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COMPORTAMENTO, HORMÔNIOS FECAIS E CONDIÇÃO ENERGÉTICA: UMA AVALIAÇÃO SOBRE COMO MICOS-LEÕES-DA-CARA-DOURADA LIDAM COM FLORESTAS DEGRADADAS

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BEHAVIOR, FECAL HORMONES AND ENERGETIC CONDITION: AN ASSESSMENT OF HOW GOLDEN-HEADED LION TAMARINS COPE WITH DEGRADED FORESTS

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RESUMO

Os ajustes comportamentais e fisiológicos às mudanças ambientais são essenciais para os primatas não humanos já que permitem responder à perturbação do habitat. Entre os fatores que são alterados devido a degradação antrópica do habitat, a qualidade e a quantidade de alimentos determinam em grande proporção a viabilidade de populações ameaçadas de primatas. Os glicocorticóides (GCs) e os hormônios tireoidianos (THs) coordenam as respostas dos vertebrados às demandas energéticas e, portanto, são úteis para avaliar o impacto da variação na disponibilidade de alimentos assim como os mecanismos de enfrentamento que possibilitam a resiliência a perturbações ambientais imprevisíveis. O primeiro capítulo desta tese explora as relações bidirecionais entre GCs e diversos atributos comportamentais, com base na literatura disponível sobre primatas cativos e selvagens. Ao agrupar tais relações, procuro desvendar os fatores que tendem a promover ou inibir as respostas ao estresse. Os resultados desta análise demonstram que a categoria de forrageamento, a qual compreende atributos que afetam diretamente o balanço energético, se relaciona a uma redução do estresse mediado pelos GCs. Essa redução é menor do que a associada a categoria de afiliação, que reúne atributos relativos ao suporte social e outras interações sociais positivas. As categorias que agrupam interações agonísticas entre coespecíficos e, por outro lado, comportamentos autodirigidos que denotam frustração sugerem um aumento nos níveis de GCs, onde a primeira categoria tem um aumento mais acentuado nas respostas ao estresse. Os próximos capítulos foram dedicados ao estudo de micos-leões-da-cara-dourada (*Leontopithecus chrysomelas*, MLCD) que habitam fragmentos degradados de Mata Atlântica inseridos em uma matriz agrícola. O segundo capítulo oferece uma validação biológica para a medição de metabólitos de GCs (fGCs) e triiodotironina (fT3) em fezes secas de MLCDs. Para os fGCs, foram comparadas as concentrações entre as amostras coletadas durante os procedimentos de captura e durante os dias regulares de monitoramento dos grupos. Essa comparação mostrou uma elevação significativa dos fGCs em amostras coletadas na captura e, portanto, verificou a influência de estressores agudos. Por outro lado, os níveis mais elevados de fT3 em indivíduos com uma melhor condição corporal, confirmou a ligação entre os THs e o estado nutricional desta espécie. Finalmente, o terceiro capítulo examina os padrões comportamentais e os níveis de fGCs e fT3 em MLCDs durante o monitoramento regular dos grupos, ao mesmo tempo que considera as flutuações temporais na disponibilidade das espécies vegetais preferidas pelos MLCDs. Particularmente, os níveis de fGCs se relacionaram positivamente ao tempo de descanso e negativamente ao tempo gasto em permanecer parado. Por outro lado, o tempo dedicado a se alimentar de frutos e a disponibilidade de flores de *Symphonia globulifera* foram associados, respectivamente, a um aumento e uma redução nos níveis de fT3. Em conjunto, estes resultados revelam um claro contraste entre estratégias energéticas, onde a secreção de GCs modula as respostas relativas à conservação da energia enquanto os THs regulam a aquisição energética. No geral, este trabalho fornece uma visão sobre as respostas comportamentais e fisiológicas em primatas, destacando seu papel em lidar com os desafios energéticos específicos enfrentados por uma pequena espécie ameaçada em um habitat altamente fragmentado.

Palavras-chave: respostas ao estresse, glicocorticoides, triiodotironina, balanço energético, disponibilidade de frutos, *Leontopithecus*, florestas fragmentadas, sul da Bahia, meta-análise, validação biológica, captura

SUMMARY

Behavioral and physiological adjustments to environmental change are essential for non-human primates because they allow responding to habitat disturbance. Among the factors altered by anthropogenic habitat degradation, food quality and quantity determine, to a great extent, the viability of threatened primate populations. Glucocorticoids (GCs) and thyroid hormones (THs), mediate vertebrate responses to energetic demands and thereby, are useful to assess the impact of variation in food availability and the potential coping mechanisms that allow resilience to unpredictable perturbations. In the first chapter of this thesis, I explore the bidirectional relationships between GCs and diverse behavioral traits, based on available literature on captive and wild primates. By grouping such correlates, I attempt to unveil the factors that show a tendency to promote or, conversely, inhibit the stress responses. The results of this analysis demonstrate that the foraging domain, comprising traits that directly affect energy balance, relate to a reduction in the stress response mediated by GCs. This reduction was smaller in comparison to the effect of the affiliative domain, used to categorize social support and other positive social interactions. Behavioral domains that pooled agonistic interactions between conspecifics and, on the other hand, self-directed and frustrative behaviors suggest a rise in the stress responses, with the first domain associated to a greater increase in GC levels. The next chapters were devoted to the study of golden-headed lion tamarins (*Leontopithecus chrysomelas*, GHLT) inhabiting degraded fragments of Atlantic Forest embedded in an ever-changing agricultural matrix. The second chapter offers a biological validation for the measurement of GC and triiodothyronine metabolites (fGCs and fT3) in dried feces of GHLTs. For fGCs, this was accomplished by comparing the concentrations between samples collected during capture procedures and regular group monitoring days. This comparison showed a significant elevation of fGCs in samples taken during capture, and thus the influence of severe stressors in mounting the stress response of this species. Conversely, I found higher fT3 levels in individuals with a better body condition which confirmed the known link between THs and nutritional status. Finally, in third chapter, I examine the behavioral patterns and fGC and fT3 levels of GHLTs during regular group monitoring, while considering the temporal fluctuations in the availability of preferred plant food species. Notably, time spent resting was related positively and remaining stationary negatively to fGCs. Conversely, time dedicated to feeding on fruits and availability of *Symphonia globulifera* flowers were respectively associated to an increase and a reduction in fT3. Jointly, these results reveal a clear contrast between energetic strategies where secretion of GCs modulate energy-saving responses and THs energy-intake responses in the GHLTs of this study. Overall, this work provides an insight into the primate behavioral and physiological responses highlighting their role in coping with the specific energetic challenges confronted by a small, endangered species in a highly fragmented habitat.

Keywords: stress responses, glucocorticoids, triiodothyronine, energetic balance, fruit availability, *Leontopithecus*, fragmented forests, south of Bahia, meta-analysis, biological validation, capture

GENERAL INTRODUCTION

Degradation and fragmentation of habitats and their impact on plant food sources

Changes in land use, and ensuing habitat modifications to accommodate human activity have altered the landscape, and consequently the distribution and abundance of species, disrupting the biological interactions that sustain functioning ecosystems (Bennett and Saunders, 2010). For instance, when habitat fragmentation changes the microclimatic condition around forest patches, so called edge effects (Saunders et al., 1991), it increases tree damage and mortality in the edges in comparison to the forest interior (Laurance et al., 2000), promoting the expansion of secondary vegetation dominated by pioneer trees and vines (Laurance et al., 1998). In addition to that, fragmentation and the associated edge effects can modify flower production and, thereby, maximize or minimize the availability of fruits for forest consumers (Laurance et al., 2003; Morellato et al., 2016). This carries consequences for the establishment and recruitment of fruiting trees, as many of those consumers fulfill the important role of seed dispersers (Nathan and Muller-landau, 2000), further exacerbating the effect of fragmentation and degradation on the structure and diversity of plant community (Murcia, 1995). Habitat disturbance can affect forest biodiversity by many other means, for example landscape deforestation can drive forest succession to early stages where biotic community is less complex and diverse (Rocha-Santos et al., 2016). One group of forest dwelling animals that may be particularly challenged by changes in food access because of habitat disturbance are primates. For primates, living in modified forest fragments implies facing a less suitable habitat of reduced size and increased isolation where food availability may depend on an altered vegetation composition (Arroyo-Rodríguez and Dias, 2010; Cristóbal-Azkarate and Arroyo-Rodríguez, 2007). Consequently, in order to meet daily energetic demands, primates need to adapt their diet and behavioral patterns to the environmental conditions imposed by their degraded habitat (Bicca-Marques, 2003).

Energy balance and primate behavioral flexibility

Energy is a critical factor that links organisms to their environment. Therefore, the study of the ways in which primates acquire, process, and use energy can help behavioral ecologists define more accurately what strategies may allow adaptation and long-term survival in the face of habitat disturbance and fragmentation (Emery Thompson, 2016; Karasov, 1986). To consider the energy framework, Emery Thompson (2016) indicates that studies can tackle their questions by evaluating three dimensions: 1) food quality, distribution and availability which overall refer to the spatial and temporal possibilities of accessing energy and nutrients in the environment (Strier, 2017), 2) energy intake and expenditure, the first of which estimates the absorption of calories through food while the second represents the use of calories to perform daily physical activities, reproduce, grow and maintain bodily functions (McGrosky and Pontzer, 2023) and finally 3) energy balance which captures the difference between energy intake and expenditure. Time is also another limiting factor influenced by food supply that may require primates to adjust their activity budgets in response to habitat disturbance or seasonal food scarcity (Ménard et al., 2013). Nevertheless, when it comes to managing energy and time allocation, most primates exhibit a broad range of responses to variations in food access. Behavioral flexibility in primates can be represented by dietary adjustments such as feeding on a high diversity of food items, switching among different food items on diverse timescales, or including exotic plants and agricultural crops in their diet (Lambert and Rothman, 2015; McLennan et al., 2017). Additionally, as most primate species live in social groups and form strong bonds with group members (van Schaik, 1983), they also make use of socioecological adjustments including changes in activity and ranging patterns, group organization and size, and mating systems (McLennan et al., 2017) which may allow balancing the costs of intra- and intergroup competition (Markham and Gesquiere, 2017).

Non-invasive physiological markers of energetic stress

When assessing primates' behavioral responses to environmental perturbations, it may be difficult to predict the outcome of such responses due to the lack of a proximate mechanism to explain individual fitness (Behringer and Deschner, 2017; Cooke et al., 2013). This problem in particular can be addressed by integrating the study of physiological responses to ecological models about environmental factors and behavior (Cooke et al., 2014). Thus, physiology may not only provide a mechanistic understanding about the impact of habitat disturbance and behavioral strategies at individual level but also permit anticipating their potential consequences at population level (Dantzer et al., 2014). Among the physiological mediators that participate in the metabolic processes to regulate energy balance (Kim, 2008; Sapolsky et al., 2000), two of them, glucocorticoids (GCs) and thyroid hormones (THs), have attracted the attention of researchers, partly due to the possibility of measuring their respective metabolites in samples collected non-invasively from wild primate populations (Behringer and Deschner, 2017).

Although both GCs and THs are associated to energetic stressors, they offer complementary information given that they may respond differently to certain stimuli (Dias et al., 2017; Wasser et al., 2010) and reflect different aspects of energy metabolism (Touitou et al., 2021). GCs, on the one hand, constitute one of the most extensively investigated hormones used to study stress responses. GC secretion is activated by the hypothalamic-pituitary-adrenal (HPA) axis to mobilize energy necessary to face a wide variety of physical, metabolic, and psychological challenges (Emery Thompson, 2016). Hence, GCs respond to predictable changes that occur routinely (e.g.: digestion, reproduction, daily and seasonal cycles), and unpredictable ones which, if not overcome, could lead to chronically elevated GC levels, and have deleterious health and fitness effects (Boonstra, 2013; Reeder and Kramer, 2005; Wingfield, 2013). In response to energetic constraints, specifically, GC increases are expected to amplify the use of energy reserves (Sapolsky et al., 2000).

On the other hand, triiodothyronine (T3), the biologically more active form of THs, modulates the metabolic rate in vertebrates through the hypothalamic-pituitary-thyroid (HPT) axis (Behringer et al., 2018) and is sensitive to alterations in the quantity and quality of ingested food (Eales, 1988). However, unlike GCs, T3 appears to be less sensitive to psychosocial stressors and thus, is probably more directly related to nutritional deficits (Dias et al., 2017; Emery Thompson, 2016; Wasser et al., 2010), in response to which it normally decreases as a means of saving energy (Cristóbal-Azkarate et al., 2016).

When analyzing sample matrices such as feces and urine, it is important to consider that measurements reflect the concentration of metabolites which were largely metabolized by the liver from circulating hormones before being excreted (Palme et al., 2005; Sheriff et al., 2011). For this and other technical reasons (see Touma and Palme, 2005), assays used to measure hormone metabolites need an additional step to show their biological significance for the matrix and species investigated. Given that fecal and urine samples can accumulate the metabolites produced over several hours, non-invasive physiological measurements soften the effect of short-term elevations (Emery Thompson, 2016) and provide an integrated picture of hormone variation (Dantzer et al., 2014). This may be desirable if the aim is to explore the influence of stressors that act on a broader time scale such as food shortages or the potential effects of chronic stress for the conservation of threatened populations.

Golden-headed lion tamarins of fragmented habitats

Golden-headed lion tamarins (GHLTs, *Leontopithecus chrysomelas*) are small callitrichids endemic to the Southern Bahian Atlantic Forest in Brazil (Pinto and Rylands, 1997). They are listed by the International Union for Conservation of Nature (IUCN) as

Endangered (Oliveira et al., 2021) given their restricted geographic distribution and the constant reduction and degradation of their habitat due to forest clearance for timber, agriculture, and cattle ranching (De Vleeschouwer and Raboy, 2013). In fact, a recent study about GHLT's current distribution indicated that previous demographic parameters were underestimated and with this, it demonstrated a reduction of 42% and 60% of GHLTs' area of occupancy and population size, respectively, over the past 30 years (Teixeira, 2022).

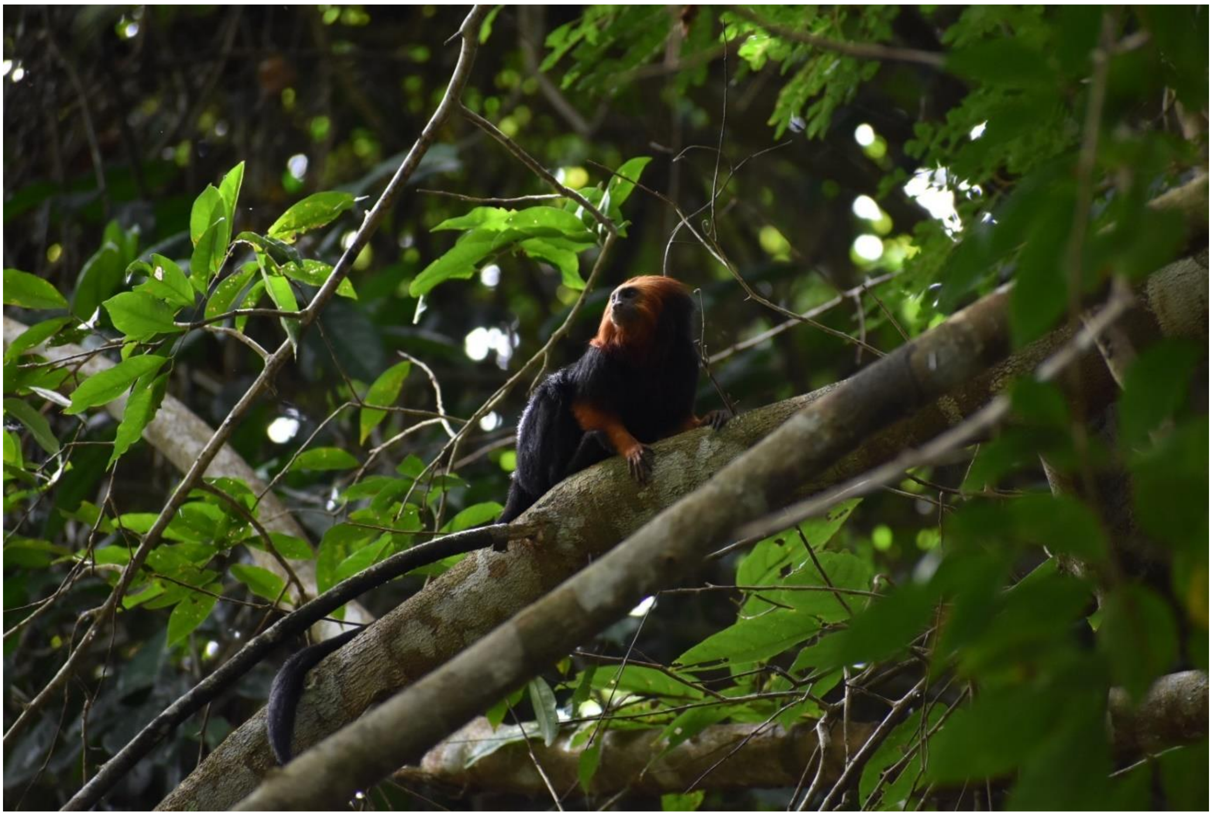

Figure 1. Adult golden-headed lion tamarin (*Leontopithecus chrysomelas*) living in a fragmented forest in Una - Bahia, Brazil (personal photo collection).

GHLTs are classified as frugi-faunivores since their diet comprises mostly fruit and animal prey with smaller amounts of flower nectar and gum (Catenacci et al., 2016; Raboy and Dietz, 2004). Most GHLT populations are found in the eastern portion of their distribution range (Zeigler et al., 2010), where they normally occupy shade-cocoa agroforests, locally known as cabrucas, with interconnecting patches of mature and regenerating forests, although both cabrucas and forest patches have been unceasingly jeopardized by land-use intensification (De Vleeschouwer and Raboy, 2013; Raboy et al., 2010). Activity patterns and resource use in this species largely depends on the habitat type being used (Oliveira et al., 2011; Raboy et al., 2004). In general, however, GHLTs devote a major part of their time budget to foraging for prey in different substrates, especially bromeliads (Catenacci et al., 2016) and sleep in tree holes which are more frequently found in mature forests (Oliveira et al., 2010; Raboy et al., 2004). In severely fragmented habitats, GHLTs may have to deal with additional energetic constraints given the low floristic richness of these areas which may lead to more significant variations in fruit availability (Catenacci et al., 2016; Pessoa et al., 2012). Additionally, the diminished vagility of non-forest matrix in human-dominated landscapes (Raboy et al., 2010) may force GHLTs to settle in low quality habitats or ecological traps with limited food sources and reproductive options (Robertson and Hutto, 2006) where they may be more susceptible to local extinction. Studies of GHLTs living in degraded forests and cocoa agroforests have reported the smallest home ranges for the species, the consumption of crop plantations, the habitual use of different agricultural areas to forage and the smallest proportions of time dedicated to resting in exchange to higher rates of foraging substrates (Coutinho, 2018; Oliveira et al., 2011). These results may highlight the species behavioral flexibility to cope with degraded habitats, as long as sufficient resources are available. However, Costa et al. (2022, 2020) have documented the presence of intestinal parasites in GHLTs living in fragmented forests in contrast to individuals occupying shaded cocoa agroforests or cabrucas, where this parasitic infection was absent. This difference could be related to the restricted home range and therefore, re-infection of individuals in more disturbed habitats, through the repeated use of the same travel routes. Furthermore, Costa (2019) found hemoparasites in the blood samples of 68% of tested individuals (N = 21), including those from fragmented forests, although none of the infected GHLTs showed alterations in hematological parameters. Taken together, these findings suggest that GHLTs residing in fragmented landscapes may already suffer the consequences of a poor habitat quality which may be taking its toll on the individuals’ health despite their apparent capacity to adapt to such environmental conditions. Non-invasive endocrine monitoring is an ethically appropriate way to better understand the complex link between energy balance, the expression of behavior and ultimately individual fitness and survival in disturbed habitats.

Figure 2. View of forest and agricultural matrix close to a lake and Project BioBrasil’s field station (photo by K. De Vleeschouwer).

Overall objective, chapters, and goals

The overall objective is to understand the energetic strategies adopted by GHLTs to cope with the environmental challenges associated with living in degraded and fragmented habitats. To this end, I explore some of their behavioral and physiological responses, focusing primarily on GCs and T3.

CHAPTER 1 synthesizes, through a metanalysis, the correlations between GC levels and diverse behavioral traits compiled from the literature on wild and captive primates. By evaluating the strength and direction of cumulative effect sizes, I uncover which factors have a more profound impact on GC-behavior interactions and may lead to an intensification or alleviation of the stress responses associated to GC secretion.

The following two chapters are devoted to the study of GHLT groups inhabiting a highly anthropized and fragmented landscape. These groups are part of a long-term research program conducted by Project BioBrasil and were also studied by Costa et al. (2022), (2020) and Coutinho (2018).

CHAPTER 2 validates the use of enzyme immunoassays (EIA) to measure fecal metabolites of GCs and T3 in samples from wild GHLTs. In this chapter, my goal is to demonstrate the biological validity of these measurements by showing the response of fecal GCs and T3 to the stress of capture and investigating the influence of body condition and other intrinsic factors.

Finally, **CHAPTER 3** combines data on the behavior and the levels of fecal GCs and T3 with estimates for the availability of preferred plant food species composing the diet of the GHLTs. In this chapter, I assess the behavioral and physiological adjustments to daily and seasonal energetic demands while controlling for changes in food availability in an effort to reveal the strategies that may help GHLTs to cope with the ecological challenges in degraded forest remnants.

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CHAPTER 1

Behavioral responses to glucocorticoids in non-human primates: a meta-analytic approach to unveil potential coping mechanisms

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ABSTRACT

Glucocorticoids (GCs) have allowed researchers to investigate how vertebrates respond to challenges in their physical and social environment. Research efforts, however, have been disproportionately concentrated on the detrimental consequences of sustained GC secretion and often overlooked the adaptive outcome of stress responses. Exploring the interaction between GCs and behavior can also offer insights into the potentially beneficiary effects of certain behavioral responses and help identify coping mechanisms that may enhance individual fitness. We conducted a meta-analysis of the behavioral correlates of GC variation described in the literature devoted to captive and wild primates. By grouping these correlates within behavioral domains while controlling for differences in sex, age, sample matrix, method, setting and species, we assessed the overall magnitude and direction of such GC-behavior interactions and revealed what type of behavior tends to promote or inhibit the stress response as shown by GC change. We found that foraging and affiliative behaviors facilitate stress alleviation, with the former having a stronger negative effect on GC levels. This suggests that although social support may enable group-living primates to cope with some stressors, energy balance permeates stress responses and likely prevails over social support in circumstances where stressors are linked to severe habitat degradation and food scarcity. Conversely, agonistic and anxiety-like behaviors mediate an intensification of the stress response, which may indicate that the increased expression of such behaviors is connected to environments that are perceived as challenging by primates.

Keywords: stress response, behavior, coping, cortisol, stress alleviation, non-human primates, captive, wild, correlations, meta-analysis

1. INTRODUCTION

In the face of unpredictable environmental perturbations, vertebrates rely on a set of behavioral and physiological responses that diverts them from their normal life history stage and increases their chances for survival in the best adaptive way (Charmandari et al., 2005; Wingfield and Kitaysky, 2002). These perturbations, which can be short- or long-termed, are known as stressors (Levine and Ursin, 1991). Such stressors activate response mechanisms, that may result in physiological and/or behavioral changes called stress responses (Beehner and Bergman, 2017). From the perception of a stressor to its associated stress response, there are specific physiological systems involved, one of the most studied is the hypothalamic-pituitary-adrenal (HPA) axis (McEwen and Wingfield, 2003). As soon as the HPA-axis is activated by sensory stimuli in the brain, it triggers a cascading reaction that leads to the release of neuroendocrine factors which in turn stimulate the adrenal glands to synthetize and secrete many hormones, including glucocorticoids (GCs) (McCormick and Romero, 2017). When an individual is confronted with environmental and/or social challenge, GCs enter the bloodstream causing the allocation of glucose through the conversion of protein and lipids to usable carbohydrates and the restoration of homeostasis by momentarily restricting non-essential processes such as growth, reproduction, and immune function (Markham and Gesquiere, 2017; McEwen and Wingfield, 2003). However, GCs' role is much more elemental than solely coordinating vertebrate stress response: they are also involved in basic energy acquisition, deposition, and mobilization (Busch and Hayward, 2009) linked to normal daily and seasonal energetic demands.

At the behavioral level, GCs along with other hormones and neurotransmitters motivate or inhibit the expression of multiple behaviors. For instance, they can promote locomotion and food-seeking behaviors by stimulating the appetite for food (Leibowitz and Wortley, 2004; Sapolsky et al., 2000), thereby influencing the activity budget. Another effect of GCs is associated with moving away from a stressor or finding a shelter during potential life-threatening situations (Wingfield et al., 1998) such as the presence of a predator or anthropogenic disturbances. They also enhance the state of vigilance caused by fear or anxiety about well-defined or imaginary adversities (Korte, 2001; McEwen and Wingfield, 2003). Finally, GCs can modulate social behavior in both non-social and social species by influencing territorial defense, sexual reproduction, aggression, parental care and affiliation (Creel et al., 2013; Raulo and Dantzer, 2018). This cursory list of associations between GCs and behavioral responses only highlights the GCs potential to ensure survival and lifetime breeding success, as long as GC levels are produced within the normal reactive range needed to respond to predictable and unpredictable environmental changes (Romero et al., 2009).

Despite many fitness-enhancing benefits, research effort devoted to GCs has been overly focused on establishing the causes of variation and the detrimental effects of GC secretion (Beehner and Bergman, 2017). Particularly, the negative side of a stress response emerges when an elevation in GCs is above the normal reactive range and extends over long-time intervals because one or more stressors persist, or the individual is unable to escape such stressors (Wingfield and Kitaysky, 2002). This range of GC levels is referred to as homeostatic overload (Romero et al., 2009). In addition to somatic damage linked to immunosuppression, muscle wasting, and reduced fertility (Sapolsky, 2002), this type of chronic GC elevation gradually impairs an individual's ability to face additional perturbations (Edes and Crews, 2017). Consequently, short-term increases of GCs will likely generate adaptive stress responses, while long-term increases may result in damaging health effects for the individual facing chronic stressors.

Concerning stress research dedicated to primates, Beehner and Bergman (2017) indicate that most studies conducted in wild populations evaluated the influence of dominance rank and anthropogenic disturbances on GC variation. The relationship between dominance rank and GCs has often provided contradictory results. However, most reviews examining these relationships agree that both high- and low-ranking positions entail energetic demands associated with different challenges which for dominants involve attaining and maintaining their high rank, whereas for subordinates they imply limited access to food sources and reduced opportunities for social support (Abbott et al., 2003; Emery Thompson, 2017; Goymann and Wingfield, 2004). These differences among social status can be further elucidated if male and female social competition are separately examined and temporal dynamics of HPA axis regulation taken into consideration (Cavigelli and Caruso, 2015). Conversely, regarding anthropogenic disturbances, Kaisin et al. (2021) have shown, through a global meta-analysis, that primates living in disturbed areas exhibit higher GC levels especially where habitat loss and hunting are the predominant human pressures. In that regard, GCs study can contribute to addressing other conservation questions, such as which behavioral responses are used by primates to cope with the unrelenting and severe anthropogenic impacts on their habitats.

