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Determining adrenocortical activity as a measure of stress in African elephants (*Loxodonta africana*) in relation to human activities in Serengeti ecosystem

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Abstract

African elephants (*Loxodonta africana*) play a vital role in most African ecosystems, with their opportunity to alter the entire ecosystem by their sheer numbers. Defining and measuring animal welfare has been much discussed. One potential way of determining an animal's welfare is to record the absence or presence of stress. Little research on elephant welfare has so far been performed in the Serengeti ecosystem. The aim of this study was to record the faecal glucocorticoid metabolite levels of African elephants in areas with high or with minimum human interference. A total of 117 faecal samples were collected from randomly located single elephants as well as family herds in the northern, central and western Serengeti National Park (SNP) as well as in Grumeti Game Reserve and Ikoma Open Area, northern Tanzania in 2010. Elephants had higher levels of faecal glucocorticoid metabolites in the areas outside, compared with areas inside SNP. No single males were observed outside SNP, and in general, higher abundance of elephants was observed inside SNP. This suggests that elephants may prefer to reside in the potential safer areas inside the national park, demonstrating the importance of protected areas to improve the welfare of elephants.

Key words: African elephant, conservation, faecal glucocorticoid metabolite, human activity, Serengeti National Park, stress

Résumé

Les éléphants africains *Loxodonta africana* jouent un rôle vital dans la plupart des écosystèmes africains et ils ont le pouvoir de modifier tout un écosystème du simple fait de leur nombre. La définition et la mesure du bien-être animal ont été largement discutées. Une façon de déterminer le bien-être animal pourrait être d'enregistrer la présence ou l'absence de stress. Jusqu'à présent, peu d'études de ce type ont été faites sur les éléphants de l'écosystème du Serengeti. Le but de la présente étude était de déterminer le taux de métabolites fécaux des glucocorticoïdes des éléphants africains dans des zones où l'interférence des hommes est élevée ou minimale. Au total, 117 échantillons fécaux ont été prélevés au hasard venant d'éléphants solitaires et de familles, dispersés dans le nord, le centre et l'ouest du parc National de Serengeti (SNP) ainsi que dans la Réserve de Faune de Grumeti et dans l'*Ikoma Open Area*, dans le nord de la Tanzanie, en 2010. Les éléphants avaient un taux de métabolites des glucocorticoïdes plus élevé dans les régions situées à l'extérieur du SNP qu'à l'intérieur. Aucun mâle solitaire ne fut observé à l'extérieur du SNP et, de manière générale, les éléphants étaient plus abondants à l'intérieur du SNP. Cela laisse penser que les éléphants pourraient préférer résider dans les zones potentiellement plus sûres de l'intérieur du parc national, ce qui prouve l'importance des aires protégées pour améliorer le bien-être des éléphants.

Introduction

Biodiversity conservation faces several challenges, among which are human population growth and habitat loss. Humans and wildlife are progressively competing for space and resources as human activity is intensifying in most landscapes. Animals generally manage anthropogenic

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disturbances or predation risk in the same way, namely by altering their behaviour. However, under some circumstances this alternation may not be possible and will consequently lead to chronic stress (Wielebnowski & Watters, 2007). Chronic stress is reported among others to lower the reproductive success and survival of an animal (Adkins-Regan, 2005; Cabezas *et al.*, 2007).

Existing protected areas are often too small and isolated to provide a sufficient habitat size for long-term conservation of species that require large home ranges (Kikoti, Griffin & Pamphil, 2010). For example, home range size of elephants reflects their requirements of resources and how they obtain it. Edges of protected areas are often focal points of human–elephant conflict as human population around these protected areas are quickly growing and is suffering economic and social costs (Hart & O'Connell, 1998, Stokes *et al.*, 2010). Costs such as denied access to previously accessible resources, property damage through crop raiding, livestock depredation, transmission of wildlife diseases and the risk of being killed are some factors contributing to negative attitudes among locals (Røskaft *et al.*, 2007). This makes it harder for locals to contribute in conservational work and avoid harming animal populations. Hence, instead of participation in community-based conservation projects they engage in actions adding to the human-wildlife conflict (Holmern *et al.*, 2002).

