Behavioral and physiological stress responses to local spatial disturbance and human activities by howler monkeys at Los Tuxtlas, Mexico

S. Cañadas Santiago[1,2], P. A. D. Dias[1] (iD), S. Garau[1,2], A. Coyohua Fuentes[1],
D. R. Chavira Ramírez[3], D. Canales Espinosa[1] & A. Rangel Negrín[1]

1 Primate Behavioral Ecology Lab, Instituto de Neuroetología, Universidad Veracruzana, Xalapa, México
2 Posgrado en Neuroetología, Instituto de Neuroetología, Universidad Veracruzana, Xalapa, México
3 Departamento de Biología de la Reproducción, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Ciudad de México, México

Abstract

To develop effective conservation and management actions, it is important to examine anthropogenic disturbance patterns and their impact on wildlife. We examined variation in the behavioral and physiological stress response of the Critically Endangered Mexican mantled howler monkeys *Alouatta palliata mexicana* in relation to two types of anthropogenic disturbance, habitat spatial patterns and presence of humans or their livestock (noise and presence nearby primates). We studied four groups (42 individuals) in two forest fragments at the Los Tuxtlas Biosphere Reserve for a total of 1100 observation hours, during which we recorded the howler monkeys' vocalizations, locomotion and vigilance. We additionally collected fecal samples to determine the concentrations of glucocorticoid metabolites (fGCM). Howler monkeys vocalized less and moved more when in more spatially disturbed locations and when exposed more time to nearby noise but spent less time in locomotion when humans were present. fGCM were only related to human presence, suggesting that habitat spatial patterns are less of a challenge than human presence. This may be related to the generally unpredictable nature of the latter. Although our study does not allow determining whether the behavioral and physiological responses of howler monkeys to disturbance are costly, from a conservation standpoint it may be more prudent to assume that they are and design strategies to mitigate them. In this sense, actions aimed at reducing anthropogenic noise could benefit the conservation of Mexican mantled howler monkeys.

Introduction

Biodiversity is under threat, mainly due to habitat loss and degradation, overexploitation, pollution, invasive species and climate change (Secretariat of the Convention on Biological Diversity, 2010). It is estimated that current species extinction rates are exceptionally fast (McCauley *et al.*, 2015; Ceballos, Ehrlich & Dirzo, 2017), and that, if maintained, a very high proportion of species will become extinct before the end of the century (Pimm & Raven, 2000). Under this scenario, it is critical to examine anthropogenic disturbance patterns and their impact on wildlife.

The impact of anthropogenic disturbance on wildlife has been mainly studied in two ways. First, some studies focus on investigating how variation in habitat spatial patterns caused by human activities affects animals (i.e. anthropogenic spatial disturbance). These studies usually analyze how variation of one or several spatial attributes (e.g. habitat size, habitat shape) measured on local, landscape or regional scales affect population parameters, behavior or physiology of animals (e.g. Cormont *et al.*, 2014; Ferreira *et al.*, 2017). Second, other studies concentrate on examining how the presence of humans and/or domestic animals (e.g. grazing, recreational activities, traffic) inside or in the vicinity of a natural habitat affects population parameters, behavior or physiology (e.g. Tablado & Jenni, 2017; Karimov, Kachel & Hackländer, 2018). More rarely, the effects of both variation in habitat spatial patterns and human presence on wildlife are analyzed (e.g. Fernández-Juricic, 2000; Gill, 2007; Bötsch *et al.*, 2018). These latter studies suggest that variation in habitat spatial patterns and human presence have synergistic effects: usually human presence has a stronger effect on populations living in habitats with higher spatial disturbance (e.g. with more trails). Still, the paucity of

studies with this design, and the fact that the existing studies have been almost exclusively conducted with birds, limit our ability to understand how different anthropogenic disturbance types affect wildlife, and as a consequence, to develop effective conservation and management actions.

In order to better understand how different types of anthropogenic disturbance affect wildlife, we examined variation in the behavioral and physiological stress response of mantled howler monkeys *Alouatta palliata* in the Los Tuxtlas Biosphere Reserve. A diet that includes a large array of food items and energy-conservative time budgets seem to support the persistence of howler monkeys in degraded habitat, and their frequent presence in forest fragments where other mammals are absent (Rangel-Negrín *et al.*, 2014) has led to claims that they are resilient to anthropogenic disturbance (Schwarzkopf & Rylands, 1989; Terborgh *et al.*, 2001). However, there is ample evidence that population parameters, behavior and physiology of howler monkeys are negatively affected by anthropogenic disturbance (Arroyo-Rodríguez & Dias, 2010). Still, this evidence comes almost exclusively from studies focusing on human-induced variation in habitat spatial patterns and its effect on resource availability (Arroyo-Rodríguez & Dias, 2010). The few studies that have related human presence with howler monkey behavior and physiology (Behie, Pavelka & Chapman, 2010; Aguilar-Melo *et al.*, 2013; McKinney, Westin & Serio-Silva, 2015) yielded contrasting results. Contrasting results may be due to differences across species (i.e. different howler monkey species may respond differently), group size (e.g. mean ± SD: 4.8 ± 1.2 individuals vs. 38.5 ± 6.4: Behie *et al.*, 2010; Aguilar-Melo *et al.*, 2013) and study design (e.g. counts vs. occurrence of humans: Behie *et al.*, 2010; Aguilar-Melo *et al.*, 2013). In addition, none of these studies controlled for the spatial attributes of the habitat. Therefore, we currently do not know how different sources of anthropogenic disturbance affect howler monkeys, and as a consequence, if their presence in disturbed habitat is linked to resilience in coping with variation in habitat spatial patterns and/or human presence, and if their persistence in disturbed habitats could be viable.

