Translocation stress and faecal glucocorticoid metabolite levels in free-ranging African savanna elephants

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There are local populations of African elephants (*Loxodonta africana*) which have increased to levels where they are implicated in altering vegetation types. The local reduction of elephant numbers for wildlife management objectives can involve contraception, killing excess animals, or translocation to alternative habitats. The effects these management decisions can have on the physiological stress response of free-ranging African savanna elephants are still not fully understood. We examined the effect of translocation on faecal glucocorticoid metabolite levels of an African elephant family group, which was translocated within the Kruger National Park, South Africa. We found that translocation resulted in a significant increase in faecal glucocorticoid metabolite levels (up to 646 ng/g wet weight) compared to (1) pre-translocation levels in this group, (2) post-translocation levels in this group, and (3) levels measured in undisturbed 'control' groups in the area. However, the faecal glucocorticoid metabolite levels had returned to <100 ng/g by the time the translocated animals had navigated their way back to their previous home range, covering 300 km in 23 days.

Key words: *Loxodonta africana*, elephant, capture stress, translocation, glucocorticoids, Kruger National Park**.**

INTRODUCTION

Although the global population of African elephants (*Loxodonta africana*) has declined sharply in the last 20 years, from an estimated 1.3 million to 580 000 (Blanc *et al.* 2003), there are places where local populations of elephants have increased to the level where they are implicated in altering vegetation types (Trollope *et al.* 1998). The local reduction of elephant numbers for wildlife management objectives can involve contraception (Fayrer-Hosken *et al.* 2001), killing excess animals (van Aarde *et al.* 1999), or translocation to alternative habitats. Translocation has the advantage of not disrupting the age structure of the population, which contraception does, and of having fewer ethical complications than killing animals (Butler 1998; Whyte *et al.* 1998). However, there are potential drawbacks with the translocation of any wild animal, and one of these is the stress associated with capture, transportation, and post-translocation adjustment (Goymann *et al.* 1999; Möstl *et al.* 2002). For example, Millspaugh *et al.* (2007) recently demonstrated that faecal glucocorticoid metabolite **(**FGM) concentrations of African elephants living in a hands-on environment increased as a response to transportation.

A free-ranging animal is in a state of stress if it is required to make abnormal or extreme adjustments in its physiology or behaviour in order to cope with adverse aspects of its environment or management (Friend 1980). Several endocrine responses are involved during stress situations. Some of the frontline hormones produced in these situations are the glucocorticoids, which are indicators of adrenocortical activity and thus are elevated in times of stress (Möstl & Palme 2002). As a physiological mechanism, stress is not inherently negative (Moberg 2000). However, prolonged high concentrations of glucocorticoids might decrease

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individual fitness (Munck *et al.* 1984) and reproductive success (Liptrap 1993), and also permanently alter behaviour.

Glucocorticoid levels can be measured from blood samples, but the collection procedure itself can elicit a cortisol response (Möstl & Palme 2002). Measuring glucocorticoid metabolite levels in urine or faeces is a non-invasive technique that has been used in a variety of mammalian species (Wasser *et al.* 2000; Touma & Palme 2005), including elephants (Brown *et al.* 1995; Stead *et al.* 2000; Wasser *et al.* 2000; Foley *et al.* 2001; Ganswindt *et al.* 2003, 2005). Faecal samples offer the advantage that they can be more easily collected than urine.

It is assumed that in elephants, stress can be triggered by certain stimuli including environmental factors (availability of food and water and its quality), as well as behavioural (courtship or mating behaviour) and psychosocial causes (e.g. translocation, or culling). The extreme fear of, or aggression towards, humans, sometimes registered in elephants (Garaï *et al.* 2004), may be both cause and consequence of stressful events and can pose safety problems for both elephants and humans. However, the measurement of possible effects of human and natural disturbances on elephant populations has so far been restricted to the detection of behavioural indicators (Whyte 1993). Therefore, quantitative measures of physiological parameters, such as measurements of changes in glucocorticoid metabolite levels, would be an important addition to our ability to determine the effects management actions have on animals. Burke *et al.* (2008) stated that physiological stress assays were more effective than behavioural responses in detecting effects of human intervention.

In this paper we describe FGM levels of an elephant family group before, during, and after translocation to provide a reliable basis for monitoring and managing elephant stress levels during management actions in the future.

MATERIALS AND METHODS

Study area

The study area was located in the Kruger National Park (KNP) which covers an area of approximately 19 000 km². The KNP can be longitudinally divided into resistant granites in the west succeeded by ecca shales, basalt and rhyolites in the east that give rise to different soil types and the associated flora and fauna (Venter *et al.* 2003). Data during the pre-and post-translocation phases were collected in the southern KNP within the Lebombo Arid Mountain Bushveld, Sweet Lowveld Bushveld and Mixed Lowveld Bushveld vegetation types (Low & Rebelo 1996). During the translocation phase data were collected in the northern KNP within the Mopane Bushveld and Mopane Shrubveld vegetation types (Low & Rebelo 1996).

