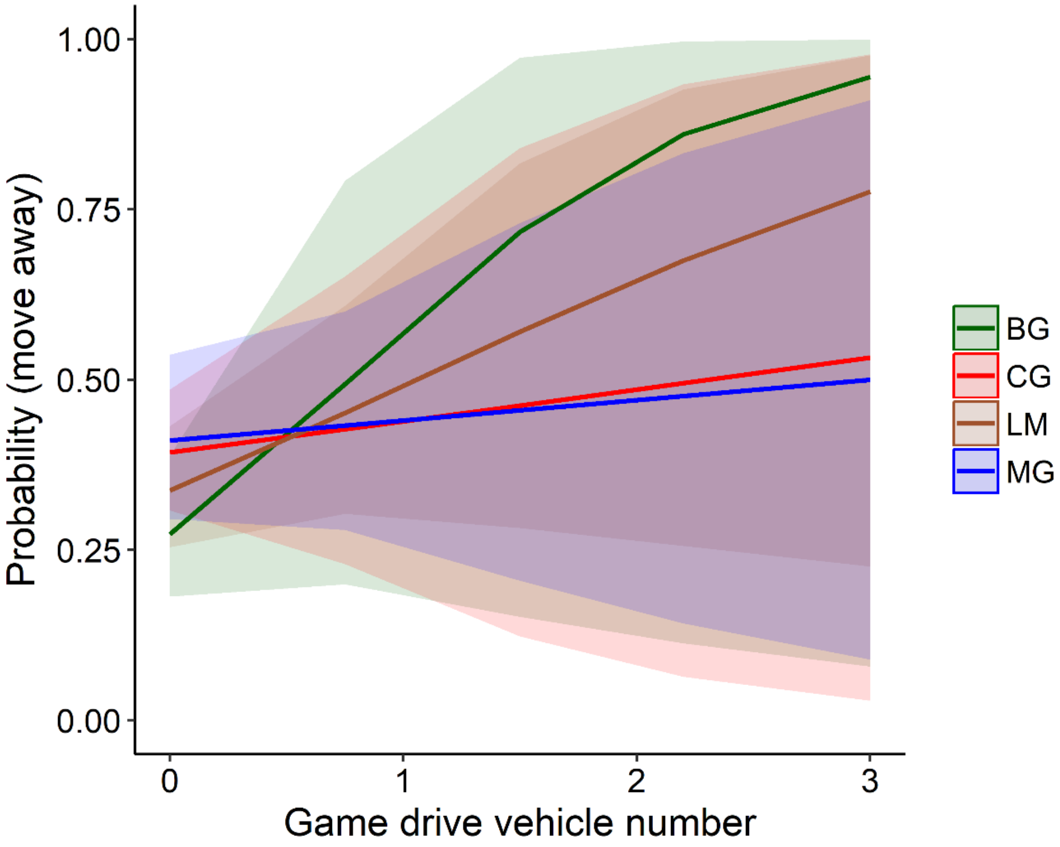

Figure 4.2.

Predicted mean probability of a Generalized Linear Model analysing the effect of game drive vehicle presence on the probability of African elephant herds, *Loxodonta africana*, in Madikwe Game Reserve moving away from observation points in different herd types. BG: bull group; CG: cow-calf group; LM: lone male; MG: mixed group. Coloured areas represent 95% confidence intervals.

4.4 Discussion

This presents the first report of any behavioural measure of the Madikwe elephant population. The study found that wildlife tourism pressure, assessed as total number of tourists within the reserve each month, and game drive vehicle presence influenced the behaviour of African elephants in Madikwe Game Reserve and adds to a small but growing body of literature monitoring the effects of tourist viewing on wildlife (*e.g.* Dyck & Baydack, 2004). Elephants were more likely to move away from tourists with increasing numbers of GDs present. High tourist pressure was related to increased conspecific-directed aggression, but not to changes in stress-related or vigilance behaviour. Results showed effects of habitat type on vigilance behaviour, and sex and herd size on conspecific-directed aggressive behaviour.

Asian elephants displayed more fear, stress, vigilance, and aggressive behaviours during tourist presence in a recent study (Ranaweerage *et al.*, 2015). The results presented here align with the increased aggression, but not other findings of that study. However, Ranaweerage and colleagues (2015) studied elephant behaviour during immediate presence and absence of tourists, whilst the research presented in this chapter focused on overall tourist pressure in Madikwe during that month, but during behavioural observations no tourists were present. This means that all behaviours observed and analysed in the study presented here were during the presence of the researcher only, whilst data from Ranaweerage and colleagues (2015) was collected whilst tourists were present. As such, different findings between the studies are not surprising. Further, Ranaweerage and colleagues (2015) noted that tourist noise, proximity and running vehicle engines were closely associated with changes in elephant behaviour; factors we were unable to assess in this study presented here. As the results from Ranaweerage's study indicate that elephants perceived immediate presence of tourists as a stressor (Ranaweerage *et al.,* 2015), future research should assess direct responses in African elephants to further our understanding of how they react.

In contrast to the study on Asian elephants, Madikwe's guides extensively briefed guests on appropriate behaviour, engines were generally switched off during sightings and the majority of guides maintained an appropriate distance between their vehicles and the elephants (I. Szott, *pers.obs*.). Albeit this impression is based on personal observations from the field and could not be assessed statistically, extreme behavioural reactions (such as charging at vehicles or ears out threats) in response to tourist presence as reported in Asian elephants (Ranaweerage *et al.,* 2015) were rarely observed in Madikwe by the principal investigator. However, although these specific data were not collected, observations from the field confirmed that rare occurrences of high speed of approach by GDs as well as close distance or GDs to elephants did result in elephants displaying aggression towards tourists as well as stress-related and vigilance behaviours (I. Szott, *pers.obs.*). For example, elephants would turn and face vehicles which approached them at a close distance, displaying 'ears out' threats or occasionally even taking a few steps towards vehicles (I. Szott, *pers.obs.*). This reaction was most often observed in adult and juvenile bulls, as well as older females (I. Szott, *pers.obs.*). Additionally, elephants would cease to forage and retreat quickly when vehicles approached with high speed (I. Szott, *pers.obs.*).

Elephant herds became increasingly likely to move away from vehicles with increasing numbers of vehicles present. Whilst this effect was noticeably large in bull groups and lone males, it was only very small in cow-calf and mixed herds. It is possible that the factors related to vehicle presence which affected behaviour in Asian elephants (Ranaweerage *et al.*, 2015), such as noise or close proximity, played a role in how fast or how far herds in the study presented here retreated. Unfortunately, we did not collect information on noise and proximity of vehicles to elephants, and detailed differences affecting herd movement remain unknown. Nonetheless, herds displayed a short-term coping mechanism by removing themselves from the vicinity with increasing numbers of game drive vehicles present. This indicates that elephant herds perceived GDs as stressors.

Travel direction of herds was significantly affected by an interaction between number of GDs present and herd type. More GDs were always related to an increased likelihood of herds moving away from GDs, but this effect was most marked in bull groups compared to mixed groups (Fig. 4.2). This supports the hypothesis that elephants may remove themselves from a tourist stressor and is in line with other studies that found flight responses were affected by tourist presence, where animals moved away from areas with increasing presence of tourists (Lott & McCoy, 1995; Taylor & Knight, 2003; see Chapter 1). It is interesting that this effect was most pronounced in adult males (bull groups and lone males) compared to groups composed of large numbers of adult females (cow-calf groups and mixed groups). It is possible that this reflects a difference in willingness to, or the ability to quickly move away from a resource (Stokke & du Toit, 2002; Woolley *et al.*, 2009). Cow-calf groups and mixed groups contain neonates and young calves, smaller individuals that have reduced mobility. Further, this may potentially indicate a trade-off between the perceived risk and the value of a resource from which groups containing adult females and dependent young move away. Additionally, bull elephants have been shown to travel farther and have larger home ranges compared to female elephant herds (Ngene *et al.,* 2017). Hence, the increased probability of male elephants to move off, could reflect the increased movement of this sex overall. Unfortunately, we do not have data on proximity to water sources or nutrient content of forage during GD events to investigate this possibility.

Tourist pressure effects on elephants’ behaviour produced mixed effects. Conspecific-directed aggression was more likely to occur during high tourist pressure, similar to reported effects of tourism on aggressive behaviour in sea lions and Asian elephants (Lovasz *et al.*, 2008; Ranaweerage *et al.*, 2015). Contrary to our expectation, high tourist pressure did not increase vigilance or stress-related behaviour.

No increase in vigilance during high tourist pressure may be due to elephants using auditory and olfactory mechanisms in order to remain vigilant rather than expressing vigilance behaviour. Elephants have an acute sense of hearing and the ability to perceive seismic vibrations in the ground (O'Connell-Rodwell, 2007). Therefore, the noise of game drive vehicle engines and accumulations of tourists at lodges may be detected by elephants using those means, which could not be easily observed and therefore remained undetected by the data collected in this study. Further, elephant's keen sense of smell (Rasmussen & Wittemyer, 2002) may not require a trunk being extended in the air (which was included as a vigilance behaviour in the ethogram used here) and was thereby not detected by the methodology used for this study. Furthermore, the behavioural category of 'smell down' occurred numerous times during our observations and was classed as vigilance. However, it is possible that this behaviour was performed to smell the downwind scent of a nearby individual. Without experimental manipulation it is impossible to know which exact scent an elephant is picking up during such a behavioural display. Also, as tourists were present all across Madikwe, auditory and olfactory stimuli may have been present in all directions and from all angles, which may be too much to allow elephants to focus specifically on one single direction of the stimulus reaching them. Hence, vigilance behaviour for a stimulus occurring from all directions such as indirect tourist pressure, may not be an appropriate measure to collect for this specific analysis.

Vigilance behaviour may have been an inappropriate type of behaviour to assess in response to indirect measure of tourist pressure as such behaviour is usually associated with immediate potential threats. Because tourists were not immediately present during behavioural observations, this may have been the wrong measure to use. Additionally, vigilance behaviour is known to be affected by spatial position in the herd (Burger & Gochfeld, 1993; Hunter & Skinner, 1998; Beauchamp, 2007) and it is possible that this influenced our findings. Unfortunately, we did not have data on spatial position to control for this possibility.

As we found increased concentrations of faecal glucocorticoid metabolites during times of high tourist pressure in our study population (see Chapter 3) we expected to observe increased stress-related behaviour. It is unlikely that we did not detect stress-related behaviour due to the specific behaviour not being recorded, as the *ad libitum* sampling method used for this study recorded all occurring behaviour (Altmann, 1974). However, elephants could only be observed and hence included in the study if they remained in close vicinity. This means that elephants which may perceive not only tourists but also the research vehicle and researcher as a stressor, could have left the area immediately. Running away from a specific stimulus or running in general was classed as a stress-related behaviour in this study but could only be observed if it occurred during an observation. In the case where elephants moved off quickly, potentially running, before an observation started, this would have been a stress-related behaviour but not been included as a behavioural observation.

In a study of Barbary macaques, faecal glucocorticoid levels were not related to measures of tourist pressure (number of tourists present, duration of tourist presence, proximity of tourists to macaques) but were related to aggressive interactions with tourists, whilst a behavioural indicator of anxiety (scratching) was positively related to the maximum number of tourists present (Maréchal *et al.*, 2011). Similarly, Humboldt penguins, *Sphenicus humbolti*, have responded to tourism with an elevated heart rate whilst no behavioural changes were observed (Ellenberg *et al.*, 2006). Thus, whilst these behavioural measures have been found to be useful indicators of GC concentrations in many species (Fowler, 1999; Rehnus *et al.*, 2014) it is possible that behavioural expression and physiological response are triggered by different aspects of the stressor such as proximity or direct and indirect stressors such as immediate tourist presence and tourist pressure within the habitat (Higham *et al.*, 2009; Mandalaywala *et al.*, 2014; Young *et al.*, 2014). Further, increased concentrations of glucocorticoids make energy available for individuals, for example in order to perform coping behaviours (see Chapter 1). Therefore, although we did not find an increase in specific stress-related behaviours, aggression may have been a coping behaviour (see Chapter 1; Romero *et al.,* 2009).

It may be possible that stress-related behaviour was not observed here, as observations were carried out when no tourists were immediately in the vicinity during the observation. Rather, tourist pressure in this study was an indirect measure. It is possible that stress-related behaviours are performed as a direct response by elephants during immediate presence of tourists, as has been reported for Asian elephants (Ranaweerage *et al*., 2015). Unfortunately, the amount of data collected during the study period did not allow statistical analyses of behaviour during immediate game drive vehicle presence. Future studies should observe stress-related behaviour during tourist presence in order to assess whether stress-related behaviour is displayed as an immediate response to tourists. Further, stress-related behaviour may be found to reflect in increased GC concentrations if behaviour is observed in direct response to immediate presence of a stressor such as GDs.

As predicted, habitat type had an effect on vigilance behaviour and sex and herd size affected conspecific-directed aggression. Waterholes are a point resource (Archie *et al.*, 2006b) where vigilance behaviour was significantly more likely to occur, and male elephants have repeatedly been shown to be the more aggressive sex (Ganswindt *et al.*, 2005). Further, increasing herd size increases the potential for conflict with conspecifics as well as the number of individuals which may display conspecific directed aggression.

There are several limitations to this study which should be considered. Firstly, the elephants included in this study were those we were able to observe repeatedly in order to collect data. This could have resulted in a bias toward elephants which are more comfortable with the presence of a human observer or GDs to begin with, and therefore elephants which are less likely to perceive tourists as stressors. Indeed, during collection of data elephants which immediately moved away were encountered occasionally. However, elephants which were not observed due to the previous reasons likely perceived tourism or vehicle presence as a stronger stressor compared to elephants which were observed in this study. Therefore, if this were the case, tourism would be a stronger stressor than indicated by this study, rather than no stressor at all. Further, although we aimed to collect data on immediate behavioural responses during tourist presence, these data were not sufficient for statistical analyses.

Changes in the frequency and increased or decreased occurrence of behaviours can indicate chronic stress and poor welfare (Jennings & Prescott, 2009; Williams *et al.,* 2018). In captive animals, for example, increases in repetitive behaviour and decreased locomotion and feeding have been used as indicators of poor welfare (Koyama *et al.,* 2012; Williams *et al.,* 2018). In order to be able to use behaviour as an indicator of chronic stress, researchers need to collect repeated observations of known individuals over time. If tourist presence is related to a consistent change in behaviour which does not, in turn, have potential negative welfare implications, it could indicate a successful coping mechanism (Romero *et al.,* 2009). However, if an individual changes its behaviour in response to tourist presence over time in a way that itself can have negative welfare effects, for example by becoming increasingly aggressive, vigilant, or restless, this could indicate chronic stress.

Future studies may consider collecting data using video cameras, albeit in the present study this was trialled originally, but restricted the observable area and did not produce high enough resolution to reliably assess and score behaviours (I. Szott, *pers.obs*.). Where field conditions allow researchers to control for those potential issues, video cameras may offer an effective alternative to being physically present to collect behaviour observations. Without immediate physical presence, potential confounding effects of human presence would not affect animal behaviour and further, video footage can be repeatedly analysed and allow assessment of inter- and/ or intra-observer reliability. Drones have been used recently in wildlife conservation studies and their use warrants further investigation (Koh & Wich, 2012) but would likely present a disturbance to elephants, interfering with their behaviour (I.Szott, *pers.obs.)*, as well as limiting the view to one from an aerial viewpoint. One method which may provide useful, would be to set up hides at strategically placed observation points such as waterholes, from where elephants can be observed without making the presence of human observers noticeable.

Another restriction to this study was the limited number of observations collected for some individuals, and the relatively short duration of five-minute sampling. Future research should aim to collect a larger amount of longer observations, spread evenly throughout the seasons and between sexes and ages. Longer observations may detect further behavioural changes which were missed during the five-minute observations carried out for the research presented here. Such changes in sampling will allow for stronger arguments to be made based on the results.

Lastly, the movement of elephant herds was assessed visually in this study, by assessing the core of a herd at the beginning and end of an observation. This may have introduced a bias, especially in dense shrub areas, where observations of herds' cores were more difficult. Nonetheless, because a distance of ten meters was used in this study, the difference in herd core placement was easily noticeable. If vegetation was very dense and only individuals close to the road were visible, movement of herds away from the road resulted in fewer individuals being visible at the end of the observation, which made it possible to assess core movement. Further, by classing herd travel as either 'away' or 'stay', we used a broad estimate rather than fine scale differences in movement, adding confidence to our methodology.

4.4.1 Practical implications

Behavioural studies highlight the contribution that behavioural indicators of welfare can make to the management and assessment of welfare of wild populations (Goldenberg *et al.*, 2017). Our results show that, even with regulations in place, where wildlife viewing is carried out exclusively from GDs driven by qualified guides and overall numbers of tourists viewing elephants at any time are restricted, high tourist pressure and tourist presence led to changes in behaviour of elephants. However, the changes in behaviour were relatively limited, possibly because elephants were able to move away from the stressor or possibly because some aspects of behaviour were not observed during immediate presence of tourists. The limited effects suggest that, with careful management, wildlife tourism can be conducted in a welfare focused manner and that wildlife tourism holds a promising future as a conservation measure.

The Code of Conduct in Madikwe did not stipulate a minimum distance to be kept from elephants. As elephants performed more aggressive behaviours during times of high tourist pressure, and because waterholes are a point resource over which elephants compete, we highlight the increased chance of conflict with nearby GDs during times of high tourist pressure and at waterholes. Elephants at waterholes could experience frustration as well as being the target of aggressive behaviour from conspecifics, increasing the possibility that they will display redirected aggressive behaviours (Rajaram, 2006) towards bystanders such as vehicles. At Madikwe, field guides were aware of elephant behaviours signalling aggression (I. Szott, *pers. obs.*), and such ability to interpret behaviour is of value when approaching wild animals, as distances can be adjusted early on, when signs of aggression are detected, to mitigate potential negative effects. We suggest that a consistent minimum distance from the nearest individual, especially upon first approach, should be introduced to guidelines for wildlife viewing to alleviate the potential for conflict between tourist vehicles and wildlife. This will ensure not only the safety of guests but would also alleviate the potential additional stressor of increased agonistic interactions which could otherwise lead to repeated or long-term perception of stress (McEwen & Wingfield, 2003; Romero *et al.,* 2009; Pinter-Wollman *et al.*, 2009; Jachowski *et al.*, 2013b). It would further provide elephant herds, or indeed other wildlife, with an increased distance to humans and may reduce the likelihood of animals moving off, giving tourists longer, more natural viewing experiences.

Due to strict regulations in Madikwe, tourist pressure is based on maximum availability of lodges hosting tourists and GDs are restricted to three vehicles at sightings. However, demand to view animals in their natural habitat is growing and most wildlife viewing worldwide, not only of elephants but a broad range of species, is carried out in areas where fewer/ no restrictions apply (see Chapter 1; World Tourism Organization, 2014). Research into effects of non-consumptive wildlife tourism on animals, where no direct interactions between human and non-human animals take place, is scarce but has consistently reported aggressive, stress-related or vigilance-related responses by wildlife (Dyck & Baydack, 2004; Lovasz *et al.*, 2008; Ranaweerage *et al.*, 2015). Consideration of personality traits of animals (Lee & Moss, 2012; Goldenberg *et al.*, 2017), controlled for through repeated sampling of known individuals, would further inform our understanding of the effects of wildlife tourism on wild animals, as individuals which are bolder, for example, may cope with tourism stressors better than those which are shy (Nelson & Kriegsfeld, 2017). Such knowledge could inform how guides approach known individuals during times of high tourist pressure, for example. It is important that future research investigates whether animals in other reserves react in a similar manner, showing changes in aggressive behaviour and herd movement. This will allow management decisions to be guided by up-to-date, quantitative, and qualitative findings and allow reserves to advertise that they consider implications of tourism on the welfare of animals in their care.

Author contributions

I.S. carried out data collection, statistical analysis, conceptualized the project and wrote the paper.

Y.P. co-supervised the project and edited the final draft of the paper.

N.K. supervised and conceptualized the project and edited drafts of the paper.

Summary of Chapter 4

In Chapter 3 I found a relationship between high tourist pressure and elevated GC concentrations and Chapter 4 built on results from Chapter 3. Contrary to my expectation, high tourist pressure was not found to be related to increased stress-related or vigilance behaviour in elephants. However, high tourist pressure was related to an increase in the probability of conspecific-directed aggression. Further, elephant herds became increasingly likely to retreat from tourists observing them with increasing numbers of vehicles present. As elephants showed some behavioural and physiological indicators that high tourist pressure and tourist presence in form of game drive vehicles presented a stressor to them, I further investigated the spatial behaviour of three adult female elephants in relation to high tourist pressure in Madikwe in Chapter 5.

Chapter 5 – Impact of wildlife tourism on elephant spatial behaviour

In the previous two chapters, I investigated faecal glucocorticoid metabolite concentrations as well as changes in behaviour in free-ranging African elephants in relation to high tourist pressure. In Chapter 4, I observed that elephant herds were more likely to move away from vehicles with increasing numbers of vehicles present, as well as to show increased conspecific-directed aggression during months with high tourist pressure. In Chapter 3, I reported increased GC concentrations during high tourist pressure. Those results suggest that elephants perceived wildlife tourism as a stressor. African elephants have been shown to alter spatial behaviour in response to human activity, roads, and human settlements (Chase & Griffin, 2009; Munshi-South et al., 2008; Orrick, 2018). Therefore, I expected that elephants would alter their spatial behaviour during times of high tourist pressure. I used geographical positioning service (GPS) data from satellite collars from three adult female elephants in Madikwe during the same study period as in Chapters 3 and 4 to investigate any changes in spatial behaviour related to wildlife tourism.

