RESEARCH ARTICLE

Preliminary Evidence of Accumulation of Stress During Translocation in Mantled Howlers

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Translocation—an extensively used conservation tool—is a potentially stressful event, as animals are exposed to multiple stressors and cannot predict or control the changes in their environment. Therefore, it may be expected that during a translocation program stress accumulates and social behavior changes. Here, we present data from a translocation of four adult mantled howlers (Alouatta palliata), which was conducted in southern Veracruz (Mexico). We found that stress (measured in fecal corticosterone) increased during translocation, but that the rate of both affiliative and agonistic interactions remained unchanged. Females showed higher levels of corticosterone than males throughout translocation, although no sex differences were observed in social interactions. Our findings provide a preliminary evidence for accumulation of physiological stress during translocation in primates, and may have implications for decisions concerning releasing practices. Am. J. Primatol. 72:805-810, 2010. © 2010 Wiley-Liss, Inc.

Key words: Alouatta palliata; Mexico; sex differences; stress; translocation; social interactions

INTRODUCTION

Translocation involves moving wild animals from one part of their distributional range to another, and usually aims at re-establishing self-sustaining populations and maintaining their viability [Griffith et al., 1989]. Translocations are potentially stressful events, as individuals cannot predict or control the changes that are occurring in their immediate environment. Acute stress responses to translocation are defined as rapid and transient increases in glucocorticoid levels following the capture of individuals, and have been reported in several animal species [e.g. sharpnose sharks, Rhizoprionodon terraenovae; Hoffmayer & Parsons, 2001]. Even when translocations involve hard releases (i.e. individuals are not held in enclosures before release, except during transportation), temporary captivity can significantly stress animals [e.g. tuatara, Sphenodon punctatus; Tyrrell & Cree, 1998; loggerhead sea turtles, Caretta caretta: Gregory et al., 1996]. For example, confinement of only 3hr for wild tuatara and 6 hr for loggerhead turtles results in significant increases in plasma corticosterone [Gregory et al., 1996; Tyrrell & Cree, 1998]. Currently, there are no published stress hormone data on translocated freeranging nonhuman primates.

Teixeira et al. [2007] reviewed the relationship between translocation and stress in animal relocations, and concluded that stress is a probable causal

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factor for the failure of some translocations. For example, they found evidence that stress during translocations may have adverse effects on cognitive abilities, such as memory, which are critical to the survival of released animals in the wild. Building on the framework proposed by Moberg [2000], Teixeira et al. [2007] proposed that during translocation individuals face multiple stressors (i.e. factors that tend to alter homeostasis), which may have additive or cumulative effects: environmental disturbance, rescue/capture, captivity, veterinary examinations, transportation to release site, release, adaptation to new environment, and being monitored by humans. Thus, animals may not be able to recover from a perturbing situation before another emerges, which may lead to an overactivation of the hypothalamicpituitary-adrenal axis. This cumulative effect, in turn, may affect negatively the immune competence,

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reproduction, metabolism, and behavior of individuals [Moberg, 2000].

The aim of this study was to examine if translocation affects the stress levels and social interactions of mantled howlers (Alouatta palliata). Howlers have been the subject of multiple translocations. For instance, there are reports of translocations of red [A. seniculus; Richard-Hansen et al., 2000] and black [A. pigra; Ostro et al., 1999] howlers, but to date, no assessment of the impact of translocation on stress hormones and social interactions has been conducted in howler monkeys. The Mexican populations of mantled howlers (A. palliata *mexicana*) are classified as critically endangered by the IUCN [Cuarón et al., 2008]. In particular, we (1) document whether stress increases throughout a translocation program and (2) examine if during translocation howlers change their patterns of affiliation and agonism. Additionally, as males and females may respond differently to stressors, we (3)explore sex differences in stress and social interactions in relation to translocation.

METHODS

Subjects and Handling

In March 2005, we learned that a 4.9 ha forest patch in Cascajal del Río, Mexico (17°59' N, 95°10 W), would be slashed and burned. This patch was occupied by an A. palliata group composed of two adult males and two adult females. Based on the direct inspection of individuals during capture, two individuals (one male and one female) were aged 7-8 years and the other two animals were 4-5 years. During examination, we observed evidence that the animals were in bad physical condition, such as alopecia, dehydration, and the animals were thin (as demonstrated by low body weight: males 6.5 kg, females 4.7 kg; approximately 78% of what Estrada [1982] found in the nearby Los Tuxtlas region) and had protruding bones; additionally, one female had a missing eye. Following the guidelines of IUCN [1998] and of the Mexican authorities, we implemented a translocation program for this group that included four stages: Pre-translocation: before being translocated, animals were followed to collect behavioral data and fecal samples (May 10–June 19); Captivity: after capture animals were housed socially in a $12 \times 5.5 \times 2$ m cage for close veterinary monitoring (June 20-August 18). This cage was located at the Parque de la Flora y Fauna Silvestre Tropical (PAFFASIT), a 220 ha preserve of tall evergreen forest, managed by the Universidad Veracruzana in Catemaco (Veracruz state). This site was ca. 50 km from the capture site and ca. 1 km from the release site. During this period, animals were provisioned with food (commercial fruit and fresh leaves from several species, which were collected from the adjacent forest) once each day; Semi-captivity: the

