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Linking glucocorticoid variations to monthly and daily behavior in a wild endangered neotropical primate

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Abstract

Identifying the factors swaying physiological stress levels in wild animals can help depict how they cope with environmental and social stressors, shedding light on their feeding ecology, behavioral plasticity, and adaptability. Here, we used noninvasive methods to explore the link between glucocorticoid levels and behavior in an endangered neotropical primate facing habitat fragmentation pressure, the black lion tamarin (*Leontopithecus chrysopygus*). We investigated monthly and day-to-day glucocorticoid variations independently to attempt to disentangle the complex nature of the adrenocortical activity. Between May 2019 to March 2020, we followed two groups of black lion tamarins in two different areas, a continuous forest and a small fragment, and gathered behavioral data (over 95 days in total; 8.6 ± 3.9 days/month) and fecal samples ($N_{samples}$ = 468; 4.93 ± 3.5 samples/day) simultaneously. Preliminary analyses enabled us to identify circadian variations linked to the biological rhythm, which were taken into account in subsequent models. Monthly analyses revealed that black lion tamarin fecal glucocorticoid metabolite levels vary according to changes in activity budget associated with the fruit consumption, movement, and resting time of the groups. At a day-to-day level, while intergroup encounters led to increases in fecal glucocorticoid metabolite concentrations, we found that changes in food intake or activity level did not trigger physiological stress responses. These findings suggest that diet and ranging patterns, driven by food availability and distribution, influence physiological stress at a seasonal scale, while acute stressors such as interspecific competition trigger short-term stress responses. Exploring fecal glucocorticoid metabolite variations over different timescales can help uncover the predictive and reactive facets of physiological stress in wild species. Moreover, having a comprehensive understanding of the physiological state of species is a valuable conservation tool for evaluating how they cope in changing environments.

KEYWORDS
black lion tamarin, cortisol, diet, energetics, fecal glucocorticoid metabolites, physiological stress

Abbreviations: fGM, fecal glucocorticoid metabolites; GC, glucocorticoids; HPA, hypothalamic–pituitary–adrenocortical.

1 | INTRODUCTION

Understanding how environmental and social factors influence animals' physiological states is a key question of behavioral endocrinology and ecology. In the wild, animals deploy behavioral and physiological responses to overcome both abiotic and biotic challenges (Kamilar & Beaudrot, 2018; Wingfield, 2013). To maintain homeostasis, vertebrates possess broad physiological artillery, including stress responses (Romero, 2004; Wingfield et al., 1997). The perception of a stressor first activates the sympathetic nervous system, leading to the secretion of catecholamines (i.e., epinephrine and norepinephrine), and then stimulates the hypothalamic–pituitary–adrenocortical (HPA) axis, which thenceforth triggers the secretion of glucocorticoids (GCs). The primary function of GCs, which are often labeled *stress hormones*, is to mediate energetic demands necessary to overcome predictable and unpredictable environmental and social challenges (Sapolsky et al., 2000). Identifying how and which stressors affect GC levels is a fundamental aspect of conservation physiology that ultimately helps to understand how a species will respond to a changing environment. This is particularly relevant for endangered species such as the endangered black lion tamarin (*Leontopithecus chrysopygus*), a small neotropical primate living in the fragmented Atlantic Forest of Brazil (Culot et al., 2015; Rezende et al., 2020).

Biologically, an increase in GC concentrations regulates immune function, mobilizes stored energy, promotes escape behaviors, suppresses nonvital activities such as social interactions or reproduction, and also enhances the effects of catecholamines, which, in turn, induce the fight or flight response (Sapolsky et al., 2000; Wingfield, 2005; Wingfield et al., 1997). As described in Romero's reactive scope model, an increase in GC levels is not always synonymous with a short-term response to an acute stressor since GC levels are also expected to vary within a normal circadian and seasonal range (Figure 1), defined as predictive homeostasis (Romero et al., 2009). Throughout the day, GC levels follow a specific circadian rhythm governed by photoperiod and a species' activity period. In diurnal species, GC concentrations are expected to increase in the morning, characterizing the cortisol awakening response (Figure 1; Fries et al., 2009). At a seasonal scale, GC levels also vary to withstand predictable changes linked to resource availability, reproduction, seasonal climate, or photoperiod (Romero, 2002). Concurrently with predictable GC variations, a sharp increase in GC levels due to an unpredictable stressor (e.g., predation, unexpected harsh weather, injury, aggression) is called a stress response, defining reactive homeostasis (Figure 1; Wingfield, 2005). This acute elevation of GC levels is rapidly autoregulated through negative feedback and baseline levels are restored. These baseline levels (i.e., the starting point of a stress response) vary according to the predictable circadian

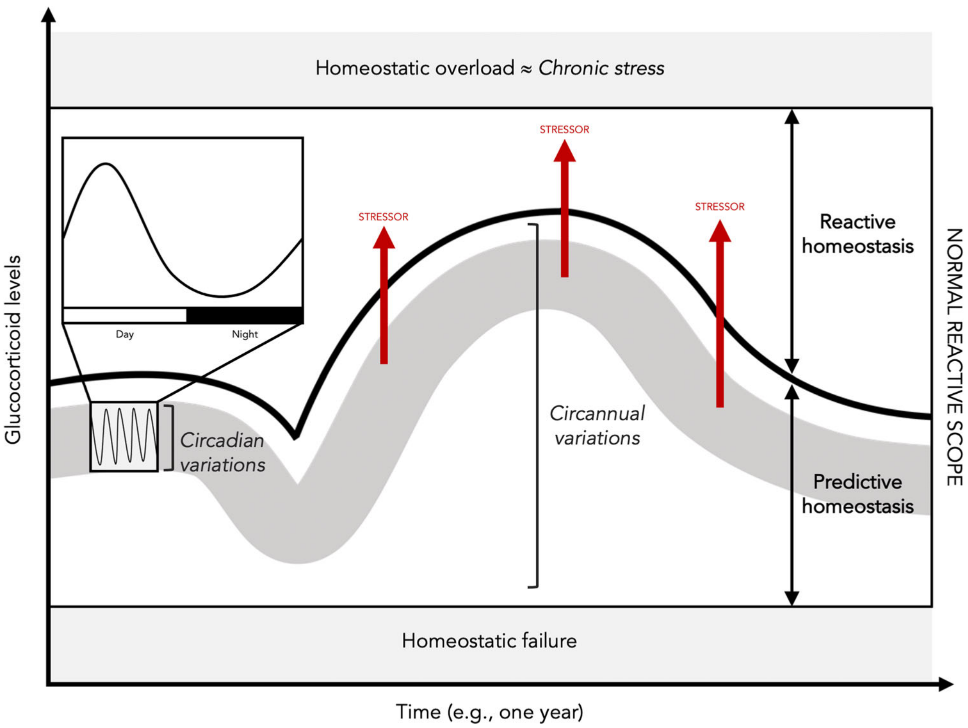

FIGURE 1 Graphical model depicting theoretical glucocorticoid (GC) variations in an individual over a year. Within the predictive homeostasis range, GC levels vary depending on the time of day (i.e., circadian variation) and season (i.e., circannual variations). Throughout the year, an unpredictable challenging stressor (in red) may trigger a stress response, representing the reactive homeostasis range. Together, the predictive and reactive homeostasis ranges compose the normal reactive scope of an individual. Homeostatic failure occurs when GC levels are too low to maintain homeostasis. At the opposite extreme, homeostatic overload arises when GC levels surpass a threshold at which they start having detrimental effects on an individual. Figure and terminology modified from Romero et al. (2009).

and circannual rhythms. Together, predictive homeostasis and reactive homeostasis constitute the normal reactive scope of an organism (Figure 1; Romero et al., 2009). Impeded by the difficulty of deconstructing the complex mechanisms underlying physiological stress in an uncontrolled and varying environment, to our knowledge, no studies to date have managed to explore simultaneously the different elements of the reactive scope model and behavior in a wild primate species.

Over the past two decades, the development of noninvasive methods to monitor physiological markers has considerably increased the number of studies in the wild (Palme, 2019; Sheriff et al., 2011). GCs released into the plasma, via the activation of the HPA axis, are metabolized by the liver and their metabolites end up in urine, passing through the kidneys, or are excreted in feces via the gut (Möstl & Palme, 2002). Fecal GC metabolites (fGMs) reveal information on an animal's short-term adrenocortical activity, over a distinct amount of time, specific to each species. Indeed, the excretion of GC metabolites in the feces is governed by two different timelines. First, an explicit time elapses between the secretion of GCs into the bloodstream by the adrenal glands and the transit of the metabolites into the feces via the liver (Palme et al., 1996). Second, metabolite levels measured in feces represent a cumulative secretion of these biomarkers over a certain time lag, depending on the gut passage time and defecation rate of the species (Touma et al., 2003). Hence, to establish if changes in plasma GCs are accurately measured in the feces and to determine the time lag between variations in circulating GCs and detection in the feces, it is crucial to perform a physiological or biological validation (Palme, 2019). Defining this time lag provides essential knowledge of a species biology, allowing researchers to calibrate and align the behavioral and hormonal timeframes as well as consider circadian variations linked to the biological rhythm of individuals. Surprisingly, the number of studies without appropriate validation remains high in primate research (Kaisin et al., 2021; Palme, 2019).

Numerous studies have set out to comprehend the complex relation between variations in GC levels on the one hand and environmental and social factors faced by primates in the wild on the other. Across a wide diversity of primate species, scientists have explored changes in adrenocortical activity associated with hierarchy, social behavior, parasite prevalence, environmental, and ecological factors, as well as anthropogenic disturbances (Beehner & Bergman, 2017; Kaisin et al., 2021). Given the energy metabolizing function of GCs, higher levels of these hormones have repeatedly been associated with nutritional stress, low food availability, poor diet quality, and energy expenditure. Multiple studies compared monthly GC levels to identify seasonal trends. For instance, a study on forest-living olive baboons (*Papio anubis*) revealed that lower food availability may require the mobilization of stored energy, demonstrated by higher GC levels (MacLarnon et al., 2015). Similar trends (i.e., higher GC levels during the season with low fruit availability) were also observed in yellow baboons (*Papio cynocephalus*; Gesquiere et al., 2008), red colobus monkeys (*Piliocolobus tephrosceles*; Chapman et al., 2006), black howler monkeys (*Alouatta pigra*; Behie et al., 2010), spider monkeys (*Ateles geoffroyi*; Ordóñez-Gómez et al., 2016), and ring-tailed lemurs (*Lemur catta*; Pride, 2005). Furthermore, studies on chimpanzees (*Pan troglodytes*; Emery Thompson et al., 2010; Muller & Wrangham, 2004), Sykes' monkeys (*Cercopithecus mitis*; Foerster & Monfort, 2010), and red colobus monkeys (Chapman et al., 2007) discovered that poor diet quality or low availability of favored food resources yielded higher GC levels as well. Conversely, a study found that the amount of food intake did not influence fGM levels in howler monkeys, suggesting that diet may not impact generalist species that exploit different food items (Martínez-Mota et al., 2016). Regarding energy expenditure, Dunn et al. (2013) showed that travel time predicted fGM levels in mantled howler monkeys (*Alouatta palliata*). Collectively, these studies highlight the substantial potential of GCs as indicators of nutritional and energetic stress. Accordingly, measuring GC levels can provide precious insight on primate energetics, understanding the cascading effects of food availability on diet quality, energy intake, foraging effort, and energy expenditure (Emery Thompson, 2017). Evaluating physiological stress side by side with behavior helps to depict how wild primates cope with environmental and social stressors, highlighting their degree of adaptability, and behavioral plasticity, as well as the potential consequences on their health and fitness.