We focused on the Critically Endangered Mexican mantled howler monkeys *Alouatta palliata mexicana* (Cuarón *et al.*, 2008) living in a protected area, and examined variation in vigilance, locomotion, vocalizations and physiological stress in relation to habitat spatial patterns and human presence. Vigilance and locomotion are linked to the detection and avoidance of potential threats respectively (Tablado & Jenni, 2017), and should be influenced by both spatial disturbance (e.g. increased vigilance and locomotion when individuals are near areas where encounters with humans are more likely) and presence of humans (increased vigilance and locomotion in response to human noise or when humans are actually present). Alternatively, locomotion should decrease if individuals have a passive antipredatory strategy (wildlife usually perceives humans as predators: Frid & Dill, 2002), whereby they hide or remain cryptic when humans are present (Steen, Gabrielsen & Kanwisher, 1988; Stankowich & Blumstein, 2005). Under this strategy, individuals should

also vocalize less to avoid detection. In the absence of habituation (Cyr & Romero, 2009) to anthropogenic disturbance, individuals should activate the physiological stress response when humans are present, but the magnitude of this activation may depend on coping style (i.e. a set of behavioral and physiological responses to challenges that is consistent overtime and particular to a certain group of individuals: Koolhaas *et al.*, 1999). Passive (or reactive) coping is associated with more activation, and corresponding higher levels of adrenocortical hormones, than proactive coping (Koolhaas *et al.*, 1999; Carere, Caramaschi & Fawcett, 2010). In contrast, if individuals have a proactive coping style, when humans are present vocalizations should be more frequent and levels of adrenocortical hormones should be similar to those at other times (Koolhaas *et al.*, 1999). Finally, adrenocortical hormone levels should also be affected by spatial disturbance if individuals associate location within their habitat with risk of encountering humans (i.e. a 'landscape of fear' *sensu* Brown, Laundré & Gurung, 1999), or if habitat quality is influenced by spatial disturbance (e.g. less food resources in some areas; Busch & Hayward, 2009).

Materials and methods

Ethical note

This study was non-invasive and followed the 'Guidelines for the treatment of animals in behavioural research and teaching' (Guidelines for the Use of Animals, 2019). Research protocols were approved by the Secretaria de Medio Ambiente y Recursos Naturales (permits SGPA/DGVS/10637/11 and SGPA/DGVS/04999/14) and adhered to the legal requirements of the Mexican law (NOM-059-SEMARNAT2010).

Study sites and subjects

We conducted the study in the Los Tuxtlas Biosphere Reserve, in southeastern Mexico. The reserve was created in 1998 and encompasses 155 122 ha. In this reserve there is a distinction between core and buffering areas, with the former representing areas where human activities are highly restricted (i.e. a land sparing conservation strategy), whereas the use and exploitation of natural resources are allowed in the latter (i.e. land sharing; Diario Oficial, 2009). To increase the likelihood of observing human activities close to mantled howler monkeys, we studied four groups living in two forest fragments, La Flor de Catemaco (18°26′43″N, 95°02′49″W) and Montepío (18°37′43″N, 95°05′05″W) located in the buffering area of the reserve (Fig. 1). These fragments have approximately the same area (*c.* 120 ha), altitude (*c.* 200 m a.s.l.) and vegetation type (tracts of old-growth evergreen rainforest interspersed with secondary forest, crops and pasturelands). In addition, there are several constructions bordering the fragments, including small huts where cattle are milked (Montepío), warehouses where ornamental plants are processed and stored before being sold (La Flor de Catemaco) and roads (both fragments).
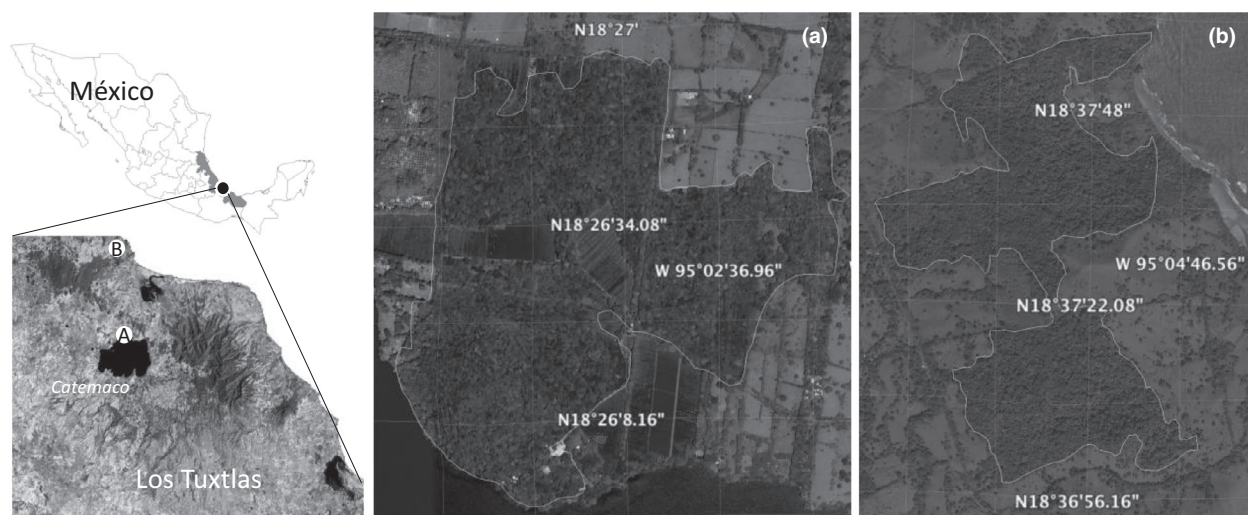
Figure 1 The study was conducted in southeastern Mexico (top left), specifically at the Los Tuxtlas region (botton left). In this region we sampled groups of Mexican mantled howler monkeys in two locations (white dots in bottom left): La Flor de Catemaco (a) and Montepío (b).

Fifty-two individuals lived in the four study groups. Groups had approximately the same size and composition (mean ± SD): 4.3 ± 2.1 adult males; 6.0 ± 2.4 adult females; 2.8 ± 1.5 immatures. In this study we concentrated on the 42 adult individuals, as the behavior of immatures (<3 years old) is usually highly dependent on the behavior of their mothers. Howler monkeys were fully habituated to the presence of researchers (i.e. ignored our presence: Williamson & Feistner, 2011). We identified each individual monkey easily through anatomical and physiognomic characteristics, including body size and proportions, scars, broken fingers and genital morphology and pigmentation.

