



Original Article

## Faecal Glucocorticoids Metabolite Response in Giraffes (*Giraffa camelopardalis tippelskirchi*) in Relation to Protected Area Management Objectives in Tanzania

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#### Keywords:

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The increase in the human population and the demand for natural resources and recreational activities poses insurmountable threats to the welfare and survival of wildlife. Human disturbance negatively impacts wildlife populations. A prospective way of determining wildlife welfare is to assess stress. To manage and conserve giraffes, it is vital to understand their stress factors and their responses to stressors. This study used a non-invasive (faecal collection) technique to evaluate the Faecal Glucocorticoid Metabolite (FGM) levels of giraffes depending on the protected area type, poaching risk, group size, age and sex. The study took place at the Serengeti National Park and Selous Game Reserve where a total of 63 faecal samples were randomly collected from 272 giraffe groups. A significant difference in the FGM levels between the sexes was found, as females had higher concentrations compared to males. In addition, a significant difference was found in relation to group size and age; however, protection type and poaching risk did not have any significant effect. Stressful conditions when prolonged can result in deteriorating animal welfare especially in calves and young animals thus their survival. However, the level of impaired FGMs and the amount of time required to produce damage are not known. In this regard, conservation strategies should seek to minimize the occurrence of stressful events in protected areas.

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## INTRODUCTION

One in five vertebrate species is threatened with extinction where the threatened animals are categorized as vulnerable, endangered or critically endangered species (IUCN, 2016). The understanding hormonal activity provides an insight into animal and species biology and thus enhancing conservation initiatives (Kersey & Dehnhard, 2014). The ever increasing human population coupled with increasing demands for bushmeat, human encroachment into wildlife areas, lack of alternative economic activities, political instability, poor governance and demand for wildlife body parts for traditional medicine has recently amplified threats towards wildlife (Challender et al., 2015; Holmern et al., 2004; Lee et al., 2014; Lindsay, 2012; Muller, 2011). There is little doubt that giraffe populations are under threat across Africa due to increasing human-wildlife conflict, loss of habitat and poaching (Marealle et al. 2010; Muller 2011).

Understanding the stress response of animals is essential to conservation because it will provide insights in the means to correct management problems before the outcomes become too severe as well as to ensure that the objectives of wildlife conservation are met (Wasser et al., 1997). Recently, many studies on stress physiology have been conducted on mammals (Hing et al., 2014). However, compared to other taxa, relatively little is known about the response of Masai giraffe (*Giraffa camelopardalis tippelskirchi*) to stressors and their

conservation implications. Marealle et al. (2010) found that the sex ratio was female-skewed in potential stressful habitats or areas. In other similar studies, Lunde et al. (2016) found that human disturbances caused by traffic had a negative influence on the FGM levels on impala (*Aepyceros melampus*). Similarly, Tingvold et al. (2013) reported an increased level of stress hormones of African elephants (*Loxodonta africana*) living outside of protected areas (PAs) where human settlements were found compared to core protected areas.

Stress involves physiological and neurophysiological changes in an organism (Barja et al. 2007). Stress can be stimulatory, preparative or inhibitory (Creel et al., 2009; Hing et al., 2014). When an animal is stressed, hormones glucocorticoids (GCs) and catecholamines are released that may help in some positive situations such as mating, parturition, fleeing from predators and hunting for food. In contrast, stress may also have deleterious effects such as lowering the immune system, suppressing reproduction, affecting development and in some cases altering the behaviour of animals (Creel et al., 2002; Hing et al., 2014; Möstl & Palme, 2002; Omsjoe et al., 2009; Palme 2012; Touma & Palme, 2005). When stressed, an individual portrays a stress response to cope with the challenge (Touma & Palme, 2005).

Stressors can be natural environmental factors or may result from human activities (Cabezas et al., 2007). Understanding the stress response to major threats, such as habitat loss and climate change is

critical to conservation planning and also has economic implications (Hing et al., 2014; Millspaugh & Washburn, 2004). Human disturbances are one of the major stressors to different species of wild animal populations (Creel et al., 2002; Moen et al., 1982) where direct chase or hunt by humans induces a stress response (Bateson & Bradshaw, 1997). Giraffes (*G. camelopardalis Tippelskirchi*) are exposed to a number of stressors such as but not limited to habitat loss, predation, climatic changes and anthropogenic disturbances (Cabezas et al., 2007). Assessing stress is fundamental for understanding and improving animal welfare, health, and reproduction (Touma & Palme, 2005).

