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Endocrine correlates of musth and the impact of ecological and social factors in free-ranging African elephants (*Loxodonta africana*)

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ABSTRACT

Sexual activity in mature male African elephants is predominantly associated with the occurrence of musth, a state or condition which refers to a set of physical, physiological and behavioral characteristics, including an elevation in androgen levels. Although musth appears to be energetically costly, the degree to which it is associated with changes in adrenal endocrine function (e.g., glucocorticoid output) is still unclear. To investigate the possible effect of musth on adrenocortical function, and the impact of socioecological changes on androgen and glucocorticoid levels, six adult African elephant bulls were followed for 13 months in the Kruger National Park, South Africa, and observations and fecal sample collection for endocrine monitoring was carried out about twice weekly. Our data showed that the occurrence of musth was associated with reduced glucocorticoid output, suggesting that musth does not represent a physiological stress mediated by the hypothalamic–pituitary–adrenal axis. This confirms previous findings in captive-housed animals, providing evidence for a suppressive effect of the musth condition on adrenocortical activity. Furthermore, a seasonal effect on androgen and glucocorticoid levels was found, which appears to vary depending on the reproductive status of the animal. The results also indicate a relationship between the presence or absence of social partners and changes in testicular and adrenal endocrine activity. Finally, the data confirm previous findings in captive-housed elephants, that an elevation in androgen concentrations usually occurs before the onset of physical musth signs, and therefore support the idea that the change in androgen levels represents the initial stimulus for the musth condition.

Introduction

Sexual activity in male African elephants is mostly associated with the occurrence of musth, a state or reproductive condition of variable duration, which can be considered a competitive reproductive tactic, predominantly seen in older individuals (Poole, 1987, 1994; Rasmussen, 2005; Rasmussen et al., 2008a). Although musth is not an explicit requirement for male reproductive success, paternity analyses have revealed that approximately 75–80% of offspring can be attributed to male elephants in musth (Hollister-Smith et al., 2007; Rasmussen et al., 2008b). Elephant bulls in musth can be identified by a greenish discoloration of the penis and sheath, continuous discharge of urine (urine dribbling), and a typical, strong odor (Hall-Martin, 1987; Poole and Moss, 1981; Poole, 1987). A further visual signal is the copious secretion from and enlargement of the temporal glands (Hall-Martin, 1987; Poole and Moss, 1981; Poole, 1987; Rasmussen and Schulte, 1998). Musth is also associated with an increase in aggression, dominance displays and unpredictability, especially towards other bulls in musth (Poole and Moss, 1981; Poole, 1989). There is an abundance of information about musth and elevated androgen levels (Poole et al., 1984; Rasmussen et al., 1996, 2008a; Ganswindt et al., 2002, 2005a). In contrast, the relationship between musth and adrenal endocrine function is less clear. Although the state of musth appears to be energetically costly and therefore may be a stressful condition (Poole, 1989; Rasmussen and Perrin, 1999), data on the activity of the hypothalamic–pituitary–adrenal (HPA) axis during musth are inconsistent (Brown et al., 2007; Ganswindt et al., 2003, 2005a,b; Rasmussen et al., 2008a). For example, a recent study carried out in three captive African bulls exhibiting musth demonstrated a modest (but significant) positive correlation (average $r = 0.35$) between circulating testosterone and cortisol levels (Brown et al.,

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2007), thus providing some support for the hypothesis that musth may represent a stressful condition. In contrast, a study performed on a larger number of captive African bulls ($n = 12$) using fecal glucocorticoid measurements found the opposite, i.e. that the occurrence of musth was associated with a decrease in glucocorticoid output (Ganswindt et al., 2005b). By providing additional data on the temporal relationship between endocrinological changes and the occurrence of the various physical signs of musth, the results of the latter study support an alternative hypothesis, namely a suppressive effect of the musth condition on adrenal endocrine function (Ganswindt et al., 2005b). Interestingly, the only study so far conducted on free-ranging animals found no musth-related changes in glucocorticoid concentrations (Ganswindt et al., 2005a; Rasmussen et al., 2008a). This study, however, included more individuals at the expense of fewer samples per animal compared to the studies carried out on captive animals (Brown et al., 2007; Ganswindt et al., 2005a,b; Rasmussen et al., 2008a). Further studies using a higher temporal resolution of endocrine status are therefore needed to provide more detailed information about the possible effect of musth on adrenocortical function in free-ranging elephants.

In order to clarify the association between musth and gonadal and adrenal endocrine status, a better understanding of the interaction between environmental (ecological) and social factors, and endocrine activity is also necessary. Studies of bulls in captivity are only of limited use because their ecological and environmental conditions differ markedly from those in the wild, and this may affect the relationship between musth and endocrine activity. However, information on the impact of extrinsic factors such as seasonal changes in the environment (e.g., rainfall and food abundance) or presence of social partners, on endocrine gonadal or adrenal function in free-ranging African elephants is extremely limited. Season has been shown to influence progestogen and glucocorticoid levels in wild female African elephants (Wittemyer et al., 2007; Foley et al., 2001; Viljoen et al., 2008). The only study available for free-ranging African elephant bulls showed no overall seasonal changes in androgen or glucocorticoid metabolite levels (Rasmussen et al., 2008a). A possible explanation for the sex-specific effects of season on endocrine activity may be the difference in body size and contrasting reproductive strategies, which have a significant influence on the foraging behavior and ultimately the spatial segregation of males and females (Shannon et al., 2006; Stokke and du Toit, 2002). However, under exceptional ecological conditions such as a prolonged dry season, a 50% increase in individual androgen and glucocorticoid baseline concentrations was found even in bulls (Rasmussen et al., 2008a), suggesting that extrinsic factors can indeed influence on the endocrine state of males. More data are, however, required in order to understand the impact of ecological changes on testicular and adrenal endocrine activity, and their relationship to musth.

