

## Birth season glucocorticoids are related to the presence of infants in sifaka (*Propithecus verreauxi*)

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The responses of plural breeding mammals to environmental stressors are little understood in free-ranging populations, but recent studies of singular breeders suggest that ecological factors and social milieu influence the variable physiological stress responses observed among individuals. Our previous studies examining faecal glucocorticoid (fGC)–behaviour interactions in plural breeding male sifaka (*Propithecus verreauxi*) show that fGC elevations coincide with specific dispersal events, particularly the eviction of subordinates by resident alpha males. This study examined the utility of fGC assays for assessing the stress responses of male sifaka to demographic changes in the population during the birth season. Analyses were based on 889 faecal samples collected over five birth seasons from 124 adult males residing in 55 different groups at Beza Mahafaly, Madagascar. Results show that fGC levels in males are unrelated to age, residence, group stability or rank, but are significantly and positively correlated with the presence of infants, annual elevations in weekly mean fGC concentrations in males paralleling increasing numbers of infants born annually in the population. These data are the first to show that in seasonal plural breeding species such as sifaka, elevated fGC in males reflects specific events related to reproduction rather than states or social context during the birth season.

**Keywords:** sifaka; glucocorticoids; plural breeding; demography; infants; infanticide

### 1. INTRODUCTION

Stressors are ubiquitous in nature, and as a consequence animals have developed adaptive physiological and behavioural responses for coping with acute stressors. One of these is the hypothalamic–pituitary–adrenal axis that releases glucocorticoid (GC) steroids from the adrenal cortex into the blood stream, mobilizing energy reserves needed to counteract the effects of the stressor (Nelson 2000). While clearly adaptive in the short term, GC elevations that are prolonged can have serious detrimental physiological effects, including gastric ulcers, hypertension and immune suppression (Sapolsky 2005).

Many vertebrate taxa seasonally modulate GC release, with peak GC concentrations occurring during the breeding season, when increased energy mobilization is required to support breeding effort and territorial defence. These annual rhythms in GC concentrations appear to be adaptive when considered in the context of mating competition and reproduction (reviewed in Romero 2002). In mammals, on the other hand, the stress response is linked to an array of social traits, states and events that characterize individuals and their daily lives, a subset of these being reliable predictors of elevated GC levels. For example, *behavioural traits* such as aggression (Muller & Wrangham 2004), *current states* such as increased age (Sapolsky *et al.* 1986) and reproductive status (Fichtel *et al.* 2007) and *social context* such as numbers of resident males (Gould *et al.* 2005) and social instability (Brockman

*et al.* 2001) correlate with elevated GC responses. Likewise, *demographic events* such as male immigration (Alberts *et al.* 1992), dispersal (Bergman *et al.* 2005) and *specific events related to reproductive success* such as mating (Strier *et al.* 1999), birth of offspring (Berg & Wynne-Edwards 2001) and male infanticide (Engh *et al.* 2006) have all been shown to be associated with elevated GC levels. In singular breeding carnivores (i.e. cooperative social species with high reproductive skew and communal care of young, e.g. dwarf mongoose *Helogale parvula*; African wild dog *Lycaon pictus*; grey wolf *Canis lupus*), GC levels are most often associated with social status and, in some cases, severity of aggression. Dominant individuals have higher GC concentrations than subordinates, particularly during the breeding season when individuals aggressively compete for mating opportunities (African wild dog, dwarf mongoose; Sands & Creel 2004; Creel 2005). Among African wild dogs and dwarf mongoose, alpha females are known to suppress reproduction in subordinates through infanticide, but no direct fGC–infanticide link has been demonstrated for these species thus far, though aggression-related fGC elevations occur in females (but not males) during the non-mating season. In plural breeding species (i.e. social species having low reproductive skew and little/no cooperation; Goymann & Wingfield 2004) such as the spotted hyena (*Crocuta crocuta*), fGC concentrations in dominant males (but not females) are likewise elevated above those of subordinates, but factors other than social status affect fGC levels, including the proportion of males per clan or clan size

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(Goymann *et al.* 2003 in Goymann & Wingfield 2004). These fGC–rank effects have been variously interpreted to reflect the cost of dominance (rather than the consequence of subordination, Creel 2005) or allostatic load (e.g. the increased energy requirements associated with dominant or subordinate status, Goymann & Wingfield 2004).