Study population

The KNP elephant population currently exceeds 10 000 animals, and translocation is one of the methods used by the park managers to locally reduce elephant densities in KNP while reintroducing elephants to parts of their former range (Whyte *et al.* 2003). A family group was randomly selected in the southern region of KNP and the matriarch was fitted with a VHF radio collar. This group (experimental) was followed on foot so as not to bias sampling close to roads. The distance field crews maintained from the animals to minimize disturbance varied from a 100 to 500 m, and they always avoided being positioned upwind of the herd. Owing to safety issues and visual impairment caused by vegetation, samples were only collected during daylight hours and therefore contact with these family groups was not continuous. Thus, the possible occurrence of stress-causing-factors during the night could not be excluded. Faecal samples were collected as soon as possible after animals had defecated and the same sampling protocol was used for the experimental group as well as for family herds randomly located within the study area (control groups). The exact number of control groups is unknown but estimated to be at least 20. The number of individuals in the experimental and control groups varied throughout the study period, with a median of 12 animals in the experimental group and 13 across all the control groups (Table 1).

While wearing rubber gloves we collected ~100 g of faecal material which was then placed in a plastic bag. We tried to exclude individual bias by collecting samples throughout each feeding patch used by a family herd. The time lapsed between defecation and freezing of a collected sample was two hours or less. Each sample was marked with the date of collection, a GPS coordinate, and the estimated age class (adult, subadult, juvenile) of the subject using a field method based on the bolus diameter as developed by Wimberger (2001).

Table 1. Median of number of individuals (all, adults, subadults, and juveniles and calves) for the experimental and control elephant family groups involved in the study from March 2003 to August 2005.

Group-type	Median	Adult	Subadult	Juveniles & calves
Experimental	12	2	5	3
Control	13	3	6	3

The study was divided into three phases and faecal samples were concurrently collected from experimental and control groups throughout the pre-translocation, translocation, and post-translocation phases. This allowed us to determine, through FGM levels, the effect of the translocation process on physiological stress within the experimental group. Seasonal factors were also taken into account based on the current findings of Viljoen *et al.* (2008) which show that the variability in baseline concentrations of FGM is dependent on seasonal changes, rather than on the age class of the subject. This enabled us to account for any variations in stress caused by environmental factors such as differences in seasonal diet selection.

We were also able to confirm that the experimental group's pre- and post-translocation FGM levels were consistent with other undisturbed elephants in the same area, because control groups were sampled in immediate vicinity of the experimental group. In October 2004 the experimental group (three adult and five subadult females, one juvenile female, and two male calves) was captured in the Lower Sabie section, transported approximately 300 km, and offloaded north of the Letaba Rest Camp within the KNP. They were located from a helicopter and immobilized with a drug dosage of M99 and Azaperone. The dosage varied from 3 mg of M99 up to 12 mg of M99 depending on the size and age of the individual, with 5000 i.u of Hyaluronidase included in the dart dosage irrespective of the age of the individual. Ataxia set in after approximately 8 min where after the darts were removed and the vital signs of all individuals monitored. The antidote (M5050 and Naltrexone) was administered intravenously as soon as the individual were loaded into the transport crate. The antidote dosages were also adapted from 1 ml of M5050 up to 3 ml of M5050 according to the age of the elephant. After two hours the transport vehicle stopped for 30 min for an inspection as well as to give the elephants a rest. After four hours of travel the elephants were safely offloaded. These elephants then navigated their way back and re-entered their pre-translocation home range after 23 days. The post-translocation phase was defined as the day the experimental group re-entered the pre-translocation home range. The entire period of faecal sampling from experimental and control groups (n = 439 samples) was from March 2003 to August 2005 (Table 2).

Faecal extraction and hormone assays

Hormones and their metabolites were extracted from faecal samples according to the procedure

Table 2. Number (n) of samples collected from the experimental- and control groups during March 2003 to August 2005, as well as mean, standard deviation and standard error of faecal glucocorticoid metabolite levels (ng/g WW) for the experimental- and control groups during the pre-translocation, translocation and post-translocation phases.

