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Stress in Yucatan spider monkeys: effects of environmental conditions on fecal cortisol levels in wild and captive populations

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Abstract

In the Yucatán Peninsula, spider monkeys *Ateles geoffroyi yucatanensis* are generally found in two contrasting conditions: large tracts of conserved forest or small fragments surrounded by human populations. In the present study, we analyzed fecal cortisol levels of spider monkeys to investigate whether environmental conditions have an influence on stress; specifically, we compared fecal cortisol across individuals living in conserved forests, fragmented forests and captive conditions (zoos and pets). Radioimmunoanalysis of fecal samples from 121 individuals indicated significant differences in mean cortisol for *A. g. yucatanensis* based on habitat type, with the lowest levels found in the conserved forest condition. The higher cortisol levels in both fragmented forest populations and in captive individuals may be the result of metabolic and behavioral stress. The mean male fecal cortisol concentration was significantly higher than that of females, and the fecal cortisol concentration was higher in the dry season compared with the wet season in a conserved habitat. Therefore, we emphasize the importance of considering sex and seasonality when monitoring fecal cortisol concentrations of spider monkeys, and more generally of frugivores, as they face a seasonal variation in food availability. Finally, our results suggest that forest fragmentation may create long-term stressors for spider monkeys, affecting the viability of populations living under such conditions.

Introduction

Individual physiology is regulated by periodical environmental cues. Environmental perturbations such as ecological or social events disrupt homeostasis (Reeder & Kramer, 2005), promoting immediate physiological adjustments to cope with such stimuli (Wingfield, 2005). One such process is the stress response, which is an adaptive reaction to acute stressors over short periods of time. Long-term stress can be related to disease and parasite load, to dietary or metabolic stress and/or to behavioral stressors (Lasley & Kirkpatrick, 1991; Schwarzberg *et al*., 1996). The stress response is physiologically costly and can have negative effects on essential catabolic body processes when sustained over extended periods (Sapolsky, 2002). For instance, long-term high levels of cortisol can have negative effects on the health of an organism by inhibiting growth, suppressing the functioning of the immune system or inhibiting reproductive functions (Sapolsky & Pulsinelli, 1985; Sapolsky, 1990; McEwen, 2000; DeVries, 2002). Stress can affect both individual fitness and population viability.

The Yucatán spider monkey *Ateles geoffroyi yucatanensis*, endemic to the Yucatán Peninsula, México, is found in extensive areas of conserved forest, in small patches of fragmented forest (Watts & Rico-Gray, 1987; Serio-Silva, Rico-Gray & Ramos-Fernández, 2006) and in captivity, both in zoos and as illegal pets (Duarte-Quiroga & Estrada, 2003). This subspecies is currently considered 'endangered' by both the IUCN (Cuarón *et al*., 2008) and the Mexican Environmental Laws (NOM-059-ECOL-2001), due to various threats, including hunting, habitat destruction and the encroachment of populations by agricultural lands (SEMARNAT, 2002). In order to predict the long-term sustainability of wild populations of *A. g. yucatanensis*, it is important to understand the effects of such threats on physiology.

In this study, we measured fecal cortisol levels of *A. g. yucatanensis* in forests of different sizes and levels of disturbance, as well as in individuals living under captive conditions, to determine the effect of habitat disturbance and captive conditions on stress. Fecal cortisol has been validated as an appropriate measure of stress for several primates (Norcross & Newman, 1999; Muller &

Wrangham, 2004; Chapman *et al.*, 2006; Martínez-Mota *et al.*, 2007).

We hypothesized that spider monkeys living in fragmented forests and captive conditions would have significantly higher levels of cortisol compared with individuals in conserved forests, because: (1) fragmentation acts as a stressor for wild fauna (e.g. Franceschini *et al.*, 1997; Romero, 2004; Wikelski & Cooke, 2006); (2) proximity to humans and changes in social organization have important impacts on stress (e.g. Davis, Schaffner & Smith, 2005). In addition, food availability may be an important stressor in primates (Cavigelli, 1999; Chapman *et al.*, 2006), so it might be expected that spider monkeys' cortisol levels vary seasonally. As spider monkeys living under captive conditions are food provisioned, we predicted that seasonal differences should occur only under free-ranging conditions.