In addition, detailed knowledge of the impact of social factors on testicular and adrenal endocrine activity could be helpful to interpret the endocrine correlates of musth in African elephants. In elephant cows, a positive correlation between glucocorticoid concentrations and group size has been shown during dry season, indicating that intra-group competition may influence adrenocortical activity under non-favourable (stressful) conditions (Foley et al., 2001). Female elephants stay in small, stable social groups throughout their lives, whereas elephant bulls leave their natal families at the onset of puberty at about 14 years of age. They then live alone or temporarily in small groups with other males, except during sexually active periods when they predominantly associate with females (Lee, 1997; Moss, 1983; Poole, 1994). Association with estrous females has been shown to result in elevated androgen concentrations and unchanged glucocorticoid levels in males in musth, but elevated glucocorticoid concentrations and unchanged androgen levels in reproductively active non-musth bulls (Rasmussen et al., 2008a). However, the potential influence of male social partners on endocrine activity has not been studied in free-ranging African elephant bulls. The presence of social partners can inhibit or ameliorate the neuroendocrine response to various types of stressors in a variety of animals (Hennessy et al., 2006). This effect, often referred to as social buffering of the stress response, has been found in New World primates, guinea pigs, hamsters, and zebra finches (Hennessy et al., 2006; Kikusui et al., 2006; Remage-Healey et al., 2003; Rukstalis and French, 2005). Because elephants are highly social animals (Moss and Poole, 1983; Wittemyer et al., 2005; Wittemyer and Getz, 2007), the presence of a non-competitive partner may possibly reduce glucocorticoid levels in musth bulls.

The overall aim of this study was to investigate the impact of local environmental and social factors on endocrine activity in wild male African elephants during musth and non-musth. More specifically, we hypothesized that (i) the occurrence of musth is associated with changes in glucocorticoid levels, (ii) seasonal environmental changes affect androgen and glucocorticoid concentrations, and (iii) the presence or absence of social partners influences endocrine activity in free-ranging African elephant bulls.

Material and methods

Study area and animals

The study area covered approximately 5500 km^2 in the northern part of the Kruger National Park (KNP), South Africa, and consisted predominately of open shrub to fairly dense bush savannah and partly open to moderately dense tree savannah (Ganswindt, 2008; Venter et al., 2003). Rainfall in this region averages approximately 450–500 mm/year on approximately 47 days (Zambatis, 2003), and occurs predominantly (>80% of annual precipitation) between November and April (Venter et al., 2003). Rainfall data were recorded throughout the study period (June 2007 to June 2008) at three different locations within the study area (Shingwedzi, Mooiplaas, and Letaba) and a total of 430–648 mm of precipitation was recorded (see Fig. 1). The total elephant population within the KNP is estimated at about 12,500 individuals and is the largest free-ranging elephant population in South Africa (van Aarde et al., 2008).

The study was conducted on six adult male African elephants which were aged on the basis of facial shape, tusk size, and general physical appearance (Moss, 1996; Poole, 1987) as follows: Bull 1 (40–45 years), Bull 2 (30–35 years), Bull 3 (35–40 years), Bull 4 (~35 years), Bull 5 (~35 years), and Bull 6 (30–35 years). The animals were already fitted with GPS/radio tracking devices by Save the Elephants' Transboundary Elephant Research Programme in December 2006 as part of an ongoing project in KNP.

The study was conducted with the permission of the South African National Parks' Conservation Services, and the Animal Use and Care and Research Committees (V012/06) of the University of Pretoria, South Africa.

Observations and sample collection

The six elephant bulls were monitored continuously for 13 months from June 2007 to June 2008, and were located via GPS/radio tracking. Behavioral observations took place regularly (approximately twice weekly per animal), for at least 30 min/session, using ad libitum sampling (Altmann, 1974). The presence and degree of musth signs, i.e. urine dribbling (UD), penis coloration, odor, temporal gland swelling (TG) and temporal gland secretion (TGS) were rated according to the procedure described by Poole (1987). Observations further included recording of geographical position, association (alone, with other bulls, or with cows) and group composition. Furthermore, all sightings of other elephant bulls showing visible signs of musth (see above) were recorded during the 2357 h spent on 328 days in the field.

