

RESEARCH ARTICLE

The Effect of Energetic and Psychosocial Stressors on Glucocorticoids in Mantled Howler Monkeys (*Alouatta palliata*)

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The proximate causes of variation in glucocorticoids (GCs) of many free-ranging primates are still unclear, and in some cases, the available evidence is contradictory. Such is the case of mantled howler monkeys. In the present study, we tested whether variation in GC levels in this species could be predicted by energetic challenges or by psychosocial stressors. We focused on two groups living in Los Tuxtlas (Veracruz, Mexico) that differed in a number of parameters including: group size, habitat size, number of groups, and solitary males within the same habitat. Furthermore, one of the groups experienced changes in composition during our observations. From March to December 2009 we determined food availability in each group's habitat, studied the behavior of all adult individuals ($N = 17$), including, feeding, time budgets, ranging, and social interactions ($N = 426.6$ h), and measured weekly GCs in fecal samples ($N = 160$ individual/weeks) of both females and males. We found that participation in agonistic interactions, which were more frequent in the group that lived in the smaller habitat, was associated with increased weekly GCs, particularly in pregnant and lactating females. During the dry season weekly GCs were also higher in the group that lived in the smaller habitat. Although in this group individuals significantly increased travel time during the dry season, weekly GC levels were unrelated to time-budgets or ranging distances, contrasting with previous findings on mantled howler monkeys' GC response. We found no evidence that weekly variation in GC levels between groups resulted from differences in food availability. Our results indicate that mantled howler monkey GC levels respond to the effects of agonism, reproductive state, and the influence of a seasonal stressor, which may be attributable to anthropogenic disturbance. We conclude that psychosocial stressors affect the GC response of mantled howler monkeys, and that this response is modulated by reproductive state. *Am. J. Primatol.* © 2013 Wiley Periodicals, Inc.

Key words: agonism; cortisol metabolites; mantled howlers; Mexico; reproductive state; stress

INTRODUCTION

Glucocorticoids (GCs) are the main modulators of physiological stress responses in vertebrates [Sapolsky et al., 2000]. Depending on their actions during a typical stress response [as described by Sapolsky et al., 2000], GCs may alter an organism's response to an ongoing stressor or may prepare an organism's response to a subsequent stressor. Independently from the nature of the stressful stimuli, during the stress response GCs increase circulating glucose through a number of mechanisms, contributing to the depletion of present and, when the action of a stressor is prolonged, future energy stores [Sapolsky et al., 2000]. Therefore, the role of GCs in energy allocation in both females and males makes them good indicators for the energetic consequences of stress, and their measurement may provide insights into the selective forces acting on animal populations that face variation in the availability of energy.

Non-invasive measurement of GCs have been used to assess the stress response of wildlife, including female and male primates, to environmental [e.g., temperature: Weingrill et al., 2004], ecological [e.g., food availability: Pride, 2005; predation: Arlet & Isbell, 2009], psychosocial [e.g., social rank:

Contract grant sponsor: Dirección General del Área Académica de Ciencias Biológicas y Agropecuarias (UV); contract grant sponsor: CONACyT; contract grant number: 26848.

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Received 15 July 2013; revised 6 November 2013; revision accepted 6 November 2013

DOI: 10.1002/ajp.22240
Published online XX Month Year in Wiley Online Library (wileyonlinelibrary.com).

Abbott et al., 2003; social instability: Engh et al., 2006], or metabolic [e.g., pregnancy: Setchell et al., 2008] stressors, as well as the interactions among different stressors. For instance, in ring-tailed lemurs (*Lemur catta*) cortisol levels of females increase during periods of food scarcity, but the best predictor of cortisol variation is dominance rank [Cavigelli, 1999]; and in blue monkeys (*Cercopithecus mitis*) GCs increase during periods of low availability of preferred foods, although this effect is particularly pronounced in females that are pregnant or in early lactation [Foerster et al., 2012]. Despite these efforts, the proximal causes of variation in the stress response of many free-ranging primates are still unclear, and in some cases, the available evidence is contradictory.

There is currently evidence that female mantled howler monkeys (*Alouatta palliata*) that live in forest fragments with high densities of solitary males, have higher GC levels, presumably because these represent psychological stressors due to takeover-infanticide possibilities [Cristóbal-Azkarate et al., 2007]. However, it has also been found that in this species GC levels are negatively related to food availability, either directly through a reduction in the abundance of food sources in small forest fragments [Aguilar-Cucurachi et al., 2010], or indirectly, through an increase in time traveling to reach food (fruits) sources, which are scarcer in small forest fragments [Dunn et al., 2011, 2013]. Conversely, GC levels of reproductive females (i.e., pregnant and lactating) are higher than those of both non-reproducing females and males, independently of habitat characteristics [Dunn et al., 2011]. In Central American black howler monkeys (*A. pigra*), a species closely related to mantled howlers [Cortés-Ortiz et al., 2007], GCs are also higher in more disturbed habitats [i.e., small forest fragments vs. large continuous forests: Martínez-Mota et al., 2007] as well as in central males [Van Belle et al., 2009]. To date, no study has assessed the relationship between GC and rates of social interactions in mantled howler monkeys or directly measured the effects of variation in food availability among different habitats on behavior and GC levels.

Here we examined the GC levels in both sexes of mantled howler monkeys across two groups inhabiting the Los Tuxtlas region of Veracruz, Mexico. More generally, we were interested in how energetic (food availability) and psychosocial stressors (feeding competition, takeover-infanticide probabilities) affected physiological stress profiles. One group lived in a large forest fragment with multiple groups and solitary males (hereafter, Montepío), whereas the other group included less individuals and lived in a small forest fragment without other groups or solitary individuals (hereafter, Balzapote). In addition, two individuals (one male and one female) immigrated into Montepío during our study. Specifi-

cally, we tested the following competing predictions. First, we predicted that variation in GC levels would be mainly determined by energetic stressors. Food availability should be higher for a group living in a larger habitat [Arroyo-Rodríguez & Dias, 2010], thus preferred seasonal foods [i.e., young leaves and fruits: Milton, 1980] should be consumed more frequently in Montepío. In turn, this abundance of preferred foods should result in an energy saving time-budget (i.e., more time spent resting, less time spent feeding and traveling as well as shorter ranging distances) and in lower within group feeding competition (more affiliation and less agonism). As a consequence, GC levels should be lower in Montepío. Second, variation in GC levels would be mainly determined by psychosocial stressors. Independently from differences between habitats in food availability, by sharing its habitat with other groups Montepío faces higher potential within and between group direct and indirect feeding competition [Arroyo-Rodríguez et al., 2011], thus individuals should present an energy demanding time-budget (i.e., less time spent resting, more time spent feeding and traveling as well as longer ranging distances) and more agonism in feeding contexts. Furthermore, the immigration of new individuals and the presence of solitary males in Montepío should represent additional strong stressors for females, particularly for females that are pregnant and lactating. As a consequence, GC levels should be higher in Montepío.

METHODS

This research adhered to the American Society of Primatologists principles for the ethical treatment of primates.

Study Sites and Subjects

We selected two groups of howler monkeys (*Alouatta palliata*) that inhabited two forest fragments (separated by ca. 1 km) in the Los Tuxtlas region, in the southeast of the state of Veracruz, Mexico (Fig. 1). In this region the climate is warm and humid, with a mean annual temperature of 25°C and annual rainfall that oscillates between 3,000 and 4,600 mm [Soto, 2004]. There is a marked seasonal difference in rainfall levels, with ca. >80% of total annual rainfall falling from June to January (wet season) and ca. <20% falling in the months of February–May (dry season) (data for the period 1989–2012 from the nearest weather station—ca. 2.5 km from the study sites: Estación Biológica Los Tuxtlas, Instituto de Biología, UNAM). The original dominant vegetation at altitudes below 700-m asl was tropical rainforest, but this region has been severely disturbed over the past 60 years and the remaining forest is surrounded by a matrix of pastures and croplands.