Abstract

Several species are negatively affected by wildlife tourism, where tourists view animals in their natural environment without directly interacting with them. Long-term, such effects may negatively impact on an animal‘s fitness, health and welfare, and lead to lasting changes in behaviour. African elephants, *Loxodonta africana*, are commonly viewed by tourists across Africa, yet studies assessing the impact of tourist pressure, in the form of visitor numbers, on free-ranging elephants are scarce. The spatial behaviour of three collared adult female elephants, each belonging to a separate herd in Madikwe Game

Reserve, South Africa, was investigated over 12 months. We investigated whether tourist pressure had an impact on females‘ movement using Akaike Information Criterion for an information theoretic approach. Generalised Linear Mixed Effects Models (controlling for month as a random effect) were applied to monthly 95% isopleth home range sizes and to mean hourly journey length during five time periods throughout the day and night. Fixed effects were tourist pressure (monthly visitor numbers), season, and either Normalized Difference Vegetation Index (NDVI) or temperature. Females‘ home ranges were larger during the wet season, compared to the dry season. Average hourly journey length was affected by a positive interaction effect between the different periods and temperature, where increasing temperature increased the distance travelled during the morning (6 – 11 am), afternoon (3 – 8 pm), and early night (8 pm – 1 am), whilst it decreased distance travelled during midday (11 am – 3 pm) and late night (1 – 6 am). Tourist pressure did not affect females‘ movement in this study. Managers should monitor potential impacts of wildlife tourism on populations and we have demonstrated that large-scale movement data can be used for such analyses.

5.1 Introduction

Tourists observe wild animals in their natural habitat for recreational purposes on a global scale (Burger & Gochfeld, 1993; Orams, 2002; Newsome *et al.*, 2005; see Chapter 1). Such tourism, here termed wildlife tourism, can protect habitat, biodiversity, and natural ecological processes, but only if carried out in a sustainable manner (Reynolds & Braithwaite, 2001; Maciejewski & Kerley, 2014). However, to make wildlife tourism sustainable, animal welfare needs to be prioritized over another goal in management: tourist satisfaction (Novellie, 1991). Wildlife tourism has been shown to have several negative impacts on animal welfare (Moorhouse *et al.*, 2015) as animals perceive tourism as a negative stressor which challenges their predictive homeostatic state (Romero *et al.,* 2009). When such negative impacts of tourism persist over time and individuals cannot cope with them within their normal reactive scope, this can lead to decreased reproductive output, increased risk of predation (for example by increasing the risk of predation of young when breeding behaviour is disturbed (Reynolds & Braithwaite, 2001) or through a decrease in antipredator investment (Teixeira *et al.,* 2007)), increased chances of starvation, increased susceptibility to diseases, and can have lasting effects on behavioural patterns in animals (see Chapter 1; Reynolds & Braithwaite, 2001; McEwen & Wingfield, 2003; Teixeira *et al.*, 2007; Romero *et al.,* 2009).

Across Africa, one of the most popular species being viewed in the context of wildlife tourism, is the African elephant (Lindsey *et al.*, 2007), a species threatened with a drastic decline in population numbers and number of countries in which they occur due to habitat loss and poaching (Bouche *et al.*, 2011; Maisels *et al.*, 2013; Chase *et al.*, 2016). Observing elephants from vehicles is in high demand by tourists and used widely for wildlife tourism purposes (World Tourism Organization, 2014; Chase *et al.*, 2016; Arbieu *et al.*, 2017). Although revenue from such tourism aids in the protection of habitat and biodiversity (see Chapter 1; Reynolds & Braithwaite 2001), research assessing the impact of wildlife viewing on animals is scarce (Wardle *et al.*, 2018).

An animal‘s seasonal range is influenced by energetic constraints, social factors and the abundance and distribution of resources (Osborn, 2004; Cerling *et al.*, 2006; Wittemyer *et al.*, 2008). For example, the size of elephant home ranges in unfenced and fenced areas is influenced by water distribution (Jarman, 1972; Ottichilo, 1986; Lindeque & Lindeque, 1991; Purdon & van Aarde, 2017). Home ranges in unfenced reserves are generally larger during the wet season, compared to the dry season when elephants are restricted to areas close to water sources (Lindeque & Lindeque, 1991; Shannon *et al.*, 2006; Chase & Griffin, 2009). Studies in fenced areas have found different effects of season on home range sizes (Shannon *et al.*, 2006; Orrick, 2018). In fenced areas, restriction of movement forces elephants to revisit foraging patches more frequently, and waterholes holding water throughout the year further alter elephant spatial patterns by allowing elephants access to foraging patches which otherwise would be inaccessible (Loarie *et al.*, 2009a).

Elephants have been shown to alter their behaviour to avoid contact with humans (Chase & Griffin, 2009) and an individual's spatial location may also be dependent on past experiences (Roever *et al.*, 2014). For example, elephants travelled faster through unprotected areas compared to protected areas, and more often used unprotected areas during the night (Douglas-Hamilton *et al*., 2005; Graham *et al*., 2009). Elephants also avoided areas of high human density and areas near main roads (Roever *et al*., 2013) and, at a certain density threshold of humans, elephants leave human dominated landscapes entirely (Hoare & Du Toit, 1999). Additionally, elephants have been observed to increasingly raid crops during the night, compared to during the day, and to increasingly drink at night when abundance of available water sources is limited to those close to human settlements during the dry season (Jackson *et al.,* 2008). Elephant abundance is usually higher further away from human settlements (Blake *et al.*, 2007; Remis & Kpanou, 2010; Stokes *et al.*, 2010). Previous research suggested that elephants avoid roads (Orrick, 2018; Molina-Vacas *et al.,* 2019) and prefer habitat away from roads during the day when they are in use, but forage there at night (Munshi-South *et al.*, 2008). This suggests that elephants actively seek out areas of decreased human disturbance. However, in Etosha National Park, Namibia, elephants actively seek out roads, thought to be due to easier and more direct travel between water sources (Tsalyuk *et al.,* 2019). Additionally, use of roads increased during the high tourist season and authors suggested this may be due to a better condition of roads during that time, indicating elephants were not currently disrupted in their ranging behaviour by tourist vehicles (Tsalyuk *et al.,* 2019). Further, perceived risk, such as high poaching pressure, has led to shifts in home ranges of elephants away from areas of high poaching risk (Goldenberg *et al.,* 2018).

Elephants with elevated GC concentrations exhibit refuge behaviour, where they restrict their use of space, stay near refuges and display less exploratory behaviour (Jachowski *et al.*, 2012). Perceived stressors can be linked to ecological factors, such as long periods of drought, dry seasons or large fires (Woolley *et al.,* 2008), or they can be linked to antrhopogenic factors, such as high human density.

Refuge areas (areas with no- or very limited human disturbance) not only increase elephant welfare, but also human safety during times when elephants perceive multiple stressors. This is because elephants experiencing stress responses long-term and exhibiting refuge behaviour have also been implicatd in being hyperaggressive, where elephants have attacked humans, sometimes resulting in injury or even death of elephants or humans involved (Slotow *et al.*, 2008; Jachowski *et al.*, 2012). With provision of refuge areas, elephants exhibiting refuge behaviour as a means to coping with perceived stressors, the risk of elephants exhibiting hyperaggressive behaviour towards humans could be decreased. Such use of a refuge area has, for example, has been observed in elephants in Pilanesberg National Park, South Africa, following a large fire (Woolley *et al.*, 2008).

Previously, Asian elephants have been reported to perform alert (gaze fixed at tourists, guarding position), stress (flapping ears, tossing soil, swaying head and shoulders), and aggressive behaviours (running towards or attacking tourist vehicles) when tourists were viewing them (Ranaweerage *et al.*, 2015). In African elephants, evidence reported in this thesis suggests that high tourist pressure is related to increased glucocorticoid concentrations (see Chapter 3), increased conspecific-directed aggression, and elephant herds moving away from tourists observing them from vehicles with increasing numbers of vehicles present, especially lone males and bull groups (see Chapter 4). Additionally, studies have shown that wildlife tourism affected the spatial behaviour of a range of species, where animals increased their speed of travel, the distance between themselves and tourists, or retreated in response to tourist presence (Lott, 1992; Burger & Gochfeld, 1993; Lott & McCoy, 1995; Taylor & Knight, 2003; Lusseau & Higham, 2004; Burgin & Hardiman, 2015; see Chapter 1). However, to our knowledge, no studies have assessed the impact of tourist pressure on elephant spatial behaviour.

Experiencing and encountering unpredictable stressors is a normal occurrence in the lives of wild animals, however, prolonged or frequent stressors and inability to cope with them (assessed through using a variety of measures such as glucocorticoid concentrations and changes in behaviour) can lead to homeostatic overload and wide-reaching negative effects for animal welfare and survival (Liptrap, 1993; Mendl, 1999; O'Connor *et al.*, 2000; Reynolds & Braithwaite, 2001; Möstl & Palme, 2002; Sapolsky, 2002; McEwen & Wingfield, 2003; Teixeira *et al.* 2007; Romero *et al.,* 2009; see Chapter 1). In terms of spatial behaviour, home range size and the total distance travelled (journey length) could be affected if elephants perceived tourists as a stressor (see hypotheses below). Faster or further travel is energetically costly as it increases locomotion, decreases foraging time, and eventually impacts on the fitness of individuals (McComb *et al.*, 2014). Given that elephant populations are declining (Chase *et al.*, 2016) whilst wildlife tourism offers possibilities to preserve elephant habitat, it is increasingly important to understand whether wildlife tourism affects the spatial behaviour of elephants.

We aimed to investigate whether wildlife tourism is a stressor for African elephants (Romero *et al.,* 2009) and whether elephants cope with the stressor through changes in their ranging behaviour, by studying the spatial movements of three adult female elephants in Madikwe Game Reserve, South Africa. Madikwe is a fenced reserve with a founding population of African elephants introduced from various traumatic backgrounds, such as culling and poaching, effects of which can be long-lasting (Bradshaw *et al.*, 2005; Gobush *et al*., 2009). As prior research, presented above, has reported that elephants actively avoid human features such as roads and settlements, we hypothesized that tourist pressure (monthly number of visitors in Madikwe), would influence elephant spatial behaviour. We hypothesised that elephants would aim to avoid contact with humans and therefore their monthly home range would be smaller during times of high tourist pressure (Orrick, 2018). In order to examine potential shifts in distance travelled throughout the day, we compared mean distance travelled during five time periods. As we found that elephants became increasingly likely to move away during presence of game drive vehicles (see Chapter 4), we expected journey length to be longer during high tourist pressure, due to the potential of more frequent encounters with tourists within the reserve which displaced elephants more frequently. We further hypothesised that, during the dry season, elephant home range would be smaller, and that hourly journey length would be affected by season, although we were unable to predict directionality of this effect. We therefore included the effect of season, as well as a measure of productivity, the Normalized Difference Vegetation Index (NDVI; Young *et al.*, 2009), in our analysis. Further, because elephants have been reported to rest during the hottest hours of the day (Shannon *et al*., 2008), we assessed the effect of mean monthly temperature in our analyses.

5.2 Materials and methods

5.2.1 Study site

Madikwe Game Reserve is approximately 680 km^2 in size and managed by a state/private/communal partnership. The reserve is fenced and holds an estimated 1348 ± 128 elephants (1.9 elephants per km^2, July 2017, P. Nel, *pers. comm.*), representing one of the highest population densities of elephants in South Africa. Following culls in Kruger National Park (operations where herds of adult individuals were lethally wounded and youngsters translocated to other reserves as a measure of population control; Dickson & Adams, 2009), 25 orphaned juvenile elephants were first introduced in 1992. Then, in 1994, entire herds (194 individuals) were introduced from Zimbabwe, having experienced two bush wars, heavy poaching, and severe drought in Zimbabwe. The last elephants were introduced in 1998 and 1999, with six and two adult bulls (minimum 3.2 m shoulder height) from Kruger National Park, respectively.

A total of 33 lodges were situated in Madikwe, each with a waterhole providing water year-round. Additionally, the reserve was bordered by the Marico River along the east and contained several large artificial dams, which pumped water year-round. Use of private vehicles was restricted to roads connecting the lodges and gates. Wildlife viewing occurred almost exclusively from game drive vehicles. Game drive vehicles were large, open vehicles driven by qualified guides, seating up to ten people and a ‘spotter’ at the front of the vehicle. A maximum number of three game drive vehicles were allowed at an elephant sighting at any given time.

North West Parks Board fitted three adult females from three separate herds with satellite collars (African Wildlife Tracking, Pretoria, South Africa; see Chapter 2 for more details). Collars were fitted by veterinarians holding Threatened or Protected Species permits (permit #S21200, unique registration M132/6502195751085). The veterinarian, upon locating a breeding herd from a helicopter, darted a large adult female with 1.2 ml Etorphine (M99) in combination with Azaperone. On the ground, the collar was fitted, and the female's sedation reversed with Naltrexone before she re-joined her herd. In all cases, the procedure on the ground lasted no more than 20 minutes and the female was observed re-united with a herd on the same day. Because *Mia* repeatedly moved her collar up-side-down, the collar was removed by the North West Parks Board in April 2017. The remaining two collars were removed by the North West Parks Board in September 2018 without complications.

5.2.2 Data collection

Data collection received ethical clearance from Liverpool John Moores University (NK_IS/2016-6) as well as permission from the North West Parks Board, South Africa.

Satellite collars provided hourly readings for the three females (*Ivy* from the 22nd June 2016 – 30th June 2017 (12 months, 8 days), *Joy* from the 1st July 2016 – 30th June 2017 (12 months), and *Mia* from the 1st September 2016 – 28th February 2017 (6 months)), which we used for home range and hourly journey length analyses.

We used Landsat 8 (Roy *et al.*, 2014) eight-day composites and Moderate Resolution Imaging Spectrometer (MODIS) daily NDVI (Lunetta *et al.*, 2006) images to calculate monthly mean NDVI. Images were downloaded using Google Earth Engine on the 8th of January 2018 with a 30 m and 250 m resolution for Landsat 8 and MODIS images, respectively. For monthly average temperature, we extracted temperatures in hindsight from historical records (in ° Celsius) online (https://www.worldweatheronline.com/madikwe-weather-history/north-west/za.aspx). Average monthly temperature ranged between 16° C and 30° C.

Dry and wet season were defined based on average monthly rainfall where wet season was defined as the period in which 95% of precipitation for the study period fell (Loarie *et al.*, 2009a, b). Rainfall data were provided by the South African Weather Service measured at four stations across Madikwe. Dry season mean (± SD) rainfall was 6.13 ± 11.8 mm (June 2016 – September 2016 and March 2017 – June 2017) and mean (± SD) wet season rainfall was 118.89 ± 63.51 mm (October 2016 – February 2017).

Time periods were classed as morning (6 – 11 am), midday (11 am – 3 pm), afternoon (3 – 8 pm), early night (8 pm – 1 am), and late night (1 – 6 am). The morning and afternoon time periods were chosen as they coincided with times during which lodges in Madikwe carry out game drives and we aimed to have a relatively equal number of hours within each period. Total number of tourists visiting Madikwe each month was provided by the North West Parks Board.

5.2.3 Data analysis

Hourly journey length was extracted using QGIS v. 2.18.12 (QGIS Development Team, 2015), where we extracted the mean distance travelled (in km) during times corresponding with the time periods defined above. We then calculated mean distance travelled during each time period per month for each female. All remaining data analyses were carried out in R v.3.4.1 (R Core Team, 2000). We extracted monthly 95% isopleth home ranges (home ranges containing 95% of all GPS locations from the collars) for all females using packages *sp* (Pebesma & Bivand, 2005), *rgdal* (Bivand *et al.*, 2014), and *TLoCoH* (Lyons *et al.*, 2013).

For all analyses, we assessed factors to rule out collinearity using variance of inflation factor (VIF) analysis (Fox & Monette, 1992; see Chapter 2), with a cut-off value of four. Numerical variables were scaled and centred. VIF values of models including NDVI, temperature, tourist pressure and, in the case of hourly journey length, time periods were higher than the cut-off value of four (5.02 for home range and 4.46 for hourly journey length). This indicated that models including season instead of NDVI were a better fit (VIF values of 2.07 and 2.53 for home range and hourly journey length, respectively). In addition to this, we also assessed which model yielded a better fit to the data by comparing the Akaike Information Criterion (AIC; Burnham & Anderson, 2002) of a global model containing all possible two-way interactions with either NDVI or season included. In each case, this confirmed the findings from VIF values, where models including season had lower AIC values than those including NDVI.

We used the ‘lmer’ command (*lme4* package; Bates *et al.*, 2014) to run the following global model on home range:

$$\begin{aligned} lmer\ (formula & \\ &= Home\ range \sim Tourist * Season\ +\ Tourist * Temperature \\ &+\ Season * Temperature +\ ID\ +\ (1|Month), data\ =\ Data) \end{aligned}$$

And the following global model on hourly journey length, excluding the interaction between tourist pressure and time period, as tourists are only present on roads during the day:

$$lmer\ (formula = Hourly\ journey\ length \sim Tourist * Season + Tourist * Temperature + Temperature * Season + Season * Time\ period + Temperature * Time\ slot + ID + (1|Month), data = Data)$$

As data came from only three individuals, we could not include ID as a random factor (Bolker *et al.*, 2009) and instead we included animal ID as a control fixed factor. We included month as a random effect to control for repeated observations. We used the ‘dredge’ function in the MuMIn package (Barton, 2018) for comparison of all possible models, which ranked them by their conditional AIC (AICc, Burnham & Anderson, 2002) to determine the top model. However, due to the small sample size of the home range data (n=30) we would only consider the top model for these data if this did not result in an overly complex model (see Chapter 2). In case several models were within 2 AICc of each other, we further followed the criteria provided in Leroux (2019) to identify the best model (see Chapter 2).

AIC methods fail to distinguish between how strong or weak each effect in the top ranked model is, respectively (Harrison *et al.,* 2018). In order to gain more detailed understanding on the importance of each individual predictor indicated in the top model (Mundry, 2011; Symonds & Moussalli, 2011), we bootstrapped the confidence intervals of each top model with 1000 iterations with the *lme4* package to account for possible temporal autocorrelation in residuals (Bro-Jørgensen *et al.*, 2008), and assessed which of the fixed effects’ 95% confidence intervals did not cross zero, indicating significance of this effect. We plotted graphs using the packages *effects* (Fox, 2003) and *ggplot2* (Wickham, 2016; see Chapter 2).

5.3 Results

5.3.1 Home range

A total of *n*=30 monthly 95% isopleth home ranges for three females were extracted (*n*=12 for *Ivy* and *Joy*, *n*=6 for *Mia*, Table 5.1). The top model included only season, according to the AICc values (Table 5.2).

Table 5.1. Descriptive statistics of monthly 95% isopleth home range sizes in km^2 of three adult female African elephants, Loxodonta Africana, in Madikwe Game Reserve.

Female	Season	Month and year	95% isopleth home range size (km^2)
Ivy	Dry	August 2016	4.70
		September 2016	3.63
		March 2017	9.87
		April 2017	7.24
		May 2017	6.48
		June 2017	8.39
	Wet	October 2016	5.35
		November 2016	2.94
		December 2016	21.96
		January 2017	7.71
		February 2017	11.17
Joy	Dry	August 2016	5.82
		September 2016	8.38
		March 2017	7.66
		April 2017	7.85
		May 2017	6.10
		June 2017	3.71
	Wet	October 2016	8.41
		November 2016	9.77
		December 2016	25.82
		January 2017	10.44
		February 2017	15.37
Mia	Dry	September 2016	7.07
	Wet	October 2016	17.89
		November 2016	13.04
		December 2016	18.84
		January 2017	11.43
		February 2017	9.08

Table 5.2. Fixed effects included in the top models for the effect of various factors on African elephant, *Loxodonta africana*, monthly home range size of three adult females in Madikwe Game Reserve, South Africa, as ranked by conditional Akaike Information Criterion (AICc). Degrees of freedom (df), log likelihood, delta value and weight for each model are also reported.

Fixed effects included	**df**	**Log Likelihood**	**AICc**	**delta**	**weight**
Season	4	-35.064	79.7	0.00	0.605
Null model	3	-38.129	83.2	3.45	0.108

Elephant home range size was significantly larger during the wet, compared to the dry season (Table 5.3, Fig. 5.1, Fig. 5.2).

Table 5.3. Results of a bootstrap (1000 iterations) of an LMM for the impact of season on monthly home range size of three adult female African elephants, *Loxodonta africana*, in Madikwe Game Reserve, South Africa. Fixed effects' estimates and standard errors from the model summary. Significance was assessed as bootstrapped 95% Confidence Intervals not crossing zero. Significant effects are shown in bold.

Fixed effect (reference level)	**Level**	**Estimate (± SE)**	**Levels (reference level vs. comparison level)**	**95% Confidence Intervals**
Intercept		-0.752(± 0.30)		
Season (Dry)	**Wet**	**0.998(± 0.41)**	**Dry: Wet**	**0.130 to 1.839**
ID (Ivy)	Joy	0.374(± 0.25)	Ivy: Joy	-0.125 to 0.849
	Mia	0.535(± 0.33)	Ivy: Mia	-0.120 to 1.163
			Joy: Mia	-0.469 to 0.834

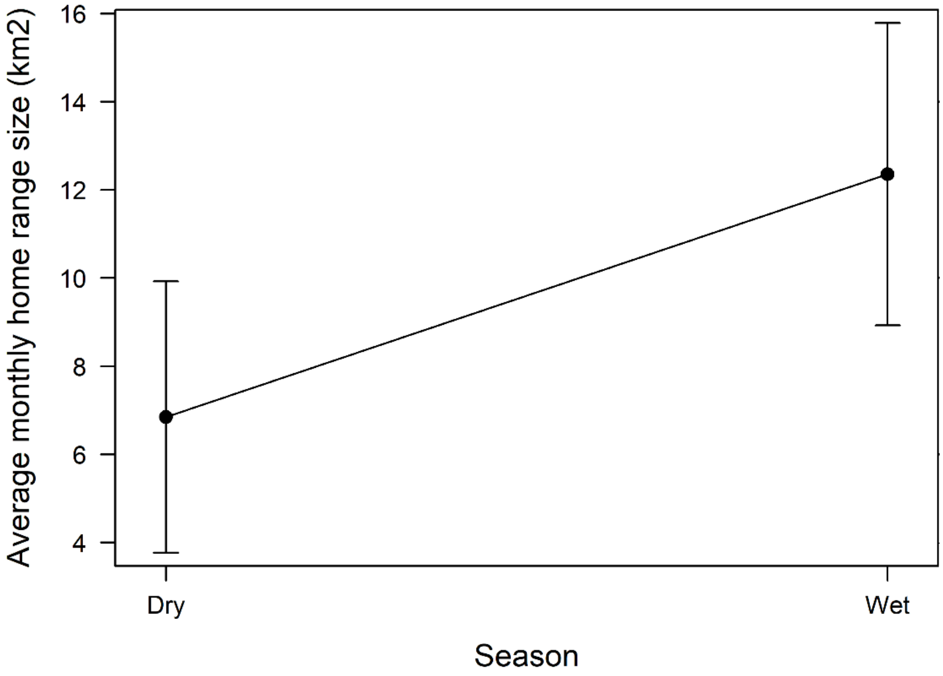

Figure 5.1

Effect of season, as assessed by a Linear Mixed Effects Model, on average monthly home range size (km^2) of three adult female African elephants, *Loxodonta africana*, in Madikwe Game Reserve. Error bars represent 95% confidence intervals.