Among plural breeding primates, *no consistent* relationship has been observed between social status and stress response hormones (reviewed in Weingrill *et al.* 2004). However, Abbott *et al.*'s (2003) meta-analysis of rank differences in cortisol (CORT) shows that subordinate males residing in overtly aggressive societies elevate CORT concentrations when subjected to higher rates of stressors and reduced opportunities for social support. Among strepsirrhine primates, fGC–rank effects vary by species and season. Alpha male sifaka (*Propithecus verreauxi*) at Kirindy Forest, Madagascar exhibit significantly elevated fGC levels above those of subordinates during the pre-breeding/breeding season of increased reproductive competition but not during the following birth season (Fichtel *et al.* 2007). Although no effect of rank on fGC concentrations has been observed in male red-fronted lemurs (*Eulemur fulvus rufus*: Ostner *et al.* 2008) at the same site, increases in both faecal testosterone (fT) and fGC during the birth season suggest that these hormonal changes may reflect preparation for defence against infanticide (Ostner *et al.* 2008). Our previous studies examining fGC interactions with behaviour in male *P. verreauxi* at Beza Mahafaly Special Reserve (BMSR) showed that high fGC levels are not a predictable cost of high rank during the birth season, and that elevated fGC concentrations coincide with specific behavioural traits and social contexts, including social instability (Brockman *et al.* 2001) and the aggressive eviction of subordinates by resident alpha males (Brockman *et al.* 2003). We found that alpha males living in unstable groups had significantly higher fGC levels than their subordinates, and among subordinates, those residing in unstable groups exhibited significantly higher fGC levels than those residing in stable groups (Brockman *et al.* 2001).

This study extends those findings with analyses of fGC responses to demographic changes in a larger sample of male sifaka, including, for the first time, investigations of the relationship between fGC levels in males, annual variation in the numbers of infants born in groups and male caregiving behaviour. The aim of this study was to investigate further the relationship of male fGC concentrations to specific traits, states and events, including age, group stability, residence patterns, rank and the presence of infants in wild sifaka, *P. verreauxi*, a diurnal, monomorphic, strepsirrhine primate from south and southwest Madagascar (Tattersall 1982). The studies noted above and the present study are important for the new insights they provide into the role and function of endocrine mechanisms in mediating male coping strategies to natural sources of stress in plural breeding species, including the birth of infants and infanticide risk.

We examined whether variations in weekly mean fGC levels in males would be associated with increased age (Sapolsky *et al.* 1986), group stability, residence, rank and the presence of infants. Specifically, we predicted that weekly mean fGC levels would be: (i) higher in middle-aged and old males than in young adult and adult males,

(ii) higher in males residing in unstable groups than in those residing in stable groups, (iii) higher in immigrant than in resident males, (iv) higher in alpha than in subordinate males in unstable groups, and (v) higher in males residing in groups with infants than in those residing in groups without infants.

## 2. MATERIAL AND METHODS

### (a) *Study site and subjects*

This study was conducted at the BMSR, Madagascar (site, climate, habitat are described in Brockman *et al.* 2008). The sifaka study population currently comprises approximately 278 marked and habituated individuals residing in 38 core social groups (average of approx. five to six individuals; range 2–16), their 4–6 ha overlapping home ranges being confined within the boundaries of parcel 1 at BMSR. The ecology, demography and social behaviour of this population has been studied continuously by Richard and colleagues since 1984, yielding unparalleled insights into the social life, reproduction and life history of this species (reviewed in Brockman *et al.* 2008). Sifaka here live in multimale/multifemale social groups in which females are philopatric and socially dominant to males. Of the 182 groups censused during the study, 54 per cent were composed of multiple adult females, 27 per cent of these groups experiencing multiple births. Of the 140 infants born in core groups during the study period, 47 per cent died before 1 year of age (D. K. Brockman & M. Schwartz 2008, unpublished data). Adult sex ratios within groups were biased towards males and averaged 1.29. Mating is polygamous, characterized by high rates of male reproductive competition during the breeding season (e.g. physiological and behavioural suppression of subordinates by alphas: Brockman *et al.* 1998; D. K. Brockman 2001, unpublished data), moderate reproductive skew (Lawler 2007; R. R. Lawler 2008, personal communication) and consequently increased variation in paternity certainty. Moderate reproductive skew in the presence of intense reproductive competition in this population ( $n=134$  males, 1989–1999) derives from extragroup paternities (Lawler 2007), variation in the degree to which oestrus is hormonally (a)synchronous and the presence of strong mate choice and multiple mating by females (Brockman & Whitten 1996), resulting in some resident males not reproducing at all, some siring offspring within their resident groups and others successfully reproducing in groups adjacent to their own groups (Lawler 2007). Reproduction is seasonal in the wild with 96 per cent of births occurring during the late June to late August austral winter (Richard & Dewar 1991). In this species, males are the dispersing sex, most often leaving their natal group at 3 years of age, secondary and tertiary transfers representing the norm for this population (Richard *et al.* 1993). Thirty-three per cent of adult males transfer annually, one-third of these transfers occurring during the birth season, resulting in as many as 60 per cent of groups undergoing a change in male membership at this time. Transfer-related aggression by adult males, including invasions by neighbouring group males, group takeovers and infanticide, has been known to occur at this time, suggesting that immigrant males pose a risk to neonates (reviewed in Brockman *et al.* 2001).

