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BIODIVERSITY RESEARCH

Physiological indicators of stress in African forest elephants (*Loxodonta africana cyclotis*) in relation to petroleum operations in Gabon, Central Africa

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ABSTRACT

Aim Human activities are major determinants of forest elephant (*Loxodonta africana cyclotis*) distribution in Gabon, but the types and intensity of disturbance that elephants can tolerate are not known. We conducted dung surveys within the Gamba Complex of Protected Areas in SW Gabon to examine (1) the feasibility of noninvasive faecal analyses for monitoring stress physiology, and (2) the influence of petroleum operations on stress levels in forest elephants.

Location Gabon, Central Africa.

Methods We identified multiple dung piles from the same individual by matching their eight-locus microsatellite genotypes, and measured faecal concentrations of glucocorticoid metabolites as an indicator of stress in areas subject to different levels of disturbance: (1) Loango National Park (2) an 'industrial corridor' dominated by oil fields, and (3) a nearby area of human settlements.

Results We obtained unique microsatellite genotypes and faecal glucocorticoid metabolite (FGM) concentrations for 150 forest elephant individuals, which is the largest hormonal data set for wild African forest elephants to date. Adults exhibited higher mean FGM concentrations than juveniles, and in contradiction of our expectations of chronic stress around oil fields, elephants in Loango National Park exhibited significantly higher FGM concentrations than elephants in the industrial corridor.

Main conclusions We argue that forest elephants in the industrial corridor of the Gamba Complex have become acclimated to oil fields, resulting in part from oil company regulations that minimize stressful interactions between elephants and petroleum operations. Our findings for a flagship species with substantial ecological requirements bode well for other taxa, but additional studies are needed to determine whether oil operations are compatible over their life span with rain forest ecosystems in Central Africa.

Keywords
Conservation physiology, disturbance ecology, faecal DNA, faecal glucocorticoid metabolites, Gamba Complex of Protected Areas, oil fields.

INTRODUCTION

Widespread poaching has caused catastrophic declines in forest elephant (*Loxodonta africana cyclotis*) populations despite extensive forest cover and new protected areas in Central Africa (Blake *et al.*, 2007). These declines have gone largely undocumented as a result of notorious difficulties in surveying forest elephants. Aerial counts are ineffective in habitats with dense canopy cover, and systematic dung counts often require a daunting amount of effort by field teams (Walsh & White, 1999). Central Africa may contain 137,000 forest elephants, but less than half of these elephants have been counted by reliable survey methods (Blanc *et al.*, 2007). Gabon harbours more than half (70,637 individuals) of the estimated forest elephant population

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despite accounting for only 5% of the entire area of Central Africa and 22.5% of forest elephant range (Blanc *et al.*, 2007). National characteristics in favour of Gabon remaining an elephant stronghold include a small human population, current political stability, economic prosperity from petroleum and mining, and the designation of 13 national parks in 2002 that encompass 11% of the country's total area. However, timber production has accelerated as petroleum production has declined, leading to increased forest access for hunters and subsistence farmers (Laurance *et al.*, 2006a).

The Gamba Complex of Protected Areas, estimated to contain 11,200 forest elephants (Blanc *et al.*, 2007), is a microcosm of the promises and challenges for elephant conservation in Gabon. The Gamba Complex consists of two national parks that are longitudinally divided by an 'industrial corridor' of active oil fields (Lee *et al.*, 2006). Strict regulations protect the largest oil concession from hunting, but oil and timber roads outside the concession perimeter facilitate bushmeat hunting. Elephants and forest ungulates decline in abundance and exhibit stronger avoidance of roads in hunted areas outside the oil concession (Laurance *et al.*, 2006b). Even within the Gamba Complex, elephant distributions are negatively associated with human presence and infrastructure (Buij *et al.*, 2007). However, hundreds of elephants still permanently or seasonally inhabit the oil fields of the industrial corridor. The extent to which the industrial corridor represents marginal habitat for elephants deserves investigation. Oil, mineral and logging interests exert an increasingly dominant influence on Congo Basin rain forests, but the implications for Central Africa's iconic megafauna are poorly understood.