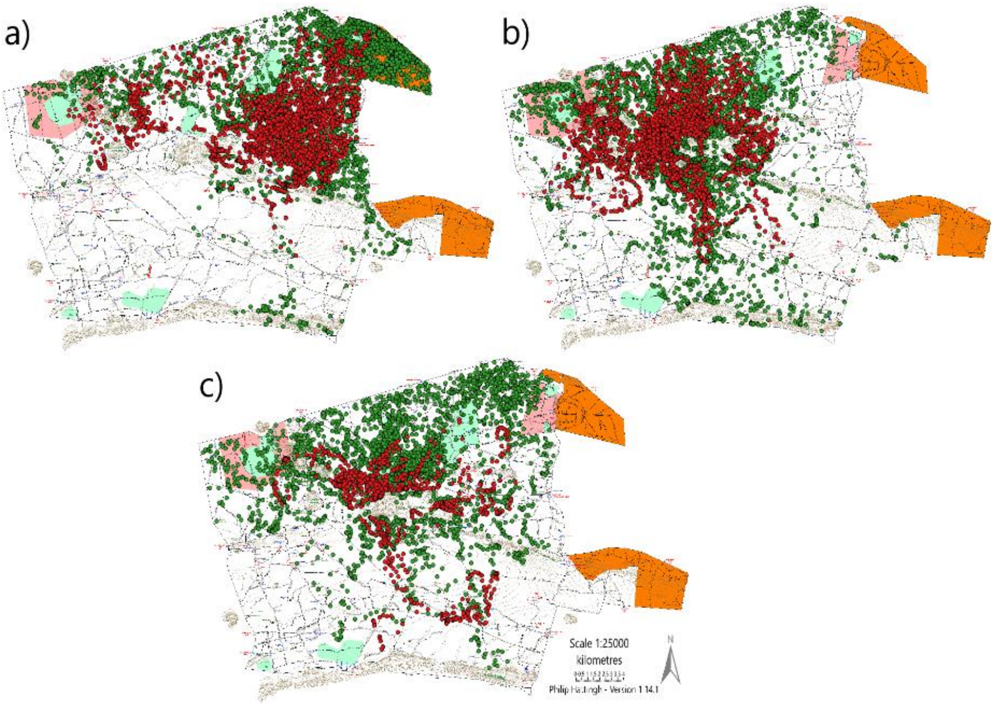

Figure 5.2

Locations of GPS collar readings during the dry season (June 2016 – September 2016 and March 2017 – June 2017, red circles) and wet season (October 2016 – February 2017, green circles) for three adult female African elephants, *Loxodonta africana*, in Madikwe

Game Reserve, South Africa. Readings were transmitted hourly. Figure shows readings for a) *Ivy*, b) *Joy,* and c) *Mia.* For additional information on the map please refer to Figure 2.2 in Chapter 2.

5.3.2 Hourly journey length

We extracted *n*=155 periods of mean hourly journey length for three adult females (*n*=65 for *Ivy*, *n*=60 for *Joy*, *n*=30 for *Mia*). The top model, as assessed by AICc, included an interaction of temperature and time period (Table 5.4).

Table 5.4. Fixed effects included in the top models for the effect of various factors on African elephant, *Loxodonta africana*, average hourly journey length of three adult females in Madikwe Game Reserve, South Africa as ranked by conditional Akaike information criterion (AICc). Degrees of freedom (df), log likelihood, delta value and weight for each model are also reported.

Fixed effects included	**df**	**Log Likelihood**	**AICc**	**delta**	**weight**
Temperature*Time period	12	-65.195	156.6	0.00	0.597
Temperature*Time period + Season	13	-65.443	159.5	2.88	0.141

We found a significant positive interaction effect between temperature and time period on mean hourly journey length. As temperature increased, distance travelled during the early night (8 pm – 1 am), late night (1 – 6 am), morning (6 – 11 am), and afternoon (3 – 8 pm) increased, whilst distance travelled during midday (11 am – 3 pm) did not change (Table 5.5, Fig. 5.3).

Table 5.5. Results of a bootstrap (1000 iterations) of an LMM for the impact of several fixed effects on average monthly day journey length of three adult female African elephants, *Loxodonta africana*, in Madikwe Game Reserve, South Africa. Fixed effects' estimates and standard errors from the model summary. Significance was assessed as bootstrapped 95% Confidence Intervals not crossing zero. Significant effects are shown in bold. Temp: Temperature.

Fixed effect (reference level)	**Level**	**Estimate (± SE)**	**Levels (reference level vs. comparison level)**	**95% Confidence Intervals**
Intercept		0.142 (± 0.09)		
Temp		**0.230 (± 0.09)**		**0.069 to 0.397**
Time (Afternoon)	Midday	-0.676 (± 0.08)	**Afternoon: Midday**	**-0.815 to -0.511**
	Early night	1.322 (± 0.08)	**Afternoon: Early night**	**1.165 to 1.471**
	Late night	-1.318 (± 0.08)	**Afternoon: Late night**	**-1.472 to -1.158**
	Morning	-0.335 (± 0.08)	**Afternoon: Morning**	**-0.490 to -0.186**
			Midday: Early night	**1.853 to 2.151**
			Midday: Late night	**-0.801 to -0.493**
			Midday: Morning	**0.181 to 0.497**
			Early night: Late night	**-2.805 to -2.472**
			Early night: Morning	**-1.813 to -1.507**
			Late night: Morning	**0.839 to 1.139**
ID (Ivy)	Joy	0.115 (± 0.06)	Ivy: Joy	-0.003 to 0.225
	Mia	0.074 (± 0.08)	Ivy: Mia	-0.073 to 0.219
			Joy: Mia	-0.187 to 0.100
Temp* Time period (Temp* Afternoon)	Temp* Midday	-0.208 (± 0.08)	**Temp* Afternoon: Temp* Midday**	**-0.371 to -0.052**
	Temp* Early night	0.275 (± 0.08)	**Temp* Afternoon: Temp* Early night**	**0.113 to 0.429**
	Temp* Late night	-0.050 (± 0.08)	Temp* Afternoon: Temp* Late night	-0.209 to 0.108
	Temp* Morning	0.005 (± 0.08)	Temp* Afternoon: Temp* Morning	-0.153 to 0.166
			Temp* Midday: Temp* Early night	**0.318 to 0.639**
			Temp* Midday: Temp* Late night	**0.001 to 0.320**
			Temp* Midday: Temp* Morning	**0.048 to 0.367**
			Temp* Early night: Temp* Late night	**-0.482 to -0.175**
			Temp* Early night: Temp* Morning	**-0.422 to -0.106**
			Temp* Late night: Temp* Morning	-0.092 to 0.204

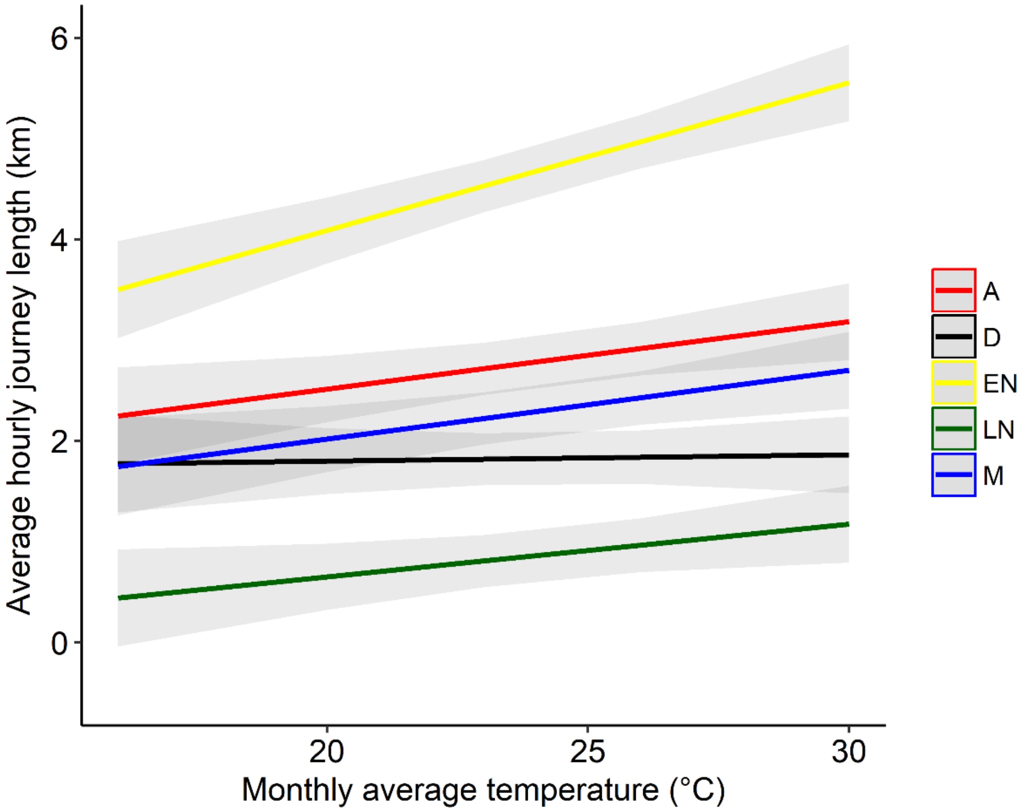

Figure 5.3.

Effect of temperature, as assessed by a Linear Mixed Effects Model, on mean hourly journey length (km), of three adult female African elephants, *Loxodonta africana*, in Madikwe Game Reserve during different time periods. A: Afternoon (3 – 8 pm), D: Midday (11 am – 3 pm), EN: Early Night (8 pm – 1 am), LN: Late Night (1 – 6 am), and M: Morning (6 – 11 am). Error bars represent 95% confidence intervals.

5.4 Discussion

Our study did not find an effect of wildlife tourism on elephant spatial behaviour. Monthly home range size was affected by season, with home ranges larger during the wet, compared to the dry season. Hourly journey length differed between time periods throughout the day and night, depending on the monthly average temperature. As monthly average temperature increased, elephants increased the distance travelled during the early night (8 pm – 1 am), morning (6 – 11 am), and afternoon (3 – 8 pm), and decreased the distance travelled during midday (11 am – 3 pm) and late night (1 – 6 am).

Although all these time periods were statistically significant, Figure 5.3 indicates that the increase in distance travelled during the early night was the only biologically meaningful effect. Irrespective of temperature, elephant travelled the furthest distance during the early night, followed by the afternoon, the morning, midday, and late night, respectively (Table 5.5).

Elephants have previously been shown to avoid human settlements and roads, and change day- and night-time behaviour in response to human activity (Hoare & Du Toit, 1999; Douglas-Hamilton *et al.*, 2005; Jackson *et al.,* 2008; Graham *et al.*, 2009; Roever *et al*., 2013; Molina-Vacas *et al.,* 2019). Previous research reported that individuals increasingly utilised areas close to humans or unprotected areas during the night, rather than during daylight hours, and this has been suggested to be in response to perceived risk of encountering humans (Jackson *et al.*, 2008; Munshi-South *et al.,* 2008; Graham *et al.*, 2009). We expected that elephants would perceive wildlife tourism as a stressor and that elephants would alter their spatial behaviour as a coping mechanism (Romero *et al.,* 2009). However, the research presented here did not find such an effect of tourist pressure on the spatial behaviour of three adult female elephants in Madikwe.

Tourist pressure is always present to some extent in Madikwe as tourists reside within the reserve year-round. It is possible that the elephants have already adapted flexibly to the constant pressure within a relatively small and fenced habitat by increasing movement during the night overall, throughout the year, instead of showing responses to short term fluctuations in tourists, which resulted in higher or lower levels of tourist presence at various times throughout the year. In line with previous studies which suggest that elephants shift to increased movement or utilisation of water sources during the night in areas with human disturbance (Douglas-Hamilton *et al.,* 2005; Jackson *et al.,* 2008; Graham *et al.,* 2009), the increased distance travelled at night rather than during the day (Fig. 5.3) in the Madikwe elephant population could present active avoidance of encounters with tourists on the roads during the day. If this were the case, such a long-term alteration of behaviour could be a successful coping mechanism in response to a stressor (Romero *et al.,* 2009). However, when behaviour is altered long-term it could pose a new stressor in itself. For example, if elephants spent significantly more time moving overall it could simultaneously decrease time available for other essential behaviours such as resting and, due to it being long-term, this could then become a chronic stressor for elephants. However, as an overall shift in increased ranging behaviour during the night remains speculative, so do the potential implications for this to be a long-term or chronic stressor. Further, if elephants had adapted to tourist pressure overall in such a way, observed changes in GCs and behaviour related to high and low tourist pressure (see Chapters 3 and 4) would have been unlikely to have occurred.

In contrast to the results presented here, recent research found that elephants did, in fact, use roads more often during high tourist season, thought to be due to easier and more direct movement between water sources, and it was suggested that tourism did not affect elephant behaviour (Tsalyuk *et al.,* 2019). However, this study was carried out in the much larger Etosha National Park in a semi-arid savanna environment and distance to roads decreased most during the cold-dry season, when rainfall was low, whilst distance to roads increased during months with increased rainfall (see Fig. 5 in Tsalyuk *et al.,* 2019). Further, differences in topography of the landscape vary and may affect the extent to which elephants use roads as easily accessible travel routes when traversing through hilly or rocky areas. The effect of tourism was not statistically assessed in the study from Etosha (Tsalyuk *et al.,* 2019), but rather the authors plotted number of tourist vehicles in the reserve each month in comparison to the distance of elephants to roads. Further, as noted by the authors, artificial waterholes are located along the roads, which results in roads being the most direct and fastest route between those waterpoints

(Tsalyuk *et al.,* 2019). As mentioned in Chapter 1, differences in environmental factors such as accessibility to water or seasonality in rainfall are likely to be related to different results. Additionally, the authors did suggest that elephant behaviour was not affected by high tourist pressure during the cold-dry season but did not collect or analyse any specific behavioural data related to tourism specifically (Tsalyuk *et al.,* 2019). In order to assess whether elephants were, in fact, unaffected by high tourist pressure itself, statistical analysis should consider a single statistical model in which season or water availability is controlled for, whilst including tourist pressure or high and low tourist season as a variable.

It is possible that ranging behaviour in Madikwe was not affected by tourist pressure at all, as the reserve presents only a small fraction of the natural home range sizes of unfenced elephants (see Chapter 1). The available space in Madikwe may not have been large enough to allow elephants to alter their space use significantly in relation to fluctuations in tourist pressure. This may be due to the fact that tourist accommodation is spread all across the reserve and tourist activities are carried out throughout. Therefore, the tourism stressor would be present throughout the elephant’s habitat and there would be no part of the habitat into which to alter the focus of their range in order to avoid tourists. Further, this would depend on location and distribution of key resources which elephants need to access such as water and forage. However, the small sample size from Madikwe means that further research is needed to investigate fine scale changes in elephant ranging behaviour. Currently, high tourist pressure did not appear alter elephant ranging behaviour.

In this study, elephant home ranges were larger during the wet, than the dry season. Elephants should be able to find naturally occurring water and more widespread forage more easily during the wet season, allowing them to spread their foraging efforts more evenly throughout the reserve, hence avoiding competition with conspecifics over point resources. However, previous studies have not reported one consistent result with regard to home ranges of fenced elephant populations; some reported larger home range size during the dry season (Shannon *et al.*, 2006), whilst others presented results in line with the ones presented here (Orrick, 2018). It is likely that elephant home range size, especially in fenced reserves, is affected by factors specific to the habitat of each population rather than following a general trend. Such specific characteristics could be related to the size of the reserve, elephant population density, whether forage quality and water is distributed evenly throughout the reserve or not, and whether forage quality and water availability varies throughout the seasons or not. Further specific characteristics could be related to the number of tourist accommodations and density of roads, as well as presence of other anthropogenic features such as settlements. Conservation biologists and population managers should aim to understand their specific population in relation to ecological, as well as anthropogenic factors in order to gain an informed understanding upon which to base management decisions.

Osborn (2004) pointed out that elephant home range sizes, although correlated with rainfall, seem to vary greatly between areas, and that humans have profound impacts on home range sizes of elephants. He further suggests that, historically, rainfall may have been a significant driver of home range size, but that human encroachment, fences, settlements, and activities are more likely to be the major driving force behind elephant home range sizes recently. However, in the study presented here, tourism did not affect elephant home range sizes or hourly journey length. Future research should investigate whether elephants may avoid roads or lodges at a fine scale. For example, elephants may avoid coming within a certain distance to roads or lodges and maintain a buffer zone, radius, or minimum distance from anthropogenic features which they do not utilise. Unfortunately, such fine scale analyses were not possible with the data collected here.

Although elephants sleep little for a mammal of their size (Gravett *et al.*, 2017), they have been shown to rest most during the late night/ early morning hours between 2 – 7 am, as well as between 11 am – 2 pm (Wyatt & Eltringham, 1974). Additionally, high temperatures have been shown to decrease day-time activity in African elephants (Loarie *et al.,* 2009a). This aligns with the results presented here, as elephants travelled the smallest distance during the early morning hours (‘late night’), followed by the hours between 11 am – 3 pm (‘midday’, Fig. 5.3). Further, with increasing average monthly temperature, elephants increased the distance travelled during the night, but the distance travelled during the day did not increase when temperatures were high.

Madikwe adhered to strict regulations, as tourists were restricted to game drive vehicles and a maximum of three vehicles were allowed in an elephant sighting at a time. Other reserves do not have a maximum number of vehicles within a sighting and allow visitors to enter just for a day and to drive their own vehicle without supervision by qualified guides. Therefore, tourist pressure, in terms of maximum daily numbers as well as immediate presence of tourists within a sighting, could be far larger in other reserves. Such unregulated tourism could have different effects on elephants’ spatial use. Additionally, tourists are always present in Madikwe, whereas other reserves could experience greater fluctuations between high and low tourist pressure (Pilanesberg, ZA, for example, which contains no lodges within the main area of the reserve and allows no visitors within the reserve during the night). Future research should investigate whether elephants increase early nocturnal (following sunset) ranging behaviour in such areas during times of high tourist pressure or respond in other ways.

Although this study did not find an effect of tourist pressure on elephant spatial behaviour, our data here comes from only three adult females. No other information on elephant home range sizes of the Madikwe elephant population were available, so no comparisons can be drawn. Future research should include collar data of both sexes, a larger sample size, as well as elephants ranging in fenced and unfenced areas for comparison. For example, elephant bulls more often raid crops (Jackson *et al.,* 2008) and therefore effects of anthropogenic disturbance could differ between the sexes, with bulls being bolder and less likely to avoid humans. Those areas which carry out tourism activities also differ in road density, type of tourism (viewing and/ or hunting, self-driven or guided) and whether tourists stay within the elephants' habitat over night at lodges and camp sites, or are restricted to access during the day only. This adds additional factors which could affect elephant ranging patterns differently and to a different extent.

As demand from wildlife tourism grows and elephant population numbers decline, understanding impacts of tourism on elephant welfare and adverse impacts on health is crucial in order to mitigate negative welfare effects on elephants and ensure tourist and elephant safety. Economic gain from wildlife tourism aids in habitat protection, aiding to conserve this iconic mega-herbivore. Effects of fluctuating tourist pressure on elephant ranging behaviour, when tourism is regulated as in our study population, appear to remain marginal but require further investigation given our small sample size and limitations of this study.

Author contributions

I.S. carried out data collection, statistical analysis, conceptualized the project and wrote the paper.

Y.P. co-supervised the project and approved the final draft of the paper.

N.K. supervised and conceptualized the project and edited drafts of the paper.

Summary of Chapter 5

In Chapter 5, I used GPS data to analyse the spatial behaviour of three adult female African elephants in Madikwe. I found that monthly 95% isopleth home ranges increased during the wet, compared to the dry season. Further, I found that increasing monthly average temperature was related to an increase in hourly journey length during the night hours. Unlike Chapters 3 and 4, these results did not indicate that wildlife tourism was related to changes in large-scale movement of African elephants at Madikwe. However, the small sample size and an all-female data set limits the conclusions which can be drawn from this without further research.

Chapter 6 - General discussion

In the research presented in this thesis, I have investigated whether wildlife tourism is perceived as a stressor by free-ranging African elephants and if so, how it affects their GC concentrations, behaviour, and large-scale movement. In my final chapter, I summarise the results related to wildlife tourism as a stressor and discuss how they relate to the scheme presented in Chapter 1. I further discuss the direct implications of these results for the management of the Madikwe elephant population and the broader implications for elephants in fenced and potentially unfenced reserves, as well as future research needs.

6.1 Wildlife tourism impact on the Madikwe elephant population

Investigating wildlife tourism effects on African elephants, I predicted that elephants would perceive tourism as a stressor and, in response to high tourist pressure and presence of tourists observing them from vehicles, would alter their behaviour and space use and have increased glucocorticoid concentrations. Within the previous chapters, it has been discussed that high tourist pressure was related to increased concentrations of faecal glucocorticoid metabolites (see Chapter 3), that elephants were more likely to perform conspecific-directed aggression during high tourist pressure (see Chapter 4), and that elephant herds moved away from tourists observing them from game drive vehicles with increasing numbers of vehicles present (see Chapter 4). However, high tourist pressure was not related to changes in spatial use, in form of home range size and distance travelled (see Chapter 5). I conclude that wildlife tourism is perceived as a stressor by free-ranging African elephants in Madikwe, South Africa.

