American Journal of Primatology

WILEY

AMERICAN JOURNAL OF PRIMATOLOGY

RESEARCH ARTICLE OPEN ACCESS

Sources of Variation in Fecal Haptoglobin in a Population of Wild Capuchin Monkeys (*Cebus imitator*)

Raquel Hernández-Rojas[1] | Hadjira Hamou[1] | Ronald Lopez Navarro[2] | Katharine M. Jack[3] | Fernando A. Campos[4,5] | Amanda D. Melin[1,6,7] | James P. Higham[8]

[1]Department of Anthropology and Archaeology, University of Calgary, Calgary, Alberta, Canada | [2]Área de Conservación Guanacaste, Hacienda Naranjo, Costa Rica | [3]Department of Anthropology, Tulane University, New Orleans, Louisiana, USA | [4]Department of Anthropology, University of Texas at San Antonio, San Antonio, Texas, USA | [5]Department of Management Science and Statistics, University of Texas at San Antonio, San Antonio, Texas, USA | [6]Department of Medical Genetics, University of Calgary, Calgary, Alberta, Canada | [7]Alberta Children's Hospital Research Institute, University of Calgary, Calgary, Alberta, Canada | [8]Department of Anthropology, New York University, New York, New York, USA

Correspondence: Raquel Hernández-Rojas (raquel.hernandezroja@ucalgary.ca)

Received: 31 July 2025 | **Revised:** 12 April 2026 | **Accepted:** 25 May 2026

Funding: Natural Sciences and Engineering Research Council of Canada, Grant/Award Numbers: DGECR-2023-00272, RGPIN-2023-04399, RGPIN-2017-03782; Canada Research Chairs program, Grant/Award Number: 950-231257; National Institutes of Health, Grant/Award Number: R61-AG078529-01

Keywords: biomarker | haptoglobin | immune system | longitudinal study | non-invasive sampling | seasonality

ABSTRACT

The ongoing decline of non-human primates places renewed emphasis on monitoring health in wild populations. Non-invasive monitoring of reliable biomarkers of inflammation and immune activation allows researchers to assess individual health status without capturing or interfering with wild animals, but studies are limited by the availability of such biomarkers that are measurable from fecal and urine samples. In the present study, we aimed to validate the measurement of fecal haptoglobin, a biomarker primarily studied in relation to gut-associated inflammation, in wild white-faced capuchin monkeys (*Cebus imitator*), and to evaluate the relationship between fecal haptoglobin concentrations and age, sex, dominance rank, circadian effects and environmental factors including temperature and rainfall. We analytically validated the measurement of fecal haptoglobin in white-faced capuchins. Our subsequent results did not demonstrate a relationship between haptoglobin concentrations and age, sex, dominance rank or circadian effects. However, we found significant influences of environmental conditions on fecal haptoglobin concentrations, with an increase and more variation observed during drier conditions in the early dry season, when the animals are typically under greater environmental stress. We conclude that haptoglobin measurement is feasible in wild white-faced capuchin monkeys, and its concentrations vary in our study population, reflecting seasonal patterns of inflammation that are consistent with changes to environmental stressors associated with lower access to food and water.

1 | Introduction

The world population of wild non-humans primates is decreasing due to habitat loss, climate change, and trade in live primates (Carvalho et al. 2019; Estrada et al. 2017; Norconk et al. 2020), creating greater urgency of efforts to monitor the health of primates as a crucial component of their conservation (Bicca-Marques et al. 2022). Monitoring the health of wild primates can also aid in the early identification of zoonotic and anthroponotic diseases (Balansard et al. 2019). This highlights the importance of longitudinal studies that enable the monitoring of health over time, helping us to understand the fundamental biology of aging and variation in

Abbreviations: ACG, Área de Conservación Guanacaste; ELISA, Enzyme-linked immunosorbent assay; GAMM, Generalized additive mixed model.

Summary

- Fecal haptoglobin measurement is feasible in wild white-faced capuchin monkeys.
- Fecal haptoglobin concentrations show significant seasonal variation, and are highest in the early dry season when food is typically scarce and water sources are small and often pathogen rich.
- There is no relationship between haptoglobin concentrations and age, sex, dominance rank or circadian effects.

lifespan (Campos et al. 2024). Longitudinal studies allow researchers to link variation in ecological and social pressures to changes in health and disease across the lifespan, as well as seasonal or long-term environmental changes on nutrition and health, social behaviour, and the ecological roles of primates (Alberts and Altmann 2012; Gonçalves et al. 2022; Harrison and Van De Waal 2022; Lopresti-Goodman and Villatoro-Sorto 2022).

Accordingly, there is continued interest in expanding the number of non-invasive health biomarkers that are validated for use in wild and naturalistic primate populations (Lucore et al. 2022). Haptoglobin is a glycoprotein used as a health biomarker whose blood concentrations change during the acute phase of inflammatory responses (Hooijberg and Cray 2023). Haptoglobin has three major phenotypes: Hp1-1, Hp2-1 and Hp2-2, with Hp1-1 found in almost all animal species (Carter and Worwood 2007; Lai et al. 2008). In humans, these can have biological implications for interpretation because baseline circulating concentrations can depend on the phenotype, with Hp2-1 and Hp1-1 showing higher reference values than Hp2-2 (Kasvosve et al. 2000). In humans, blood concentrations of haptoglobin are considered a biomarker of inflammation and can be used in the monitoring of many systemic diseases, including parasitic, bacterial and viral infections (Naryzhny and Legina 2021; Quaye 2008;). Haptoglobin concentrations in blood can also rise during chronic diseases, including cardiovascular diseases and cancer, which has led to its growing use in human medical research (Cheng et al. 2024). Beyond human medicine, elevated blood haptoglobin has been observed during subclinical infections, providing a valuable tool for detection in wildlife, particularly when agent-specific methods are impractical (Vicente et al. 2019). This protein can also be found in other body fluids across a variety of mammals (Wan et al. 2021). Fecal levels of haptoglobin have been described as an important tool for predicting the presence of bowel lesions and the early diagnosis of colorectal cancer in humans, which are local affections limited to the gut (Chalkias et al. 2011; Shiotani et al. 2014). In rhesus macaques (*Macaca mulatta*), lymph-node extirpation and intestinal biopsy sampling lead to an increase in fecal excretion of haptoglobin, representing a biological validation of the assessment of fecal haptoglobin as a measure of gut inflammation in non-human primates (Higham et al. 2015). Despite the clinical importance of haptoglobin, the literature on non-human primates is limited, creating a need for studies that help to understand sources of variation of this biomarker in the wild. Fecal haptoglobin has primarily been associated with conditions of the intestinal tract and has not been reported to be necessarily associated with systemic circulating inflammation in humans and non-human primates.

