Stressed-out Primates?

Physiological and behavioural responses of primates to habitat disturbances: a focus on black lion tamarins (*Leontopithecus chrysopygus*) in the Brazilian Atlantic Forest

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Dissertation submitted in fulfilment of the requirements for the degree of Doctorate in Sciences

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Primates stressés?

Réponses physiologiques et comportementales des primates face aux perturbations de leur habitat : focus sur les tamarins-lions noirs (*Leontopithecus chrysopygus*) dans la forêt atlantique brésilienne

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Primatas estressados?

Respostas fisiológicas e comportamentais de primatas a distúrbios de habitat: um foco em micos-leões-pretos (*Leontopithecus chrysopygus*) na Mata Atlântica brasileira

Olivier Kaisin

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“An understanding of the natural world is a source of not only great curiosity, but great fulfilment.”

Sir David Attenborough

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Summary

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The unsustainable exploitation of Earth's resources, driven by the exponential human population growth, has led to the destruction of more than a tenth of natural areas across the globe over the past two decades alone. Occurring across tropical and subtropical regions, including major hotspots for biodiversity, primates are suffering from habitat loss, degradation, and fragmentation. Predictive models estimate that 75 percent of primate habitats will undergo intense agricultural expansion during this century. Thus, evaluating and understanding the effect of anthropogenic disturbances on primate populations is paramount for their conservation. In this context, measuring glucocorticoid (GC) concentrations is a powerful tool to assess animals' welfare, giving precious insight on their behaviour, physiology, and ecology. GCs are metabolic hormones that mediate energetic demands necessary to respond to predictable and unpredictable environmental and social challenges. Given their pivotal role in the stress response and the potentially deleterious effect of chronic stress they have been increasingly used as indicators of animal health, survival, and fitness. To improve current knowledge of how primates cope in changing environments, in this thesis we explored GC variations at a global scale before focusing on an endangered neotropical primate, the black lion tamarin (*Leontopithecus chrysopygus*), at a regional and local scale.

Due to the development of non-invasive methods to monitor GCs in wildlife, the number of studies exploring physiological stress in free-ranging primates has considerably increased over the past decades. Here, we started by performing a global meta-analysis to provide an overview of how physiological stress levels vary in primate populations subject to anthropogenic disturbances and uncover general trends. We established that, overall, primates inhabiting disturbed areas exhibited higher GC levels than their conspecifics in undisturbed sites. Furthermore, habitat loss and hunting were the main threats influencing physiological stress in primate populations. Comparing responses across primate species, living in different biomes and exposed to different disturbances of varying intensities, enabled us to identify important confounding factors that must be considered in future research. Additionally, investigating responses across various primate taxa revealed that species, populations, and even individuals may have independent tolerance thresholds and degrees of plasticity when dealing with disturbances.

After evaluating primates' physiological responses at a global scale, we focused our research on the black lion tamarin, a frugivorous and faunivorous primate endemic to the Brazilian Atlantic Forest. This biome has suffered extensive deforestation and is currently highly fragmented, composed of small islands of forest within a human-degraded matrix of agricultural exploitations, pastures, and urban areas. To assess how this small primate copes

in a degraded landscape, we evaluated the influence of habitat quality on GC levels in lion tamarin groups living in six different Atlantic Forest fragments of varying sizes. We measured GC levels in hair samples to obtain information on the overall physiological stress state of black lion tamarins. We found that low habitat quality negatively affects their GC levels since cortisol levels were higher in sites with lower total tree basal area (i.e., a proxy for fruit availability and habitat structure), vegetation diversity, and fragment size. Yet, the arthropod biomass of each fragment did not influence physiological stress levels. Our results also revealed that higher levels of frugivory may reduce cortisol levels, implying that fruits represent an important and limiting resource for black lion tamarins. This study suggests that healthy and protected Atlantic Forest fragments may represent suitable habitats for this neotropical primate with a broad diet and a relatively small home range.

Finally, we looked deeper into the factors influencing physiological stress in black lion tamarins by studying faecal GC levels over multiple timescales. We collected faecal samples and behavioural data from two groups in two different sites: a continuous forest and a small 100 ha fragment. Working with faecal samples enabled us to unravel the complex nature of physiological stress in relation to activity budget and diet. We analysed GC variations at different time scales to understand the predictive and reactive facets of physiological stress. Given the high defecation rate of black lion tamarins, we were able to identify typical circadian patterns and account for them in our analyses. At a monthly scale, our results showed that diet and ranging patterns, driven by food availability and distribution, influenced GC levels as part of the predictive role of physiological stress. Then, to identify which acute stressors triggered a reactive stress response in this neotropical primate, we explored GC levels at a day-to-day level, while taking into account the predictive range of physiological stress (i.e., the anticipated hourly and monthly variations). We discovered that, although fundamental behaviours (i.e., feeding, foraging, moving, resting) did not influence daily GC levels, encounters with neighbouring lion tamarin groups sparked a stress response in the group living in the small fragment. This suggests that intergroup competition and maintaining access to precious resources may be more challenging for black lion tamarins constrained to small habitats. The distinctive theoretically-grounded approach used in this study allowed for an in-depth understanding of GC variations in black lion tamarins, making a meaningful contribution to the study of physiology stress in relation to the ecology of wild primates.

In this thesis, we started with a global overview and progressively made our way to an in-depth analysis of an endangered, little-studied, and opportunist primate. This allowed us to further understand the complex elements underlying the physiological stress of wild primates in changing environments subjected to human pressure. While the physiological stress response enables species to overcome and adapt to challenges, the key question for future studies in conservation physiology is determining the threshold at which elevated GC levels may become maladaptive. The ability of a species to respond to anthropogenic stressors will

depend on their tolerance thresholds, behavioural flexibility, and dietary plasticity. Thus, to establish well-founded and species-specific conservation management plans it is crucial to have an adequate understanding of species' life history traits, diet, behaviour, habitat, ecology, and physiology.

Resumé

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Au cours des deux dernières décennies, l'exploitation non-durable des ressources de notre Terre, nourrie par la croissance exponentielle de la population humaine, a causé la destruction de plus d'un dixième des écosystèmes naturelles à travers le monde. Au sein des régions tropicales et subtropicales, comptant plusieurs points chauds majeurs de biodiversité, les primates sont affectés par la perte, la dégradation, et la fragmentation de leur habitat. Des modèles prédictifs estiment qu'au cours du 21^{e} siècle 75% des habitats occupés par les primates feront face à une reconversion agricole intense. Pour leur conservation, il est dès lors impératif d'évaluer et de comprendre l'effet de ces perturbations anthropiques sur les populations de primates. Dans ce but, la mesure de glucocorticoïdes (GCs) constitue un outil efficace pour évaluer le bien-être d'un animal, en offrant des informations précieuses sur son comportement, sa physiologie et son écologie. Les GCs sont des hormones métaboliques qui régulent les demandes énergétiques nécessaires pour faire face aux facteurs de stress environnementaux et sociaux, à la fois prévisibles et imprévisibles. Étant donné leur rôle central dans la réponse au stress et l'effet potentiellement délétère de celui-ci s'il s'avère chronique, les GCs sont de plus en plus utilisés comme indicateurs de la santé, de la survie et de la condition physique des animaux. Afin d'améliorer nos connaissances actuelles sur la façon dont les primates font face à des environnements changeants, nous avons, au cours de cette thèse, exploré les variations de GCs chez les primates à l'échelle mondiale avant de nous concentrer plus particulièrement sur une espèce néotropicale menacée, le tamarin-lion noir (*Leontopithecus chrysopygus*).

Grace au développement de méthodes non-invasives de quantification des GCs au sein de la faune, le nombre d'études explorant le stress physiologique chez les primates en milieu naturel a considérablement augmenté depuis 2010. Dans le cadre de cette recherche, nous avons commencé par réaliser une méta-analyse globale. Ce premier chapitre a permis d'analyser la variation des niveaux de stress physiologique au sein des populations de primates soumises à des perturbations anthropiques et de mettre en évidence les tendances générales. Nos résultats ont montré que, dans l'ensemble, les primates vivant dans des sites perturbés présentaient des niveaux de GCs plus élevés que leurs congénères issus de sites moins perturbés. La perte d'habitat et la chasse représentent les principales menaces accentuant le stress physiologique chez les primates. Cette étude a également révélé que les espèces, les populations et même les individus présentent des seuils de tolérance et des degrés de plasticité différents lorsqu'ils font face aux mêmes perturbations. En comparant les réponses physiologiques de nombreuses espèces de primates vivant dans divers biomes et soumises à différentes perturbations, nous avons pu identifier plusieurs facteurs confondants à prendre en compte lors de futures recherches dans ce domaine.

Après avoir évalué les réponses physiologiques des primates à l'échelle mondiale, nous avons focalisé nos recherches sur le tamarin-lion noir, un petit primate frugivore et faunivore endémique de la forêt atlantique brésilienne. Ce biome ayant subi une déforestation importante, il est actuellement très fragmenté et composé de petits îlots de forêt répartis au sein d'une matrice d'exploitations agricoles, de pâturages et de zones urbaines. Afin d'évaluer l'adaptation de ce primate au sein de ces paysages dégradés, nous avons étudié l'influence de la qualité de l'habitat sur les niveaux de GCs pour plusieurs groupes de tamarins-lions vivant dans six fragments de forêt de différentes tailles. Nous avons quantifié les GCs dans des échantillons de poils afin d'obtenir une mesure intégrée de l'activité corticosurrénale et ainsi l'état de stress physiologique général des tamarins-lions noirs. Nos résultats ont démontré que lorsque la qualité de l'habitat diminue, les taux de GCs augmentent. En effet, les niveaux de GCs sont plus élevés dans les fragments de plus petite taille, avec une surface terrière totale (i.e., un indicateur de la disponibilité des ressources et de la structure de l'habitat) et une diversité végétale. Cependant, la biomasse d'arthropodes au sein de chaque fragment n'a aucun impact sur les niveaux de stress physiologique. Nos résultats ont également mis en évidence que des niveaux plus élevés de frugivorie peuvent réduire les niveaux de GCs, ce qui confirme que les fruits représentent une ressource importante et limitante pour les tamarins-lions noirs. De manière générale, cette étude suggère que des fragments sains et protégés de la forêt atlantique pourraient représenter des habitats appropriés pour ce primate néotropical au régime alimentaire diversifié et au domaine vital relativement petit.

Pour terminer, sur base de modèles théoriques et en mesurant les niveaux de GCs dans les fèces à différentes échelles de temps, nous avons pu étudier en détails les facettes prédictives et réactives du stress physiologique chez les tamarins-lions. Ceci nous a permis de mieux comprendre la nature complexe du stress physiologique et de lier ce dernier au budget d'activité et à l'alimentation de cette espèce. Dans ce but, nous avons collecté les échantillons fécaux ainsi que les données comportementales de deux groupes au sein de deux sites distincts : une forêt continue et un petit fragment de 100 ha. Grâce au taux de défécation élevé des tamarins-lions noirs, nous avons pu identifier leurs variations circadiennes typiques des tamarins-lions et tenir compte de l'heure de défécation dans nos analyses. À l'échelle mensuelle, nos résultats ont montré que le régime alimentaire et le mouvement des tamarins-lions, gouvernés par la distribution et la disponibilité des ressources, influençaient les niveaux de GCs dans le cadre de l'étendue prédictive du stress physiologique. Ensuite, afin d'identifier les facteurs de stress aigus qui déclenchent une réponse au stress chez ce primate, nous avons analysé les niveaux de GCs journaliers, tout en tenant compte du rôle prédictif du stress physiologique (i.e., les variations circadiennes et mensuelles anticipées). Nos résultats montrent que, même si les comportements fondamentaux (i.e., le mouvement, l'alimentation, la recherche de nourriture et le repos) n'ont pas d'influence sur les niveaux quotidiens de GCs, les rencontres avec d'autres groupes de tamarins-lions déclenchent une réponse au stress dans le groupe vivant au sein du plus petit fragment. Ceci suggère que la compétition intergroupe

ainsi que le maintien de l'accès aux ressources sont certainement plus difficiles pour les tamarins-lions restreints à de plus petits habitats. L'approche singulière utilisée dans cette étude, basée sur des modèles théoriques et combinant des données hormonales, comportementales et écologiques, a permis la compréhension approfondie des variations de GCs chez les tamarins-lions noirs, représentant une contribution significative à l'étude de la physiologie du stress écologique chez les primates sauvages.

Cette thèse a débuté par un aperçu global du stress physiologique chez les primates, suivi de l'étude d'une espèce menacée, peu étudiée et opportuniste. Cela nous a permis de mieux comprendre les éléments complexes qui sous-tendent le stress physiologique chez les primates au sein d'environnements en évolution rapide face aux pressions anthropiques. Bien que la réponse au stress permette aux espèces de surmonter les défis et de s'y adapter, une question clé pour les futures études en physiologie de la conservation consiste à déterminer le seuil auquel les niveaux élevés de GCs peuvent devenir délétères. La capacité des espèces à répondre aux facteurs de stress anthropiques dépend de leur seuil de tolérance, leur flexibilité comportementale et leur plasticité alimentaire. Ainsi, pour établir des plans de gestion de la conservation fondés et spécifiques aux espèces, il est essentiel d'avoir une compréhension adéquate du cycle biologique, du régime alimentaire, du comportement, de l'habitat, de l'écologie et de la physiologie des espèces.

Resumo

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A exploração insustentável dos recursos da Terra, alimentada pelo crescimento exponencial da população humana, levou à destruição de mais de dez porcento das áreas naturais em todo o mundo nas últimas duas décadas. Ocorrendo em regiões tropicais e subtropicais, incluindo grandes hotspots de biodiversidade, os primatas vêm sofrendo com a perda, degradação e fragmentação do habitat. Modelos preditivos estimam que 75% dos habitats ocupados por primatas enfrentarão intensa expansão agrícola ainda neste século. Assim, avaliar e compreender o efeito das perturbações antropogênicas nas populações de primatas é primordial para a sua conservação. Nesse contexto, a medição das concentrações de glucocorticóides (GCs) é uma ferramenta poderosa para avaliar o bem-estar de um animal, fornecendo informações preciosas sobre seu comportamento, fisiologia e ecologia. Os GCs são hormônios metabólicos que mediam as demandas energéticas necessárias para responder a desafios ambientais e sociais previsíveis e imprevisíveis. Dado seu papel fundamental na resposta ao estresse e o efeito potencialmente deletério do estresse crônico, GCs têm sido cada vez mais usados como indicadores de saúde, sobrevivência e condicionamento animal. Desta forma, para aprofundar o conhecimento atual acerca de como os primatas lidam com alterações ambientais, nesta tese nós inicialmente exploramos as variações de GCs em escala global, para então focar em um primata neotropical ameaçado de extinção, o mico-leão-preto (*Leontopithecus chrysopygus*), nas escalas regional e local.

Graças ao desenvolvimento de métodos não invasivos para quantificar GCs in situ, o número de estudos explorando o estresse fisiológico em primatas na natureza aumentou consideravelmente desde 2010. Neste estudo, inicialmente realizamos uma meta-análise global para analisar como os níveis de estresse fisiológico variam em populações de primatas submetidas a distúrbios antropogênicos, revelando tendências gerais. Nossos resultados mostram que, em geral, os primatas que vivem em áreas perturbadas exibiram níveis mais altos de GCs do que seus coespecíficos em locais não perturbados. A perda de habitat e a caça foram as principais ameaças que influenciaram o estresse fisiológico em primatas. Ao comparar as respostas fisiológicas de vários primatas, vivendo em diferentes biomas e submetidos a diferentes distúrbios de intensidades variadas, conseguimos identificar fatores de confusão importantes que precisarão ser considerados em pesquisas futuras nessa área. Além disso, nós indicamos que espécies, populações e até indivíduos podem ter diferentes limiares de tolerância e graus de plasticidade ao lidar com distúrbios.

Após avaliar as respostas fisiológicas dos primatas em escala global, focamos nossa pesquisa no mico-leão-preto, um pequeno primata frugívoro e faunívoro endêmico da Mata Atlântica brasileira. Este bioma, significativamente degradado, atualmente é altamente

fragmentado e composto por pequenas ilhas de floresta dentro de uma matriz de fazendas, pastagens e áreas urbanas. Para avaliar como este pequeno primata sobrevive nessa paisagem degradada, avaliamos a influência da qualidade do habitat nos níveis de GCs em vários grupos de micos-leões que vivem em seis fragmentos de Mata Atlântica de diferentes tamanhos. Medimos GCs em amostras de pelo para avaliar o estado fisiológico geral dos micos-leões-pretos. Nossos resultados demonstraram que quando a qualidade do habitat diminui, os níveis de GCs aumentam. De fato, os níveis de GCs foram maiores em fragmentos com menor área basal total (i.e., um indicador de disponibilidade de recursos e de estrutura de habitat), diversidade da vegetação e tamanho do fragmento. No entanto, a biomassa de artrópodes dentro de cada fragmento não teve efeito sobre os níveis de estresse fisiológico. Nossos resultados também revelaram que níveis mais altos de frugivoria podem reduzir os níveis de cortisol, confirmando que os frutos representam um recurso importante e limitante para os micos-leões-pretos. Neste estudo, nós apontamos que remanescentes de Mata Atlântica saudáveis e protegidos podem representar habitats adequados para este primata neotropical com uma dieta diversificada e área de vida relativamente pequena.

Finalmente, examinando os níveis de GCs medidos nas fezes durante diferentes escalas de tempo, pudemos estudar em detalhes os fatores que influenciam o estresse fisiológico em micos-leões-pretos. Nós coletamos amostras fecais e dados comportamentais de dois grupos em dois locais: uma floresta contínua e um fragmento de 100 ha. Trabalhando com amostras fecais, fomos capazes entender melhor a natureza complexa do estresse fisiológico e vinculá-lo ao orçamento de atividade e dieta dos micos-leões. Nós analisamos as variações dos GCs em diferentes escalas de tempo para então entender as facetas preditivas e reativas do estresse fisiológico. Dada a alta taxa de defecação dos micos-leões-pretos, nós identificamos variações circadianas típicas e, assim, contabilizamos o tempo de defecação para nossas análises subsequentes. Na escala mensal, nossos resultados mostraram que a dieta e o movimento do mico-leão, determinados pela disponibilidade e distribuição de recursos, influenciaram os níveis de GCs como parte do papel preditivo do estresse fisiológico. Em seguida, a fim de identificar quais estressores agudos desencadearam respostas reativas neste primata, nós exploramos os níveis diários de GCs, levando em consideração o papel preditivo do estresse fisiológico (i.e., variações horárias e mensais previstas). Nossos resultados mostram que, embora os comportamentos fundamentais (i.e., movimento, alimentação, forrageio) não tenham influenciado os níveis diários de GCs, encontros com outros grupos de micos-leões desencadearam respostas de estresse no grupo restrito ao pequeno fragmento. Isso sugere que a competição entre grupos, bem como a manutenção do acesso a recursos valiosos, pode ser um maior desafio para micos-leões em pequenos habitats. A abordagem distinta utilizada neste estudo, baseada em modelos teóricos, permitiu o aprofundamento do entendimento acerca das variações do GCs em micos-leões-pretos, contribuindo significativamente para o estudo da ecologia do estresse fisiológico em primatas selvagens.

Nesta tese, começamos com uma visão global do estresse fisiológico em primatas e depois avançamos para o estudo de uma espécie ameaçada, pouco estudada e oportunista. Isto nos permitiu entender melhor os elementos complexos relacionados ao estresse fisiológico em primatas em ambientes sujeitos a pressões antrópicas. Embora a resposta fisiológica ao estresse permita que as espécies se adaptem e superem desafios, uma questão-chave para estudos futuros em fisiologia da conservação é determinar o limite em que altos níveis de GCs possam se tornar deletérios. A capacidade das espécies de responder aos estressores antropogênicos dependerá de seu limiar de tolerância, sua flexibilidade comportamental e sua plasticidade alimentar. Assim, a fim de estabelecer planos de gestão de conservação bem fundamentados e espécie-específicos, é essencial que tenhamos uma compreensão adequada da história de vida, dieta, comportamento, habitat, ecologia e fisiologia das espécies de interesse.

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List of Abbreviations and Acronyms

-

ACTH: adrenocorticotropic hormone

AIC: Akaike information criterion

AVP: arginine vasopressin

CAR: cortisol awakening response

CGB: corticosteroid-binding globulin

CRH: cortisol releasing-hormone

CV: coefficient of variation

EIA: enzyme immunoassay

fGM: faecal glucocorticoids metabolites

GC: glucocorticoids

GR: glucocorticoid receptor

HCC: hair cortisol concentrations

HPA: hypothalamus-pituitary-adrenal

IUCN: International Union for Conservation of Nature

IRMS: isotope ratio mass spectrometer

LM: linear model

LMM: linear mixed model

MDSP: Morro do Diabo State Park

MR: mineralocorticoid receptor

PVN: paraventricular nucleus

REML: restricted maximum likelihood

RIA: radioimmunoassay

SNS: sympathetic nervous system

TEF: trophic enrichment factor

VIF: variance inflation factor

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State of the Art

1. Primates in peril

1.1. Why primates matter

Non-human primates (hereafter 'primates') are one of the richest and diverse mammal orders, gathering a total of 521 extant species, spread in 91 countries (IUCN, 2022). These species are distributed across three biogeographic realms, including the Neotropics, the Afrotropics (i.e., mainland Africa and Madagascar), and Indomalaya (Fig. 1), and inhabit multiple hotspots of biodiversity (i.e., regions with exceptional degrees of endemism that are enduring severe habitat loss; Myers et al., 2000; IUCN, 2022). Although a large portion of primates inhabit tropical forests, they also occur in subtropical and temperate forests, woodlands, and savannahs (Estrada et al., 2022). Nevertheless, primates are mainly forest-dependent, with 97 percent of species inhabiting areas with forested environments (IUCN, 2022). In order to thrive and survive in a wide range of different habitats and climates, primate species have evolved to occupy a variety of ecological niches, which is further demonstrated by their diversity in body sizes, locomotor adaptations, diets, ranging patterns, and social systems (Mitani et al., 2012; Rylands & Mittermeier, 2014). Given their important reliance on forested habitats, primates are particularly vulnerable to habitat disturbances and are thus considered excellent ecological indicators of ecosystem health (Isaac & Cowlishaw, 2004; Cavada et al., 2016; Estrada et al., 2022).

As Humans' closest relatives, primates have always fascinated anthropologists, behaviouralists, biologists, and ecologists. On top of providing unparalleled insights on human evolution, cognition, behaviour, and sociality, they fulfil a range of crucial ecological functions (Marshall & Wich, 2016). Primates play key roles in the balance, structure, and resilience of ecosystems, in which they act as prey, predators, competitors, and hosts to parasites (Setchell, 2019). Moreover, primates provide precious ecosystem services including the pollination and seed dispersal of vascular plants (Janson et al., 1981; Goldingay et al., 1991; Lambert & Garber, 1998; Chapman, 2005; Heymann, 2011; Bufalo et al., 2016). Multiple studies have highlighted that many of these mutualist flower-pollinator and fruit-frugivore interactions may have co-evolved or at least share evolutionary history (Sussman, 1991; Kress et al., 1994; Gómez & Verdú, 2012; Fuzessy et al., 2022). Due to their function as gardeners of the forest, the local extinction of primate populations has been linked to important changes in forest structure, biodiversity, and recruitment potential (Nunez-Iturri et al., 2008; Brodie et al., 2009; Federman et al., 2016; Culot et al., 2017). For all these reasons, including their role as flagship, umbrella, and indicator species, studying primate ecology and promoting their conservation is paramount (Marshall & Wich, 2016; Estrada et al., 2017).

1.2. Main threats

Unsustainable exploitation of Earth's resources, exacerbated by exponential human population growth, has spurred the unprecedented destruction of habitat (Díaz et al., 2019), leading to global declines in primate populations (Estrada et al., 2017; Fernández et al., 2022). To date, approximately 93 percent of primate species have declining populations and about 68 percent are threatened with extinction (i.e., classified as vulnerable, endangered, or critically endangered by the International Union for Conservation of Nature (IUCN); IUCN, 2022; Estrada et al., 2022). Across geographic regions, Madagascar has the largest percentage of threatened species (96.3%) followed by Indomalaya (83.7%), mainland Africa (50.9%), and lastly the Neotropics (40.2%; Fig. 1; Fernández et al., 2022).

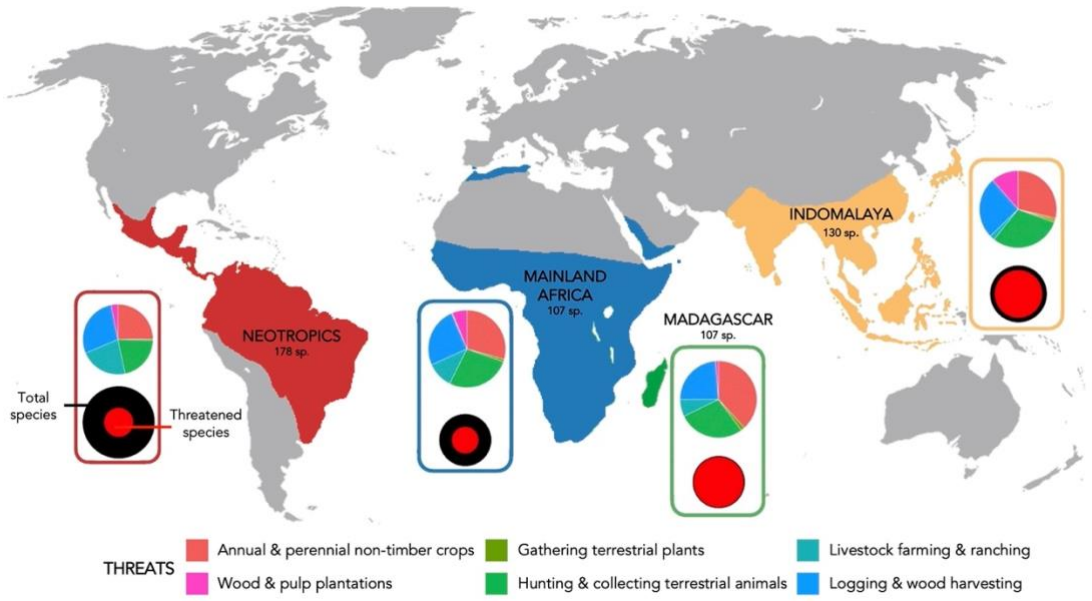

***Figure* 1:** Main threats and conservation status of primate species within the Neotropic, Afrotropic (i.e., mainland Africa and Madagascar), and Indomalayan biogeographic realms. The pie chart represents the proportion of species affected by distinct threats and the bottom circle represents the proportion of threatened species (i.e., classified as vulnerable, endangered, or critically endangered by the IUCN; modified from Fernández et al., 2022).

Unsurprisingly, habitat loss, caused by the overexploitation of natural resources, landscape conversion, agricultural activity, and urban development, is the leading cause of defaunation (Laurance, 2007; Maxwell et al., 2016). Primates are unfortunately not immune to the loss and degradation of natural habitats, which are the main threats hanging over their populations (Estrada et al., 2017). Between 2001 and 2018, deforestation rates reached ~11 million ha per year across primates' range (Estrada et al., 2019, 2020). Habitat loss, degradation, and fragmentation have disastrous synergistic impacts on primate populations, threatening their survival by limiting their ranging patterns, access to resources, dispersal, and

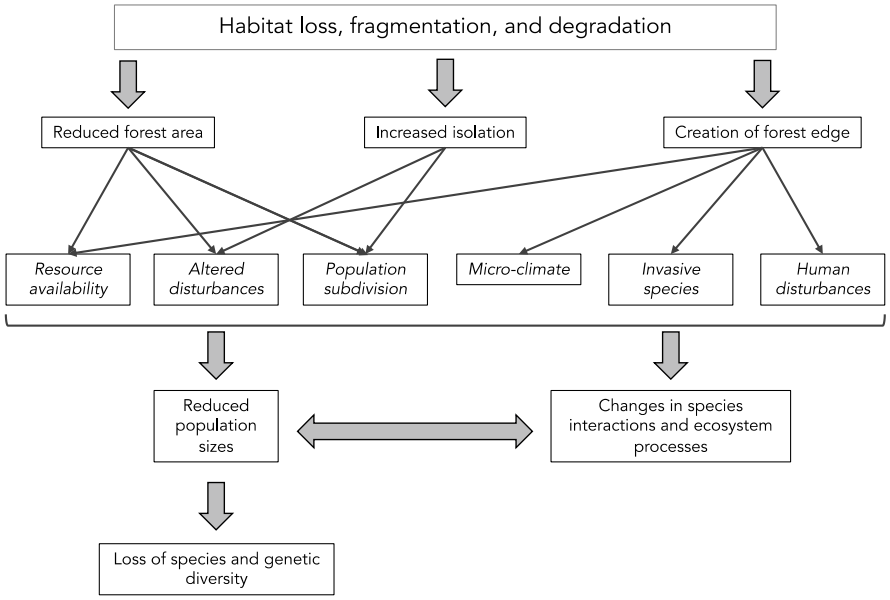

***Figure* 2:** Synergistic effects of habitat loss, fragmentation, and degradation on species (modified from Kupfer et al., 2006).

gene flow but also increasing intra- and interspecific competition as well as disease transmission (Fig. 2; Chapman et al., 2005; Kupfer et al., 2006; Benítez-Malvido & Arroyo-Rodríguez, 2008; Köndgen et al., 2008; Gardner et al., 2009). A pantropical meta-analysis assessing biodiversity metrics in disturbed versus undisturbed areas showed that primate diversity, species richness, and abundance are negatively affected by agriculture and logging (de Almeida-Rocha et al., 2017). Large-scale agricultural expansion is the principal cause of habitat loss affecting primate species (76% of species), followed by logging and wood harvesting (60%), and livestock farming and ranching (31%; Estrada et al., 2017). This commodity-driven deforestation consists in the clearing of forested areas and their replacement by monoculture plantations (e.g., oil palm, soybean, sugarcane) or pastures for cattle ranching (*Global Forest Watch*, 2022). For example, the increase in global demand for palm oil has devasted and still threatens Bornean orangutan (*Pongo pygmaeus*) populations, a flagship species in conservation biology and primatology (Swarna Nantha & Tisdell, 2009). In order to meet global demands for hardwood and to increase their economic growth, countries in primate range regions have also expanded their logging activities at staggering rates (Estrada et al., 2017). Road building, urban expansion, mining, and fossil fuel extraction represent additional causes of habitat loss and degradation.

Habitat loss is often associated with coinciding disturbances due to the intensified contact with human populations, which can also lead to changes in the behaviour, diet, and physiology of primates (see Section 2.4; Beehner and Bergman, 2017; McLennan *et al.*, 2017; Gould *et*

al., 2020). The exploitation of natural resources, such as fossil fuel extraction and mining, spurs the expansion of road networks, which often facilitates access to forests by poachers, promoting illegal hunting and trade (Laurance et al., 2014; Alamgir et al., 2017; Harfoot et al., 2018). Hunting for bushmeat and culturally valued body parts is a major driving force of primate population declines, affecting 60 percent of primate species (Estrada et al., 2017). This issue is particularly critical in Africa and Southeast Asia (Meijaard et al., 2011; Remis & Jost Robinson, 2012; Fa et al., 2015). In the Congo Basin, extensive bushmeat hunting has devastated populations of multiple primate species, including eastern gorillas (*Gorilla beringei*) and chimpanzees (*Pan troglodytes*; Remis & Jost Robinson, 2012; Spira et al., 2019). On top of the direct loss of primates to hunting and trade, increased contact with human populations enhances risks of zoonotic disease transmission, especially in great apes (Köndgen et al., 2008; Genton et al., 2012; Molyneaux et al., 2021).

Climate change has become an emerging threat to tropical ecosystems and consequently primates. While the Amazon, the Brazilian Atlantic Forest, Central America, and Southeast Asia are hotpots of biodiversity, they are also the areas predicted to be the most vulnerable to climate change (Graham et al., 2016). Paradoxically, while tropical forests act as world climate regulators, being important carbon dioxide sinks and significant terrestrial carbon stocks, they are expected to be the most impacted by climate change (Dixon et al., 1994). As tropical organisms, primates have narrower thermal tolerance levels than temperate mammals and are likely to be more impacted by global rising temperatures (Khaliq et al., 2014). Recent biogeographic models have projected that climate change will considerably reduce suitable habitats for multiple species, putting primate populations at significant risk (Meyer et al., 2014; Brown & Yoder, 2015; Struebig et al., 2015).

1.3. Habitat loss in Brazil

Brazil is the country with the second largest forest area, as well as the highest primate richness, home to 116 species (FAO, 2020; IUCN, 2022). However, it is infamous for being the world leader in terms of deforestation (Turubanova et al., 2018; Estrada et al., 2019). The main causes of deforestation in Brazil are livestock farming, agriculture, wood and pulp plantations, and mining. In 2016, Brazil was the world leader in the exportation of commodities that directly affect to primates habitats (i.e., forestry products, beef, and soybean; Estrada et al., 2019). In the Neotropics, it was also the leading exporter of metals and minerals. Spurred by growing global demands, Brazilian beef production has increased exponentially since the 1980s, accelerating deforestation (França et al., 2021). While most of Brazil's agriculture production is presumably deforestation-free, illegal forest clearing, to be replaced by pastures, has reached alarming rates. This is illustrated by the catastrophic wildfires that hit Brazil in 2019, which were directly linked to deforestation (Escobar, 2019).

Among the four countries considered essential for primate conservation (i.e., Brazil, Madagascar, Indonesia, and the Demographic Republic of Congo; Estrada et al., 2018), Brazil showed the highest rate for tree cover loss between 2011 and 2016 (Potapov et al., 2017). Over the past 20 years, 27.8 million ha of Brazil's primary forest has disappeared, representing an 8.1 percent decline. Furthermore, just in 2021, 2.9 million ha of natural forest was clearcut, an area nearly equivalent to the size of Belgium (*Global Forest Watch*, 2022). Currently, due to increasing global market demands, mining concessions already cover 21 percent of the Amazon (Bebbington et al., 2018). Projections are alarming, estimating that areas inhabited by primates in Brazil could potentially decrease by 78 percent in 2050, under a worst-case-scenario of agriculture expansion (Estrada et al., 2018).

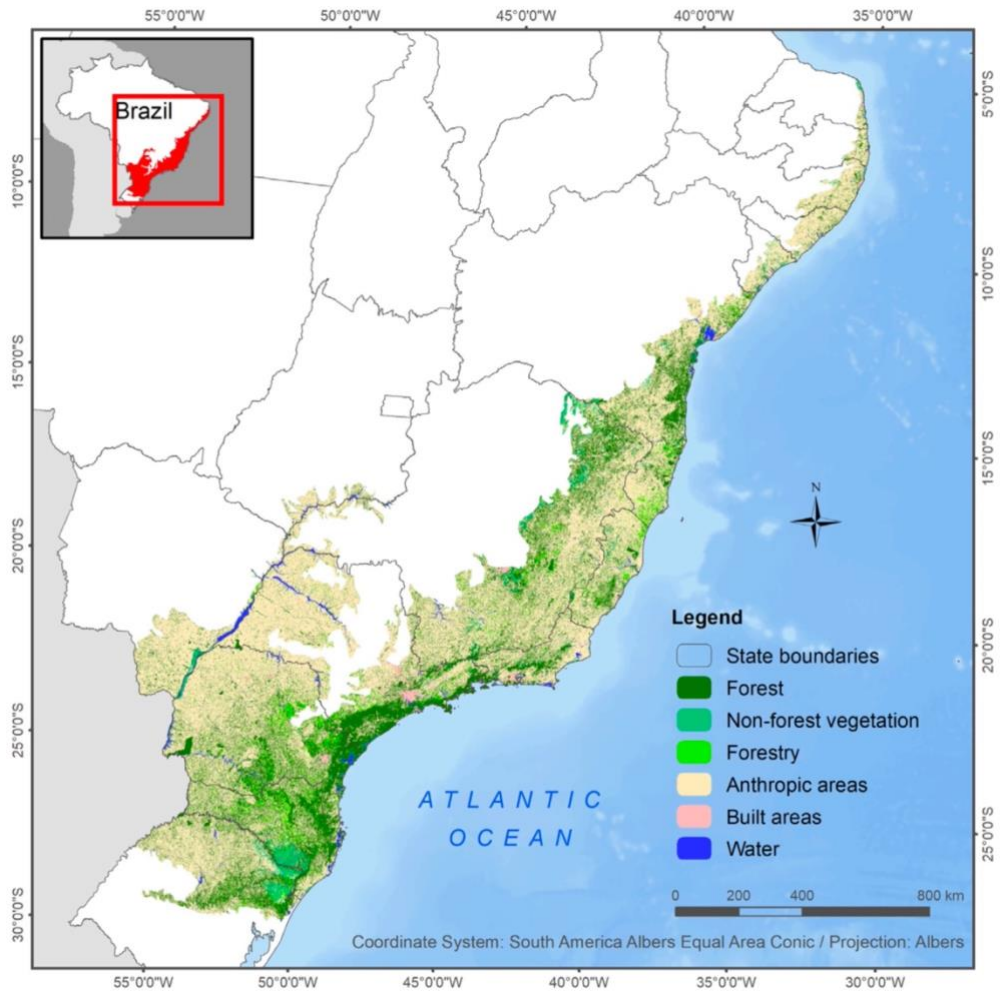

***Figure* 3:** Land cover in the Atlantic Forest biome in eastern Brazil. Native forests cover 26% of the area, 2% is covered by water bodies, 2% by urban areas, 3% by tree monocultures, and 65% by other anthropogenic areas, including agriculture, pastures, mining, and degraded areas (Rezende et al., 2018).

1.3.1. The Brazilian Atlantic Forest

The Brazilian Atlantic Forest, is one of the Earth's 25 biodiversity hotspots, hosting an exceptional degree of endemic flora and fauna (Myers et al., 2000; Mittermeier et al., 2011). It is home to 26 primate species, of which 19 are endemic (Culot et al., 2019). While it used to be one of the largest rainforest of the Neotropics, stretching over 150 million ha, only 26 percent of the native forest cover remains (Fig. 3; Rezende et al., 2018). The Brazilian Atlantic Forest has been highly impacted by habitat loss due to land conversion for agriculture, plantation forestry, cattle ranching, and unsustainable and illegal logging (Joly et al., 2014). This has transformed this biome into a landscape of endless archipelagos of small forest fragments surrounded by a matrix of pastures and plantations (Ribeiro et al., 2009). Indeed, the Brazilian Atlantic Forest is highly fragmented, with 83 percent of forest remnants having areas inferior to 50 ha (Ribeiro et al., 2009). In other words, only 9 percent of the Brazilian Atlantic Forest is farther than 1 km away from a forest edge (Haddad et al., 2015). Furthermore, the remaining vegetation is presumably mainly secondary or degraded forest (Rezende *et al.*, 2018). Within the State of São Paulo, 2.8 million ha of the Atlantic Forest remains, representing only 16.2 percent of its natural area (SOS Mata Atlântica, 2019).

1.4. Effects on behaviour, ecology, and physiology of primates

Widespread habitat loss has led to the fragmentation of subtropical and tropical forest (Haddad et al., 2015), forcing primate populations to live in increasingly disconnected, irregularly shaped, and degraded fragments (Laurance et al., 2002; Hill & Curran, 2003; Benítez-Malvido & Arroyo-Rodríguez, 2008). Altogether, habitat loss, fragmentation, and degradation have synergistic, multidimensional, and cascading effects on primate populations (Fig. 2; Kalbitzer & Chapman, 2018), impeding resource acquisition by impairing their ranging patterns, but also by increasing the prevalence of anthropogenic disturbances (Benítez-Malvido & Arroyo-Rodríguez, 2008; de Almeida-Rocha et al., 2017). Indeed, fragmentation, along with enhanced edge effects, alters vegetation diversity, composition, and structure, as well as resource availability, quality, and abundance for primates (Arroyo-Rodríguez & Mandujano, 2006; Marsh & Chapman, 2013). These changes in habitat can influence the behaviour and ecology of primates (Arroyo-Rodríguez et al., 2007; Arroyo-Rodríguez & Mandujano, 2009; da Silva Júnior et al., 2009). Accordingly, habitat quality (i.e., resources and conditions that influence the presence, abundance, and fitness of a certain species) is a major factor driving the occurrence and abundance of primate populations in fragments (Estrada and Coates-Estrada, 1996; Cristóbal-Azkarate *et al.*, 2005; Benchimol and Peres, 2014). On top of habitat change, direct anthropogenic disturbances, such as hunting, mining, or logging, are detrimental for primates and may also restrain their movement and access to resources.