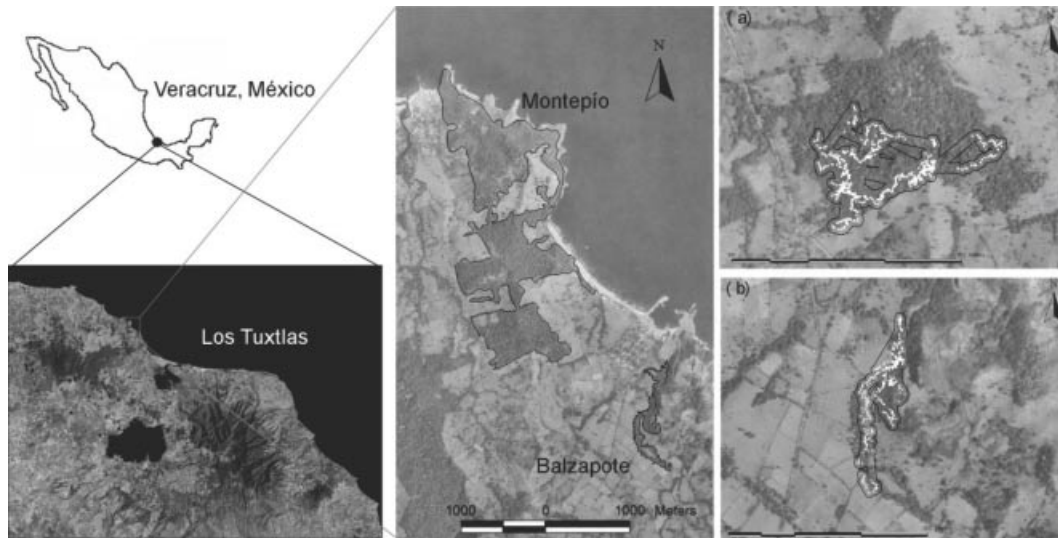


Fig. 1. Location of the study area in the Veracruz state (left), of the study sites in the Los Tuxtlas region (center), and of each group's home range in its forest fragment: (a) Montepío; (b) Balzapote.

The first group (Montepío hereafter) occupied a large forest fragment located near the town of Montepío, whereas the second group (Balzapote hereafter) occupied a riparian forest fragment near the town of Balzapote that was *ca.* 15 times smaller than Montepío (Fig. 1). These groups were selected to maximize differences in potential within and between group competition, as assessed by group size

and the number of groups inhabiting the same habitat, respectively, as well as in habitat size (Table I). During the first sampling period (see below) one female with an infant about 2 months of age entered the Montepío group, and during the second half of the second sampling period an adult male immigrated to this group. We studied all adult individuals from the two groups, which were known

TABLE I. Demographic Characteristic of Two Groups of Howler Monkeys Living in Two Forest Fragments in Los Tuxtlas and the Characteristics of Their Habitats and Home ranges

	Montepío	Balzapote
Characteristics of the fragments		
Size (ha)	230.6	15.6
Number of groups ^a	12	1
Number of solitary males ^a	3	0
Population density (individuals/ha)	1.8	1.56
Characteristics of the study groups		
Number of adult males	6 (5) ^f	3
Number of adult females	5 (4) ^f	3
Number of juveniles	3	2
Number of infants	2	2
Characteristics of the home ranges		
Size (ha) ^b	15.94	9.04
Mean DBH ^c	19.3	23.3
No. <i>Ficus</i> spp. (individuals/ha) ^c	0	9
Density of BTTFT (individuals/ha) ^{c,d}	0	30
Estimated overall BTTFT (individuals)	0	271.2
Biomass of FrTFT (kg/ha) ^{c,e}	57,363,753.6	346,403,712.9
Estimated overall FrTFT (kg)	914,378,231.7	3,131,489,565.0

^aDetermined by direct counts of individuals [following Cristóbal-Azkarate et al., 2005].

^bCalculated with the digitized polygons method [Ostro et al., 1999].

^cCalculated from Gentry [1982] transects.

^dBTTFT = big trees (DBH > 60 cm) from top food taxa [Dunn et al., 2009].

^eFrTFT = fruits from top food taxa [Dunn et al., 2009].

^fTwo individuals (one female and one male) immigrated during the study.

and easily identified by their natural anatomical and physiognomic characteristics, such as body size and proportions, scars, broken fingers, genital morphology and pigmentation, as well as blond hairs and skin pigmentation on the feet, hands and tail. Following Dunn et al. [2013], we classified females as pregnant (defined as starting from the day of estimated conception to the day before parturition), lactating (defined by either observations of lactation, or, as starting from the day of parturition until 20 months, when latest weaning occurs), or in other reproductive state (neither pregnant nor lactating). We based this classification on observations of births and lactation during the study and during periodical visits to the study groups up to 7 months after the end of the study.

Behavioral Observations

Observations were performed during complete days (i.e., 6:00–7:00 to 17:00–18:00, depending on the time of year). We used focal-animal sampling with continuous recording (1-h samples) to study time budgets, feeding behavior, and ranging distances. All adults in a group were randomly chosen as focals, with no animals sampled twice until all were sampled once. We recorded time-budgets using the software EZrecord for Palm Pilot, and categorized behavioral observations into the following: feeding, resting and traveling. During feeding we recorded the time focal animals dedicated to the consumption of fruits, mature leaves, young leaves and flowers. All trees used by focal animals were marked and located with a handheld global positioning system, and subsequently digitized as points with ArcView 3.2 (Environmental System Research Institute, USA). Ranging distances were calculated as the sum of the lengths (in meters) of straight lines connecting individual tree points used by individuals during each focal sample. These straight lines were also used to calculate the home range of each study group with the digitized polygons method [Ostro et al., 1999]. Agonistic and affiliative social interactions exchanged among adults were recorded with the all occurrences sampling by one observer (E.G.E.) with the help of one field assistant. High visibility in both sites allowed seeing all group members at all times, and therefore, using this sampling method. Each time an interaction occurred, we recorded the identities of both actor and receiver, as well as the description of the social interaction (Table II). The sampling of social interactions was performed simultaneously with focal sampling described above.

We collected a total of 426.6 focal hours, 231.9 in Montepío and 194.7 in Balzapote, with an average (\pm SD) of 21.1 ± 3.7 focal hours per individual in Montepío and 32.5 ± 2.9 focal hours in Balzapote.

TABLE II. Frequencies of Social Interactions Observed Per Group

Behavior ^a	Montepío	Balzapote
Affiliation		
Embrace	8	1
Greeting ceremony	5	2
Groom	34	5
Hold hands	5	1
Play	110	26
Sit in contact	159	56
Touch	42	16
Agonism		
Avoid	5	3
Charge	4	8
Chase	1	1
Cower	1	3
Displace	15	28
Fight	4	0
Flee	0	1
Push	0	1
Slap	0	1
Supplant	25	42
Threat	11	22
Total	429	217

^aBehaviors described in Dias [2002].