Group	Phase	n	Mean	S.D.	S.E.	95% Confidence interval for mean		Minimum	Maximum
						Lower bound	Upper bound		
Experimental	Pre-translocate	120	83.82	42.876	3.914	76.07	91.57	3	214
	Translocate	45	173.22	161.002	24.001	124.85	221.59	26	646
	Post-translocate	158	97.07	57.094	4.542	88.10	106.04	7	300
	Total	323	102.76	81.574	4.539	93.83	111.69	3	646
Control	Pre-translocate	56	64.54	35.050	4.684	55.15	73.92	4	141
	Translocate	20	56.20	31.210	6.979	41.59	70.81	11	122
	Post-translocate	40	44.58	30.254	4.784	34.90	54.25	10	156
	Total	116	56.22	33.760	3.135	50.01	62.42	4	156

Table 3. Mean difference and statistical characteristics (standard error and significance level) of faecal glucocorticoid metabolite levels (ng/g WW) between the experimental and control groups in the pre-translocation, translocation and post-translocation phases.

Group	(I) period	(J) period	Mean difference (I–J)	S.E.	Significance	95% Confidence interval	
						Lower bound	Upper bound
Experimental	Pre-translocate	Translocate	–89.406*	13.366	0.001	–121.57	–57.24
		Post-translocate	–13.253	9.259	0.460	–35.54	9.03
	Translocate	Pre-translocate	89.406*	13.366	0.001	57.24	121.57
	Post-translocate		76.153*	12.921	0.001	45.06	107.25
	Post-translocate	Pre-translocate	13.253	9.259	0.460	–9.03	35.54
	Translocate		–76.153*	12.921	0.001	–107.25	–45.06
Control	Pre-translocate	Translocate	8.336	8.551	0.995	–12.45	29.12
	Post-translocate		19.961*	6.796	0.012	3.45	36.48
	Translocate	Pre-translocate	–8.336	8.551	0.995	–29.12	12.45
	Post-translocate		11.625	8.990	0.596	–10.22	33.47
	Post-translocate	Pre-translocate	–19.961*	6.796	0.012	–36.48	–3.45
	Translocate		–11.625	8.990	0.596	–33.47	10.22

*Mean difference is significant at the 0.05 level.

described by Merl *et al.* (2000), and the resulting extracts were measured for immunoreactive glucocorticoid metabolites using an 11-oxoaetiocholanolone enzyme immunoassay measuring a group of glucocorticoid metabolites with a 3α-hydroxy-11-oxo-structure (Möstl *et al.* 2002), which has previously been shown to provide reliable information on adrenocortical function in African elephant bulls (Ganswindt *et al.* 2003; 2005). The sensitivity of the assay at 90% binding was 3.0 pg/well and intra- and interassay coefficients of variation, determined by repeated measurements of high and low value quality controls, ranged between 3.0% and 12.5%.

Data comparison

We compared data from the experimental group and control groups throughout the pre-translocation, translocation and post-translocation phases using ANOVA (StatSoft 2006) followed by the Bonferroni *post hoc* range test using SPSS version 13 (SPSS Inc. 2004). The independence of data cannot be guaranteed as one individual may have contributed more than one bolus to the data set and ANOVA's are known to be relative robust to violations of independence of the data. This potential lack of independence is compensated by the relatively large sample sizes, field collection of single boluses and the Bonferroni significance levels. The ANOVA's were conducted under the assumption of equality of variance. FGM concentrations can vary between individuals in the same herd depending on the behaviour they have been engaged in prior to collection of samples. Owing to the difficulty in obtaining continuous behavioural data on focal animals over a 30–50-hour period, faecal samples were collected from as many individuals in each group as possible and mean values were calculated for entire herds. Differences at $P \leq 0.05$ were considered significant.

RESULTS

There were high fluctuations in 3α-hydroxy-11-oxo cortisol metabolites concentrations visible in the profile of the experimental, as well as the control group. However the mean and median FGM values ($\bar{x}$ = 173 ng/g wet weight (WW), med = 116 ng/g WW) of the experimental group during the translocation phase were significantly higher than that of the control herds ($\bar{x}$ = 56.2 ng/g WW, med = 50 ng/g WW) during the same period (Tables 2 & 3). The mean FGM level of the experimental group reached a maximum (646 ng/g WW) the day after the translocation and remained elevated for five days (average of 540 ng/g WW) after which it showed a sharp diminution (Fig. 1). By contrast, the FGM profile of the control groups showed no corresponding peak (Fig. 1). When comparing the translocation phase with the pre- and post-translocation phases within the experimental group there were significant differences (both cases P = 0.001), whereas within the control groups there were no significant differences (P = 0.995 and P = 0.596, respectively) (Table 3). The