Human–elephant conflict (HEC) is defined by the International Union for Conservation of Nature – African Elephant Specialist Group (IUCN, 2012) as: 'Any humanelephant interaction which results in negative effects on human social, economic, or cultural life, on elephant conservation or on the environment'. As a part of this HEC, illegal hunting for ivory conceivably constitutes the utmost detrimental effect on African elephants *(Loxodonta africana)*. Reducing illegal hunting for meat and ivory in Serengeti National Park (SNP) has been the main duty of law enforcement department ever since SNP was declared a national park in 1951 (Hofer *et al.*, 1996; Loibooki *et al.*, 2002). The general abundance of wildlife inside SNP has made the park and its surrounding areas attractive for game meat hunting for centuries (Arcese, Hando & Campbell, 1995). The possible benefits of hunting combined with poverty and lack of profits for participation in conservational efforts among local people, may lead to a reduction of the human appreciation for elephants and eventually contribute to disadvantageous effects for conservation (De Boer & Baquete, 1998; Røskaft *et al.*, 2007). Thus, HEC is identified as one of five high priority issues to address for sustainable conservation of African elephants. Further conservational work to advance the knowledge and improve the coexistence between humans and wild animals is an important aspect when planning the best possible conservation strategies for both species.

Hunting risk is known to be related to elevated concentrations of stress hormones in elephants (Burke *et al.*, 2008). Improving the knowledge of how and to what extent the impact of anthropogenic disturbance (especially illegal hunting) has on the welfare of elephants in SNP may provide a 'missing piece to the puzzle' and provide key information to managers and planners in conservational work.

Elephants have few natural enemies. However, the harmful impacts applied by humans may contribute to an elevated level of stress hormones (glucorticoids, GC). This includes HEC and almost all interactions that evolve from loss of habitat (Burke *et al.*, 2008). Previous studies on stress in elephants have given variable results concerning how elephants react to different anthropogenic disturbances. However, Barnes (1983) found an inverse relationship between elephant density and human pressure in Ruaha-Rungwa ecosystem, Southern Tanzania. The author reasoned that an increase in elephant density inside the national park could be a result of increased human pressure around the park.

Earlier studies in SNP found that flight initiation distance (FID) (the distance from an approaching object at the time the animal flees) reflected the hunting-risk level (or a general anthropomorphic disturbance) in an area (Setsaas *et al.*, 2007; Nyahongo, 2008; Marealle *et al.*, 2010). The proposed reason for the higher FID is thought to be a result of long-ongoing hunting activity, which has led the animals to associate humans and vehicles to detrimental effects which has further triggered the development of anti-predator strategies in these animals. This is in contrast to animals living in the central areas of the park that are habituated to tourist vehicles (Hofer *et al.*, 2000; Nyahongo, 2008).

The absence or presence of stress is a potential way of measuring an animal's welfare. There are many ways to define stress. Commonly, stress is defined as 'an environmental stimuli that leads to an imbalance of homeostasis as the "stressor", and the corresponding defence reaction of an animal as the "stress response"' (Möstl & Palme, 2002). Consequences of stress are changes in behaviour and physiology which can reduce an animal's fitness,

affect population performance and reduce resistance to diseases (Millspaugh & Washburn, 2004).

There are several methods used to monitor stress related changes in adrenocortical function. However, determining the level of the faecal glucocorticoid metabolites (FGM) is by now a common and effective method that reflects the adrenal responsiveness to a number of potential stressors without disturbing the animal (Wasser *et al.*, 2000). The FGM method mirrors the average glucocorticoids (GC) levels in the elephants from the last two-three days (Laws *et al.*, 2007). For the purpose of this study, the use of this noninvasive method is by far the most convenient, if not the only one applicable.

Although there are several advantages by using the noninvasive faecal sampling method, there are several factors that may affect its potential as well as the invasive methods potentials. Such factors are for example seasonal and daily changes in glucocorticoid excretion (Creel *et al.*, 2002), reproductive status (Gobush, Mutayoba & Wasser, 2008; Rasmussen *et al.*, 2008), diet (Millspaugh & Washburn, 2004; Woolley *et al.*, 2009), sex (Huber, Palme & Arnold, 2003; Touma *et al.*, 2003) and food availability (Huber, Palme & Arnold, 2003; Viljoen *et al.*, 2008), which all should be considered when working with GC analyses (Millspaugh & Washburn, 2004).