Field methods

Behavioral sampling

We studied each group for 2 months during the dry season (i.e. March–May) and 2 months during the rainy season (i.e. June–February) in 2016. We conducted the same number of consecutive 4-day follows per week for each group in each season (24 days/group/season). During each day, we recorded all occurrences (Altmann, 1974) of loud calls, which are vocalizations that howler monkeys use mainly for inter-group communication but have also been described to function as alarm calls (Kitchen *et al.*, 2015). We also used focal animal sampling with continuous recording (1-h samples; Altmann, 1974) to determine locomotion (movement > 0.5 m from an individuals' current position) and vigilance time. Individuals were scored as vigilant if their heads were up, their eyes were open and their attention was not focused on a competing activity (e.g. foraging, socializing; Barrett, Halliday & Henzi, 2006). Subjects were randomly chosen as focal animals, with no individual sampled again until all others were sampled once. We performed a total of 1100 observation hours, with a mean (±SD) of 24.2 (±1.8) hours per week per group.

Assessment of physiological stress

To assess physiological stress, we measured fecal glucocorticoid metabolites (fGCM hereafter). We collected fecal samples opportunistically whenever they could be matched with individuals. We only collected samples that were not contaminated with urine or water. We deposited samples in polyethylene bags and stored them in a cooler with ice packs while in the field, and in a freezer at −20°C at the field station. Variation in this freezing procedure did not affect fGCM measurements (Supporting Information). Samples were freeze-dried (FreeZone 2.5 Liter Benchtop Freeze Dry System; Labconco, Kansas, MO, USA) to constant weight within a mean (±SD) of 2 ± 1 months after collection. We then pulverized samples using a porcelain mortar and pestle, removed seeds and bulks of undigested fiber with forceps, homogenized samples in the mortar with disposable plastic spatulas and stored samples in 15 mm polypropylene tubes at −20°C until extraction. To extract fGCM from fecal samples we shook 0.6 g of lyophilized, pulverized and homogenized feces for 24 h in 4.0 mL of analytical-grade methanol. Then, we centrifuged (2500 g for 30 min at −40°C) samples, collected supernatants containing steroids with pipettes, put supernatants in borosilicate glass tubes and evaporated them overnight in a water bath (PolyScience WA20, Niles, IL, USA) at 60°C. We added 3 mL of albumin buffer to the tubes and shook them for 1 min in a vortex (Vibrax, Serono, Geneva, Switzerland) and for 15 s in an ultrasonic cleaner (Cole-Parmer 08895-21, Vernon Hills, IL, USA).

We determined fGCM concentrations in extracts with a chemiluminescent immunoassay using a commercial kit (Cortisol, Immulite, Siemens, Los Angeles, CA, USA;

sensitivity = 5.5 nmol L^{-1}; calibration range = 28–1380 nmol L^{-1}) and an automated immunoassay system (Immulite 1000 analyzer, Siemens, Munich, Germany). The antibody in this kit is highly specific to cortisol, showing low (8.6%) cross-reactivity with corticosterone. Pooled fecal extracts, when added to the standard curve points, exhibited a similar slope ($R^2 = 0.97$, $N = 10$, $P < 0.001$), and serial dilutions of a fecal pool from howler monkeys yielded results that ran parallel to the kit's standards ($t = 0.7$, $P = 0.673$). Recovery rate was (mean ± SE) 96.5 ± 3.9% ($N = 5$). Glucocorticoid intra-assay variation averaged 9.5% (fecal extract pool, $N = 6$) and inter-assay variation was 7.7%. We performed a biological validation that showed that our technique reliably detected changes in fGCM in response to an emulated stressor (see Supplementary methods in Supporting Information Appendix S1). We analyzed a total of 611 fecal samples, with a mean (±SD) of 12.7 (±2.1) samples per week per group. fGCM concentrations are expressed in ng g^{-1}.

Assessment of habitat spatial patterns

To assess variation in habitat spatial patterns associated with anthropogenic disturbance, we used a point-quarter sampling (Krebs, 1999). At the beginning of each focal animal sampling, we used the tree in which the focal was located as the center of four compass directions that divided the sampling point into four 90° quadrants that extended 30 m (26 m is the maximum mean group spread for the species: Bezanson *et al.*, 2008) from the central tree (Ganzhorn 2011). In each quadrant we determined the predominant (i.e. cover type that occupied > 0.035 ha) land cover type as forest, crops, pasture or constructions. Therefore, in each sampling point a particular land cover type could have a score of between 0 (was not predominant in any quadrant) and 4 (was predominant in all quadrants). When forest was the predominant land cover in a quadrant, we estimated the abundance of young leaves, mature leaves and fruits (the main food items of howler monkeys: Dias & Rangel-Negrín, 2015) in all trees with a diameter at breast height (DBH) ≥ 10 cm (howler monkeys do not use trees with DBH < 10 cm) using semiquantitative scores: 0 = total absence; 1 = presence of the item in 0–25% of the total coverage of the frond of the tree; 2 = 26–50%; 3 = 51–75%; and 4 = 76–100% (Fournier, 1974). We then calculated the relative abundance of each item in the quadrant as:

$$A_x = \frac{\left(\sum_1^N \left(S_x \times \mathrm{DBH} \right) \right)}{N},$$

where A_x is the relative abundance of item x in a given quadrant, S_x is the abundance score of x in each tree in that quadrant, DBH is the diameter at breast height of each tree in that quadrant and N is the number of trees sampled in the quadrant. We performed a total of 1100 point-quarter samplings.

Assessment of human presence

To assess anthropogenic disturbance associated with the presence of humans, we recorded the duration of all human-associated noises as well as of the actual presence of humans or domestic animals. Concerning noise, we noted whether it occurred within (hereafter nearby noise) or outside (hereafter distant noise) a circle with a 30 m radius (maximum group spread) surrounding the focal animal being sampled at the moment of occurrence of each noise. We recorded the following noises: vocalizations (people talking to each other or vocalizing towards howler monkeys; dogs barking); cellphone ringing; work tools (machete, chainsaw, hammer); engine sound (car, boat, airplane, helicopter); and music (played from loudspeakers in town or from loudspeakers mounted on the top of a car). Concerning the actual presence of humans or their domestic animals, we only recorded it (hereafter intruder presence) when they were < 30 m from the focal animal and did not produce noise. There was variation within each human presence category in terms of stimuli intensity. For instance, in the category intruder presence, sometimes humans passed by without paying attention to howler monkeys, but other times they threw objects at them. Although we acknowledge the possible influence of such variation in our study, the short duration of several disturbances precluded a more detailed analysis. We recorded a total of 1134 events of human presence, with a mean ± SD of 23.9 ± 18.8 disturbances per group per week. Given the observed delay between the exposure of individuals to an acute challenge and peak fGCM concentrations (see Supplementary methods in Supporting Information Appendix S1), we assumed a 24-h delay between disturbance associated with the presence of humans and the associated fGCM excretion response. Thus, we scheduled both weekly behavioral observations and fecal sample collection accordingly: fecal sample collection began the day after behavioral sampling began and ended one day after the end of behavioral sampling.