Assessments of Food Availability

Previous research at Los Tuxtlas indicated that mantled howler monkeys have folivore–frugivore diets, but that the relative proportions of consumed leaves and fruits vary seasonally [Cristóbal-Azkarate & Arroyo-Rodríguez, 2007; Dunn et al., 2009]. When fruits (end of the dry season–beginning of the rainy season, and wet season) and young leaves (dry season) are more abundant, mantled howler monkeys show a marked preference for consuming them [e.g., Dunn et al., 2010].

We measured food availability in three ways. First, following Dunn et al. [2009], we determined the availability of big trees [i.e., diameter at breast weight (DBH) >60 cm] and fruit of top food taxa [i.e., the 10 most-consumed tree taxa by howlers at Los Tuxtlas] by sampling vegetation in each site with the Gentry [1982] protocol. Specifically, at the end of the first sampling period we located ten 50 m × 2 m transects in the areas that were occupied by each group, and measured the DBH of all trees ≥ 10 cm, which we identified at the species level. From these samplings we determined both the density and the overall availability big trees from top food taxa, as well as the density and the overall availability of fruits from top food taxa following the calculations proposed by Dunn et al. [2009], corrected for the area that was sampled in the present study. We additionally calculated the mean DBH (by averaging the DBH of all sampled trees) and the number of *Ficus* spp. per site. Second, to assess the abundance of fruits, mature leaves, young leaves and flowers in the

habitat of each group, in each of the Gentry transects described before we located the four nearest trees with DBH ≥ 10 cm to each 10 m of the transect, resulting in 24 sampling trees per transect and 240 trees per site. We assessed the presence and coverage of the plant parts mentioned above in each tree using the following abundance scores: 0 = none, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–100%. Phenological sampling was conducted at the end of each behavioral sampling period (i.e., one sampling per season per group). Third, with the abundance scores described above, we determined the abundance of fruits, mature leaves, young leaves and flowers in each tree that was used as a food source by focals.

Fecal Sample Collection and GC Analyses

Fecal samples were collected opportunistically whenever they could be matched with individuals. Fresh samples uncontaminated by urine were collected from the forest floor and deposited in polyethylene bags labeled with the identity of each individual. We analyzed 285 fecal samples (192 from Montepío and 93 from Balzapote), with an average (\pm SD) of 16.7 ± 3.67 samples per individual, 3.2 ± 1.2 samples per week per individual and 8.4 ± 4.6 samples per individual per season. Fecal samples were kept in a cooler with frozen gel packs while in the field and stored at the end of the day in a freezer at -20°C at the field station until extraction was performed. Samples were stored at constant temperature (-20°C) for a maximum of 12 months until the extractions were conducted. The freezing procedure used for storing the samples has been reported to have a weak time-storage effect on fecal hormone metabolites in other primate species [Khan et al., 2002].

Steroid metabolites were extracted following a modification of the method described in Wasser et al. [2000]. Briefly, 0.6 g of homogenized, lyophilized, and pulverized feces was shaken for 24 h in 6.0 ml of analytical-grade methanol. Extracts were then centrifuged (460g for 30 min at -40°C), and the supernatant containing the steroids was recovered. After complete evaporation of the solvent, pellets were reconstituted with 3-ml albumin buffer and used for the GC analyses.

To date, all studies addressing variation in GC levels of howler monkeys as a function of ecological and social factors have used fecal samples and have measured either cortisol [Aguilar-Melo et al., 2013; Behie et al., 2010; Martínez-Mota et al., 2007; Van Belle et al., 2009] or corticosterone [Aguilar-Cucurachi et al., 2010; Cristóbal-Azkarate et al., 2007; Dunn et al., 2011, 2013] metabolites. GC assays were conducted at the Instituto de Ciencias Médicas y Nutrición Salvador Zubirán, in Mexico City. Fecal GC levels in all samples were measured using a commercial radioimmunoassay ^{125}I cortisol kit (Izo-

top, Institute of Isotopes Ltd, Budapest, HU; sensitivity = 2.9 nmol/l; calibration range = 0–1,600 nmol/L) and gamma counter (Cobra Model E5005, Packard, Inc., Downers Grove, IL, USA). The manufacturer reports low cross-reactivity with corticosterone (4.3%).

As a biological validation of our assay, we determined the short-term effect of capture (an acute stressor) and anesthesia (ketamine) on the GC excretion profile in feces of three howler monkeys (one male and two females) following the procedures described above. We collected all fecal samples ($N=23$; 7.7 ± 2.5 SD samples per individual) from 5 h before to 96 h after capture, and compared pre-capture levels with peak concentrations (i.e., the highest post-stressor values that were $\geq 2^*$ SD above the mean concentration before capture), using a Wilcoxon signed-rank test. GC levels peaked at a mean (\pm SD) of 21.5 ± 0.4 h after capture. Peak GC levels (mean \pm SD = $1,497 \pm 290$ ng/g) were significantly higher than pre-capture levels (809.4 ± 187.5 ng/g; $H_{1,7} = 4.5$, $P = 0.034$), indicating that our GC assays reliably measured adrenal responses of mantled howlers to stressors. This capture was part of a concurrent project on the genetics of mantled howler monkeys, and our capture and handling procedures [Canales-Espinosa et al., 2011] were approved by Mexican authorities (SEMARNAT, SGPA/DGVS/10637/11).

Howler monkeys' pooled fecal extracts, when added to the standard curve points, exhibited an accuracy of $R^2 = 0.99$ ($N=4$, $P < 0.001$), and serial dilutions of a fecal pool from howler monkeys yielded results that ran parallel to the GC standards ($R^2 = 0.91$, $N=4$, $P < 0.01$). Fecal pools with three different total GC levels (low, medium, and high) were used as quality controls. A total of 48 assays were run for each GC level, and the intra- and inter-assay coefficients of variation were 8.4% and 7.2%, respectively. The mean recovery of GC extracted from fecal samples, as described above, was 61.7% ($N=6$). All samples were run in duplicate, and mean GC values are reported as ng/g dry feces.

Data Organization and Analyses

The study was performed between March and December 2009 and was divided into four sampling periods, corresponding to one sampling period per season (wet and dry season) per group. Each sampling period was divided into five weeks, during which for each individual we collected behavioral data, fecal samples, and determined abundance scores of plant parts in trees used as food sources. The female that immigrated to the Montepío group joined the group during the third week of the dry season sampling period, whereas the immigrating male joined the group in the fourth week of the wet season sampling period. Therefore, for Montepío we

analyzed a total of 100 individual/weeks (48 in the dry season and 52 in the wet season), whereas in Balzapote we analyzed a total of 60 individual/weeks (30 in the dry season and 30 in the wet season).

We compared the abundance of each plant part (i.e., young and mature leaves, fruits and flowers; assessed through phenological transects) in each sampled tree ($N = 240$) between seasons within and between sites with repeated-measures MANOVA tests. In these tests we nested tree identity within transect identity, and transect identity within sites to account for spatial autocorrelation. To compare the abundance of plant parts in trees used by individuals as food sources between seasons and sites we first calculated weekly mean abundance scores per individual. We then performed linear mixed models (LMM) to analyze the effects of site and season (independent fixed factors) in weekly variation in mean abundance scores of plant parts in trees used by each individual as food sources.