To manage and conserve giraffes, it is vital to understand their stress factors and their responses to stressors (Hing et al., 2014). This understanding can act as a proactive management tool enabling wildlife managers to optimize the trade-offs between resource utilization and the propagation of species at risk (Wasser et al., 1997). Therefore, we hypothesized that stress hormone levels (FGMs) are higher in the less protected areas that involve more human stressors. Furthermore, because poachers are constantly disturbing wildlife, the poaching risk influences the levels of FGMs; therefore, higher levels of FGMs were expected in areas with higher risks of poaching. It was also hypothesized that the FGM levels are influenced by group size with higher levels in smaller groups compared to larger ones and that there is no difference in the FGM levels between the age and sex of giraffes.

## RESEARCH METHODOLOGY

### Study Area

This study was conducted in the Serengeti National Park (SNP), 14,763 km<sup>2</sup> (Estes et al., 2012) and the northern part of the Selous Game Reserve (SGR) where giraffes are found in approximately 6% or 800 km<sup>2</sup> (Brink et al. 2013) of the total 47,500 km<sup>2</sup>. In the SNP, any type of utilization is strictly forbidden. In the SGR, sustainable utilization is allowed and trophy hunting is practised under specified regulations (URT, 2002) except for giraffes. However, in both areas, illegal hunting has no exception. The density of human population is highest at the western and north-western borders of

the Serengeti where poaching and other detrimental land-use practices have long been considered a serious threat in this ecosystem (Kideghesho et al., 2006; Marealle et al., 2010; Nyahongo, 2008; Setsaas et al., 2007). Likewise, a huge population of over 47,000 people resides around the buffer zones of the SGR, hence the pressure on the resources (Gillingham & Lee 1999). The study areas were demarcated according to the illegal hunting risk areas as defined in (Marealle et al., 2010; Marealle et al., 2020) and the same considerations were taken into account while categorizing the risk areas in the SGR.

### Study Species

Giraffes are the tallest savanna megaherbivore exploiting mainly tree leaves and twigs, which determine their distribution (Owen-Smith, 1988). Giraffes are ecologically adapted to go several days without water (Okello et al., 2015). They are sexually dimorphic (Marealle et al., 2010), non-territorial and move in loose herds of which they form fluid groups (Leuthold & Leuthold, 1978; Pratt & Anderson, 1982). Giraffes have a major role in shaping savanna woodlands that impact the ecosystem structure and tourists are highly attracted to them (Bond & Loffell, 2001).

Giraffes are exposed to a suite of potential human-induced stress factor such as human population growth, illegal hunting, habitat loss and habitat fragmentation (GCF, 2014; Marealle et al., 2010; Muller, 2011) and naturally through increased predation pressure (Hayward & Kerley, 2005; Owen-Smith, 2008; Sheepers & Gilchrist, 1991). These factors contributed to the region-wide decline and extinction of native species (Corlett, 2007).

Although the giraffe range in the SGR falls within an area recently set aside for photographic tourism (Brink et al., 2013), the population is still subjected to the fear of the hunting disturbance. Similarly to many other PAs in the world, human population growth tends to be higher around protected areas (Caro, 1999; Mwamfupe, 1998; Packer et al., 2011). Surveys in the SNP showed a significant drop in giraffe densities especially in areas that previously supported large herds of giraffes. This was also reported in the SNP snare reports of which

nearly 45% of victims were giraffes (Strauss et al., 2015). Vigilance studies evidently showed that humans and other ecological stressors such as predation posed a threat to giraffes (Marealle et al., 2010; Marealle et al., 2020; Periquet et al., 2010). When vigilant, an individual's head is raised up with a neck angle of 135° or 180° that lasts for at least 1 second without showing any other behaviour (Cameron & Du Toit, 2005). Vigilance acts are used as an anti-predator strategy during courtship and for monitoring conspecifics (Cameron & Du Toit, 2005). There was a positive relationship between vigilance and stress (Lima, 1998; Wiepkema & Koolhaas, 1993) and therefore, elevated FGMs during vigilance (Arthur 1987).

