

# Season and reproductive activity influence cortisol levels in the Malagasy primate *Lepilemur edwardsi*

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## Funding information

Evangelisches Studienwerk Villigst e.V.; Universität Hamburg

## Abstract

Throughout the year, wild animals are exposed to a variety of challenges such as changing environmental conditions and reproductive activity. These challenges may affect their stress hormone levels for varying durations and in varying intensities and impacts. Measurements of the glucocorticoid hormone cortisol in the hair of mammals are considered a good biomarker for measuring physiological stress and are increasingly used to evaluate stress hormone levels of wild animals. Here, we examined the influence of season, reproductive activity, sex, as well as body condition on hair cortisol concentrations (HCC) in *Lepilemur edwardsi*, a small Malagasy primate species. *L. edwardsi* lives in the seasonal dry forests of western Madagascar, which are characterized by a strongly changing resource availability throughout the year. We hypothesized that these seasonal changes of resource availability and additionally the reproductive cycle of this species would influence HCC of *L. edwardsi*. Results revealed that hair cortisol concentration of females did not change seasonally or with the reproductive cycle. However, we found a significant increase of hair cortisol levels in males from the early wet season during the early dry season (mating season). This increase is presumably due to changed behavior during the mating season, as sportive lemurs travel more and show aggressive behavior during this time of the year. This behavior is energy-costly and stressful, and presumably leads to elevated HCC. As elevated cortisol levels may impair immune function, *L. edwardsi* males might also be more susceptible to parasites and diseases, which is unfavorable in particular during a period of low resource availability (dry season).

## KEYWORDS

cortisol, dry forest, *Lepilemur edwardsi*, Madagascar, physiological stress, reproduction, season

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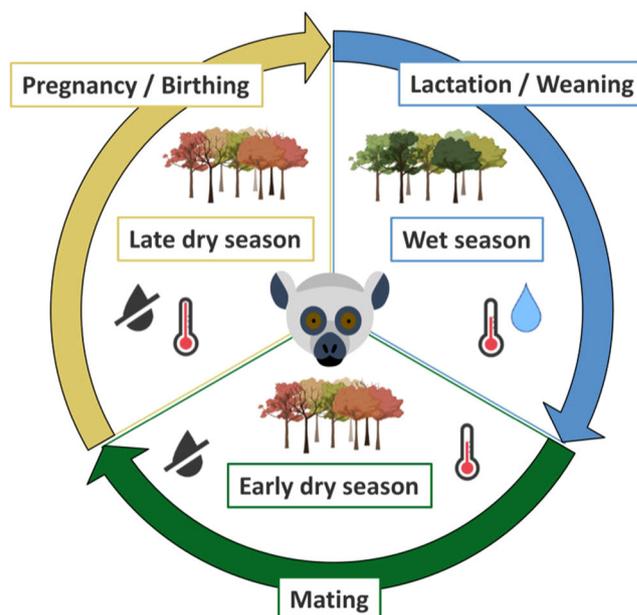
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## 1 | INTRODUCTION

Animals are exposed to various physiological challenges throughout the year which are caused by seasonal changes in environmental conditions (Chesson & Huntly, 1997; Ruf et al., 2006; Varpe, 2017). These challenges can be extrinsic, such as changes in resource availability (Chapman et al., 2015; Ruf et al., 2006) or seasonally occurring weather events (e.g., droughts and cyclones) (Fardi et al., 2018; Sapolsky, 1986). Additionally, animals experience intrinsic challenges, such as changes of reproductive activity and costs due to parental investment (Brunton et al., 2008; Havenstein et al., 2021) as well as parasitic infections (Triki et al., 2016) and diseases (Mormède et al., 2007). In response to these challenges, animals release glucocorticoid (GC) hormones via the hypothalamic–pituitary–adrenal (HPA) axis which promotes the availability and allocation of energy when demands are high due to high activity levels, or food scarcity (Dallman et al., 1999; Sapolsky et al., 2000). The HPA-axis comprises the hypothalamus, the anterior pituitary and the adrenal cortex. When activated, neurons in the hypothalamus synthesize corticotropin-releasing hormone (Johnson et al., 1992; Spencer & Deak, 2017; Spiga et al., 2014). In the anterior pituitary, the corticotropin-releasing hormone stimulates the release of the adrenocorticotropic hormone into the blood; which in turn, triggers the production and release of GCs in the adrenal cortex (Spencer & Deak, 2017; Spiga et al., 2014). GC-levels in animals change throughout the year due to a multitude of interacting stressors of varying impacts, durations and intensities (Romero, 2004; Sapolsky et al., 2000; Spiga et al., 2014). Short-term elevation of GC-levels are crucial for survival, as GC for example enhances the flight response to avoid predation. Long-term or chronic elevation of GC-levels, however, can impair various body functions (e.g., immunosuppression and accelerated aging) and can divert resources away from other biological functions (e.g., reproduction, growth) and may ultimately reduce fitness (Havenstein et al., 2016, 2021; Johnson et al., 1992; Lebl et al., 2011; Moberg & Mench, 2000).

In mammals, the GC hormone cortisol (corticosterone in rodents) normally follows diurnal and ultradian rhythms and is considered a good hormonal biomarker to measure short-term as well as long-term physiological stress (Mormède et al., 2007; Ralph & Tilbrook, 2016; Spiga et al., 2014), as its production and release is directly triggered by the HPA-axis (Johnson et al., 1992). In animal conservation efforts, measurements of GC hormones have proven useful to monitor the impact environmental or anthropogenic stressors have on the health of wild animals (Ralph & Tilbrook, 2016; Romero, 2004).