Once populating the whole continent, African elephants are now reduced to small isolated protected areas (Kikoti, Griffin & Pamphil, 2010). Tanzania (945,090 km^2) has the largest population of elephants in Eastern Africa with at least 109,000 elephants. The elephant population in Serengeti was close to 1,500 in 2006, which gives an average density of 0.1 elephants per km^2 (Blance *et al.*, 2002).

Human activities impact the elephant distribution in Tanzania, but the types and intensity of disturbance that the elephants can tolerate are not known. The aim of this study was to assess if there is a relation between FGM level and location of sampling in the African elephant in relation to human interference, using a noninvasive measure of GC. This study was conducted as a comparative study in SNP and the adjoining partially protected areas, Grumeti Game Reserve and Ikoma Open Area.

Materials and methods

Study area

The study was conducted from March to July, 2010. The study area cover more than 16,000 km^2 (Arcese, Hando & Campbell, 1995) of savannah and woodland, and includes SNP (14,763 km^2), Ikoma Open Area (OA) (c. 600 km^2) and Grumeti Game Reserve (GR) (c. 400 km^2; Fig. 1) (Sinclair, 1995; Setsaas *et al.*, 2007).

The confounding effect of illegal hunting threat (Hofer *et al.*, 1996, 2000; Setsaas *et al.*, 2007; Marealle *et al.*, 2010) and the possible lack of habituation to vehicles (Nyahongo, 2008) cannot easily be segregated. Therefore, this study explores the general effect of human disturbances on elephants. The study area was divided into four smaller areas based on historical and expected risk of hunting and human interference: northern (moderate), central (low), western Corridor (moderate), Ikoma Open Area and the Grumeti Game Reserve (high; Fig. 1) (Setsaas *et al.*, 2007; Marealle *et al.*, 2010).

Human settlements and use of natural resources without permission are prohibited also in the above mentioned game reserves, while trophy hunting is allowed six months a year on some species excluding elephants. The open areas have several types of use, all controlled by the Wildlife division. Legal hunting is strictly controlled, but illegal hunting continues close to and in these areas. No fences separate the wildlife from humans in Serengeti, so animals as well as illegal hunters can pass the boarders at any time. The absence of fences makes it easier to hunt wildlife in and around these 'commonplace' areas (Knapp *et al.*, 2010).

The economic benefit of illegal hunting has been and is still attracting people to settle down in villages close to the boarders (Hofer *et al.*, 1996). Serengeti boarders are occupied by settlements of almost two million people (Arcese, Hando & Campbell, 1995). People here live in poverty, so illegal hunting for protein-rich food and cash is common (Kaltenborn, Nyahongo & Tingstad, 2005). Loibooki *et al.* (2002) found that approximately 60,000 local villagers (living within 45 km of the park) admitted that they were hunting or were involved in hunting activities inside the national park and game reserves.

Focal animals

Collection of group and individual data when encountering the elephants was performed according to Jachmann (1996), with some modifications described below. All data were collected by the use of a vehicle.

Elephants were primary located and identified from the main roads and the smaller by-ways that extended up to 10 km from the main roads. Elephants moving away from