Using noninvasive methods, we examined a physiological indicator of stress (i.e. faecal glucocorticoid metabolites, FGM) of forest elephants in relation to anthropogenic disturbance in the Gamba Complex. The emerging discipline of 'conservation physiology' seeks to understand the physiological responses of animals to environments altered by human disturbance (Wikelski & Cooke, 2006), and endocrinology is a crucial component of this field. FGMs measured from animal faeces are powerful indices of physiological stress in wild populations subject to human disturbance (e.g. snowmobiles and stress in wolves and elk, Creel *et al.*, 2002; primates in rain forest fragments, Martinez-Mota *et al.*, 2007). Stress hormones play an adaptive role in vertebrate behaviour (Boonstra, 2005), but chronically high glucocorticoid concentrations brought about by psychological disturbance have a myriad of negative physiological effects (e.g. suppression of reproduction and immune function in primates, Sapolsky, 2005). Noninvasive indicators of stress are even more useful when combined with faecal DNA techniques. Fresh elephant dung is relatively easy to collect and can be used for genetic surveys of population size, sex ratio, genetic variability and relatedness (Eggert *et al.*, 2003; Archie *et al.*, 2007). In this study we identify males and females using sex-specific faecal DNA sequences, and identify multiple dung samples from the same individual using multilocus microsatellite genotypes.

We conducted dung collection surveys for noninvasive faecal DNA and FGM analyses in oil fields within the industrial corridor, north of the industrial corridor where roads have facilitated human settlement, and in a relatively undisturbed national park. By linking a unique faecal DNA genotype, sex and FGM concentration to the location of a dung sample collected in the field, we examined (1) the feasibility of noninvasive faecal analyses for monitoring stress physiology; and (2) the influence of petroleum operations on FGM concentrations in forest elephants. We hypothesize that elephants within oil concessions experience chronic stress. Alternatively, elephants remaining within oil concessions (now operating for 20+ years in the Gamba Complex) may acclimate to human presence and infrastructure and fail to show an elevated stress response. Our study is the first analysis of stress physiology in forest elephants and has direct relevance to the conservation of this taxon throughout the Congo Basin.

METHODS

Study areas

We conducted dung surveys within the Gamba Complex in SW Gabon (1°55′ S, 9°50′ E) from August to October 2006. This area is characterized by lowland tropical rain forest and consists of two national parks created in 2002: Loango (1550 km^2) along the western Atlantic coastline and Moukalaba-Doudou to the east (4500 km^2). These two parks are longitudinally separated by the Rabi-Ndogo Protected Area (3500 km^2), a forested area colloquially known as the 'industrial corridor' as a result of the major oil fields that occur throughout. Biological inventories have revealed high species richness throughout the Gamba Complex (Alonso *et al.*, 2006), including an intact community of rain forest megafauna and one of Africa's largest forest elephant populations (Blanc *et al.*, 2007). Our focal study sites were (1) a relatively undisturbed area in SE Loango National Park (2) an area in the industrial corridor centred around the Rabi oil field, and (3) an area north of Rabi where roads have facilitated human settlement (see Laurance *et al.*, 2006b; Buij *et al.*, 2007; for site maps).

Elephants concentrate along Loango National Park's coastline during the wet season (January to April), but retreat inland during dry periods to feed on riparian vegetation (Buij *et al.*, 2007; Morgan & Lee, 2007). We conducted dung surveys in an inland area between Sounga village and the Ngové River. For logistical reasons, we centred our surveys along an abandoned road that is frequented by elephants foraging on *Sacoglottis gabonensis* and other preferred fruit trees. We considered Loango samples as a control for stress as a result of human disturbance. To examine adrenal function of elephants in relation to petroleum development, we conducted dung surveys within the industrial corridor. The corridor is still under forest cover but was selectively logged before the development of oil fields in the 1980s. Our surveys were centred on the Rabi oil concession maintained by Shell-Gabon (130 km^2). Sources of disturbance at Rabi include 800 workers, large pumping terminals and platforms, natural gas flares, and a vast network of unpaved production roads. However, Shell-Gabon's health and safety policies provide incidental protection for wildlife in the concession (Laurance *et al.*, 2006b).

Vehicles are mechanically restricted to speeds less than 40 km h^{-1}, all workers are confined to camp from 18.00 hours to 06.00 hours, and concession access is restricted by 24-h checkpoints. Shell-Gabon has also banned the hunting and consumption of bushmeat by its employees. Outside of the concession, hunting intensity is highest around logging camps and small villages. Villagers and logging company employees place snares along trails (including research transects) and hunt with firearms. Only a few elephant carcasses have been found by Smithsonian researchers in this area, but Shell-Gabon employees regularly report elephants wounded by snares and gunfire. We considered this site to be heavily disturbed.

Dung surveys and sample collection

We collected dung in eight contiguous blocks (approximately 5×5 km) in each of the three study areas. Two researchers on foot searched for dung in each block using 'recce' sampling (Hedges & Lawson, 2006). Recce sampling increases dung encounters over transect methods because the search team follows paths of least resistance that are likely to be used by elephants (e.g. fresh elephant trails or abandoned roads). The team navigated across the block using a GPS receiver during the morning, and then followed a different set of paths to return to the starting point in the afternoon. After completing the first collection for all blocks, we conducted a second round of surveys in the same order.

