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RESEARCH ARTICLE

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The relative effects of reproductive condition, stress, and seasonality on patterns of parasitism in wild female black howler monkeys (*Alouatta pigra*)

Rodolfo Martínez-Mota[1,2] | **Paul A. Garber**[2] | **Rupert Palme**[3] | **Thomas R. Gillespie**[4,5]

[1] Department of Biology, University of Utah, Salt Lake City, Utah

[2] Department of Anthropology, University of Illinois at Urbana-Champaign, Urbana, Illinois

[3] Department of Biomedical Sciences, University of Veterinary Medicine, Vienna, Austria

[4] Program in Population Biology, Ecology, and Evolution and Department of Environmental Sciences, Emory University, Atlanta, Georgia

[5] Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, Georgia

Correspondence
Rodolfo Martínez-Mota, Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, Utah 84112.
Email: rmarti39@illinois.edu

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Parasitic infections in wildlife are shaped by host-related traits including individual reproductive condition. It has been argued that female primates are more susceptible to infectious diseases during pregnancy due to short-term changes in immune function that result in reduced ability to combat infections. Likewise, lactation, which is the most energetically expensive state, may affect immunity and infection risk due to tradeoffs between milk production and maintenance of immune function. Here, we examine the degree to which parasite prevalence and parasite richness are affected by female reproductive condition and stress levels in wild female black howler monkeys (*Alouatta pigra*). Over the course of one year, we collected fresh fecal samples from 15 adult females belonging to seven black howler groups living in Escárcega, Mexico. Fecal samples were used for parasitological analysis and for measuring fecal glucocorticoid metabolites (i.e., stress biomarker). We found that the prevalence of intestinal parasites and parasite richness did not differ among non-pregnant, pregnant, and lactating females. Fecal glucocorticoid metabolite levels increased significantly during pregnancy and during the first month of lactation, and positively predicted the probability of *Controrchis biliophilus* infection. Parasite prevalence and richness decreased during the months of increased rainfall. We conclude that reproductive physiology has limited consequences on intestinal parasitic infection risk in female black howler monkeys and that seasonal environmental fluctuations have greater effects.

KEYWORDS
gastrointestinal parasites, glucocorticoids, immune system, Mexico, pregnancy

1 | INTRODUCTION

Patterns of parasite infection in human and animal populations are influenced by factors associated with host sex, age, and reproductive condition (Wilson et al., 2002). For example, in humans, chimpanzees, and domesticated animals (e.g., dogs), adult females are more susceptible to intestinal macroparasites (e.g., helminths) and/or intracellular microparasites (e.g., protozoans) during pregnancy and lactation than during non reproductive periods (Beasley, Kahn, & Windon, 2010; Jamieson, Theiler, & Rasmussen, 2006; Lloyd, Amerasinghe, & Soulsby, 1983; Vleugels, Brabin, Eling, & Graaf, 1989). Several authors have indicated that the physiological and endocrine changes that females experience during late pregnancy or early lactation, including an increase in the production of estradiol, progesterone, and glucocorticoids, may temporarily suppress female immune function (Henriquez, Menzies, & Roberts, 2010; Robinson & Klein, 2012). In this regard, increased levels of progesterone and estradiol in women during the last trimester of pregnancy are reported to inhibit the production of Th1 lymphocytes and macrophages resulting in greater susceptibility to intracellular pathogens (e.g., *Plasmodium* spp., *Toxoplasma* spp.) (Jamieson et al., 2006; Roberts, Satoskar, & Alexander, 1996). Moreover, changes in immune function and increased susceptibility to infection in pregnant women usually occur from the latter half of pregnancy to the time of parturition (Robinson & Klein, 2012). Similarly, studies of nonhuman primates have shown that pregnant Sanje mangabeys (*Cercocebus sanjei* [McCabe,

2012]), mandrills (*Mandrillus sphinx* [Setchell et al., 2007]), and chimpanzees (*Pan troglodytes verus* [De Nys et al., 2014]) have higher parasitic infection rates than non-pregnant females. For example, De Nys et al. (2014) found that the probability of *Plasmodium* spp. (malaria causative agent) infection in wild female chimpanzees increased with gestational age, with females more susceptible to malaria in the final trimester of pregnancy. The inhibition of specific immune responses during pregnancy (e.g., IL-2, IL-12, Th1, NK immune cells) serves to reduce the likelihood that the fetus, which is recognized by the mother's body as a genetically foreign organism, is aborted (Roberts et al., 1996; Robinson & Klein, 2012). Despite the fact that this physiological response is adaptive in that it helps maintain pregnancy and avoids miscarrying, it may increase parasite infection risk (Robinson & Klein, 2012).

During lactation, females experience the highest energetic demand due to costs related to an extended period of milk production (Emery Thompson, 2013; Gittleman & Thompson, 1988). Veterinary research indicates that in the case of Merino sheep and dogs, females experience a decrease in immune cell responses and an increase in susceptibility to gastrointestinal parasites during the early stage of lactation (i.e., 1–4 weeks postpartum) (Beasley et al., 2010; Lloyd et al., 1983). For example, helminth intensity increased by ~3-fold and >100-fold, and mean eosinophil concentrations (e.g., a type of white blood cells that fight parasitic infections) declined by ~50% and ~70% in pregnant and early lactating Merino sheep compared to non-pregnant females (Beasley et al., 2010). A reduction in the immune response to gastrointestinal parasites during lactation is considered to be the result of nutritional trade-offs, with energy and macronutrients allocated to the costs of milk production at the expense of immune system function (Barger, 1993; Houdijk, Kyriazakis, Jackson, Huntley, & Coop, 2003; Lochmiller & Deerenberg, 2000). Although in human and non-human primates lactation results in lower energetic costs compared to other mammals (e.g., rodents) (Gittleman & Thompson, 1988; Hinde & Milligan, 2011), it is unclear whether lower energetic costs associated with primate lactation facilitate a more robust immune response to parasitic infections.