In Chapter 1, I presented an overarching scheme of the potential interactions between the different components assessed in this study (Fig. 1.4). An unpredicted stressor leads to increases in physiological mediators, which brings these mediators into the range of reactive homeostasis (Romero *et al.,* 2009). High tourist pressure was related to an increase in fGCM concentrations and increased conspecific-directed aggression and presence of game drive vehicles was related to elephant herds moving away from those vehicles. Changes in hormone concentrations affect effectors such as muscles and make energy available to respond to stressors, whilst they are regulated by the input of stimuli through the sensory system (see Chapter 1). The increase in fGCMs (see Chapter 3) could relate to an increased amount of energy being made available to perform aggressive behaviour and moving away from GD vehicles (see Chapter 4), or vice versa, the increase in aggression may have been related to the increase in fGCMs. Short-term, such physiological and behavioural changes may have been adaptive coping mechanisms (see Fig. 1.4; Romero *et al.,* 2009; Busch & Hayward, 2009; Sheriff *et al.*, 2011; Nelson & Kriegsfeld, 2017).

Although I reported an increase in GC concentrations during high tourist pressure (see Chapter 3), elephants did not perform more stress-related behaviour during high tourist pressure (see Chapter 4). Discrepancies between physiological and behavioural measures of the stress response are not uncommon (Ellenberg *et al.*, 2006; Maréchal *et al.*, 2011). For example, Barbary macaques did show behavioural stress responses related to high tourist numbers present and high rates of occurrence of neutral, feeding or aggressive interactions, but only high rates of aggressive interactions between tourists and macaques were associated with increased fGCMs (Maréchal *et al.,* 2011). Similarly, high tourist pressure in Madikwe was related to increased fGCMs alongside increased occurrence of aggressive behaviours.

Elevated GC concentrations make animals more prone to aggression, but it is also possible that engaging in aggression is psychologically stressful, affecting GC concentrations (Muller & Wrangham, 2004), and animals may become increasingly anxious with the threat of potential occurrence of aggression (Maréchal *et al.*, 2011). The connection between aggressive behaviour and GC concentrations has been established elsewhere. Creel (2001) discussed that high levels of aggression are related to increased concentrations of GCs in a broad range of mammals and birds. However, in humans, rhesus macaques, *Macaca mulatta*, yellow-eyed penguins, and African elephants, aggression has been linked to lower cortisol levels (De Bellis *et al.,* 1999; Dettling *et al.,* 1999; Hart *et al.,* 1995, 1996; Westergaard *et al.,* 2003; Grand *et al.,* 2012; Ellenberg *et al*., 2007). For example, captive chimpanzees that received more aggression had higher cortisol concentrations measured in hair, whilst individuals which initiated aggression had lower concentrations of hair cortisol (Yamanashi *et al.*, 2016). The authors concluded that receiving aggression may be an important contributor to long-term stress (Yamanashi *et al.*, 2016). Similarly, Scheun and colleagues (2015) found increased fGCM concentrations in female African lesser bushbabies, *Galago moholi*, living in urban environments, and suggested this may have been the result of increased conspecific-directed aggression.

Muller and Wrangham (2004) stated that it is unlikely that performing aggressive behaviours, even though it may present a metabolically significant demand on animals, is the driving factor of GC production. It appears that receiving aggression increases GCs, whilst being aggressive may present a type of coping mechanism which reduces GCs. Following results reported in previous studies, it is possible that individuals who performed more aggressive behaviour had reduced GC concentrations compared to those who did not perform aggressive behaviour or received aggression from conspecifics. Although it was not possible with the data collected for the research presented in this thesis, a fine scale comparison between elephants who perform aggressive behaviours, compared to those who receive it, and associated fGCM concentrations of those individuals would be interesting to investigate to assess whether this hypothesis holds and fGCM concentrations of individuals who are more aggressive are lower.

I suggest that increases in fGCM concentrations and increased conspecific-directed aggression in the Madikwe elephant population during high tourist pressure were a coping mechanism in response to a perceived stressor (tourism). The results reported in Chapter 3 and 4 are in line with previous studies which suggested increased perceived stress to be related to aggression towards other species such as rhinoceros, *Ceratotherium simum*, as well as conspecifics (Slotow & van Dyk, 2001; Bradshaw *et al.*, 2005; Jachowski *et al.*, 2012). Further, elevated fGCM concentrations have been linked to hyperaggression of elephants towards humans (Slotow *et al.,* 2008), so-called refuge behaviour where elephants restrict their movement to specific areas of their habitat with lower anthropogenic disturbances such as tourism (Viljoen *et al.*, 2008b; Jachowski *et al.*, 2012, 2013b, c) and direct interactions with humans (Millspaugh *et al.*, 2007). For any reserve depending on tourist satisfaction and income from tourism, aggressive elephants which cannot be approached for viewing purposes or actively attack human observers, pose a serious threat to human safety and likely lead to a negative reputation for elephant viewing amongst tourists. Further, this would provide poorer welfare and likely poorer health for those elephants. Reserves should therefore monitor elephant behaviour and aim to reduce tourist impacts to a minimum.

In addition to increased fGCM concentrations and aggressive behaviour, elephants also became increasingly likely to move away from GDs with increasing numbers of vehicles present (see Chapter 4). This increase in movement likely presents a minor energetical cost if only performed for a short distance or occasionally, but, if it occurs at a high frequency, it may become significantly more costly to individuals as they incur a cost, for example to their foraging time and also in terms of energy expenditure. Higher numbers of tourists require higher numbers of vehicles to accommodate them on game drives. As one vehicle can fit a maximum number of 10 tourists, lodges need to utilise more vehicles when they accommodate more tourists (K. Potgieter, C. Catton, P. Hattingh, *pers.comm*.). This increases the chances and frequency of elephant herds encountering GDs and hence herds may be moving away from present GD vehicles more frequently during high tourist pressure. It is possible that the cost of performing aggressive behaviour alongside increased movement away from vehicles is related to an increase in GCs to make energy available for such additional movement (see Chapter 1; Fig. 1.4). Further, high tourist pressure was related to increases in three different mediators (GCs, aggressive behaviour, movement). Nevertheless, this remains speculative and fine-scale measurements would be necessary to make a more detailed analysis of which of the coping mechanisms and reactions to the perceived stressor occur first and whether increases in all three above mentioned mediators do occur simultaneously and incur a high enough cost on, for example, foraging behaviour.

Chapter 5 reported on the fact that elephants did not appear to alter ranging behaviour in relation to high tourist pressure. This was unexpected, given that wildlife tourism was assumed to be a stressor to elephants based on the results presented in Chapters 3 and 4. However, the data stems from only three female elephants and, as discussed in Chapter 5, Madikwe may not be a large enough reserve to allow elephants to alter large-scale movement such as habitat size and distance travelled. Additionally, large areas of Madikwe contain dense habitat within close vicinity of the road (I. Szott, *pers.obs.*) and elephants could have been a short distance from the road whilst being removed from the sight of tourists. This could have further reduced the need for large-scale alterations of spatial use for elephants. However, more data is required before either a conclusion or alternative explanation can be made. At this point, the observed increase in GCs (see Chapter 3) did not appear to be related to increased amounts of energy related to changes in large-scale spatial movement of elephants in Madikwe (Fig. 1.4).

The coping mechanisms performed by elephants in response to wildlife tourism which have been reported in Chapters 3 and 4 may have been successful to cope with the perceived stressor (Romero *et al.,* 2009). Therefore, large-scale alterations of spatial movement were not necessary for elephants to cope with the perceived stressor. As seen in the scheme in Fig. 1.4, I suggested that, if the stressor cannot be avoided by alterations in spatial behaviour, changes in behaviour may be necessary in order to cope, and this is what was reported in Chapter 4.

In response to a perceived stressor, physiological mediators increase (Romero *et al.,* 2009). This itself is adaptive in the short-term and does not present an immediate concern for the individual’s welfare. However, high tourist pressure was observed for several months at a time and elephants may perceive stress long-term given the increase in GCs, aggressive behaviour, and movement away from the stressor. Even this may not be a welfare concern in itself. However, if individuals encountered additional stressors, such as extreme drought and increased competition over resources, they would be less well equipped to react and adapt to such additional stressors (see Fig. 1.1C; Romero *et al.,* 2009). Further, when individuals perceive stress for a prolonged period of time, this can be chronic and lead to the associated negative implications discussed in Chapter 1, such as impaired immune function, loss in body condition or reproduction.

Overall, the results relating to high tourist pressure in Madikwe suggest some degree of negative impact of wildlife tourism on elephants, potentially chronic for several months each year, when tourist numbers are above a certain threshold. However, taking into consideration the lack of changes in large-scale spatial behaviour and average fGCM concentrations comparable to reported values of other elephant populations, I suggest that the results presented in Chapters 3 to 5, at this current stage, do not present negative welfare concerns related to the effects of chronic stress. However, high tourist pressure was related to fGCM concentrations comparable to those of elephants with foot injuries (Chapter 3), as well as increases in aggressive behaviour (Chapter 4), both of which have been identified as stressors. Therefore, the results of the research presented here do warrant consideration and continued monitoring in terms of future management at Madikwe.

6.2 Management implications

Based on the results of this study and findings of other studies of negative effects on animals related to tourism, some direct management implications for Madikwe will be suggested in the following. However, as stated above, there did not appear to be negative effects of tourism related to chronic stress in the Madikwe population and evidence is limited. Nonetheless, the small size of the reserve, high elephant population density and high tourist presence across the reserve are factors that may predispose the population to susceptibility to future chronic stress and so warrant caution with increased monitoring required in order to implement action at an early stage. Even though further research is needed (discussed below) in order to assess the situation better, managers could introduce measures to mitigate or reduce potential existing or future problems and I will give suggestions.

First, based on personal observations in the field and personal communication with elephant experts (Y.Pretorius, T.Eggeling, J.Selier), I suggest that a minimum distance between the nearest elephant and a game drive vehicle should be added to the Code of Conduct. Although no data were collected on this, and no analyses carried out, the reactions of elephants to vehicles which approached to less than 10 m upon first approach or at high speed indicated that this was a strong stressor and elephants often reacted with aggressive or stress-related behaviour (see Chapter 4, I. Szott, *pers.obs*.). Burke (2005) proposed a minimum distance of 50 m from elephants for tourists, as this distance was observed to substantially reduce stress-related behaviours in elephants in Pilanesberg National Park. As behavioural reactions by elephants in Madikwe were mostly observed within 10 m of approach, and other research has suggested distances of 50 m (Burke, 2005), I suggest that at least a minimum distance of 10 m on first approach should be introduced, which still allows tourists to observe elephants at very close distance. Implementation of such a minimum distance should then be assessed for its effectiveness in reducing strong negative behavioural responses by approached elephants as reported in Chapter 4.

A minimum distance may affect the probability of retreat of elephant herds, as seen in Chapter 4. If, as suggested above, high tourist pressure is related to increased frequency of herds moving away from GDs and to an increased concentration of GCs, then such a minimum distance may additionally aid in reducing perceived stress in elephants. Furthermore, during times of high tourist pressure, increased perceived stress may not only result in conspecific-directed aggression, but also human-directed aggression in other areas (Slotow *et al.,* 2008; Jachowski *et al.,* 2012). A minimum distance could increase tourist safety. However, these are hypothetical suggestions and require further study.

Tourist satisfaction is a driving goal for tourism providers and managers of protected areas (Novellie, 1991; Maciejewski & Kerley, 2014), and guides aim to give their guests a rewarding and positive experience (P.Hattingh, C.Catton, K.Potgieter, *pers.comm.*). However, guests paying prices associated with luxury accommodation such as found in Madikwe, may have high expectations and sometimes demand to be as close to an animal as possible (P. Hattingh, C. Catton, K. Potgieter, *pers.comm.*). Guests who request close approaches to animals may have no inhibition to put themselves at risk and may lack awareness of welfare issues. Other research suggests that guests may reconsider their request once they are educated about the associated welfare implications for the animals (Higham & Shelton, 2011). Guides should aim to educate guests on potential negative effects on animals' behaviour and welfare linked to close approaches by tourists and vehicles. Further, if included in the Code of Conduct, guides could refer to the minimum distance when guests ask them to approach more closely. Whether this is a successful method to deter guests from putting pressure on guides to approach wildlife closely in Madikwe, remains to be investigated.

Previous research has repeatedly highlighted the value of refuge areas for elephants during times of elevated GC concentrations (Jachowski *et al.*, 2012, 2013b). Although game drive vehicles do not go off-road in order to view elephants, off-roading occurs to view other animals and guided walks take place across the reserve. In line with suggestions reported in previous studies and following the example of other reserves (*e.g.* Pilanesberg National Park), I suggest a strict no off-roading zone in Madikwe, within which no guided walks are carried out. This would resemble a refuge area, with decreased human disturbance, as well as present elephants and other species with an area in which they may experience an increased sense of control to remove themselves from the stressor (Nelson & Kriegsfeld, 2017). This has been shown to aid in reducing and controlling animals' stress response, as discussed in Chapter 1 (Nelson & Kriegsfeld, 2017). In Madikwe, such a zone could incorporate an area in which off-roading is prohibited already, due to soft soil, and where road density is lower overall. Areas in the southern part of Madikwe fit those criteria and establishing such a refuge area there would therefore result in the least amount of area lost to tourist viewing. Future research should then investigate whether individual elephants do move into such a refuge area during sporadic events such as fires, whether there are individual differences in whether elephants do use refuge areas and whether those differences may be based on personality traits.

Relevant not only to Madikwe but other reserves as well, is the consideration by managers about timings of potentially stressful operations. During periods when individuals are coping with unpredictable stressors such as high tourist pressure, additional stressors which lie within conservation managers’ control should be avoided in order to reduce the potential cumulative impact of multiple stressors which may negatively affect animal health and well-being. Such additional stressors could be large-scale construction of roads or lodges, intensive management operations involving other species in the reserve (such as game captures) or other operations such as translocations.

6.3 Limitations of study and future research

Although I did not find any effects of tourist pressure on stress-related or vigilance behaviour, these findings require confirmation, ideally using repeated behavioural observations of identified elephants during immediate game drive vehicle presence. As my sample sizes were not sufficient for this analysis, immediate behavioural responses by elephants to GDs were not analysed and the effects of immediate tourist presence on the behaviour of elephants remain unknown. Factors such as speed of approach, proximity, number of vehicles and tourists present, and whether engines are switched off could then be included and assessed as potential stressors. In addition, continued behavioural observations and hormone sampling in line with the research carried out here would allow investigation across several years with larger fluctuations of tourist pressure, as well as environmental factors such as rainfall and NDVI. Such continued collection of faecal samples would also allow a baseline to be established for the Madikwe population, which would make interpretation of periods of potential homeostatic overload more accurate and reliable. Additionally, collection of faecal samples for this study was limited to samples from individuals encountered close to roads, which could represent a sample of elephants less averse to roads and tourists. Future research should aim to include faecal samples collected throughout the reserve by employing local guides to accompany researchers on foot.

As research of wildlife tourism impacts on African elephants is scarce and because tourism at Madikwe is more strictly regulated (with the exception of distance and speed of approach), further research in areas of unregulated wildlife tourism is required. Further research is especially needed in areas where no maximum number of vehicles is given in an elephant sighting, and where daily numbers of tourists far exceed what we have observed in Madikwe. Tourists who drive private vehicles and have little or no understanding of elephant behaviour often approach elephants at extremely close distances, without being able to identify signs of distress or aggression (I. Szott, *pers.obs.*, Dr Y. Pretorius, *pers.comm.*). This could have implications for those elephant populations, as elephants may be exposed to close approaches by tourists more frequently and may therefore experience increased or more frequent stress responses related to wildlife tourism. In addition, there are various private game reserves which carry out tourism exclusively from game drive vehicles as in Madikwe. Observations from those reserves would further our understanding on whether restrictions and strict regulations can be used to minimise the impacts of wildlife tourism consistently. Lastly, data from additional sites will allow us to form a more complete picture and gain better understanding of wildlife tourism effects on elephants. However, direct comparison between study sites will remain difficult, due to differences such as types of tourism, climate, presence of other species, vegetation types, availability of water, or population density and history.

Age of sampled elephants in this study was assessed as adult, juvenile, or calf, whilst other publications use known ages of elephants or further classification of calf, juvenile, and small-, medium- or large adult (e.g. Lee & Moss, 1986; van Aarde *et al.,* 2008; Poole & Granli, 2009). However, such detailed classification requires extensive experience (Poole & Granli, 2009) and it was not possible for the research presented in this thesis due to the experience of the observer (I. Szott). As such, classification of calf, juvenile and adult was the most reliable method of aging elephants.

A previous study that used the same three age categories as this thesis found an effect of age on fGCM concentrations (Pinter-Wollman *et al.,* 2009), albeit using slightly different ages for each category (calf 0 – 5 years, juvenile 5 – 15 years, adult >15 years) compared to this thesis (calf 0 – 3 years, juvenile 4 – 12 years, adult >13 years). In contrast, a study which categorised elephants as adults, sub-adults, and juveniles (Viljoen *et al.,* 2008b), did not find an effect of those age categories on fGCM concentrations. In order to best assess the effect of age, researchers should aim to assess elephant age as reliably as possible, ideally using known ages based on birth records. For faecal samples, bolus size could be used as an indicator of elephant size and hence age, in future analyses (Morrison *et al.,* 2005), although this poses difficulties as the bolus size of an old adult female may be similar to that of a juvenile or young bull (Dr Y.Pretorius, *pers.comm.*). If age does have an effect on fGCM concentrations, I suggest that the lack of an effect of age in the case of the research presented in Chapter 3 may more likely be due to the limited sample size, rather than the age-classification system used.

Analysing behavioural differences with regard to age would most likely show the largest differences in behaviour between calves and juveniles compared to adults (e.g. play behaviour; Lee & Moss, 2012). Additionally, calves classified as juveniles and juveniles classified as adults may have biased against finding an effect of a behaviour increasing or decreasing with age. The results of vigilance, stress-related and aggressive behaviours in Chapter 4 were unlikely to be affected by the lack of the additional sub-adult age category. If any of these behaviours did increase or decrease with age, there should have been, at least, a statistical difference between the adult and calf category, which was not the case. Nonetheless, knowledge of exact ages of elephants for future studies would be preferable in order to assess or control for age-related effects on behaviour as would be a larger sample size of observations of calves and juveniles.

The results obtained from elephant satellite collars in Madikwe showed no effect of tourist pressure on spatial use, but this warrants further investigation. There are various published studies which have used satellite data of elephant collars and these data would present an opportunity to investigate tourism impacts on elephants without having to fit new collars. Any researcher in possession of such data could identify which measures of tourist pressure (such as total number of visitors, road networks used almost exclusively by tourists, areas of high and low tourist activity) they could analyse in order to further our understanding. Although those areas may not have accompanying data on behaviour or endocrine markers, reporting results of spatial use of elephants in relation to anthropogenic disturbance in form of tourism would give more detailed insight. Further, it is uncommon for studies to report and consider as many measures as used for the research presented here (hormones, spatial use, and behaviour) at once. Types of tourist activities, such as self-driven or guided, hunting of elephants, as well as density of roads and accommodation could yield insight into how those activities are related to elephant space use. This may further inform on the idea of elephants shifting movement behaviour from the day to the night overall in response to tourist pressure within their habitat, as hypothesized in Chapter 5.

As data collected for this study were restricted to those which could be collected from the road, future studies should aim to explore other ways of data collection, in order to be less restricted spatially. This would allow sampling of elephants which remain further away from roads. However, this requires increased resources, such as money to hire a qualified ranger carrying a weapon to accompany the researcher on foot. Further, elephants may react different to researchers approaching on foot compared to approaches by car (Goldenberg *et al.,* 2017). Additionally, it is possible that myself or the research vehicle was affecting animals or presenting a stressor, however, I did not observe behaviours indicating this (behavioural observations were the only method available to me to assess this). Therefore, collection methods should either be consistent within a study or assess for confounding effects of observation methods specifically.

Collecting data remotely, for example using video cameras, restricts the researcher to a specific area where the camera is set up. Further, analysing elephant behaviour from video material may be highly inaccurate if the elephant is not close enough to be reliably observed (I. Szott, *pers.obs*.). The use of drones has become increasingly valuable and may be advanced enough to observe animal behaviour without disturbing the animal with the drone in the future and remains a promising tool for conservationists (Koh & Wich, 2012; Ivošević *et al.,* 2015). Nevertheless, at the current point it appears impractical to utilise drones and highly unlikely to be able to fly close enough to an elephant to reliably score behaviour (other than behaviour involving the full body such as immediate movement responses toward or away from a stimulus, or to assess ranging behaviour on a day-by-day basis) without disturbing the animal. Lastly, if possible, researchers could establish hides from where they can observe animals, although this restricts data collection to certain points. Long-term studies which aim to habituate animals over time may likely present the most promising future for the study of animal behaviour.

Data for the research presented in this thesis were collected whilst driving selected routes throughout Madikwe, rotating different areas based on a schedule (see Chapter 2). However, as field work is less controlled than the laboratory environment, the selection of routes had to be adapted to unforeseen circumstances, such as when an animal had been poached and the associated area had to be closed off for a period of time to allow all criminal evidence to be collected (I. Szott, *pers.obs.*). Poaching itself was limited to rhinoceros and occurred on foot as vehicle access to the reserve is controlled and hence was unlikely to be a significantly different stressor from guided walks which occur throughout Madikwe. Nevertheless, there is evidence of empathy in Asian elephants (Plotnik & de Waal, 2014) and hunting of African elephants has previously been shown to be a stressor to conspecifics (Burke *et al.,* 2008) and, therefore, poaching of other species may be a stressor and may result in elephants becoming more wary of humans. Further, there was a likely bias of increased sampling around the area of my accommodation, as all data collection started and ended at this same point. Truly random data collection schedules may be developed but are unlikely to be practical in the field. In turn, if known elephants are sampled repeatedly, such selection of random sampling areas is not needed as they can be located based on known last location or potentially through use of tracking collars.