group was captured again and released into a 0.18 ha outdoor enclosure of trees surrounded by an electric fence (August 18-September 17), also located at the PAFFASIT (ca. 25 m from the cage). In this enclosure, the howlers not only foraged from natural vegetation, but also were given a supplement of fruit every day (approximately 250 g/individual); Posttranslocation: the group was recaptured and released into an 80 ha protected forest (September 17-October 12). Once released into the protected forest, no further provisioning or direct contact with humans occurred. Another group of howlers lived in this forest patch, but during our observations it had no visual or vocal contact with the translocated group. The capture and handling techniques used in our study have been described elsewhere [Rodríguez-Luna et al., 1993]. The howlers spent 90 days in captivity, which is the time Mexican authorities require primates to be quarantined before being released. The housing conditions of animals adhered to the ethical and legal requirements of Mexico [Diario Oficial de la Federación, 1999].

Behavioral Sampling

We performed focal animal observations (1hr samples) with a continuous recording of all occurrences of affiliative (grooming, huddling, and friendly contact) and agonistic (supplants, threats, and fights) interactions exchanged among the howlers, during all stages of the translocation. We discarded any samples interrupted by temporary loss of visual contact with the focal animal, if the monkey remained out of view for >15 min. Individuals were easily identifiable by their natural marks, such as blond hairs on the feet and tails, skin pigmentation on the hands and feet, and scars. Behavioral data were collected during 17 days in each stage and observations were performed during daylight hours (0700-1900 hr). All animals were observed for similar proportions of time during each of the four stages: M1 $(mean \pm SD) = 22.8 \pm 2.06 hr;$ $M2 = 23.4 \pm 3.45$ hr; $F1 = 22.9 \pm 2.29$ hr; and F2 = 24.4 ± 2.95 hr. We collected 374 hr of focal observations (94.5 hr in pre-translocation, 92.5 hr in captivity, 106 hr in semi-captivity, and 81 hr in post-translocation).

Fecal Sampling

Fecal samples were collected opportunistically during all phases of the study. Fresh samples uncontaminated by urine were collected from the cage or forest floor, deposited in polyethylene bags labeled with the ID of each individual, 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 Catemaco field station of the Universidad Veracruzana. To assess variation in stress throughout translocation, we analyzed the fecal concentrations of corticosterone, a glucocorticoid that is released in response to psychological as well as physical stressors across a wide variety of vertebrates [Sapolsky, 2002]. Although cortisol is the primary glucocorticoid for primates, corticosterone antibodies have shown to be superior to other antibodies for measuring glucocorticoid metabolites in feces of a large array of species [Wasser et al., 2000]. Corticosterone extractions and radioimmunoassayes were performed, as described in Cristóbal-Azkarate et al. [2007], at the Departamento de Biología de la Reproducción, Instituto de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico DF. All hormone analyses were conducted 4–9 months after the collection of samples.

To determine the short-term effect of capture (an acute stressor) on the corticosterone excretion profile, we collected all fecal samples for the 46 hr after the first capture (i.e. from pre-translocation to captivity). As shown in Figure 1, there was a delay (ca. 20-30 hr) between the acute stressor and the corresponding increase in corticosterone, as this hormone peaked at 22 hr post-capture for females and 28 hr post-capture for males. Therefore, to control the effects of the acute stressor of capture in our analyses, we excluded all fecal samples collected within 36 hr of captures. One hundred and twenty-eight samples (61 from males and 67 from females) were analyzed: 28 in pre-translocation, 59 in captivity, 15 in semi-captivity, and 26 in posttranslocation. No differences were found between corticosterone levels of samples collected in the morning (N = 94, i.e. 07:00-13:00 hr) and in the afternoon (N = 37, i.e. 13:00-19:00 hr) from the same individual (paired *t*-tests P > 0.05 for the four tests); therefore, collection time was not considered in further analyses.

Our research adhered to all institutional guidelines of the Universidad Veracruzana, government of



Fig. 1. Mean $(\pm 95\%$ confidence intervals) corticosterone levels (ng/g) in male (n = 14) and female (n = 12) fecal samples during the 46 hr after capture. Arrows indicate peaks in hormone concentrations.

Mexico (permit SEMARNAT SGPA/DGVS/05417), and the American Society of Primatologists principles for the Ethical Treatment of Non-human Primates.