Statistical analysis

We calculated the following variables at the group level on a weekly basis: (1) number of times individuals vocalized (i.e. frequency of vocalizations); (2) sum of time spent in locomotion in all focal samples; (3) sum of time spent in vigilance in all focal samples; (4) mean fGCM for all samples collected per group (Supporting Information Table S1). We also calculated the weekly mean presence of land cover types in the locations used by each group each week by dividing the sum of scores obtained by each land cover type in all point-quarter samplings in a given week by the total number of point-quarter samplings recorded in that week. We calculated mean weekly abundance of fruits and both young and mature leaves in areas used by each group by dividing the sum of abundance scores of each food item in a given week by the total number of point-quarter samplings collected in that week. Concerning human presence, we calculated weekly proportions of time each group was exposed to disturbance by dividing the sum of durations of all disturbances in each category (i.e. distant and nearby noises and intruder presence) in a given week by the total observation time in that week.

To analyze the impact of anthropogenic disturbance on mantled howler monkeys, we built four mixed models (West, Welch & Galecki, 2014), one for each of the following dependent variables: frequency of vocalizations; time in locomotion; time in vigilance; mean fGCM. Fixed factors were measures of habitat spatial patterns (land cover types in areas used by the group: forest, crops, pasture and constructions) and human presence (nearby and distant noise and intruder presence), whereas the term group id nested in site id was added as a random factor to account for the repeated sampling of different groups living in the same site. Given previous evidence suggesting that habitat spatial patterns and human presence may have synergistic effects on wildlife behavior (Fernández-Juricic, 2000; Gill, 2007; Bötsch *et al.*, 2018), we also added two-way interaction terms between both types of disturbance to models. However, models including interaction terms failed to converge, and we therefore only analyzed additive effects.

We included as control fixed factors in all models food abundance (i.e. fruits, young leaves and mature leaves), to account for the alternative hypothesis that variation in howler monkey behavioral and physiological stress response was mainly linked to ecological factors and group id, to account for inter-group variation in responses to disturbance. Food abundance variables were collinear (variance inflation factors > 10), so we reduced them to an orthogonal variable using principal components analysis. This component (hereafter food availability) had an eigenvalue of 2.8 and explained 94.5% of the variance in the original variables. It was positively related with fruit ($r = 0.95$), young leaf (0.98) and mature leaf (0.98) abundance. All other predictors were not collinear (Kutner *et al.*, 2004).

We used a Poisson error distribution and log link function for the vocalizations and locomotion models. Although in our study vigilance was a count variable (i.e. weekly total seconds) we obtained a better fit, as assessed using Q-Q plots of residuals fitted against predicted values and a Shapiro-Wilk test for normality of model residuals, with a model with Gaussian error distribution and identity link function on square root transformed vigilance time than with a model with Poisson error distribution on original values. In models of vocalizations, locomotion and vigilance we included weekly observation time as an offset variable, and in the locomotion model we also included an observation-level random effect to correct for overdispersion (Harrison, 2014). We used a Gaussian error distribution and identity link function to analyze variation in log transformed mean fGCM.

We followed an information-theoretic approach and multi-model inference to generate set models based on all combinations of predictors and then average model parameters across all resulting models (Burnham & Anderson, 2013; see Supplementary results in Supporting Information Appendix S2). We calculated the relative importance of each predictor based on its Akaike weights across all possible models. As model weights represent the probability of a model to be the best model in the model set and thus reflect model uncertainty, importance can be understood as the likelihood of a predictor to be included in the best model

(Burnham & Anderson, 2013). We used R package 'MuMln' (Barton, 2018) for model selection and averaging, package 'lme4' (Bates *et al.*, 2018) to obtain model parameters and confidence interval for fixed factors and package 'sjstats' to calculate pseudo-R-squared to assess the effect size of each model (Lüdecke, 2019). In all cases the best models had a significantly different effect on dependent variables than null models including only the intercept and random factors, as assessed using a likelihood ratio test (R function 'ANOVA': R Core Team, 2019).

Results

Howler monkeys vocalized more frequently in locations with a lower proportion of pasture and crops, and when they were exposed to less nearby noise (Table 1; Fig. 2). In contrast, they vocalized more when in locations with more constructions, although this factor had a relatively lower importance ($\Sigma W_i = 0.55$). Individuals spent more time in locomotion in locations with more crops and constructions and when exposed to more nearby noise. However, the model-averaged parameter estimate of crops was smaller than its SE, suggesting that caution is needed in the interpretation of the result for this predictor. In contrast, locomotion time was lower when intruder presence was higher. Howler monkeys were more vigilant when exposed to more nearby noise and when in locations with more pasture (although its parameter estimate was smaller and the estimate's SE), whereas vigilance was lower with more distant noises. fGCM concentrations were higher when howler monkeys were exposed to more nearby noise and were negatively related with distant noise.

Discussion

We investigated how variation in habitat spatial patterns and human presence affected the behavioral and physiological stress response of mantled howler monkeys living in two forest fragments within the Los Tuxtlas Biosphere Reserve. Both disturbance types affected the behavior of individuals, but not their fGCM concentrations, which were only influenced by human presence. Thus, our results highlight the importance of using different response (i.e. behavioral and physiological) and disturbance (i.e. habitat spatial patterns and human presence) variables when studying the relationships between anthropogenic disturbance and wildlife.