### (b) *Data collected*

Behavioural observations and faecal samples were collected from 124 individually marked focal males in 55 social groups

Table 1. Effects of state, demographic and social factors on weekly mean fGC concentrations in adult male sifaka during five birth seasons. (Ages of individuals include young adult (4–8 years), adult (9–13 years), middle-aged (14–18 years), and old (19 years and above) based on patterns of dental wear/known birth dates (Richard *et al.* 2002). *Unstable groups* are defined as those having experienced a transfer event within the previous one to six weeks, and *immigrant versus resident* males refer to those non-natal males that have and have not recently migrated since the previous census, respectively. *Alpha-subordinate positions* were assessed based on the consistent direction and outcome of aggressive (supplants, cuffs, grabs, bites and fights) and submissive (fear chatter/grimace) behaviour (Brockman *et al.* 2001). *Infants present/absent* refers to those groups with and without infants, respectively, and *caregiving versus non-caregiving* males refer to those males that do and do not engage in caregiving behaviour (e.g. hold, groom, carry, retrieve) with infants. Differences in male sample size across comparisons reflect uncertain age or unsettled rank for some males, male emigration or the absence of a resident male in some groups, the absence of infants in half of the focal groups and opportunistic sampling of males not in the focal groups. We employed *t*-tests, Mann–Whitney *U* tests and Kruskal–Wallis ANOVA on ranks to detect variable-related differences in fGC levels; significance was set at  $p < 0.05$ .)

variable	weekly mean fGC (ng g <sup>-1</sup> ) ± s.d.	no. of males sampled	<i>p</i> -value
age			
young adult	62.12 ± 39.15	56	0.99
adult	59.63 ± 32.95	42	
middle-aged	64.34 ± 43.21	18	
old	69.10 ± 47.75	8	
group stability			
stable	61.86 ± 41.35	45	0.94
unstable	62.28 ± 34.92	79	
residence			
resident	66.18 ± 38.48	81	0.34
immigrant	59.74 ± 36.42	47	
rank			
alpha	60.13 ± 38.25	62	0.49
subordinate	65.55 ± 36.07	68	
infants			
present	71.39 ± 37.56	73	0.009
absent	53.92 ± 36.04	56	
males in infant groups			
caregiving	69.85 ± 40.16	30	0.90
non-caregiving	70.03 ± 37.65	43	

during the July–August birth season in 1998, 2000, 2001, 2003 and 2005. Continuous and ad libitum sampling methods were used to collect data on male dispersal and social behaviour for 7- to 10-day periods. Behavioural data focused on indices of rank, such as aggressive and submissive interactions (table 1), as well as scent-marking and caregiving behaviours. Indices of male caregiving included: grooming: licking/using the toothcomb to scrape the fur of an infant; holding/grooming: embracing and grooming an infant out of contact with its mother; holding: embracing an infant out of contact with its mother; carrying: unassisted transporting of an infant; and retrieving: rescuing an infant who lost contact with its mother and returning it to her. Rates of caregiving behaviour were derived by dividing the total number of each index of caregiving behaviour by total number male focal

animal hours in caregiving groups (i.e. 681.73 hours). D.K.B., P.L.W. and 15 graduate field assistants conducted eight to ten 30 min focal follows on focal males between 08.00 and 18.00 hours (with a 2-hour midday break), totalling 1220.82 focal animal hours of observation. Randomized focal samples were evenly distributed between morning and afternoon. We conducted biweekly censuses to identify groups experiencing male transfer and infant births and deaths. These changes in group composition were used to select focal groups to compare for the effects of group stability and infant presence (see table 1 for definitions). A total of 867 faecal samples were collected from the 124 focal males, averaging seven samples per male. Faecal samples were also opportunistically collected from non-focal co-resident males ( $n = 22$  faecal samples, 14 males). Early morning faecal samples weighing 5–10 g were collected immediately after voiding, and oven-dried as described in Brockman *et al.* (1998). Our previous studies of diurnal variation show that while fGC concentrations decline throughout the day, there is no significant effect of hour of sample on fGC (ANOVA:  $F = 0.985$ ,  $p = 0.415$ ; Brockman *et al.* 2003). Faecal sampling is ideal for assessing stress responses by providing an integrative measure of chronic steroid release without disturbing the natural population (Whitten *et al.* 1998). Faecal immunoassays using a corticosterone antibody that reacts with several cortisol metabolites have been shown to reflect stress responses in a number of species (reviewed in Wasser *et al.* 2000), and our previous studies of sifaka have shown that faecal corticosterone (fCCOS) is a better assay than fCORT for measuring stress in long-term studies. In this regard, a CCOS radioimmunoassay was previously validated and found to detect GC metabolites reliably in sifaka faeces and to index stressful states and events (Brockman *et al.* 2003).

### (c) Faecal extraction

A 0.1 g sample of faeces was homogenized in 2 ml methanol:acetone (8:2, v/v) and filtered with a 0.2 µm nylon centrifuge filter (Centrex MF; Schleicher & Schuell, Keene, NH). The filtrate was extracted on Sep-Pak VAC C18 columns (500 mg; Waters Corp. Milford, MA). The sample was diluted with an equal volume of water and then layered onto a column primed according to the manufacturer's instructions. The column was washed with 5 ml water, and the steroid fraction was eluted with 3 ml methanol and stored at  $-80^{\circ}\text{C}$ .