In humans, recent studies show that intestinal inflammation increases with age. Contributing factors may include changes to intestinal microbiota with aging (Heston et al. 2023). Sex related differences in inflammatory bowel disease have also been reported: Depleted estrogen has been proposed to increase intestinal permeability and decrease mucin production, contributing to inflammation. Indeed menopausal women with low estrogen levels tend to exhibit greater inflammation (Xu et al. 2022). In men, androgens have anti-inflammatory effects (Mohamad et al. 2019; Xu et al. 2022). Men with higher androgen levels experience lower inflammation because these hormones reduce intestinal permeability, support epithelial barrier integrity, and decrease microbiome dysbiosis (Brettle et al. 2022). Observational evidence also suggests that circadian disruption is associated with intestinal inflammatory conditions (Niu et al. 2024). However, there is limited evidence regarding diurnal variation in fecal inflammatory biomarkers. In vervet monkeys (*Chlorcebus aethiops sabaeus*), older monkeys exhibit higher systemic inflammation and poorer intestinal barrier function than younger individuals (Mitchell et al. 2017).

Climatic seasonality has been documented to impact immune function in nonhuman primates. In baboons (*Papio* spp.), a seasonal immune rhythm is described, with an increase of markers of inflammation such as Interleukin-6 and C-reactive protein reported during months with the higher temperatures (McFarlane et al. 2012). Additionally, a study conducted in the tropical dry forest in Guanacaste, Costa Rica, concluded that mantled howler monkeys (*Alouatta palliata*) have more labile body temperatures compared to humans under a similar range of temperatures (Thompson et al. 2014), suggesting that at least some non-human primates are responsive to changes in temperature. Seasonality can also impact triggers of inflammation indirectly. For example, gastrointestinal parasite species richness is higher during the dry season in red howler monkeys (*Alouatta seniculus*), brown spider monkeys (*Ateles hybridus*), and variegated capuchins (*Cebus versicolor*) (Rondón et al. 2017), which may lead to increased gut inflammation.

In the present study, we investigated variation in fecal haptoglobin excretion in a population of wild capuchin monkeys. The population inhabits the tropical dry forest of the Santa Rosa Sector of the Área de Conservación Guanacaste (ACG), in Guanacaste, Costa Rica. The white-faced capuchin monkeys (*Cebus imitator*) present in the ACG experience a long dry season of approximately 5 months, with temperatures that can exceed 37°C, with low humidity and scarce precipitation (Campos and Fedigan 2009), which impact the resource availability. The middle of the dry season is typically associated with low food abundance while water scarcity is an important stressor of the late dry season, especially as remaining water sources become small, heavily used and contaminated with feces of many animals (Hogan and Melin 2018; Melin et al. 2014; Orkin et al. 2019). As water sources become scarce, capuchin monkeys must drink from the few remaining

watering holes, which are considered an important source of parasitic infections such as *Strongyloides*, and with fewer fruits available, they eat more insects, which could serve as intermediate host for gastrointestinal parasites such as cestodes and acanthocephalans (Henriquez et al. 2025).

We analyzed haptoglobin concentrations from 185 fecal samples collected from 68 different individuals over a period of 8 months, spanning the dry and wet seasons. Our first aim was 1) to perform an analytical validation of the measurement of fecal haptoglobin in wild white-faced capuchin monkeys. To achieve this, we used a commercial direct sandwich enzyme-linked immunosorbent assay (ELISA), designed for haptoglobin detection in serum, which has been successfully applied to the analysis of fecal extracts in rhesus macaques (Higham et al. 2015). As a second aim, we 2) investigated the relationship between haptoglobin concentrations and a range of intrinsic, social, and environmental covariates including age, sex, dominance rank, circadian effects, temperature, and rainfall.

2 | Methods

2.1 | Ethics Statement

This animal study was reviewed and approved by the Animal Care Committee (ACC) of the University of Calgary in Canada (AC19-0167), Tulane's Institutional Animal Care and Use Committee (Protocol #2432) and by the Sistema Nacional de Áreas de Conservación (SINAC) and the Área de Conservación Guanacaste (ACG: R-SINAC-ACG-PI-059-2022/ACG-PI-033-2023ACG-PI-011-2024/, and CONAGEBIO (R-013-2022-OT-CONAGEBIO/R-042-2023-OT-CONAGEBIO)/R-021-2025-OT-CONAGEBIO) in Costa Rica. Fecal samples were imported to Canada under Canadian Food Inspection Agency (CFIA) permits A-2023-06194-1 and A-2022-05488-4.

2.2 | Study Site and Subjects

This study was conducted in the tropical dry forest of Sector Santa Rosa, ACG, Guanacaste, Costa Rica (10.836° latitude; −85.615° longitude), at an elevation of 300 meters above sea level and in a region that experiences distinct dry and wet seasons. The study population consisted of wild adult white-faced capuchin monkeys (*Cebus imitator*) from five social groups, including males and females, that have been followed and documented in ACG for more than 40 years.

2.3 | Sample Collection

We collected fecal samples from 68 different individuals (26 males and 42 females) from five different social groups. The age range was from 5 to 30 years old. A total of 185 samples (2.72 ∓ 1.41 per individual) were collected from October 2022 to June 2023, between 6:00 am to 5:00 pm. Trained field technicians collected the samples non-invasively and opportunistically immediately after observing an individual defecating, avoiding contact with rainwater. Samples were collected from the ground or leaves and transferred into capped 2 mL cryovials to minimize drying prior to preservation. In the field, fecal samples were temporarily stored in a portable cooler until they arrived at the field facilities, where they were transferred to a cryogenic liquid nitrogen dewar before being shipped to the University of Calgary for analysis. All samples arrived frozen to the laboratory and were immediately stored at −80°C.