When facing habitat changes, primates, as well as other species, have three alternatives: dispersion (i.e., migrating to a more suitable habitat), evolutionary change (i.e., developing genetic adaptations), or phenotypic plasticity (i.e., behavioural responses to cope with changing conditions; Wong & Candolin, 2015). In fragmented landscapes, the grass is rarely greener elsewhere and movement is often hampered due to physical barriers, making dispersion hazardous for primates (Arroyo-Rodríguez et al., 2013). As for evolutionary change, human-induced habitat disturbances are rapid and exponential, not leaving time for long-lived animals, such as primates, to develop adaptations (Wong & Candolin, 2015). This leaves primates with the ultimate option of promoting behavioural plasticity in order to cope with environmental and social challenges (Kalbitzer & Chapman, 2018). To respond to habitat quality changes and anthropogenic disturbances, primates adapt their ranging patterns, activity budgets, and diet (McLennan et al., 2017; Kalbitzer & Chapman, 2018). For example, multiple primate species shift their diets and feed on fallback food in order to survive during periods of food scarcity (McLennan et al., 2017). Other species will increase their ranging patterns to reach other food sources (Dunn et al., 2010, 2013). To cope with changes in food abundance and available range, some primate species will modify their group size and composition (van Schaik et al., 1983; Snaith & Chapman, 2007; Mbora et al., 2009). Multiple primate species also exhibit fission-fusion dynamics to mirror resource availability and habitat conditions (Symington, 1990; Lynch Alfaro, 2007; Bowler & Bodmer, 2009; Coles et al., 2012).

Primates' degree of ecological and behavioural plasticity is species-specific and is contingent on the species' biology and life-history traits. For example, generalist and opportunist primates (e.g., *Papio* sp. and *Macaca* sp.), with highly diversified diets often display higher levels of ecological and behavioural flexibility (Jaman & Huffman, 2013; Albert et al., 2014; McLennan et al., 2017). Moreover, a species' aptitude to cope with disturbances is governed by the balance between the costs and benefits of modifying their behaviour or diet. This equilibrium influences their energetic balance and physiology (see Section 3.4). In order to evaluate the resilience, tolerance, and plasticity of a primate species, it is crucial to comprehend its ecology (i.e., diet, ranging patterns, social structure). Understanding how primates respond to changing environments and evaluating the potential consequences for their health, fitness, and survival is paramount for their conservation.

2. Physiological stress

The term stress is used to refer to multiple concepts, in multiple fields, making its definition arduous and often ambiguous. In biology, stress can be defined as a condition that affects an animal's performance and welfare (i.e., animal's state regarding its attempts to cope with its environment; Broom & Johnson, 2019), and prompts both behavioural and physiological responses (Kamilar & Beaudrot, 2018). In the physiological context, *stress* can be defined as the loss of homeostasis of an organism due to intrinsic or extrinsic challenges (i.e., stressors) (Kagias et al., 2012). Accordingly, a *stressor* is a noxious or unpredictable stimulus that affects an organism and triggers a stress response. Thenceforth, the *stress response* represents the array of physiological and behavioural responses deployed by an animal to face and overcome a stressor (Romero, 2004). Succinctly, stress is the effect, the stressor is the cause, and the stress response is the coping mechanism.

2.1. The stress response, the HPA axis, and the reactive scope model

To cope with environmental and social challenges, vertebrates deploy a series of behavioural and physiological responses (Wingfield, 2013). Fundamentally, these responses are adaptive and increase an animal's chances of survival (Charmandari et al., 2005). Behavioural responses may involve heightened vigilance, enhanced cognition, and focused attention (Beehner & Bergman, 2017). The two main physiological responses to a stressor are the stimulation of the sympathetic nervous system (SNS) and the activation of the hypothalamic-pituitary-adrenal (HPA) axis (Sapolsky et al., 2000). The response of the SNS is practically instantaneous and induces the secretion of catecholamine hormones (i.e., norepinephrine and epinephrine) by the adrenal medulla. Catecholamines elevate heart rate, increase arousal, and stimulate glycogenesis and lipolysis, delivering extra energy necessary to overcome a challenge (Reeder & Kramer, 2005). This characterises the well-known fight-flight-freeze response (Axelrod & Reisine, 1984; Wingfield et al., 1997). Simultaneously with the SNS, the perception of a stressor activates the HPA axis (Fig. 4). The hypothalamic paraventricular nucleus (PVN) is stimulated and triggers the release of corticotropin-releasing hormone (CRH) and other related hormones such as arginine vasopressin (AVP). In turn, CRH and AVP stimulate the anterior pituitary gland, which produces and releases adrenocorticotropin hormone (ACTH). The latter is secreted into the bloodstream and induces the release of glucocorticoids (GCs), a class of corticosteroids, by the adrenal cortex (de Kloet et al., 2005; Sheriff et al., 2011). In mammals, it takes 2-3 minutes to witness a significant rise in GC levels after the activation of the HPA axis (Romero, 2004). The HPA pathway is autoregulated through a negative feedback loop (Fig. 4). When GCs reach above baseline concentrations, they bind with receptors in the hippocampus, hypothalamus, and pituitary that suppress the HPA axis. On average, stress-induced GC levels reach their maximum 15-20 minutes after a stressor and return to baseline levels after an hour (de Kloet et al., 2005).

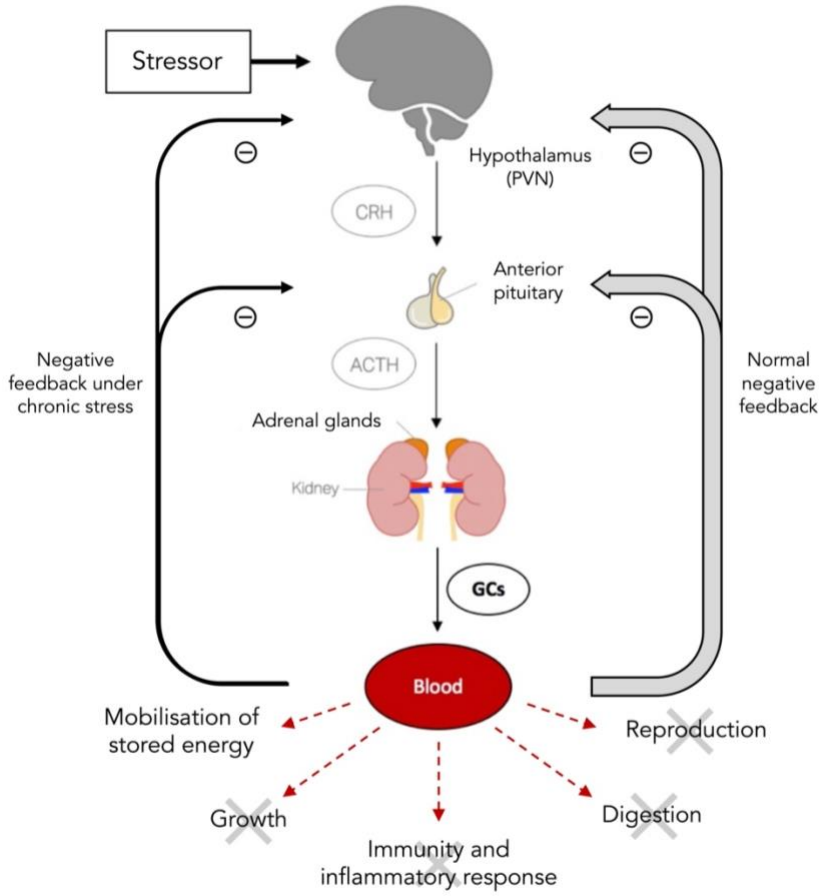

***Figure* 4:** Physiological pathway of the adrenocortical stress response. The hypothalamic-pituitary-adrenal (HPA) axis is activated in response to an external stressor. The hypothalamic paraventricular nucleus (PVN) triggers the release of corticotropin-releasing hormone (CRH), which in turn stimulates the anterior pituitary to produce adrenocorticotropin hormone (ACTH). ACTH induces the secretion of glucocorticoids (GCs) into the blood stream by the adrenal glands. Stress-induced GCs mobilise stored energy and suppress non-vital activities. GC levels are autoregulated by a rapid negative feedback, suppressing the HPA axis. Animals suffering from chronic stress, after being exposed to prolonged stressors, may have a dysregulated HPA axis due to a poor negative feedback (modified from Boonstra et al., 1998).

GCs are metabolic hormones that regulate gluconeogenesis and mediate energy demands necessary to overcome predictable and unpredictable challenges (Sapolsky et al., 2000). Given their role in the physiological responses to stressors, they are ambiguously called *stress hormones.* Cortisol is the main GC in primates whereas corticosterone is predominant in other taxa such as amphibians and birds (Spencer & Deak, 2017). The effects prompted by GCs are moderated by two types of receptors, mineralocorticoid (MR) and glucocorticoid (GR) receptors. While MRs have a high affinity to GCs and will bind at baseline levels, GRs have a lower affinity and will only bind when circulating GCs reach stress-induced levels and MRs are saturated (Romero, 2004; Wingfield, 2013). Consequently, GCs have distinct behavioural

and physiological effects at baseline versus stress-induced levels. In 2009, Romero and colleagues proposed the reactive scope model, a novel theoretical model depicting the different aspects of GC variations in animals. At baseline levels, GCs regulate glucose metabolism to meet predictable circadian and circannual demands in what is called *predictive homeostasis* (Fig. 5; Romero et al., 2009). Depending on a species' activity period and photoperiod, GCs follow a specific circadian rhythm. For example, in diurnal species GC concentrations habitually increase in the morning, characterizing the cortisol awakening response (CAR), and then diminish as the day goes on. The CAR provides the necessary energy surge to promote glucose availability during an animal's waking hours but may also prepare the HPA axis to face anticipated stress during the animal's activity period (Fries et al., 2009).

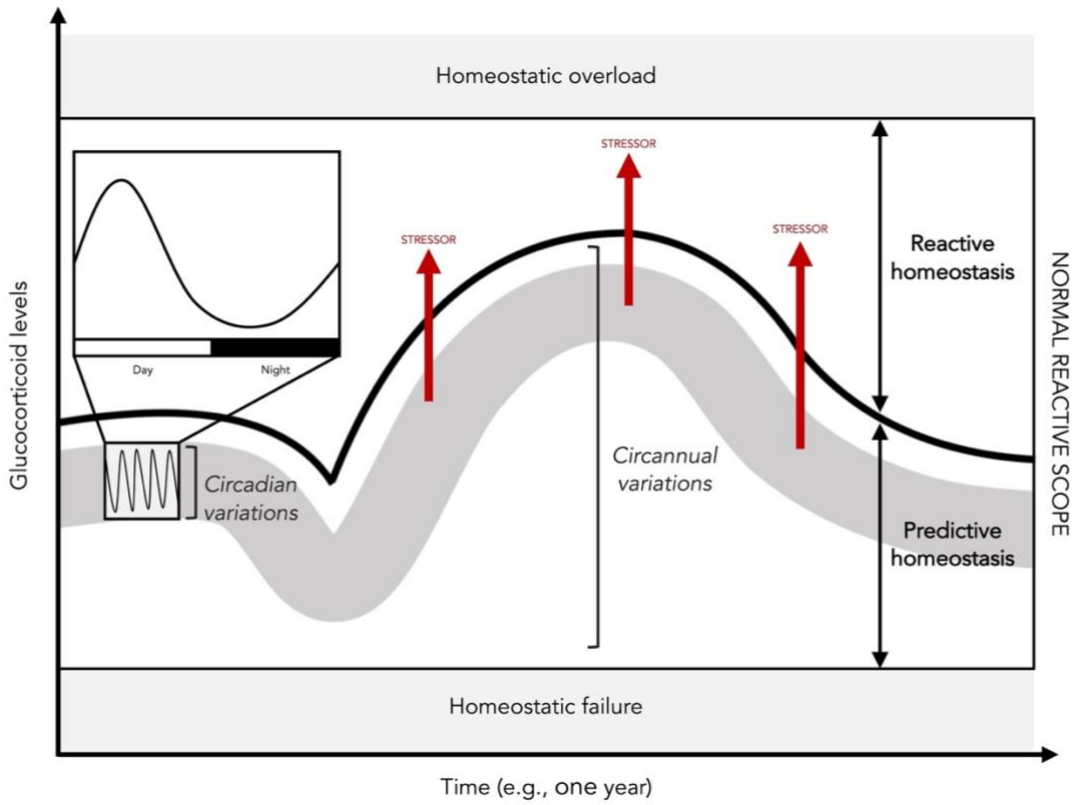

***Figure* 5:** Graphical model depicting theoretical glucocorticoid (GC) variations in an animal over a year. Within the predictive homeostasis range, GC levels vary depending on the time of day (i.e., circadian variation) and season (i.e., circannual variations). Throughout the year, an unpredictable 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. Homeostasis failure occurs when GC levels are too low to maintain homeostasis. In opposition, homeostasis overload arises when GC levels surpass a threshold at which they start having detrimental effects on an individual (modified from Romero et al., 2009).

Seasonally, GCs vary to cope with predictable challenges such as variations in resource availability, reproductive status, seasonal climate, or photoperiod (Romero, 2002). For example, a study on African elephants (*Loxodonta africana*) found that GC levels decreased during the dry season due to lower water and food availability (Foley et al., 2001). While seasonal GC variations are needed to maintain homeostasis, an acute surge is necessary to restore homeostasis after an unpredictable stressor. This adrenocortical stress response is termed *reactive homeostasis*. For example, the attack from a predator will prompt an animal's stress response. Stress-induced GC levels induce the mobilisation of stored energy, promote escape behaviours, and suppress non-vital activities such as growth, digestion, reproduction, and inflammatory responses (Fig. 4; Sapolsky et al., 2000; Wingfield, 2005). The magnitude and intensity of a stress response mirror the severity and duration of a stressor. Furthermore, according to the level of baseline GCs at a given time, the starting point of the response varies. Together, reactive and predictive homeostasis represent the *normal reactive scope* of an animal (Fig. 5; Romero et al., 2009).

The stress response is essentially adaptive and a rise in GC levels promotes survival. In the short run, the costs of temporary inhibition of non-vital activities are outweighed by the benefits of increasing survival chances when facing an acute stressor. However, animals subjected to prolonged and intense stressors may exhibit sustained elevated GC levels, which may in turn have noxious repercussions on the health and fitness of individuals (Juster et al., 2010; Dantzer et al., 2014). This chronic stress response (i.e., *homeostatic overload*; Fig. 5) can cause immunosuppression, muscle breakdown, reduced growth, and reproductive suppression, leading to an overall decrease in survival (Romero et al., 2009). While these detrimental effects of homeostatic overload have been established in captive animals (Sapolsky et al., 2000), the potentially harmful effects of chronic stress on wild animals are debated given the adaptive nature of the stress response (Sapolsky, 1994; Boonstra, 2013). At the antipodes of homeostatic overload, an animal enters a state of *homeostatic failure* when GC levels cannot be maintained within the normal reactive scope and reach levels below the lower limit of predictive homeostasis (Fig. 5). Homeostatic failure is pathological and can lead to energy dysregulation, water balance failure, and catecholamine insufficiency, causing death (Romero et al., 2009).

2.2. Stress in wildlife

One of the key questions of behavioural endocrinology and ecology is understanding how environmental, climatic, and social factors influence animals' physiological states, and subsequently their welfare and fitness. Over the past three decades, the development of non-invasive methods to monitor physiological markers has boosted research on wild species (Sheriff et al., 2011; Palme, 2019). Within their natural habitat, animals are exposed to a

multitude of abiotic, biotic, and anthropogenic stressors, which often have synergistic effects (Kamilar & Beaudrot, 2018).

Abiotic stress includes changes in climate, such as rainfall and temperature, which can in turn affect resource availability. Climate variations modify the energetic demands to maintain optimal body temperature through thermoregulation. Due to the minor climatic variations in the tropics, species living closer to the equator are expected to have lower thermal tolerance levels than species in temperate regions (Khaliq et al., 2014). Drastic short-term changes in climate may cause heat or cold stress in animals, triggering a proportionate stress-response. Seasonal changes in rainfall and temperature influence food abundance and distribution, which affect the diet, ranging patterns, and feeding behaviour of species. For example, Galapagos marine iguanas show elevated baseline and stress-induced GC levels during periods of starvation caused by El Niño events (Romero & Wikelski, 2001).

Biotic stress encompasses both inter and intra-specific interactions. Predation risk is one of the major stressors encountered by wild animals. Higher predator abundance has been linked to increased GC levels in tropical stonechats (*Saxicola torquata*; Scheuerlein et al., 2001), snowshoe hares (*Lepus americanus*; Boonstra et al., 1998), and ground squirrels

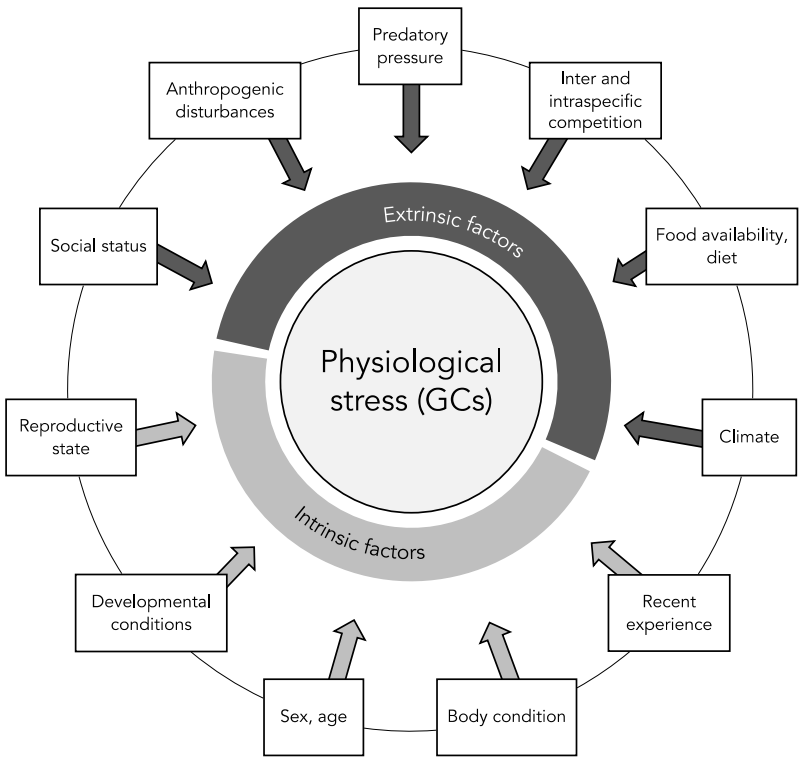

***Figure* 6:** A multitude of intrinsic (light grey) and extrinsic (dark grey) factors potentially influence glucocorticoid (GC) concentrations in wild species (modified from Dantzer et al., 2014 and Beehner & Bergman, 2017).

(*Spermophilus beldingi*; Mateo, 2006). Between species, stressors also include competition for common resources. Similarly, intraspecific competition for food or mates has been linked to higher GC levels. For example, group size and dominance rank was linked to higher cortisol concentrations in African elephants (Foley et al., 2001).

On top of biotic and abiotic stress, animals are increasingly prone to anthropogenic disturbances due to the expanding and widespread encroachment of natural habitats by humans (Peres et al., 2006). Anthropogenic disturbances include both direct (e.g., hunting, pollution, logging, wildlife tourism) and indirect (e.g., habitat loss, fragmentation) stressors which have been linked to higher adrenocortical activity in different taxa. For example, red deer (*Cervus elaphus*) hunted down by humans exhibited high cortisol concentrations (Bateson & Bradshaw, 1997). Forestry logging also revealed higher GC levels in spotted owls (*Strix occidentalis*; Wasser et al., 1997). Overall, a multitude of intrinsic and ecological factors may influence an animal's GC levels, forcing researchers to adopt a comprehensive and multidisciplinary approach when studying physiological stress in the wild (Fig. 6; Dantzer et al., 2014). Understanding how species cope with anthropogenic disturbances is a major question of conservation physiology, which aims to evaluate the welfare and fitness of species subjected to environmental challenges using physiological markers (Busch & Hayward, 2009; Cooke et al., 2013).

2.3. Sampling matrices and methods

After activation of the HPA axis, GCs are released into the blood stream and are assimilated in multiple biological sampling matrices through different pathways (Fig. 7; Sheriff et al., 2011). Each sampling matrix reveals different insights on an animal's physiological stress (Table 1). GCs circulating in the blood are affected by circadian and seasonal variations, immediate prior stressors (e.g., predator attack), and long-term stressors (e.g., nutritional stress). Therefore, measuring GC levels directly in the plasma reveals an instantaneous snapshot of an animal's physiological state and allows a minute-by-minute monitoring of its adrenocortical activity. In the blood, most GCs bind with corticosteroid-binding globulin (CGB), a transport protein that guides them to their target cells, while a smaller portion remains free (Wingfield, 2013). Unbound GCs pass freely from the blood to the saliva of vertebrates (Riad-Fahmt et al., 1982). Similar to blood samples, GCs measured in the saliva provide an immediate sampling of physiological stress. While these two sampling methods have been extensively used in captivity, they are rarely used in wildlife studies because they are prone to research-induced biases caused by handling and capture of individuals (Sheriff et al., 2011).

Faecal and urine samples are usually favoured when studying free-ranging animals because these methods are completely non-invasive and therefore not affected by research-

induced bias (Sheriff et al., 2011). GCs released into the plasma are metabolized by the liver and end up in urine, passing through the kidneys, or are excreted in the gut, via the bile duct, and can be measured in faeces (Möstl & Palme, 2002; Palme, 2005). While both metabolised and native GCs are found in urine, only metabolites end up in faecal samples (Touma & Palme, 2005). Both these sampling matrices provide information on an animal's short-term adrenocortical activity, over a distinct species-specific amount of time (i.e., hours to days; Palme et al., 1996; Novak et al., 2013). Faecal glucocorticoid metabolites (fGMs) represent a cumulative secretion of unbound circulating GCs over a definite time lag, depending on the gut passage time and defecation rate of the species (Touma et al., 2003). It is therefore essential to carry out a physiological or biological validation in order to determine the time lag between variations in circulating GCs and their detection in faecal samples (Palme, 2019).

Although most wildlife endocrinological studies employ faecal and urine samples, hair cortisol concentrations (HCCs) have been increasingly used as an indicator of chronic stress (Sheriff et al., 2011; Carlitz et al., 2014; Dettmer et al., 2014). GCs circulating in neighbouring capillaries diffuse into the hair shaft during its growth. Thus, HCCs reflect a long-term accumulation of GCs (Meyer & Novak, 2012; Shimamoto, 2022). However, studies have revealed that GCs also diffuse into the hair through excretions from surrounding tissues (e.g.,

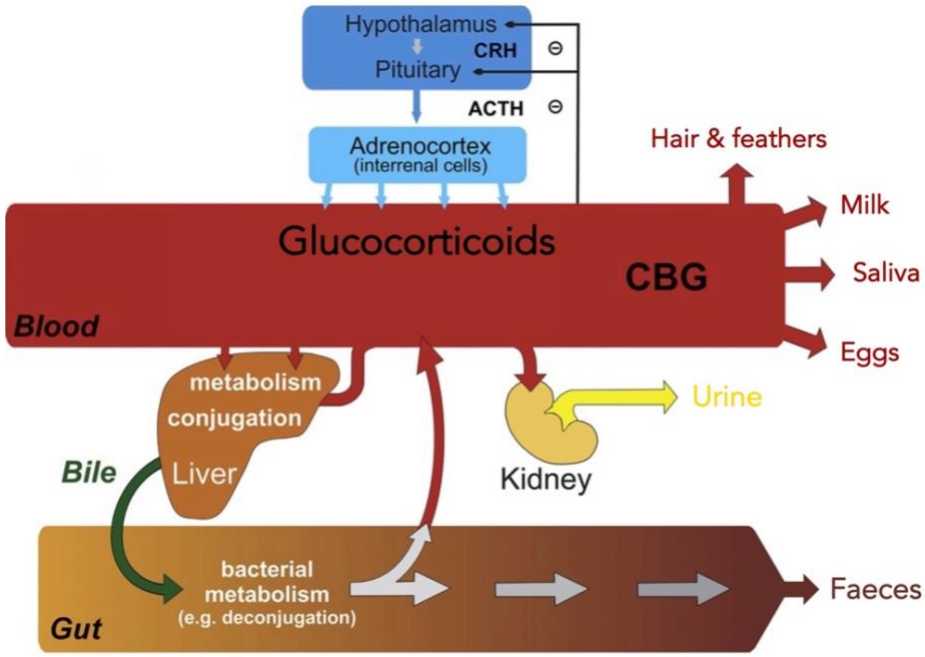

***Figure 7**: Secretion of glucocorticoids (GCs), after activation of the hypothalamus-pituitary-adrenal (HPA) axis, and pathway of the assimilation of glucocorticoids in different biological sampling matrices. CBG: corticosteroid binding globulin; CRH: cortisol releasing hormone; ACTH: adrenocorticotropic hormone (modified from Palme et al., 2019).*

sebum, sweat) and at minor level from external contaminations (e.g., urine, faeces, saliva), suggesting that HCCs may represent a blend of long-term hormone accumulation during hair growth and transient levels from auxiliary sources (Koren et al., 2019). HCCs represent a stable measure of adrenocortical activity and are a useful tool to study the effects of ongoing stressors (Kalliokoski et al., 2019).

Depending on the study species' biology, additional miscellaneous sampling matrices have been used to quantify GC levels such as feathers (Dominoni et al., 2021), eggs (Sopinka et al., 2017), or even whale baleens (Fernández Ajó et al., 2018) and blowhole water (Thompson et al., 2014). Regardless of the chosen biological matrix, it is essential to perform a physiological or biological validation when exploring adrenocortical activity in order to ascertain that the changes in measured GCs truly reflect the changes in circulating GCs (Palme, 2019). These validations must not only be matrix-dependent but also species-specific (Sheriff et al., 2011). Physiological validations are achieved by performing an ACTH challenge. The injection of ACTH in the animal triggers a rise in circulating GC levels. To validate a method, GC levels measured in the sampling matrix must correspond to the predicted changes in the blood (Touma & Palme, 2005). When studying wild, and potentially endangered species, it is not systematically possible to undertake a physiological validation. In this case, biological validations, which consist in measuring GC levels before and after an acute stressor (e.g., capture, translocation, predation event), are favoured (Palme, 2019). In both methods, GC concentrations are generally quantified using radioimmunoassay (RIA) or enzyme immunoassay (EIA) techniques. These assays must also go through analytical validations, including measures of precision, sensitivity, specificity, and accuracy (Sheriff et al., 2011; Palme, 2019).

***Table* 1.** Different aspects of glucocorticoid sampling matrices (modified from Novak et al., 2013).

Matrix	Time frame	Cortisol fraction	Circadian variability	Invasiveness
Blood	Minutes	Total	Yes	Considerable
Saliva	Minutes	Free	Yes	Reduced
Urine	Hours to days	Free	Yes*	None
Faeces	Hours to days	Free	Yes*	None
Hair	Months	Free	No	Reduced

*depending on a species gut passage time and defecation rate.

2.4. Primate energetics and physiological stress

In primatology, evaluating adrenocortical activity has been widely used to explore the proximate effects of diverse ecological and social factors. The physiological profile of free-ranging primates is swayed by both intrinsic and extrinsic stressors (see Fig. 6; Beehner & Bergman, 2017). Intrinsic factors, such as age, reproductive state, and health, may influence

an animal's predictive homeostasis, and potentially the intensity of its responsiveness to additional stressors (Romero et al., 2009). For example, females have heightened metabolic demands during both gestation and lactation, resulting in elevated GC levels (Carnegie et al., 2011; Dias et al., 2017; Charpentier et al., 2018), and are potentially more sensitive to agonistic social interactions (Maestripieri et al., 2008; Gómez-Espinosa et al., 2014). Regarding health, parasite load and richness has been linked to higher GC levels in multiple species (Chapman et al., 2006, 2007; Behie & Pavelka, 2013; Foerster et al., 2015). Several studies have also revealed that older individuals often exhibit higher GC levels due to senescence and their inability to efficiently regulate HPA activity (Sapolsky & Altmann, 1991; Seraphin et al., 2008; Hämäläinen et al., 2015). As a whole, it is crucial to consider the potential influence of intrinsic factors when exploring physiological stress in primates as they may obscure interpretations.

Given the ethological essence of primatology, the relationship between social behaviours and physiological stress levels has been the most explored in past literature. Multiple studies investigated the link between social rank and adrenocortical activity but the trends are equivocal (Sapolsky, 2005; Beehner & Bergman, 2017). While high-ranking individuals may have high GC levels due to the energetic costs of maintaining dominance, lower-ranking individuals may also exhibit high GC levels due to resource inequity or harassment (Muller & Wrangham, 2004; Sapolsky, 2005). On the other hand, dominant individuals generally have priority access to food resources and should thus not suffer from nutritional stress. In general, studies exploring the effect of dominance rank conclude that GCs are higher in individuals occupying a position involving greater energetic demands, linked to increased expenditure or reduced intake (Abbott et al., 2003). A rise in GC levels has also been associated to other social predictors such as aggression (Westergaard et al., 2003), death of kin (Engh et al., 2006), male immigration (Beehner et al., 2005), and the presence of infants (Brockman et al., 2009). Contrariwise, social bonds and grooming have been shown to reduce GC levels (Young et al., 2014; Sonnweber et al., 2015). Interestingly, the individuals grooming show lower physiological stress levels than the receivers (Shutt et al., 2007).

Since GCs are first and foremost metabolic hormones that mediate energy demands, studying the impact of ecological predictors on physiological stress necessitates an understanding of primate energetics. GCs fluctuate according to the intricate balance between energy expenditure and intake. *Energy intake* is the amount of calories absorbed when feeding on specific food items while *energy expenditure* refers to quantity of calories consumed by the body. Both these notions are affected by *food distribution* (i.e., accessibility of food in the habitat) and *availability* (i.e., temporal and spatial abundance of food) which directly influence *diet quality* (Fig. 8; Emery Thompson, 2017). Thus, diet quality depends on both the true energetic value of food resources but also the presence of resources in the habitat. On top of *foraging effort* (i.e., the amount of time spent searching for food and

manipulating/extracting food), energy expenditure is also affected by other energy-consuming behaviours such as reproduction, territory monitoring, anti-predator behaviours, and inter- and intragroup competition.

Multiple studies have explored the tie between primate energetics, physiological stress, and the influence of ecological factors. Lower food availability and diet quality have been linked to increased GC concentrations across various taxa (Muller & Wrangham, 2004; Chapman et al., 2007; Foerster & Monfort, 2010; Emery Thompson et al., 2010; MacLarnon et al., 2015). Similar findings were also observed regarding seasonality, with higher physiological stress levels during the season with lower food availability (Pride, 2005; Chapman et al., 2006; Gesquiere et al., 2008; Behie et al., 2010; Ordóñez-Gómez et al., 2016). According to the energy-maximizing strategy (Hall, 1962), a rise in GC levels may provide additional energy for primates to increase their ranging patterns in order to tolerate degraded habitats or periods of food scarcity (Dunn et al., 2010, 2013). Naturally, at a time when the human impact on the environment is extraordinary, multiple studies have explored the impact of anthropogenic disturbances on physiological stress levels (i.e., increased GC levels). Global trends reveal that disturbances, including tourism, habitat loss and degradation, hunting, urbanisation, and logging, provoke a positive physiological stress response across various primate species. These trends are discussed thoroughly in Chapter 1: *A meta-analysis of anthropogenic impacts on physiological stress in wild primates* (Kaisin et al., 2021).

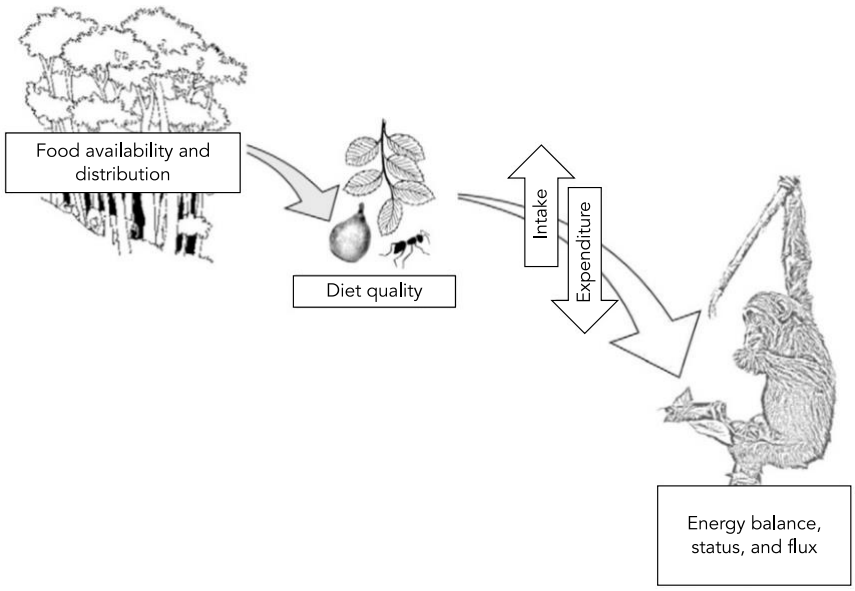

***Figure* 8:** Primate energetics are influenced by different factors that may in turn influence their physiology and glucocorticoid levels (modified from Emery Thompson, 2017).

3. Study species: Leontopithecus chrysopygus

3.1. Taxonomy and morphology

Order: Primates

Suborder: Haplorrhini

Infraorder: Simiiformes

Parvorder: Platyrrhini

Family: Callitrichidae

Genus: *Leontopithecus*

Species: *chrysopygus*

***Figure* 9:** Adult black lion tamarin.

Platyrrhines regroup all New World primates, endemic to central and south America and characterized by flat noses and sideways-facing nostrils (Groves, 2001). Among this taxon, the callitrichids are a diverse family of small neotropical primates, regrouping marmosets (23 species), tamarins (22 species), and lion tamarins (4 species) (Rylands & Mittermeier, 2009; Rylands et al., 2012, 2016). The genus *Leontopithecus* sp., vernacularly named lion tamarins, comprises 4 allopatric species that are endemic to the Brazilian Atlantic Forest: the golden lion tamarin (*L. rosalia*), the black-faced lion tamarin (*L. caissara*), the golden-headed lion tamarin (*L. chrysomelas*), and the black lion tamarin (*L. chrysopygus*) (Rylands & Mittermeier, 2009; Meyer et al., 2014).

Primates of the Callitrichidae family possess long fingers with specialised claw-like nails (i.e., tegulae) that enable them to efficiently climb trees and forage for arthropods and small vertebrates (Sussman, 2000). The species of the *Leontopithecus* genus are the largest callitrichids, weighing around 500-700 g, have a long non-prehensile tail, and are called lion tamarins due to their lion-like mane. As indicated by its name, the black lion tamarin is characterized by a black pelage with a golden patch on their rump and the base of their tail (Fig. 9). Despite the lack of apparent sexual dimorphism in this species, pelage can vary inter-individually (Garbino et al., 2016).

3.2. Geographic distribution and habitat

The black lion tamarin, which is the focal species of this research, is endemic to the State of São Paulo in south-eastern Brazil (Kierulff et al., 2008). Unlike the other lion tamarin species, they only occur inland. Their range is delimited to the south by the Paranapanema River, to the north by the Tietê River, to the west by the Paraná River, and finally to the east by the Serra do Mar mountain range. The current total population size of this species is estimated at 1600 individuals, with the large majority (i.e., 1200 individuals) inhabiting the Morro do Diabo State Park (MDSP) at the extreme west of the State of São Paulo (Rezende, Knogge, et al., 2020). The remaining subpopulations inhabit small highly isolated Atlantic Forest remnants, scattered within the Tietê-Paranapanema interfluve (Fig. 10; Hershkovitz, 1977; Culot et al., 2015). These fragments are islands of forest within a highly deforested and human-degraded matrix of plantations (e.g., eucalyptus, sugar cane, citrus, soybean), pastures, and urban areas.

The habitat of black lion tamarins is exclusively seasonal semideciduous Atlantic Forest, ranging from 260 to 913 m in altitude (Valladares-Padua, 1993; Joly et al., 2014). The Brazilian Atlantic Forest possesses a high degree of floral and faunal endemism and is therefore considered to be one of the Earth's 25 biodiversity hotspots (Myers et al., 2000; Mittermeier et al., 2011). The structure of primary Atlantic Forest typically includes multiple canopies with a lush vegetation and an important diversity of epiphytes (Joly et al., 2014).

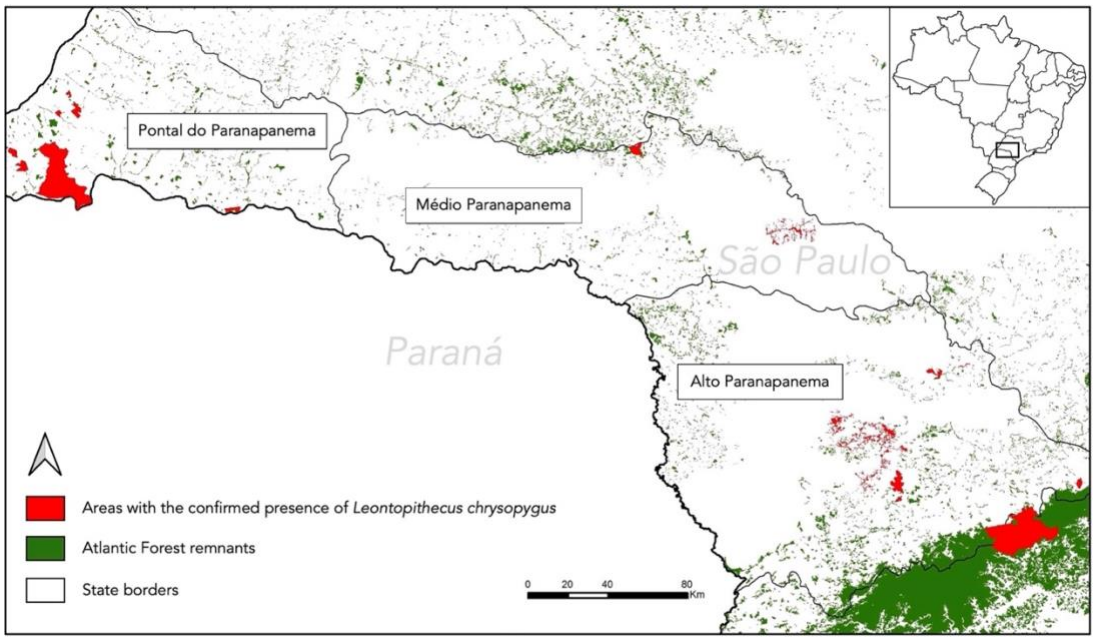

***Figure* 10:** Brazilian Atlantic Forest remnants with confirmed occurence of black lion tamarin subpopulations within the State of São Paulo, Brazil.

Nonetheless, the occurrence of black lion tamarins is not constrained to primary forest as they also inhabit degraded fragments and riparian forest with secondary vegetation (Rylands & Kierulff, 2002; Culot et al., 2015).

3.3. Social structure and communication

Black lion tamarin group size varies between 2 to 8 individuals (Coimbra-Filho & Mittermeier, 1973; Valladares-Padua, 1993). Although lion tamarins are habitually monogamous (Sussman & Garber, 1987), with a unique reproductive pair, some populations may exhibit polyandry (Garber et al., 2016). A group usually comprises an adult pair and one or two generations of offspring. The female has a four-month gestation period and typically gives birth to twins during the rainy season (Rezende, Knogge, et al., 2020). As cooperative breeders, parental care is carried out by both the mother and the father (Garber et al., 2016).

As many monogamous primate species, black lion tamarins display territorial behaviours including long call vocalisations (Bury, 2020). Long calls serve both to maintain intra-group cohesion and to communicate with conspecifics (Snowdon et al., 1986). Long calls can be used to notify neighbouring groups of their presence and may often lead to intergroup encounters. Lion tamarins also possess specialised glands in the anogenital, suprapubic, and sternal region, which they rub on branches to deposit their scent (Heymann, 2006). Scent marking is used to advertise sexual status but also for spatial orientation and food resource relocation (Heymann, 2022). A recent study concluded that black lion tamarins mainly use scent markings for intra-group communication rather than for territorial purposes (Bury, 2020).

3.4. Feeding ecology and ranging patterns

Black lion tamarins can be designated as a generalist species. Although they are mainly frugivorous and arthropodivorous, they complement their diet with gum, flowers, buds, and may occasionally prey on small vertebrates (e.g., frogs, bird chicks, and lizards) (Coimbra-Filho & Mittermeier, 1973; Valladares-Padua, 1993; Garbino et al., 2020). Based on behavioural focal sampling data, their diet is composed of 78.5 percent fruits, 13.5 percent arthropods, and 7.8 percent plant exudates (Valladares-Padua, 1993). Black lion tamarins consume a wide variety of fruit species, which is influenced by the diversity and availability of trees in their habitat. Among these, *Syagrus ramonzoffiana*, a palm tree species, represents a crucial resource for black lion tamarins and is systematically part of their diet (Valladares-Padua, 1993).

Lion tamarins are eclectic foragers that actively search for animal prey in epiphyte bromeliads, under tree bark, in tree hollows, and in palm sheaths. They prey on both mobile (e.g., phasmids, grasshoppers, katydids) and non-mobile (e.g., cockroaches and coleoptera

and lepidoptera larva) insect prey (Valladares-Padua, 1993). Following the abundance and availability of resources, black lion tamarins adapt their daily, monthly, and seasonal foraging and feeding behaviours. During the dry season, lion tamarins increase their foraging effort and animal prey consumption to compensate the lower availability of fruits (Keuroghlian & Passos, 2001). A recent study also revealed that black lion tamarins mainly feed on frogs during the dry season when fruits are scarce (Garbino et al., 2022). This species demonstrates a significant level of diet plasticity, enabling them to use a variety of microhabitats and live in different forest types (Valladares-Padua, 1993; Passos & Keuroghlian, 1999).

