



Physiological and Behavioral Effects of Capture Darting on Red Colobus Monkeys (*Procolobus rufomitratus*) with a Comparison to Chimpanzee (*Pan troglodytes*) Predation

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Abstract Understanding how human activities affect wild primates is critical to the design of effective conservation strategies. Despite this need, few studies have examined the physiological and behavioral effects of field research methods in the wild. Here, we examine how the stress response, i.e., fecal cortisol, and behavior of Ugandan red colobus monkeys (*Procolobus rufomitratus*) in Kibale National Park are affected by chemical immobilization and collaring, i.e., capture. We compare this anthropogenic stressor to a naturally occurring stressor: a chimpanzee (*Pan troglodytes*) predation attack. Two adult males had peak cortisol levels of 283 and 284 ng/g 2–3 d after capture, which were 190% and 182% above their baseline levels, i.e., the first sample taken immediately after capture, but these peak levels did not remain elevated. Using long-term data, i.e., 11 mo of data, we found no difference in fecal cortisol levels between 10 darted and collared individuals and 14 individuals living in the same social group that

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were not darted or collared. For the chimpanzee attack, peak fecal cortisol levels (147–211% above baseline) were recorded 1–4 d after the attack, but these levels also did not remain elevated for long. These data show that darting and collaring and a chimpanzee predation attempt caused an acute stress response, but neither leads to sustained elevated cortisol levels. Thus, in situations in which research contributes significantly to the conservation of primates and cannot be conducted noninvasively, capture darting appears to be a useful technique with minimal long-term effects as long as injury and mortality are avoided. However, we encourage researchers to make similar physiological and behavioral comparisons in other field studies using similar techniques to provide a better understanding of the effects of research practices on the stress physiology and social behavior of wild primates.

Keywords Chemical immobilization · Environmental endocrinology · Fecal cortisol · Predator effects · Primate conservation · Research effects

Introduction

Understanding how human activities, including research, affect wild primates is critical to the design of more ethical research practices and effective conservation strategies. Increasingly in primatology, researchers are using invasive field techniques with the claim that such studies are necessary to understand better the biology of wild primates, with consequent conservation benefits. In particular, remotely delivered chemical immobilization (“darting” hereafter) and similar methods, e.g., trapping, are being used to capture primates and affix radio collars to monitor movement patterns (Crofoot *et al.* 2009; Juarez *et al.* 2011) or identifying collars to distinguish among individuals (Fernandez-Duque and Rotundo, 2003; Glander *et al.* 1991), obtain blood samples for genetics (Milton *et al.* 2009) or disease studies (Goldberg *et al.* 2009), or collect morphological measurements (Glenn and Bensen 1998). Despite this, few studies have examined the physiological and behavioral effects of darting on primates.

The use of such invasive procedures in primatology should weigh the costs to the health and well-being of focal individuals, groups, and populations against the conservation benefits of the knowledge gained from the research. As addressed in *Animal Behaviour*’s “Guidelines for the treatment of animals in behavioral research and teaching,” “A range of behavioural research techniques, including capture, handling and marking of wild animals, fitting with data logging or transmitting devices (e.g., radiotransmitters, geolocators), collection of physiological data (e.g., blood or tissue samples) or the experimental manipulations themselves, may have adverse consequences, such as reduced probability of survival and reproduction,” which would ultimately threaten the fitness of the endangered species we are attempting to help (*Animal Behaviour*, 2012, p. 303). Animal care committees share the responsibility for predicting the costs and benefits of research, along with the researchers themselves, but often decisions are based on little data. In fact, there is little consensus on the appropriate methods to dart and capture arboreal primates, which makes an evaluation of the risks involved difficult for both researchers and animal care committees (Fedigan 2010).