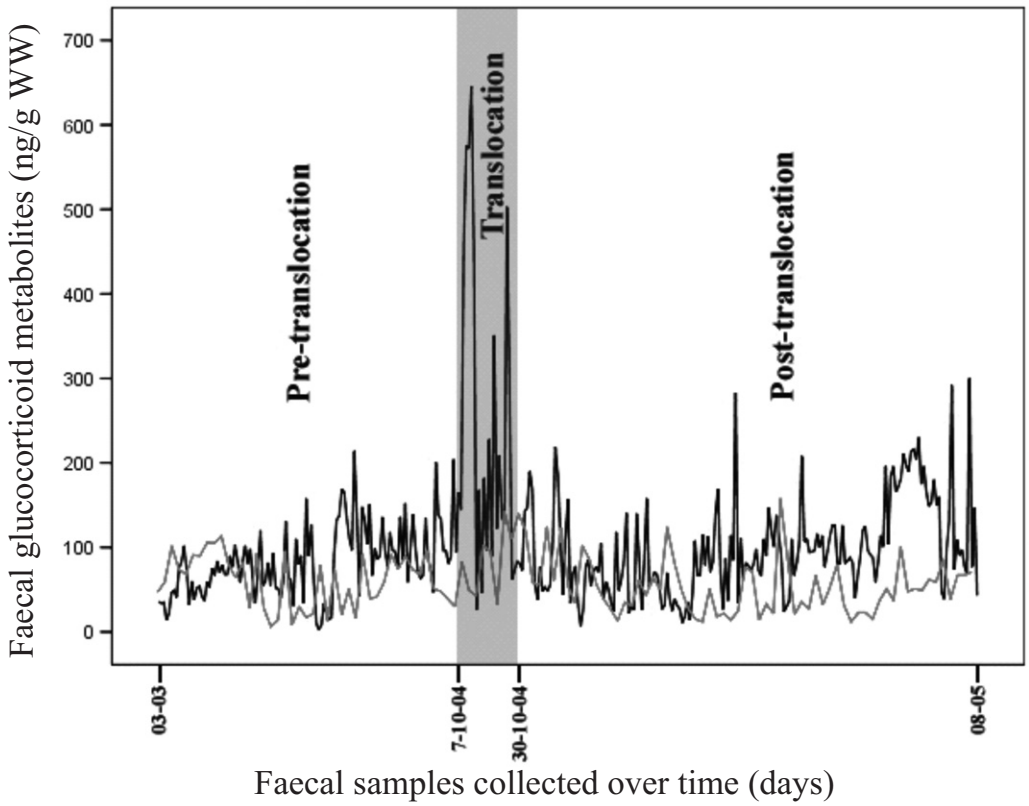

Fig. 1. Concentrations (mean) of faecal glucocorticoid metabolites (ng/g WW) of the experimental group (black line) and the control groups (grey line) of free-ranging family herds of elephants in KNP from March 2003 to August 2005. Experimental group (pre-translocation $n = 120$, translocation $n = 45$ and post-translocation phase $n = 158$), control groups (pre-translocation $n = 56$, translocation $n = 20$ and post-translocation phase $n = 40$).

pre-translocation phase compared to the post-translocation phase in the experimental group does not show a significant difference ($P = 0.460$), which indicates that the experimental group's glucocorticoid metabolite levels had settled at the pre-translocation baseline values upon return to their former home range. For the pre-translocation phase there were no significant differences between the control groups and a randomized sample of the experimental group ($F = 3.96$, $P = 0.352$).

DISCUSSION

The observation that FGM levels peaked during and immediately after translocation in the experimental group, while no corresponding spike was seen in the control group, indicates that factors other than capture and translocation can be excluded as causal stimuli for the observed glucocorticoid metabolite response. However, FGM levels did not remain elevated, but returned to pre-translocation levels by the time the animals had returned to their normal home range. It is uncertain how post-translocation FGM levels would have changed if the elephants were translocated to an area from where they could not have returned to their former home range. It is not known as to how long the FGM levels would remain elevated before returning to pre-translocation baseline values, and also whether it would be to a baseline level similar to the pre-translocation level. Burke *et al.* (2008) found that although hunt events induced a stress response in elephants that were present at the hunting of the targeted animal, FGM levels returned to baseline levels within four days.

From this study it is evident that the chosen family group of elephants recovered relatively quickly from the immobilization, loading and transportation as indicated by levels of FGMs. However, due to the fact that we only focused on a herd level, further studies are necessary to identify

possible existing differences in physiological stress response between different herd members.

The present study clearly affirmed that the measurement of FGMs represents a reliable tool to generate information about adrenal activity in individuals of African elephant herds, which enables us to conduct long term monitoring on the effect of ethological and environmental factors on the stress potentiality for elephants without interfering with the result. Fruitful areas for future studies include how post-translocation FGM levels are affected when elephants are translocated to an area from where they cannot return to their former home range.

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