### (d) Corticosterone radioimmunoassay

The fGCs were assayed using a modification of the <sup>125</sup>I corticosterone RIA kit (MP Biomedicals, Irvine, CA, formerly ICN Diagnostics). The corticosterone antibody in this assay has high cross-reactivity with the major faecal metabolites of cortisol (Wasser *et al.* 2000). Working buffer was 0.1 per cent gelatine phosphate-buffered saline (pH 7.4). <sup>125</sup>I tracer (50 µl) and antiserum (200 µl), diluted 1:3 in working buffer, were added to 100 µl aliquots of the standards (diluted 1:3) to give concentrations of 20–1670 pg ml<sup>-1</sup>, samples (diluted 1:4) and controls (diluted 1:3). Each was vortexed and incubated for 2 hours at room temperature. After incubation, 500 µl of second antibody (diluted 1:3) was added, and the incubates were vortexed, incubated an additional 15 min at room temperature, and centrifuged at 1500g for 30 min at room temperature. Following decanting of the supernatant, the radioactivity of the precipitate was determined by 5 min counts in a gamma counter.

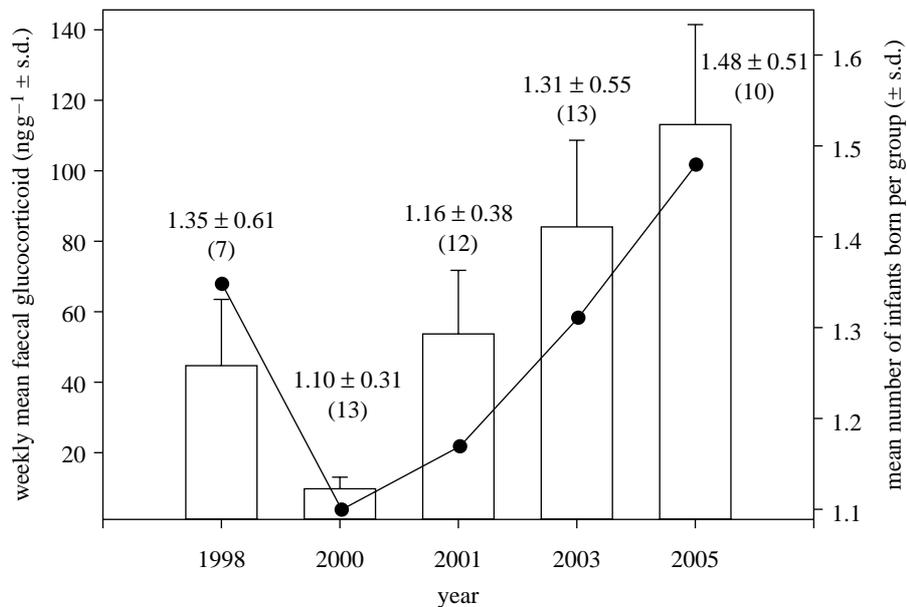


Figure 1. Annual variation of weekly mean fGC concentrations in focal males is strongly linked to the presence of infants, annual changes in male weekly mean fGC paralleling annual changes in mean number of infants born per group  $\pm$  s.d. Values in parentheses indicate number of groups sampled.

#### (e) *Statistical tests and notes on normality testing*

Statistical analyses were performed using SIGMASTAT v. 3.5 (Systat Software, Inc., Point Richmond, CA). Multi- and univariate statistical tests were used to identify which variable(s) best predicted variation in weekly mean fGC concentrations. The effects of age, group stability, residence, rank and infant presence on fGC were tested using multivariate (multiple linear and linear regression) and univariate (*t*-test, ANOVA, Wilcoxon signed-rank test, Mann–Whitney *U*-test) analyses (Brockman *et al.* 2001). Pairwise multiple comparison procedures (Bonferroni, Dunn's tests) were used if an ANOVA/ANOVA on ranks showed a significant difference among the variables. Variables with departures from normality were adjusted by removal of outliers (samples with more than two standard deviations from the mean) or were square-root transformed. Results are reported as means and s.d., with significance set at  $p < 0.05$ .

### 3. RESULTS

#### (a) *Validation of corticosterone radioimmunoassay*

Parallelism was tested by serially diluting faecal extracts and comparing the slope of the plot of the expected dose versus per cent bound to the slope of the standard curve. The regression equations showed that the serial dilutions ( $y = -11.5 \log x + 44.1$ ) paralleled the standard curve ( $y = -11.9 \log x + 45.8$ ). Method recovery, assessed by the addition of standards to a low sample, averaged 90 per cent. Intra- and interassay coefficients of variation averaged 7–12 and 5–6%, respectively.

