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2016

A Coprological Survey of Golden Mantled Howler Monkeys (*Alouatta palliata palliata*) in the Osa Peninsula, Costa Rica

Jess M. Conatser conatser@marshall.edu

## A COPROLOGICAL SURVEY OF GOLDEN MANTLED HOWLER MONKEYS (ALOUATTA PALLIATA PALLIATA) IN THE OSA PENINSULA, COSTA RICA

A thesis submitted to the Graduate College of Marshall University in partial fulfillment of the requirements for the degree of Master of Science In Biological Sciences: Organismal, Evolutionary and Ecological Biology by Jess M. Conatser

> Approved by Dr. Anne Axel, Committee Chairperson Dr. Shane Welch Kimberly Dingess

> > Marshall University December 2016

#### APPROVAL OF THESIS/DISSERTATION

We, the faculty supervising the work of Jessyca Mariah Conatser, affirm that the thesis, A Coprological Survey of the Golden Mantled Howler Monkey (Alouatta palliata palliata) in the Osa Peninsula, Costa Rica, meets the high academic standards for original scholarship and creative work established by the Biological Sciences Program and the College of Science. This work also conforms to the editorial standards of our discipline and the Graduate College of Marshall University. With our signatures, we approve the manuscript for publication.

Anne CArd

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UL.

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15 December 2016

**Committee Chairperson** 

Committee Member

Date

Dec. 15, 2016

Committee Member

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## ABSTRACT

Interactions between free-ranging primates and their associated parasitic forms continue to be a point of interest in ecology for several reasons. External and internal parasitism is not atypical for wild populations of primates, and the repercussions of these relationships can range from benign to life-threatening and capable of altering the structure of naturally occurring groups. The ecological relationship between the golden mantled howler monkey (*Alouatta palliata palliata*) and its host-specific ectoparasite, the howler monkey bot fly (*Alouattamyia baeri*) poses significant potential detriment to the overall body quality and success of the host primate. *A. p. palliata* has also been recorded as a host to a number of gastrointestinal parasites. We suspect that the colonization of a primate by one parasite species may prove to diminish host condition and increase susceptibility for additional parasitic establishment.

The objectives of this research project are to (1) measure the density and species richness of both external and internal parasites inhabiting golden-mantled howler monkeys, (2) provide information on the overall parasite load for howler monkeys in a protected region of Costa Rica, (3) provide information on the relative densities of internal parasites in the presence or absence of howler monkey bot flies, and (4) document any existing relationships between parasite presence, density, and species richness as a function of host primate demographics including age class and sex.

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#### Chapter I

## INTRODUCTION

Relationships between host animals and their parasitic counterparts are variable and a popular topic of interest in many fields—parasitism of free-ranging animals is frequently studied in the interest of assessing zoonotic disease risk to humans [Helenbrook et al., 2015b]. Understanding the interactions between primate health and their environment can also be of critical importance when assessing a number of ecological or anthropological interactions. Interactions between primates and parasites can often illuminate connections greater than what is readily apparent, including deviations in host behavior, survival, and any number of environmental relationships including fragmentation and climate change [Lafferty, 2009; Cristóbal-Azkarate et al., 2010; Helenbrook et al., 2015]. Howler monkeys of the genus *Alouatta* have been documented as hosts to a variety of both external and internal parasites, including but not limited to the howler monkey botfly (*Alouattamyia baeri*), protozoan coccidian oocysts, threadworms, and roundworms of the phylum Nematoda [Milton, 1996; Phillips et al., 2004; Cristóbal-Azkarate et al., 2010; Behie et al., 2014].

Previous studies concerning external parasitism in particular have determined that the burdens of ectoparasites devastate the overall health and body quality of primate hosts. Evidence has been provided suggesting that persistent bot fly infestations place significant ecological and physiological stress on howler monkeys, as these parasites seek to extract as much nutrition and energy possible without directly killing the host [Milton, 1996; Kowalewski & Zunino, 2005]. External infestations can result in a loss of host blood and tissue, stunted host growth, lowered reproductive

potential, and increased risk of mortality due to secondary infections [Milton, 1996; Colwell, D. D., Milton, 1998]. Internal parasites have also been documented as a determinant in the growth and reproductive success of free-ranging primates, and transmission is thought to be especially likely for primates that exist in close social groups or limited spaces [Stoner, 1996].