When a dung pile was encountered, we recorded the location using GPS and measured the circumference of the three largest boli. We classified samples as 'juvenile' or 'adult' as previously defined for forest elephants in Ghana (mean boli circumference < 32 cm as a juvenile sample, Eggert *et al.*, 2003). Next, a sample for genetic analysis was collected from the exterior of the most-fresh bolus. Approximately 10 g of wet, sticky intestinal mucus with associated faecal material was deposited in a 50-mL centrifuge tube, and then mixed with 10–20 mL of Queen's College buffer for preservation at ambient temperature (Frantzen *et al.*, 1998). If the sample was fresh, then we also collected material for FGM analyses. The entire dung pile was thoroughly mixed by hand to homogenize the FGM distribution, and then several subsamples totalling 100–200 g were sealed in a sterile bag. Samples were kept on ice during the day and then frozen. Previous research indicates that freezing preserves FGMs in elephant dung for at least 2 years (Hunt & Wasser, 2003). We aimed to collect only 'fresh' dung samples (< 48 h old) as defined by the collection standards of the Monitoring of Illegal Killing of Elephants program (MIKE, Hedges & Lawson, 2006). At the beginning of the study, we also collected several older samples for genetic analyses to avoid under-sampling as we learned to identify 'fresh' dung piles. Using this screening procedure, we collected a total of 316 dung samples, 279 of which we classified as fresh.

Molecular genetic methods

We extracted faecal DNA using QIAamp DNA Stool Kits (Qiagen, Valencia, CA) with protocol modifications for elephant dung described in Archie *et al.* (2003). Extractions were carried out in a separate room under quasi-clean conditions to prevent contamination. We used multilocus microsatellite genotypes to associate each dung sample with a unique elephant. Eight microsatellite loci were amplified from each sample, including the previously described loci FH67 (Comstock *et al.*, 2000), FH126 (Comstock *et al.*, 2002), and LafMS02 (Nyakaana & Arctander, 1998). We genotyped samples at five other loci using primer pairs that amplify the same tandem repeat regions as published loci FH19, FH48, FH60, FH94 (Comstock *et al.*, 2000), and LA6 (Eggert *et al.*, 2000), but that were redesigned to amplify less of the flanking region (Eggert *et al.*, 2007). PCR amplifications were performed in MJ Research PTC-100/200 thermocyclers in 10 μL volumes containing 0.5 U Ampli*Taq* Gold DNA Polymerase (Applied Biosystems, Foster City, CA), 1X Ampli*Taq* Buffer II, 0.4 μM fluorescently labelled forward primer, 0.4 μM unlabelled reverse primer, 2 mM $MgCl_2$, 0.2 mM each dNTP, 10X BSA (New England Biolabs, Ipswich, MA), and 1 μL of template DNA. PCR profiles consisted of denaturation at 95 °C for 5 min, followed by 45 cycles of denaturation at 95° for 45 s, annealing at locus-specific temperatures (FH19R and FH48R at 60 °C, FH60R, FH94R, FH126, and LA6R at 58 °C, LafMS02 at 55 °C, and FH67 at 54 °C) for 35 s, and primer extension at 72 °C for 35 s. We separated and visualized PCR products in an ABI 3100 automated sequencer and allele fragment sizes were scored using Genotyper 2.5 (Applied Biosystems). A savanna elephant sample was included as a positive control to standardize allele scoring across PCRs in this study. In all reactions we included a control without DNA to detect PCR contamination.

Microsatellites amplified from dung are particularly susceptible to allelic dropout, so we controlled for data quality using approaches advocated by Paetkau (2003) and Wasser *et al.* (2004). First, we eliminated samples from the study if they did not produce clear, repeatable genotypes for at least five of eight loci within two attempts (2.5%, or 8/320, were eliminated). Second, we amplified each sample at each locus at least twice for a heterozygote genotype, and at least three times for a homozygote genotype. Although our methods were not as stringent as the multiple-tubes approach (Taberlet *et al.*, 1996), our high genotyping success rate (Table 1) and data screening

Table 1 Numbers of faecal DNA samples collected, successfully genotyped, and identified as samples from unique individuals during two collection periods at each site.