Data Analyses

Hormone concentration data was log transformed to normalize the distribution and equalize the variances (Kolmogorov-Smirnov and Levene's tests: P > 0.05). To analyze overall and per sex variation in corticosterone across translocation stages, we used one-way ANOVAs. Social interactions data exhibited a Poisson distribution, so we used analyses of deviance (ANODEV) through generalized linear models by fixing a Poisson distribution to compare across translocation stages and between sexes the rates of affiliative and agonistic interactions. As we had multiple hormonal and behavioral measures from each individual in each translocation stage, in these tests we included as random factors [Zar, 2009]: (1) individual values nested in stages when analyzing for variation throughout translocation and (2) individual values nested by sex when analyzing for differences between sexes in each translocation stage. Therefore, besides reporting the result of the relationship of primary interest, for each test we provide the statistical significance of within-subject variation. A nonsignificant result indicates that intraindividual variation in hormonal concentrations and social interactions does not exert an important effect in the relationship being tested. Interaction rates were calculated as the number of interactions exchanged by an individual on a particular day divided by the observation hours accumulated for that individual on that day. Data on interaction rates was corrected for overdispersion using the Pearson χ^2 estimate. Post hoc analyses for ANOVA were performed with the Student's t means comparisons test, and for ANO-DEV we used analysis with contrasts [Crawley, 1993]. Statistical analyses were carried out using JMP (SAS Institute Inc., Cary, NC), and the statistical significance was set at P values less than 0.05.

RESULTS

When analyzing males and females together, corticosterone levels varied significantly across the four stages of translocation ($F_{15,112} = 1.968$, P = 0.023). Within-subject variation in corticosterone levels in each stage was not significant ($F_{12} = 1.580$, P = 0.107). As expected, corticosterone increased progressively from pre-translocation (mean \pm s.e.: 98.7 \pm 0.019 ng/g) to captivity (106.9 \pm 0.013 ng/g) and to semi-captivity (140.8 \pm 0.022 ng/g; Fig. 2). However, after the release of individuals into their 80 ha protected environment, corticosterone decreased to levels below those recorded at the beginning of the study (79.6 \pm 0.021 ng/g). Post hoc

analysis revealed significant differences between pretranslocation and both captivity and semi-captivity, and between post-translocation and both captivity and semi-captivity (Student's t P < 0.05). When analyzing males and females separately, we found that only females showed significant differences in corticosterone across stages (females: $F_{7,53} = 2.732$, P = 0.01; males: $F_{7,59} = 1.231$, P = 0.301). For both sexes, within-subject variation in corticosterone levels in each stage was not significant (females: $F_4 = 3.488, P = 0.201;$ males: $F_4 = 0.464, P = 0.762).$ Post hoc analysis revealed that female corticosterone was significantly higher in captivity and semicaptivity than in post-translocation (Student's tP < 0.05). With the exception of the pre-translocation stage, during which there were no differences between sexes in corticosterone levels, females had significantly higher hormone concentrations than males in each stage (Table I).

As can be observed in Table II, the rates of social interactions were generally low during the study. Although both affiliation and agonism were more frequent in captivity and semi-captivity than in pre- and post-translocation, the rates of affiliation $(\chi^2 = 6.39, \text{ df} = 16, P = 0.983)$ and agonism $(\chi^2 = 15.139, \text{ df} = 16, P = 0.515)$ did not vary significantly across translocation stages. Within-subject variation in social interactions in each stage was not



Fig. 2. Mean ($\pm 95\%$ confidence intervals) corticosterone levels (ng/g) in fecal samples in the four stages of translocation. Pretranslocation n = 28; captivity n = 59; semi-captivity n = 15; post-translocation n = 26.

significant (affiliation: $\chi^2 = 3.413$, df = 13, P = 0.996; agonism: $\chi^2 = 2.781$, df = 13, P = 0.999). There were no significant differences between sexes in the frequency or types of social interactions in the different stages of translocation (P > 0.05 for the eight tests).

DISCUSSION

Although increases in stress levels based on measures of glucocorticoid concentrations have been reported earlier for mammal translocations, this is the first systematic evidence for cumulative effects of stress during translocation in primates. Corticosterone levels increased in captivity and semi-captivity, but decreased after the release of individuals in their new habitat. This suggests that mantled howlers may sustain elevated glucocorticoid concentrations over periods of up to 90 days and then return to baseline levels 1–4 weeks after release. Additionally, these data suggest that based on corticosterone levels, the process of acclimation to a new environment represented a less stressful situation to individuals than living in a disturbed habitat (i.e. 4.9 ha pre-translocation forest) or in captivity and semi-captivity. Corticosterone has been shown to be an accurate physiological measure of the stress response of individuals [Wasser et al., 2000]. Therefore, stress during translocations may not be linked to novelty per se, but rather to being captured, handled, confined, and in close proximity to people. These results are consistent with observations on translocated rhinoceros (Ceratotherium simum and Diceros bicornis) and Grevy's zebras (Equus grevyi), where a pronounced decrease in fecal glucocorticoids occurred 4-6 and 11-18 weeks after being released from captivity, respectively [Franceschini et al., 2008; Turner et al., 2002].