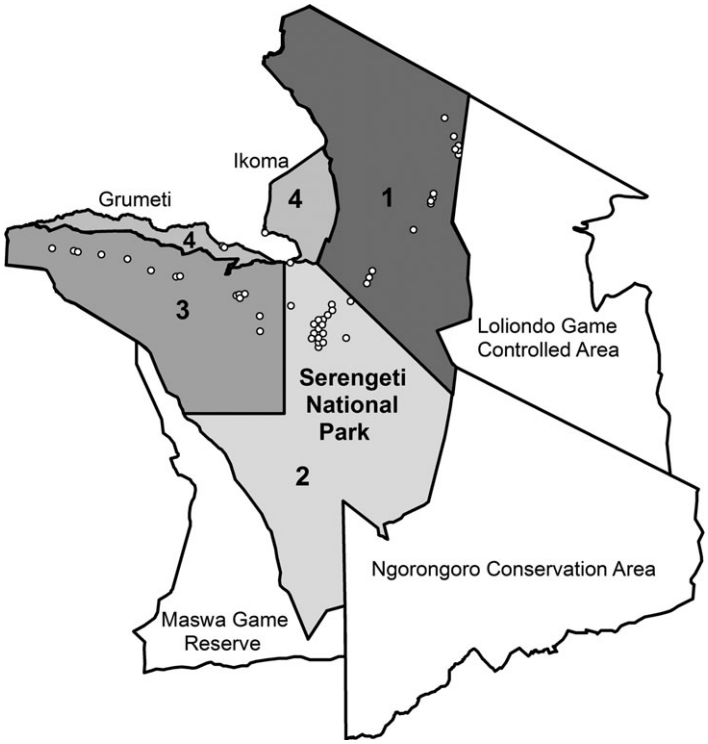

Fig 1 Map of sampling areas in Serengeti National Park (areas 1–3) and outside the park in Ikoma OA and Grumeti GR (area 4). The green triangles represent the location of each of the 117 samples collected between February and June 2010

the roads were followed off-road so that the best possible view of the herd could be obtained at all times. When an animal or a herd was located, time of day and GPS coordinates were recorded.

The number of individuals in the group was counted. Groups that were on the move and difficult to count were recorded as X+ (X is the number of individuals actually counted and + indicating five or less additional individuals). Large groups (more than 50 individuals) were recorded as '50+ individuals', due to difficulties counting moving elephants in such large groups.

Instant data on weather, temperature and habitat were included as baseline data, although not used in this study. Weather and habitat data collected at a particular time of day cannot yield as a general impact on stress, as both are continuously changing for the elephants and would not be correctly reflected in the faecal hormone samples.

We recorded 74 elephant observations and classified their social structure. These included a total number of 626 elephants of which 417 individuals were size estimated, 287 were sexed and 218 individuals were both sized and sexed (Table 1). From all the sexed elephants, there was a practically equal gender distribution ($n_{females} = 146$, $n_{males} = 141$).

An average of 1.42 groups was observed daily during our 52 days in the field. The number of groups encountered during 36 days inside the park was 70 (1.89 groups per day), while we observed 4 groups during 16 observation days outside SNP (0.25 groups per day). The encounter rate differed statistically significantly between the two areas ($\chi^2 = 25.5$, df = 1, $P < 0.001$).

Individual identification

After recording the group data, identification of focal individuals took place. Each individual was identified by its size. The size was determined relative to the size of the matriarch (or the largest female) in the group, which was set to be 100% in size. Likewise accompanying adult males (including solitary roaming males) were set up to a maximum of 200%, and the youngest elephants down to 5% (new born). In addition to size estimation, we observed physical development, eruption of tusks and the size of tusks to improve the individual recognition according to Moss (1996). Photos to help determining size and special recognition signs of each individual in retrospect were taken of all individuals.

Table 1 Number of sampled groups in relation to group structure and the number of samples collected from each group type

Group structure	Number of individuals	Number of groups	Number of body sized individuals	Number of faeces sampled individuals
Single bulls	1	31	31	28
Bull groups	2–5	10	26	12
Family groups	4–27	24	257	64
Mixed groups	5–50+	9	103	13
Total	1–50+	74	417	117

Determining the sex of each animal was relatively easy on the older elephants compared to the younger individuals. Three main methods for determining the sex were used: (i) head shape (ii) body shape and (iii) sexual characteristics such as mammary glands, breasts and genitalia.

Distinct recognition marks on individuals (especially the matriarch) in the herd were drawn by hand and/or captured by photos, to remember special characteristics of individuals in the group as described by Moss (1996).