In this study, we investigated the seasonal cortisol levels in a wild population of the nocturnal sportive lemur species, *Lepilemur edwardsi*. This species lives in the highly seasonal dry deciduous forests of western Madagascar which is characterized by a drastic decrease in food availability and precipitation during the dry season (Louis et al., 2020; Mittermeier et al., 2010). *L. edwardsi* mates during the early dry season (May/June; Figure 1) and females gestate throughout the less resourced dry season (June–September) until they give birth in the early wet season, when food becomes abundant (October/November). Young are weaned at the end of the wet season (April) (Mittermeier et al., 2010; Randrianambinina et al.,



**FIGURE 1** Reproductive cycle of *Lepilemur edwardsi* throughout the year. At the beginning of the wet season females give birth and lactate their young throughout this season. Precipitation, ambient temperatures, and resource availability are high. Mating occurs in the early dry season when no rainfall occurs and resource availability decreases. During the late dry season females are pregnant, ambient temperatures during the day are extremely high, and resource availability is low. Source: Some of the icons are provided by [biorender.com](https://www.biorender.com).

(2007) (Figure 1). Males and females are assumed to be monogamous, but live in dispersed pairs and males do not have permanent access to their females (Randrianambinina et al., 2007; Warren & Crompton, 1997). To examine the influence of seasonal changes in environmental conditions on cortisol levels in *L. edwardsi*, we measured hair cortisol concentrations (HCC) during different seasons and linked them to seasonal changes of environmental conditions (food availability and climate) and their reproductive cycle. We hypothesized that HCC in both sexes increase at the beginning of the dry season, when food quantity and quality start to decline and females are pregnant. This effect should especially affect individuals in poor body condition (Havenstein et al., 2021). Second, we assumed that females have higher HCC than males during the periods of gestation and lactation due to their high maternal investment. Thirdly, due to changes in behavior associated with mating (e.g., higher activity) males should have higher HCC than females during the mating season (Havenstein et al., 2021).

## 2 | MATERIALS AND METHODS

### 2.1 | Study species and study site

*L. edwardsi*, one of the bigger sportive lemurs with a body mass of 800–1000 g and a head-body length of 26–29 cm, lives in the

Ankarafantsika National Park in western Madagascar. During the wet abundant season from November to April precipitation is high (annual mean precipitation of 1467.4 mm) (Ito et al., 2013), ambient temperature ( $T_a$ ) rarely drops below 20°C and increases daily up to 30°C. In the less resourced dry season, there is almost no precipitation for about 6 months, and daily maximum  $T_a$  increases from May to October.  $T_a$  during the late dry season, rises to around 33°C during the day and 23°C during the night (Klein et al., 2018; Randrianambinina et al., 2007). These climatic conditions lead to a continuing decrease in quantity and quality of food resources over the dry season for folivorous and frugivorous species, as plant growth is generally reduced and many plants decrease their nutritional content (e.g., protein) and increase their protection against herbivores in the leaves with increasing  $T_a$  (Dearing, 2013; Ganzhorn & Wright, 1994; Ganzhorn, 1995). As *L. edwardsi* is mainly folivorous (Mittermeier et al., 2010), this constitutes an increasingly challenging period in the seasonal cycle for this species. During the day, *L. edwardsi* rests in tree hollows and sometimes share their resting sites with their mating partners. Current and continuing habitat loss due to anthropogenic impacts constitute severe threats for this species, as well as global climate change (Craul et al., 2009; Steffens & Lehman, 2018).

## 2.2 | Animal captures and sampling

We conducted the study in the Ankarafantsika National Park (S 16° 19', E 46° 48') in Jardin Botanique A, a 30.6 ha research area. We captured 34 individuals of *L. edwardsi* during three capture periods in 2018 and 2019. We located the individuals in their resting sites in the mornings (9:00–12:00 h), captured them by hand and briefly anesthetized them with 0.1 ml/kg ketamine hydrochloride (Ketamidol® 100 mg/ml; WDT). We weighed the individuals to the nearest 1 g (Clatronic® International GmbH), sexed them and measured the tarsus length (from the heel to the end of the metatarsal bones between the third and the forth toe) with a caliper (Horex®; Helios-Preisser GmbH) before we individually marked them with subcutaneously injected passive integrated transponders (Trovan; EURO I.D. Usling GmbH). We only sampled adult individuals with a body mass of more than 500 g. As HCC varies between sample areas on the body (Acker et al., 2018; Lavergne et al., 2020), we always sampled the hair in the dorsocaudal region. We took 47 hair samples of 14 individuals (9 males and 5 females) in February 2018, 15 individuals (8 males, 7 females, and 8 recaptures) in May–June 2019 and 18 individuals

(7 males, 11 females, and 7 recaptures) in August–September 2018 (Supporting Information: Table 1). Measurements of HCC in lemurs has proven accurate in members of other lemur species of the family Cheirogaleidae (Rakotoniaina et al., 2016, 2017). The hair was cut by hand as closely as possible to the skin, with an industrial razorblade (Gillette Platinum) and stored dry at  $T_a$  in little sealable plastic bags. We were able to assess the hair growth rate of *L. edwardsi* through recaptures and determined it at approximately 0.5–1 cm in 6 months. The sampled full-length hair had a length of about 1–2 cm, therefore depicting the previous 6–12 months. As there is a wash-out effect of cortisol from the proximal to the distal hair segment (Kirschbaum et al., 2009), we assumed that the measured HCC represents approximately 1–6 months before sampling. Hence, reflecting the early wet season (sampling in February 2018), the late wet season (sampling in May/June 2019) and the early dry season (sampling in August/September 2018; Table 1). Using HCC to assess stress hormone levels has the advantage that they reflect cortisol turnover of a comparatively long time, as the incorporation of cortisol into the growing hair happens through passive diffusion (Russell et al., 2012). Hence, HCC represents responses to long-term stress exposure of several months, which is necessary to evaluate continuing environmental challenges.