Changes in the patterns of social interactions in response to spatial confinement have been reported in several primate species [de Waal et al., 2000], but in this study no such variation was observed. This result could be explained by our small sample size (i.e. only four individuals in the group and an average of 93.5 hr of observation per stage). However, mantled howlers exhibit very low rates of social interactions [Cristóbal-Azkarate et al., 2004], and although socio-ecological conditions vary

TABLE I. Overall and Stage Comparisons of Corticosterone Levels (mean ± SE) Between Sexes (ng/g)

Stage	Male samples		Female samples		F^{a}	Р
Pre-translocation	97.2 ± 0.028	n = 13	$99.9 \! + \! 0.026$	n = 15	0.024	0.994
Captivity	75.4 ± 0.022	n = 27	133.6 ± 0.015	n = 32	2.635	0.051
Semi-captivity	97.7 ± 0.034	n = 9	205.5 ± 0.028	n = 6	5.108	0.019
Post-translocation	67.2 ± 0.035	n = 12	90.3 ± 0.028	n = 14	3.379	0.036
All	81.7 ± 0.014	n = 61	123.4 ± 0.011	n = 67	2.944	0.036

^aWithin subject variation was not significant in all tests (P > 0.05).

	Pre-translocation	Captivity	Semi-captivity	Post- translocation
Total affiliation	0.034	0.094	0.171	0.131
Total agonism	0.025	0.341	0.150	0.055
Female affiliation	0.057	0.156	0.123	0.126
Male affiliation	0.012	0.031	0.218	0.136
Female agonism	0.049	0.403	0.115	0.043
Male agonism	0.000	0.279	0.185	0.067

TABLE II. Mean Daily Rates of Interactions (Interactions/hr) in Each Translocation Stage: Total and per Sex Rates According to the Type of Interaction (n = 17 Days per Stage)

significantly across populations that have been studied, the patterning of social behavior of this species is very constant [e.g. Dias et al., 2008]. This is a probable consequence of the energy-saving strategy used by howlers to cope with their folivorous-frugivorous diet and lack of a specialized digestive system [Milton, 1980]. Therefore, either the ability of howlers to adjust their social behavior to the varying environmental conditions of translocation is limited or we were unable to detect subtle variations in their behavioral patterns.

Earlier studies described that when facing conspecific threats (e.g. infanticide), female mantled howlers increase glucocorticoid production, whereas males increase androgen production [Cristóbal-Azkarate et al., 2007]. Similarly, in our study, females had consistently higher corticosterone levels than males after the first capture. Although we note our limited sample size, according to our results female mantled howlers seemed to be more sensitive to both acute (i.e. capture) and sustained (e.g. proximity to humans) stressors, and showed accumulation of stress during translocation. In contrast, males seem to have recovered quickly from the stress of capture and their corticosterone levels remained quite stable throughout translocation. There is only limited evidence of a sex-dependent stress response in primates [e.g. Saimiri sciureus; Coe et al., 1978] and our results pose the question of whether sexes should be handled differently during translocations.

This translocation involved the soft release of animals, which is defined as the reintroduction of individuals after a period of captivity in enclosures at or near the release site [IUCN, 1998]. Although soft releases may have the advantage of allowing animals to acclimatize to certain conditions of their new environment (e.g. climate), they prolong the exposure of individuals to several stressors, such as crowding [e.g. Nephew et al., 2005] or human presence [e.g. Davis et al., 2005]. Stress responses alter biological functions [e.g. cellular responsivity; Gasser et al., 2009] and can have negative physiological effects, such as reduced immune competence or reproductive suppression when sustained over extended periods [Moberg, 2000; Sapolsky, 2002]. In the future, we will need to assess if the high levels of corticosterone in our sample of two females truly represent a sex-based difference in response to translocation for mantled howlers, and if so, whether this has a potentially negative effect on female fertility. This assessment should allow researchers to make better cost-benefit analyses of whether translocation is "worth it," and of the advantages/ disadvantages of soft vs. hard releases to assure the success and sustainability of translocation programs for mantled howlers. Our observations suggest that this translocation did not compromise the survival and reproduction of individuals, as all animals are still alive (more than 4 years after translocation) and three individuals have been born to earlier translocated females. Therefore, the transitory increases in corticosterone of females may not have an important short- or medium-term impact on this species, suggesting that there may be more benefits (i.e. acclimation of individuals to a new environment and prevention of disease transmission between populations) than costs associated with the hard release of translocated mantled howlers.

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