Faecal sample collection

When defecation was observed, photos were taken and drawings were made of the sample location and land marks at the time of deposition, to document the exact place of defecation. Faecal samples were always collected within two hours after defecation. Samples from fresh dung were collected including the muculus layer on the dung surface into 50-ml plastic vials (Ganswindt *et al.*, 2005). Material from at least three and maximum four different boli were collected to help obtain reliable and unbiased results as there is a possibility of uneven distribution of corticosteroids and their metabolites in the faeces. The faecal samples collected were for dual purposes, one part for FGM analysis and the second part for genetic analyses (to be published elsewhere). As such the samples were not homogenized prior collection to increase the chance of collecting cells for mucus lining. In the field, all samples were immediately stored on ice for a maximum of 24 h. After returning to the Serengeti Wildlife Research Centre (SWRC) in Seronera, the samples were transferred to a freezer (−18°C) until they were processed in the laboratory.

A total of 117 dung samples were successfully collected in the study area comprising 116 unique elephants. There was a significant skewed sex ratio among adults (body size 100 – 200%) with more females being faecal sampled ($n_{females} = 41$, $n_{males} = 16$; $\chi^2 = 10.97$, df = 1, $P = 0.001$), while among the juveniles and young adults (body size 5–90%), equal numbers of females and males were faecal sampled (n = 26 for both sexes).

Extraction of faecal glucocorticoid metabolites in the field

Processing of the faecal samples was performed during or after the field period within 60 days after collection. The extraction process took place at SWRC (TAWIRI-Veterinary laboratory) in SNP. Prior to processing, the samples were defrosted and mixed throughout by hand. To each sample (range 0.48–0.52 g), 5 ml of 90% ethanol was added and homogenized for roughly one minute with a micro homogenizer (OMNI international, Kennesaw, GA, USA). Then, each sample was centrifuged for 20 min at 503 g, to separate the faecal matter from the supernatant with hormone extract. This was followed by transferring the supernatant to a new vial before homogenizing thoroughly. Further, 1 ml of each hormone extract was transferred to a third vial and placed in a pelican case for drying. To shorten the drying time, the case was partially filled with silica gel (Merck KGaA, 2–5 mm). Samples were dried for about one week at the prevailing ambient temperature, until there was no fluid left in the tubes. The samples were then capped and stored dried at room temperature for subsequent laboratory processing and analysis.

Enzyme immunoassay

The hormone analysis was performed within six months after collection. All samples were analysed at University of Veterinary Medicine in Vienna, Austria. The enzyme immunoassay (EIA) was performed as described by Möstl & Palme (2002) and Palme & Möstl (2009), using the faecal cortisol metabolite 11-oxoaetiocholanolone to reflect the GC level. The assay detects glucocorticoid metabolites with a 5β-3a-ol-11-one structure, a method which earlier

has been validated for African elephants (Ganswindt *et al.*, 2003; Viljoen *et al.*, 2008).

Statistical analyses

All statistical analyses were performed using SPSS (IBM Statistics, Version 19, for Windows, New York, NY, USA). Two of the collected samples were obtained from the same individual at different times and the mean FGM concentration of these two samples was consequently calculated and used in all analyses. FGM concentrations were log transformed before the statistical analysis to normalize the data. To compare variance of hormone concentrations between individuals, one-way ANOVA (analysis of variance) and Tukey's *post hoc* tests were performed with sex, body size, group size and area as model effects. Nonparametric tests were used when data were not normal distributed. A general linear model analysis of FGM level as dependent variable and with area, group size, body size and the interaction between group size and area as independent variables was performed as the final test. Significance levels were set at $P \leq 0.05$.

Results

Faecal glucocorticoid concentrations in the sample collection ranged between 11 and 275 ng g^{-1}, with a mean of 69.7 ng g^{-1} ($\pm$ 42.7, n = 116). A small number of elephants (n = 6) exhibited extreme FGM concentrations (>150 ng g^{-1}). These extremes are spread among sex and age groups, and hence do not comprise a special group of individuals. Although adult bulls generally showed a lower level of hormone levels than family groups, there were no significantly difference in hormone level between bulls and family groups inside SNP (Fig. 2). No samples of bulls could be obtained outside SNP.

There was a statistically significant difference in FGM concentration among elephants from the different areas investigated (ANOVA; $F = 8.01$, df = 3, $P < 0.001$). Grumeti GR and Ikoma OA had the highest mean concentration (115.7 ng g^{-1}) of FGM. This high value differed significantly from the values obtained inside SNP with average mean FGM concentration of 62.6 ng g^{-1} (Fig. 2). As we did not record any adult male outside SNP, this difference was solely due to differences between family groups (Fig. 2). No statistically significant differences in hormone levels in the three areas inside SNP were found (Fig. 2).