#### (b) *Weekly mean fGC levels and annual variation*

Weekly mean fGC concentrations in the 124 focal males during the birth season averaged  $62.13 \text{ ng g}^{-1} \pm 37.22$  s.d. (range: 5.1–144.10  $\text{ng g}^{-1}$ ), and were sixfold higher than baseline weekly mean fGC levels ( $9.05 \text{ ng g}^{-1} \pm 2.08$  s.d.,  $p = 0.003$ , derived from focal males ( $n = 4$  in year 2000) residing in stable groups without infants in which rank relationships were stable). Weekly mean fGC levels varied across the years, pairwise multiple comparison

procedures showing that fGC differed significantly in all years except 2005 versus 2003 and 1998 versus 2001 ( $p < 0.001$ ).

#### (c) *Weekly mean fGC, age, group stability, residence and rank*

Our multiple linear regression analyses showed that 'the presence of infants' was the only independent variable that predicted mean weekly fGC in males ( $p < 0.001$ ; see below), a result that was confirmed using a linear regression model ( $R = 0.23$ ,  $p = 0.014$ ,  $n = 118$ , derived from pooling data across the 5 years and then regressing male fGC against number of infants born per group). Weekly mean fGC levels were not related to age, group stability, male residence or rank (table 1). A within-group examination of fGC–rank effects in stable versus unstable groups containing matched pairs of alpha and subordinate males showed that fGC was unrelated to rank regardless of the degree of group stability (stable groups: alpha fGC:  $72.35 \text{ ng g}^{-1}$ , subordinate fGC:  $70.50 \text{ ng g}^{-1}$ , Wilcoxon signed-rank test:  $W = 32.000$ ,  $p = 0.43$ ,  $n = 16$  groups; unstable groups: alpha fGC:  $56.20 \text{ ng g}^{-1}$ ; subordinate fGC:  $51.40 \text{ ng g}^{-1}$ ,  $W = -55.000$ ,  $p = 0.56$ ,  $n = 33$  groups).

#### (d) *Weekly mean fGC, presence of infants and male caregiving*

fGC levels were significantly higher in males residing in groups containing infants than in those in which infants were absent (table 1), annual changes in male fGC paralleling annual changes in infant birth rates within groups (figure 1). Forty-one per cent ( $n = 30$ ) of males residing in infant groups exhibited caregiving behaviour, rate of male–infant contact totalling 10.4 per cent. Male caregiving included primarily grooming (7.5%,  $n = 24$  males), as well as grooming/holding (2%,  $n = 9$  males), holding (0.3%,  $n = 1$  male) and carrying (0.3%,  $n = 1$  male; 45 min, 7 min). One male retrieved an infant that slipped off his mother's back and lifted him onto his mother's ventrum (0.1%). The fGC levels in these males were, however, nearly identical to those in males that were

Table 2. History of sifaka groups invaded by neighbouring males and outcomes, 1998–2005.

date	group of origin/male no.	group invaded	weekly mean fGC (ng g <sup>-1</sup> )	infants present?	outcome
29 and 30 Jul 1998	Rengor (RG) 9	Kashka (KK)	35.2	yes	invasions repulsed
	Rengor 211		37.0		
2 Aug 2001	Nify (NI) 9096	Felix (FX)	51.0	no	FX alpha M156 mortally wounded/dies
	Nify 9100		40.0		
15 Aug 2001	unmarked immigrant into the population	Maka (MK)	n.a.	yes	infanticide
15 Aug 2003	Lanto (LA) 9141	Borety (BT)	82.0	yes	infant disappears next day
2 Aug 2005	Lanto 288	Borety	110.7	yes (2)	BT alpha M9093 mortally wounded/dies; infants disappear; M288 becomes BT alpha male; mortally wounded/dies Aug 2007
	Rengor 427		104.1		
	Rengor 387		116.3		

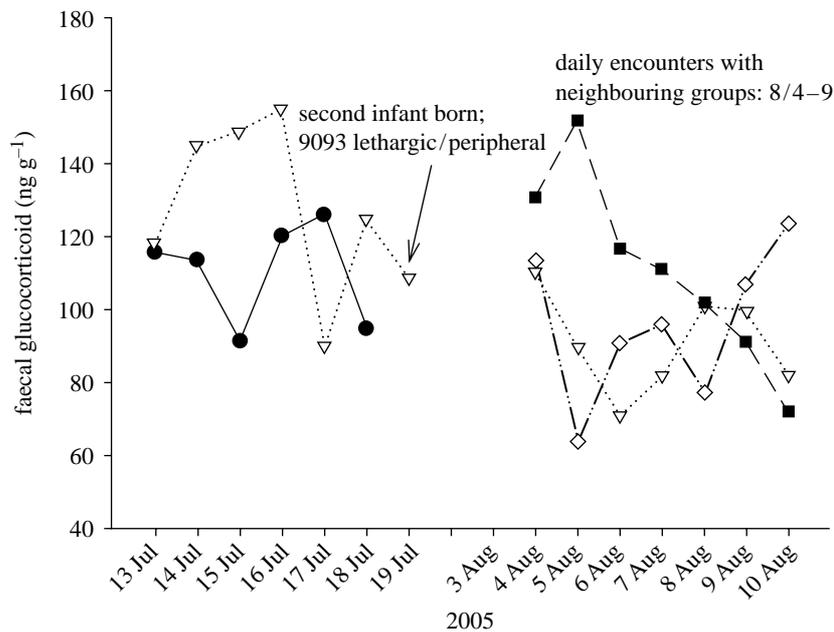


Figure 2. FGC profiles of resident alpha (M9094) and subordinate (M430) males in Borety (BT) group before and after an invasion and group takeover by Lanto (M288) and Rengor (M387) males (assuming a 24 hours serum excretion lag time). Circles, M9093 ResAlpha (10 years); triangles, M430 ImmSub (4 years); squares, M288 InvaderAlpha (17 years); diamonds, M387 InvaderSub (13 years). 1 August: 9093 dead; infants gone.

