Spring 2021

Ecological and Anthropogenic Influences on a Sentinel Species' Gut Microbiome

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Recommended Citation

Black, Carson E., "Ecological and Anthropogenic Influences on a Sentinel Species' Gut Microbiome" (2021). *All Master's Theses*. 1515.
https://digitalcommons.cwu.edu/etd/1515

ECOLOGICAL AND ANTHROPOGENIC INFLUENCES ON A SENTINEL SPECIES' GUT MICROBIOME

A Thesis
Presented to
The Graduate Faculty
Central Washington University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Primate Behavior and Ecology

by
Carson Elizabeth Black
May 2021

CENTRAL WASHINGTON UNIVERSITY

Graduate Studies

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ABSTRACT

ECOLOGICAL AND ANTHROPOGENIC INFLUENCES ON A SENTINEL SPECIES' GUT MICROBIOME

by

Carson E. Black

May 2021

Central and South American primates face augmented challenges due to increased rates of forest fragmentation. Among these challenges are implications on the nonhuman primate gut microbiome. Wild Argentine black and gold howler monkeys (*Alouatta caraya*) are listed as "least concern" with a population that is decreasing. Despite their propensity to occupy habitats with varying degrees of degradation and disturbance, they are not immune from the stressors that forest fragmentation places on them. Their increased exposure to humans and livestock leaves them vulnerable to cross-species transmission of emerging infectious diseases and pathogens. Therefore, their role as sentinels of ecosystems, as well as a greater comprehension of what aspects of habitat are driving the gut microbiome, merits conservation and research. I seek to add to our understanding of what habitat factors are driving a sentinel species' health via the gut microbiome. Here I examine fragment size, the subsequent exigency of ground movement, and overlapping home ranges to determine whether there is variation in the gut microbiome of Argentine black and gold howler monkeys due to these factors.

ACKNOWLEDGMENTS

I extend my sincerest gratitude to my thesis committee chair and advisor, Dr. Jessica Mayhew for her brilliant mentorship and motivation, and for her unwavering support and belief in me. I also thank the rest of my committee, Dr. Katherine Amato and Dr. April Binder for their extensive insights, guidance, and time on this project. Many, many thanks to Dr. Martin Kowalewski for being flexible when the world turned upside down and also for his enormous amounts of kindness and deep knowledge that he shared with me, as well as the entire team at the Estación Biológica de Corrientes, and Sahana Kuthyar for sample collection. Profound thanks, also, to Bill Glessner with Information Services at Central Washington University for his patience, kindness, and many lessons in coding and supercomputers. I am grateful to my family for their continual support and understanding of all my academic endeavors, and to the many friends who have seen me through this degree. Funding was provided by the Pete and Sandra Barlow Award (CWU Department of Anthropology and Museum Studies), the Debra and Arlen Prentice Award (CWU Primate Behavior and Ecology Program), and the School of Graduate Studies and Research at Central Washington University.

This thesis is dedicated to the irreplaceable monkeys and incalculable biodiversity losses that were incurred at Corrientes in August and October of 2020. This research would not have been possible without their existence.

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CHAPTER I

INTRODUCTION

Central and South American primates face unique, multifaceted conservation challenges. Among these challenges are the ecological and anthropogenic implications of forest fragmentation on the nonhuman primate (henceforth: primate) gut microbiome. Research from the past two decades has revealed complex relationships between microbes and the hosts they inhabit, particularly how these microbes shape host behavior, nutrition, and health (Amato et al., 2016). Additionally, there is growing interest in the relationship that emerging pathogens share with bacteria in the gut as it pertains to ecological overlap in anthropogenic areas (Kuthyar et al., 2020). Gut bacteria may also play a role in host vulnerability to pathogenic infection and presentation of disease (Berrilli et al., 2012; Amato et al., 2016; Kuthyar et al., 2020). Howler monkeys (*Alouatta* spp.) present a unique model for investigating patterns and relationships in the gut microbiome due to their leaf-heavy diet and ability to occupy habitats that vary in degree of disturbance. It is because of these attributes that they are often described as resilient primates (Zunino et al., 2007; Amato et al., 2013; Kowalewski et al., 2015).