### ***Faecal Glucocorticoid Metabolites (FGMs)***

Hormonal studies are currently being incorporated into wildlife research as a means to evaluate the health and physiology of individuals (Tarlow & Blumstein, 2007). There are several methods for measuring stress in wildlife (Hing et al., 2014; Keay et al., 2006; Tingvold et al., 2013). However, this study focused on GCs as physiological measures of stress because they provide a mechanistic understanding of the physiological stress response compared to catecholamines, which are unstable with quite a short half-life (Hing et al., 2014; Palme et al., 2005). Faecal cortisol metabolites are a non-invasive method of measuring GCs that is particularly useful because samples can be easily obtained without disturbing the studied animals or interfering with the results (Millsbaugh & Washburn, 2004; Möstl & Palme, 2002).

### **Sample Collection**

After identifying a target group for faecal collection we stopped the vehicle and waited for 15-20 minutes and patiently observed the group while simultaneously recording the initial behaviour of individuals until members of the group defecated. Immediately after observing fresh dung, one person collected the pellets while another observer remained in the car and recorded the age and sex and counted individuals. A few pellets from the whole defecation that were not contaminated with dirt or urine were collected. After collection, we placed the sample(s) in a mobile portable freezer for temporary storage, which was later transferred to a

stationed 20 °C freezer in the laboratory to reduce the possible metabolism by bacterial enzymes before analysis (Möstl & Palme, 2002). A total of 63 faecal samples from 63 groups that were composed of 272 individuals were randomly collected taking into consideration age and sex over a 2-month period that is June - August 2015.

### **Sample Processing**

The sample processing occurred at the Department of Biology, Norwegian University of Science and Technology (NTNU). Samples were thawed and pellets from a single individual were thoroughly crushed and mixed together to assure that the hormones were readily distributed. Then, measured 5g (wet) and homogenised the sample was measured following (Lunde et al., 2016; Millsbaugh & Washburn, 2004; Palme, 2012; Palme et al., 2013; Tingvold et al., 2013). After homogenisation, 5 ml of 80% ethanol was added to the sample and shaken for 30 minutes. This was followed by centrifuging the samples for 20 minutes at 3300 rpm. A 0.5-ml aliquot of the supernatant was later transferred from each sample to a 0.5-ml Eppendorf cup. In total, 63 faecal samples from 63 individuals were collected. Each sample consisted of 4-5 pellets of varying shape and drop time; therefore, samples were treated differently when the 134 (1 control) faecal samples were processed. Samples were packed in a dry icebox and the hormone analysis was conducted in the laboratory of Dr. Rupert Palme, Institute of Physiology, Pathophysiology and Biophysics, University of Veterinary Medicine, Vienna, Austria. The enzyme immunoassay (EIA) used was the 11-oxo-aetiocholanolone EIA as described in detail by Möstl & Palme (2002) and it was found to be suitable for measuring FGMs in several ruminant species.

### **Data analysis**

The data was analysed using SPSS 21.0 software for windows. A logistic linear regression analysis was used to test for interaction effect with the GC level used as a dependent variable with the following independent variables: protected area type, poaching risk, group size, age and sex. Descriptive statistics were used to determine the mean FGM levels on the same variables. For

calculation purposes, the FGM levels were log-transformed ( $\log_{10}$ ) to normalize the data. The statistical significance was set at  $P < 0.05$ .

## RESULTS

The FGM levels were recorded from a total of 272 groups. All of the hormone data were log-transformed before the statistical analyses and ranged from  $4.01 \text{ ng g}^{-1}$  to  $7.22 \text{ ng g}^{-1}$  faeces, with a log mean of  $6.1 \text{ ng g}^{-1}$  and a median of  $6.2 \text{ ng g}^{-1}$ . A logistic regression analysis examining the log FGM

level (0 to 1) as the dependent variable was tested over the five explanatory independent variables of protected area type, poaching risk, group size, age and sex (Table 1). However, only two of the independent variables were highly significant contributors to the variation, altogether explaining 25% of this variation. The variable explaining most of the variation in FGMs was sex, and the second most important predictor was group size. Type of protection, age and poaching risk were insignificant.