Climatic factors may also influence physiological stress in primates (MacLarnon et al., 2015). Extreme cold or hot temperatures as well as extreme drought may potentially represent thermoregulatory stressors and trigger significant GC increases (Beehner & McCann, 2008; Chowdhury et al., 2021; Guo et al., 2018; MacLarnon et al., 2015; McFarland et al., 2014). Moreover, rainfall has also been shown to indirectly affect GC levels due to its influence on food availability, causing nutritional stress in various species (Behie et al., 2010; Gesquiere et al., 2008; Rangel-Negrín et al., 2009).

Shifting perspective to a shorter timeframe, from months to days, studies have documented increases in GC levels linked to acute stressors, sparking an ephemeral stress response. Indeed, a surge in fGM levels has been observed in primates after captures in the wild (Aguilar-Cucurachi et al., 2010; Hämäläinen et al., 2014), predation attempts (Wasserman et al., 2013), intergroup aggressions (Young et al., 2014), or death of kin (Engh et al., 2006). In spider monkeys, direct human disturbances, such as days of logging and hunting, also induced a short-term rise in GCs (Ordóñez-Gómez et al., 2016). Given the multiple aspects and abundance of factors influencing of physiological stress, adopting a holistic approach by exploring GC variations at hourly, daily, and monthly scales can aid in better understanding the predictive and reactive dimensions of adrenocortical activity in a wild primate.

In this study, for the first time, we explore the link between behavior and physiological stress responses of a wild and endangered callitrichid using noninvasive techniques. By gathering both behavioral data and fecal samples, we explored whether monthly (a) and daily (b) variations in activity budget and weather conditions influenced GC levels in black lion tamarins, small frugivorous and faunivorous primates endemic to the State of São Paulo. To explore how black lion tamarins cope and adapt their behavior in changing habitats, we measured physiological stress responses in two groups living in different areas: a continuous forest and a small 100-ha fragment. Looking at GC variations over two different timescales enabled us to identify the factors influencing both reactive and predictive homeostasis, respectively, in lion tamarins. Before our analyses, we tested for sex and age-class differences to appropriately consider for potential variations. We also conducted a series of preliminary analyses on hourly GC variations to take into account predictable circadian GC fluctuations linked to the lion tamarin's normal biological rhythm. In the daily models, we also accounted for predictable variations between months in order solely to extract significant day-to-day changes in adrenocortical activity.

(a) Across the year, we expected GC levels to increase during the dry season. First, considering the energy-regulating role of GCs and previous studies on primates, we anticipated fruit consumption (i.e., a proxy of energy intake) to decrease during the dry season, which is characterized by a lower fruit availability (Staggemeier et al., 2017), causing nutritional stress mirrored by higher GC levels. Since diet quality itself may influence GC levels, we also looked into the different fruit species consumed by the black lion tamarin groups to further understand the influence of fruit consumption on fGM levels. Depending on resource availability and distribution, we also expected the balance between movement, foraging, and resting time to vary between months and influence the lion tamarins' activity level (i.e., a proxy of energy expenditure). Accordingly, we expected an increase in movement and a decrease in resting time to lead to higher GC levels. Since black lion tamarins also feed on arthropods and small vertebrates, we predicted an increase in prey foraging effort, linked to a lower fruit availability (Keuroghlian & Passos, 2001), to be associated with elevated GC levels. Finally, climatic variables (i.e., temperature and rainfall) may also influence resource availability and perhaps thermoregulation (Chaves et al., 2019; Touitou et al., 2021). During the dry season, characterized by lower temperatures and rainfall, extra energy may be necessary to overcome periods of food scarcity and potentially to maintain body temperature, resulting in an increase in GC levels.

(b) Across the day, above and beyond seasonal monthly variations, we anticipated GC levels to increase with metabolically challenging events associated with energy demand (e.g., foraging and traveling) and/or social challenge (e.g., intergroup encounters). Extreme daily rainfall or temperatures may also be linked to increases in GC levels due to energy demanding thermoregulation or impaired behavior caused by unpredictable weather.

Combining behavioral and physiological data, and exploring variations over two distinct timescales, will shed light on how activity budget, diet, and weather conditions influence GC levels and help identify the environmental and social stressors affecting black lion tamarins.

2 | MATERIALS AND METHODS

This study was conducted completely noninvasively and with the relevant authorizations from the Sistema de Autorização e Informação em Biodiversidade (SISBIO; N°68253-4), the Comissão Técnico-Científica do Instituto Florestal (COTEC; N°153/2021 D28/2021 PH), and the ethics committee of the São Paulo State University (UNESP; N°16/2019).

2.1 | Study sites and groups

This research was conducted between May 2019 and March 2020 in two notably different study sites within the state of São Paulo in Brazil: the Morro do Diabo State Park (MDSP; 33,845 ha) and a private fragment in the municipality of Guareí (100 ha; Figure 2). MDSP is the largest Atlantic Forest remnant in western São Paulo state and hosts to the largest population of black lion tamarins, approximately 1200 individuals (Rezende et al., 2020). It is located in the Pontal do Paranapanema region and, other than an imposing hill culminating at 600 m, has an altitude ranging between 250 and 450 m. During the study period, the mean temperature in MDSP was 25.3°C, and the total rainfall was of 1065.6 mm (Agritempo, 2022; TRMM 1018). The Guareí fragment is located in the municipality of Guareí, which is of the Alto Paranapanema region, where Atlantic Forest fragments only represent 23.6% of the total area (Pinto, 2017). The fragment has a mean elevation of 680 m and is surrounded by a highly fragmented matric of pastures and sugar cane and eucalyptus plantations (Figure 2). The mean temperature and total rainfall during the study period were of 21.4°C and 912 mm, respectively (Agritempo, 2022; TRMM 892). Monthly rainfall and temperature ranges (mean, maximum, and minimum) are available for Guareí and MDSP as Supporting Information: Appendix S1). All temperature and rainfall data used in this study were based on Tropical Rainfall Measuring Mission (TRMM) satellite data.

We studied a group of fully habituated wild black lion tamarins in each study site. This period encompassed both the cold dry (April to September) and hot rainy (October to March) seasons in southeast Brazil. When we started our observations, the MDSP group comprised eight individuals: four adult males, two adult females, and two juveniles (sex unidentified). In August 2019, two adult males and one adult female emigrated from that group and we continued to follow the group of five individuals with the two juveniles. During the study period, the MDSP group had intergroup encounters with one group at the north of their home range. In Guareí, the group initially comprised five individuals, two adult males and three adult females. In January 2020, after the birth of two infants in September 2019, the group split into two new groups. We continued following the group that retained the original home range, which comprised two adult males, one adult female, and one adult individual whose sex could not be identified. In Guareí, the group shared the fragment with at least two other groups, to the south and to the west of the fragment, and another group to the northwest that occasionally

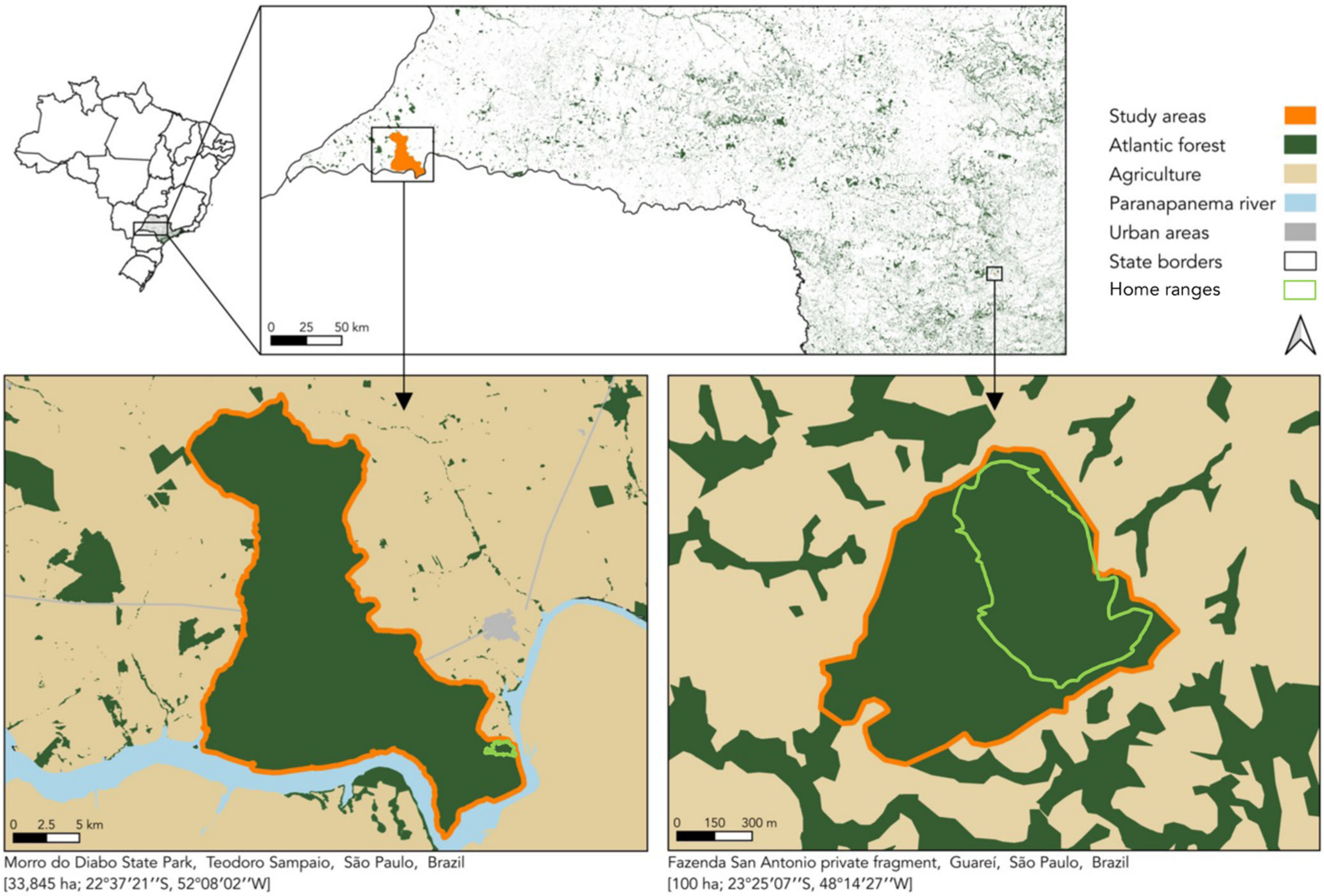

FIGURE 2 Location of the two study sites, the Morro do Diabo State Park (MDSP) (left) and Guareí (right), within the State of São Paulo, including the surrounding land use and the home range of both groups estimated using the Time Local Convex Hull method.

crossed from a neighboring fragment. Overall, the groups of MDSP and Guareí showed noteworthy differences in activity budget (see Supporting Information: Appendix S2), diet, and ranging patterns. The groups' average active day length (i.e., from sleeping site to sleeping site) was greater in MDSP (11h07 ± 58 min) than in Guareí (9h09 ± 53 min). The group in MDSP also used an area considerably larger than in Guareí, 99 versus 40 ha, respectively.

