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Article

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

Isabelle D. Szott[1], Yolanda Pretorius[2], Andre Ganswindt[3], Nicola F. Koyama[1]

[1] School of Natural Sciences and Psychology, Liverpool John Moores University, U.K., Byrom Street Campus, L3 3AF, Liverpool, UK.

[2] Centre for Wildlife Management, University of Pretoria, South Africa.

[3] Mammal Research Institute, University of Pretoria, South Africa. Endocrine Research Laboratory, Department of Anatomy and Physiology, University of Pretoria, South Africa.

Author for correspondence:

Isabelle Szott (I.Szott@2011.ljmu.ac.uk)

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 km^2 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 km^2) 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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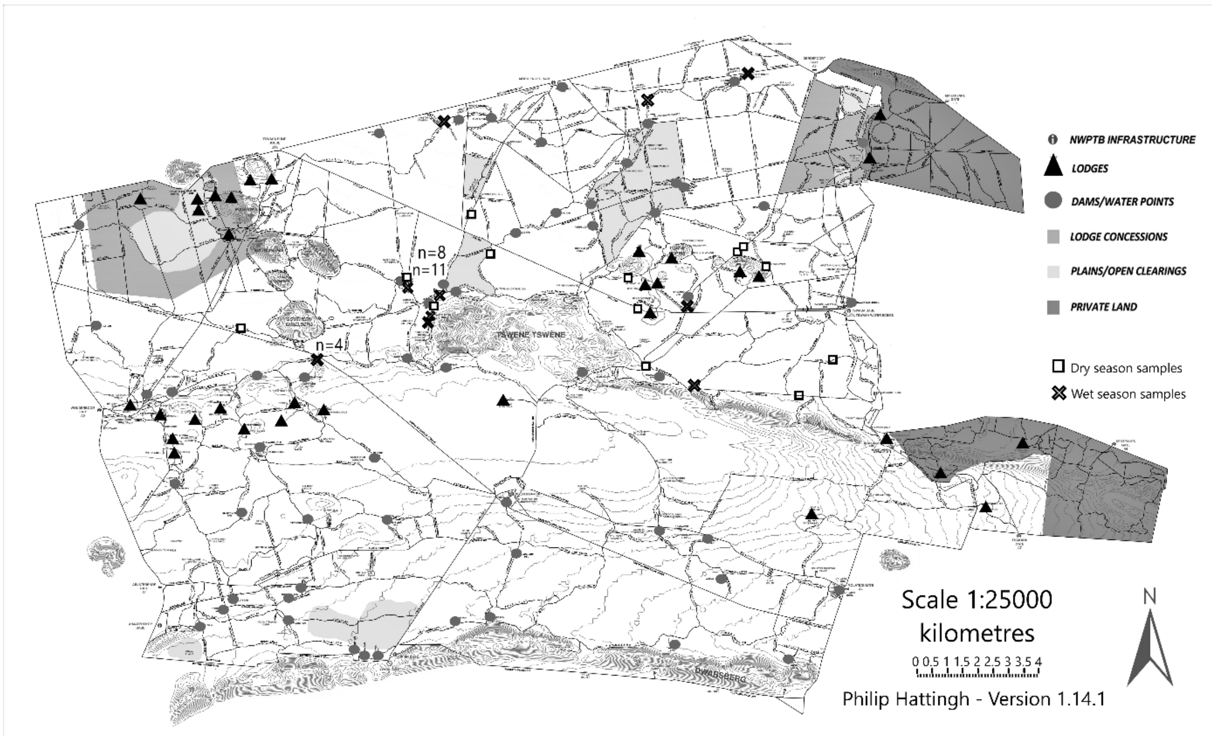

Figure 1.

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).