The time each individual spent in each time-budget component (i.e., feeding, resting, and traveling) during focal samples was transformed into proportion of total observation time per week, and time dedicated to feeding on mature and young leaves, fruits and flowers was transformed into proportions of total feeding time per week. We calculated individual ranging distances per week as the sum of distances ranged by each individual during focal samples in a particular week divided by the total observation time for each individual in that week. For each individual, we calculated weekly rates of affiliation and agonism by dividing its frequency of participation as both actor or receiver in interactions by the total number of observation hours conducted for its group in each week. These rates were corrected for group size, by dividing individual interaction rates by the number of adults in each subject's group, not including the subject. We used LMM to analyze the effects of: (1) site, season, reproductive state (i.e., males, pregnant, lactating, and females in other reproductive state) and the abundance of plant parts in trees used as food sources (fixed predictive factors) on weekly variation in feeding behavior; (2) site, season and reproductive state (fixed predictive factors) on weekly variation in time budgets, ranging distances and social interactions; (3) feeding behavior (i.e., time spent consuming each plant part) site, season and reproductive state (fixed predictive factors) on weekly variation in time budgets, ranging distances and social interactions. GC levels were averaged for a given individual within each week. To analyze variation in GC levels, we ran a LMM including all time budget behaviors, ranging distances, social interactions, site, season and reproductive status and two-way interactions among these variables as predictive fixed factors and individual GC levels per week ($N = 160$ individual/weeks) as a dependent variable. We used Akaike's information criterion to select the most parsimonious model, that

is, the combination of predictive fixed factors that best explained variation among individuals in GC levels [Motulsky & Christopoulos, 2003]. To reduce collinearity among the predictor variables, we checked the variance inflation factor. Redundant variables were deleted where variance inflation factor values were above 4, which indicate possible multicollinearity [Chatterjee et al., 2000]. Specifically, we excluded time spent resting because it was highly correlated with feeding time ($r = -0.88$, $P < 0.001$) and travel time ($r = -0.75$, $P < 0.001$). In all LMM, we specified weeks within individuals and individuals within sites as hierarchical random effects to account for the repeated sampling of several individuals belonging to the same group through time. Proportional data were normalized using the arcsine square root transformation, whereas ranging distances, social interaction rates and GC levels were normalized using a logarithmic (\ln) transformation. Following transformation all variables showed normal distributions and homogeneous variances (Kolmogoro–Smirnov tests and Levene's tests: $P > 0.05$). For each dependent variable we report means \pm standard errors (SE). All tests were two-tailed and were performed with SPSS 12.0 (SPSS, Inc., Chicago, IL, USA.). Significance was set at $P < 0.05$.

RESULTS

Feeding Behavior Depends on Food Availability

We assessed food availability for each group through vegetation transects, phenology transects and by measuring food abundance in trees used as food sources. Vegetation transects indicated that, although the home range size of Balzapote was *ca.* 43% smaller than that of Montepío, the number of big trees for top food taxa and the biomass of fruits from top food taxa were greater in the former than in the latter (Table I). In Balzapote there were also more large trees and more *Ficus* spp. trees than in Montepío.

Data from phenology transects indicated that at both sites the wet season was a period of higher abundance of mature leaves, fruits and flowers, whereas young leaves were more abundant in the dry season (Table III). Seasonal variation in mature ($F_{1,18} = 27.1$, $P < 0.001$) and young leaf abundance ($F_{1,18} = 12.1$, $P = 0.003$) was significant in both sites; young leaf abundance during the wet season was significantly higher in Montepío than in Balzapote ($F_{1,18} = 19.2$, $P < 0.001$). In both sites fruits and flowers were absent from sampled trees during the dry season.

The mean weekly abundance scores of fruits in trees used by individuals as food sources (Table III) was not different between groups ($F_{1,12.87} = 0.02$, $P = 0.891$), but was overall higher in the wet season than in the dry season ($F_{1,62.11} = 8.3$, $P = 0.005$). In contrast, the abundance of mature leaves in feeding

TABLE III. Variation (Mean ± SE) Within and Between Sites in the Abundance of Plant Parts

	Montepío		Balzapote	
	Wet season	Dry season	Wet season	Dry season
Phenology transects ^a				
Mature leaves	3.2 ± 0.08	1.3 ± 0.04 ^c	3.9 ± 0.03	0.9 ± 0.06 ^c
Young leaves	1.4 ± 0.07	3.5 ± 0.04 ^c	0.8 ± 0.05 ^d	3.4 ± 0.05 ^c
Fruits	0.2 ± 0.04	0.0 ± 0.00	0.2 ± 0.04	0.0 ± 0.00
Flowers	0.5 ± 0.07	0.0 ± 0.00	0.4 ± 0.06	0.0 ± 0.00
Phenology of feeding trees ^b				
Mature leaves	2.5 ± 0.11	2.3 ± 0.11	3.8 ± 0.04 ^d	2.9 ± 0.06 ^e
Young leaves	2.3 ± 0.10	2.3 ± 0.08	1.1 ± 0.07	1.6 ± 0.05 ^e
Fruits	0.7 ± 0.12	0.2 ± 0.03	1.2 ± 0.11	0.5 ± 0.06
Flowers	0.01 ± 0.01	0.3 ± 0.05	0.1 ± 0.03	0.05 ± 0.02

^aN = 240 trees per site.

^bCalculated from mean weekly abundance scores of trees used by individuals as food sources. Montepío dry season N = 83 feeding trees; Montepío wet season N = 60 feeding trees; Balzapote dry season N = 85 feeding trees; Balzapote wet season N = 86 feeding trees.

^cSignificant difference between seasons in the marked group.

^dSignificant difference between groups in the marked season.

^eSignificant difference between groups in both seasons.

trees was significantly higher in Balzapote than in Montepío in both seasons ($F_{1,10.1} = 40.1, P < 0.001$), and was also higher in the wet season ($F_{1,53.27} = 5.8, P = 0.019$). Young leaf abundance was higher in Montepío than in Balzapote ($F_{1,13.66} = 29.7, P < 0.001$) but did not vary between seasons ($F_{1,66.54} = 3.4, P = 0.079$). Flower abundance did not vary according to site ($F_{1,17.71} = 0.09, P = 0.771$) or season ($F_{1,57.77} = 0.8, P = 0.366$).

For each plant part, we analyzed variation in the time each individual spent feeding from it with respect to its respective abundance in trees used as food sources, site, season, and reproductive state. Independently from season of the year or reproductive state, the proportion of time spent consuming fruits increased when individuals fed in trees with more fruits ($F_{1,70.21} = 21.9, P < 0.001$) and was higher in Balzapote than in Montepío ($F_{1,2.7} = 20.3,$

$P = 0.026$; Table IV). There were no differences between groups, seasons, or among reproductive states in the proportion of time spent eating both mature and young leaves ($P > 0.05$). Time spent eating flowers could not be analyzed due to many missing cases (i.e., several individuals from both groups did not consume flowers in several observation weeks), although flower consumption tended to be higher in Balzapote.