resident in infant groups but had no contact with infants (table 1). Males exhibited equal rates of total caregiving interactions regardless of rank (alpha males:  $0.003 \pm 0.001$  acts per hour,  $n=16$ ; subordinate males:  $0.003 \pm 0.005$  acts per hour,  $n=14$ ) and no effect of rank on fGC concentrations among caregivers was observed ( $t$ -test:  $t = -0.33$ , d.f. = 28,  $p = 0.75$ ). Paternity is currently unknown for caregiving males.

#### (e) Weekly mean fGC, male immigration, group turnover and infanticide risk

The birth season at BMSR is characterized by frequent male transfers between groups (Brockman *et al.* 2003), some of these involving aggressive immigration (e.g. invasions) by multiple males, the death of resident males and the death or disappearance of the targeted group's infants (table 2). In the five cases in which we observed aggressive immigration/group takeovers (table 2), fGC concentrations in invading males and subsequent infant disappearance in targeted groups were significantly higher than those in males whose invasion

attempts were repulsed (mean fGC in invading males + infant disappearance:  $102.58 \text{ ng g}^{-1}$ ,  $n=36$  samples, 4 males (M9141, M288, M427 and M387); mean fGC in invading males whose invasions were repulsed:  $35.92 \text{ ng g}^{-1}$ , 22 samples, 2 males (M9 and M211); Mann-Whitney  $U$ -test:  $T = 253.000$ ,  $p < 0.001$ ). The 2005 Borety group (BT) case study illustrates the links between fGC and male immigration, group turnover, infant disappearance and mortality during the birth season. Figure 2 shows fGC profiles of resident alpha (M9093) and subordinate (M430) males in the BT group before and after the invasion and group takeover by neighbouring Lanto M288 and Rengor M387. BT also contained two females, a mother and her 4-year-old daughter, and two infants (born on 9 and 19 July).

Weekly mean fGC levels varied across males (ANOVA,  $F = 3.41$ ,  $p = 0.02$ ), pairwise multiple comparison procedures (Bonferroni  $t$ -test) showing that M430 exhibited significantly higher fGC levels prior to group takeover than afterwards (fGC pre-takeover:  $127.18 \text{ ng g}^{-1}$ ; fGC post-takeover:  $90.75 \text{ ng g}^{-1}$ ,  $t = 3.33$ ,  $p = 0.0024$

Table 3. Allostatic load of dominance and subordination and relative GC levels in wild singular and plural breeders in which co-resident females or immigrant males commit infanticide (modified from [Goymann & Wingfield 2004](#)). (GC, glucocorticoids; C, creatinine; \*  $p=0.49$ .)

species	breeding system	social status	sex	sum allostatic load <sup>a</sup>	ratio of allostatic load (dom/sub)	GC levels (sample size of subjects)	ratio of GC (dom/sub)	perpetrator of infanticide	source
dwarf mongoose	singular	dominant	f	5	1.25 <sup>b</sup>	48.04 (ng ml <sup>-1</sup> ) C	2.53	co-resident alpha	Goymann & Wingfield (2004)
		subordinate		4		18.96 (ng ml <sup>-1</sup> ) C		female	
African wild dog	singular	dominant	f	5	1.25	225.66 (ng g <sup>-1</sup> )	2.13	co-resident alpha	Goymann & Wingfield (2004)
		subordinate		4		106.19 (ng g <sup>-1</sup> )		female	
spotted hyena	plural	dominant	m	3	1.00 <sup>c</sup>	37.15 (ng g <sup>-1</sup> ) (33)	1.20	immigrant male	East <i>et al.</i> (2003) and Goymann & Wingfield (2004)
		subordinate		3		30.90 (ng g <sup>-1</sup> ) (9)			
Verreaux' sifaka	plural	dominant	m	5	1.25	60.13 (ng g <sup>-1</sup> ) (62)*	0.92	immigrant male	Brockman <i>et al.</i> (1998, 2001)
		subordinate		4		65.55 (ng g <sup>-1</sup> ) (68)			

<sup>a</sup>Sum calculated for dominants from aspects of costs of rank acquisition (1, low; 2, moderate) and maintenance (1, low; 2, moderate; 3, high); sum calculated for subordinates from degree of threat from dominants (1, low; 2, moderate; 3, high) and potential outlet for threat from dominants (1, low; 2, moderate; see [Goymann & Wingfield 2004](#)).