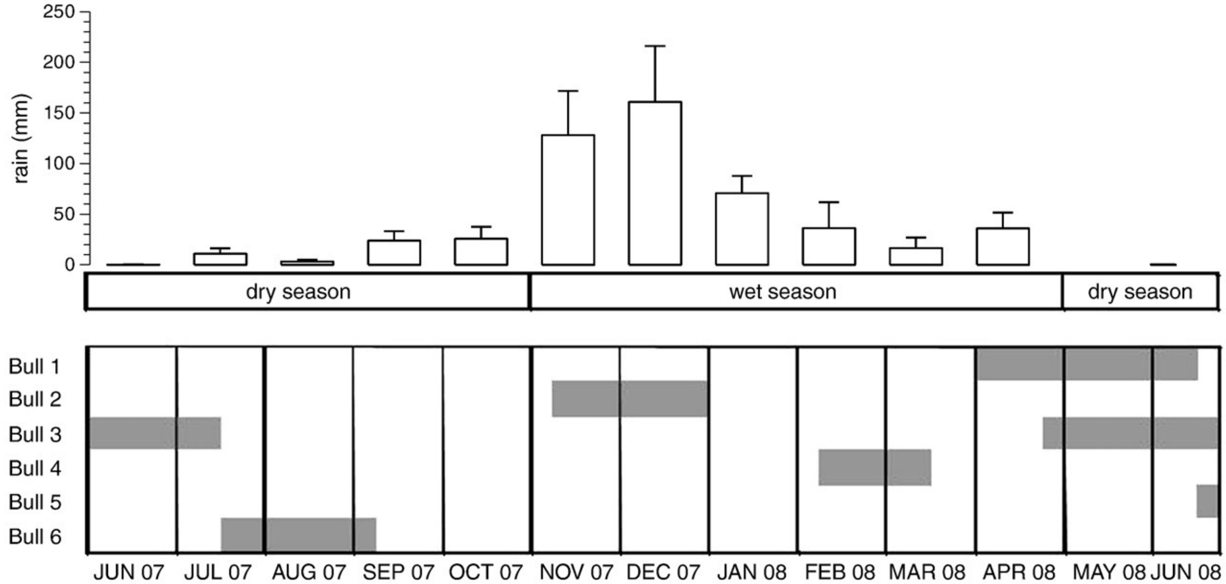

Fig. 1. Monthly rainfall in mm (mean, SD) over the 13-month study period, including categorization of season (white boxes). The graph below shows the occurrence of periods of musth (gray bars) in six free-ranging adult male African elephants during the study period.

During each observation session, approximately 50 g of feces was collected from the study animal shortly after it had defecated and moved away. Using rubber gloves, well homogenized aliquots of each fecal sample were taken and stored in glass vials. The samples were placed on ice and frozen within 1 h after collection at −20 °C until steroid analysis. A total of 537 observations were made and an equal number of corresponding fecal samples collected.

Fecal extraction and steroid assays

Fecal samples were lyophilized, pulverized and sifted using a nylon mesh strainer to remove fibrous material as described by Fieß et al. (1999). Approximately 0.05 g of the fecal powder was then extracted with 80% ethanol in water (3 ml) according to the procedure described by Ganswindt et al. (2005b). The resulting extracts were measured for immunoreactive androgen and glucocorticoid metabolites (GCM) using enzyme immunoassays for epiandrosterone (EA; Palme and Möstl, 1994) and 11-oxoetiocholanolone (Möstl et al., 2002), which have been shown to reliably reflect testicular and adrenocortical endocrine function respectively in male African elephants (Ganswindt et al., 2002, 2003). Assay procedures followed the protocols published by Ganswindt et al. (2002). Sensitivities (90% binding) of the assays were 7.5 pg/well for EA and 3.0 pg/well for GCM, respectively. Intra- and interassay coefficients of variation, determined by repeated measurements of high and low value quality controls ranged between 7.3% and 14.7% for EA, and 4.2% and 13.8% for the GCM measurements.

Sample categorization

Due to the gut passage time in African elephants of approximately 24–38 h (Ganswindt et al., 2003), a time lag exists between circulating hormone levels and the corresponding hormone concentrations in feces. This effect as well as the time lag and its variability between consecutive observations should be considered when linking fecal hormone levels to specific behavioral events in a longitudinal data set. Although the study animals were monitored on a regular basis, the time lag between consecutive observations was generally too long and inconsistent to allow a temporary adjustment regarding the gut passage time. Therefore fecal samples were categorized in relation to signs of musth, season, and association based on observations made on the same day.

Sample categorization in relation to signs of musth

Samples were categorized regarding the occurrence of common physical signs of musth (TG, TGS, and UD) according to the procedure described by Ganswindt et al. (2005b). In this regard, UD was classified as present if either urine discharge in a series of discrete drops or a thin stream, wet hind legs, greenish sheath coloration, musth odor, or any combination of these signs were recorded. The onset of a musth period was defined as the first date on which an elephant exhibited signs of both TG/TGS and UD on at least two consecutive observations. The end of such a period was defined as the last date before an elephant no longer showed signs of UD on at least two consecutive observations, regardless of the occurrence of TG/TGS. Samples from elephants exhibiting signs of TG/TGS only (without UD) which were collected directly before onset of a musth period were classified as pre-musth samples, whereas those collected directly after a musth period were classified as post-musth samples. Samples collected from bulls which were not in pre-, post-, or musth, were classified as samples from non-musth animals.

Sample categorization in relation to season

For season, samples were categorized into two groups (dry and wet season) according to Viljoen et al. (2008). We verified the categorization using rainfall data recorded throughout the study period (see Fig. 1). Between 52 and 80 mm of rain fell within the study area during June–October 2007 and May–June 2008 (dry season), whereas 378–588 mm of was recorded between November 2007 and April 2008 (wet season).

Sample categorization in relation to association

During each observation, the study bull was classified as either (1) alone, no signs of association with other elephants, (2) in association with other bulls only, or (3) in association with mature females, regardless of the presence of other bulls (Ganswindt et al., 2005a). An individual was regarded as associating if it was in close proximity (up to 10 body lengths) to other elephants and moving temporarily in a coordinated manner at the time of observation. No further subclassifications were made regarding e.g. the reproductive state of the individual(s) with which the study animal was associating.