Site	No. samples collected	No. samples genotyped	No. unique genotypes (%)
Rabi oil concession	1st Survey: 84	80	49 (61.3%)
	2nd Survey: 52	52	16 (30.8%)
North of Rabi oil concession	34	34	22 (64.7%)
	24	24	12 (22%)
Loango National Park	62	62	36 (58.1%)
	60	60	32 (53.3%)
Total	316	312	167 (53.5%)

described in succeeding discussions indicates that the resulting genotypes were highly reliable.

We identified multiple dung samples that came from the same individual (i.e. recaptures) by searching for matching microsatellite genotypes using the Excel Microsatellite Toolkit (Park, 2001). When two samples differed at only one or two alleles across loci, we re-analysed their fragment signatures in Genotyper to resolve scoring errors. Matching sex assignments (see succeeding discussions) and bolus circumference were used as additional confirmation that matching genotypes belonged to the same individual. We tested for linkage disequilibrium and deviations from Hardy–Weinberg using FSTAT 2.9.3.2 with Bonferroni procedures for multiple statistical tests (Goudet, 2002).

We established the sex of the individual that produced each dung sample using a modified version of the methods described by Fernando & Melnick (2001). First, we PCR-amplified a 141-bp fragment of the X- and Y-linked zinc-finger protein genes using the primers LaZFXYF2 (5′-CTCACACTGGGGCTTTGTTT-3′) and LaZFXYR (5′-TCTTGCTATGGACTGCCAAA-3′; L. S. Eggert *et al.* unpublished data). PCR conditions were the same as previously, but were performed in 25 μL volumes and consisted of denaturation at 95 °C for 10 min, 45 cycles of denaturation at 94° for 1 min, annealing at 57 °C for 1 min, and primer extension at 72 °C for 1 min. We then digested 5 μL of the PCR products with *Bam*HI (New England Biolabs, Ipswich, MA) and ran out the digested products next to the original PCR products on a 3% agarose gel containing Gelstar® Nucleic Acid Stain (Cambrex, San Diego, CA). Males produce the expected three bands after digestion, whereas females show only the original band of the PCR product.

Faecal glucocorticoid metabolite (FGM) analysis

To measure FGM concentrations from dung, we used methods validated for the African elephant (Wasser *et al.*, 2000; Brown *et al.*, 2004; Brown *et al.*, 2007). Faecal samples (~100 g) were lyophilized and crushed, taking care to remove fibrous material. In brief, 4.5 mL ethanol and 0.5 mL distilled water were added to the well-mixed, powdered dung samples. Then samples were mixed using a multitube vortexer for 30 min, and centrifuged at 2200 r.p.m. ($g = 596$) for 20 min to pellet the faecal material. We then poured off the supernatant into a separate tube and repeated the procedure for a final extract volume of 10 mL. These samples were dried under air, reconstituted in 1 mL methanol, and diluted at 1 : 16 into phosphate buffer saline. The efficiency of steroid extraction was evaluated by adding a radiolabelled glucocorticoid (^{3}H-corticosterone; 4000 dpm) to faecal samples before extraction and exceeded 80%. We then measured FGM concentrations using a commercially available double-antibody corticosterone ^{125}I radioimmunoassay (ICN Biomedicals, Costa Mesa, CA). Sensitivity of the assay at maximum binding was 12.5 ng mL^{-1}. Intra- and interassay coefficients of variation were < 10% and 15%, respectively. We converted final FGM concentrations from ng FGM mL^{-1} of liquid extract to ng FGM g^{-1} dung.

Statistical analyses

To compare the Gamba Complex elephants to other populations, we computed the range, mean and standard error of FGM concentrations for adults and juveniles of both sexes. To examine whether FGM concentrations differed between adults and juveniles, we used a nonparametric Van der Waerden test (Conover, 1999) after excluding six adult outliers with FGM concentrations greater than 100 ng g^{-1}. We $\log_{10}$-transformed FGM concentrations to improve normality and homogeneity of variances for subsequent parametric analyses. We were interested in the repeatability of hormone measurements, so we calculated all pairwise correlations between the first, second and third FGM concentrations from separate dung piles produced by the same individual in the same day. When multiple dung piles were collected for an individual on different days, we subtracted the FGM concentration of the first sample from the FGM concentration of the last sample, and then tested if the differences were significantly different from zero using Wilcoxon signed rank tests. We also calculated Spearman's nonparametric correlation coefficient between the difference in the first and last FGM concentrations and the number of days between collection of the first and last samples.