Black lion tamarins are almost exclusively arboreal, although they occasionally descend to the ground to forage or cross open-areas. Like other *Leontopithecus* species, they spend the majority of their time in the middle layer of the canopy, between 5-10 m, and will ascend to the upper levels of the canopy to reach preferred fruit resources (Coimbra-Filho & Mittermeier, 1973). At night, black lion tamarins have the particularity of sleeping in tree hollows, palm tree sheath, or dense tangles of lianas, protecting them from predators and helping thermoregulation. This primate may travel ~ 1.3 to 2.3 km per day and groups have home ranges covering 40 to 400 ha (Albernaz, 1997; Keuroghlian & Passos, 2001; Rezende, Knogge, et al., 2020).

3.5. Conservation status and main threats

After being thought to be extinct for more than 60 years, black lion tamarins were rediscovered in the 1970s in the Morro do Diabo State Park (Coimbra-Filho & Mittermeier, 1973). Currently, *L. chrysopygus* is listed as endangered by the IUCN (Rezende, Knogge, et al., 2020). In 2008, it was downgraded from critically endangered after the discovery of new populations in the upper region of the Paranapanema River basin (Culot et al., 2015) and the success of conservation efforts. At present the total population of black lion tamarins is estimated to reach 1600 individuals, including a minimum of 250 adults. Approximately 75 percent of black lion tamarins inhabit the MDSP. While this population is considered genetically viable, the other subpopulations are estimated to be too small to survive in the longer-term (Rezende, Knogge, et al., 2020). The latter occur in small and isolated fragments, scattered throughout the Paranapanema River's watershed (Culot et al., 2015).

The main threat hanging over *L. chrysopygus* populations is the loss of habitat. The Brazilian Atlantic Forest is highly fragmented and subjected to intense habitat loss spurred by agricultural and urban expansion (Ribeiro et al., 2009; Joly et al., 2014). Currently, only 2.3 percent of the black lion tamarin's historical distribution is considered suitable for this species, the rest has disappeared. It is estimated that the black lion tamarins occur in less than 40 percent of this suitable area, representing 838.4 ha (Rezende, Sobral-Souza, et al., 2020). On top of the negative effects that trickle down from habitat loss and fragmentation (see

Section 2.3. of the Introduction), black lion tamarins are threatened by forest fires, road kills, predation by dogs, and the re-emergence of the yellow fever pandemic which affects multiple primate species in the south of Brazil (Mares-Guia et al., 2020; Rezende, Knogge, et al., 2020). Conservation actions are currently directed toward the protection of remaining forest fragments and the creation of biological corridors between isolated subpopulations (Rezende, Knogge, et al., 2020). Evaluating the health and welfare of black lion tamarins in small and potentially degraded fragments is essential to have a better understanding of their ecology in order to make well-founded conservation decisions.

4. Objectives and organisation of the thesis

This research aims to improve our current knowledge of how primates cope in changing environments. As human pressure on natural ecosystems continues to intensify, studying the effects of anthropogenic disturbances on the behaviour, diet, and health of species is a key question of conservation biology. In this framework, quantifying physiological stress levels is a powerful tool to assess an animal's welfare, giving precious insight on their behaviour, physiology, and ecology. In this thesis, we start by reviewing current research exploring the effect of anthropogenic disturbances on glucocorticoid levels in wild primates, in order to uncover general trends. Then we shift our focus to the species level, identifying the factors responsible for glucocorticoid variations in an endangered neotropical primate, the black lion tamarin (*Leontopithecus chrysopygus*). Using two different sampling matrices (i.e., hair and faeces), each providing different insight into this species' physiological state, we explore the impact of their habitat before looking at the influence of behaviour on GC variations.

Chapter 1 – *A meta-analysis of anthropogenic impacts on physiological stress in wild primates*, compiles previous studies comparing the glucocorticoid levels of primates inhabiting areas subjected to anthropogenic disturbances with their undisturbed conspecifics. The goal is to derive global trends and understand how distinct primate species respond to diverse disturbances of varying intensities, bringing to light their resilience and behavioural plasticity. [Published in *Conservation Biology*; https://doi.org/10.1111/cobi.13656]

Chapter 2 – *Impact of habitat quality on physiological stress in an endangered neotropical primate*, attempts to understand how habitat quality, including the abundance of food resources, influences the diet of black lion tamarins living in six different Atlantic Forest fragments, and how this influences their hair cortisol concentrations. [To be submitted to *Animal Conservation*]

Chapter 3 – *Linking glucocorticoid variations to monthly and daily behaviour in a wild endangered neotropical primate* – identifies the factors swaying faecal glucocorticoid levels in two groups of black lion tamarins inhabiting different fragments. This chapter investigates physiological stress over different timescales in order to unravel the complex nature of adrenocortical activity in relation to activity budget and diet. [Submitted to the *American Journal of Primatology*]

The Conclusions and Perspectives section brings together the different ideas and findings discussed in each chapter, suggests advances and directions for future research, outlines the diverse conservation implications, and reveals the impending threats for primate populations.

Two first author papers that stemmed from side projects on tool use by black lion tamarins and camera-trap evidence of zoopharmacognosy by multiple Atlantic Forest mammals are made available in the General Appendix.

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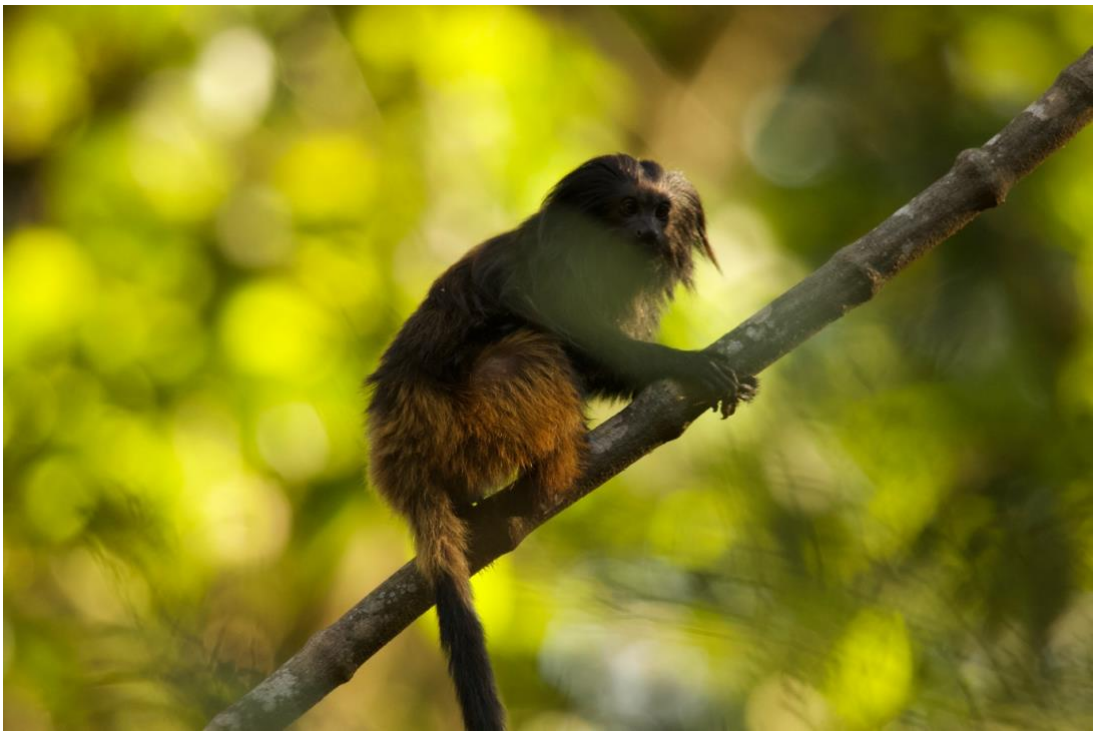
CHAPTER 1



A meta-analysis of anthropogenic impacts on physiological stress in wild primates

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A meta-analysis of anthropogenic impacts on physiological stress in wild primates

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ABSTRACT

As humanity continues to alter the environment extensively, comprehending the effect of anthropogenic disturbances on the health, survival, and fitness of wildlife is a crucial question for conservation science. Many primate populations occupy suboptimal habitats prone to diverse anthropogenic disturbances that may be sources of acute and chronic stress. Quantification of glucocorticoid (GC) concentrations has repeatedly been used to explore the impact of disturbances on physiological stress. Although it is still debated, prolonged elevation of GC levels may impair reproduction, growth, and immune system activity of individuals. We quantified the effect of anthropogenic disturbances on physiological stress in primates with a global meta-analysis based on data from 26 articles, covering 24 distinct species in 13 different countries. Anthropogenic disturbances were classified into 6 distinct categories: habitat loss, habitat degradation, ongoing logging, hunting, tourism, and other human activities. We calculated effect sizes (Hedges' g) with the standardized mean difference in GC concentrations between primates affected by human activity and their undisturbed conspecifics. We ran random-effects models and subgroup analyses to estimate the overall effect as well as a cumulative effect size for each disturbance category. Overall, primates inhabiting sites subject to anthropogenic disturbances exhibited significantly higher GC levels (g = 0.60; 95% CI: 0.28–0.93). Habitat loss and hunting were overall associated with increased GC concentrations, whereas the cumulative effects of the other disturbances were not statistically significant. Biologically, high GC levels may increase fitness by enabling individuals to overcome the challenges linked to anthropogenic disturbances. However, primates in disturbed environments may have sustained elevated GC levels. To strengthen future research, it is necessary to control confounding factors systematically (e.g., diet, reproductive status, predatory pressure, and resource availability) and improve understanding of the link between GC levels and the health, fitness, and survival of animals.

INTRODUCTION

The widespread human encroachment into the natural habitats of other species, spurred by exponential human population growth, results in multiple disturbances on ecosystems (Peres et al., 2006). Human-induced habitat change, caused by the over-exploitation of natural resources, landscape conversion, agricultural activity, and urban development, is a leading cause of faunal biodiversity loss (Laurance, 2007). However, before witnessing the impact of anthropogenic disturbances at the population level (*e.g.*, birth rates, death rates), disturbances affect both the behaviour and physiology of individuals (Madliger & Love, 2014). Indeed, in human-modified habitats, on top of being prone to natural abiotic (*e.g.*, extreme temperatures, droughts) and biotic (*e.g.*, intra- and inter-specific competition, predation) stressors, animals often have to deal with increased anthropogenic pressure (Kamilar & Beaudrot, 2018). Understanding how animals respond to disturbances and identifying the ultimate consequences for the health, survival, and fitness of individuals is a key question of conservation biology (Isaac & Cowlishaw, 2004).

Many primates are forest-dependent, making them particularly vulnerable to anthropogenic disturbances and consequently good ecological indicators (Cavada et al., 2016). Currently, 75% of primate species are experiencing population declines and about 60% are threatened with extinction (IUCN, 2018). Habitat loss, degradation, and fragmentation are the main threats for primate populations (Estrada et al., 2017). Recently, a pantropical meta-analysis assessing biodiversity metrics in disturbed versus undisturbed areas found that primate diversity, species richness, and abundance were negatively impacted by agriculture and logging (de Almeida-Rocha et al., 2017). Habitat change directly affects primates by limiting ranging patterns, access to resources, dispersal and gene flow, and increasing intra- and interspecific competition and disease transmission (Chapman et al., 2005; Benítez-Malvido & Arroyo-Rodríguez, 2008). On top of large-scale habitat change, primates in anthropogenically modified areas often suffer higher rates of hunting (Peres, 2001). Primates are also increasingly subject to wildlife tourism which intensifies the encroachment of their habitat by humans (Borges de Lima & Green, 2017). Increased and close contact to humans can lead to changes in behaviour, diet, and physiology, and other consequences such as anthropozoonotic disease transmission (Köndgen et al., 2008; McLennan et al., 2017; Beehner & Bergman, 2017).

The impact of anthropogenic stressors on individuals can be explored through different biological responses: behavioural, autonomic, endocrine, and immunological (Honess & Marin, 2006). The assessment of adrenocortical activity through hormone analysis is a valuable tool to explore the stress response of individuals. Glucocorticoids (GCs) are metabolic hormones that mediate the energetic demands needed to overcome predictable and unpredictable environmental and social challenges (Sapolsky et al., 2000; Romero et al.,

2009). The main GC in primates, as in many mammals, is cortisol (Palme, 2019). Exposure to a stressor activates the hypothalamic-pituitary-adrenocortical (HPA) axis which then triggers the secretion of GCs. An endogenous increase in these physiological biomarkers leads to the mobilization of stored energy reserves, increases reactivity, and suppresses non-immediately critical activities such as digestion, growth, or copulation (Sheriff et al., 2011). Within their normal reactive scope, GCs regulate glucose metabolism to meet daily and seasonal energetic demands (Romero et al., 2009). For example, GC levels of chimpanzees (*Pan troglodytes*) were higher during periods with lower-quality diets (Muller & Wrangham, 2004). However, a prolonged elevation of GC levels may have deleterious impacts on health and fitness, by impairing reproduction, growth, and immune system activity of individuals (Romero et al., 2009; Juster et al., 2010). While a normal response to a stressor is characterized by an acute increase in GC levels, followed by a rapid negative feedback, chronically stressed animals may have dysregulated and dampened HPA axes (Sapolsky et al., 2000; Romero et al., 2009). Although the negative impact of a chronic elevation of GC levels stress is well defined in captive animals (Sapolsky et al., 2000; Boonstra, 2013), few studies have established this for wild populations. The adaptive nature of the stress response is still poorly understood and past research has too often focused on the causes of GC variations rather than exploring the influence of GC secretion on fitness (Beehner & Bergman, 2017). In wild primates, only two studies have linked high GC concentrations to higher mortality rates (Pride, 2005b; Rakotoniaina et al., 2017). Boonstra (2013) argued that when facing natural stressors (*i.e.*, predatory pressure, low food availability, extreme weather) a sustained elevation of GCs is part of an animal's normal adaptive response, despite the potential fitness costs. Indeed, from an evolutionary standpoint, deleterious chronic elevations in GCs should not exist in the wild (Boonstra, 2013). However, this may not be the case regarding abrupt and intense anthropogenic disturbances to which most species have not had the time to adapt to (Kalbitzer & Chapman, 2018).

In ecological and conservation research, GC concentrations have been increasingly used as indicators of animal health, survival, and fitness (Busch & Hayward, 2009; Sheriff et al., 2011). Different biological samples, each providing insight on adrenocortical activity over different time frames, have been used to measure GCs and their metabolites in free-ranging primates. Contrary to plasma and salivary samples, measuring GCs in faeces, urine, and hair has the benefit of not being prone to research induced biases caused by handling and capture (Novak et al., 2013). In wildlife studies, faecal and urine samples are often favoured due to the non-invasiveness of the collecting methods. GCs in the plasma are metabolized by the liver and subsequently excreted in urine or faeces (Palme 2019). The level of GCs, or their metabolites, measured in urine or faeces represent a cumulative secretion of hormones over a species-specific amount of time (Novak et al., 2013; Palme, 2019). In faeces, this time delay corresponds approximately to the species' gut passage time, varying from a few hours to more than a day (Palme 2019). Urine and faecal GCs reflect an animal's short-term stress response,

unveiling circadian fluctuations, but will also be influenced by sustained higher levels of GCs linked to long-term stressors. In contrast, hair samples offer information on long-term adrenocortical activity (over months), recounting the animal's stress levels over longer periods (Sheriff et al., 2011). Although it is still unclear how GCs are incorporated into the hair shaft as well as the exact time frame of physiological stress they represent, this method has great potential and remains minimally-invasive (Kalliokoski et al., 2019). Here, GC levels will hereafter refer to any measures of adrenocortical activity (*e.g.*, hair cortisol, faecal glucocorticoid metabolites, and urinary cortisol).

In the past decades, understanding the impact of habitat change and human pressure on primate populations has been the purpose of a number of studies (Estrada et al., 2017; Galán-Acedo et al., 2019). The growing number of studies on the influence of anthropogenic disturbances on stress in primates allows for a global analysis. Most studies compare the impact of a unique disturbance by comparing two groups in different conditions. While such comparisons have some statistical limitations to demonstrate the effect of a disturbance, it is possible to derive global trends by combining multiple studies. Here we present a meta-analysis pooling current research exploring the effects of habitat loss, habitat degradation, ongoing logging, hunting, tourism, and other human activities on GC levels in primates. We survey all studies comparing GC levels between primates inhabiting less disturbed sites and their conspecifics living in more disturbed sites. Furthermore, we discuss preliminary trends in the results, identify substantial confounding factors that influence GC levels, highlight discrepancies regarding results and methods, and suggest advances for future research. Primate populations living in disturbed habitats may have altered ranging patterns, lower resource availability, higher intra- and interspecific competition and/or frequent human encounters, which may have energetic impacts on primates and affect their GC secretion. We expect human-induced disturbances to increase the stress response in primates and therefore to find higher GC levels in disturbed than in undisturbed sites. By reviewing existing literature in this field, we aim to better comprehend the complex relationship between anthropogenic disturbance and stress in primates as well as identify directions for future research.

METHODS

The methods listed below summarize the protocol we used to perform this meta-analysis (see *Supporting Information*, Appendix S1.1, for a full and detailed version of the methods).

Literature review

This meta-analytical review was based upon articles published until April 2020 that investigated the effects of anthropogenic disturbances on GC levels in wild primate populations. We conducted extensive and systematic bibliographic research through the

databases *Scopus* and *Google Scholar*. Since the goal of this study was to evaluate the influence of anthropogenic disturbances on physiological stress in primates, we restricted our database to studies that measured GC concentrations in individuals inhabiting an undisturbed (or less disturbed) site versus individuals living in a disturbed (or more disturbed) site, within the same study area and on the same species. Although undisturbed areas, completely exempt of any type of human disturbance, are very rare, the undisturbed or less disturbed groups used in this meta-analysis will hereafter be referred to as the undisturbed groups. Depending on the study design, different matrices were used to quantify GC levels: faecal glucocorticoid metabolites (ng/g), hair cortisol (ng/mg), and urinary cortisol (μg/dl). Due to the limited number of studies, we included all the studies, regardless of the sampling method used.

For every study, we recorded whether the authors performed a biological or physiological validation of their GC quantification method prior to, as part of their study, or if they refer to a previous validation for the same species. We also checked if they did or did not control for potential confounding factors that may influence GC levels and lead to misinterpretations when interpreting the effect of disturbances: sex, age, reproductive status, diet, activity budget, and resource availability.

Data analysis

We calculated effect sizes to estimate the impact of anthropogenic disturbances on GC levels. Effect sizes were estimated by calculating Hedges' *g* which is a standardized mean difference that accounts for the fact that the sampling variance of the undisturbed group and the disturbed group may differ (Hedges, 1981). Accordingly, we did not compare the absolute values obtained in each study but rather a standardized mean difference between the GC concentrations measured in the undisturbed and the disturbed group. One distinct effect size was calculated for each pairwise comparison. A higher GC concentration in the disturbed group compared to the undisturbed group was expressed as a positive effect size. Inversely, a negative effect size implied that the GC levels were higher in the undisturbed group than in the disturbed group. Estimates of the effect sizes are considered significant if the calculated ninety-five percent confidence intervals (CI (95%)) did not overlap zero.

To calculate the overall effect of anthropogenic disturbances on GC levels, we pooled the effect sizes of all studies, regardless of their disturbance category, using a random-effects model, taking into account that studies are from different populations and used different data collection methods (Harrer et al., 2019). Furthermore, in order to have a better understanding of how different anthropogenic disturbances influence GC levels in primates, we classified the disturbances considered in the studies in 6 separate categories: *habitat loss*, *habitat degradation*, *ongoing logging*, *hunting*, *tourism*, and *other human activities.* To estimate the effect of each disturbance on GC levels, we ran subgroup random-effects model, with study

identity as random-effect (Borenstein & Higgins, 2013; Harrer et al., 2019). We also ran subgroup random-effects models to test the influence of sampling matrices, primate families, and geographic regions on GC levels.

We used forest plots to represent the distribution of effect sizes along with the calculated overall effect. Since each study comprised one or more pairwise comparisons, we calculated combined effects within each study to have a unique cumulative effect size per study to facilitate the comparison of studies with one another and the interpretation of the forest plots.

To determine if effect sizes varied significantly between studies, within each category, and between categories, we tested for heterogeneity. We quantified heterogeneity by calculating the I^2 statistic derived from Cochran's Q. Publication bias was evaluated by calculating fail-safe numbers and using the funnel plot and *P*-curve methods.

RESULTS

Dataset

The final dataset included 26 articles, published between 2006 and 2019, which evaluated glucocorticoid levels in relation to anthropogenic disturbance in one or two primate species (see *Supporting Information*, Appendix S1.2-1.3). When articles studied two different species, we considered each species to be a distinct study. In this case, this meta-analysis included a total of 30 studies. Half of the studies compared a unique undisturbed group with a unique disturbed group, resulting in a unique comparison. However, the 15 other studies collected and compared GC concentrations in several undisturbed and disturbed groups, generating multiple comparisons. Hence, in total, the studies yielded a total of 178 pairwise comparisons (mean ± SD = 5.93 ± 10.54 comparisons per study). The studies were conducted in 13 different countries across five geographic regions (Fig. 1.1) and spanned 24 different primate species belonging to 8 families (Fig. 1.1). Three different sampling matrices were used to quantify GC levels. Faecal samples were the most commonly used (23 studies) while urine (3 studies) and hair (4 studies) samples were less employed. A biological or physiological validation was performed for 57% of the articles included in this meta-analysis. While studies often controlled for sex (80%), age (77%), and reproductive status (50%), less studies measured diet (23%), activity budget (20%), and resource availability (30%) (Fig. 1.2).

Overall effect of anthropogenic disturbances

In general, primates living in disturbed habitats showed higher levels of GCs than in undisturbed habitats, indicated by a significant positive overall effect (Fig. 1.3). Between-

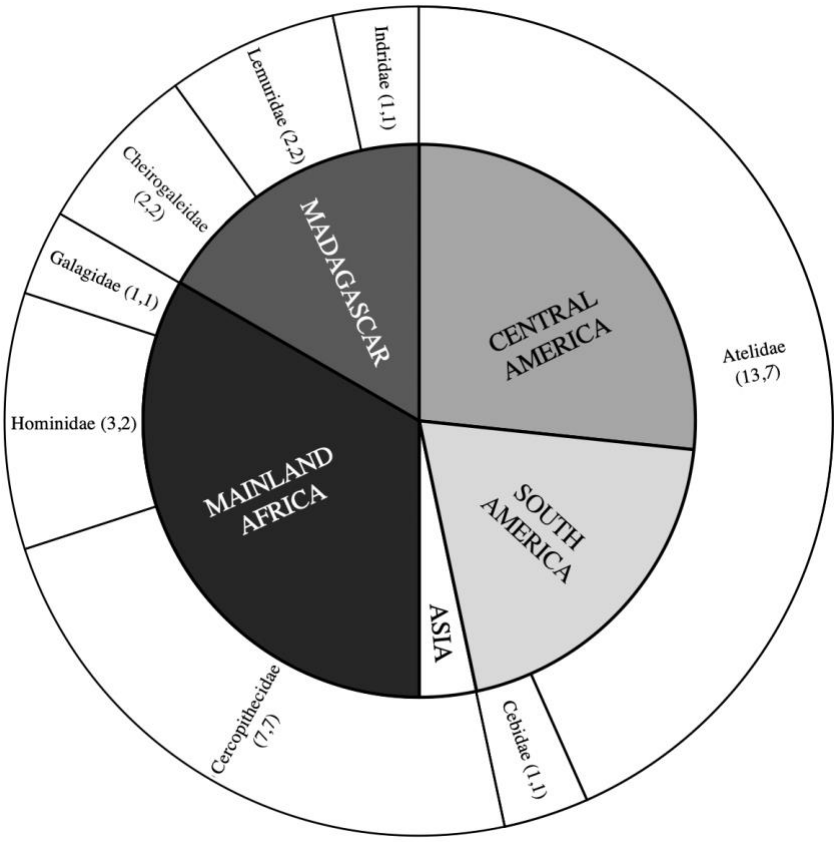


***Figure* 1.2:** *:* Distribution of the 30 studies included in the dataset across geographic regions and primate families. The numbers between parentheses indicate the number of studies (#,) and the number of species (,#) in each families.

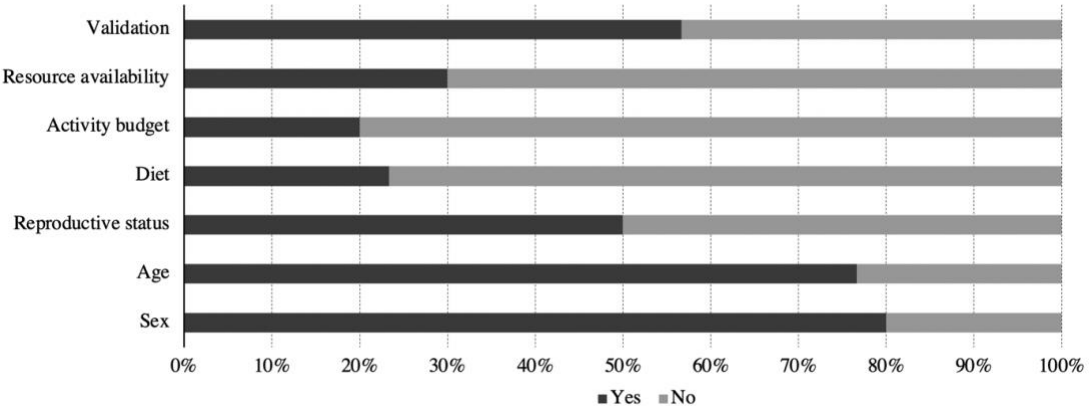


***Figure* 1.2:** Histogram showing the percentage of studies which did or did not take into account confounding factors (sex, age, reproductive status, diet, activity budget, and resource availability) as well as which did or did not perform a biological or physiological validation.

study heterogeneity was significant, indicating that the variation in effect sizes is not only linked to sampling error. As the dataset spans multiple primate species, geographic regions, sites, and groups, subject to singular or multiple anthropogenic disturbances of different intensities, it was ex pected to have a high level of heterogeneity. This is also revealed by the broad prediction interval that overlaps zero (Fig. 1.3), indicating that future studies may still find both positive and negative effect sizes. Out of the 30 studies included in this meta-analysis, 22 had significant effect sizes, including 6 negative (*i.e.*, lower GC levels in the disturbed than in the undisturbed habitat) and 16 positive (*i.e.*, higher GC levels in the disturbed than in the undisturbed habitat) effects. The overall effect calculated by running a random-effects model only including the studies that used faecal samples (112 out of 178 comparisons) was comparable (g = 0.79; 95% CI = -0.41 to 1.17). The random-effect models on two subsets, one including studies with a validation and another including studies that controlled for sex, age, and reproductive status, produced similar overall effect sizes (g = 0.98; 95% CI = 0.54 to 1.43, and g = 0.80; 95% CI = 0.37 to 1.24, respectively).

Effect of disturbance categories, sampling matrices, geographic regions, and primate families

Since some sites were subject to more than one disturbance, the disturbance category subgroup analysis compiled a total of 203 pair-wise comparisons. The subgroup random-effects model revealed that *habitat loss* and *hunting* had a significant positive cumulative effect size (Fig. 1.4). Albeit positive, the cumulative effect of *tourism, ongoing logging* and *other human activities,* were not significant (Fig. 1.4). The effect size of *habitat degradation* was almost null and non-significant. Effect sizes within all disturbance categories were significantly heterogeneous, with moderate to high heterogeneity, and had I^2 values ranging between 40-96%. Cumulative effects differed significantly between disturbance categories (Fig. 1.4). We ran a *post hoc* disturbance subgroup analysis including only the studies with a validation and another including the studies that controlled for sex, age, and reproductive status. Both these analyses also showed that *habitat loss* and *hunting* had significant positive effects and all other disturbances had no significant effects (see *Supporting Information*, Appendix S1.4-1.5).

The subgroup analyses also revealed that effect sizes were influenced by sampling matrix, geographic region, and primate family (Fig. 1.5-1.7), indicating that GC differences varied significantly across these different categories. Hominids and atelids were the only primate families that showed significantly higher GC levels in sites subject to disturbances.

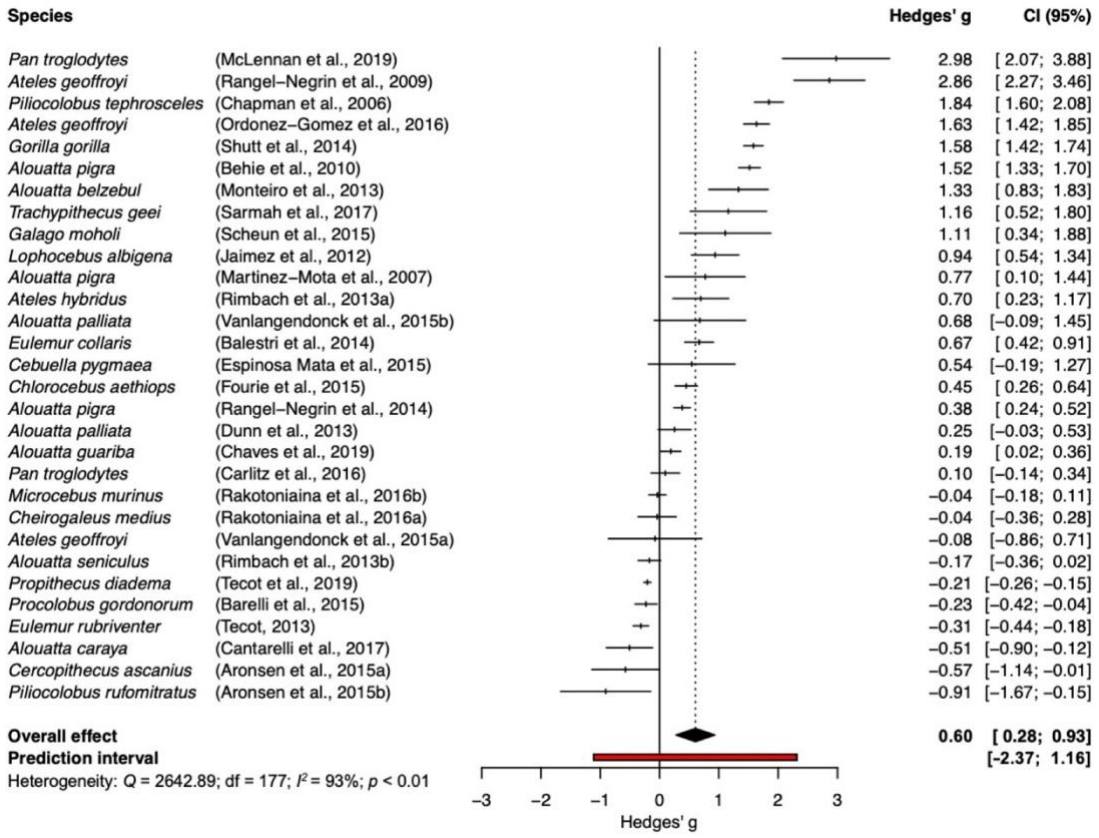
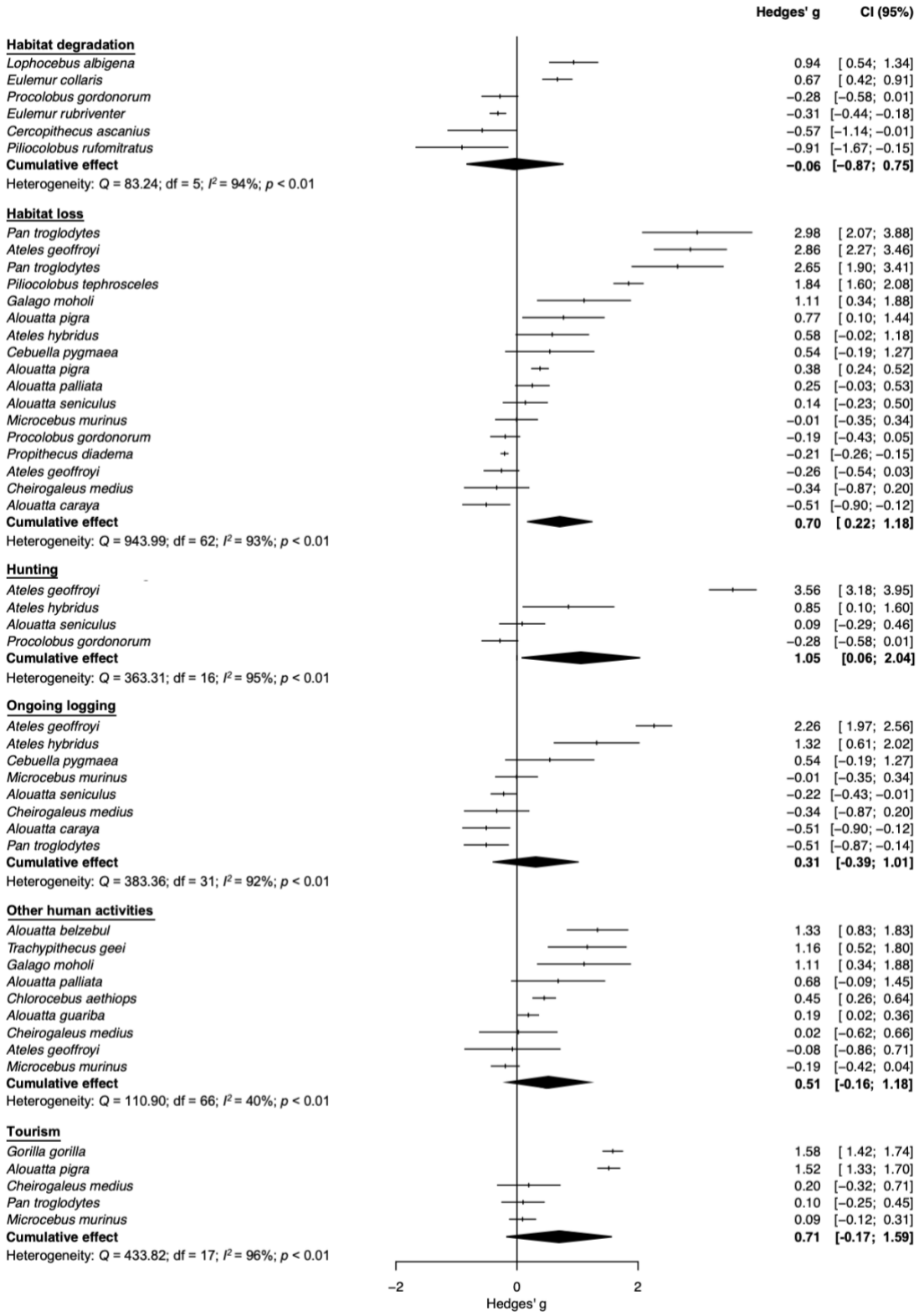


***Figure* 1.3:** Forest plot representing the overall effect (black diamond), estimated using a random-effects model. The effect sizes of each study are shown for interpretation purposes and were calculated using a subgroup fixed-effect model. Effect-sizes represent the standardized mean difference (Hedges' *g*) of GC concentrations between disturbed and undisturbed habitats. A positive (or negative) effect indicates that GC levels are higher (or lower) in disturbed than in undisturbed habitats. Effect sizes are represented with their corresponding 95% confidence intervals and are considered significant if the 95% confidence intervals do not overlap zero. The prediction interval (red line) indicates the estimated distribution of the effect sizes of future studies.

***Figure* 1.4:** Forest plot representing the cumulative effect size of each disturbance category (black diamond) estimated using a subgroup random-effects model. The effect sizes of each study are shown for interpretation purposes and were calculated using a subgroup fixed-effect model. Effect-sizes represent the standardized mean difference (Hedges' *g*) of GC concentrations between disturbed and undisturbed habitats. A positive (or negative) effect indicates that GC levels are higher (or lower) in disturbed than in undisturbed habitats. Effects are considered significant if the 95% confidence intervals do not overlap zero.


Hedges' g
CI (95%)
Habitat degradation
Lophocebus albigena 0.94 [0.54; 1.34]
Eulemur collaris 0.67 [0.42; 0.91]
Procolobus gordonorum −0.28 [−0.58; 0.01]
Eulemur rubriventer −0.31 [−0.44; −0.18]
Cercopithecus ascanius −0.57 [−1.14; −0.01]
Piliocolobus rufomitratus −0.91 [−1.67; −0.15]
Cumulative effect −0.06 [−0.87; 0.75]
Heterogeneity: Q = 83.24; df = 5; I² = 94%; p < 0.01
Habitat loss
Pan troglodytes 2.98 [2.07; 3.88]
Ateles geoffroyi 2.86 [2.27; 3.46]
Pan troglodytes 2.65 [1.90; 3.41]
Piliocolobus tephrosceles 1.84 [1.60; 2.08]
Galago moholi 1.11 [0.34; 1.88]
Alouatta pigra 0.77 [0.10; 1.44]
Ateles hybridus 0.58 [−0.02; 1.18]
Cebuella pygmaea 0.54 [−0.19; 1.27]
Alouatta pigra 0.38 [0.24; 0.52]
Alouatta palliata 0.25 [−0.03; 0.53]
Alouatta seniculus 0.14 [−0.23; 0.50]
Microcebus murinus −0.01 [−0.35; 0.34]
Procolobus gordonorum −0.19 [−0.43; 0.05]
Propithecus diadema −0.21 [−0.26; −0.15]
Ateles geoffroyi −0.26 [−0.54; 0.03]
Cheirogaleus medius −0.34 [−0.87; 0.20]
Alouatta caraya −0.51 [−0.90; −0.12]
Cumulative effect 0.70 [0.22; 1.18]
Heterogeneity: Q = 943.99; df = 62; I² = 93%; p < 0.01
Hunting
Ateles geoffroyi 3.56 [3.18; 3.95]
Ateles hybridus 0.85 [0.10; 1.60]
Alouatta seniculus 0.09 [−0.29; 0.46]
Procolobus gordonorum −0.28 [−0.58; 0.01]
Cumulative effect 1.05 [0.06; 2.04]
Heterogeneity: Q = 363.31; df = 16; I² = 95%; p < 0.01
Ongoing logging
Ateles geoffroyi 2.26 [1.97; 2.56]
Ateles hybridus 1.32 [0.61; 2.02]
Cebuella pygmaea 0.54 [−0.19; 1.27]
Microcebus murinus −0.01 [−0.35; 0.34]
Alouatta seniculus −0.22 [−0.43; −0.01]
Cheirogaleus medius −0.34 [−0.87; 0.20]
Alouatta caraya −0.51 [−0.90; −0.12]
Pan troglodytes −0.51 [−0.87; −0.14]
Cumulative effect 0.31 [-0.39; 1.01]
Heterogeneity: Q = 383.36; df = 31; I² = 92%; p < 0.01
Other human activities
Alouatta belzebul 1.33 [0.83; 1.83]
Trachypithecus geei 1.16 [0.52; 1.80]
Galago moholi 1.11 [0.34; 1.88]
Alouatta palliata 0.68 [−0.09; 1.45]
Chlorocebus aethiops 0.45 [0.26; 0.64]
Alouatta guariba 0.19 [0.02; 0.36]
Cheirogaleus medius 0.02 [−0.62; 0.66]
Ateles geoffroyi −0.08 [−0.86; 0.71]
Microcebus murinus −0.19 [−0.42; 0.04]
Cumulative effect 0.51 [-0.16; 1.18]
Heterogeneity: Q = 110.90; df = 66; I² = 40%; p < 0.01
Tourism
Gorilla gorilla 1.58 [1.42; 1.74]
Alouatta pigra 1.52 [1.33; 1.70]
Cheirogaleus medius 0.20 [−0.32; 0.71]
Pan troglodytes 0.10 [−0.25; 0.45]
Microcebus murinus 0.09 [−0.12; 0.31]
Cumulative effect 0.71 [-0.17; 1.59]
Heterogeneity: Q = 433.82; df = 17; I² = 96%; p < 0.01
−2
0
2
Hedges' g

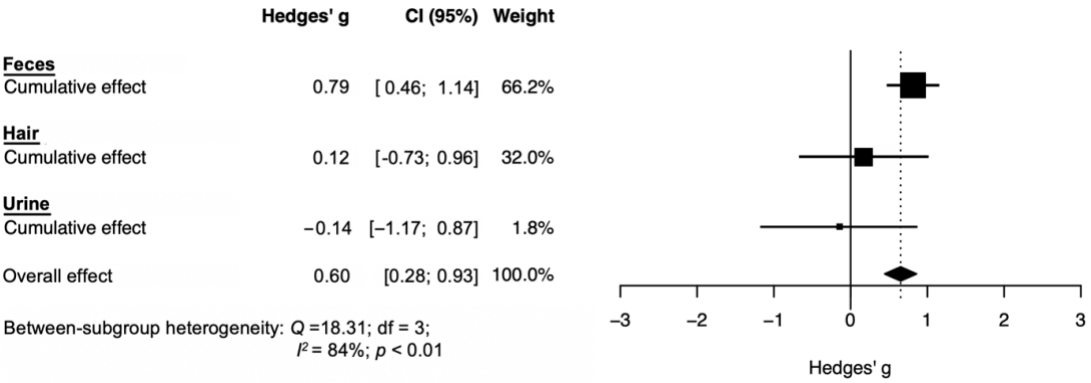


***Figure* 1.5:** Forest plot of cumulative effect sizes for each sampling matrix (faeces, urine, hair), estimated using a subgroup random-effects model. Effect-sizes represent the standardized mean difference (Hedges' *g*) of GC concentrations between disturbed and undisturbed habitats. A positive (or negative) effect indicates that GC levels are higher (or lower) in disturbed than in undisturbed habitats. Effects are considered significant if the 95% confidence intervals do not overlap zero. The respective weight of each sampling matrix subgroup in the overall effect is represented by the black squares.

