Habitat Degradation and Seasonality Affect Physiological Stress Levels of *Eulemur collaris* in Littoral Forest Fragments

Michela Balestri¹,², Marta Barresi², Marco Campera¹,², Valentina Serra², Jean Baptiste Ramananjato³, Michael Heistermann⁴, Giuseppe Donati¹*  

¹ Department of Social Sciences, Oxford Brookes University, Oxford, United Kingdom, ² Department of Biology, University of Pisa, Pisa, Italy, ³ QIT Madagascar Minerals, Rio Tinto, Tolagnaro, Madagascar, ⁴ Endocrinology Laboratory, German Primate Center, Leibniz Institute for Primate Research, Goettingen, Germany

Abstract

The littoral forest on sandy soil is among the most threatened habitats in Madagascar and, as such, it represents a hot-spot within a conservation hot-spot. Assessing the health of the resident lemur fauna is not only critical for the long-term viability of these populations, but also necessary for the future re-habilitation of this unique habitat. Since the Endangered collared brown lemur, *Eulemur collaris*, is the largest seed disperser of the Malagasy south-eastern littoral forest its survival in this habitat is crucial. In this study we compared fecal glucocorticoid metabolite (fGCM) levels, a measure of physiological stress and potential early indicator of population health, between groups of collared brown lemurs living in a degraded forest fragment and groups occurring in a more preserved area. For this, we analysed 279 fecal samples collected year-round from 4 groups of collared brown lemurs using a validated 11-oxoetiocholanolone enzyme immunoassay and tested if fGCM levels were influenced by reproductive stages, phenological seasons, sex, and habitat degradation. The lemurs living in the degraded forest had significantly higher fGCM levels than those living in the more preserved area. In particular, the highest fGCM levels were found during the mating season in all animals and in females during gestation in the degraded forest. Since mating and gestation are both occurring during the lean season in the littoral forest, these results likely reflect a combination of ecological and reproductive pressures. Our findings provide a clear indication that habitat degradation has additive effects to the challenges found in the natural habitat. Since increased stress hormone output may have long-term negative effects on population health and reproduction, our data emphasize the need for and may add to the development of effective conservation plans for the species.

Citation: Balestri M, Barresi M, Campera M, Serra V, Ramananjato JB, et al. (2014) Habitat Degradation and Seasonality Affect Physiological Stress Levels of *Eulemur collaris* in Littoral Forest Fragments. PLoS ONE 9(9): e107698. doi:10.1371/journal.pone.0107698

Editor: Jason M. Kamilar, Midwestern University & Arizona State University, United States of America

Received February 4, 2014; Accepted August 22, 2014; Published September 17, 2014

Copyright: © 2014 Balestri et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work received funding from a Rufford Small Grant (Reference number: 8953-2) and Oxford Brookes University (Mentoring Scheme: HRSU-ASQL). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors declare that the affiliation of one of them to a commercial company (QIT Madagascar Minerals) does not alter their adherence to PLOS ONE policies on sharing data and materials.

* Email: gdonati@brookes.ac.uk

Introduction

The term “stressor” is used to refer to any internal or external stimuli that perturb homeostasis of living organisms [1], [2]. There are several potential stressors for free-ranging animals. These can be natural, such as adverse climatic factors [3], high predation pressure [4], decrease in food availability [5], social aggression or competition [6], or anthropogenic, such as habitat degradation and fragmentation [7], [8], logging and hunting [9], and noise pollution [10].

In support of behavioural studies, hormonal studies have been recently used to assess animals’ adaptability to such challenges and their welfare [11–13]. In vertebrates, stressors, depending on their severity, may cause a physiological response that entails an increase in glucocorticoid (i.e., a class of steroid hormones) secretion from the adrenal cortex [1], [14]. This response is considered adaptive in helping the animal to face critical periods that are threatening to homeostasis [13], [15], [16].

In spite of the positive short-term effects, the action of glucocorticoids may cause severe problems when animals are exposed to long-term (chronic) stressors [14], [17]. Among the most common problems caused by long-term elevated glucocorticoid levels are immune suppression, atrophy of tissue, reproductive suppression, gastric ulcers, and muscle wasting [18–21]. Furthermore, species with slow life histories, such as primates, may be particularly strongly impacted by lost reproductive opportunities resulting from chronic stress [22]. Since chronic stressors can lead to health risks [19], [23], high glucocorticoid levels are often assumed to indicate lower individual fitness or population viability [17]. However, this generalized view is debated, since recent studies suggest a complex relationship between glucocorticoids, stressors, and fitness [5], [24–26]. Despite the associated fitness costs, chronic stress has been proposed to evolve in species where it has an adaptive role in helping to face long-term stressors [13]. Reproductive stages may considerably affect glucocorticoid levels in vertebrates [27]. In the case of primates, physiological stress levels have been shown to increase in males during mating seasons as a consequence of high reproductive competition [28–34], but not in species where mate competition is low [22], [35],...
Physiological Stress Levels in *Eulemur collaris*

[36]. Females, on the other hand, have higher energetic demands during gestation and lactation and this may also lead to higher physiological stress [34], [37–41]. This seems to hold true despite the fact that, in primates, females have been shown to have lower energetic costs during reproductive stages than other mammals of similar size [42–44]. Furthermore, females are expected to have higher glucocorticoid levels during gestation than during lactation because of (1) placental release of corticotropin-releasing hormone which directly affects both fetal and maternal HPA-axis activity (reviewed by [45]), (2) increased synthesis of cortisol binding corticosteroid-binding globulin, (e.g. [46]) and (3) pregnancy-related increases in levels of estrogens (e.g. [47]).

Habitat disturbance has also been found to be associated with physiological stress in primates [48–51] and in other vertebrate species [52–55]. Anthropogenic disturbance is a widespread phenomenon in Madagascar where a large proportion of the original habitats have been lost [56], [57]. Forest disturbance due to anthropogenic pressure has been shown to reduce food availability and diversity, emphasizing the ecological unpredictability of the island [58]. In fact, Madagascar has been shown to differ from other primate habitats, due to its relatively unpredictable rainfall which leads to irregular fruiting patterns, making these environments challenging especially for frugivores [58], [59]. Despite this, recent studies have highlighted some degree of flexibility in frugivorous lemurs, which demonstrate a level of tolerance to habitat disturbance [60], [61]. Several frugivorous lemurs respond to habitat disturbance by integrating fallback food species and/or by shifting to a more folivorous diet [62], [63]. Additionally, activity patterns and ranging behaviour may be modified in order to maximize resource access or, alternatively, to conserve energy [64], [65].