A coping mechanism can be defined as a behavioral response that has been shaped across evolution to alleviate the effects of a stressor and induce a reduction in a physiological measure of stress like GCs (Cheney and Seyfarth, 2009; Wechsler, 1995). It describes a hormone-behavior interaction that enables an individual not only to respond to physical and psychological perturbations but also to avoid the deleterious reverberations of stress (McEwen and Wingfield, 2003). Yet a coping mechanism may only be successful if the setting does not prevent the individual from performing the coping behavior (Wechsler, 1995). Aside from fight-flight (Cannon, 1915) and freeze responses (Engel and Schmale, 1972) which facilitate an immediate and short-lived action against a threat (Wingfield et al., 1998), most authors recognize three groups of behaviors that have an enduring development and can serve as coping strategies, namely affiliative, aggressive, and self-directed behaviors (Gustison et al., 2012). For example, under naturalistic conditions, fecal glucocorticoid levels of female chacma baboons (*Papio ursinus*) who lost a close relative returned to base levels after they increased their grooming frequency and expanded the number of grooming partners (Engh et al., 2006a). Moreover, lactating females of the same species that formed close affiliative relationships with long-term resident males exhibited lower GCs than females without a male friend when a potential infanticidal dominant male immigrated to the group (Beehner et al., 2005; Engh et al., 2006b). Similarly, wild male olive baboons (*Papio anubis*) that rely on certain behavioral strategies, such as pair-bonding interactions, displacement aggression redirected to bystanders, and having the ability to distinguish threatening from neutral rival interactions or positive from negative outcomes following a fight, have lower basal GC concentrations irrespective of their dominance rank (Ray and Sapolsky, 1992; Sapolsky and Ray, 1989; Virgin and Sapolsky, 1997). Evidence of this type has led authors to conclude that in addition to strengthening social support, coping mechanisms may help individuals to regain some predictability and control over their social environment (Cheney and Seyfarth, 2009; Levine and Ursin, 1991; Sapolsky, 2004).

As with any hormone-behavior interaction, GCs and coping behaviors have a bidirectional and indirect relationship, meaning that they affect each other by altering the probability of a certain behavioral or physiological outcome in the presence of a specific stimulus and context (Korte, 2001; Nelson and Kriegsfeld, 2017; Romero and Wingfield, 2016). These interactions likely involve complex feedback loops between the individual’s internal and external environment (Romero et al., 2009). In fact, Johnstone et al. (2012) indicates that the associations between stress physiology and behavior in vertebrate research generally lack consensus and suggests that both mechanisms may be largely modulated by contextual components such as season, stressor type, adaptation to local conditions, life-history stage, and others (Davis et al., 2008). Furthermore, given that GC production can be triggered by a variety of metabolic, physical, and psychological challenges, the difficulty of isolating the influence of a particular stressor (Emery Thompson, 2017) or finding causal relationships between GCs and behavior outside the laboratory (Cheney and Seyfarth, 2009) comes as no surprise.

Here we examine how GCs are linked to a set of different behavioral traits found in research articles dealing with both captive and wild primates. By uncovering the patterns that arise from these interactions, we aim at better understanding how behaviors relate to stress alleviation or intensification, as measured by GC variation. To do so, we first classify certain behavioral traits (see Table 2) into four domains: affiliative, agonistic, anxiety-like, and foraging. Subsequently, we evaluate the strength and direction of each domain. We predict that affiliative and anxiety-like behaviors will produce negative cumulative effect sizes since these domains generally comprise coping reactions to psychosocial stressors (Cheney and Seyfarth, 2009; Maestripieri et al., 1992). Similarly, given the role of GCs in energy acquisition (McEwen and Wingfield, 2003), foraging domain will likely generate a negative effect size. In contrast, we expect that agonistic domain will reveal a positive effect size because of the cost of engaging in agonism that may become more apparent as aggressive interactions increase (Creel et al., 2013). In addition to behavioral domain, we test other factors that may affect GCs and behavior interactions, including sex, age, sample matrix, type of setting and type of method (see Table 1).

2. METHODS

2.1. Datasets

We performed systematic searches in Web of Science [v. 5.35] and Scopus indexing databases until November 17th and 11th, 2021, respectively. In both databases, we used the search string '**primate* AND (cortisol OR glucocorticoid*)**' and limited the search to research articles published in English. This simple combination of terms allowed a more comprehensive inclusion of articles that studied some aspect of GCs secretion in primates with different aims over a great breadth of research fields. This initial search yielded 1206 articles in Web of Science and 1176 in Scopus. To refine this collection of articles, we employed a first structured selection criteria described in Suppl. text 1, after removing duplicates the number of articles reduced considerably to 444. Each of these was thoroughly examined in order to detect and extract correlational data on the association between a diverse array of behavioral traits and GCs levels.

As a second structured selection criteria, we kept articles that measured quantitatively behavioral traits through frequency or time engaged in certain behavior, and personality scores that captured continuous behavioral traits. As a result, most of the data extracted were correlation coefficients (Pearson - r, Spearman - r_s and Kendall - r_k), partial coefficients (r_p), coefficients of determination (R^2) from simple linear regressions, and slopes or estimates (ß) along with their standard errors (SE) or t-values obtained from regression models. Whenever available we also took note of the significance values (p-value) tied to all measures. While extracting these measures, we recorded the sex, age, and the number of individuals from whom the GCs were determined and used the latter as the sample size for the meta-analytical techniques. Each statistical measure was further encoded with the respective primate species, type of sample matrix involved in the measurement of GCs, type of method applied and the type of setting that surrounded the subjects in study (see Table 1 for more details). Within the "captive" category, we included semi-free ranging and introduced populations since we consider that these populations are largely dependent on humans to survive, like the colony of rhesus macaques in Cayo Santiago that although living freely on this island, it is regularly provisioned with monkey chow (Higham and Maestripieri, 2014). After applying these criteria, the number of scientific articles reduced to 156, which altogether comprised 936 measures about the association between GCs and target behavioral traits.

Table 1. Factors that may affect GC-behavior interactions with the respective categories assigned to each statistical measure and used to conduct both the descriptive analysis and the meta-analysis.

FACTORS	CATEGORIES
Behavioral domain	Affiliative, Agonistic, Anxiety-like, or Foraging
Sex	Females, Males, or Males and females
Age	Adults, Adults and juveniles, Adults and subadults, Infants, Juveniles, Subadults, Subadults and juveniles, or All ages
Sample matrix	Blood, Cerebrospinal fluid, Feces, Hair, Saliva, or Urine
Type of setting	Captive or Wild
Type of method	Experimental or Observational

Following this procedure, the whole dataset was further refined by the third exclusion criteria, first to retain only measures that reported the direction of their relationship, and second to remove measures involving behavioral traits that did not fit into the domains, later described. Hence, the new dataset included 138 studies comprising 756 measures in total. We used this dataset to describe the overall characteristics of the published literature on primates that correlated the secretion of GCs with the expression of the behavioral traits here considered.

With the sole purpose of carrying out the meta-analysis, we created a second dataset that included only correlation coefficients, coefficients of determination, partial correlations, and t-values. We selected these measures based on their prevalence in the complete dataset (62.5% of all measures) and the availability of additional information in the primary studies to be able to perform the pertinent conversions. Thus, we transformed r_s (Eq. 1), r_k (Eq. 2), and R^2 (Eq. 3) to Pearson correlations and t-values (Eq. 4) to partial correlations (Lajeunesse, 2013).

$r = 2\sin\left(\frac{\pi r_s}{6}\right)\ if\ n < 90\ \ or\ \ r = r_s\ if\ n \geq 90\ where\ n\ is\ the\ sample\ size$ (Eq. 1)

$r = \sin\left(\frac{\pi r_k}{2}\right)$ (Eq. 2)

$|r| = \sqrt{R^2}\ if\ sign\ of\ correlation\ was\ reported$ (Eq. 3)

$r_p = \frac{t}{\sqrt{t^2+df}}\ where\ df\ is\ degrees\ of\ freedom$ (Eq. 4)

Our measures now consisting of Pearson correlations and partial correlations (Harrison, 2011) were standardized to Fisher's z scores (Z-scores) to obtain the point effect sizes used in the meta-analysis (Eq. 5). To obtain the sample variance (V_z) for every point effect size, we excluded measures estimated with a sample size of less than four individuals and calculated the variance for each Z-score with Eq. 6 (Rosenberg et al., 2013).

$Z = \frac{1}{2} ln\left(\frac{1+r}{1-r}\right)$ (Eq. 5)

$V_z = \frac{1}{n-3}$ (Eq. 6)

This final cut resulted in the inclusion of 103 research articles with 585 measures. The complete procedure of this section and selection criteria employed to achieve the two datasets are summarized in Figure 1.

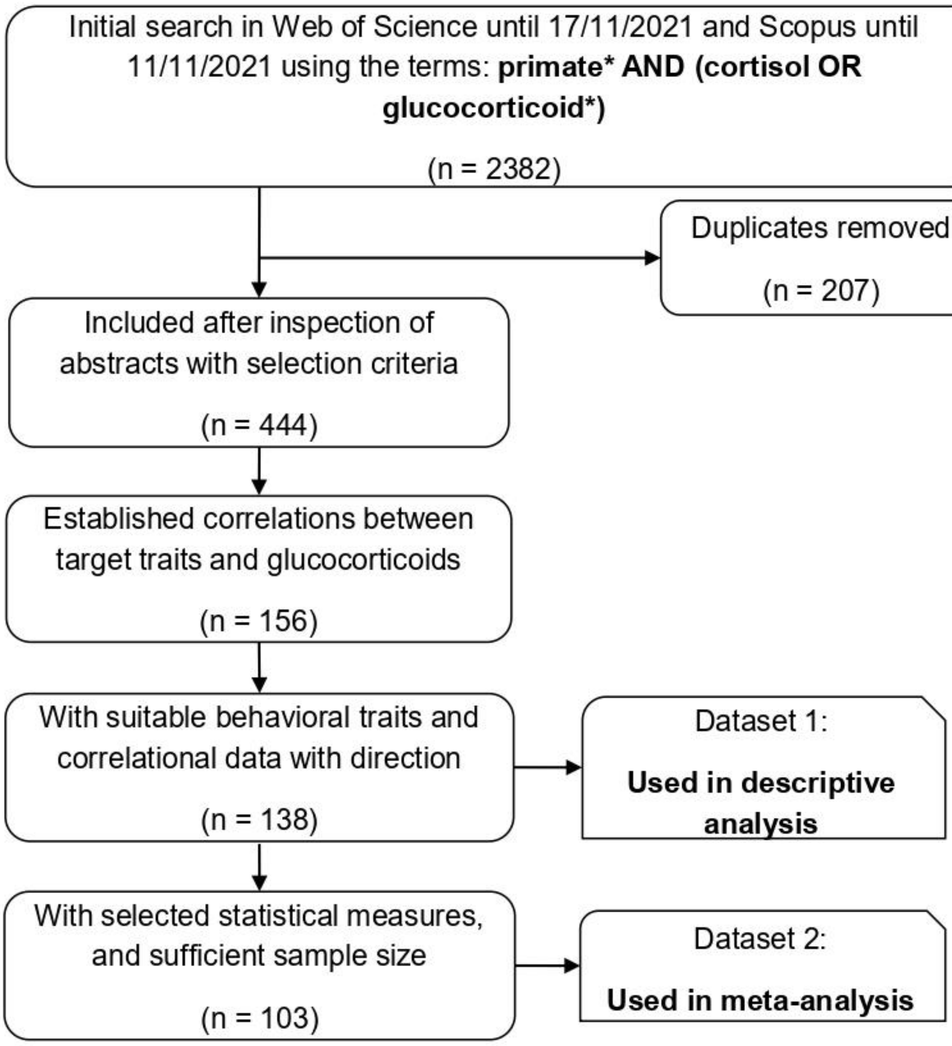

Figure. 1. Flowchart describing search terms, inclusion, and exclusion criteria used to create two datasets with distinct purposes, where n is the number of studies.

2.2. Behavioral domains

The different behavioral traits used in this meta-analysis to choose studies and extract data were sorted into four broad-sense domains: “Affiliative”, “Agonistic”, “Anxiety-like” and “Foraging”. Each domain is described in Table 2, together with the selected traits and our expectations. We tried as much as possible to offer an objective categorization of traits. For that, we consulted the ethograms and methods in the primary studies and excluded traits that failed to be incorporated into a category because of ambiguity. We acknowledge that pooling such wide range of traits into a single category may lead to missing out many of the nuances and particular influences of behavior on the physiological response. However, the aim here was to reveal the general magnitude and direction of effect that emerge from such pooled domains.

Table 2. Description of the behavioral domains used to classify the selected traits found in the literature and expected direction of their relationship with glucocorticoid variation.

BEHAVIORAL DOMAIN	SELECTED TRAITS	EXPECTED DIRECTION
AFFILIATIVE: depicts social support as a positive interaction between two conspecifics, also including parental care and sexual pair-bonding.	Grooming received or given, social plucking, social play, time spent in contact or proximity of a neighbor, embracing, group huddling, tension-reducing behaviors, exchanging affiliative vocalizations, index of sociality or sociability, time spent near mother, infant-carrying, infant-cradling, nursing, infant handling, licking or sniffing, copulating, mounting, rate of genital contact, etc.	NEGATIVE
AGONISTIC: groups aggressive or submissive interactions denoting conflict between conspecifics within or outside social group.	Aggression received or given, agonistic interactions, contact or noncontact aggression, submission, aggressive or submissive displays, frequency of chases, escapes, threats or fear grimaces, intergroup offense or defense, number of visible injuries, dominance, confidence or aggressiveness index, maternal or paternal aggression, etc.	POSITIVE
ANXIETY-LIKE: pools self-directed and frustrative behaviors or stereotypies that normally indicate impaired welfare and/or psychological distress.	Scratching, self-grooming, scent marking, locomotion, exploration of novel environment, approaching or moving away from an external stimulus, alarm calling, self-plucking, self-mutilation, self-biting, solitary rocking, stereotypic movements, rate of certain vocalizations (cry, screech, phee call, peep call, twitter, etc.), piloerection, yawning, urine wash rate, neuroticism or anxiousness index, time spent depressed or alone, maternal responsiveness to infant distress, maternal protectiveness, infant anxiety, frustrative behaviors, etc.	NEGATIVE
FORAGING: includes any maintenance behavior or environmental factor that increases the likelihood of obtaining a positive energy balance.	Energy balance, energy intake, foraging effort, feeding time, feeding on certain food items (leaves, fruits, insects, flowers, vines, etc.), protein intake, non-protein intake, travel time or distance, home range, resting, food availability, diet quality, fruit or herbaceous biomass, fruit content in feces, feeder use, etc.	NEGATIVE

2.3. Meta-analytic procedure

We performed the analyses in R 4.2.0 with different statistical packages. To overcome problems of nonindependence in the data caused by the use of multiple effect sizes from the same study and species, as well as the phylogenetic relatedness among species (Garamszegi et al., 2013), we applied hierarchical meta-analysis models to our data. With this statistical approach, we consistently relied on defining categorical random interactions to fit a model structure that could effectively deal with the correlated data within different levels, this was made possible by using the MCMCglmm package for generalized linear mixed models based on Bayesian statistics (Hadfield, 2010). To control the phylogenetic effect, we downloaded a consensus tree with all the species in the dataset from 10kTrees Primates (Version 3) (Arnold et al., 2010), converted it to an ultrametric tree with FigTree (v 1.4.4) and added the resulting matrix with phylogenetic distances into the MCMCglmm function. Before fitting the model with the moderators, we tested the combination of random effects, priors, and other model specifications by running random models including only the intercept as fixed effect while checking the model convergence until both intercept and variances of random effects would mix well (Hadfield, 2014). We found that adding the phylogenetic tree improved the deviance information criteria (DIC without phylogeny = 765.24) of our models, so we maintained it throughout the model fitting procedure. Accordingly, the random effect terms in the formula comprised study identity, primate species, the phylogenetic tree and the sample variance for each point effect size which is fundamental for the meta-analysis approach (Hadfield, 2010). Priors were set at $V = 1$ and $\nu = 0.02$, which specify an inverse Gamma distribution with a degree of belief of 0.02, widely used in the literature (Gelman and Hill, 2006). For all the models, we employed a Markov chain Monte Carlo (MCMC) algorithm with 60000 iterations, a burn-in of 10000 and thinning interval of 20, this configuration produced an effective sample size of 2500.

The categorical moderators encoded during the literature review, namely behavioral domain, sex, age, sample matrix, method, and setting, were defined as the fixed effects for the selection of the best model. We started with a full model incorporating all fixed effects and backward selected the models depending primarily on their DIC and the significance values (pMCMC) in the outputs. After each consecutive run, we retained the fixed effects that had at least one significant category. We also obtained separate models for each fixed effect removed while fitting the model and calculated the difference between the null model DIC and the DIC of the fitted models (ΔDIC). In addition to that, we ran univariate models without intercept for every fixed effect term in order to understand how the moderators considered in this study explain the variation in effect sizes. The resulting summaries of this subgroup analysis were used to build a forest plot that presents the cumulative effect size of each moderator category and the pooled effect size of all the studies derived from the null model constructed only with the intercept and the random effects.

2.4. Heterogeneity and publication bias

Because meta-analysis combines multiple effect sizes into a single mean effect size, one must examine the extent to which such measures vary between studies, as well as the bias toward studies with large effect sizes. Consequently, to inspect the influence of heterogeneity, we first created analog models considering the same combination of fixed and random terms with the package metafor (Viechtbauer, 2010) and applied multilevel linear mixed-effects models. We present the values of Cochran's Q-test for residual heterogeneity (Q_E) and the F-test for moderators (F_{mod}) retrieved from the model's outputs. Furthermore, we calculated the values of Higgins & Thompson's I^2 using these analog models, the results were interpreted following Higgins and Thompson (2002). Similarly, to detect the influence of publication bias in our complete set of Z-scores, we once more relied on the tools metafor offers, thus we visually verified the funnel plot asymmetry and obtained the Egger's regression analysis. When the intercept of Egger's analysis is significantly different from zero, we assumed our data show evidence of publication bias. Since we conducted a subgroup analysis to evaluate the influence of the moderators on the correlational measures, we decided to focus on calculating the fail-safe N (Rosenthal, 1979) for categories that generated significant cumulative effect sizes. To do so we split up the set of Z-scores into different subsets based on the respective categories and used the fail-safe N from metafor to estimate the number of missing or unpublished studies needed to render the cumulative effect sizes nonsignificant.

3. RESULTS

3.1. Descriptive analysis

The following analysis was performed based on the statistical measures that evaluated the relationship between primate GCs levels and the behavioral traits of interest. Many primary studies present multiple measures that belong to more than one moderator's category and, consequently, the total sum of studies among categories does not coincide with the total number of studies in the first dataset (138 studies). Most studies measured GCs in adults (95 studies), other less frequent age clusters were adults and subadults (11 studies), infants (10 studies), juveniles (10 studies) and adults and juveniles (nine studies). The rest of the age categories amount to a total of eight studies, which include the categories of subadults, subadults and juveniles, and all ages. Number of studies were balanced between sex categories: males were represented in 53 studies, males and females in 52 studies, and females in 48 studies. The most common sample matrix was feces (72 studies), then comparably fewer studies measured GCs in blood (31 studies), urine (21 studies), hair (11 studies), saliva (five studies) and cerebrospinal fluid (two studies). This dataset included 42 primate species of which *Macaca mulatta* was the most sampled species (22 studies), then *Pan troglodytes* (12 studies) and *Callithrix jacchus* (10 studies). Other species were present in six or fewer studies. Figure 2 accumulates the number of studies per genus and shows how uneven taxonomic representation was.

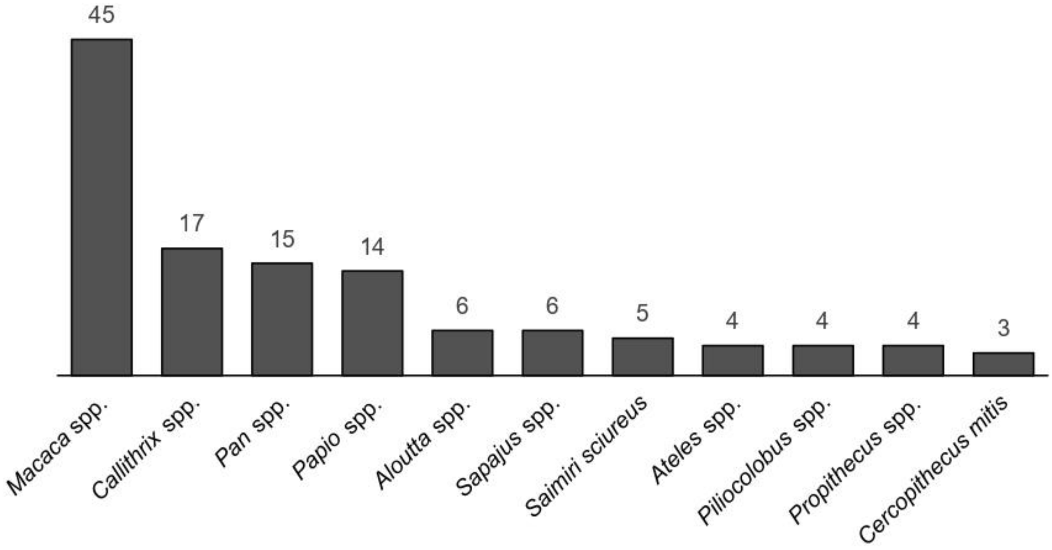

Figure 2. Number of studies used in the descriptive analysis grouped by primate genus (top edge of each bar). Genus represented by one or two studies are not displayed in this graph. Our dataset included 21 primate genera.

Regarding behavioral domains, the number of studies that evaluated the correlation between GCs and behavioral traits in the affiliative (54 studies), agonistic (57 studies) and anxiety-like (55 studies) domains were comparable, while fewer studies addressed foraging domain (39 studies). As for the direction of the behavioral correlates of GCs (i.e. measures), domains showed clear contrasting directions. While agonistic and anxiety-like domains had more measures indicating a positive relationship with GCs, affiliative and foraging domain had more measures with a negative relationship. Most measures in every domain were non-significant, except for the foraging domain and the negative relationship for the affiliative domain (Fig. 3).

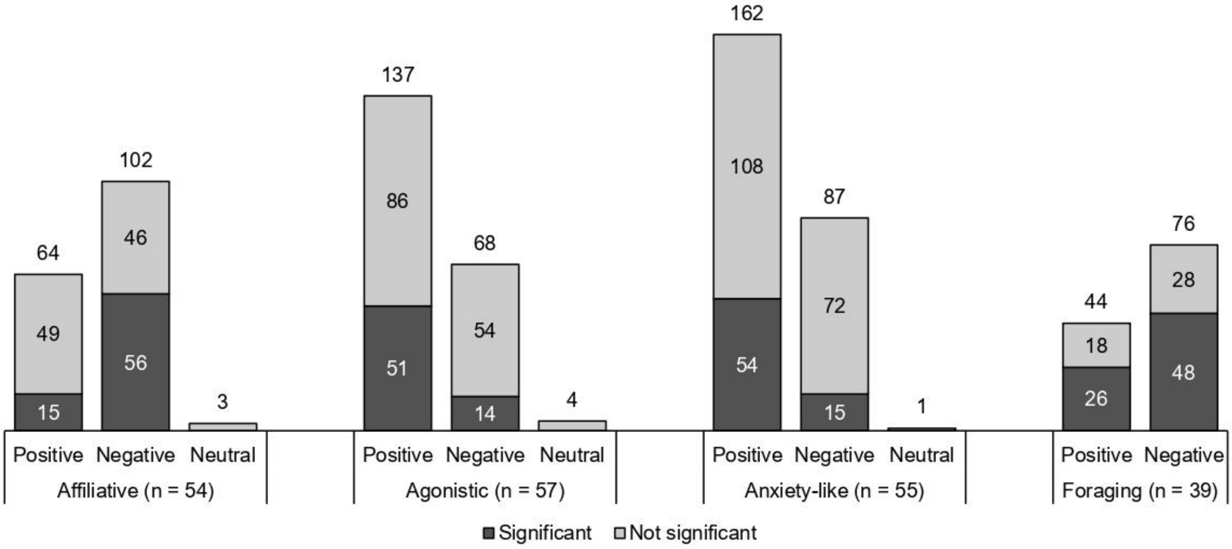

Figure 3. Total number of positive, negative, and neutral measures (top edge of each bar) that established a correlation between studied behavioral traits and glucocorticoid levels. Bars are grouped by behavioral domain, where n represents the number of primary studies. Each bar is further differentiated in number of significant and not significant measures.

Nearly all studies in the foraging domain were conducted in the wild using observational methods, only three did not fall into this combined category. Whereas in the other domains, most studies were carried out with animals in captivity, being experimental the most frequent approach for anxiety-like and observational for affiliative and agonistic domains (Fig. 4a). Some of the experimental approaches found in the literature were relocation to a different setting or social group (e.g.: Dettmer et al., 2012; Smith et al., 1998), social separation (e.g.: Bardi et al., 2005; Byrne and Suomi, 1999; Laudenslager et al., 1995), exposition to recordings (e.g.: Barbosa et al., 2017), injection of synthetic compounds (e.g.: Qin et al., 2019; Tiefenbacher et al., 2003), routine procedures in captivity (e.g.: de Menezes Galvão et al., 2016) and sleep deprivation (e.g.: Zeitzer et al., 2007). Interestingly, males was the sex category most studied in the agonistic domain, the rest of sex categories, excluding foraging domain, have similar number of studies across domains (Fig. 4b).

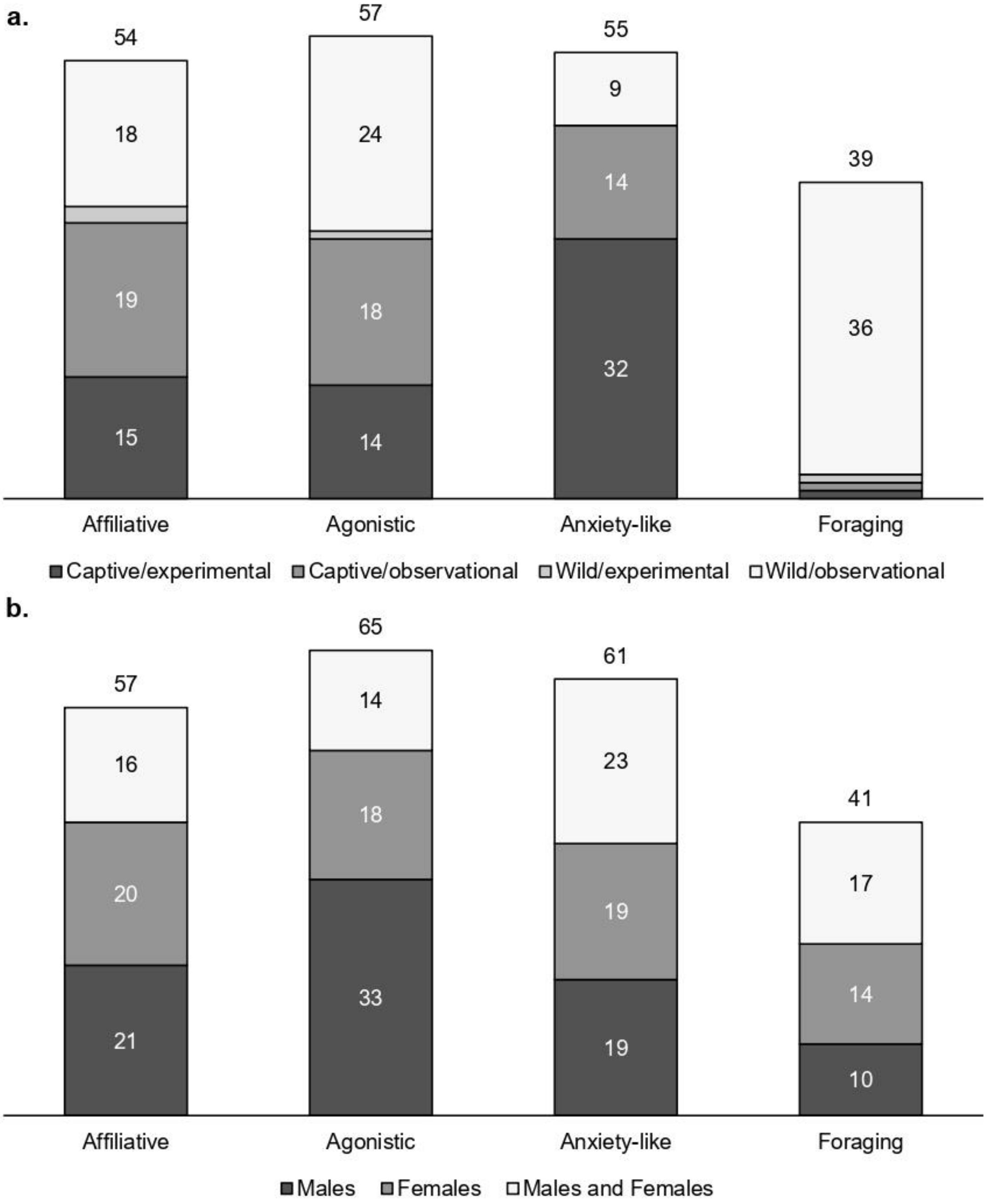

Figure 4. Total number of primary studies assessed per behavioral domain (top edge of each bar), where a) differentiates the combination of type of setting and method and b) the sex of individuals.