Methods

Sites and conditions

Collection locations categorized as 'conserved habitat' were large extensions of continuous forest (>30 000 ha) more than 20 km away from any human settlements and without internal highways or roads. These included Petcacab (or Muxucux reserve) in the Felipe Carrillo Puerto municipality (19°17′N, 88°13′W) and Tres Garantías, in the Othon P. Blanco municipality (18°12′N, 89°00′W) in Quintana Roo State (Fig. 1).

Collection locations categorized as 'fragmented habitats' were heavily touristed forests of <200 ha in size, with the presence of human settlements and domestic animals within 1 km of the forest, and frequently used roads that divided the habitat into patches. These included the Otoch Ma'ax Yetel Kooh Reserve (20°38′N, 87°40′W) in Yucatán State and Puerto Morelos Botanical Garden (20°50′N, 86°50′W) in Quintana Roo State.

Fecal samples from captive monkeys came from zoos and private parks. Collection locations included the Chetumal Zoo (18°30′N, 88°18′W) and Aktun Chen Park (21°30′N, 88°20′W), near Akumal, both in Quintana Roo State. All individuals in zoos were kept in cages of <200 m^2, with small trees available for climbing. Finally, samples from pets were collected in four private homes in different localities (Puerto Morelos: 20°50′N, 86°50′W; Petcacab: 19°17′N, 88°13′W; Ak-tun Chen: 21°30′N, 88°20′W; Felipe Carrillo Puerto: 19°35′N, 88°03′W) in Quintana Roo State. To ascertain the effect of sex on cortisol levels, we collected and quantified fecal samples from six males and six females at the Centenario Zoo in Merida, Yucatán, during the wet season.

Sample collection methods

Spider monkey's distinct genitalia makes it easy to differentiate males and females in the wild. However, it was often

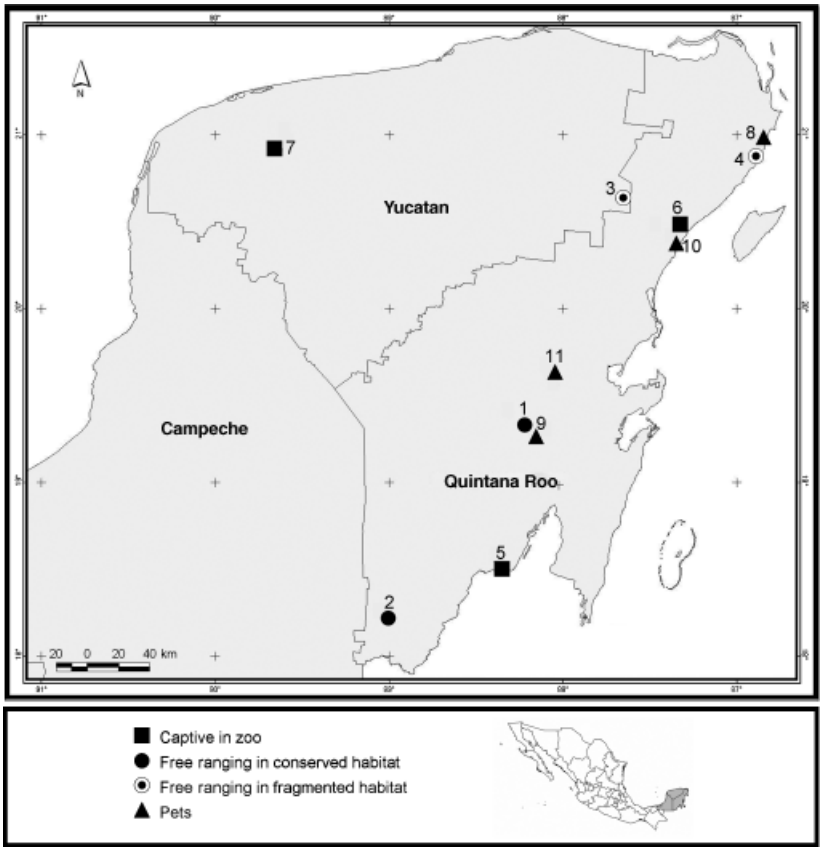

Figure 1 Locations where spider monkey *Ateles geoffroyi yucatanensis* fecal samples were collected at Yucatán Peninsula, Mexico. (1) Petcacab; (2) Tres Garantías; (3) Otoch Ma'ax Yetel Kooh reserve; (4) Puerto Morelos Botanical garden; (5) Chetumal Zoo; (6) Aktun Chen Park; (7) Centenario Zoo; (8) Puerto Morelos town; (9) Petcacab town; (10) Akumal town; (11) Felipe Carrillo Puerto town.