Time Budgets, Ranging Distances, and Social Behavior Depend on Feeding Behavior

We compared the time budgets, ranging distances, and social behavior of individuals between sites, seasons, and among reproductive states. The proportion of time spent traveling was higher in the dry season in Balzapote than in Montepío

TABLE IV. Variation (Mean ± SE) Between Groups and Seasons in Feeding Behavior, Time Budgets, Ranging Distances, Social Interactions and GC Levels

Behavior and cortisol	Montepío		Balzapote	
	Wet season	Dry season	Wet season	Dry season
Fruits (%)	41.1 ± 1.5	14.6 ± 0.6	63.0 ± 3.2	33.9 ± 3.2 ^a
Mature Leaves (%)	0.6 ± 0.1	6.7 ± 0.8	1.7 ± 0.3	2.3 ± 0.3
Young leaves (%)	58.3 ± 1.6	78.7 ± 2.1	28.1 ± 0.9	49.9 ± 2.7
Flowers (%)	0.0 ± 0.0	<0.01 ± 0.0	7.2 ± 0.05	14.0 ± 0.5
Feeding (%)	14.7 ± 2.1	14.8 ± 2.1	19.5 ± 3.4	26.0 ± 3.9
Resting (%)	72.0 ± 3.3	77.6 ± 2.8	71.1 ± 4.1	63.5 ± 4.8
Traveling (%)	13.3 ± 2.3	7.6 ± 0.9	9.5 ± 1.4	10.5 ± 1.5 ^b
Ranging distances (m/h/wk)	85.6 ± 11.7	81.5 ± 21.8	74.6 ± 10.8	93.2 ± 15.6
Affiliation (interactions/h/individual)	0.03 ± 0.01	0.04 ± 0.00	0.01 ± 0.00	0.02 ± 0.00 ^a
Agonism (interactions/h/individual)	0.006 ± 0.01	0.007 ± 0.00	0.01 ± 0.00	0.02 ± 0.01 ^a
Cortisol (ng/g)	416.5 ± 67.9	450.2 ± 57.9	397.6 ± 64.8	698.9 ± 118.6 ^b

^aSignificant difference between groups in both seasons.

^bSignificant difference between groups in the marked season.

($F_{1,63.82} = 4.4$, $P = 0.039$; Table IV). There were no differences between groups in the proportion of time spent feeding, time spent resting, and in ranging distances. Affiliation rates were significantly higher in Montepío than in Balzapote ($F_{1,41.65} = 4.1$, $P = 0.049$), whereas rates of agonism were higher in Balzapote than in Montepío ($F_{1,13.61} = 6.5$, $P = 0.024$) and in males than in females, independently from their reproductive state ($F_{3,34.68} = 5.3$, $P = 0.004$; Fig. 2).

We analyzed whether variation in time budgets, ranging distances, and social interactions was explained, in addition to site, season, and reproductive state, by feeding behavior. At both sites, when individuals spent more time eating fruits they spent less time traveling ($F_{1,122.2} = 8.4$, $P < 0.001$), and this relationship was more marked in the wet season ($F_{1,44.16} = 7.2$, $P = 0.011$). Time spent consuming fruits did not predict time spent feeding and resting, ranging distances or social interactions ($P > 0.05$). At both sites and seasons, when individuals ate more mature leaves they fed for less time ($F_{1,119.8} = 21.0$, $P < 0.001$), traveled less ($F_{1,115.7} = 9.8$, $P = 0.002$), ranged for shorter distances ($F_{1,121.6} = 16.2$, $P < 0.001$) and rested longer ($F_{1,121} = 74.8$, $P < 0.001$). The consumption of young leaves positively predicted: (1) feeding time ($F_{1,112.5} = 9.8$, $P = 0.002$), particularly at Montepío ($F_{1,13.64} = 4.9$, $P = 0.045$); (2) travel time ($F_{1,116.9} = 14.5$, $P < 0.001$), particularly during the wet season ($F_{1,36.79} = 8.8$, $P = 0.005$); and (3) ranging distances ($F_{1,116} = 5.3$, $P = 0.023$). Young leaf consumption negatively predicted time spent resting ($F_{1,114.1} = 9.2$, $P = 0.003$). Time spent consuming both mature and young leaves did not predict rates of social interactions.

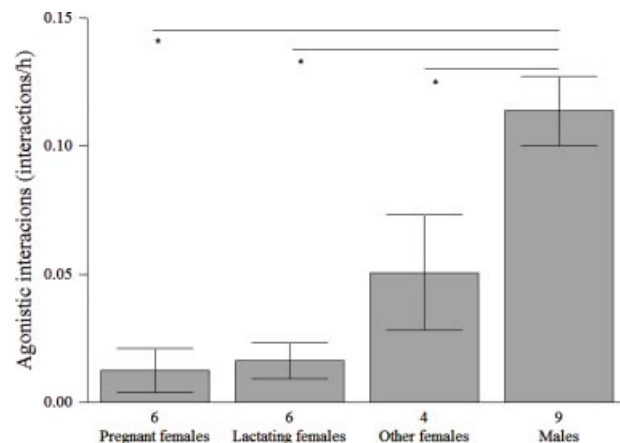


Fig. 2. Mean (\pm SE) rates of agonistic interactions per sex (males $N = 82$ individual/weeks) and female reproductive state (pregnant females $N = 30$; lactating females $N = 28$; other females $N = 20$) in the two groups. The number of individuals contributing to each mean is provided below bars. Significant differences are denoted by an asterisk.

GC Levels Depend on Time Budgets, Ranging Distances, and Social Interactions

We modeled variation in weekly GCs as a function of time budgets, ranging distances, and social interactions. Weekly GC levels were not predicted by time budgets or ranging distances. The best model (i.e., lowest AIC value) explaining variation in weekly GC levels included the interaction between site and season ($F_{1,68.67} = 16.8$, $P < 0.001$; Fig. 3), rates of agonism ($F_{1,114.8} = 15.2$, $P < 0.001$) and the interaction between reproductive state and agonism ($F_{3,106.5} = 2.9$, $P = 0.038$; Fig. 4). According to these results, weekly GC levels were significantly higher: (1) in Balzapote than in Montepío during the dry season; (2) when individuals interacted more agonistically; and (3) in pregnant and lactating females that were involved in agonistic interactions than in males and females in other reproductive states.

DISCUSSION

We tested whether weekly variation in GC levels between two groups of mantled howlers could be predicted by differences in energetic challenges or by psychosocial stressors. We found that participation in agonistic interactions, which were more frequent in Balzapote that lived in the small forest fragment, was associated with an increase in weekly GCs, particularly in pregnant and lactating females. During the dry season weekly GCs were also higher in Balzapote. Although in this group individuals significantly increased travel time during the dry season, weekly GC levels were unrelated to time-budgets or ranging distances, contrasting with previous findings on mantled howler monkeys' GC response [Dunn et al., 2013].

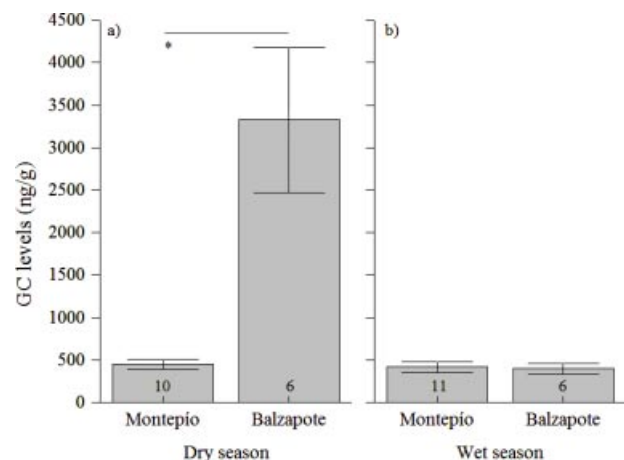


Fig. 3. Mean (\pm SE) GC levels per group in each season: (a) dry season (Montepío $N = 48$ individual/weeks; Balzapote $N = 30$); (b) wet season (Montepío $N = 52$; Balzapote $N = 30$). The number of individuals contributing to each mean is provided inside bars. Significant differences are denoted by an asterisk.