Data analysis

Any sample which were collected from an animal which was obviously affected by a physical injury (Bull 1: June–October 2007, 30 samples; Bull 3: end of August–October 2007, 19 samples) were excluded from the analysis. Health problems are known to potentially affect steroid levels, particularly glucocorticoid output (Muenscher et al., 2009). The temporal relationship between onset and end of physical signs of musth and changes in hormone levels, as well as the occurrence of periods of elevated EA and reduced GCM levels was determined as described by Ganswindt et al. (2005b). In this respect, an increase of 50% above the individual steroid baseline was used to define a threshold level above which hormone levels were considered

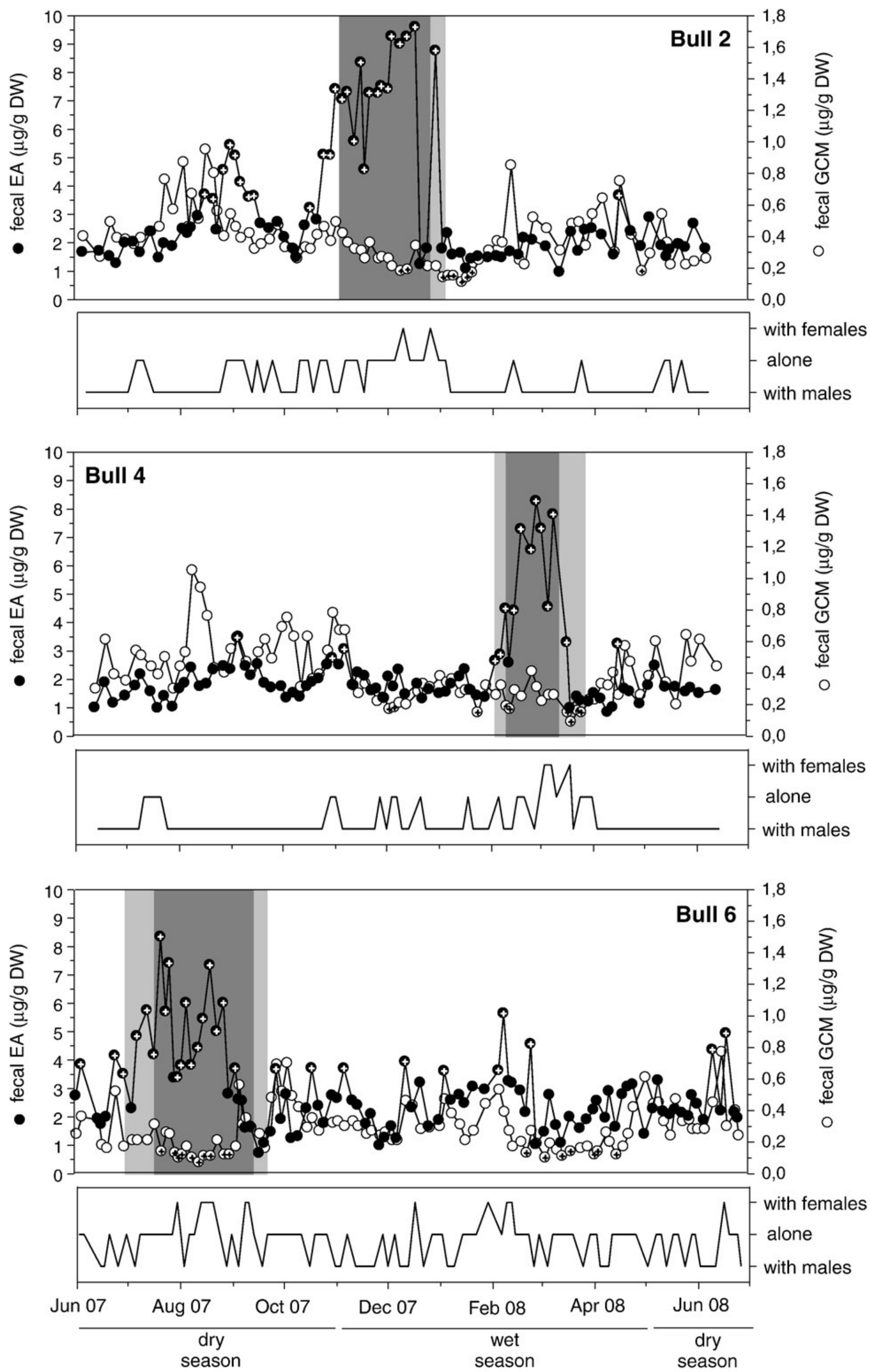

Fig. 2. Profiles of fecal EA (●) and GCM (○) immunoreactivity over a 13-month period in three free-ranging adult male African elephants (Bull 2, Bull 4, and Bull 6). Values exceeding the respective individual EA baseline or falling below the individual GCM threshold are indicated with a cross. Gray bars indicate the periods during which signs of TG/TGS (light) and UD (dark) were recorded and the black line below indicates the type of association (with females, alone, or with males).

Table 1
Onset and end of temporal gland swelling and secretion (TG/TGS), urine dribbling (UD) and reduced glucocorticoid levels (redGC) in relation to onset and end of periods of elevated androgen levels in six free-ranging African elephant bulls.