## 2.3 | Lab analysis

The analysis of HCC was performed in the lab of Prof. Dr. C. Kirschbaum at the Technical University of Dresden following the protocol by Gao et al., 2013. Hair samples were washed in isopropanol and dried for at least 12 h under a fume hood. Thereafter, 7.5 mg of the total hair sample were removed and 40 µl internal standard and 2.4 ml methanol were added, followed by an incubation for 18 h to extract the steroids. After centrifugation, the clear supernatant was separated, and the samples dried under a constant stream of nitrogen using high temperatures of 65°C until the alcohol had completely evaporated. Finally, the residues were resuspended with 175 µl distilled water and an aliquot of 100 µl was used for the determination of cortisol concentration with liquid chromatography tandem mass spectrometry (LC-MS/MS; LC-20AD HPLC unit with SIL-20AC autosampler and CTA-20AC column temperature oven; Shimadzu) coupled to a Turbo-ion-spray® triple quadrupole tandem mass spectrometer (API 5000; ABSciex) with purification by on-line solid-phase extraction.

**TABLE 1** Sampling period and time of incorporation of hair cortisol concentrations (HCC) of male and female *Lepilemur edwardsi*

Incorporation of HCC	Sampling period	Median HCC (pg mg <sup>-1</sup> )	
		Males	Females
Early wet season	January–February 2018	3.98 (1.49)	3.3 (0.99)
Late wet season	May–June 2019	4.33 (1.57)	5.37 (1.75)
Early dry season	August–September 2018	18.99 (21.92)	5.0 (1.66)

Note: Median HCC with interquartile range during the early wet season (N = 14; 9 males, 5 females), late wet season (N = 14; 7 males, 7 females) and early dry season (N = 18; 7 males, 11 females).

## 2.4 | Data analyses and statistics

We analyzed the data using Cran R (Version 4.0.3, R Core Team, 2019) and the packages “plyr” (Wickham, 2011), “dplyr” (Wickham et al., 2021) and “ggplot2” (Wickham, 2009).

Body mass (g) data were dependent and showed normality (Shapiro–Wilk Test:  $p = 0.45$ ). We applied a generalized linear mixed model (GLMM) with the explanatory variables season (3 levels: wet season, early dry season, and late dry season), sex (2 levels), tarsus length (cm), and animal ID as random effect. We included tarsus length to correct for the body size of individuals.

We examined and modeled the influence of season (3 levels: wet season, early dry season, and late dry season), and sex on the HCC ( $\text{pg mg}^{-1}$ ) of *L. edwardsi*. After thorough data exploration, we excluded the extremely low HCC-value of one individual in the early dry season from the model, as it is beyond biologically realistic values (Supporting Information: Table 1). Due to a high variance, we log<sub>10</sub> transformed the cortisol data and applied a GLMM using the package “glmmTMB” (Brooks et al., 2017). We modeled GLMMs with animal ID as random effect, as the HCC of some individuals were measured in multiple

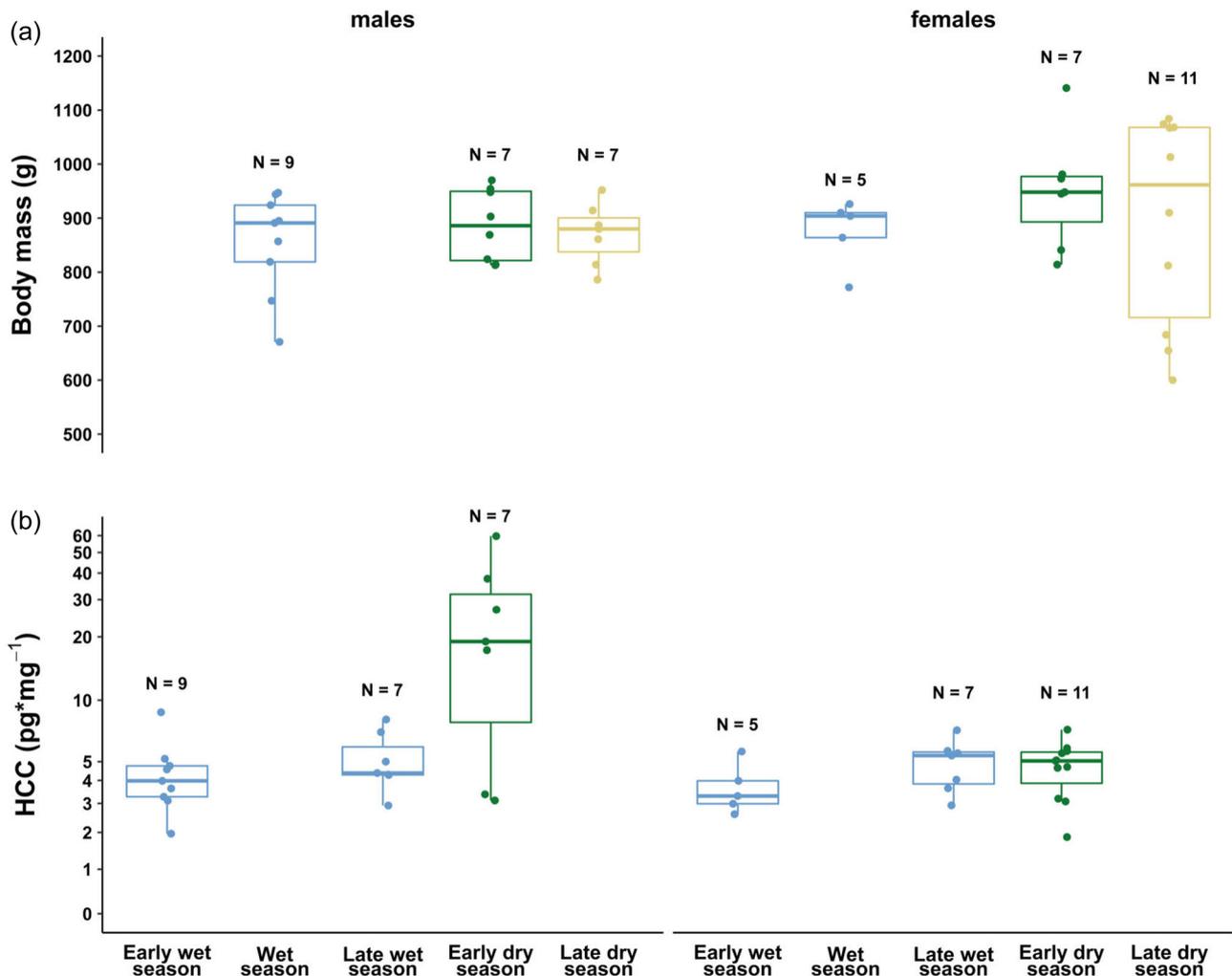
seasons. After model selection using the dredge()-function in package “MuMIn” (Barton, 2020), we selected the “best model” based on Akaike's information criterion (AIC) (Burnham & Anderson, 2002). HCC was best explained by the explanatory variables season and sex (and the interaction term) as AIC of the full model was lowest.