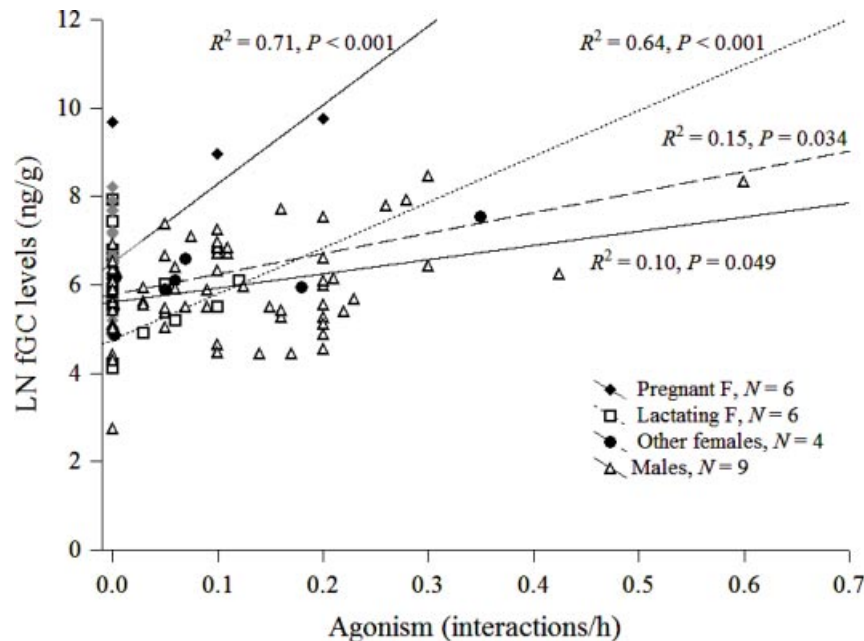


Fig. 4. The relationship between participation in agonistic interactions and GC levels per sex ($N = 160$ individual/weeks).

Overall, both groups experienced similar seasonal variation in the abundance of foods, but the group that lived in the small forest fragment had higher food availability as measured by the occurrence of big trees, fruits and figs. However, we found no evidence that variation in weekly GC levels between groups resulted from differences in food availability. Therefore, our results indicate that GC levels of mantled howler monkeys respond to the effects of agonism, reproductive state, and the influence of a seasonal stressor in the small fragment that could not be definitively determined in the present study.

Our study is the first to analyze the relationship between rates of social interactions and GC variation in howler monkeys. Agonistic interactions in this genus are in general infrequent and of low-intensity, mostly involving spatial displacements and ritualized behaviors [e.g., Dias et al., 2008; Van Belle et al., 2008], which have been associated with the energetic constraints of a primarily folivorous diet [Milton, 1980]. However, dominance-related agonistic behavior has been reported among females [Jones, 1980; Zucker & Clarke, 1998], and direct confrontations between males may lead to serious physical injury or death [Dias et al., 2010]. Social interactions have the potential to represent strong stressors, because they may entail both a high degree of unpredictability [Creel, 2001] and an increase in metabolic demands [Muller & Wrangham, 2004]. In our study, it is unlikely that agonistic interactions caused an increase in energy expenditure, because they were mostly displacements and threats. Therefore, we propose that, as reported for other primates

[e.g., Crockford et al., 2008; Ostner et al., 2008], in mantled howler monkeys participation in agonistic interactions, although infrequent, may result in psychological stress. As we could not define a dominance hierarchy in these groups because many dyads (37% in Montepío and 25% in Balzapote) did not exchange agonistic interactions, it remains for future research to determine whether the observed relationship between GC response and participation in agonistic interactions in mantled howler monkeys depends on individual rank [Abbott et al., 2003].

In primates the relationship between agonism and stress responses is primarily associated with unpredictability about the nature and quality of social relationships [Foerster et al., 2012], as usually happens in the context of immigrations or rank reversals [e.g., Engh et al., 2006]. We therefore expected that the changes in group composition that occurred in Montepío would result in higher weekly GC levels in this group. Further, a previous study documented that female mantled howler monkeys increase their GC levels in response to higher takeover probabilities by extra-group males [Cristóbal-Azkarate et al., 2007], which were higher at Montepío. However, the relationship between weekly GCs and agonism did not vary according to site, but rather depended on sex and reproductive state. Pregnant and lactating females, which in general were less involved in agonistic interactions, showed more significant weekly GC responses to agonism than non-reproductive females and males. As GCs are expected to be elevated during moments of increased metabolic demands [Sapolsky et al., 2000], such as

those associated with gestation and lactation [Gittleman & Thompson, 1988], it is possible that GC responsiveness to negative social interactions is higher in reproducing females [Hoffman et al., 2010; but see Brunton et al., 2008]. In addition, negative social interactions with conspecifics may lead to miscarriage or infant loss [e.g., Pereira, 1983; Shopland & Altmann, 1987], therefore representing strong psychological stressors for females. For instance, the female that entered the Montepío group had a 2-month-old infant. Soon after her immigration we observed on several occasions other females trying to separate the infant from its mother, and these attempts usually involved the exchange of agonistic interactions. In weeks when such interactions occurred this female had some of the highest GC levels registered among Montepío females, whereas in weeks without agonistic interactions involving infant handling her GC levels were lower, also suggesting that her elevated GC levels were not related to her recent immigration. Still, in many mammals pregnancy is a transient period of hypercortisolism [Mastorakos & Ilias, 2003], which could account for the higher GC levels of pregnant females observed in this and in a previous study with mantled howler monkeys [Dunn et al., 2011]. Therefore, in addition to psychosocial stress, other mechanisms could be associated with increases in GCs during pregnancy in this species. Measuring variation in female hormonal profiles within reproductive states and its relationship with ecological (e.g., food availability) and behavioral (e.g., agonism) variables will allow a better understanding of the results that were found here.

We posed that if variation in GC levels of mantled howler monkeys was mainly determined by energetic stressors, weekly GC levels should be higher in Balzapote than in Montepío. Accordingly, we found such differences, although these were significant only in the dry season. However, and contrary to our prediction, these differences were not caused by an increased foraging effort in Balzapote associated with lower food availability or quality compared to Montepío. Actually, overall food availability and fruit consumption were higher in Balzapote (discussed below) and although in the dry season individuals in this group spent more time traveling, travel was not a significant predictor of weekly GCs, as found in previous studies with howler monkeys [Dunn et al., 2013; Martínez-Mota et al., 2007]. Other potential stressors that could have caused higher weekly GC levels in Balzapote include walking on the ground or increased human presence in the fragment. During the dry season individuals traveled on the ground on three occasions to reach a nearby area where several trees had fruits. For arboreal primates like howler monkeys, terrestrial locomotion represents an atypical situation that may increase susceptibility to predation [Rangel-Negrín et al., 2011], and there-

fore could represent a stressful situation. Furthermore, people capture freshwater prawns (*Macrobrachium* spp.) in the river that crosses the Balzapote forest fragment, and we saw more people visiting the river during the dry season, probably because freshwater prawns are more abundant throughout rivers in this season [Mejía-Ortíz & Alvarez, 2010]. The frequent presence of people near groups of black howler monkeys has been associated with higher GC levels [Behie et al., 2010], suggesting that a similar effect could explain the increased weekly GC levels of mantled howler monkeys living in Balzapote in the dry season. Therefore, we speculate that differences in anthropogenic stressors that vary seasonally between the two groups explain the higher weekly GC levels found in Balzapote in the dry season.

