Animal Conservation ZSL

ORIGINAL RESEARCH

Lower Habitat Quality Increases Physiological Stress in an Endangered Neotropical Primate

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ABSTRACT

Understanding how habitat quality affects wildlife is one of the fundamental questions of conservation biology and ecology. Across the tropics, habitat loss and degradation threaten arboreal species, such as primates. To establish well-founded, species-specific conservation management plans, it is crucial to have an adequate understanding of a species' diet, behaviour, habitat, ecology and physiology. Measuring physiological stress in these species offers exclusive insight into how they cope and adapt within their environment. Here, we evaluated the influence of habitat quality on cortisol levels in black lion tamarins (*Leontopithecus chrysopygus*), an endangered frugivorous–faunivorous primate endemic to the state of São Paulo, Brazil. We compared hair cortisol concentrations among six different black lion tamarin populations inhabiting forest fragments of varying quality. We adopted a patch-landscape approach and measured forest cover to estimate habitat availability for each population. To estimate forest quality in each study, we calculated total tree basal area, a proxy for forest structure and maturity that is positively correlated to fruit availability. Our model revealed that cortisol levels increased as the amount of available habitat and tree basal area decreased. Lower forest cover may alter resource acquisition and disrupt ranging patterns of black lion tamarins, as well as increase the degree of anthropogenic disturbances. Furthermore, forests with smaller trees might impair their movement and decrease fruit and sleeping site availability. Given that small, unprotected fragments and riparian forests represent important habitats in its geographic range, protecting such areas, while increasing inter-fragment connectivity and limiting human encroachment, is crucial for the conservation of this species.

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1 | Introduction

Habitat transformation is the main threat to vertebrate populations across the world (Maxwell et al. 2016; Díaz et al. 2019). Along with most large-bodied mammals in the tropics, non-human primate populations suffer the consequences of habitat loss, degradation and fragmentation (de Almeida-Rocha et al. 2017; Estrada et al. 2017). Habitat conversion has multidimensional impacts on primates by not only limiting their ranging patterns, access to resources, dispersal and gene flow, but also increasing intra- and inter-specific competition and disease transmission (Chapman et al. 2005; Köndgen et al. 2008; Galán-Acedo et al. 2019). Furthermore, habitat loss is often associated with synergic disturbances due to increased contact with human populations, which can lead to changes in the behaviour, ecology, health and physiology of primates (Beehner and Bergman 2017; McLennan et al. 2017; Gould et al. 2020). Among the multiple consequences of habitat loss, degradation and fragmentation, changes in habitat quality are a major factor driving the presence and abundance of primate populations in habitat patches (Benchimol and Peres 2014). Indeed, forest cover loss, along with exacerbated edge effects, can alter the abundance and diversity of food resources and therefore influence the behaviour and ecology of occurring species (Arroyo-Rodríguez and Mandujano 2006; Galán-Acedo, Arroyo-Rodríguez, et al. 2021; Galán-Acedo et al. 2023). Understanding how primates living in forest fragments respond to disturbances and evaluating the potential consequences for their health, fitness and survival is crucial for their conservation.

Conservation physiology aims to identify the intrinsic and proximal effects of habitat disturbances on species to better understand how they cope with environmental challenges (Busch and Hayward 2009; Cooke et al. 2013). Evaluating adrenocortical activity, by measuring glucocorticoid (GC) levels, is a powerful tool to assess an animal's welfare, as it gives insights into their behaviour, physiology and ecology (Sheriff et al. 2011; Novak et al. 2013). These steroid hormones mediate energetic demands required to face predictable and unpredictable environmental and social challenges (Romero et al. 2009). The perception of a stressor activates the hypothalamic–pituitary–adrenal (HPA) axis that ultimately prompts the secretion of elevated GC levels in the bloodstream (Sapolsky et al. 2000). A surge in GC levels not only induces the mobilisation of stored energy, promotes escape behaviours and suppresses non-vital activities (e.g., social interactions, digestion, reproduction), but also heightens the effects of catecholamines, which trigger the fight or flight response (Wingfield 2005). Within their normal range, GCs regulate glucose metabolism to meet daily and seasonal energetic requirements (Romero et al. 2009). Although a rise in GC levels is adaptive and promotes survival, chronic stress (i.e., extended increases in GC levels) may have nocent consequences on the health and fitness of individuals (Juster et al. 2010; Dantzer et al. 2014). Sustained elevated GC levels in response to intense and unprecedented anthropogenic disturbances may negatively impact wild primates (Pride 2005; Rakotoniaina et al. 2017; Kalbitzer and Chapman 2018). Consequently, these physiological biomarkers have repeatedly been used to assess the physiological state and welfare of primates in disturbed habitats (Kaisin et al. 2021).

In wildlife endocrinological studies, urine and faecal samples are often favoured to measure GC levels because they are collected non-invasively and are, therefore, not prone to biases linked to handling and capture (Sheriff et al. 2011). However, unlike faecal and urine samples that may reveal hourly and/or daily fluctuations in some primate species (Kaisin et al. 2023), hair samples are thought to present a more stable and integrated measure of cortisol (Meyer and Novak 2012; Shimamoto 2022), the predominant glucocorticoid in primates (Spencer and Deak 2017). Therefore, hair has been increasingly used in wildlife studies as an indicator of chronic stress (Sheriff et al. 2011; Carlitz et al. 2014; Dettmer et al. 2014). While the precise pathway by which cortisol is incorporated into the hair shaft is still unknown, it is assumed that hair cortisol concentrations (HCCs) reflect the long-term accumulation of GCs that are integrated into the hair shaft during its growth (Stalder and Kirschbaum 2012). Recent studies have revealed that GCs may also diffuse into the hair through excretions from surrounding tissues (e.g., sebum, sweat) and at a minor level from external contaminations (e.g., urine, faeces, saliva), representing a blend of long-term hormone accumulation during hair growth and transient levels from auxiliary sources (Koren et al. 2019). Nonetheless, HCCs have proved to be consistent with integrated salivary cortisol level (Short et al. 2016) and show high intra-individual stability (Stalder et al. 2012). When comparing HCCs inter-individually, it is however recommended to systematically sample from the same body region. As with other GC biomarkers, it is also important to consider important intrinsic variables (e.g., sex and age) and extrinsic factors (e.g., seasonality and food availability; Dantzer et al. 2014). Overall, HCCs provide valuable information on HPA axis functioning and are adequate to explore physiological stress and determine the influence of persisting environmental or social stressors (Heimbürge et al. 2019; Kalliokoski et al. 2019).