There was a statistically significant difference in FGM concentrations of elephants with different body size (ANOVA; $F = 5.75$, df = 3, $P < 0.001$). Highest mean FGM concentrations (98.1 ng g^{-1}) were found in elephants with body size 40–60% and adult females (100%; 88.8 ng g^{-1}), while lowest FGM concentrations were found in adult males (110–170%; 55.5 ng g^{-1}) and those with body size (70–90%; 66.9 ng g^{-1}) respectively (Fig. 3).

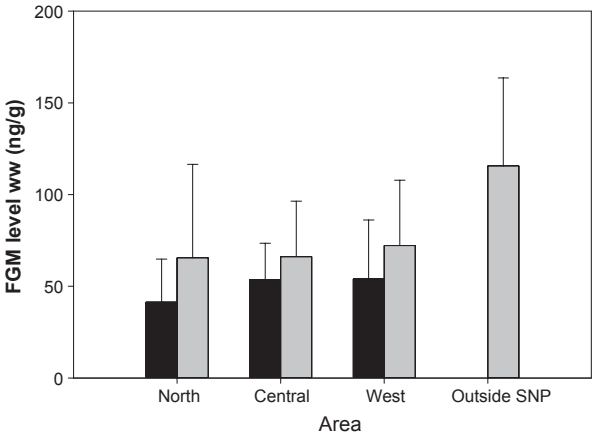

Fig 2 Faecal glucocorticoid metabolites concentration (mean + SD) of all samples from the free-ranging African elephants grouped according to where they were sampled; north, central and west SNP, and outside SNP (Grumeti GR and Ikoma OA). The bars represent FGM means ($\pm$SD). Results are divided among groups of single bulls (dark bars) and family groups (light bars) of elephants sampled in the four study areas. Those elephants from outside are significantly different from all other groups ($P \leq 0.05$) using Tukey's post hoc tests. None of the groups inside the park differed statistically from each other

There was a statistically significant difference in FGM concentrations among groups of different sizes (ANOVA; $F = 6.13$, df = 5, $P < 0.0001$). The groups with 1 and 2–5 individuals (91% of which were male groups) had significant lower FGM concentrations than the groups with 6–10 and 21–49 individuals (Fig. 4).

A general linear model analysis of FGM level as dependent variable and with area, group size, individual size and the interaction between group size and area as independent variables turned out to be statistically significant ($F = 4.64$, df = 3 and 113, $P < 0.001$). However, the only independent variable explaining this variation significantly was the interaction group size/area ($F = 3.04$, $P < 0.001$).

Discussion

We found no differences in FGM concentration between the two sexes. However, groups consisting of 1 and 2–5

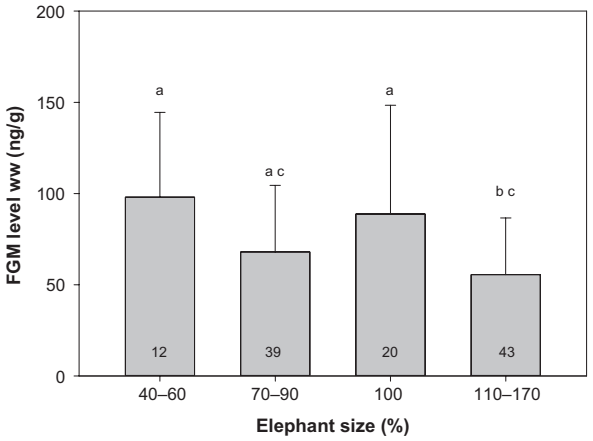

Fig 3 Faecal glucocorticoid metabolite concentration (mean + SD) for free-ranging elephants. The elephants are divided into groups according to size (see materials and method). Numbers in the bars are sample sizes, while bars with different letters are significantly different from one another ($P \leq 0.05$) using Tukey's *post hoc* tests