We tested for significant differences in FGM concentrations using ANOVA with sex, site and age as model effects. For individual elephants that were sampled multiple times, we calculated their mean FGM concentration for use in the ANOVA. We used $\alpha = 0.05$ as our acceptable probability of a Type I error (i.e. significance level), and computed the power and least significant number (LSN) for the sex, site and age model effects. To examine differences in least-square means between specific sites, we used posthoc Tukey's Honest Significant Difference (HSD) tests that correct for multiple comparisons. All statistical analyses were performed in JMP 5.0.1.2 (SAS Institute, Cary, NC).

RESULTS

We collected samples from 316 dung piles and successfully genotyped all but four (98.7%, Table 1). More than half of these genotypes were unique, but the rate of new genotypes identified from the first to second sampling period dropped by one-half to two-thirds in and just north of the Rabi oil concession. We recovered a similar percentage of unique genotypes in the two sampling periods in Loango National Park, indicating a higher turnover of individuals in this area (Table 1).

FGM concentrations were obtained for 283 samples comprising 150 unique elephants. Males and females exhibited similar mean FGM concentrations, although the most extreme values were measured in bulls (Table 2). The distributions of FGM concentrations were similar for adults and juveniles, but adults exhibited significantly greater concentrations than juveniles (Van der Waerden test statistic $S = -9.94$, $P = 0.036$; Fig. 1). A small number of elephants ($N = 6$) exhibited extreme FGM concentrations (> 100 ng g^{-1}, Fig. 1): two adult males and two adult females in Loango National Park, and two adult females in the Rabi oil concession that were sampled on the same day.

Table 2 Mean, standard error, and range of faecal glucocorticoid metabolite concentrations (ng FGM g^{-1} dung) recorded for elephants in the Gamba Complex of Protected Areas.

	N	Mean ± SE (ng g^{-1})	Range (ng g^{-1})
Adults			
Female	74	51.4 ± 3.2	20.5–154.2
Male	46	46.4 ± 4.6	18.4–200.8
Juveniles			
Female	22	36.4 ± 3.3	18.1–76.4
Male	8	35.4 ± 4.5	19.4–51.5

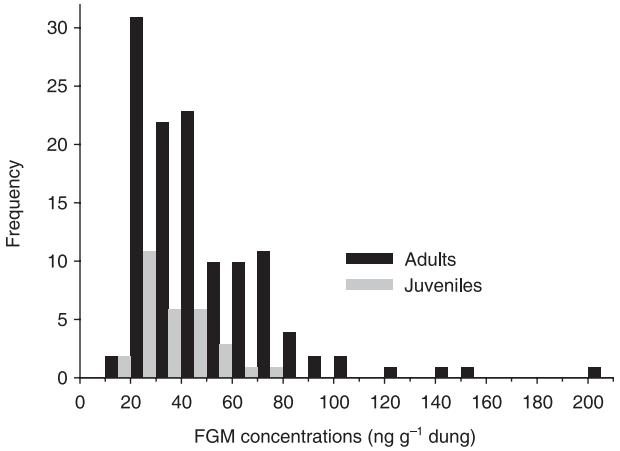

Figure 1 Histogram of adult and juvenile faecal glucocorticoid metabolite (FGM) concentrations.

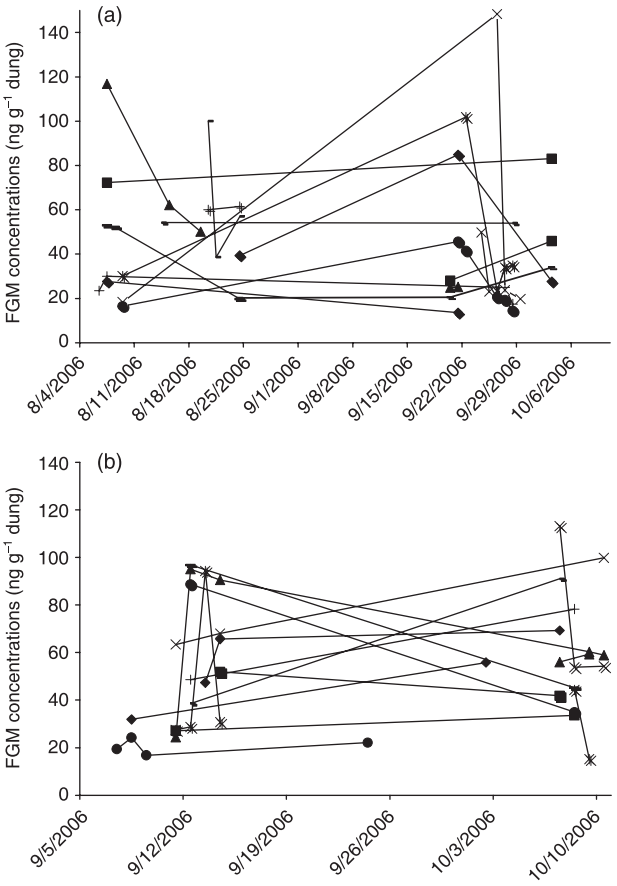

Figure 2 Temporal variation in faecal glucocorticoid metabolite (FGM) concentrations among (a) 17 elephants in the Rabi oil concession and (b) 15 elephants in Loango National Park.