Because GCs are fundamentally metabolic hormones, they fluctuate according to the intricate balance between energy intake and expenditure, contingent on food availability, diet quality and foraging effort (Emery Thompson 2017). Consequently, the indirect effects of habitat disturbances can often be depicted in GC concentrations. Multiple primatological studies have highlighted the impacts of forest cover loss and degradation on GC levels, with primates inhabiting areas subject to anthropogenic disturbances repeatedly presenting higher GC levels than conspecifics in less disturbed environments (Kaisin et al. 2021). Furthermore, nutritional stress linked to food scarcity often yields higher GC levels in primate populations inhabiting fragments (Chapman et al. 2006; Martínez-Mota et al. 2007; McLennan et al. 2019), because resource availability and distribution are frequently lower in such habitats due to enhanced edge effects and poor plant recruitment (Cordeiro and Howe 2001; Marsh and Chapman 2013). Nonetheless, fragments are not consistently characterised by lower food availability and therefore higher GC levels (Marsh and Chapman 2013; Ordóñez-Gómez et al. 2016), emphasising the importance of evaluating and quantifying resource availability when comparing primate populations in habitats of varying size.

Black lion tamarins are frugivorous–faunivorous primates that are primarily monogamous and live in small groups, usually

comprising two to eight individuals (Valladares-Padua 1993). Given their rich carbohydrate, vitamin and mineral contents, and being the central food category in the diet of black lion tamarins (Kaisin et al. 2023), fruits are considered the main limiting resource for this primate (Raskin et al. 2025). After being considered extinct for several decades, this species was rediscovered in the 1970s and currently has a wild population of about 1600 individuals (Rezende, Knogge, et al. 2020; Rezende, Sobral-Souza, et al. 2020). Endemic to the state of São Paulo, in south-eastern Brazil, black lion tamarins occur within inland Brazilian Atlantic Forest (Coimbra-Filho and Mittermeier 1973; Culot et al. 2015; Rezende, Knogge, et al. 2020; Rezende, Sobral-Souza, et al. 2020), one of the Earth's 25 biodiversity hotspots (Mittermeier et al. 2011). Sadly, this biome is also highly fragmented and subject to intense forest cover loss that is spurred by agricultural and urban expansion, especially the inland and seasonal vegetation (Ribeiro et al. 2009; Joly et al. 2014). Although latest estimates suggest that about 23% of the native forest cover remains, the remaining vegetation is presumably mainly secondary or degraded forest (Vancine et al. 2024).

In this study, we explore the influence of habitat quality on cortisol levels in black lion tamarins (*Leontopithecus chrysopygus*), a rare and endangered neotropical primate. Prior to exploring the effect of habitat quality, we tested the influence of potential confounding factors that may sway cortisol levels of this primate (i.e., age, sex, region, season). Given that an age-related decline in HCCs has been previously reported in primates (e.g., *Callithrix jacchus*: Garber et al. 2020; *Macaca mulatta*: Dettmer et al. 2014; *Chlorocebus aethiops* and *Papio hamadryas papio*: Fourie and Bernstein 2011), we expect juveniles to present higher HCCs than adults. Due to the co-dominance observed between adult males and females, shared parental care and high cohesion of black lion tamarin groups (Valladares-Padua 1993; Kaisin et al. 2023), we expect sex to have no effect on HCCs. As witnessed in common marmosets (*C. jacchus*: Garber et al. 2020), we anticipate individuals to exhibit higher HCCs during the dry season, when fruit productivity is lower (Morellato et al. 2024), revealing potential nutritional stress. Finally, since black lion tamarins have established populations in both study regions (i.e., Pontal do Paranapanema and Alto Paranapanema), we expect them to be adapted to the general geographic abiotic and biotic conditions of these regions. Therefore, we expect HCCs to be influenced by area-specific habitat quality characteristics rather than region.

To assess how black lion tamarins respond to living in different habitat quality contexts, we assessed HCCs in six populations inhabiting different Atlantic Forest fragments varying in size, forest cover, forest quality and resource availability. In each study site, we assessed both forest cover and calculated total tree basal area (TBA), a proxy for forest structure and maturity that is positively related to fruit availability (Chapman et al. 1992; Arroyo-Rodríguez and Mandujano 2006). Because habitat loss may impair ranging patterns, restrict access to resources and increase the prevalence of anthropogenic disturbances, we expect individuals in areas with lower forest cover to exhibit higher HCCs. As a fallout of nutritional stress, we also predict individuals to have higher HCCs in areas with lower TBA. By assessing the physiological state of black lion tamarins living in fragments, we will reach a better understanding of how they cope in these environments and support future well-founded and species-specific conservation strategies related to habitat management.

2 | Materials and Methods

2.1 | Ethics Statement

This study was carried out with the relevant authorisations from the Sistema de Autorização e Informação em Biodiversidade (SISBIO; no. 68253-4 and no. 41375-8), the Comissão Técnico-Científica do Instituto Florestal (COTEC; no. 153/2021 D28/2021 PH) and the ethics committee of the São Paulo State University (UNESP; no. 16/2019). *Leontopithecus chrysopygus* being listed under Appendix S1 of the Convention on International Trade in Endangered Species (CITES), the necessary exportation (no. 21BR036613/DF) and importation (no. 2021/BE00456/PI) permits were obtained.