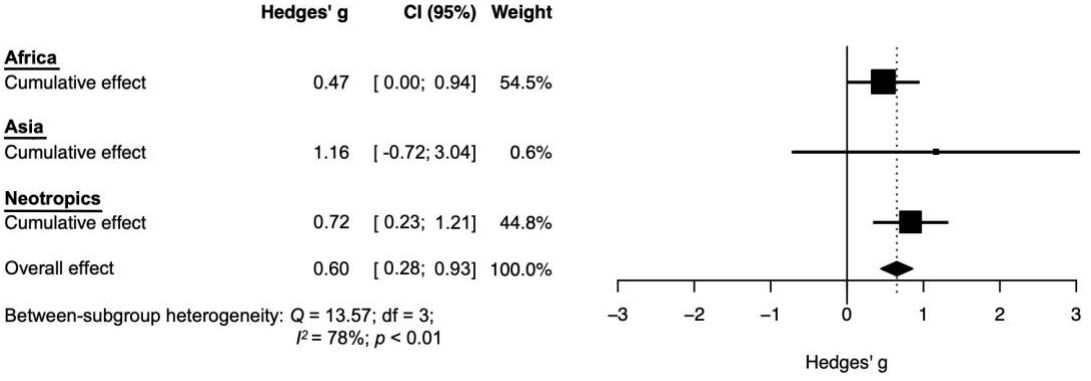


***Figure* 1.6:** Forest plot of cumulative effect sizes for each geographic region (Africa, Asia, Neotropics), estimated using a subgroup random-effects model. Effect-sizes represent the standardized mean difference (Hedges' *g*) of GC concentrations between disturbed and undisturbed habitats. A positive (or negative) effect indicates that GC levels are higher (or lower) in disturbed than in undisturbed habitats. Effects are considered significant if the 95% confidence interval do not overlap zero. The respective weight of each geographic region subgroup in the overall effect is represented by the black squares.

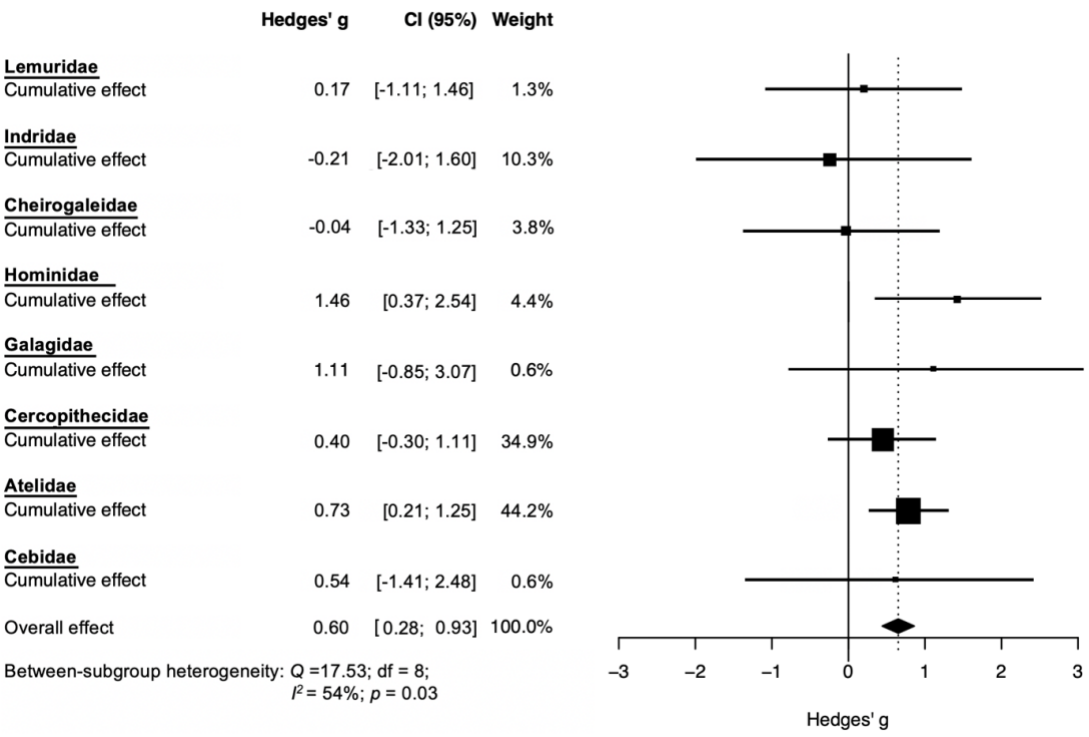


***Figure* 1.7:** Forest plot of cumulative effect sizes for each primate family, estimated using a sub-group random-effects model. Effect-sizes represent the standardized mean difference (Hedges' *g*) of GC concentrations between disturbed and undisturbed habitats. A positive (or negative) effect indicates that GC levels are higher (or lower) in disturbed than in undisturbed habitats. Effects are considered significant if the 95% confidence interval do not overlap zero. The respective weight of each primate family subgroup is represented by the black squares.

Publication bias

The studies included in this systematic review compared average GC concentrations between disturbed and undisturbed groups, pooling the respective GC levels of various primate individuals. Rosenthal's fail-safe number (*i.e.*, number of non-significant, unpublished, or missing studies that would need to be added to a meta-analysis to change the results from significant to non-significant) for the effect of anthropogenic disturbances (25291) was far larger than the number of comparisons included in the meta-analysis (178), indicating that results are robust. While *habitat degradation* had a fail-safe number of 0, the remaining disturbance categories displayed no bias: *habitat loss* (1068), *tourism* (3580), *ongoing logging* (354), *other human activities* (1024), and *hunting* (736). Since the cumulative effect size of *habitat degradation* was close to null with broad confidence intervals (g = -0.06; 95% CI = -0.87 to 0.75) no additional studies were needed to make the effect size non-significant. The funnel plot was asymmetrical which may indicate potential publication bias. However, this is probably linked to the important heterogeneity of the data

set. Hence, in the funnel plot the effect sizes of studies with greater samples sizes (*i.e.*, lower standard error) did not converge towards a common true effect size (see *Supporting Information*, Appendix S1.6). The *p*-curve plot included 22 statistically significant studies of which 19 had *p*-values lower than 0.025 (see *Supporting Information*, Appendix S1.7). The *p*-curve reveals that the meta-analysis is statistically powerful (99%) with 73% of the 22 studies having *p*-values of 0.01. These results indicate that the results of this meta-analysis are not skewed by publication bias linked to data dredging.

DISCUSSION

Overall, anthropogenic disturbances were linked to increased GC levels. Biologically, a greater secretion of GCs by primates inhabiting disturbed sites enables them to mobilize extra energy to overcome the challenges linked to disturbances. However, long-term elevations in GC levels may be deleterious. Although GC levels are often considered as indicators of health and fitness, it is important to keep in mind that the maladaptive nature of long-term elevated GC concentrations is still debated. Additional research is still needed to understand the ultimate biological impact of prolonged high GC levels the wild. Since the adrenocortical stress response is species-specific, it is currently not possible to predict which levels of GCs would be potentially detrimental for individuals.

Although the studies included in this meta-analysis focused on different species, in different habitats, and subject to distinctive disturbances of varying intensities, overall *habitat loss* and *hunting* were associated with elevated adrenocortical activity. We found that the physiological stress responses to anthropogenic disturbances varied significantly between studies and across sampling matrix, geographic region, and primate family. Since only a few studies used hair and urine to quantify GC levels compared to faeces, the differences linked to the sampling matrix shouldn't be overinterpreted and are probably linked to the heterogeneity of the dataset. Although we found results to be significantly different between geographic region, Africa and the Neotropics both had significant positive effects. Furthermore, albeit not significant, the unique study in Asia also found a positive effect. Since primates are behaviourally and ecologically diverse, certain families are likely to be more resilient than others to disturbances. For example, our results revealed that cercopithecines and cebids, which are known to be generalist primates, did not show significant changes in GC levels. On the other hand, hominids, that are known to depend heavily on preserved habitats, and atelids, that are arboreal and rely on a plant-based diet, were significantly affected. Due to the small sample size and the global heterogeneity of the dataset, only preliminary conclusions can be made on the effect of geographic region and phylogenetic signal. As the number of studies in this field increases, clearer trends may appear, linking the inherent behavioural and ecological characteristics of species with how they react to anthropogenic disturbances.

Since the studies included in this meta-analysis were performed on numerous species and populations, variability in response was expected. Nonetheless, in order to improve future study designs, it is crucial to control for potential covariates when trying to relate an anthropogenic disturbance to variations in GC levels. In this meta-analysis, almost half of the studies did not perform a physiological or biological validation for their study species. Such validations are essential to ensure that the GC levels measured in the samples reflect *in-situ* variations (Palme, 2019). Hereafter, we discuss global patterns of GC levels in primates living in disturbed environments, while shedding light on diverging results in several studies, and identify confounding factors that may influence GC levels.

Habitat loss

Our results show that there was an overall significant positive effect of habitat loss on GC levels across the studies examined. Primates living in smaller fragments regularly have limited ranging patterns, restrained access to resources, and reduced genetic diversity (Benítez-Malvido & Arroyo-Rodríguez, 2008). Since, many disturbances are directly correlated to habitat loss (*e.g.*, hunting, logging, human habitat encroachment; Arroyo-Rodríguez et al., 2013) the impact on primates can be multidimensional. While most of the studies included in this meta-analysis focus on the size of fragments, future studies should also measure the effect of landscape metrics such as the number of neighbouring fragments, isolation, and connectivity (Arroyo-Rodríguez & Fahrig, 2014).

In general, lower fruit consumption in the diet of frugivorous primates is concurrent with increases in GC levels (Foerster et al., 2012). Since fruit accessibility and availability are frequently lower in fragments (Cordeiro & Howe, 2001), resource scarcity triggered nutritional stress in several fragment-dwelling primates (Chapman et al., 2006; Martínez-Mota et al., 2007; Dunn et al., 2013; Chaves et al., 2019; McLennan et al., 2019; Tecot et al., 2019). However, smaller fragments do not automatically present lower resource availability (Ordóñez-Gómez et al., 2016), which highlights the necessity to directly measure this variable. A study also showed that, due to the higher energy intake, crop-raiding significantly reduced GC levels in baboons (Lodge et al., 2013). It is important to keep in mind that diet itself (*e.g.*, fibre content) can have a non-biological influence on GC concentrations by increasing the weight and water content of faecal samples (S. K. Wasser et al., 1993). Although having detailed knowledge of the species diet during the sampling period is ideal, this bias can be partially overcome by drying and sieving the faecal samples (Dantzer et al., 2011). Canopy discontinuity and resource dispersion due to habitat loss can also force primates to increase their travel times (González-Zamora et al., 2011), which may be accompanied by higher GC levels (Dunn et al., 2013). Together, the studies mentioned above reveal the important energy mobilizing role of GCs to overcome environmental challenges.

While most primate species were negatively impacted by habitat loss, some species seemed to show resilience that enabled them to bypass environmental stressors linked to habitat loss. For example, hair cortisol concentrations, parasitism, and body condition of two species of sympatric lemurs did not vary in accordance to habitat loss (Rakotoniaina et al., 2016). Similarly, Cantaralli et al. (2017) found that habitat loss combined to logging activity did not influence adrenocortical activity in black-and-gold howler monkeys (*Alouatta caraya*). Since large predators are often the first species to vanish in degraded habitats (Terborgh, 2001), the low predation risk in the fragment may explain why the residing howler monkeys were less stressed than the ones inhabiting the undisturbed site (Cantarelli et al., 2017).

Ongoing logging

Although positive, our results indicate that the overall effect of ongoing logging on GC levels was not significant. Logging can have a twofold effect on primate populations, which depends on the intensity of logging activities (Burivalova et al., 2014). First, logging leads to habitat degradation which is often linked to habitat impoverishment and decreased resource availability (Gardner et al. 2009; see section 4.3: *Habitat degradation*). Second, in sites where logging activities are ongoing, primates are subject to direct disturbances due to increased human encroachment. Accordingly, GC levels were significantly higher in Geoffroy's spider monkeys during days where logging activities were recorded (Ordóñez-Gómez et al., 2016). Similar results were found in brown spider monkeys (Rimbach et al., 2013) and pygmy marmosets (*Cebuella pygmeae*; Espinosa Mata et al. 2014). However, the differences from other studies were not as evident. In order to make studies comparable, future research should try to include supplementary information on the frequency, severity, or intensity of the logging activities.

Habitat degradation

Habitat degradation had no overall significant impact on GC levels. Nevertheless, it is expected that, due to low food availability, populations living in degraded habitats may suffer nutritional stress that triggers the adequate physiological response, an increase in GC levels to mobilize stored energy (Busch & Hayward, 2009). This was demonstrated in collared brown lemurs (*Eulemur collaris*; Balestri et al. 2014), brown spider monkeys (Rimbach et al., 2013, 2014), and grey-checked mangabeys (*Lophocebus albigena*; Jaimez et al. 2012). However, several studies found that food availability and predation pressure explained unexpected lower GC levels in degraded habitats. In Madagascar, an invasive tree species (*Psidium* sp.), only present at the degraded site, provided an important food resource during the fruit scarcity season and may have buffered the nutritional stress of red-bellied lemurs

(*Eulemur rubriventer*) in this habitat (Tecot, 2013). A study on two species of cercopithecines showed that habitat degradation was offset by reduced predatory risk (Aronsen et al., 2015).

Hunting

Overall, hunting was associated with higher GC levels. On top of the direct threat of hunting, encroachment of their habitat by hunters focusing on other mammalian prey may also cause stress to primates due to gun fire noises and human presence. Hunting is perceived as an acute stressor that triggers an adaptive reactive stress response. For example, spider monkeys in Mexico showed elevated GC levels during hunting days (Ordóñez-Gómez et al., 2016). A temporary rise in GC levels may enhance an individual's vigilance and consequently increase its chance of survival (Voellmy et al., 2014). However, prolonged and intense hunting pressure might lead to sustained elevated GC levels. As hunting was always associated to one or more anthropogenic disturbances in this meta-analysis, future research should try to clearly quantify the intensity of hunting in their field site (*e.g.*, number of snares, traps, gunshots).

Tourism

Tourism had no overall significant effect despite being linked to elevated GC levels in golden langurs (*Trachypithecus geei*; Sarmah et al. 2017), black howler monkeys (*Alouatta pigra*; Behie et al. 2010), and western gorillas (*Gorilla gorilla*; Shutt et al. 2014). In many sites, tourism is intrusive and requires primates to modify their behaviour. For example, in Morocco, aggressive interactions where tourists threatened, pushed, or threw objects at Barbary macaques (*Macaca sylvanus*) induced a significant endocrine stress response (Maréchal et al., 2011). In the same population, the frequency of self-scratching, a widely used behavioural index of anxiety, increased with the number of tourists (Maréchal et al., 2011).

Although tourism can have deleterious effects on primates, it is noteworthy that simple mitigating rules reduce or eliminate a stress response in apes. In the Central African Republic, a study on gorillas showed that although human contact provoked an increase in GC levels, the process of habituation progressively reduced this response (Shutt et al., 2014). Similar results were found in orangutans (*Pongo pygmaeus*; Muehlenbein et al. 2012). Shutt et al. (2014) also found that the infringement of the 7-meter tourist-gorilla distance limit triggered an increase in GC levels. Comparably, tourism did not provoke a stress response at the Budongo Forest Reserve in Uganda where a 10m distance was kept between tourists and chimpanzees (Carlitz et al., 2016). Jointly, these studies highlight the effectiveness of long-term habituation and the importance of adopting appropriate regulations in human-primate

contacts. Although strict guidelines are often applied when observing great apes, similar rules still need to be brought into force regarding other primate species (Russon & Wallis, 2014).

Other human activities

Overall, other human activities did not have a significant effect on GC levels. Habitat loss due to agriculture, urbanization, mining, and industry restrains the ranging of primate populations which increases human-primate contact, proximity with human infrastructures, and the consequential conflicts (Galán-Acedo et al., 2019). In Brazil, noise pollution due to mining activities (*e.g.*, machinery and detonations) was linked to higher physiological stress in red-handed howlers (*Alouatta belzebul*; Monteiro et al. 2013). On the other hand, in western Madagascar, fat-tailed dwarf lemurs (*Cheirogaleus medius*) and grey mouse lemurs (*Microcebus murinus*) showed resilience to human habitat encroachment for food and fire wood gathering (Rakotoniaina et al., 2016).

A number of studies focused on the multifactorial impacts of urbanization on primates. Although some species, such as baboons (*Papio* sp.) and macaques, are able to thrive in urban environments, other primates do not have sufficient ecological and behavioural plasticity (McLennan et al., 2017). Urban primates are often viewed as pests and experience high rates of human aggression (McLennan et al., 2017). In two studies on cercopithecines, although the groups in urban habitats were provisioned and may not have suffered from nutritional stress, high intra-group feeding competition was linked to a rise of GC levels (Scheun et al., 2015; Fourie et al., 2016). Additional studies in Asia, where primates commonly co-exist with humans, would help to have a better understanding of the physiological stress levels in urban-dwelling primate populations.

Conclusions and perspectives

This meta-analysis provides the first overview of the physiological stress levels in primate populations across the world in response to different anthropogenic disturbances. Overall, despite important variations between and within disturbance categories, disturbances were mainly associated with higher GC levels. If sustained, increases in GC levels may potentially impair the survival and fitness of primates in disturbed environments. However, it is important to keep in mind that a series of intrinsic (*e.g.*, age, sex, reproductive status) and behavioural (*e.g.*, diet, activity budget, ranging patterns, dominance rank) factors can influence adrenocortical activity and complicate the interpretation of GC variations (Goymann, 2012; Beehner & Bergman, 2017). Other potential confounding factors have been identified such as predatory pressure, social dynamics, parasitism, and food availability. To better comprehend the causes of GC level fluctuations and when logistically and financially feasible, we urge researchers to control or acknowledge as many relevant covariates as possible and to perform

a physiological or biological validation. Since anthropogenic disturbances are often linked to other disturbances, future studies should also increase the number of study sites and evaluate the presence and intensities of all disturbances within each site. In order to make accurate conclusions on physiological stress levels, it is best to have an exhaustive and in-depth comprehension of our study species' behaviour, diet, and environment.

This meta-analysis also highlighted the important heterogeneity of the physiological responses to anthropogenic disturbances by primates. It is crucial to keep in mind that species, populations, and even individuals, have different tolerance thresholds and degrees of plasticity when dealing with anthropogenic disturbances (Rimbach et al., 2013; Aronsen et al., 2015). Since primatological research often focuses on particular well-known species (Bezanson & McNamara, 2019), upcoming studies should target unexplored taxa in new geographic regions, primarily in Asia where very few studies have investigated this question. Increasing the number of studies in this field would help to have a broader overview of the impact of disturbances on physiological stress in primates and allow for a well-founded comparative phylogenetic approach. In this meta-analysis, studies focused on identifying anthropogenic disturbances as potential stressors for primate populations. The next step for research efforts in endocrinological primatology is to understand the effect of prolonged increases in GC levels by understanding the role of GCs as mediators for survival and fitness (Beehner & Bergman, 2017). Accordingly, researchers should promote long-term studies of groups and populations (Clutton-Brock & Sheldon, 2010). As the adaptive nature of the adrenocortical response is still not well understood, future research should focus on relating GC levels with fitness indicators such as mortality, growth, reproductive success, survival, and population metrics.

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SUPPORTING INFORMATION

Appendix S1.1: *Full and detailed version of the methods used to perform the meta-analysis*

Literature review

This meta-analytical review was based upon articles published until April 2020 that investigated the effects of anthropogenic disturbances on physiological stress in wild primate populations. We conducted extensive and systematic bibliographic research through the databases *Scopus* and *Google Scholar* using the following query: [(primate*) AND (“logging” OR “disturbance” OR “degradation” OR “fragment*” OR “tourism” OR "hunting") AND (“stress”) AND (“cortisol" OR “glucocorticoid”)]. The literature list was supplemented with studies cited in the reference lists of the surveyed articles. Since the goal of this study was to evaluate the influence of anthropogenic disturbances on physiological stress in primates, we restricted our database to studies that measured GC concentrations in individuals inhabiting an undisturbed (or less disturbed) site versus individuals living in a disturbed (or more disturbed) site, within the same study area and on the same species. Undisturbed groups were exempt of known direct intrusive disturbances (*e.g.*, hunting, ongoing logging, tourism). Nonetheless, solely when examining primates living in fragments, a group inhabiting an undisturbed fragment would become a potential undisturbed site for a conspecific group in another fragment of the same size impacted by an additional disturbance (*e.g.*, hunting, ongoing logging). Depending on the study design, different matrices were used to quantify GC levels: faecal glucocorticoid metabolites (ng/g), hair cortisol (ng/mg), and urinary cortisol (μg/dl). Due to the limited number of studies, we included all the studies, regardless of the sampling method used.

In order to ascertain that the *in-situ* fluctuations in GC levels are reflected in the measured GC concentrations, it is essential to perform a validation of the assay to quantify the GC levels. Physiological validations consist in inducing an increase in GC levels by injecting a stimulant (e.g., ACTH), and measuring the response in the samples. When studying wild primates, it is often difficult to perform a physiological validation because individuals need to be captured. As an alternative, researchers can opt for a biological validation by measuring GC levels before and after a stressful event (Palme, 2019). For every article, we recorded whether the authors performed a biological or physiological validation of their GC quantification method prior or as part of their study, or if they refer to a previous validation for the same species. We also checked if they did or did not control potentially important confounding factors: sex, age, reproductive status, diet, activity budget, and resource availability. We considered articles to have controlled a covariate if they used a specific experimental design (*e.g.*, gathering faecal samples from adults only to control for age) or if they included these cofounding factors as explanatory variables in their analytical models.

Data analysis

Overall effect of anthropogenic disturbances

Effect sizes were estimated by calculating Hedges' *g* which is a standardized mean difference that accounts for the fact that the sampling variance of the undisturbed group and the disturbed group may differ (Hedges, 1981). Hedges' *g* is calculated using the following equation:

$$g = \frac{\mu_d - \mu_c}{\sigma_{pooled}}$$

with

$$\sigma_{pooled} = \sqrt{\frac{\sigma_d^2(n_d-1)+\sigma_c^2(n_c-1)}{n_d+n_c-2}}$$

where *g* is the standardized mean difference, μ_d and μ_c are the means of the disturbed and undisturbed groups respectively, σ_{pooled} is the pooled variance, and σ_d, σ_c, n_d, and n_c are the variances and sample sizes of the disturbed and undisturbed groups respectively. Accordingly, we did not compare the absolute values obtained in each study but rather a standardized mean difference between the GC concentrations measured in the undisturbed and the disturbed group. The means, standard deviations, and sample sizes for every study were obtained from text, tables, and graphs or otherwise directly provided by the authors. The means represented the mean GC levels found in the disturbed and the undisturbed groups. For studies with insufficient available data, appropriate data transformations were used to calculate Hedges' *g* from t-test values (*i.e.*, t-score, degrees of freedom) (Thalheimer & Cook, 2002). One distinct effect size was calculated for each pairwise comparison. For interpretation purposes, we multiplied all effect sizes by a negative reversal marker in order that a higher GC concentration in the disturbed group than in the undisturbed group was expressed as a negative effect size. Inversely, a positive effect size implied that the GC levels were higher in the undisturbed group than in the disturbed group. For each effect size, ninety-five percent confidence intervals (CI) were calculated. Estimates of the effect sizes are considered significant if the CI (95%) do not overlap zero (Hedges et al., 1999). As a rule of thumb, the strength of an effect size can be classified in three categories: small (0.2-0.5), medium (0.5-0.8), and large (>0.8) effects (Cohen, 1992).

To calculate the overall effect of anthropogenic disturbances on GC levels, we pooled the effect sizes of all studies using a random-effects model. Unlike fixed-effect models, random-effects models assume that studies do not stem from the same unique population.

Hence, on top considering sampling error, random-effects model meta-analyses include another source of variance accounting for the fact that there is not a unique true effect size but rather a distribution of true effect sizes (Borenstein et al., 2010). This considers that studies are from different populations and used different data collection methods (Schwarzer et al., 2015). Some studies generated multiple pairwise comparisons because they compared GC concentrations between several undisturbed and disturbed groups. In order to take repeated measures within-study into account, we added study identity as a random-effect (*i.e.*, ~ 1| studyID). We estimated the variance of the distribution of true effect sizes (*i.e.*, between-study variance; τ^2) using the Restricted Maximum Likelihood (REML) method (Viechtbauer, 2005). Accordingly, effect sizes were combined across studies and weighted (w_i) according to the reciprocal of the within-study variance (v_i) and the between-study variance (τ^2).

$$w_{i(rand)} = \frac{1}{(v_i + \tau^2)}$$

A prediction interval, which takes into account the between-study variance of the meta-analysis, was estimated for the overall effect size. Prediction intervals present a range of effect sizes between which future studies are expected to fall. All statistical analyses were performed using the *metafor*, *meta*, and *detmar* packages in R (version 3.6.1) (Schwarzer, 2007; Viechtbauer, 2010; Harrer et al., 2019).

Effect of disturbance categories, sampling matrices, geographic regions, and primate families

In order to have a better understanding of how different anthropogenic disturbances influence GC levels in primates, we classified the disturbances considered in the studies in 6 separate categories: *habitat loss*, *habitat degradation*, *ongoing logging*, *hunting*, *tourism*, and *other human activities*. *Habitat loss* included studies that explored the influence of reduced fragment size by comparing continuous forests or large fragments (≥ 500 ha) with smaller fragments. A fragment of habitat can be defined as a remnant of a species' suitable habitat which is surrounded by a matrix of modified habitat (Arroyo-Rodríguez & Mandujano, 2009). Although the relative size of a fragment is often species-specific, depending on the ranging patterns and group sizes, we set distinction between a disturbed and undisturbed site at 500 ha, based on Marsh (2003) and considering the limits chosen by the authors of each studies exploring this disturbance in our meta-analysis. In the case of *ongoing logging*, *habitat degradation*, *hunting*, *tourism*, and *other human activities*, we compared a site where the disturbance was present with an undisturbed site that was not subject to this specific disturbance. *Ongoing logging* included study sites with ongoing selective logging, conventional logging, and reduced-impact logging (Burivalova et al., 2014). *Habitat degradation* included study sites that suffered logging in the recent past (< 20 years) leading

to important changes in forest structure and diversity. *Hunting* included study sites where primate hunting or capture occurred during the time of the study. *Tourism* included studies comparing a group visited by tourists and an undisturbed group in the same or another study site. *Other human activities* characterized study sites with anthropogenic disturbances that could not be included in the other categories hereinabove such as mining, agriculture, access to rubbish, and urbanization.

While some studies explored the impact of a unique disturbance, others investigated multiple disturbances. In a few studies, since the same group was subject to more than one disturbance (*e.g.*, Cantarelli et al., 2017: habitat fragmentation and ongoing logging) in the same site, only one effect size could be calculated. For such studies, in the framework of this subgroup analysis testing the effect of the disturbance category, the same we used the same effect size for each category (*e.g.*, Cantarelli et al., 2017: habitat fragmentation and ongoing logging). We ran a subgroup random-effects model, with study identity as random-effect (*i.e.*, ~ disturbance + (1| studyID)), to estimate a cumulative effect size for each disturbance category (Borenstein & Higgins, 2013; Harrer et al., 2019). Subgroup analyses with more than two groups can be seen as meta-regressions with categorical variables (Harrer et al., 2019). In summary, a subgroup analysis consists of pooling the effect of each subgroup and then comparing the effects of the subgroups (Borenstein & Higgins, 2013). For this analysis, we also used the REML method to estimate the between-study variance ($\square^2$) for each category.

We also ran subgroup random-effects models to test if effect sizes varied according to sample matrices, primate families, and geographic regions (*i.e.*, ~ subgroup + (1| studyID)).

Cumulative effect per study

Since each study comprised one or more pairwise comparisons, we calculated combined effects within each study to have a unique cumulative effect size per study to facilitate the comparison of studies with one another and the interpretation of the forest plots (Borenstein et al., 2009). Forest plots are used in meta-analyses to represent the distribution of effect sizes along size the calculated overall effect. Since we can assume that, within a study, the samples were gathered from a unique population, we ran a subgroup fixed-effect model with the study as the grouping variable. The effect size of studies with a single comparison remained the same.

Heterogeneity

To determine if effect sizes varied significantly between studies, within each category, and between categories, we tested for heterogeneity (Gurevitch & Hedges, 1999). Heterogeneity analyses partition variance between studies and act as an indicator of the

variation in effect sizes across studies (Gurevitch & Hedges, 1999). We quantified heterogeneity by calculating the I^2 statistic derived from Cochran's Q (Higgins & Thompson, 2002).

$$I^2 = \frac{Q\text{-df}}{Q} * 100$$

Cochran's Q test, based on chi-squared statistics, is the difference between the observed effect sizes and the fixed-effect model estimates (Borenstein et al., 2011; Harrer et al., 2019). As I^2 values range from 0 to 100%, I^2 of 25, 50, and 75% are interpreted as low, moderate, and high heterogeneity respectively (Higgins & Thompson, 2003).

Publication bias

Publication bias was evaluated by calculating fail-safe numbers (*i.e.*, number of non-significant, unpublished, or missing studies that would need to be added to a meta-analysis to change the results from significant to non-significant) using Rosenthal's method (Rosenthal, 1979). Results are considered to be robust for fail-safe numbers exceeding $5n + 10$, where n is the number of comparisons in the meta-analysis (Møller & Jennions, 2001). In addition, we assessed publication bias using the funnel plot method in which a symmetrical funnel indicates the absence of publication bias (Palmer, 1999). Since the interpretation of a funnel plot may be subjective, we used Egger's method to test for funnel plot asymmetry (Egger et al., 1997). Rosenthal's fail-safe numbers and funnel plots are small sample bias methods that assume that small studies with small effect sizes are less likely to be published, generating publication bias. In order to asses potential bias linked to data dredging, which is based on the fact that some researchers may modify their data and use many statistical tests in order to attain significance, we performed a *P*-curve analysis (Simonsohn et al., 2014).

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Appendix S1.2

Reference list of the articles included in this meta-analysis

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Appendix S1.3

***Table* 1.1.** Articles included in the meta-analysis that investigated the effects of anthropogenic disturbances on physiological stress (GC levels quantified from different sampling matrices) in wild primate populations by comparing groups in disturbed and undisturbed sites.

Reference	Study species	Matrix	Region	Disturbance	# of groups	Comparisons
Shutt et al., 2014	*Gorilla gorilla*	Faeces	Africa	Tourism	2	3
Behie et al., 2010	*Alouatta pigra*	Faeces	Central America	Tourism	8	12
Tecot, 2013	*Eulemur rubriventer*	Faeces	Madagascar	Habitat degradation	5	1
Balestri et al., 2014	*Eulemur collaris*	Faeces	Madagascar	Habitat degradation	4	1
Aronsen et al., 2015a	*Cercopithecus ascanius*	Urine	Africa	Habitat degradation	4	1
Aronsen et al., 2015b	*Piliocolobus rufomitratus*	Urine	Africa	Habitat degradation	4	1
Jaimez et al., 2012	*Lophocebus albigena*	Urine	Africa	Habitat degradation	11	1
Monteiro et al., 2013	*Alouatta belzebul*	Faeces	South America	Other human activities	2	1
Fourie et al., 2015	*Chlorocebus aethiops*	Hair	Africa	Other human activities	16	54
Vanlangendonck et al., 2015a	*Ateles geoffroyi*	Faeces	Central America	Other human activities	13	1
Vanlangendonck et al., 2015b	*Alouatta palliata*	Faeces	Central America	Other human activities	13	1
Sarmah et a l., 2017	*Trachypithecus geei*	Faeces	Asia	Other human activities	2	1
Chaves et al., 2019	*Alouatta guariba*	Faeces	South America	Other human activities	6	6
Scheun et al., 2015	*Galago moholi*	Faeces	Africa	Habitat loss, Other human activities	2	1
Barelli et al., 2015	*Procolobus gordonorum*	Faeces	Africa	Habitat loss, Habitat degradation, Hunting	30	2
Rakotoniaina et al., 2016a	*Cheirogaleus medius*	Hair	Madagascar	Habitat loss, Ongoing logging, Tourism, and Other human activities	4	3
Rakotoniaina et al., 2016b	*Microcebus murinus*	Hair	Madagascar	Habitat loss, Ongoing logging, Tourism, and Other human activities	4	3
Carlitz et al., 2016	*Pan troglodytes*	Hair	Africa	Habitat loss, Ongoing logging, Tourism	4	3
Ordóñez-Gómez et al., 2016	*Ateles geoffroyi*	Faeces	Central America	Habitat loss, Ongoing logging, Hunting*	6	10

Rimbach et al., 2013a	*Ateles hybridus*	Faeces	South America	Habitat loss, Ongoing logging, Hunting*	8	9
Rimbach et al., 2013b	*Alouatta seniculus*	Faeces	South America	Habitat loss, Ongoing logging, Hunting*	31	24
Espinosa Mata et al., 2014	*Cebuella pygmaea*	Faeces	South America	Habitat loss, Ongoing logging	2	1
Cantarelli et al., 2017	*Alouatta caraya*	Faeces	South America	Habitat loss, Ongoing logging	2	1
Chapman et al., 2006	*Piliocolobus tephrosceles*	Faeces	Africa	Habitat loss	9	8
Martínez-Mota et al., 2007	*Alouatta pigra*	Faeces	Central America	Habitat loss	4	4
Rangel-Negrín et al., 2009	*Ateles geoffroyi*	Faeces	Central America	Habitat loss	4	1
Rangel-Negrín et al., 2014	*Alouatta pigra*	Faeces	Central America	Habitat loss	5	6
Dunn et al., 2013	*Alouatta palliata*	Faeces	Central America	Habitat loss	2	1
McLennan et al., 2019	*Pan troglodytes*	Faeces	Africa	Habitat loss	2	1
Tecot et al., 2019	*Propithecus diadema*	Faeces	Madagascar	Habitat loss	8	16

Appendix S1.4

***Table* 1.2.** Disturbance category subgroup analysis only including studies with a physiological or biological validation (17 studies, 156 comparisons).

Disturbance category	Hedges' *g*	Lower CI (95%)	Upper CI (95%)
Habitat degradation	0.67	-1.54	2.87
Habitat loss*	0.87	0.19	1.55
Human activity	0.71	-0.42	1.83
Hunting*	1.52	0.22	2.82
Ongoing logging	0.67	-0.33	1.68
Tourism	1.55	-0.01	3.10

Appendix S1.5

***Table* 1.3.** Disturbance category subgroup analysis only including studies that controlled sex, age, and reproductive status (15 studies, 121 comparisons).

Disturbance category	Hedges' *g*	Lower CI (95%)	Upper CI (95%)
Habitat degradation	0.17	-1.33	1.68
Habitat loss*	0.72	0.03	1.41
Human activity	0.86	-0.40	2.12
Hunting*	1.52	0.25	2.78
Ongoing logging	1.11	-0.14	1.36
Tourism	1.52	-0.61	3.65

Appendix S1.6

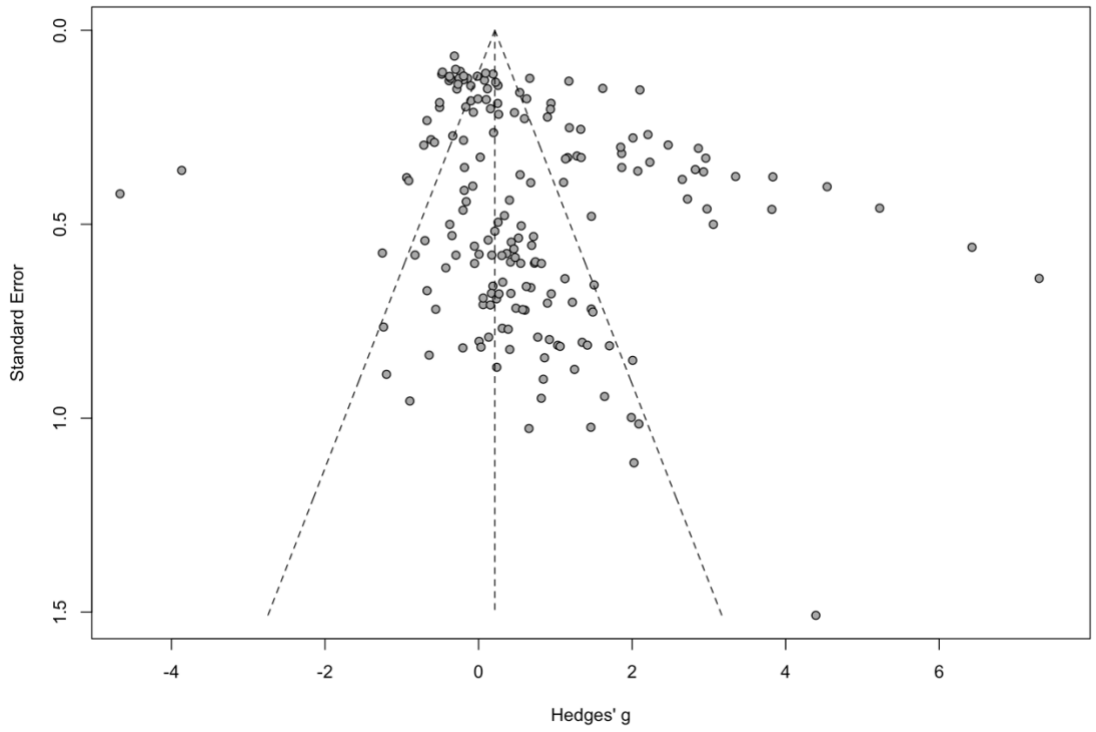

***Figure* 1.8:** Funnel plot of effect sizes (Hedges' *g*) against standard error for all 178 pair-wise comparisons included in this meta-analysis. In the absence of publication bias, studies with lower standard error should tend towards the overall effect ($g = 0.60$; vertical dotted line), displaying a funnel-shape distribution (dotted triangle). This funnel plot is asymmetrical (Egger's test: $t = -4.71$; $p < 0.01$).

Appendix S1.7

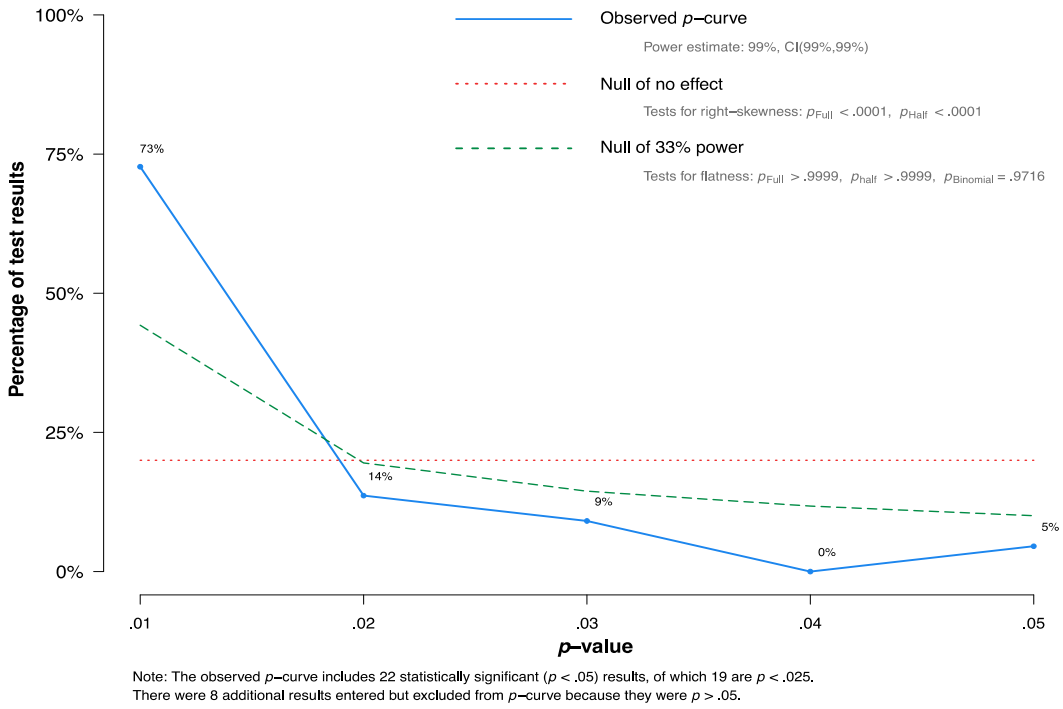

***Figure* 1.9:** P-curve plot including 22 statistically significant studies out of the 30 studies of the meta-analysis.