Table 1 Characteristics and sample sizes of each environmental condition studied

Environment	Condition	Habitat size (ha)	Wet season (*n*)	Dry season (*n*)	Total samples (*n*)
Conserved	Wild	>30 000	23	35	58
Fragments	Wild	<200	28	5	33
Zoos	Captive	<0.02	16	10	26
Private homes	Captive	<0.002	3	1	4

difficult to assign with accuracy a particular fecal sample to the individual that defecated it, because subgroup members tend to defecate in synchrony. As we collected fecal samples opportunistically from non-habituated groups, we did not recognize group members individually. For these reasons, fecal samples collected in the wild were not identified by sex or individual. This sampling regime could lead to re-sampling of some individuals, and so we sampled each subgroup only once, during a single defecation event. After each defecation event, we always moved >1 km from the sampled subgroup to reduce the probabilities of re-sampling the same individuals.

Fecal samples from spider monkeys were collected immediately after defecation. Samples were collected from adult animals only, with a clean spatula to avoid manual contact, and were placed in zip-lock plastic bags.

We registered the following data for each sample: location, date, time of collection, forest condition (conserved or fragmented), presence of human settlements and captive condition (pet or zoo). All the fecal samples collected were preserved on dry ice (without direct contact) at approximately −20 °C, or in a mix of ice and acetone (for 1 day only), to maintain a temperature of −4 °C. These samples were subsequently stored in a freezer at −4 °C.

Samples were analyzed in the Physiology Department, CINVESTAV-IPN, México City. Once the fecal samples arrived to the laboratory, each sample was mixed until homogenized, deposited into a plastic tube and refrozen at −20 °C. Samples were then dried in a speed vac (Speed Vac Rotatory Evaporator, Savant Instruments Inc., Farmingdale, NY, USA) for 15–38 h, for the subsequent extraction of steroids.

We have separated the sample collection in each environmental condition into 'wet' (May–October) and 'dry' (November–April) seasons (Vidal-Zepeda, 2005). Fecal samples from a total of 121 spider monkeys were collected from continuous forest, fragmented forest, zoos and pets (Table 1).

Fecal cortisol assays

Cortisol extraction

The protocol for fecal cortisol extraction used was described by Brown *et al.* (1994) and modified by Brousset *et al.* (2005). Dried samples were pulverized and 0.2 g was weighed and placed in sterile 16 × 125 mm tubes. One milliliter of distilled water and 4 mL of 100% ethanol were added to each tube, and the mixture was vortexed for 1 min. The tubes were placed on a water bath at 93–100 °C for 20 min. The samples were then centrifuged for 20 min at 460 *g* and the liquid extract was decanted into another tube. These tubes were dried with pressurized air in a water bath at 36 °C, for 2–5 h, until the sample was completely dry. One milliliter of ethanol was added, and tubes were vortexed for 1 min. After 30 min of incubation at room temperature, samples were centrifuged for 20 min at 460 *g* Finally, the supernatant was placed into polycarbonate tubes, and 2 mL of diluted RIA buffer was added to each tube. The samples were stored at −20 °C until assayed by a RIA. Assays were performed within 1 week of the initial extraction.

Fecal cortisol radioimmunoassays

The RIA determination of cortisol was carried out following the method described by Parrott, Misson & Baldwin (1989). Rabbit anticortisol (3/CMO Chemicon, Billerica, MA, USA) was used as the antibody. The antibody cross-reacted 3.63% for corticosterone, 0.96% for progesterone, 0.96% for pregnenolone; 0.17% for androstenedione, 0.44% for testosterone and 0.15% for 17β-estradiol. The tracer was tritiated hydrocortisone [1,2,6,7-^{3}H(N)] 70–100 Ci mmol^{-1}, 1 mCi mL^{-1} of ethanol] (NEN Life Science products Inc., Boston, MA, USA).

Validation of fecal cortisol assays

The analytic validations were run as recommended by Zambrano & Díaz (1996) and Beehner & Whitten (2004). The extraction method recovered 56% of the cortisol in the original samples. To validate the fecal extraction method, the samples were diluted with buffered solution. The linearity was: $r^2 > 0.90$. The intraassay and interassay coefficients of variation were 6.25 and 11.5%, respectively.