2.2 | Study Sites

This research was carried out in six study sites distributed within two regions of the Paranapanema River Basin, within the state of São Paulo, Brazil: (1) Pontal do Paranapanema and (2) Alto Paranapanema.

1. Pontal do Paranapanema, the westernmost region of the state, comprises the Morro do Diabo State Park (MDSP; 33,845 ha; 22°37′21″ S, 52°08′02″ W) and the fragments of Ponte Branca (1306 ha; 22°24′53″ S, 52°30′48″ W) and San Maria (520 ha; 22°14′20″ S, 52°18′23″ W; Figure 1; 1-3). Pastures for cattle ranching, as well as sugarcane and soy plantations, are the main agricultural exploitations in this region. MDSP is the largest inland Atlantic Forest remnant in the state of São Paulo and harbours the largest population of black lion tamarins, with approximately 1200 individuals (Rezende, Knogge, et al. 2020; Rezende, Sobral-Souza, et al. 2020). Apart from an imposing hill culminating at 600 m, which gives its name to the park, MDSP has an altitude ranging between 250 and 450 m. Similarly, the Ponte Branca and San Maria fragments have altitudes varying between 300 and 450 m.
2. Alto Paranapanema, located in the southeast region of the state, encompasses the Guareí fragment (100 ha; 23°25′07″ S, 48°14′27″ W) and the riparian forests in Angatuba (~79 ha; 22°27′26″ S, 48°23′57″ W) and Buri (~78 ha; 23°47′19″ S, 48°35′20″ W; Figure 1; 4–6). The sizes of riparian forest fragments were estimated based on the areas known to be occupied by the tamarins and delimited by physical barriers such as urban areas, dams or plantations. Agriculture in this region is characterised by pastures and sugarcane, eucalyptus, pine tree and orange plantations. The altitude in these study sites varies between 591 and 680 m.

Typically, the inland and seasonal semideciduous forest of south-eastern Brazil is characterised by a dry winter (April–September) and a rainy summer (October–March; Alvares et al. 2013), holding typical regional plant species (Eisenlohr and de Oliveira-Filho 2015; Alves-Araújo et al. 2022), few epiphytes (Marcusso

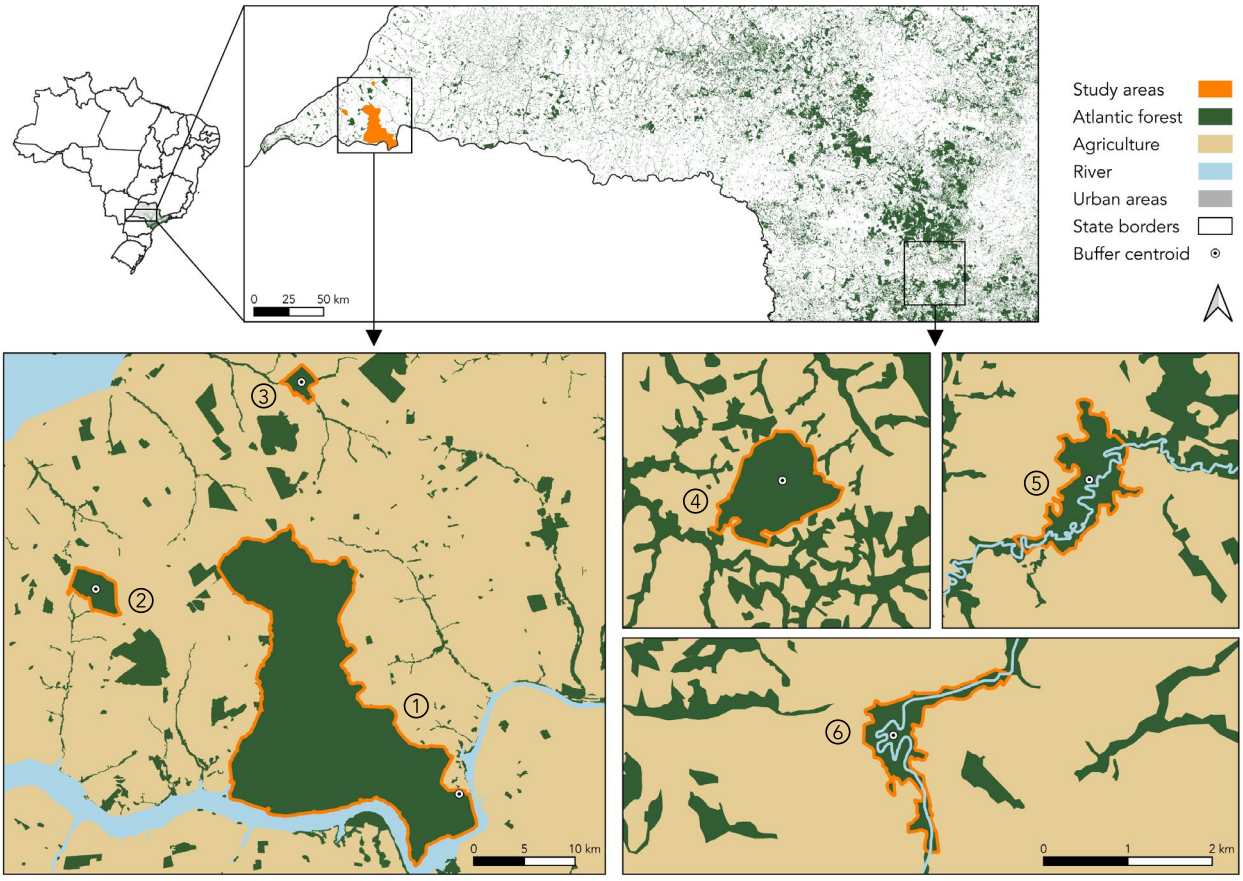

FIGURE 1 | Location of the six study sites within the state of São Paulo (Brazil) including the peripheral land use. The sites are located in two regions of the Paranapanema River Basin, which partially delimits the border between the São Paulo and Paraná States. The Pontal do Paranapanema region (in the west) includes the (1) Morro do Diabo State Park (MDSP; 33,845 ha) and the fragments of (2) Ponte Branca (1306 ha) and (3) San Maria (520 ha). The Alto Paranapanema region (in the east) encompasses the (4) Guareí fragment (100 ha) and the riparian forests in (5) Angatuba (79 ha) and (6) Buri (78 ha).

et al. 2022) and a high prevalence of climbing plants (da Vargas et al. 2018), when compared to coastal tropical forests.