	Parameter	*N* of cases	Categories of occurrence		
			Before	Together	After
Onset of elevated androgens	TG/TGS	6	0	50%	50%
	UD	6	0	0	100%
	redGC	4	0	0	100%
End of elevated androgens	TG/TGS	5	100%	0	0
	UD	5	80%	20%	0
	redGC	4	75%	0	25%

to be elevated. To define periods of decreased hormone excretion, the threshold used was 50% below the individual baseline median (Ganswindt et al., 2005b).

Median fecal hormone concentrations were initially compared between stages of musth (non-musth, pre-musth, musth and post-musth) using Kruskal–Wallis one-way ANOVA on ranks, with multiple comparisons using the Kruskal–Wallis *z*-test. Differences in association pattern between musth and non-musth bulls were assessed using a chi-squared test. In this analysis, each observation was weighted by the inverse of the number of observations for that animal–musth combination in order that differences in sample size between animals did not bias results.

We then used mixed effects multiple linear regression models to assess the effect of musth, season and association pattern on fecal hormone concentrations. Animal was modeled as a random effect and, in order to account for the serial autocorrelation of measurements within animal, a first-order autoregressive disturbance term was included, with the autocorrelation parameter (ρ) estimated based on a single-lag regression of residuals. Interaction terms between musth and season, and musth and association pattern were also included in the models. A significance level of $\alpha = 0.05$ was used for all analyses. Data analysis was done using Stata 10.1 (StataCorp, College Station, TX).

Results

Occurrence of musth

Bulls exhibiting physical signs of musth were observed on 121 occasions (39% during the dry season, 61% during the wet season) during the 13-month study period. Musth was recorded in all six study animals and was distributed throughout the year (see Fig. 1). A complete musth cycle was recorded for four of the six bulls. The musth cycles lasted 28 to 70 days.

Individual hormone profiles and temporal relationship between endocrinological changes and musth-related physical parameters

Fig. 2 shows the profiles of immunoreactive EA and GCM, the association patterns, and the occurrence of TG/TGS and UD over the whole study period for three study subjects (Bulls 2, 4, and 6). The profiles of the remaining bulls are not shown because of injury (Bulls 1 and 3) or incomplete recording of musth period (Bulls 3 and 5). All three bulls showed one clear period of musth which was associated with a period of elevated EA and 1–2 periods of reduced GCM levels. During musth, EA levels were elevated up to 2-fold above baseline.

The musth period of Bull 2 occurred during the wet season between the beginning of November and the end of December 2007. Associated periods of elevated EA levels (54 days) and of reduced GCM concentrations (17 days) were identified, and a short period of “TG/TGS only” was recorded after his musth period. An additional period of elevated EA levels (29 days) occurred approximately 6 weeks prior to his musth period. Bull 2 spent most of his time in association with other bulls, but increased his time alone prior to and during his musth period. He was only observed with cows during musth.

Bull 4 showed a 4-week musth period during the wet season between the beginning of February and the beginning of March 2008. A period of “TG/TGS only” was recorded before and after his musth period, as well as associated periods of elevated EA levels (42 days) and of reduced GCM concentrations (8 days). Bull 4 was mostly observed in association with other bulls, except during his musth period, when he was alone or in association with females.

The musth period of Bull 6 occurred during the dry season between mid July 2007 and mid September 2007. Before and after musth, short periods of “TG/TGS only” were recorded, and associated periods of elevated EA and reduced GCM concentrations lasted 71 and 32 days, respectively. Within the 13-month study period, Bull 6 spent his time almost equally either alone or in association with other bulls. During the musth period he associated with cows a few times but also occasionally unrelated to musth in December 2007 as well as in January, February and June 2008.

Table 1 shows the results regarding the temporal relationship between the onset/end of periods of elevated androgen levels and the three parameters TG/TGS, UD and reduced GCM concentrations. The onset of the musth period was recorded in all six study animals,

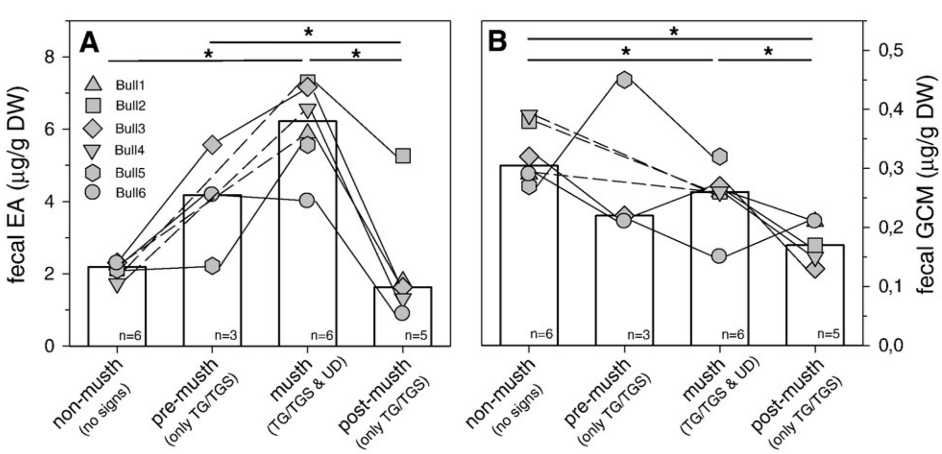

Fig. 3. Dot-bar plots of grouped concentrations of EA (A) and GCM (B) representing data derived from fecal samples ($n = 486$) of six bulls. Each symbol represents the median hormone value of an individual which showed either no physical signs, only TG/TGS, or TG/TGS and UD. Values from the same individual are indicated by the same symbol connected by a line (solid line for consecutive groups, dashed line for non-consecutive groups). Each bar shows the median of the individual medians. Asterisks indicate statistically significant differences between groups, determined using Kruskal–Wallis one-way ANOVA on ranks (see text).