Additionally, to determine whether our presence would influence the cortisol levels of fecal samples collected from wild spider monkeys, an experimental protocol was applied at the Centenario Zoo, during which the effect of an acute stressor (the capture) on the fecal cortisol excretion profile was studied. Two adult individuals (one male and one female) were captured by the zoo's veterinarian, and were maintained in isolation for the duration of the experiment (32 h). All fecal samples were collected throughout this period, and analyzed for fecal cortisol concentrations. Fecal cortisol peaked for the male subject at 7–8 h after the stressful stimulus and at 20–25 h for the female (Fig. 2a and b). Therefore, as each sampled group was followed for a maximum of 2 h before fecal collection, the cortisol levels that we measured were not affected by our presence in the field.

Statistical analyses

The Kruskal–Wallis test was applied to analyze differences in cortisol levels across environmental conditions, and the Mann–Whitney test as a *post hoc* analysis to determine which pairs of conditions differed significantly. We used Mann–Whitney tests to compare the cortisol levels between seasons and between sexes. All statistical analyses were performed with Statistica 6.0 (StatSoft Inc., 2001).

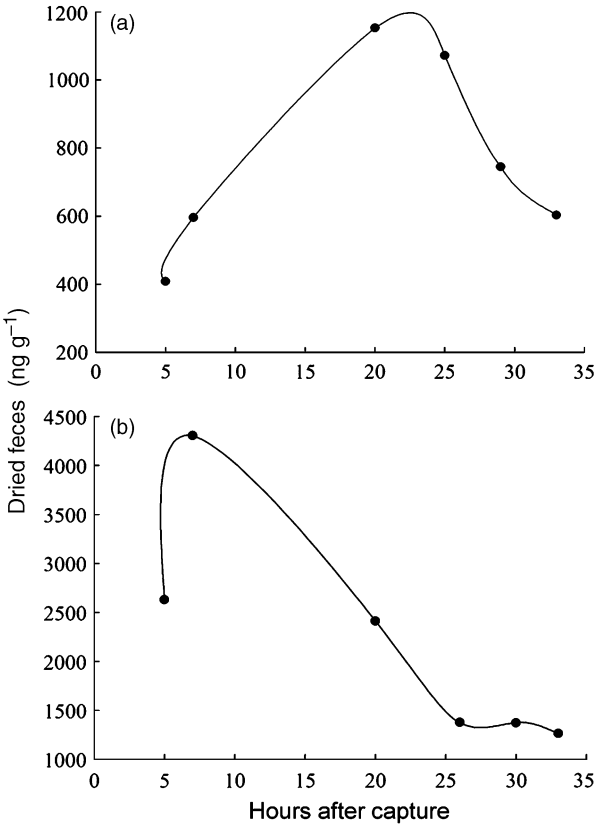

Figure 2 Fecal cortisol concentrations for captive spider monkeys *Ateles geoffroyi yucatanensis* in 'El Centenario' Zoo, Yucatán, Mexico, during 32 h after capture: (a) adult female; (b) adult male. Note the different scales on the *Y*-axis for cortisol concentration.

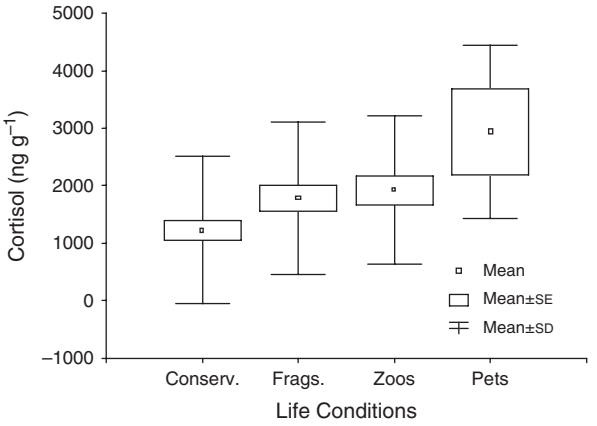

Figure 3 Mean fecal cortisol concentrations of spider monkeys *Ateles geoffroyi yucatanensis* under different environmental conditions. Data indicate mean ± SD. Conserved habitat ($n=58$); fragmented habitat ($n=33$); zoo animals ($n=26$); pets ($n=4$).