Fortunately, relatively simple noninvasive tools are available to track the physiological reaction of wild primates to invasive procedures in the field. In particular, noninvasive

endocrinological techniques can provide insight into the physiological state of an animal. The hypothalamo–pituitary–adrenal (HPA) axis plays a central role in allowing an animal to return to homeostasis in the face of external disturbances, with the steroid hormone cortisol released in response to stressors (Sapolsky 2005). After performing its role in the stress response, cortisol is degraded and excreted from the body (Palme *et al.* 1996). Steroid hormones are fairly stable and once cortisol and its metabolites are excreted in feces, they can be reliably measured to quantify the stress response if collected immediately after defecation (Whitten *et al.*, 1998a,b). Cortisol metabolite measurement can therefore be used to assess both the more immediate effect of an acute stressor, such as darting, and the delayed effect of a long-term chronic stress response that may occur with re-darting or collaring (Sapolsky 2005). If frequent re-darting of individuals or collaring causes a chronic stress response, then it should not be used in primate field studies. However, procedures that cause an acute stress response with no negative long-term effects on health or reproduction may be acceptable (Creel *et al.* 1997).

The objective of our study was to examine the physiological, i.e., acute and chronic stress response, and behavioral effects of a common research procedure used in primatology, darting and collaring, for the Ugandan red colobus monkey (*Procolobus rufomitratus*) of Kibale National Park. We compare this anthropogenic stressor to that of a naturally occurring stressor: a predation attack by chimpanzees (*Pan troglodytes*). This red colobus is an endangered folivorous primate, with the last remaining viable population found in Kibale National Park (Struhsaker 2005, 2008). They live in large multimale–multifemale groups of *ca.* 65 individuals (Snaith *et al.* 2008). Adult males aggressively defend their group from predation by blocking the path of the chimpanzees to the rest of group or chasing and attacking the chimpanzees (Stanford 1995). Previous studies of red colobus HPA activity have been conducted (Chapman *et al.* 2006, 2007; Snaith *et al.* 2008), but this is the first to document their acute stress response. Kibale contains the largest known community of chimpanzees (*Pan troglodytes*) in the world with *ca.* 150 individuals living in the Ngogo region (Teelen 2007). This community conducts frequent, successful hunting of red colobus with 15–53% of the population killed each year (Teelen 2008). This level of predation has been suggested to be unsustainable and a threat to the Kibale red colobus, evidenced by a *ca.* 40% decline in red colobus population size at Ngogo over the past 30 yr (Struhsaker 2005; Teelen 2008). Local extirpation of red colobus by chimpanzee hunting has also been predicted for Gombe National Park (Fourrier *et al.* 2008; Struhsaker 2005). Given this situation, chimpanzee predation is likely among the most severe acute stressors that red colobus naturally encounter.

Methods

Adult Male Red Colobus Stress Response to Darting and Collaring

In June 2006, we darted 31 red colobus (*Procolobus rufomitratus*) from two social groups for a health inspection and to give them unique collar color/tag shape combinations for individual recognition to aid in long-term studies. For details of the darting procedure and ethical considerations, see Goldberg *et al.* (2009). We immobilized individuals using tiletamine hydrochloride–zolezepam (Telazol, Fort Dodge Animal Health, Fort Dodge, IA). Anaesthetic cocktails containing telazol may influence

cortisol levels (Clapper 2008), but any positive affect appears to be due to ketamine, whereas telazol appears to decrease or have no effect on cortisol in primates (Bentson *et al.* 2003). Similarly, telazol had no effect on cortisol levels of adult northern elephant seals, whereas physical constraint did elevate cortisol (Champagne *et al.* 2012). We received approval for darting and collaring from the McGill University Animal Care Committee, Uganda Wildlife Authority, and Uganda National Council for Science and Technology. Research conducted during this study complied with all regulations regarding the study of wild animals and with Ugandan law.