CHAPTER 2

Impact of habitat quality on physiological stress in an endangered neotropical primate

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Impact of habitat quality on physiological stress in an endangered neotropical primate

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Short running title: Physiological stress and habitat quality in lion tamarins

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ABSTRACT

Understanding the impact that habitat quality has on species is one of the fundamental questions of conservation biology and ecology. Given their high sensitivity to habitat changes and other anthropogenic disturbances, primates are considered excellent ecological indicators. In this study, we evaluated the influence of habitat quality on physiological stress in black lion tamarins (*Leontopithecus chrysopygus*), a frugivorous and faunivorous primate, endemic to the State of Sao Paulo, Brazil. Glucocorticoids (GCs), repeatedly termed stress hormones, are metabolic hormones that play a key role in regulating energy mobilization and maintaining homeostasis. Measuring these hormones in wild animals provide exclusive insight on the physiological state of individuals and how they cope with their environment. Here, we compare hair cortisol concentrations (HCCs) between six distinct black lion tamarin populations, sampling 31 individuals, inhabiting different fragments of varying size and quality. To estimate habitat quality, we calculated tree total basal area (TBA) as a proxy of fruit abundance in the different fragments. Since insects represent a significant part of the diet of black lion tamarins, we also evaluated insect abundance by setting up 100 pitfall traps per site. Furthermore, using stable isotope analyses on hair samples, we estimated the proportion of frugivory in the diet of each individual. To discover which ecological variable influences HCCs, we ran linear models to discover which ecological variable influence HCCs, while considering intrinsic factors (*i.e.*, sex and age). Our results revealed that low habitat quality negatively affects their cortisol levels since decreasing TBA, vegetation diversity, and fragment size increased cortisol levels. While arthropod abundance did not affect HCCs, higher frugivory may reduce cortisol levels. These results suggests that fruits represent a preferred and limiting resource for this species. Altogether, this study suggests that healthy Atlantic Forest remnants may represent suitable habitats for this small neotropical primate with a broad diet and relatively small home ranges. Protecting such areas is therefore crucial for the conservation.

INTRODUCTION

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 limiting their ranging patterns, access to resources, dispersal, and gene flow but also increasing intra- and interspecific competition as well as disease transmission (Chapman et al., 2005; Benítez-Malvido and Arroyo-Rodríguez, 2008; Köndgen et al., 2008). Furthermore, habitat loss is often associated to synergic disturbances due to the increased contact with human populations, which can lead to changes in the behaviour, ecology, 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 occurrence and abundance of primate populations in fragments (Estrada and Coates-Estrada, 1996; Cristóbal-Azkarate et al., 2005; Benchimol and Peres, 2014). Indeed, reduced habitat size, along with exacerbated edge effects, can alter the abundance and diversity of food resources in fragments and therefore influence the behaviour and ecology of occurring species (Arroyo-Rodríguez and Mandujano, 2006, 2009; Arroyo-Rodríguez et al., 2007; da Silva Júnior et al., 2009). 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 in order 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, giving relevant insight on their behaviour, physiology, and ecology (Sheriff et al., 2011; Novak et al., 2013). GCs are steroid hormones that 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 GCs in the bloodstream (Sapolsky et al., 2000). A surge in GC levels induces the mobilization of stored energy, promotes escape behaviours, 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 et al., 1997; Sapolsky et al., 2000; 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). Although, the potentially negative effect of chronic stress is still debated for wild animals given the adaptive nature of the stress response (Boonstra, 2013), studies suggest that 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 precious 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 completely noninvasively and are therefore not prone to biases linked to handling and capture (Sheriff et al., 2011). However, hair samples also present significant advantages because they are easily and painlessly collected with limited invasiveness, can be simply stored in paper bags at room temperature, and are stable over several years (Koren et al., 2002; Macbeth et al., 2010). 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 (Meyer and Novak, 2012; Stalder & Kirschbaum, 2012; Shimamoto, 2022). In this sense, hormones are thought to be incorporated during the anagen growth phase, during which the hair follicle remains connected to the bloodstream (Kapoor et al., 2018). In primates, using data from 6 species (i.e., *Cercocebus chrysogaster*, *Allenopithecus nigroviridis*, *Theropithecus gelada*, *Symphalangus syndactylus*, *Gorilla gorilla*, *Hylobates gabriellae*) a study postulated that the time archived in hair samples varies across species, ranging from 3.4 to 7.1 months with growth rates of 0.7 to 3.5 cm/months (Fourie et al., 2016). However, 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). HCCs may therefore represent a blend of long-term hormone accumulation during hair growth and transient levels from auxiliary sources (Koren *et al.*, 2019). Accordingly, HCCs may not be stationary in the hair shaft once incorporated. Until future research determines precisely how GCs are incorporated into hairs and how long they remain post-deposition, it is therefore not recommended to interpret HCCs as historical records of physiological stress (Kalliokoski et al., 2019). Keeping these limitations in mind, HCC measures do show high intra-individual stability (Stalder et al., 2012). Hence, hair cortisol analysis remains a promising tool to assess adrenocortical activity in wild animals, providing precious information on their overall physiological stress state (Heimbürge et al., 2019). Unlike faecal and urine samples, that may be prone to hourly and daily fluctuations, hair samples present a more stable and intergrated measure of cortisol. Overall, HCCs provide information on HPA axis functioning and are adequate to explore physiological stress and determine the influence of persisting environmental or social stressors (Kalliokoski et al., 2019).

A recent meta-analysis showed that primates inhabiting areas subject to anthropogenic disturbances repeatedly present higher GC levels than their conspecifics in less disturbed environments (Kaisin et al., 2021). 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 habitat loss and degradation on GC levels (see Kaisin et al., 2021). 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; Behie et al., 2010; Dunn et al., 2013; Chaves et al., 2019; McLennan et al., 2019; Tecot et al., 2019) because resource availability and distribution are typically lower in such habitats due to enhanced edge effects and poor plant recruitment (Laurance et al., 2000; Cordeiro and Howe, 2001; Marsh and Chapman, 2013). Nonetheless, other studies have also demonstrated that fragments are not consistently characterized by lower food availability and therefore higher GC levels (Tecot, 2013; Ordóñez-Gómez et al., 2016), emphasizing the importance of evaluating and quantifying resource availability when comparing primate populations in habitats of varying quality.

In this study, we explore the influence of habitat quality and diet on HCCs in black lion tamarins (*Leontopithecus chrysopygus*), an endangered Atlantic Forest primate. The Brazilian Atlantic Forest is one of the Earth's 25 biodiversity hotspots, hosting countless endemic species (Myers et al., 2000; Mittermeier et al., 2011). Sadly, this biome is also highly fragmented and subject to intense habitat loss spurred by agricultural and urban expansion (Ribeiro et al., 2009; Joly et al., 2014). Although latest estimates suggest that 26% of the native forest cover remains, the remaining vegetation is presumably mainly secondary or degraded forest (Rezende et al., 2018). Black lion tamarins are one of the endemic inhabitants of this highly disconnected landscape. Their range is restricted to the State of São Paulo in south-eastern Brazil (Culot et al., 2015). Here, we assess cortisol concentrations in six different black lion tamarin populations, inhabiting six different Atlantic Forest fragments of varying size and quality. Given that these callitrichids are both frugivorous and arthropodivorous, we assess the availability of these two resources. In each site, we estimate vegetation diversity using Simpson's diversity index (D_s) and calculate total tree basal area (TBA), a proxy for fruit resource availability (Chapman et al., 1992; Stevenson et al., 1998; Worman and Chapman, 2006; Brūmelis et al., 2020). To evaluate arthropod prey abundance, we set up pitfall traps and measure total arthropod biomass in each fragment. In addition, we use the results of a sister study (Raskin, 2021) which analysed the isotopic composition of the same hair samples, as a proxy to estimate the proportion of animal matter in the diet of the lion tamarin populations. Collectively, we explored the influence of habitat quality and diet on HCC levels in black lion tamarins in order to assess how this species responds to habitat change. We started by testing whether the size of the fragments influenced vegetation

characteristics and arthropod abundance. While exceptions exist, we expect smaller fragments to be more degraded and therefore have lower resource availability. Regarding HCC levels, we expect lower habitat quality (i.e., smaller fragment size and lower resource availability) to lead to increased concentrations. Furthermore, we predict fruits to be a limiting resource for lion tamarins and therefore that cortisol levels will be influenced by vegetation abundance and diversity rather than arthropod abundance. In this sense, we also expect individuals with a greater proportion of fruits in their diet to have lower cortisol levels. Assessing the physiological state of tamarins living in fragments will help identify how they cope in these environments and potentially support future conservation strategies.

METHODS

Ethics statement

This study was conducted with the relevant authorisations 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 State University of São Paulo (UNESP; N°16/2019). *Leontopithecus chrysopygus* being listed under Appendix I of the Convention on International Trade in Endangered Species (CITES), the necessary exportation (N°21BR036613/DF) and importation (N°2021/BE00456/PI) permits were obtained.

Study sites

This research was conducted in 6 different study sites distributed within two distinct regions of the Paranapanema River Basin, within the State of São Paulo in Brazil. Typically, southeast Brazil is characterized by a dry winter (April-September) and rainy summer (October-March).

The Morro do Diabo State Park (MDSP; 33,845 ha; 22°37'21"S, 52°08'02"W), and the fragments of Ponte Branca (1,306 ha; 22°24'53"S, 52°30'48"W) and Santa Maria (520 ha; 22°14'20"S, 52°18'23"W) are located in the Pontal do Paranapanema region (Fig. 2.1; 1-3). Pastures as well as sugarcane and soy plantations are the main agricultural exploitations in this region. MDSP is the largest Atlantic Forest remnant in the western part of the São Paulo state and is host to the largest population of black lion tamarins, approximately 1200 individuals (Rezende et al., 2020a). Aside from an imposing hill culminating at 600 m, which gave its name to the park, MDSP has an altitude ranging between 250-450 m. Similarly, the Ponte and Santa Maria fragments have altitudes varying between 300 to 450 m. During the study period, the mean temperature and average monthly total rainfall in the Pontal region were 25.2°C and 110.9 mm, respectively (Agritempo 2022; TRMM 1018).

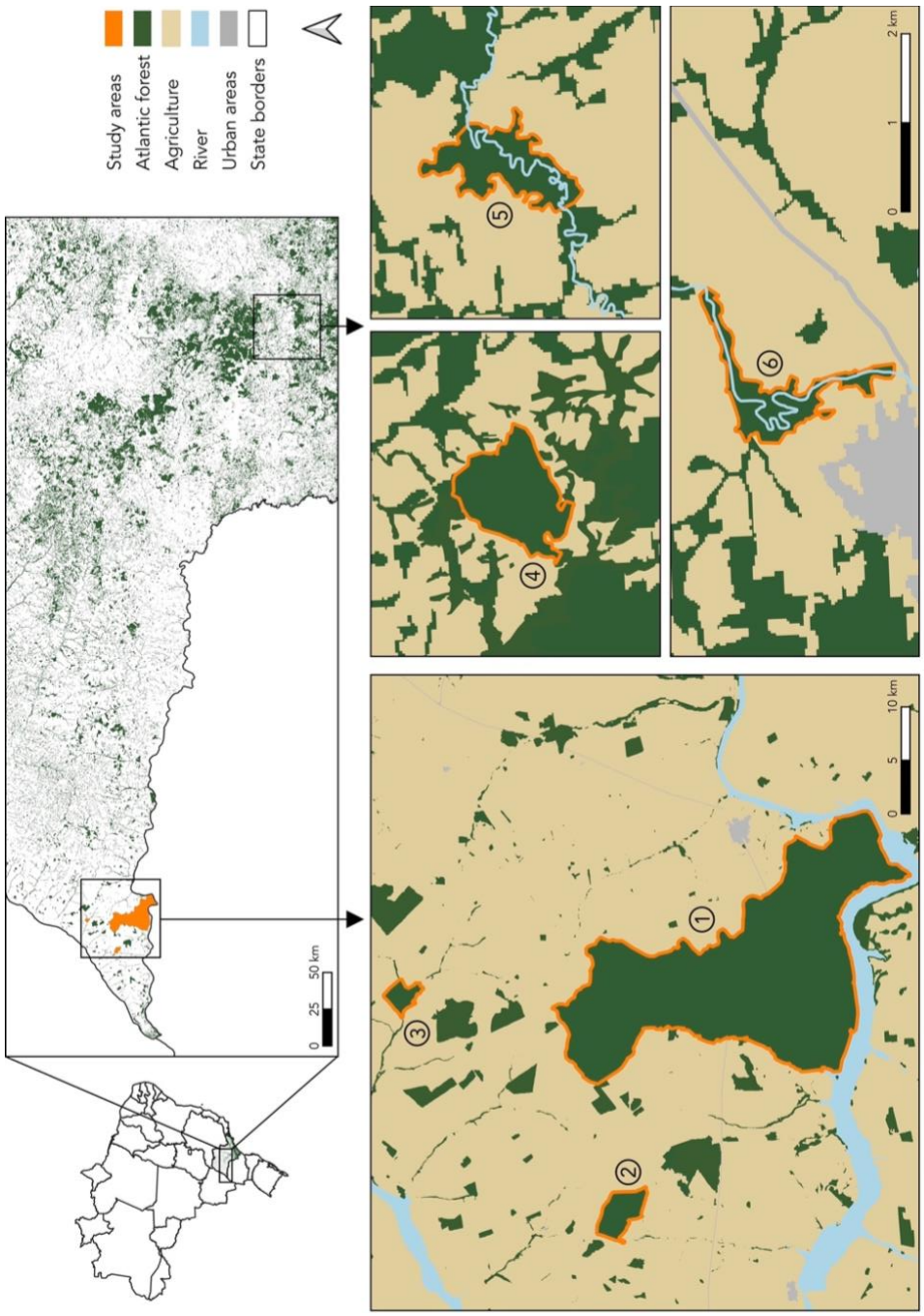

***Figure* 2.1:** Location of the six study sites within the State of São Paulo, including the peripherical land use. The sites are located in two distinct regions of the Paranapanema River Basin which partially delimits the border of the Sao Paulo State. The Pontal Paranapanema region (West) includes the (1) Morro do Diabo State Park [MDSP; 33,845 ha] and the fragments of (2) Ponte Branca [1,306 ha] and (3) Santa Maria [520 ha]. The Alto Paranapanema region (East) encompasses the (4) Guareí fragment [100 ha] and the riparian forest fragments of (4) Angatuba [79 ha] and (6) Buri [78 ha].

The Alto Paranapanema region encompasses the Guareí fragment (100 ha; 23°25’07"S, 48°14’27"W) and the riparian forests of Angatuba (79 ha; 22°27’26"S, 48°23’57"W) and Buri (78 ha; 23°47’19"S, 48°35’20"W; Fig. 2.1; 4-6). The size of riparian forests was established 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 characterized by pastures and sugarcane, eucalyptus, pine tree and orange plantations. The altitude in these study sites varies between 591-680 m. The mean temperature and average monthly total rainfall were 22.2°C and 93.1 mm during the study period (Agritempo 2022; TRMM 892). All temperature and rainfall data used in this study were based on Tropical Rainfall Measuring Mission (TRMM) satellite data.

Captures and hair sample collection

***Table* 2.1.** Distribution of hair samples collected in the 6 study sites according to sex and age-class

Region	Site	Adults		Juveniles		Total
		♀	♂	♀	♂	
Pontal do Paranapanema	MDSP	2	3	-	-	5
	Ponte Branca	2	2	2	-	6
	Santa Maria	1	-	-	-	1
Alto Paranapanema	Guareí	2	3	1	-	6
	Angatuba	1	3	-	2	6
	Buri	1	4	-	2	7
	Total	9	15	3	4	**31**

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

Hair samples for endocrinal 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 radio-telemetry tracking collars (Holohil RI-2D) in order to collect behavioural data and monitor the groups. To ensure the welfare of the tamarins, a trained veterinarian was present at all captures. The tamarins were captured using three different methods (i.e., inside their sleeping site, using *Tomahawk* traps, or using a snare pole; see *Supporting Information*, Appendix S2.1). To avoid potential HCC variations linked to the body region (Carlitz et al., 2015), the hair was systematically clipped from the interscapular region as close to the skin as possible, while taking special precautions not to damage the skin.

Furthermore, this region is the less accessible by primates and therefore minimises potential contaminations (Fourie et al., 2016). Samples were then placed into small plastic bags and in opaque paper envelopes to protect them from UV degradation. In total, we collected samples from 31 different individuals in 6 different areas (Table 2.1).

Hormone analysis

Sample extraction and analysis were performed at the Laboratory of Experimental Endocrinology, at the University of Veterinary Medicine of Vienna, Austria. For extraction, 100 mg sub-samples from each sample 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, which 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).

HCC concentrations were measured using a homemade enzyme immunoassay (EIA) sensible to 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 sample pools and comparing the slope of the curve with that of the standard curve (see *Supporting Information*; Appendix S2.2). Unfortunately, performing a physiological validation is hard to achieve when using hair samples since hormones are thought to be incorporated over long time window. Furthermore, such validations require repeated administration of adrenocorticotropic hormone (ACTH), which is hard to perform on endangered species for both methodological and ethical reasons. While a hair growth experiment on captive black lion tamarins was planned at the Primate Center of Rio de Janeiro, it was not completed due to the COVID-19 pandemic.

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, 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.

Habitat quality estimation

Vegetation characterisation

Between 2020 and 2021, we set up 20 (10 x 10 m) botanical plots in each study site. We calculated the total tree basal area (TBA) as a proxy for fruit resource availability (Chapman et al., 1992; Stevenson et al., 1998; Brūmelis et al., 2020). The plots were distributed as a grid, 200 to 400 m apart, within the estimated home range of each captured black lion tamarin group (see *Supporting Information* for a representation of the set up; Appendix S2.3). Inside every botanical plot, we calculated the TBA of every tree with a circumference at breast height (CBH) ≥ 15 cm, corresponding to a diameter at breast height (DBH) ≥ 4.8 cm. The total TBA for each site was calculated by summing the TBA of every tree within the 20 plots. Given that we only have feeding behaviour data for two sites (i.e., Guarei and MDSP; Kaisin et al., 2022) and that black lion tamarins are eclectic frugivores and have highly variable diets, we calculated total TBA rather than the TBA of known consumed species. Furthermore, total TBA may be used to evaluate general habitat quality and forest structure (Sagar & Singh, 2006; da Silva Júnior et al., 2009).

Vegetation diversity and richness are also valuable measures of habitat quality since higher species diversity may offer a greater variety of food sources (Rovero and Struhsaker, 2007; Cavada *et al.*, 2016). In the 20 botanical plots of each site, we identified to the species level all the trees with a DBH ≥ 4.8 cm. We estimated tree diversity by calculating Simpson's diversity index (D_s), which takes into account the number of species present as well as their respective abundance. Additional metrics on tree abundance, diversity, and abundance in each site, are available as *Supporting Information* (Appendix S2.4).

Arthropod biomass

We used pitfall traps to estimate arthropod biomass between March and June 2022, months during which arthropod abundance is relativity stable in Atlantic Forests (Develey and Peres, 2000; Leiner and Wesley, 2007; Hohbein and Conway, 2018). In each site, 10 pitfall traps were set up in 10 of the 20 botanical plots, one and not the other, totalising 100 traps per site (see *Supporting Information* for a representation of the set up; Appendix S2.5). Within each site, the traps were installed in two parallel lines, 2 m apart, of five traps 1 m apart. To construct the traps, we buried 500 ml plastic pots and filled them with 150 ml of 70% ethanol to preserve the arthropods (Lamperty et al., 2020). We used the lid of the pots as suspended roofs to prevent rain and debris from falling into the traps. The traps were left in the field for 5 full consecutive days. The pitfall traps were then sieved to remove any detritus and all arthropods were collected and sorted. Since, based on behavioural observations, black lion tamarins do not seem to prey upon small arthropods, and

macroinvertebrates have repeatedly been used to evaluate environmental changes (Haskell, 2000; Hodkinson and Jackson, 2005; Walters et al., 2009; Lamperty et al., 2020), we only considered arthropods >5 mm. Arthropods were then classified by order and then sorted into morphospecies (Derraik et al., 2002, 2010). When available, six individuals from each morphospecies were then dried for two weeks at 50°C in a laboratory oven. The arthropods were then weighed using an analytical balance. Based on the weight of these six representatives, we calculated a mean dry weight for each morphospecies. The mean dry weight of each morphospecies was then extrapolated according to the abundance (i.e., number of individuals) collected at each study site. Finally, for each site we summed the total weight of each morphospecies to calculate the total arthropod biomass. The abundance and diversity of the main arthropod orders consumed black lion tamarins (i.e., Araneae, Blattodea, Coleoptera, Dermaptera, Hemiptera, Hymenoptera, and Orthoptera) are available for each site in the *Supporting Information* (Appendix S2.6).

Frugivory

Since GCs are metabolic hormones that are influenced by diet and energy intake (Emery Thompson, 2017), we estimated the proportion of frugivory in the diet of black lion tamarins by using isotope data for hair samples. The isotopic composition and fractionation of a consumer are influenced by its diet (Deniro and Epstein, 1981). Essentially, the nitrogen isotope ratio ($\delta^{15}N$) increases with trophic level in a given food chain (Caut et al., 2009). In other words, as the $\delta^{15}N$ increases, the prevalence of animal matter in the diet increases. Similarly, carbon isotope ratios ($\delta^{13}C$) provide information on the amount and type of fruits consumed by a species (Herrera et al., 2003). Therefore, $\delta^{15}N$ and $\delta^{13}C$ have previously been used to estimate the proportions of fruits and arthropods in the diet of primates (Schoeninger et al., 1997; Sandberg et al., 2012). A recent sister study used $\delta^{15}N$ and $\delta^{13}C$ isotope data from black lion tamarin hair to estimate the diet of our study groups (Raskin, 2021). The hair samples in the latter study were collected on the same individuals and during the same captures as the ones used in this study. This study used Bayesian isotopic mixing models to estimate the relative contribution of fruits and arthropods in the diet of each individual (Phillips et al., 2014; Stock et al., 2018). In our models, we included the contribution of frugivory as an explanatory variable of HCC variation. Additional information on the methods used to estimate frugivory are available as *Supporting Information* (Appendix S2.7).

Statistical analysis

To test whether arthropod biomass, TBA, or D_s varied according to the size of the fragments (N = 6), we started by running Spearman correlations. Then, we square root transformed HCCs to achieve a normal distribution (Shapiro-Wilk test: $W = 0.96$, $p = 0.376$) and standardised all variables. To preliminary control for potential inter-individual and

geographic differences in HCCs, we ran *t*-tests to compare mean HCCs across sex, age-classes, and geographic regions (Alto vs. Pontal do Paranapanema). Then, to explore the influence of habitat quality on cortisol levels in black lion tamarins, we ran linear models (LMs). HCCs were set as the response variable while TBA, D_s, fragment size, arthropod biomass, and frugivory were included as fixed effects in the model. Due to the relatively small sample size (N = 31) and the fact that HCCs were not significantly influenced by age, sex, and region, we avoided including random effects to keep our model simple.

We conducted all statistical analyses using R 3.6.1 with a significance level of $\alpha = 0.05$. Models were fitted using the restricted maximum likelihood (REML) method and compared using the Akaike information criterion (AIC) which measures the relative quality of models (Burnham and Anderson, 1998). For each model, we tested for multicollinearity using the variance inflation factor (VIF) and all variables included in a model had a VIF < 5 (Menard, 2002; James et al., 2013). We compared the goodness-of-fit of the best model with the null model using a likelihood-ratio test (Wilks, 1938). Due to the uneven sampling between the rainy (N = 5) and dry (N = 26) seasons, we also ran models with only the samples from the dry season.

RESULTS

The size of the study sites was not correlated with arthropod biomass (r_s = 0.77, p = 0.103), TBA (r_s = -0.54, p = 0.297), or D_s (r_s = 0.14, p = 0.803; Fig. 2.2). We found no significant differences in HCCs between males and females ($t_{(10.5)}$ = 0.13, p = 0.899), adults and juveniles ($t_{(8)}$ = -1.72, p = 0.141), or the Alto and Pontal do Paranapanema geographic regions ($t_{(29)}$ = 0.20, p = 0.843). Mean HCCs per study site and explanatory variable values are listed in Table 2.2. Boxplots representing the distribution of HCC, square root transformed to achieve a normal distribution, across sexes, age-classes, regions, and study sites are available as *Supporting information* (Appendix S2.8).

The best model included the TBA, D_s, fragment size, and frugivory, which all had a negative effect on HCC levels (Table 2.3). In other words, as TBA, D_s, fragment size, and frugivory decreased, cortisol levels increased. These trends are plotted in Figure 2.3 to help interpretation. This model fitted our data better than the null model (F = 4.24, p = 0.009). However, the best model was not significantly better than the second-best model that included TBA, D_s, and fragment size (F = 4.24, p = 0.098). This suggests that the effect of frugivory on HCCs is weak. The full model and AIC model selection are detailed in the *Supporting Information* (Appendix S2.9). The model including only the samples from the dry season showed similar results (see *Supporting Information*; Appendix S2.10).

***Table* 2.2.** Mean hair cortisol concentrations (HCCs) and explanatory variables: fragment size, total tree basal area (TBA), arthropod biomass, Simpson's diversity index (D_s), and frugivory for each of the six study sites.

Site	Size (ha)	TBA (m^2)	Arthropod biomass (g)	D_s	Frugivory (%)	HCC (ng/g)
MDSP	33 845	5.49	141.51	0.032	58.6	1047.2
Ponte Branca	1 306	4.25	65.42	0.171	52.8	589.6
Santa Maria	520	2.91	147.85	0.073	63.7	2156.6
Guareí	100	8.52	8.91	0.031	64.2	579.6
Angatuba	79	7.36	6.85	0.052	61.3	1375
Buri	78	6.8	8.2	0.051	54.7	1411.8

MDSP: Morro do Diabo State Park.

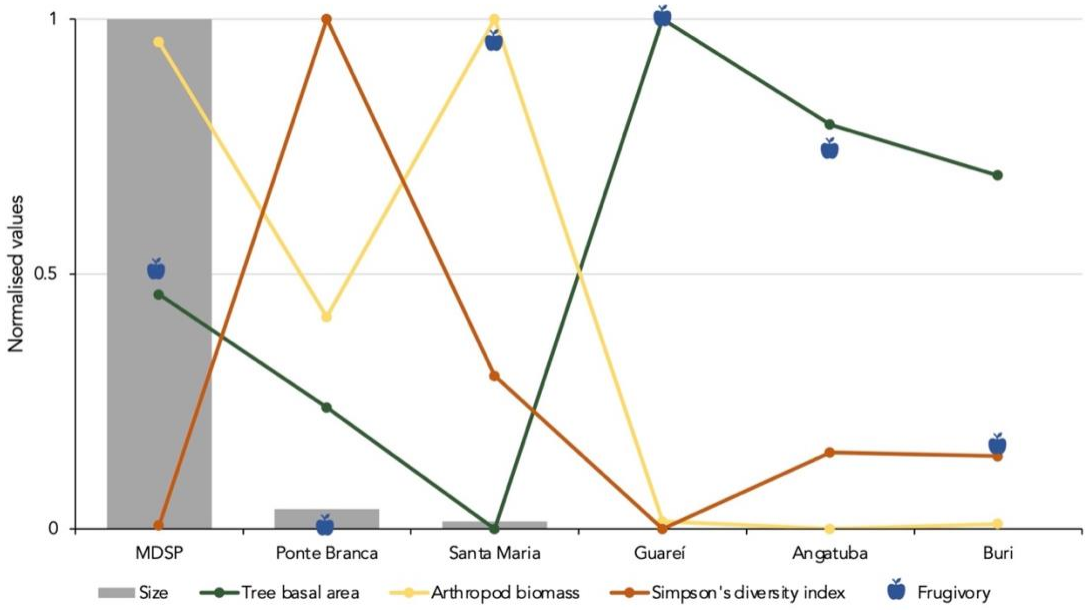

***Figure* 2.2:** Normalised habitat quality variables, including fragment size, total tree basal area, arthropod biomass, Simpson's diversity index, and the normalised proportion of frugivory in tamarins' diets for each of the six study sites. MDSP: Morro do Diabo State Park.

***Table* 2.3.** Best fit linear model testing the effect of habitat quality (total tree basal area (TBA), Simpson's diversity index (D_s), fragment size and diet (frugivory) on hair cortisol concentration levels

Effect	Estimate	SE	*t* ratio	*p*-value
D_s	-1.25	0.33	-3.84	$< 0.001^{***}$
Frugivory	-0.36	0.21	-1.71	0.098
TBA	-1.04	0.31	-3.36	0.002^{**}
Size	-0.57	0.23	-2.55	0.017^{*}

Estimate of the coefficient and the corresponding standard error (SE), *t* statistic and *p*-value calculated using the T distribution.

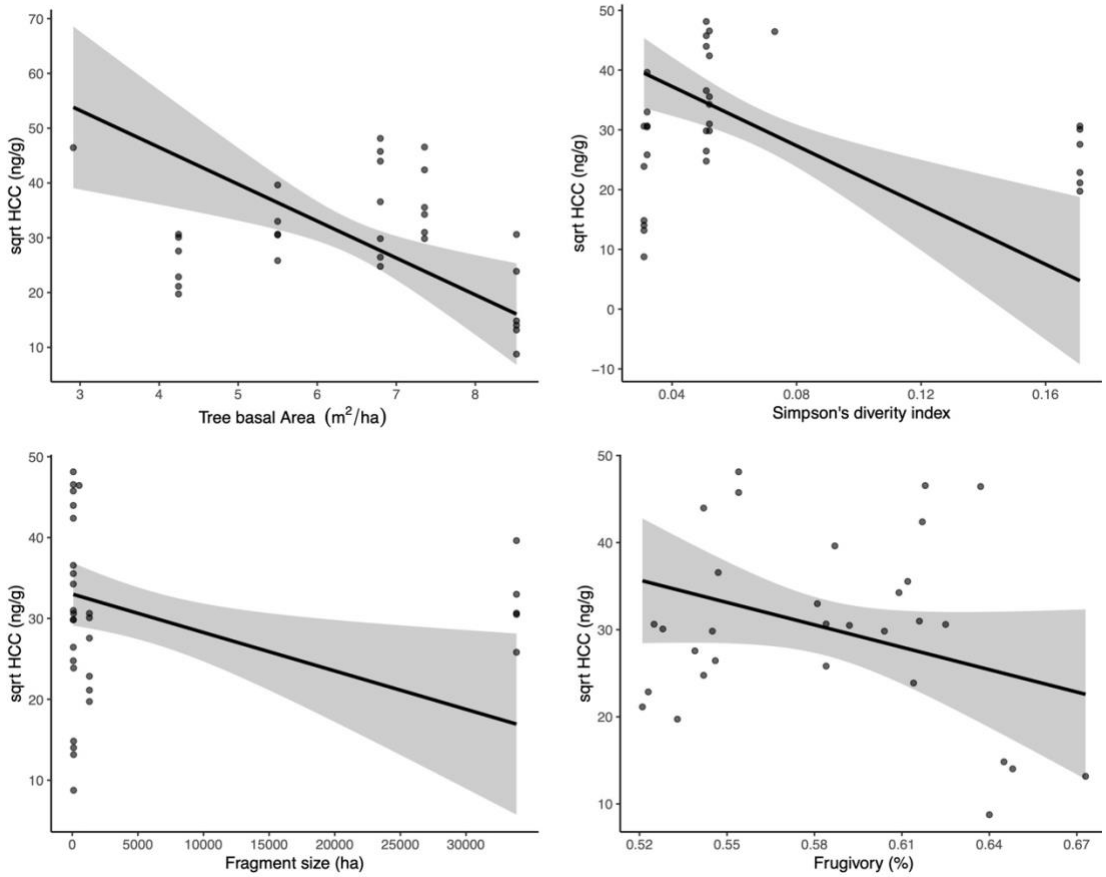

***Figure* 2.3:** Correlation between hair cortisol concentrations (HCC) (square root transformed to achieve a normal distribution) and habitat quality variables (total tree basal area (TBA), Simpson's diversity index (D_s), fragment size) and diet (frugivory).

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 gauged physiological stress in different black lion tamarin populations to evaluate how these small primates cope in disturbed fragments of the Brazilian Atlantic Forest. Our results suggest that low habitat quality negatively affects their cortisol levels since decreasing total tree basal area (TBA), vegetation diversity (D_s), and fragment size increased hair cortisol levels. Our model also revealed that higher levels of frugivory may reduce cortisol levels. However, arthropod biomass had no significant effect.

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. 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, 2002; Romero 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). Since lion tamarins are primarily monogamous, groups include a unique reproductive pair. The female has a four-month gestation period and typically gives birth to twins during the rainy season (Rezende et al., 2020a). Despite the reduced sample size, among the females sampled in our study, while two individuals were presumably pregnant, they did not display higher HCCs than the other adult females (see *Supporting Information*; Appendix S2.8). Furthermore, we found no significant differences in cortisol levels between males and females. Similarly, age class did not influence HCCs. This may be due to the absence of a dominance hierarchy and the 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 synchronization of activities and homogeneity of their diets (Kleiman & Ryland, 2002; Dixson, 2012). Therefore, their energy balance and metabolic needs are susceptible of being similar. In regard to circannual changes in cortisol linked to food availability or climate, our sampling is uneven with most of the captures occurring during the dry season for practical reasons (N = 26 out of 31). Expanding sampling effort to both seasons and over several years could reveal circannual fluctuations in GC levels. Nonetheless, when exploring the effect of habitat quality and diet, we ran a linear model regrouping only the samples from the dry season and found similar results than the model including all samples (see *Supporting Information*; Appendix S2.10). Keeping in mind 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 repeated measures

on the same individuals (e.g., every month), to better understand interindividual or temporal variations in HCCs linked to intrinsic or seasonal factors.

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., 2002; Hill and Curran, 2003; Benítez-Malvido and Arroyo-Rodríguez, 2008). Fragmentation alters vegetation diversity, composition, and structure, modifying resource availability, quality, and abundance for primates (Arroyo-Rodríguez and Mandujano, 2006; Marsh and Chapman, 2013). In this study, we measured physiological stress in black lion tamarin populations inhabiting Atlantic Forest remnants ranging from 33,845 to 78 ha. The size of fragments is a fundamental measure of habitat quality, altering the acquisition of resources by species by impairing their ranging patterns, but also by increasing the prevalence of anthropogenic disturbances (Benítez-Malvido and Arroyo-Rodríguez, 2008; de Almeida-Rocha et al., 2017). Combined with the other habitat quality variables, we found that, as the size of the fragments decreased, cortisol levels increased, suggesting that lion tamarins in smaller fragments are more stressed. The first assumption would be that resource availability is lower in smaller fragments, causing nutritional stress in lion tamarins. However, the vegetation characterization of the areas used by the lion tamarin groups revealed that TBA and tree species diversity were not correlated with fragment size. The second assumption is related to social competition because smaller fragments may have potentially greater inter and intra-species competition that could cause a rise in cortisol levels. A recent study discovered that overcrowding was the main cause of increased GCs in fragment-dwelling ring-tailed lemurs (*Lemur catta*; Gabriel et al., 2018). We also suspect that black lion tamarins living in smaller fragments are exposed to intensified human pressure (e.g., cattle ranching, agricultural machinery, domestic dogs, hunting) linked to the proximity of agricultural exploitations and urban areas, which increases physiological stress levels. This was the case in Buri and Angatuba, which are riparian forests with higher levels of human disturbances despite having greater TBAs than the study sites in the Pontal do Paranapanema region. Higher cortisol levels have also been found in other mammal species due to the proximity to human settlements (*Alouatta palliata*; Vanlangendonck et al., 2015), roads (*Aepyceros melampus*; Lunde et al., 2016), noise pollution (*Alouatta belzebul*; Monteiro et al., 2013), and domestic dogs (*Galago moholi*; Scheun et al., 2015). Although other primate species, such as baboons (*Papio* sp.), macaques (*Macaca* sp.), and other callitrichid species (e.g., *Callithrix penicillata*; Secco et al., 2018) seem to be capable to adapt 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).

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). Therefore, because lion tamarins are both frugivorous and arthropodivorous, we

estimated the availability of both these resources in each study site. While arthropod biomass had no significant effect, our model revealed that lion tamarins living in habitats with an impoverished flora, with lower diversity and basal area, had higher cortisol levels. Fruit availability seems to represent an important and limiting resource for this species. Despite the effect being weak, our results also suggest that as the proportion of fruits in the diet decreased, cortisol levels increased. Expanding the sampling effort to new areas and more individuals per area may uncover robust trends regarding the influence of frugivory.

Given that GCs play a key role in glucose metabolism regulation, they vary according to the energy balance of individuals, which signifies energy intake and expenditure (Emery Thompson, 2017). In degraded habitats, where resources are sparse and dispersed, energy intake may decrease due to diet quality while expenditure may amplify due to increased travelling time between food patches. In accordance with our results, multiple studies have revealed that lower fruit availability triggers nutritional stress in primates (see MacLarnon et al., 2015). Likewise, diet quality and the availability of preferred food also enhance GC secretion (Muller and Wrangham, 2004; Chapman *et al.*, 2007; Foerster and Monfort, 2010). Since black lion tamarins are both generalists and opportunists (Keuroghlian and Passos, 2001), a higher tree diversity within the fragments may offer them a larger variety of food sources (Rovero and Struhsaker, 2007; Cavada et al., 2016), therefore reducing their cortisol levels. Nevertheless, in one of our study sites (i.e., MDSP), black lion tamarins exhibited higher faecal GC levels when feeding on non-preferred fruits during the dry season, characterized by lower fruit availability (Kaisin et al., 2022). Feeding on fallback food has also been linked to elevated physiological stress in Sykes' monkeys (*Cercopithecus mitis*; Foerster and Monfort, 2010) and yellow baboons (*Papio cynocephalus*; Gesquiere et al., 2008). While feeding on non-preferred fallback food may not be optimal and increase metabolic demands (Dunn et al., 2012), feeding behaviour plasticity and the capacity of consuming fallback items during times of food shortage can promote survival (McLennan et al., 2017).

On top of food availability, forest structure is another key habitat quality variable that governs primate diversity and abundance (Pyritz et al., 2010; Gouveia et al., 2014). Forest structure can influence the movement of arboreal primates (Bicca-Marques, and Calegaro-Marques, 1995; Dagosto and Yamashita, 1998; Cheyne et al., 2013). For example, the number of large trees dictated the occurrence of howler monkeys in tropical forest fragments (Arroyo-Rodríguez et al., 2007). 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, TBA was highly correlated to the density of large trees (DBH > 20cm; see *Supporting Information*; Appendix S2.11), suggesting that habitat structure may also influence physiological stress by impairing movement.

As eclectic consumers (Keuroghlian and Passos, 2001), black lion tamarins are able to adapt their diet according to food availability and may consume low-quality fallback items during periods of food shortage. Nonetheless, this dietary plasticity comes at a cost as it has been linked to higher GC levels in black lion tamarins (Kaisin et al., 2022). In this study, our results revealed that their physiological stress levels were swayed by vegetation quality, despite tree abundance and diversity not being correlated with fragment size. Nonetheless, smaller fragments are often subject to heightened human disturbances, which increases their cortisol levels and may potentially lead to chronic stress. In this sense, healthy and undisturbed Atlantic Forest remnants may represent suitable habitats for black lion tamarins, regardless of their size. 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 et al., 2020b). Protecting and preserving small fragments is therefore paramount for their conservation while increasing connectivity between fragments and limiting human encroachment.

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SUPPORTING INFORMATION

Appendix S2.1: *Captures and hair sample collection*

Hair samples for endocrinal 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 radio-telemetry tracking collars in order to collect behavioural data and monitor the groups. To evaluate the health of individuals and fully exploit a capture opportunity, we also collected various biological samples, took morphological measurements, and estimated the age and body condition of individuals. To ensure the welfare of the tamarins, a trained veterinarian was present at all captures.

When targeting a new group, lacking radio-telemetry collars, the first step is locating the group in the fragment. Essentially, we survey the study area using playback of pre-recorded tamarins calls from other groups. Once a group is sighted, different methods are used to capture the tamarins. The most widely used method to capture callitrichid primates is using live-traps (Jolly, Phillips-Conroy, & Müller, 2011). In this study, we used up to 10 *Tomahawk* traps (48 x 20 x 20 cm) that we set up within a tree on different large branches. Installing multiple traps directly on a natural support, rather than on a platform, helps with the required habituation by limiting novel unknown objects. To attract the tamarins towards the location of the traps we used playback of diverse pre-recorded calls (e.g., long calls, alarm calls, agonistic calls). Moreover, the traps were mainly baited with *Psidium guajava* fruits (i.e., guavas) although we also used bananas, apples, mangoes, and quail eggs. Before attempting a capture, the traps were disarmed and left open for 4-5 days with food bait to habituate the tamarins. After capturing a maximum of individuals, while respecting a maximum two-hour delay after the first capture, we brought down the traps and covered the cages with cloth bags prior sample collection.