In order to assess tourist pressure at a finer scale, game drive vehicles could be equipped with tracking devices, recording vehicles’ routes and allowing researchers to establish maps with high and low use tourist areas. Additionally, lodges should be encouraged to keep an exact record of the number of vehicles used during any given game drive, as well as the number of tourists on the vehicle. This could yield insight into differences in behavioural reactions of elephants within different areas, avoidance of areas by elephants depending on tourist use, as well as indicate suitable areas to establish as refuge areas due to low tourist use. In Madikwe, game drives start at the respective lodges and lodge clusters exist in the north east, north west, and south west, which likely resulted in the south and south east to be used less frequently by vehicles, although no data were collected on this. It would be interesting to assess elephants' responses to approaching vehicles as well as compare behaviour from elephants encountered in such areas. As fGCMs represent an average concentration of GCs for a species-specific gut-passage time, collecting a specific sample within an area does not relate to the potential stressor experienced within that area; the sampled individual may have only entered the sampling area a short time before the sample was obtained. Therefore, sampling of fGCMs can only be linked to specific areas if individual location prior to sampling is known and controlled for.

Citizen science has been used by researchers, especially for identification of species (Simpson *et al.,* 2014). Reserves could encourage tourists to identify certain individuals, for example by providing photographic material. Tourists could then record when and where they saw those animals, herd sizes or group compositions. However, this requires reserves to provide material, either digital or in print, which is associated with additional costs. Further, it requires tourists to be able to reliably assess factors such as sex and age, which is not possible without extensive experience and training. Guides who are trained could be more easily involved in such data collection and may be enthusiastic about being involved in research (I. Szott, *pers.obs*.). However, guides' ability to spend time on recording such data depends on whether tourists are not opposed to them doing so, rather than investing their time in educating and guiding the paying tourist. Additionally, individual identification of elephants may be feasible where the population is relatively small, but in cases such as Madikwe, with over 1000 individuals, it is unlikely that tourists or guides have the time to identify and observe the individuals they encounter.

Assessing effects of wildlife tourism can be done at a finer scale and with greater accuracy, if the identity of the sampled animals is known, and repeated observations are collected from known individuals. This is due to differences in life history, such as previous experiences, reproductive state, or age, to differences between different populations, such as a history of culling or poaching, and to differences in personality traits between animals which can affect how they respond to certain stimuli. Because individual personality differences are held constant over time, collecting data over time from the same individuals allows us to account for such individual variation (Goldenberg *et al.,* 2017). Future research should aim to identify the sampled animals and collect repeated samples over time. This is relevant to all measures, including hormone concentrations, behaviour, or spatial use.

Although the results presented in this thesis present, to my knowledge, some of the first insights into wildlife tourism effects on African elephants, I was unable to distinguish between several potential stimuli causing these effects. High tourist pressure could be associated with a larger number of game drive vehicles on the roads, more noise (from lodges and increased numbers of vehicles), more olfactory stimuli (from tourists and vehicles), as well as potentially more frequent encounters between elephants and tourists. Which of those stimuli, or others, are the cause of the observed effects on elephants remains unknown and warrants further investigation.

6.4 Conclusion

As no studies, to my knowledge, have previously assessed how wildlife tourism affects wild African elephants, the research presented in this thesis provides first evidence that wildlife tourism may present a stressor to African elephants. Further, high tourist pressure may be a long-term stressor during several months each year to the Madikwe elephant population. The results presented in this thesis align with results reported in other species. However, strict regulations, such as limiting numbers of vehicles in a sighting or total number of tourists within a reserve at any given time, have the potential of restricting negative impacts whilst increasing sighting quality for tourists. Such regulations should aim to maximise animal welfare in order to be able to have long-term sustainable populations, exhibiting species appropriate behaviour which tourists can witness. More research is needed in order to understand which regulations prove to be effective in reducing or limiting potential stressors for animals.

Because tourism contributes to a large extent towards the protection of species and habitat, as well as countries' economies, it is crucial for local management decisions to be guided by scientific research into how wildlife tourism may affect animals. Several suggestions have been made for management actions relevant not only to Madikwe, but also to other reserves. As high tourist pressure has been identified as a stressor to the Madikwe elephant population, it is likely that other populations perceive wildlife tourism as a stressor, too. Managers should limit additional stressors under their control, during times when populations already experience stress, for example through ecological constraints during the dry season. Further, I have identified several gaps and weaknesses in the presented research and made recommendations for future research.

By working toward high welfare standards for animals, managers can advertise this to tourists, achieving a good reputation for their care of animals and our natural world. By educating tourists as to why such things are important, future generations will hopefully value non-consumptive wildlife tourism as a way to experience animals within their natural environment.

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Appendix I – Identification of individual elephants

Individual elephants were identified based first on sex (female, male) and approximate age (calf, adult, juvenile). Then additional distinguishing features such as the patterns of notches and holes in their ears, lack of tusk/s, direction of growth of tusk/s, and other identifying features such as scars, folded ears or missing part of their trunks were noted. Additionally, note was made whether individuals were left- or right-trunked, synonymous with humans being left or right handed. This means that an elephant will have a predominant direction in which it wraps the trunk around object, either grasping clockwise (right-trunked) or anti-clockwise (left-trunked). I aimed to obtain as many good quality photographs as possible upon sighting an elephant, especially of their ears and a frontal view of their head. Length of tusks was not noted, as tusks may break at any point. The following are examples of identified individuals.

Example 1: Collared female *Ivy:* Adult female, right-trunked, notched in both ears, collar

Ivy right ear

Ivy left ear

Example 2: *Zeus:* Adult male, outwards facing tusks, notches in both ears, hole in bottom half of left ear, left-trunked

Zeus, front shot

Example 3: *Tip:* Adult male, outwards facing tusks, notches in both ears with *v*-shaped notches in both ears, part of trunk tip missing, left-trunked

Tip, right ear

Tip, front shot

Tip, trunk close-up

Example 4: *Emma:* Adult female, tuskless, notches in both ears

Emma, right ear

Emma left ear

Examples 5 and 6: *Flip and Flop:* Adult males, both left-trunked, right and left ear folded, respectively

Flip, right ear folded backward

Flop, left ear folded backward

Example 6: *Assasin:* Adult male, markings in both ears with three small holes in bottom left ear, only right tusk present, diagonal wrinkle across face, right-trunked

Assasin, front shot

Behavioural changes in African elephants in response to wildlife tourism

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Abstract

Eco-tourism and human-wildlife interaction can lead to increases in stress, vigilance and aggression in many species, however, studies investigating wildlife viewing are scarce. We present the first study investigating the impact of wildlife tourism on African elephant, *Loxodonta africana,* behaviour. Over 15 months, we studied the effect of monthly tourist pressure (tourist numbers) on the occurrence of stress-related, vigilance and conspecific-directed aggressive behaviour in 27 individually identified elephants and the effect of up to 3 vehicles on the direction of travel of non-identified herds using five-minute continuous focal observations. We analysed the effect of tourist pressure and vehicle presence using generalised linear mixed models, including habitat type, herd type and size, and season, as well as sex and age for behaviour models, as additional factors. We found no effect of factors on stress-related behaviour, but elephants were more likely to perform vigilance behaviours at waterholes compared to other habitat types. As tourist pressure increased, conspecific-directed aggression in elephants increased and male elephants were more likely to perform conspecific-directed aggression compared to female elephants. Further, we found that elephant herds became increasingly likely to move away with increasing numbers of vehicles present. Results suggest that reserves should monitor elephant behaviour to identify when tourist pressure has potential effects on elephant welfare and train guides to monitor behaviour and adjust minimum distances flexibly to ensure high welfare standards and tourist safety. This study further contributes to a small but growing body of literature on non-consumptive wildlife tourism impacts on wild animals.

Keywords: eco-tourism, conservation, stress, animal welfare, game drive, wildlife-viewing

1. Introduction

Observing wildlife as a non-consumptive tourist attraction for recreational purposes has become increasingly popular (Orams, 2002) and plays a key role in global wildlife conservation (Burger & Gochfeld, 1993; Newsome, Dowling & Moore, 2005). Wildlife viewing, where carried out sustainably, facilitates protection of wildlife habitats, biodiversity and natural ecological processes worldwide (Reynolds & Braithwaite, 2001; Maciejewski & Kerley, 2014). In terms of the management of such protected wildlife habitats, tourist satisfaction is usually the driving goal (Novellie, 1991). Negative impacts on animal welfare caused by wildlife tourism have been reported (Moorhouse *et al.*, 2015). Where negative impacts elicit chronic stress, they can potentially lead to decreased reproduction, increased risk of predation, starvation, susceptibility to diseases, dispersing away from release site (Reynolds & Braithwaite, 2001; Teixeira *et al.*, 2007; Bhattacharjee *et al.*, 2015) and lasting effects on behavioural patterns (McEwen & Wingfield, 2003). Impacts of wildlife tourism on animals are not well understood (Wardle *et al.*, 2018) and the few studies that have assessed viewing impact on animals found increases in fear, alert, aggressive, vigilance and stress behaviour (*Elephas maximus* (Ranaweerage, Ranjeewa & Sugimoto, 2015), *Rhinoceros unicornis* (Lott & McCoy, 1995), *Phoca groenlandica* (Kovacs & Innes, 1990), *Ursus maritimus* males (Dyck & Baydack, 2004), *Sula spp.* (Burger & Gochfeld, 1993)), reduced reproductive fitness (*Pygoscelis adeliae* (Giese, 1996), *Haliaeetus leucocephalus* (Grubb & King, 1991)), increased probability of retreat (*Bison bison*, *Odocoileus hemionus*, *Antilocapra americana* (Taylor & Knight, 2003), *Oreamnos americanus* (Lott, 1992)) and increased physiological stress responses (*Loxodonta africana* (Szott *et al.*, sub.), *Spheniscus magellanicus* (Fowler, 1999)).

Mega-fauna, such as African elephants, *Loxodonta africana,* are among the most popular species for wildlife viewing, particularly for international tourists (Lindsey *et al.*, 2007), yet research assessing the impact of tourist pressure, in form of monthly numbers of tourists, or tourist presence, in form of vehicle presence, on elephant behaviour is scarce. Elephants in unfenced areas have been reported to avoid human roads and settlements by altering their behaviour and movement (Hoare & Du Toit, 1999; Douglas-Hamilton, Krink & Vollrath, 2005; Jackson *et al.*, 2008; Graham *et al.*, 2009; Roever, van Aarde & Leggett, 2013) suggesting active avoidance of human contact by some herds. Only one study has investigated viewing-induced disturbance in elephants (Asian elephants, *Elephas maximus* (Ranaweerage *et al.*, 2015)) in a relatively large population of over 1000 individuals in a fenced national park. Tourist behaviour and vehicle presence increased the likelihood of elephants switching their behaviour from feeding to fear, alert, stress-related or aggressive behaviour. Additionally, increasing tourist pressure has been shown to be related to increased physiological stress levels of individuals in our study population of African elephants (Szott *et al.*, sub.).

The most widely used sustainable method to conserve elephant habitat is to allow wildlife tourism to take place in the form of viewing animals from vehicles, either self-driven or guided (World Tourism Organization, 2014). Tourist demand to view elephants is high (Chase *et al.*, 2016; Arbieu *et al.*, 2017). Human population growth in Africa is rapidly increasing and, by 2050, the population in Africa is predicted to double, with South Africa predicted to increase from an estimated population of 57.7 million people in 2018, to 81.8 million in 2050 (Population Reference Bureau, 2018). Such increases in human populations no only cause habitat loss but also increase possibilities of interactions between elephants and humans (Armbruster & Lande, 1993; Pozo *et al.*, 2017) Given the increasing populations of both humans and elephants in South Africa, it is important to investigate the impact of tourist pressure on elephant welfare. To our knowledge no published research has assessed the impact of tourist pressure or vehicle presence on the behaviour of African elephants.

In elephants physiological stress levels have previously been shown to be affected by season, where low availability of water and key nutrients during the dry season increased elephant stress levels (Foley, Papageorge & Wasser, 2001; Viljoen*et al.*, 2008). In a fenced area, elephants are forced to revisit foraging patches more frequently (Loarie, van Aarde & Pimm, 2009) and overcrowding and the increased frequency of interactions with unrelated individuals are thought to present a consistent social stressor for elephants (Munshi-South *et al.*, 2008). Elephants compete over access to resources, where agonistic interactions have been reported to occur at point resources such as fruiting trees, waterholes (Archie *et al.*, 2006), or mineral rich soil (*pers. obs.*). Further, bulls regularly come into musth, a reproductive state during which testosterone levels are heightened (Hollister-Smith *et al.*, 2007). Even when not in musth, males have been shown to be the more aggressive sex (Ganswindt *et al.*, 2005; Hollister-Smith *et al.*, 2007), often engaging in dominance interactions with each other (Goldenberg *et al.*, 2014) or bullying younger males (Buss & Smith, 1966). Stress-related, vigilance or aggressive behaviour in elephants may therefore be caused by a variety of factors other than tourism.

Our aim was to investigate the effect of wildlife tourism on elephants displaying aggressive, stress-related and vigilance behaviours as well as direction of herd movement in relation to tourists viewing them. Madikwe Game Reserve (Madikwe) in South Africa provided a suitable population to study the effects of tourist pressure on elephant behaviour. The founding population was introduced from various backgrounds, such as culling and poaching, and as the effects of such events can be long-lasting (Bradshaw *et al*., 2005; Gobush, Kerr, & Wasser, 2009; Jachowski, Slotow, & Millspaugh, 2013) these elephants may be particularly sensitive to the presence of vehicles. Additionally, such traumatic experiences are not an exception for elephant populations across Africa (Chase *et al.*, 2016). Given that previous research found effects of wildlife tourism on stress-related, vigilance and aggressive behaviour in viewed animals, we predicted that tourists would be a stressor for elephants and that increasing tourist pressure would increase vigilance to avoid the stressor and, if avoidance was not possible, increased stress-related and aggressive behaviour. Because point resources, season and sex are known to influence stress and aggression in elephants, we included these factors in our analysis as control factors, alongside age and herd type. Lastly, we predicted that elephant herds would be more likely to retreat from tourists observing them from vehicles with increasing numbers of vehicles present.

2. Materials and Methods

Study site and driving regulations

Madikwe Game Reserve (Madikwe) is a reserve managed by a state/private/communal partnership (Fig. 1). The reserve, approximately 680km^2 in size, was fenced and held an estimated 1348±128 elephants (July 2017, P. Nel, *pers. comm.*) that is, 1.9 elephants per km^2, representing one of the highest population densities of elephants in South Africa. Elephants were first introduced to Madikwe in 1992 when 25 orphaned juvenile elephants from Kruger National Park culls (operations where herds of adult individuals were lethally wounded, and youngsters translocated to other reserves as a measure of population control) were introduced. In 1994, entire herds (194 individuals) from Zimbabwe were introduced from a background of severe drought, two bush wars and heavy poaching. In 1998 and 1999, six and two adult bulls (measured by a minimum 3.2 m shoulder height) were introduced from Kruger National Park, respectively.

Figure 1 here

Private vehicles are restricted to a few roads to travel between lodges and gates in Madikwe. Hence, elephant viewing occurs almost exclusively from game drive vehicles (GDs) where they encounter elephants on roads. A GD is a large, open vehicle, driven by a qualified field guide, that seats up to ten people as well as a 'spotter' at the front of the vehicle. No more than three GDs were allowed at an elephant sighting. The researchers' vehicle was not included in this number. All vehicles were obliged to park leaving an unobstructed exit before switching the engine off but were not limited in how close they could approach. Vehicles were not permitted to position themselves between individuals of a herd and had to remain on roads. All guests were briefed on appropriate behaviour. Standing up, loud noise or use of camera flash was not permitted. Eating, drinking and smoking were strictly forbidden during game drives. All these regulations are part of Madikwe's Code of Conduct and no regulations were amended for the purpose of this study.

Data collection

Data were collected from the 18th of April 2016 until the 28th of June 2017. The mean (±SD) number of observation days per month was 14 ±5. The area was sampled by driving random routes as well as communicating with field guides about elephant presence. Thirty-one lodges were spread across the reserve and conducted game drives in the morning from sunrise until approximately 11am, and in the afternoon between approximately 3.30pm until 8pm.

The primary investigator collected all data in the field. For the behaviour analysis, we identified 27 individuals (14 males, 13 females) based on distinguishing features. Herds included in travel direction analyses were not individually identified herds, but those encountered throughout data collection. Upon spotting an elephant, the researcher aimed to keep 30 m distance from the nearest elephant. If the animal was spotted at <30 m distance, the vehicle was slowly reversed to 30 m before the engine was switched off. When animal/s moved parallel to the road without displaying signs of distress (such as vigilance, body posture changes such as 'ears out' threats or moving away whilst repeatedly looking back at the vehicle), the researcher followed at a distance before switching the engine off again. A bull group was defined as such when several bulls were within a 500m radius of each other, whilst a mixed group was defined as such when an adult bull was within 200m of a cow-calf group.

We collected data on a Lenovo TAB 2 A8-50F tablet using the Prim8 app (McDonald & Johnson, 2014). We classed elephants as juvenile or adult based on size (elephantvoices.org, 2018). Adult females had mammary glands and an angled forehead, whilst adult males had a rounded forehead, wider skulls and could be twice the size of adult females. Juveniles were smaller than adult females, moving and foraging independently of their mothers and had tusks of approximately ten centimetres in length. Once a sighting was made from the road, we randomly selected a focal elephant to observe using continuous sampling (Altmann, 1974) for five minutes and noted identity if known, along with additional factors (Table 1).

Table 1 here

We noted the direction of travel of the whole herd by visually comparing herd location at the start and end of the focal observation and inferring direction of travel. If the centre of the herd increased its' distance from the observer or, if present, the closest GD ≥10 m (without simultaneously approaching another GD), we classed it as 'retreat', otherwise we classed it as 'stay'. We only recorded one herd movement observation per encounter, during the first five-minutes after a herd was encountered or after a GD(s) arrived, as a measure of immediate reaction of herd movement to the potential stressor.

Following previously published ethograms (Langbauer, 2000; McComb *et al.*, 2014; elephantvoices.org, 2018), behaviours were categorised (see supplementary material for full ethogram) as stress-related, vigilance or aggressive. Because several aggressive behaviours could be directed at either humans or conspecifics, we made note of the direction of the recipient of the threat, and we included all aggression not explicitly directed at a human in the analysis of conspecific-directed aggression.

Season was defined as wet or dry based on average monthly rainfall measured at four stations within Madikwe by the South African Weather Service. Average total rainfall during the study period was 189.69 mm. Wet season was defined as the period in which 95% of precipitation for the study year fell (Loarie, van Aarde & Pimm, 2009a) and therefore wet season lasted from October 2016-February 2017 and dry season lasted from April 2016-September 2016 and March 2017-June 2017. North West Parks Board provided the total number of tourists visiting Madikwe each month and this number was defined as tourist pressure per month.

Data analysis

Only focal observations where the animal was visible for ≥4 mins 30 s were retained for analysis. For the analysis of monthly tourist pressure, we selected observations with only the research vehicle present. This was to avoid the possibly confounding effect of game drive vehicle presence on behaviour. We included individuals that had a minimum of n=2 observations. For herd movement direction analysis, we included observations with game drive vehicles present. Where GDs arrived or left within the five-minute observation but were present for less than 60 s, the observation was excluded from analysis. If GDs were present for more than 60 s, the herd movement was considered to be in response to the number of GDs present for that time. This means that, if one GD was present from the beginning, but a second GD arrived and stayed for over 60 s, herd movement was in response to two GDs present. If a second GD arrived but left in under 60 s, the whole observation was considered in response to one GD.

We analysed data using R v. 3.4.1 (R Core Team, 2000). We scored each behaviour as occurring or not, and elephant herd travel as retreat or stay, forming binary response variables. First, we assessed factors to rule out collinearity using variance of inflation factor analysis (Fox & Monette, 1992), using a cut-off value of 2. We specified three General Linear Mixed Effects Models (package *lme4*, (Bates *et al.*, 2014)) to analyse the effect of tourist pressure on stress-related, vigilance and conspecific-directed aggression:

glmer (formula = Behavioural category ~ Tourist pressure + Herd type + Sex + Habitat type + Season + Herd size + Age + (1|ID), family = binomial, data = Data)

We scaled and centred the tourist pressure and herd size variables and included animal ID as a random effect to control for repeated observations from known elephants. We analysed significance of fixed effects with a type II ANOVA (Langsrud, 2003). Where categorical fixed effects were significant, we assessed differences between the levels using a Tukey post-hoc test in the *multcomp* package (Hothorn, Bretz & Westfall, 2008), checking that 95% confidence intervals did not cross zero.

For the direction of travel dataset, we excluded the open grassland habitat type from analysis as only n=5 observations had one GD present, resulting in poor model fit. The following Generalised Linear Model was used:

glm (formula = Travel ~ Herd type * GD number + Habitat type * GD number + Season * GD number + Herd size, family = binomial, data = Data)

We scaled GD number and herd size. We included an interaction with GD number and herd type as we predicted that different age and sex classes may have been affected differently by GD presence. Further, we included an interaction between habitat type and GD number, as well as season and GD number, as we predicted that differences in thickness of vegetation and varying constraints during the seasons may have affected individual's reaction to GD presence. To account for non-independence in the data due to potential pseudoreplication, we performed 1000 iterations of bootstrapping, using the package *boot* (Canty & Ripley, 2018) to obtain bootstrapped 95% confidence intervals. We considered fixed effects significant if confidence intervals did not cross zero. We plotted all graphs using the *effects*- (Fox, 2003) and *ggplot2* (Wickham, 2016) packages.

3. Results

A total of 156 observations of known individuals were collected (mean ±SD = 6 ±6 per individual). These observations were from 10 adult males (18 observations as lone males, 8 in bull groups, 3 in mixed groups), 10 adult females (56 observations in cow-calf groups, 37 in mixed groups), 3 juvenile females (8 observations in cow-calf groups, 2 in mixed groups) and 4 juvenile males (16 observations in cow-calf groups, 8 in mixed groups). Removal of individuals with a small sample size did not change the effect of tourist pressure below. We collected travel direction of herds during 479 observations (81 bull groups, 141 cow-calf groups, 100 mixed groups and 157 lone males).