We then followed two adult males for 5 consecutive days (08:00–18:00 h), starting on the day of darting, and again 3 wk after darting. Individual 14 (LM group) was darted at 07:51 h on June 13, 2006, and individual 40 (BSH in SC group) was darted at 09:52 h on June 19, 2006. We collected fecal samples ($N = 29$) to examine each individual's fecal cortisol levels as an index of its stress response. We compared the two males' initial fecal cortisol levels, i.e., sample collected within 5 h of being darted, to their peak levels and determined how long this peak remained elevated. We collected behavioral data *ad libitum*, continuously focusing on behaviors related to the experimental procedure, i.e., pulling at collar, giving a stress squeal vocalization, intragroup agonistic interactions (chasing and fighting), all reported as frequency of the behavior per hour of observation.

We also examined long-term effects of darting and collaring by comparing fecal cortisol levels of 10 collared individuals to 14 individuals from the same social group that were not darted and collared using fecal samples collected from August 2007 to June 2008. These two sets of samples were collected simultaneously, from both adult males and females, only between 08:30 and 12:30 h, and across wet and dry seasons. A two-tailed *t*-test was used for this comparison (IBM SPSS Statistics Version 20). To control for the possibility that sex influenced these results, we also conducted this analysis on adult males and females separately. The *t*-tests were conducted at the level of fecal sample, with each one categorized as either from a collared individual or individual with no collar. Although intraindividual variation was not controlled for, we decided that the issue of pseudo-replication was not problematic for detecting differences between collared individuals and individuals with no collar because cortisol levels can vary dramatically over time for one specific individual as conditions change.

Red Colobus Stress Response to a Chimpanzee Predation Attack

During the early evening of October 18, 2008, a group of chimpanzees (*Pan troglodytes*) attacked our focal red colobus group and killed two juveniles and one subadult. From October 20 through October 25, 2008, we followed this red colobus group and collected fecal samples ($N = 30$) between 08:00 and 12:00 h from six known individuals to examine their stress response. We compared each individual's cortisol profile after the attack to baseline levels estimated using 11 mo of data on each individual's long-term cortisol levels (except for one individual lacking long-term sampling, mean samples per individual with long-term sampling: $N = 51.2 \pm 10.5$) collected from August 2007 to June 2008. Samples greater than 2 standard deviations above the mean baseline were considered elevated to a biologically significant level. One of the adult males, individual 40 (BSH), was examined after both the darting and collaring event and the chimpanzee predation attack. Data from this male are used to compare the red colobus stress response between the two events.

Hormone Analyses

Fecal samples were collected immediately on defecation, placed in sterile vials, and stored in a cooler with ice packs until brought back to the field station later that day, where they were stored in a -20°C freezer. On the day of extraction, samples were thawed, homogenized using a spatula, and 0.5 g of each sample was weighed to a test tube. We added 5 ml each of 5.0 pH citrate buffer and 95% ethanol to the sample and mixed all samples on a homogenizer for 24 h. Steroid hormones were separated from the fecal pellet using a centrifuge, and 2 ml of supernatant were passed through a preconditioned solid phase extraction cartridge at a flow rate of 4 ml/min. Capped cartridges were stored out of direct light until analysis via enzyme immunoassay (EIA) for cortisol content at the Wisconsin National Primate Research Center (WNPRC). The final hormone values are given in nanograms of steroid hormone per gram of dry feces. The dry matter content of all fecal samples was calculated by drying 0.5 g of each sample to a constant weight.