Results

Comparison of cortisol levels across environmental conditions

Environmental condition had a significant effect on the mean fecal cortisol concentration (Kruskal–Wallis test, $P<0.01$; Fig. 3). The mean cortisol concentrations were lower in the conserved habitat (mean ± SD: $1224.2 \pm 168.5\,ng\,g^{-1}$), and higher in fragments (mean ± SD: $1782.8 \pm 231.3\,ng\,g^{-1}$), zoos (mean ± SD: $1925.4 \pm 252.2\,ng\,g^{-1}$) and finally in pets, which showed the highest values (mean ± SD: $2941.1 \pm 755.8\,ng\,g^{-1}$). In the *post hoc* comparisons, the only significant difference was found between the conserved habitat and all other conditions (Mann–Whitney *U*–tests, $P<0.05$).

Sex and seasonal variation on cortisol levels

In the conserved habitat, the mean fecal cortisol was significantly lower in the wet season (mean ± SD: $940.5 \pm 285.3\,ng\,g^{-1}$) than in the dry season (mean ± SD: $1410.68 \pm 178.7\,ng\,g^{-1}$), Mann–Whitney $Z=2.98$, $P<0.01$. However, there were no seasonal differences in the mean cortisol concentrations in the fragmented habitat (Mann–Whitney $Z=0.20$, $P>0.05$), and in zoos (Mann–Whitney $Z=1.63$, $P>0.05$). We did not have sufficient samples to analyze this effect among pets.

When comparing samples from the dry season across the conserved and fragmented habitat, there was no difference in the mean fecal cortisol concentrations (Mann–Whitney $Z=0.96$, $P>0.05$). In contrast, for samples collected during the wet season, fecal samples in the fragmented habitat had a significantly higher mean fecal cortisol concentration (mean ± SD: $1814.0 \pm 270.7\,ng\,g^{-1}$) than those in the conserved habitat (mean ± SD: $940.5 \pm 323.14\,ng\,g^{-1}$, Mann–Whitney $Z=3.10$, $P<0.01$).

Controlling for season, fecal cortisol levels were significantly higher in captive males at the zoo (mean ± SD: $2229.3 \pm 479.4\,ng\,g^{-1}$; $n=6$) compared with captive females at the zoo (mean ± SD: $717.5 \pm 151.5\,ng\,g^{-1}$; $n=6$, Mann–Whitney: $Z=2.88$, $P<0.01$).

Discussion

As predicted, our results suggest that spider monkeys living in a conserved habitat have lower cortisol levels than individuals living in fragmented habitats and in captivity. Additionally, we expected to observe seasonal differences in cortisol concentrations because the animals were exposed to natural fluctuations in food availability, in both conserved and fragmented conditions. However, this trend was only confirmed for the conserved habitat, where cortisol was significantly higher during the dry season.

Several factors may explain why spider monkeys living under non-conserved conditions show higher stress. First, forest fragmentation has multiple consequences for primates, such as spatial restrictions to their activities (Bicca-Marques, 2003), reduced food availability (Arroyo-Rodríguez & Mandujano, 2006), altered demographics and population dynamics

(Bicca-Marques & Calegaro-Marques, 1998; Rodríguez-Toledo, Mandujano & García-Orduña, 2003), increased susceptibility to diseases (Chapman, Gillespie & Goldberg, 2005; Chapman *et al*., 2006) and changes in social structure (Bicca-Marques & Calegaro-Marques, 1998). These factors may unbalance the homeostasis of individuals, and result in stress. Previous comparative research on the differences in stress levels between conserved and disturbed habitat corroborates our findings. For instance, Martínez-Mota *et al*. (2007) found higher fecal cortisol levels in black howlers *Alouatta pigra* living in highly fragmented landscapes than in individuals living in a continuous forest. Second, captive animals (in zoos and pets) face several disturbances to their natural behavioral patterns and ecological conditions that may increase stress. Among these, the continued presence of humans (Davis *et al*., 2005), the artificial provisioning of food resources (Waitt & Buchanan-Smith, 2001) and changes in grouping patterns (Beehner *et al*., 2005; Honess & Marin, 2006) may be important stressors.

As in many other vertebrates (Romero, 2002), cortisol seems to vary seasonally in spider monkeys. Our results suggest that cortisol is relatively high for all spider monkeys in the dry season, but was significantly lower for those living in conserved forests during the wet season. There is a reduction in the number of fruiting trees during the dry season in the forests of the Yucatán Peninsula (Valero & Byrne, 2007), explaining why spider monkeys may face reduced food availability during this period. This scarcity may obligate individuals to increase their foraging effort (e.g. by increasing day ranges) in order to maintain net food intake levels. In other primate species, such seasonal influences in activity (e.g. *Lemur catta*: Cavigelli, 1999; *Pan troglodytes*: Muller & Wrangham, 2004) and food supply (e.g. *L catta*: Pride, 2005; *Procolobus rufomitratus*: Chapman, Saj & Snaith, 2007) have been related to increases in stress. In contrast, the mean cortisol concentrations remained high throughout the year for spider monkeys in the fragmented habitat. This may indicate that, whereas spider monkeys living in conserved habitat may experience seasonal increases in metabolic stress, in the fragmented habitat, individuals may suffer from long-term stress.