Howler monkeys reduced the frequency of vocalizations and increased locomotion time when they were in more disturbed locations (i.e. areas where forest was not the predominant land use type) and were exposed to more nearby noises. The likelihood of encountering humans should increase with proximity to crops, pasturelands, constructions and anthropogenic noise, so it is possible that howler monkeys try to remain unnoticed and move around more to evade humans under higher encounter risk but tend to freeze (i.e. no locomotion) when intruders are actually present. Additionally, compared to forested areas, less forested locations were infrequently used, supporting previous evidence that wildlife avoids disturbed areas (e.g. Pfister, Harrington

Table 1. Information-theoretic based mixed models of the factors influencing the behavior and fecal glucocorticoid metabolite (fGCM) concentrations of four mantled howler monkey groups studied in Los Tuxtlas (Mexico)

Predictor	Vocalizations			Locomotion			Vigilance			Mean fGCM		
	β	SE	95% CI	β	SE	95% CI	β	SE	95% CI	β	SE	95% CI
Group identity	0.21	0.06	−0.58 to 0.21	0.05	0.22	−0.46 to 1.43	0.12	0.21	−0.12 to 1.54	−0.07	0.90	−1.83 to 1.68
Forest	−0.38	0.24	−0.86 to 0.10	0.47	0.51	−1.24 to 1.45	−0.73	1.23	−3.15 to 1.69	−1.52	1.07	−3.61 to 0.58
Crops	**−0.65**	**0.25**	**−1.15 to −0.15**	**0.15**	**0.17**	**0.14 to 0.33**	0.20	0.17	−0.13 to 0.52	0.09	0.16	−0.23 to 0.41
Pasture	**−0.60**	**0.13**	**−0.86 to −0.33**	−0.03	0.39	−0.93 to 0.87	**0.46**	**0.88**	**1.27 to 2.18**	−1.14	0.77	−2.64 to 0.36
Constructions	**0.75**	**0.36**	**0.02 to 1.48**	**0.36**	**0.18**	**0.05 to 0.72**	0.17	0.32	−0.47 to 0.80	0.02	0.29	−0.54 to 0.58
Food availability	0.15	0.08	−0.02 to 0.32	0.04	0.05	−0.04 to 0.14	−0.01	0.08	−0.16 to 0.14	−0.03	0.07	−0.16 to 0.10
Nearby noise	**−4.14**	**0.95**	**−6.07 to −2.20**	**3.90**	**2.88**	**0.05 to 9.66**	**6.05**	**4.39**	**2.55 to 14.65**	**6.72**	**3.81**	**0.74 to 14.17**
Distant noise	0.09	0.14	−0.20 to 0.38	−0.49	0.36	−1.17 to 0.12	**−1.69**	**0.69**	**−3.03 to −0.35**	**−0.72**	**0.10**	**−1.39 to −0.95**
Intruder presence	0.11	0.07	−0.03 to 0.25	**−0.38**	**0.18**	**−0.80 to −0.01**	−0.42	0.34	−1.09 to 0.25	−0.57	0.30	−1.15 to 0.01

Numbers in bold represent the cases in which predictor beta values did not include zero.

& Lavine, 1992; Constantine, 2001). The fact that vigilance was positively related with the imminence of encounters with humans (i.e. nearby noise) is consistent with the possibility that risk perception is the mechanism underlying behavioral responses to disturbance (Tablado & Jenni, 2017). Therefore, we provide evidence that, similar to other wildlife (e.g. Visser et al., 2016; Bötsch et al., 2018), howler monkeys adjust their behavior to different types of anthropogenic disturbances, and it is possible that the intensity of their responses (i.e. avoid vs. freeze) is coupled with the perceived risk of encounters with humans. It remains for future research to address whether interactions between habitat spatial patterns and human presence, which could not be tested in this study, may act synergistically on mantled howler monkeys (Fernández-Juricic, 2000; Gill, 2007; Bötsch et al., 2018).

Whereas the behavior of howler monkeys was affected by both habitat spatial patterns and human presence, fGCM concentrations were only related to the latter, suggesting that habitat spatial patterns are less of a challenge than human presence (Bötsch, Tablado & Jenni, 2017; Bötsch et al., 2018). It is possible that, following spatial disturbance (e.g. habitat loss), individuals adjust to the new spatial attributes of their habitat through, for instance, behavioral flexibility (Hockings et al., 2015). In this sense, spatial disturbance would represent a predictable environmental challenge, to which howler monkeys respond with behavioral coping strategies (Lowry, Lill & Wong, 2013). In contrast, human presence could be usually unpredictable, and howler monkeys reacted to it by activating the physiological stress response.

Interestingly, although intruder presence has been generally found to affect the physiological stress response of wildlife (Busch & Hayward, 2009), in our study its importance was not particularly high. This result supports the suggestion that freezing in the presence of intruders may be a sufficient behavioral coping strategy to buffer the physiological stress response. Alternatively, this could result from habituation to human intrusion. If this was the case, given the involvement of glucocorticoids in metabolic processes (Sapolsky, Romero & Munck, 2000), the fGCM response to nearby noises could result from the energetic costs of increasing locomotion (Dias et al., 2017), rather than from psychological activation of the physiological stress response associated with fear. In any case, howler monkeys are responding to anthropogenic disturbance, and both behavioral and physiological modulation may entail costs.