To better understand how parasitism affects primates in a geographic area, it is necessary to collect baseline data on the parasites present in a given population. This investigation aims to record and evaluate the occurrence of parasites co-existing on and within the golden mantled howler monkey (*Alouatta palliata palliata*) populaces surrounding Estación Biológica Piro. The primary interest of this study was to evaluate parasite presence, richness, and density among howler monkeys in a tropical humid forest.

#### **Parasite Behavior Hypothesis**

A secondary interest of this study was to record primate arboreal stratification data during daily activity and to assess that information as it applies to the parasite behavior hypothesis [Kowalewski & Zunino, 2005]. It has been evidenced that howler monkeys, particularly those restricted to forest patches, are susceptible to parasite infection and re-infection due to space limitations and proximity [Kowalewski & Zunino, 2005]. Several hypotheses have been offered to suggest mitigation strategies that primates may use to decrease the likelihood of initial or repeated parasitic infection. One of these hypotheses proposes that howler monkeys will select sites for feeding, resting, and travelling that are different from those where they defecate—in particular, this study investigates the suggestion that howlers will defecate from lower branches than the

ones they use for other activities. The ideology behind this hypothesis is that defecating at a lower vertical site will decrease the likelihood of contaminating feeding, resting, or travelling strata with potentially parasitized feces [Kowalewski & Zunino, 2005].

In this study, I expected that female howlers would exhibit a higher rate of internal parasitism than males because of their frequent proximity to offspring and other female monkeys. Female howler monkeys have been observed to attempt alliance establishment with other females, likely in an effort to create a space for themselves in a new social group and to increase the likelihood of reproductive success [Pope, 2000]. Additionally, female howlers practice cooperative infant care [Silk, 2007]. This hypothesis was rejected previously by K.E. Stoner (1996) when she investigated the parasites of pregnant and lactating howler females in Northeastern Costa Rica. Stoner subsequently called for additional research to investigate sexual differences in hosts infected by internal parasites, particularly with larger samples of both male and female monkeys. This study aims in part to contribute to the available information on sexual differences in host parasite richness and density as it applies to Southwestern Costa Rica. Rica.

Secondly, I hypothesized that the primates that were externally parasitized would have a higher density of internal parasites than those with no external parasites. During larval development, howler monkey bot flies rely on discretion and tactical growth to avoid extreme host irritation or premature death. I suspect that this type of larval development may diminish the quality of host health or immune strength over time, potentially increasing the primates' susceptibility to additional parasite infestation, external or otherwise.

Information collected on parasite diversity and prevalence in a protected forest will be useful in studies concerning primate body condition as it relates to parasite presence. A better understanding of inter-parasite relationships, parasite avoidance strategies, and host selection may serve to promote the better management and continued success of this and other non-human primate species.

## Ecology of Alouatta

Golden mantled howler monkeys (Alouatta palliata palliata) are large-bodied New World primates, and one of four subspecies of howler monkey (Alouatta palliata) endemic to Central and South America. Howler monkeys live in multi-male multi-female social groups that are subject to near constant change. They are polygynous breeders and both sexes have been documented to disperse from their natal groups as juveniles or adults [Glander, 1980]. In the past, howler groups have been considered to be comprised of non-related adult males and females along with their offspring [Clarke et al., 1998]. More recent evidence has suggested that males in a group may be closer in kinship than previously thought—this relatedness within groups may aid resource and mate acquisition for allied males [Milton et al., 2016]. Mantled howler social composition reflects these mating and dispersal behaviors as there is typically a maximum of four female howlers to a single male with an overall group composition of 1-3 adult males to 5-10 adult females [Fedigan & Jack, 2001; DeGama-Blanchet, H. N., Fedigan, 2006]. Howler monkeys have also been documented to frequent edge habitat and territorial buffer zones. In Costa Rica where ecotourism is a major economic stimulant, monkeys are often in close proximity to humans and agricultural areas [Estrada et al., 2002; Daily et al., 2003; DeGama-Blanchet, H. N., Fedigan, 2006]. In addition, the proximity of

humans and domestic animals to wildlife such as the howler monkey can facilitate parasite and pathogen transmission between groups, particularly when howlers are found in degraded landscapes [Phillips et al., 2004; Helenbrook et al., 2015].