The littoral forest of South-Eastern Madagascar is one of the most threatened habitats on the island with only a few hundred hectares of fragmented forest left [66], [67]. The Endangered collared brown lemur, *Eulemur collaris*, is the largest frugivorous lemur living in these forests, where it shows high social and ecological flexibility [61], [68]. However, a reduction of food availability and quality [61], [69] and an increase in parasite load [70] in the more disturbed areas indicate that these lemurs may be exposed to high physiological stress which, in turn, may lead to increased health risks. Since *Eulemur collaris* is also the largest seed disperser in the littoral forest [71] its survival in this habitat is crucial. Thus, assessing the impact of habitat disturbance on the stress physiology and welfare of this species is not only important for the long-term viability of the local populations, but also necessary for the future re-habitation of this unique habitat [72].

In this paper, we examined how reproductive and phenological seasons, habitat degradation, and sex affect the physiological responses of collared brown lemurs. This response was investigated by comparing fecal glucocorticoid metabolite (fGCM) levels [73] between groups living in a degraded fragment (Mandena) and groups living in a more preserved fragment of littoral forest (Sainte Luce) in South-Eastern Madagascar. Fecal samples can be easily collected without disturbing the animal, thereby allowing frequent sampling, even over a long time period [74], thus they can be used as a powerful non-invasive measure of physiological stress levels in free-ranging animals [1], [12], [17], [37].

This study aims to elucidating whether the behavioural and ecological flexibility previously recorded in collared brown lemurs living in littoral forest fragments [61] may be sufficient to compensate for the non-optimal environment or whether the animals show increased signs of physiological stress.

Against this background we predicted higher fGCM levels:

1. In males during the mating season compared to other reproductive stages, because the mating season represents a period with pronounced reproductive competition in many species of primates;
2. In females during gestation and lactation compared to other reproductive stages, because of the expected higher energy demands;
3. During the lean season than during the season of abundance since the former is expected to be a time of food shortage in forest fragments due to low levels of fruit availability;
4. In the more degraded forest during stressful reproductive stages and the lean season, since anthropogenic disturbance may amplify fruit shortage and potentially increase exposure to climatic fluctuations and predators.

**Materials and Methods**

**Ethics Statement**

We conducted this study with the authorization of the Commission Tripartite of the Direction des Eaux et Forêts de Madagascar (Autorisation de recherche n.29/11/MEF/SG/DGF/DCF/SAP/SCB du 20/01/11) and this research was ethically approved by the University of Pisa (Animal Care and Use Board). We captured the adult individuals via cages, using banana slices as bait, and we rapidly anesthetized them with Zoletil 100 (5 mg/kg of tiletamine hydrochloride). All animals recovered from anesthesia within 1.5 hours and were subsequently released at the site of capture. The lemurs were followed until regaining full mobility in trees, and there were no injuries as a consequence of the captures.

**Study Sites and Subjects**

The data were collected in two littoral forest areas from February 2011 to January 2012: Mandena (MAN) and Sainte Luce (STL) in South-East Madagascar. The Conservation Zone of MAN (24°57’S, 47°0’E) consists of two fragments of around 240 ha of degraded littoral forest [75]. The average canopy height in MAN is 8.9±SD 4.4 m [69]. The second study site, the littoral forest of STL (24°46’S, 47°10’E), around 30 km North of Fort Dauphin, is among the most intact littoral ecosystems in Madagascar and contains a very high diversity of vegetation [66]. The study area was located in a 252-ha-fragment of well-preserved littoral forest and swamp, 190 of which are included in the Conservation Zone [75]. The average canopy height in STL is 14.7±SD 4.3 m [69]. Previous botanical analyses illustrate that floristically MAN and STL represent the same habitat although structural differences indicate higher degradation in the former area [69]. Several lemur-focused studies confirm that MAN contains lower quality resources than STL in terms of fruit nutritional values and size of feeding trees [61], [65].

*Eulemur collaris* is a medium-sized lemur with body mass of 2.15±SD 0.25 kg and body length of 46.1±SD 2.6 cm [61]. These lemur live in multi-male multi-female groups and show no clear dominance of one sex [61], [76]. In this study we analysed hormonal data collected from all adult individuals of four different groups (*n* = 22): two groups in MAN (group AB and group C), and two groups in STL (group A and group B).

To ensure continuous observations of the groups, four animals (one for each group) were captured and equipped with radio-collars in order to monitor them via the use of radio-telemetry (Biotrack). Collection of fecal samples began approximately one month after capturing the animals to minimize the risk that fGCM levels were influenced by the capture event itself. Individuals were
Fecal sample collection and GC analysis

Each habituated group was followed four days (from 6 a.m. to 6 p.m.) per month in order to collect fecal samples and behavioral observations. A total of 279 fecal samples were collected from 22 subjects (mean per individual: 12.7 ± SE 0.3; range: 2–25). Each individual was sampled every 18.0 ± SE 1.9 days (range: 8.3–44.3 days). The samples were collected immediately after defecation. Site, group, date, time, and identity of the donor were recorded. Fecal samples were preserved in 10 ml tubes with 96% ethanol and stored at room temperature for 7–12 months before further processing for hormone analysis [77].

We collected 12 additional fecal samples to evaluate possible degradation of fGCM concentrations over one-year storage as reported for other species [78], [79]. For this, each fecal sample was divided into three aliquots and stored in ethanol as described above. Aliquots were kept at the field station at ambient temperature before being processed (see below), thus simulating the conditions under which the study samples were collected and stored. The first aliquot was extracted after 3 months, while the other two aliquots were extracted after 6 and 12 months, respectively, in order to match the longest storage time study samples were stored in ethanol. Fecal extracts were stored at −20°C before the final hormone analysis. The results showed no significant effect of storage duration on fGCM levels (RM ANOVA: Storage effect: F2,22 = 1.44, p = 0.258), a finding in line with what was found in Propithecus verreauxi [32] and in Eulemur rufifrons [33]. Thus, there was no indication that variation in storage duration biased our hormone data.

Fecal samples for hormone analyses were homogenized in their original ethanolic solvent by mechanical squashing of the fecal pellets with a metal stick. The ethanolic fecal suspension (including a 2-mL ethanol rinse) was decanted into a 50-mL propylene tube, pellets with a metal stick. The ethanolic fecal suspension (including a 2-mL ethanol rinse) was decanted into a 50-mL propylene tube, pellets with a metal stick. The ethanolic fecal suspension (including a 2-mL ethanol rinse) was decanted into a 50-mL propylene tube, pellets with a metal stick. The ethanolic fecal suspension (including a 2-mL ethanol rinse) was decanted into a 50-mL propylene tube, pellets with a metal stick. The ethanolic fecal suspension (including a 2-mL ethanol rinse) was decanted into a 50-mL propylene tube, pellets with a metal stick. The ethanolic fecal suspension (including a 2-mL ethanol rinse) was decanted into a 50-mL propylene tube, pellets with a metal stick. The ethanolic fecal suspension (including a 2-mL ethanol rinse) was decanted into a 50-mL propylene tube, pellets with a metal stick.