**Table 1: A logistic regression analysis with log FGM in  $\text{ng g}^{-1}$  as the dependent variable and protection type, poaching risk, group size sex and age as independent variables**

Faecal glucocorticoid metabolite (FGM)			
Independent variables	Rank	t	P=
Sex	1	4.672	0.0001
Group size	1	-3.631	0.0001
Type of protection	2	0.983	0.327
Age	3	0.854	0.395
Poaching risk	4	-0.809	0.420
R <sup>2</sup>		0.246	0.0001
Constant		19.147	0.0001

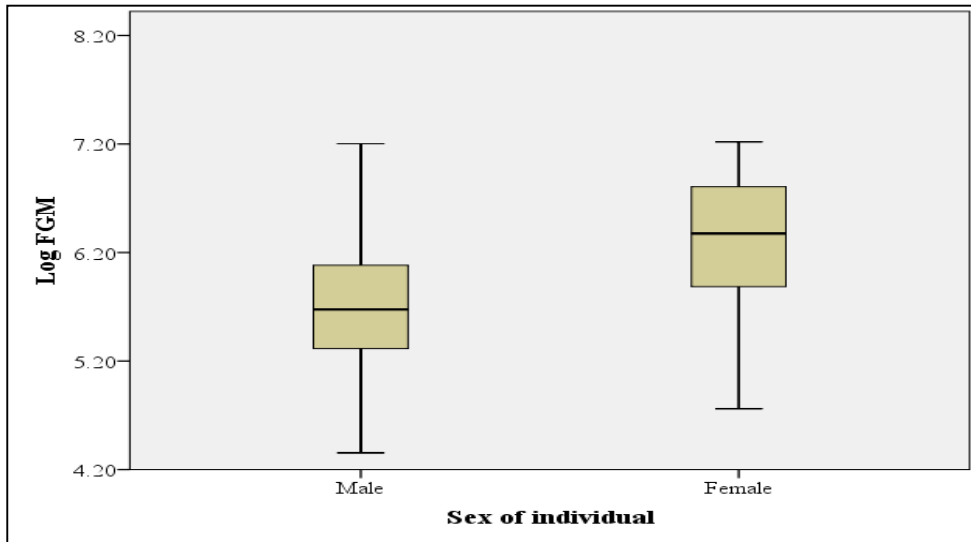
The concentration levels of the mean FGM levels in different variables (sex, age, group size, protection type and poaching risk) are shown in Table 2 and Figures 1-3. The mean FGM level was found to be significantly higher in females compared to males (Figure 1). Furthermore, calves had higher FGM

levels followed closely by young animals when compared to adults (Figure 2). The group size of 2-5 individuals had slightly higher levels of mean FGMs than the other groups (Figure 3). However, FGM level was not influenced by the type of protection.

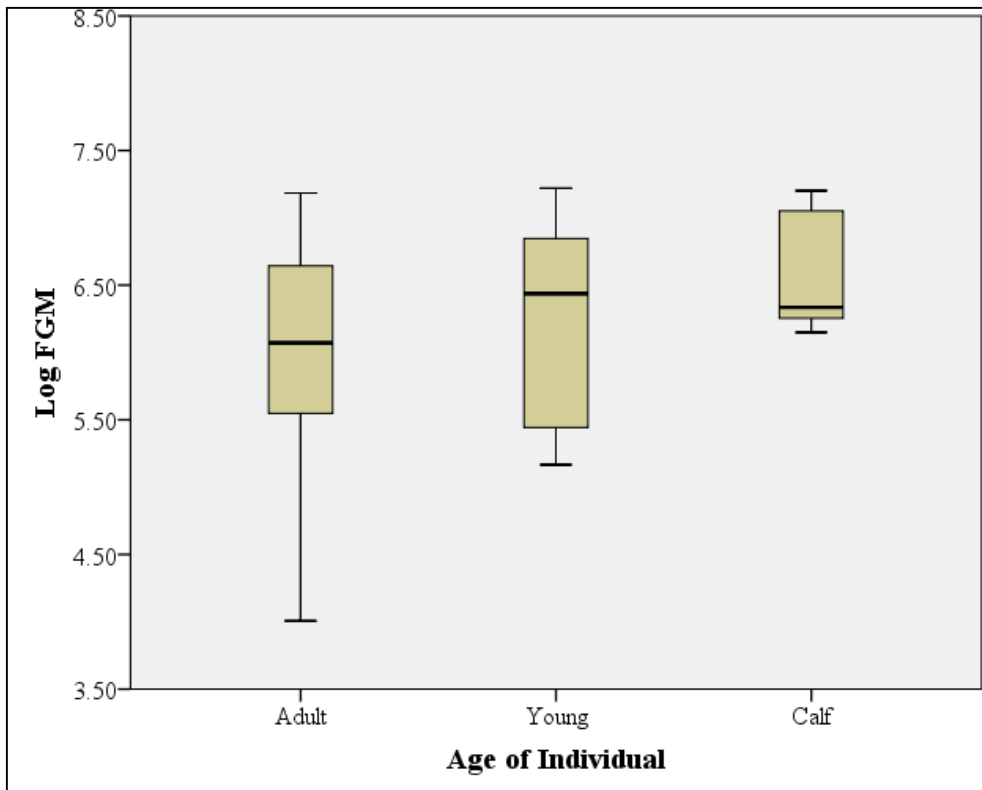
**Table 2: Mean log FGM test for different variables**