2.4 | Fecal Analysis

At the University of Calgary, we undertook extractions by freeze-drying fecal samples overnight then pulverizing them. We added 1 mL of extraction buffer from the ELISA kit to 20 mg of pulverized fecal sample, vortexed, and centrifuged the mixture. We then collected the supernatant and discarded the pellet.

We measured fecal haptoglobin using a commercial ELISA kit (Monkey Haptoglobin ELISA Kit, Catalog No. HAPT-3, Life Diagnostics Inc.), following the manufacturer's instructions. The kit is designed for haptoglobin detection in serum and has been successfully used to quantify haptoglobin in fecal extracts collected from rhesus macaques, in a study which provided a biological validation for the measurement of haptoglobin in nonhuman primate feces as indicating gut inflammation (Higham et al. 2015). We read the absorbance at 450 nm using Synergy HTX multi-mode reader (Biotek, Ref: S1LFA) with the software Gen5 3.11 (BioTek Instruments 2020).

The fecal extracts were measured undiluted; however, fecal extractions with results outside the linear portion of the standard curve (typically 20%–80% binding) were remeasured. A total of 141 samples did not need remeasurement. For 23 extracts with low concentrations, including 10 samples collected on rainy days, we freeze-dried them overnight and concentrated the sample by resuspending them in 0.25 mL of extraction buffer from the ELISA kit. For 21 extracts with high concentrations, collected on dry days with scarce rainfall, we diluted them with the extraction buffer from the ELISA kit using higher dilution factors: 10 samples were diluted 1:4, 1 sample was diluted 1:8, 9 samples were diluted 1:16, and 1 sample was diluted 1:32. To determine the final concentration we incorporated the dilution or concentration factor. Samples with coefficient of variation (CV) greater than 10% were remeasured. The inter-assay variation was 7.98% for the high concentration quality control and 12.65% for the low concentration quality control. The final fecal haptoglobin concentration is expressed in ng/g of feces.

2.5 | Analytical Validation

We performed a parallelism test using a pooled sample consisting of ten individual samples. We serially diluted the pooled sample at 1:2, 1:4, 1:8, and 1:16, and each dilution was run and analysed in duplicate alongside the assay standard curve to evaluate if the pooled sample followed the same pattern across the different concentrations. The analysis was undertaken using an analysis of covariance (ANCOVA) of log optical density vs log concentration to assess whether the slopes of the dilution curve and the standard curve were parallel.

We also conducted a recovery experiment to assess assay accuracy in the same pooled sample matrix used for the parallelism test. The pooled sample was spiked with the assay kit

standard that had a concentration of 25 ng/mL, and was then serially diluted within the same matrix at 1:2, 1:4, 1:8, and 1:16 dilutions. These dilutions were run and analysed to evaluate standard recovery across the dilution series. Recovery was calculated by comparing observed to expected concentration and reporting the result as a percentage.

2.6 | Environmental and Social Covariates

Temperature data were collected every half hour throughout the study period using an environmental meter (Kestrel, Model: 5000) located in a protected location at the research station, which is approximately in the center of the study groups' home ranges. We later determined that the temperature data from the Kestrel meter were moderated by its location, resulting in lower maximum temperatures and higher minimum temperatures relative to conditions in the field. We therefore made use of a second temperature data source, a HOBO Weather Station (Onset Corp) with a S-THC-M002 temperature sensor protected by a solar radiation shield, for which data collection began in August 2023, after the period in which fecal samples were collected for this study. Using 15 months of subsequent overlapping simultaneous temperature recordings between the Kestrel and the HOBO instruments (from August 2023 to March 2025), we fit a linear model of HOBO temperature data as a function of Kestrel data, and we used this model to predict temperature values during the sampling period from the Kestrel data collected during that time (Figure S1). To measure rainfall, we used a Metric Rain Gauge (Cole-Parmer Instrument Company, Model: 03319-10), recording accumulated rainfall once every 24 h.

White-faced capuchins form dominance hierarchies in which a clear alpha male and alpha female can be identified in each social group (Bergstrom and Fedigan 2013; Muniz et al. 2010). We assigned a dominance rank as alpha or non-alpha for each individual, based on patterns of agonism and submission following our standard ethograms and protocols (Bergstrom and Fedigan 2013; Hall and Fedigan 1997). Females and males were assessed separately.

2.7 | Statistical Analysis

All statistical analyses were performed using R software, version 4.4.2 (R Core Team 2024). We modeled log-transformed haptoglobin concentrations using a generalized additive mixed model (GAMM). We included smooth (non-linear) terms for the following continuous predictors: age in years (separately by sex), the average maximum temperature over the 15 days prior to and including the day of sample collection, the sum of rainfall over the 30 days prior to and including the day of sample collection, and the number of minutes into the day starting from midnight. We also included fixed effects of dominance rank (alpha vs. non-alpha), sex, and the interaction between rank and sex.

As random effects, we included individual ID, because each individual had multiple measurements, allowing each individual to have their own intercept, as well as the study group that the individual belonged to when the sample was collected. The study group was the same for all the samples from a given individual in this dataset.

To fit the models, we used the mgcv R package, version 1.9-1 (Wood 2011), and for generating and visualizing the predictions and partial effects, we used the R packages marginaleffects, version 0.24.0 (Arel-Bundock et al. 2024), and gratia, version 0.10.0 (Simpson 2024).

3 | Results

3.1 | Aim 1: Analytical Validation

In the parallelism test, we found that the trendline of the pooled samples exhibited parallel behaviour to the trendline of the standard curve ($F = 1.348$, $p = 0.310$, $N = 8$) with minimal deviation, indicating consistent assay performance across different sample concentrations (Figure S2). In the recovery test the standard showed recoveries of 83%, 80% and 86% at 1:2, 1:4 and 1:8 dilutions respectively, whereas the recovery dropped to 33% at the very low 1:16 dilution. This drop is likely to be due to the amplified impact of absolute error at very low standard concentrations (Figure S3). The average recovery between the higher dilutions was ~83% and the correlation coefficient between observed and expected values was $r = 0.87$. Overall, the ELISA recovery test showed acceptable performance confirming the reliability of the assay for our samples.

3.2 | Aim 2: Intrinsic, Social, and Environmental Influences on Haptoglobin

Fecal haptoglobin concentrations do not exhibit a significant relationship with age, and do not differ by sex, dominance status of the monkeys or circadian effects (Table 1), as assessed using GAMM (Figure 1). There were significant effects of environmental conditions on fecal haptoglobin concentrations, which were higher during drier conditions, where the rainfall is low over the previous 30 days (Table 1). During the early dry season, specifically between January and mid-March, fecal haptoglobin concentrations were especially high, particularly in some individuals (Figure 2).