The differences in cortisol levels between conserved and non-conserved habitat could alternatively be associated with altered metabolic or dietary activity across habitat types that might alter the fecal cortisol concentration but not necessarily the circulating levels of cortisol. The differences might also be a result of other stressors, such as parasites. Increases in parasite infections may affect survival and reproduction through a number of processes (e.g. Chapman *et al*., 2005, 2007), resulting in stress. In a parallel study for gastrointestinal parasite presence and richness in *A. g. yucatanensis*, Bonilla-Moheno (2002) analyzed a subsample of the fecal samples analyzed here. In her study, neither parasite presence nor richness was significantly different between fragmented and conserved habitat. Therefore, it is unlikely that intestinal parasitosis represents a significant cause for the increased cortisol levels of spider monkeys in fragmented forests. Instead, higher cortisol levels were found not only in fragmented forest populations, where dietary stress is high, and human contact is relatively low, but also in captive individuals, with high dietary intake, low parasite infections (due to antiparasitic treatments), but increased human activity or proximity. Therefore, the higher cortisol concentrations found in these groups of spider monkeys may be the result of both metabolic and behavioral stress related to increased proximity to humans (Davis *et al*., 2005; Martínez-Mota *et al*., 2007). However, further studies that address the interaction among stress, food supply and parasites for wild populations are required, as they may have synergistic effects that vary through time (Chapman *et al*., 2007).

One important limitation to our results is that field data were pooled independently from individual variation in sex, reproductive condition or social status, which are factors with demonstrated effects on cortisol profiles (e.g. Wasser, Risler & Steiner, 1988; Barrett *et al*., 2002; Cavigelli *et al*., 2003; Muller & Wrangham, 2004; Honess & Marin, 2006). Our captive data show that male spider monkeys had significantly higher cortisol levels compared with females. Several aspects may explain this sex difference: (1) females in captivity may be less fertile, and so cortisol, as well as reproductive hormones, may be low (Bethea *et al*., 2005); (2) as male spider monkeys are usually philopatric in the wild, the modified social structures of groups living in zoos may affect them more acutely (Waterhouse & Waterhouse, 1971; Ziegler *et al*., 1997); (3) males may have higher cortisol under both wild and captive conditions (Wasser, Risler & Wasser, 1986). These questions can be addressed only through further research. However, there is no reason to assume that our sampling regime of wild spider monkeys was significantly biased towards collecting more fecal samples from one of the sexes, females with similar reproductive conditions or individuals with particular social status. We consider our results to be representative of the cortisol levels of Yucatán spider monkeys, and the present study establishes a range of values for fecal cortisol for wild and captive populations. These data will facilitate further use of non-invasive fecal sampling techniques for the rapid assessment of stress in spider monkey populations throughout their range.

Another limitation to our study was uneven sample sizes from different environmental conditions. The small number of samples collected from pets reflects the fact that it is illegal in México to have spider monkeys as pets, and people were reluctant to admit that they had them in their houses. Although there were no significant differences between pets and zoo animals, we found a tendency towards higher levels of fecal cortisol in pets, suggesting perhaps that proximity to humans and isolation may be important stressors for spider monkeys. However, our small sample size for fecal samples from pets requires these results to be interpreted with caution.

Finally, it is important to highlight that our results for wild populations converge with previous evidence that spider monkeys living in disturbed habitat may be at risk. The habitat loss and fragmentation that has occurred in the Yucatán Peninsula during the past 50 years have reduced habitat availability and quality for spider monkeys,

affecting the patterns of forest occupancy (Watts, Rico-Gray & Chan, 1986; Serio-Silva *et al*., 2006) and population characteristics (Gonzalez-Kirchner, 1999; Ramos-Fernández & Ayala-Orozco, 2003). Our study suggests that forest fragmentation may be inducing long-term stress in spider monkeys and limiting the long-term viability of those populations.

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