Fecal extracts were analyzed for immunoreactive 11oxoetiocholanolone (3α,11oxo-CM), a group specific measurement of 5-reduced cortisol metabolites with a 3α,11oxo-structure [81]. The assay has been successfully applied to monitor adrenocortical activity and glucocorticoid output from fecal samples in various primate species (e.g. [81]), including other species of lemurs [32], [82]. It has also been used successfully to monitor physiological stress in the redfronted lemur (Eulemur rufifrons) [33], a species closely related to the collared brown lemur. We used reverse-phase high pressure liquid chromatography analysis (HPLC) to characterize the immunoreactive metabolites measured by the 11oxoetiocholanolone EIA. HPLC was carried out as described by Heistermann et al. [81]. To evaluate possible sex differences in the 11oxoetiocholanolone immunoreactivity profiles, we performed HPLC on both a male and a female sample. HPLC also allowed us to evaluate whether certain fecal androgens, which could potentially be detected by antibodies raised against cortisol metabolites [81], [83], were co-measured by the 11oxoetiocholanolone EIA.

HPLC analysis indicated that almost all immunoreactivity was identified via collars as well as individual characteristics such as age, sex, size, canine length, tail shape, fur colour, and other distinctive traits.

where cortisol metabolites in our HPLC system elute (Fig. 1) [81].

The similarity between HPLC glucocorticoid immunoreactivity profiles from the collared brown lemur samples and those derived from fecal samples of other primate species [81], including the redfronted lemur [33], strongly suggests that the 11oxoetiocholanolone assay is reliable in detecting glucocorticoid output in our study species. In this respect, the presence of only small amounts of immunoreactivity measured after fraction 40 (positions where certain potentially cross-reacting androgen metabolites elute at [81]) suggests a low degree of co-measurement of these androgens in our assay (Fig. 1). Furthermore, HPLC profiles were almost identical between the male and female sample in terms of both number and elution position (i.e. characteristic) of metabolites measured, indicating that the 11oxoetiocholanolone assay measures the same immunoreactive compounds in both males and females.

Data analyses

In order to evaluate the effect of food availability on fGCM levels, we distinguished between a lean season (April–October) and a season of food abundance (November–March). The two seasons were distinguished on the basis of previous multi-annual studies in STL [84] and phenological data collected in MAN during our study period [65].

In order to evaluate the effect of the reproductive stages, we distinguished between four main stages: mating (May to mid-June), gestation (mid-June to September), lactation (October to December), and non-reproductive (January to April) [85].

All 279 fecal samples collected from the 22 adult individuals were used in the analyses. All adult females gave birth during the study period. Statistical comparisons were conducted using a General Linear Mixed Model (GLMM) with reproductive stages (nesting in phenological seasons), sites, and sexes as fixed factors, and individuals as a random factor. Both main effects and two-way interaction effects were evaluated in the model. We controlled for the time of the day (morning or afternoon) when each sample was collected by including it in the model as fixed factor, since it has been shown to potentially affect fGCM levels [41], [86]. In fact, the fGCM levels were higher during the afternoon (median: 766.5 ng/g, quartiles: 438.5–1439.8 ng/g, n = 181) than during the morning (median: 600.3 ng/g, quartiles: 424.9–1013.3 ng/g, n = 98) (GLMM, Time of day: F1,265 = 6.14, P = 0.014). We also included in the model the sample weight as a covariate (fixed
effect) to control for the potential effects of the fecal mass on hormone concentrations [86]. The sample weight was negatively correlated with the fGCM levels (GLMM, Weight: \( F_{1,265} = 67.13, p<0.001 \)).

We used Duncan’s tests as post hoc analyses. We tested for normal distribution of residuals (Kolmogorov-Smirnov test) and equality of variances (Levene’s test) as underlying assumptions of the GLMM. Residual values of fGCM levels were not normally distributed and therefore the data were ln transformed. We performed all tests with SPSS 21.0 considering \( p<0.05 \) as threshold of significance.

**Results**

The fGCM levels excreted by the 22 adult individuals (Fig. 2) did not differ between males (median: 666.1 ng/g, quartiles: 361.4–1318.5 ng/g, \( n = 164 \)) and females (median: 740.1 ng/g, quartiles: 487.2–1309.1 ng/g, \( n = 115 \)) (GLMM, Sex: \( F_{1,265} = 1.54, p = 0.216 \)).

The fGCM levels differed between the four reproductive stages (GLMM, Reproductive stage: \( F_{3,265} = 2.62, p = 0.048 \)), with a median of 1307.6 ng/g (quartiles: 710.9–2291.2 ng/g, \( n = 19 \)) during mating, 766.5 ng/g (quartiles: 445.2–1473.1 ng/g, \( n = 111 \)) during gestation stage, 612.4 ng/g (quartiles: 358.6–1132.4 ng/g, \( n = 115 \)) during lactation stage, and 638.7 ng/g (quartiles: 474.6–990.3 ng/g, \( n = 34 \)) during the non-reproductive stage. The fGCM levels during the mating season were higher than during gestation (Duncan: \( p = 0.003 \)), lactation (\( p<0.001 \)), and non-reproductive stage (\( p<0.001 \)). Females during lactation had lower fGCM levels than during mating (\( p = 0.008 \)) and gestation (\( p = 0.030 \)). Interaction effects indicated that the two sexes did not show a different pattern between the two sites (GLMM, Sex*Site: \( F_{1,265} = 0.10, p = 0.755 \)) and between the two phenological seasons (GLMM, Sex*Phenological season: \( F_{1,265} = 0.11, p = 0.742 \)) (Table 1 and Table 2).

Results of pair-wise comparisons of mean differences between sites, reproductive stages, and phenological seasons for ln transformed fGCM values in males and females are shown in Table 3 and Table 4, respectively.

**Discussion**

Consistent with our predictions, the highest fGCM values were found in females during the gestation period in the degraded forest of MAN and in males during the mating season in both sites. Phenological season also played a role in shaping the fGCM output with higher values exhibited during the lean season, whilst sex had no significant effect. Fecal glucocorticoid metabolite levels were higher during the afternoon rather than during the morning, a characteristic of nocturnal animals which, from a chronobiological perspective, *Eulemur* species belong to [87]. The covariate sample weight also significantly influenced the fGCM output.