Stress-related behaviour

We found no effects of any variables on stress-related behaviour (Table 2).

Table 2 here

Vigilance behaviour

Vigilance behaviour was significantly more likely to occur at waterholes, compared to all other habitat types (Table 3).

Table 3 here

Conspecific-directed aggression

Increasing tourist pressure was significantly related to increased conspecific-directed aggression (Table 4, Fig.2). Male elephants were significantly more likely to perform conspecific-directed aggression compared to female elephants (Table 4). Although conspecific directed aggression appeared to be affected by habitat type (Table 4), Tukey post-hoc tests between habitat types revealed that the confidence intervals crossed zero.

Table 4 here

Figure 2 here

Herd movement

Increasing numbers of GDs present was related to increased likelihood of elephant herds moving away (Table 5, Fig. 3). None of the other variables affected herd movement (Table 5).

Table 5 here

Figure 3 here

4. Discussion

Our study found that wildlife tourism pressure and game drive vehicle presence influenced the behaviour of African elephants in Madikwe Game Reserve and adds to a small but growing body of literature monitoring the effects of tourist viewing on wildlife (e.g. Dyck & Baydack, 2004). Elephants were more likely to move away from tourists with increasing numbers of GDs present. High tourist pressure was related to increased conspecific-directed aggression. Our results showed effects of habitat type on vigilance behaviour, and sex on conspecific-directed aggressive behaviour. We further present the first report of any behavioural measure of the Madikwe elephant population.

We found a significant interaction between number of GDs and herd type on travel direction. More GDs were related to an increased likelihood of herds moving away from tourists (Fig. 3); this effect was most marked in bull groups compared to mixed groups. This supports the idea that elephants may remove themselves from a tourist stressor as a coping mechanism and is in line with other studies that found flight responses were affected by tourist presence (Lott & McCoy, 1995; Taylor & Knight, 2003). It is interesting that this effect was most pronounced in adult males (bull groups and lone males) compared to largely adult females (cow-calf groups and mixed groups). It is possible that this reflects a difference in willingness to, or the ability to quickly, move away from a resource (Stokke & du Toit, 2002; Woolley *et al.*, 2009). Cow-calf group and mixed groups contain neonates and young calves, smaller individuals that have reduced mobility and higher rates of water turnover. This may constrain the movements of lactating cows, growing juveniles and calves, and may present a trade-off between the perceived risk and the value of a resource from which groups containing adult females and dependent young move away. Unfortunately, we do not have data on proximity to water sources or nutrient content of forage during GD events to investigate this possibility.

We found mixed effects of tourist pressure on individuals' behaviour. Conspecific-directed aggression was more likely during high tourist pressure, supporting a similar effect in sea lions (*Neophoca cinerea*) and Asian elephants (Lovasz, Croft & Banks, 2008; Ranaweerage *et al.*, 2015). Contrary to our expectation, high tourist pressure did not increase vigilance or stress-related behaviour. As vigilance behaviour is known to be affected by spatial position in the herd (Burger & Gochfeld, 1993; Hunter & Skinner, 1998; Beauchamp, 2007), it is possible that this influenced our findings. Unfortunately, we did not have data on spatial position to control for this possibility. The lack of an effect on stress-related behaviour was surprising as we previously found increased physiological stress levels were likely when tourist pressure was high in our study population (Szott *et al.*, sub.). However, in a study of Barbary macaques, faecal glucocorticoid levels were not related to measures of tourist pressure (number, duration, proximity) but were related to aggressive interactions with tourists, whilst a behavioural indicator of anxiety (scratching) was positively related to the maximum number of tourists present (Marechal *et al.*, 2011). Thus, whilst these measures have been found to be useful indicators of physiological stress in many species (Fowler, 1999; Rehnus, Wehrle, & Palme, 2014) it is possible that behavioural expression and physiological response are triggered by different aspects of the stressor (Higham *et al.*, 2009; Mandalaywala *et al.*, 2014; Young *et al.*, 2014).

As predicted, habitat type had an impact on vigilance behaviour and sex impacted on conspecific-directed aggression. Waterholes are a point resource (Archie *et al.*, 2006) where vigilance behaviour was significantly more likely to occur, and male elephants have repeatedly been shown to be the more aggressive sex (Ganswindt *et al.*, 2005).

Prior research has demonstrated the effect of consumptive tourism, such as elephant trophy hunting, on stress levels of non-targeted herds in the population, leading to changes in behaviour that could potentially be fatal for humans (Burke *et al.*, 2008). From our findings, it appears that regulated non-consumptive tourism has the potential to be carried out in a more ethical manner than trophy hunting, with fewer welfare implications (no effect on vigilance or stress-related behaviour) for elephant populations. Although consumptive use generates larger amounts of money within a short amount of time, issues persist such as false hunting quota, corruption, and inequity of distribution of money (Lindsey, Roulet, & Romañach, 2007), in addition to welfare concerns for the wider population.

Practical implications

Studies highlight the contribution that behavioural indicators of welfare can make to the management and success of wild populations (Goldenberg *et al.*, 2017). Our results show that even with regulations in place, where wildlife viewing is carried out exclusively from GDs driven by qualified guides and overall numbers of tourists viewing elephants at any time are restricted, tourism led to changes in behaviour of the viewed elephants. However, the changes in behaviour were relatively limited, possibly because elephants were able to move away from the stressor, and suggest that, with careful management, wildlife tourism can be conducted in a welfare focused manner and hold a promising future for wildlife tourism as a conservation measure.

The Code of Conduct in Madikwe did not stipulate a minimum distance to be kept from elephants. Due to individuals performing more aggressive behaviours during high tourist pressure, and because waterholes are a point resource over which elephants compete, we recommend consideration of the increased chance of conflict with nearby GDs during times of high tourist pressure and at waterholes. Elephants at waterholes could experience frustration and stress, as well as being the target of aggressive behaviour from conspecifics, increasing the possibility that they will display redirected aggressive behaviours (Rajaram, 2006) towards bystanders such as vehicles. At Madikwe, field guides were aware of elephant behaviours signalling aggression (I. Szott, *pers. obs.*), highlighting the value of the ability to interpret behaviour when approaching wild animals. We suggest that a consistent minimum distance from the nearest individual, especially upon first approach, should be introduced to guidelines for wildlife viewing to alleviate the potential for conflict between tourist vehicles and wildlife. This will ensure not only the safety of guests but would also alleviate potential stress caused by increased agonistic interactions which could otherwise lead to elevated physiological or even chronic stress (McEwen & Wingfield, 2003; Pinter-Wollman, Isbell & Hart, 2009; Jachowski *et al.*, 2013).

It would further give elephant herds, or indeed other wildlife, more space and may reduce the likelihood of animals moving off, giving tourists longer, more natural viewing experiences.

Due to strict regulations in Madikwe, tourist pressure is based on maximum availability of lodges hosting tourists and GDs are restricted to small numbers in sightings. However, most wildlife viewing, not only of elephants but a broad range of species, is carried out in areas where fewer/ no restrictions apply and is under growing demand worldwide (World Tourism Organization, 2014). Research into non-consumptive wildlife tourism, where no direct interactions between human and non-human animals take place, is scarce but has consistently reported aggressive, stress-related or vigilance-related responses by wildlife (Dyck & Baydack, 2004; Lovasz *et al.*, 2008; Ranaweerage *et al.*, 2015). Consideration of personality traits (Goldenberg *et al.*, 2017) would further inform our understanding of the differential effects of wildlife tourism on wild animals. It is important that future research investigates whether animals in other areas react in a similar manner, showing changes in behaviour. This will allow management decisions to be guided by up-to-date, quantitative and qualitative findings and allow reserves to advertise high animal welfare standards.

Ethical Statement

All data collected were non-invasive and received ethical clearance from Liverpool John Moores University (NK_IS/2016-6) as well as permission from the North West Parks Board. This research adhered to the Association for the Study of Animal Behaviour guidelines for ethical treatment of animals.

Data Accessibility

Prim8 Software can be accessed and downloaded here: http://www.prim8software.com/
R Statistical Software can be accessed and downloaded here: https://www.R-project.org/
Data and code can be accessed here: Link to data on figshare will be made available here upon MS acceptance

Author's Contributions

I.S. carried out data collection, statistical analysis, conceptualized the project and wrote the paper.

N.K. supervised and conceptualized the project and edited several drafts of the paper. Y.P. co-supervised and conceptualized the project and edited the final draft.

Conflict of Interest

We have no conflict of interest.

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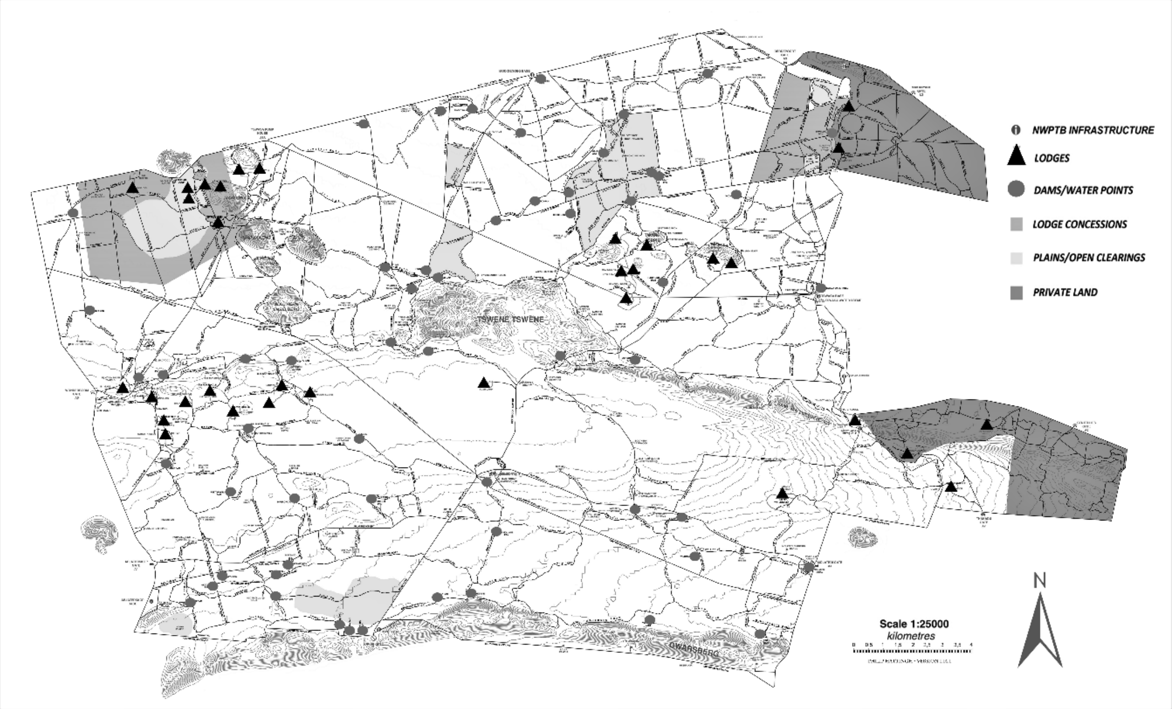

Figure 1.
Map of Madikwe Game Reserve, South Africa, in 2014. Dark grey areas are private concessions, grey areas are private concessions used with lodge permission, and light grey areas are open plains where off-roading was prohibited. Lines are roads, triangles are locations of lodges, and all waterholes (containing water either year-round or during the wet season) are indicated as circles. Map courtesy of P. Hattingh (2014).

Table 1. Factors recorded for five-minute continuous behavioural observations of African elephants, *Loxodonta africana*, carried out in Madikwe Game Reserve, South Africa.

Factor	**Levels**	**Description**
Sex	Female, male	Sex of focal individual
Age	Adult, juvenile	Age of focal individual
Herd type	Lone male, bull group, cow-calf group, mixed group	Type of herd in which focal individual was observed
Herd size	1-100	Number of animals in the herd
Habitat type	Shrub, dense shrub, open grassland, waterhole[a]	Type of habitat the focal individual was observed in
Season	Dry, wet	Season in which observation took place.
Vehicle	0-3	Number of GD vehicles present during the focal observation
Travel direction	Retreat, stay	The direction of movement of the core of an elephant herd in relation to present GD vehicles. If a herd moved parallel to, or towards vehicles, it was classes as stay, if the distance of the core of the herd increased by ≥10 m from vehicles, it was classed as retreat

[a] Shrub= various bushes and trees in observed area but not obscuring observation noticeably; dense shrub= shrub and trees in observed area, growing so densely that observation only possible at close distance and dense enough to cover view of large areas of the body of the focal animal; open grassland= observation area vastly open with only occasional bushes or trees; waterhole= water accumulated either naturally or pumped artificially with enough water for one or more elephants to drink

Table 2: Results of a type II ANOVA on a GLMM for the occurrence of stress-related behaviour in known African elephants, *Loxodonta africana*, in Madikwe Game Reserve, South Africa. Fixed effects' estimates and standard errors (SE) are from the model summary and X^2 values, degrees of freedom (df) and p-values are from a type II ANOVA.

Fixed effect	**Levels**	**Estimate (± SE)**	X^2	**df**	***p*-value**
Intercept		-0.581(±1.41)			
Tourist		-0.138(±0.23)	0.378	1	0.539
Herd type (Bull group)	Cow-calf group	-0.871(±1.29)	1.74	3	0.628
	Lone male	-1.418(±1.09)			
	Mixed group	-0.938(±1.3)			
Sex (Female)	Male	-0.235(±0.83)	0.081	1	0.777
Habitat (Dense shrub)	Open grassland	0.067(±1.12)	1.372	3	0.712
	Shrub	-0.168(±0.66)			
	Waterhole	0.425(±0.69)			
Season (Dry)	Wet	-0.106(±0.5)	0.045	1	0.833
Herd size		-0.19(±0.3)	0.406	1	0.524
Age (Adult)	Juvenile	0.199(±0.77)	0.068	1	0.795

Table 3: Results of a type II ANOVA on a GLMM for the occurrence of vigilance behaviour in known African elephants, *Loxodonta africana*, in Madikwe Game Reserve, South Africa. Fixed effects' estimates and standard errors (SE) are from the model summary and X^2 values, degrees of freedom (df) and p-values are from a type II ANOVA. Significant effects in bold, where significance was assigned at $p \leq 0.05$.

Fixed effect	**Levels**	**Estimate (± SE)**	X^2	**df**	***p*-value**
Intercept		-0.238(±1.38)			
Tourist		-0.091(±0.21)	0.199	1	0.656
Herd type (Bull group)	Cow-calf group	-0.880(±1.31)	1.858	3	0.602
	Lone male	-0.002(±1.01)			
	Mixed group	-1.318(±1.31)			
Sex (Female)	Male	-0.258(±0.80)	0.103	1	0.749
Habitat (Dense shrub)	Open grassland	0.654(±0.89)	26.758	3	**<0.001**
	Shrub	0.187(±0.54)			
	Waterhole	2.924(±0.69)			
Season (Dry)	Wet	0.420(±0.44)	0.899	1	0.343
Herd size		0.118(±0.28)	0.184	1	0.668
Age (Adult)	Juvenile	0.627(±0.77)	0.667	1	0.414

Table 4: Results of a type II ANOVA on a GLMM for the occurrence of conspecific-directed aggressive behaviour in known African elephants, *Loxodonta africana*, in Madikwe Game Reserve, South Africa. Fixed effects' estimates and standard errors (SE) are from the model summary and X^2 values, degrees of freedom (df) and p-values are from a type II ANOVA. Significant effects in bold, where significance was assigned at $p \leq 0.05$.

Fixed effect	**Levels**	**Estimate (± SE)**	X^2	**df**	***p*-value**
Intercept		-6.506(±2.19)			
Tourist		0.704(±0.30)	5.439	1	**0.02**
Herd type (Bull group)	Cow-calf group	2.496(±1.83)	2.980	3	0.395
	Lone male	-0.494(±1.61)			
	Mixed group	1.984(±1.76)			
Sex (Female)	Male	2.843(±1.22)	5.409	1	**0.02**
Habitat (Dense shrub)	Open grassland	1.359(±1.72)	7.915	3	**0.048**
	Shrub	1.176(±1.21)			
	Waterhole	2.729(±1.23)			
Season (Dry)	Wet	0.049(±0.61)	0.006	1	0.936
Herd size		0.567(±0.33)	3.050	1	0.081
Age (Adult)	Juvenile	-1.046(±1.14)	0.836	1	0.361

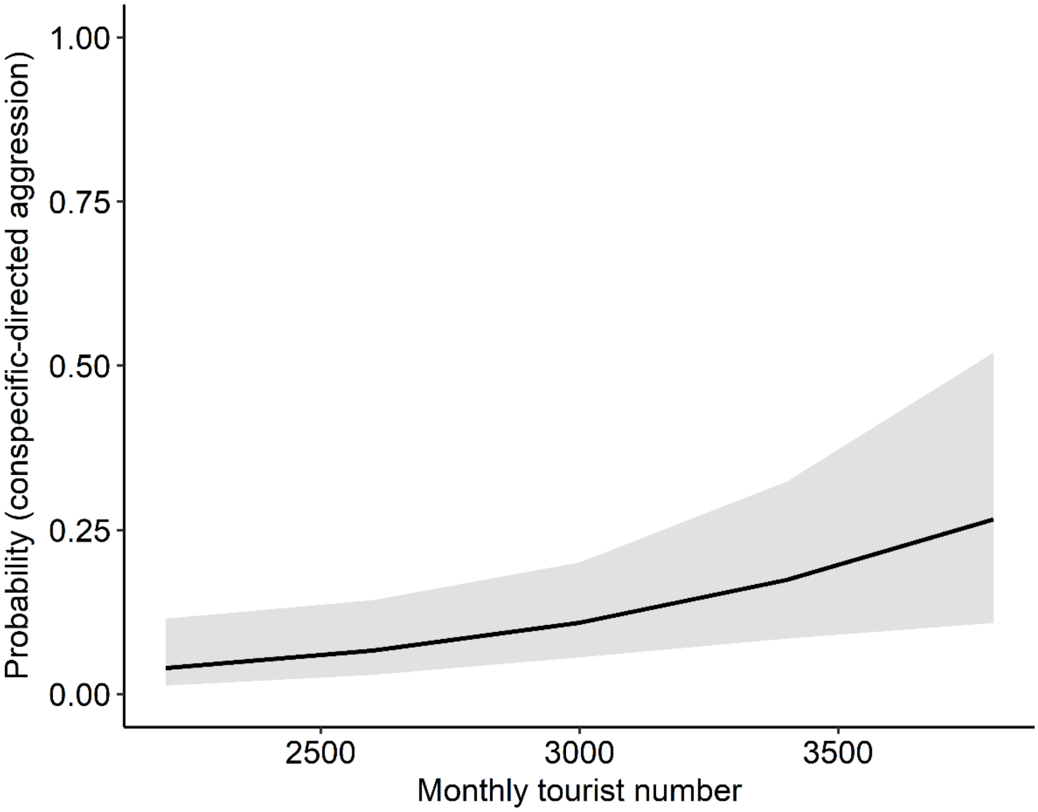

Figure 2.
Predicted mean probability of a Generalized Linear Mixed Model analysing the effect of tourist pressure on the probability of known African elephants, *Loxodonta africana*, in Madikwe Game Reserve displaying conspecific-directed aggressive behaviour. Grey areas represent 95% confidence intervals.

Table 5: Results of a nonparametric bootstrap (1000 iterations) of a GLM for the impact of several fixed effects on the probability of African elephant herds, *Loxodonta africana*, in Madikwe Game Reserve, South Africa, moving away from observers. Fixed effects' estimates and standard errors are from the model summary, and level comparisons and 95% Confidence Intervals are from bootstrapped confidence intervals. Significant effects are shown in bold.