Our study does not address the fitness consequences of the responses of howler monkeys to anthropogenic disturbance, but there is sufficient evidence in the literature to infer that they may be costly. First, given that time is inelastic (Dunbar, Korstjens & Lehmann, 2009), changes in one time-budget component, as in the case of locomotion and vigilance in our study, imply trade-offs with other components (Beckmann & Berger, 2003; Fleischer, Bowman & Woolfenden, 2003; Ordóñez-Gómez et al., 2016). Therefore, even if behavioral flexibility allows coping with time-budget adjustments without incurring fitness costs (Abrams
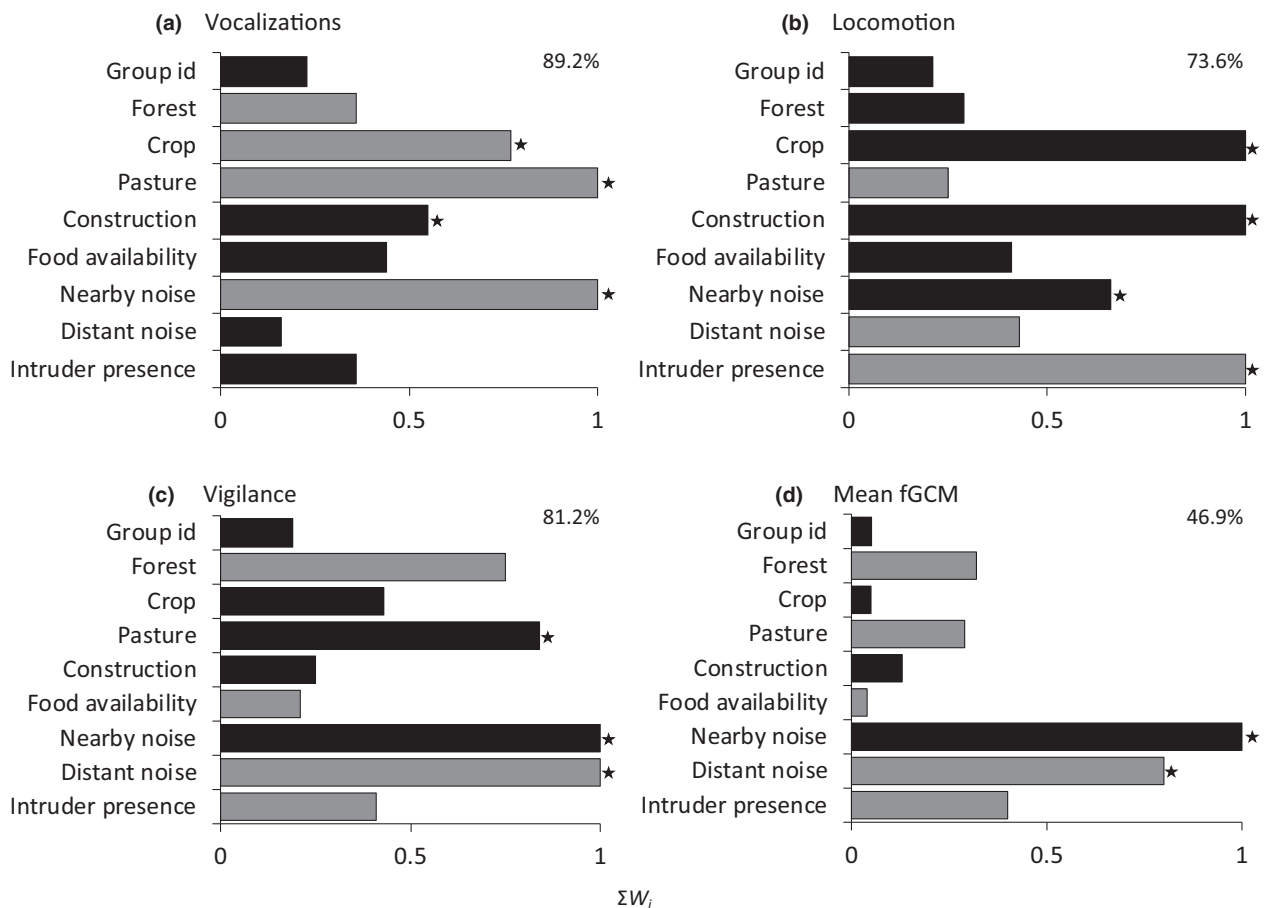
Figure 2 Models of behavioral and physiological responses of mantled howler monkeys to anthropogenic disturbance: (a) vocalizations; (b) locomotion; (c) vigilance); (d) mean fecal glucocorticoid metabolite (fGCM) concentrations. The relative importance (W_i) of each predictor is represented in bars as the sum of Akaike weights ($\sum W_i$). We also indicate the values of pseudo-R^2, i.e. the percentage of deviance explained by complete models. Bar color represents the relationship between predictors and dependent variables (black = positive; gray = negative), and stars represent cases in which the 95% confidence interval of model-averaged parameter estimate did not include zero.

& Schmitz, 1999), the long-term impact of such human-induced variation is less clear. It has been predicted, for instance, that under certain climate change scenarios, variation in time-budgets may be insufficient to allow for survival (Lehmann, Korstjens & Dunbar, 2010), and that energy-demanding time-budgets may reduce the fecundity of howler monkeys living in small forest fragments (Rangel-Negrín *et al.*, 2018). Second, although the fitness consequences of glucocorticoid modulation in wildlife in response to anthropogenic disturbance are still unclear (Bonier *et al.*, 2009; Boonstra, 2013; Beehner & Bergman, 2017), there is evidence that stress physiology may be critical for individual survival when facing unpredictable challenges (Romero & Wikelski, 2010). Third, noise could interfere in the long-distance communication of howler monkeys, affecting spatial regulation among groups and ultimately, social organization. Thus, from a conservation standpoint, it may be more prudent to promptly employ measures to reduce the behavioral and physiological responses of wildlife to anthropogenic

disturbance than to wait for more evidence of fitness consequences to become available before starting to employ such measures.

Our results have implications for the management of this population. The most obvious recommendation is that howler monkeys could benefit from a reduction of anthropogenic noise, which could be accomplished through an environmental education program to increase awareness on the impact of human activities on howler monkeys. This may be facilitated by the fact that one of the aims defined in the management plan of this biosphere reserve is to 'mitigate the impacts of anthropogenic disturbances that are detrimental for the ecosystems' (Diario Oficial, 2009: pp. 50).

In sum, as many other protected areas, Los Tuxtlas is a complex mosaic of original and anthropic habitats where humans and wildlife interact in many ways. We found that howler monkeys respond to such interaction by modulating their behavioral and physiological stress response. Therefore, our study provides avenues for future investigation on the

relative effects of different types of human disturbance on wildlife, as well as for the management of the endangered Mexican mantled howler monkeys.

References

Abrams, P.A. & Schmitz, O.J. (1999). The effect of risk of mortality on the foraging behaviour of animals faced with time and digestive capacity constraints. *Evol. Ecol. Res.* **1**, 285–301.

Aguilar-Melo, A., Andresen, E., Cristóbal-Azkarate, J., Arroyo-Rodríguez, V., Chavira-Ramírez, D.R., Schondube, J., Serio-Silva, J.C. & Cuarón, A. (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.

Altmann, J. (1974). Observational study of behaviour: sampling methods. *Behaviour* **49**, 227–267.

Arroyo-Rodríguez, V. & Dias, P.A.D. (2010). Effects of habitat fragmentation and disturbance on howler monkeys: a review. *Am. J. Primatol.* **72**, 1–16.

Barrett, L., Halliday, J. & Henzi, S.P. (2006). The ecology of motherhood: the structuring of lactation costs by chacma baboons. *J. Anim. Ecol.* **75**, 875–886.

Barton, K. (2018). *Multi-model inference. R package version 1.42.1*. Available at https://CRAN.R-project.org/package=MuMIn.