Multiple FGM concentrations for the same individual on the same day were significantly correlated (first and second sample, $N = 54$, $r = 0.55$, $P < 0.00001$; first and third sample, $N = 12$, $r = 0.77$, $P = 0.003$; second and third sample, $N = 12$, $r = 0.80$, $P = 0.002$). We collected multiple faecal samples for the same individual on different days for 31 elephants. Individuals in Loango National Park exhibited relatively stable FGM concentrations over the course of a month (mean difference in FGM concentration between first and last sample collected = –1.16 ± 8.74 ng g^{-1}; Wilcoxon $P = 0.98$; Fig. 2(b)). The pattern was similar in the Rabi oil concession (mean difference in FGM concentration between first and last sample = –9.54 ± 5.79 ng g^{-1}; Wilcoxon $P = 0.13$), but four elephants exhibited substantially higher FGM concentrations in late September than measured before or after (Fig. 2(a)). Differences in FGM concentrations between the first and last sample collected for the same individual were not correlated with the number of days between collection in either Loango National Park (Spearman's $r = –0.35$, $P = 0.21$) or the Rabi oil concession (Spearman's $r = –0.39$, $P = 0.13$).

The overall ANOVA model for FGM concentration in relation to sex, site and age was significant ($F_{4,145} = 3.86$, $P = 0.005$, $R^2 = 0.10$). FGM concentrations were significantly different between sites and ages, but not between sexes, in this model (Table 3). Adults had significantly higher FGM concentrations than juveniles (Figs 1 and 3, Table 3). Post-hoc Tukey's HSD tests showed that FGM concentrations were significantly higher in Loango National Park than in the Rabi oil concession (difference ± SE in least squares means = 0.094 ± 0.038; $P < 0.05$), but not between other site pairs. Statistical power was relatively high for site and age differences, but nearly twice as much data would be needed to find differences between males and females given the current distribution of FGM concentrations among the sexes (Table 3).

Table 3 Model effect tests, statistical power, and Least Significant Number (LSN) from ANOVA of sex (male versus female), site (Loango national park, Rabi oil concession, and area north of Rabi), and age (adult versus juvenile) on faecal glucocorticoid metabolite (FGM) concentrations of 150 forest elephants.

Effect	Levels	*F*	*P*	Power	LSN
Sex	2	1.96	0.16	0.29	296
Site	3	3.38	0.04	0.63	135.9
Age	2	7.03	0.009	0.75	84.5

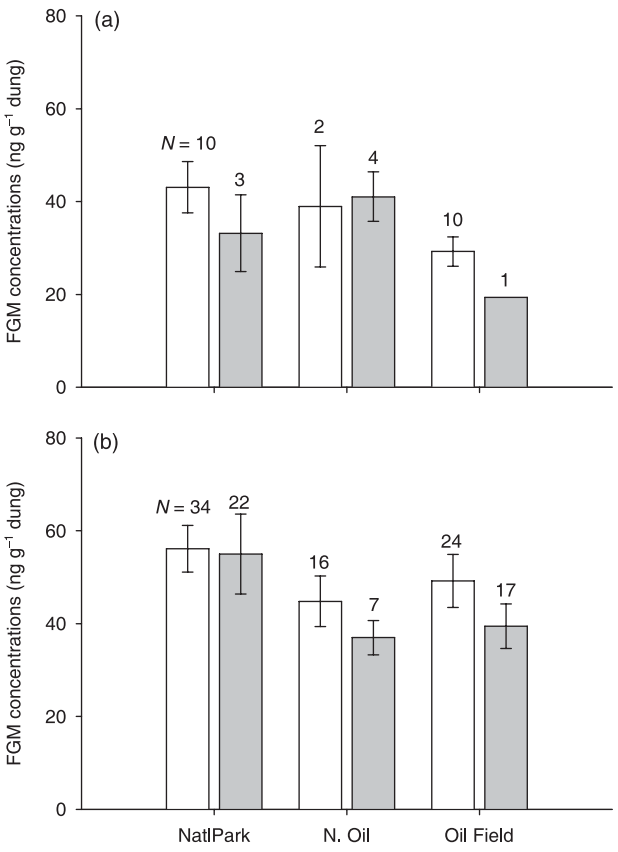

Figure 3 Mean faecal glucocorticoid metabolite (FGM) concentrations for female (white bars) and male (grey bars) (a) juveniles and (b) adults across three study sites (NatlPark = Loango National Park, N. Oil = disturbed areas north of the Rabi oil concession; Oil Field = Rabi oil concession in the Industrial Corridor). Error bars represent one standard error of the mean, and numbers above each bar are sample sizes.