2.2 | Behavioral data and fecal sample collection

In both sites, we followed the groups throughout their activity period and gathered behavioral data using scan sampling at 5-min intervals (Altmann, 1974). We followed the groups during full days, from sleeping site to sleeping site, and recorded the behavior of every visible individual at scan time. We gathered behavioral data in Guareí and MDSP for 9 and 8 months, following the lion tamarins during 5.44 ± 2.45 and 5.75 ± 2.44 full days per month, respectively. In total, throughout the study period, we followed the MDSP and Guareí group for 46 and 49 full days, respectively, covering both the dry and rainy seasons (Table 1; a monthly distribution of full days and fecal sample collection is available as Supporting Information: Appendix S3). Behavioral observations were categorized into mutually exclusive behaviors: feeding (i.e., chewing, ingesting, manipulating food items), traveling (i.e., movement from one tree to another or within a tree), foraging for animal prey (i.e., active search for animal prey in lianas, under bark, inside tree hollows, under palm tree sheath, etc.), resting (i.e., laying down or sleeping on a branch), and other (i.e., grooming, inactive, and intergroup encounters). An intergroup encounter is an event during which the face-to-face presence of a neighboring group induces a chase, fight, and/or vocalization from the study group. Individuals are considered to be inactive when they are static and not displaying any noticeable behavior. Grooming is a behavior involving at least two individuals in which a group member cleans or maintains another member's body/fur by removing dirt, dead skin, or parasites. For feeding behaviors, we also identified the food category of the items consumed (e.g., fruit, invertebrate, vertebrate, gum) as well as the species for fruiting trees. We estimated the contribution of plant species in the diet of the black lion tamarin groups by calculating the percentage of scans spent feeding on fruits from different tree species.

Despite knowing the individuals of each group, due to the limited visibility in tropical forests and the lack of dimorphism in this species, it was not possible to systematically identify individuals during scan observations. However, because black lion tamarins live in small

TABLE 1 Fecal sample and behavioral data sampling effort in the Morro do Diabo State Park (MDSP) and Guareí.

Site	Full days		Hours	Scans	Individuals/scan	Fecal samples	Samples/day
MDSP	Rainy	25	160	1924	3.2 ± 1.3	127	6.4 ± 3.5
	Dry	21	109	1304	4.2 ± 1.5	75	3.8 ± 2.4
Guareí	Rainy	19	151	1806	2.8 ± 1.1	51	4.3 ± 2.9
	Dry	30	237	2846	3 ± 1.2	215	6.9 ± 3.7

monogamous or polyandrous groups with high intragroup cohesion, characterized by synchronization of their activities and homogenization of their diets (Dixson, 2012; Kleiman & Rylands, 2002; Valladares-Padua, 1993), the activity level and food intake of group members are likely to be similar. Taking this into consideration, we estimated group activity budgets by calculating a percent of daily and monthly scan observations for each behavioral category. Data was not collected on infant individuals. All analyses were performed using behavioral data gathered during full days.

While following the lion tamarins, we also collected fecal samples in 10 mL polypropylene containers immediately after defecation. The samples were directly stored on ice in a thermal bag before being stored at −20°C within 10–12 h of the collection time. For every sample, we noted the date, time, group, and the sex and age of the individual when possible. However, it was not possible to systematically identify individuals when gathering fecal samples. Therefore, fecal samples from the same day were not necessarily independent. A total of 468 fecal samples were collected, 202 in MDSP and 266 in Guareí (Table 1).

2.3 | Steroid analysis

Before extraction, fecal samples were thawed at ambient temperature and then dried at 70°C in a laboratory oven for ±24 h. Once dried, the samples were pulverized using a mortar and pestle and sieved to remove all solid inert materials (e.g., seeds and undigested fibers). A subsample of 0.05 g from each sample was suspended in 1 mL of 80% methanol for extraction. Samples were then vortex-mixed (1000 rpm, 30 min) and centrifuged (14,000 rpm, 5 min). Aliquots of 0.5 mL of the supernatant were transferred to plastic microtubes and dried in a dry bath coupled to an airflow (70°C, ±4 h). The stable dried-down extracts were then shipped to the University of Veterinary Medicine of Vienna, Austria, for further analysis.

In Austria, the dried-down extracts were resuspended in 80% methanol and dissolved using an ultrasonic bath. We quantified fGM concentrations using a homemade cortisol enzyme immunoassay (EIA), measuring fGMs with a 11β,17α,21-triol-20-one structure, according to the procedures described in Palme and Möstl (1997). Cross-reactivity for this assay was reported as 100% for cortisol, 6.2% for corticosterone, 4.6% for 5α-dihydrocortisol, 0.8% for allotetrahydrocortisol, 0.1% for tetrahydrocortisol, and <0.01 for other relevant compounds (Palme & Möstl, 1997). This EIA has been physiologically validated using an adrenocorticotropic hormone (ACTH) challenge on captive black lion tamarins at the Primate Centre of Rio de Janeiro (Bertoli et al., 2019), revealing that the average latency time to witness a peak in fGM after an ACTH injection was between 20 and 25 h. Therefore, since fGM excretion is governed by two different timelines, it is estimated that there is an initial 20–25 h time-lag until GC variations are detected in the collected feces. Second, due to the short gut-passage time in our species (i.e., 92.8 ± 56.1 min, unpublished data), fGM measured in the samples reflect GC levels accumulated during a short timeframe. Sensitivity of the EIA used in this study was 0.33 pg/well. Inter-assay coefficients of variation (CVs), assessed by running a two high and a two low-level quality control in each assay, were 9.3% (high) and 9.5% (low). All samples were analyzed in duplicates. The mean intra-assay CV for control samples was 1.92 ± 1.89% (N_{plates} = 15). All sample duplicates with intra-assay CV > 10% were re-analyzed to obtain a CV < 10%.

2.4 | Statistical analysis

Given the distinct behavioral differences between the two groups (i.e., activity budget, diet, and day length), we explored temporal GC variations for both sites independently, allowing us to make well-founded group and site-specific interpretations and discuss potential between-site differences. We conducted all statistical analyses using R 3.6.1 with a significance level of $\alpha = 0.05$. To achieve a normal distribution, fGM concentrations were log-transformed for all statistical analyses (Shapiro–Wilk test: $W = 0.99$, $p = 0.212$). We ran linear mixed models (LMMs) to explore the effect of both monthly and daily behavior on fGM variations. Before constructing our LMMs, we ran preliminary analyses to verify if we had an effect of sex and age-class as well as circadian variations. Based on the results of these initial analyses, we controlled for these potential confounding factors in our models. Before constructing our models, all variables were standardized (i.e., rescaled to have a mean of 0 and a standard deviation of 1). All models were fitted using the restricted maximum likelihood (REML) method and the best models were selected using the Akaike information criterion (AIC), which measures the relative quality of models (Burnham & Anderson, 1998). The best model is the one with the lowest AIC, representing the one that best predicts the values of the response variable with as few predictors possible. We tested for multicollinearity using the variance inflation factor (VIF) for each model. All variables included in the models had a VIF < 5 (James et al., 2013; Menard, 2002). To determine the estimate and standard error of every variable, we ran 1000 bootstrap iterations of the best models. Using this random resampling method, we accounted for the fact that the fGM samples were not necessarily independent as feces were not systematically associated with an individual (Fieberg et al., 2020).

2.4.1 | Preliminary analyses

Sex and age-class differences

As mentioned in the methods, identifying individuals was difficult due to low visibility in tropical forests and the lack of dimorphism and small size of black lion tamarins. Therefore, the following analyses were performed on a smaller subset of samples for which sex or age-class was confirmed. Since no significant differences ($U = 28{,}504$, $p = 0.26$) were observed between mean fGM levels in Guareí ($N_{samples} = 266$) and MDSP ($N_{samples} = 202$), we pooled individuals from both sites to explore the effect of the sex and age-class on fGM levels. Using adult individuals only, we compared mean fGM levels between males ($N_{samples} = 19$) and females ($N_{samples} = 5$) with a Mann–Whitney *U* test and found no significant difference ($U = 29$, $p = 0.21$). Consistently, no significant difference was identified between juvenile ($N_{samples} = 20$) and adult ($N_{samples} = 76$) fGM levels ($U = 753$, $p = 0.95$). Boxplots for these analyses are available as Supporting Information: Appendix S4. Since fGM concentrations did not differ significantly with sex and age-class, we pooled all samples in subsequent analyses.

Group fission was observed in both sites during the study period, but no clear trend was observed regarding changes in mean group GC levels. Levels decreased in MDSP while they slightly increased in Guareí.

Circadian variations

Given an animal's normal circadian rhythm, GC concentrations are expected to vary throughout the day as part of predictive homeostasis. Based on the latency period defined by the physiological validation (i.e., 20–25 h; Bertoli et al., 2019), we considered the fGM concentrations of a fecal sample to represent GC levels at approximately the same period as defecation but the previous day. Hence, to explore mean hourly variations in GC levels, we assigned an hour time slot to each sample, corresponding to the hour of defecation. Since no significant differences in fGM levels were observed between males and females and the different age-classes, we pooled all samples per hour for each site during the study period to test if mean GC levels were higher or lower during specific hours of the day (see Supporting Information for hourly sample sizes, Appendix S5). To test whether black lion tamarins showed a similar circadian fGM profile in both sites, we tested if their hourly fGM profiles were correlated using a Pearson's correlation test. We compared hourly mean fGM levels in both sites using a Kruskal–Wallis rank sum test. We then tested for pair-wise hourly differences using Dunn's test to identify significant peaks in fGM levels.