3.2. Meta-analysis

Following successive runs, the model that resulted in the lowest DIC and the highest ΔDIC, hence deemed as the most competitive, included only behavioral domain (model 5 of Suppl. table 1). Besides behavioral domain, sex of individuals showed significant categories along the process of model selection and was the last moderator to be removed before achieving model 5 (model 4 of Suppl. table 1). Models constructed with a single moderator indicate that, apart from behavioral domain, type of method and setting are important to explain variation in effect sizes as suggested by their improvement in DIC with respect to the null model. Sex of individuals, however, failed to enhance the data fit in this step, which could hint that statistical power of this moderator is lost when isolated from behavioral domain. All models without exception showed an excess of variation in effect sizes as all Q_E are highly significant. I^2 which is a more informative estimate of between-study heterogeneity indicates that only models created with behavioral domain stay within the limit of moderate heterogeneity (I^2<75%). Moderators were significantly different when models included behavioral domain or type of setting as F_{mod} points out (see Suppl. table 1). This finding not only reveals how significant the differences across categories are, but also which moderators handle better the high variability in our data.

The forest plot in Figure 5, compiles the essential information about the moderators and the univariate models without intercept. It is very clear that confidence intervals (CIs) in the plot reflects the number of studies that contributed to the cumulative effect size of each category. CIs are especially broader in age of individuals and sample matrix. Most categories considered in this study overlapped zero and as a result, produced nonsignificant effect sizes. However, similarly to what the previous model selection revealed, behavioral domain and type of setting contain significant effect sizes. Age of individuals and sample matrix also have significant effect sizes but should be interpreted with caution given their wide CIs. Regarding behavioral domain, agonistic traits have the strongest positive effect with respect to GC levels (Z-score = 0.25, 95% CI = [0.13 – 0.38], pMCMC < 0.001), followed by anxiety-like traits (Z-score = 0.20, 95% CI = [0.07 – 0.31], pMCMC < 0.01) in that same direction. In opposite direction, we have foraging traits that show the strongest negative effect (Z-score = -0.21, 95% CI = [-0.36 – -0.06], pMCMC < 0.05), followed by affiliative traits (Z-score = -0.16, 95% CI = [-0.29 – -0.04], pMCMC < 0.05). Furthermore, captive settings yielded a positive effect (Z-score = 0.13, 95% CI = [0.003 – 0.29], pMCMC < 0.05), as well as the categories of hair within sample matrix (Z-score = 0.25, 95% CI = [0.02 – 0.5], pMCMC < 0.05) and infants within age of individuals (Z-score = 0.26, 95% CI = [0.05 – 0.48], pMCMC < 0.05). It is worthwhile noting that the overall mean effect size (Z-score = 0.06, 95% CI = [-0.07 – 0.19], pMCMC = 0.27), derived from the null model, crosses the zero-effect line, indicating that studies that relate GCs to the behavioral traits here considered will continue to find oscillating measures. This nonsignificant mean effect size is usually expected when performing a subgroup analysis where the purpose is to identify why certain type of studies, coded in the analysis with a moderator, generates higher or lower effect sizes than other studies (Harrer et al., 2021).

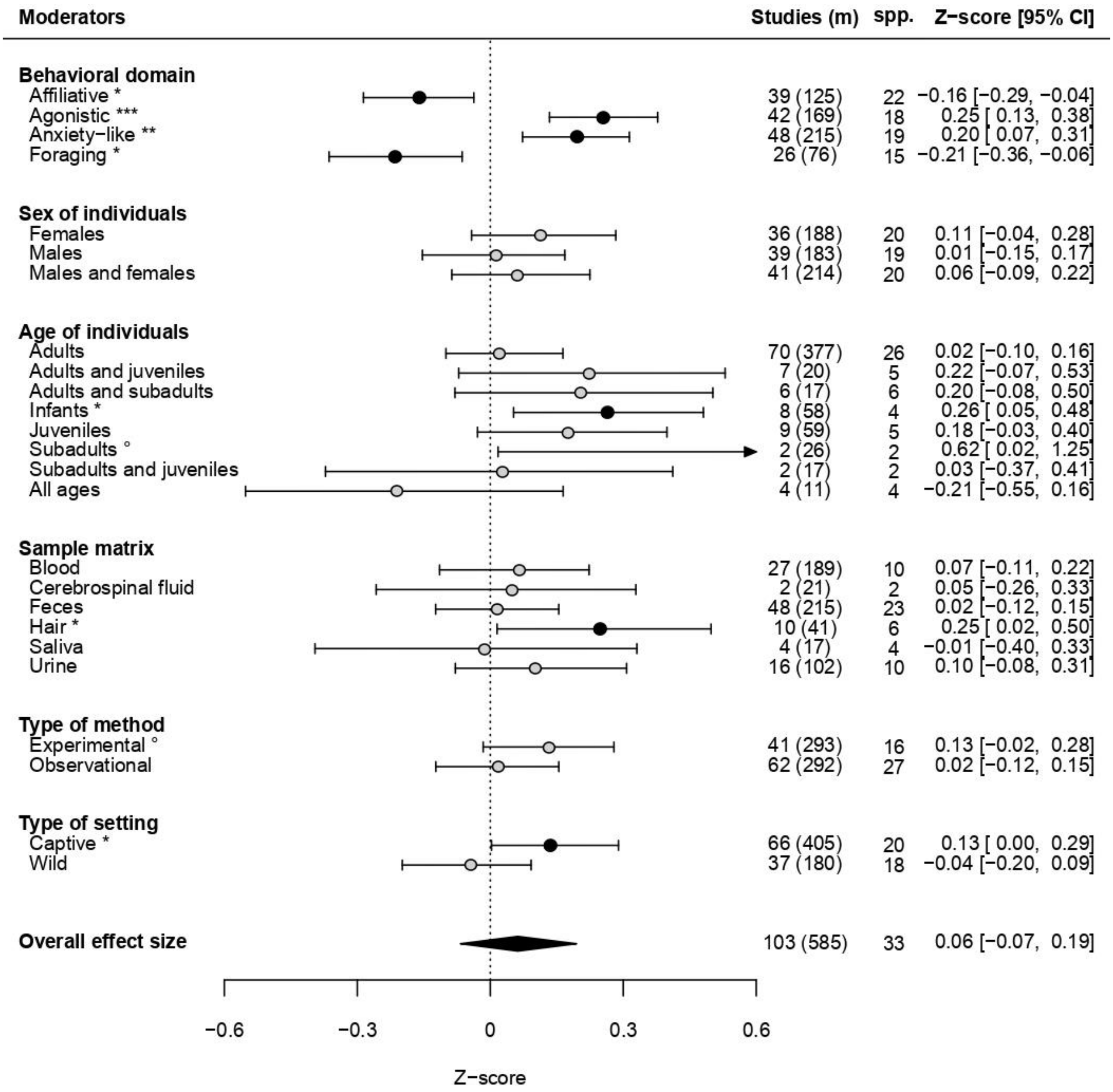

Figure 5. Forest plot of subgroup analysis displaying the overall and cumulative effect sizes (Z-scores) with confidence intervals (95% CI) for the null model, polygon at the bottom, and moderators separated in their respective categories. When CI's whiskers overlap zero-line, the category has a neutral effect. Significant effect sizes are denoted with black dots. In the columns, Studies (m) is the number of research articles and number of measures in parenthesis that contributed to each category, also "spp." designates the number of primate species. In the moderator column, when pMCMC of category is < 0.001: ***, < 0.01: **, < 0.05: * and marginally significant: °.

Egger's analysis intercept (b = 0.03, p = 0.45) suggests that our dataset is not affected by publication bias, although the intercept approximates to zero and many Z-scores in the funnel plot sit outside the idealized funnel shape (Suppl. figure 1), this however is likely another evidence of the high heterogeneity in our data caused by the wide amplitude of Z-scores and not a case of funnel plot asymmetry. According to the fail-safe N, among the behavioral domains, anxiety-like (5745) has the most robust cumulative effect size, followed by foraging (5748), then agonistic (4128) and finally affiliative (1208). Concerning other categories, captive setting produced the most robust effect size (6160), followed by the age category of infants (1056) and the sample matrix of hair (458).

4. DISCUSSION

Our systematic review offers some insights into the factors that modulate the reciprocal relationship between the behavioral traits studied and GCs. Behavioral domain and type of setting proved to be relevant in explaining the variation of such associations. Their cumulative effects suggest that foraging and affiliative behaviors inhibit the stress responses mediated by GCs. This reveals the expected coping role of affiliative behaviors and the energetic impact of foraging behaviors on GC levels, as GCs are major regulators of energy allocation. Furthermore, the negative relationship between the foraging domain and GCs indicates that in certain instances, like in environments with abundant food sources, foraging behaviors may function as coping mechanisms and lead to overeating as a means of relieving stress. On the contrary, agonistic and anxiety-like behaviors, as well as captive settings may in general promote GC production or relate to contexts that enhance the stress response in primates. Although vertebrate stress research has several methodological and interpretational issues that complicate the application of GCs results to primate conservation, as thoroughly reviewed by some authors that helped clarifying these issues (e.g.: Busch and Hayward, 2009; Johnstone et al., 2012; Millspaugh and Washburn, 2004), integrating behavioral aspects in the study of GC variation has the potential to improve our understanding of how primates mediate stress responses through behavioral strategies (Cooke et al., 2014) and therefore, how GC secretion could translate into fitness consequences (Beehner and Bergman, 2017). We stress that by focusing on coping mechanisms and their effects on stress alleviation, we do not intend to undermine the adaptive value of GC production. It is already well established that high GC levels do not automatically represent something negative for an individual’s health or well-being. In fact, without GC rise in response to a stressor, coping mechanisms may not unfold adequately (Busch and Hayward, 2009). In the following sections, we discuss the possible interpretations and caveats for the results concerning the defined behavioral domains and their associations with GC levels.

4.1. Agonistic behaviors and coping mechanisms

To disentangle the costs and benefits of an individual's social environment is especially difficult with primates, as most species live in groups where competition between group members largely defines their access to food sources and mates. However, group living can also provide certain advantages such as protection against predators (e.g.: Oliveira and Dietz, 2011) , collective resource defense against neighboring groups (e.g.: Raboy and Dietz, 2004) and information exchange about food quality and location (Markham and Gesquiere, 2017). To grasp the complexity of the social dimension in the scope of stress physiology, researchers usually compare the impact of dominance rank on the secretion of GCs. As previously mentioned, each intersection between dominance rank and sex involves different energetic and social challenges which can be associated to distinct GC production and consequently specific physiological and behavioral responses (Cavigelli and Caruso, 2015). For instance, males in general engage more frequently in agonistic interactions than females and often climb hierarchy through fighting and coalitions (Bernstein, 1976; Clutton-Brock and Huchard, 2013). This tendency is reflected by the higher number of studies with measures of male aggression in the descriptive analysis (Fig. 4b). In addition to this sex difference, low-ranking males will usually secrete more GCs than high-ranking males, if they have less access to food and social support and experience a more unpredictable social environment. However, in periods of social instability this difference could be reversed if high-ranking males engage more in group defense (Anestis, 2010; Muller and Wrangham, 2004).

Many other factors besides dominance rank and sex can influence GCs and aggression relationship. Honess and Marin (2006) describe in detail these sources of variation and review which changes in resource distribution; group formation, composition, and density; and social stability can lead to stress and aggression modification in captive and wild primates. We found a significant positive interaction between GCs and agonistic behaviors, suggesting that the increased occurrence of such behaviors may likely stimulate GC secretion and consequently promote the stress response. However, agonistic domain captured both display and receipt of aggression which could have diluted their differences in relation to GC levels or clouded the negative effect of known coping behaviors like redirected aggression normally used by individuals that lose a contest (Virgin and Sapolsky, 1997). Nonetheless, there is evidence that both winners and losers exhibit higher GCs when subjected to experimental contests and although the effect may be stronger for losers, this finding supports the notion that engaging in agonistic interactions entails costs for both group members involved in a contest (Creel et al., 2013; Rubenstein and Shen, 2009). Furthermore, since social hierarchies and aggressive behaviors evolve to minimize these costs and avoid the potential risks of physical aggression (Bernstein and Gordon, 1974; Honess and Marin, 2006), it seems plausible that GC production does not decrease as aggression escalates, given that GCs may help individuals overcome physically and psychologically challenging periods with minimal losses. On the other hand, agonism should be identified as a social stressor which, unless it is resolved, could result in chronically elevated GC levels (Schrock et al., 2019).

4.2. Anxiety-like behaviors and coping mechanisms

Displacement activities are described as behaviors that often appear irrelevant to the ongoing biological context or to the stimuli that would normally bring them about, their occurrence can be linked to an animal’s uncertainty about the outcome of a decision, or frustration when it is prevented from reaching its goal (Maestripieri et al., 1992; Tinbergen, 1952). Thus, given that these behaviors denote internal conflict, displacement activities can be associated to an animal’s emotional state or for that matter, anxiety (Maestripieri et al., 1992). The anxiety-like domain tested in this analysis not only included displacement activities which are typically seen in wild primates like self-grooming and scratching, but also behaviors quantified in experiments that manipulated the physical, social, and hormonal environment of captive primates in order to activate their stress response (Honess and Marin, 2006). Additionally, anxiety-like domain also contained stereotypes and abnormal behaviors that emerge when the captive individuals are unable to cope with a stressor especially due to social and spatial constraints of captivity (Novak et al., 2013; Wechsler, 1995).

Most of the correlations in the anxiety-like domain resulted from studies that combined captivity and experimental approaches (Fig. 4a) which may have contributed to the positive interaction found between anxiety-like behaviors and GCs, countering therefore our prediction. Furthermore, given the marginally significant effect of experimental procedure, it is possible that the type of method may have confounded this result (Fig. 5). Contrary to this result, anxiety-like behaviors are sometimes thought to represent a coping strategy that can lead to GC reduction (e.g.: Kaplan et al., 2012; Watson et al., 1999). One well founded example comes from studying self-injurious behaviors in adult rhesus macaques, individuals that display these behaviors have an abnormal response to stressors by which they bite themselves to reinforce the arousal reduction, seemingly subsiding the physiological stress response (Novak, 2003). However, primates kept in captivity, and especially those that engage in abnormal behaviors, may present an HPA-axis dysfunction that can influence their stress response in unexpected ways (Millspaugh and Washburn, 2004). If and how meaningful anxiety-like behaviors are for stress alleviation in wild primate populations is still unclear and results obtained in captive settings should be applied with extreme care, given that behaviors displayed in such contexts, like scent-marking, may have a completely different interpretation in the wild. In fact, some authors have found no evidence of correlation between self-directed behaviors and GCs in free living primates (Ellis et al., 2011; Higham et al., 2009). This is still an underexplored association that may become increasingly relevant as habitat disturbance continues to threat wild populations, limiting their chances to avoid stressors.

4.3. Affiliative behaviors and coping mechanisms

Affiliative interactions are by large the most studied coping mechanism: like agonistic interactions, they generally occur within a complex network of social interrelationships that can promote both stress alleviation and augmentation (Cheney and Seyfarth, 2009). In a recent review examining how HPA-axis activity contributes to the variation of vertebrate social behavior, Raulo and Dantzer (2018) found a nonlinear relationship between GCs and parental care, and moderate elevations in GCs leading to the highest amount of parental care. Furthermore, the authors showed that independent of the sexual context, a rise in GCs encourages the expression of affiliative behaviors that in turn fulfill a coping role in reducing these elevations (Raulo and Dantzer, 2018). In our analysis, however, we merged parental care, and sexual and nonsexual affiliation within the same category, and still detected a negative association between affiliative domain and GC levels of captive and wild primates. This could indicate that performing affiliative behaviors reduces the concentration of GCs and therefore, inhibits the stress response. Nonetheless, we do not dismiss the idea that, in many situations, affiliative behaviors may initiate because the individual previously experienced a GC increase, as shown by Raulo and Dantzer (2018). For instance, female rhesus macaques from different matrilines sharing a relatively cramped space engaged in more aggressive contacts which subsequently increased grooming between these subgroups, although such affiliative exchanges rarely happen given the strong cohesion of matrilines in this species (de Waal et al., 2000). This covariation of behaviors may serve not only to reconcile with the opponent subgroups but also to generate a reduction in GCs of both groomer and receiver (Gust et al., 1993). Yet, as witnessed in wild female baboons living in large social groups, affiliative interaction may be focused on a smaller number of group members usually composed of kin and close companions, which renders more predictability to social bonds (Cheney and Seyfarth, 2009; Sapolsky, 1992). Additionally, the role affiliative behaviors play in decreasing GCs may depend on the species sociality, the stressor's severity, and whether an environmental stressor, such as food scarcity, superimposes on a social one, in which case affiliation would likely be ineffective (Lemos et al., 2012; Raulo and Dantzer, 2018).

4.4. Foraging behaviors and coping mechanisms

Overall, we found fewer publications with statistical measures for the relationship between GCs and foraging behaviors (26 studies), or food availability (16 studies) compared to the other behavioral domains with at least 50 studies each. The majority of correlations in the foraging domain were drawn from observations under naturalistic conditions (Fig. 4a), which is reasonable due to the central interest of predicting the consequences of energy balance variation in free ranging primates. Both paucity of studies and special attention to wild populations might be related to the fairly recent development of non-invasive techniques to measure hormones in the field, mainly in feces and urine (Busch and Hayward, 2009). Despite that, correlative studies on GCs and foraging behaviors do not need to be restricted to natural environments. The use of certain environmental enrichment practices to feed captive primates, like different floor substrates or automated feeders, could allow measuring food intake and foraging effort, as well as testing their effect on the intrinsic stress of captivity (Baker, 1997; Chamove et al., 1982; Line et al., 1990).

The behavioral correlates of GCs captured in the foraging domain yielded the largest negative effect size when compared to the affiliative domain, which suggests that while affiliative behaviors may help primates to cope with the psychosocial stressors that originate from social interactions, they may not be sufficient to offset the impacts of environmental stressors that threaten food provision of wild populations. In such case, foraging behaviors are better suited to dampen the stress response regulated by GCs and deal with such energetic demands. A similar negative covariation was in a review by de Bruijn and Romero (2018) who showed that decreasing food availability is predominantly associated with higher GC levels in vertebrates. However, some species may not always exhibit an HPA-axis activation in relation to changing food availability if they switch to energy conserving strategies in order to prevent prolonged exposure to high GCs (Romero et al., 2009). Likewise, foraging effort may not automatically reduce GC levels if intra- and inter-group competition impedes energy intake (Martínez-Mota et al., 2016), although temporal and spatial availability of food sources can, to a substantial degree, determine how social groups will be structured so that costs of group-living diminish. For instance, grouping patterns of chimpanzees and spider monkeys normally reflect fluctuations in food availability (reviewed by Markham and Gesquiere, 2017).

Interestingly, to build the foraging domain we assumed that any maintenance behavior had the potential to optimize energy balance and lessen the stress response. Thus the more an individual forages, travels in order to forage, rests, or ingests food the higher the probability it will achieve a positive energy balance that will flatten the physiological stress response, unless food shortage in its environment is so severe that it is not able to compensate for the energetic demands with any coping strategy (de Bruijn and Romero, 2018). Conversely, in captivity, where food access is normally unlimited, the alleviatory effect of foraging behaviors may result in chronically elevated food consumption in individuals constantly exposed to psychosocial stressors (McEwen and Wingfield, 2003). Therefore, the resulting effectiveness of a coping mechanism will depend on how manageable a stressor is in a specific environmental and social context.

4.5. Potential confounding effects in the glucocorticoid-behavior interactions

We want to point out some limitations in the interpretation of our results. One of our goals was to compare the influence of the designed behavioral domains on the stress response. However, as shown in the descriptive analysis, these domains suffer from a strong bias towards one combination of type of setting and method, especially anxiety-like and foraging domain. Therefore, their resulting cumulative effect sizes should be interpreted with caution. Furthermore, we found that the categories of infants and hair have a positive effect on the behavioral correlates of GCs, these influences, however, are merely a consequence of the type of method, setting and behavioral domain that these categories incorporate. To be precise, all the measures in both moderator categories were acquired from studies conducted in captivity that mainly correlated anxiety-like or agonistic behaviors to GCs and, more specifically for infants, usually involved social or maternal separation. Accordingly, behaviors evaluated in captive environments or linked to laboratory procedures tend to be positively correlated to GCs, showing an overall intensification of stress responses associated to GC secretion, which probably proves a concern that many authors in the field warn about (e.g.: Honess and Marin, 2006; Millspaugh and Washburn, 2004). Given the limited space and companionship, the regular presence of unfamiliar humans, and the routine management; captivity can be stressful for non-human primates and alter HPA-axis activation, likely leading to a dysfunctional stress response (Hosey, 2005; Novak et al., 2013). Nonetheless, captive and experimental studies allow for a much higher control of the stressor of interest and the factors that might confound its effect on the stress response (de Bruijn and Romero, 2018). So, although investigating primates’ coping mechanisms in the wild offers a better representation of how they normally respond to external stimuli (de Bruijn and Romero, 2018), leaving out captive studies in this analysis would be a missed opportunity to integrate results that attempt establishing causal relationships between hormones and behavior. The fact that the effect size for captive primates is positive while the effect size for wild primates intersects the zero-effect line may suggest that behavioral responses in nature have more flexible outcomes and are more prone to promote fitness (Boonstra, 2013). However, adaptive stress responses in the wild largely depend on environmental gradients that may drastically change in anthropogenically-disturbed environments (Dantzer et al., 2016).

Other sources of variation, like sex differences, were less important in defining the strength and direction of GCs-behavior interactions. However, Reeder and Kramer (2005) indicate that, in general, female mammals have higher basal GCs and show a more robust stress response than males (Handa et al., 1994). Such differences are likely caused by the dramatic changes of female reproductive status, which was not assessed in this analysis. During gestation and lactation, for instance, females undergo a period of high metabolic demand (Gittleman and Thompson, 1988) that leads to sustained elevations in GCs, as more energy is needed, mounting the stress response. One potential problem of exploring physiology and behavior simultaneously is to temporally synchronize their estimators, as they may operate on varying timescales. Consequently, many physiological changes might manifest more slowly than neurologically based behaviors (Cooke et al., 2014). Additionally, the choice of sample matrix, which often depends on whether we assess the impact of chronic or acute stressors as well as the desirable degree of invasiveness (Sheriff et al., 2011), could also cause a temporal mismatch since different sample matrix accumulate GCs over different timescales.

Despite the intrinsic and extrinsic influences of possible confounding factors, in general, the behavioral domains tested in this meta-analysis provide an overall picture of how certain set of behaviors promote certain physiological outcomes that may lead to stress alleviation and, therefore, be considered coping mechanisms.

CONCLUSION

The present study has shown that the behavioral correlates of GC levels, found in the primatological literature, reveal specific influences on the stress response in terms of both magnitude and direction of effect when they are sorted into general behavioral domains. Assuming behavior and GCs maintain a bidirectional relationship, we focused on the potential consequences for stress alleviation, indicated as a GC reduction, which may arise from engaging in certain behaviors. We discussed the role that such behaviors have as coping mechanisms. Foraging and agonistic correlates generated the largest negative and positive effect, respectively, and therefore can be considered key drivers of the physiological stress response. Affiliative and anxiety-like correlates yielded an intermediate negative and positive effect, respectively. These findings suggest that both foraging and affiliative behaviors inhibit the production of GCs and that foraging strategies have a stronger effect on stress alleviation, highlighting not only the metabolic function of GCs but also the importance of energy balance for stress responses. Furthermore, the contrast between these two coping mechanisms leads us to believe that wild populations of primates that face unpredictable changes in resource abundance and distribution may be able to cope with social conflicts that originate from them. However, if such changes result in serious food scarcity that prevent the alleviatory effects of foraging and affiliative behaviors, wild populations may be subject to chronic stress and lower chances of survival. We share the understanding that GCs mediate stress responses which help an individual overcome physical and psychosocial challenges, sometimes at the expense of its own health, depending on the stressor's nature. Consequently, the positive effect of agonistic and anxiety-like behaviors on GCs suggests that aggressive and frustrative environments, the latter linked to captivity, are perceived as challenging situations that induce an intensification of the stress response. Moreover, given the positive trend of the behavioral correlates in captive settings, we expect that coping mechanisms used to deal with psychosocial stressors will tend to be less effective in captivity than in the wild. Finally, the impact of the stress responses coordinated in part by GCs depends substantially on the social and ecological context in which these physiological and behavioral responses unfold. Non-humane primates may only be able to cope with unpredictable stressors when their context provides options for stress alleviation.

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Declaration of interest

None

Appendix A. Supplementary material

Appendix B. List of analyzed studies

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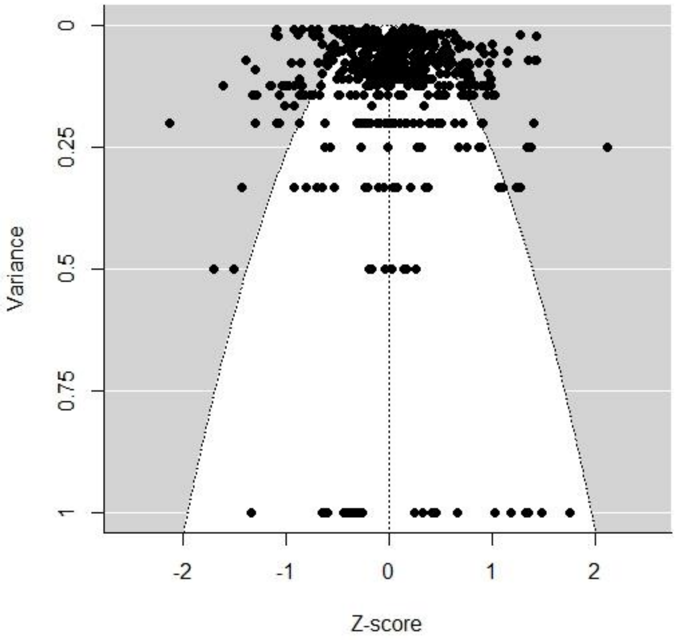
Appendix A. Supplementary material

Suppl. text 1. As a first structured selection criteria, we carefully inspected the titles, keywords and abstracts, and selected those that could have potentially evaluated the relationship between GCs and the expression of any type of behavior. Given the small number of studies measuring feeding or foraging behaviors and GCs within primate literature, which was apparent through exploratory searches in both databases and Google Scholar, we extended our inclusion criteria to articles assessing food availability or quality as proxies of feeding behavior. Furthermore, studies that linked personality or behavioral styles to GCs were also included, since they are typically scored based on quantitative observations of behavior. We disregarded studies devoted solely to the association between GCs and sex, reproductive state, or dominance rank since this type of interactions are outside the scope of the present study and have already been reviewed by other authors (Abbott et al., 2003; Cavigelli and Caruso, 2015; Sapolsky, 2005). Certainly, such intrinsic factors exert great influence on behavioral and physiological responses in primates, but they do not directly represent the deliberate expression of behavior in individuals attempting to cope with external stimuli.



Suppl. figure 1. Funnel plot of the complete set of Z-scores versus sample variance used in the detection of publication bias.

Suppl. table 1. Combination of fixed effect parameters specified for the model selection with the resulting deviance information criteria (DIC), difference of DIC from the null model (ΔDIC), Cochran's Q-statistic for the residual heterogeneity (Q_E), percentage of between-study heterogeneity (I^2) and F-test for moderators (F_{mod}) for each model.