Another physiological factor mediating the immune response is represented by circulating glucocorticoids. Glucocorticoid hormones are a class of steroids that are released from the adrenal cortex through activation of the hypothalamic-pituitary-adrenal (HPA) axis (Sapolsky, 2002). During a stress response, increased production of glucocorticoids helps the body mobilize and increase energy from body reserves (i.e., stimulates glucose formation from muscle or tissue) (Sapolsky, 2002). In the case of short-term or acute stress responses lasting from minutes to hours, increased glucocorticoid levels are beneficial in increasing energy available for metabolism, and brain and body function (McEwen, 2002). However, chronic exposure to social, environmental, and physiological stressful situations can inhibit the production of immune cells that function in defense against viral, bacterial, and parasitic infections (Segerstrom & Miller 2004; Webster Marketon & Glaser, 2008). Because of this, it is suggested that chronic glucocorticoid production can result in increased susceptibility to parasites (Chapman, Saj, & Snaith, 2007; Pedersen & Greives, 2008). For example, positive correlations between glucocorticoid levels and blood and gastrointestinal parasite intensity and richness have been found in humans and chimpanzees (Bouyou-Akotet et al., 2005; Muehlenbein, 2006; Vleugels et al., 1989), suggesting a link between long-term glucocorticoid output and parasite infection risk. Moreover, several studies document a significant increase in glucocorticoid concentrations during the second half of pregnancy to support fetal growth and development (Singh, Cuffe, & Moritz, 2012) and during lactation as a physiological response to energetic (Foerster, Cords, & Monfort, 2012) and psychosocial stress (Maestripieri, Hoffman, Fulks, & Gerald, 2008). However, in most primate species it remains unclear whether elevated levels of glucocorticoids exhibited by females throughout pregnancy and lactation negatively affect their immune responses resulting in increased susceptibility to parasitic infection.

In the present study, we examine the degree to which parasite prevalence and parasite richness are affected by reproductive condition and stress levels (measured by fecal glucocorticoid metabolites [fGCM]) in wild female black howler monkeys (*Alouatta pigra*). *A. pigra* is currently listed as "Endangered" in the International Union for Conservation of Nature Red List of Threatened Species (IUCN, 2017). This is due to the fact that, across its restricted distribution, which encompasses Southern Mexico, Guatemala, and Belize, *A. pigra* populations have been severely impacted by habitat loss and fragmentation (Estrada, 2015). Presently, black howler monkeys exploit a range of habitats, from large tracts of continuous forests characterized by low levels of deforestation and minimal human land-use change to small forest fragments (<10 ha) characterized by low diversity of primary forest trees, decreased abundance of large trees, and surrounded by agricultural fields and pastureland (Van Belle & Estrada, 2006). Given the current threats to the survival of *A. pigra* populations, and the potential risk that non-human primates face in terms of increased pathogen exposure in altered habitats (Nunn & Altizer, 2006), there is a need to understand the degree to which female howler reproductive physiology (i.e., host-related traits) modifies parasite infection patterns, overall health, and population viability.

Assuming that immunity to parasite infection is compromised during the most energetically demanding periods of reproduction (Beasley et al., 2010; Lloyd, 1995), we predict that during late pregnancy and the 1st month of lactation, black howler females will exhibit a higher prevalence and richness of gastrointestinal parasites compared to non-pregnant females, females in their first half of pregnancy, and females during the later stages of lactation. Since increased glucocorticoid concentrations may inhibit the action/production of immune cells (Sapolsky, Romero, & Munck, 2000), and given that glucocorticoid levels tend to increase as pregnancy progresses and during lactation, we predict a positive relationship between levels of fGCM and measures of parasitic infection (i.e., prevalence and richness).

2 | METHODS

2.1 | Study site

This study was conducted in fragments of tropical deciduous forest at Escárcega, State of Campeche, Mexico (18°16′N, 90°43′W). Forest fragments differed in size ranging from a large forested area (2,140 ha) to small forest fragments (range = 2–9 ha). The distance between the two nearest forest fragments was <1 km and between the most distant fragments was 13 km. This region is characterized by a rainy season from June to September (~900 mm of rain accumulated during 4 months) and a dry season from October to May (~390 mm of rain accumulated during 8 months). Mean annual temperature is 26.0°C (CONAGUA, 2017).

2.2 | Study subjects

Fifteen adult female black howlers belonging to seven social groups were monitored from February 2011 to February 2012. Three groups (JK, MN, and BS) inhabited the large forest fragment, and the other four groups (FE, PD, CN, and CH) each inhabited a different small forest fragment. Average group size was 7.6 ± SD 3.0 individuals. Each group had two adult females with the exception of group CH, which had three adult females. Females were classified as adults based on size (i.e., fully grown individuals >40-month old) and were individually recognized by body features such as scars, broken fingers, and by color-coded anklets in the case of three individuals. Individuals with anklets were darted and marked in August 2010, 6 months prior to the start of this investigation.