<sup>b</sup>Ratio > 1.00: more costly for dominants to acquire and maintain alpha status; predicts higher GC levels in dominants.

<sup>c</sup>Ratio = 1.00: equally costly to be dominant or subordinate; predicts no rank-related difference in GC levels.

(Bonferroni adjusted)). Prior to group takeover, subordinate M430 had higher weekly mean fGC levels than alpha M9093 (M430: 127.18 ng g<sup>-1</sup>; M9093: 93.60 ng g<sup>-1</sup>), the former being significantly higher than the 2005 weekly mean fGC levels for non-caregiving males (2005 weekly mean: 97.81 ng g<sup>-1</sup>,  $p=0.03$ ). The days 14–20 July were noteworthy for the absence of male–male aggression, frequent female-directed aggression towards males (e.g. lunging, cuffing, grabbing, biting), a dramatic increase in scent-marking by M430 on 17 July and alpha M9093's apparent illness (profound lethargy, e.g. long periods of resting, curled; not feeding; lagging behind group), which coincided with the birth of F411's infant on 19 July. Male 430 was scent-marking more than a subordinate male normally does (i.e. almost never), perhaps reflecting anticipation of group takeover by Lanto/Rengor males. However, as recently as 20 July, there had been a peaceful encounter with Rengor, including a brief reciprocal grooming bout between M430 and Rengor M320. We recensused BT on 1 August and found M9093's fresh body, his femoral artery severed and both infants gone and presumed dead. After group takeover, the newly dominant Lanto M288 initially had higher fGC concentrations than M387 and M430. This may reflect residual elevation from the takeover event (and possible infanticide) since his fGC levels declined markedly over the next week in spite of frequent aggressive encounters with neighbouring groups.

#### 4. DISCUSSION

Contrary to predictions, population-wide fGC levels did not vary with male age, group stability, residence or rank during the birth season. The fGC concentrations varied little in males regardless of whether they were young adult, adult, middle aged, old, whether they resided in unstable versus stable groups or whether they were residents or immigrants. In contrast to singular and plural breeding carnivores (African wild dog, dwarf mongoose: [Creel 2005](#); spotted hyena, grey wolf: [Goymann & Wingfield 2004](#)) but concordant with previous findings for this species ([Brockman \*et al.\* 2003](#); [Fichtel \*et al.\* 2007](#)), fGC was not higher in alpha than in subordinate males for all years combined, lending support to the idea that high fGC levels are not a predictable cost of high or low rank in this species during the birth season (see also [Ostner \*et al.\* 2008](#) for red-fronted lemurs). In the context of the GC-mediated allostatic load hypothesis ([Goymann & Wingfield 2004](#)), sifaka are concordant with singular and plural breeding carnivores exhibiting infanticide ([table 3](#)), i.e. it is more costly for dominants to acquire and maintain dominant status, and perhaps suppress the reproduction of co-resident subordinates, than to be subordinate. However, in contrast to singular breeding carnivores, allostatic load does not predict GC levels in plural breeding spotted hyenas or sifaka. Among spotted hyenas it is equally costly to be dominant or subordinate, but alpha males nevertheless exhibit elevated fGC levels above those of subordinates, and in sifaka, higher allostatic load in alpha males is not associated with higher fGC levels in the birth season. Thus, it appears that factors other than rank are influencing GC levels in these plural breeders (reviewed in [Goymann & Wingfield 2004](#)), including for spotted hyenas, sex ratio and clan size, and for sifaka, aggressive male immigration, group takeover and

increased infanticide risk (see below and Alberts *et al.* 1992 for population-wide GC elevations in baboons in response to aggressive male immigration).

The absence of fGC–rank effects in male sifaka contrasts with our finding of marked within-group rank effects on weekly mean fT concentrations during the birth season: alpha males had significantly higher fT concentrations than their subordinates (alpha fT: 80.90 ng g<sup>-1</sup>, subordinate fT: 16.90 ng g<sup>-1</sup>, Wilcoxon signed-rank test:  $W = -904.000$ ,  $p = 0.001$ ,  $n = 49$  male pairs), suggesting a role for androgen-mediated behaviours in the maintenance of dominance in sifaka. Additionally, some males in this population are known to elevate fT facultatively in anticipation of birth and while defending against aggressive challenges of transferring males in the presence of newborns, suggesting that some individual males may be particularly sensitive to parturition events and the risk of infanticide (Brockman *et al.* 2001).