Model	**Z ~ Fixed effects**	**DIC**	**ΔDIC**	**Q_E^a**	**I^2 [%]**	**F_{mod}**
Null model	Z ~ 1	762.08		2676.74	78.58	
	Model fitting procedure					
Full model	Z ~ Behavior + Sex + Age + Matrix + Setting + Method	739.78	22.29	1970.20	74.37	4.35^{+}
1)	Z ~ Behavior + Sex + Age + Matrix + Setting	737.53	24.55	1982.30	74.34	4.57^{+}
2)	Z ~ Behavior + Sex + Age + Matrix	737.86	24.22	1984.45	74.26	4.85^{+}
3)	Z ~ Behavior + Sex + Age	730.38	31.70	2033.46	74.27	6.53^{+}
4)	Z ~ Behavior + Sex	728.21	33.87	2126.54	74.47	14.32^{+}
5)	Z ~ Behavior	725.78	36.29	2150.48	74.48	22.49^{+}
	Testing separate moderators					
6)	Z ~ Sex	763.25	-1.18	2596.38	78.72	1.18
7)	Z ~ Age	767.61	-5.53	2491.93	77.54	1.81°
8)	Z ~ Matrix	766.95	-4.87	2472.42	78.21	0.95
9)	Z ~ Setting	755.20	6.88	2445.08	77.87	5.22*
10)	Z ~ Method	759.04	3.04	2536.48	78.30	2.35

When p-value of F_{mod} is < 0.0001: $^{+}$, < 0.05: * and marginally significant: °.

^{a} All Q_E values reached significance at < 0.0001.

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Appendix B. List of analyzed studies

Author(s) and year	**Title of primary study**	**Used in both descriptive analysis and meta-analysis***
Amrein et al., 2014	The Effect of Fission-Fusion Zoo Housing on Hormonal and Behavioral Indicators of Stress in Bornean Orangutans (*Pongo pygmaeus*)	X
Anestis, 2005	Behavioral style, dominance rank, and urinary cortisol in young chimpanzees (*Pan troglodytes*)	X
Anestis et al., 2006	Age, rank, and personality effects on the cortisol sedation stress response in young chimpanzees	X
Arlet et al., 2009	Reproductive tactics influence cortisol levels in individual male gray-cheeked mangabeys (*Lophocebus albigena*)	
Barbosa and da Silva Mota, 2013	Alloparental Responsiveness to Newborns by Nonreproductive, Adult Male, Common Marmosets (*Callithrix jacchus*)	X
Barbosa and da Silva Mota, 2014	Do newborn vocalizations affect the behavioral and hormonal responses of nonreproductive male common marmosets (*Callithrix jacchus*)?	X
Barbosa et al., 2017	The effect of infant vocalization in alloparental responsiveness of common marmosets (*Callithrix jacchus*)	X
Bardi and Huffman, 2005	Maternal behavior and maternal stress are associated with infant behavioral development in macaques	X
Bardi et al., 2004	The role of the endocrine system in baboon maternal behavior	X
Bardi et al., 2017	Physiologic Correlates of Interactions between Adult Male and Immature Long-tailed Macaques (*Macaca fascicularis*)	X
Berghaenel et al., 2016	Prenatal stress effects in a wild, long-lived primate: predictive adaptive responses in an unpredictable environment	X
Bergman et al., 2005	Correlates of stress in free-ranging male chacma baboons, Papio hamadryas ursinus	X
Boinski et al., 1999	Environmental enrichment of brown capuchins (*Cebus apella*): Behavioral and plasma and fecal cortisol measures of effectiveness	X
Boinski et al., 1999b	Terrestrial predator alarm vocalizations are a valid monitor of stress in captive brown capuchins (*Cebus apella*)	X
Bosc et al 2017	Checking behavior in rhesus monkeys is related to anxiety and frontal activity	
Brand et al., 2016	Hair plucking, stress, and urinary cortisol among captive bonobos (*Pan paniscus*)	X

Brent et al., 2011	Social capital and physiological stress levels in free-ranging adult female rhesus macaques	
Byrne and Suomi, 1999	Social separation in infant *Cebus apella*: Patterns of behavioral and cortisol response	X
Cañadas et al., 2019	Behavioral and physiological stress responses to local spatial disturbance and human activities by howler monkeys at Los Tuxtlas, Mexico	
Capitanio et al., 1999	The relationship of personality dimensions in adult male rhesus macaques to progression of simian immunodeficiency virus disease	X
Cavigelli, 1999	Behavioural patterns associated with faecal cortisol levels in free-ranging female ring-tailed femurs, *Lemur catta*	X
Chapman et al., 2006	Do food availability, parasitism, and stress have synergistic effects on red colobus populations living in forest fragments?	X
Chapman et al., 2007	Temporal dynamics of nutrition, parasitism, and stress in Colobus monkeys: Implications for population regulation and conservation	X
Chapman et al., 2015	Competing pressures on populations: long-term dynamics of food availability, food quality, disease, stress and animal abundance	X
Chaves et al., 2019	Assessing the influence of biotic, abiotic, and social factors on the physiological stress of a large Neotropical primate in Atlantic forest fragments	
Cicini et al., 2014	Social isolation disrupts hippocampal neurogenesis in young non-human primates	X
Cleveland et al., 2004	Physiological predictors of reproductive outcome and mother-infant behaviors in captive rhesus macaque females (*Macaca mulatta*)	X
Crockford et al., 2008	Social stressors and coping mechanisms in wild female baboons (*Papio hamadryas ursinus*)	X
Cubicciotti et al., 1986	Differences between *Saimiri sciureus* and *Callicebus moloch* in physiological responsiveness: implications for behavior.	X
de Menezes Galvão et al., 2016	Physiological and behavioral responses to routine procedures in captive common marmosets (*Callithrix jacchus*)	X
Dettling et al., 1998	Physiological responses to parental separation and a strange situation are related to parental care received in juvenile Goeldi's monkeys (*Callimico goeldii*)	X
Dettmer et al., 2012	Physiological and behavioral adaptation to relocation stress in differentially reared rhesus monkeys: Hair cortisol as a biomarker for anxiety-related responses	X
Dias et al., 2017	Hormonal correlates of energetic condition in mantled howler monkeys	
Duarte et al., 2018	Behavioral and cortisol responses of adult marmoset monkeys (*Callithrix penicillata*) to different home-cage social disruption intervals	X
Dunn et al., 2013	Travel Time Predicts Fecal Glucocorticoid Levels in Free-Ranging Howlers (*Alouatta palliata*)	

Elder and Menzel, 2001	Dissociation of cortisol and behavioral indicators of stress in an orangutan (*Pongo pygmaeus*) during a computerized task	X
Ellis et al., 2011	The social correlates of self-directed behaviour and faecal glucocorticoid levels among adult male olive baboons (*Papio hamadryas anubis*) in Gashaka-Gumti National Park, Nigeria	X
Emery Thompson et al., 2010	Dynamics of social and energetic stress in wild female chimpanzees	
Emery Thompson et al., 2020	Wild chimpanzees exhibit humanlike aging of glucocorticoid regulation	
Feng et al., 2016	Social correlates of the dominance rank and long-term cortisol levels in adolescent and adult male rhesus macaques (*Macaca mulatta*)	X
Fernández-Lázaro et al., 2019	Nonhuman primate welfare: Can there be a relationship between personality, lateralization and physiological indicators?	X
Ferreira et al., 2018	Hormonal correlates of behavioural profiles and coping strategies in captive capuchin monkeys (*Sapajus libidinosus*)	
Fichtel et al., 2007	Influence of reproductive season and rank on fecal glucocorticoid levels in free-ranging male Verreaux's sifakas (*Propithecus verreauxi*)	X
Foerster et al., 2011	Social behavior, foraging strategies, and fecal glucocorticoids in female blue monkeys (*Cercopithecus mitis*): Potential fitness benefits of high rank in a forest guenon	X
Foerster et al., 2012	Seasonal Energetic Stress in a Tropical Forest Primate: Proximate Causes and Evolutionary Implications	
Fontani et al., 2014	Fecal Concentrations of Cortisol, Testosterone, and Progesterone in Cotton-Top Tamarins Housed in Different Zoological Parks: Relationships Among Physiological Data, Environmental Conditions, and Behavioral Patterns	
Fuller et al., 2018	Behavioral and hormonal responses to the availability of forage material in Western lowland gorillas (*Gorilla gorilla gorilla*)	
Fürtbauer et al., 2014	Low female stress hormone levels are predicted by same- or opposite-sex sociality depending on season in wild Assamese macaques	
Gabriel et al., 2018	Crowding as a primary source of stress in an endangered fragment-dwelling strepsirrhine primate	
Galvão-Coelho et al., 2008	Common Marmosets (*Callithrix jacchus*) as a Potential Animal Model for Studying Psychological Disorders Associated with High and Low Responsiveness of the Hypothalamic-Pituitary-Adrenal Axis	X
Galvão-Coelho et al., 2012	The Influence of Sex and Relatedness on Stress Response in Common Marmosets (*Callithrix jacchus*)	X

Galvão-Coelho et al., 2017	Common marmosets: A potential translational animal model of juvenile depression	X
Girard-Buttoz et al., 2009	Seasonal and social influences on fecal androgen and glucocorticoid excretion in wild male long-tailed macaques (*Macaca fascicularis*)	X
Girard-Buttoz et al., 2014	Costs of mate-guarding in wild male long-tailed macaques (*Macaca fascicularis*): Physiological stress and aggression	X
Girard-Buttoz et al., 2014b	Costs of and Investment in Mate-Guarding in Wild Long-Tailed Macaques (*Macaca fascicularis*): Influences of Female Characteristics and Male-Female Social Bonds	X
Gomez-Espinosa et al., 2014	The Effect of Energetic and Psychosocial Stressors on Glucocorticoids in Mantled Howler Monkeys (*Alouatta palliata*)	X
Gustinson et al., 2012	An experimental study of behavioural coping strategies in free-ranging female Barbary macaques (*Macaca sylvanus*)	X
Hannibal et al., 2018	Intermittent pair-housing, pair relationship qualities, and HPA activity in adult female rhesus macaques	
Henkel et al., 2010	Infants as costly social tools in male Barbary macaque networks	
Higham et al., 2009	Rates of self-directed behaviour and faecal glucocorticoid levels are not correlated in female wild olive baboons (*Papio hamadryas anubis*)	X
Hohmann et al., 2009	The relationship between socio-sexual behavior and salivary cortisol in bonobos: Tests of the tension regulation hypothesis	X
Huck et al., 2005	Characterization and social correlates of fecal testosterone and cortisol excretion in wild male Saguinus mystax	
Inoue-Murayama et al., 2018	Common marmoset (*Callithrix jacchus*) personality, subjective well-being, hair cortisol level and AVPR1a, OPRM1, and DAT genotypes	X
Kalbitzer et al., 2015	Social behavior and patterns of testosterone and glucocorticoid levels differ between male chacma and Guinea baboons	
Kaplan et al., 2012	Stress and stress reduction in common marmosets	X
Kohn et al., 2016	Dominance rank causally affects personality and glucocorticoid regulation in female rhesus macaques	X
Kutsukake et al., 2012	Individual Variation in Behavioural Reactions to Unfamiliar Conspecific Vocalisation and Hormonal Underpinnings in Male Chimpanzees	X
Laudenslager et al.,	The influences of perinatal challenge persist into the adolescent period in socially housed bonnet macaques (*Macaca radiata*)	X

2012		
Laudenslager et al., 1995	Total cortisol, free cortisol, and growth-hormone associated with brief social separation experiences in young macaques	
Line et al., 1990	Responses of female rhesus macaques to an environmental enrichment apparatus	X
Lyons et al., 1999	Postnatal experiences and genetic effects on squirrel monkey social affinities and emotional distress	X
MacLarnon et al., 2014	Assessing adaptability and reactive scope: Introducing a new measure and illustrating its use through a case study of environmental stress in forest-living baboons	
Maestripieri, 2005	Effects of early experience on female behavioural and reproductive development in rhesus macaques	X
Maestripieri et al., 2009	Mother-infant interactions in free-ranging rhesus macaques: Relationships between physiological and behavioral variables	X
Malalaharivony et al., 2021	Maternal stress effects on infant development in wild Verreaux's sifaka (*Propithecus verreauxi*)	
Maninger et al., 2017	Imaging, behavior and endocrine analysis of "jealousy" in a monogamous primate	
Maréchal et al., 2011	Impacts of tourism on anxiety and physiological stress levels in wild male Barbary macaques	X
Markham et al., 2015	Optimal group size in a highly social mammal	X
Martensz et al., 1987	Relation between aggressive behaviour and circadian rhythms in cortisol and testosterone in social groups of talapoin monkeys	X
Martinez-Mota et al., 2016	Fluctuations in daily energy intake do not cause physiological stress in a Neotropical primate living in a seasonal forest	X
Marty et al., 2017	Is social dispersal stressful? A study in male crested macaques (*Macaca nigra*)	
McFarland et al., 2013	Physiological stress hormone levels and mating behaviour are negatively correlated in male rhesus macaques (*Macaca mulatta*)	X
McLennan et al., 2019	Are human-dominated landscapes stressful for wild chimpanzees (*Pan troglodytes*)?	X
Miller et al., 2004	A mu-opioid receptor single nucleotide polymorphism in rhesus monkey: Association with stress response and aggression	X
Miller et al., 2008	Why do captive tufted capuchins (*Cebus apella*) urine wash?	X
Moreira et al., 2016	Variation in glucocorticoid levels: survival and reproductive demands in wild black capuchins	X

	(*Sapajus nigritus*)	
Muller and Wrangham, 2004	Dominance, cortisol and stress in wild chimpanzees (*Pan troglodytes schweinfurthii*)	X
Mustoe et al., 2014	Gestational Cortisol and Social Play Shape Development of Marmosets' HPA Functioning and Behavioral Responses to Stressors	X
Nguyen et al., 2008	Late pregnancy glucocorticoid levels predict responsiveness in wild baboon mothers (*Papio cynocephalus*)	X
Nunes et al., 2001	Interactions among paternal behavior, steroid hormones, and parental experience in male marmosets (*Callithrix kuhlii*)	X
Ordóñez-Gómez et al., 2016	Proximal and distal predictors of the spider monkey's stress levels in fragmented landscapes	X
Ostner et al., 2008	Dominance, aggression and physiological stress in wild male Assamese macaques (*Macaca assamensis*)	X
Parker et al., 2006	Maternal mediation, stress inoculation, and the development of neuroendocrine stress resistance in primates	
Patterson et al., 2021	Effects of early life adversity on maternal effort and glucocorticoids in wild olive baboons	
Pflueger et al., 2016	Allelic variation of the COMT gene in a despotic primate society: A haplotype is related to cortisol excretion in *Macaca fuscata*	X
Pizzutto et al., 2015	Relation between the level of self-mutilation and the concentration of fecal metabolites of glucocorticoids in captive chimpanzees (*Pan troglodytes*)	X
Pride., 2005	Foraging success, agonism, and predator alarms: Behavioral predictors of cortisol in Lemur catta	X
Qin et al., 2019	Chronic Glucocorticoid Exposure Induces Depression-Like Phenotype in Rhesus Macaque (*Macaca Mulatta*)	X
Ramirez et al., 2004	Hormonal correlates of changes in interest in unrelated infants across the peripartum period in female baboons (*Papio hamadryas anubis* sp.)	X
Rangel-Negrin et al., 2014	Primates Living Outside Protected Habitats Are More Stressed: The Case of Black Howler Monkeys in the Yucatan Peninsula	X
Rimbach et al., 2014	Behavioral and Physiological Responses to Fruit Availability of Spider Monkeys Ranging in a Small Forest Fragment	X
Rodrigues, 2017	Female Spider Monkeys (*Ateles geoffroyi*) Cope with Anthropogenic Disturbance Through Fission-Fusion Dynamics	X
Ross et al., 2011	Female Marmosets' Behavioral and Hormonal Responses to Unfamiliar Intruders	X

Rudolph et al., 2019	One size fits all? Relationships among group size, health, and ecology indicate a lack of an optimal group size in a wild lemur population	
Sánchez et al., 2005	Alterations in diurnal cortisol rhythm and acoustic startle response in nonhuman primates with adverse rearing	X
Sapolsky, 1992	Cortisol concentrations and the social significance of rank instability among wild baboons	
Sapolsky et al., 1997	Hypercortisolism associated with social subordinance or social isolation among wild baboons	X
Scallet et al., 1981	Sex differences in adrenocortical response to controlled agonistic encounters in rhesus monkeys	X
Schoof et al., 2016	Male endocrine response to seasonally varying environmental and social factors in a neotropical primate, *Cebus capucinus*	
Schrock et al., 2019	Aggression and social support predict long-term cortisol levels in captive tufted capuchin monkeys (*Cebus* [*Sapajus*] *apella*)	X
Schülke et al., 2014	Lack of Evidence for Energetic Costs of Mate-Guarding in Wild Male Assamese Macaques (*Macaca assamensis*)	X
Seyfarth et al., 2012	Variation in personality and fitness in wild female baboons	X
Shively et al., 2008	Depressive behavior and coronary artery atherogenesis in adult female cynomolgus monkeys	X
Shutt et al., 2007	Grooming in Barbary macaques: better to give than to receive?	X
Smith et al., 1998	Close proximity of the heterosexual partner reduces the physiological and behavioral consequences of novel-cage housing in black tufted-ear marmosets (*Callithrix kuhlii*)	X
Soltis et al., 2003	Adult cortisol response to immature offspring play in captive squirrel monkeys	X
Sonnweber et al., 2015	Rank-dependent grooming patterns and cortisol alleviation in Barbary macaques	
Surbeck et al., 2012	Social correlates of variation in urinary cortisol in wild male bonobos (*Pan paniscus*)	
Takeshita et al., 2017	Effect of castration on social behavior and hormones in male Japanese macaques (*Macaca fuscata*)	
Takeshita et al., 2018	Beneficial effect of hot spring bathing on stress levels in Japanese macaques	
Taylor et al., 2014	Behavioral Responses to Social Separation Stressor Change Across Development and Are Dynamically Related to HPA Activity in Marmosets	X
Taylor et al., 2015	Reunion behavior after social separation is associated with enhanced HPA recovery in young marmoset monkeys	X
Tecot et al., 2019	Faecal glucocorticoid metabolite profiles in diademed sifakas increase during seasonal fruit scarcity with interactive effects of age/sex class and habitat degradation	X

Tennenhouse et al., 2017	Relationships between steroid hormones in hair and social behaviour in ring-tailed lemurs (*Lemur catta*)	X
Thompson et al., 2020	Energy balance but not competitive environment corresponds with allostatic load during development in an Old World monkey	
Tiefenbacher et al., 2003	Fenfluramine challenge, self-injurious behavior, and aggression in rhesus monkeys	X
Tkaczynski et al., 2019	Repeatable glucocorticoid expression is associated with behavioural syndromes in males but not females in a wild primate	X
Touitou et al., 2021	Triiodothyronine and cortisol levels in the face of energetic challenges from reproduction, thermoregulation and food intake in female macaques	X
Vadeleest et al., 2019	Sex differences in the impact of social status on hair cortisol concentrations in rhesus monkeys (*Macaca mulatta*)	
Van Schaik et al., 1991	A pilot-study of the social correlates of levels of urinary cortisol, prolactin, and testosterone in wild long-tailed macaques (*Macaca fascicularis*)	X
Wasserman et al., 2012	Estrogenic plant consumption predicts red colobus monkey (*Procolobus rufomitratus*) hormonal state and behavior	X
Westergaard et al., 2003	Physiological correlates of aggression and impulsivity in free-ranging female primates	X
Wittig et al., 2016	Social support reduces stress hormone levels in wild chimpanzees across stressful events and everyday affiliations	X
Wood et al., 2021	Stress-induced plasma cortisol concentrations in infancy are associated with later parenting behaviors in female rhesus macaques (*Macaca mulatta*)	X
Yamanashi et al., 2013	Cortisol analysis of hair of captive chimpanzees (*Pan troglodytes*)	X
Yamanashi et al., 2016	Effects of Relocation and Individual and Environmental Factors on the Long-Term Stress Levels in Captive Chimpanzees (*Pan troglodytes*): Monitoring Hair Cortisol and Behaviors	X
Yamanashi et al., 2017	Social relationship and hair cortisol level in captive male chimpanzees (*Pan troglodytes*)	X
Zeitzer et al., 2007	Increasing length of wakefulness and modulation of hypocretin-1 in the wake-consolidated squirrel monkey	X

* Unmarked studies were used only in the descriptive analysis.

CHAPTER 2

A biological approach to validating the measurement of fecal glucocorticoid and triiodothyronine metabolites in free-ranging golden-headed lion tamarins

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ABSTRACT

Glucocorticoids (GCs) and thyroid hormones (THs), along with other physiological mediators, modulate the responses that allow long-life adaptation to recurring energetic and psychosocial challenges, including exceptional environmental perturbations. This has sparked the interest of primatologists who, with the advent of non-invasive sampling techniques, have been able to explore changes in GCs and, more recently, in THs under field conditions. However, to fulfill their role in understanding behavioral adaptations, these techniques need a validation process showing that measurements are biologically meaningful for the species and the matrix being studied. We here validate the measurement of GC and triiodothyronine (T3) metabolites (fGCs and fT3) in the feces of wild golden-headed-lion-tamarins (GHLTs; *Leontopithecus chrysomelas*) inhabiting highly disturbed forest patches in South Bahia, Brazil. We did so by testing the effect of capture, body condition (weight and score), sex, social status, and group identity on the levels of fGCs and fT3 in samples collected during capture events and regular group-monitoring days. Our results reveal a significant impact of capture and handling on both metabolites whereas the specific body condition score applied in this study was relevant only to the variation in fT3 levels. These findings confirm the notion that the procedure of capture is a suitable acute stressor to biologically validate non-invasive hormone measures, especially fGCs, and that fT3 is a promising marker of the fluctuations in energetic condition. Additionally, while neither sex nor dominance hierarchy affected significantly measures for both metabolites, fT3 concentrations suggested that metabolic rate differs according to group identity which may be associated with differences in food access within groups' home range. Overall, we demonstrate that a simple biological approach may be sufficient to verify the applicability of non-invasive GC and TH determinations in fecal samples of GHLTs, adding to the literature that shows the usefulness of such techniques.

Keywords: hormones, non-invasive, validation, glucocorticoids, thyroid hormones, lion tamarins

1. INTRODUCTION

Hormones are chemical messengers released by specialized glands into the bloodstream where they reach and act on target tissues and organs to regulate a multitude of bodily functions that allow achieving internal stability in the face of environmental challenges (McEwen and Wingfield, 2003; Nelson and Kriegsfeld, 2017). In an evolutionary sense, hormones coordinate a mechanism of physiological and behavioral adjustments by which animals appropriately respond to environmental and social cues resulting in certain fitness outcomes adapted to the specific environmental context (Higham, 2016). Such is the central role of hormones that they have attracted the attention of scientists from various fields (Touma and Palme, 2005), including primatologists who, thanks to the development of non-invasive and field-friendly techniques, can now measure diverse endocrine markers in matrices like feces or urine of wild primates, without altering their behavior and/or hormonal status through sample collection (Schwarzenberger, 2007). Moreover, with these advances, field observations can be complemented to better address questions about the significance of behavioral traits or the impact of human activities on primate physiology (Hodges and Heistermann, 2011).

Glucocorticoids (GCs) are the preferred hormones to conduct research on conservation issues threatening natural populations (Busch and Hayward, 2009). This is due to their mediation of the physiological stress response that initiates with the activation of the vertebrate hypothalamic-pituitary-adrenal (HPA) axis trigged by predictable or unpredictable perturbations in the environment, ultimately leading to the secretion of GCs (Dantzer et al., 2014). These GC elevations then modulate energy allocation necessary to cope with a variety of physical, metabolic, and psychological challenges (Emery Thompson, 2016). If increases in GCs are short-term, they enhance survival by promoting escape from noxious stimuli (Wingfield et al., 1998). However, chronic elevations of GCs caused by prolonged exposure to stressors may entail reductions in individual health and longevity (Beehner and Bergman, 2017; Sheriff et al., 2011).

Other hormones also involved in vertebrate energy balance, but for which non-invasive studies are more recent compared to GCs, are thyroid hormones, hereafter THs (Wasser et al., 2010). The synthesis of the two major forms of THs, thyroxine (T4) and triiodothyronine (T3), is controlled by the hypothalamic-pituitary-thyroid (HPT) axis. While the latter form is biologically more active, the former serves as a peripheral reservoir for T3 production (Behringer et al., 2018; reviewed by Deschner et al., 2020). Given THs’ particular sensitivity to nutritional deficits which results in a lower metabolic rate (Eales, 1988), it has been suggested that joint TH and GC measures may enable to differentiate energetic from psychological influences as well as distinct strategies to maintain energy balance (Dias et al., 2017; Wasser et al., 2010).

However, to perform such studies, it is of critical importance to validate the hormonal assay for the species and matrix being investigated (Sheriff et al., 2011), for several reasons. Regarding fecal samples, once GCs and THs are released into the bloodstream, they are metabolized by the liver and excreted into the gut via bile ducts (Palme, 2005; Visser et al., 2017), hence substances present in the feces are not native or active forms of the circulating hormones but instead metabolites of GCs and THs (Touma and Palme, 2005). Therefore, all immunoassays, typically chosen to measure fecal metabolites, need to be validated in analytical terms with respect to precision, accuracy, sensitivity, and specificity (Higham, 2016). This not only ensures that potential effects of storage, extraction, and laboratory protocols are accounted for in measurements for the sample in question, but also that assay antibodies cross-react with the metabolites of interest without major interference of other substances present in the sample matrix (Hodges and Heistermann, 2011).

In addition to analytical validation, studies should test assays to determine whether they show biologically meaningful variations in the physiology of the species (Touma and Palme, 2005). This can either be done through a physiological or a biological approach (Touma and Palme, 2005). The former type of validation normally involves inducing changes in circulating hormone levels by administering a specific drug to later evaluate whether such changes are reflected by the measures of related metabolites in excreta (Behringer and Deschner, 2017). Furthermore, if repeated sample collection is possible, this type of experiment offers an opportunity to explore the effects of the lag-time between the circulation of hormones in plasma and their excretion in feces or urine (Behringer and Deschner, 2017; Hodges and Heistermann, 2011). However, physiological validation can be very invasive, a concern particularly if the species is threatened with extinction or if test subjects are not in captivity. In that case, a method of biological validation may be more appropriate (Behringer and Deschner, 2017). This alternative examines the levels of the non-invasive markers in relation to a state or event known to alter the secretion of target hormones, thus proving the biological relevance of the proposed technique (Touma and Palme, 2005). For GC metabolites, specifically, biological validation may be performed through procedures like capture, confinement, translocation, new housing conditions, disturbances by human presence or natural diurnal fluctuations of GCs in excreta (reviewed by Higham, 2016 and Touma and Palme, 2005). For TH metabolites, this has been achieved by assessing the influence of caloric restriction or low body mass (reviewed by Behringer et al., 2018) as well as the impact of infectious diseases (Dias et al., 2017).

In this study, we explore the effect of some intrinsic and stress-related factors on the levels of fecal GC and T3 metabolites, hereafter simply referred to as fGCs and fT3. This with the aim of validating the quantification of these markers in wild golden-headed lion tamarins - GHLTs (*Leontopithecus chrysomelas*), an endangered primate species with a distribution range restricted to the Southern Bahian Atlantic Forest in Brazil. More specifically, to test the biological applicability of two commercial enzyme immunoassays (EIA), we evaluate the stress response of GHLTs to temporary capture and handling, which we predict will raise the fGC levels between the moment captured individuals are removed from nature until they are released back to it (see Methods). We further examine the impact of body condition, expecting that higher body mass and better nutritional status (reflected by a qualitative body score) will both correspond with higher fT3 levels. Additionally, we explore the differences in fGCs and fT3 in relation to GHLTs' group identity, sex, and social status. Currently, there is no published study on the levels of THs in lion tamarins and only few studies used GCs in wild and captive populations of lion tamarins to address different hypotheses (Bales et al., 2002, 2006, 2005; Costa et al., 2020; Henry et al., 2013; Kaisin et al., 2022), including those that focused exclusively on the validation of the techniques (Bertoli et al., 2019; Wark et al., 2016). Therefore, conducting the present study will add to the literature that shows the potential of non-invasive hormone analyses to understand the physiological responses of endangered and non-endangered primate species to environmentally induced stress and energetic constraints, and thus better inform conservation actions.