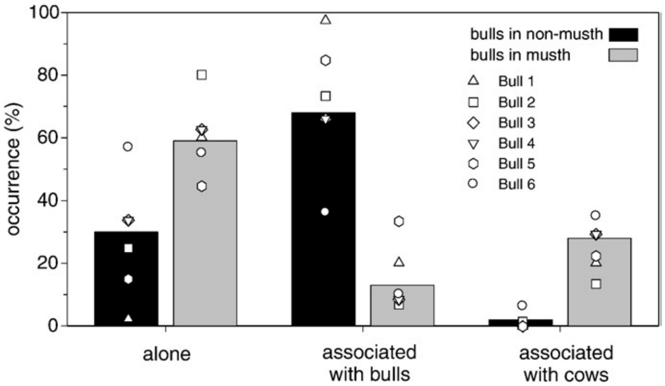

Fig. 4. Dot-bar plots of observations (%) of male African elephants in association with other bulls, alone, or with females when in musth or non-musth. Each symbol represents the percent of observations within an individual of the respective association/condition combination. Values from the same individual are indicated by the same symbol in the different groups. Each bar shows the mean of the six individual observation values.

whereas the end was only observed in five of the six bulls. In four of the six study animals a period of reduced GCM concentrations was detected simultaneously with the occurrence of musth. Periods of UD and reduced GCM levels occurred only after the onset of elevated EA, whereas TG/TGS occurred equally either after or simultaneously with the onset of elevated androgen levels. With one exception, TG/TGS and UD terminated after the end of elevated EA levels, whereas reduced GCM concentrations continued beyond the period of elevated androgens in three of the four cases.

General endocrine changes during the stages of musth

Fig. 3 shows the individual median EA and GCM levels during different states of musth for the six study bulls. The median EA value when no physical signs of musth were present (2.13 μg/g) was about half that of the median EA level when animals showed pre-musth (4.18 μg/g), about one third of the corresponding level when TGS and UD (musth) were present (6.06 μg/g), and almost the same when bulls showed post-musth (1.63 μg/g). EA levels differed significantly between the non-musth and musth, the pre-musth and post-musth and the musth and post-musth group ($P<0.001$). In contrast, GCM levels were higher in non-musth bulls (0.32 μg/g), compared to animals in all three other conditions (pre-musth: 0.21 μg/g; musth: 0.26 μg/g; post-musth: 0.15 μg/g). GCM levels between the non-musth and musth, the non-musth and post-musth and the musth and post-musth group were significantly different ($P<0.001$).

Association patterns in musth and non-musth bulls

Fig. 4 shows the percentage of observations of the six study males in association with other bulls, alone, or with females when in musth or not in musth. The low number of observations for animals in pre- and post-musth (showing only signs of TG/TGS) prevented analysis of the association pattern of bulls in these stages. There were significant differences in the association patterns of the study animals when they were in musth compared to non-musth. Adjusted for differing numbers of observations between individuals, the elephants of the study group were found in association with other bulls in 68% of the cases when in non-musth compared to 13% when in musth ($P<0.001$). In contrast, animals in non-musth were found alone in 30% of the cases compared to 59% when the bulls showed musth ($P<0.001$). Finally, non-musth bulls were very seldom seen in association with females (2%) compared to musth bulls, which were associated with cows in 28% of the observations ($P<0.001$).

Endocrinological changes associated with musth, season and association pattern

The results of the mixed effects multiple linear regression models to estimate the effect of musth, season and association on fecal EA and GCM concentrations are shown in Tables 2 and 3 respectively. Due to low numbers, observations of animals in pre-musth ($n=6$) and post-musth ($n=15$) were not included in the models. In both models, the musth*season interaction term and for GCM also the musth*association interaction term tended towards significance ($P<0.1$). Since these interactions were of particular interest, rather than to obscure their possible existence, the effects of association and season are presented separately for bulls in musth and bulls not in musth.

Overall, fecal EA concentrations (Table 2) were higher for bulls in musth than for bulls not in musth ($P<0.001$). Adjusted for season, fecal EA in musth bulls tended to be lower when associated with cows compared to when being alone ($P=0.053$). Adjusted for association, fecal EA in musth bulls tended to be higher during the wet season ($P=0.090$). The autocorrelation parameter (ρ) for fecal EA in the model was estimated to be 0.234.

Fecal GCM concentrations (Table 3) were lower for bulls in musth than for bulls not in musth ($P<0.001$). Adjusted for season, fecal GCM in musth bulls tended to be lower when associated with bulls than when alone ($P=0.094$). In non-musth bulls associated with cows, fecal GCM was higher than when associated with bulls ($P=0.042$)

Table 2
Effect of musth, association and season on fecal EA concentrations in six free-ranging African elephant bulls: results of a mixed-effects multiple regression model with serial autocorrelation parameter.