2.3 | Reproductive state classification

Adult female howler monkeys were classified into five reproductive conditions: early pregnant, late pregnant, early lactating, late lactating, and "other." Early pregnancy was defined as the first 3 months of pregnancy. The period from the 4th month of pregnancy to the time of parturition was classified as late pregnancy. Gestation length for *Alouatta* spp. is estimated to be 180–190 days (Di Fiore, Link, & Campbell, 2011), thus, the start of pregnancy was calculated by subtracting the previous 6 months (~180 days) from parturition. Females were classified as early lactating when they were in the 1st month of lactation. This 4-week lactation period was chosen because it is the stage during which temporal post-partum immune inhibition most likely occurs (Lloyd, 1995). Later lactation was defined as the period from the 2nd month of lactation until the infant stopped suckling (i.e., 8–12 months [Dias, Rangel-Negrín, & Canales-Espinosa, 2011; Domingo Balcells & Veà Baró, 2009]). In *A. pigra*, the interbirth interval is estimated to be 15.5 months (Dias et al., 2011), and the ovarian cycle length is 18.3 days (Van Belle, Estrada, Ziegler, & Strier, 2009). Females were classified as "other" when individuals failed to exhibit any apparent signs of pregnancy (e.g., increased body mass), were not lactating, and were confirmed to remain in either of these conditions for a period of at least 6 months based on our field observations.

2.4 | Sampling

Each group of howler monkeys was followed from approximately 0600–1800 hr at least 2 days per month during the 13-month study period. A total of 258 fresh fecal samples were collected non-invasively from recognized adult females. We collected ~2 samples per individual per month (1.8 ± SD 0.4 samples, range = 1–4). Given that our study design was meant to survey parasite infections in females at a population level (i.e., monitoring a large number of individuals instead of sampling few subjects), we restricted the number of samples per individual/month. Although this approach might limit the potential for detecting variability in parasite egg production within each female, we think that surveying several individuals within the same month compensates for methodological restrictions, providing a good estimate of the parasite infections hosted by females in this population. Immediately after each fecal deposition, fecal samples were collected and divided in two aliquots. One aliquot consisted of approximately 5 g of thoroughly mixed feces that were placed in a 20 ml tube and temporarily stored in a cooler with frozen gel packs. These samples were transported to the field laboratory at Escárcega, Mexico and stored for 3–5 days in a domestic freezer until glucocorticoid metabolites were extracted from the feces. The other aliquot consisted of ~3 g of fresh feces and was used for parasitological analysis. These samples were fixed in 10% buffered-formalin solution and kept in a domestic refrigerator until shipped to the Department of Environmental Sciences at Emory University for analysis. Each tube containing samples was labeled with the individual's ID, hour and date of collection.

2.5 | Parasitological analysis

Helminth eggs and protozoan cysts were recovered using flotation (e.g., $NaNO_3$ solution) and fecal sedimentation techniques described in detail by Gillespie (2006). One slide per fecal sample was systematically scanned using a compound microscope. Parasite eggs were counted under the 10× objective lens and measured under the 40× objective lens to the nearest 0.1 μm with an ocular micrometer. One drop of Lugol's iodine solution was added to facilitate identification. Photographs of representatives were taken and used in the identification of parasite taxa. Counts of eggs and cysts were used to determine parasite prevalence and richness as measures of parasitic infection. Parasite prevalence was defined as the proportion of fecal samples infected with at least one parasite taxon. Parasite richness was defined as the number of parasite taxa found in each fecal sample.

2.6 | Extraction and analysis of fGCM

In the field laboratory at Escárcega, Mexico, glucocorticoid metabolites were extracted from the feces following the technique reported in Palme, Touma, Arias, Dominchin, and Lepschy (2013). For each fecal sample (0.5 g wet weight), 5 ml of 80% methanol were added. This suspension was vortex-mixed for 10 min, and then centrifuged at 1600 *g* for 20 min. After this, 1 ml of the supernatant (i.e., hormone extract) was diluted in 9 ml of distilled water. This 10 ml diluted extract was passed slowly through a solid-phase extraction (SPE) cartridge

(MaxiClean Prevail C18 SPE Cartridges Alltech®). SPE cartridges were air-dried after being loaded with the hormone extracts and stored in a refrigerator until they were transported to the Department of Biomedical Sciences at the University of Veterinary Medicine, Vienna, Austria for analyses.

At the University of Veterinary Medicine, the hormone extracts were eluted from the SPE cartridges using methanol. Concentrations of fGCM were measured with a group specific 11-oxoetiocholanolone enzyme-immunoassay that quantifies glucocorticoid metabolites with a 5β-3α-ol-11-one structure (Möstl, Maggs, Schrotter, Besenfelder, & Palme, 2002). This enzyme-immunoassay has been successfully validated to monitor adrenocortical activity in black howler monkeys (Martínez-Mota, Valdespino, Rivera Rebolledo, & Palme, 2008). Sensitivity was 5 ng/g. Intra- and inter-assay coefficient of variation for a high and low concentration pool sample was 2.6% and 2.9% (intra), and 9.7% and 12.5% (inter), respectively. Concentrations are reported in ng/g wet weight.

2.7 | Data analysis

The effects of female reproductive state and fGCM on parasite prevalence and parasite species richness were analyzed with generalized linear mixed-effects models (GLMMs) (Bolker et al., 2009). In each model, female reproductive state (i.e., early pregnant, late pregnant, early lactation, late lactation, and "other") and fGCM were considered predictor variables (fixed effects). Seasonality was included as a covariate due to the fact that parasitic infection patterns may show year-round fluctuations (Altizer et al., 2006). The variable seasonality was represented by the sine and cosine of the sampling date within the year, which was previously transformed into a circular variable (day × 2 × π/365).