Mantled howlers exhibit a great degree of dietary plasticity, being opportunistically frugivorous and regularly folivorous—feeding on leaves to varying degrees at different times of year or depending on geographic location [Estrada & Coates-Estrada, 1996; Silver et al., 1998]. Because their diet is often comprised primarily of leaves, howler monkeys spend more than half of each day (approximately 61.2%) resting [Silver et al., 1998; Cristobal-Azkarate, J., Arroyo-Rodriguez, 2007]. Despite being largely folivorous, the golden mantled howler also serves as a dispersal agent for plants and facilitates seed germination [Estrada & Coates-Estrada, 1996].

## **Documented Parasites of Alouatta**

The howler monkey bot fly (*Alouattamyia baeri*) is monospecific member of the subfamily Cuterebrinae. Cuterebrids include large-bodied, myiatic flies which depend on a mammalian host for nutrition over the duration of their larval stage (Figure 1.1). Female Cuterebrid flies do not always deposit their eggs directly on a host; rather, they secure their ova onto intermediate mosquito or fly vectors. When these intermediate vectors land on a primate, the host's body temperature induces larval emergence and burrowing [Milton, 1996]. *A. baeri* is unique in that it exclusively relies on the subcutaneous tissue of hosts within the genus *Alouatta* to sustain its growth and development. This particular species of bot fly is endemic to the Neotropic ecozone.

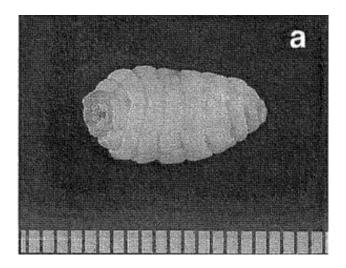


Figure 1.1 Larval Alouattamvia baeri (3<sup>rd</sup> instar), Scale bar = 1mm (Colwell & Milton) Howler monkey bot flies pose significant potential threat to the overall body quality of the host primate and may hinder population growth [Milton, 1996]. Cuterebrid bot flies characteristically rely on a single host throughout their larval development. This discretion is thought to facilitate parasite transmission and continued bot fly growth while avoiding parasite removal or premature host elimination [Frank, 1996]. Cuterebrid larvae such as the howler monkey bot fly burrow into existing abscesses or may simply penetrate the host animal's skin (Figure 1.2), developing subcutaneously for three instars before exiting at larval maturity [Milton, 1996]. The host serves as a source of nutrition and protection for the six weeks of bot fly development. The presence of these larval parasites can have severe and lasting physiological effects on the howler monkey [Milton, 1996; Stuart et al., 1998]. The ectoparasite larvae are capable of reducing their host's physical condition via blood loss and tissue damage, potentially reducing the host's reproductive potential and offspring viability [Milton, 1996]. These types of infestations often predispose the host to secondary infections and in some casesparticularly in young monkeys-induce mortality.

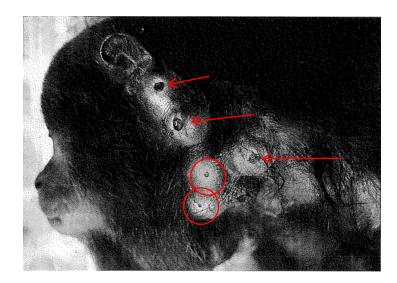


Figure 1.2 Adult male Panamanian howler monkey exhibiting five occupied warbles of *Alouattamyia baeri*. Three late 3<sup>rd</sup> instar (arrows) and two early 3<sup>rd</sup> instar warbles (circles) (Milton, 1995).

*Necator americanus* (New World Hookworm) is a soil-dwelling parasitic helminth that relies on warm, moist soil conditions. *N. americanus* is often thought to exclusively parasitize humans, but other mammals, including several species of non-human primate such as chimpanzees and Patas monkeys, harbor this parasite as well [Ghai, Chapman, Omeja, Davies, Goldberg, 2014]. This species of hookworm is widely distributed, spanning Central and South America, Africa, China, and Southeast Asia.

Hookworms are typically transmitted via eggs in fecal matter or through contaminated soils and substrates [May & Anderson]. Hosts of helminth worms can experience physiological repercussions including anemia, diarrhea and accompanying discomfort of the digestive system, and potentially diminished cognitive function [Martinez-Mota, 2015].

*Enterobius vermicularis* is a small nematode that functions as the contributing species of enterobiasis [Cook, 1994]. Commonly referred to as the threadworm or pinworm, *E. vermicularis* is normally considered a nuisance parasite rather than a

severe disease causing agent. Infection results from direct contact with the perianal region, contaminated digits, or the ingestion of nematode eggs, either through contaminated food or water [Cook, 1994]. The most commonly recognized effect of these parasites relate to their migration habits which cause a condition commonly called *priuritis ani*: irritation of the anus and surrounding areas.