Variable	Level	*b*	SE(*b*)	95% C.I.	*P*-value
Musth	musth vs. non-musth	3.607[a]	0.283	3.052, 4.162	<0.001
Association	Musth bulls				
	with bulls vs. alone	−0.602	0.439	−1.464, 0.259	0.170
	with cows vs. alone	−0.663	0.342	−1.334, 0.008	0.053
	with cows vs. with bulls	−0.060	0.502	−1.044, 0.924	0.905
	Non-musth bulls				
	with bulls vs. alone	−0.225	0.155	−0.529, 0.079	0.146
	with cows vs. alone	0.494	0.456	−0.399, 1.387	0.278
	with cows vs. with bulls	0.720	0.453	−0.169, 1.608	0.113
Season	Musth bulls				
	wet vs. dry	0.549	0.324	−0.085, 1.184	0.090
	Non-musth bulls				
	wet vs. dry	−0.195	0.145	−0.480, 0.090	0.180
Musth*association interaction		–	–	–	0.112
Musth*season interaction		–	–	–	0.040

Estimated autocorrelation coefficient $r=0.234$.
[a] Applicable to a lone bull during the dry season.

Table 3
Effect of musth, association and season on fecal GCM concentrations in six free-ranging African elephant bulls: results of a mixed-effects multiple regression model with serial autocorrelation parameter.

Variable	Level	*b*	SE(*b*)	95% C.I.	*P*-value
Musth	musth vs. non-musth	−0.148[a]	0.032	−0.210, −0.086	<0.001
Association	Musth bulls				
	with bulls vs. alone	−0.079	0.047	−0.171, 0.013	0.094
	with cows vs. alone	−0.044	0.037	−0.117, 0.029	0.235
	with cows vs. with bulls	0.035	0.055	−0.072, 0.141	0.525
	Non-musth bulls				
	with bulls vs. alone	−0.017	0.017	−0.050, 0.016	0.310
	with cows vs. alone	0.083	0.049	−0.014, 0.179	0.092
	with cows vs. with bulls	0.100	0.049	0.004, 0.196	0.042
Season	Musth bulls				
	wet vs. dry	0.020	0.037	−0.052, 0.093	0.578
	Non-musth bulls				
	wet vs. dry	−0.086	0.016	−0.118, −0.054	<0.001
Musth*association interaction		–	–	–	0.077
Musth*season interaction		–	–	–	0.009

Estimated autocorrelation coefficient $r = 0.451$.
[a] Applicable to a lone bull during the dry season.

and tended to be higher than when solitary ($P = 0.092$). Adjusted for association, fecal GCM in non-musth bulls was lower during the wet season ($P < 0.001$), but no such effect was found for bulls in musth. The autocorrelation parameter (ρ) for fecal GCM in the model was estimated to be 0.451.

Discussion

This study provides new information on the relationship between the state of musth and glucocorticoid output and the impact of season and association patterns on endocrine gonadal and adrenocortical activity (as measured by fecal androgen (EA) and glucocorticoid metabolite (GCM) concentrations) in free-ranging male African elephants. Apart from analyzing the impact of extrinsic and intrinsic factors on endocrine activity, our study also supports previous findings on the temporal relationship between hormonal changes and the various physical signs of musth.

Previous long-term studies have demonstrated that periods of musth in African elephants are often synchronized between years in individual bulls, but that the occurrence of musth is not in synchrony at a population level (Hall-Martin, 1987; Hollister-Smith et al., 2008; Poole, 1987). Our data are consistent with the latter, as in only two cases was there a partial overlap between musth periods in our study animals. Owing to the small number of animals investigated here, no definite conclusion about the degree of musth synchrony between bulls can be made. Future studies using a larger sample size are needed to explore this issue further. Although musth has been shown to occur throughout the year (Hall-Martin, 1987; Poole, 1987, 1994), there is evidence that its occurrence in the Kruger National Park is correlated with the onset of the wet season (Hall-Martin, 1987). Our results are consistent with this. Although musth was distributed throughout the year, two thirds of our bulls showed musth during the wet season.

Our data confirm previous findings in captive-held elephants (Ganswindt et al., 2005b) showing an elevation in androgen concentrations before the onset of the physical signs of musth (UD and TGS/TG). Once again, an increase in androgen levels appears to be the initial stimulus for the occurrence of the physical musth-related signs, in particular urine dribbling (UD). The trigger or triggers for the increase in androgen levels, however, remain unclear. It may well be that internal energy stores act as the initial trigger for the musth-related elevation in androgen concentrations or that an internal annual clock exists, which announces the individual onset of musth (Rasmussen et al., 2008a).