To analyze the effects of reproduction on parasite prevalence, we created a 0–1 response variable in which 0 represented non-infected (i.e., absence) and 1 represented infected samples (i.e., presence). We ran GLMMs separately for each parasite taxon. We selected for analysis only those parasite taxa that were present in at least 50 samples. Additionally, we ran a GLMM that included all parasites combined in a single database (from here on "overall prevalence"). These models were fitted with a binomial error distribution (Crawley, 2007). To analyze if reproduction, fGCM, and seasonality affected the probability of having multiple infections, we ran a GLMM using the number of parasite taxa found in each female's fecal sample (i.e., parasite richness) as the response variable. This model was fitted with a Poisson error distribution. The effects of reproductive state on fGCM were analyzed in a linear mixed-effects model (LMM) using the identity link function (Crawley, 2007). Data on fGCM were log-transformed to achieve a normal distribution. Data from 258 fecal samples were used in the statistical analyses.

Given that fecal samples were collected repeatedly overtime from the same females belonging to stable social groups, in all analyses the individual's ID and the group were set as random factors (Crawley, 2007). The fit of each full model was compared with a null model that included only the intercept and random terms using a likelihood ratio test. All parasite analyses (GLMMs) were run using the package lme4. We used the package nlme for running the LMM that tested the effects of reproductive state on fGCM, and the package multcomp to run a multiple comparison test for detecting significant differences among levels of the reproductive condition variable. All analyses were run in the R statistical software (version 3.2.4). Fecal glucocorticoid metabolite values are reported as mean ± SE.

2.8 | Legal permits

This study complied with the legal requirements of Mexico (SEMARNAT- DGVS/09084/10), was approved by the Institutional Animal Care and Use Committee (IACUC) of University of Illinois at Urbana-Champaign (protocol #10054), and complied with the American Society of Primatologists principles for the ethical treatment of primates.

3 | RESULTS

3.1 | Effects of reproductive state on parasite prevalence and richness

In this study, 8 out of 15 females were observed to experience each of the five reproductive states (i.e., early pregnant, late pregnant, early lactation, late lactation, and other). Fifteen births were recorded throughout the study period. The distribution of births encompassed the period from August to February, with the majority of the births occurring during the drier months of the year (Figure 1). Five parasite morphotypes were recovered from the adult female howler fecal samples. These included two nematodes (*Trypanoxyuris* sp. and *Parabronema* sp.), two trematodes (*Controrchis biliophilus* and an unidentified trematode referred to as Trematode I), and a protozoan parasite (*Entamoeba coli*). The proportion of samples infected with these parasites was as follows: *Trypanoxyuris* sp. = 7.7%, *Parabronema* sp. = 0.7%, *C. biliophilus* = 49.2%, Trematode I = 34.4%, and *E. coli* = 9.7%. Overall, 44.5% of samples were infected with one parasite taxon, 22.8% were infected with two taxa, 3.8% contained three taxa, and 28.6% of the samples showed no signs of parasite infection.

Each full model and the corresponding likelihood ratio test results are shown in Table 1. Based on the GLMMs, we found that female reproductive state did not have a significant effect on the prevalence of gastrointestinal parasites (Table 2). The prevalence of *C. biliophilus*, Trematode I, and the overall prevalence (i.e., all parasite taxa analyzed in a single database) were similar among females classified as "other" (i.e., non-pregnant and non-lactating), pregnant, and lactating (Table 3). *Trypanoxyuris* sp., *Parabronema* sp., and *E. coli* were excluded from individual statistical analyses due to the fact that few fecal samples were infected with these parasites (<25 samples). We also found that female reproductive state did not have significant effects on parasite richness (Table 2). In general, female howler monkeys showed low parasite richness across all phases of reproduction, averaging 1.02 ± SD 0.8 parasite taxa per fecal sample.

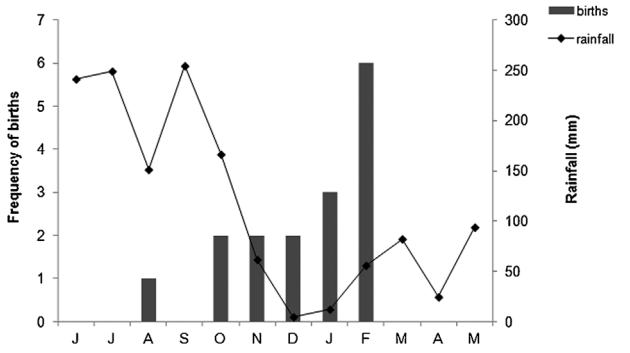

FIGURE 1 Monthly rainfall and birth distribution of black howler monkeys (*Alouatta pigra*) at Escárcega, Mexico. A birth period was recorded from August to February

3.2 | Effects of fecal glucocorticoid metabolites on parasite prevalence and richness

We found that fGCM levels did not have a significant effect on the prevalence of Trematode I and on overall parasite prevalence (Table 2). Similarly, fGCM levels did not have a significant effect on parasite richness (Table 2), with comparable fGCM levels in females showing no parasite infections and females harboring one taxon, two taxa, or three taxa. However, fGCM levels positively predicted *C. biliophilus* infection prevalence (estimate = 0.41, SE = 0.20, *t*-value = 2.10, $p = 0.03$, Table 2).

3.3 | Effects of seasonality on parasite prevalence and richness

Seasonality had significant effects on the prevalence of Trematode I (estimate = 0.95, SE = 0.27, *z*-value = 3.60, $p = 0.0003$) and overall parasite prevalence (estimate = 0.80, SE = 0.28, *z*-value = 2.83, $p = 0.004$) both of which decreased during the months corresponding to the onset of the rainy season (Figure 2). Although not statistically significant, the prevalence of *C. biliophilus* also was reduced during the months of increased rainfall (Figure 2). The likelihood ratio test indicated that none of the predictors in the full model, including seasonality, affected parasite richness (Table 1). However, considering different terms of the model separately, we found that seasonality had an effect on parasite richness, this being lower during July and August which are months that corresponded to the middle of the rainy season (estimate = 0.26, SE = 0.11, *z*-value = 2.25, $p = 0.024$; Figure 3).