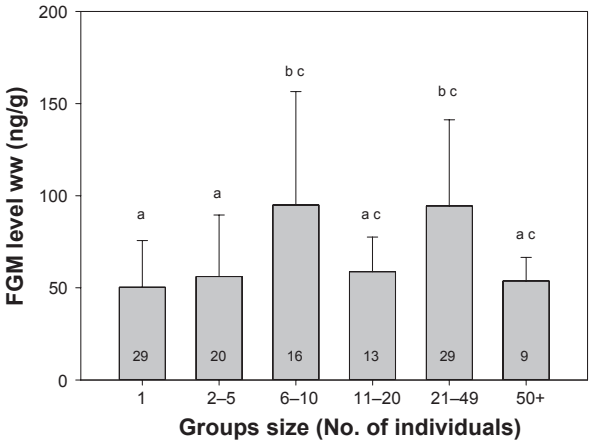

Fig 4 Distribution of mean faecal glucocorticoid metabolite concentration (mean + SD) from free-ranging African elephants. The elephants are divided into groups according number of individuals in the family group. Numbers in the bars are sample sizes, while bars with different letters are significantly different from one another ($P \leq 0.05$) using Tukey's *post hoc* tests

individual(s) (mostly males) had a significantly lower FGM level than individuals belonging to larger family groups. The relative low FGM level in the adult single males might be a reflection of them having awareness that they are located in a protected area of low hunting risk. Elephant bulls periodically come into musth, a state of sexual activation and heightened testosterone levels where they can be perceived as aggressive towards humans and conspecifics. Data obtained from free-ranging male elephants suggest that there is no relationship between the HPA-axis activity and musth (Ganswindt *et al.*, 2003, 2005; Rasmussen *et al.*, 2008), or that there is actually a decrease in FGM output (Ganswindt *et al.*, 2005, 2010).

Elephant abundance inside SNP was higher than on outside. This suggests that elephants probably prefer to reside inside SNP with fewer disturbances. This is in accordance with Remis & Kpanou (2011), Blake *et al.* (2007), and Stokes *et al.* (2010) who found that elephants had the highest abundances farthest away from human settlements.

Estimates of the home range of the African elephants give values in excess of 10,000 km^2 (Thouless, 1995, 1996; Whyte, 1996). Such large areas are often difficult to conserve exclusively by protected areas and requires alternative conservation methods. Interconnecting corridors to join the mosaic of protected area patches is a common strategy employed. Elephants are therefore often forced to move outside protected areas to obtain adequate resources.

The results in the present study suggest a demographic difference in chronic stress level in African elephants due to geographical variances of human disturbance. The differences in FGM levels of the elephants indicate that there is a difference in human activities between the study areas. Elephants residing outside the SNP had significantly higher FGM concentrations than the elephants in all the three areas inside the park. These differences in FGM level may be interpreted to indicate that elephants somehow must 'know' that they are in an area of higher risk than when residing in the protection of the national park. Several studies on foraging behaviour, daily movement and ranging have generally shown that animals avoid areas with human disturbances (Douglas-Hamilton, Krink & Vollrath, 2005; Aleper & Moe, 2006; Remis & Kpanou, 2011) or unfamiliar areas Druce, Pretorius & Slotow (2008). The relative high FGM level reported in elephants outside SNP with higher human disturbances, compared to the elephants roaming in more protected areas, may alternatively be explained by a lack of habituation outside the park. Inability to habituate to human disturbances may lead to chronic stress in animals. Environmental stressors causing such stress may lead to nonadaptive levels of GC secretion and can cause damaging effects that decreases the animals general fitness (Tarlow & Blumstein, 2007).

Conclusion

This study is an assessment to evaluate the elephant welfare as a key species in the Serengeti ecosystem, and to monitor the effectiveness of areas which represent different levels of protection for our study species. Significant support for the hypothesis that elephants have higher FGM level in high-risk areas in SNP was found in this study. A higher abundance of elephants inside SNP compared with outside was also observed. This suggests that elephants prefer to reside inside the protected area, which demonstrates the importance of protected areas to improve the welfare of elephants. The reason for the higher FGM level in the high-risk areas are thought to be a result of long-ongoing hunting activity, which has led the animals to associate humans and vehicles with detrimental effects. This has probably triggered the development of anti-predator strategies in the elephants, such as avoidance of areas of lower protection.

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