## MATERIALS AND METHODS

## **Study Site**

Estación Biológica de Piro (08°24.12' N, 83°20 W) is located on the southern Pacific coast of Costa Rica's Osa Peninsula [Singletary, 2013]. The forested area surrounding EBP is composed of tropical wet forest and mature tropical forest mixed with agricultural areas, livestock pastures, and sparse residential areas [Sanchez-Azofeifa et al., 2002]. The study site is comprised of 1,700 hectares of mixed primary and secondary forest with an elevation ranging from sea level beaches and riparian areas to 300 meters above sea level (Osa Conservation). Annually, the Osa Peninsula receives about 5,500mm of rainfall; because this region has little variability in temperature, with a yearly average of 27 °C, seasons are marked by amount of rainfall [Roberts, N., Fanning, E., Walters, H., Levac, A., Morris, A. Carpani, 2013]. My study took place during the area's first annual rainy season (July-August 2015), which is characterized by more frequent rainfall and comparatively high relative humidity [Frankie, G. W., Baker, H. G., Opler, 1974].

## **Parasite Sampling**

We conducted gross examination surveys to inspect three groups of howler monkeys at my study site for external bot fly lesions. In addition, we collected fecal samples to quantify endoparasite ova and calculated parasite richness and density for each primate. Here, we define *richness* as the count of parasite species and *density* as the relative abundance of parasite species. We gathered photographs or field notes on each monkey so that we could consider covariates such as age class and sex during data analysis.

Age class determination was an important identifier for this study. We split monkeys into the adult or sub adult age class based on physical characteristics. This was determined in several ways: for males, a larger body size and developed mane around the head and throat were recognized as adult characteristics. Females are smaller than males of the same age so we relied on genital physiognomies and observed relationships with infant and juvenile monkeys to determine age class. Females reach sexual maturity at 36 months and typically produce their first offspring around 42 months of age—a female that habitually carried an infant or that exhibited genital swelling and color variability was classified as an adult [Glander, 1980].

### Visual Assessment of Bot Fly Presence

The best way to determine if a monkey was presently parasitized by bot flies was through visual examinations. Bot fly larva typically burrow into their host near the face, chest, or neck and remain there for roughly six weeks. Warbles or raised lumps in the areas of interest were interpreted as positive IDs for bot fly presence. In all, a total of 19 monkeys were visually examined and analyzed.

## **Collection of Stratification Data**

During behavioral events (Defecation and Feeding) we collected positional data based on the focal primate's arboreal location. Using a Bushnell Yardage Pro Sport 450 range finder, we measured the distance of the primate from the ground.

## **Collection of Fecal Samples**

We followed each of three focal groups for 6-12 hours during mornings and early afternoons (0400-1700). Fecal collection was possible immediately after defecation events, which typically occur after feeding bouts or long periods of inactivity. Following each observed defecation event, we collected fecal samples from individuals that could be positively identified by age class and sex. After primate identification, we immediately collected the sample in a plastic bag and labelled the bag with the primate ID, time, and GPS waypoint [Eckert et al., 2006; Cristóbal-Azkarate et al., 2010]. The time from sample collection to sample preservation ranged from one hour to eight hours.

Data collected were only considered usable if the individual monkey was able to be identified by age class, and sex [Eckert et al., 2006]. We could often find coloration or pattern differences between individuals of the same age category and sex to serve as individual identifiers. If we collected multiple samples from the same individual over the six-week sampling period, we processed and scanned each fecal sample. During statistical organization and analysis, any multiple samples were averaged to get the mean parasite count—standard deviation for all individuals was recorded to account for sampling bias.

## **Treatment and Processing of Fecal Samples**

Following data collection on each day of field work, we took bagged fecal samples to the Estación Biológica Piro lab station. Reproducing the methodology of previous coprological studies, we preserved each sample in a 10% formalin solution [Dryden et al.; Gillespie et al., 2004; Trejo-Macías et al., 2007; Parr et al., 2013]. We filled 45ml Falcon tubes with 20ml 10% formalin and added feces until the sample measured 25ml [Cristóbal-Azkarate et al., 2010]. After placing samples into the fixative solution, we labeled and stored them in a sealed container.