2.3 | Captures and Hair Sample Collection

Hair samples for endocrine analyses were collected during black lion tamarin capture campaigns between January 2019 and June 2021. These captures were performed in the framework of multidisciplinary studies to explore the behaviour, ranging, physiology and health of this species. The primary objective of these captures was to equip one or two adult individuals of each captured group with radiotelemetry tracking collars (Holohil RI-2D) in order to collect behavioural data and monitor the groups. To ensure the welfare of the lion tamarins, a trained veterinarian was present at all captures. The lion tamarins were captured using two different methods—inside their sleeping site or using *Tomahawk* traps (see Appendix S1). Individuals were placed in separate cages or holding bags and hair samples were systematically clipped from the interscapular region to consider any potential HCC variations linked to the body region. To avoid contamination, a new pair of latex gloves was used when sampling each individual, and the scissors were systematically disinfected with 70% alcohol. Samples were then placed into small plastic bags and in opaque paper envelopes to protect them from UV degradation. Some individuals were sampled twice, once in the rainy season and once in the dry season. In total, we collected 37 samples from 32 individuals in six areas (Table 1; Appendix S2).

TABLE 1 | Number of individuals from which hair was collected to assess the effect of habitat quality on cortisol levels of adult (> 18 months old) and juvenile (3–9 months old) black lion tamarins across six study sites in São Paulo, Brazil.

		Adults		Juveniles		
Region	**Site**	♀	♂	♀	♂	**Total**
Pontal do Paranapanema	MDSP	2	3	—	—	5
	Ponte Branca	2	2	2	—	6
	San Maria	1	—	—	—	1
Alto Paranapanema	Guareí	2	3	1	1	7
	Angatuba	1	3	—	2	6
	Buri	1	4	—	2	7
	Total	9	15	3	5	32

Abbreviations: ♀, females; ♂, males; MDSP, Morro do Diabo State Park.

Hair samples were collected from both adults and juveniles. Following Carvalho and Carvalho (de Carvalho and de Carvalho 1989), adults (i.e., > 18 months old; Troisi 2021) showed complete body development, full sexual activity as well as permanent and complete dentition. Juveniles (i.e., 3–9 months old; Troisi 2021) represented young individuals that were half the size of adults, not well characterised sexually and with incomplete dentition (de Carvalho and de Carvalho 1989). Description of infant (i.e., < 3 months old) and sub-adult (i.e., 9–18 months old) age classes is available as Supporting Information (Appendix S1).

2.4 | Hormone Analysis

For extraction, 100 mg of entire hair strands from each individual were placed in glass test tubes and suspended in 5 mL of 100% methanol. As an organic solvent, methanol dissolves the neutral and lipophilic compounds in the hair and releases the cortisol (Xiang et al. 2016). The test tubes were plugged tightly to avoid evaporation of the methanol and thermo-mixed for 24 h at 37°C. The samples were then centrifuged (2500 *g*, 15 min). Aliquots of 2.5 mL of the supernatant were transferred into new glass test tubes and dried down at 60°C. The samples were then re-dissolved in 0.5 mL of assay buffer and vortex-mixed (1000 rpm, 30 min; Agradi et al. 2023).

HCCs were measured using a homemade cortisol enzyme immunoassay (EIA), which measures cortisol and GC metabolites with a 11β,17α,21-triol-20-one structure (Palme and Möstl 1997). The EIA had a sensitivity of 0.33 pg/well and the cross-reactivity with relevant steroids is described in Palme and Möstl (1997). A parallelism analytical validation was achieved by performing serial dilutions of two hair sample pools and comparing the slope of the curve with that of the standard curve (see Appendix S3).

We assayed 50 μL of all samples following a 1:1000 dilution in assay buffer. We estimated the precision of our analyses by measuring the inter-assay coefficients of variation (CV), assessed by running high- and low-level quality controls in each assay, which were 1.2% and 3.9% respectively. We ran all samples in duplicates and the mean intra-assay CV for control samples was 2.23% ± 1.74%. All samples with intra-assay CV > 10% were re-analysed.

2.5 | Habitat Quality Estimation

2.5.1 | Forest Cover

We assessed the impact of habitat loss and fragmentation on HCCs using a patch-landscape approach as described by Arroyo-Rodríguez and Fahrig (2014). Since black lion tamarins are forest dependent, forest cover was used as a proxy of available habitat. For each study site, we calculated forest cover within buffers of different radii (i.e., 500 m, 1 km, 1.5 km, 2 km, 3 km, 5 km, 7.5 km, 10 km—see Appendix S4). To do this, we used Sentinel-2 satellite images from the European Space Agency Copernicus mission, taken on 1 January 2021. As suggested by Jackson and Fahrig (2015), the largest buffer exceeded the maximum home range size of black lion tamarins (i.e., 400 ha; Rezende, Knogge, et al. 2020; Rezende, Sobral-Souza, et al. 2020). In each study site, the centroid of the buffer zones was positioned in the area where we captured the black lion tamarins (Figure 1). To determine the scale at which habitat loss most affected the physiological stress levels of our study species (i.e., scale of effect; Arroyo-Rodríguez and Fahrig 2014), we ran a linear model between HCCs and the percentage of forest cover at different buffer radii. We compared these models and included in our habitat quality models the forest cover values from the buffer that yielded the highest R^2 in each region (see Appendix S5). We found that forest cover within a 500-m buffer most influenced HCC levels, ranging from 60.7% to 100% of forest cover (see Appendices S4–S6).