4 | Discussion

In this study we undertook an analytical validation of the measurement of fecal haptoglobin in wild white-faced capuchin monkeys. We did not find a relationship between haptoglobin concentrations and age, sex, dominance rank or circadian effects, variables that had never before been explored in relation to fecal haptoglobin in wild primate populations. However, we found significant influences of environmental conditions on fecal haptoglobin levels, with an increase during drier conditions, when the animals are generally exposed to greater environmental stressors.

The study of fecal haptoglobin as a nonspecific biomarker of inflammation in platyrrhine primates is limited, and there has been no exploration of its relation to age, sex, dominance rank, circadian effects, or climate variability. In humans, differences in serum haptoglobin concentrations have been reported according to age and sex. In neonates haptoglobin levels are extremely low, often making them undetectable (Jacob 2016). It

TABLE 1 | Summary of parametric and smooth term results from the generalized additive mixed model.

Parameter	Coefficient	SE	95% CI	t/F	df	df (error)	*p*
Parametric terms							
(Intercept)	5.74	0.47	(4.82, 6.67)	12.28		147.53	< 0.001
Rank [Sub]	−0.16	0.47	(−1.09, 0.76)	−0.35		147.53	0.725
Sex male	0.22	0.57	(−0.91, 1.36)	0.38		147.53	0.701
rank [Sub] × sexMale	−0.13	0.62	(−1.35, 1.10)	−0.21		147.53	0.836
Smooth terms							
Smooth term (age) × sexFemale				0.59	1.00		0.445
Smooth term (age) × sexMale				6.88e-03	1.00		0.934
Smooth term (temperature)				13.81	1.00		< 0.001
Smooth term (rainfall)				18.07	2.89		< 0.001
Smooth term (time of day)				0.04	1.00		0.842
Smooth term (monkey id)				0.65	24.45		0.006
Smooth term (study group)				2.52	2.13		0.073

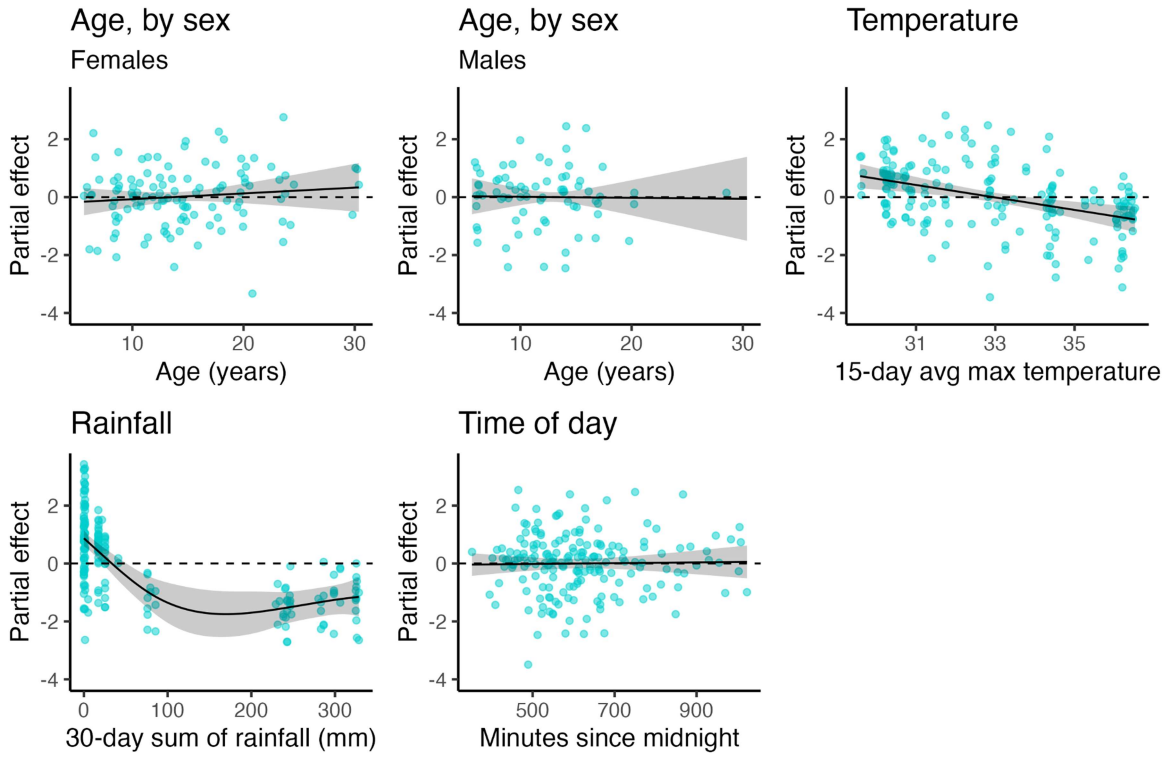

FIGURE 1 | Partial effects and partial residuals of age (by sex), temperature, rainfall, and time of day on fecal haptoglobin concentration (ng/g feces) in wild white-faced capuchin monkeys.

has also been described that women and older individuals tend to exhibit higher concentrations; however, serum haptoglobin concentration can also be affected by the proportions of haptoglobin phenotypes, which may affect baseline levels and could explain why age and sex effects are not observed in other studies when some phenotypes are overrepresented (Kasvosve et al. 2000; Lei et al. 2023). The ELISA assay used in the present study measures total haptoglobin, and does not differentiate between haptoglobin phenotypes. We detected no difference in fecal haptoglobin with respect to age, although there are reports indicating that intestinal inflammation is affected by age in both humans and non-humans primates (Heston et al. 2023; Mitchell et al. 2017). No sex differences or effects of dominance rank were detected, despite evidence that androgens are associated with reduced intestinal inflammation (Brettle et al. 2022; Xu et al. 2022). We found no circadian effects on fecal haptoglobin; however, given the limited research on this fecal biomarker in wild populations, we examined circadian effects to exclude this as a source of variation. Due to the scarce research available on this biomarker in platyrrhine primates, we suggest that additional studies with larger sample sizes are needed to determine whether these effects can be detected.