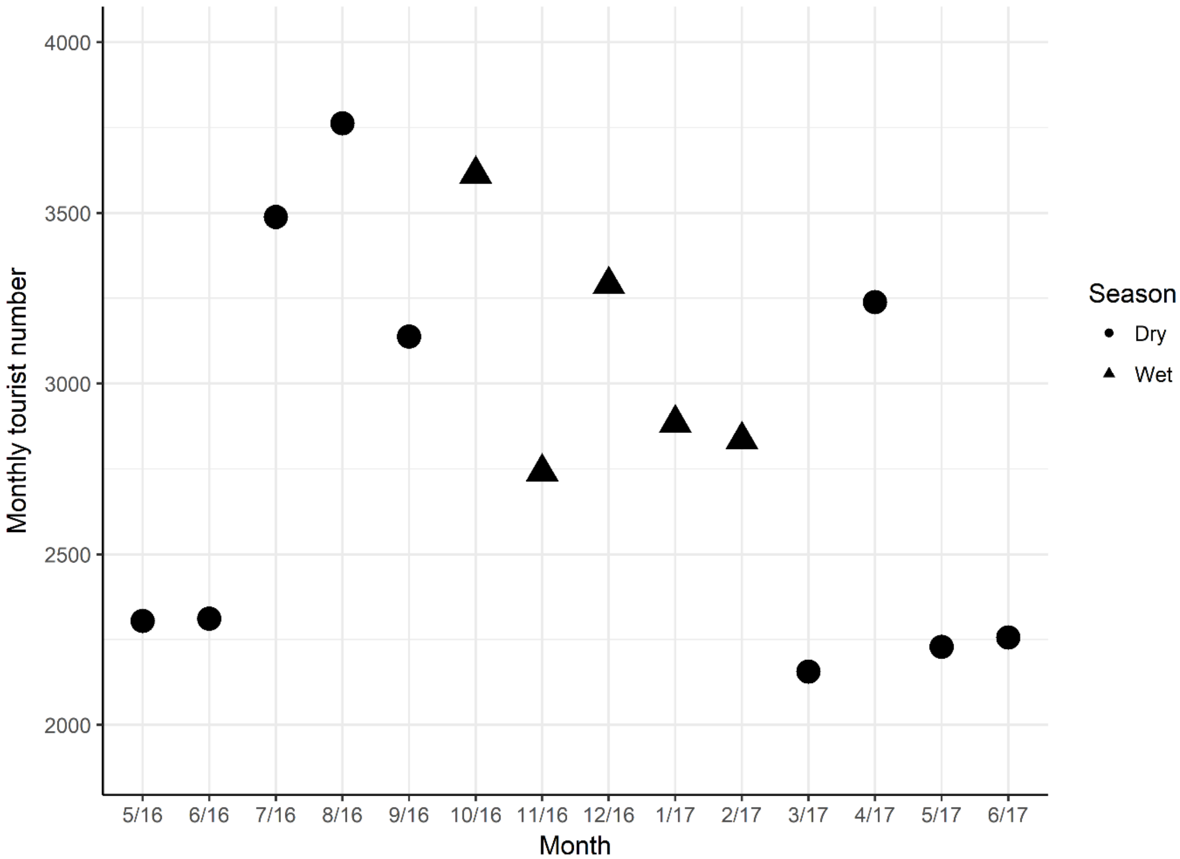

Figure 2.

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

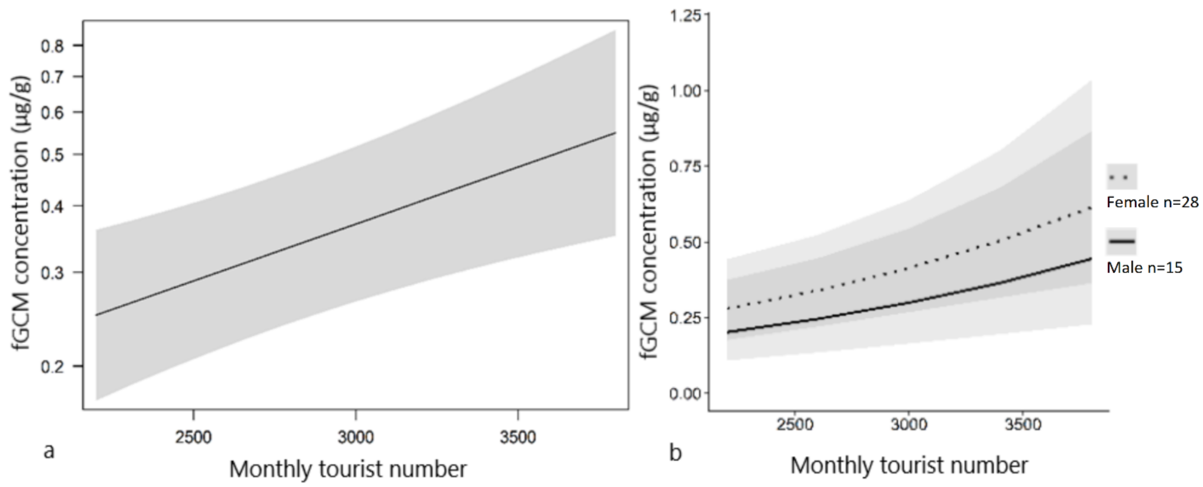

Figure 3.

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