As predicted, in males we found higher fGCM levels during the mating season than during the other reproductive stages. Mating season appears to be a stressful period also for male mouse lemurs (*Microcebus murinus* [28]), male ring-tailed lemurs (*Lemur catta* [31], [34]), male sifakas (*Propithecus verreauxi* [32]), and male red-fronted lemurs (*Eulemur rufifrons* [33]). In fact, during this study, collared brown lemur males showed higher aggression rates during the mating season as compared to the other reproductive stages (Serra et al., unpublished data). It is reasonable to assume that the higher fGCM levels found during this time of the year may be related to a general increase of aggression rates and high reproductive competition [88]. Increased aggression rates have been shown to affect fGCM levels in males of other primate species, such as Eastern chimpanzees (*Pan troglodytes* [29]), chacma baboons (*Papio ursinus* [30]), Verreaux’s sifakas (*Propithecus verreauxi* [32]) and in males of other vertebrates (wolves, wolves, wolves...
Canis lupus, [89]; bison bulls, Bison bison, [90]; American alligators, Alligator mississippiensis, [91]). Conversely, low physiological stress levels have been observed in species where mate competition is low, such as males of muriquis (Brachyteles arachnoides, [35]), tufted capuchin monkeys (Cebus apella, [36]), and red-bellied lemurs (Eulemur rubriventer [22]), while no differences were found between mating and non-mating seasons in male rhesus macaques (Macaca mulatta, [92]).

In our study, we also found high fGCM levels in females during the mating season, suggesting that this period may also be stressful for them. This is in accordance with findings on other primates (e.g. South-american squirrel monkeys, Saimiri sciureus, [93], [94]) and mammals (e.g. giant pandas, Ailuropoda melanoleuca, [95], [96]) where the GC elevation has been attributed to the effects of ovarian cycling and general anxiousness. However, we must consider that our results indicate a very high variability within the mating season, which may be due to two confounding factors. Firstly, we had a small sample size due to the very short mating season [58], and we may have accidentally included samples belonging to the previous and/or the subsequent reproductive stage. Secondly, individuals may have different levels of physiological stress depending on their dominance status [97].

By far, the highest fGCM levels were found in females in the more degraded site during gestation (see Fig. 2). It is well known that gestation represents a serious challenge for females, as during this period they have increased energetic demands, as previously shown for females of ring-tailed lemurs (Lemur catta, [37]), white faced capuchins (Cebus capucinus, [40]), and other mammalian species [little brown myotis, Myotis lucifugus, [98]; red squirrels, Tamiasciurus hudsonicus, [99]]. Higher glucocorticoid levels during gestation may not necessarily correspond to higher physiological stress, however, since placental hormones and fetal estrogens also stimulate cortisol production [100]. In the Malagasy littoral forest, the physiological increase in glucocorticoid levels due to gestation may be enhanced by the additional physiological stress due to the concomitant lean season [84]. However, the significantly lower fGCM levels found in the females inhabiting the less disturbed area strongly suggests that the degraded habitat conditions are largely responsible for this effect.

Interestingly, during gestation in the degraded forest males had similar fGCM levels as the females within the same site. This supports the idea that habitat degradation and seasonal food availability override the potential effect of female reproductive state on fGCM levels. The fact that pregnant females showed relatively low levels of fGCM in the less disturbed area is also in line with previous studies showing that lemurs minimize maternal energetic investment during gestation [100].

Contrary to other studies, collared brown lemur females did not show high fGCM levels during lactation, a potentially stressful period for females due to the burden of infant carrying and maternal care (ring-tailed lemurs, Lemur catta, [34], [38], but see [101]; Assamese macaques, Macaca assamensis, [102]; rhesus macaques, Macaca mulatta, [39]). The lack of elevated fGCM levels in lactating females of collared brown lemur may have been caused by the overriding effect of the concomitant increase in food availability. Similar results have been shown in Lemur catta at Beza Mahafaly which also showed low physiological stress levels during lactation [37]. In fact, lactation in collared brown lemur is synchronized with the transition from the lean season to the season of food abundance, when young leaves and ripe fruits increase in

Figure 2. Fecal glucocorticoid metabolite levels of Eulemur collaris over the study period. The figure shows standardized residuals of lnfGCM after controlling for the sample weight. MAN: Mandena, STL: Sainte Luce. Lean: May–October 2011, Abundance: February–April 2011 and November 2011–January 2012. Mating: 1st May–15th July, Gestation: 16th July–30th September, Lactation: 1st October–31st December, Non-reproductive: 1st January–30th April. Values are means and standard errors.
doi:10.1371/journal.pone.0107698.g002
### Table 1. Fecal glucocorticoid metabolite levels (ng/g) in males of *Eulemur collaris* over the study period.

|       | MAT (11) | GES (68) | LAC (68) | NRE (17) | LEA (109) | ABU (55) | Total |
|-------|----------|----------|----------|----------|-----------|----------|-------|
| MAN   | 1856     | 1227     | 673      | 572      | 1015      | 601      | 837   |
|       | (82)     | 1308–3151| 683–1822 | 355–1006 | 376–842   | 376–1011 | 500–1440|
| STL   | 1019     | 430      | 844      | 571      | 587       | 486      | 498   |
|       | (82)     | 602–1618 | 298–590  | 300–1495 | 329–990   | 282–1002 | 303–1002|
| Total | 1564     | 611      | 704      | 572      | 770       | 512      |       |
|       | (82)     | 770–3151 | 412–1314 | 310–1280 | 376–842   | 306–1011 |       |

Values are medians and quartiles.

MAN: Mandena (more degraded site); STL: Sainte Luce (more preserved site); MAT: mating; GES: gestation; LAC: lactation; NRE: non reproductive; LEA: lean season; ABU: period of food abundance.  
[doi:10.1371/journal.pone.0107698.t001](https://doi.org/10.1371/journal.pone.0107698.t001)

### Table 2. Fecal glucocorticoid metabolite levels (ng/g) in females of *Eulemur collaris* over the study period.

|       | MAT (11) | GES (68) | LAC (68) | NRE (17) | LEA (109) | ABU (55) | Total |
|-------|----------|----------|----------|----------|-----------|----------|-------|
| MAN   | 711      | 1981     | 593      | 822      | 1621      | 561      | 1012  |
|       | (82)     | 676–2291 | 1621–2683| 386–846  | 397–982   | 679–2462 | 386–982|
| STL   | 1265     | 713      | 588      | 813      | 729       | 630      | 713   |
|       | (82)     | 1091–1580| 505–882  | 439–1060 | 566–1168  | 507–1060 | 475–931|
| Total | 1178     | 976      | 588      | 822      | 896       | 609      |       |
|       | (82)     | 694–1936 | 596–1844 | 421–1013 | 559–1000  | 551–1621 | 392–957|

Values are medians and quartiles.