2.5.2 | Total Tree Basal Area

We calculated TBA as an indicator of general habitat quality for black lion tamarins. On top of serving as a proxy for forest structure and maturity (Arroyo-Rodríguez and Mandujano 2006; da Silva Júnior et al. 2009), it shows a positive correlation with fruit availability (Chapman et al. 1992; Stevenson et al. 1998; Worman and Chapman 2006). In each study site, vegetation characterisation was carried out the same year as hair sample collection. We set up 20 botanical plots (10 × 10 m) in each study site. The plots were distributed as a grid, 200–400 m apart, within the area used by each captured black lion tamarin group. Inside every botanical plot, we calculated the basal area of every living tree and palm tree with a circumference at breast height (CBH) ≥ 15 cm, corresponding to a diameter at breast height (DBH) ≥ 4.8 cm. We calculated TBA for each study site by summing the basal area of every tree within the 20 plots. Detailed vegetation characteristics (i.e., number of individuals, species and families, as well as overall density, density of large trees—DBH > 20 cm and mean DBH) are available as Appendix S7.

2.6 | Statistical Analysis

HCCs were square root transformed (sHCC) to achieve a normal distribution (Shapiro–Wilk test: $W = 0.97$, $p = 0.559$). Prior to exploring the effect of habitat quality, we tested the potential effect of confounding factors on HCCs by running a generalised linear mixed model (GLMM) including sex, age, season and region as explanatory variables. In this model, we added both individual ID and study site as random effects to consider the effect of individual replicates and habitat quality respectively. This first model enabled us to identify which confounding factors influenced HCCs, which were then considered in the habitat quality model. Then, to explore the influence of habitat quality on HCCs, we ran a second model with TBA and forest cover as explanatory variables and individual ID and significant confounding factors as random effects (i.e., age and season).

The significance level considered for all analyses was $\alpha = 0.05$. Models were fit using the restricted maximum likelihood (REML) method and the effects of variables were calculated using a frequentist approach (i.e., null hypothesis significance

TABLE 2 | Variable included in the model that tested the effect of habitat quality—forest cover and total tree basal area (TBA)—on square root transformed hair cortisol concentration (sHCC ± SD) in black lion tamarins and number of collected hair samples for each of the six study sites in São Paulo, Brazil.

Region	Site	Forest cover (%)	TBA (m^2)	sHCC (ng/g) ± SD	# of hair samples
Pontal do Paranapanema	MDSP	96.3	5.49	32.1 ± 4.54	6
	Ponte Branca	100	4.25	26.4 ± 6.82	8
	San Maria	100	2.91	46.4	1
Alto Paranapanema	Guareí	97.2	8.52	21.4 ± 11.8	8
	Angatuba	73.5	7.36	36.6 ± 6.58	6
	Buri	60.7	6.80	36.5 ± 9.66	7

Note: Forest cover calculated within a 500-m buffer.
Abbreviation: MDSP, Morro do Diabo State Park.

TABLE 3 | Generalised linear mixed model testing the effect of confounding factors—sex, age, region and season—on square root transformed hair cortisol concentrations (sHCC) in black lion tamarins, with individual ID and study site as random effects.

Effect	Estimate	SE	*t* ratio	*p*
Season_Rainy	−7.25	2.29	−3.17	0.032*
Age_Juvenile	11.71	2.71	4.32	< 0.001***
Sex_Male	3.25	2.55	1.27	0.217
Region_Pontal	5.02	5.67	0.89	0.455

Note: Estimate of the coefficient and the corresponding standard error (SE), *t* statistic and *p* value calculated using T distribution. Season: rainy versus dry. Age: adults (> 18 months old) versus juveniles (3–9 months old). Sex: male versus female. Region: Pontal do Paranapanema versus Alto Paranapanema. Level of significance: *$p < 0.05$, ***$p < 0.001$.

testing; Mundry 2011). For each model, we tested for multicollinearity using the variance inflation factor (VIF) and all variables included in a model had a VIF ≤ 2.5 (Johnston et al. 2018). We tested the goodness of fit of our models by comparing them to the corresponding null models using a likelihood ratio test (LRT). We performed all statistical analyses using R 4.3.2 (R Core Team 2023) with the packages *stats* (R Core Team 2023), *lme4* (Bates et al. 2015), *lmtest* (Zeileis and Hothorn 2002) and plotted figures using *jtools* (Long 2024) and *ggplot2* (Wickham 2016).

3 | Results

Mean HCCs and habitat quality values for each site are listed in Table 2. First, regarding the effect of confounding variables, we found that, while region and sex had no significant effect, HCCs were significantly higher during the dry season and juveniles had significantly higher HCCs than adults (Table 3; Figure 2). Our model was significantly different from the null model ($\chi^2_{(4)} = 25.9$, $p < 0.001$). A boxplot representing the distribution of sHCCs across study sites is available as Appendix S8. Second, our model exploring the influence of TBA and forest cover revealed that both variables had negative effects on HCCs (Table 4). In other words, as TBA and forest cover decreased, HCCs increased (Figure 3). This model was significantly different from the null model ($\chi^2_{(2)} = 6.68$, $p = 0.034$).

4 | Discussion

Neotropical primates are forest dependent and generally highly sensitive to habitat change and other anthropogenic disturbances, making them excellent ecological indicators (Cavada et al. 2016; Estrada et al. 2017). In this study, we measured HCCs as an indicator of physiological stress in different black lion tamarin populations to evaluate how these small primates cope in disturbed fragments of inland Brazilian Atlantic Forest. We show that low habitat quality negatively affects cortisol levels, since both decreasing forest cover and TBA were correlated with increased hair cortisol levels.