Since tamarin species use sleeping sites (e.g., tree hollows, clumped lianas, or palm tree sheaths) to spend the night, an alternative method to capture an entire group is to round up individuals when they occupy an easily accessible tree hollow. To identity a sleeping site, the target group is followed until they enter a sleeping site. The following day, the team returns to the sleeping site before dawn. Once the hollow is identified, the tree is inspected and all potential exits are sealed with cloth bags stuffed with dry leaves. The tamarins are then manually pulled out one by one through the main hollow with leather gloves and directly stowed in individual cloth bags awaiting sample collection and manipulation.

Finally, on rare occasions, when following a group until their sleeping site and the use of traps was impossible due to the lack of habituation, a unique individual was captured using a snare pole. To avoid hurting the animals, the lasso was tightened around the neck (i.e., cervical vertebra) and the axilla of the individual. The captured individual was equipped with a radio-telemetry collar to be able to follow and habituate the targeted group.

Regarding all capture methods, once the individuals were physically restrained in cloth bags, they were anesthetized through isoflurane inhalation anaesthesia. The use of isoflurane was preferred over other deeper anaesthetic methods (e.g., ketamine) due to the straightforward administration (i.e., using an oxygen tank, a vaporiser, and an adequate face-mask), rapid induction of anaesthesia, and smooth control of anaesthesia depth (Calle & Ott Joslin, 2015). Once under anaesthesia, we collected extensive biometric data (e.g., weight, body length), collected biological samples, evaluated the tamarin's general body condition, and inspected its body for any scars, wounds or ectoparasites. To avoid contamination and potential HCC variations linked to body region (Carlitz, Kirschbaum, Miller, Rukundo, & van Schaik, 2015), the hair was systematically clipped from the interscapular region as close to the skin as possible, while taking special precautions not to damage the skin. Samples were then placed into small plastic bags and in opaque paper envelops to protect from UV degradation. In total, we collected samples from 31 different individuals.

The advantage of using an inhalatory anaesthetic is that animals can usually be released a few minutes after they are withdrawn from the isoflurane flow. Nevertheless, to ensure the safety and welfare of the subjects, the tamarins were placed in separate traps after anaesthesia until they were completely awake. On average, the individuals were kept under anaesthesia for 30-45 min. When more than one individual was captured, we released all tamarins at the same time to maintain group cohesion.

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Appendix S2.2

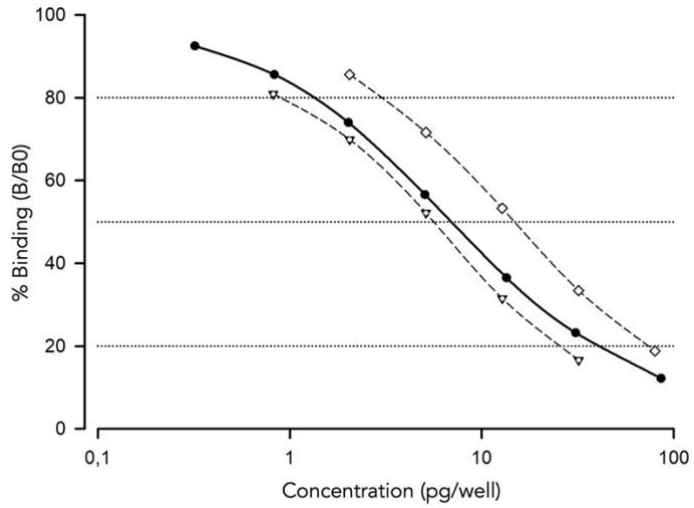

***Figure* 2.4:** Parallelism between the standard curve (circle line) and two serially diluted pool samples (triangle and square dotted lines).

Appendix S2.3

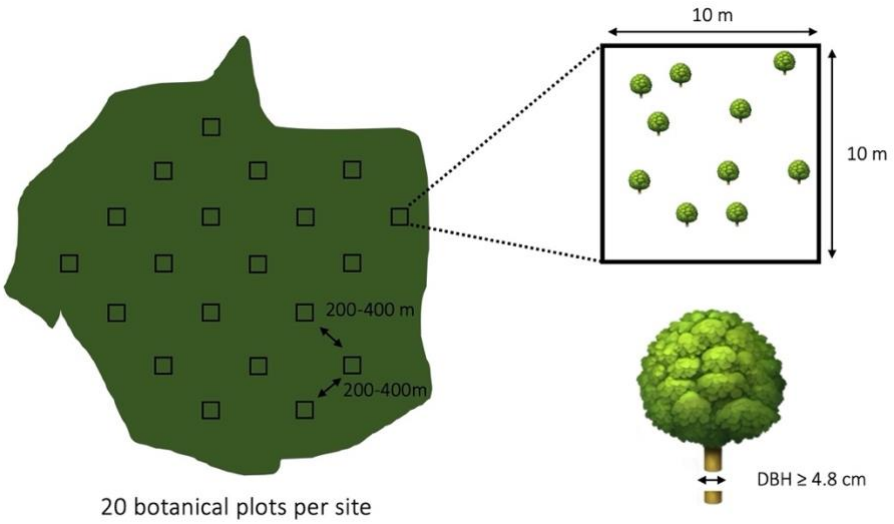

***Figure* 2.5:** Schematic representation of the vegetation characterisation.

Appendix S2.4

***Table* 2.4.** Tree abundance, diversity, and abundance in each site, estimated by our vegetation characterisation.

Site	*N* individuals	*N* species	*N* families	Density (ind/ha)	Large tree density (ind/ha)	DBH (cm)
MDSP	266	78	26	1266	210	13.11 ± 10.2
Ponte Branca	280	48	21	1473	110	10.85 ± 8.1
Santa Maria	315	50	22	1575	95	9.49 ± 5.3
Guareí	317	87	28	1585	315	12.83 ± 8.4
Angatuba	442	83	31	2210	275	12.49 ± 7.5
Buri	283	63	24	1415	235	13.82 ± 10.7

MDSP: Morro do Diabo State Park, DBH: diameter at breast height.

Appendix S2.5

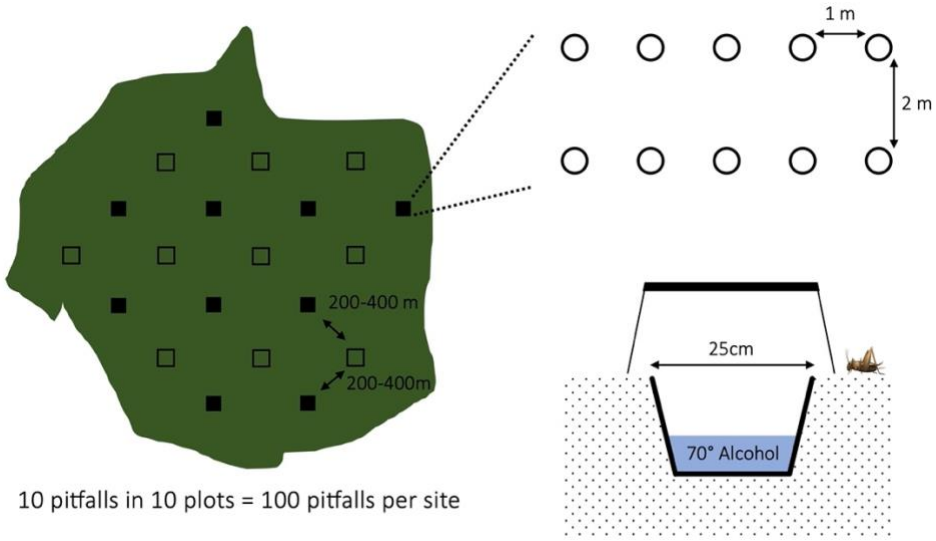

***Figure* 2.6:** Schematic representation of the vegetation characterisation.

Appendix S2.6

***Table* 2.5.** Arthropod abundance (# of individuals) and diversity (# of morphospecies) in each site estimated using pitfall traps.

Order	MDSP	Ponte Branca	Santa Maria	Guareí	Angatuba	Buri
Aranaea	93 (26)	53 (9)	114 (15)	138 (14)	116 (11)	66 (18)
Blattodea	203 (17)	104 (10)	65 (12)	75 (9)	126 (14)	74 (12)
Coleoptera	427 (53)	85 (25)	427 (27)	54 (21)	78 (24)	44 (13)
Dermaptera	276 (3)	54 (2)	39 (3)	13 (2)	39 (3)	7 (2)
Hemiptera	66 (27)	13 (6)	16 (9)	27 (11)	38 (17)	56 (12)
Hymenoptera	3828 (31)	536 (18)	1175 (19)	213 (21)	120 (16)	274 (12)
Orthoptera	169 (19)	107 (13)	53 (11)	135 (6)	119 (15)	18 (6)

MDSP: Morro do Diabo State Park. # of morphospecies is between parentheses.

Appendix S2.7: *Stable isotope data analysis*

Stable isotope analyses, which are based on the principle that "you are what you eat", represent a powerful tool to assess the diet of wild primates (Crowley et al., 2016). In another study conducted by our lab, we used isotopic ratios from hair samples to determine the proportion of frugivory in the diet of black lion tamarins (Raskin, 2021). To estimate their diet, we looked at their carbon ($^{13}C/^{12}C$) and nitrogen ($^{14}N/^{15}N$) isotope ratio. A sample's ratio, noted δ, is expressed in parts per thousand (‰) and is calculated relatively to an internationally recognised standard using the following formula:

$$\delta X = [\frac{R\ sample}{R\ standard} - 1] \times 1000 \ \ (‰)$$

Where X is the element measured (e.g., carbon or nitrogen). Typically, the nitrogen isotope ratio ($\delta^{15}N$) increases between each trophic level as you move up the food-chain, following trophic enrichment (Crowley, 2012). In this sense, $\delta^{15}N$ can be used to estimate the proportion of animal matter in the diet of primates (Schoeninger et al., 1997). Similarly, carbon isotope ratios ($\delta^{13}C$) can also provide information on a species diet as they vary according to the photosynthesis pathway of plants (Blumenthal et al., 2016). By running mixing models including $\delta^{15}N$ and $\delta^{13}C$ values, it is possible to partition the dietary proportion of different food sources in an animal's diet (Phillips et al., 2014).

To estimate resource contribution in the diet of black lion tamarins, we compared the $\delta^{15}N$ and $\delta^{13}C$ measured in their hairs with the ratios found in fruits and arthropods in their diet. Based on data from literature and field observations, we gathered fruits from a total of 139 trees, representing 24 species of 13 families, from all six study areas. To represent the arthropod diversity in the diets of black lion tamarins, we captured insects and spiders opportunistically and using pitfall traps in each study area. In total, we collected 625 arthropods from more than 50 different morphospecies, from 7 orders, known to be consumed by black lion tamarins (i.e., Araneae, Blattodea, Coleoptera, Dermaptera, Hemiptera, Hymenoptera, and Orthoptera). Fruit and arthropod samples were dried in a laboratory oven (50°C) for two weeks and then pulverized using a mortar and a pestle. All hair, fruit, and arthropod samples were then shipped to Belgium for isotope analyses at the Laboratory of Oceanology (OceanoBio) at the University of Liège.

Prior to analysis, hair samples were washed three times using dichloromethane to remove potential exogeneous contaminants, chopped finely, and then put to dry for 24 h in a laboratory oven (60°C). The samples were then placed in tin capsules, weighed, and analysed using an isotope ratio mass spectrometer (IRMS) coupled to an elemental analyser to determine their isotope ratios. For calibration, we followed the same method and certified materials as described in Lepoint et al. (2016). The weight of fruit samples was between 2.6-3.2 mg while arthropods and hairs were between 1.5-2 mg (Das et al., 2016).

Using the raw isotope ratios, we ran Bayesian mixing models using the MixSIAR package to estimate the contribution of fruits and arthropods in the diet of black lion tamarins (Stock et al., 2018). We divided food sources into two arthropod and two fruit groups according to their ecological characteristics and isotopic values (i.e., Arth1: majority of predatory arthropods, Arth2: primary consumer arthropods, Fruit1: all C3 plant species and *Pereskia* sp., Fruit2 : CAM plants). Despite being a CAM plant, *Pereskia* sp. had similar isotopic values as C3 plants and was therefore included in Fruit1. To account for trophic enrichment between the consumer and the sources, we used the mean of the trophic enrichment factors (TEFs) determined for carnivorous and herbivorous species (Post et al., 2002). We ran the models with 310^5 iterations and burn-in size was set as $2x10^5$ (Lepoint et al., 2016). The model estimated the proportion of frugivory and arthropodivory in the diet of each black lion tamarin individual in each study area (Table 2.6).

***Table* 2.6.** Mean proportion of frugivory and arthropodivory in the diet of black lion tamarins according to study area

Site	*N*	Frugivory (%)	Arthropodivory (%)
Angatuba	6	61.3 ± 0.5	38.7 ± 0.5
Buri	7	54.7 ± 0.5	45.3 ± 0.5
Guarei	6	64.1 ± 2.1	35.9 ± 2.1
MDSP	5	58.6 ± 0.4	41.4 ± 0.4
Ponte Branca	6	52.8 ± 0.7	47.2 ± 0.7
Santa Maria	1	63.7	36.3

MDSP: Morro do Diabo State Park. *N* = number of individuals per site

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Appendix S2.8

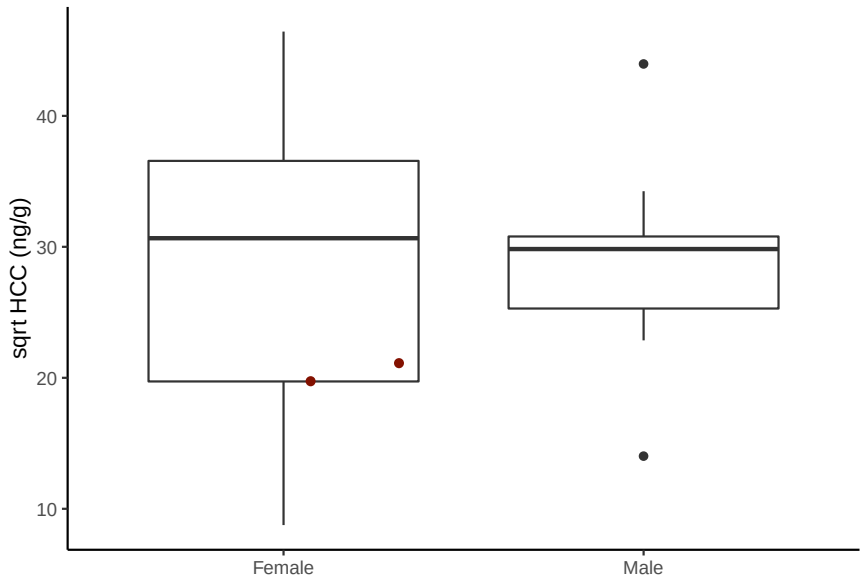

***Figure* 2.7:** Boxplot representing the median and distribution of hair cortisol concentrations (HCC) (square root transformed to achieve a normal distribution) in adult females ($N = 9$) and males ($N = 15$). No significant difference was observed ($t_{(19.6)} = 1.23$, $p = 0.233$). Pregnant females are highlighted in red and outliers are represented by black dots.

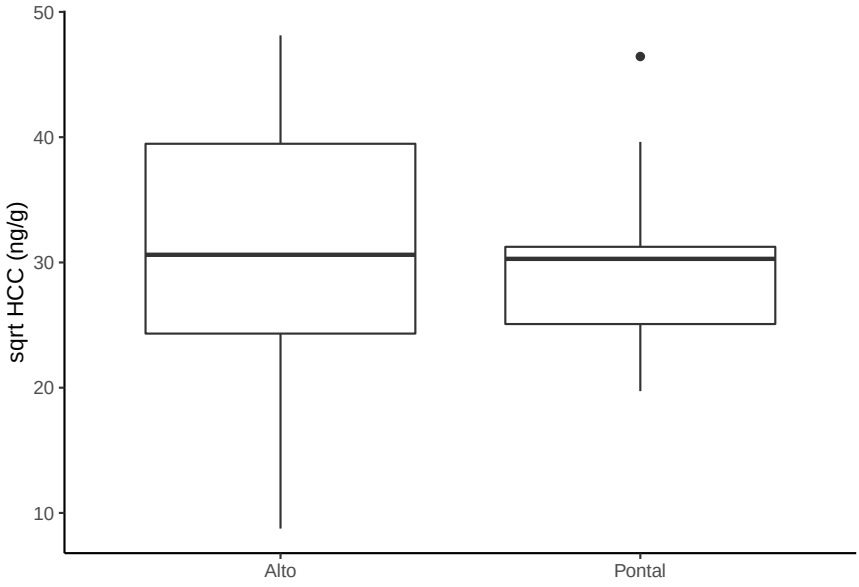

***Figure* 2.8:** Boxplot representing the median and distribution of hair cortisol concentrations (HCC) (square root transformed to achieve a normal distribution) in adults (N = 24) and juveniles (N = 7). No significant difference was observed ($t_{(8)}$ = -1.72, p = 0.141). Outliers are represented by black dots.

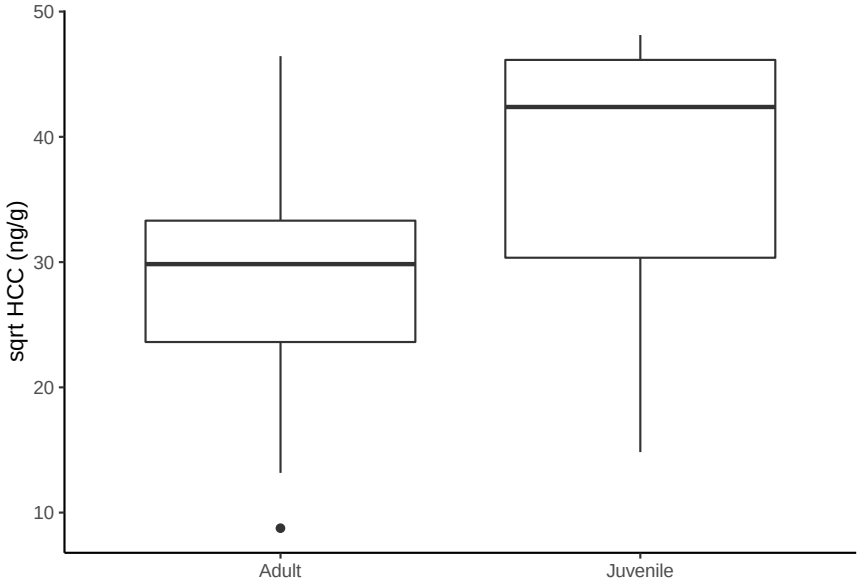

***Figure* 2.9:** Boxplot representing the median and distribution of hair cortisol concentrations (HCC) (square root transformed to achieve a normal distribution) between the two geographic regions: Alto Paranapanema (N = 19) and Pontal do Paranapanema (N = 12). No significant difference was observed ($t_{(29)}$ = 0.20, p = 0.843).

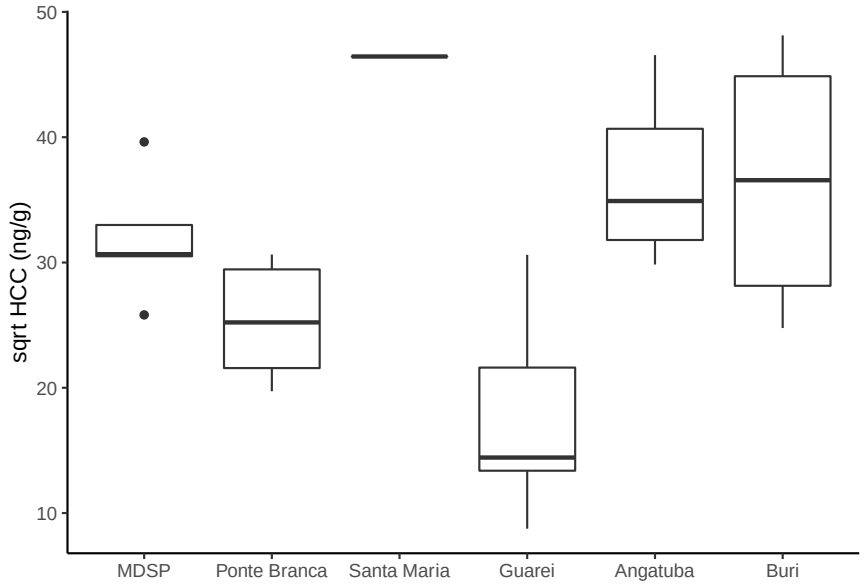

***Figure* 2.10:** Boxplot representing the median and distribution of hair cortisol concentrations (HCC) (square root transformed to achieve a normal distribution) in the study sites: Morro do Diabo State Park (MDSP; $N = 5$), Ponte Branca ($N = 6$), Santa Maria ($N = 1$), Guarei ($N = 6$), Angatuba ($N = 6$), Buri ($N = 7$). No significant difference was observed ($t_{(29)} = 0.20$, $p = 0.843$). Outliers are in black.

Appendix S2.9

***Table* 2.7.** Selection of linear mixed models, exploring the influence of habitat quality, including total tree basal area (TBA), Simpson's diversity index (D_s) size of the study sites (Size), arthropod biomass, and frugivory, on hair cortisol concentrations in six different study sites. The best model is shown in bold.

Model	***K***	**df**	**AIC**	**ΔAIC**	w_i
(1) 1	0	2	48	7.6	0.01
(2) D_s	1	3	49.6	9.2	0.01
(3) TBA + D_s	2	4	45.5	5.1	0.05
(4) TBA + D_s + Size	3	5	41.7	1.3	0.32
(5) TBA + D_s + Size + Frugivory	**4**	**6**	**40.4**	**0**	**0.61**

K is the number of parameters in the model, df: degrees of freedom, ΔAIC is the absolute difference in the AIC values between the best fit model and the model under consideration, w_i is the Akaike weight which provides a measure of relative support for each model. Arthropod biomass was removed from model selection due to issues of multicollinearity with TBA and fragment size (VIF > 5). Models including biomass instead of TBA and fragment size had no significant variables.

Appendix S2.10

***Table* 2.8.** Best fit linear mixed model testing the effect of habitat quality and diet, including total tree basal area (TBA), Simpson's diversity index (D_s), and fragment size, on hair cortisol concentrations levels of samples collected during the dry season ($N = 26$).

Effect	Estimate	SE	*t* ratio	*p*-value
D_s	-0.89	0.26	-3.39	0.002**
TBA	-0.63	0.28	-2.29	0.032*
Size	-0.48	0.19	-2.56	0.018*

Estimate of the coefficient and the corresponding standard error (SE), *t* statistic and *p*-value calculated using the T distribution.

Appendix S2.11

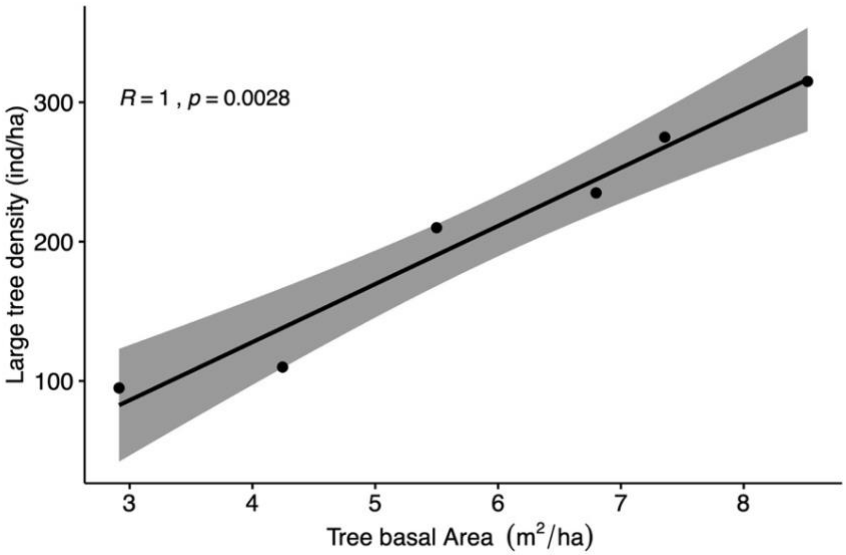

***Figure* 2.11:** Correlation between tree basal area (TBA) and the density of large trees (DBH > 20 cm) in each of the study sites. MDSP: Morro do Diabo State Park.

CHAPTER 3

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

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

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Key words: black lion tamarin, physiological stress, cortisol, faecal glucocorticoid metabolites, diet, energetics

Short running title: Physiological stress and behaviour in lion tamarins

Author contributions: O.K., F.B., and L.C. contributed to the design and implementation of the research, to the analysis and interpretation of the results, and to the writing of the manuscript. F.B., L.C., and P.P. supervised the research and led project administration and funding acquisition. O.K., R.A., and F.B., contributed to data collection. R.P. supervised hormonal analysis

ABSTRACT

Identifying the factors swaying physiological stress levels in wild animals can help depict how they cope with environmental and social stressors, highlighting their feeding ecology, behavioural plasticity, and adaptability. Here, we used non-invasive methods to explore the link between glucocorticoid levels and behaviour in an endangered neotropical primate facing habitat fragmentation pressure, the black lion tamarin (*Leontopithecus chrysopygus*). Guided by theoretical concepts of Romero's reactive scope model, which depicts expected glucocorticoid variations in an animal, we investigated monthly and day-to-day glucocorticoid variations independently to attempt to disentangle the complex nature of 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 behavioural data (over 95 days in total; 8.6 ± 3.9 days/month) and faecal samples (N = 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 glucocorticoid levels vary according to changes in activity budget associated with the energy balance of the groups (e.g., fruit consumption, movement, resting time). At a day-to-day level, while intergroup encounters led to increases in glucocorticoid concentrations, we found that changes in energy intake or energy expenditure did not trigger physiological 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 glucocorticoid variations over different time scales 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 precious conservation tool to evaluate how they cope in changing environments.

INTRODUCTION

Understanding how environmental and social factors influence animals' physiological states is a key question of behavioural endocrinology and ecology. In the wild, animals deploy behavioural and physiological responses to overcome both abiotic and biotic challenges (Wingfield, 2013; Kamilar & Beaudrot, 2018). To maintain homeostasis, vertebrates possess a broad physiological artillery, including stress responses (Wingfield et al., 1997; Romero, 2004). The perception of a stressor first activates the sympathetic nervous system leading to the secretion of catecholamines (i.e., epinephrine and norepinephrine) and subsequently stimulates the hypothalamic-pituitary-adrenocortical (HPA) axis which thenceforth triggers the secretion of glucocorticoids (GCs). GCs, often labelled *stress hormones*, are physiological markers that 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 behaviours, suppresses non-vital activities such as social interactions or reproduction, and also enhances the effects of catecholamines, which in turn induce the fight or flight response (Wingfield et al., 1997; Sapolsky et al., 2000; Wingfield, 2005). As described in Romero's reactive scope model, an increase in GC levels is not always synonymous with stress as GC levels are expected to vary within a normal circadian and seasonal range (Fig. 3.1), defined as predictive homeostasis (Romero et al., 2009). Throughout the day, GC levels follow a specific circadian rhythm governed by photoperiod and species' activity period. In diurnal species, GC concentrations are expected to increase in the morning, characterizing the cortisol awakening response (Fries et al., 2009)(Fig. 3.1). 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 (Wingfield, 2005) (Fig. 3.1). This acute elevation of GC levels is rapidly autoregulated through a 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 and circannual rhythms. Together, predictive homeostasis and reactive homeostasis constitute the normal reactive scope of an organism (Romero et al., 2009) (Fig. 3.1). Impeded by the difficulty of deconstructing the complex mechanisms underlying physiological stress

in an uncontrolled and varying environment, few studies have managed to explore simultaneously the different elements of the reactive scope model in a wild species.

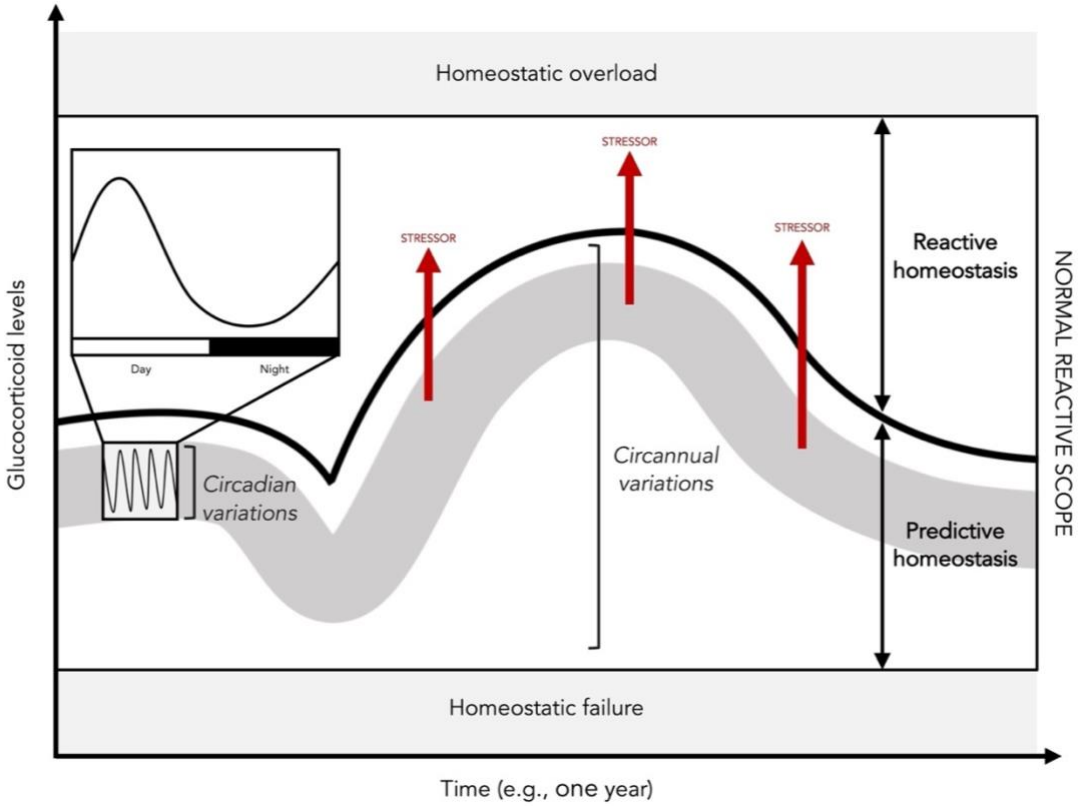

***Figure* 3.1:** Graphical model depicting theoretical glucocorticoid (GC) variations in an individual over a year. Within the predictive homeostasis range, GC levels vary depending on the time of day (i.e., circadian variation) and season (i.e., circannual variations). Throughout the year, an unpredictable 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. Homeostasis failure occurs when GC levels are too low to maintain homeostasis. In opposition, homeostasis 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.

Over the past two decades, the development of non-invasive methods to monitor physiological markers has considerably increased the number of studies in the wild (Sheriff et al., 2011; Palme, 2019). 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 faeces via the gut (Möstl & Palme, 2002). Faecal 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 faeces 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 faeces via the liver (Palme et al., 1996). Second, metabolite levels measured in faeces 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 faeces and to determine the time lag between variations in circulating GCs and detection in the faeces, it is crucial to perform a physiological or biological validation (Palme, 2019). Defining this time lag provides essential knowledge on a species biology, allowing researchers to calibrate and align the behavioural 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 (Palme, 2019; Kaisin et al., 2021).

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 hand. Across a wide diversity of primate species, scientists have explored changes in adrenocortical activity associated with hierarchy, social behaviour, 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*; Muller & Wrangham 2004; Emery Thompson et al. 2010), Sykes' monkeys (*Cercopithecus mitis*; Foerster & Monfort 2010), and red colobus monkeys (Chapman et al., 2007) discovered that poor diet quality or low availability of favoured food resources yielded higher GC levels as well. Conversely, a study found that the amount of energy 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 together with

behaviour helps to depict how wild primates cope with environmental and social stressors, highlighting their degree of adaptability, behavioural plasticity, as well as the potential consequences on their health and fitness.

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, we investigated the link between physiological stress levels and changing behavioural profiles over time in a small frugivorous and faunivorous callitrichid endemic to the State of São Paulo, the black lion tamarin. By gathering behavioural data and faecal samples, we explored whether daily and monthly variations in activity budget and weather conditions influenced GC levels. Looking at GC variations over two different timescales, days and months, enabled us to identify the factors influencing both reactive and predictive homeostasis, respectively, in lion tamarins. In parallel, predictable circadian GC fluctuations, such as the cortisol awakening response, linked to the lion tamarin's normal biological rhythm, were expected and taken into account in the analyses. Thus, we first conducted a series of preliminary analyses on circadian hourly variations in GCs, as well as exploring sex and age-class differences in order to appropriately control for these factors in the subsequent models. Then, we analysed the influence of (a) monthly and (b) daily behaviour and climate on GC levels. In the daily models, we accounted for predictable variations between months in order solely to extract significant day-to-day changes in adrenocortical activity.

(a) Across the year, we expect GC levels to increase during the dry season. First, considering the energy regulating role of GCs and previous studies on primates, we anticipate fruit consumption (i.e., a proxy of energy intake) to decrease during the dry season that is characterized by a lower fruit availability (Staggemeier et al., 2017), causing nutritional stress mirrored by higher GC levels. Depending on resource availability and distribution, we also expect the balance between movement, foraging, and resting time to vary between months and influence the lion tamarins' energy expenditure. Accordingly, we expect 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 predict 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, we expect that extra energy may be necessary to maintain body temperature, resulting in an increase in GC levels.

(b) Across the day, above and beyond seasonal monthly variations, we anticipate GC levels to increase with metabolically challenging events associated with energy demand (e.g., foraging and travelling) 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 behaviour caused by unpredictable weather.

Combining behavioural 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.

METHODS

This study was conducted completely non-invasively 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). The data that support the findings of this study are available from the corresponding author upon reasonable request.

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; Fig. 3.2). MDSP is the largest Atlantic Forest remnant (33,845 ha) 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 250-450 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 (100 ha) is located in the Alto Paranapanema region and has a mean elevation of 680 m. Surrounded by pastures and sugar

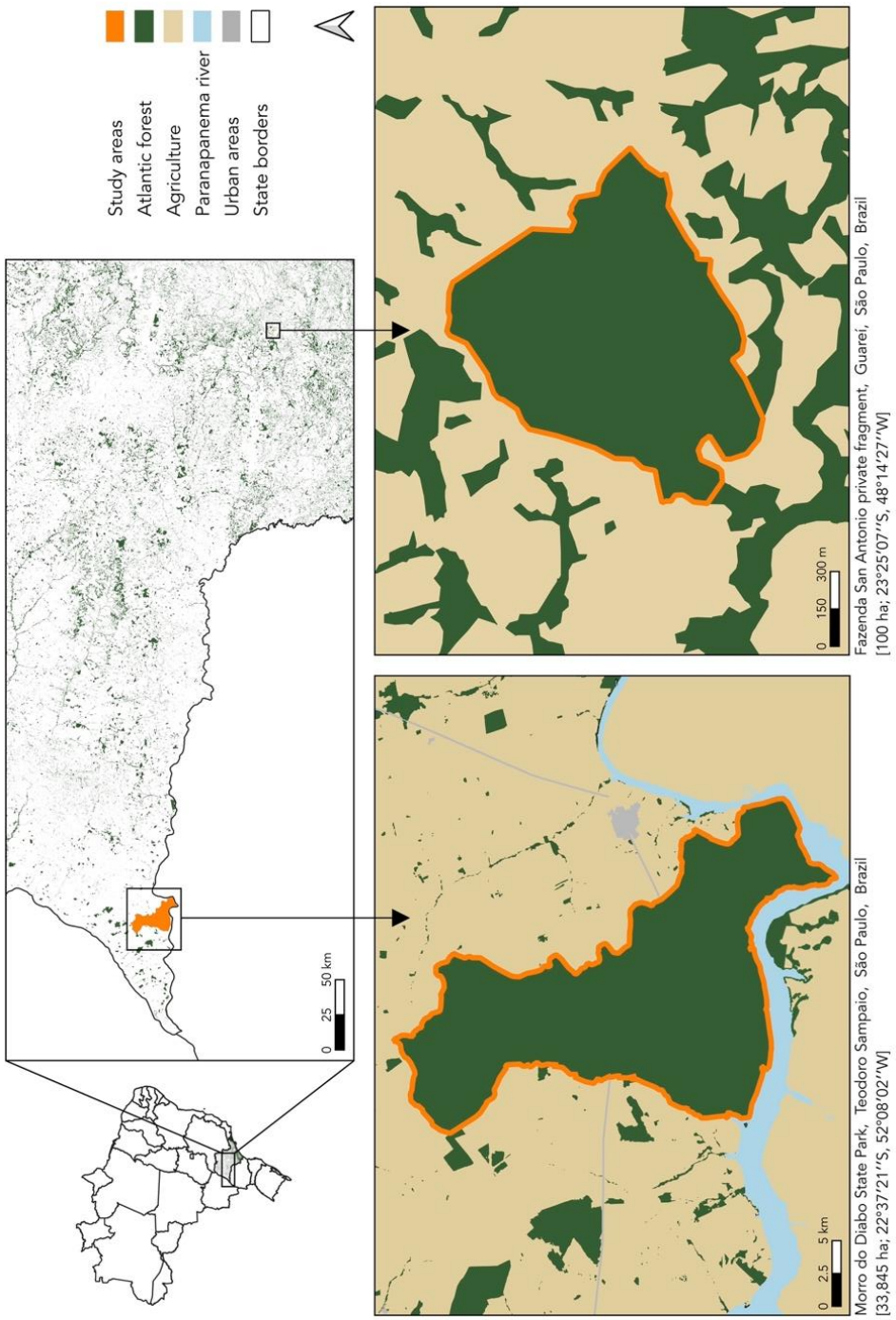

***Figure* 3.2:** Location of two study sites, the Morro do Diabo State Park (MDSP) (left) and Guareí (right), within the State of São Paulo, including the peripherical land use and the home range of both groups estimated using the Time Local Convex Hull method.

cane and eucalyptus plantations, it is part of a highly fragmented landscape. The mean temperature and total rainfall during the study period were of 21.4°C and 912 mm, respectively (Agritempo 2022; TRMM 892). All temperature and rainfall data used in this study was 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 dry (April-September) and rainy (October-March) seasons in southeast Brazil. When we started our observations, the MDSP group comprised eight individuals: 4 adult males, 2 adult females, and 2 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. In Guareí, the group initially comprised five individuals, two adult males and three adult females. In February 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. Overall, the two groups showed noteworthy differences in activity budget (see *Supporting Information*, Appendix S3.1), 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.

Behavioural data and faecal sample collection

In both sites, we followed the groups throughout their activity period and gathered behavioural 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 behaviour of every visible individual at scan time. We gathered behavioural 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 season (Table 3.1; a monthly distribution of full days and faecal sample collection is available as *Supporting Information*, Appendix S3.2). Behavioural observations were categorized into mutually exclusive behaviours: feeding (i.e., chewing, ingesting, manipulating food item, foraging for fruits on a feeding tree), 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), inactive (i.e., static and not displaying any noticeable behaviour), and other (i.e., marking territory, long calls, fur-rubbing, and social behaviours). For feeding behaviours, we also identified the food category of the items consumed (e.g., fruit, invertebrate, vertebrate, gum) as well as the species when possible. 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 difficult to identify individuals during scan observations. Therefore, we estimated group activity budgets by calculating a percent of daily and monthly scan observations for each behavioural category. All analyses were performed using behavioural data gathered during full days.

***Table* 3.1.** Faecal sample and behavioural data sampling effort in the Morro do Diabo State Park (MDSP) and Guareí

Site	Full days		Scans	Hours	Individuals/scan	Faecal samples		Samples/day
MDSP	Rainy	25	3232	269	3.6 ± 1.5	Rainy	127	5 ± 3.3
	Dry	21				Dry	75	
Guareí	Rainy	19	4652	387	2.9 ± 1.2	Rainy	51	6.2 ± 3.7
	Dry	30				Dry	215	

While following the lion tamarins, we also collected faecal samples in 10ml 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 hours of the collection time. For every sample, we noted the date, time, group, and the sex and age of the individual when possible. As for behavioural data collection, it was not possible to systematically identify individuals when gathering faecal samples. A total of 468 faecal samples were collected, 202 in MDSP and 266 in Guareí (Table 3.1).

Steroid analysis

Prior to extraction, faecal 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 sub-sample of 0.5 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 (14000 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 re-suspended 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 & 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 20-25h. Sensitivity of the EIA used in this study was 0.33 pg/well. Inter-assay coefficients of variation (CVs), assessed by running high and low-level quality controls in each assay, were 9.3% (high) and 9.5% (low). All samples were analysed in duplicates. The mean intra-assay CV for control samples was 1.92 ± 1.89%. All samples with intra-assay CV > 10% were re-analysed to obtain a satisfying CV.

Statistical analysis

Given the inherent behavioural differences between the two groups (i.e., activity budget, diet, and daylength) we explored GC variations for both sites independently, allowing us to make well-founded group and site-specific interpretations and discuss potential deviations in the results. 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. Prior to model selection, all variables were standardized. 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). We tested for multicollinearity using the variance inflation factor (VIF) for each model. All variables included in the model had a VIF < 5 (Menard, 2002; James et al., 2013). To determine the estimates and *p*-values for every variable, we ran 1000 bootstrap iterations of the best models. Using this random resampling method, we overcame the fact that the fGM samples were not independent as faeces were not systematically associated with an individual (Fieberg et al., 2020).