The circadian fGM profiles were similar in both sites ($r_{10} = 0.73$, $p < 0.01$; Figure 3). Although MDSP's circadian profile was flatter, mean hourly fGM levels showed an increase during the first hours of the day, followed by a slight decrease and stabilization of fGM levels during the afternoon. Since only one sample was collected at 5 p.m. in Guareí, this hour slot was removed from the analysis for this site. For the same reason, we also removed 5 a.m. and 6 p.m. from the analysis in MDSP. Hourly mean fGM levels varied significantly in both Guareí ($\chi^2 = 66.2$, $df = 10$, $p < 0.01$) and MDSP ($\chi^2 = 20.1$, $df = 11$, $p = 0.04$). In both sites, the highest mean hourly levels were recorded at 9 a.m. In Guareí, the 9 a.m. peak was significantly higher than in the early morning hours (6 h: $p < 0.01$; 7 h: $p < 0.01$; 8 h: $p < 0.01$). Revealing circadian variations in fGM concentrations enabled us to consider the potential bias linked to the time of defecation in our subsequent models for both sites.

2.4.2 | Effect of monthly behavior and climate on fGM levels

In accordance with the reactive scope model, circannual long-term GC variations are expected as part of the predictive homeostasis. Rather than calculating mean monthly fGM levels, we associated every fecal sample to the corresponding monthly climatic and behavioral data (see Supporting Information for the monthly distribution of full days and fecal samples, Appendix S3). Although we initially ran a global LMM, including all of the variables in a unique model yielded VIF > 5 due to multicollinearity between climatic and behavioral variables (see Supporting Information: Appendix 6). Therefore, we explored the effect of these factors separately in two sets of LMMs.

First, we ran LMMs to evaluate the link between fGM levels and both monthly rainfall and temperature. Second, to analyze the link between fGM levels and monthly behavioral data, we ran additional LMMs. Since GCs are first and foremost metabolic hormones that regulate energetic demands, we selected fruit consumption, foraging, resting, and movement as the main categorical behaviors likely influencing activity level and henceforth GC levels over the duration of a month. For both sets of models, we added a random-effect of an hour of defecation to take into account the determined circadian rhythm variations (see Section 2.4.1.). In MDSP, monthly movement was significantly negatively correlated with resting ($r_{(200)} = -0.91$, $p < 0.01$), yielding a VIF > 5. Therefore, for MDSP, we did not include resting, and we constructed our models using movement, foraging, and fruit consumption. To further understand the influence of fruit consumption on fGM levels in black lion tamarins, we also ran correlation tests to explore whether the consumption of specific fruit species influenced the time spent feeding on fruits in both black lion tamarins groups.

2.4.3 | Effect of daily behavior and climate on fGM levels

To understand the effect of daily behavior and climate changes on short-term increases in physiological stress levels (i.e., reactive homeostasis), we ran LMMs with fGM levels as the response variable and behavioral categories and weather (i.e., mean, maximum, and minimum daily temperature, and rainfall) as explanatory variables. Since black lion tamarins live in cohesive groups, we calculated the percentage of scan observations of each behavior and determined daily group behaviors. Based on the latency period defined by the

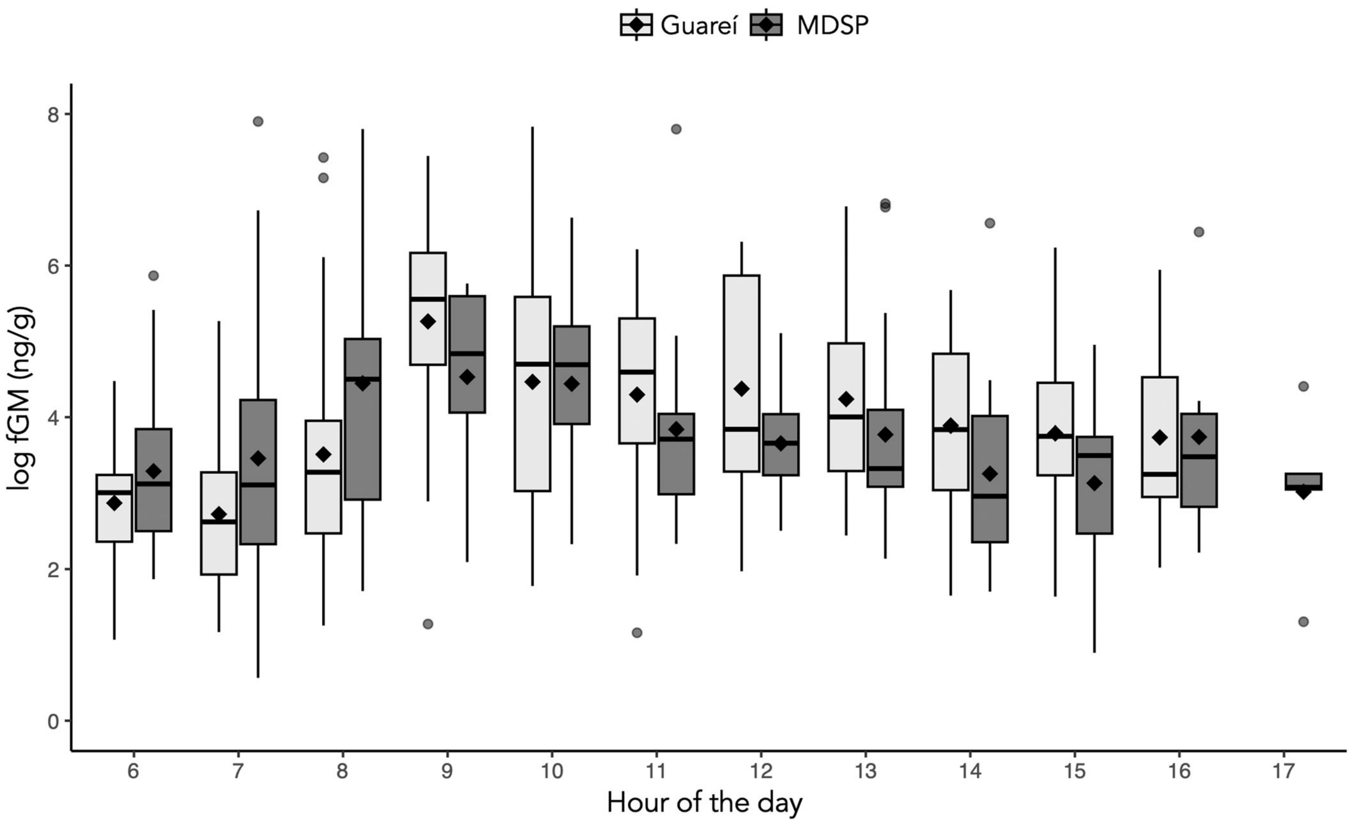

FIGURE 3 Boxplots representing the median and distribution of hourly fecal glucocorticoid metabolites (fGM) in the Morro do Diabo State Park (MDSP) and Guareí fragment black lion tamarin groups. Mean values are represented by black diamonds.

physiological validation, we attributed to each fecal sample the percentages for each behavior as well as the total rainfall and mean temperature of the previous day. For the daily models, we only included behaviors that may potentially influence activity level and food intake when acute and unprecedented (i.e., resting, foraging, movement, fruit consumption). For example, a day with abnormally low fruit consumption or high movement may be perceived as a short-term stressor and lead to a transient rise in GC levels. We also included sporadic and less frequent behaviors (i.e., invertebrate consumption, intergroup encounters, grooming) that may influence short-term GC levels. We only included fecal samples with full-day continuous behavioral data the previous day, 80 in MDSP and 225 in Guareí. To account for the anticipated influence of circadian and monthly variations (Romero et al., 2009), the hour of defecation and the month were included as random effects in the models.

3 | RESULTS

3.1 | Effect of monthly behavior and climate on fGM levels

Regarding climatic variables, for both sites, the best models only included mean monthly temperature (Figures 4–5). While fGMs were analyzed at the fecal sample level, Figures 4 and 5 display mean monthly fGM levels for interpretation purposes. In Guareí, the temperature had a significant positive effect on fGM levels (Estimate = 0.15, SE = 0.04), while the opposite was found in MDSP (Estimate = −0.33, SE = 0.05). Exploring behavioral variables revealed that in MDSP the best model included monthly movement and fruit consumption (Table 2). In Guareí, the best LMM also included monthly movement and fruit consumption (Table 2). At both sites, monthly fruit consumption had a positive effect, implying that fGM levels were higher in months where the percentage of scans spent feeding on fruit increased (Figures 4–5). In MDSP, fGM levels increased when the group spent more time moving, while in Guareí, they decreased when movement increased (Figure 4). Full models and AIC model selections are detailed in the Supporting Information: Appendix 7.

Since monthly fruit consumption had a significant positive effect on fGM levels at both study sites, we explored the link between the contribution of fruit species in the diet and the time spent feeding. Although some Brazilian Atlantic Forest keystone species, such as *Syagrus romanzoffiana*, were present in the diet of both groups, the black lion tamarin in Guareí and MDSP consumed different species (see Supporting Information: Appendix 8). We found that mean monthly fGM levels in MDSP were positively correlated with the time spent feeding on *Tapirira guianensis*, Anacardiaceae ($r_{(7)} = 0.744$, $p = 0.03$; see Supporting Information: Appendix 8). In Guareí, fGM levels were not correlated with the consumption of any particular fruit species.