DISCUSSION

Achieving the goal of scientifically rigorous management of forest elephants in Central African rain forests has challenged the creativity and resourcefulness of conservation biologists. New survey methods and faecal DNA technologies have mitigated many of the problems of measuring forest elephant population size. However, these methods only detect population declines after development and poaching have exacted their toll. Foley *et al.* (2001) promoted the use of FGMs for monitoring long-term effects of social disruption in an elephant population. Our study indicates that noninvasive approaches can produce high-quality, reliable data for monitoring forest elephant populations on regional scales.

Appropriate field preservation and screening of dung for deposition time allowed us to obtain genetic and hormonal data from nearly all samples collected during this study. Our genotyping success rate (98.7%) was much higher than previously reported from studies that included older samples (60% in Ghana, Eggert, Eggert *et al.*, 2003; 72% in an earlier collection in the Gamba Complex, Eggert *et al.* unpublished data). Dung surveys that target hormonal data require very fresh samples, so they may reduce problems inherent in faecal DNA studies that use samples of varying age (particularly allelic dropout, Paetkau, 2003). We were also able to recover measurable levels of FGMs in all samples save one lost as a result of lab error, and FGM measurements were repeatable across multiple samples collected from the same individual (Fig. 2). Microbial activity or other environmental factors such as rain may cause FGM concentrations to increase in older samples (i.e. seven-day-old dung from white-tailed deer, Washburn & Millspaugh, 2002), but we collected dung during the dry season in Gabon and judged samples as unacceptable if they had obvious fungal growth. Furthermore, FGM concentrations reported here fall within the 'normal' range previously recorded for wild African elephants. In Tanzania, 16 adult females exhibited mean FGM concentrations from 22 to 35 ng g^{-1} during the rainy season months, and 42–75 ng g^{-1} during the dry season months, using the same immunoassay technique (Fig. 4 in Foley *et al.*, 2001). Both adult males and females in Gabon exhibited FGM concentrations within the higher dry season range for Tanzania. FGM concentrations in forest elephants were also similar to those observed in males (46 ng g^{-1}, $N = 15$), females (40 ng g^{-1}, $N = 29$), and calves (38 ng g^{-1}, $N = 20$) living in a small, fenced game reserve in NW South Africa that experiences substantial tourist activity (Figs 5.5, 5.6 in Pretorius, 2004).

We did not detect elevated FGM concentrations in forest elephants because of human disturbance. During our surveys north of Rabi we encountered logging, hunters with firearms, transects with active snares and one recently constructed camp for smoking bushmeat. However, FGM concentrations were very similar to those recorded within the Rabi oil concession and Loango National Park (Fig. 3). Our search effort was equal across sites, but we collected less than half the number of samples north of Rabi as in the other two sites (Table 1), and all but one dung sample from here was collected in a block directly adjacent to the oil concession. Elephants may avoid, or have been driven out of, areas immediately outside the Gamba Complex.

Elephants within one of Africa's largest onshore oil reserves exhibited lower FGM concentrations than elephants in a nearby national park. Shell-Gabon regulations may provide protective benefits to elephants within the concession (Laurance *et al.*, 2006b). These regulations prohibit outside visitors, restrict vehicles from 18.00 hours to 06.00 hours, provide stiff penalties for bushmeat hunting, and minimize unnecessary deforestation. If direct human disturbance is limited, then forest elephants may become habituated to petroleum production. The Rabi concession has been producing for over two decades now, and the elephants we sampled have likely spent most or all of their lives around oil fields. Offspring born in or near the concession may become habituated even more quickly, as indicated by our finding of lower FGM concentrations among juveniles in Rabi than within other sites (Fig. 3(a)).

Other evidence suggests that forest elephants have become year-round residents of the Rabi oil concession. South of

Rabi, genetic capture-recapture analyses indicated that many elephants migrate across the Industrial Corridor in response to wet and dry seasons (L. S. Eggert, unpublished data). However, two adult cows fitted with satellite collars at Rabi remained within the oil concession for up to 13 months (A. Henderson *et al.* unpublished data). Sources of minerals, water, and preferred elephant foods such as *Sacoglottis* fruits and secondary vegetation are plentiful within the concession. Forest elephants also seem to have behaviourally adapted to vehicular traffic at Rabi. We rarely encountered elephants along the concession roads, but often found fresh elephant dung in the morning. These elephants may spend the day in the interior forest of the concession, but feed along the roads at night when vehicles are restricted to camp.