Preliminary analyses

Prior to constructing our linear mixed models for both the daily and monthly analyses, 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.

Sex and age-class differences

We started by running a Mann Whitney *U* test to compare the mean fGM levels between the two study sites and found no significant difference (U= 28504, p = 0.26). Since no significant differences were observed between mean fGM levels in Guareí (N = 266) and MDSP (N = 202), we pooled individuals from both sites to explore the effect of the sex and age-class on fGM levels. 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. Using adult individuals only, we compared mean fGM levels between males (N = 19) and females (N = 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 = 20) and adult (N = 76) fGM levels (U= 753, p = 0.95). Boxplots for these analyses are available as *Supporting Information* (Appendix S3.3). Since fGM concentrations did not differ significantly between sex and age-class we pooled all samples in subsequent analyses.

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.*, 20-25h; Bertoli et al. 2019), we considered the fGM concentrations of a faecal sample to represent GC levels at 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 S3.4). To test whether black lion tamarins showed a similar circadian fGM profile in both sites, we tested if their hourly fGM values co-varied using a Spearman correlation. We compared hourly mean fGM levels in both sites using a Kruskal-

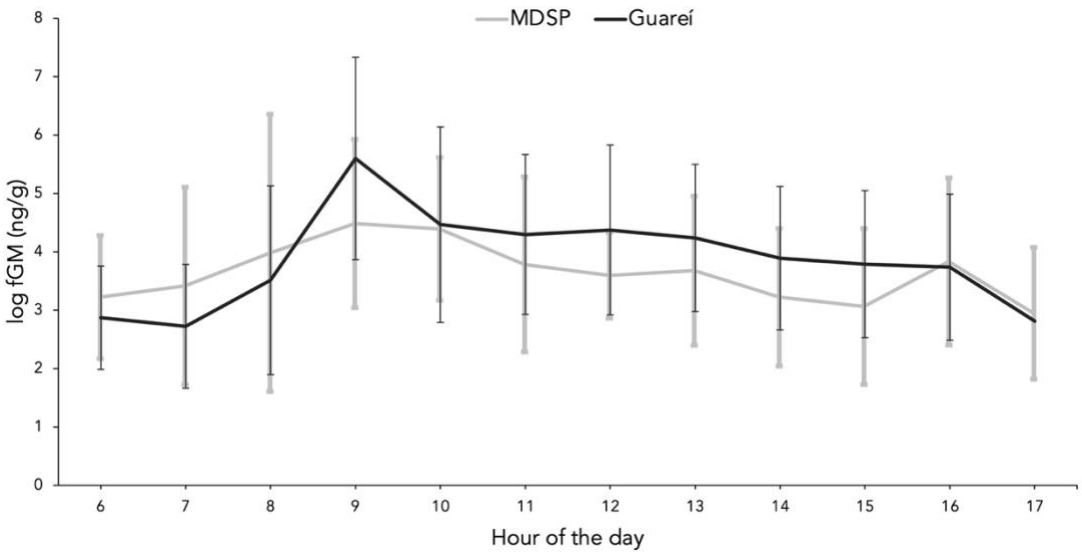

***Figure* 3.3**: Circadian profile of mean hourly faecal glucocorticoid metabolites (fGM) and their corresponding standard deviations in the Morro do Diabo State Park (MDSP) and Guareí fragment black lion tamarin groups.

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_s = 0.58, p = 0.05; Fig. 3.3). Although MDSP's circadian profile was flatter, mean hourly fGM levels showed an increase during the first hours of the day and a peak at 9 am, followed by a slight decrease and stabilization of fGM levels during the afternoon. While hourly mean fGM levels varied significantly in Guareí (X^2 = 66.9, df = 11, p < 0.01), no significant differences were observed in MDSP (X^2 = 18.9, df = 13, p = 0.13). In both sites, the highest mean hourly levels were recorded at 9 am. In Guareí, the 9 am peak was significantly higher than in the early morning hours (6h: p < 0.01; 7h: p < 0.01; 8h: p < 0.01). Revealing circadian variations in fGM concentrations enabled us to account for the potential bias linked to the time of defecation in our subsequent models.

Influence of monthly behaviour 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. We associated every faecal sample to the corresponding monthly climatic and behavioural data (see *Supporting Information* for monthly full days and faecal samples; Appendix S3.2). Although we initially ran a global LMMs, due to collinearity between climatic and behavioural variables 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 analyse the link between fGM levels and monthly behavioural 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 categorial behaviours likely influencing energy expenditure and henceforth GC levels over the duration of a month. For both sets of models, we added a random-effect on hour of defecation to take into account the determined circadian rhythm variations (see *2.5.1. Preliminary analyses*). In MDSP, monthly movement was significantly negatively correlated with resting ($r_{(200)}$ = -31.6, p < 0.01), yielding a VIF > 5. Therefore, we constructed our models using movement only.

Influence of daily behaviour and climate on fGM levels

To understand the effect of daily behaviour 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 behavioural categories and weather (i.e., mean, maximum and minimum daily temperature, and rainfall) as explanatory variables. Since black lion tamarins live in cohesive groups and no significant differences in fGM levels between sex and age-classes were observed, we calculated the percentage of scan observations of each behaviour

and determined daily group activity budgets. Based on the latency period defined by the physiological validation, we considered the fGM concentrations to represent GC levels at the same time the previous day. Hence, we attributed to each faecal sample the activity budget percentages for each behaviour as well as the total rainfall and mean temperature of the previous day. For the daily models we only included behaviours that, when acute, may potentially influence energy expenditure and intake (i.e., resting, foraging, movement, fruit consumption) as well as sporadic and less frequent behaviours (i.e., insect consumption, intergroup encounters, grooming) that may influence transient GC levels. We only included faecal samples with full day continuous behavioural data the previous day, 80 in MDSP and 225 in Guareí. To account for the anticipated influence of circadian and monthly variations, the hour of defecation and the month were included as random effects in the models.

RESULTS

Influence of monthly behaviour and climate on fGM levels

Regarding climatic variables, for both sites, the best models only included mean monthly temperature (Fig. 3.4-3.5). In Guareí, temperature had a significant positive effect on fGM levels (Estimate = 0.15, SE = 0.04, df = 263.8, t = 3.95, $p < 0.01$), while the opposite was found in MDSP (Estimate = -0.33, SE = 0.05, df = 192.6, t = -7.1, $p < 0.001$). Exploring behavioural variables revealed that in MDSP the best model included monthly movement and fruit consumption (Table 3.2). In Guareí, the best LMM also included monthly fruit consumption and movement (Table 3.2). At both sites, monthly fruit consumption had a positive significant effect, implying that fGM levels were higher in months where the percentage of time spent on feeding on fruit increased (Fig. 3.4-3.5). In MDSP, fGM levels increased when the group spent more time moving, while in Guareí they decreased when movement increased. Full models and AIC model selections are detailed in the *Supporting Information* (Appendix 3.5).

***Table* 3.2.** Best fit linear mixed model of the effect of monthly behaviour, including fruit consumption (FC) and movement (M), on faecal glucocorticoid metabolite levels in the Morro do Diabo State Park (MDSP) and Guareí

Site	Effect	Estimate	SE	df	*t* value	*p* value
MDSP	M	0.737	0.209	196.2	3.529	$< 0.001^{***}$
	FC	0.79	0.224	186.9	3.531	$< 0.001^{***}$
Guareí	M	-0.702	0.191	254.5	3.682	$< 0.001^{***}$
	FC	1.141	0.205	260	5.554	$< 0.001^{***}$

Estimate of the coefficients and the corresponding standard error (SE), df: degrees of freedom, t statistic and p value calculated using the T distribution.

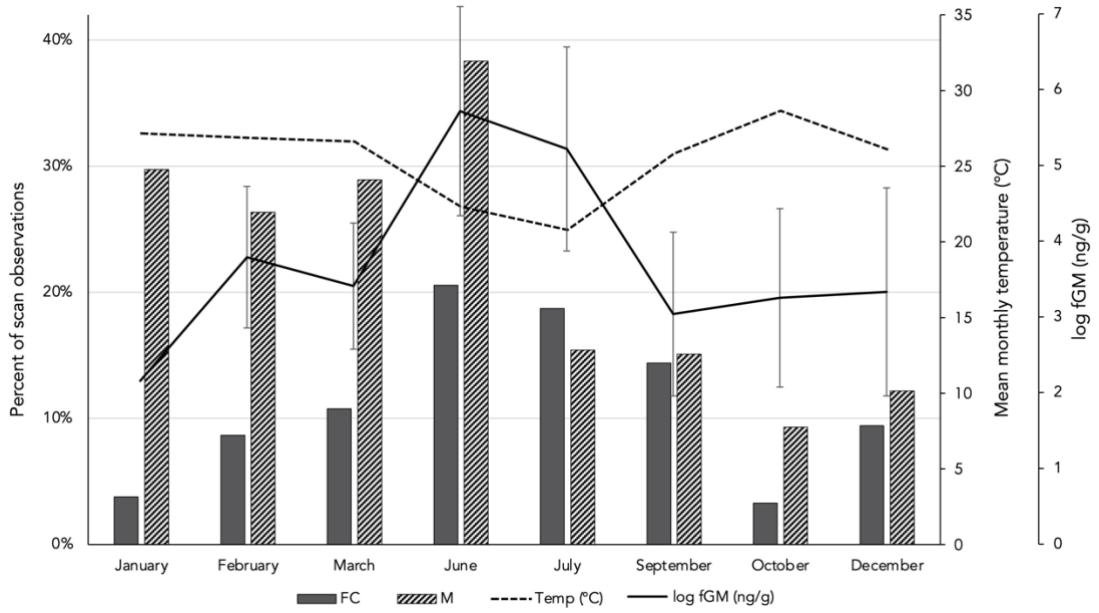

***Figure* 3.4:** Mean monthly faecal glucocorticoid metabolite (fGM; log-transformed to achieve a normal distribution) levels and their standard deviation in the Morro do Diabo State Park (MDSP) in relation to mean temperatures and significant activity budget categories: fruit consumption (FC) and movement (M).

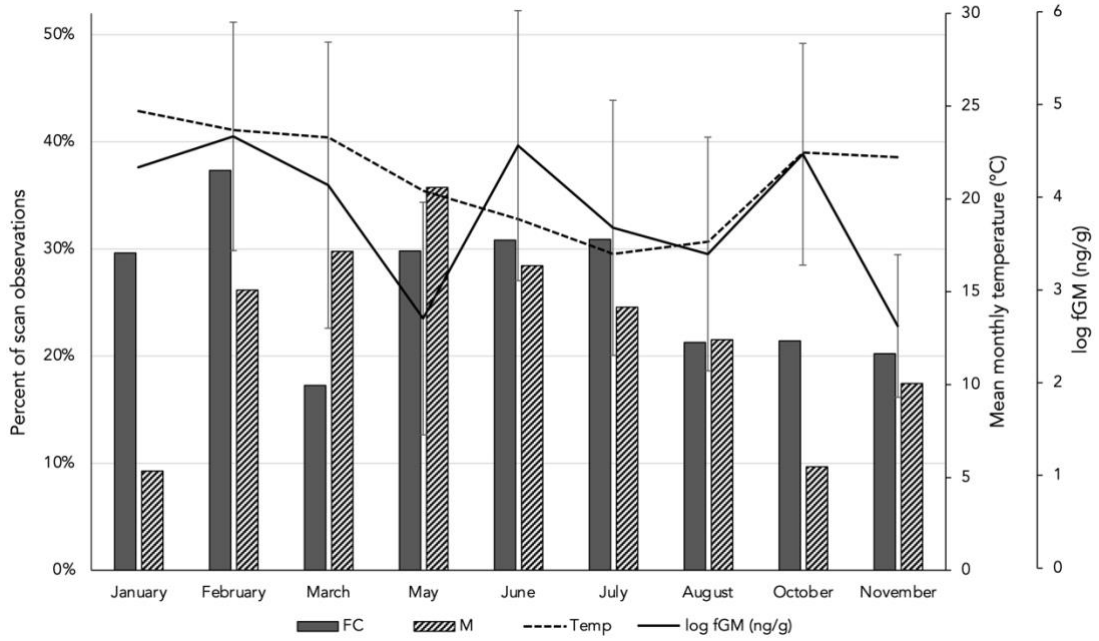

***Figure* 3.5:** Mean monthly faecal glucocorticoid metabolite (fGM; log-transformed to achieve a normal distribution) levels their standard deviation in Guareí in relation to mean temperatures and significant activity budget categories: fruit consumption (FC) and movement (M).

Since monthly fruit consumption had a significant positive effect on fGM levels at both study sites, we explored the influence of variation in dietary content on the time spent feeding and on monthly fGM levels. Although some 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 S3.6). We found that mean monthly fGM levels in MDSP were positively correlated with the time spent feeding on *Tapirira guianensis*, Anacardiaceae ($r_{(6)}$ = 0.744, p = 0.03) (see *Supporting Information*, Appendix 3.6). In Guareí, fGM levels were not correlated with the consumption of any particular fruit species.

Influence of daily behaviour and climate on fGM levels

In MDSP, none of the variations in daily behaviours, temperature, or rainfall had a significant effect on fGM levels. However, in Guareí, the best model (REML = 755.9, AIC = 765.9, w_i = 0.657) included the time spent involved in intergroup encounters, which was linked to higher fGM levels (Table 3.3). The full model and AIC model selection are detailed in the *Supporting Information* (Appendix S3.7).

***Table* 3.3:** Best fit linear mixed model of the effect of daily behaviour, including intergroup encounters (ENC), on mean daily faecal glucocorticoid metabolite levels in Guareí

Site	Effect	Estimate	Std error	df	*t* ratio	*p*-value
Guareí	ENC	0.593	0.196	186.9	3.019	< 0.01**

Estimate of the coefficient and the corresponding standard error (SE), df: degrees of freedom, *t* statistic and *p* value calculated using the T distribution.

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 (Sapolsky et al., 2000; Romero et al., 2009), we looked at black lion tamarin adrenocortical activity at three different timescales (i.e., including circadian variations) to attempt to uncover the influence of behaviour and climate on predictive and reactive homeostasis, respectively. Our results indicate that, depending on the chosen timeframe, months or days, different factors influence GC levels. At a monthly scale, GC levels are influenced by movement and fruit consumption in different ways according to 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 GC levels, but only in Guareí.

Analyzing GC levels throughout the day, we found that black lion tamarins in both sites experience daily GC fluctuations associated with their circadian rhythm, part of the predictive homeostasis range, exemplified by a coinciding cortisol awakening response. In line with current research (Palme, 2019), this finding supports and validates the use of non-invasive 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 faeces. 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 in order to put the extracted concentrations into context.

By identifying behavioural 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, GC levels increased during colder months. Unexpectedly, the opposite was observed in Guareí, with higher GC 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 (McFarland et al., 2014; Guo et al., 2018; Chowdhury et al., 2021). On the contrary, high humidity and extreme hot temperature may also cause heat stress, as observed in baboons inhabiting a semi-arid savannah (Gesquiere et al., 2008), 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 GC levels, we believe that the effect 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 distinguish the influence of fruit availability from other seasonal variables such as weather.

In both sites, an increase in fruit consumption led to higher GC 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., 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 non-preferred food was greater (Foerster et al., 2012). Breaking down the monthly diet of our study species, we discovered that the consumption of non-preferred fruits, with potentially lower nutritional value, may be behind the positive relation between GC levels and fruit consumption. In MDSP, *Tapirira 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 GC levels. In Guareí, fruit consumption was also positively associated with monthly GC concentrations although it was not possible to single-out a specific fruit species potentially responsible for these unintended 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 behaviour 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 S3.6). 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 in order 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., amount of calories spent by the body). Our models based on monthly GC variations showed that, for the MDSP group, time spent moving was positively related to GC levels. Since resting was negatively correlated to movement in this group's monthly activity budget, resting time was associated with a decrease in GC 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 S3.1). Our results revealed that, in Guareí, GC levels were negatively influenced by the time in movement. Although the inverse was expected, the shorter resting time may influence the energetics of the group, who 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 m^2 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 MDSP, the 100 ha fragment in Guareí may not provide predictable resource availability and distribution for the lion tamarins, which is portrayed by the fact that monthly fruit consumption and GC levels do not follow a clear seasonal trend.

To identify the short-term behavioural or climatic factors triggering an acute stress response (i.e., reactive homeostasis) in black lion tamarins, we changed timeframe and compared daily GC 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 GCs in MDSP, intergroup encounters sparked a short-term increase in GC 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 behaviours and vocalizations (Cooper et al., 2004; Koch et al., 2016; Cooksey et al., 2020; Van Belle et al., 2021). 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 (Ross et al., 2004; Huck et al., 2005; Cristóbal-azkarate et al., 2007; Van Belle et al., 2009). 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 *versus* 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.

In many primate species, due to their long gut passage times, only severe GC peaks are detected in faeces, dampening other transient variations (Heistermann et al., 2006; Heistermann, 2010; Behringer & Deschner, 2017; Christensen et al., 2022). However, in this study, because black lion tamarins are small-bodied primates with high defecation rates, we were able to identify and account for circadian patterns in subsequent analyses. At a monthly level, our results reveal that lion tamarin GC concentrations vary according to changes in activity budget associated with the energy balance of the groups (e.g., fruit consumption, movement). However, day-to-day changes in major behavioural categories do 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. The novel twofold approach adopted in this study helped us understand how behavioural and climatic factors influence both the predictive and reactive homeostasis separately. As demonstrated here, the complex mechanisms underlying physiological responses are often species and site-specific, stressing the importance of gathering simultaneous behavioural data to unravel and best explain the sources of GC variations. Future studies should therefore attempt to adopt a similar extensive approach, to assess the ecological stress physiology in other wild species and better understand their response in changing and challenging environments. Such research may prove to be pivotal to guide species management and conservation efforts.

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SUPPORTING INFORMATION

Appendix S3.1

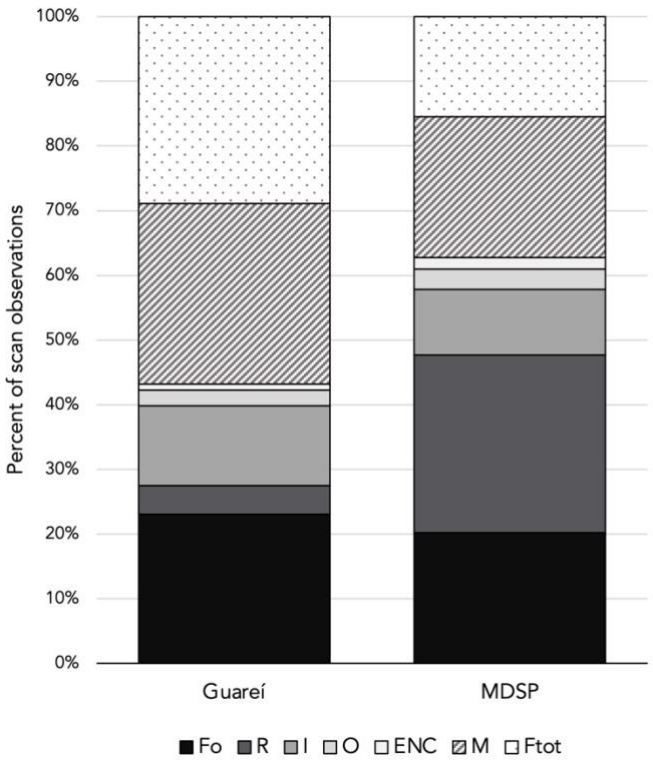

***Figure* 3.6:** Overall activity budget, based on all full day scan observations, in Guareí and the Morro do Diabo state Park (MDSP) including foraging (Fo; i.e., active search for animal prey), resting (R), inactive (I), other (O), intergroup encounters (ENC), movement (M), and total feeding (Ftot; i.e., consumption of fruits, gum, invertebrates, vertebrates).

Appendix S3.2

***Table* 3.4.** Temporal distribution of faecal sample and behavioural data in the Morro do Diabo State Park (MDSP) and Guareí

Site		2019								2020		
		May	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**
MDSP	Full days	-	6	6	-	9	8	-	3	1	7	6
	Faecal samples	-	10	19	-	46	12	-	17	1	54	43
Guareí	Full days	8	5	9	8	-	4	5	-	1	6	3
	Faecal samples	28	46	55	86	-	4	8	-	1	34	4

Appendix S3.3

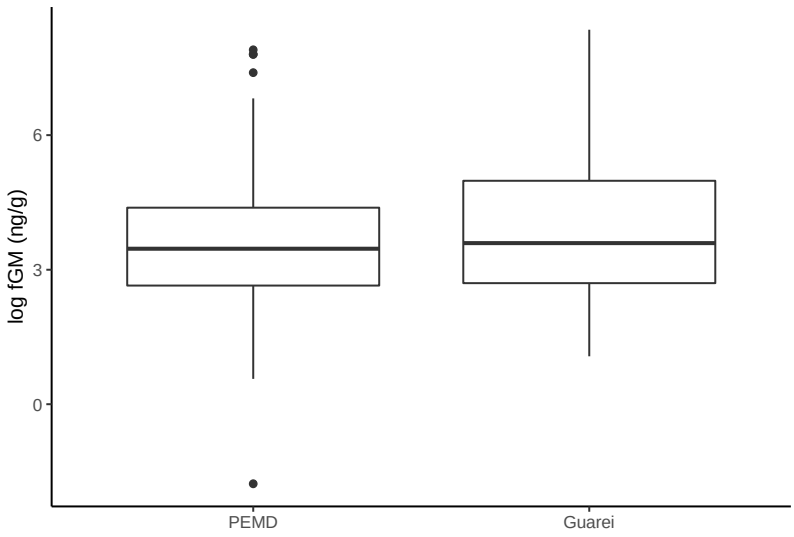

***Figure* 3.7:** Boxplot representing the distribution of faecal glucocorticoid metabolite (fGM) levels (log-transformed to achieve a normal distribution) in Guareí (N = 266) and the Morro do Diabo State Park (MDSP; N = 202). No significant difference was observed.

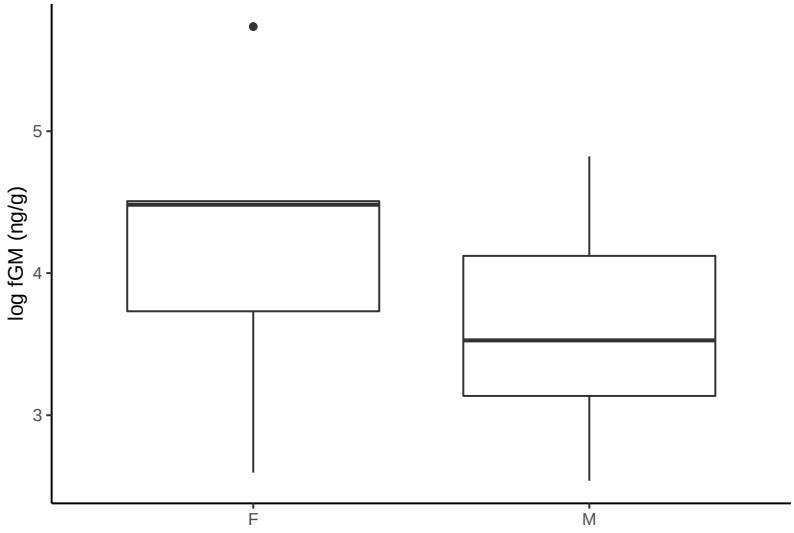

***Figure* 3.8:** Boxplot representing the distribution of faecal glucocorticoid metabolite (fGM) levels (log-transformed to achieve a normal distribution) in female (F; $N = 5$) and males (M; $N = 19$). No significant difference was observed.

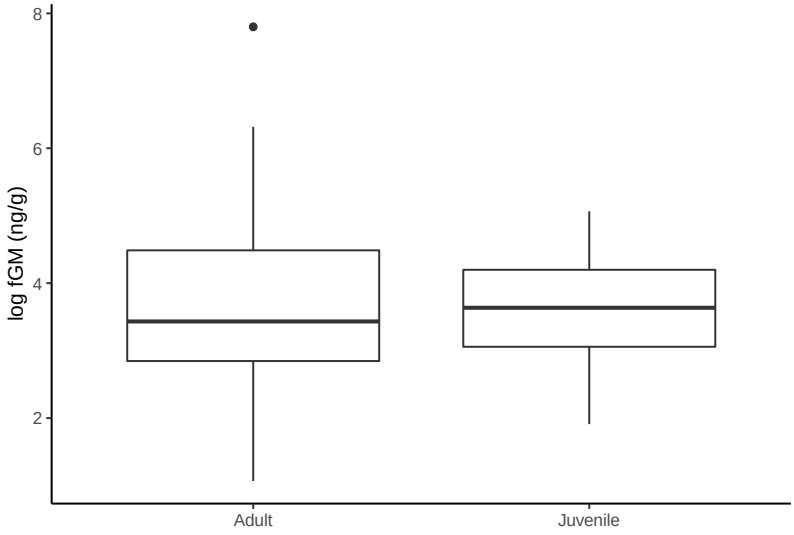

***Figure* 3.9:** Boxplot representing the distribution of faecal glucocorticoid metabolite (fGM) levels (log-transformed to achieve a normal distribution) in Adult ($N = 76$) and juveniles ($N = 20$). No significant difference was observed.

Appendix S3.4

***Table* 3.5.** Number of faecal samples collected at every hour in Morro do Diabo State Park (MDSP) and Guareí

Site	6:00	7:00	8:00	9:00	10:00	11:00	12:00	13:00	14:00	15:00	16:00	17:00
MDSP	29	41	16	6	18	12	13	23	20	11	6	5
Guareí	16	52	30	18	30	22	25	27	16	19	10	1

Appendix S3.5

***Table* 3.6.** Selection of linear mixed models, exploring the influence of monthly behaviour, including fruit consumption (FC), foraging (Fo; i.e., active search for animal prey), resting (R), movement (M), on mean monthly faecal glucocorticoid metabolite levels in the Morro do Diabo State Park (MDSP) and Guareí, with hour of defecation as a random effect. R was not included in the MDSP model because it was correlated to M. The best model is shown in bold.

Site	Model	*K*	df	AIC	ΔAIC	w_i
MDSP	(1) FC + (1|Hour)	2	4	706	1.7	0.31
	(2) FC + M + (1|Hour)	**3**	**5**	**704.3**	**0**	**0.69**
	(3) FC + M + Fo + (1|Hour)	4	6	715.1	10.8	0
Guareí	(1) FC + (1|Hour)	2	4	923.5	0.5	0.39
	(2) FC + M + (1|Hour)	**3**	**5**	**923.0**	**0**	**0.49**
	(3) FC + M + R + (1|Hour)	4	6	932.7	9.7	0
	(4) FC + M + R + Fo + (1|Hour)	5	7	925.8	2.8	0.12

K is the number of parameters in the model, df: degrees of freedom, ΔAIC is the absolute difference in the AIC values between the best fit model and the model under consideration, w_i is the Akaike weight which provides a measure of relative support for each model.

Appendix S3.6

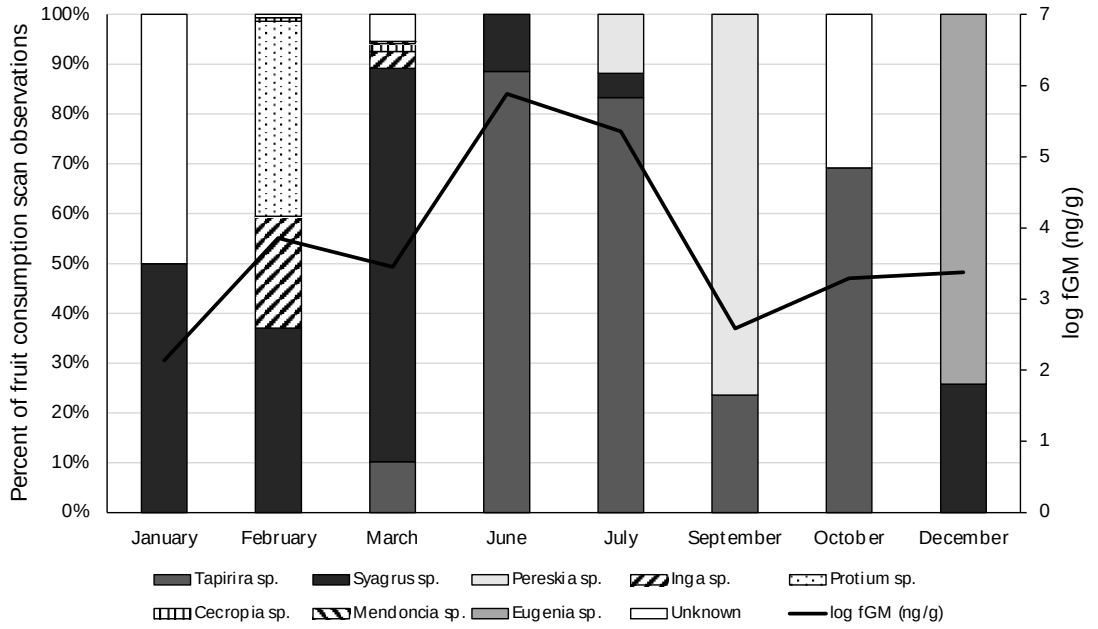

***Figure* 3.10:** Mean monthly faecal glucocorticoid metabolite (fGM) levels (log-transformed) in MDSP in relation to monthly fruit consumption per species.

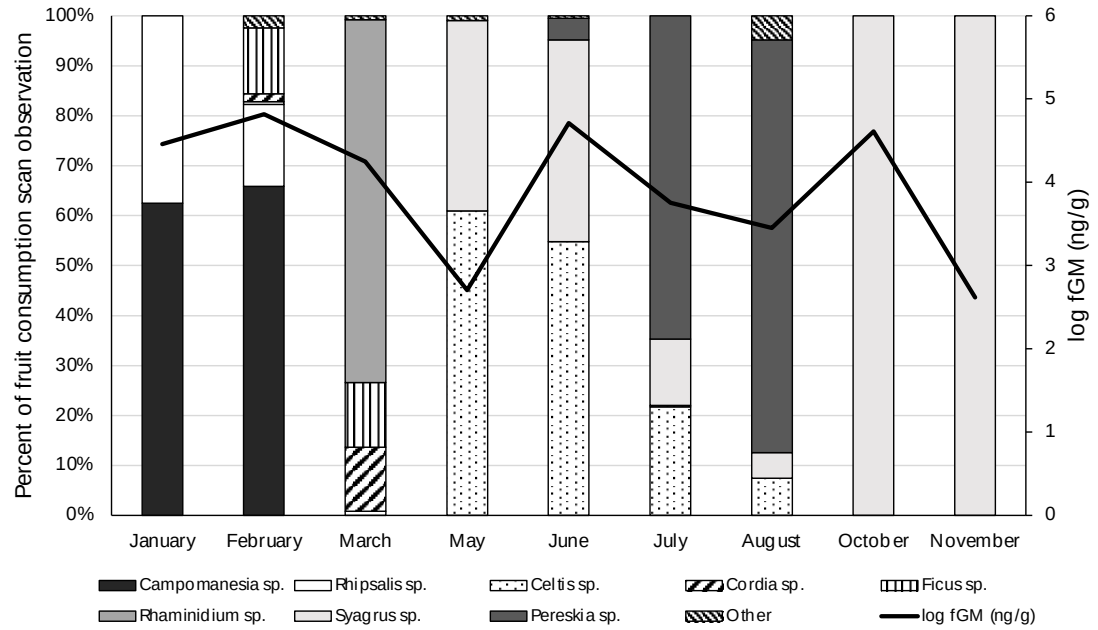

***Figure* 3.11:** Mean monthly faecal glucocorticoid metabolite (fGM) levels (log-transformed) in Guareí in relation to monthly fruit consumption per species.

Appendix S3.7

***Table* 3.7.** Selection of linear mixed models, exploring the influence of daily behaviour, including fruit consumption (FC), foraging (Fo; i.e., active search for animal prey), resting (R), movement (M), intergroup encounters (ENC), invertebrate consumption (IC) and grooming (Gr), on mean daily faecal glucocorticoid metabolite levels in Guareí, with hour of defecation and month as random effects. The best model is shown in bold.

Site	Model	*K*	df	AIC	ΔAIC	w_i
Guareí	**(1) ENC + (1|Hour) + (1|Month)**	**3**	**5**	**771**	**0**	**0.66**
	(2) ENC + Fo + (1|Hour) + (1|Month)	4	6	772.3	1.3	0.34
	…	...	...	…	…	…
	(4) ENC + Fo + FC + M + R + IC + Gr + Rainfall + T_{mean} + (1|Hour) + (1|Month)	9	11	785.5	14.5	0

K is the number of parameters in the model, df: degrees of freedom, ΔAIC is the absolute difference in the AIC values between the best fit model and the model under consideration, w_i is the Akaike weight which provides a measure of relative support for each model.

CONCLUSIONS & PERSPECTIVES

1. Main findings and implications

Fuelled by exponential human population growth and the unsustainable exploitation of Earth's resources, forests continue to disappear at alarming rates and human pressure on ecosystems keeps intensifying (Venter et al., 2016; Díaz et al., 2019; *Global Forest Watch*, 2022). In this chaos, understanding how animals cope and react to anthropogenic disturbances is paramount for their conservation (Sih et al., 2016). Among these threats, the loss of habitat is by far the most devastating for species as it brings on a series of direct and indirect synergistic impacts (Maxwell et al., 2016; Kalbitzer & Chapman, 2018). Primates occur across tropical and subtropical regions, including major hotspots for biodiversity such as the West African Forests, Madagascar, Southeast Asia, and the Brazilian Atlantic Forest (Myers et al., 2000; IUCN, 2022). Within these ecosystems, primates play key roles as forest gardeners and ecological indicators (Estrada et al., 2017). Studying the effect of anthropogenic disturbances on primates will help shed light on their behavioural flexibility and estimate their resilience to changing environments. This thesis set out to explore the impact of disturbances on primates using glucocorticoids (GCs), physiological biomarkers that provide precious insight on energetics and that can help estimate how primates respond to environmental challenges (Emery Thompson, 2017; Beehner & Bergman, 2017). To obtain a thorough understanding of the physiological stress in wild primates, we explored GC variations at a global scale before focusing on a species we know little about, the black lion tamarin (*Leontopithecus chrysopygus*).

To provide an exclusive overview of physiological stress levels in primate populations subject to diverse anthropogenic disturbances (i.e., habitat loss, ongoing logging, hunting, habitat degradation, tourism, and other human activities), we performed a global meta-analysis, including 26 articles on 24 different primate species (**Chapter 1**). This statistical analysis revealed that primates inhabiting disturbed areas exhibited higher GC levels than their conspecifics in less disturbed sites. Habitat loss and hunting were identified as the main threats, triggering physiological responses in primate populations. A pantropical meta-analysis, assessing biodiversity metrics in disturbed versus undisturbed areas, also found that habitat loss due to agriculture was the main cause of decline in primate diversity, species richness, and abundance (de Almeida-Rocha et al., 2017). To cope with disturbances, primates activate their HPA axis to trigger stress responses that are adaptive over the short-term but increasingly unsustainable over time (Wingfield, 2005; Romero et al., 2009). While the maladaptive nature of the stress response is still debated (Boonstra, 2013), studies suggest that sustained elevated GC levels in response to intense and unprecedented anthropogenic disturbances might be negatively impacting wild primate populations (Pride, 2005; Rakotoniaina *et al.*, 2017; Kalbitzer and Chapman, 2018). Our meta-analysis also enabled us to identify a series of important confounding factors (e.g., predatory pressure, social dynamics, parasitism, and food availability) and highlight the important heterogeneity of the

physiological responses to anthropogenic disturbances by primates. Despite the positive global trend, our study demonstrated that species, populations, and even individuals may have independent tolerance thresholds and degrees of plasticity when dealing with anthropogenic disturbances.

As habitat loss is the main threat hanging over primates, affecting their diversity, richness, abundance, and physiology (de Almeida-Rocha et al., 2017; Kaisin et al., 2021), we assessed the GC concentrations of black lion tamarin populations inhabiting six fragments of varying sizes in order to estimate how they cope in different habitats (**Chapter 2**). For this study, we chose to dose cortisol in hair samples because they reflect adrenocortical activity over a longer period of time and are not biased by hourly or daily variations. Thus, analysing a single sample offers valuable information on the general physiological stress state of an animal (Heimbürge et al., 2019; Kalliokoski et al., 2019). Since food sources act as strong selective forces in a species' behaviour, ecology, and physiology, we evaluated habitat quality in the different fragments according to lion tamarins' main dietary categories (i.e., arthropods and fruits). Our models showed that, although lion tamarins in smaller fragments tended to have higher cortisol levels, tree abundance and diversity were the main factors influencing physiological stress. Lower vegetation quality, a proxy for fruit availability, was linked to increased cortisol levels suggesting that fruits represent an important and limiting resource for black lion tamarins. This assumption was also confirmed in a sister study in which we evaluated the diet of different groups and compared the contribution of arthropods with similar habitat quality variables. We found that lion tamarins increased their arthropod consumption in fragments with lower fruit availability, regardless of arthropod biomass (Raskin, 2021). Surprisingly, vegetation quality was not correlated with fragment size, highlighting the importance of quantifying habitat quality using species-relevant features.

After establishing that habitat quality influenced physiological stress in our focus species, we attempted to understand how activity budgets and diet influenced their GC levels over days or months (**Chapter 3**). Rather than hair samples, we collected faecal samples simultaneously with behavioural data to explore adrenocortical activity at a finer scale. Based on the theoretical concepts depicted in Romero's reactive scope model (see *Section 2.1* of the State of the Art), this study aimed to explore GC variations over different time scales to identify the multiple facets of adrenocortical activity. We studied two black lion tamarin groups living in different areas: a continuous forest and a small 100 ha fragment. Given that lion tamarins are small-bodied primates with high defecation rates, we were able to identify circadian patterns and account for them in the subsequent analyses. Both groups shared a similar circadian profile with a soft rise in the morning, corresponding to the cortisol awakening response (CAR). Our results reveal that lion tamarin GC concentrations varied according to monthly changes in activity budget associated with the energy balance of the groups. In other words, diet and ranging patterns, driven by food availability and distribution,

influenced GC levels at a circannual scale as part of the lion tamarins' predictive homeostasis. To identify acute stressors triggering a stress response (i.e., reactive homeostasis) in this neotropical primate, we explored GC levels at a day-to-day level and muted hourly and monthly variations. While changes in the main behavioural categories (e.g., feeding, moving, foraging) did not influence daily GC levels, intergroup encounters sparked a physiological stress response in a group living in a restricted 100 ha fragment. The novel theoretically-grounded approach used in this study enabled a broad-based exploration of Romero's reactive scope model, making a meaningful contribution to the study of ecological stress physiology in wild primates or, more generally, in mammals.

Starting this thesis with a global overview and progressively making our way to an in-depth analysis of an endangered and little-studied primate, allowed us to better understand the complex mechanisms underlying physiological stress in wild primates facing anthropogenic disturbances. One of the key outcomes of our meta-analysis is that physiological signals are highly variable across primate species, depending on their tolerance thresholds and plasticity. Regarding black lion tamarins, our results suggest that, as eclectic and opportunist omnivores, they can adapt their behaviour and diet, according to food availability and habitat quality, in order to ride out periods of food shortage and subsist in degraded habitats. Nonetheless, fragment size and vegetation quality did seem to influence physiological stress levels. Long-term monitoring of the physiological state of black lion tamarins in fragments would be necessary to link GC levels with survival and fitness indicators, such as reproductive success, mortality, and health. Nevertheless, small healthy Atlantic Forest fragments, with sufficient and adequate resources, may represent suitable habitats for this small neotropical primate, which has a diversified diet and a relatively small home range. Since black lion tamarins already inhabit 40% of areas suitable for them (Rezende et al., 2020b) and their range spreads across highly fragmented landscapes, protecting small fragments while ensuring their connectivity may be paramount for the conservation of this species.

2. Outlooks for future research

Fauna across the world is increasingly subject to anthropogenic disturbances due to mankind's insatiable encroachment of natural habitats. In mammals, birds, amphibians and reptiles, these disturbances are predominantly associated with significant increases in GC levels (Dantzer et al., 2014). Similarly, our meta-analysis concluded that primates in disturbed habitats had higher physiological stress levels. Allegedly, animals facing anthropogenic disturbances, which demonstrate elevated GC concentrations, may have diminished health and fitness levels. Yet, because of the dual nature of the stress response, interpreting the effect of adrenocortical activity is not as straightforward. In essence, the stress response prioritizes immediate energy usage over future energy storage (Wingfield & Sapolsky, 2003). Hence, while a short-term activation of the HPA axis promotes adaptive physiological and behavioural changes to overcome punctual challenges, chronic stress is potentially deleterious for animals (Korte et al., 2005; Landys et al., 2006; Romero et al., 2009). However, chronic elevations in GC levels have also been shown to promote adaptive phenotypic plasticity in animals exposed to ecological challenges (Middlemis Maher et al., 2013; Dantzer et al., 2013), further complexifying the implications of sustained GC levels. From an evolutionary standpoint, the deleterious effects of chronic stress should not exist in the wild (Boonstra, 2013). However, abrupt and intense anthropogenic disturbances submit animals to unforeseeable pressures to which most species do not have the time to adapt (Kalbitzer & Chapman, 2018). Future research should focus on exploring the influence of GC secretion on the health of individuals and populations over time (Beehner & Bergman, 2017). When does chronic stress become a problem? Identifying the threshold at which GCs concentrations become deleterious for a species is a key question for imminent studies in physiological conservation.