We did post hoc testing of the GLMMs with a Tukey's test using the package “emmeans” (Lenth et al., 2021). Data that showed non-normal distribution are presented as median (interquartile range), normally distributed data are presented as mean  $\pm$  standard deviation.

## 3 | RESULTS

### 3.1 | Body mass

Body mass of males showed no significant changes among seasons (GLMMs, Supporting Information: Table 2) and amounted to  $856 \pm 94$  g ( $N = 9$ ) in the wet season,  $887 \pm 66$  g ( $N = 8$ ) in the early dry season, and  $871 \pm 57$  g ( $N = 7$ ) in the late dry season (Figure 2). Female body mass was  $875 \pm 62$  g ( $N = 5$ ) in the wet season, and significantly increased to  $949 \pm 107$  g ( $N = 7$ ) in the early dry season, and  $897 \pm 194$  g ( $N = 10$ ) in



**FIGURE 2** Body mass in gram (a) and hair cortisol concentration (HCC) in  $\text{pg mg}^{-1}$  on a logarithmic scale (b) of male and female *Lepilemur edwardsi* during the wet and dry season (bold line, median; box limits, first and third quartiles; whiskers,  $1.5 \times$  interquartile range; all data points are included).

**TABLE 2** Results of the generalized linear mixed effects model (GLMM) with the log-transformed hair cortisol concentration (HCC) as response variable: Estimate, standard error (SE), z value, p value, and the 95% confidence intervals (CI) for each explanatory variable

Explanatory variable	Estimate	SE	z Value	p Value	CI 2.5%	CI 97.5%
Intercept	0.57	0.07	7.93	<0.001***	0.43	0.72
Late wet season	0.15	0.10	1.47	0.14	-0.06	0.36
Early dry season	0.60	0.09	6.37	<0.001***	0.40	0.79
Females	-0.02	0.11	-0.19	0.85	-0.27	0.22
Late wet season: females	-0.00	0.15	-0.03	0.98	-0.31	0.30
Early dry season: females	-0.51	0.14	-3.60	<0.001***	-0.82	-0.20

Note: The asterisk represents the respective significance levels with \*\*\* $p < 0.001$  for each variable.

**TABLE 3** Estimated marginal means of the GLMM using the tukey method for p value adjustment: Pairwise seasonal comparison for males and females, estimate, standard error (SE), degrees of freedom (df), t ratio, the p value, and the effect sizes (Cohen's d) for each pairwise comparison

Sex	Pairwise comparison	Estimate	SE	df	t ratio	p value	Effect size
Males	Early wet season-late wet season	-0.15	0.10	38	-1.47	0.32	-0.87
	Early wet season-early dry season	-0.60	0.09	38	-6.37	<0.001***	-3.43
	Late wet season-early dry season	-0.45	0.11	38	-4.21	<0.001***	-2.56
Females	Early wet season-late wet season	-0.15	0.11	38	-1.28	0.42	-0.84
	Early wet season-early dry season	-0.08	0.11	38	-0.78	0.72	-0.48
	Late wet season-early dry season	0.06	0.10	38	0.63	0.80	0.36

Note: The asterisk represents the respective significance levels with \*\*\* $p < 0.001$  or each variable.

the late dry season (Supporting Information: Table 2, Figure 2). Presumably due to pregnancy during the dry season.

### 3.2 | HCC

Median HCC of male *L. edwardsi* in the early wet season amounted to 3.98 (1.49)  $\text{pg mg}^{-1}$  ( $N = 9$ ), to 4.33 (1.57)  $\text{pg mg}^{-1}$  ( $N = 7$ ) in the late wet season and to 18.99 (21.92)  $\text{pg mg}^{-1}$  ( $N = 7$ ) in the early dry season (mating season; Table 1; Figure 2). Female HCC was 3.3 (0.99)  $\text{pg mg}^{-1}$  ( $N = 5$ ) in the early wet season, 5.37 (1.75)  $\text{pg mg}^{-1}$  ( $N = 7$ ) in the late wet season (lactation and weaning) and 5.0 (1.66)  $\text{pg mg}^{-1}$  ( $N = 11$ ) in the early dry season (mating; Table 1; Figure 2). The best model showed a strong influence of season and sex on HCC, as HCC was 15% higher in the late wet season, than in the early wet season and 60% higher in the early dry season than in the early wet season (Table 2).

Five out of seven males showed a three to elevenfold higher median HCC during the early dry season (mating) compared to the early and late wet season (Supporting Information: Table 1; Figure 2). The GLMM showed no significant increase in HCC in both sexes from the early wet season to the late wet season ( $p = 0.1$ ) but a significant increase from the wet to the early dry season ( $p < 0.001$ ; Table 2). Post hoc testing showed a strong significant increase of HCC from the early and late wet season to the early dry season in males, but not in females (Table 3; Tukey's test, males:  $p < 0.001$ , females:  $p = 0.9$ ; Figure 2).

## 4 | DISCUSSION

In this study, we present the first data on the HCC of wild sportive lemurs. We show a significant seasonal increase of the HCC of male *L. edwardsi*, from the well-resourced early wet season to the less-resourced early dry season, which is characterized by low precipitation and in which mating occurs. In contrast, season had no influence on HCC of females, when females give birth in the early wet season and lactate during the late wet season. Body mass did not change among the seasons in both sexes.