2. METHODS

2.1. Study subjects

We studied 32 individuals belonging to four habituated groups of GHLTs, named ELI, MRO, OZA and RIB, all of which are monitored with radiotelemetry and captured routinely as part of the ongoing long-term research project BioBrasil (De Vleeschouwer and Oliveira, 2017). The GHLT groups move freely in an area that comprises various privately owned farms located in the municipality of Una in South Bahia (15°17'7.46"S, 39° 8'1.64"W). The landscape in this area is a mosaic composed of disturbed forests fragments of diverse sizes and an agricultural matrix that includes crop plantations (mostly cocoa, rubber, banana, cassava, and vegetables), pastures, open fields, and unpaved roads (De Vleeschouwer and Oliveira, 2017). The predominant natural vegetation in the region is classified as the Southern Bahian Moist Forest (Gouvêa et al., 1976) and the climate is characterized by an annual average temperature of 24 °C and precipitation of 2,500 mm, with no marked seasonality (Mori et al., 1983).

Following Miller et al. (2003), we categorized age of individuals into infants (<3 months), juveniles (3 – 12 months), subadults (12 – 18 months) and adults (>18 months). Dominance hierarchy and age of adult and subadult lion tamarins was assessed based on historic group composition data available from Project BioBrasil, observations of their behavior and information shared by BioBrasil’s field assistants, who were able to recount the breeding history and relatedness of GHLTs on a longer period. According to studies with golden lion tamarins (*Leontopithecus rosalia*), dominant and subordinate breeders (males or females) from the same group rarely display aggressive interactions with each other but instead frequently engage in mutual affiliation, which is something typical of a cooperative-breeding social structure (Baker et al., 1993; Baker et al., 2002). However, we can still classify the dominance relationship of both males and females by examining a set of behaviors that indicate their status and roles within their social context (Baker et al., 2002). Therefore, to define the social status of adult and subadults, we considered their involvement in chases during intergroup encounters and intragroup aggression, their access to mates when females are expected to be fertile, the frequency of mounts, copulations and arch-walks, and the participation in carrying and nursing infants (Baker et al., 2002; Bales et al., 2006, 2005).

All the activities here described involving captures, sample collection and monitoring were approved by the International Committee for the Conservation and Management of the Lion Tamarins and the Brazilian Environmental Agency (ICMBio/SISBIO permit no. 23457-6).

2.2. Capture procedure

Study groups are routinely captured twice a year by a multidisciplinary and trained team of biologists, veterinarians, and field assistants to replace radio-collars (model RI-2D, Holohil Systems Ltd., Ontario) on one or two adult individuals per group and provide all individuals with a tattoo number and a unique dye mark (Nyanzol Dye). This way captures not only allow for the use of radiotelemetry to locate groups in the field but also facilitate identifying individuals during subsequent behavioral observations and sample collections. Before capturing the lion tamarins, platforms, baited with banana, are assembled in an area regularly used by the group, and gradually armed with Tomahawk traps, which are activated once there is evidence the group frequently visits the platform (Catenacci et al., 2022; De Vleeschouwer and Oliveira, 2017). Trapped lion tamarins are taken to a nearby field laboratory and, following a fasting period (three to four hours), anesthetized with a combination of Ketamine Hydrochloride (dose 8–10 mg/kg) and Midazolam (dose 0.25–0.5 mg/kg) to perform examination (Catenacci et al., 2016; Costa et al., 2022). No infant lion tamarin is anesthetized or kept separated from its mother or caregiver (Catenacci et al., 2022). Due to the COVID-19 pandemic which coincided with the present study and the risk of spreading the disease to the animals (Fedigan, 2010), we reinforced biosafety measures adhered by all team members and shortened the handling time by limiting the collection of biometric data. Nonetheless, relevant variables such as body mass, weighed with a one-gram digital scale after containment, and body condition score (see Table 1), determined based on Clingerman and Summers (2005), were still collected. All animals were released the following day in the morning at the same place where they were caught (Costa et al., 2020). For a more comprehensive description on the methods used during captures and examinations of GHLTs consult Catenacci et al. (2022).

Table 1. Description of the body condition scores used during two capture events to categorize nutritional status (body fat and muscle) of all captured golden-headed lion tamarins.

Body condition score	**Definition**
1	**Emaciated**: very prominent and easily palpable bones (hips, ribs, and spinal processes), very low to no palpable muscle mass over the ilium or ischium, subcutaneous fat layer is absent, very angular body, sunken anus between ischial callosities, and protruding facial bones.
2	**Thin**: prominent and palpable bones, low muscle mass over the hips and back, low fat reserves and subcutaneous fat layer, and angular body.
3	**Optimum**: bones are generally not visible but palpable to soft pressure, both muscle mass and fat layer are well-developed giving the spine and hips a firm but smooth touch.
4	**Overweight**: bones are not visible and only palpable to firm pressure, abundant subcutaneous fat layer, and smooth and less defined body contour.
5	**Obese:** bones are not visible at all and difficult to palpate, abundant fat deposits (abdominal, axillary, and inguinal region), difficult abdominal palpation due to large amount of mesenteric fat, and body contour without definition.

Adapted from Clingerman and Summers (2005)

2.3. Sample collection and preservation

Collection of fresh uncontaminated feces was carried out in two different circumstances: 1) during two capture events, one in April and the other in November 2021, including all four GHLT groups and 2) along 11 months of full-day observations between December 2020 and October 2021, only involving MRO, OZA and RIB. In both cases, we focused our sampling effort on adult and subadult individuals (see Table 2) given that GC production increases and GC negative feedback becomes less responsive with age (Gust et al., 2000; Sapolsky and Altmann, 1991). However, we incidentally took samples of juvenile and infant lion tamarins which represent 6.25% of capture and 4.67% of monitoring samples. During group monitoring days, feces were collected any time we observed an individual defecate. During captures, traps installed in the field were verified every two hours and taken to the lab if an animal was caught. Time between the moment an individual was found trapped in a cage (between 8:00 to 17:00) to the moment of collection was on average 3.84 ± 2.19 h. This time interval falls within the gut passage time demonstrated for most callitrichids (2.5-4.5 h; Power and Oftedal, 1996; Heymann and Smith, 1999), which increases the probability of detecting changes in hormonal markers due to the procedures of capture. We stored samples inside 15-ml polypropylene screw-cap tubes prefilled with 4 ml of 80% ethanol (Hodges and Heistermann, 2011), which is the preferred field preservation technique to prevent microbial degradation when immediate freezing is not available (Schwarzenberger, 2007). We made sure that stored samples were completely submerged in ethanol before closing the lid tightly (Hunt and Wasser, 2003) and then labelled the tube with the date, the time of collection and the donor identity. We transferred tubes, the same day of collection, to a freezer at -20 °C where samples remained for 30 to 149 days until oven dried (Khan et al., 2002). To dry samples, we first let the alcohol evaporate overnight for about 12 hours (Terio et al., 2002) and then placed samples inside a laboratory oven at 50 °C for 4 h (Gholib et al., 2018), at such point feces became crumbly suggesting complete water loss. Finally, we transferred samples to small, labelled plastic bags containing oxygen absorbers, sealed them, and took them back to a freezer at -20 °C until all samples were shipped to the lab for further processing.

Table 2. Number of fecal samples per sex and social status collected during capture events and regular monitoring days.

Collection type	N° of groups	N° of individuals	N° of fecal samples						
			♀D	♀S	♂D	♂S	J & I	♂U	**Total**

Capture	4	22	10	10	3	5	2	2	32
Monitoring	3	25	39	54	68	83	12	1	257

Where adults and subadult lion tamarins are differentiated in dominant females (♀D) and males (♂D), subordinate females (♀S) and males (♂S) or males with undetermined social status (♂U). Juveniles and infants are represented by “J & I”.

2.4. Hormone extraction and assay

Extraction of fecal metabolites and quantification of fGCs and fT3 were conducted in the Interdisciplinary Laboratory of Clinical Analyses at University of Murcia (Interlab-UMU), Spain between April and June 2022. To extract the metabolites, we followed a method similar to that described in Wasser et al. (2000). First, we removed large seeds, insect parts and plant debris from crushed dried feces (Foerster and Monfort, 2010), and weighed an aliquot of approximately 0.06 g (95.7% of aliquots: 0.0599 g ± SD 0.0023). We pulverized aliquots and let them shake for 15 h in 1 ml of analytical-grade methanol (Gómez-Espinosa et al., 2014; Rangel-Negrín et al., 2015). Extracts were then centrifuged (3500 rpm for 5 min) and 0.6 ml of supernatant recovered. Supernatants were evaporated inside a vacuum concentrator for 2 h, reconstituted with 0.15 ml, vortexed, and stored at -80°C until analysis.

We used commercial EIA kits from IBL International GmbH for the determination of cortisol (RE52061) and total triiodothyronine (RE55251) to respectively measure fGCs and fT3 in our sample extracts. While the chosen T3 kit has already been validated for T3 metabolites excreted in feces and urine of non-human primates (Behringer et al., 2014; Cristóbal-Azkarate et al., 2016; Deschner et al., 2020; Sadoughi et al., 2021; Touitou et al., 2021), the cortisol kit has thus far been tested in teleosts (e.g.: Cerqueira et al., 2017; Mazzoni et al., 2020; Nilsson et al., 2012) and some mammals (e.g.: Brain et al., 2015; Kaiser et al., 2023; Seyed Almoosavi et al., 2021) but not in non-human primates, based on a Google Scholar search of published articles on May 2023. To prevent alterations in the assays, we strictly followed manufacturer’s instructions of use. The standard curves for calibration of all 10 plates tested (5 for fGCs and 5 for fT3) exhibited an accuracy of $R^2 = 0.98 – 1$. Besides the coefficients of variation (CV) for repeatability already provided by the kits (cortisol: intra-assay CV = 2.5 – 3.5% and inter-assay CV = 2.1 – 5.2%; total T3: intra-assay CV = 3.59 – 6.61% and inter-assay CV = 5.23 – 6.73%), we performed, prior to the analysis of main samples, an analytical validation on a small set of fecal samples collected from captive GHLTs from Terra Natura in Benidorm (Spain), following the same sample processing previously exposed. Both for fGCs and fT3 quantification, we found that intra and inter-assay CVs were less than 15% and displacement curves obtained from serial dilutions of fecal samples ran parallel to the standard hormone curves with a R^2 close to 1.

2.5. Data analysis

We compared the levels of fGCs and fT3 between groups within the predictor variables by applying ANOVA to multiple mixed-effect models fitted with the ‘lmer’ function from the R package lme4 (Bates et al., 2015). Because data contained repeated measures for the same individual within and, in some cases, between groups, we consistently defined individual identity as random factor throughout this analysis. Both fGC and fT3 levels were transformed to logarithm with base 10 to conform with assumptions of normality of residuals and homogeneity of variance, verified at each time data was reorganized. However, when dealing with fT3 levels measured in monitoring samples, we used ANOVAs with Aligned Rank Transformed Data from package ARTool (Wobbrock et al., 2011). If we detected a significant effect for a certain variable, we performed a post-hoc analysis by least-square means from package emmeans (Searle et al., 1980) or a contrast test provided by ARTool. We began evaluating the effect of capture stress on both metabolites considering the total number of samples ($n = 289$) and then we split up the dataset to test the influence of sex, social status, and social status in interaction with sex (status*sex) on capture and monitoring collections without juveniles and infants. Body condition score and body weight were assessed only with capture samples, for the latter variable we excluded juveniles from the dataset as body weight would not be comparable. To explore the differences in group identity, we solely considered samples from regular monitoring including all age categories. All statistical tests were run in R version 4.2.2. (R Core Team, 2022), and considered significant at $p < 0.05$.

3. RESULTS

3.1. Effects of capture stress and body condition

Concentrations of fGCs in capture samples were significantly higher than in monitoring samples ($F_{1,\ 261.67} = 36.67$, $p < 0.001$) with mean levels of 6462.14 ± 921.76 ng/g and 2712.3 ± 197.88 ng/g, respectively for each collection type. Although the range of fGC levels observed in monitoring samples (181.81 – 22065.65 ng/g) was wider than in capture samples (1020.81 – 21712.85 ng/g), they nearly overlapped each other’s scope of variation (Figure 1). On the contrary, fT3 values measured in capture samples were significantly lower than in monitoring samples ($F_{1,\ 259.67} = 11.74$, $p < 0.001$) with mean levels of 29.7 ± 2.73 ng/g and 89.55 ± 7.98

ng/g, respectively. Also in contrast to fGCs, the range of fT3 levels was much narrower in capture (9.86 – 74.44 ng/g) than in monitoring samplings (10.76 – 848.61 ng/g), as shown in Figure 1. We found no significant effect of body weight on either metabolite. However, body condition score of captured individuals was significantly associated to variation in fT3 levels ($F_{1, 29} = 5.84$, $p = 0.02$), this distinction related higher fT3 concentrations to individuals with a better nutritional status (optimum versus thin), see Figure 2a.

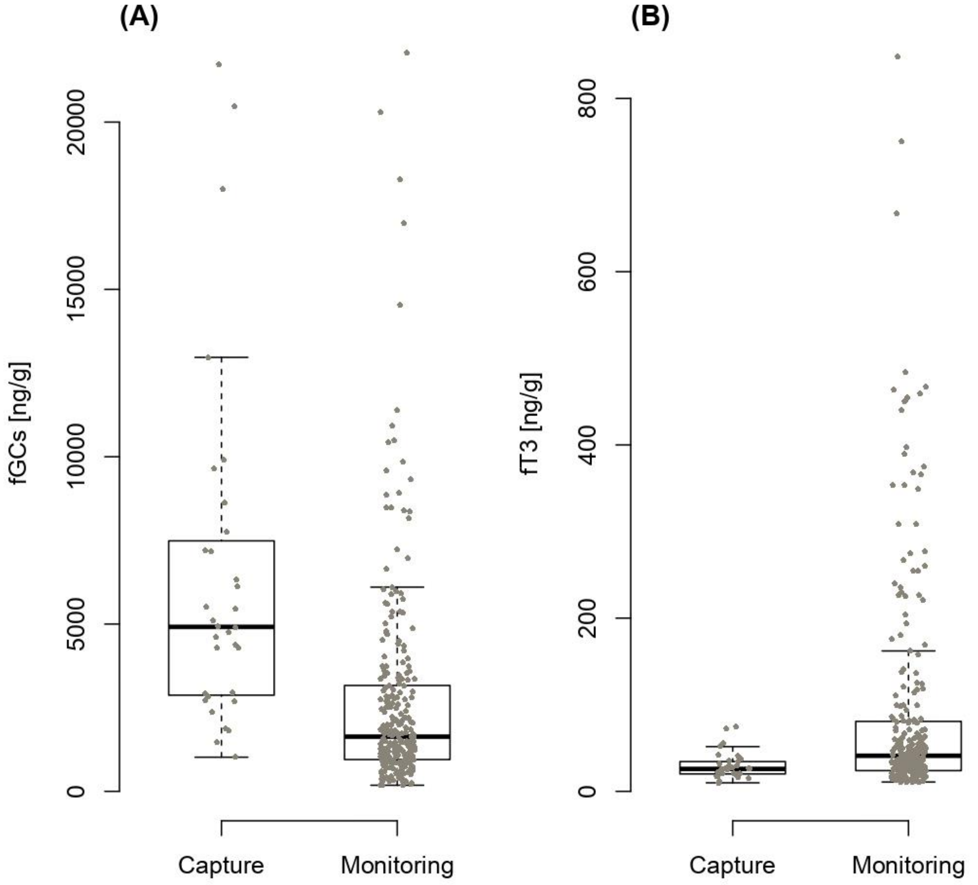

Figure 1. Levels of fecal glucocorticoid – fGC (A) and fecal triiodothyronine – fT3 (B) metabolites measured in samples collected in capture events and groups' monitoring. Data points are plotted as grey dots and boxplots displaying the median in the center line, the 75th and 25th percentiles at the box limits, and 1.5 times the interquartile range from the 25th and 75th percentiles at the whiskers.

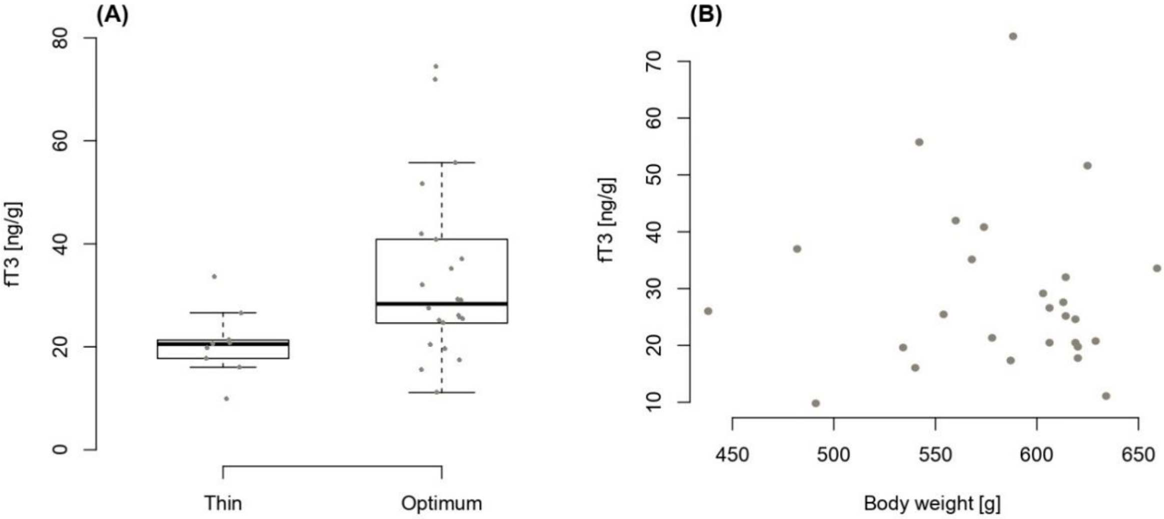

Figure 2. Fecal triiodothyronine (fT3) levels measured in capture samples as a function of body condition score (A) and weight in grams (B). Data points are plotted as grey dots and boxplots displaying the median in the center line, the 75th and 25th percentiles at the box limits, and 1.5 times the interquartile range from the 25th and 75th percentiles at the whiskers.

3.2. The effect of sex, social status, and group identity

Neither sex nor social status was associated to significant differences in the levels of fGCs or fT3 for any of the collection types. Similarly, the interaction between sex and social status was not significant for neither of the metabolites measured in the monitoring samples (see Figure 3), although there was a marginally significant effect of these crossed variables on the fGCs levels from capture samples ($F_{1,\ 17.28} = 3.58$, $p = 0.075$). Regarding only monitoring samples, group identity had a marginally significant effect on fGC values ($F_{2,\ 27} = 2.69$, $p = 0.09$), whereas for fT3 this effect was significant ($F_{2,\ 25.3} = 6.08$, $p = 0.007$) with fT3 levels in MRO being higher than in OZA and RIB (Figure 4). Given that the variation in fT3 among GHLT groups could potentially bias the previous results regarding stress of capture, we ran an additional test for fT3 and type of collection, including individual identity nested within group membership as a random factor in the model configuration. This test confirmed that the procedure of capture has a significant effect on fT3 levels ($F_{1,\ 120} = 9{,}81$, $p = 0.002$) regardless of group identity.

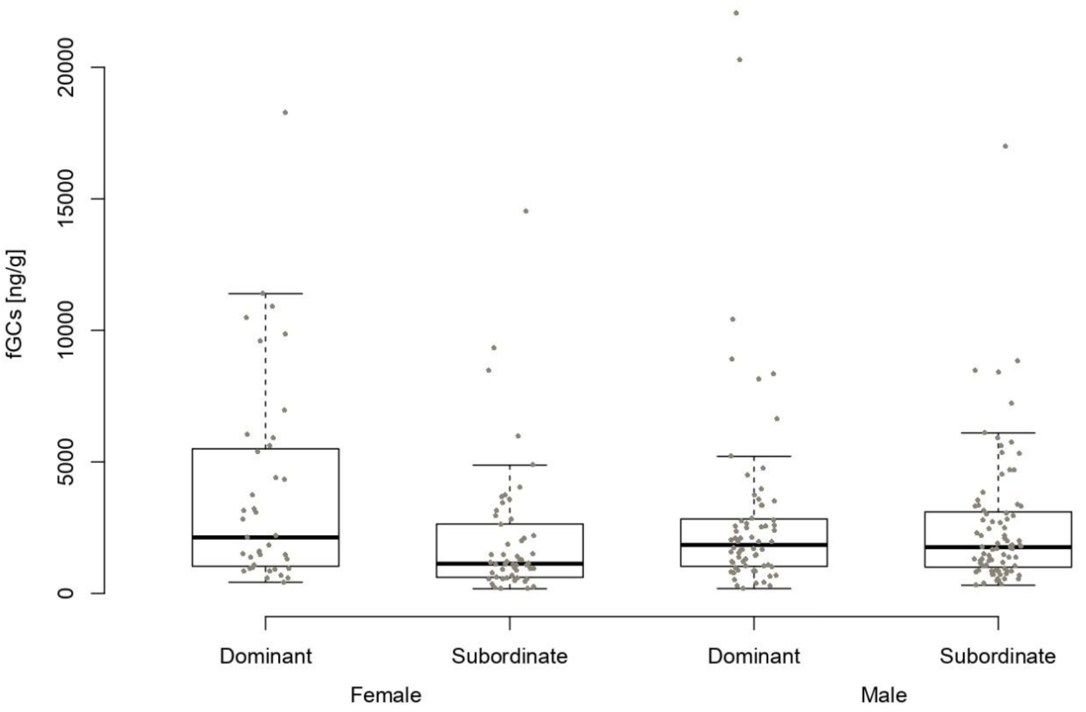

Figure 3. Fecal glucocorticoid (fGCs) levels measured in monitoring samples across different sex and social status of adult and subadult golden-headed lion tamarins. Data points are plotted as grey dots and boxplots displaying the median in the center line, the 75th and 25th percentiles at the box limits, and 1.5 times the interquartile range from the 25th and 75th percentiles at the whiskers.

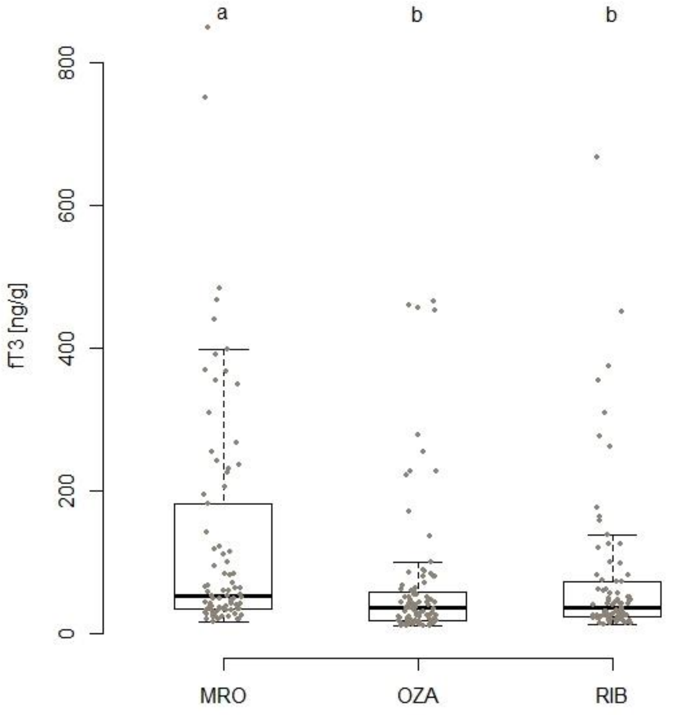

Figure 4. Fecal triiodothyronine (fT3) levels as a function of group identity evaluated for monitoring samples only. Small letters indicate significantly different groups of data (p<0.05).

Data points are plotted as grey dots and boxplots displaying the median in the center line, the 75th and 25th percentiles at the box limits, and 1.5 times the interquartile range from the 25th and 75th percentiles at the whiskers.

4. DISCUSSION

Here we demonstrated, through a simple biological approach, that fGCs and fT3 can be reliably quantified in dried feces of wild GHLTs using commercially available cortisol and total triiodothyronine EIA kits. To do so, we compared their physiological response to a stressful and a natural situation and assessed the relationship between their body condition and the metabolites of interest.

As expected, GC excretion in individuals during capture and handling procedures were, on average, higher than the levels measured during the GHLT's daily activity throughout 11 months of sampling. This finding confirms the notion that the activation of the HPA axis during procedures such as physical restraints, captures and transportations allow detecting GC alterations that show the capacity of individuals to mount their stress response (Touma and Palme, 2005; Wikelski and Cooke, 2006). Our results show that changes in the HPA-axis induced by severe stressors may be detected in fecal samples way before the peak of fGC excretion, between 20 to 27.7 h, determined for congeneric species in studies that conducted a physiological validation (Bertoli et al., 2019; Wark et al., 2016). On the other hand, the repeated captures and constant human presence on the study site may lead to the habituation of individuals and, consequently, lower reactivity to such type of stressors (Johnstone et al., 2012). However, this seems not to be the case for our study subjects who reside in groups with occasional changes in composition (personal observation) which may result in more individuals without the previous experience of a capture. Nonetheless, reduced fear response to human proximity and physical contact has been reported in captive animals that have been habituated through training and positive reinforcement to procedures such as venipuncture (Dettmer et al., 1996) and routine husbandry (Clay et al., 2009). Despite that, regular handling and reduced avoidance towards humans does not necessarily imply lower cortisol levels, as shown in wild-caught, captive wombats (Hogan et al., 2011). Notably, the amplitude of fGC response to capture was similar to the one detected during monitoring days, especially regarding upper fGC concentrations. This may indicate that GHLTs in our study area face environmental stressors (see Chapter 3) that trigger a stress response as intense as the one induced by capture and contention (Johnstone et al., 2012). Our study groups occupy fragments of disturbed forest interspersed with agricultural areas (De Vleeschouwer and Oliveira, 2017), an environment that probably presents particular environmental challenges that could stimulate or even sustain a GC elevation in the long term (Kaisin et al., 2021). It would be interesting to compare our results with those from GHLTs in different landscapes and explore the possible health consequences linked to an over-stimulation of the stress response (Romero et al., 2009) in lion tamarins occupying disturbed forests.

Contrary to our expectations, fT3 levels did vary in relation to the stress of capture, exhibiting an opposite response pattern compared to fGCs. This raises the possibility of a cross-talk between the HPA- and HPT-axis, although levels of fGCs and fT3 during capture were not correlated with each other. This lack of correlation could be due to the small sample size of capture collections, or likely, due to the potentially different excretion time of GCs and THs in matrices such as feces which reflect a wider time frame of hormone production (Touitou et al., 2021). Nevertheless, it is frequently suggested that TH levels are downregulated by the release of GCs associated to stressful situations (Behringer et al., 2018; Burr et al., 1976). Physiological pathways to this TH suppression include the inhibition of the thyroid stimulating hormone and the decrease in the conversion of T4 to T3 (Charmandari et al., 2005). For instance, Helmreich et al. (2005) found in male Sprague-Dawley rats that mild-electric foot-shocks led to significantly lower levels of serum T3 and, although corticosterone levels were not altered by this experiment, certain HPA-axis components may participate in TH regulation. However, this relationship may not be so straightforward even when an acute stressor is involved, as demonstrated in Guadalupe fur seals by DeRango et al. (2019) who associated a simultaneous reduction in T3 and an integrated stress response comprising cortisol and corticosterone levels to a capture event. Furthermore, this potential cross-talk between T3 and GCs may have been confounded by the effect of the food restriction performed in order to anesthetized trapped individuals. In fact, various studies have successfully validated the measurement of T3 in primates by linking reduced food intake to lower levels of T3 in urine and feces (Sadoughi et al., 2021; Schaebs et al., 2016; Wasser et al., 2010). Regardless of the possible explanations, our results for the stress of capture may indicate that, under fearful situations, GCs increase to promote alertness and a freezing response (Charmandari et al., 2005; Korte et al., 2005) whereas T3 decreases as a mechanism to modulate the metabolic rate and save energy (Behringer and Deschner, 2017; Gesquiere et al., 2018). Both changes likely occur in preparation to future demands or additional stressors (Sapolsky et al., 2000).