At WNPRC, the cartridges were washed with 1 ml of 5% methanol and the steroid hormones were collected using 2 ml of 100% methanol passed through the cartridge at a 1 ml/min flow rate. The methanol was then evaporated off and steroids hormones were reconstituted in 1 ml of 100% ethanol and stored in a 4°C refrigerator until analysis. For the cortisol EIA, 50 μl of sample was used and recovery was $125.27\% \pm 3.18\%$. Parallelism was demonstrated using serial dilution curves, with no significant difference between the sample pool and standards ($P > 0.05$). Interassay variation for the high pool was 18.83% and for the low pool was 16.62%, whereas intra-assay variation was 6.24% for the high pool and 6.26% for the low pool. Details of the cortisol antibody are given in Ziegler *et al.* (1995). In brief, R4866 (anti-cortisol-bovine serum albumin) developed by Stabenfeldt and Munro at the University of California, Davis, with 60% cross-reactivity to cortisone and 2.5% to corticosterone

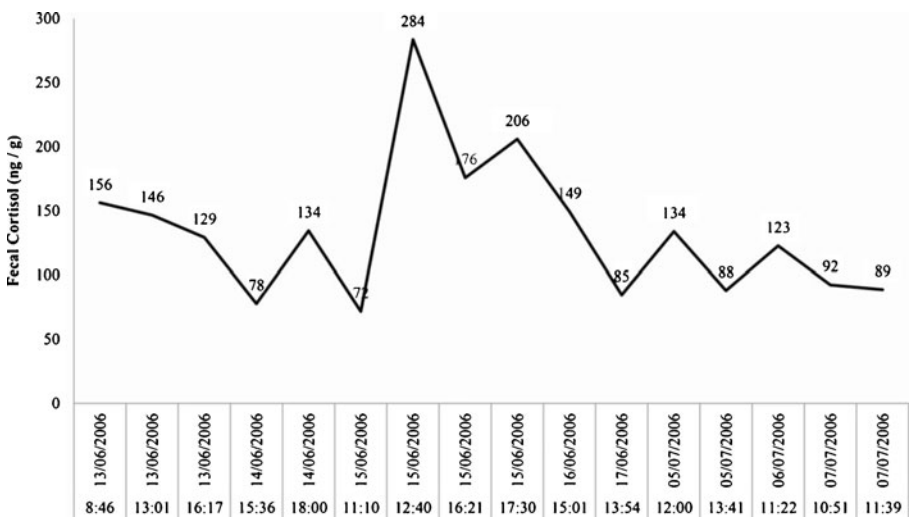


Fig. 1 Fecal cortisol profile for individual 14 (adult male red colobus) darted at 07:51 h on June 13, 2006, in Kibale National Park, Uganda.

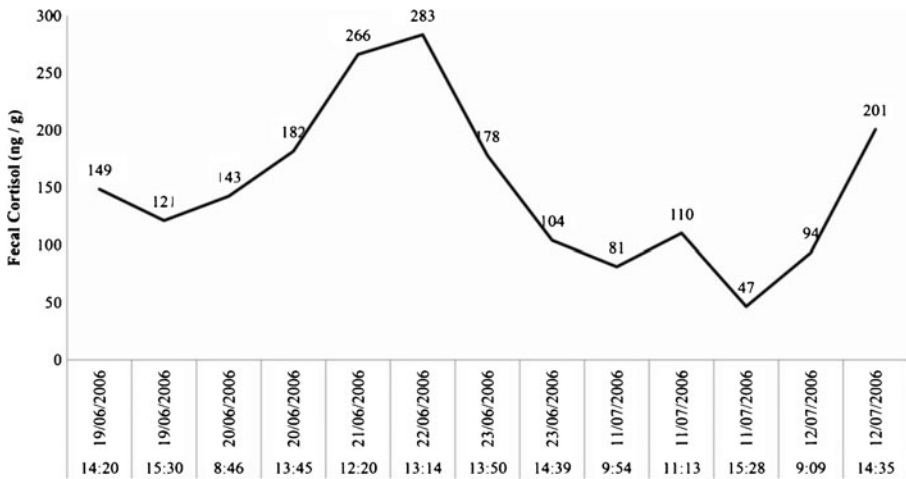


Fig. 2 Fecal cortisol profile for individual 40 (adult male red colobus) darted at 09:52 h on June 19, 2006, in Kibale National Park, Uganda.

was used. It was diluted to 1:22,000 with 50 mM bicarbonate buffer (pH 9.6). Before this study, neither an adrenocorticotropic hormone challenge nor a biological validation using a known stressor had been conducted on this species.