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2018). *Linear mixed-effects models using 'Eigen' and S4. R package version 1.1-19*. Available at http://CRAN.R-project.org/package=lme4

Beckmann, J.P. & Berger, J. (2003). Rapid ecological and behavioural changes in carnivores: the responses of black bears (*Ursus americanus*) to altered food. *J. Zool.* **261**, 207–212.

Beehner, J.C. & Bergman, T.J. (2017). The next step for stress research in primates: to identify relationships between glucocorticoid secretion and fitness. *Horm. Behav.* **91**, 68–83.

Behie, A.M., Pavelka, M.S.M. & Chapman, C.A. (2010). Sources of variation in fecal cortisol levels in howler monkeys in Belize. *Am. J. Primatol.* **72**, 600–606.

Bezanson, M., Garber, P.A., Murphy, J.T. & Primo, L.S. (2008). Patterns of subgrouping and spatial affiliation in a community of mantled howling monkeys (*Alouatta palliata*). *Am. J. Primatol.* **70**, 282–293.

Bonier, F., Martin, P.R., Moore, I.T. & Wingfield, J.C. (2009). Do baseline glucocorticoids predict fitness? *Trends Ecol. Evol.* **24**, 634–642.

Boonstra, R. (2013). Reality as the leading cause of stress: rethinking the impact of chronic stress in nature. *Funct. Ecol.* **27**, 11–23.

Bötsch, Y., Tablado, Z. & Jenni, L. (2017). Experimental evidence of human recreational disturbance effects on bird-territory establishment. *Proc. Roy. Soc. Lond. Ser. B Biol. Sci.* **284**, 20170846.

Bötsch, Y., Gugelmann, S., Tablado, Z. & Jenni, L. (2018). Effect of human recreation on bird anti-predatory response. *Peerj* **6**, e5093.

Brown, J.S., Laundré, J.W. & Gurung, M. (1999). The ecology of fear: optimal foraging, game theory, and trophic interactions. *J. Mammal.* **80**, 385–399.

Burnham, K.P. & Anderson, D.R. (2013). *Model selection and multimodel inference*. New York: Springer.

Busch, D.S. & Hayward, L.S. (2009). Stress in a conservation context: a discussion of glucocorticoid actions and how levels change with conservation-relevant variables. *Biol. Conserv.* **142**, 2844–2853.

Carere, C., Caramaschi, D. & Fawcett, T.W. (2010). Covariation between personalities and individual differences in coping with stress: converging evidence and hypotheses. *Curr. Zool.* **56**, 728–740.

Ceballos, G., Ehrlich, P.R. & Dirzo, R. (2017). Biological annihilation via the ongoing sixth mass extinction signaled by vertebrate population losses and declines. *Proc. Natl. Acad. Sci. USA* **114**, E6089–E6096.

Constantine, R. (2001). Increased avoidance of swimmers by wild bottlenose dolphins (*Tursiops truncatus*) due to long-term exposure to swim-with-dolphin tourism. *Mar. Mamm. Sci.* **17**, 689–702.

Cormont, A., Vos, C.C., Verboom, J., van Turnhout, C.A.M., Foppen, R.P.B. & Goedhart, P.W. (2014). Population dynamics of Great Bittern (*Botaurus stellaris*) in the Netherlands: interaction effects of winter weather and habitat fragmentation. *Reg. Environ. Change* **14**, 943–952.

Cuarón, A.D., Shedden, A., Rodríguez-Luna, E., de Grammont, P.C. & Link, A. (2008). *Alouatta palliata ssp. mexicana*. The IUCN Red List of Threatened Species: e.T925A13095701. https://doi.org/10.2305/IUCN.UK.2008.RLTS.T925A13095701.en.

Cyr, N.E. & Romero, L.M. (2009). Identifying hormonal habituation in field studies of stress. *Gen. Comp. Endocrinol.* **161**, 295–303.

Diario Oficial. (2009). Programa de manejo de la Reserva de la Biosfera Los Tuxtlas. Available at https://simec.conanp.gob.mx/pdf_pcym/138_DOF.pdf.

Dias, P.A.D. & Rangel-Negrín, A. (2015). Diets of howler monkeys. In *Howler monkeys: behavior, ecology, and conservation*: 21–56. Kowalewski, M.M., Garber, P.A., Cortés-Ortiz, L., Urbani, B. & Youlatos, D. (Eds). New York: Springer.

Dias, P.A.D., Coyohua-Fuentes, A., Canales-Espinosa, D., Chavira-Ramírez, D.R. & Rangel-Negrín, A. (2017). Hormonal correlates of energetic condition in mantled howler monkeys. *Horm. Behav.* **94**, 13–20.

Dunbar, R.I.M., Korstjens, A.H. & Lehmann, J. (2009). Time as an ecological constraint. *Biol. Rev.* **84**, 413–429.

Fernández-Juricic, E. (2000). Local and regional effects of pedestrians on forest birds in a fragmented landscape. *Condor* **102**, 247–255.

Ferreira, D.F., Rocha, R., López-Baucells, A., Farneda, F.Z., Carreiras, J.M.B., Palmeirim, J.M. & Meyer, C.F.J. (2017). Season-modulated responses of Neotropical bats to forest fragmentation. *Ecol. Evol.* **7**, 4059–4071.

Fleischer, A.L., Bowman, R. & Woolfenden, G.E. (2003). Variation in foraging behavior, diet, and time of breeding of Florida scrub-jays in suburban and wildland habitats. *Condor* **105**, 515–527.

Fournier, L.A. (1974). Un método cuantitativo para la medición de características fenológicas en árboles. *Turrialba* **24**, 422–423.

Frid, A. & Dill, L.M. (2002). Human-caused disturbance stimuli as a form of predation risk. *Conserv. Ecol.* **6**, 11.

Ganzhorn, J.U. (2011). Habitat description and phenology. In *Field and laboratory methods in primatology*: 41–56. Setchell, J.M. & Curtis, D.J. (Eds). Cambridge: Cambridge University Press.

Gill, J.A. (2007). Approaches to measuring the effects of human disturbance on birds. *The Ibis* **149**, 9–14.

Guidelines for the Use of Animals. (2019). Guidelines for the treatment of animals in behavioural research and teaching. *Anim. Behav.* **147**, I–X.

Harrison, X.A. (2014). Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ* **2**, e616.