Another aspect that links THs' secretion to metabolic activity in relation to energy balance is their response to weight gain or loss (Chatzitomaris et al., 2017). Specifically, when high food quantity and quality is accompanied by high T4 and T3 levels, bodyweight will normally increase (Behringer et al., 2018). However, in our study, we found no association between adults and subadults' body mass and fT3 levels, which could be attributed to the fact that weight values were not scaled to specific body length of each individual (DeRango et al., 2019). The latter variable was not measured in all sampled individuals because of the limitation in handling time during capture imposed by pandemic restrictions. Nonetheless, by using a semiquantitative body condition score, we did identify a tendency of lower fT3 levels to be related with individuals presenting a bonier structure and lesser amount of palpable muscle and fat (Clingerman and Summers, 2005). This goes in line with evidence that THs play a direct role in regulating the metabolism of brown adipose tissues and skeletal muscles (López et al., 2013).

Primate males and females normally differ in their strategies to achieve and maintain social dominance which, as reviewed by Cavigelli and Caruso (2015), results in dominant males having elevated metabolic demands due to the costs of competition over access to mates, especially during periods of social instability, whereas exclusion of quality food sources entails reduced energy intake, particularly for subordinate females. Accordingly, one may predict that dominant males and subordinate females will probably exhibit high GC but low T3 levels. However, fGC and fT3 concentrations across sex and social status in adult and subadult GHLTs were statistically undistinguishable. Such absence of a social status effect is consistent with studies on free-ranging male and female golden lion tamarins, at least concerning fGC metabolites (Bales et al., 2006, 2005). Furthermore, the pattern found in our data about fGCs coincides with a hierarchy system, commonly attributed to cooperatively breeding species, where subordinates are not subjected to high rates of aggression and usually rely on close kin support (Abbott et al., 2003). On the other hand, although not investigated here, it is likely that differences in fGCs in relation to female reproductive status may be present in wild GHLTs as demonstrated in several other primate species (e.g.: Bales et al., 2005; Dias et al., 2017; Rimbach et al., 2013). To address such question and more accurately define reproductive condition in females, GC measures should be accompanied with the determination of estrogen conjugates and pregnanediol glucuronide (De Vleeschouwer et al., 2000; French et al., 2003). During group monitoring, we observed the MRO group going through various changes in composition which initiated with the death of the eldest dominant male, then the emigration of females and finally, the disintegration of the group with a single subordinate male using the original home range and attempting to enter a neighboring group. Considering such a dramatic and likely stressful group dynamic, one may presume higher fGC levels in MRO than in the other two groups. However, this was not the case for fGCs but it was instead for fT3 levels. The significantly higher level of fT3 in MRO may point to the potential influence of ecological factors, such as the availability of space and the associated access to resources or perhaps even key food sources. Previous studies on GHLTs have reported differences in home range and feeding behavior among groups using distinct habitats (Costa et al., 2020; Oliveira et al., 2011), as may be generally expected. However, Coutinho (2018) showed that our target groups, which live in the same type of habitat and have a substantial proportion of their home range overlapping, differ in the time devoted to feeding on plants and animals. Although this requires additional investigation, extrinsic factors may potentially lead to constraints in energy intake that may explain this variation in metabolic rate between different groups (see Chapter 3).

CONCLUSION

This study validates the use of two commercial EIA kits to measure fGCs and fT3 in fecal samples of GHLTs. It also provides further evidence that biological validation of hormonal measurements in wild individuals is an effective alternative to the traditional pharmacological challenge when careful consideration of the factors being tested is taken. Nonetheless, physiological and biological validations conducted in captive settings facilitate the elucidation of the lag time between a drug administration or a stressful event and the appearance of the target marker in the feces (Touma and Palme, 2005), through the analysis of temporal profiles of metabolite excretion. This step is particularly difficult to perform in naturalistic settings given the uncertainty of obtaining repeated samples of tested individuals following their release into the wild. Taken together, our results show that fGCs respond appropriately to the stress of capture and possibly, under acute stressors, downregulate the levels of fT3. On the other hand, fT3 responded to changes in body condition and therefore may have a more direct connection to energetic challenges. A study combining physiological markers with behavioral and ecological data, within and across different habitat types, may help to not only compare the amplitude and temporal dynamics of the physiological responses of wild GHLTs but also answer questions about the specific nature of the environmental perturbations that activate such responses.

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CHAPTER 3

Glucocorticoids and thyroid hormones reveal distinct energetic strategies in golden-headed lion tamarins inhabiting disturbed fragments of the Atlantic Forest

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ABSTRACT

Managing energy allocation to meet daily and seasonal demands while adjusting for changes in social and environmental context can in large part define fitness success. For animals inhabiting areas affected by habitat disturbance, this may be particularly challenging given additional energetic constraints resulting from food scarcity or resource competition. Primates possess an unmatched behavioral flexibility which, in coordination with physiological processes, enables them to cope with both predictable and unpredictable stressors. However, the extent to which these response mechanisms are effective in degraded environments is for many species still unknown. Here we examined behavioral and hormonal adjustments adopted by golden-headed lion tamarins (*Leontopithecus chrysomelas*) living in a highly anthropogenic habitat, while taking into account the variation in the availability of preferred plant food sources. We focused on two non-invasive hormonal markers that contribute to energy balance: fecal glucocorticoids (fGCs) and fecal triiodothyronine (fT3). Overall, our results showed that fGC levels were associated to behaviors that reflect energy conservation, more specifically fGCs related positively to the time spent resting and negatively to remaining stationary, suggesting that recovery time between demanding activities is crucial for the attenuation of the stress response. Conversely, fT3 levels are linked positively to rates of feeding on ripe fruits and negatively to the abundance *of S. globulifera* flowers providing nectar, thus fT3 variation is associated with important aspects of energy acquisition which to some degree depended on the interchange in the availability of such food items. Additionally, the positive relation between fGCs and fT3, the absence of a clear effect of ripe fruit availability and the potentially negative influence of agonistic interactions on fruit intake and foraging time, suggest that food access for our study groups is constrained by the intensity of intergroup encounters and the presence of cultivated species. This study is the first to simultaneously analyze fGCs and fT3 in golden-headed lion tamarins and demonstrates the benefits of using both markers to discern the energetic challenges faced by endangered primate species.

Keywords: primates, stress response, resting, stationary, fruit, flowers, habitat disturbance

1. INTRODUCTION

Forest destruction as a result of human activity is generally connected to three distinct but highly intertwined processes: habitat loss, habitat fragmentation and land-use conversion (Bennett and Saunders, 2010). Because tropical forests harbor nearly two-thirds of all terrestrial biodiversity (Gardner et al., 2009), these aspects of landscape change are in large part responsible for the rapid decline of species (WWF, 2020), disrupting the stability of ecosystems upon which life depends (Costanza et al., 2017). Alterations in species' distribution and abundance directly compromise food resources for forest dwelling consumers with implications for their health, survival, and reproduction (Acevedo-Whitehouse and Duffus, 2009), especially for those with a restricted habitat and diet (Birnie-Gauvin et al., 2017). Shifts in food availability linked to habitat disturbance can arise from changes in physical properties such as the solar radiation and wind or water fluxes which affect the vegetation structure and composition of the forest (Benítez-Malvido and Martínez-Ramos, 2003; Chaves and Bicca-Marques, 2013). For example, habitat fragmentation affects forest structure by producing forest edges (Murcia, 1995), in which the microclimatic conditions differ from the forest interior (Harper et al., 2005; Laurance et al., 2006), causing certain fruiting trees, especially shade-tolerant species, to die out. This in turn reduces food resources available to frugivores (Arroyo-Rodríguez and Mandujano, 2006; Kirika et al., 2008). Additionally, habitat disturbance can hamper the biological interactions that ensure normal food supply for certain group of organisms. For instance, habitat fragmentation disturbs interactions between pollinators and plants reducing the final seed set (Rathcke and Jules, 1993) which diminishes seedlings recruitment, and ultimately affects the consumers that feed on such plants (Birnie-Gauvin et al., 2017). In practice, these cause-effect relationships are not always obvious because habitat disturbance may sometimes increase food availability particularly if productive pioneer plants become novel food resources (Emery Thompson, 2016; Johns, 1991). Consequently, to face habitat disturbance, wild animals inhabiting tropical forests must rely on individual flexibility to access available food sources despite the potential energetic constraints of their environment (Ménard et al., 2013; Onderdonk and Chapman, 2000).

Most non-human primates have an omnivorous diet consisting of a variety of food items that include more than one trophic level (Garber, 1987). As consumers, primates have adapted their feeding preferences according to the phenology of plants and insects (Lambert and Rothman, 2015) which in the tropics, where most primate species live, is believed to be less driven by climatic variations than in temperate regions (Boyle and Bronstein, 2012).

Nonetheless, seasonal availability of plant reproductive parts in tropical ecosystems is a common phenomenon that can be influenced both by abiotic factors like rainfall (reviewed by Mendoza et al., 2017) or day length and temperature (e.g.: Pessoa et al., 2012) as well as biotic factors like the presence of pollinators and seed dispersers (van Schaik et al., 1993). Therefore, when searching for food items, primates use their ability to retain and combine spatial and temporal information about the clumped distribution and relatively synchronous fruiting patterns of many tropical trees (Garber, 1987; Zuberbühler and Janmaat, 2010). Furthermore, primates exhibit a wide range of behavioral adaptations that may allow them to mitigate the costs of intra- and intergroup foraging competition (Markham and Gesquiere, 2017) and face periodic or even prolonged food scarcity (van Schaik et al., 1993). For instance, in response to low food availability, primates may alter total foraging time and movement patterns, change habitat use and home range size, shift group size and dietary preferences, limit their activity patterns to minimize energy expenditure or exploit fallback foods (reviewed by Cristóbal-Azkarate and Arroyo-Rodríguez, 2007 and Lambert and Rothman, 2015) which are alternative items often containing lower usable energy but more plant secondary compounds than preferred items (Foerster et al., 2012). In view of this, it is tempting to presume that primate behavioral flexibility will enable them to cope with the reduced food availability of human-impacted habitats. However, the opposite seems true, as more than half of primate species are endangered (Estrada et al., 2017) with forest conversion into intensive agriculture, cattle production and natural resource extraction as the main anthropogenic activity affecting primate communities (Almeida-Rocha et al., 2017; Estrada et al., 2020). The extent to which habitat disturbance brings about changes in food quality and quantity that surpass primate behavioral flexibility and produce adverse consequences for group size and composition, reproductive patterns, and social relationships (reviewed by Kalbitzer and Chapman, 2018) may, to a considerable degree, explain such unprecedented decline in primate species.

To better understand how primates' behavioral responses contribute to matching their phenotypes to the novel environmental context and thus to their persistence (Maspons et al., 2019), researchers can integrate the non-invasive monitoring of physiological markers into studies of primate behavioral ecology. Hormones are chemical messengers that play a key role in individual's homeostasis by coordinating both physiological and behavioral responses in the face of diverse environmental challenges (Higham, 2016). Consequently, hormones can provide valuable information to evaluate not only the proximate mechanisms that influence behavior but also the strategies to acquire and use energy which may in turn impact individual fitness and ultimately the persistence of wild populations (Behringer and Deschner, 2017; Emery Thompson, 2016). Furthermore, with the advent of modern non-invasive approaches, sample collection and hormonal measurement no longer pose an adverse effect for animal welfare (Fedigan, 2010) and allow repeated sampling, necessary to minimize the influence of short-term responses to environmental perturbations (Emery Thompson, 2016). Among the endocrine markers that participate in the regulation of metabolism (Norman and Litwack, 1997), two of them, glucocorticoids (GCs) and triiodothyronine (T3), can be applied to investigate how energetic condition is associated with individual behavior.

GCs mediate the stress response necessary to overcome the predictable and unpredictable challenges that vertebrates encounter in their physical and social environment (Romero et al., 2009). GCs are secreted by the adrenal glands following a cascade reaction that takes place when the hypothalamic-pituitary-adrenal (HPA) axis is activated (Sapolsky et al., 2000) in response to a noxious or threatening stimuli or stressor (Romero and Wingfield, 2016). Circulating GCs then help restore homeostasis by supplying energy to tissues and redirecting behavior and physiology towards survival (Wingfield et al., 1998). However, GC actions are far more primary than that: they also modulate energy allocation to meet daily and seasonal demands (Busch and Hayward, 2009; Markham and Gesquiere, 2017). Chronic elevations or insufficient levels of GCs, on the contrary, can have detrimental consequences for the individual’s immune system, growth, reproduction, energy regulation and, ultimately, survival (Romero et al., 2009). Given their importance in coping with energetic and psychosocial stressors, GCs have become quite popular in wild primate research. According to Beehner and Bergman (2017), stress research on wild primate GCs has focused largely on the effects of rank and anthropogenic disturbances, and the negative health consequences of chronic stress and less on the adaptive outcomes of the stress response. Energetic challenges, such as food scarcity or restriction, are correlated with increased levels of GCs in most vertebrates irrespective of the stressor’s duration (de Bruijn and Romero, 2018). In primates specifically, numerous studies have indicated that food availability and food intake, linked to seasonality and/or habitat disturbance, correlate negatively with GC levels (e.g.: Balestri et al., 2014; Cavigelli, 1999; Dunn et al., 2013; Foerster et al., 2012; Muller and Wrangham, 2004; Pride, 2005; Tecot et al., 2019). Such associations highlight the severity of food shortage as a stressor and the participation of GCs in triggering stress coping mechanisms (see Chapter 1; Fiorini-Torrico et al., in review). Nonetheless, given that GCs can be equally sensitive to intrinsic (e.g.: age, sex, reproductive and immune condition) and extrinsic (e.g.: weather, anthropogenic disturbance, social and ecological context) factors (Dantzer et al., 2014), isolating the effects of energetic challenges that derive from changes in food availability and feeding behavior may be difficult, especially under naturalistic conditions (Emery Thompson, 2016).

Thyroid hormones have received considerably less attention than GCs although they are essential for the metabolic activity throughout all life stages of vertebrates (Behringer and Deschner, 2017). T3, which is the biologically more active form of thyroid hormones, can be used as an index to measure the differences in basal metabolic rate that reflect cellular activity at rest and has been positively associated with enhanced fitness (Cristóbal-Azkarate et al., 2016; Hulbert and Else, 2004). In addition to metabolism, T3 coordinates adjustments in heart rate, blood pressure, brain development, nutritional balance, and heat production unconnected to movement or locomotion (Silva, 2006; Wasser et al., 2010). Contrary to GCs, depletion of food resources will decrease the concentration of T3 which as a result reduces metabolic rate, promoting energy conservation until more favorable times permit higher food intake (Eales, 1988; Markham and Gesquiere, 2017). Also, in contrast to GCs, T3 appears to be unresponsive to psychological stressors, offering the opportunity to discern non-energetic from energetic challenges when GCs measures are combined with T3 (Emery Thompson, 2016; Wasser et al., 2010). To the best of our knowledge, only few studies have integrated the analysis of both GCs and T3 with such aim in wild primates (Dias et al., 2017; Liu et al., 2022; Touitou et al., 2021).

Golden-headed lion tamarins (*Leontopithecus chrysomelas*, GHLTs) are small-bodied primates, belonging to the family Callitrichidae, and endemic to the Southern Bahian Atlantic Forest in Brazil. Most of their diet consists of ripe fruit and insects, in addition to small quantities of nectar, gum, soil, and small vertebrates (Catenacci et al., 2016; Raboy and Dietz, 2004). A large part of their activity budget is spent manipulating different substrates to locate animal prey, particularly epiphytic bromeliads (Catenacci et al., 2016; Oliveira et al., 2010; Raboy and Dietz, 2004). The geographic distribution of wild GHLT populations can be divided into two contrasting areas based on predominant land use patterns (Pinto and Rylands, 1997). The western range is dominated by pastures and intense cattle ranching with predominantly small and highly isolated forest fragments, whereas in the eastern range the landscape is characterized by the presence of shaded cocoa agroforests (locally known as 'cabrucas') with embedded patches of mature and regenerating forests (De Vleeschouwer and Raboy, 2013; Raboy et al., 2010). Although the characteristics and better connectivity of the eastern portion support the majority of GHLT populations (Teixeira, 2022), particularly due to the presence of key-stone food and foraging resources as well as tree holes for sleeping available in cabrucas and mature forests (Oliveira et al., 2011, 2010; Raboy et al., 2004), conversion of cabrucas to more intensive land use forms and sustained deforestation throughout the GHLTs' geographic distribution range have caused a gradual decline in population numbers, and the species is still considered endangered (Oliveira et al., 2021). With ongoing anthropogenic disturbance in the region, degraded forests and the surrounding agricultural matrices will become proportionally more prevalent, and increasingly more important for GHLT's long-term survival.

In degraded forest fragments, GHLTs feed on a great diversity of plants (Catenacci et al., 2016) which may indicate the inclusion of secondary species to cope with habitat fragmentation and modification of food availability (Cristóbal-Azkarate and Arroyo-Rodríguez, 2007; Pinto et al., 2003 and others). Additionally, Pessoa et al. (2012) have shown that richness and abundance of fruiting trees and fruit biomass in the Atlantic Forest of Southern Bahia are negatively affected by forest cover, suggesting consequences for frugivorous species. Despite the absence of a marked dry season in the region, GHLTs' feeding behavior in degraded areas is largely modulated by acute changes in monthly fruit availability (Catenacci et al., 2016) which in agriculture-dominated areas may force GHLTs to supplement their diet with cultivated species (Costa et al., 2022). Endoparasite loads of GHLTs living in degraded forest fragments within an agricultural matrix are higher to those living in cabrucas, and not linked to differences in fruit availability and feeding behavior (Costa et al., 2022). Investigating how endocrine traits mediate the expression of phenotypes that influence fitness (Dantzer et al., 2016) can shed light on the proximate mechanisms that affect immune competence (Acevedo-Whitehouse and Duffus, 2009). Here, we propose exploring the behavioral and hormonal adjustments associated with changes in food availability experienced by GHLTs using degraded forest fragments. More specifically, we evaluate the associations between the lion tamarins' activity patterns and the levels of fecal GC and fecal T3 metabolites (herein fGCs and fT3) while keeping track of the monthly variations in food availability, measured separately for three GHLT groups. By doing so, we aim to understand how differences in energy acquisition and expenditure at the individual level are linked to an attenuation in the hormonal response, and a positive energy balance.

1.1. Theoretical framework

We envision two possible scenarios leading to different physiological and behavioral responses (Figure 1). Under a scenario of high food availability, we expect lower levels of fGCs and higher levels of fT3 to correlate with higher proportions of time devoted to feeding and less so to travelling and foraging resulting in high energy acquisition. In the second scenario, we predict that low food availability will accompany higher levels of fGCs and lower levels of fT3 which will be associated with more time dedicated to travelling and foraging which will nonetheless lead to lower proportions of feeding and therefore low energy acquisition. For both scenarios, we foresee two possible socially induced changes that counterbalance energy acquisition, one relates to an energy saving time-budget (Gómez-Espinosa et al., 2014), where lion tamarins rest and socialize more, likely resulting in low energy expenditure, and another that characterizes an energy demanding time-budget (Gómez-Espinosa et al., 2014), where lion tamarins engage more in intergroup aggression but spend more time stationary to counterbalance energy use. Within this framework, we expect that foraging strategies will be effective when food abundance is either high or when low energy acquisition matches low expenditure generating in both cases a positive energy balance. However, if low energy acquisition coincides with high expenditure, then lion tamarin’s stress responses will be reinforced and, consequently, risk bypassing the reactive homeostasis range (Romero et al., 2009).

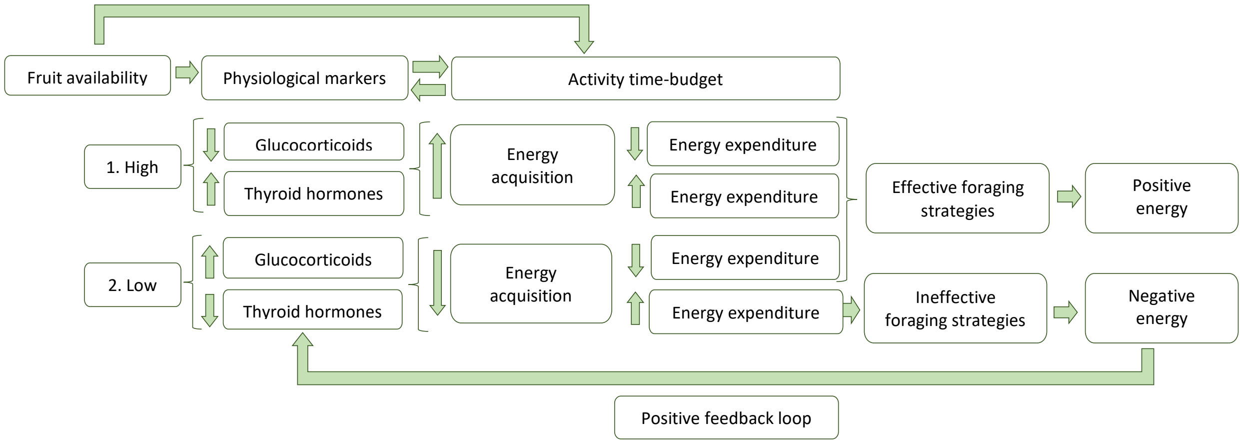

Figure 1. Conceptual scheme of predicted relationships between two scenarios of fruit availability and changes in physiological markers and activity time-budget, resulting in distinct energetic outcomes.

The proposed framework was built upon certain assumptions. First, although behavioral patterns have the potential to alter the physiological markers of stress (Dantzer et al., 2016; Nelson and Kriegsfeld, 2017 and others), the association between food availability and GCs and T3 remains overall fixed even when high energy expenditure or effective foraging strategies may affect this relation. Second, we assume that high energy expenditure in a scenario of high food abundance will not exceed energy acquisition and therefore will still generate a positive energy balance. Third, other extrinsic factors which are not considered in this study could influence this framework such as climatic fluctuations, human noise and presence of predators and domestic animals. We do however contemplate some intrinsic factors like sex and group identity for the model selection. We assume that differences in dominance rank and female reproductive stage will have a weak impact on the behavioral and hormonal responses to energy balance, although Bales et al. (2005) showed the effect of reproductive condition on the cortisol levels of female golden lion tamarins. We discuss some of these intrinsic differences in Chapter 2.

2. METHODS

2.1. Study area and subjects

Sampling was conducted between December 2020 and October 2021 in an area located in the municipality of Una in the south of Bahia state, Brazil (Field base: 15°17'7.46"S, 39° 8'1.64"W). This area spans across various private farms where landscape is composed of disturbed remnants of Atlantic Forest interspersed with an agricultural matrix that encompasses perennial and ephemeral plantations, pastures, and dirt roads. Forest remnants in our study area are constantly changing due to intense human activity, leading to different regeneration stages among patches. Following Catenacci et al. (2016), we classified vegetation inside fragments in advanced (AS), medium (MS), initial (IS) secondary forests and swamps (SW). The regional climate is hot and humid, and characterized by the absence of a seasonal dry period (Mori et al., 1983). Average rainfall is approximately 2000 mm/yr and average temperatures are around 24 °C with little variation over the year (Thomas et al., 1998).

We studied three neighboring groups of previously habituated GHLTs: MRO, RIB and OZA. These groups are part of project BioBrasil’s ongoing research program which, in addition to regular monitoring, includes capturing individuals every 6 months to check health status, take biometric measures, as well as to change radio collars and renew tail dye marks, enabling the use of radio telemetry to locate the groups and the identification of individuals during field observations (see Costa et al., 2022; Coutinho, 2018; De Vleeschouwer and Oliveira, 2017). For more details on the capture and examination procedures see Catenacci et al. (2022). Encounters between groups were frequent, we recorded intergroup aggression and territorial defense on 58% of all the days we followed target groups which corresponded to a total of 0.185 encounters per hour of observation. This encounter rate is likely connected to the high amount of home range overlap previously documented for all three groups (Costa et al., 2022; Coutinho, 2018). High rates of intergroup encounters have also been reported for GHLT groups using cabrucas and mosaic landscapes (Oliveira et al., 2011). However, for groups living inside a biological reserve consisting of mature and regenerating forests, the encounter rate was comparatively lower – average of 0.022 encounters per hour of observation (Raboy and Dietz, 2004).

Since the HPA axis in mammals tends to be more sensitive as they grow older (Sapolsky et al., 1985; Sapolsky and Altmann, 1991), we focused our sampling effort on male and female adults (age > 18 months) and subadults (between 12 and 18 months of age; Dietz and Baker, 1993; Miller et al., 2003). Throughout this study, group size and composition varied due to occasional predation or death, and mostly disappearance and migratory events (total number of changes in composition per group: MRO = 7, RIB = 8, OZA = 7). In Table 1, we describe individuals included in this study that resided within target groups for at least two of the 11 months of field activities.

Table 1. Group characteristics and details of sampling effort

Group	Group composition	Group size	N of days followed	N of fecal samples
MRO	Adult males: 115[D], 130[d], 131 Adult females: 138[e], 516[i] Subadult females: 157[e], 507[i]	3 - 5	23	81
OZA	Adult males: 124, 128[d], 155 Subadult males: 156[d] Adult females: 511[i], 512[i], 518[i] Subadult females: 501[d]	6 - 8	18	95
RIB	Adult males: 85[D], 139[i], 140[i], 146[d] Adult females: 148[d], undet.[i] Subadult females: 507[e], 510[i]	3 - 5	21	81
Total			**62**	**257**

Note: D = died, d = disappeared, e = emigrated, i = immigrated

2.2. Food availability assessment

To assess the monthly abundance of potential food sources per group, we calculated a fruit availability index (FAI) and a flower availability index for *Symphonia globulifera* (SAI). We established 28 vegetation plots of 20mx10m evenly distributed across different vegetation types and group home ranges and determined the floristic composition of trees with a diameter at breast height (DBH) of more than 10 cm inside the plots. Based on this, we selected trees from 21 species (see supplementary Table 1) commonly consumed by GHLTs following previous studies that documented feeding time and dietary preferences (Catenacci et al., 2016; Coutinho, 2018; Oliveira et al., 2010). With the aim of later monitoring the phenology score of those tree species, we marked 3 to 12 randomly selected specimens (130 specimens in total) depending on the relative abundance of each species in our study area. We then calculated the average basal area (BAi) and total density per ha (Di) of every tree species separated by group. We determined the monthly abundance of flower buds, flowers, ripe and unripe fruits of marked trees by assigning a score between 0 and 4 to the percentage of tree crown area covered with each plant item (where 0 = 0% of the crown area, 1 = 1 to 25%, 2 = 26 to 50%, 3 = 51 to 75%, and 4 = 76 to 100%) following Fournier's semiquantitative scale (Fournier, 1974). For further calculations, we only considered the abundance of ripe fruits and flowers of *S. globulifera* since unripe fruits were rarely eaten and, among the species included in our phenology monitoring, *S. globulifera* was the only species from which GHLTs sipped the nectar. We observed GHLTs feeding on other flowers, especially some vines and climbers, however these species were not considered in the selection of preferred plant food sources. We then obtained the mean phenology score for each tree species in a given month (Pi) and multiplied this value with the respective Di and BAi within each group home range. Finally, to obtain FAI we summed the product of Di, BAi and Pi across all species as indicated in equation 1 (Huang et al., 2015). SAI, on the other hand, was the individual product of *S. globulifera* per month.

Eq. 1

$$FAI = \sum_{i=1}^{21} DiBiPi$$

Where: Di = total density of tree species per ha, Bi = average basal area per tree species, Pi = phenology score per tree species in a given month

2.3. Collection of behavioral data

Observations were performed on full days from the moment the groups left a sleeping den at dawn until they took shelter before dusk. We located groups using a radio telemetry system with the help of an experienced research assistant who also assisted in the identification of individuals and collection of fecal samples. To study the time budget of different behaviors, we used continuous focal samples (Altmann, 1974) of 10 min on all adult and subadult individuals in the group (see Table 1) and randomly alternated individuals after a 10 min interval, making an effort to balance out the number of focal samples among individuals throughout the day. All observations were voice-recorded, pausing whenever we lost sight of the focal individual. We resumed the recording once the same individual was visible within a 2-min search, otherwise we aborted the focal sample and started a new one with the closest individual. Field observations were conducted by RFT. Transcriptions of voice notes were split with an assistant with whom we held regular meetings to ensure equal criteria.