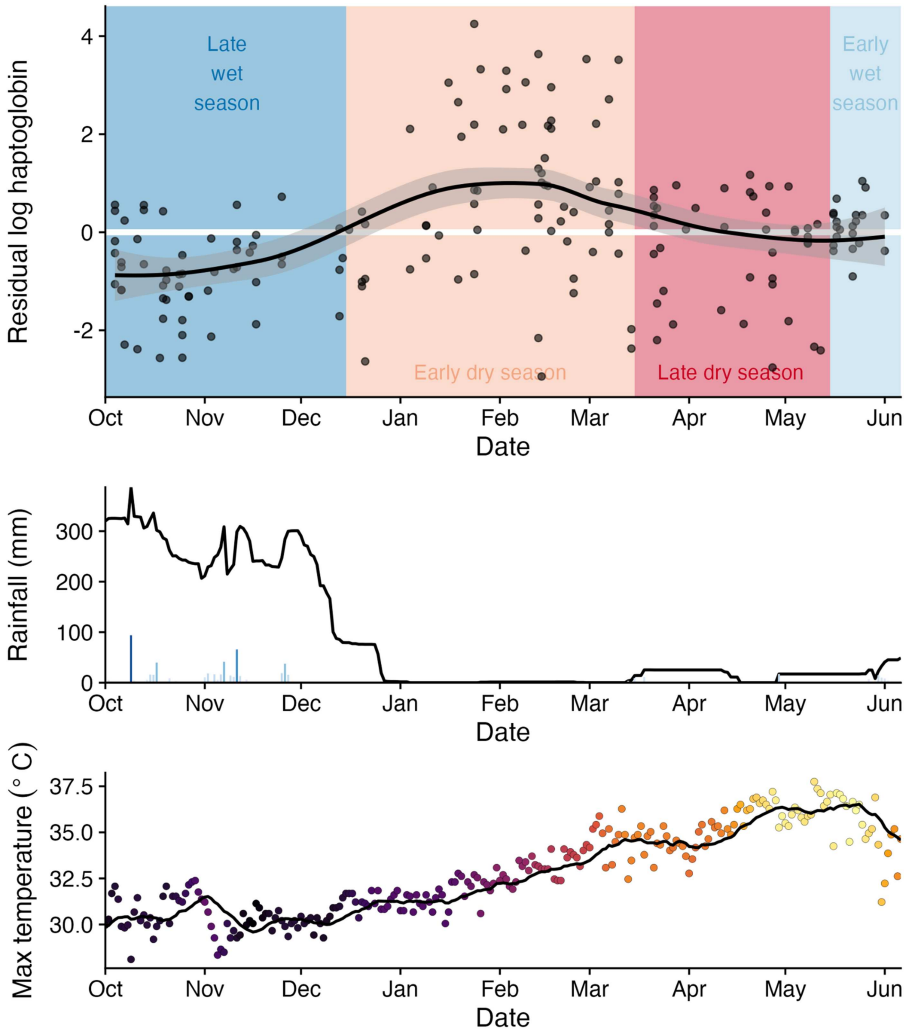

FIGURE 2 | Top: Seasonal variation in residual (log) haptoglobin concentrations, based on a linear mixed model that includes only random effects of individual ID and group ID (to account for repeated and unbalanced sampling across units), but with no environmental predictors included. For visualization, we divide the year into four seasons with distinct climate conditions: the late wet season is typically cool and rainy (dark blue), the early dry season has moderate temperatures and dry conditions (orange), the late dry season is hot and dry (red), and the early wet season is warm and rainy (light blue). Middle: daily rainfall totals (bars) and 30-day rolling sum of rainfall (black line) during the study period. Bottom: daily maximum temperature (colored points) and 15-day rolling average of maximum temperature (black line) during the study period.

We found significant influences of environmental conditions on fecal haptoglobin concentrations, which were higher during the months of January, February and March in the early to mid dry season. This is a time when water is generally available through standing pools in quebradas (small river beds) but becoming increasingly scarce and contaminated (Henriquez et al. 2025; Melin et al. 2014; Orkin et al. 2019). Scarcity of clean water impacts intestinal homeostasis, leading to dysbiosis of the gut microbiome and reduction in the number of certain immune cells, which decreases the ability to eliminate microorganisms and can contribute to inflammation (Bekkevold et al. 2013; Sato et al. 2024). While it is perhaps surprising that haptoglobin concentrations did not peak at the end of the dry season, when temperatures are highest and water availability is lowest, it is important to highlight that the early to mid dry season is also consistently a time of very low fruit availability in the dry forest while in the late dry season several species of preferred fruits are available, which provide a clean source of hydration as well as carbohydrates and nutrition (Hogan and Melin 2018; Melin et al. 2014 a,b). Low food availability contributes to a decrease in the intestinal transit speed, increases epithelial permeability in the gut, causes an imbalance in the intestinal microbiota, and consequently leads to intestinal inflammation (Genton et al. 2015). A separate study of our population demonstrated that seasonality influences the composition and function of their gut microbiome. When the fruit availability was scarce, microorganisms such as *Campylobacter, Enterococcus, Helicobacter, Haemophilis, Pseudomonas*, and *Streptococcus*, which are associated with human dysbiosis, ill health, and irritable bowel syndrome, were higher (Orkin et al. 2019).

Previous research on capuchins in ACG has also found higher rates of infection by some gastrointestinal parasites during the dry season, when animals are more exposed to parasitic infections with *Strongyloides*, cestodes, and acanthocephalans (Henriquez et al. 2025; Henriquez et al. 2025). These infections can cause diarrhea and weight loss due to improper nutrient absorption. Consequently, this affects gut health and leads to inflammation

(Dib et al. 2023). Thus, the rise in fecal haptoglobin levels we found in association with drier conditions could be explained by the damage and onset inflammation that the parasites can cause to the gut of the host. Another study in wild black capuchin monkeys demonstrated that parasite loads decreased when the animals had higher food availability, which could indicate that nutritional status affects the parasite dynamics in non-human primates (Agostini et al. 2017). This may be relevant for our population, as haptoglobin levels are lower in the months when food availability is higher (Melin et al. 2014).