Results

Adult Male Red Colobus Stress Response to Darting and Collaring

Individual 14 had a mean fecal cortisol level of 134 ± 55 ng/g during the darting study (June 13–July 12, 2006), while individual 40 had a mean cortisol level of 151 ± 70 ng/g. Individual 14 had a peak cortisol level of 284 ng/g occurring 2 d after capture, which was 182% above his initial baseline level of 156 ng/g (Fig. 1). Individual 40 had a peak cortisol level of 283 ng/g occurring 3 d after capture, which was 190% above his initial baseline level of 149 ng/g (Fig. 2). These peak levels did not remain elevated for either male, although individual 40 did have a high fecal cortisol level (201 ng/g) at the end of the study, likely unrelated to the darting or collaring as indicated by his long-term data collected in 2007 and 2008.

Although both males initially behaviourally responded to the collars by pulling at them and emitting stress squeals, these behaviors quickly subsided after the day of darting and they ceased for both males by day 3 (day 1 = day of darting; Fig. 3A, B). In contrast, elevated levels of agonistic interactions occurred after darting (Fig. 3C) as compared to an average of 0.1 ± 0.14 agonistic acts per hour calculated from 11 mo of data from red colobus males.

From data collected over 11 mo, beginning 14 mo after the darting event, we found no difference in mean fecal cortisol levels between 10 collared individuals (68 ± 28 ng/g) and 14 other individuals living in the same social group that were neither darted nor collared (71 ± 30 ng/g; $t = 1.575$, $P = 0.116$, $df = 1108$). The same results were found when analyzing data only from adult males (collared = 68 ± 23 ng/g, not collared = $72 \pm$