MAN: Mandena (more degraded site); STL: Sainte Luce (more preserved site); MAT: mating; GES: gestation; LAC: lactation; NRE: non reproductive; LEA: lean season; ABU: period of food abundance.  
[doi:10.1371/journal.pone.0107698.t002](https://doi.org/10.1371/journal.pone.0107698.t002)
### Table 3. P values of pair-wise comparisons of mean differences between sites in ln transformed fGCM values across different reproductive stages in males of *Eulemur collaris* (Duncan post-hoc).

| Site | Stage | MAN | MAN | MAN | MAN | STL | STL | STL | STL | STL | STL |
|------|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|      | MAT-L | GES-L | LAC-L | LAC-A | NRE-A | MAT-L | GES-L | LAC-L | LAC-A | NRE-A | NRE-A |
| MAN  | MAT-L | - | - | - | - | - | - | - | - | - | - |
| MAN  | GES-L | 0.12 | - | - | - | - | - | - | - | - | - |
| MAN  | LAC-L | 0.00 | 0.06 | - | - | - | - | - | - | - | - |
| MAN  | LAC-A | 0.01 | 0.23 | 0.46 | - | - | - | - | - | - | - |
| MAN  | NRE-A | 0.00 | 0.11 | 0.74 | 0.65 | - | - | - | - | - | - |
| STL  | MAT-L | 0.07 | 0.71 | 0.12 | 0.37 | 0.21 | - | - | - | - | - |
| STL  | GES-L | 0.00 | 0.02 | 0.58 | 0.23 | 0.41 | 0.04 | - | - | - | - |
| STL  | LAC-L | 0.10 | 0.85 | 0.08 | 0.29 | 0.15 | 0.82 | 0.03 | - | - | - |
| STL  | LAC-A | 0.00 | 0.04 | 0.84 | 0.37 | 0.62 | 0.09 | 0.70 | 0.06 | - | - |
| STL  | NRE-A | 0.00 | 0.07 | 0.92 | 0.50 | 0.79 | 0.14 | 0.55 | 0.10 | 0.78 | - |

Median sample size: 15 (range: 5–36).
MAN: Mandena (more degraded site); STL: Sainte Luce (more preserved site); MAT: mating; GES: gestation; LAC: lactation; NRE: non reproductive; L: lean period; A: period of food abundance.

doi:10.1371/journal.pone.0107698.t003

### Table 4. P values of pair-wise comparisons of mean differences between sites in ln transformed fGCM values across different reproductive stages in females of *Eulemur collaris* (Duncan post-hoc).

| Site | Stage | MAN | MAN | MAN | MAN | STL | STL | STL | STL | STL | STL |
|------|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|      | MAT-L | GES-L | LAC-L | LAC-A | NRE-A | MAT-L | GES-L | LAC-L | LAC-A | NRE-A | NRE-A |
| MAN  | MAT-L | - | - | - | - | - | - | - | - | - | - |
| MAN  | GES-L | 0.07 | - | - | - | - | - | - | - | - | - |
| MAN  | LAC-L | 0.15 | 0.00 | - | - | - | - | - | - | - | - |
| MAN  | LAC-A | 0.08 | 0.00 | 0.73 | - | - | - | - | - | - | - |
| MAN  | NRE-A | 0.23 | 0.00 | 0.77 | 0.55 | - | - | - | - | - | - |
| STL  | MAT-L | 0.60 | 0.17 | 0.06 | 0.03 | 0.10 | - | - | - | - | - |
| STL  | GES-L | 0.23 | 0.00 | 0.78 | 0.56 | 0.97 | 0.10 | - | - | - | - |
| STL  | LAC-L | 0.64 | 0.03 | 0.31 | 0.19 | 0.42 | 0.35 | 0.42 | - | - | - |
| STL  | LAC-A | 0.11 | 0.00 | 0.84 | 0.86 | 0.65 | 0.04 | 0.66 | 0.24 | - | - |
| STL  | NRE-A | 0.65 | 0.03 | 0.30 | 0.19 | 0.42 | 0.36 | 0.42 | 0.98 | 0.24 | - |

Median sample size: 9 (range: 3–25).
MAN: Mandena (more degraded site); STL: Sainte Luce (more preserved site); MAT: mating; GES: gestation; LAC: lactation; NRE: non reproductive; L: lean period; A: period of food abundance.

doi:10.1371/journal.pone.0107698.t004
their availability [56], [84], [103]. Additionally, primates produce some of the most dilute milk of all mammals [42] and, in particular, milk-producing costs for *Eulemur* species are among the lowest amongst primates [104].

As expected, we found that seasonality had a strong effect on fGCM output in collared brown lemurs. This was in the expected direction as we found higher fGCM values during the lean season, when fruit availability was low [65]. In primates physiological stress levels have been repeatedly found to be shaped by fruit availability (olive baboons, *Papio anubis*, [105]; ring-tailed lemurs, *Lemur catta*, [37], [38]; chimpanzees, *Pan troglodytes*, [29]; black howler monkeys, *Alouatta pigra*, [106]; yellow baboons, *Papio cynocephalus*, [107]; Mexican howler monkeys, *Alouatta palliata*, [51]) and periods of presumed nutritional stress (Eastern red colobus, *Procolobus rufomitratus*, [108]). In particular, in MAN, the percentage of tree species with ripe fruits averaged 4.4% during the mating season and the gestation period while it stands at 14.2% during lactation and non-reproductive stages [65]. Thus, low fruit availability seems to have an additive effect on reproductive stages and habitat degradation, and the combination of these three factors is likely to reflect fGCM output in our study species.

This study clearly indicates that levels of fGCM were higher for collared brown lemurs in the degraded forest fragment of MAN when compared to lemurs in the more preserved forest of STL, suggesting a higher level of physiological stress in animals living in disturbed areas. The most likely explanation for this difference may be found in the lower levels of food availability [65] and quality [61] recorded in MAN as compared to STL which may result in increased nutritional stress. Other stressors, such as a higher predation risk in the disturbed, more open MAN forest, may have contributed to the recorded difference. In support of this, the lemurs’ primary predator, the fossa (*Cryptoprocta ferox*), was reported several times in MAN but not in STL over the last decade [109].