Besides finding differences in GC between groups residing in habitats of varying size, previous research on howler monkeys has reported that GC levels are higher in females than males [Aguilar-Cucurachi et al., 2010] and in reproductive than non-reproductive females [Dunn et al., 2011], but we did not find such effects. In contrast to what would be expected under the energy expenditure prediction, our GC model did not include the interaction among reproductive state and season or site as significant predictors. However, in the dry season two females were pregnant and one was lactating in Balzapote, whereas in Montepío two females were lactating and three were in other reproductive states (i.e., in ovulatory or anovulatory cycles). Conversely, during the wet season in Balzapote there was one pregnant female, one lactating female and one female in other reproductive state, whereas in Montepío three females were pregnant, one was lactating and one was in other reproductive state. Therefore, the difference between groups in the proportion of females in energy demanding reproductive states was higher during the dry season, which could account for the observed differences in weekly GC levels. Larger sample sizes will be required in the future to reliably test these effects.

Contrary to our prediction, there was not a positive relationship between habitat size and food availability. It is noteworthy that the potential negative effects of reduced food availability in small habitats for mantled howler monkeys' metabolic balance (and associated GC response) has been commonly mentioned [Aguilar-Cucurachi et al., 2010; Arroyo-Rodríguez & Dias, 2010; Dunn et al., 2011, 2013], but actual food availability had not been previously measured. Young leaves were more abundant in Montepío in the wet season, a period during which fruit abundance was high, and therefore young leaves could be of lower importance to the diet of howler monkeys, but the overall patterns of variation in the abundance of plant parts were similar between sites. However, in Balzapote there were more big trees, figs, and fruit, all of which have been described as key food resources for howlers [Behie et al., 2010; Dunn

et al., 2009; Serio-Silva et al., 2002] and other primates [e.g., Fashing, 2001; Felton et al., 2008], and independently from season of the year, howlers in Balzapote consumed more fruit than in Montepío. These patterns contrast with previous suggestions that howlers living in small habitats face reduced food availability [Arroyo-Rodríguez & Dias, 2010] and highlight the importance of considering the specific structure and composition of the vegetation when assessing the interplay among forest size, food availability, behavior, and stress. Finally, our results support previous findings that howler monkeys' time budgets show little variation among groups living in fragments with different sizes [e.g., Cristóbal-Azkarate & Arroyo-Rodríguez, 2007], as in both sites young leaf consumption associated with energy-demanding time budgets and ranging, mature leaf consumption associated with energy-saving time budgets and ranging, and fruit consumption associated with less traveling.

In conclusion, we found that psychosocial stressors significantly affect the GC response of mantled howler monkeys, and that this response is modulated by reproductive state. Specifically, participation in agonistic interactions increased weekly GC levels, especially in pregnant and lactating females. However, documented seasonal differences in weekly GC levels between the two groups suggest that other factors, which could not be fully assessed in the present study, also affect the GC response in mantled howler monkeys. In contrast with previous studies on the behavioral and physiological consequences of the disturbance of mantled howler monkeys' habitat, we found that food availability is not always positively related to habitat size and does not predict, either directly through changes in feeding patterns or indirectly through constraints on time budgets, weekly GC levels. These conclusions leave a number of unanswered questions on the interplay among the ecology, reproductive physiology, and behavior of howler monkeys. To resolve these questions, data on the association between reproductive state and CG levels from more groups living in a more diverse array of social and ecological settings, together with long-term data on the behavioral responses of howlers to fluctuations in food availability, are needed.

ACKNOWLEDGMENTS

We thank all the students and volunteers that helped with data collection, in particular to A. Coyohua-Fuentes and R. Rivera-Bialas. Permission to work at Montepío was granted by the Asociación Civil Montaña y Mar Montepío, and at Balzapote by the Promotor-Díaz and Mendoza-Ramírez families. We are indebted to L. Cortés-Ortiz for permission to sample the mantled howlers that were used for the biological validation of our cortisol measurements. Dr. R. Coates (Instituto de Biología, UNAM) kindly provided climate data. We thank C.M. Schaffner, K.

Bales, and two anonymous reviewers for very helpful comments that improved this manuscript. Our study adhered to the legal requirements of Mexico for scientific research.

REFERENCES

- Abbott DH, Keverne EB, Bercovitch FB, et al. 2003. Are subordinates always stressed? *Horm Behav* 43:67–82.
- Aguilar-Cucurachi MS, Dias PAD, Rangel-Negrín A, et al. 2010. Preliminary evidence of accumulation of stress during translocation in howlers. *Am J Primatol* 72:805–810.
- Aguilar-Melo AR, Andresen E, Cristóbal-Azkarate J, et al. 2013. Behavioral and physiological responses to subgroup size and number of people in howler monkeys inhabiting a forest fragment used for nature-based tourism. *Am J Primatol* 75:1108–1116.
- Arlet ME, Isbell LA. 2009. Variation in behavioral and hormonal responses of adult male gray-cheeked mangabeys (*Lophocebus albigena*) to crowned eagles (*Stephanoaetus coronatus*) in Kibale National Park, Uganda. *Behav Ecol Sociobiol* 63:491–499.
- Arroyo-Rodríguez V, Dias PAD. 2010. Effects of habitat fragmentation and disturbance on howler monkeys: A review. *Am J Primatol* 72:1–16.
- Arroyo-Rodríguez V, Dias PAD, Cristóbal J. 2011. Group size and foraging effort in mantled howlers at Los Tuxtlas, Mexico. In: Gama L, Pozo G, Contreras WM, Arriaga SL, editors. *Perspectivas en primatología mexicana*. Villahermosa: UJAT. p 103–116.
- Behie AM, Pavelka MS, Chapman CA. 2010. Sources of variation in faecal cortisol levels in howler monkeys in Belize. *Am J Primatol* 72:600–606.
- Brunton PJ, Russell JA, Douglas AJ. 2008. Adaptive responses of the maternal hypothalamic-pituitary-adrenal axis during pregnancy and lactation. *J Neuroendocrinol* 20:764–776.
- Canales-Espinosa D, Dias PAD, Rangel-Negrín A, et al. 2011. Translocación de primates mexicanos. In: Dias PAD, Rangel-Negrín A, Canales-Espinosa D, editors. *La conservación de los primates en México*. Xalapa: Covecyt. p 81–107.
- Cavigelli SA. 1999. Behavioural patterns associated with faecal cortisol levels in free-ranging female ring-tailed lemurs, *Lemur catta*. *Anim Behav* 57:935–944.
- Chatterjee S, Hadi AS, Price B. 2000. *Regression analysis by example*. New York: Wiley. 424 p.
- Cortés-Ortiz L, Duda TF, Canales-Espinosa D, et al. 2007. Hybridization in large-bodied New World primates. *Genetics* 176:2421–2425.
- Creel S. 2001. Social dominance and stress hormones. *Trends Ecol Evol* 16:491–497.
- Cristóbal-Azkarate J, Arroyo-Rodríguez V. 2007. Diet and activity patterns of howler monkeys (*Alouatta palliata*) in Los Tuxtlas, Mexico. *Am J Primatol* 69:1–17.
- Cristóbal-Azkarate J, Veà JJ, Asensio N, Rodríguez-Luna E. 2005. Biogeographical and floristic predictors of the presence and abundance on mantled howlers (*Alouatta palliata mexicana*) in rainforest fragments at Los Tuxtlas, Mexico. *Am J Primatol* 67:209–222.
- Cristóbal-Azkarate J, Chavira R, Boeck L, Rodríguez-Luna R, Veà JJ. 2007. Glucocorticoid levels in free ranging resident howlers. *Am J Primatol* 69:1–11.
- Crockford C, Wittig RM, Whitten PL, Seyfarth RM, Cheney DL. 2008. Social stressors and coping mechanisms in wild female baboons (*Papio hamadryas ursinus*). *Horm Behav* 53:254–265.
- Dias PAD. 2002. Alterações na estrutura das relações sociais num grupo de macacos uivadores de manto (*Alouatta palliata mexicana*). MSc Thesis. Lisboa: ISCSP, Universidade Técnica de Lisboa.