We processed the preserved samples in the parasitology laboratory at Marshall University located in Huntington, West Virginia. Following the methodology of Trejo-Macías et al., 2007, we used a saturated sodium solution to separate parasite eggs by their specific gravity (sp. gr. 1.05-1.20) from digested material and sediment (NaCl sp. gr. 1.20) [Dryden et al.]. We filtered a small amount of feces through a cheesecloth screen to remove sediment and incompletely digested material. We combined the screened sample with a saturated sodium solution in a straight wall vial, adding solution until a positive meniscus formed at the top of the vial. We then placed a coverslip over the meniscus and waited 15 minutes for the floatation process to complete. We systematically scanned each 22x22mm coverslip at 10x (150x)—any potential ova were looked at under 40x (600x) magnification. We measured any ova that we found and compared them to standard references, using size and shape description to place eggs as narrowly as possible, usually into a family or genus.

#### RESULTS

## Group Pooling (G test of goodness-of-fit)

While the three focal howler groups in our study may be considered socially independent, we cannot say with certainty that there is spatial independence between them. In order to best detect trends in our complete data set and to compensate for the small values associated with our data sets, we conducted a G test of goodness-of-fit to assess whether the three focal groups could be pooled by age class and sex. We determined that these three groups were homogeneous enough in both sex and age class composition to be pooled and analyzed together (Pooled P-Values of 0.467 and 0.850, respectively).

## Assessment of Normality

After pooling data for sex composition and age class, we conducted tests to determine whether these data followed a normal distribution. Our probability plot of richness gave a P-Value of 0.011 and our probability plot for density resulted in a P-Value of <0.010, each indicating that our data do not follow a normal distribution. Due to the lack of normality, we were unable to further test our data with T-Tests.

## Visual Assessment of Bot Fly Presence

We observed apparent warbles on two of 19 howler monkeys among our three focal groups. We defined warbles as raised bumps or lesions anywhere on the body of the primate, with special attention to common areas of infestation such as the neck and chest of monkeys. Each of the primates parasitized by bot flies was a sub adult female belonging to the third focal group (Group C).

## **Analysis of Vertical Stratification Data**

We created histograms to look at the trends in arboreal space utilization by behavior (defecating or feeding) in five meter vertical ranges (Figures 2.2 and 2.3). Because the data were collected *ad libitum*, there were not enough observations to conduct a true statistical measure of comparison between behavioral stratification.

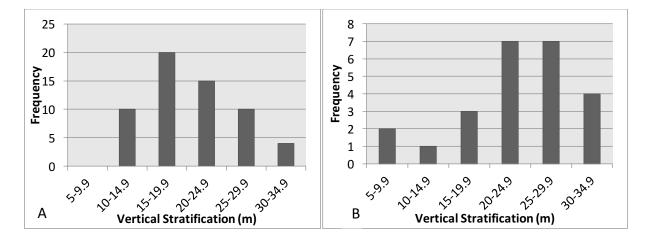


Figure 2.2 Vertical stratification of howlers during (A) Defecation events Figure 2.3 Vertical stratification of howlers during (B) Feeding events

## **Analysis of Fecal Samples**

We analyzed a total of 37 fecal samples (19 individuals) from three groups of monkeys. We assumed that each sample from an individual was not independent from the next, even though multiple samples were not collected from an individual on the same day. The mean parasite count for all fecal samples was 5.22 (+/- 8.58) with a minimum count of 0.00 and a maximum count of 30.50 (Minitab 17 Statistical Software, 2016).

		Coccidian	Strongylid	Enterobius	Necator	Alouattamyia
	n	oocyst	oocyst	vermicularis	americanus	baeri
Adults	14	74.8	3	4.4	1	0
Subadults	5	15.53	0	1.67	0	2
Males	3	30.67	1	0	0	0
Females	16	59.66	2	6.07	1	2
Total	19	90.33	3	6.07	1	2

Table 1.1 Parasite count by host demographic

The majority of internal parasites identified through coprological sampling and microscopic assessment were coccidian oocysts (90.33 occurrences), *Enterobius vermicularis* was the second most frequent parasite observed in these fecal samples (6.07 occurrences). Strongylid (nematode) oocysts were observed three times, *Alouattamyia baeri* appeared twice, and *Necator americanus* was observed only once.