Sportive lemurs inhabit almost all habitats in Madagascar and can survive even in the driest and harshest environments on this island, such as the extremely dry spiny forests in southern Madagascar and the seasonal deciduous dry forests in the West. However, individuals seem to be affected by the unfavorable conditions of the dry season, as previous studies show that *L. edwardsi* increases its resting metabolism to cope with these conditions and has a higher parasite prevalence towards the end of the dry season (Bethge et al., 2021, 2022).

### 4.1 | HCC of *L. edwardsi* were generally low

In general, HCC of *L. edwardsi* was extremely low, compared to those of other lemur species such as the diurnal ring-tailed lemurs (*Lemur catta*) where the HCC ranged from 407 to 2258  $\text{pg mg}^{-1}$ , depending on weather events, age and body mass (Fardi et al., 2018). Also other

mammalian species, such as the Egyptian mongoose (*Herpestes ichneumon*) showed distinctly higher HCC levels (median HCC:  $18.98 \pm 5.42 \text{ pg mg}^{-1}$ ) (Azevedo et al., 2021). Low HCC in *L. edwardsi* is in concordance with previous findings which show that plasma GC decreases with decreasing mass-specific resting metabolic rates of mammals (Haase et al., 2016). Sportive lemurs have one of the lowest mass-specific resting metabolic rates measured in mammals so far (Bethge et al., 2017, 2021; Schmid & Ganzhorn, 1996). Indeed, other nocturnal lemur species, such as the fat-tailed dwarf lemur (*Cheirogaleus medius*) and the gray mouse lemur (*Microcebus murinus*), which also have comparatively low resting metabolic rates, also showed comparatively low HCC levels ranging from 0.15 to  $24.33 \text{ pg mg}^{-1}$  and 0.15 to  $42.35 \text{ pg mg}^{-1}$  HCC respectively (Rakotoniaina et al., 2016).

Arboreal folivores, such as *L. edwardsi*, depend on low nutritional food resources (particularly during the dry season), which seems to select for a more sedentary lifestyle and low metabolism (Bethge et al., 2017, 2021; Lovegrove, 2000; McNab, 2008). As an adaptation to these energetic limitations, *L. edwardsi* may further have to inhibit the release of GC by their HPA-axis.

## 4.2 | HCC of males increase in the early dry season

The reproductive cycle of *L. edwardsi* is tightly linked to the seasonality of its habitat. Mating occurs in the early dry season and females give birth and nurse their offspring in the wet season, when high quality food becomes abundant. During the mating season, 70% of the males (five out of seven) extremely elevated their HCC levels, presumably due to behavior associated with reproduction (e.g., high activity levels and aggressive behavior). This, however, is quite unusual for a monogamous species and could be an indicator for strong intrasexual competition for receptive females and the occurrence of extracopulations in *L. edwardsi*. Males of other promiscuous primates, such as mangabeys (*Lophocebus albigena*), also showed elevated fecal GC levels during the mating period. Here, males aggressively compete for females, and dominant males show higher fecal GC level than subordinates (Arlet et al., 2009). Group hierarchy often seems to affect GC levels of males in primates. For example, in the golden lion tamarins (*Leontopithecus rosalia*) mating does not affect fecal cortisol levels of reproducing dominant males and unrelated subordinate males (Bales et al., 2006). To avoid intrasexual aggression, subordinate *L. rosalia* males presumably suppress their androgen secretion and therefore reproduction to avoid aggressions from the dominant males (Bales et al., 2006).

Females of *L. edwardsi*, however, showed no significant changes in HCC among seasons, contrary to our expectations. However, this can be explained by a suppression of the HPA-axis, which might avoid detrimental effects of high HCC on the mother and her offspring (Reeder & Kramer, 2005). Furthermore, the circadian release of GCs is downregulated, which secures a stable energy supply to the fetus or young, as it limits the catabolic effects that are generally a result of surges in secretion (Brunton et al., 2008).

Conversely, the HCC of female *Callithrix jacchus* was significantly higher than that of males, however, independent of reproduction, potentially due to a combination of environmental and social stressors (Garber et al., 2020). Dominant female *C. jacchus* have a high reproductive output (e.g., can produce two litters per year) and often suppress the reproduction of subordinate females, which may result in higher HCC of dominant females (Garber et al., 2020). Two of our sampled *L. edwardsi* males did not show elevated HCC. Both males shared resting sites with another pair (male–female) or an already paired female during the dry season. These behavioral observations in combination with the low HCC levels, suggests that these two males were not reproductively active during the early dry season and could be the offspring from a previous breeding event.

In conclusion, *L. edwardsi* generally seem to have low HCC which are presumably only affected by higher activity due to reproductive behavior in males and not by seasonal changes associated with food availability and quality. Generally, there are a lot of inter and intraspecific sex-differences in HCC and the influence of sex on HCC seems to be inconsistent between species and additionally depends on other factors, such as age (Heimbürge et al., 2019). However, as elevated cortisol levels may impair the immune response of animals, this might in turn result in a higher susceptibility to parasites and diseases (Pruett, 2003).

## ACKNOWLEDGMENTS

This study was carried out under the “Accord de Collaboration” between Madagascar National Parks (MNP), the University of Antananarivo, and the Universität Hamburg, and all procedures comply with the current laws of Madagascar. The research was approved by the Direction du Système des Aires Protégées, Ministère de l'Environnement, the Université d'Antananarivo (Autorisation de recherche no. 305/17/MEEF/SG/DGF/DSAP/SCB.Re, no. 106/18/MEEF/SG/DGF/DSAP/SCB.Re and no. 086/19/MEDD/SG/DGF/DSAP/SCB.Re) and the Bundesamt für Naturschutz in compliance with CITES (no. DE-E-07783/17 and no. 1028C-EA12/MG18, no. DE-E-05323/18, no. DE-E-01666/19 and no. 497C-EA07/MG19). We acknowledge the authorization and support of this study by the Ministère de l'Environnement des Forêts, MNP and the University of Antananarivo. We thank MNP, J. B. Kennedy, T. Andrianasolo, J. Rakotondravony, S. Reher, A. Wulff and J. U. Ganzhorn for their collaboration and support. We thank the Dresden Lab Service GmbH and Prof. Dr. C. Kirschbaum und I. Kaden for performing the laboratory work. This study was funded by the Evangelisches Studienwerk Villigst e.V. and the Universität Hamburg. Open Access funding enabled and organized by Projekt DEAL.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## REFERENCES