Previous studies show that collared brown lemurs in the degraded forest shape their ranging, feeding, and activity pattern to cope with a decrease in food abundance [61], [65]. In particular, lemurs in MAN during our study period had larger home ranges and traveled shorter daily distances than lemurs in STL [65]. Our results indicate that, although the collared brown lemurs seem to cope with habitat degradation by changing their behavioral ecology, living in a degraded forest area nevertheless increases physiological stress. This may have an effect on the long-term viability of the population. These effects may include higher vulnerability to diseases, reduced reproduction, and even a higher mortality rate [17]. Our finding of a higher parasite burden for the species known to shape its 24-h activity depending on luminosity [52], [53]) and periods of presumed nutritional stress (Eastern red colobus, *Procolobus rufomitratus*, [108]; black howler monkeys, *Alouatta pigra*, [49]; Yucatan spider monkeys, *Ateles geoffroyi*, [50]; Mexican howler monkeys, *Alouatta palliata*, [51]; but see [9] for effect of logging and hunting on brown spider monkeys, *Ateles hybridus* and red howler monkeys, *Alouatta seniculus*) and other animal species (American redstarts, *Serophaga ruticilla*, [52]; spotted salamanders, *Ambystoma maculatum*, [53]; African savanna elephants, *Loxodonta africana*, [54]; agile antechinus, *Antechinus agilis*, [55]; see also [110] for effects of human disturbance on elk, *Cervus elaphus*, and wolves, *Canis lupus*). Conversely, the only previous study which compared lemurs’ fGCs levels in disturbed and undisturbed habitats [22] showed opposite results. In the latter study, red-bellied lemurs (*Eulemur rubriventer*) in the undisturbed forest of Ranomafana showed higher fecal cortisol levels than those in the disturbed habitat during the lean period. This result might be explained by an attenuated response to prolonged stress to reduce costs of continued stress hormone production [14]. In fact, *Eulemur rubriventer* in the disturbed habitat showed higher infant mortality [22]. Conversely, lemurs in MAN have birth and mortality rates similar to those in STL [84] and in other more preserved forests [111]. Thus, lemurs in MAN do not give indications of an attenuated response to habitat degradation, but they do not seem to exhibit any clear negative effects at the population level. Furthermore, *Eulemur rubriventer* in Ranomafana relied on the exotic *Psidium cattleianum* in the disturbed area, and this might have shielded them during lean periods [22]. In fact, exotic fruits are known to sometimes provide a nutritionally higher resource and a longer temporal availability than native species [112]. Contrary to Tecot’s study [22], collared brown lemurs do not seem to rely on exotic species in MAN [61] and this may also explain the high fGCM levels detected during the lean season.

In addition to Tecot [22], studies on other animals (bighorn sheep, *Ovis canadensis* [113]; Canadian grizzly bears, *Ursus arctos*, [114]; African forest elephants, *Loxodonta cyclotis*, [115]; spotted salamanders, *Ambystoma maculatum*, [53]) found higher fGCM in undisturbed habitats. Thus, the hypothalamic–pituitary–adrenal axis response may differ between species [13] and the relationship between GC levels and stressors may be not so clear-cut.

Another possible stressor that may have influenced the higher fGCM levels found in lemurs living in MAN is their proximity to a mining site. The area of MAN in which the study took place is in fact very close to the machinery set up in the region to extract titanium deposits [116]. This may have exposed the lemurs to chronic stressors such as anthropogenic noise and light pollution. In particular, noise pollution may lead to behavioural changes [10] and to an increase in fGCM levels [117]. *Eulemur collaris* is a species known to shape its 24-h activity depending on luminosity [68], [118]. Hence, artificial light pollution may potentially alter the species behaviour. For example, human activities have been shown to alter the activity budget of bighorn sheep [113]. Further studies focusing on these aspects may give a clearer insight on the impact of mining on the area.

**Conclusions**

By comparing fGCM levels in collared brown lemurs living in a degraded and in a more preserved forest fragment, we found higher fGCM levels occur in those individuals living in the former situation, which appears to be a stressful environment. Higher fGCM levels in disturbed habitats suggest that coping with a harsher environment has a cost of increased physiological stress in these lemurs. This study underlines the importance of physiological investigations to assess population health of threatened species and the potentially detrimental effect of habitat loss on animal welfare. Because of the paucity of studies comparing lemurs living in disturbed environments over the long-term, more research is urgently required to evaluate the consequences of chronic physiological stress on the highly threatened lemur populations.

**Acknowledgments**

This work was carried out under the collaboration agreement with the Department of Animal Biology and Anthropology of the University of
Antananarivo and QIT Madagascar Minerals (QMM). We thank the Madagascar Institute for the Conservation of Tropical Environments (MICET), the Association of Managers of Forests of Ambatoorinsina (FIMPIA), the Mandena Management Committee (COGEMA), and the Ministère des Eaux et Forêts for their collaboration and permission to work in Madagascar. We thank the Costwold Wildlife Park & Gardens for giving us precious help for the validation test. We are grateful to Silvana Borgognini for her support during the early stages of this research. We thank Rachel Sawyer and Lauren Lansdowne for the language revision of this manuscript. Our gratitude goes to Sergio Tofanelli, Stefania Bertontini and Giulio Petroni for giving us the opportunity to work in their laboratories. We acknowledge the QMM biodiversity staff, especially Manon Vincellette, Julhy Rabenantoandro, Christophe Rambolamanana, Laza Andriamandimbirisa, Faly Randiatrafika, David Rabehreva, Sylvio Angelico, Claude Sonnay, and the field assistants (Kadlofa, Joffete, Faris, Fléhéron, German, Crescint) in MAN and STL. We thank Murielle Ravaolalhy and Aristide Andrianarimina for their contribution to this research and Andrea Heistermann for conducting the hormone analyses.

Author Contributions
Conceived and designed the experiments: GD MH. Performed the experiments: M Barresi M Balestri MC VS. Analyzed the data: M Barresi M Balestri MC VS MD GD. Contributed reagents/materials/analysis tools: JBR MH GD. Wrote the paper: M Balestri MC JBR MH GD.