TABLE 1 Full models and results of the likelihood ratio test for each model

Full model	Likelihood ratio test
Controrchis biliophilus prevalence ~ reproductive state + **fGCM** + seasonality	$\chi^2 = 15.5$, df = 7, $p = 0.029$
Trematode I prevalence ~ reproductive state + fGCM + **seasonality**	$\chi^2 = 20.8$, df = 7, $p = 0.004$
Overall prevalence ~ reproductive state + fGCM + **seasonality**[a]	$\chi^2 = 15.7$, df = 7, $p = 0.027$
Parasite richness ~ reproductive state + fGCM + seasonality	$\chi^2 = 10.1$, df = 7, $p = 0.18$
fGCM ~ **reproductive state**	$\chi^2 = 46.4$, df = 4, $p < 0.0001$

fGCM, fecal glucocorticoid metabolites. Variables in bold were significant predictors.

[a]Refers to the prevalence of all parasite taxa found in the study and analyzed in a single data set.

3.4 | Effects of reproductive state on fecal glucocorticoid metabolite concentrations

We found that reproductive condition had a significant effect on fGCM (likelihood ratio test: $\chi^2 = 46.4$, df = 4, $p < 0.0001$; Table 4). Levels of fGCM increased over the course of pregnancy, reaching a peak during late pregnancy, and early lactation (Figure 4). During the first 3 months of pregnancy, fGCM levels increased by a factor of 1.7 compared to females classified as "other." However, fGCM levels were threefold higher in females during late pregnancy and the 1st month of lactation compared to the period of non-pregnancy and during later lactation (multiple comparisons: late pregnant vs. "other": estimate = 1.4, SE = 0.22, *t*-value = 6.1, $p < 0.0001$; early lactating vs. "other": estimate = 1.2, SE = 0.30, *t*-value = 3.9, $p < 0.0001$; late pregnant vs. late lactating: estimate = 1.3, SE = 0.25, *t*-value = 5.3, $p < 0.0001$; late lactating vs. early lactating: estimate = −1.2, SE = 0.31, *t*-value = −3.5, $p < 0.01$, Figure 4). Black howler females did not show significant differences in fGCM levels during early pregnancy compared to females during later lactation (Figure 4). Similarly, there were no significant differences in fGCM levels between the period of non-pregnancy and the period of later lactation (Figure 4).

4 | DISCUSSION

In this study, we examined whether the reproductive condition of wild female black howler monkeys affected patterns of gastrointestinal parasitic infection. Based on studies of other mammalian taxa, we predicted that measures of parasitic infection would increase across late pregnancy and early lactation, due to the fact that during these reproductive periods the female immune system may be temporarily inhibited (Jamieson et al., 2006; Lloyd et al., 1983). However, we found no support for this prediction, since the prevalence of two parasite taxa (*C. biliophilus* and an unidentified trematode (Trematode I)), the overall parasite prevalence (i.e., prevalence of all parasite taxa analyzed in a single database), and parasite richness did not differ among non-pregnant, pregnant, and lactating black howler females. Few studies in nonhuman primates have explored variation in parasite infection risk in relation to female reproductive physiology. Research conducted on nine primate taxa represented by three species of New World monkeys and six species of Old World primates (Table 5), indicates that parasite infection risk in females does not follow a consistent pattern across pregnancy and lactation. Our results together with previous findings summarized in Table 5 highlight the complex

TABLE 2 Results of the GLMMs examining the effects of reproductive states, fGCM, and seasonality on parasite prevalence and parasite richness in female black howler monkeys (*Alouatta pigra*) at Escárcega, Mexico

Reproductive state	Estimate	Standard error	*z*-value	*p*
Controrchis biliophilus				
Intercept	0.25	0.32	0.77	0.44
Early pregnant	−0.49	0.49	−1.00	0.32
Late pregnant	−0.41	0.54	−0.76	0.45
Early lactating	0.47	0.71	0.65	0.51
Late lactating	−0.54	0.48	−1.13	0.26
fGCM	**0.41**	**0.20**	**2.10**	**0.037**
Sine (seasonality)	0.17	0.22	0.78	0.43
Cosine (seasonality)	0.33	0.25	1.34	0.18
Trematode I				
Intercept	−0.75	0.27	−2.80	0.005
Early pregnant	0.19	0.48	0.39	0.70
Late pregnant	0.43	0.52	0.83	0.40
Early lactating	−0.50	0.66	−0.75	0.45
Late lactating	−0.01	0.46	−0.03	0.98
fGCM	−0.00	0.17	−0.03	0.97
Sine (seasonality)	**0.42**	**0.22**	**1.92**	**0.054**
Cosine (seasonality)	**0.95**	**0.27**	**3.6**	**0.0003**
Overall prevalence[a]				
Intercept	1.50	0.31	4.80	<0.0001
Early pregnant	−0.48	0.47	−1.02	0.31
Late pregnant	−0.30	0.60	−0.50	0.61
Early lactating	−0.03	0.88	−0.03	0.97
Late lactating	−0.75	0.52	−1.43	0.15
fGCM	0.17	0.22	0.75	0.45
Sine (seasonality)	0.28	0.25	1.11	0.27
Cosine (seasonality)	**0.80**	**0.28**	**2.83**	**0.004**
Parasite richness				
Intercept	0.02	0.10	0.25	0.80
Early pregnant	−0.16	0.22	−0.73	0.46
Late pregnant	−0.03	0.22	−0.12	0.90
Early lactating	0.00	0.27	0.03	0.97
Late lactating	0.00	0.20	0.00	0.99
fGCM	0.06	0.72	0.83	0.40
Sine (seasonality)	0.03	0.09	0.39	0.70
Cosine (seasonality)	**0.26**	**0.11**	**2.25**	**0.024**