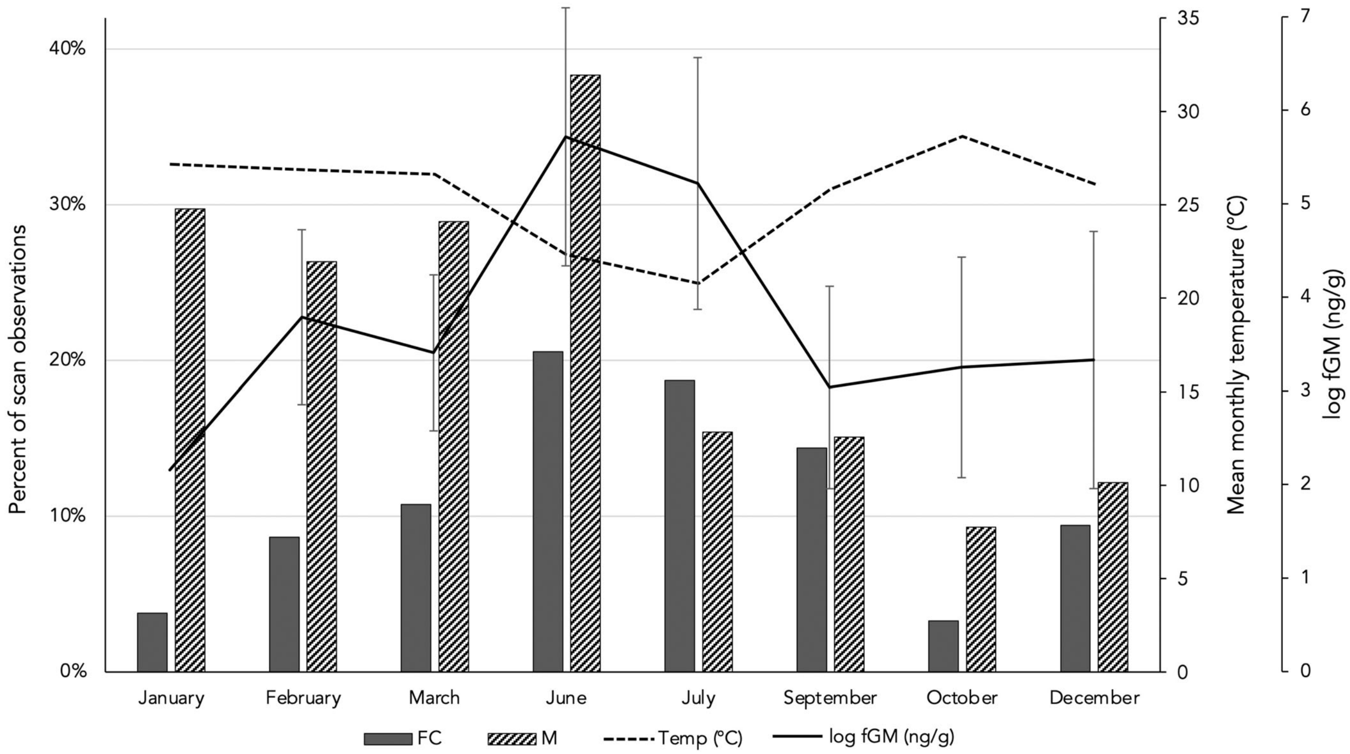

FIGURE 4 Mean monthly fecal glucocorticoid metabolite (fGM; log-transformed to achieve a normal distribution) levels and their standard deviation in the Morro do Diabo State Park (MDSP) in relation to mean temperatures and significant activity budget categories: fruit consumption (FC) and movement (*M*).

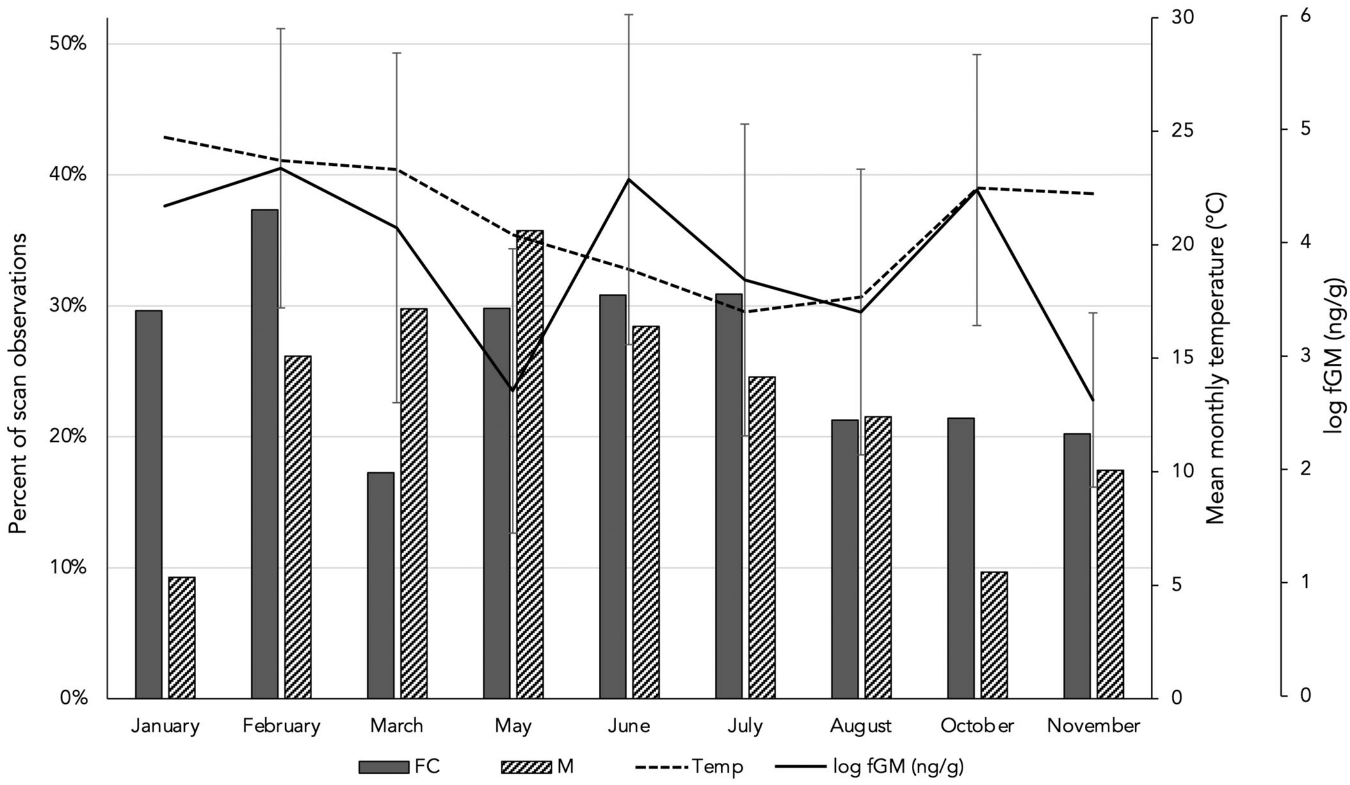

FIGURE 5 Mean monthly fecal glucocorticoid metabolite (fGM; log-transformed to achieve a normal distribution) levels their standard deviation in Guareí in relation to mean temperatures and significant activity budget categories: fruit consumption (FC) and movement (*M*).

TABLE 2 Best fit linear mixed model testing the effect of monthly behavior, including fruit consumption (FC) and movement (*M*), on fecal glucocorticoid metabolite levels in the Morro do Diabo State Park (MDSP) and Guareí.

Site	Effect	Estimate	SE
MDSP	*M*	0.737	0.209
	FC	0.79	0.224
	(1|Hour): Variance = 0.08, SD = 0.29		
Guareí	*M*	−0.702	0.191
	FC	1.141	0.205
	(1|Hour): Variance = 0.04, SD = 0.19		

Note: Estimate of the coefficients and the corresponding standard error (SE). Variance and standard deviation (SD) of the random effect (1|Hour).

TABLE 3 Best fit linear mixed model testing the effect of daily behavior, including intergroup encounters (ENC), on fecal glucocorticoid metabolite levels in Guareí.

Site	Effect	Estimate	Standard error
Guareí	ENC	0.593	0.196
	(1|Hour): Variance = 0.47, SD = 0.68; (1|Month): Variance = 0.47, SD = 0.69		

Note: Estimate of the coefficient and the corresponding standard error (SE). Variance and standard deviation (SD) of the random effects (1|Hour) and (1|Month).

3.2 | Effect of daily behavior and climate on fGM levels

In MDSP, none of the variations in daily behaviors, temperature, or rainfall had a significant effect on fGM levels. However, in Guareí, the best model (AIC = 765.9, $w_i = 0.657$) included the time spent involved in intergroup encounters, which was linked to higher fGM levels (Table 3). The full model and AIC model selection are detailed in the Supporting Information: Appendix 9.

4 | DISCUSSION

Given the multiple factors influencing endocrine responses, studying physiological stress (i.e., identifying the social and environmental factors swaying GC fluctuations) in a wild primate is intricate and challenging. Guided by theoretical concepts (Romero et al., 2009; Sapolsky et al., 2000), we looked at black lion tamarin adrenocortical activity at three different timescales (i.e., including circadian variations) to attempt to uncover how both predictive and reactive homeostasis change with behavior and climate. Our results indicate that, depending on the chosen timeframe, months, or days, different factors are associated with changes in fGM levels. At a monthly scale, movement and fruit consumption are linked to variations in fGM levels in different ways depending on the study site (i.e., Guareí and MDSP). At a daily scale, an increase in the day-to-day incidence of intergroup encounters was accompanied by an increase in fGM levels, but only in Guareí.

Regarding intrinsic factors, we found no significant differences in fGM concentrations between males and females or between adults and juveniles. These results are coherent with the fact that black lion tamarins live in small monogamous or polyandrous groups, characterized by the absence of a dominance hierarchy, shared parental care, and high intragroup cohesion (Valladares-Padua, 1993). Studies on a sister species, the golden lion tamarin (*Leontopithecus rosalia*), found no significant differences in GC concentrations between dominant and subordinate males or females (Bales et al., 2005, 2006). While pregnant golden lion tamarins did show significantly higher GC levels during their third trimester (Bales et al., 2005), in our study, there is very limited, or no, overlap between the third trimester of the pregnant female in Guareí and data collection. Typically, the costs related to within-group conflicts are offset by the benefits of strong group cohesion, expressed through the synchronization of activities and homogenization of diets in lion tamarins (Dixson, 2012; Kleiman & Rylands, 2002; Valladares-Padua, 1993). In this study, given the specific social organization of lion tamarin groups, we were able to make the assumption that group activity budgets were good representation of the general activities performed by each group members. This enabled us, together with the use of a random resampling method, to consider the lack of individual identification, which is one of the hurdles of studying black lion tamarins in the wild (Rezende et al., 2020). Nevertheless, future studies on this species should attempt to explore potential interindividual differences in adrenocortical activity. Through long-term studies, future research may also be able to estimate the potential effect of group size and intragroup competition on GC levels in lion tamarins.

Analyzing fGM levels throughout the day, we found that black lion tamarins in both sites experienced daily GC fluctuations associated with their circadian rhythm, part of the predictive homeostasis range. In both sites, keeping in mind that some margins of error exist, a morning increase in fGM levels was detected, which is in alignment with the anticipated cortisol awakening response observed in diurnal vertebrates (Fries et al., 2009). In many primate species, due to their long gut passage times, only severe GC peaks are detected in feces, dampening other transient variations (Behringer & Deschner, 2017; Christensen et al., 2022; Heistermann, 2010; Heistermann et al., 2006). However, in this study, because black lion tamarins are small-bodied primates with high defecation rates, we were able to detect and consider circadian variations in our subsequent analyses. In line with current fGM research (Palme, 2019), this finding supports and validates the use of noninvasive methods to assess transient GC levels but also further accentuates the importance of determining the time lag between the initial GC secretion in the plasma and their excretion in the feces. Together with the prior physiological validation (Bertoli et al., 2019), our preliminary analysis on circadian rhythm allowed us to account for a potential bias linked to defecation time in our subsequent models. When working with biological samples that provide a snapshot of hormonal activity, it is indeed essential to consider the existing temporal misalignment to put the extracted concentrations into context.