As mentioned in Introduction, the relationship between the state of musth and activity of the HPA axis is unclear due to inconsistent results (Brown et al., 2007; Ganswindt et al., 2003, 2005a,b; Rasmussen et al., 2008a). Musth is known to be associated with increased restlessness and reduced feeding activities, often leading to a progressive loss of condition (Poole, 1987, 1989). This has led to the hypothesis that musth represents a form of physiological stress (Wingate and Lasley, 2002). The idea is supported by Brown and colleagues (2007) who reported a positive correlation between testosterone and cortisol levels in captive bulls exhibiting musth. In contrast, Ganswindt et al. (2003, 2005b) found a reduction in glucocorticoid output during musth in captive elephants, suggesting that musth does not represent a physiological stress mediated by increased adrenocortical activity, but rather exerts a suppressive effect on adrenocortical function. Apart from the necessity to resolve the apparent conflict in current knowledge regarding musth-related effects on glucocorticoid output in captive animals, the findings for bulls in captivity should not simply be extrapolated to free-ranging bulls. The different ecological and environmental settings between captive and free-ranging animals are apparently accompanied by differences in reproductive success and health status (Clubb et al., 2008), and this could also include differences in musth-related adrenocortical activity. Neither of the two contrary explanations hypothesized for the scenario in captivity has yet received support in free-ranging elephants, since no change in glucocorticoid concentrations was found in the only study conducted on free-ranging animals thus far (Ganswindt et al., 2005a; Rasmussen et al., 2008a). Although this result already supports the idea that the musth condition does not represent a physiological stressor for animals in the wild, our present data using more frequent and high-resolution monitoring of endocrine function in free-ranging bulls clearly confirm that musth is not associated with increased glucocorticoid output. Moreover, for the first time our data demonstrate that GCM concentrations are significantly lower during the period of musth in wild elephant bulls, similar to the finding reported for captive-housed animals (Ganswindt et al., 2005b). Furthermore, the fact that musth-related periods of reduced glucocorticoid levels occurred after the elevation in androgen concentrations as well as after the occurrence of physical signs of musth (UD and TG/TGS) provides evidence for a negative feedback mechanism between the HPG and HPA axes during musth, and therefore lends support for the hypothesis of a suppressive effect of the musth condition on adrenocortical function.

Previous reports of seasonal changes in progesterone and GCM levels in African elephant cows have already shown that variation in the seasonal environment can affect hormone levels in this species (Foley et al., 2001; Viljoen et al., 2008; Wittemyer et al., 2007). The only data available thus far for African elephant bulls, however, shows no overall seasonal pattern in EA and GCM levels, although exceptional conditions, such as drought led to increases in androgen and glucocorticoid levels (Rasmussen et al., 2008a). Our present data extend these findings by demonstrating a seasonal pattern in both hormones, when including reproductive activity as covariate in the analysis. Musth bulls are known to show increased aggression, especially towards other bulls in musth (Hall-Martin, 1987), and aggressive elephant bulls have higher androgen levels (Ganswindt et al., 2005b). The tendency for lower EA levels during the dry season found in musth bulls may therefore be at least partly explained by the less frequent occurrence of musth bulls during this time of the year, and therefore a lower probability of exposure to social and reproductive competition. Furthermore, significantly lower GCM levels in non-musth individuals were found during the wet compared to the dry season, a finding similar to that reported for females (Foley et al., 2001; Viljoen et al., 2008). Presumably this is due to less nutritional stress during this resource abundant period of the year. However, interestingly no such effect of season on GCM concentration was found in bulls exhibiting musth. At present there is no definitive explanation for this, but the possible suppressive effect of the musth condition on adrenocortical activity may diminish any seasonal effect on GCM levels in musth males.

For an interpretation of the relationship between sociality and gonadal and adrenocortical function it is advisable to distinguish between bulls in a competitive (musth) and non-competitive (non-musth) mode. Although rarely observed during this study, non-musth bulls associated with cows showed significantly higher concentrations of GCM compared to non-musth bulls in association with other bulls. The fact that females are often accompanied by a musth bull may explain the elevated GCM levels in non-musth bulls associated with cows. They would have an increased probability of encountering a competitive musth bull, which are clearly dominant, and therefore presumably act as a stressor to a non-musth male. Although the occurrence of musth is generally characterized by an increase in EA and a decrease in GCM levels (Fig. 3), musth bulls associated with cows tended to have lower EA levels compared to musth bulls alone. The reason for this is not clear, but may represent an outcome of a trade-off between the amount of calmness needed for engaging in matings with the female(s) and the level of competitiveness necessary for maintaining the musth status and outcompeting other males. In terms of stress hormones, musth bulls associated with other bulls tended to have lower GCM levels compared to when they were alone, independent of the suppressive effect of the musth condition on adrenal function. This is somewhat unexpected since, on first thought, the presence of other bulls should have increased inter-male competition which, in turn, would be expected to result in heightened adrenocortical activity. However, given that in almost all cases the associated bull would have been in a non-musth condition and thus not competitive, his presence presumably was not a threat and a stressor to the musth male. In contrast, our data suggest that association with non-musth bulls may result in a social buffering effect in musth bulls displaying the presumably energetically challenging state of musth. That the presence of specific social partners can have an inhibiting or ameliorative effect on HPA responses (Hennessy et al., 2006; Kikusui et al., 2006) is well-known for many species. This effect is clearly seen in the context of the mother–infant relationship (Hennessy et al., 2006; Kikusui et al., 2006), but similar results have also been found in relationships between adult individuals in a variety of species such as rodents, birds, non-human primates and also in humans (Hennessy et al., 2006; Kikusui et al., 2006; Remage-Healey et al., 2003; Rukstalis and French, 2005; Sachser, 1998). For example, the presence of a preferred partner effectively buffers HPA responses in adult male guinea pigs during exposure to a novel environment (Hennessy et al., 2006), and in female prairie voles a reduction in circulating glucocorticoid levels in the presence of a male may be necessary for forming a bond (DeVries et al., 1996). Although a social buffering effect is likely to occur in a highly social species like the elephant, further research would be necessary to support this hypothesis.

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