Notwithstanding their ability to thrive relatively undeterred in anthropogenic habitats, howler monkeys are not exempt from the pressures that fragmentation places on them, and their role at the species- and ecosystem-level in maintaining overall ecosystem health merits conservation and research efforts (Estrada, 2006). Northeastern Argentina presents a noteworthy locality for investigating forest fragmentation and how it drives the primate gut microbiome due to increased agricultural expansion and forest exploitation in the area, including the development of plantations and highways, selective logging practices, and presence of livestock (Zunino & Kowalewski, 2008; Holzmann et al., 2010; Kowalewski, 2010; Kuthyar et al., 2020). These changes also affect primate dispersal and resource availability, which can hinder genetic diversity and increase the likelihood of pathogenic swapping, ultimately leading to the risk of local extinction events (Oklander et al., 2017).

Argentine black and gold howler monkeys (*Alouatta caraya*) utilize a broad range of these ecological settings that can expose them to humans and domesticated animals like dogs and cattle. In these disturbed habitats, one of the ways howlers encounter humans and livestock is the demand to move terrestrially between forest fragments (Kuthyar et al., 2020), a dangerous alternative to their usual arboreal movements through trees, which has been affected by continual deforestation and resulting forest patches. This subjection to contact and shared susceptibility to zoonoses with humans and livestock increases their vulnerability to cross-species transmission of emerging infectious diseases, most notably yellow fever (Kowalewski & Gillespie, 2009; Garber et al., 2015), making howlers important for investigating the components of these diseases. Moreover, howlers may additionally provide early detections of viral outbreaks before human populations become infected (Holzmann et al., 2010; Oklander et al., 2017; Kuthyar et al., 2020).

Previous studies have revealed how primate gut microbiomes are negatively influenced by increasing rates of forest fragmentation across the globe (Zunino et al., 2007; Amato et al., 2013; Clayton et al., 2015; Barelli et al., 2015), and all point to the importance of conservation efforts and a broader understanding of microbes to curb future infectious outbreaks in an ever-changing global system. I seek to add to our understanding of what habitat factors are driving a sentinel species' health via the gut microbiome. Here I examine fragment size and the subsequent exigency of ground movement and overlapping home ranges to determine whether there is variation in the gut microbiome of Argentine black and gold howler monkeys due to these factors.

CHAPTER II

LITERATURE REVIEW

Howler Monkeys

Alouatta, in the subfamily Alouattinae, represents the most diverse genus of the platyrrhines, consisting of 12 species (Fleagle, 2013; Garber & Kowalewski, 2015). Howlers are slow, quadrupedal monkeys and are well-studied (Fleagle, 2013). They have the largest geographic range of the Neotropical primates, extending from Mexico through Brazil and into Argentina (Bicca-Marques, 2003; Garber & Kowalewski, 2015) with populations found in semideciduous, gallery, evergreen, and flooded forests (Zunino et al., 2007). Group composition is influenced by numerous variables, including species and habitat as well as human-caused land alteration and disease (Zunino et al., 2007). Most often, howler groups are comprised of one or more adult males, multiple adult females, and their respective offspring. They give birth to a single infant and receive help rearing offspring from other adult females in the group (Fleagle, 2013). There is genus-typical bisexual dispersal (Collevatti et al., 2013) that usually varies with age (Oklander et al., 2010; Oklander et al., 2014). *Alouatta caraya* (hereafter, black and gold howler monkeys) are the southernmost limits of the Meso- and South American primate species (Garber et al., 2015) and get their name from the sexual dichromatism in their species: males are black whereas females are blonde or golden in color. Compared to other howler species, black and gold howlers have a relatively small group composition of 2 to 23 individuals (Crockett & Eisenberg, 1987; Zunino et al., 2007), although these numbers tend to fluctuate with the availability of preferred food sources and habitat quality (Zunino et al., 2007; Collevatti et al., 2013).