Prior to discussing the impact of habitat quality, it is opportune to address the potential influence of other proximal and distal factors that may sway physiological stress in primates. In this study, we tested the effect of confounding factors, including age, sex, season and study region. We found that juveniles showed higher HCC than adults, which may be due to age-specific physiological differences—juveniles have lower concentrations of corticosteroid-binding globulin, resulting in higher levels of free cortisol in the plasma, which is then deposited in the hair during growth (Grant et al. 2017). However, this may also be linked to higher energetic demands during development (Emery Thompson 2017) or increased mortality risks in juvenile primates, inducing physiological stress (Rakotoniaina et al. 2017; Garber et al. 2020). Consistent with what has been recently observed in black lion tamarin faecal glucocorticoids (Kaisin et al. 2023), we found no significant difference in HCCs between males and females. This is presumably due to the co-dominance of adult males and females, lack of sexual dimorphism, shared parental care and high cohesion of black lion tamarin groups (Valladares-Padua 1993). In lion tamarins, the costs related to conflicts of interest between group members are offset by the benefits of strong group cohesion, expressed through synchronisation of activities and homogeneity of their diets (Kleiman and Rylands 2002; Dixson 2012; Kaisin et al. 2023; Raskin et al. 2025). Therefore, their energy balance and metabolic needs are likely to be consistent across sexes.

As part of the predictive homeostasis of individuals, GC levels may vary seasonally to cope with predictable stressors linked to resource availability, reproduction or seasonal climate (Romero

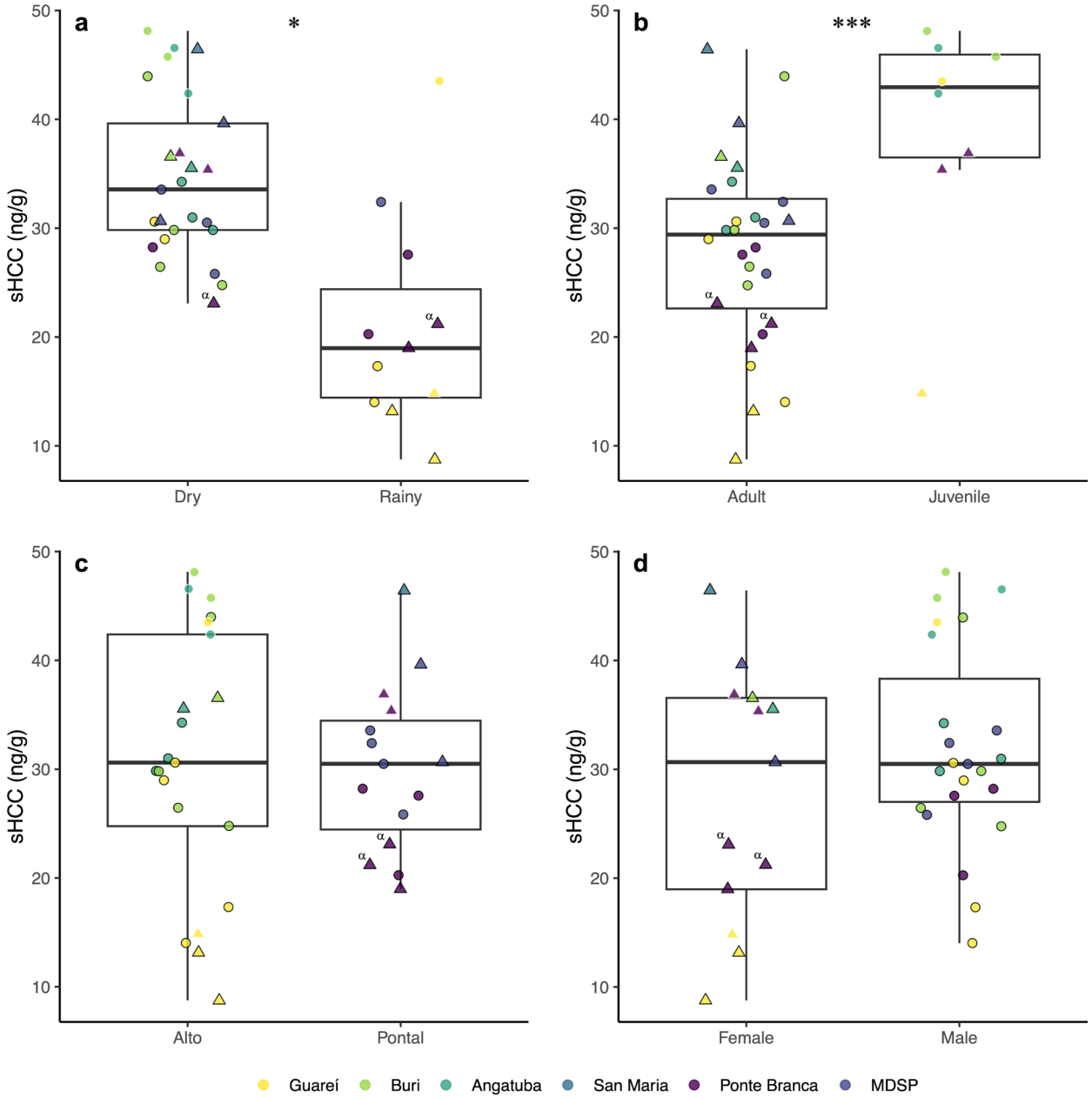

FIGURE 2 | Median and distribution of square root transformed hair cortisol concentrations (sHCC) in black lion tamarins according to (a) season, (b) age–class, (c) region (Alto Paranapanema versus Pontal do Paranapanema, São Paulo, Brazil) and (d) sex. Adults (>18 months old) = black outline; juveniles (3–9 months old) = white outline; males = circles; females = triangles; pregnant females = α. MDSP, Morro do Diabo State Park. Level of significance from generalised linear mixed model: *$p < 0.05$, ***$p < 0.001$.