In sum, we found that fecal haptoglobin measurement is viable in wild white-faced capuchin monkeys, and that concentrations of this biomarker vary with seasonal climatic variation in our study population. Future studies integrating this biomarker of health and inflammation could combine data on the gut microbiome and other measures of animal condition as part of integrated studies addressing the sources of variation in individual health across the lifespan.

Author Contributions

Raquel Hernández-Rojas: writing – original draft, validation, data curation, investigation, writing – review and editing, methodology, formal analysis, visualization. **Hadjira Hamou:** validation, methodology, investigation. **Ronald Lopez Navarro:** methodology, investigation. **Katharine M Jack:** investigation, funding acquisition, conceptualization, writing – review and editing, project administration, resources. **Fernando A Campos:** conceptualization, investigation, funding acquisition, visualization, writing – review and editing, formal analysis, project administration, data curation, resources. **Amanda D Melin:** conceptualization, investigation, funding acquisition, writing – review and editing, project administration, resources, supervision; writing – original draft. **James P Higham:** conceptualization, Investigation; funding acquisition, writing – review and editing; validation, project administration, supervision, resources, writing – original draft.

Acknowledgments

We thank Roger Blanco, Maria Marta, Staff and Administration of ACG at Sector Santa Rosa. Linda M Fedigan, Saul Cheves Hernández, Danielka Rugama Taylor, Wendy Tellez Arias, Philippine Delga, Lindy Wolhuter, Cielo De La Rosa Meza, Ann-Kathrin Pohle, Peyton Schmidli, Suheidy Romero Morales, and all members of the Santa Rosa field team. We thank Patricia Ströher for administrative support. This project was supported by funding from the Natural Sciences and Engineering Research Council of Canada (NSEC, RGPIN-2017-03782 to ADM), the Canada Research Chairs program (950-231257 to ADM), and the National Institutes of Health Aging Research In Animals (NIH ARIA) program (R61-AG078529-01 to FC, ADM, KJ, JPH). RGPIN-2023-04399. We thank two anonymous reviewers for constructive comments, which improved the manuscript.

Data Availability Statement

The data that support the findings of this study are openly availble at https://doi.org/10.5281/zenodo.20635999.

References

Agostini, I., E. Vanderhoeven, M. S. Di Bitetti, and P. M. Beldomenico. 2017. "Experimental Testing of Reciprocal Effects of Nutrition and Parasitism in Wild Black Capuchin Monkeys." *Scientific Reports* 7, no. 1: 12778. https://doi.org/10.1038/s41598-017-12803-8.

Alberts, S. C., and J. Altmann. 2012. "The Amboseli Baboon Research Project: 40 Years of Continuity and Change." In *Long-Term Field Studies of Primates*, edited by P. M. Kappeler and D. P. Watts, 261–287. Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-22514-7_12.

Arel-Bundock, V., N. Greifer, and A. Heiss. 2024. "How to Interpret Statistical Models Using Marginal Effects for R and Python." *Journal of Statistical Software* 111, no. 9: 1–32. https://doi.org/10.18637/jss.v111.i09.

Balansard, I., L. Cleverley, K. L. Cutler, M. G. Spångberg, K. Thibault-Duprey, and J. A. Langermans. 2019. "Revised Recommendations for Health Monitoring of Non-Human Primate Colonies (2018): FELASA Working Group Report." *Laboratory Animals* 53, no. 5: 429–446. https://doi.org/10.1177/0023677219844541.

Bekkevold, C. M., K. L. Robertson, M. K. Reinhard, A. H. Battles, and N. E. Rowland. 2013. "Dehydration Parameters and Standards for Laboratory Mice." *Journal of the American Association for Laboratory Animal Science: JAALAS* 52, no. 3: 233–239.

Bergstrom, M. L., and L. M. Fedigan. 2013. "Dominance Style of Female White-Faced Capuchins." *American Journal of Physical Anthropology* 150, no. 4: 591–601. https://doi.org/10.1002/ajpa.22231.

Bicca-Marques, J. C., R. M. Rabelo, M. A. B. De Almeida, and L. P. Sales. 2022. "The Risks of Yellow Fever to Asian Primates." *International Journal of Primatology* 43, no. 1: 74–91. https://doi.org/10.1007/s10764-021-00223-5.

BioTek Instruments. 2020. *Gen5* (Version 3.11).[Computer software]. https://www.agilent.com/en/product/microplate-instrumentation/biotek-instruments.

Brettle, H., V. Tran, and G. R. Drummond, et al. 2022. "Sex Hormones, Intestinal Inflammation, and the Gut Microbiome: Major Influencers of the Sexual Dimorphisms in Obesity." *Frontiers in Immunology* 13: 971048. https://doi.org/10.3389/fimmu.2022.971048.

Campos, F. A., and L. M. Fedigan. 2009. "Behavioral Adaptations to Heat Stress and Water Scarcity in White-Faced Capuchins (*Cebus capucinus*) in Santa Rosa National Park, Costa Rica." *American Journal of Physical Anthropology* 138, no. 1: 101–111. https://doi.org/10.1002/ajpa.20908.

Campos, F. A., E. C. Wikberg, J. D. Orkin, et al. 2024. "Wild Capuchin Monkeys as a Model System for Investigating the Social and Ecological Determinants of Ageing." *Philosophical Transactions of the Royal Society, B: Biological Sciences* 379, no. 1916: 20230482. https://doi.org/10.1098/rstb.2023.0482.

Carter, K., and M. Worwood. 2007. "Haptoglobin: A Review of the Major Allele Frequencies Worldwide and Their Association With Diseases." *International Journal of Laboratory Hematology* 29, no. 2: 92–110. https://doi.org/10.1111/j.1751-553X.2007.00898.x.

Carvalho, J. S., B. Graham, H. Rebelo, et al. 2019. "A Global Risk Assessment of Primates under Climate and Land Use/Cover Scenarios." *Global Change Biology* 25, no. 9: 3163–3178. https://doi.org/10.1111/gcb.14671.

Chalkias, A., G. Nikotian, A. Koutsovasilis, et al. 2011. "Patients With Colorectal Cancer Are Characterized by Increased Concentration of Fecal Hb-Hp Complex, Myeloperoxidase, and Secretory IgA." *American Journal of Clinical Oncology* 34, no. 6: 561–566. https://doi.org/10.1097/COC.0b013e3181f9457e.