[a]Refers to the prevalence of all parasite taxa found in the study analyzed in a single data set. All reproductive states are compared with the condition classified as "other." Significant or marginally-significant terms are highlighted in bold.

interplay of reproduction, immunity, and parasitism in female primates. It seems plausible that infection patterns of gastrointestinal parasites in female primates may be best explained by local ecological conditions such as seasonal climate or habitat attributes rather than reproductive physiology.

We also predicted a positive relationship between stress hormones and measures of parasitic infection in black howler females. In support of this prediction, we found that fGCM levels positively predicted the probability of being infected with *C. biliophilus*, which was the most common taxon present in fecal samples. Although not significant, the prevalence of these parasites tended to be higher in females during the 1st month of lactation which corresponded to a period of increased fGCM levels. *C. biliophilus* (Dicrocoeliidae) are trematodes that presumably use ants as intermediate hosts. It is assumed that howler monkeys are infected with these parasites by inadvertently consuming ants present on leaves or fruits (Kowalzik,

TABLE 3 Fecal glucocorticoid metabolites (fGCM), parasite richness, and parasite prevalence (%) in female black howler monkeys (*Alouatta pigra*) across different reproductive states at Escárcega, Mexico

	Glucocorticoids		Gastrointestinal parasites			
	Mean (SE) fGCM (ng/g)	Median fGCM (ng/g)	Mean (SE) parasite richness	Overall prevalence[a]	*Controrchis biliophilus* prevalence	Trematode I prevalence
Other	494 ± 66	335	1.0 ± 0.07	74.8 (66–82)	49.6 (41–59)	32.5 (24–42)
Early pregnant	870 ± 136	684	0.8 ± 0.11	61.5 (45–77)	35.9 (21–53)	25.6 (13–42)
Late pregnant	2064 ± 238	1771	1.1 ± 0.13	78.9 (63–90)	52.6 (36–69)	44.7 (29–62)
Early lactating	2116 ± 440	2234	1.3 ± 0.18	88.9 (65–99)	77.8 (52–94)	27.8 (10–54)
Late lactating	547 ± 99	386	1.1 ± 0.14	72.5 (56–85)	45.0 (29–62)	42.5 (27–59)

Trematode I refers to an unidentified trematode from the Dicrocoeliidae family.
[a]Refers to the prevalence (%) of all parasite taxa found in this study and analyzed in a single data set. The prevalence of two parasite taxa across different reproductive states is shown. Values in parenthesis are 95% confident intervals of parasite prevalence.

Pavelka, Kutz, & Behie, 2010), but it remains unclear whether these parasites have detrimental effects on howler health (Pastor-Nieto, 2015). Our study suggests that the endocrine changes experienced by female howlers during an energetically demanding reproductive phase (i.e., early lactation) are an additional factor that increases susceptibility to *C. biliophilus* infections.

Our findings with *C. biliophilus* are supported by other studies conducted on red colobus monkeys (*Piliocolobus tephrosceles* [Chapman et al., 2007]) and Eastern chimpanzees (*P.t. schweinfurthii* [Muehlenbein, 2006]), which reported positive relationships between levels of fGCM and measures of gastrointestinal parasitic infection. However, research on Sykes' monkeys (*Cercopithecus mitis* [Foerster, Kithome, Cords, & Monfort, 2015]), white-handed gibbons (*Hylobates lar* [Gillespie, Barelli, & Heistermann, 2013]), and Belizean black howler monkeys (*A. pigra* [Behie, Kutz, & Pavelka, 2014]) have found that variation in fGCM levels did not influence the probability of parasitic infection. In agreement with these studies, we also found no relationship between fGCM levels and the prevalence of the unidentified trematode, overall parasite prevalence, and parasite richness. Controlled experimental research conducted in other wild mammals (raccoons, *Procyon lotor* [Monello, Millspaugh, Woods, & Gompper, 2010]; bighorn sheep, *Ovis canadensis* [Goldstein, Millspaugh, Washburn, Brundige, & Raedeke, 2005]) shows that even when parasitic infections are significantly reduced via administration of antiparasitic drugs (e.g., 50–80% reduction), there is no concomitant reduction in glucocorticoid concentration. Our results together with these previous studies suggest that the interaction between host immune and endocrine function may operate differently on specific parasitic infections, and that certain parasites are more sensitive than others to the host's HPA activation.