- Acker, M., Mastrodonato, G., & Schulte-Hostedde, A. I. (2018). The effects of body region, season and external arsenic application on hair cortisol concentration. *Conservation Physiology*, 6(1), 1–9. <https://doi.org/10.1093/conphys/coy037>
- Arlet, M. E., Grote, M. N., Molleman, F., Isbell, L. A., & Carey, J. R. (2009). Reproductive tactics influence cortisol levels in individual male gray-cheeked mangabeys (*Lophocebus albigena*). *Hormones and Behavior*, 55(1), 210–216. <https://doi.org/10.1016/j.yhbeh.2008.10.004>
- Azevedo, A., Bailey, L., Bandeira, V., Fonseca, C., Wauters, J., & Jewgenow, K. (2021). Decreasing glucocorticoid levels towards the expansion front suggest ongoing expansion in a terrestrial mammal. *Conservation Physiology*, 9(1), 1–14. <https://doi.org/10.1093/conphys/coab050>
- Bales, K. L., French, J. A., McWilliams, J., Lake, R. A., & Dietz, J. M. (2006). Effects of social status, age, and season on androgen and cortisol levels in wild male golden lion tamarins (*Leontopithecus rosalia*). *Hormones and Behavior*, 49(1), 88–95. <https://doi.org/10.1016/j.yhbeh.2005.05.006>
- Barton, K. (2020). Package “MuMIn”: Multi-Model Inference (1.43.17). CRAN. <https://cran.r-project.org/package=MuMIn>
- Bethge, J., Razafimampandra, J. C., Wulff, A., & Dausmann, K. H. (2021). Sportive lemurs elevate their metabolic rate during challenging seasons and do not enter regular heterothermy. *Conservation Physiology*, 9(1), 1–15. <https://doi.org/10.1093/conphys/coab075>
- Bethge, J., Razafimampandra, J. C., Wulff, A., & Dausmann, K. H. (2022). Seasonal changes of the parasite prevalence of a small Malagasy lemur species (*Lepilemur edwardsi*). *Integrative Zoology*. <https://doi.org/10.1111/1749-4877.12647>
- Bethge, J., Wist, B., Stalenberg, E., & Dausmann, K. (2017). Seasonal adaptations in energy budgeting in the primate *Lepilemur leucopus*. *Journal of Comparative Physiology B*, 187(5–6), 827–834. <https://doi.org/10.1007/s00360-017-1082-9>
- Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Mächler, M., & Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, 9(2), 378. <https://doi.org/10.32614/RJ-2017-066>
- Brunton, P. J., Russell, J. A., & Douglas, A. J. (2008). Adaptive responses of the maternal hypothalamic-pituitary-adrenal axis during pregnancy and lactation. *Journal of Neuroendocrinology*, 20(6), 764–776. <https://doi.org/10.1111/j.1365-2826.2008.01735.x>
- Burnham, K. P., & Anderson, D. R. (2002). *Model Selection and Multimodel Inference* (K. P. Burnham & D. R. Anderson (Eds.); Issue 2). Springer. <https://doi.org/10.1007/b97636>
- Chapman, C. A., Schoof, V. A. M., Bonnell, T. R., Gogarten, J. F., & Calme, S. (2015). Competing pressures on populations: Long-term dynamics of food availability, food quality, disease, stress and animal abundance. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, 370, 20140112(1669). <https://doi.org/10.1098/rstb.2014.0112>
- Chesson, P., & Huntly, N. (1997). The roles of harsh and fluctuating conditions in the dynamics of ecological communities. *American Naturalist*, 150(5), 519–553. <https://doi.org/10.1086/286080>
- Craul, M., Chikhi, L., Sousa, V., Olivieri, G. L., Rabesandratana, A., Zimmermann, E., & Radespiel, U. (2009). Influence of forest fragmentation on an endangered large-bodied lemur in northwestern Madagascar. *Biological Conservation*, 142(12), 2862–2871. <https://doi.org/10.1016/j.biocon.2009.05.026>
- Dallman, M. F., Akana, S. F., Bhatnagar, S., Bell, M. E., Choi, S. J., Chu, A., Horsley, C., Levin, N., Meijer, O., Soriano, L. R., Strack, A. M., & Viau, V. (1999). Starvation: Early signals, sensors, and sequelae. *Endocrinology*, 140(9), 4015–4023. <https://doi.org/10.1210/endo.140.9.7001>