Cheng, C.-H., W.-R. Hao, and T.-H. Cheng. 2024. "Multifaceted Role of Haptoglobin: Implications for Disease Development." *World Journal of Hematology* 11, no. 3: 98807. https://doi.org/10.5315/wjh.v11.i3.98807.

Dib, L. V., A. Barbosa, B. Da Silva, et al. 2023. "Gastrointestinal Parasites Affecting Non-Human Primates That Are Kept Ex Situ and Their Handlers in Different Brazilian Institutions: Diagnosis and Analysis of Risk Factors." *Pathogens* 12, no. 12: 1410. https://doi.org/10.3390/pathogens12121410.

Estrada, A., P. A. Garber, A. B. Rylands, et al. 2017. "Impending Extinction Crisis of the World's Primates: Why Primates Matter." *Science Advances* 3, no. 1: e1600946. https://doi.org/10.1126/sciadv.1600946.

Genton, L., P. D. Cani, and J. Schrenzel. 2015. "Alterations of Gut Barrier and Gut Microbiota in Food Restriction, Food Deprivation and

Protein-Energy Wasting." *Clinical Nutrition* 34, no. 3: 341–349. https://doi.org/10.1016/j.clnu.2014.10.003.

Gonçalves, B. A., L. C. P. Lima, and L. M. Aguiar. 2022. "Diet Diversity and Seasonality of Robust Capuchins (*Sapajus* sp.) in a Tiny Urban Forest." *American Journal of Primatology* 84, no. 8: e23396. https://doi.org/10.1002/ajp.23396.

Hall, C. L., and L. M. Fedigan. 1997. "Spatial Benefits Afforded by High Rank in White-Faced Capuchins." *Animal Behaviour* 53, no. 5: 1069–1082. https://doi.org/10.1006/anbe.1996.0392.

Harrison, R. A., and E. Van De Waal. 2022. "The Unique Potential of Field Research to Understand Primate Social Learning and Cognition." *Current Opinion in Behavioral Sciences* 45: 101132. https://doi.org/10.1016/j.cobeha.2022.101132.

Henriquez, M. C., F. A. Campos, and H. Hamou, et al. 2025. "Temperature and Rainfall Affect Patterns of Parasitism in Free-Ranging Capuchin Monkeys (*Cebus imitator*)." *American Journal of Primatology* 88: e70111. https://doi.org/110.1002/ajp.70111.

Henriquez, M. C., H. Hamou, J. Churcher, et al. 2025. "Gastrointestinal Parasites in Wild Capuchins (*Cebus imitator*): Insights From Morphology, Infection Patterns, and Progress in Molecular Identification." *International Journal of Primatology* 46, no. 2: 475–500. https://doi.org/10.1007/s10764-024-00467-x.

Heston, M. B., K. L. Hanslik, K. R. Zarbock, et al. 2023. "Gut Inflammation Associated With Age and Alzheimer's Disease Pathology: A Human Cohort Study." *Scientific Reports* 13, no. 1: 18924. https://doi.org/10.1038/s41598-023-45929-z.

Higham, J. P., C. Kraus, C. Stahl-Hennig, A. Engelhardt, D. Fuchs, and M. Heistermann. 2015. "Evaluating Noninvasive Markers of Nonhuman Primate Immune Activation and Inflammation." *American Journal of Physical Anthropology* 158, no. 4: 673–684. https://doi.org/10.1002/ajpa.22821.

Hogan, J., and A. D. Melin. 2018. "Intra- and Interannual Variation in the Fruit Diet of Wild Capuchins: Impact of Plant Phenology." In *Primate Life Histories, Sex Roles, and Adaptability*, edited by U. Kalbitzer and K. M. Jack, 193–212. Springer. https://doi.org/10.1007/978-3-319-98285-4_10.

Hooijberg, E. H., and C. Cray. 2023. "Acute Phase Reactants in Nondomesticated Mammals—A Veterinary Clinical Pathology Perspective." *Veterinary Clinical Pathology* 52, no. S1: 19–36. https://doi.org/10.1111/vcp.13189.

Jacob, E. A. 2016. "Hematological Differences in Newborn and Aging: A Review Study." *Hematology & Transfusion International Journal* 3, no. 3: 178–190. https://doi.org/10.15406/htij.2016.03.00067.

Kasvosve, I., Z. A. R. Gomo, I. T. Gangaidzo, et al. 2000. "Reference Range of Serum Haptoglobin Is Haptoglobin Phenotype-Dependent in Blacks." *Clinica Chimica Acta* 296, no. 1–2: 163–170. https://doi.org/10.1016/S0009-8981(00)00225-4.

Lai, I. H., K. Y. Lin, M. Larsson, et al. 2008. "A Unique Tetrameric Structure of Deer Plasma Haptoglobin—An Evolutionary Advantage in the Hp 2-2 Phenotype With Homogeneous Structure." *FEBS Journal* 275, no. 5: 981–993. https://doi.org/10.1111/j.1742-4658.2008.06267.x.

Lei, D., S. Hu, and M. Guo, et al. 2023. "Genotype-Specific Reference Interval of Haptoglobin Tests in a Chinese Population on the BN II System." *Scientific Reports* 13, no. 1: 577. https://doi.org/10.1038/s41598-022-20496-x.

Lopresti-Goodman, S. M., and B. Villatoro-Sorto. 2022. "The Benefits and Challenges of Conducting Primate Research in Different Settings." *Animals* 13, no. 1: 133. https://doi.org/10.3390/ani13010133.

Lucore, J. M., A. J. Marshall, S. F. Brosnan, and M. E. Benítez. 2022. "Validating Urinary Neopterin as a Biomarker of Immune Response in Captive and Wild Capuchin Monkeys." *Frontiers in Veterinary Science* 9: 918036. https://doi.org/10.3389/fvets.2022.918036.

McFarlane, D., R. F. Wolf, K. A. McDaniel, and G. L. White. 2012. "The Effect of Season on Inflammatory Response in Captive Baboons." *Journal of Medical Primatology* 41, no. 6: 341–348. https://doi.org/10.1111/j.1600-0684.2012.00560.x.

Melin, A. D., C. Hiramatsu, N. A. Parr, Y. Matsushita, S. Kawamura, and L. M. Fedigan. 2014. "The Behavioral Ecology of Color Vision: Considering Fruit Conspicuity, Detection Distance and Dietary Importance." *International Journal of Primatology* 35: 258–287. https://doi.org/10.1007/s10764-013-9730-8.