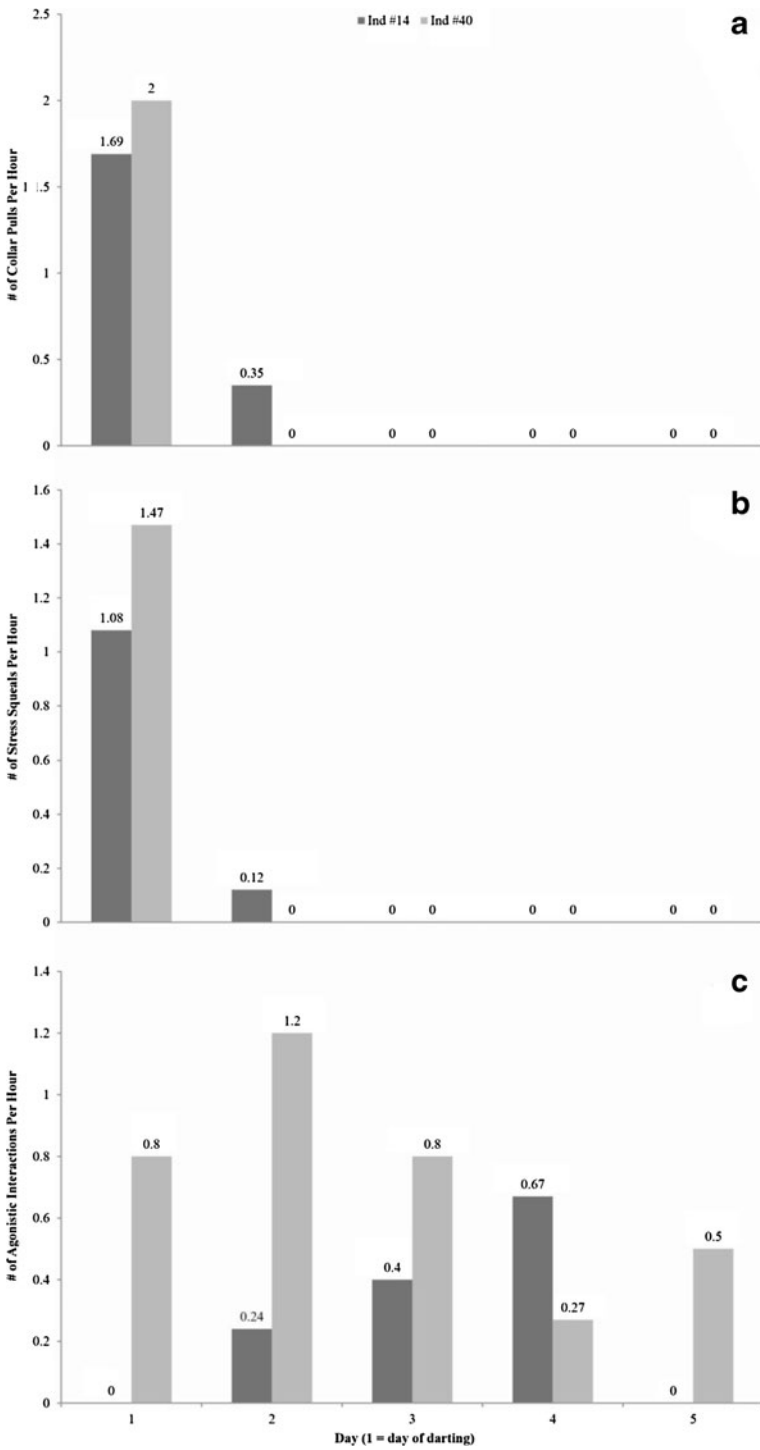


Fig. 3 Frequency of stress-related behaviours observed during the first 5 d after being darted and collared for two adult male red colobus in Kibale National Park, Uganda. **(A)** The number of times each individual pulled at their collar per hour. **(B)** The number of stress squeal vocalizations emitted by each subject per hour. **(C)** The number of intragroup agonistic interactions, e.g., fighting, chasing, involving each focal subject per hour.

32 ng/g; $t = 0.768$, $P = 0.443$, $df = 405$) and only from adult females (collared = 68 ± 28 ng/g, not collared = 70 ± 27 ng/g; $t = 0.663$, $P = 0.508$, $df = 701$).

Red Colobus Stress Response to a Chimpanzee Predation Attack

Mean fecal cortisol levels for each individual varied from 95 ± 19 ng/g to 136 ± 24 ng/g after the chimpanzee attack (Table I). These levels were significantly elevated, i.e., greater than 2 standard deviations above the 11-mo baseline mean calculated from samples collected from August 2007 through June 2008, for four of the six individuals, with peak levels occurring from 1–4 d after the attack that were 147–211% above baseline (Fig. 4). However, these peak levels did not remain elevated 5 d after the attack. For the two individuals that did not show elevated cortisol levels, one was a male that we did not have long-term data on to determine his baseline levels, so we could not evaluate if he had elevated levels after the attack. The other was a female that did not show an elevation in cortisol after the attack. The fecal cortisol response of individual 40 to the chimpanzee attack displayed a 192% peak 3 d after the attack, compared to the 190% peak that occurred 3 d after being darted and captured.

Discussion

Darting and collaring caused an acute stress response in adult red colobus males, as indicated by a short-term *ca.* 2-fold increase in fecal cortisol levels 2–3 d after the stressor. The quick return to baseline cortisol levels, along with the lack of difference in fecal cortisol levels between collared and non-collared individuals over a year after capture, supports the idea that collaring did not cause chronic stress. These results suggest that primate researchers studying red colobus monkeys need not be concerned that collaring of individuals causes chronic stress when all other risks are minimized. Data on the physiological and behavioral responses to darting and collaring such as those presented here are rare. However, one notable supporting study on African wild dogs (*Lycaon pictus*) found no differences between fecal

Table I Fecal cortisol levels (ng/g) for six red colobus individuals living in a group that was attacked by chimpanzees on October 18, 2008, in Kibale National Park, Uganda
Fecal samples were collected starting on October 20 and ending October 25, 2008.