Fixed effect	**Levels**	**Estimate (± SE)**	**Levels (reference level vs. comparison level)**	**95% Confidence Intervals**
Intercept		-0.500(±0.39)		
Herd type (Bull group)	Cow-calf group	0.394(±0.35)	Bull group: Cow-calf group	-0.068 to 0.233
	Lone male	0.215(±0.32)	Bull group: Lone male	-0.095 to 0.169
	Mixed group	0.457(±0.40)	Bull group: Mixed group	-0.069 to 0.269
			Cow-calf group: Lone male	-0.187 to 0.093
			Cow-calf group: Mixed group	-0.112 to 0.159
			Lone male: Mixed group	-0.118 to 0.225
Vehicle		0.540(±0.66)		-0.230 to 0.481
Habitat (Dense shrub)	Shrub	-0.318(±0.27)	Dense shrub: Shrub	-0.221 to 0.060
	Waterhole	-0.340(±0.33)	Dense shrub: Waterhole	-0.241 to 0.086
			Shrub: Waterhole	-0.117 to 0.101
Season (Wet)	Dry	-0.128(±0.21)	Dry: Wet	-0.130 to 0.063
Herd size		0.005(±0.13)		-0.055 to 0.065
Herd type * GD (Bull group * GD)	Cow-calf group *GD	-0.485(±0.47)	Bull group*GD: Cow-calf group*GD	-0.360 to 0.038
	Lone male *GD	-0.283(±0.44)	Bull group*GD: Lone male*GD	-0.304 to 0.079
	Mixed group *GD	-0.515(±0.41)	**Bull group*GD: Mixed group*GD**	**-0.340 to -0.001**
			Cow-calf group*GD: Lone male*GD	-0.111 to 0.236
			Cow-calf group*GD: Mixed group*GD	-0.147 to 0.197
			Lone male*GD: Mixed group*GD	-0.174 to 0.063
Habitat type * GD (Dense shrub * GD)	Shrub *GD	0.034(±0.50)	Dense shrub*GD: Shrub*GD	-0.268 to 0.308
	Waterhole *GD	0.103(±0.52)	Dense shrub*GD: Waterhole*GD	-0.271 to 0.349

			Shrub*GD: Waterhole*GD	-0.092 to 0.138
Season * GD (Dry * GD)	Wet *GD	-0.044(±0.22)	Dry*GD: Wet*GD	-0.108 to 0.118

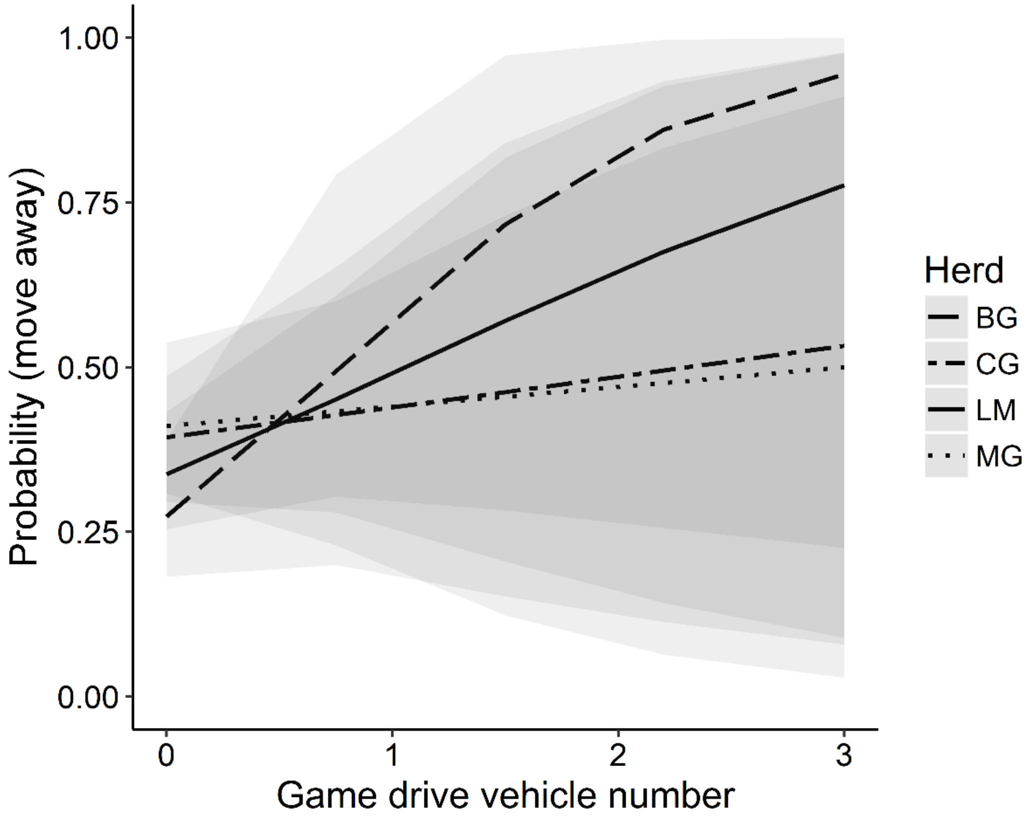

Figure 3.
Predicted mean probability of a Generalized Linear Model analysing the effect of game drive vehicle presence on the probability of African elephant herds, *Loxodonta africana*, in Madikwe Game Reserve moving away from observation points in different herd types. BG: bull group; CG: cow-calf group; LM: lone male; MG: mixed group. Grey areas represent 95% confidence intervals.

Table S1. Full ethogram of all behaviours included in this study of African elephants, *Loxodonta africana*, in Madikwe Game Reserve. Each behaviour was assigned to a specific category for statistical analysis.

Behaviour category	**Behaviour included**	**Description of behaviour**
Stress-related behaviour	Run	Animal is moving fast without feeding and often with the whole herd moving away from a specific stimulus such as a predator
	Trunk to body	Animal is touching own body with trunk. Different from scratching. Can happen during locomotion
	Trunk to face	Animals' trunk is touching its face for very short duration. Can happen during locomotion
	Trunk twirl	Animal is curling its trunk in a swift motion. Can happen during locomotion
Vigilance behaviour	Smell	Animal extends the trunk down or up, with the tip of the trunk curled horizontally. Often the trunk is rotated in several directions to pick up scent. Can happen during locomotion
	Trunk to mouth	Animal puts the tip of its trunk into its own mouth without ingesting any food or water possibly processing chemicals using its vomeronasal organ. Can happen during locomotion
	Vigilance	Animals' head is held high and ears are spread out. Often the head is moved from one side to another such as to listen to the surrounding
Conspecific-directed aggressive behaviour	Charge	Fast walk, often with ears out and head held high, towards a conspecific. Can be accompanied by a trumpet
	Displace given	Focal animal is approaching a conspecific which leaves the currently occupied spot
	Ears flapping	Animal is moving ears in and out resulting in a loud noise when the ears hit the body. Can happen during locomotion
	Ears out	Animal is spreading its ears outwards, away from the body, making it appear larger. Can happen during locomotion
	Head shake	Animal rapidly moves the head in a flowing motion tilting it from the right to the left, resulting in the ears flapping against the body and making a loud sound. Often, this is done whilst turning towards the stimulus at which the head shake is directed
	Pushing object	Animal is pushing an object such as a tree with its body
	Redirected aggression	Animal often will have received aggression by a dominant individual or was the loser of a play or aggressive sparring interaction. Often redirected aggression can be throwing around leaves or sticks or turning rapidly from the dominant individual and push a tree over or uproot a bush
	Slap	Animal is using its trunk or head to strike a conspecific
	Sparring aggressive	Animal is pushing with conspecific head to head often with their trunks entwined and tusks clashing against each other

	Standing tall	Animal is standing with its head held high up and glancing forwards over the trunk
	Trunk swing	Animal is swinging trunk backwards and forwards between the front legs, often whilst exhaling or stepping forward. Can happen during locomotion
	Tusk	Animal is pushing its tusks into conspecifics body. Can happen during locomotion

Short summary:

Wildlife tourism can increase stress in a variety of species and affect welfare and behaviour. We assessed whether wildlife tourism affected African elephants' physiological stress levels and found that greater tourist numbers were positively correlated with stress. Reserve managers should provide potential alleviation measures for elephants during periods of high tourist pressure, for example, by ensuring refuge areas are available.

Physiological stress response of African elephants to wildlife tourism in Madikwe Game Reserve, South Africa

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Running head: Stress response of African elephants to tourism

Abstract

Context: Wildlife tourism has been shown to increase stress in a variety of species and can negatively affect individuals' survival, reproduction, welfare, and behaviour. In African elephants *Loxodonta africana* increased physiological stress has been linked to use of refugia, rapid movement through corridors, and heightened aggression towards humans. However, we are unaware of any studies assessing the impact of tourist pressure (tourist numbers) on physiological stress in elephants.

Aims: We used faecal glucocorticoid metabolite (fGCM) concentrations to investigate whether tourist numbers in Madikwe Game Reserve, South Africa, were related to changes in physiological stress in elephants.

Methods: We repeatedly collected dung samples (n=43) from 13 individually identified elephants over 15 months. Using a Generalised Linear Mixed Model and a Kenward-Roger approximation, we assessed the impact of monthly tourist numbers, season, age, and sex on elephant fGCM concentrations.

Key results: High tourist numbers were significantly related to elevated fGCM concentrations. Overall, fGCM concentrations increased by 112% (from 0.26 to 0.55 µg/g dry weight) in the months with highest tourist pressure, compared to months with lowest tourist pressure.

Conclusions: Managers of fenced reserves should consider providing potential alleviation measures for elephants during high tourist pressure, for example, by ensuring refuge areas are available. This may be of even higher importance if elephant populations have had traumatic experiences with humans in the past, such as poaching or translocation. Such management action will improve elephant welfare and increase tourist safety.

Implications: Whilst tourism can generate substantial revenue to support conservation action, careful monitoring of its impact on wildlife is required to manage potential negative effects.

Keywords: conservation, faeces, stress endocrinology, physiology, wildlife management, welfare, African elephant

Introduction

Wildlife conservationists can use stress-related hormone measurements to assess welfare, translocation success, and the ability to cope with injury, disease, and environmental challenges (Millspaugh & Washburn 2004; Teixeira *et al.* 2007; Ganswindt *et al.* 2010a). Perceiving stress is a normal process and may even be adaptive in the short term. However, prolonged or chronic stress, and the inability to cope with it, can lead to changes in an individual's behaviour and cognition, which might detrimentally affect reproduction, welfare, and survival (Sapolsky 2002; McEwen & Wingfield 2003; Bhattacharjee *et al.* 2015).

What an individual perceives as a stressor, depends on past experiences, personality traits and the amount of control an individual perceives to have in a given situation (Koolhaas *et al.* 1999; Bradshaw *et al*. 2005; Nelson & Kriegsfeld 2017). When a perceived stressor disrupts homeostasis, an organism's stable physiological state, the neuroendocrine systems and/or behavioural responses are activated to cope with the stressor and re-establish homeostasis (McEwen & Wingfield 2003; Palme 2019). The neuroendocrine response involves activation of what is called the hypothalamic-pituitary-adrenal axis, resulting in increased secretion of hormones referred to as glucocorticoids (GCs; Nelson & Kriegsfeld 2017). Increased glucocorticoid concentrations over longer periods of time are related to suppression of reproductive hormones and the immune system, as well as muscle loss and reduced growth (Nelson & Kriegsfeld 2017). If a stressor becomes chronic, individuals may therefore become more susceptible to predation, starvation, disease, and decreased reproduction, as well as experiencing lasting changes of behaviour (Reynolds & Braithwaite 2001; McEwen & Wingfield 2003; Teixeira *et al.* 2007). Therefore, changes in GC concentrations are often measured as a physiological response to stress (Möstl & Palme 2002; Sapolsky 2002; Touma & Palme 2005) and used as a welfare indicator.

GCs can be measured using faecal glucocorticoid metabolite (fGCM) concentrations excreted in dung. This approach is advantageous as it does not require restraint or capture of animals and thus does not interfere with an animal's natural behaviour (Sheriff *et al.* 2011). FGCM monitoring therefore allows us to noninvasively assess animal welfare, effects of environmental conditions, as well as human induced disturbance (Millspaugh & Washburn 2004; Millspaugh *et al.* 2007; Palme 2012; Scheun *et al.* 2015). One potential stressor that has been studied across various wildlife species is tourism, which can take several forms, such as watching, feeding, petting, or animals being transported (Orams 2002; Millspaugh *et al.* 2007; Sarmah *et al.* 2017). Tourism has been linked to elevated fGCMs in a range of species, e.g. gray wolf *Canis lupus,* and red deer *Cervus elaphus* (Creel *et al.* 2002), African elephant *Loxodonta africana* (Millspaugh *et al.* 2007), western capercaillie *Tetrao urogallus* (Thiel *et al.* 2008), black howler monkey *Alouatta pigra* (Behie, Pavelka & Chapman 2010), wildcat *Felis silvestris* (Piñeiro *et al.* 2013), Tatra chamois *Rupicapra rupicapra tatrica* (Zwijacz-Kozica *et al.* 2013), western lowland gorilla *Gorilla gorilla gorilla* (Shutt *et al.* 2014), and mountain hare *Lepus timidus* (Rehnus, Wehrle & Palme 2014).

Funding from wildlife tourism, or tourists visiting protected areas, can aid in the protection of habitat, biodiversity, and ecological processes (Reynolds & Braithwaite 2001), and has become increasingly common over the past few years (Orams 2002). However, assessing how wildlife tourism impacts the behaviour, physiological stress, and welfare of the wildlife being viewed is difficult and studies doing so are relatively scarce. African elephants, *Loxodonta africana,* are one of the most popular species viewed by tourists across Africa (Lindsey *et al.* 2007), and are threatened with a drastic decline in numbers due to habitat loss and poaching (Chase *et al.* 2016).

To carry out wildlife tourism in a sustainable and welfare focused manner, it is important to understand whether overall tourist pressure, in form of number of tourists within an elephant's habitat, increases elephant GC concentrations. Further, as elevated GC concentrations in elephants from reintroduced populations have been linked to human fatalities (Slotow *et al.* 2008; Jachowski *et al.* 2012), it is important that managers monitor stress levels in their elephant population to increase tourist safety. Even so, we know of only three studies assessing the effects of wildlife tourism on elephants. A recent study has found that wildlife tourist presence was related to increased alert, fear, stress and aggressive behaviours in Asian elephants *Elephas maximus* (Ranaweerage, Ranjeewa & Sugimoto 2015). In working African elephants, fGCM concentrations were slightly higher on days with human interaction compared to days without interaction (Millspaugh *et al.* 2007). Further, high tourist pressure, in form of total number of tourists in the reserve each month, was related to increased conspecific-directed aggressive behaviours in the population of African elephants in Madikwe Game Reserve, South Africa (Szott, Pretorius & Koyama 2019).

Concentrations of fGCMs provide estimates of circulating steroid levels for an estimated two to three days prior to when the sample was collected; this roughly corresponds with the gut passage time of an elephant (Ganswindt *et al.* 2003; Laws *et al.* 2007). Further, fGCM concentrations in African elephant dung have been shown to be stable for up to twenty hours before collection (Webber *et al.* 2018). Yet, elephant fGCMs must be interpreted with care, as elephants secrete GCs in response to many factors. For example, an elephant's GC secretion may shift according to ecological changes, increasing during low availability of key nutrients, during the dry season, and following large fires within their habitat (Foley, Papageorge & Wasser 2001; Viljoen *et al.* 2008; Woolley *et al.* 2008). Social and environmental stressors may increase elephant fGCM concentrations, such as following trophy hunting of conspecifics (Burke *et al.* 2008), during injury (Ganswindt *et al.* 2010a), living outside of protected areas (Hunninck *et al.* 2017), living in areas of high poaching risk, being in herds with weak social bonds or lacking older matriarchs (Gobush, Mutayoba & Wasser 2008), and increased intra-group competition (Foley *et al.* 2001). Reintroduced or translocated herds have also been found to have increased fGCM concentrations for six to ten years following the intervention (Jachowski, Slotow & Millspaugh 2012) and, at a population level, an even longer-term stress response for over ten years has been suggested (Jachowski, Slotow & Millspaugh 2013a).

Here, we investigated the effect of monthly tourist numbers on fGCM concentrations in a large population of elephants in Madikwe Game Reserve, South Africa (henceforth Madikwe). We hypothesised that high tourist pressure would cause greater stress in elephants and therefore predicted that fGCM concentrations would be elevated during times of high tourist pressure. We further included season as a potential covariate, as it has been shown that fGCM concentrations are elevated during the dry season (Viljoen *et al.* 2008; Jachowski *et al.* 2012). However, because water is artificially pumped at

Madikwe and available throughout the year, we expected season to have a minimal effect. No hunting of elephants took place in Madikwe, or other potential impacting sporadic events such as large fires, and no elephants with visible injuries were sampled. Madikwe has strict driving regulations in place, with a maximum of three game drive vehicles at an elephant sighting at a time, and private vehicles are restricted to main roads. Given these restrictions, we expected tourism to have a minimal effect on elephant fGCM concentrations.

Materials and methods

Study area

Madikwe is a fenced reserve, managed by a state/private/communal partnership and is 680 km² in size (Fig. 1). A total of 228 elephants were introduced to Madikwe between 1992 and 1999 from various traumatic backgrounds (Bradshaw *et al.* 2005). First, 25 orphaned elephants between 8 - 12 years of age were introduced following culling operations in Kruger National Park (Davis & Brett 2003). This was followed by 194 individuals in entire herds from Zimbabwe, aged between a few months to over 50 years, from an area experiencing extreme drought and heavy poaching (Davis & Brett 2003; P.Nel *pers.comm*.). Today, this founding population has grown to 1348 ± 128 elephants (July 2017, North West Parks Board, P. Nel *pers.comm*.), representing one of the highest population densities (1.9 elephants per km²) in South Africa.

Wildlife viewing in Madikwe is carried out from game drive vehicles, which are large, open vehicles driven by qualified field guides, seating up to ten people. Game drives are mainly carried out in the morning (sunrise-11am) or afternoon (3.30pm-sunset). No more than three vehicles were permitted at a given sighting at a time and guests were briefed on appropriate behaviour, such as no shouting or eating, which guides enforced (see Szott *et al.* 2019 for further details). A higher number of tourists in Madikwe directly relates to higher numbers of game drive vehicles on the roads. The current Code of Conduct in Madikwe does not stipulate a minimum distance between elephants and game drive vehicles. There is no limitation to the total number of game drive vehicles conducting game drives within Madikwe. Offroading in Madikwe occurred when viewing certain animals such as leopard *Panthera pardus*, lion *Panthera leo*, buffalo *Syncerus caffer*, or cheetah *Acinonyx jubatus*. As offroading did not occur to view elephants, this meant that elephants could encounter vehicles off-road. Madikwe is accessible for tourists throughout and contains no restricted areas.

Each of the 33 lodges at Madikwe has their own waterhole, providing water all year round (Fig. 1). The reserve is also bordered by the Marico River on the eastern side and contains large artificial dams that pump water throughout the year. According to Mucina and Rutherford (2006), Madikwe contains three main vegetation types: Dwaalboom thornveld contains ultramafic clay plains with a nearly continuous herbaceous layer dominated by grass species, deciduous microphyllous trees and shrubs and a few broadleaf species. Madikwe dolomite bushfeld contains a continuous herbaceous layer dominated by grass species and a woody layer dominated by deciduous trees. The Dwaarsberg-Swartruggens mountain bushveld has various combinations of tree and shrub layers as well as dense grass layers (Mucina & Rutherford 2006). Elephants have access to the whole reserve and can be encountered across all the previously mentioned vegetation types.

Figure 1 here

Data and sample collection

The principal investigator collected the faecal samples between April 2016 and June 2017 throughout Madikwe, spending similar amounts of time in the different areas of the reserve searching for individuals that could be observed defaecating (Fig. 1). As no previous information on Madikwe's elephant population was available, the number of sampled elephants was limited to individuals we were able to identify reliably, so we could collect repeated faecal samples from each. We identified elephants based on distinguishing characteristics such as holes and notches in their ears, wrinkles across the face and orientation of tusk growth (elephantvoices.org 2018), resulting in 12 known individuals of four different cow-calf groups as well as from one solitary adult male. The cow-calf individuals included five adult females, three juvenile males, three juvenile females, and one male calf. Sampling for this study was restricted to elephants encountered near roads, which led to a relatively low rate of sightings of known elephants and consequently a low number of faecal samples collected. A total of 43 faecal samples were collected (mean ± SD per individual = 3.31 ± 1.9, Table 1), with a mean ± SD of 3 ± 3 samples per month.

Samples were collected with sterile gloves following previously published protocols (Ganswindt *et al.* 2010a,b). We stored approximately 50 g of faecal matter in a sterile vial in a cooler box on ice and transferred it to a freezer at -18 °C no longer than four hours after collection. For each sample we recorded the sex, age class (calf (0 - 3 years), juvenile (4 - 12 years), or adult (13 years or older), Moss 1996; elephantvoices.org 2018), and ID of the defaecating individual, the time, and the longitude and latitude on a Lenovo TAB 2 A8-50F tablet. The average time between observing an elephant defaecating and sample collection was 16 min (±12mins).

We defined wet and dry season based on average monthly rainfall measured at four stations in Madikwe by the South African Weather Service. Average total rainfall in Madikwe during the study period was 189.69 mm. We classed wet season as the period in which 95% of precipitation for the study year fell (Loarie, van Aarde & Pimm 2009). During the dry season (May 2016 - September 2016 and March 2017 - June 2017) mean (± SD) monthly rainfall was 6.79 ± 7.79 mm, and during the wet season (October 2016 - February 2017) mean monthly rainfall was 118.89 ± 63.51 mm.

South African North West Parks Board provided the total number of tourists visiting Madikwe each month. Tourist number was assessed as the number of guests counted at the gate to the reserve and the total number of tourists per month, within each season, is shown in Figure 2.

Figure 2 here

Steroid extraction and faecal glucocorticoid metabolite analysis

Steroid extraction and analysis was carried out at the Endocrine Research Laboratory, University of Pretoria, South Africa, and followed previously published protocols (Fieß, Heistermann & Hodges 1999; Ganswindt *et al.* 2003; Ganswindt *et al.* 2010b). In short, faecal matter was lyophilized and pulverized before being sieved through a mesh to remove any undigested faecal matter. Between 0.050 – 0.055 g of the remaining powder was extracted with 3 ml 80% ethanol in water. The suspension was vortexed for 15 minutes and then centrifuged for 10 minutes at 1500 g and the supernatant then transferred to a microcentrifuge tube. An 11-oxoaetiocholanolone enzyme immunoassay (EIA; detecting fGCMs with a 5β-3α-ol-11-one structure (Möstl *et al.* 2002)) was used to measure immunoreactive fGCMs in diluted extracts (1:10 or 1:50 in aqueous buffer). This EIA has been validated and repeatedly used to monitor adrenocortical activity in elephants (Ganswindt *et al.* 2003; 2005; 2010a). Sensitivity of the assay at 90% binding was 1.2 ng/g dry faecal mass. Repeated measurements of high- and low-value controls determined intra-assay variance of 3.3% and 5.6% (15 and 16 plates used for high- and low-quality control respectively) and inter-assay variance (13 plates used) of 9.5% and 12.3%.

Data analysis

We analysed data in R v.3.4.1 (R Core Team 2000) and assessed factors to rule out collinearity using variance of inflation factor (VIF) analysis (Fox & Monette 1992) in the *car* package (Fox & Weisberg 2011), using a cut-off value of 2. Tourist number was scaled and centred and all VIF values were below 2. We analysed the samples with a Generalized Linear Mixed Effects Model with a gamma error structure and log link because data resembled a normal distribution with a log10 transformation. Using the 'glmer' command (*lme4* package) we ran the following model:

$$glmer\ (formula\ =\ fGCMs \sim Tourist\ +\ Season\ +\ (1|ID), data\ =\ data, family\ =\ Gamma\ (link\ =\ \text{“}log\text{”}))$$

To control for the relatively small sample size of our study, we used a Kenward-Roger approximation (Kenward & Roger 1997; Luke 2017) with the *afex* package (Singmann *et al*. 2018) to obtain *p*-values for our fixed effects. Significance was assigned at *p*<0.05. Due to the low sample sizes, we excluded the hour in which the sample was collected, sex and age from this analysis. However, a model including time of sample collection, sex and age did not find significant effects of these factors (see supplementary Table S1).