Variable		Mean log FGM ( $\text{ng g}^{-1}$ )	SD ( $\text{ng g}^{-1}$ )	N
Sex	Males	5.8	0.7	54
	Females	6.3	0.6	77
Age	Calves	6.6	0.5	6
	Sub adult	6.2	0.7	23
	Adults	6.0	0.7	104
Group size	1	6.1	0.5	16
	2-5	6.3	0.6	64
	6-10	6.0	0.8	37
	11-30	5.6	0.5	16
Protection type	Game Reserve-SGR	6.0	0.8	57
	National Park-SNP	6.1	0.6	76
Poaching risk	High	6.2	0.6	75
	Low	6.3	0.8	58

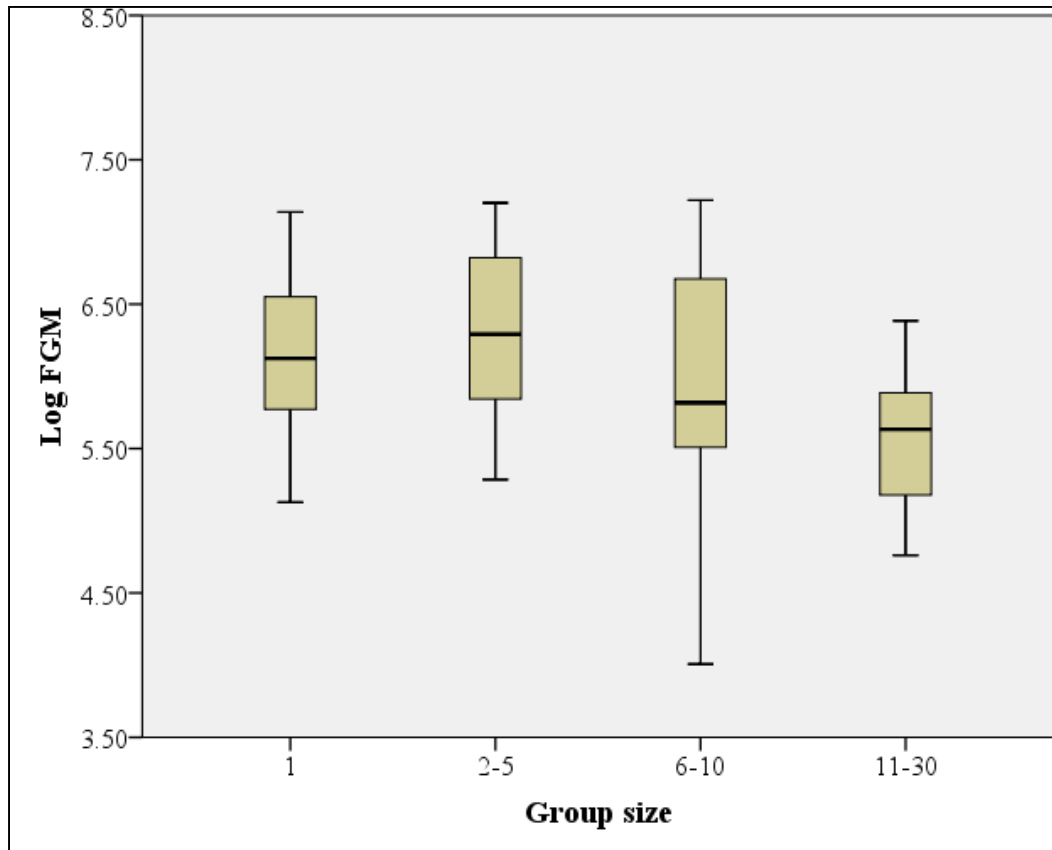
**Figure 1: FGM concentrations (mean) between males and females**