All timed behaviors were categorized based on an ethogram proposed by Raboy and Dietz (2004). Each category grouped together behaviors according to the descriptions provided in Table 2.

Table 2. Behavioral categories used to classify focal observations of free-ranging adult and subadult golden-headed lion tamarins.

Category	Description
Traveling	Individual moves from one place to another covering a small or long distance, normally in order to change of foraging site or food item.
Stationary	Individual remains in one place observing, scratching, self-grooming, scent marking or vocalizing. It usually occurs in between traveling.
Foraging	Individual manipulates or bits a substrate (bromeliad, dead trunk, dried leaves, tree bark, branches, palm tree, tangled vines, and lianas) in search for animal prey. It includes simply looking at a substrate, and manipulating or smelling some fruit, possibly not ripe.
Feeding	Individual brings a food item to its mouth or holds it and takes it to another place to begin or continue ingesting it. It ends when individual leaves a fruiting tree or engages in a different activity. Food items were distinguished in fruit, animal prey (small vertebrates and invertebrates), flowers, gum, and mushrooms.
Resting	Individual lies down on a surface in proximity or apart from the rest of the group with closed eyes or while observing the surroundings.
Agonism	Individual chases or is being chased by another individual during inter or intragroup conflicts. It is usually accompanied by loud vocalizations from focal individual or other group members.
Affiliation	Individual engages in social grooming (received or given) and playing. It includes mounting and copulating.
Inside a substrate	Individual remains concealed inside big bromeliads, tangled vines, or palm trees, either foraging, resting or in affiliation.

Adapted from Raboy and Dietz (2004)

Although we initially intended to monitor each group for two full days each month (6 days in total per month), we had some unforeseen issues because batteries of some radio collars died prematurely, complicating the location of groups in the study area. This together with the strict measures to prevent the spread of COVID-19 between field team members during the pandemic reduced the amount of field observations between April (4 full days) and May (4 full days). Overall, RFT conducted 1165 10-min focal samples which adds up to approximately 194.2 hours of focal observations registered during 11 consecutive months of field activity. Observation time per individual was on average 1.3 ± SD 0.6 hours per month, variation in this value was caused in large part by the constant changes in group composition (see Table 1).

2.4. Fecal sample collection, processing, and hormone assay

Fecal samples were collected from known individuals, immediately after defecation concurrently with group observations. We collected a total of 257 samples and an average of 23.26 ± SD 8.6 samples per month. In the field, we stored samples inside polypropylene screw-cap tubes prefilled with 80% ethanol (Hodges and Heistermann, 2011) which were labelled with date, collection time, and individual identity, and later, the same day, taken to a freezer at -20 °C (Whitten et al., 1998). Consecutive steps to preserve samples in the long-term combined ethanol evaporation at room temperature (Terio et al., 2002), desiccation in an oven at 50 °C (Gholib et al., 2018) and storage of samples at -20 °C. Samples were shipped to the Interdisciplinary Laboratory of Clinical Analyses at University of Murcia (Interlab-UMU) in Spain, where they were further processed between April and June 2022. Based on a modification of Wasser et al. (2000) for dried feces, we extracted fGCs and fT3 from 0.06 g of pulverized aliquots in 1 ml of analytical-grade methanol (Gómez-Espinosa et al., 2014), resulting supernatants (0.6 ml for each sample) were evaporated, then reconstituted with a PBS buffer and finally stored at -80°C. We employed commercial EIA kits from IBL International GmbH to quantify fGC (cortisol: RE52061) and fT3 (total T3: RE55251) metabolites in the fecal extracts. Before measurements, we conducted a small analytical validation regarding precision (intra- and inter-assay coefficients of variation < 15%), accuracy and linearity (R^2 close to 1). Additionally, we carried out a biological validation by assessing the stress of capture and body condition of examined individuals in relation to differences in fGC and fT3 levels. We strictly followed manufacturer’s instructions throughout hormone determinations. Hormone metabolite concentrations are presented in ng per g of dried feces. For more details on any procedure described in this section see Chapter 2.

2.5. Data organization and analysis

To assess the relationship between the multiple variables measured in this study, we applied linear mixed effects models (LMMs) to the data when the dependent variable in the model equation was fGCs and generalized linear mixed effect models (GLMMs) when the dependent variable was fT3. All statistical analyses were performed in a R environment, version 4.2.2. (R Core Team, 2022), where we set the statistical significance threshold at $p \leq 0.05$. We used the R package 'lme4' (Bates et al., 2015) that allowed us defining LMMs for fGCs with the function 'lmer' and GLMMs for fT3 with the function 'glmer'. We used a log10 transformation for both hormones, which improved the fitting of a normal distribution on fGC levels and a Gamma distribution on fT3 levels. As many of the explanatory variables differed largely in scale, especially food availability indices, we scaled the equation terms in the models whenever needed using the function 'scale' from R package 'base'. These transformation steps not only minimized overparameterization but also enhanced model convergence particularly when dealing with Gamma distribution. Concerning only fGC models, we verified that the assumption of homogeneous and normally distributed residuals was met by visually inspecting Q-Qplots. We selected the best models based on the Akaike's information criterion (AIC), with this approach, we decided which combination of explanatory variables (fixed effects) best explained the variation in fGCs or fT3. To make that choice, we compared the AIC of a respective model with the lowest AIC obtained, if such difference was greater than two, we considered the model with the lowest AIC still better than the model being assessed (Burnham and Anderson, 2002). However, if difference was smaller than two, we employed a Likelihood ratio test (LRT) with the function 'lrtest' from the R package 'lmtest' to compare which model significantly outperformed the other in terms of fitting the data.

Prior to testing our main hypothesis, we evaluated the possible confounding effect of sex of individuals, group identity, collection time and interval of days before sample drying on fGCs and fT3. The latter two predictors have been shown to be a major concern in various studies that explored the influence of diverse ecological factors on hormonal cues, such as fGCs (e.g.: Chaves et al., 2019; Dunn et al., 2013; Foerster and Monfort, 2010), owing to the normal circadian rhythms in vertebrates (Felig and Frohman, 2001) and how storage method can change fGCs concentrations over time (Gholib et al., 2018; Khan et al., 2002). For this preliminary analysis, we constructed models with identity of individual as random factor, the potential confounding effects as fixed factors and either fGCs or fT3 as dependent variables. Before starting the stepwise model selection, we controlled for multicollinearity between variables with the function 'vif' from R package 'car', whereby variables with a Variance inflation factor (VIF) higher than three were excluded from the model equation.

With the purpose of interconnecting the three datasets about availability of plant food sources, hormone levels, and time budget of activities, and yet, losing as little information as possible; we combined the primary data on fGCs and fT3 of each fecal sample with the monthly values of FAI and SAI per group, and the monthly proportions of each behavioral category per individual. In such a configuration, we considered one of the hormones as the response variable, while the other hormone together with food availability and behaviors served as predictor variables or fixed factors. Random factors for all main models were individual identity and number of focal samples per individual per month, this later factor was included as a mean of controlling for the differences in total observation time per individual. To obtain the proportion of a behavioral category per individual, we aggregated the time devoted to a certain behavior and divided it by the total time of focal observation on a specific month. Given the large number of predictors, we first ran single fixed effect models with the aim of detecting the predictors with significant interactions. We then constructed full models, checked for multicollinearity and selected the best models according to previously exposed criteria. To show the amount of variability explained by the resulting models, we obtained pseudo R^2s with the function ‘r2’ provided by the R package ‘performance’. Finally, we assessed the associations between the average monthly values of the multiple variables considered in this study (where $N = 11$ months for each variable) with Spearman correlations. This with the aim of taking advantage of predictors that may be excluded during model selection but could potentially enrich our analysis.

3. RESULTS

3.1. Confounding factors of hormonal variation

The best-supported model for fGC levels included collection time and group identity and differed significantly from the second-best one (LRT: $X^2 = 7.03$, d.f. = 2, $p = 0.03$). Furthermore, this model indicated that collection time had a slight negative effect on fGC levels ($b = -0.025$, se = 0.0089, $t = -2.78$, $p = 0.006$), showing that fGC excretion reduces with time of the day. A post hoc ANOVA of aligned rank transformed data corrected for individual identity showed that there is no significant difference in the sample collection time across the 11 months of field activity ($F[10,229.65] = 1.3$, $p = 0.23$, Tuckey for 11 months indicates $p>0.05$ for all repetitions). Thus, assuming that mean collection time per month does not have affect fGCs, we decided to disregard this factor as a possible term in our main models. On the other hand, the best-supported model for fT3 levels, which significantly outperformed the second-best one (LRT: $X^2 = 6.89$, d.f. = 1, $p = 0.0089$), also included group identity but instead of collection time, it indicated a positive effect of days of storage prior to drying on fT3 ($b = 0.038$, $se = 0.014$, $t = 2.64$, $p = 0.008$). A post hoc analysis showed that days of storage prior to drying is strongly correlated to the date of sample collection ($r_s = -0.69$, $p < 0.001$) and that both factors have a strong positive influence on fT3 levels (GLMM - date of collection: $b = 0.13$, $se = 0.02$, $t = 6.56$, $p < 0.001$; days of storage: $b = 0.09$, $se = 0.016$, $t = 5.6$, $p < 0.001$) . This may likely indicate that days of storage reflects a natural increase in fT3 levels that occurred throughout the study period rather than demonstrating the confounding effect of the storage technique on hormonal stability. Testing whether days of storage before desiccating in fact changed fT3 concentrations in GHLTs fecal samples would require a different approach (see Gholib et al., 2018; Khan et al., 2002; Terio et al., 2002). We nonetheless consider highly advisable to limit the time between collection of sample and hormonal measurement to the minimum possible. Therefore, in order not to interfere with the temporal variation in fT3 that could potentially be related to other seasonal and ecological factors, we omitted days of storage prior to drying from further analysis. The influence of group identity on fGC and fT3 concentrations were already addressed in Chapter 2. Therefore, to avoid complex structures that may hamper model convergence, we will not consider group identity as a term in our main models.

3.2. Food availability, hormones, and behavior

Mean (±SD) FAI between the months of data collection was 1623.48 ± 1183.83, ranging from 18.65 to 5222.36 at group level and showing two separate peaks in fruit production, one that lasted three months between March and May 2021 and the other that took place only in September 2021. Mean SAI, on the other hand, was 8796.86 ± 11828.06 with a range between 0 and 113095.29 and a single peak in flower production in June 2021 (Figure 2).

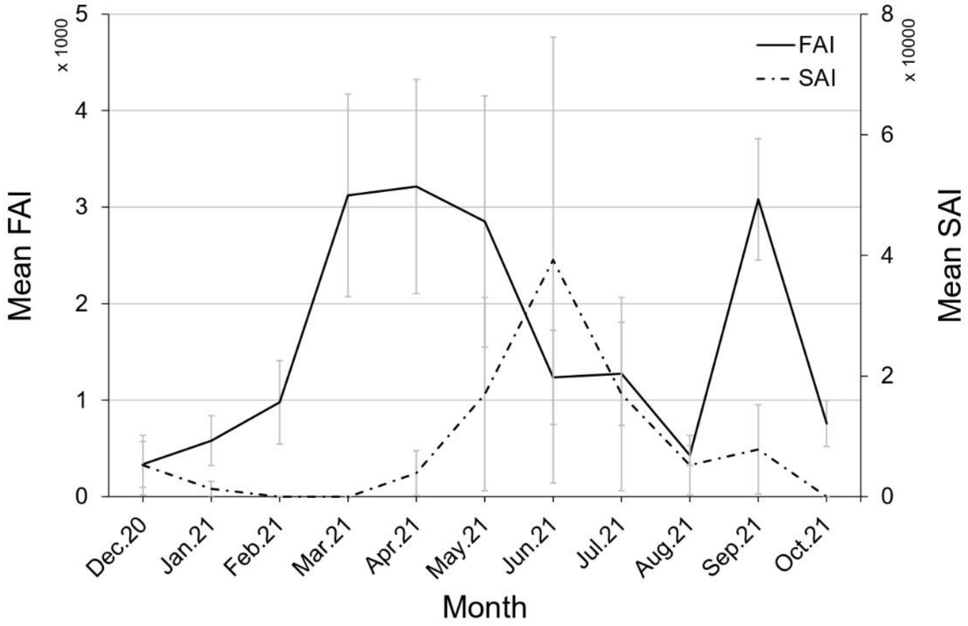

Figure 2. Temporal variation in the mean fruit availability index (FAI) and the mean flower availability index for *Symphonia globulifera* (SAI). Whiskers indicate the standard error for each mean value.

Mean fGC and fT3 levels of the GHLTs in our study area were, respectively, 3184.7 ± 1196.9 ng/g and 90.33 ± 50.44 ng/g throughout the months of sample collection. Both hormone levels varied widely among sampled individuals (fGCs range = 377.85 – 22065.65 ng/g and fT3 range = 13.84 – 570.29 ng/g). Regarding the overall behavioral patterns of GHLTs, almost 46% of the recorded time was devoted to traveling (24.83 ± 2.49%) and foraging substrates (21.03 ± 4.36%), whereas remaining stationary (17.13 ± 4.58%) and resting (6.81 ± 3.50%) together concentrated approximately 24% of the time. Feeding time accounted for 14% of the activity budget which was mostly spent feeding on fruits (9.22 ± 3.17%), considerably less consuming animal prey (3.30 ± 2.01%) and flowers (1.28 ± 1.93%) and below 1% of the time eating gum (0.10 ± 0.27%) and mushrooms (0.12 ± 0.21%). GHLTs consumed flowers, fruits, and gum from 31 known and at least 14 undetermined species. The total number of plant species that composed the GHLT's diet was 49, including four crop species. Throughout the months, GHLTs dedicated more time to behaviors involving affiliation (11.19 ± 5.54%) than agonism (3.57 ± 4.66%), although intergroup encounters were frequently observed during our study period. Finally, percentage in which GHLTs stayed inside a substrate was 1.41 ± 1.38% of total observation time.

3.3. Correlates of hormonal variation

Initial inspection of univariate models for fGC concentrations generated a significant interaction with fT3, SAI, Resting and Stationary. We constructed a model with those fixed factors (max. VIF = 1.12) and started model selection. This process showed that the best-supported model was more parsimonious (ΔAIC = 2.4) and differed significantly from the third-best model (LRT: $X^2 = 4.39$, d.f. = 1, p = 0.036). However, second-best and best-supported models were not significantly different (LRT: $X^2 = 0.39$, d.f. = -1, p = 0.53) and explained almost equal amounts of variation in fGCs (pseudo $R^2 = 0.36$ vs. 0.363). Then, we decided to present model that retained all the initial predictors (the second-best one) which demonstrated that fGCs interacts positively with fT3 and Resting, and negatively with Stationary and SAI, although nonsignificant for the latter factor (see Table 3).

Table 3. Linear mixed effects models (LMMs) investigating the variation in fecal glucocorticoid levels (fGCs) of golden-headed lion tamarins (GHLTs) using fecal T3 levels (fT3), flower availability index of *S. globulifera* (SAI), proportion of time spent resting and stationary as predictors (n = 251 samples from adult and subadult individuals). Individual identity and number of focal samples per individual were included as random factors. Likelihood ratio tests (LRT) compare models' performance. Bolded texts indicate statistical significance (P<0.05).

LMM fGCs								
Selection rank	**Fixed effects**	**ß**	**SE**	**t-value**	**P**	**AIC**	**LRT**	**R^2**
m2nd	Intercept	2.980	0.132	22.551	**<0.0001**	215.96	m2nd vs. m1st	0.36
	fT3^1	0.150	0.056	2.700	**0.0074**			
	SAI^2	-0.018	0.029	-0.633	0.528			
	Resting	1.703	0.393	4.332	**<0.0001**			
	Stationary	-0.642	0.310	-2.073	**0.04**			
m1st						214.35	**m1st vs. m3rd**	0.363
m3rd						216.75		

Note: values transformed to log 10 ^{1} or scaled ^{2}.

Based on the number of individuals per month, the mean level of fGCs and the mean proportion of time dedicated to resting exhibit a parallel variation pattern across the months (Figure 3A), whereas the same estimates for fGCs and remaining stationary display an opposite pattern (Figure 3B). Similarly, Figures 5 represents the positive relationship between fGCs and resting time (A) and negative between fGCs and stationary time (B).

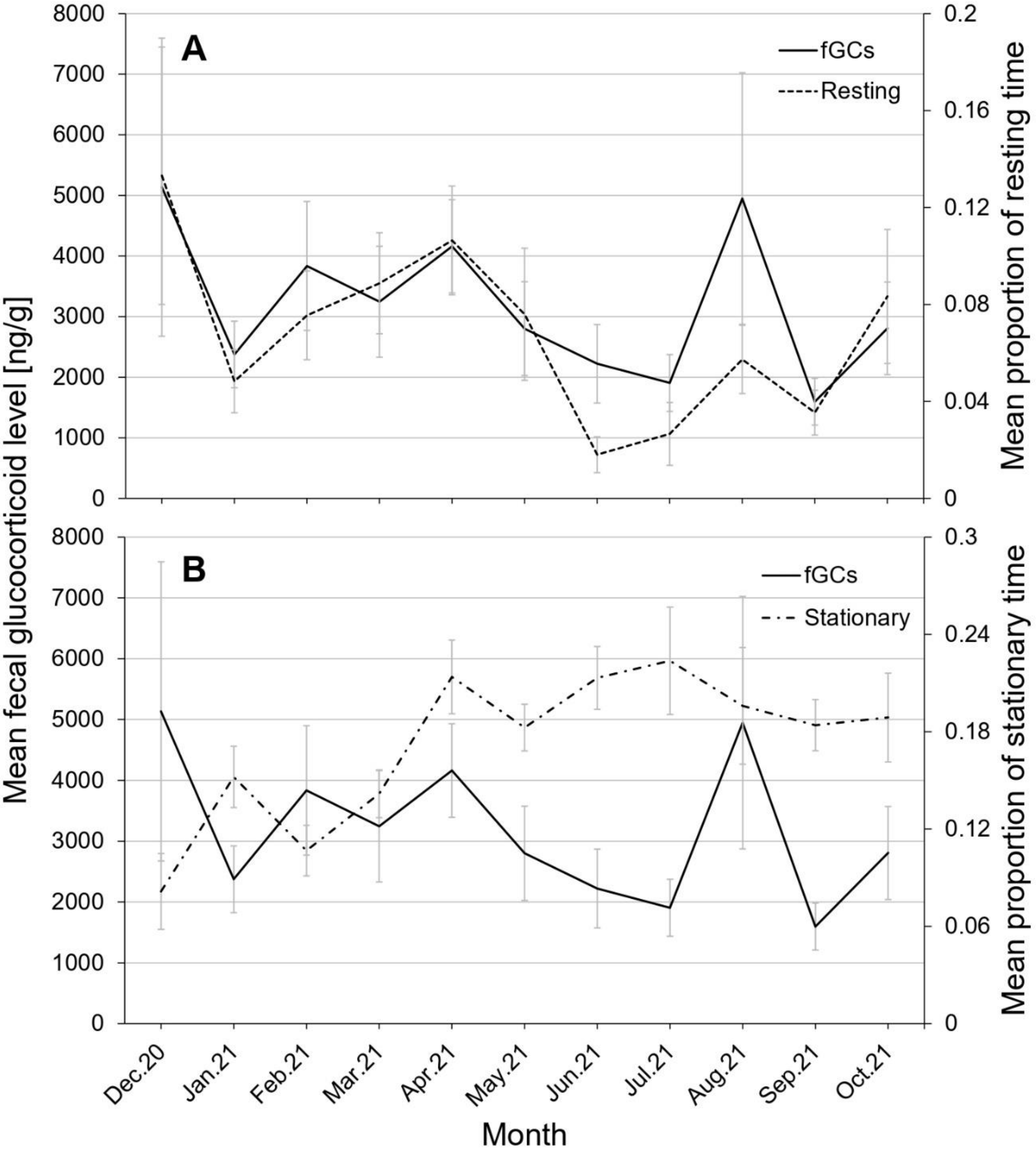

Figure 3. Temporal variation in the mean levels of fecal glucocorticoids (fGCs) plotted against the mean proportion of time devoted to resting (A) and remaining stationary (B). Whiskers indicate the standard error for each mean value.

Following the same procedure for fT3, single fixed effect models indicated significant coefficients for fGCs, SAI, FAI and Feeding on fruits. We then initiated model selection with those fixed effects and a VIF below 1.05. Similarly to fGC modelling, difference of AIC between best-supported and third-best models was greater than two (ΔAIC = 8.92), resulting in a significant LRT among them (LRT: $X^2 = 10.92$, d.f. = -1, $p < 0.001$). Second-best and best-supported models were again not different (LRT: $X^2 = 0.46$, d.f. = -1, $p = 0.50$) and explained similar amounts of variation in fT3 (pseudo $R^2 = 0.356$ vs. 0.36). The final model, we present, indicated that fT3 levels correlated positively with fGCs, Feeding on fruits and FAI, nonsignificant for the latter factor, whereas negatively with SAI (see Table 4).

Table 4. Generalized linear mixed effect models (GLMMs) investigating the variation in fecal T3 (fT3) of golden-headed lion tamarins (GHLTs) using fecal glucocorticoid levels (fGCs), flower availability index of *S. globulifera* (SAI), ripe fruit availability index (FAI) and proportion of time spent feeding on fruits as predictors (n = 251 samples from adult and subadult individuals). Individual identity and number of focal samples per individual were included as random factors. Likelihood ratio tests (LRT) compare models' performance. Bolded texts indicate statistical significance (P<0.05).

GLMM fT3								
Selection rank	**Fixed effects**	**ß**	**SE**	**t-value**	**P**	**AIC**	**LRT**	**R^2**
m2nd	Intercept	0.056	0.138	0.406	0.685	201.92	m2nd vs. m1st	0.360
	fGCs[1]	0.123	0.037	3.336	**0.0008**			
	SAI[2]	-0.045	0.018	-2.480	**0.013**			
	FAI[2]	0.011	0.016	0.680	0.496			
	Feeding on fruits	0.938	0.241	3.885	**0.0001**			
m1st						200.38	**m1st vs. m3rd**	0.356
m3rd						209.30		

Note: values transformed to log 10 [1] or scaled [2].

Monthly means of fT3, proportion of fruit feeding time and SAI are illustrated in Figure 4, where fT3 level and feeding on fruits have a nearly parallel variation (Figure 4A). In Figure 5, however, the direct association between fT3 and feeding on fruits (C) and the inverse association between fT3 and SAI (D) are more obvious.

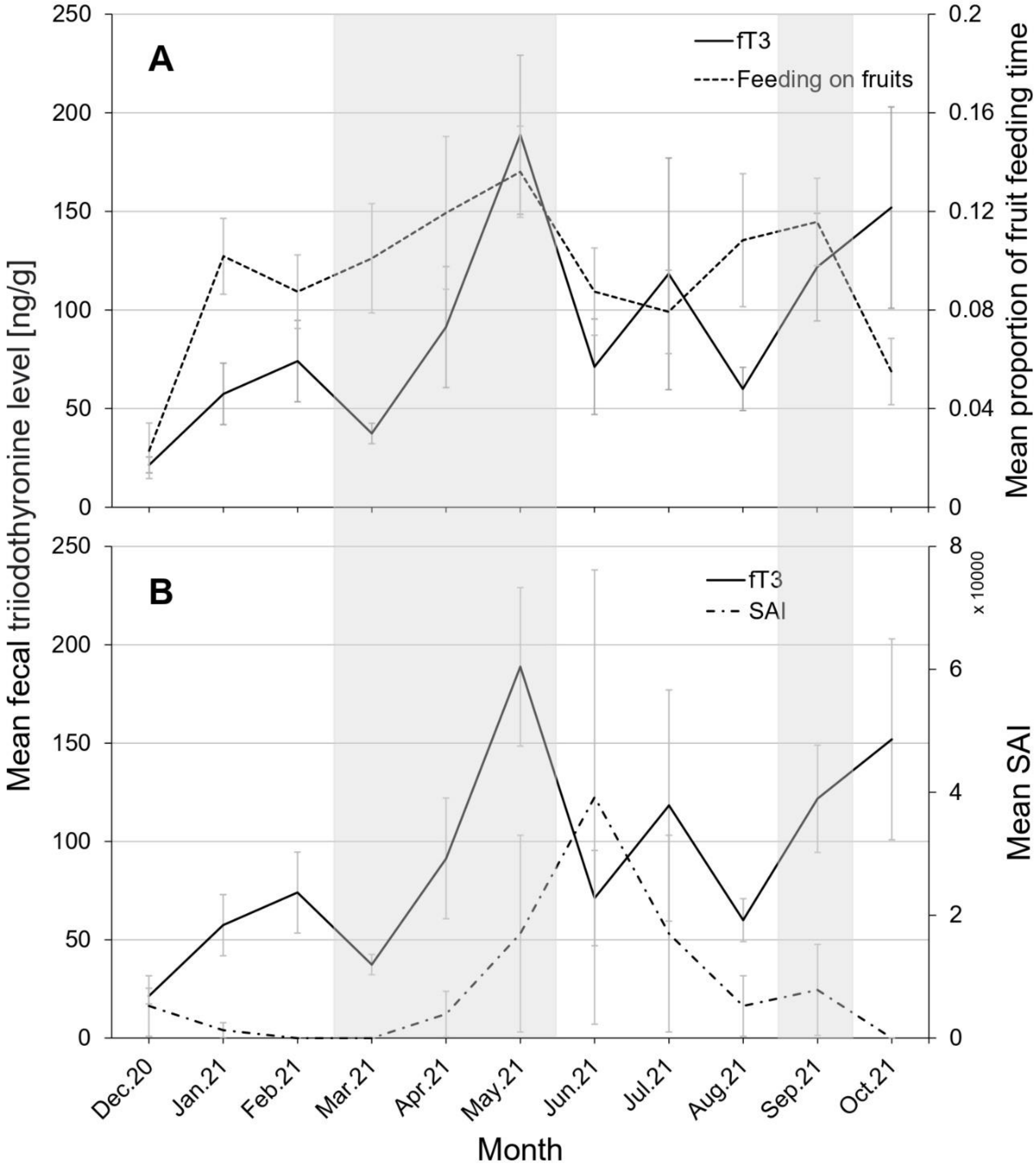

Figure 4. Temporal variation in the mean levels of fecal triiodothyronine (fT3) plotted against the mean proportion of time devoted to feeding on fruits (A) and the mean flower availability index for *Symphonia globulifera* (SAI) (B). Whiskers indicate the standard error for each mean value. Shaded areas encompass months of peak fruit availability.