Melin, A. D., H. C. Young, K. N. Mosdossy, and L. M. Fedigan. 2014. "Seasonality, Extractive Foraging and the Evolution of Primate Sensorimotor Intelligence." *Journal of Human Evolution* 71: 77–86. https://doi.org/10.1016/j.jhevol.2014.02.009.

Mitchell, E. L., A. T. Davis, K. Brass, et al. 2017. "Reduced Intestinal Motility, Mucosal Barrier Function, and Inflammation in Aged Monkeys." *Journal of Nutrition, Health & Aging* 21, no. 4: 354–361. https://doi.org/10.1007/s12603-016-0725-y.

Mohamad, N.-V., S. K. Wong, W. N. Wan Hasan, et al. 2019. "The Relationship between Circulating Testosterone and Inflammatory Cytokines in Men." *Aging Male* 22, no. 2: 129–140. https://doi.org/10.1080/13685538.2018.1482487.

Muniz, L., S. Perry, J. H. Manson, H. Gilkenson, J. Gros-Louis, and L. Vigilant. 2010. "Male Dominance and Reproductive Success in Wild White-Faced Capuchins (Cebus capucinus) at Lomas Barbudal, Costa Rica." *American Journal of Primatology* 72, no. 12: 1118–1130. https://doi.org/10.1002/ajp.20876.

Naryzhny, S. N., and O. K. Legina. 2021. "Haptoglobin as a Biomarker." *Biomeditsinskaya Khimiya* 67, no. 2: 105–118. https://doi.org/10.18097/pbmc20216702105.

Niu, Y., M. Heddes, B. Altaha, et al. 2024. "Targeting the Intestinal Circadian Clock by Meal Timing Ameliorates Gastrointestinal Inflammation." *Cellular & Molecular Immunology* 21, no. 8: 842–855. https://doi.org/10.1038/s41423-024-01189-z.

Norconk, M. A., S. Atsalis, G. Tully, et al. 2020. "Reducing the Primate Pet Trade: Actions for Primatologists." *American Journal of Primatology* 82, no. 1: e23079. https://doi.org/10.1002/ajp.23079.

Orkin, J. D., F. A. Campos, M. S. Myers, S. E. Cheves Hernandez, A. Guadamuz, and A. D. Melin. 2019. "Seasonality of the Gut Microbiota of Free-Ranging White-Faced Capuchins in a Tropical Dry Forest." *ISME journal* 13, no. 1: 183–196. https://doi.org/10.1038/s41396-018-0256-0.

Quaye, I. K. 2008. "Haptoglobin, Inflammation and Disease." *Transactions of the Royal Society of Tropical Medicine and Hygiene* 102, no. 8: 735–742. https://doi.org/10.1016/j.trstmh.2008.04.010.

R Core Team. 2024. *R: A Language and Environment for Statistical Computing* (Version 4.4.2) [Computer software]. R Foundation for Statistical Computing. https://R-project.org/.

Rondón, S., M. Ortiz, C. León, N. Galvis, A. Link, and C. González. 2017. "Seasonality, Richness and Prevalence of Intestinal Parasites of Three Neotropical Primates (*Alouatta seniculus*, *Ateles hybridus* and *Cebus versicolor*) in a Fragmented Forest in Colombia." *International Journal for Parasitology: Parasites and Wildlife* 6, no. 3: 202–208. https://doi.org/10.1016/j.ijppaw.2017.07.006.

Sato, K., M. Hara-Chikuma, M. Yasui, J. Inoue, and Y.-G. Kim. 2024. "Sufficient Water Intake Maintains the Gut Microbiota and Immune Homeostasis and Promotes Pathogen Elimination." *iScience* 27, no. 6: 109903. https://doi.org/10.1016/j.isci.2024.109903.

Shiotani, A., K. Tarumi, K. Honda, et al. 2014. "Application of Fecal Hemoglobin–Haptoglobin Complex Testing for Small Bowel Lesions." *Scandinavian Journal of Gastroenterology* 49, no. 5: 539–544. https://doi.org/10.3109/00365521.2014.891260.

Simpson, G. 2024. gratia: Graceful ggplot-Based Graphics and Other Functions for GAMs Fitted Using mgcv *(Version 0.10.0) [Computer software]*. R Foundation. https://gavinsimpson.github.io/gratia/.

Thompson, C. L., S. H. Williams, K. E. Glander, M. F. Teaford, and C. J. Vinyard. 2014. "Body Temperature and Thermal Environment in a Generalized Arboreal Anthropoid, Wild Mantled Howling Monkeys (*Alouatta palliata*)." *American Journal of Physical Anthropology* 154, no. 1: 1–10. https://doi.org/10.1002/ajpa.22505.

Vicente, J., J. Martinez-Guijosa, A. Tvarijonaviciute, et al. 2019. "Serum Haptoglobin Response in Red Deer Naturally Infected With Tuberculosis." *Comparative Immunology, Microbiology and Infectious Diseases* 64: 25–30. https://doi.org/10.1016/j.cimid.2019.01.021.

Wan, B.-N., S.-G. Zhou, M. Wang, X. Zhang, and G. Ji. 2021. "Progress on Haptoglobin and Metabolic Diseases." *World Journal of Diabetes* 12, no. 3: 206–214. https://doi.org/10.4239/wjd.v12.i3.206.

Wood, S. N. 2011. "Fast Stable Restricted Maximum Likelihood and Marginal Likelihood Estimation of Semiparametric Generalized Linear Models." *Journal of the Royal Statistical Society Series B: Statistical Methodology* 73, no. 1: 3–36. https://doi.org/10.1111/j.1467-9868.2010.00749.x.

Xu, L., G. Huang, Y. Cong, Y. Yu, and Y. Li. 2022. "Sex-Related Differences in Inflammatory Bowel Diseases: The Potential Role of Sex Hormones." *Inflammatory Bowel Diseases* 28, no. 11: 1766–1775. https://doi.org/10.1093/ibd/izac094.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Figure S1: Comparison of HOBO and Kestrel temperature measurements.

Figure S2: Assessment of assay parallelism using serial dilutions of a pooled sample.

Figure S3: Recovery of the assay standard in a pooled sample matrix across serial dilutions.