**Figure 2: Mean levels of FGM in different age classes of observed giraffes.**



**Figure 3: Distribution of mean FGM levels from giraffes divided into groups according to the number of individuals**



## DISCUSSION

The results of the study did not indicate any significant differences in the levels of FGM concentrations of giraffes between SNP and SGR. However, in both areas, the mean stress levels were high. The study sites fall under different management objectives, specifically a National Park and a Game Reserve (Dudley, 2008; Phillips, 2002). Nevertheless, the part of the SGR where giraffes are found was converted from hunting blocks to a photographic tourism zone (Brink et al., 2013). The probable explanation for both areas having the same high levels of FGM was the presence of touristic activities (road networks) and incidences of poaching that took place within these areas. Similar effects were observed in other studies (Creel et al., 2002; Fowler, 1999; Lunde et al., 2016; Marealle et al., 2010; Müllner et al., 2004; Thiel et al., 2008; Walker et al., 2006) as well as in a recent behavioural study (Strauss et al., 2015). Tourist activities were spread almost equally in

both high and low risk poaching areas. The FGM levels did show the same results in these two areas and had no significant difference and rather high FGM levels. These results were contrary to the hypothesis, as the anticipated differences were not observed.

Living in a group is very important for animal survival; however, it may also have some fitness consequences (Marealle et al., 2020; Pride, 2005; Shrader et al., 2006). In behavioural studies, evidence showed that stress in some cases increased costs such as agonistics while in other cases, costs were reduced such as vigilance with group size (Marealle et al., 2020; Pride, 2005). Our results conformed to our hypothesis, where significantly higher FGM levels were found in lower groups of <10 individuals and FGM levels decreased in extremely larger groups of >11 individuals. Animals tended to relax when they were in larger groups compared to smaller groups or singletons or they fled immediately when a threat was detected

due to the many eyes effect (Burger & Gochfeld, 1988; Ebensperger et al., 2006; Marealle et al., 2020; Setsaas et al., 2007) and with the relaxed state as a result of larger group sizes, the FGM levels also decreased.

Calves and young animals had higher FGM levels compared to adult individuals; however, most of the individuals in this category were found in larger herds of 2-30 individuals. Calves' FGM levels may be a direct result of ingesting maternal hormones during nursing and in young individuals, the FGM levels may be due to the effect of weaning (Hunt et al., 2006). It was also reported that tourism induced stress resulting in high FGM levels in younger animals that stayed longer in young individuals while adults can regulate FGM levels toward lower concentrations in a very short time (Hunt et al., 2006; Müllner et al., 2004; Walker et al., 2006). Higher levels of stress in young individuals may lead to higher mortality (Hatchwell, 1989; Müllner et al., 2004).

Different studies reported that gender could affect cortisol levels found in faeces (Keay et al., 2006; Touma & Palme, 2005) and that the actions of stress varied with sex and were influenced by the predominance of particular sex steroids (Tilbrook et al., 2002). In our case, the level of FGMs was higher in females than males, which was contrary to our hypothesis. The results are supported by the study of Pride (2005) that found that females had higher FGM levels. The probable explanation for this result is that males were fluidly moving from one group to another and were able to maintain their FGM levels. In addition, male stress is group size dependant only during the mating season due to mate competition (Norscia et al., 2016) although giraffes are seasonal breeders. During this study, any mating behaviour was nor recorded and this might be the reason why the results did not show higher levels in males. The potential effect of stress in females was previously reported by Love et al. (2005) and Marealle et al (2010) where it influenced the female sex bias in calves in the SNP, meaning that stress was much more pronounced in females and its effect was evident during reproduction compared to males (James, 1996).

## CONCLUSION

The non-invasive monitoring of stress hormones by using faecal samples has been largely applied to various species and has shown reliable results (Touma & Palme, 2005). Tourism is of economic importance for Tanzania; however, mass tourism can jeopardise conservation goals (Zwijacz-Kozica et al., 2013). From our results, the variables that best explained the higher FGM levels in giraffes were group size, age and sex. However, like Lunde et al. (2016), the effect on fitness consequences associated with it was unknown, although a previous finding indicated that it affects the sex-ratio in calves (Marealle et al., 2010). The stressful conditions, when prolonged can result in deteriorating animal welfare and a declining population (Sapolsky, 2004). However, the level of impaired FGM and the amount of time required to produce damage are not known (Fowler, 1999). In this regard, conservation strategies should seek to minimize the occurrence of stressful events in protected areas, in particular, the human-induced stressors as their impacts are irreversible and stay longer especially in calves and young individuals.

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