By identifying behavioral and climatic factors provoking seasonal physiological changes, exploring circannual GC variations in primates can reveal very useful information on their predictive homeostasis. Based on monthly analyses, our results revealed that, in MDSP, fGM levels increased during colder months. Unexpectedly, the opposite was observed in Guareí, with higher fGM levels in hotter months. Considering the effect of temperature independently, colder months may induce a shift in metabolic demands linked to thermoregulation and, therefore, an increase in GC levels. Respectively, previous studies on primates inhabiting temperate regions revealed that GC levels increased with lower temperatures (Chowdhury et al., 2021; Guo et al., 2018; McFarland et al., 2014). On the contrary, high humidity and extreme hot temperature may also cause heat stress, as observed in baboons inhabiting a semiarid savannah or dense woodland (Gesquiere et al., 2008; MacLarnon et al., 2015), giving rise to elevated GC concentrations. However, in the tropics, due to the minor temperature variations throughout the year (e.g., around 7°C between mean monthly temperatures during our study period), changes in temperature may not be significant enough to necessitate a physiological response (Dias et al., 2017). Additionally, the existing synchrony between the colder dry season and lower fruit productivity in our study region makes it challenging to untangle the effect of temperature from resource availability. In both study sites, monthly shifts in fruit consumption were indeed associated with changes in HPA axis activity. Altogether, and because our day-to-day analysis revealed no effect of hotter or colder daily temperatures on fGM levels, we believe that the association of monthly temperature is probably due more directly to changes in diet linked to fruit availability. In the future, systematically evaluating the phenology of consumed fruiting tree species will help to separate the influence of fruit availability from other seasonal variables such as temperature and rainfall.

In both sites, an increase in fruit consumption time led to higher fGM concentrations. While this result may seem counterintuitive according to the theoretical physiological framework and previous studies on other species, it is important to consider that feeding time does not systematically correlate with food and energy intake (i.e., the amount of calories absorbed from food consumption). A study on Amboseli yellow baboons uncovered that, despite a rise in GC levels caused by lower resource availability throughout the dry season, time spent feeding was higher during this period due to an increase in the food processing time of fallback resources (Gesquiere et al., 2008). Likewise, Sykes' monkeys exhibited higher GC levels when the feeding time on nonpreferred food was greater (Foerster et al., 2012). Breaking down the monthly diet of our study species, we discovered that the consumption of nonpreferred fruits, with potentially lower nutritional value, may be behind the positive relation between fGM levels and fruit consumption. In MDSP, *T. guianensis* represented more than 80% of the group's diet at the peak of the dry season, when fruit availability is customarily lower (Staggemeier et al., 2017). Given their low pulp ratio, these small fruits probably represent a low energy intake resource for black lion tamarins. Furthermore, the lion tamarins only ingest the proportionally large pulp-coated seed and remove the undesirable exocarp, considerably increasing fruit processing time and perhaps resulting in a low energy balance (Emery Thompson, 2017). Consequently, even though we witnessed an increase in fruit consumption time at the height of the dry season in MDSP, the absolute energy intake may be lower and thus yield higher fGM levels. In Guareí, fruit consumption was also positively associated with monthly fGM concentrations, although it was not possible to single-out a specific fruit species potentially responsible for these unexpected results. Nevertheless, we expect diet composition to influence adrenocortical activity in a similar way as in MDSP, with the tamarins feeding longer on low-quality fallback fruits. Feeding behavior analysis also revealed that, in this small Atlantic Forest fragment, lianas, such as *Pereskia aculeata* and *Celtis iguanaea*, seem to represent an important food resource during the dry season to help buffer nutritional stress (see Supporting Information: Appendix S8). Lianas are valuable fallback resources for frugivorous primates but may increase foraging effort (Dunn et al., 2012). As eclectic and opportunist omnivores (Keuroghlian & Passos, 2001), black lion tamarins are capable of shifting diet to consume low-quality fallback food in times when preferred resources are absent. Their dietary decisions are expected to follow an optimal foraging strategy based on the complex balance between energy intake, foraging and handling time, and food distribution and availability (Lambert & Rothman, 2015). Therefore, while keeping in mind the difficulties of accurately evaluating food intake when studying free-ranging primates in the tropics (Rothman et al., 2012), quantifying the nutritional value of items consumed by lion tamarins, would be a significant step forward in understanding diet quality, energy balance, and consequently the effect on physiology.

The concept of energy balance comprises two major factors: energy intake as discussed above and energy expenditure (i.e., the amount of calories spent by the body). Our models based on monthly fGM variations showed that, for the MDSP group, time spent moving (i.e., a behavioral proxy for energy expenditure) was positively related to fGM levels. Since resting was negatively correlated to movement in this group's monthly activity budget, resting time was associated with a decrease in fGM levels. Given that GC are metabolic hormones that regulate energetic requirements, these results are in agreement with our predictions. Accordingly, when the lion tamarins spend more time moving, energy expenditure increases, energy demand is higher, and thus GC levels rise. Inversely, when the group spend more time resting, they reduce energy expenditure and GC levels remain low. Similar results were found in howler monkeys, where greater travel times were linked to elevated GC levels (Dunn et al., 2013). Unlike in MDSP, where the group spent 27.5% of their global activity budget resting, in Guareí, the group rarely rested (i.e., 4.5% of their global activity budget; see Supporting Information: Appendix S2). Our results revealed that, in Guareí, fGM levels increased as the time in movement increased. Although the inverse was expected, the shorter resting time may influence the energetics of the group, which is accustomed to travel and forage within their smaller home range in search of fruits and other resources. By increasing their movement and foraging time, the lion tamarins in Guareí may enhance their chances of reaching preferred food and, thus, the reward of traveling is perhaps greater than its expense, resulting in a positive energy balance (Chapman et al., 2012; Emery Thompson, 2017). Since Guareí is a small fragment in which the total basal area (TBA) of tree species consumed by lion tamarins is considerably lower than in MDSP (i.e., 2.05 vs. 3.04 m^2, respectively; unpublished data), the dissimilitude observed between the two groups may potentially be linked to the habitat quality of the study sites themselves. Furthermore, in Guareí, edge effects may enhance the presence of lianas that reduce canopy tree fruit productivity (García León et al., 2018). In Guareí, the group may thus be adopting an energy-maximizing strategy (Hall, 1962), by increasing their ranging patterns to obtain sufficient food intake. Unlike in MDSP, where both fruit consumption and fGM levels increase during the dry season (see Figure 4), data from Guareí does not show a clear seasonal trend (see Figure 5). Due to the abundance of lianas and altered forest structure, resource availability and distribution may not follow a typical seasonal pattern in this fragment. Additional data on fruit phenology, distribution, and energy content would be necessary to have a deeper understanding of the link between movement, fruit consumption, and GC levels.

To identify the short-term behavioral or climatic factors triggering an acute stress response (i.e., reactive homeostasis) in black lion tamarins, we changed the timeframe and compared daily fGM levels with the corresponding daily activity budgets and climate. Controlling for temporal variations linked to the predictive homeostasis, our models revealed that, while none of the included variables had an effect on daily fGMs in MDSP, intergroup encounters sparked a short-term increase in fGM levels in the black lion tamarin group of Guareí. In primates, intergroup encounters are a recurrent outcome of competition over resources and territory, often accompanied by aggressive agonistic behaviors and vocalizations (Van Belle et al., 2021; Cooksey et al., 2020; Cooper et al., 2004; Koch et al., 2016). Although encounters are stressful, energetically costly, and are thus expected to elicit a physiological response, research on this topic is equivocal. Several studies found no increase in GC levels after intraspecific encounters (Belle et al., 2009; Cristóbal-azkarate et al., 2007; Huck et al., 2005; Ross et al., 2004). However, a study on white-faced capuchins (*Cebus capucinus*) reported that, as intergroup encounter rates increased, male capuchins expressed higher levels of GCs (Schoof & Jack, 2013). In the case of our study, the Guareí group inhabits a restricted 100-ha fragment that it shares with at least two other black lion tamarin groups. Consequently, in Guareí, the group has a limited home range, less than half of the size of the group in MDSP (i.e., 40 vs. 99 ha, respectively). Intergroup competition is thus expected to be enhanced in Guareí because maintaining access to precious resources may be more challenging. Congruently, intergroup encounters stimulated a rise in GC levels in Guareí, while no significant physiological response was observed in the MDSP, where the lion tamarins live in a continuous forest. A study on spider monkeys in Mexico also found that acute anthropogenic disturbances (i.e., daily logging and hunting activities) cause significant increases in daily fGM concentrations (Ordóñez-Gómez et al., 2016). While considering the predictive aspect of the adrenocortical activity, future studies in primatology should continue to explore the influence of short-term stressors on fecal GCs.

In general, our monthly analysis revealed that lion tamarin GC concentrations vary according to changes in the activity budget associated with the activity level and food intake of the groups. However, day-to-day changes in major behavioral categories did not elicit an acute physiological response in daily GC levels. In line with the reactive scope model (Romero et al., 2009), this finding suggests that diet and ranging patterns, driven by food availability and distribution, influence GC levels at a circannual scale as part of predictive homeostasis. Despite the potential limitations of this study given the lack of individual identification and the margins of error in the circadian and daily analyses, the novel twofold approach adopted here helped us understand how behavioral and climatic factors influence both the predictive and reactive homeostasis separately. As demonstrated, the complex mechanisms underlying physiological responses are often species and site-specific, stressing the importance of gathering simultaneous behavioral data to unravel and best explain the sources of GC variations. Bearing in mind the challenges of studying wild species in uncontrolled environments, future studies should attempt to adopt a similar holistic approach. Assessing the ecological stress physiology in wild species will allow for a better understanding of their resilience in changing and challenging environments. Such research may prove to be pivotal to guide species management and conservation efforts.

AUTHOR CONTRIBUTIONS

Olivier Kaisin: Conceptualization (lead); data curation (lead); formal analysis (lead); funding acquisition (lead); investigation (lead); methodology (lead); project administration (lead); visualization (lead); writing—original draft (lead); writing—review and editing (lead). **Felipe Bufalo**: Data curation (equal); methodology (equal); writing—review and editing (equal). **Rodrigo Amaral**: Data curation (equal); methodology (equal); writing—review and editing (supporting). **Rupert Palme**: Investigation (equal); methodology (equal); resources (equal); validation (equal); writing—review and editing (equal). **Pascal Poncin**: Project administration (equal); resources (equal); supervision (equal). **Fany Brotcorne**: Conceptualization (lead); Funding acquisition (lead); investigation; methodology (equal); project administration (lead); resources (lead); supervision (lead); writing—review and editing (lead). **Laurence Culot**: Conceptualization (lead); funding acquisition (equal); investigation; methodology; project administration (lead); resources (equal); supervision (lead); writing—review and editing (lead).