References
1. Cred S (2001) Social dominance and stress hormones. Trends Ecol Evol 16: 491–97.
2. Citronus GP (2009) Stress and disorders of the stress system. Nat Rev Endocrinol 5: 374–381.
3. Wingfield J (2013) Ecological processes and the ecology of stress: the impacts of abiotic environmental factors. Func Ecol 27: 37–44.
4. Clancy M, Sherryf MJ, Zanette I (2013) Predator-induced stress and the ecology of fear. Func Ecol 27: 56–65.
5. Busch DS, Hayward LS (2009) Stress in a conservation context: a discussion of glucocorticoid actions and how levels change with conservation-relevant variables. Biol Conserv 142: 2044–2853.
6. Creel S, Danzter B, Goymann W, Rubenstein DR (2013) The ecology of stress: an overview of the field. Func Ecol 27: 66–80.
7. Wingfield JC, Hunt K, Breuner C, Dunlap K, Fowler GS, et al. (1997) Adrenocortical responses to stress and their modulation in free-living vertebrates. In: McEwen BS, Goodman HM, editors. Behavioral Approaches to Conservation in the Wild. Cambridge University Press, Cambridge. pp. 95–131.
8. Faligu L (2003) Effects of habitat fragmentation on biodiversity. Annu Rev Ecol Evol Syst 34: 487–515.
9. Rimbach R, Link A, Heistermann M, Gomez-Pozada C, Galvis N, et al. (2013) Effects of logging, hunting, and forest fragmentation on physiological stress levels of two sympatric olive baboons in Colombia. Conserv Physiol 1: doi:10.1093/comphys/cto031.
10. Francis CD, Barber JR (2013) A framework for understanding noise impacts on wildlife: an urgent conservation priority. Front Ecol Environ 11: 305–213.
11. Kreu JM, Singh J, Gastric MG, Kaar T (2006) Fecal glucocorticoids and their metabolites as indicators of stress in various mammalian species: a literature review. J Zoo Wildl Med 37: 234–244.
12. Goymann W (2012) On the use of non-invasive hormone research in ecological studies. Trends Ecol Evol 27: 56–65.
13. Boonstra R (2013) Reality as the leading cause of stress: rethinking the impact of abiotic environmental factors. Trends Ecol Evol 28: 37–44.
14. Romero LM (2004) Physiological stress in ecology: lessons from biomedical research. Ecol Evol Syst 34: 487–515.
15. Creel S, Dantzer B, Goymann W, Rubenstein DR (2013) The ecology of stress: an overview of the field. Func Ecol 27: 66–80.
16. Wingfield JC, Hunt K, Breuner C, Dunlap K, Fowler GS, et al. (1997) Adrenocortical responses to stress and their modulation in free-living vertebrates. In: McEwen BS, Goodman HM, editors. Behavioral Approaches to Conservation in the Wild. Cambridge University Press, Cambridge. pp. 95–131.
17. Faligu L (2003) Effects of habitat fragmentation on biodiversity. Annu Rev Ecol Evol Syst 34: 487–515.
18. Rimbach R, Link A, Heistermann M, Gomez-Pozada C, Galvis N, et al. (2013) Effects of logging, hunting, and forest fragmentation on physiological stress levels of two sympatric olive baboons in Colombia. Conserv Physiol 1: doi:10.1093/comphys/cto031.
19. Francis CD, Barber JR (2013) A framework for understanding noise impacts on wildlife: an urgent conservation priority. Front Ecol Environ 11: 305–213.
20. Kreu JM, Singh J, Gastric MG, Kaar T (2006) Fecal glucocorticoids and their metabolites as indicators of stress in various mammalian species: a literature review. J Zoo Wildl Med 37: 234–244.
21. Goymann W (2012) On the use of non-invasive hormone research in ecological studies. Trends Ecol Evol 27: 56–65.
22. Boonstra R (2013) Reality as the leading cause of stress: rethinking the impact of abiotic environmental factors. Trends Ecol Evol 28: 37–44.
23. Creel S, Dantzer B, Goymann W, Rubenstein DR (2013) The ecology of stress: an overview of the field. Func Ecol 27: 66–80.
24. Creel S, Danzter B, Goymann W, Rubenstein DR (2013) The ecology of stress: an overview of the field. Func Ecol 27: 66–80.
74. Palme R, Rettenbacher S, Touma C, El-Bahr SM, Möstl E (2005) Stress in Yucatan spider monkeys: effects of environmental conditions on cortisol levels in wild and captive populations. Anim Conserv 12: 496–502.

61. Donati G, Kesch K, Ndremifidy K, Schmidt SL, Ramanamanjato JB, et al. (2010) Lemurs of Madagascar. Washington DC: Conservation International. 767 p.

59. Dewar RE, Richard AF (2007) Evolution in the hypervariable environment of populations: the effects of a 2-year drought on a naturally occurring population of ring-tailed lemurs (Lemur catta) in southwestern Madagascar. Int J Primatol 31: 49–69.

60. Gould L, Sussman RW, Sauther ML (1999) Natural disasters and primate populations: a test of the challenge and social stress hypotheses. Behav Ecol Sociobiol 67: 19–30.

58. Wright PC (1999) Lemur traits and Madagascar ecology: coping with an island environment. Yearb Phys Anthropol 42: 31–72.

66. Bollen A, Donati G (2006) Conservation status of the littoral forest of Sainte Luce, southeastern Madagascar. Biochem Zool 79: 918–928.

67. Bollen A, Donati G, Lazdane K, Broll A, Theisinger O, Bearder SK, Donati G (2014) Susceptibility to gastrointestinal parasite infections in remnant fragments of Malagasy littoral forest. Ital J Zool. doi:10.1080/11250003.2014.915993.

57. Chapman CA, Wasserman MD, Gillespie TR, Speirs ML, Lawes ML, et al. (2004) Do food availability, parasitism, and stress have synergistic effects on Red Colobus populations living in forest fragments? Am J Phys Anthropol 131: 525–34.

62. Irwin MT (2007) Living in forest fragments reduces group cohesion in Propithecus diadema. Int J Primatol 34: 246–259.

55. Johnstone CP, Lill A, Reina RD (2012) Does habitat fragmentation cause stress in the agile antechinus? An haematological approach. J Comp Physiol 182: 327–34.

50. Rangel-Negrín A, Alfaro JL, Valdez RA, Romano MC, Serio-Silva JC (2009) Evaluating adrenal stress in the agile antechinus (Antechinus stuartii) in Eastern Australia. J Comp Physiol 182: 327–34.

48. Chapman CA, Wasserman MD, Gillespie TR, Speirs ML, Lawes ML, et al. (2004) Do food availability, parasitism, and stress have synergistic effects on Red Colobus populations living in forest fragments? Am J Phys Anthropol 131: 525–34.

53. Martinez-Mota R, Valdespino C, Sánchez-Ramos MA, Serio-Silva JC (2007) Effects of forest fragmentation on the physiological stress response of black howler monkeys. Anim Conserv 10: 374–379.

49. Chapman CA, Wasserman MD, Gillespie TR, Speirs ML, Lawes ML, et al. (2004) Do food availability, parasitism, and stress have synergistic effects on Red Colobus populations living in forest fragments? Am J Phys Anthropol 131: 525–34.