- Dias PAD, Rodríguez-Luna E, Canales-Espinosa D. 2008. The functions of the "greeting ceremony" among male mantled howlers (*Alouatta palliata*) on Agaltepec Island, Mexico. *Am J Primatol* 70:621–628.
- Dias PAD, Rangel-Negrín A, Veà JJ, Canales-Espinosa D. 2010. Coalitions and male-male behavior in *Alouatta palliata*. *Primates* 51:91–94.
- Dunn JC, Cristóbal-Azkarate J, Veà JJ. 2009. Differences in diet and activity pattern between two groups of *Alouatta palliata* associated with the availability of big trees and fruit of top food taxa. *Am J Primatol* 71:654–662.
- Dunn JC, Cristóbal-Azkarate J, Veà JJ. 2010. Seasonal variations in the diet and feeding effort of two groups of howlers in different sized forest fragments. *Int J Primatol* 31:887–903.
- Dunn JC, Cristóbal-Azkarate J, Veà JJ, Chavira R. 2011. Effects of fruit consumption and foraging effort on stress levels in two groups of *Alouatta palliata mexicana* in forest fragments. In: Gama L, Pozo G, Contreras WM, Arriaga SL, editors. *Perspectivas en primatología mexicana*. Villahermosa: UJAT. p 39–53.
- Dunn JC, Cristóbal-Azkarate J, Schulte-Herbrüggen B, Chavira R, Veà JJ. 2013. Travel time predicts fecal glucocorticoid levels in free-ranging howlers (*Alouatta palliata*). *Int J Primatol* 34:246–259.
- Engh AL, Beehner JC, Bergman TJ, et al. 2006. Female hierarchy instability, male immigration and infanticide increase glucocorticoid levels in female chacma baboons. *Anim Behav* 71:1227–1237.
- Fashing PJ. 2001. Feeding ecology of guerezas in the Kakamega forest, Kenya: The importance of Moraceae fruit in their diet. *Int J Primatol* 22:579–609.
- Felton AM, Felton A, Wood JT, Lindenmayer DB. 2008. Diet and feeding ecology of *Ateles chamek* in a Bolivian semi-humid forest. *Int J Primatol* 29:379–403.
- Foerster S, Cords M, Monfort SL. 2012. Seasonal energetic stress in a tropical forest primate: Proximate causes and evolutionary implications. *PLoS ONE* 7:e50108.
- Gentry AH. 1982. Patterns of neotropical plant species diversity. *Evol Biol* 15:1–85.
- Gittleman JL, Thompson SD. 1988. Energy allocation in mammalian reproduction. *Am Zool* 28:863–875.
- Hoffman CL, Ayala JE, Mas-Rivera A, Maestripieri D. 2010. Effects of reproductive condition and dominance rank on cortisol responsiveness to stress in free-ranging female Rhesus macaques. *Am J Primatol* 72:559–565.
- Jones CB. 1980. The functions of status in the mantled howler monkey, *Alouatta palliata* Gray. *Primates* 21:389–405.
- Khan MZ, Altmann J, Isani SS, Yu J. 2002. A matter of time: Evaluating the storage of fecal samples for steroid analysis. *Gen Comp Endocrinol* 128:57–64.
- Martínez-Mota R, Valdespino C, Sánchez-Ramos MA, Serio-Silva JC. 2007. Effects of forest fragmentation on the physiological stress response of black howler monkeys. *Anim Conserv* 10:374–379.
- Mastorakos G, Ilias I. 2003. Maternal and fetal hypothalamic-pituitary-adrenal axes during pregnancy and postpartum. *Ann NY Acad Sci* 997:136–149.
- Mejía-Ortiz LM, Alvarez F. 2010. Seasonal patterns in the distribution of three species of freshwater shrimp, *Macrobrachium* spp., along an altitudinal river gradient. *Crustaceana* 83:385–397.
- Milton K. 1980. The foraging strategy of howler monkeys: A study of primate economics. New York: Columbia University Press. 165 p.
- Motulsky HJ, Christopoulos A. 2003. Fitting models to biological data using linear and nonlinear regression. San Diego: GraphPad Software. 351 p.
- Muller MN, Wrangham R. 2004. Dominance, cortisol and stress in wild chimpanzees (*Pan troglodytes schweinfurthii*). *Behav Ecol Sociobiol* 55:332–340.
- Ostner J, Kappeler PM, Heistermann M. 2008. Androgen and glucocorticoid levels reflect seasonally occurring social challenges in male redfronted lemurs (*Eulemur fulvus rufus*). *Behav Ecol Sociobiol* 62:627–638.
- Ostro LET, Young TP, Silver SC, Koontz FW. 1999. A geographic information system method for estimating home range size. *J Wildl Manag* 63:748–755.
- Pereira ME. 1983. Abortion following the immigration of an adult male baboon (*Papio cynocephalus*). *Am J Primatol* 4:93–98.
- Pride RE. 2005. Foraging success, agonism, and predator alarms: Behavioral predictors of cortisol in *Lemur catta*. *Int J Primatol* 26:295–319.
- Rangel-Negrín A, Dias PAD, Canales-Espinosa D. 2011. Impact of translocation on the behavior and health of black howlers. In: Gama L, Pozo G, Contreras WM, Arriaga SL, editors. *Perspectivas en primatología mexicana*. Villahermosa: UJAT. p 271–288.
- Sapolsky RM, Romero LM, Munck AU. 2000. How do glucocorticoids influence stress responses? *Endocr Rev* 21:55–89.
- Serio-Silva JC, Rico-Gray V, Hernández-Salazar LT, Espinosa-Gomez R. 2002. The role of *Ficus* (Moraceae) in the diet and nutrition of a troop of Mexican howler monkeys, *Alouatta palliata mexicana*, released on an island in southern Veracruz, Mexico. *J Trop Biol* 18:913–928.
- Setchell JM, Smith T, Wickings EJ, Knapp LA. 2008. Factors affecting fecal glucocorticoid levels in semi-free-ranging female mandrills (*Mandrillus sphinx*). *Am J Primatol* 70:1023–1032.
- Shopland JM, Altmann J. 1987. Fatal intragroup kidnapping in yellow baboons. *Am J Primatol* 13:61–65.
- Soto ME. 2004. El clima. In: Guevara S, Laborde J, Sánchez G, editors. *Los Tuxtles. El paisaje de la sierra*. Xalapa: INECOL and Unión Europea. p 195–198.
- Van Belle S, Estrada A, Strier KB. 2008. Social relationships among male *Alouatta pigra*. *Int J Primatol* 29:1481–1498.
- Van Belle S, Estrada A, Ziegler TE, Strier KB. 2009. Social and hormonal mechanisms underlying male reproductive strategies in black howler monkeys (*Alouatta pigra*). *Horm Behav* 56:355–363.
- Wasser SK, Hunt KE, Brown JL, et al. 2000. A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. *Gen Comp Endocrinol* 120:260–275.
- Weingrill T, Gray DA, Barrett L, Henzi SP. 2004. Fecal cortisol levels in free-ranging female chacma baboons. *Horm Behav* 45:259–269.
- Zucker EL, Clarke MS. 1998. Agonistic and affiliative relationships of adult female howlers (*Alouatta palliata*) in Costa Rica over a 4-year period. *Int J Primatol* 19:433–449.