## DISCUSSION

This study was successful in that we were able to collect some information on the internal and external parasites present in a previously unstudied group of golden mantled howler monkeys. Due to low power, however, we were unable to assess much of the collected data with intended statistical assessments including generalized linear models. It is surprising that we observed so few botfly infestations given the number of individuals surveyed. Given the regularity of bot fly studies in howler monkeys, we expected to observe warbles in several primates over the course of this study. One potential explanation for the low bot fly density may be seasonality—local residents mentioned that they had observed lesions on the area's monkeys in previous years but that there seemed to be a greater prevalence in the dry season. It should be noted that bot flies were only documented for Group C, which leads us to believe there may be a geographic component that should be considered in future studies. Since bot flies

typically use mosquito vectors to transport their eggs, it can be hypothesized that groups of monkeys with a range encompassing more stagnant water bodies may be at greater risk of contracting bot fly larva. Geographic data was not a crucial point in this study but it may be worthwhile to investigate territories of Estación Biológica Piro's howlers and to investigate differences in habitat. There is sufficient evidence to suggest that fragmented or altered habitats increase the risk of primate exposure to internal and external parasites [Behie et al., 2014]. The howlers investigated in this study may be largely unaffected by external parasites due to their placement in a largely continuous tract of forest. Comparing the howler monkeys in this study to a group of similar composition in a fragmented or otherwise altered landscape would likely reveal significant differences in parasitism.

Another variable that may be useful in determining significant relationships related to parasite load is the frequency of physical contact or grooming behaviors for the golden mantled howler monkey. Howler troops do not usually intermingle—they maintain separation from one another almost exclusively through territorial calls. Physical aggression between members of this species has been very rarely documented [Martinez-Mota, 2015]. For this reason, we can expect that there will be no transmission of parasite forms between groups due to physical interaction. However, we cannot say with certainty that these primates do not share physical space or that there is not spatial overlap due to monkeys using the same trees or strata at different times.

Studies regarding the social grooming behaviors of the mantled howler monkey are few and existing documents suggest that allogrooming and autogrooming is infrequent if it occurs at all [Milton, 1996]. For other species of howlers, hierarchies and

grooming roles have been more solidly established. Female brown howler monkeys have been observed to groom significantly more often than males and black howler males are recorded to groom females more often during ovarian cycles in an effort to establish positive relationships with potential reproductive partners [Chiarello, 1995; Van Belle et al., 2009]. The ladder of authority in mantled howler monkeys is generally understood as young adults holding more dominant positions and older adults in more submissive roles. Based on the recorded behavior of other howler species, we can predict that those in a higher position of dominance would groom subordinate individuals [Glander, 1980; Nunn et al., 2003]. First instar bot fly larvae have been described as minute and are unlikely to be physically removed before they burrow. For this reason, it cannot be said with certainty if monkeys that are more frequently groomed would have a lower parasite load. Significantly more research on the hierarchy, contract frequency, and social grooming behavior of *A. palliata* is necessary, especially as it pertains to parasitic density.

Our results appear to indicate a trend in vertical stratification—it looks as though golden mantled howlers use lower strata when defecating compared to the strata used when feeding. Low statistical power, unequal variance, and potentially skewed observations prevent a true determination of stratification trends in this particular study. Because we conducted stratification data collection according to *ad libitum* sampling techniques, there is an unintentional bias of data points during defecation events compared to feeding events. Future studies should conduct scan sampling techniques to collect more consistent group data on vertical stratification during behavioral events. In addition, there may be some benefit to collecting horizontal stratification information

as this would allow a three-dimensional understanding of arboreal space use during behavioral observations.

The interconnection between wildlife, parasites, humans, and the environment in which they coexist is irrefutable. Assessments of assorted relationships between freeranging primates and their environments are necessary if we wish to understand these complex connections and their implications. Parasite ecology, socioecology, and ethnoprimatology are three of many potential approaches that have been used to assess primate interconnectedness, function, and wellness in a region [Loudon et al., 2005]. Future studies concerning the golden mantled howler, their physical interactions with group members, utilization of arboreal space, and variability of parasite load in different habitat types may prove to illuminate aspects of host-parasite dynamics that have been previously overlooked.