Models of infectious disease risk and parasitism in primates indicate that parasite infection patterns may also be affected by fluctuating climatic conditions (Masi et al., 2012; Nunn & Altizer, 2006). In agreement with this, we found that parasite prevalence and richness showed seasonal fluctuations, declining at the onset of the rainy season. Moreover, it appears that a process of re-infection occurred at mid-rainy season, since parasite prevalence and richness were elevated by the end of the rainy period. Several primate studies report that gastrointestinal parasite prevalence and species richness increased during periods of higher rainfall (Benavides et al., 2012; Gonzalez-Moreno et al., 2013; Valdespino, Rico-Hernández, &

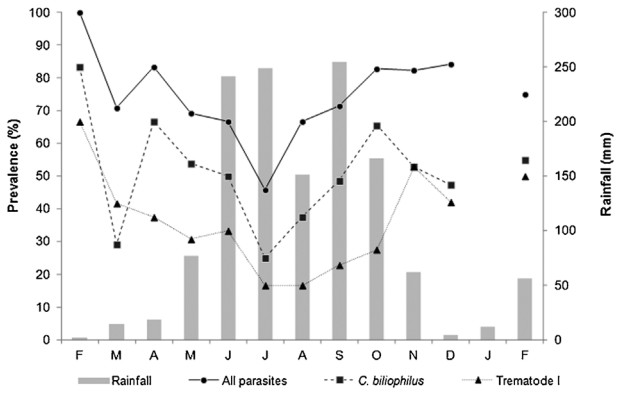

FIGURE 2 Parasite prevalence (%) of female black howler monkeys (*Alouatta pigra*) across a year at Escárcega, Mexico. Females showed reduced parasite prevalence during the months corresponding to the onset of the rainy season. "All parasites" refers to the prevalence of *Trypanoxyuris* sp., *Parabronema* sp., *Controrchis biliophilus*, Trematode I, and *Entamoeba coli* lumped in a single data set

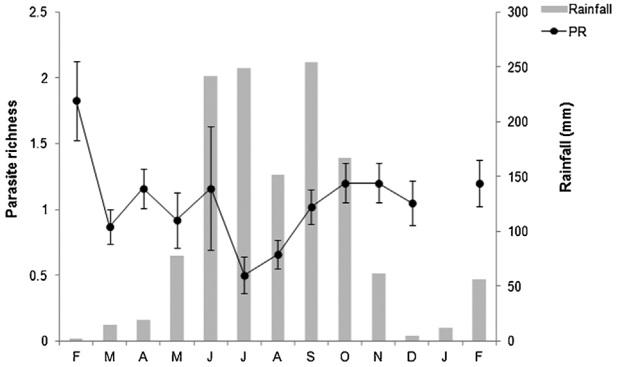

FIGURE 3 Parasite richness (PR) of female black howler monkeys (*Alouatta pigra*) across a year at Escárcega, Mexico. Females showed reduced parasite richness during July and August which are months corresponding to mid-rainy season

TABLE 4 Results of the GLMM examining the effects of reproductive states on fecal glucocorticoid metabolites in female black howler monkeys (*Alouatta pigra*) at Escárcega, Mexico

Reproductive state	Estimate	Standard error	*t*-value	*p*
Intercept	5.76	0.21	26.10	<0.0001
Early pregnant	**0.38**	**0.22**	**1.70**	**0.09**
Late pregnant	**1.40**	**0.23**	**6.12**	**<0.0001**
Early lactating	**1.20**	**0.30**	**4.00**	**<0.001**
Late lactating	0.07	0.23	0.33	0.73

All reproductive states are compared with the "other" condition. Significant or marginally-significant terms are highlighted in bold.

Mandujano, 2010), when humidity may provide better conditions for larvae survival and parasite transmission (Altizer et al., 2006; Stoner, 1996). The trend we found suggests that the process of parasitic infections in black howler females follows a cyclic pattern most likely related to the life cycle of the parasites.

In the present study, female black howler monkeys were infected by a limited number of gastrointestinal parasite taxa (i.e., five) and showed low levels of multiple intestinal infections. This pattern is consistent with results from previous short-term parasitological surveys conducted on *A. pigra* populations in Belize. Females in these black howler populations were infected by a maximum of five parasite species (Eckert et al., 2006; Vitazkova & Wade, 2006). It has been observed that certain primates such as chimpanzees may reduce gastrointestinal parasite infections through ingestion of leaves that either mechanically or chemically (e.g., secondary plant metabolites) remove parasites (Huffman, Nakagawa, Go, Imai, & Tomonaga, 2013). In particular, condensed tannins are proposed to have antiparasitic properties decreasing the viability of larvae and parasite fecundity (Athanasiadou, Kyriazakis, Jackson, & Coop, 2001). In this same howler population, Righini, Garber, and Rothman (2017) found that among the top 80% of plant species that constitute the majority of the black howler monkey diet, 63% contain high levels of condensed tannins. Given that these black howler monkeys include between 30% and 50% of leaves in their yearly diet (Righini, 2014), it is possible that the regular intake of secondary plant metabolites helps limit the number of intestinal parasite infections in these primates (Janzen, 1978). This is a hypothesis that merits further investigation.

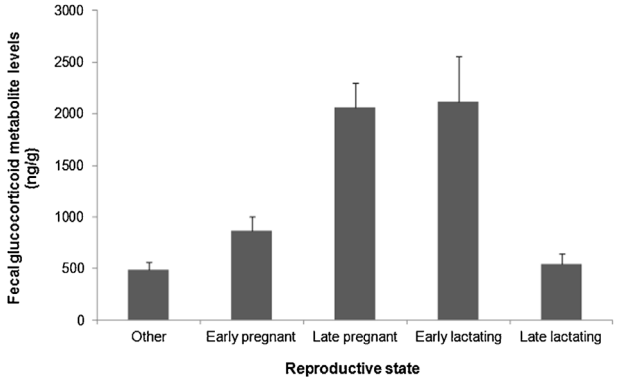

FIGURE 4 Fecal glucocorticoid metabolite levels (ng/g) of female black howler monkeys (*Alouatta pigra*) in different reproductive conditions at Escárcega, Mexico. Levels of fecal glucocorticoid metabolites increased over the course of pregnancy, reaching a peak in the second half of pregnancy and in the first month of lactation, and decreased after the first month of lactation. Bars represent means ± SE