Individual	Sex	Mean	<i>N</i>	SD
BBS	F	97	5	19.7
BEZZ	M	136	5	23.6
BSH	M	98	6	26.1
CGS	F	95	5	19.1
RGS	F	109	3	36.1
STIF	M	101	6	23.4

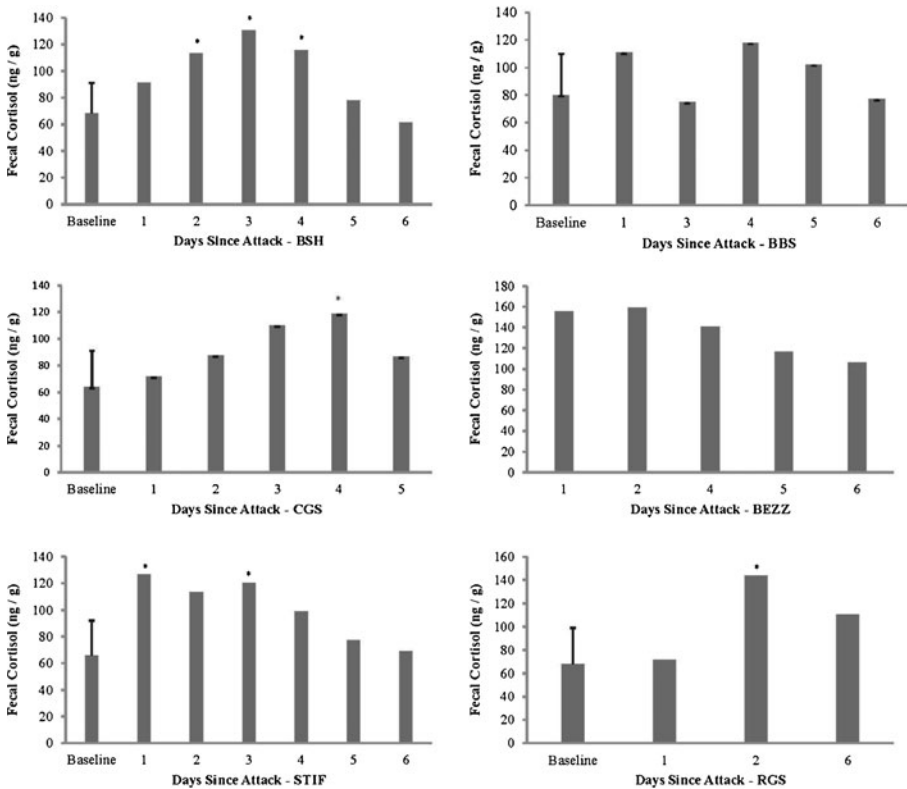


Fig. 4 Fecal cortisol response of six red colobus individuals to a chimpanzee predation attack that occurred on October 18, 2008, at Makerere University Biological Field Station in Kibale National Park, Uganda. Two juveniles and one subadult were killed during this attack. Data were collected starting 1 d after the attack and ending 6 d after the attack. *Indicates a sample that was greater than 2 standard deviations above the baseline cortisol of that particular individual. Baseline levels and standard deviation were determined using 11 mo of data on each individual.

corticosterone levels in collared and uncollared individuals and also concluded that collaring did not cause chronic stress (Creel *et al.* 1997).