## REFERENCES

- Anderson, R. C. Nematode Parasites of Vertebrates: Their Development and Transmission. Wallingford, Oxon, UK: CABI Pub, 2000. Web.
- Behie AM, Kutz S, Pavelka MS. 2014. Cascading Effects of Climate Change: Do Hurricane-damaged Forests Increase Risk of Exposure to Parasites? *Biotropica* 46:25–31.
- Van Belle S, Estrada A, Ziegler TE, Strier KB. 2009. Sexual behavior across ovarian cycles in wild black howler monkeys (*Alouatta pigra*): Male mate guarding and female mate choice. *American Journal of Primatology* 71:153–164.
- Chiarello AG. 1995. Grooming in brown howler monkeys, Alouatta fusca. American *Journal of Primatology* 35:73–81.
- Clarke MR, Glander KE, Zucker EL. 1998. Infant-nonmother interactions of free-ranging mantled howlers (*Alouatta palliata*) in Costa Rica. *International Journal of Primatology* 19:451–472.
- Colwell, D. D., Milton K. 1998. Development of Alouattamyia baeri (Diptera: Oestridae) from Howler Monkeys (Primates: Cebidae) on Barro Colorado Island, Panama. *Entomological Society of America* 35:674–679.
- Cook GC. 1994. Tropical infection of the gastrointestinal tract and liver series. *Journal of Parasitology*, The 35:1159–1162.

Cristobal-Azkarate, J., Arroyo-Rodriguez V. 2007. Diet and Activity Pattern of Howler Monkeys (*Alouatta palliata*) in Los Tuxtlas, Mexico: Effects of Habitat
Fragmentation and Implications for Conservation. *American Journal of Primatology* 69:1013–1029.

- Cristóbal-Azkarate J, Hervier B, Vegas-Carrillo S, et al. 2010. Parasitic infections of three Mexican howler monkey groups (*Alouatta palliata mexicana*) living in forest fragments in Mexico. *Primates* 51:231–239.
- Daily GC, Ceballos G, Pacheco J, Suzán G, Sánchez-Azofeifa A. 2003. Countryside
   Biogeography of Neotropical Mammals: Conservation Opportunities in Agricultural
   Landscapes of Costa Rica. *Conservation Biology* 17:1814–1826.
- DeGama-Blanchet, H. N., Fedigan LM. 2006. The Effects of Forest Fragment Age, Isolation, Size, Habitat Type, and Water Availability on Monkey Density in a Tropical Dry Forest. In: New Perspectives in the Study of Mesoamerican Primates. New York: Springer. p 165–188.
- Dryden MW, Payne PA, Ridley R, Smith V. Comparison of Common Fecal Flotation Techniques for the Recovery of Parasite Eggs and Oocysts\*. :15–28.
- Eckert KA, Hahn NE, Genz A, et al. 2006. Coprological surveys of Alouatta pigra at two sites in Belize. *International Journal of Primatology* 27:227–238.
- Estrada A, Coates-Estrada R. 1996. Tropical rain forest fragmentation and wild populations of primates at Los Tuxtlas, Mexico. *International Journal of Primatology* 17:759–783.
- Estrada A, Mendoza A, Castellanos L, et al. 2002. Population of the black howler monkey (Alouatta pigra) in a fragmented landscape in Palenque, Chiapas, Mexico. *American Journal of Primatology* 58:45–55.
- Fedigan LM, Jack K. 2001. Neotropical primates in a regenerating Costa Rican dry forest: A comparison of howler and capuchin population patterns. *International Journal of Primatology* 22:689–713.

Frank SA. 1996. Models of Parasite Virulence. Quarterly Review of Biology 71:37–78.

- Frankie, G. W., Baker, H. G., Opler PA. 1974. Frankie 1974.pdf. *Journal of Ecology* [Internet] 62:881–919.
- Gillespie TR, Greiner EC, Chapman C a. 2004. Gastrointestinal parasites of the guenons of western Uganda. *The Journal of Parasitology* 90:1356–1360.
- Glander KE. 1980. Reproduction and Population Growth in Free-Ranging Mantled Howling Monkeys. *American Journal of Physical Anthropology* 6:25–36.
- Helenbrook WD, Shields WM, Whipps CM. 2015a. Characterization of Blastocystis species infection in humans and mantled howler monkeys, Alouatta palliata aequatorialis, living in close proximity to one another. *Parasitology Research* 114:2517–2525.
- Helenbrook WD, Wade SE, Shields WM, Stehman S V, Whipps CM. 2015b.
  Gastrointestinal Parasites of Ecuadorian Mantled Howler Monkeys (Alouatta palliata aequatorialis) Based on Fecal Analysis. *The Journal of Parasitology* [Internet] 101:341–50.
- Kowalewski M, Zunino GE. 2005. The parasite behaviour hypothesis and the use of sleeping sites by black howler monkeys (*Alouatta caraya*) in a discontinuous forest. *Neotropical Primates* 13:22–26.
- Lafferty KD. 2009. The ecology of climate change and infectious diseases. Ecology 90:888–900.
- Martinez-Mota R. 2015. The Effects of habitat Disturbance, Host Traits, and Host Physiology on Patterns of Gastrointestinal Parasite Infection in Black Howler Monkeys (*Alouatta* pigra).