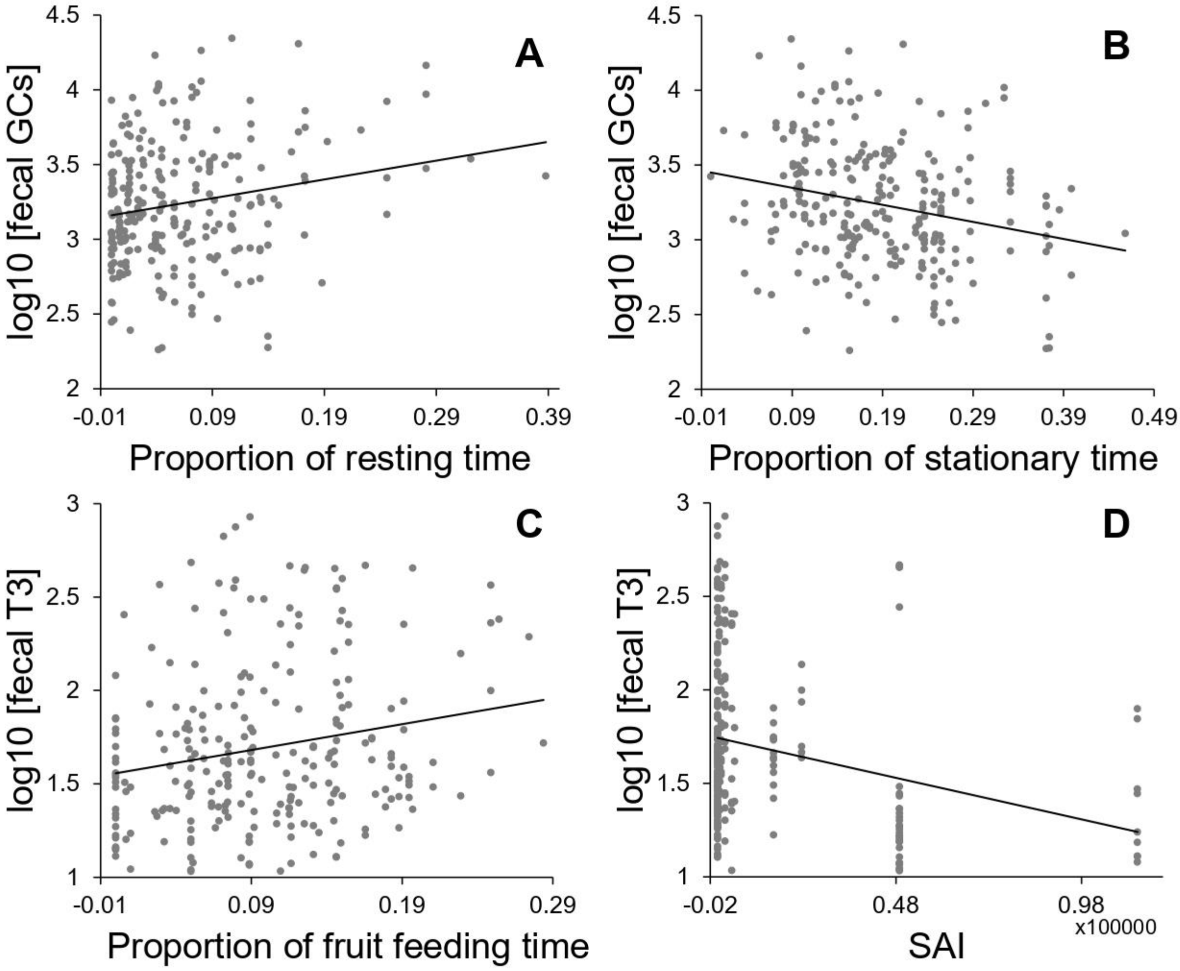

Figure 5. Bivariate plots illustrating the relationship between fecal glucocorticoid levels (GCs) transformed to log 10 and the proportion of time spent resting (A) and remaining stationary (B). The plots below display the relationship between fecal triiodothyronine levels (T3) transformed to log 10 and the proportion of time spent feeding on fruits (C) and the scaled flower availability index for *Symphonia globulifera* (SAI) (D). Data points represent fecal samples collected for adult and subadult individuals (n = 251). Trend lines shown only for visualization purposes.

4. DISCUSSION

In this study, we evaluated the associations between two hormonal measures that mediate energy balance, the behavioral patterns of GHLTs and the abundance of plant food sources in a highly anthropized and fragmented forest in South-Bahia, Brazil. Our results show a complex and, at times, unforeseen interplay among the assessed variables, and offer important insights into how lion tamarins manage their activity budget in relation to energy intake and food availability, pointing out some potential energetic constraints. Importantly, we found that fGC and fT3 levels were linked to a different set of predictors, with fGC concentrations more strongly correlated to changes in energy allocation, more specifically, the amount of time spent resting and stationary; while fT3 excretion was connected to the variation in energy intake driven primarily by the time spent feeding on fruits and the availability of *S. globulifera* flowers.

Contrary to our expectations, high fGC were linked to high fT3 levels with both metabolites having a similar influence on each other, as suggested by the coefficient estimates of the selected models (Table 2 and 3). Given the evidence from studies on captive animals that GCs relate negatively but T3 relates positively to energy intake and reserves (e.g.: Deschner et al., 2020; Romero, 2004; Sadoughi et al., 2021), researchers assume that, in wild settings, GCs and T3 are themselves negatively correlated (Jesmer et al., 2017). However, Touitou et al. (2021) and Dias et al. (2017) quantified simultaneously GCs and T3 in wild primates and reported no relation or a weak negative relationship for a collection of the sampled individuals. In our case, the positive relationship between fGCs and fT3 may indicate that although energy acquisition is sufficient to adequately maintain metabolic rate, GHLTs likely need to overcome many energetic and/or psychological challenges in order to access and use those energy sources (Gobush et al., 2014). That territorial disputes were common among study groups and that their home ranges overlapped each other’s (Costa et al., 2022; Coutinho, 2018) lends support to this explanation. Peres (1989) already demonstrated the costs of territorial defense in congeneric golden lion tamarins (*Leontopithecus rosalia*), where involvement in intergroup encounters implies less feeding, foraging, and resting especially in overlapping areas. Additionally, Kaisin et al. (2022) found that an increase in occurrence of intergroup encounters was associated with elevated fGCs in a group of black lion tamarins (*Leontopithecus chrysopygus*) living within a contested home range area.

Despite the positive relationship between fGCs and fT3, none of the measures used to characterize high energy expenditure such as the time devoted to agonism, traveling or foraging were significantly related to any of the hormonal measures. Particularly, aggression and physiological stress may covary with several intrinsic and extrinsic aspects (see Honess and Marin, 2006) which may have obscured the interaction between agonism and fGCs. For instance, Pride (2005) showed that female ring-tailed lemurs, with low food intake, engaged more in intergroup defense and had higher cortisol levels, whereas intragroup agonism by the same individuals did not produce a significant raise in cortisol. This suggests that different types of agonism do not necessarily result in similar GC secretion. Our definition of agonism lumped together both inter- and intragroup as well as given and received aggression which may have masked such differences. Other intrinsic factors, such as sex, dominance rank and female reproductive status may have allowed detecting individuals that are targets of aggression and often exhibit a more pronounced stress response (e.g.: Cavigelli et al., 2003; Emery Thompson et al., 2010; Yamanashi et al., 2016). However, as pointed out in Chapter 2, sex and social status did not correspond with significant differences in either metabolite, suggesting that stress response to aggression, if any, may not be linked to such individual distinctions. Nonetheless, the connections between agonism and time dedicated to obtaining and ingesting food should be further investigated as they may hint to the potential energetic costs of engaging in aggression (correlations of monthly agonism with feeding on fruit and foraging were, respectively, -0.52, $p = 0.1$ and -0.54, $p = 0.083$).

We expected travel time to be positively related to fGCs but negatively to fT3, given that the more GCs are secreted to meet the energetic demands of locomotion (Sapolsky et al., 2000), the slower the metabolism will become, especially in situations of restricted food availability where higher metabolic rates would be detrimental to the own individual's growth and survival (Burton et al., 2011). Although such interaction was not confirmed here, other authors have determined for Mexican howlers, *Alouatta palliata mexicana* (Dunn et al., 2013) and spider monkeys, *Ateles geoffroyi* (Ordóñez-Gómez et al., 2016) that higher fGC concentrations during periods of low fruit availability or fruit consumption, were accompanied by increases in travel time. One major characteristic of the stress response that may have precluded finding a clear association between energy demanding activities and fGCs or fT3 may be the gradual change in the environmental challenges confronted by the GHLTs. In other words, if energetic demands for territorial defense, traveling long distances or foraging during food shortages are within a controllable and predictable range, or even coincide with diurnal or seasonal cycles, we presume that such type of energetic stressors would be less challenging than unpredictable ones (Cavigelli, 1999; Sapolsky, 2005). Consequently, GHLT's stress responses to adjust time budget in relation to energy use would not result in detectable or significant changes in the physiological mediators (Romero et al., 2009).

Model fitting demonstrated that an elevation in fGC levels is generally associated with a decrease in the proportion of time GHLTs remain stationary but an increase in the time they rest. These findings provide a proximate picture of how changes in behavioral patterns possibly articulate the energy-saving strategies of this species. More specifically, stopping between high energy activities, predictably, attenuated the stress response as indicated by the fGC reduction, which in turn may help lion tamarins preserve energy. Interestingly, mean stationary time corresponded negatively with time spent consuming animal prey ($r_s = -0.65$, $p = 0.03$), suggesting that the less lion tamarins paused amid movement the higher their chances of catching a prey. In view of these results, it is likely that wild GHLTs need to offset the cost of allocating more energy to remaining active and pausing less with the benefit of gaining access to more foraging substrates and potential prey. Our data showed a tendency in GHLTs, already documented by Raboy and Dietz (2004), of investing more time in traveling, foraging and aggression during the first hours of the day and resting the following hours until approximately 13:00 h (see Figure 6B). In addition to that, diurnal fluctuations of fGC excretion were consistent with studies that show a steady decline in those levels between the morning and late afternoon (e.g.: Coe and Levine, 1995; Muller and Lipson, 2003), see Figure 6A. Although, other studies on captive marmosets showed an opposite excretion pattern (Pizzutto et al., 2015; Sousa and Ziegler, 1998), which could be related to differences in diet composition and/or metabolization and gut passage times (Schwarzenberger, 2007; Touma and Palme, 2005; Wasser et al., 1993). Notably, fGC levels peaked at 9:00 h and decreased subsequently coinciding with findings by Kaisin et al. (2022) made for congeneric black lion tamarins. Therefore, the exchange in activity budgets between high- and low-energy behaviors together with the diurnal decrease in fGC excretion suggest that resting time is activated as a response to previous energy expenditure, by which higher activity levels and consequently higher GC secretion require longer periods of recovery afterwards. This not only supports the idea that GCs respond to the diurnal cycles of resting and activity (McEwen and Wingfield, 2003) but also highlights the role of GCs as an anti-stress hormone, in that they prevent overshooting the HPA axis and exposing individuals to the deleterious effects of energetic stressors (Wingfield and Kitaysky, 2002). Resting time may also indicate time-budgeting issues. Particularly if enforced resting time, needed for the recuperation and digestion as well as to avoid the high temperature and radiation around midday (Herbers, 1981), limits free resting time which could be dedicated to other essential activities like socializing and additional foraging (Korstjens et al., 2010). The direction in the interaction between fGCs and resting time, also confirmed at monthly scale ($r_s = 0.77$, $p = 0.005$), should not be merely interpreted as an escalation of the stress response but rather as the result of our design that did not consider sampling individuals from the same group on consecutive days to test for the buffering effects of daily behavioral and physiological adjustments on a broader time frame.

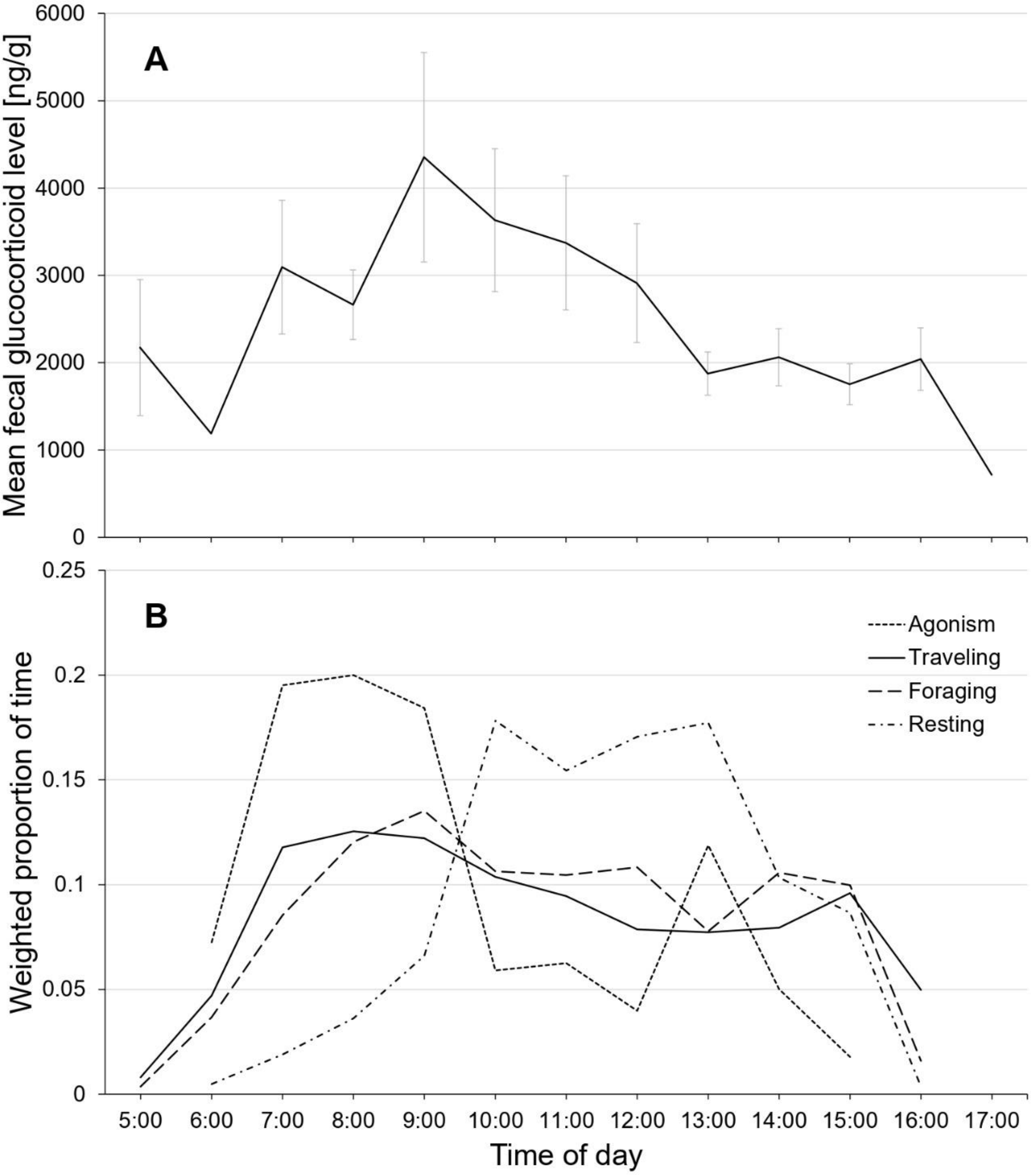

Figure 6. Diurnal fluctuation of mean fecal glucocorticoid levels (A) and selected behaviors plotted as the weighted proportion of time dedicated to agonism, traveling, foraging, and resting (B). Whiskers in A indicate the standard error for each mean value.

We found no evidence that fruit availability is linked to variation in fGC and fT3 levels or time spent feeding on fruits. Conversely, Catenacci et al. (2016) reported a positive correlation between monthly ripe fruit availability and the rate of fruit consumption by GHLTs using an area inside Una Biological Reserve containing secondary vegetation in various regeneration stages (Pessoa et al., 2012). The same authors indicated that because of the constant availability and consumption of fruits, likely connected to the high diversity of species with available fruits throughout the year, their study area may not exhibit an apparent fruit shortage (Catenacci et al., 2016). Probably the level of habitat degradation in our study area is more severe than that in the previous study, as in our case, habitat alteration is still a relentless process taking place in forest remnants, fruit availability varied strongly across months, and the number of plant species consumed by our focal groups (49 species including four crop species) was far below 114, the number of species registered by Catenacci et al. (2016). Regarding plant diversity, specifically, habitat loss and associated fragmentation is probably the main cause of impoverished floristic composition in many tropical regions (Corlett, 2016) which could lead to fewer fruiting species and, inevitably, fruit scarcity (Tabarelli et al., 2010). Our results do not permit to conclude that energy balance or its physiological mediators are modulated by fruit abundance and distribution. Instead, we may argue that, in our study, seasonal variation in fruit availability may have fallen within the predictable range of stress responses or predictive homeostasis (see Romero et al., 2009). However, as expected, increases in fruit intake were associated with an elevation in fT3 excretion. Considering previous findings, this may suggest that although thyroid hormones effectively regulate energy metabolism, fruit intake by our study groups is likely dominated by other contextual factors, like intergroup competition, presence of agricultural crops and landscape connectivity, than solely the temporal changes in fruit availability. One of the characteristics that contributes to primates' flexibility within anthropogenic habitats is their capacity to include cultivated plants in their diet (McLennan et al., 2017) which, under certain circumstances, may help them cope with the energetic challenges and the human exposure of such environments (McLennan et al., 2019). We witnessed, on several occasions, groups feeding on different crops, mainly banana, and less frequently disputing those resources with nearby groups. Since our phenological survey only comprised fruiting trees within secondary forests, perhaps a different fruit availability index integrating crop species may reflect more precisely the fluctuations in the chosen hormonal markers.

Dias et al. (2017) also obtained a positive effect for fruit consumption on fT3 concentrations but a negative one for young leaf intake in wild mantled howler monkeys (*Alouatta palliata*). We, instead, found a negative correlation between flower availability of *S. globulifera* and fT3 levels. Since the principal food item of GHLTs was ripe fruit and the *S. globulifera* peak bloom coincided with a progressive reduction in ripe fruit availability between May and July 2021 (see Figures 2 and 4), this downregulation of fT3 levels and increased abundance of *S. globulifera* flowers probably reveals a situation of nutritional deficit in lion tamarins that needed the intervention of an energy saving mechanism (Eales, 1988).

Additionally, correlation analysis showed a negative relationship between mean fGC concentrations and mean proportion of time devoted to feeding on flowers ($r_s = -0.66$, $p = 0.03$) estimated at monthly level. Thus, given that most flower feeding time was focused on *S. globulifera*, this finding further emphasizes the potential role of *S. globulifera* as a fallback food, offering a secondary option to compensate the negative energy balance caused by the decline in fruit intake and availability. However, in contrast to Foerster et al. (2012) who linked higher fGC levels to increases in the ingestion of mature leaves and other non-preferred items in female blue monkeys (*Cercopithecus mitis*), this inverse interaction may indicate that, to feed on flowers, lion tamarins face fewer stressors, especially if we assume that nectar from *S. globulifera* is less contested than ripe fruits, which consequently contributed to identifying such alleviatory effect on the physiological stress response. In fact, a recent study that compared the nutritional composition of various flower species included in the diet of red-tail monkeys (*Cercopithecus ascanius*) in Uganda, revealed the highest fat content but lower than expected proportions of sugar in *S. globulifera* (Ross et al., 2022). This suggests that GHLTs may select *S. globulifera* flowers to replenish or accumulate fat reserves rather than to obtain readily usable energy, which may be a suitable strategy for episodes of fruit scarcity.

CONCLUSION

This is the first study that measures fT3 levels and combines them with the quantification of fGCs in wild GHLTs occupying forest remnants and adjacent croplands. By doing so and matching these hormonal markers to the relative abundance of plant food sources and the general behavioral patterns of adult and subadult lion tamarins, we demonstrated that together, fGCs and fT3 can be useful for disentangling the factors that affect the daily energetic demands of GHLTs. Our results highlighted the interaction between fGCs and proxies of energy conservation, particularly resting time which generally does not attract much attention from researchers (Korstjens et al., 2010), but may play a pivotal role in facing daily and seasonal energetic challenges. Furthermore, we identified a switch in food items between ripe fruits and flowers of *S. globulifera*, which may serve as a strategy to cope with increased energy allocation, or seasonal scarcity in ripe fruits. Fruit availability is likely not the main limiting factor for energy acquisition in our study groups. However, as fruit intake reflected changes in energy metabolism, in order to fully assess the impact of the energetic stressors faced by GHLTs, it is crucial to look deeper into the implications of their social context. More specifically, future research should address questions on how intergroup competition restricts food access, complicates the success of energy strategies, and relates to space constraints that result from habitat loss and ensuing saturation.

We conclude that an appropriate interpretation of the hormonal correlates in this type of studies is only feasible when there is a thorough understanding of the subjects' physical and social environment. This may allow researchers to compare their results, despite the general lack of consensus, with other studies dealing with the associations between hormonal markers and individual aspects such as behavioral and life-history traits which are intertwined with other extrinsic and intrinsic characteristics (Crespi et al., 2013; Dantzer et al., 2016), especially when we examine GC variation. Therefore, to uncover the metabolic strategies and stress responses of GHLTs inhabiting different environments, from shaded cocoa plantations to protected areas with large portions of mature forests, researchers should consider the presence of key food and foraging elements, the impact of human-wildlife conflict and the costs of territorial defense and resource competition, among other context-dependent features.

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Appendix A. Supplementary material

Supplementary table 1. Tree species selected for the assessment of plant food availability. Ranking (range from 3 to 30) and importance score (range from 1 to 3) were obtained from Couto and Oliveira (2019), who compiled existing data on golden-headed lion tamarins' diet to evaluate the relative importance of plant food sources, based on Oliveira et al. (2010). For the former index, highest rankings include the most attractive, prevalent across habitats and commonly used species while the latter categorizes species' importance from "of interest" to "very valuable".

Family	Species	Local Portuguese name	Edible part	Ranking	Importance
Moraceae	Artocarpus heterophyllus Lam.	Jaca	Fr	27	3
Melastomataceae	Miconia mirabilis (Aubl.) L.O.Williams	Mundururu cabelo de cutia	Fr	26	3
Anacardiaceae	Tapirira guianensis Aubl.	Pau pombo	Fr	24	3
Clusiaceae	Symphonia globulifera L.f.	Landi	Fr, Ne	24	3
Melastomataceae	Henriettea succosa (Aubl.) DC.	Mundururu ferro	Fr	22	3
Urticaceae	Pourouma velutina Mart. ex Miq.	Tararanga lixa	Fr	22	3
Moraceae	Helicostylis tomentosa (Poepp. & Endl.) Rusby	Amora vermelha	Fr	18	3
Fabaceae	Inga thibaudiana DC.	Inga tabua	Fr	17	3
Melastomataceae	Miconia hypoleuca (Benth.) Triana	Mundururu de rego	Fr	17	3
Sapotaceae	Chrysophyllum splendens Spreng.	Abil da mata	Fr	17	3
Sapotaceae	Micropholis guyanensis (A.DC.) Pierre	Bapeba branca	Fr	17	3
Myrtaceae	Myrcia splendens (Sw.) DC.	Murta vermelha	Fr	*15	*3
Urticaceae	Pourouma mollis Trécul.	Tararanga vermelha	Fr	14	3
Simaroubaceae	Simarouba amara Aubl.	Pau paraiba	Fr	10	3
Urticaceae	Cecropia pachystachya Trécul.	Embaúba	Fr	*9	*2
Malpighiaceae	Byrsonima sericea DC.	Murici	Fr	8	1
Sapotaceae	Pouteria cf. grandiflora	Bapeba de nervura	Fr	8	1
Araliaceae	Schefflera morototoni (Aubl.) Maguire et al.	Matatauba	Gu	7	1
Peraceae	Pogonophora schomburgkiana Miers ex Benth.	Cocão	Fr	7	1
Lecythidaceae	Eschweilera ovata (Cambess.) Mart. ex Miers	Biriba	Fr	6	1
Melastomataceae	Miconia prasina (Sw.) Dc.	Mundururu branco	Fr	6	1

Note: Fr = fruit, Ne = nectar, Gu = gum and * denotes that scorings were obtained from congeneric species.

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CONCLUDING REMARKS

This research demonstrates the effectiveness of integrating behavioral and physiological measures to study how golden-headed lion tamarins (GHLTs), and by extension other primate species, deal with some of the environmental challenges of living in an altered habitat.

In Chapter 1, I provide an evaluation of the possible coping mechanisms that emerge from the bidirectional relationship between behavioral traits and glucocorticoid (GC) levels measured in wild and captive primates. This analysis indicates that traits associated with energy intake and expenditure (foraging domain) have a greater effect in buffering stress responses, mediated by GC secretion, than any other type of behavioral trait. This finding can have large repercussions for the ways in which we interpret the stress responses of non-human primates. First, we may affirm that affiliative behaviors are generally related to an alleviatory effect. However, their impact may be limited by energetic stressors which consequences are superimposed on the needs of social support and interaction. Second, agonistic and anxiety-like behaviors are less likely to offer stress relief and instead, motivate an intensification of the stress response. Third, contextual factors also appear to be important, here specifically, it was shown that captivity may complicate the beneficial outcome of coping mechanisms.

In addition to validating the measurements of fecal GC (fGCs) and triiodothyronine metabolites (fT3) for GHLTs, Chapter 2 illustrates how these metabolites respond to distinct influences. As predicted, capture and handling procedures caused the levels of fGCs to increase in comparison to those obtained during regular group monitoring. Also expectedly, an improved body condition was related to higher level of fT3. Additionally, group identity indicated different fT3 levels. However, to understand these response pattern, we may need to investigate the role of environmental factors, including the availability of food sources and space, and activity budgets. Although these results are valid for dried feces of GHLTs, other non-invasive studies on GCs and T3 in this species should consider performing their own validation, if collection, preservation, and assay protocols are not similar to those here exposed.

Figure 1. One of the food items rarely eaten by golden-headed lion tamarins were mushrooms (personal photo collection).

Finally, Chapter 3 shows that combining data on food availability, behavioral patterns, and hormonal markers is an effective way of revealing the main energetic strategies of wild endangered primates, such as GHLTs. Measuring two physiological markers, fGCs and fT3, regulated by different neuroendocrine axes, may be instrumental for obtaining a better understanding of a particular ecological question. In our case, variation in fGCs was more closely related to energy-saving strategies whereas variation in fT3 was associated with energy-intake strategies. Other potential stressors, like energy-consuming activities, agonism and fruit shortage were not significantly connected to fGC or fT3 levels. However, it would be careless to claim that, for instance, fruit availability does not affect the foraging decisions and physiology of GHLTs, given that fT3 was indeed correlated to changes in fruit feeding time and peaks in the availability of *S. globulifera* flowers, which in turn coincided with periods of fruit scarcity. Rather, our results indicate that fruit availability has an indirect effect on metabolic rate but is not the main energetic challenge for GHLTs in fragmented and anthropized forest remnants. Further investigation as to whether the rate of intergroup encounters, or landscape factors such as forest fragment size and matrix vagility limit food access will be essential to determine what conservation action should be prioritized in order to expand the opportunities of long-term survival and reproduction for populations of GHLTs in this type of habitat.

Figure 2. Golden-headed lion tamarin in the middle of a flowering *Symphonia globulifera* (personal photo collection).

The possible applications of physiological markers in the study of GHLTs' ecology and other species are extensive. One of them involves measuring fGCs and fT3 in GHLTs living in shaded cocoa plantations (cabrucas) and protected areas. This may provide a basis for comparing not only the specific challenges faced by GHLTs in other environments but also the intervals of fGC and fT3 response across GHLTs' habitats. Various other variables not tested in this study could improve our current understanding of the response mechanisms associated to the target markers. Those variables could be grouped into behavioral correlates (e.g.: rate and duration of intergroup encounters; frequency of self-grooming, scratching, and vocalizing; total daily distance travelled per group; comparisons between receiving and giving grooming or aggression; parental care and investment), dietary correlates (e.g.: caloric intake, nutritional composition of food items, food selectivity), intrinsic correlates (e.g.: age, female reproductive status, parasite load), extrinsic correlates (e.g.: presence of potential predators, diversity and abundance of foraging substrates, home range size and density of individuals, size and connectivity of forest fragments, human activity within forest fragments, management intensity of agricultural matrix) and abiotic correlates (e.g.: variation in ambient temperature and precipitation).

Perhaps it is timely to mention how other disciplines may contribute to understanding this species and defining adequate conservation actions for the GHLT persistence in highly fragmented forests. During my period of group monitoring, the rate of territorial encounters likely affected energy-related decisions by GHLTs. On the other hand, long-term information from Project BioBrasil, suggesting recurrent migratory events of known individuals among focal groups, further raises concerns. Therefore, more research on the dispersal patterns and the genetic structure and kinship in our study population may point out particular genetic consequences that stem from habitat fragmentation and increased isolation. If this is confirmed, well devised and interdisciplinary conservation plans aimed at increasing landscape connectivity may not only improve food access for these isolated populations but also prevent low genetic diversity.