We found that pregnancy and the 1st month of lactation significantly affected glucocorticoid concentrations in black howler females. The increased levels of fGCM during late pregnancy and early lactation in our study were comparable with concentrations shown by black howler monkeys after being subject to an acute stress induced by anesthesia (Martínez-Mota et al., 2008). Our results agree with other studies conducted in humans (Dorr et al., 1989) and in captive and free-ranging primates living under different social and ecological conditions, which indicate that glucocorticoid levels increase with gestational age (*Saguinus oedipus* [Ziegler, Scheffler, & Snowdon, 1995]; *Lemur catta* [Starling, Charpentier, Fitzpatrick, Scordato, & Drea, 2010]; *Cebus capucinus* [Carnegie, Fedigan, & Ziegler, 2011]; *C. mitis* [Foerster et al., 2012]). Variation in glucocorticoid concentrations across reproductive phases is primarily related to endocrine changes associated with female reproductive physiology (Mastorakos & Ilias, 2003). For example, increased glucocorticoid production during the last two trimesters of pregnancy has been associated with fetal development and the functional maturation of fetal organs, in particular, the lungs (Bolt, van Weissenbruch, Lafeber, & Delemarre-van de Waal, 2001). In addition, glucocorticoids may remain elevated 1–8 weeks postpartum returning to pre-pregnant levels after this period [Altmann, Lynch, Nguyen, Alberts, & Gesquiere, 2004; Dettmer, Rosenberg, Suomi, Meyer, & Novak, 2015]. This short-term increase during the postpartum period is assumed to be the result of adrenal gland hypertrophy associated with increased production of glucocorticoids occurring during pregnancy (Duthie & Reynolds, 2013). Thus, the increased fGCM levels shown by pregnant and early lactating black howler females reflect normal endocrine activity experienced by human and non-human primates over the course of gestation and their postpartum period.

Gestation and lactation are characterized by high nutritional, behavioral, and physiological costs which may include increased energy intake, increased aggression and injury risk, increased infanticide risk, high stress levels, short term decreased immunity, and increased susceptibility to diseases (Archie, Altmann, & Alberts, 2014; Emery Thompson, 2013; Foerster et al., 2015; Hoffman, Ayala, Mas-Rivera, & Maestripieri, 2010). Contrary to the latter set of assumptions, however, we found that among our population of black howler monkeys, female reproductive condition had a minimal influence on gastrointestinal parasitic infection risk. These findings provide a significant contribution to the growing understanding of how host-related physiology shapes patterns of parasitism and health in wild primates.

TABLE 5 Effects of female reproductive state on parasitic infection measures in different primate species

Primate	Type of parasites	Effects	References
Alouatta palliata	Trematode: *Controrchis* sp.	Lactating females had higher prevalence than non-lactating females	Maldonado-López et al. (2014)
	Nematode: *Trypanoxyuris* sp.	No differences in prevalence between lactating and non-lactating females	Maldonado-López et al. (2014)
	Nematode: *Strongyloides* sp.	No differences in prevalence between lactating and non-lactating females	Maldonado-López et al. (2014)
A. pigra	Trematode: *Controrchis biliophilus*	No differences in prevalence among cycling, pregnant, and lactating females	This study
	Trematode: Unidentified taxon	No differences in prevalence among cycling, pregnant, and lactating females	This study
	Overall prevalence	No differences in prevalence among cycling, pregnant, and lactating females	This study
	Multiple parasite infection	No differences in multiple infections among cycling, pregnant, and lactating females	This study
Ateles geoffroyi	Trematode: *Controrchis* sp.	No differences in prevalence between lactating and non-lactating females	Maldonado-López et al. (2014)
	Nematode: *Trypanoxyuris* sp.	No differences in prevalence between lactating and non-lactating females	Maldonado-López et al. (2014)
	Nematode: *Strongyloides* sp.	No differences in prevalence between lactating and non-lactating females	Maldonado-López et al. (2014)
Cercopithecus mitis	Nematode: *Trichuris trichiura*	Lactating females had higher egg counts than non-lactating females	Foerster et al. (2015)
	Nematode: *Oesophagostomum* sp.	Lactating females had higher egg counts than non-lactating females	Foerster et al. (2015)
	Nematode: *Trichostrongylus* sp.	No effects of reproductive state on egg counts	Foerster et al. (2015)
Cercocebus sanjei	Nematode: several taxa[a]	Higher nematode prevalence in pre-conceptive and in mid-lactating females compared to pregnant, early lactating, and late lactating females	McCabe (2012)
	Protozoan: amoebas and ciliates	No effects of reproductive state on prevalence	McCabe (2012)
Papio cynocephalus	Nematode: *Trichuris* sp. and *Trichostrongylus* sp.[b]	Cycling females had higher eggs per gram than anoestrus and pregnant females	Hausfater & Watson 1976
P. c. anubis	Trematode: *Schistosoma mansoni*	No differences in prevalence and in eggs per gram among cycling, pregnant, and lactating females	Müller-Graf et al. (1997)
Mandrillus sphinx	Nematode: several taxa[c]	Higher abundance of nematode eggs in pregnant than in lactating and cycling females	Setchell et al. (2007)
Pan troglodytes verus	Protozoan: *Plasmodium* sp.	Probability of infection increases with gestational age	De Nys et al. (2014)

[a]Parasites belonging to Ancylostomidae, Trichostrongylidae, and Physalopteridae.
[b]Results reported without making a distinction between parasite taxa.
[c]Parasites belonging to Trichostrongyloidea, Strongyloidea, and Ancylostomatoidea.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest regarding this manuscript.

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