May RM, Anderson RM. Population biology of infectious diseases. [Internet].

- Milton K. 1996. Effects of bot fly (*Alouattamyia baeri*) parasitism on a free-ranging howler monkey (*Alouatta palliata palliata*) population in Panama. *Journal of Zoology* 239:39–63.
- Milton K, Nolin DA, Ellis K, et al. 2016. Genetic, spatial, and social relationships among adults in a group of howler monkeys (*Alouatta palliata*) from Barro Colorado Island, Panama. *Primates* 57:1–13.
- Nunn CL, Altizer S, Jones KE, Sechrest W. 2003. Comparative tests of parasite species richness in primates. *The American Naturalist* [Internet] 162:597–614.
- Parr NA, Fedigan LM, Kutz SJ. 2013. A coprological survey of parasites in white-faced capuchins (*Cebus capucinus*) from Sector Santa Rosa, ACG, Costa Rica. *Folia Primatologica* 84:102–114.
- Phillips KA, Haas ME, Grafton BW, Yrivarren M. 2004. Survey of the gastrointestinal parasites of the primate community at Tambopata National Reserve, Peru. *Journal of Zoology* 264:149–151.
- Pope TR. 2000. Reproductive success increases with degree of kinship in cooperative coalitions of female red howler monkeys (Alouatta seniculus). *Behavioral Ecology and Sociobiology* 48:253–267.
- Roberts, N., Fanning, E., Walters, H., Levac, A., Morris, A. Carpani J. 2013. Frontier Costa Rica Forest Research Programme.1-64 p.
- Sanchez-Azofeifa GA, Rivard B, Calvo J, Moorthy I. 2002. Title Title of Tropical Deforestation Around National Parks. Mountain Research and Development [Internet] 22:352–358.

- Silk JB. 2007. Social components of fitness in primate groups. Science (New York, N.Y.) [Internet] 317:1347–1351.
- Silver SC, Ostro LET, Yeager CP, Horwich R. 1998. Feeding ecology of the black howler monkey (*Alouatta palliata*) in Northern Belize. *American Journal of Primatology* 45:263–279.
- Singletary L. 2013. Habitat Use and Ranging Behavior of *Saimiri oerstedii oerstedii* on the Peninsula de Osa , Costa Rica, and Implications for Conservation.
- Stoner KE. 1996. Prevalence and Intensity of Intestinal Parasites in Mantled Howling Monkeys (*Alouatta palliata*) in Northeastern Costa Rica: Implications for Conservation Biology. *Conservation Biology* 10:539–546.
- Stuart M, Pendergast V, Rumfelt S, et al. 1998. Parasites of Wild Howlers (*Alouatta spp.*). *International Journal of Primatology* 19:493–512.
- Trejo-Macías G, Estrada A, Mosqueda Cabrera MÁ. 2007. Survey of helminth parasites in populations of *Alouatta palliata mexicana* and *A. pigra* in continuous and in fragmented habitat in southern Mexico. *International Journal of Primatology* 28:931–945.

## **APPENDIX A: APPROVAL LETTER**



Animal Resource Facility

DATE: May 21, 2015 TO: Anne Axel, PhD FROM: Marshall University IACUC IACUC #: 616 PROJECT TITLE: [751187-2] Contribution of a host-specific parasite to the gastrointestinal endoparasite load of the mantled howler monkey SUBMISSION TYPE: Revision APPROVED ACTION: APPROVAL DATE: June 30, 2015 EXPIRATION DATE: August 16, 2015 REVIEW TYPE: Designated Member Review

Thank you for your submission of Revision materials for this research project. The Marshall University IACUC has APPROVED your submission. All research must be conducted in accordance with this approved submission.

This submission has received Designated Member Review.

Please note that any revision to previously approved materials must be approved by this committee prior to initiation. Please use the appropriate revision forms for this procedure.

Please report all NON-COMPLIANCE issues regarding this project to this committee.

This project requires Continuing Review by this office on an annual basis. Please use the appropriate renewal forms for this procedure.

If you have any questions, please contact Monica Valentovic at (304) 696-7332 or valentov@marshall.edu. Please include your project title and reference number in all correspondence with this committee.

Monica A. Valentovic, Ph.D. Chairperson, IACUC

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