- Dearing, M. D. (2013). Temperature-dependent toxicity in mammals with implications for herbivores: A review. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, 183, 43–50. <https://doi.org/10.1007/s00360-012-0670-y>
- Fardi, S., Sauther, M. L., Cuzzo, F. P., Jacky, I. A. Y., & Bernstein, R. M. (2018). The effect of extreme weather events on hair cortisol and body weight in a wild ring-tailed lemur population (*Lemur catta*) in southwestern Madagascar. *American Journal of Primatology*, 80(2), 1–16. <https://doi.org/10.1002/ajp.22731>
- Ganzhorn, J. U. (1995). Low-level forest disturbance effects on primary production, leaf chemistry, and lemur populations. *Ecology*, 76(7), 2084–2096. <https://doi.org/10.2307/1941683>
- Ganzhorn, J. U. & Wright, P. C. (1994). Temporal patterns in primate leaf eating: The possible role of leaf chemistry. *Folia Primatologica*, 63(4), 203–208. <https://doi.org/10.1159/000156820>
- Gao, W., Stalder, T., Foley, P., Rauh, M., Deng, H., & Kirschbaum, C. (2013). Quantitative analysis of steroid hormones in human hair using a column-switching LC-APCI-MS/MS assay. *Journal of Chromatography B*, 928, 1–8. <https://doi.org/10.1016/j.jchromb.2013.03.008>
- Garber, P. A., McKenney, A., Bartling-John, E., Bicca-Marques, J. C., De la Fuente, M. F., Abreu, F., Schiel, N., Souto, A., & Phillips, K. A. (2020). Life in a harsh environment: The effects of age, sex, reproductive condition, and season on hair cortisol concentration in a wild non-human primate. *PeerJ (Corta Madera, CA and London)*, 8(e9365), 1–18. <https://doi.org/10.7717/peerj.9365>
- Haase, C. G., Long, A. K., & Gillooly, J. F. (2016). Energetics of stress: Linking plasma cortisol levels to metabolic rate in mammals. *Biology Letters*, 12, 20150867(1). <https://doi.org/10.1098/rsbl.2015.0867>
- Havenstein, N., Langer, F., Stefanski, V., & Fietz, J. (2016). It takes two to tango: Phagocyte and lymphocyte numbers in a small mammalian hibernator. *Brain, Behavior, and Immunity*, 52, 71–80. <https://doi.org/10.1016/j.bbi.2015.09.018>
- Havenstein, N., Langer, F., Weiler, U., Stefanski, V., & Fietz, J. (2021). Bridging environment, physiology and life history: Stress hormones in a small hibernator. *Molecular and Cellular Endocrinology*, 533(March), 111315. <https://doi.org/10.1016/j.mce.2021.111315>
- Heimbürge, S., Kanitz, E., & Otten, W. (2019). The use of hair cortisol for the assessment of stress in animals. *General and Comparative Endocrinology*, 270(September 2018), 10–17. <https://doi.org/10.1016/j.ygcen.2018.09.016>
- Ito, R., Rakotondraparany, F., & Sato, H. (2013). Non-flying mammalian fauna of Ampijoroa, Ankarafantsika National Park. *Madagascar Conservation & Development*, 8(1), 4–8. <https://doi.org/10.4314/mcd.v8i1.7>
- Johnson, E. O., Kamilaris, T. C., Chrousos, G. P., & Gold, P. W. (1992). Mechanisms of stress: A dynamic overview of hormonal and behavioral homeostasis. *Neuroscience and Biobehavioral Reviews*, 16(2), 115–130. [https://doi.org/10.1016/S0149-7634\(05\)80175-7](https://doi.org/10.1016/S0149-7634(05)80175-7)
- Kirschbaum, C., Tietze, A., Skoluda, N., & Dettenborn, L. (2009). Hair as a retrospective calendar of cortisol production-increased cortisol incorporation into hair in the third trimester of pregnancy. *Psychoneuroendocrinology*, 34(1), 32–37. <https://doi.org/10.1016/j.psyneuen.2008.08.024>
- Klein, A., Zimmermann, E., Radespiel, U., Schaarschmidt, F., Springer, A., & Strube, C. (2018). Ectoparasite communities of small-bodied Malagasy primates: Seasonal and socioecological influences on tick, mite and lice infestation of *Microcebus murinus* and *M. ravelobensis* in northwestern Madagascar. *Parasites and Vectors*, 11(1), 1–18. <https://doi.org/10.1186/s13071-018-3034-y>
- Lavergne, S. G., Peers, M. J. L., Mastrodonato, G., Majchrzak, Y. N., Nair, A., Boutin, S., & Boonstra, R. (2020). Hair cortisol as a reliable