Evidence is accruing that events linked to reproductive fitness, including birth/lactation, the risk of infanticide and impending birth, elicit profound GC responses in singular and plural breeders. GC concentrations in lactating (but not cycling or pregnant) female chacma baboons (*Papio hamadryas ursinus*) increase significantly in response to male immigration and infanticide, and similar GC elevations occur in males resulting from the destabilizing effects of male immigrants on the resident male hierarchy (Beehner *et al.* 2005; Engh *et al.* 2006). These results lend support to the proposition that of the stressors encountered daily by individuals, those which are the most unpredictable and uncontrollable (e.g. predation, infanticide) elicit the strongest stress responses (Crockford *et al.* 2008). On the other hand, anticipation of often predictable future events can also elicit GC responses under certain circumstances. Males of some biparental species experience significantly elevated cortisol concentrations the week prior to the birth of an infant, suggesting that expectant fathers may anticipate birth of offspring with some degree of apprehension (humans: Berg & Wynne-Edwards 2001; cotton-top tamarins, *Saguinus oedipus*: Ziegler *et al.* 2004). In plural, seasonally breeding primates such as sifaka (Lawler 2007) the entire birth season may represent a stressor, albeit a predictable one, especially when births coincide with uncontrollable events such as increased intergroup male transfers and infanticide (Brockman & Whitten 1999).

In this population of sifaka, events related to reproduction (e.g. birth of infants) rather than states and social variables were the best predictors of weekly mean fGC levels in males during the birth season. The fGC levels increased in the presence of infants at the population, group and individual level. This response resembles the elevations in cortisol seen in biparental primates (Berg & Wynne-Edwards 2001; Ziegler *et al.* 2004) prior to the birth of offspring noted above. However, it does not seem to be a reflection of male–infant bonding since fGCs do not vary with infant caregiving (table 1). Forty-one per cent of males residing in infant groups engaged in caregiving behaviour, albeit at a fairly low rate (10.4%) with grooming and grooming/holding accounting for the vast majority of male–infant interactions (9.5%). The benefits males accrue from caregiving are unclear since infant survival contributes little to male reproductive success in this population (Lawler 2007), but may be

related to potential increased mating opportunities for resident males the following breeding season (Brockman & Whitten 1999). Alternatively, high fGCs may reflect the increased risk of aggressive transfer, group takeover and infanticide. The high rate of group transfers by sifaka males during the birth season, when 60 per cent of groups undergo male migration events, suggests that male residency is particularly vulnerable at this time, as are infants (Brockman *et al.* 2001). Of the five groups targeted for aggressive male transfers (table 2), 80 per cent suffered takeovers and 75 per cent of those groups contained one or more infants, all of which subsequently died or disappeared following group takeover. The finding that fGC concentrations were significantly higher in males successfully invading/taking over groups than those that were unsuccessful accord with studies of GCs in baboons showing that males that employ high rates of aggression during immigration have GCs equivalent to those of females that are targeted for aggression post-transfer (Alberts *et al.* 1992), suggesting that elevated GCs prepare males for increased risk of grave injury during protracted immigration-related aggressive challenges. It is currently unknown whether the infant-related GC levels observed here during the birth season reflect greater male stress associated with perceived risk to infants or the likelihood of group takeovers by immigrant males. Nevertheless, moderate reproductive skew and infanticide committed by males in this population accord with Creel's (2005) prediction that in species in which confidence of paternity is low (e.g. dwarf mongoose) or in the case of BMSR sifaka, highly variable, males are not constrained from employing infanticide to enforce reproductive suppression. Since female sifaka at this site mate with multiple resident and non-resident males during the mating season (Brockman 1999), all males are potential fathers of infants born the following birth season, the population-wide elevations in GC perhaps reflecting adrenal responses of potential 'fathers' defending against external challenges by potentially infanticidal males. The costs of maintaining high fGC levels in anticipation of possible takeover events are currently unknown in this population, but immune suppression is a documented consequence of chronically elevated GC (and T) levels (Hillgarth *et al.* 1997; Sapolsky 2005). Evidence for a possible cost of elevated fGC levels in sifaka derives from the Borety group (figure 2): alpha M9093 exhibited substantially elevated fGC (and fT) levels 3 days prior to the birth of the group's second infant; both steroids dropped precipitously the day before birth, and remained low on the day of birth associated with M9093's extreme lethargy and apparent illness.

In conclusion, these data are the first to show that in seasonal plural breeding species such as sifaka, elevated fGC in males reflects specific events related to reproduction (e.g. birth of infants) rather than states or social context during the birth season. Variation in fGC concentrations did not exhibit a simple general relationship with age, group stability, residence or rank, but were significantly and positively correlated with the presence of infants. The birth of infants provides a predictable cue for the onset of future uncontrollable events, including fT-mediated aggressive immigration that puts all males at increased risk of group takeover and injury/death. These findings are important for the new insights they provide into the role of GCs in mediating male dispersal and

reproductive strategies during the birth season. Future studies will focus on examining the degree to which the infant-related GC levels observed here reflect greater male stress associated with perceived risk to infants and the likelihood of group takeovers by immigrant males.

This research adhered to the Association for the Study of Animal Behaviour/Animal Behaviour Guidelines for the Use of Animals in research, the legal requirements of the country in which the work was carried out and all institutional guidelines. This research was approved by the Duke University and University of North Carolina–Charlotte Institutional Animal Care and Use Committees (DU protocol no. A130-01-05; UNCC protocol no. 04-002).

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