Although darting and collaring does not appear to cause chronic stress, there is some indication that this procedure may alter social dynamics. Removing males from their social group, even for a few hours, may create an opportunity for shifts in the dominance hierarchy. This may explain the frequency of agonistic interactions we observed after darting. In another instance occurring after a separate darting study in 2011, a darted young male received continued aggression for a number of days after darting, which corresponded with him leaving the group and joining a black-and-white colobus (*Colobus guereza*) group (C. A. Chapman, *unpubl. data*). Eventually, he re-joined the original red colobus group. Detailed behavioral studies are needed to test the hypothesis that acute stressors and temporary removal such as darting can disrupt primate social hierarchies, as well as how to mitigate such effects. Sapolsky and Share (1998) similarly emphasize that darting can alter social behavior and provide detailed recommendations on how to reduce this disruption for terrestrial primates, but, to our knowledge, no analogous recommendations have been proposed

for arboreal primates. We note that both males stopped pulling their collars and emitting stress squeals by the third day of the study. This parallels results from a study that affixed spider monkeys (*Ateles paniscus*) with radio collars, in which it was reported that they ceased pulling at their collars 2 d after capture (Karesh *et al.* 1998).

Comparing the effects of darting and collaring to a natural stressor, we found a similar stress response to the chimpanzee predation attack, although there was individual variation in the timing and magnitude of the cortisol response. Because adult males actively defend the group from chimpanzee attacks (Stanford 1995), they, along with the adult females that lose offspring in an attack, are likely to undergo the most severe stress response. Factors such as this may explain the variation in the severity and timing of the elevation in cortisol across the individuals, e.g., the female that did not exhibit an elevated cortisol level may have been located at a point in the group far from where the attack occurred). The direct comparison of individual 40's response to both stressors supports the conclusion that darting and collaring and chimpanzee predation both produced an acute stress response, especially because both responses were so similar (192% and 190% increase in cortisol 3 d later). It is worth noting that differences in cortisol levels between the two studies may have resulted from annual (2006 vs. 2008) and seasonal (June vs. October) variation.

Our results provided a measure of baseline and elevated fecal cortisol levels after an acute stressor for Kibale red colobus. Using both the darting and collaring event and chimpanzee predation attack, we documented a 147–211% increase in fecal cortisol following a stressor. A similar peak in fecal cortisol levels, which average 238% above baseline levels ($N = 4$), was found in a captive study of the chimpanzee acute stress response using general anesthesia (Whitten *et al.* 1998b). On average, wild baboons (*Papio anubis*) of Masai Mara National Reserve, Kenya, showed an *ca.* 170% rise in plasma cortisol after being darted (Sapolsky 1982). Thus, our finding of an approximate twofold increase in fecal cortisol after a stressor is consistent with previous primate studies. These results will help inform future studies of fecal cortisol levels in red colobus, as well as in reexamining previously collected data. For example, we previously documented mean cortisol levels up to 445 ng/g in red colobus living in degraded forest fragments outside of Kibale, which when compared to the acute stress response documented here suggests that living in certain types of forest fragments causes chronic stress (Chapman *et al.* 2006). Immunosuppression due to chronic stress from fragmentation may be partially responsible for increased levels of parasitism documented in the red colobus living in these fragments (Chapman *et al.* 2007).

Finally, although we found the invasive procedure of darting and collaring to act as an acute, but not a chronic stressor for the red colobus, a physiological stress response is not the only consideration in such studies. Darting primates is a dangerous procedure regardless of whether or not it acts as a chronic stressor. For example, in a study of the small-bodied red-tailed monkeys (*Cercopithecus ascanius*) in Kibale, of 33 individuals anesthetized, one was stuck on a tree and not captured, five kept enough muscle control to avoid capture, one was killed, another partially paralyzed, and a third suffered a broken leg from its falls (Jones and Bush 1988). Thus, darting should be performed only when absolutely necessary and with highly trained personnel on hand so that risks to both primates and researchers can be minimized (Glander *et al.* 1991; Karesh *et al.* 1998; Sapolsky and Share 1998). We also suggest that other researchers consider performing similar evaluations as those presented here

to quantify the extent to which a research procedure elicits physiological changes that could lead to detrimental effects on health and fitness.

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