Alternatively, elevations in FGM concentrations from petroleum operations could have permanently lowered the endocrine response of these elephants. Such physiological acclimation may result in a more dramatic adrenal response to novel stressors (stress facilitation, Romero, 2004), but the proportion of higher FGM concentrations was not greater in Rabi than in Loango National Park. Acclimation could be examined by giving adrenocortical challenge tests to elephants at Rabi, but these tests would be logistically difficult to administer in the wild. Once the wet season begins, elephants that concentrate around water courses during the dry season spread throughout the Gamba Complex (Buij *et al.*, 2007). These unhabituated individuals may exhibit elevated FGM concentrations in response to the novel stressors of the oil fields than resident individuals when they pass through Rabi.

We may also have failed to detect chronic stress caused by oil fields because of individual variation within each site. Circulating cortisol concentrations surge before parturition in pregnant females (Meyer *et al.*, 2004) and increase among females in larger social groups and those with a higher dominance rank (Foley *et al.*, 2001). Captive males in musth also exhibit elevated peripheral cortisol concentrations (Brown *et al.*, 2007), although these changes have not been detected as elevated FGM concentrations in wild bulls (Ganswindt *et al.*, 2005). The sex ratio and age structure were similar across sites, but we cannot rule out confounding reproductive effects. Population averages of FGM concentrations compared between different regions may also not effectively measure the influence of acute, rather than chronic, stressors on forest elephants. Elephants in South Africa exhibit detectable elevations in FGM concentrations as a result of translocation (Millspaugh *et al.*, 2007) and hunting (Burke *et al.* 2008), but in these cases individual elephants were sampled multiple times before and after the stressful events of interest.

Finally, we may have detected higher FGM concentrations in Loango National Park than in the oil concession because the park elephants were more stressed than usual. During the dry season, many elephants in the Gamba Complex migrate to water courses to feed on riparian vegetation and fruiting trees (Morgan & Lee, 2007). We encountered elephants much more frequently in Loango National Park than at other sites, and have also found that fresh dung piles collected within 100 m of each other in Loango National Park were deposited by significantly less-related elephants than dung piles collected within 100 m of each other in the Rabi oil concession (J. Munshi-South *et al.* unpublished data). A temporary surge in population density during our study may have resulted in higher FGM concentrations because of stressful encounters between unrelated groups or individuals.

Although we did not detect elevations in FGM concentrations as a result of oil fields in our study, detection of elevated concentrations may be possible where elephant social structure has been disrupted by poaching. Large bulls are often the first to be killed, and their removal can lead to extreme aggressive behaviour in younger males (Slotow *et al.*, 2000). Disruption of social groups resulting from the absence of older matriarchs also leads to aberrant social behaviour and chronic stress with long-lasting effects (Foley *et al.*, 2001; Bradshaw, Schore *et al.*, 2005). Noninvasive analysis of FGMs in high-poaching areas may elucidate the social and physiological impacts on hunted populations.

Multinational interests are increasingly opening natural areas to industrial extraction in the Congo Basin (Wilkie *et al.*, 2000), but our results show that negative impacts on forest elephants may be limited by adopting and enforcing appropriate regulations. Forest elephants are a flagship species with substantial landscape-scale requirements, and thus our finding that they are not substantially stressed by oil fields bodes well for other species. Previous studies in the same area also indicated that large- and medium-sized mammals within the oil concession are afforded some protection against hunting (Croes *et al.*, 2006; Laurance *et al.*, 2006b). However, additional studies on multiple taxa are needed to address whether the oil operations themselves are compatible over their life span with rain forest ecosystems in Central Africa. Additionally, well-known multinational corporations that are susceptible to international pressure may be replaced throughout Africa by national operations with fewer incentives to protect endangered species (Laurance *et al.*, 2006b). The influence of Chinese corporations in Africa has also grown exponentially, and these companies have followed a policy of 'noninterference' in environmental concerns (Taylor, 2006). Hunting associated with logging operations may present an even larger threat than petroleum production, but efforts to develop management plans for African logging concessions are in their infancy (Elkan *et al.*, 2006). Cooperative efforts between conservation groups, corporate interests, and local governments hold largely untapped promise for developing elephant-friendly policies throughout the region.

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