TABLE 4 | Generalised linear mixed model testing the effect of total tree basal area (TBA) and forest cover on square root transformed hair cortisol concentrations (sHCC) in black lion tamarins, with individual ID, season and age as random effects.

Effect	Estimate	SE	*t* ratio	*p*
TBA	−1.85	0.84	−2.21	0.034*
Forest cover	−0.21	0.09	−2.30	0.028*

Note: Estimate of the coefficient and the corresponding standard error (SE), *t* statistic and *p* value calculated using T distribution. Forest cover calculated within a 500-m buffer. Level of significance: *$p < 0.05$.

et al. 2009). Regarding reproduction, females are expected to have higher metabolic demands during gestation and lactation, resulting in higher GC levels than cycling females (Carnegie et al. 2011; Dias et al. 2017). Despite the reduced sample size, the two pregnant females did not display higher HCCs than the other adult females (Figure 2). These females were perhaps still in the early stages of their pregnancy, as golden lion tamarins (*Leontopithecus rosalia*) only start displaying higher cortisol levels during the third trimester of pregnancy (Bales et al. 2005). In black lion tamarins, the breeding season extends from August to March (French et al. 1996), representing most of the rainy season, when fruit productivity is at its peak (Morellato et al. 2024).

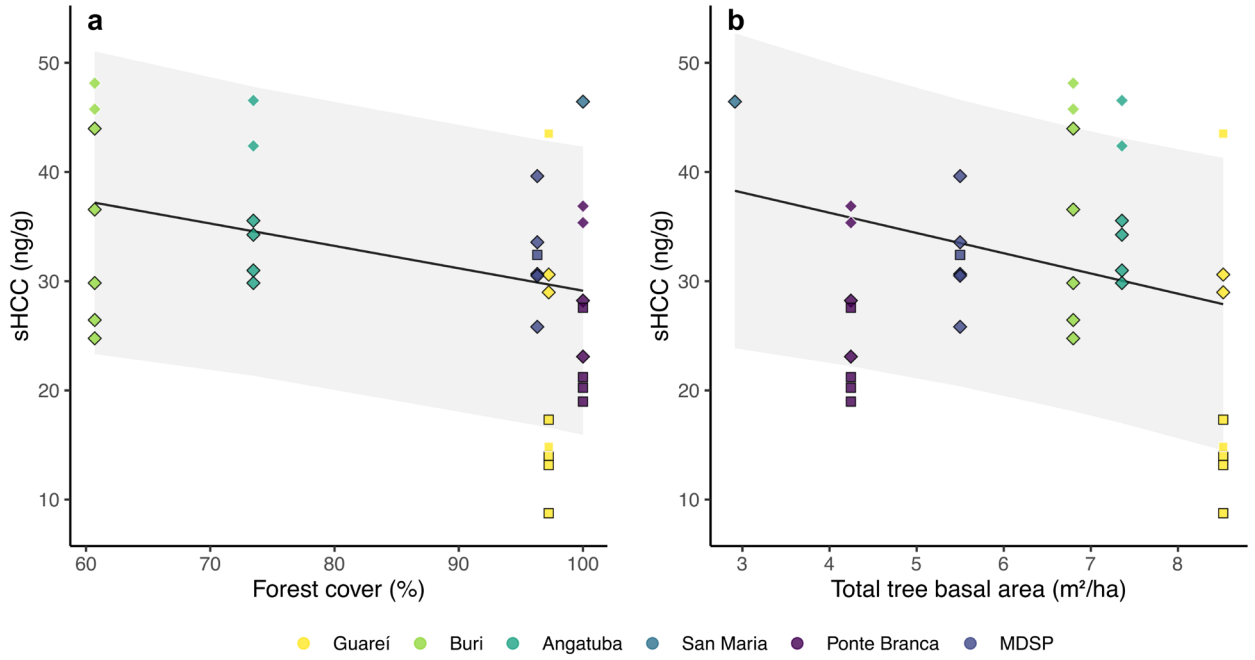

FIGURE 3 | Correlation between square root transformed hair cortisol concentrations (sHCC) in black lion tamarins and habitat quality variables—(a) forest cover and (b) total tree basal area (TBA)—based on generalised linear mixed model estimates. Model adjusted conditional $R^2 = 0.90$. Forest cover calculated within a 500-m buffer. Adults (> 18 months old) = black outline; juveniles (3–9 months old) = white outline; dry season = diamonds; rainy season = squares. MDSP, Morro do Diabo State Park.

We found significantly higher HCCs during the dry season compared to the rainy season. This suggests that resource scarcity during the dry season may induce nutritional stress in this primate, masking potential variations linked to reproduction and breeding. Similar results were found in common marmosets (*C. jacchus*), with higher HCC during the dry season across all age and sex classes (Garber et al. 2020). Finally, region had no effect on physiological stress, suggesting that black lion tamarin populations are more affected by area-specific habitat quality characteristics rather than by region. While considering the difficulties and implications of capturing an endangered primate such as the black lion tamarin, future research should attempt to amass more hair samples, with multiple repeated measures on the same individuals, to better understand inter-individual or temporal variations in HCCs linked to intrinsic or seasonal factors.