Although our sample size (n=43) was slightly lower than previously recommended for a Kenward-Roger approximation, it was close to n=45, which has been suggested to provide robust results (Arnau *et al.* 2013). Further, Arnau and colleagues (2013) showed that small to moderately skewed data (indicated by values of 0.8 and 1.6 respectively) is best assessed with a Kenward-Roger approximation. An approximate ratio of 1:2 in kurtosis between the largest and smallest group (in our case wet and dry season respectively) indicates a robustness of 60% or higher for the Kenward-Roger approximation (Arnau *et al.* 2013). In our case, wet season skewness of tourist pressure was 1.09 whilst dry season skewness was -0.02, and wet season kurtosis of tourist pressure was 3.73 whilst dry season kurtosis was 1.69.

We plotted graphs using the packages *effects* (Fox 2003) and *ggplot2* (Wickham 2016) using the unscaled data for ease of interpretation.

Results

Overall fGCM concentrations ranged from 0.05 to 1.02 µg/g dry weight (DW) with an overall mean (± SD) of 0.39 (± 0.22) µg/g DW (Table 1).

Table 1 here

Tourist numbers ranged from 2156 to 3762 tourists per month, an increase of 74.5% from lowest to highest tourist numbers and with an average (± SD) of 2831 (± 563) throughout the study period (Fig. 2). During the dry season, tourist numbers ranged from 2156 to 3762 tourists per month, and during the wet season they ranged from 2741 to 3614 tourists per month (Fig. 2).

High monthly tourist numbers in Madikwe had a significant effect on fGCM concentrations in our individually identified elephants (Table 2, Fig. 3a, b). Season did not have an impact on fGCM concentrations. Removing the adult male and calf from the data set or nesting ID in social group did not change these results. Removing six individuals (n=14 samples) that did not have samples in both high and low tourist numbers (above and below the mean tourist number) did not change the effect of tourist numbers on fGCM concentrations either.

Table 2 here

Figure 3 here

Discussion

Our aim was to investigate the physiological stress response of African elephants to tourist pressure, using fGCM concentrations of elephants and the number of visitors per month in Madikwe Game Reserve. We found that increasing tourist pressure was related to increasing fGCM concentrations. Our results indicate that wildife tourism is a stressor and are consistent with previous behavioural studies linking elevated fGCM concentrations to heightened aggression towards humans (Slotow *et al.* 2008; Jachowski *et al*. 2012), use of refugia (Jachowski *et al.* 2013b, c) and human interactions (Millspaugh *et al.* 2007). Our study thus contributes to a growing body of evidence that tourist pressure impacts physiological stress in elephants and adds to literature about the effects of wildlife tourism on stress in a range of species (Thiel *et al.* 2008; Behie *et al.* 2010; Piñeiro *et al.* 2013; Zwijacz-Kozica *et al.* 2013; Shutt *et al.* 2014; Rehnus *et al.* 2014). Such research highlights the need to monitor the potential for chronic stress in wildlife populations exposed to tourism.

Madikwe's strict regulations of only three vehicles in any sighting could have potentially limited the effect of tourist activity on fGCM concentrations in elephants and we had expected only subtle effects of tourism on stress. Further, elephants could have habituated to tourist presence throughout the years, in which case we would not see an effect of tourist pressure on fGCM concentrations. However, we found that fGCM concentrations increased from the lowest estimate of 0.26 µg/g DW when tourist pressure was low, to 0.55 µg/g DW during times of high tourist pressure, an increase of 112% (Fig. 3a). It is unknown which stimuli related to tourism may have caused an increase in elephant's GC concentrations, but possibilities include increased air traffic, vehicle noise, or vehicle encounter rate.

This study further presents the first published record of physiological stress levels of the Madikwe elephant population. The mean (±SD) fGCM concentration from samples collected for this study was 0.39 (± 0.22) µg/g DW, and values related to tourist pressure ranged from 0.26-0.55 µg/g DW (Fig. 3). No data of female African elephant's fGCM concentrations have been published with which a comparison of absolute values would be possible. This is due to, for example, differences between studies in methodologies such as sampling protocol, steroid extractions, and steroid assays used (Palme 2019). However, previous studies from Kruger National Park, South Africa, using the same collection procedure, as well as steroid extraction and assay protocols, provide an estimated fGCM concentration range of 0.29 and 0.30 µg/g DW for two adult male elephants (Ganswindt *et al.* 2010a) and a median of approximately 0.30 µg/g DW for six adult bulls (Ganswindt *et al.* 2010b), which are similar to those from Madikwe. The two adult bulls from Kruger National Park were also observed to exhibit an increase of 169% and 23% in fGCM concentrations respectively during a stressful period of injury (Ganswindt *et al.* 2010b). The values of 23% and 169% related to injury in those Kruger bulls fall above and below the increase of 112% related to tourism presented in this study, indicating that an increase in stress related to tourism is comparable to an increase in stress related to injury.

Fences have been shown to force elephants to revisit foraging patches more frequently, restrict elephant movement and increase frequency of interactions with unrelated family herds (Munshi-South *et al.* 2008; Loarie *et al.* 2009), adding to perceived stress of elephants. Nevertheless, the average fGCM concentrations of Madikwe's elephants was similar to baseline levels of Kruger bulls (Ganswindt *et al.* 2010b). This may suggest that the Madikwe population is, in terms of physiological stress, relatively unaffected by its high density at this stage.

Chronic stress has been linked to elephants becoming hyperaggressive and aggressive towards humans (Bradshaw *et al*. 2005; Slotow *et al*. 2008; Jachowski *et al.* 2012). Given the traumatic background of the originally translocated elephants in Madikwe, those individuals may be more prone to perceive humans as a negative stressor. So called "problem animals" are usually shot after attacking humans, with several such cases occuring before 2000 in Madikwe (Slotow *et al.* 2008). We did not observe elephants to be extremely aggressive towards tourists, unless game drive vehicles approached individuals at a very close distance (<10 meters; I.Szott & Y.Pretorius *pers.obs*.). However, we have recently shown that high tourist pressure in our study population was linked to increased conspecific-directed aggressive behaviours in elephants (Szott *et al.* 2019). Increases in aggression in elephants are a concern for human safety and elephant welfare. As we did not observe increases in behaviours indicating stress, such as elephants touching their own faces with their trunks or curling their trunks (Poole 1995; elephantvoices.org), in our study population (Szott *et al.* 2019), it may be possible that the increased conspecific-directed aggression observed presents a coping mechanism (Nelson & Kriegsfeld 2017) related to the increase in fGCM concentrations during high tourist pressure.

As expected from the year-round supply of artificially pumped water at Madikwe, we found that fGCM concentrations did not increase during the dry season (*cf.* Foley *et al.* 2001; Viljoen *et al.* 2008). Due to our small sample size, we did not include sex or age in our final model, but when included, neither factor was significant. Previous studies did not find an effect of age class or sex on fGCM concentrations (Viljoen *et al.* 2008; Pinter-Wollman *et al.* 2009), although Ahlering *et al.* (2013) did report female elephants had significantly lower fGCM concentrations compared to males. Nevertheless, we cannot draw a meaningful conclusion on those factors given our small sample size.

Reproductive state in the form of pregnancy or parturition can affect fGCM concentrations in animals (Palme 2019). Unfortunately, we were not able to collect information on those variables in our sampled adult females but at least three of the adult females had suckling calves and were lactating throughout the study period, thus the increase in fGCM concentrations was unlikely due to a shift from non-lactation to lactation. In addition, the effect of tourist pressure followed the same trend in all elephants regardless of sex or age (Fig. 3b), suggesting that reproductive state did not affect how females were influenced by increasing tourist pressure.

With regard to management implications in Madikwe, the authors encourage the establishment of a refuge area for elephants, as well as other wildlife. Available refuge areas and corridors with limited human disturbance are vital for elephants in altered physiological states (Jachowski *et al.* 2013b, c). Further, access to such an area could add to elephants‘ sense of control, which can reduce perceived stress (Nelson & Kriegsfeld 2017). Therefore, such refuge areas not only allow elephants to avoid contact with humans, but can also ensure human safety during when elephants have increased fGCM concentrations. A sufficiently large designated area should be established in which no guided walks are carried out, where offroading of vehicles is strictly forbidden and vehicles are restricted to roads. Due to the southern area of Madikwe having fewer roads in place already, this may present the best opportunity to establish such a refuge area. A strictly enforced refuge area would likely not only be of benefit for elephants, but also for other animals found in Madikwe during times of high tourist pressure and allow Madikwe to advertise that is prioritises animal welfare.

Our study had a relatively small sample size and so results should be interpreted with caution. However, we used a repeated measures study design, included ID of each animal to control for individual variation, and applied a Kenward-Roger correction to adjust the *p*-values. The effect of tourist pressure on fGCM concentrations reported here therefore appears to be robust, especially given that we were able to find such a distinct effect with a limited number of samples. However, further research is needed in order to identify which stimuli are perceived stressors to elephants in order to inform management of fenced reserves, especially during times of high tourist pressure, and to assess whether perceived stress in elephants is chronic. More research is also required in other fenced reserves, such as Madikwe, as well as in unfenced areas.

This study adds to a growing body of literature investigating the impacts of wildlife tourism on wildlife. Increased tourist pressure led to higher fGCM concentrations in Madikwe elephants. A refuge area, in which tourist access is restricted, would likely add to elephants' sense of control and may aid in reducing stress related to high tourist pressure. This will increase animal welfare standards as well as human safety during such times.

Conflict of Interest

The authors declare no conflicts of interest.

Ethical Statement

Our data collection was non-invasive and received ethical clearance from Liverpool John Moores University (NK_IS/2016-6) as well as permission from the South African North West Parks Board. This research adhered to the Association for the Study of Animal Behaviour guidelines for the ethical treatment of animals.

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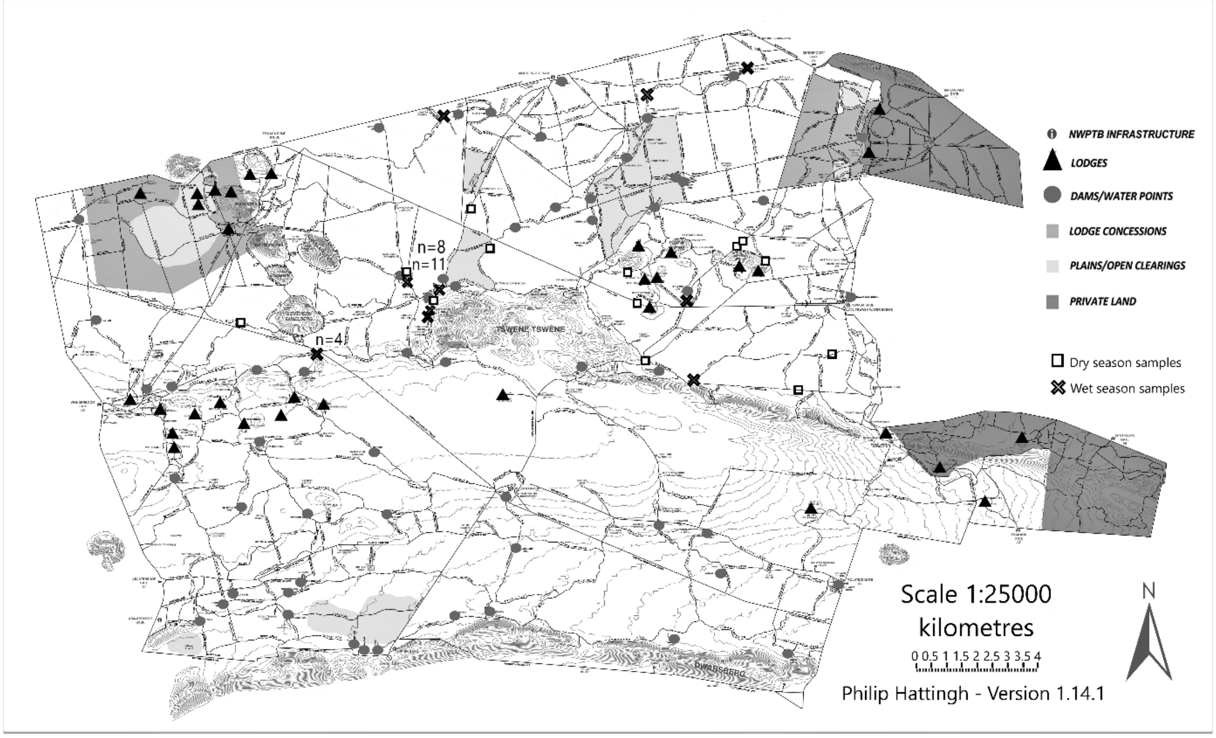

Figure 1.
Map of Madikwe Game Reserve, South Africa, as of 2014. Game drives take place throughout the whole reserve. Dark grey areas are private concessions, used for game drives only by their respective lodge, grey areas are private concessions used for game drives by any lodge with prior permission but usually restricted to three vehicles within the area at any time. Light grey areas are open plains in which off-roading is prohibited. Lines are roads, triangles are lodges, circles are waterholes (year round or during wet season). Crosses and squares are locations at which dung samples of African elephants *Loxodonta africana* were collected during the dry season (squares) and wet season (crosses). Where several dung samples were collected at the same location, the number of samples (n) is given. Map courtesy of P.Hattingh (2014).

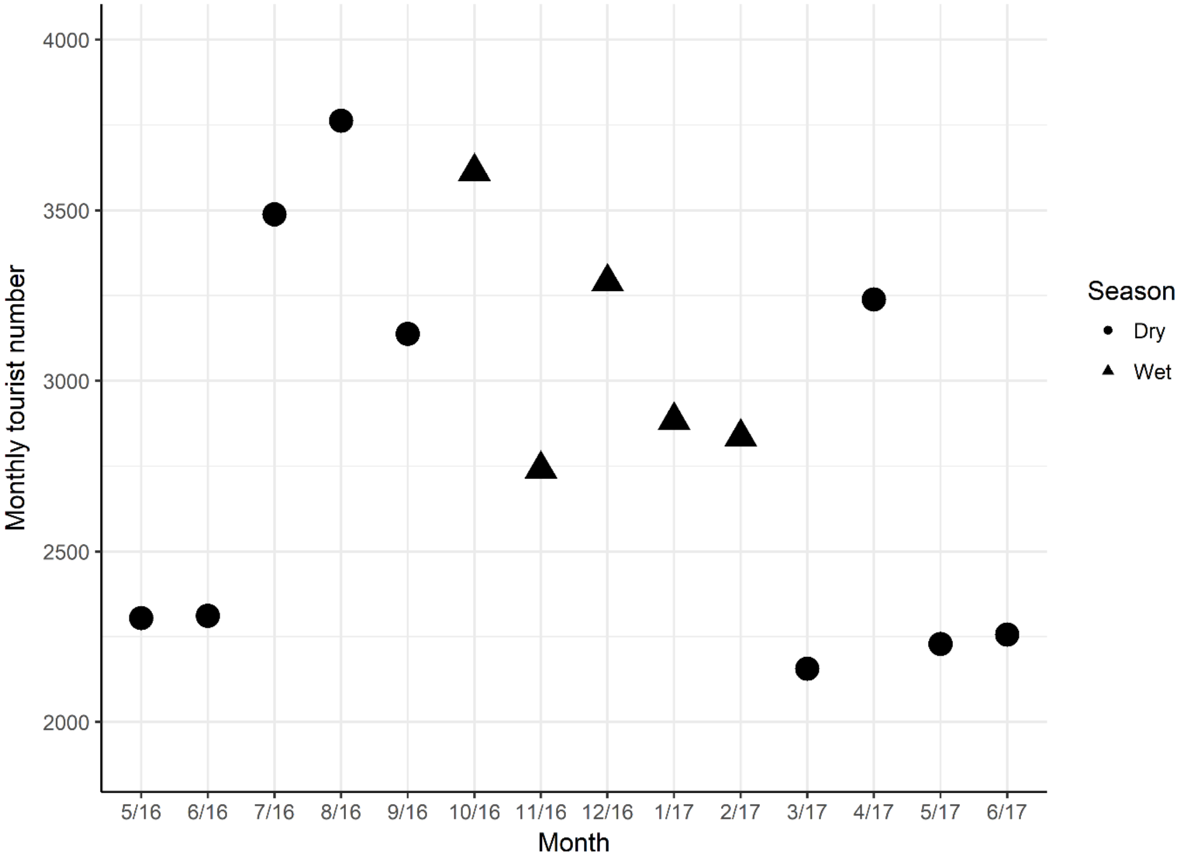

Figure 2.
Total number of tourists per month in Madikwe Game Reserve, South Africa, between May 2016 and June 2017. Dry season (circles) lasted from May 2016 to September 2016 and from March 2017 to June 2017. Wet season (triangles) lasted from October 2016 to February 2017.

Table 1. Faecal glucocorticoid metabolite (fGCM) concentrations of 13 individually identified African elephants *Loxodonta africana*, in Madikwe Game Reserve, South Africa. Concentrations are in µg/g dry weight. ID number of individuals, their age class and sex are presented (with overall mean ± SD fGCM concentrations) and a breakdown of number (n) of samples collected during the dry and wet season.

Sex	Age class	ID	fGCM concentration µg/g dry weight during dry season	fGCM concentration µg/g dry weight during wet season	N samples per individual
Female 0.38 ± 0.2	Adult 0.40 ± 0.21	1	0.46 0.58	-	2
		2	0.56	0.91	2
		3	0.2 0.22 0.64 0.23	0.17 0.34 0.4 0.19	8
		4	0.47 0.6	0.16 0.59	4
		5	0.16 0.42 0.24	-	3
	Juvenile 0.35 ± 0.23	6	0.37	0.39 0.31 0.19 0.55	5
		7	-	0.26 0.6	2
		8	-	0.09 0.38	2
Male 0.48 ± 0.28	Adult 0.10 ± 0.06	9	0.14	0. 05	2
	Juvenile 0.48 ± 0.26	1	0.53	0.57 1.02	3
		11	0.27 0.53 0.12 0.74	0.26 0.24	6
		12	0.55	0.49	2
	Calf 0.21 ± 0.12	13	-	0.29 0.12	2
N samples per season			20	23	43

Table 2. GLMM results of the fixed effects on faecal glucocorticoid metabolites of African elephants *Loxodonta africana* in Madikwe Game Reserve, assessed with a Kenward-Roger approximation.

[a]SE=Standard error, [b]df=Degrees of Freedom, significant effects in bold

Fixed effect (reference level)	**Level**	**Estimate ± SE[a]**	**df[b]**	**F**	***p*-value**
Intercept		0.400 ± 0.05			
Tourist		**0.090 ± 0.04**	**36.93**	**6.08**	**0.02**
Season (Dry)	Wet	0.057 ± 0.03	34.09	2.74	0.11

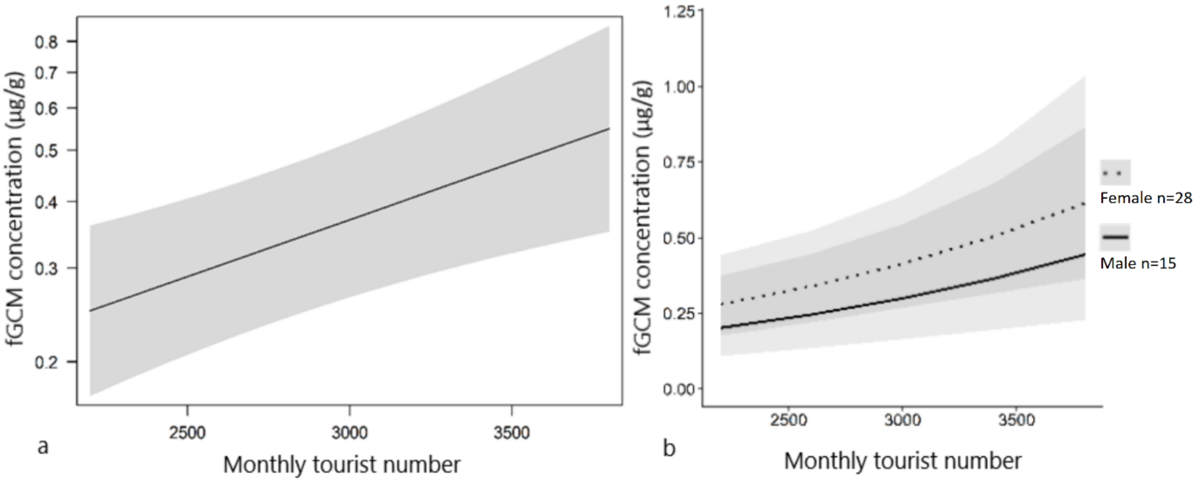

Figure 3.
Effect of total tourist numbers per month (*p*=0.02), as assessed by a Generalised Linear Mixed Effects Model, on faecal glucocorticoid metabolite concentration (µg/g dry weight) of African elephants *Loxodonta africana* in Madikwe Game Reserve, South Africa. 3a presents the overall effect of tourist pressure on elephants, whilst 3b presents the effect of tourist pressure on females (F) and males (M). Grey areas represent 95% confidence intervals.

Supplementary Table S1. GLMM results of the fixed effects on faecal glucocorticoid metabolites of African elephants *Loxodonta africana* in Madikwe Game Reserve, assessed with a Kenward-Roger approximation.

[a]SE=Standard error, [b]df=Degrees of Freedom, significant effects in bold

Fixed effect (reference level)	**Level**	**Estimate ± SE[a]**	**df[b]**	**F**	***p*-value**
Intercept		0.340 ± 0.05			
Tourist		**0.092 ± 0.04**	**32.22**	**6.23**	**0.02**
Season (Dry)	Wet	0.043 ± 0.03	29.63	1.48	0.23
Sex (Female)	Male	-0.001 ± 0.07	9.22	0.00	0.99
Age (Adult)	Calf	0.082 ± 0.11	10.15	0.50	0.62
	Juvenile	-0.161 ± 0.16			
Hour		-0.033 ± 0.03	30.29	1.14	0.29