- indicator of stress physiology in the snowshoe hare: Influence of body region, sex, season, and predator-prey population dynamics. *General and Comparative Endocrinology*, 294(January), 113471. <https://doi.org/10.1016/j.ygcen.2020.113471>
- Lebl, K., Bieber, C., Adamik, P., Fietz, J., Morris, P., Pilaastro, A., & Ruf, T. (2011). Survival rates in a small hibernator, the edible dormouse: A comparison across Europe. *Ecography*, 34(4), 683–692. <https://doi.org/10.1111/j.1600-0587.2010.06691.x>
- Lenth, R. V., Buerkner, P., Herve, M., Love, J., Miguez, F., Riebl, H., & Singmann, H. (2021). Package "emmeans": Estimated Marginal Means (1.7.1-1). CRAN. <https://doi.org/10.1080/00031305.1980.10483031%3ELicense>
- Louis, E. E., Bailey, C. A., Sefczek, T. M., Raharivololona, B., Schwitzer, C., Ratsimbazafy, J., Wilmet, L., & Borgerson, C. (2020). *Lepilemur edwardsi*. The IUCN Red List of Threatened Species.
- Lovegrove, B. G. (2000). The zoogeography of mammalian basal metabolic rate. *The American Naturalist*, 156(2), 201–219. <https://doi.org/10.1086/303383>
- McNab, B. K. (2008). An analysis of the factors that influence the level and scaling of mammalian BMR. *Comparative Biochemistry and Physiology—A Molecular and Integrative Physiology*, 151(1), 5–28. <https://doi.org/10.1016/j.cbpa.2008.05.008>
- Moberg, G. P. & Mench, J. A. (Eds.). (2000). *The biology of animal stress—Basic principles and implications for animal welfare*. CABI Publishing.
- Mittermeier, R. A., Louis, E. E., Richardson, M., Schwitzer, C., Langrand, O., Rylands, A. B., Hawkins, F., Rajaobelina, S., Ratsimbazafy, J., Rasoloarison, R. M., Roos, C., Kappeler, P. M., & Mackinnon, J. (2010). *Lemurs of Madagascar (Third Edit)*. *Conservation International*. <http://books.google.com/books?id=cn46YgECAAJ%26pgis=1>
- Mormède, P., Andanson, S., Aupérin, B., Beerda, B., Guémené, D., Malmkvist, J., Manteca, X., Manteuffel, G., Prunet, P., van Reenen, C. G., Richard, S., & Veissier, I. (2007). Exploration of the hypothalamic-pituitary-adrenal function as a tool to evaluate animal welfare. *Physiology and Behavior*, 92(3), 317–339. <https://doi.org/10.1016/j.physbeh.2006.12.003>
- Pruett, S. B. (2003). Stress and the immune system. *Pathophysiology*, 9(3), 133–153. [https://doi.org/10.1016/S0928-4680\(03\)00003-8](https://doi.org/10.1016/S0928-4680(03)00003-8)
- R Core Team. (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing. <https://www.r-project.org/>
- Rakotoniaina, J. H., Kappeler, P. M., Kaesler, E., Hämäläinen, A. M., Kirschbaum, C., & Kraus, C. (2017). Hair cortisol concentrations correlate negatively with survival in a wild primate population. *BMC Ecology*, 17(1), 1–13. <https://doi.org/10.1186/s12898-017-0140-1>
- Rakotoniaina, J. H., Kappeler, P. M., Ravoniarimbinina, P., Pechouskova, E., Hämäläinen, A. M., Grass, J., Kirschbaum, C., & Kraus, C. (2016). Does habitat disturbance affect stress, body condition and parasitism in two sympatric lemurs? *Conservation Physiology*, 4(1), cow034. <https://doi.org/10.1093/conphys/cow034>
- Ralph, C. R. & Tilbrook, A. J. (2016). Invited review: The usefulness of measuring glucocorticoids for assessing animal welfare. *Journal of Animal Science*, 94(2), 457–470. <https://doi.org/10.2527/jas.2015-9645>
- Randrianambinina, B., Mbotizafy, S., Rasoloharijaona, S., Ravoahangimalala, R. O., & Zimmermann, E. (2007). Seasonality in reproduction of *Lepilemur edwardsi*. *International Journal of Primatology*, 28(4), 783–790. <https://doi.org/10.1007/s10764-007-9158-0>
- Reeder, D. A. M. & Kramer, K. M. (2005). Stress in free-ranging mammals: Integrating physiology, ecology, and natural history. *Journal of Mammalogy*, 86(2), 225–235. <https://doi.org/10.1644/BHE-003.1>
- Romero, L. M. (2004). Physiological stress in ecology: Lessons from biomedical research. *Trends in Ecology and Evolution*, 19(5), 249–255. <https://doi.org/10.1016/j.tree.2004.03.008>
- Ruf, T., Fietz, J., Schlund, W., & Bieber, C. (2006). High survival in poor years: Life history tactics adapted to mast seeding in the edible dormouse. *Ecology*, 87(2), 372–381. <https://doi.org/10.1890/05-0672>
- Russell, E., Koren, G., Rieder, M., & Van Uum, S. (2012). Hair cortisol as a biological marker of chronic stress: Current status, future directions and unanswered questions. *Psychoneuroendocrinology*, 37(5), 589–601. <https://doi.org/10.1016/j.psyneuen.2011.09.009>
- Sapolsky, R. M. (1986). Endocrine and behavioral correlates of drought in wild olive baboons (*Papio anubis*). *American Journal of Primatology*, 11(3), 217–227. <https://doi.org/10.1002/ajp.1350110303>
- Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews*, 21(1), 55–89. <https://doi.org/10.1210/er.21.1.55>
- Schmid, J. & Ganzhorn, J. U. (1996). Resting metabolic rates of *Lepilemur ruficaudatus*. *American Journal of Primatology*, 38(2), 169–174. [https://doi.org/10.1002/\(SICI\)1098-2345\(1996\)38:2%3C169::AID-AJP5%3E3.0.CO;2-X](https://doi.org/10.1002/(SICI)1098-2345(1996)38:2%3C169::AID-AJP5%3E3.0.CO;2-X)
- Spencer, R. L. & Deak, T. (2017). A users guide to HPA axis research. *Physiology & Behavior*, 178(3), 43–65. <https://doi.org/10.1016/j.physbeh.2016.11.014>
- Spiga, F., Walker, J. J., Terry, J. R., & Lightman, S. L. (2014). HPA axis-rhythms. *Comprehensive Physiology*, 4(3), 1273–1298. <https://doi.org/10.1002/cphy.c140003>
- Steffens, T. S. & Lehman, S. M. (2018). Lemur species-specific metapopulation responses to habitat loss and fragmentation. *PLoS One*, 13(5), 1–26. <https://doi.org/10.1371/journal.pone.0195791>
- Triki, Z., Grutter, A. S., Bshary, R., & Ros, A. F. H. (2016). Effects of short-term exposure to ectoparasites on fish cortisol and hematocrit levels. *Marine Biology*, 163(9), 1–6. <https://doi.org/10.1007/s00227-016-2959-y>
- Varpe, Ø. (2017). Life history adaptations to seasonality. *Integrative and comparative biology*, 57(5), 943–960. <https://doi.org/10.1093/icb/ixc123>
- Warren, R. D. & Crompton, R. H. (1997). A comparative study of the ranging behaviour, activity rhythms and sociality of *Lepilemur edwardsi* (primates, lepilemuridae) and *Avahi occidentalis* (primates, indriidae) at Ampijoroa, Madagascar. *Journal of Zoology*, 243(2), 397–415. <https://doi.org/10.1111/j.1469-7998.1997.tb02790.x>
- Wickham, H. (2009). *ggplot2*. Springer. <https://doi.org/10.1007/978-0-387-98141-3>
- Wickham, H. (2011). The split-apply-combine strategy for data analysis. *Journal of Statistical Software*, 40(1), 1–29.
- Wickham, H., Francois, R., Henry, L., & Müller, K. (2021). Package "dplyr": A Grammar of Data Manipulation (1.0.7). CRAN. <https://cran.r-project.org/package=dplyr>

## SUPPORTING INFORMATION

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**How to cite this article:** Bethge, J., Fietz, J., Razafimampandra, J. C., Ruthsatz, K., & Dausmann, K. H. (2022). Season and reproductive activity influence cortisol levels in the Malagasy primate *Lepilemur edwardsi*. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, 1–8. <https://doi.org/10.1002/jez.2658>