By comparing physiological stress levels in black tamarins inhabiting Atlantic Forest remnants ranging from 78 to 33,845 ha, we found that cortisol levels increased as forest cover decreased. As habitat loss continues to intensify, fragmentation of the landscape spreads and the remaining forest fragments are increasingly disconnected, irregularly shaped and degraded (Laurance et al. 2000; Hill and Curran 2003; Vancine et al. 2024). Fragmentation alters vegetation diversity, composition and structure, modifying resource availability, quality and abundance for primates (Arroyo-Rodríguez and Mandujano 2006; Marsh and Chapman 2013). The amount of forest cover surrounding the area used by a forest-dependent species is a fundamental measure of habitat quality, altering the species' resource acquisition not only by disrupting their ranging patterns, but also by increasing the prevalence of anthropogenic disturbances (de Almeida-Rocha et al. 2017; Galán-Acedo et al. 2019). As expected, both habitat loss and degradation seem to physiologically stress lion tamarins. In line with the recent research exploring the effects of habitat loss on extinction thresholds in Brazilian primates (Galán-Acedo et al. 2023), our patch-landscape analysis showed that black lion tamarins are affected by the amount of forest cover at a fine scale (i.e., within a 500-m buffer), proportional to their home range. Alarmingly, habitat availability decreases rapidly in both study regions when increasing the buffer zone, with only 21.8% ± 7.4% of forest cover within a 10 km radius (see Appendix S4). Although previous research has established that species need at least 10%–30% of native habitat within a landscape to survive (Swift and Hannon 2010), this threshold generally reaches ≥ 40% for forest-dwelling species (Arroyo-Rodríguez et al. 2020; Brindis-Badillo et al. 2022). However, Brazilian Atlantic Forest primates may require up to 78% of forest cover to survive (Galán-Acedo et al. 2023).

Habitat loss may generate other synergistic effects that may impact primate physiological stress (Kaisin et al. 2021). Areas with less forest cover may potentially have greater inter- and intra-species competition, which could cause a rise in cortisol levels. For example, overcrowding was the main cause of increased GCs in fragment-dwelling ring-tailed lemurs (*Lemur catta*; Gabriel et al. 2018). Black lion tamarins living in highly fragmented landscapes with little available habitat, as observed in Buri and Angatuba, may also be exposed to intensified human pressure (e.g., cattle ranching, agricultural machinery, domestic dogs, hunting, selective logging) linked to the proximity of agricultural exploitations and urban areas, which may increase physiological stress levels. Higher cortisol levels have also been found in other primate species due to the proximity to human settlements, noise and domestic dogs (see Kaisin et al. 2021).

Although certain primate species, such as baboons (*Papio* sp.), macaques (*Macaca* sp.) and some callitrichid species (e.g., *C. penicillata*; Secco et al. 2018) seem to be capable of adapting to environments with high human presence, other primates may not be as tolerant or possess the necessary ecological and behavioural plasticity (McLennan et al. 2017).

Inferring on forest structure as well as fruit availability, TBA is a valuable forest quality index for forest-dwelling species (Chapman et al. 1992; Galán-Acedo, Arroyo-Rodríguez, et al. 2021). We showed that black lion tamarins in habitats with lower TBA exhibited higher cortisol levels. A recent study discovered that the degree of frugivory increased in black lion tamarins as the TBA in the study area increased, suggesting that fruits are the main limiting resource for this primate (Raskin et al. 2025). Given that GCs play a fundamental role in regulating glucose metabolism, they vary according to the energy balance (i.e., intake and expenditure) of individuals (Emery Thompson 2017). In degraded habitats, where resources may be sparse and dispersed, energy intake may also decrease due to diet quality, while expenditure may amplify due to increased traveling time between food patches (Dunn et al. 2012, 2013). In agreement with our results, multiple studies have revealed that lower fruit availability triggers nutritional stress in primates (see MacLarnon et al. 2015).

On top of food availability, TBA is a proxy for forest structure, another key habitat quality variable that governs primate diversity and abundance (Pyritz et al. 2010; Gouveia et al. 2014). For example, the number of large trees dictated the occurrence of howler monkeys in tropical forest fragments (Arroyo-Rodríguez et al. 2007). Forest structure can also influence the movement of arboreal primates (Bicca-Marques and Calegaro-Marques 1995; Dagosto and Yamashita 1998; Cheyne et al. 2013). Increased travelling times, translating to enhanced energy expenditure, were also linked to higher GC concentrations in this species (Dunn et al. 2013). In our study sites, TBA was strongly correlated to the density of large trees (DBH > 20 cm; see Appendix S9), suggesting that habitat structure may also influence physiological stress in black lion tamarins. Furthermore, for this small callitrichid that sleeps in tree hollows (Valladares-Padua 1993), sleeping site availability may decrease in areas with lower TBA, because such hollows occur in more mature trees (Giancola 2021). Accordingly, black lion tamarins may have to increase their energy expenditure to find suitable sleeping sites, where they are less exposed to predators.

Habitat quality is essentially species-specific and influences the distribution, survival and fitness of populations according to the abundance and availability of resources (Johnson 2007). Our results revealed that habitat availability, forest structure and fruit availability influenced physiological stress levels in black lion tamarins. The current geographic distribution of black lion tamarins is spread across isolated forest patches throughout the Rio Paranapanema watershed, including many small unprotected Atlantic Forest remnants and riparian forests. Recent climate and landscape models suggest that black lion tamarins already inhabit 40% of areas suitable for them, representing less than 1% of their original distribution due to intense habitat loss and fragmentation (Rezende, Sobral-Souza, et al. 2020). The forest cover percentages calculated within the areas inhabited by black lion tamarins were alarmingly lower than the extinction threshold recently established for Brazilian Atlantic Forest primates (Galán-Acedo et al. 2023). Protecting and preserving small fragments while increasing forest quality, availability and inter-fragment connectivity through habitat management strategies and limiting human encroachment are paramount for the conservation of this endangered species.

Author Contributions

Olivier Kaisin, Fany Brotcorne and Laurence Culot contributed to the design and implementation of the research, analysis and interpretation of the results and led the writing of the manuscript. Fany Brotcorne, Laurence Culot and Pascal Poncin supervised the research and led project administration and funding acquisition. Olivier Kaisin, Felipe Bufalo, Rodrigo Gonçalves Amaral, Gabriel Pavan Sabino and Gabriela Cabral Rezende contributed to data collection. Rupert Palme supervised hormone analysis. All authors contributed critically to the drafts and gave final approval for publication.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.