Hockings, K.J., McLennan, M.R., Carvalho, S., Ancrenaz, M., Bobe, R., Byrne, R.W., Dunbar, R.I.M., Matsuzawa, T., McGrew, W.C., Williamson, E.A., Wilson, M.L., Wood, B., Wrangham, R.W. & Hill, C.M. (2015). Apes in the Anthropocene: flexibility and survival. *Trends. Ecol. Evol.* **30**, 215–222.

Karimov, K., Kachel, S.M. & Hackländer, K. (2018). Responses of snow leopards, wolves and wild ungulates to livestock grazing in the Zorkul Strictly Protected Area, Tajikistan. *PLoS ONE* **13**, e0208329.

Kitchen, D.M., Teixeira da Cunha, R.G., Holzmann, I. & de Oliveira, D.A.G. (2015). Function of loud calls in howler monkeys. In *Howler monkeys: adaptive radiation, systematics, and morphology*: 369–399. Kowalewski, M.M., Garber, P.A., Cortés-Ortiz, L., Urbani, B. & Youlatos, D. (Eds). New York: Springer.

Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A. & Blokhuis, H.J. (1999). Coping styles in animals: current status in behavior and stress-physiology. *Neurosci. Biobehav. Rev.* **23**, 925–935.

Krebs, C.J. (1999). *Ecological methodology*. 2nd edn. Menlo Park: Addison-Wesley.

Kutner, M.H., Nachtsheim, C.J., Neter, J. & Li, W. (2004). *Applied linear statistical models*. 5th edn. New York: McGraw Hill.

Lehmann, J., Korstjens, A.H. & Dunbar, R.I.M. (2010). Apes in a changing world – the effects of global warming on the behaviour and distribution of African apes. *J. Biogeogr.* **37**, 2217–2231.

Lowry, H., Lill, A. & Wong, B.B.M. (2013). Behavioural responses of wildlife to urban environments. *Biol. Rev.* **88**, 537–549.

Lüdecke, D. (2019). *Collection of convenient functions for common statistical computations*. R package version 0.17.3. Available at https://cran.r-project.org/web/packages/sjstats/sjstats.pdf

McCauley, D.J., Pinsky, M.L., Palumbi, S.R., Estes, J.A., Joyce, F.H. & Warner, R.R. (2015). Marine defaunation: animal loss in the global ocean. *Science* **347**, 1255641.

McKinney, T., Westin, J.L. & Serio-Silva, J.C. (2015). Anthropogenic habitat modification, tourist interactions and crop-raiding in howler monkeys. In *Howler monkeys: behavior, ecology, and conservation*: 281–311. Kowalewski, M.M., Garber, P.A., Cortés-Ortiz, L., Urbani, B. & Youlatos, D. (Eds). New York: Springer.

Ordóñez-Gómez, J.D., Cristóbal-Azkarate, J., Arroyo-Rodríguez, V., Santillán-Doherty, A.M., Valdez, R.A. & Romano, M.A. (2016). Proximal and distal predictors of the spider monkey's stress levels in fragmented landscapes. *PLoS ONE* **11**, e0149671.

Pfister, C., Harrington, B.A. & Lavine, M. (1992). The impact of human disturbance on shorebirds at a migration staging area. *Biol. Conserv.* **60**, 115–126.

Pimm, S.L. & Raven, P. (2000). Biodiversity: extinction by numbers. *Nature* **403**, 843–845.

R Core Team. (2019). *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.

Rangel-Negrín, A., Coyohua-Fuentes, A., Canales-Espinosa, D. & Dias, P.A.D. (2014). Mammal assemblages in forest fragments and landscapes occupied by black howler monkeys. *Primates* **55**, 345–352.

Rangel-Negrín, A., Coyohua-Fuentes, A., Canales-Espinosa, D. & Dias, P.A.D. (2018). The influence of leaf consumption on time allocation in black howler monkeys (*Alouatta pigra*). *Folia Primatol.* **89**, 111–122.

Romero, L.M. & Wikelski, M. (2010). Stress physiology as a predictor of survival in Galapagos marine iguanas. *Proc. Roy. Soc. Lond. Ser. B Biol. Sci.* **277**, 3157–3162.

Sapolsky, R.M., Romero, M. & Munck, A.U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr. Res.* **21**, 55–89.

Schwarzkopf, L. & Rylands, A.B. (1989). Primate species richness in relation to habitat structure in Amazonian rainforest fragments. *Biol. Conserv.* **48**, 1–12.

Secretariat of the Convention on Biological Diversity. (2010). *Strategic plan for biodiversity 2011–2020 and the Aichi Targets*. Montreal: Convention on Biological Diversity.

Stankowich, T. & Blumstein, D.T. (2005). Fear in animals: a meta-analysis and review of risk assessment. *Proc. Roy. Soc. Lond. Ser. B Biol. Sci.* **272**, 2627–2634.

Steen, J.B., Gabrielsen, G.W. & Kanwisher, J.W. (1988). Physiological aspects of freezing behaviour in willow ptarmigan hens. *Acta Physiol. Scand.* **134**, 299–304.

Tablado, Z. & Jenni, L. (2017). Determinants of uncertainty in wildlife responses to human disturbance. *Biol. Rev.* **92**, 216–233.

Terborgh, J., Lopez, L., Nuñez, V.P., Rao, M., Shahabuddin, G., Orihuela, G., Riveros, M., Ascanio, R., Adler, G.H., Lambert, T.D. & Balbas, L. (2001). Ecological meltdown in predator-free forest fragments. *Science* **294**, 1923–1926.

Visser, F., Curé, C., Kvadsheim, P.H., Lam, F.A., Tyack, P.L. & Miller, P.J.O. (2016). Disturbance-specific social responses in long-finned pilot whales, *Globicephala melas*. *Sci. Rep.* **6**, 28641.

West, B.T., Welch, K.B. & Galecki, A.T. (2014). *Linear mixed models. A practical guide using statistical software*. Boca Raton: Chapman & Hall.

Williamson, E.A. & Feistner, A.T.C. (2011). Habituating primates: processes, techniques, variables and ethics. In *Field and laboratory methods in primatology: a practical guide*: 25–39. Setchell, J.M. & Curtis, D.J. (Eds). Cambridge: Cambridge University Press.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Supplementary methods.
Appendix S2. Supplementary results.