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CONFLICT OF INTEREST STATEMENT

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

Agritempo. (2022). *Agrometeorological monitoring system*. University of Campinas. https://www.agritempo.gov.br/

Aguilar-Cucurachi, M. A. S., Dias, P. A. D., Rangel-Negrín, A., Chavira, R., Boeck, L., & Canales-Espinosa, D. (2010). Preliminary evidence of accumulation of stress during translocation in mantled howlers. *American Journal of Primatology*, *72*(9), 805–810. https://doi.org/10.1002/ajp.20841

Altmann, J. (1974). Observational study of behavior: Sampling methods. *Behaviour*, *49*(3), 227–266. https://doi.org/10.1163/156853974X00534

Bales, K. L., French, J. A., Hostetler, C. M., & Dietz, J. M. (2005). Social and reproductive factors affecting cortisol levels in wild female golden lion tamarins (*Leontopithecus rosalia*). *American Journal of Primatology*, *67*(1), 25–35. https://doi.org/10.1002/ajp.20167

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

Beehner, J. C., & Bergman, T. J. (2017). The next step for stress research in primates: To identify relationships between glucocorticoid secretion and fitness. *Hormones and Behavior*, *91*, 68–83. https://doi.org/10.1016/j.yhbeh.2017.03.003

Beehner, J. C., & McCann, C. (2008). Seasonal and altitudinal effects on glucocorticoid metabolites in a wild primate (*Theropithecus gelada*). *Physiology & Behavior*, *95*(3), 508–514. https://doi.org/10.1016/j.physbeh.2008.07.022

Behie, A. M., Pavelka, M. S., & Chapman, C. A. (2010). Sources of variation in fecal cortisol levels in howler monkeys in belize. *American Journal of Primatology*, *72*(7), 600–606. https://doi.org/10.1002/ajp.20813

Behringer, V., & Deschner, T. (2017). Non-invasive monitoring of physiological markers in primates. *Hormones and Behavior*, *91*, 3–18. https://doi.org/10.1016/j.yhbeh.2017.02.001

Van Belle, S., Porter, A. M., Fernandez-Duque, E., & Di Fiore, A. (2021). Ranging behavior and the potential for territoriality in pair-living titi monkeys (Plecturocebus discolor). *American Journal of Primatology*, *83*(5), e23225. https://doi.org/10.1002/ajp.23225

Belle, S. V., Estrada, A., Ziegler, T. E., & Strier, K. B. (2009). Social and hormonal mechanisms underlying male reproductive strategies in black howler monkeys (*Alouatta pigra*). *Hormones and Behavior*, *56*(4), 355–363. https://doi.org/10.1016/j.yhbeh.2009.08.006

Bertoli, P., Culot, L., Palme, R., & Mendonça-furtado, O. (2019). Measuring fecal glucocorticoid metabolites of an endangered neotropical primate: technical details of a physiological validation. *Boletim Da Sociedade de Mastozoologia*, *80*(July), 1–6.

Burnham, K. P., & Anderson, D. R. (1998). *Model selection and interference: A practical information-theoretical approach*. Springer.

Chapman, C. A., Rothman, J. M., & Lambert, J. E. (2012). Primate foraging strategies and nutrition: Behavioral and evolutionary implications. In J. Mitani, J. Call, P. Kappeler, R. Palombit, & J. Silk (Eds.), *The evolution of primate societies* (Vol. 2014, pp. 149–168). https://www.researchgate.net/publication/258407007

Chapman, C. A., Saj, T. L., & Snaith, T. V. (2007). Temporal dynamics of nutrition, parasitism, and stress in colobus monkeys: Implications for population regulation and conservation. *American Journal of Physical Anthropology*, *134*(2), 240–250. https://doi.org/10.1002/ajpa.20664

Chapman, C. A., Wasserman, M. D., Gillespie, T. R., Speirs, M. L., Lawes, M. J., Saj, T. L., & Ziegler, T. E. (2006). Do food availability, parasitism, and stress have synergistic effects on red colobus populations living in forest fragments? *American Journal of Physical Anthropology*, *131*(4), 525–534. https://doi.org/10.1002/ajpa.20477

Chaves, Ó. M., Fernandes, F. A., Oliveira, G. T., & Bicca-Marques, J. C. (2019). Assessing the influence of biotic, abiotic, and social factors on the physiological stress of a large neotropical primate in Atlantic forest fragments. *Science of the Total Environment*, *690*, 705–716. https://doi.org/10.1016/j.scitotenv.2019.07.033

Chowdhury, S., Brown, J. L., & Swedell, L. (2021). Costs of seasonality at a Southern latitude: Behavioral endocrinology of female baboons in the Cape Peninsula of South Africa. *Hormones and Behavior*, *134*, 105020. https://doi.org/10.1016/j.yhbeh.2021.105020

Christensen, C., Bracken, A. M., Justin O'Riain, M., Heistermann, M., King, A. J., & Fürtbauer, I. (2022). Simultaneous investigation of urinary and faecal glucocorticoid metabolite concentrations reveals short-versus long-term drivers of HPA-axis activity in a wild primate (*Papio ursinus*). *General and Comparative Endocrinology*, *318*(June 2021), 113985. https://doi.org/10.1016/j.ygcen.2022.113985

Cooksey, K., Sanz, C., Ebombi, T. F., Massamba, J. M., Teberd, P., Magema, E., Abea, G., Peralejo, J. S. O., Kienast, I., Stephens, C., & Morgan, D. (2020). Socioecological factors influencing intergroup encounters in Western lowland gorillas (*Gorilla gorilla* gorilla). *International Journal of Primatology*, *41*(2), 181–202. https://doi.org/10.1007/s10764-020-00147-6

Cooper, M., Aureli, F., & Singh, M. (2004). Between-group encounters among bonnet macaques (*Macaca radiata*). *Behavioral Ecology and Sociobiology*, *56*(3), 217–227. https://doi.org/10.1007/s00265-004-0779-4

Cristóbal-azkarate, J., Chavira, R., Boeck, L., Rodríguez-luna, E., & VEÀ, J. J. (2007). Glucocorticoid levels in free ranging resident mantled howlers: A study of coping strategies. *American Journal of Primatology*, *69*(8), 866–876. https://doi.org/10.1002/ajp.20383

Culot, L., Griese, J., Knogge, C., Tonini, M., Mulato, M., Estevam, C. G., Lopes, B. P., Mantovani, B., Silva, B., Heliodora, B., Garcia, F. D. O., & Cristina, R. (2015). New records, reconfirmed sites and proposals for the conservation of black lion tamarin (*Leontopithecus chrysopygus*) in the middle and upper. *Neotropical*. *Primates*, *22*(1), 32–39.

Dias, P. A. D., Coyohua-Fuentes, A., Canales-Espinosa, D., Chavira-Ramírez, R., & Rangel-Negrín, A. (2017). Hormonal correlates of energetic condition in mantled howler monkeys. *Hormones and Behavior*, *94*, 13–20. https://doi.org/10.1016/j.yhbeh.2017.06.003

Dixson, A. F. (2012). Mating systems. In A. F. Dixson (Ed.), *Primate sexuality* (pp. 28–67). Oxford University Press. https://doi.org/10.1093/acprof:osobl/9780199544646.003.0003

Dunn, J. C., Asensio, N., Arroyo-Rodríguez, V., Schnitzer, S., & Cristóbal-Azkarate, J. (2012). The ranging costs of a fallback food: Liana consumption supplements diet but increases foraging effort in howler monkeys. *Biotropica*, *44*(5), 705–714. https://doi.org/10.1111/j.1744-7429.2012.00856.x

Dunn, J. C., Cristóbal-Azkarate, J., Schulte-Herbrüggen, B., Chavira, R., & Veà, J. J. (2013). Travel time predicts fecal glucocorticoid levels in free-ranging howlers (*Alouatta palliata*). *International Journal of Primatology*, *34*(2), 246–259. https://doi.org/10.1007/s10764-013-9657-0

Emery Thompson, M. (2017). Energetics of feeding, social behavior, and life history in non-human primates. *Hormones and Behavior*, *91*, 84–96. https://doi.org/10.1016/j.yhbeh.2016.08.009

Emery Thompson, M., Muller, M. N., Kahlenberg, S. M., & Wrangham, R. W. (2010). Dynamics of social and energetic stress in wild female chimpanzees. *Hormones and Behavior*, *58*(3), 440–449. https://doi.org/10.1016/j.yhbeh.2010.05.009

Engh, A. L., Beehner, J. C., Bergman, T. J., Whitten, P. L., Hoffmeier, R. R., Seyfarth, R. M., & Cheney, D. L. (2006). Behavioural and hormonal responses to predation in female chacma baboons (*Papio hamadryas* ursinus). *Proceedings of the Royal Society B: Biological Sciences*, *273*(1587), 707–712. https://doi.org/10.1098/rspb.2005.3378

Fieberg, J. R., Vitense, K., & Johnson, D. H. (2020). Resampling-based methods for biologists. *PeerJ*, *8*, e9089. https://doi.org/10.7717/peerj.9089

Foerster, S., Cords, M., & Monfort, S. L. (2012). Seasonal energetic stress in a tropical forest primate: Proximate causes and evolutionary implications. *PLoS One*, *7*(11), e50108. https://doi.org/10.1371/journal.pone.0050108

Foerster, S., & Monfort, S. L. (2010). Fecal glucocorticoids as indicators of metabolic stress in female Sykes' monkeys (*Cercopithecus mitis albogularis*). *Hormones and Behavior*, *58*(4), 685–697. https://doi.org/10.1016/j.yhbeh.2010.06.002

Fries, E., Dettenborn, L., & Kirschbaum, C. (2009). The cortisol awakening response (CAR): Facts and future directions. *International Journal of Psychophysiology*, *72*(1), 67–73. https://doi.org/10.1016/j.ijpsycho.2008.03.014

García León, M. M., Martínez Izquierdo, L., Mello, F. N. A., Powers, J. S., & Schnitzer, S. A. (2018). Lianas reduce community-level canopy tree reproduction in a Panamanian forest. *Journal of Ecology*, *106*(2), 737–745. https://doi.org/10.1111/1365-2745.12807

Gesquiere, L. R., Khan, M., Shek, L., Wango, T. L., Wango, E. O., Alberts, S. C., & Altmann, J. (2008). Coping with a challenging environment: Effects of seasonal variability and reproductive status on glucocorticoid concentrations of female baboons (*Papio cynocephalus*). *Hormones and Behavior*, *54*(3), 410–416. https://doi.org/10.1016/j.yhbeh.2008.04.007

Guo, S. T., Hou, R., Garber, P. A., Raubenheimer, D., Righini, N., Ji, W. H., Jay, O., He, S. J., Wu, F., Li, F. F., & Li, B. G. (2018). Nutrient-specific compensation for seasonal cold stress in a free-ranging temperate colobine monkey. *Functional Ecology*, *32*(9), 2170–2180. https://doi.org/10.1111/1365-2435.13134

Hall, K. R. L. (1962). Numerical data, maintenance activities and locomotion of the wild chacma baboon, *Papio ursinus*. *Proceedings of the Zoological Society of London*, *139*(2), 181–220. https://doi.org/10.1111/j.1469-7998.1962.tb01827.x