The development of new non-invasive methods to measure GC concentrations has considerably increased our understanding of physiological stress in wild species. Among these methods, the use of hair samples is promising but still requires further investigation. To date, the precise pathway through which cortisol is incorporated into the hair shaft is uncertain. While some research suggest that hair cortisol concentrations (HCCs) reflect the long-term accumulation of GCs that are integrated into the hair shaft during its growth (Meyer and Novak, 2012; Stalder & Kirschbaum, 2012; Shimamoto, 2022), recent studies reveal that GCs may also diffuse into the hair through excretions from surrounding tissues (Koren et al., 2019). It is therefore believed that HCCs may represent a blend of both long-term hormone accumulation during hair growth and transient levels from auxiliary sources (Koren *et al.*, 2019). Future research must be directed towards understanding how GCs are incorporated into the hair and how long they remain post-deposition in order to determine the time frame represented in hair samples. Additional studies using radioisotope-labelled GCs could substantially improve our interpretation of GCs measured in hair (Keckeis et al., 2012;

Kapoor et al., 2018). Until these questions are answered, it is therefore not recommended to interpret HCCs as historical records of physiological stress. Nevertheless, experimental studies have shown that both acute and chronic stressors produce significant elevations in HCCs compared to control groups (Kalliokoski et al., 2019). Hair cortisol analysis therefore remains a useful tool to assess adrenocortical activity in wild animals, providing precious information on HPA axis functioning and are adequate to determine the influence of persisting environmental or social stressors.

In primatology, long-term studies are extremely valuable for research and provide exceptional hindsight on the ecology and evolution of species (Clutton-Brock & Sheldon, 2010). Such studies are necessary in the field of wildlife endocrinology to put in perspective physiological stress, anthropogenic disturbance, and fitness indicators (e.g., interbirth interval, mortality, reproductive success, and population densities). Currently, only two studies have linked high GC concentrations to higher mortality rates in wild primates living in changing habitats (Pride, 2005; Rakotoniaina et al., 2017). Because long-term studies are not always an option due to funding and permit issues, alternative methods exist to explore the impact of elevated GC levels on primate health. Future studies can compare GC variations with immune parameters such as wound healing, lymphocyte counts, or cytokine concentrations (Alberts et al., 1992; Hoffman et al., 2011; Archie et al., 2012). Because physiological stress may exacerbate the clinical effects of parasitic infections, due to immunosuppression, parasite richness and abundance are good indicators of GC outcomes (Muehlenbein, 2006; Chapman et al., 2006; Behie & Pavelka, 2013; Gillespie et al., 2013). Finally, to check for signs of dysregulation of the HPA axis that may arise in chronically stressed animals, researchers should attempt to measure GC concentrations after an acute stressor (e.g., capture, environmental disasters, predator attack; Beehner & Bergman, 2017). In healthy animals, with a normally functioning HPA axis, these stressors should be accompanied by a significant increase in GC levels while a blunted response is expected in chronically stressed animals.

Ultimately, one of the main insights that arose from this thesis is that physiological stress is multifactorial and must be studied in that manner, by adopting a holistic approach that takes into consideration a maximum of intrinsic and extrinsic species-specific factors. Once more, it is important to insist that GCs are first and foremost metabolic hormones. Therefore, following a metabolic theoretical framework, such as Romero's reactive scope model that was used to explore the adrenocortical activity of black lion tamarins in Chapter 3, will help to disentangle and interpret the different roles of GCs. Chiefly, it is essential to have a thorough understanding of the study species' life history traits, diet, behaviour, habitat, and general ecology. In dealing with anthropogenic disturbances, species, populations, and even individuals demonstrate different tolerance thresholds, behavioural flexibility, and dietary plasticity (Chapman et al., 2012; Kalbitzer & Chapman, 2018; Kaisin et al., 2021). Therefore,

certain taxa are more resilient while others are more vulnerable to environmental challenges. In our meta-analysis, great apes, which depend heavily on pristine habitats, and atelids, which are almost exclusively frugivorous and folivorous, demonstrated significant changes in GC levels in response to disturbances. However, cercopithecines and cebids, which are known to be generalist and opportunist primates, were not significantly impacted by anthropogenic disturbances. Future endocrinological studies in primatology should continue to explore the effect of disturbances on species to build up a substantial dataset and establish well-founded phylogenetic trends. Moreover, since research in primatology is skewed in favour of a few emblematic species (Bezanson & McNamara, 2019), researchers should also try to focus on less studied species in new geographic regions.

3. Impending threats and primate conservation

Since the beginning of the 21st century, more than a tenth of natural areas across the globe have been transformed into anthropogenic land uses (Watson et al., 2016). Models overlapping primate global distributions and predicted agricultural demands during the 21st century expect that 75 percent of primate habitats will suffer from severe agricultural expansion (Estrada et al., 2017). Another recent study has arrived at the terrifying conclusion that if current deforestation rates persist, pristine and undisturbed tropical moist forests outside protected areas, representing crucial ecosystems for primate diversity, will have completely vanished by 2050 (Vancutsem et al., 2021). Due to their dependence on tropical ecosystems and their relatively slow life history, this projected habitat loss and degradation will have devastating consequences for primate populations around the world (Isaac & Cowlishaw, 2004; de Almeida-Rocha et al., 2017; Estrada et al., 2017; Fernández et al., 2022).

Due to the expansion of unsustainable large-scale agriculture, livestock ranching, logging, mining, and fossil fuel extraction, 25 million km of new roads are expected to be added to the transportation network in tropical forest areas by 2050 (Laurance et al., 2014). This will further impede primate dispersal and increase the risks of road kills by further fragmenting the landscape (Estrada et al., 2018; Hetman et al., 2019). The expansion of road networks will also give rise to synergistic menaces for primates and other wildlife such as increases in unrestricted and illegal logging, bushmeat hunting, and primate trade, by increasing accessibility to forest ecosystems (Wilkie et al., 2000; Laurance et al., 2015; Estrada et al., 2017). This will only exacerbate human pressures on primates given that approximately 82 and 80 percent of species are already impacted by illegal hunting and logging, respectively (IUCN, 2022; Fernández et al., 2022). Increased habitat encroachment, as well as climate change, also enhance human-primate contacts, exposing primates to emerging infectious diseases (Chapman et al., 2005). Conversely, given the close phylogenetic relationship between humans and primates, disease outbreaks may affect human populations (e.g., the infamous Ebola epidemic and HIV/AIDS pandemic; Calvignac-Spencer et al., 2012; Cooper & Nunn, 2013).

On top of habitat loss and human encroachment on the environment, primate populations are going to face an unprecedented threat, climate change. Since the industrial revolution during the second half of the 18th century, exponential increases in global greenhouse gas emissions and unsustainable anthropogenic changes in land use have been the main drivers of long-term shifts in temperatures and weather patterns. According to the latest report from the Intergovernmental Panel on Climate Change (IPCC), global warming levels of 1.5°C (i.e., the limit set by the Paris Agreement) will have catastrophic consequences for biodiversity, including the strong risks of extinction for up to 14 percent of terrestrial animals. This number may reach 48 percent if global temperatures were to rise by 5°C (IPCC, 2022). Primates are

expected to be highly exposed to extreme warming events during the 21st century, with the Brazilian Atlantic Forest, the Amazon, Central America, and Southeast Asia being severely impacted (Graham et al., 2016; Carvalho et al., 2019). Climate change will lead to drastic shifts in phenology, habitat suitability, community composition, and disease prevalence (Korstjens & Hillyer, 2016). Primates, having slow life histories and long interbirth intervals, will undoubtedly suffer from unpredictable fluctuations in resource availability and extreme reduction of their geographic ranges (Pearson et al., 2014; Kamilar & Beaudrot, 2018). In Madagascar, recent models anticipate that climate change will considerably reduce the distribution of 60 percent of lemur species (Brown & Yoder, 2015). The synergy of fragmentation, hunting, and climate change will potentially trigger further cascading effects that negatively impact primate populations (Brook et al., 2008). For instance, primate migration driven by optimal habitat shifts will be hindered by habitat loss and the lack of connectivity between forest patches.

While the impacts of the impending threats discussed hereinabove will vary across species according to their life history, social organization, diet, dispersal capacities, and current conservation status, the global response will be population declines across primates (de Almeida-Rocha et al., 2017; Estrada et al., 2017). Thus, conserving primates and preserving their habitats is paramount. Protected areas are the foundation of biodiversity conservation, providing long-term sanctuary for wildlife, preserving key habitats, allowing the migration and movement of species, and maintaining ecosystems. While the area covered by protected areas has considerably increased over the past decades, the majority of primate species currently live outside protected areas (Estrada et al., 2017, 2018, Galán-Acedo et al., 2019). In Brazil, 62 percent of primates live outside protected areas (Estrada et al., 2018). Increasing the number and surface of protected areas is therefore essential for primate conservation. To be effective, protected areas must be sufficiently large and provide adequate resources for inhabiting species. Accordingly, having a deep understanding of species' ecology, ranging patterns, and distribution is essential to establish cost-effective and well-founded conservation management plans (Kalbitzer & Chapman, 2018). Yet, a significant gap still exists between research and the implementation of conservation actions (Knight et al., 2008).

Protected areas offer exceptional opportunities for the development of eco-tourism. When correctly put into place, eco-tourism has the potential to be a valuable conservation tool for raising awareness on the value of nature, gathering funds for conservation, and improving the welfare of local communities (Russon & Wallis, 2014). However, our meta-analysis highlighted that tourism may have deleterious impacts on primates if the necessary rules are not implemented (Kaisin et al., 2021). Different studies on great apes demonstrated the effectiveness of long-term habituation and the importance of adopting appropriate regulations in human–primate contacts (Shutt et al., 2014; Carlitz et al., 2016).

In many regions, protected areas become islands of preserved habitats in highly deforested and human-degraded landscapes. Perpetual habitat loss has led to the widespread fragmentation of tropical and subtropical forests, constraining primates to live in isolated and restricted forest patches, including protected areas (Haddad et al., 2015; Estrada et al., 2017). This lack of landscape connectivity significantly hinders the dispersal of arboreal primates (Arroyo-Rodríguez & Mandujano, 2009). Therefore, maintaining landscape permeability by linking forested areas using biological corridors is essential also for primate conservation. A recent study also found that Indigenous Peoples' lands account for 30 percent of the primate range and harbour 71% of primate species (Estrada et al., 2022). Preserving these areas and encouraging community conservation is also crucial to prevent the extinction of primates across the world.

3.1. The case of black lion tamarins

Black lion tamarin subpopulations are scattered throughout the Tietê-Paranapanema interfluve, a highly fragmented landscape that suffered intense habitat loss (Culot et al., 2015; Rezende et al., 2018). Considering that the Morro do Diabo State Park (MDSP) hosts the only population estimated to be both demographically and genetically viable in the long term, increasing connectivity between subpopulations is more than ever critical. Biological corridors are currently being established to reconnect the MDSP with surrounding forest patches, such as the Ponte Branca and Santa Maria fragments studied in this thesis, as part of the black lion tamarin conservation program of the Instituto de Pesquisas Ecológicas (IPE). Similar conservation management plans are being implemented in the Alto Paranapanema region, in which black lion tamarins inhabit small Atlantic Forest remnants within a disconnected landscape. While the full regeneration of biological corridors may take 10-40 years, studies on golden lion tamarins (*Leontopithecus rosalia*) and golden-headed lion tamarins (*Leontopithecus chrysomelas*) revealed that they may move across younger forests (<10 years old) to disperse, demonstrating the short-term success of such conservation strategies for these species (Newmark et al., 2017; Dosen et al., 2017). A recent study also demonstrated that wildlife canopy bridges are effective conservation tools to help black lion tamarin dispersal and reduce road kills (Garcia et al., 2022).

Over the next decades, black lion tamarins will also suffer severe range shifts due to climate change. Predictive models estimate that, within their current range, black lion tamarins will lose most of their suitable habitat due to rising temperatures (Meyer et al., 2014). Protected areas such as the MDSP, which harbours the largest black lion tamarin populations, will no longer be suitable for this species by 2080. Black lion tamarins will be particularly vulnerable to climate change due to their limited dispersal capacities, preventing them from reaching climate refuges (Schloss et al., 2012). However, their behavioural and dietary plasticity may allow populations to adapt to these changing conditions. To make informed

and judicious conservation actions, research efforts should focus on understanding the direct impacts of climate change on their habitat and constructing a precise map of the species distribution to identify potential migration routes.

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General Appendix

List of papers that stemmed from side projects during the PhD. First author papers are available here below.

Bufalo, F., Amaral, R. G., **Kaisin, O.**, & Culot, L. (2022). A new feeding association between black-goggled tanagers (Tachyphoninae) and black lion tamarins (Primates, Callitrichinae). *Ornithology Research*, *0123456789*, 1–5. https://doi.org/10.1007/s43388-022-00099-w

Garbino, G. S. T., Rezende, G. C., Antônio, D. C., Bufalo, F., Amaral, R. G., Silva, A. D. A., **Kaisin, O.**, Culot, L. (2022). Seasonal variation in frog predation by black lion tamarins (Leontopithecus chrysopygus , Primates). *Journal of Natural History*, *56*(5–8), 449–461. https://doi.org/10.1080/00222933.2022.2078242

Kaisin, O., Amaral, R. G., Bufalo, F. S., Brotcorne, F., & Culot, L. (2020). Spontaneous Tool Use by a Wild Black Lion Tamarin (Leontopithecus chrysopygus). *International Journal of Primatology*, *41*(4), 559–561. https://doi.org/10.1007/s10764-020-00170-7

Kaisin, O., Rocha, F. C., Amaral, R. G., Bufalo, F., Sabino, G. P., & Culot, L. (2022). A universal pharmacy: Possible self-medication using tree balsam by multiple Atlantic Forest mammals. *Biotropica*, *54*(3), 576–582. https://doi.org/10.1111/btp.13095

International Journal of Primatology
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NEWS & VIEWS

Spontaneous Tool Use by a Wild Black Lion Tamarin (*Leontopithecus chrysopygus*)

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Wild nonhuman primates use a wide variety of tools, differing in size, purpose, and complexity (Sanz *et al.* 2013). For example, some chimpanzees (*Pan troglodytes*) use sticks to probe for ants while capuchins (*Cebus* sp. and *Sapajus* sp.) use stones to break open nuts (Sanz *et al.* 2013). Although tool use has multiple outcomes, it is often linked to the acquisition of important resources, such as food or water (Bentley-Condit 2010). With the exception of capuchins, few observations of tool use have been reported for New World primates. For example, in callitrichids, tool use has only been described in captive individuals of two species: captive cotton top tamarins (*Saguinus oedipus*) used tools in an experimental setup (Hauser 1997) and free-ranging captive golden lion tamarins (*Leontopithecus rosalia*) spontaneously used sticks to remove bark from trunks and search for invertebrates (Stoinski and Beck 2001).

Here, we describe the first record of spontaneous tool use in the wild by a callitrichid, the black lion tamarin (*Leontopithecus chrysopygus*). Black lion tamarins are small neotropical primates, endemic to the state of São Paulo and considered endangered because of the high fragmentation of their habitat, the Brazilian Atlantic Forest. Lion tamarins are frugivorous and insectivorous and live in cohesive groups comprising two to six individuals. We observed the tool use in a small (100 ha) private forest fragment in the municipality of Guarei, São Paulo (23°25′07″S, 48°14′27″W).

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We have studied a fully habituated group of black lion tamarins in this fragment since April 2019. The group initially comprised two adult males and three adult females. After the birth of two infants in September 2019, the group split into two new groups and we continued to study one of them, comprising four adults (two males, one female, one unidentified). We followed this group from sleeping site to sleeping site, for a minimum of 6 days a month between April 2019 to February 2020, to collect behavioral, physiological, and ranging data. During the day, while the tamarins were out of their sleeping site, we collected behavioral data using scan sampling every 5 min and recorded feeding events using all occurrence sampling. We recorded all unusual or infrequent behaviors *ad libitum*.

On August 19, 2019, at 11:52 h, we saw an adult black lion tamarin of unknown sex using a short (±15 cm) slender twig as a probe to forage for insects between the leaves of an epiphyte (*Bromelia* sp.) on the trunk of a tree, 10 m above the ground. While pushing down on the epiphyte with its body to widen the space between the leaves, the lion tamarin used the stick in its left hand to search for potential prey. The tamarin poked the stick between the leaves of the epiphyte to excavate unidentified insects. Once the insects were expelled and within reach, the lion tamarin caught them with its right hand and consumed them. The tool use lasted about 40 s, during which time the individual caught and consumed three small insects (3–4 cm). After successfully preying on the insects, the lion tamarin let go of the tool and moved on to another tree. Although we noticed the tool only when it was already in the lion tamarin's hand, we think that it was a short dead twig that the lion tamarin acquired opportunistically when already foraging for insects. Hence, we think that there was no or very little tool modification. Unfortunately, we could not take pictures or videos of the event because of its short duration and the low light that day. Although we do not see a clear causal link, we observed this behavior the day the State of São Paulo experienced a blackout due to opaque clouds of smoke, linked to intense wildfires in Brazilian Amazonia, blocking the sunlight. After some rain early in the morning, the forest became darker than usual and the light was low throughout the day. The group entered the sleeping site at 14:08 h, although they usually enter their sleeping site between 16:00 and 17:00 h during this period of the year.

Although we studied four groups, two in Atlantic Forest fragments and two in the Morro do Diabo State Park, totaling 2524 h of observation, this was the only time we observed spontaneous tool use by a black lion tamarin. Among the multiple theories on the emergence of tool use in primates, the extractive foraging theory postulates that tool use is more likely to emerge in omnivorous species that forage and feed on seasonally limited and embedded food resources (Parker and Gibson 1977). The emergence of spontaneous tool use in wild, free-ranging lion tamarins may be associated with the importance of vertebrates and invertebrates in their diet. Based on our scan data, the group spent almost as much time foraging for prey (23.1%; 3642 of 15,784 scans) as they spent feeding on fruits (27.4%; 4329 of scans). It is often difficult to witness the consumption of small prey, as this behavior is rapid and elusive. Nonetheless, we observed 213 events of invertebrate predation including spiders, caterpillars, and insects from the Blattoidea, Coleoptera, Phasmatodea, and Orthoptera orders. We also observed frog predation on 21 occasions. Although rudimentary in comparison to tool use behaviors observed in other primate genera (e.g., capuchins and chimpanzees), the

tool use we describe potentially increases predation efficiency. As black lion tamarins are omnivorous extractive foragers, this observation further supports a link between extractive foraging and the emergence of tool use.

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NATURAL HISTORY FIELD NOTE

A universal pharmacy: Possible self-medication using tree balsam by multiple Atlantic Forest mammals

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Abstract

We present camera trap evidence of 10 Atlantic Forest mammals fur-rubbing, licking, or biting balsam from *cabreúvas* (*Myroxylon peruiferum*, Fabaceae), native trees used in traditional medicine for their prophylactic and therapeutic virtues. Given the antiparasitic properties of *cabreúvas*, mammals may be using the balsam as topical self-medication to repel ectoparasites.

Abstract in Portuguese is available with online material.

KEYWORDS
cabreúva, camera trap, fur-rubbing, medicinal plants, *Myroxylon peruiferum*, zoopharmacognosy

Animal self-medication or zoopharmacognosy, the use of non-nutritional compounds by animals to prevent or control diseases or repel parasites (de Roode et al., 2013; Janzen, 1978), has been observed in a myriad of species across diverse taxa. This practice, which can have both prophylactic and therapeutic functions, encompasses a variety of behaviors such as the consumption of medicinal plants, geophagy, fur-rubbing, and anting (i.e., rubbing crushed ants on the body to appease irritated skin) (Raman & Kandula, 2008). In mammals, zoopharmacognosy has been repeatedly described in nonhuman primates (Huffman, 1997), though it has also been observed in elephants (*Loxodonta* sp.), bears (*Ursus* sp.), elks (*Cervus canadensis*), and some carnivores (Shurkin, 2014). Self-medication is often associated with the consumption of leaves, medulla, fruits, or husks of plants with antiparasitic, antimicrobial, anti-inflammatory, antioxidant, purgative, or hormonal effects (Fruth et al., 2014; Huffman & Pebsworth, 2018; Mclennan & Huffman, 2012). For example, baboons (*Papio* sp.) feed on leaves and fruits of *Balanites aegyptiaca* to control schistosomiasis, a disease caused by parasitic flatworms (Lozano, 1998). Fur-rubbing or self-anointing, the act of rubbing pungent substances on the skin or coat, is another self-medicating behavior. Although it is mainly displayed by some nonhuman primates (Huffman, 2006), this behavior is not limited to this taxon and has

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also been described in coatis (*Nasua nasua*) and interpreted as zoopharmacognosy (Gompper & Hoylman, 1993).

Fur-rubbing is generally attributed to self-medication, with a prevalence of prophylactic and therapeutic effects. For example, white-faced capuchin monkeys (*Cebus capucinus*) rub citrus fruits on their skin to repel insects (Baker, 1996), orang-outangs (*Pongo pygmaeus*) rub specific antibacterial and anti-inflammatory herbs on their joint areas (Morrogh-Bernard, 2008), while black lemurs (*Eulemur macaco*) spread toxins secreted by millipedes on their body as a repellent (Birkinshaw, 1999). Other than medicinal, fur-rubbing has also been linked to the reinforcement of social bonds (Leca et al., 2007) and territorial defense (Souza-Alves et al., 2021). It is therefore important to distinguish fur-rubbing from scent-rubbing, a behavior that is typically, although not exclusively, expressed in carnivores, in which they rub against an object in their environment to leave an olfactory signature. Scent-rubbing may have multiple purposes such as marking territory or intraspecies communication (Gosling & McKay, 1990; Hirano et al., 2008). This behavior is sometimes preceded by self-anointing where an animal impregnates its body with a specific odor (*e.g.*, urine, decaying meat, feces, etc.) (Gosling & McKay, 1990).

In this report, we describe the use of *Myroxylon peruiferum* balsam by various species of wild mammals inhabiting different fragments of Brazilian Atlantic Forest. During daily follows of black lion tamarin (Primates, Callitrichidae, *Leontopithecus chrysopygus*) groups for behavioral, endocrinal, seed dispersal, and movement ecology studies, our team witnessed tamarins fur-rubbing on a native tree species, the *cabreúva* (*M. peruiferum*, Fabaceae). *Cabreúvas*, which produce a balsam (i.e., a resinous exudate called Tolu balsam) with a typical and distinctive perfume, are recognized in traditional medicine to have both prophylactic and therapeutic properties (Lorenzi & Matos, 2002). Known to have wound-healing and anti-inflammatory properties, the balsam is used to treat a myriad of illnesses and conditions in humans such as scabies, external wounds, ectoparasite infections, bronchitis, rheumatism, urinary infections, tuberculosis, dysentery, and abscesses (Lorenzi & Matos, 2002). *In vitro* studies on *Myroxylon* sp. also revealed that the leaves have antioxidant and antibiotic actions (da Silva Junior et al., 2014; Trentin et al., 2011) and that the fruits have antimalarial activity (Muñoz et al., 2000). Furthermore, the bark is antibiotic (Gonçalves et al., 2005), antifungal (Pereira et al., 2018), inhibits leishmaniosis (Andrade et al., 2016), and is larvicidal against *Aedes aegypti*, one of the vector mosquitoes of yellow fever (Seo et al., 2012).

From March 2019 to March 2020, we studied two tamarin groups in two sites, the Morro do Diabo State Park (MDSP; 33.845 ha; 22°37′21′′S, 52°08′02′′W) and a private fragment in the municipality of Guareí (100 ha; 23°25′07′′S, 48°14′27′′W). Both study sites are composed of Brazilian Atlantic Forest, within the State of São Paulo, and are characterized by a rainy season (October–March) and a dry season (April–September). We followed the tamarins from sleeping site to sleeping site, collecting behavioral data through scan sampling every 5 min as well as all occurrence samplings for singular behaviors such as fur-rubbing. Over a total of 1246 h of direct observation, we recorded 17 events of fur-rubbing on *M. peruiferum*. In parallel, to evaluate the abundance of *cabreúva* trees in the home-range of the tamarins, we set up 20 botanical plots (10 × 10 m) in each site and identified all trees with a diameter at breast height (DBH) >4.8 cm. *M. peruiferum* density was of 20 individuals/ha in Guareí but absent from the plots in MDSP and SMA.

Generally, the tamarins directly rubbed their thoracic, abdominal, and inguinal regions on areas of the trunks with balsam exudation. They also frequently manipulated the bark of the trunk, smearing balsam on their hands before indirectly applying it over their body and tail. Moreover, on rare occasions, we observed the tamarins biting and licking the balsam, perhaps to stimulate exudation by the tree. Once, a juvenile was seen ingesting and then regurgitating the balsam, showing no signs of intoxication during the following days. In black lion tamarins, fur-rubbing was usually simultaneously performed by more than one individual (80% of the events) and sometimes performed cohesively by the entire group (20% of the events). The events of fur-rubbing only occurred during the afternoon, between 1:30 p.m. and 6:00 p.m., and lasted between 3 and 50 min (14 min 36 s ± 12 min 05 s/event). Throughout the study period, the tamarins were exclusively observed fur-rubbing on *cabreúva* balsam.

Based on the initial observations of fur-rubbing by black lion tamarins and the numerous pharmacological properties of the *cabreúva*, we decided to monitor the use of these trees placing camera traps. We set up camera traps in three different areas: the MDSP, the forest fragment in Guareí, and the fragment of Santa Maria (SMA), also inhabited by tamarins and located in the municipality of Presidente Epitácio (478 ha; 22°14′17′′S, 52°18′22′′W). In the MDSP and in Guareí, we selected a *cabreúva* tree that had previously been used by the groups of tamarins we were systematically monitoring. In Santa Maria (SMA), a fragment of Atlantic Forest in which we did not follow a group, we identified the *cabreúva* during a survey to verify the occurrence of tamarins, solely due to the strong perfume that emanated from the tree's balsam. In the MDSP, the camera trap was first set up during 130 days between February and June 2020, and then for 10 days in July 2021. In Guareí, we set up the camera traps between January and May 2020 for 65 days. In Santa Maria (SMA), the camera trap was set up between February and April 2021, totaling 41 days. We set up a single camera trap per site, 2–3m away from a *cabreúva* trunk, at 1–3 m from the ground, and oriented to the regions of the trunk with important exudation. The camera traps (Bushnell 119,435, 480p; Bushnell 119,676, 720p; Enkeeo PH760, 1080p) were running 24 h per day during the entire sampling period and programmed to record 10 s MP4 format videos with 5 s time-lags. For every video, we recorded the date, time, number, species, and described the behavior displayed by the animals present at the *cabreúvas*. To explore the daily temporal distribution of visits, we constructed radar charts for each species, revealing the different schedules during which each species visited the *cabreúvas* (Figure 2). When the time lapse between two videos of the same species was higher than 30 min, we considered this to be two distinct visits.

Camera trap footage from the different field sites unveiled novel and exclusive possible zoopharmacognosy behaviors from various Atlantic Forest mammals. The *cabreúva* trees were visited

by a mammal species every 1.77 ± 1.49 days in Guareí, 1.77 ± 2.13 days in MDSP, and 4.85 ± 3.95 days in SMA. We recorded nine additional mammal species interacting with the *cabreúva* balsam (Figure 1). Black capuchins (*Sapajus nigritus*) and Ingram's squirrels (*Guerlinguetus brasiliensis*) were also spotted at the *cabreúvas* but did not seem to interact directly with the balsam. Tayras, collared peccaries, ocelots, coatis, black lion tamarins, northern tamanduas, neotropical fruit bats, and brocket deer displayed behaviors similar to fur-rubbing on the *cabreúva* trunks (see camera trap footage in Video S1). Furthermore, mouse opossums, white-eared opossums, brocket deer, tayras, ocelots, northern tamanduas, and neotropical fruit bats were also observed sniffing the trunk and/or licking the balsam. Although numerous species visited the same *cabreúva* tree individuals, two different species were never observed simultaneously at the sites. However, different species occasionally visited the trees on the same day in Guareí (i.e., ocelot–tayra; $N_{tot} = 2$) and in MSDP (i.e., peccary–mouse opossum, tayra–mouse opossum, tayra–deer, peccary–tamarin, mouse opossum–tamarin, coati–peccary, ocelot–peccary; $N_{tot} = 10$). Excepting the presence of an ocelot 11 min after a tayra on one occasion in Guareí, these coinciding visits were separated by 4.55 ± 3.63 h. Occurrences at the *cabreúvas* followed a certain temporal distribution with species visiting the sites at different schedules of the day (Figure 2). Ocelots, bats, mouse opossums, and white-eared opossums were exclusively nocturnal (i.e., 18:00–5:59), while coatis, tayras, and black lion tamarins were mainly diurnal (i.e., 6:00–17:59) (Figure 2). Similar to what was observed during direct observations of tamarin groups, the camera trap footage showed that black lion tamarins only visited the *cabreúvas* in the afternoon. Tayras were generally present at the trees at midday.

Behaviors resembling fur-rubbing had never been documented in peccaries, tamanduas, tayras, and neotropical fruit bats. Similar to the black lion tamarin, tayras, which most frequently visited the *cabreúvas*, rubbed their jaw, neck, chest, abdomen, and inguinal region against the *cabreúva* trunk. Tamanduas used their sizable claws to pry open the bark and subsequently rub their body against the exposed trunk. Collared peccaries rubbed their costal region on the trunk but also bit the bark and the roots to anoint their snout and apply balsam on their hind legs. Furthermore, multiple videos captured pairs of peccaries mutually spreading balsam on each other in head-to-tail positions. Coatis used their hands and snout to apply resin to their body and tail. In the only video showing the use of *cabreúva* by a neotropical fruit bat, the individual seems to rub both its thoracic and abdominal regions while simultaneously licking the balsam. Ocelots rubbed the base of their mandible, neck, and chest on the trunk of the *cabreúva*. This behavior was similar to scent-marking, which is common in carnivores, and may be triggered by novel odors. Indeed, a study on gray wolfs showed that the smell of perfume and motor oil provoked scent-marking in this species (Ryon et al., 1986). Lastly, deer rubbed their antlers and forehead against the trunk in vertical movements. Such a display is common in cervids, which is assumed to be associated with territory marking (Bowyer et al., 1994; Johansson et al., 1995).

In black lion tamarins, fur-rubbing on the *cabreúva* trees stood out from territory marking, a swift behavior related to inter- and intragroup social communication and commonly displayed by tamarins (Heymann, 2006). Indeed, callitrichids possess epithelial glands in their sternal and anogenital regions that produce specific odors (Miller et al., 2003; Moraes et al., 2006). Individuals rub these specific areas against horizontal or inclined substrates to deposit their

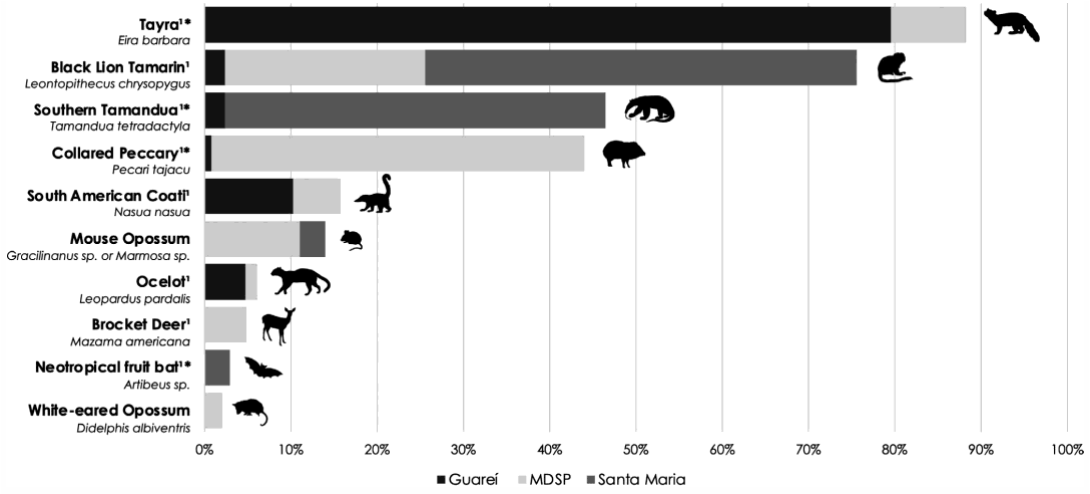
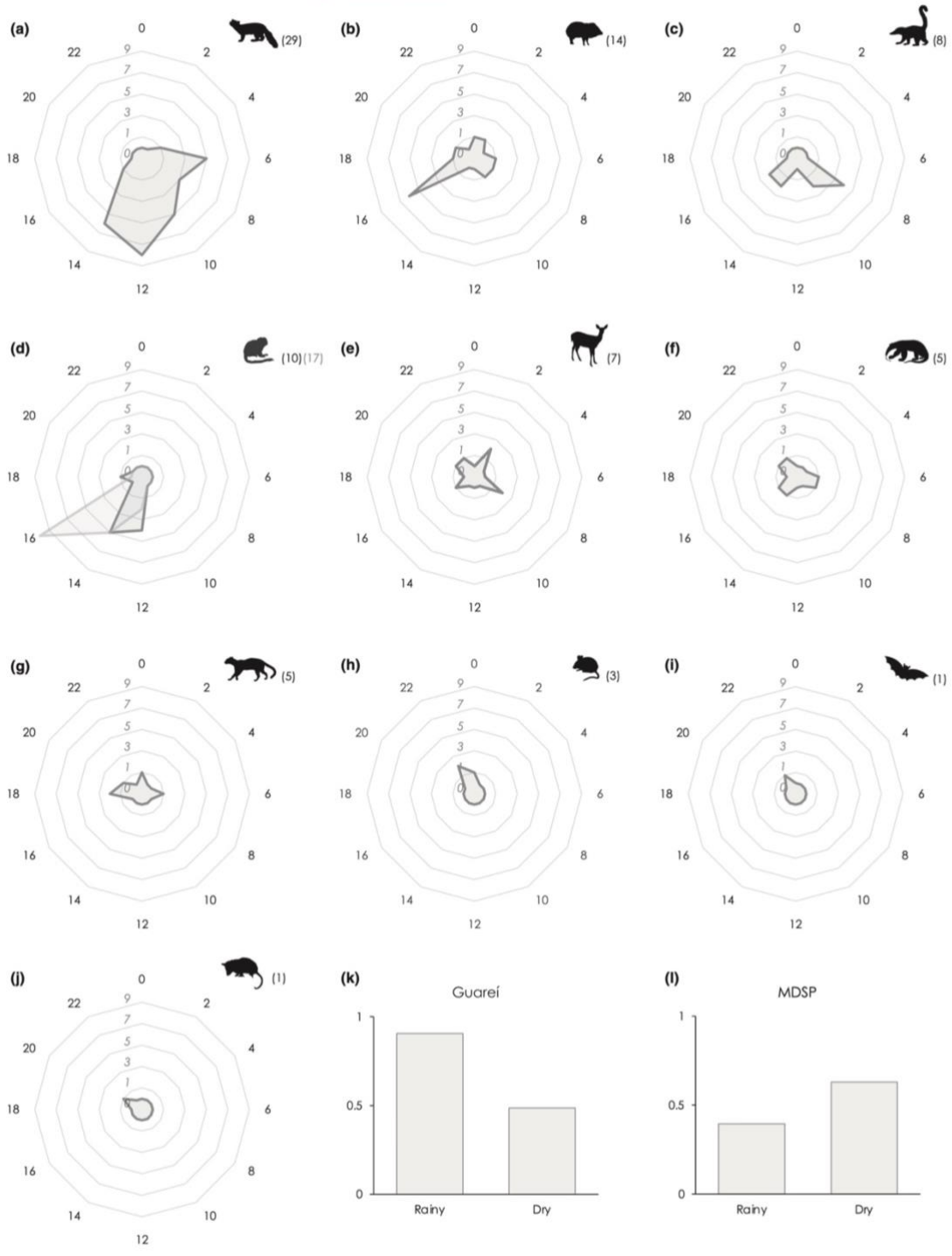


FIGURE 1 Percentage of videos captured at the *cabreúva* trees (*Myroxylon peruiferum*) per species, recorded by camera trap footage in three different field sites: a fragment in the municipality of Guareí (127 videos), the Morro do Diabo State Park (MDSP; 440 videos), and the Santa Maria (SMA) fragment (34 videos). Species that displayed fur-rubbing (1) and species for which fur-rubbing was observed for the first time (*)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(1)
(k)
Guareí
Rainy
Dry
(l)
MDSP
Rainy
Dry

FIGURE 2 Temporal distribution of visits to the *cabreúva* trees (*Myroxylon peruiferum*), based on camera trap footage, by (a) *Eira barbara*, (b) *Pecari tajacu*, (c) *Nasua nasua*, (d) *Leontopithecus chrysopygus*, (e) *Mazama americana*, (f) *Tamandua tetradactyla*, (g) *Leopardus pardalis*, (h) *Gracilinanus* sp. or *Marmosa* sp., (i) *Artibeus* sp., and (j) *Didelphis albiventris*. Direct behavioral observations, during daily follows, are also shown for *Leontopithecus chrysopygus* (d; light gray). Due to a technical problem with the camera traps, this figure is based on a subset of videos for which accurate time was available [Guareí: 93 videos, MDSP: 272 videos, Santa Maria (SMA): 34 videos]. The number of visits is specified in parentheses for each species. Frequency of *cabreúva* visits for *Leontopithecus chrysopygus* (K), based on direct observations, and for all mammal species (L), based on camera trap footage, between the rainy and dry season

olfactive signature (Heymann, 2006). In the case of the *cabreúvas*, fur-rubbing lasted up to 50 min and was performed directly and indirectly by black lion tamarins. Furthermore, unlike marking territory, which is mainly performed by adults, fur-rubbing was also accomplished by juveniles and often carried out by the entire group simultaneously. Regarding seasonality, although 70% ($N = 14$) of direct observations occurred during the dry season in Guareí, there were no significant differences in the frequencies of fur-rubbing between the rainy and dry season (chi-squared test: $N = 20$, $X^2 = 0.8$, $p = .371$; Figure 2k). Golden-headed lion tamarins (*Leontopithecus chrysomelas*) in Bahia, Brazil, displayed a similar behavior, fur-rubbing against the resin of *Myroxylon* sp. and *Thyrsodium spruceanum* (Guidorizzi & Raboy, 2009). Fur-rubbing associated to self-medication was predominant in the rainy season, presumably due to the abundance of hematophagous mosquitoes, such as *Aedes* sp., suggesting a possible use of *Myroxylon* sp. resin as a repellent (Guidorizzi & Raboy, 2009). However, during the dry season, tick nymphs, which are known to infect tamarins (Wilson et al., 1989), prevail in Brazil (Oliveira et al., 2000). Based on the camera trap footage, we found no significant difference in the frequency of visits, weighted according to the sampling effort in each site, between the rainy and dry season (chi-squared test: $N = 133$, $X^2 = 0.251$, $p = .616$; Figure 2). Nonetheless, a year-round monitoring of the *cabreúvas* would be needed to truly evaluate the effect of seasonality.

Considering the antiparasitic properties of *cabreúvas*, we suggest that the fur-rubbing displayed by the other mammal species may be regarded as prophylactic topical self-medication to repel potential ectoparasites and potentially reduce infection by parasitic diseases transmitted by mosquitoes and ticks. For black lion tamarins and other primates, the use of *cabreúva* balsam could therefore potentially play a key role in battling yellow fever infections, a mosquito-borne disease known to affect primate populations (Mares-Guia et al., 2020). Furthermore, when anointing their skin and fur, animals may also be benefiting from the multiple therapeutic, anti-inflammatory, and wound-healing properties of *cabreúvas*. Meanwhile, the species observed licking and biting the *cabreúvas* may be seeking other virtues of the balsam, used in traditional medicine to cure a myriad of diseases and infections (Souza et al., 2016). *Cabreúvas* may be a universal pharmacy for Atlantic Forest mammals in what could be described as convergent zoopharmacognosy. *M. peruiferum* may represent a valuable and disputed resource for mammal species, which may help sustain populations by promoting their health and increasing fitness. Future research should focus on gathering evidence of the use of *cabreúva* balsam and determine precisely which therapeutic or prophylactic properties may be sought out by Atlantic Forest mammals. Moreover, since *cabreúvas* are distributed in many vegetation types, occurring in both primary and secondary forests (Lorenzi, 2002; Sartori, 2015), it would be relevant to investigate the impact of forest loss and fragmentation on the abundance and distribution of this tree species, and how this may affect mammal populations.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.qnk98sfjv (Kaisin et al., 2022).

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