56. Dewar RE, Richard AF (2007) Evolution in the hypervariable environment of populations: the effects of a 2-year drought on a naturally occurring population of ring-tailed lemurs (Lemur catta) in southwestern Madagascar. Int J Primatol 31: 49–69.

52. Campera M, Serra V, Balestri M, Barresi M, Ravaolahy M, et al. (2014) Effects of varying habitat quality on the physiological stress of spotted Geoffroy’s hog-nosed monkeys. Int J Primatol 35: 537–551.

51. Chapman CA, Wasserman MD, Gillespie TR, Speirs ML, Lawes ML, et al. (2004) Do food availability, parasitism, and stress have synergistic effects on Red Colobus populations living in forest fragments? Am J Phys Anthropol 131: 525–34.

54. Johnstone CP, Lill A, Reina RD (2012) Does habitat fragmentation cause stress in the agile antechinus? An haematological approach. J Comp Physiol 182: 327–34.

47. McLean M, Smith R (1999) Corticosterone-releasing hormone in human pregnancy and parturition. Trends Endocrinol Metab 10: 174–178.

58. Wright PC (1999) Lemur traits and Madagascar ecology: coping with an island environment. Yearb Phys Anthropol 42: 31–72.

46. Chapman CA, Wasserman MD, Gillespie TR, Speirs ML, Lawes ML, et al. (2004) Do food availability, parasitism, and stress have synergistic effects on Red Colobus populations living in forest fragments? Am J Phys Anthropol 131: 525–34.

45. Chapman CA, Wasserman MD, Gillespie TR, Speirs ML, Lawes ML, et al. (2004) Do food availability, parasitism, and stress have synergistic effects on Red Colobus populations living in forest fragments? Am J Phys Anthropol 131: 525–34.

44. Chapman CA, Wasserman MD, Gillespie TR, Speirs ML, Lawes ML, et al. (2004) Do food availability, parasitism, and stress have synergistic effects on Red Colobus populations living in forest fragments? Am J Phys Anthropol 131: 525–34.
101. Gould L, Ziegler TE, Wittwer DJ (2005) Effects of reproductive and social variables on fecal glucocorticoid levels in a sample of adult male ring-tailed lemurs (Lemur catta) at the Beza Mahafaly Reserve, Madagascar. Am J Primatol 67: 5–23.

102. Fürthauer I, Heistermann M, Schülke O, Ossner J (2014) Low female stress hormone levels are predicted by same- or opposite-sex sociality depending on season in wild Assamese macaques. Psychoneuroendocrinology 48: 19–28.

103. Wright PC, Razafindratsita T, Pochron ST, Jernvall J (2005) The key to frugivory in Madagascar. In: Dew J, Boubli H, editors. Tropical Fruits and Frugivores: the Search for Strong Interactors. The Netherlands: Springer Press. pp. 121–138.

104. Tilden CD, Oftedal OT (1997) Milk composition reflects patterns of maternal care in prosimian primates. Am J Primatol 41: 195–211.

105. Sapolsky RM (1986) Endocrine and behavioral correlates of drought in wild olive baboons (Papio anubis). Am J Primatol 11: 217–227.

106. Behie AM, Pavelka MS, Chapman CA (2010) Sources of variation in faecal cortisol levels in howler monkeys in Belize. Am J Primatol 72: 600–606.

107. Gesquiere LR, Onyango PO, Alberts SC, Altmann J (2011) Endocrinology of year-round reproduction in a highly seasonal habitat: Environmental variability in testosterone and glucocorticoids in baboon males. Am J Phys Anthropol 144: 169–176.

108. Chapman CA, Saj T, Snaith TV (2007) Temporal dynamics of nutrition, parasitism and stress in colobus monkeys: implications for population regulation and conservation. Am J Phys Anthropol 134: 240–250.

109. Donati G, Ramanamanjato JB, Ravoahangy AM, Vincelette M (2007) Translocation as a conservation measure for a threatened species: the case of Eulemur collaris in the Mandena littoral forest, south-eastern Madagascar. In: Ganzhorn JU, Goodman SM, Vincelette M, editors. Biodiversity, Ecology and Conservation of the Littoral Ecosystems of South-Eastern Madagascar. Washington DC: Smithsonian Institution Press. pp. 237–245.

110. Creel S, Fox JE, Hardy A, Sandi J, Garrott B, et al. (2002) Snowmobile activity and glucocorticoid stress responses in wolves and elk. Conserv Biol 16: 809–814.

111. Overdorff DJ, Meurenlander AM, Talata P, Telo A, Forward ZA (1999) Life history of Eulemur fulvus rufus from 1988–1998 in southeastern Madagascar. Am J Phys Anthropol 108: 295–310.

112. Johnson CA, Raubenheimer D, Rodman JM, Clarke DJ, Swedell L (2013) 30 days in the life: daily nutrient balancing in a wild chacma baboon. PLOS One 8(7): e670383. doi:10.1371/journal.pone.00670383.

113. Sayre RW (1996) Ecology of Bighorn Sheep in Relation to Habitat and Oil. PhD thesis, University of North Dakota.

114. Wasser SK, Davenport R, Ramey ER, Hunt KE, Parker M, et al. (2004) Scat detection dogs in wildlife research and management: application to grizzly and black bears in the Yellowhead Ecosystem, Alberta, Canada. Can J Zoo 82: 473–492.

115. Mushiri-South J, Tchiguomba LB, Brown J, Abhoudanza N, Maldonado JE, et al. (2008) Physiological indicators of stress in African forest elephants (Loxodonta africana cyclotis) in relation to petroleum operations in Gabon, Central Africa. Divers Distrib 14: 995–1003.

116. Vincelette M, Theberge M, Randriashampa I. (2007) Evaluations of forest cover at regional and local levels in the Tolagnaro region since 1950. In: Ganzhorn JU, Goodman SM, Vincelette M, editors. Biodiversity, Ecology and Conservation of the Littoral Ecosystems of South-Eastern Madagascar. Washington DC: Smithsonian Institution Press. pp. 49–58.

117. Blickley JL, Word KR, Krakauer AH, Phillips J, Sells SN, et al. (2012) Experimental chronic noise is related to elevated fecal corticosteroid metabolites in lekking male greater sage-grouse (Centrocercus urophasianus). PLOS One 7(11): e50462. doi:10.1371/journal.pone.0050462.

118. Donati G, Baldi N, Morelli V, Ganzhorn JU, Borgognini-Taafli SM (2009) Proximate cues and ultimate determinants of brown lemur cathemerality. An Behav 77: 317–325.