Hämäläinen, A., Heistermann, M., Fenosoa, Z. S. E., & Kraus, C. (2014). Evaluating capture stress in wild gray mouse lemurs via repeated fecal sampling: Method validation and the influence of prior experience and handling protocols on stress responses. *General and Comparative Endocrinology*, *195*, 68–79. https://doi.org/10.1016/j.ygcen.2013.10.017

Heistermann, M. (2010). Non-invasive monitoring of endocrine status in laboratory primates: Methods, guidelines and applications. *Advances in Science and Research*, *5*(1), 1–9. https://doi.org/10.5194/asr-5-1-2010

Heistermann, M., Palme, R., & Ganswindt, A. (2006). Comparison of different enzymeimmunoassays for assessment of adrenocortical activity in primates based on fecal analysis. *American Journal of Primatology*, *68*(3), 257–273. https://doi.org/10.1002/ajp.20222

Huck, M., Löttker, P., Heymann, E. W., & Heistermann, M. (2005). Characterization and social correlates of fecal testosterone and cortisol excretion in wild male *Saguinus mystax*. *International Journal of Primatology*, *26*(1), 159–179. https://doi.org/10.1007/s10764-005-0728-8

James, G., Witten, D., Hastie, T., & Tibshirani, R. (2013). *An introduction to statistical learning* (Vol. 103). Springer. https://doi.org/10.1007/978-1-4614-7138-7

Kaisin, O., Fuzessy, L., Poncin, P., Brotcorne, F., & Culot, L. (2021). A meta-analysis of anthropogenic impacts on physiological stress in wild primates. *Conservation Biology*, *35*(1), 101–114. https://doi.org/10.1111/cobi.13656

Kamilar, J. M., & Beaudrot, L. (2018). Effects of environmental stress on primate populations. *Annual Review of Anthropology*, *47*(1), 417–434. https://doi.org/10.1146/annurev-anthro-102317-045949

Keuroghlian, A., & Passos, F. C. (2001). Prey foraging behavior, seasonality and time-budgets in black lion tamarins, *Leontopithecus chrysopygus* (Mikan 1823) (Mammalia, Callitrichidae). *Brazilian Journal of Biology*, *61*(3), 455–459. https://doi.org/10.1590/S1519-69842001000300015

Kleiman, D. G., & Rylands, A. B. (2002). *Lion tamarins: Biology and conservation*. Smithsonian Institute Press.

Koch, F., Signer, J., Kappeler, P. M., & Fichtel, C. (2016). Intergroup encounters in Verreaux's sifakas (*Propithecus verreauxi*): Who fights and why? *Behavioral Ecology and Sociobiology*, *70*(5), 797–808. https://doi.org/10.1007/s00265-016-2105-3

Lambert, J. E., & Rothman, J. M. (2015). Fallback foods, optimal diets, and nutritional targets: Primate responses to varying food availability and quality. *Annual Review of Anthropology*, *44*(1), 493–512. https://doi.org/10.1146/annurev-anthro-102313-025928

MacLarnon, A. M., Sommer, V., Goffe, A. S., Higham, J. P., Lodge, E., Tkaczynski, P., & Ross, C. (2015). Assessing adaptability and reactive scope: Introducing a new measure and illustrating its use through a case study of environmental stress in forest-living baboons. *General and Comparative Endocrinology*, *215*, 10–24. https://doi.org/10.1016/j.ygcen.2014.09.022

Martínez-Mota, R., Righini, N., & Palme, R. (2016). Fluctuations in daily energy intake do not cause physiological stress in a neotropical primate living in a seasonal forest. *Oecologia*, *182*(4), 973–984. https://doi.org/10.1007/s00442-016-3739-6

McFarland, R., Barrett, L., Boner, R., Freeman, N. J., & Henzi, S. P. (2014). Behavioral flexibility of vervet monkeys in response to climatic and social variability. *American Journal of Physical Anthropology*, *154*(3), 357–364. https://doi.org/10.1002/ajpa.22518

Menard, S. (2002). *Applied Logistic Regression Analysis*. SAGE Publications Inc. https://doi.org/10.4135/9781412983433

Michael Romero, L. (2002). Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. *General and Comparative Endocrinology*, *128*(1), 1–24. https://doi.org/10.1016/S0016-6480(02)00064-3

Möstl, E., & Palme, R. (2002). Hormones as indicators of stress. *Domestic Animal Endocrinology*, *23*(1–2), 67–74. https://doi.org/10.1016/S0739-7240(02)00146-7

Muller, M. N., & Wrangham, R. W. (2004). Dominance, cortisol and stress in wild chimpanzees (*Pan troglodytes schweinfurthii*). *Behavioral Ecology and Sociobiology*, *55*(4), 332–340. https://doi.org/10.1007/s00265-003-0713-1

Ordóñez-Gómez, J. D., Cristóbal-Azkarate, J., Arroyo-Rodríguez, V., Santillán-Doherty, A. M., Valdez, R. A., & Romano, M. C. (2016). Proximal and distal predictors of the spider monkey's stress levels in fragmented landscapes. *PLoS One*, *11*(2), e0149671. https://doi.org/10.1371/journal.pone.0149671

Palme, R. (2019). Non-invasive measurement of glucocorticoids: Advances and problems. *Physiology & Behavior*, *199*, 229–243. https://doi.org/10.1016/j.physbeh.2018.11.021

Palme, R., Fischer, P., Schildorfer, H., & Ismail, M. N. (1996). Excretion of infused 14C-steroid hormones via faeces and urine in domestic livestock. *Animal Reproduction Science*, *43*(1), 43–63. https://doi.org/10.1016/0378-4320(95)01458-6

Palme, R., & Möstl, E. (1997). Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. *Zeitschrift Fur Saugetierkunde*, *62*(suppl 2), 192–197.

Pinto, B. L. (2017). *Influência da paisagem e das características locais na ocorrência do mico-leão- preto* (Leontopithecus chrysopygus, *Callitrichidae)*. Universidade Estadual Paulista.

Pride, R. E. (2005). Optimal group size and seasonal stress in ring-tailed lemurs (*Lemur catta*). *Behavioral Ecology*, *16*(3), 550–560. https://doi.org/10.1093/beheco/ari025

Rangel-Negrín, A., Alfaro, J. L., Valdez, R. A., Romano, M. C., & Serio-Silva, J. C. (2009). Stress in Yucatan spider monkeys: Effects of environmental conditions on fecal cortisol levels in wild and captive populations. *Animal Conservation*, *12*(5), 496–502. https://doi.org/10.1111/j.1469-1795.2009.00280.x

Rezende, G. C., Knogge, C., Passos, F., Ludwig, G., Oliveira, L. C., Jerusalinsky, L., & Mittermeier, R. A. (2020). *Leontopithecus chrysopygus*. *The IUCN Red List of Threatened Species 2020*, *8235*, e.T11505A17935400.

Romero, L. M. (2004). Physiological stress in ecology: Lessons from biomedical research. *Trends in Ecology & Evolution*, *19*(5), 249–255. https://doi.org/10.1016/j.tree.2004.03.008

Romero, L. M., Dickens, M. J., & Cyr, N. E. (2009). The reactive scope model—A new model integrating homeostasis, allostasis, and stress. *Hormones and Behavior*, *55*(3), 375–389. https://doi.org/10.1016/j.yhbeh.2008.12.009

Ross, C. N., French, J. A., & Patera, K. J. (2004). Intensity of aggressive interactions modulates testosterone in male marmosets. *Physiology & Behavior*, *83*(3), 437–445. https://doi.org/10.1016/j.physbeh.2004.08.036

Rothman, J. M., Chapman, C. A., & van Soest, P. J. (2012). Methods in primate nutritional ecology: A user's guide. *International Journal of Primatology*, *33*(3), 542–566. https://doi.org/10.1007/s10764-011-9568-x

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

Schoof, V. A. M., & Jack, K. M. (2013). The association of intergroup encounters, dominance status, and fecal androgen and glucocorticoid profiles in wild male white-faced capuchins (*Cebus capucinus*). *American Journal of Primatology*, *75*(2), 107–115. https://doi.org/10.1002/ajp.22089

Sheriff, M. J., Dantzer, B., Delehanty, B., Palme, R., & Boonstra, R. (2011). Measuring stress in wildlife: Techniques for quantifying glucocorticoids. *Oecologia*, *166*(4), 869–887. https://doi.org/10.1007/s00442-011-1943-y

Staggemeier, V. G., Cazetta, E., & Morellato, L. P. C. (2017). Hyperdominance in fruit production in the Brazilian Atlantic rain forest: The functional role of plants in sustaining frugivores. *Biotropica*, *49*(1), 71–82. https://doi.org/10.1111/btp.12358

Touitou, S., Heistermann, M., Schülke, O., & Ostner, J. (2021). Triiodothyronine and cortisol levels in the face of energetic challenges from reproduction, thermoregulation and food intake in female macaques. *Hormones and Behavior*, *131*(April), 104968. https://doi.org/10.1016/j.yhbeh.2021.104968

Touma, C., Sachser, N., Möstl, E., & Palme, R. (2003). Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. *General and Comparative Endocrinology*, *130*(3), 267–278. https://doi.org/10.1016/S0016-6480(02)00620-2

Valladares-Padua, C. B. (1993). The ecology, behavior and conservation of the black lion tamarins (Leontopithecus chrysipygus, Mikan, 1823). http://search.ebscohost.com/login.aspx?direct=true%26db=psyh%26AN=1995-95007-060%26site=ehost-live%26scope=site

Wasserman, M. D., Chapman, C. A., Milton, K., Goldberg, T. L., & Ziegler, T. E. (2013). Physiological and behavioral effects of capture darting on red colobus monkeys (*Procolobus rufomitratus*) with a comparison to chimpanzee (*Pan troglodytes*) predation. *International Journal of Primatology*, *34*(5), 1020–1031. https://doi.org/10.1007/s10764-013-9711-y

Wingfield, J. C. (2005). The concept of allostasis: Coping with a capricious environment. *Journal of Mammalogy*, *86*(2), 248–254. https://doi.org/10.1644/BHE-004.1

Wingfield, J. C. (2013). Ecological processes and the ecology of stress: The impacts of abiotic environmental factors. *Functional Ecology*, *27*(1), 37–44. https://doi.org/10.1111/1365-2435.12039

Wingfield, J. C., Hunt, K., Breuner, C., Dunlap, K., Fowler, G., Freed, L., & Lepson, J. (1997). Environmental stress, field endocrinology, and conservation biology. In J. Clemmons, & R. Buchholz (Eds.), *Behavioral approaches to conservation in the wild* (pp. 95–131). Cambridge University Press.

Young, C., Majolo, B., Heistermann, M., Schülke, O., & Ostner, J. (2014). Responses to social and environmental stress are attenuated by strong male bonds in wild macaques. *Proceedings of the National Academy of Sciences*, *111*(51), 18195–18200. https://doi.org/10.1073/pnas.1411450111

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