Howler monkeys have typically been classified as opportunistic feeders (Zunino et al., 2007). In addition to a diet characterized by high-fibrous folivory and frugivory (Crockett & Eisenberg, 1987), over 80% of feeding time in howlers is dedicated to low-quality leaves during certain periods of the year (Amato & Garber, 2014; Amato & Righini, 2015). Preference is given to young leaves due to their high proportion of protein:fiber content (Milton, 1979). It is the howlers' enlarged colon and post-gastric (hindgut) fermentation process that mitigate the effects of toxins in different plant parts following the breakdown and digestion of plant cellulose (Milton, 1979; Fleagle, 2013; Amato & Righini, 2015).

Variability in howler monkey diets is also strongly dependent on the availability of preferred fruits. For example, for *A. seniculus*, fruits that are brightly colored and contain juicier pulps are the preferential source. These favored fruits are established as "high"-ranking due to their medium size and can be described as having what Julliot (1996) calls "primate syndrome," whereby certain species of plants heavily rely on primates for the dispersal of their seeds. The importance of primate seed dispersal, particularly of large-seeded fruits and trees that otherwise could not be dispersed by smaller animals, has been shown to have a positive influence on forest regeneration and the composition of different plant species (Arroyo-Rodríguez et al., 2015), highlighting the importance of a sentinel species in fragmented forests.

Habitat Fragmentation

Changes in land use that result in the degradation of natural ecosystems are the primary drivers of declining global biodiversity (Haddad et al., 2015). The destruction of forests leads to fragmentation, which can be defined as the breakup of natural habitats into smaller, more isolated fragmented forests, disconnected by a matrix of anthropogenic land cover (Haddad et al., 2015). While patch size shrinks with increased forest fragmentation, the number of isolated patches and inter-patch distance increases (Arroyo-Rodríguez & Dias, 2010). Additionally, increased exposure to anthropogenic land use and isolation negatively influences pertinent ecosystem functions, the lasting effects of which will be felt for generations (Haddad et al., 2015).

Another result of exacerbated rates of forest fragmentation is a decline in home range size, particularly in smaller fragments where organisms are constrained by the limitations of anthropogenically-altered habitats (Bicca-Marques, 2003). Home range is the entire area of land that a primate group uses consistently for an extended period of time; within the home range is the core area, which is defined as the part of a home range that is used the most (Fleagle, 2013). In addition to high rates of forest fragmentation in howler habitats, other limiting factors that determine home range include sufficient food sources, size and density of other conspecific groups, adequate habitat, and predation rates (Fortes et al., 2015). In these areas, howlers' isolation in smaller fragments hinders dispersal patterns (Oklander et al., 2010; Fortes et al., 2015) and further restricts the formation of larger groups. What is considered "adequate" habitat given these variables differs between forest types depending on the floristic makeup and distribution of plants, and the risks that are associated with moving in anthropogenically disturbed forest fragments, including increased predation and pathogenic infection (Fortes et al., 2015).

Day range, by contrast, is the distance travelled by primates in one day or night (Fleagle, 2013). While daily path length is likely correlated with the need to travel to obtain unevenly distributed resources, and although the pressures that are influencing home range may also affect day range, fragment size does not appear to predict daily path length, regardless of howler species (Bicca-Marques, 2003; Fortes et al., 2015). Ranges and potential group overlap will be crucial to monitor, in addition to tracking the availability of howler food resources in fragments, for a broader understanding of their ranging behavior and response to ecological and anthropogenic pressures.

The diversity of native flora and fauna is vehemently determined by the configuration of human-altered habitats, including forest fragments (Daily et al., 2003). It is consequently important to investigate different types of ecological and anthropogenic pressures on neotropical primates, since many species are essential for seed dispersal and classified as keystone species that enhance forest regeneration in both disturbed and undisturbed forests (Arroyo-Rodríguez et al., 2015; Estrada et al., 2017). Primary and secondary-level dispersal are both affected by fragmentation, especially in the absence of primate zoochorous seed dispersal. *Alouatta* spp. influence the proportion of resources obtainable to secondary dispersers such as dung beetles, which rely on howler dung for food, making them extremely sensitive to forest fragmentation (Estrada et al., 1999). Research suggests that the reduction and, in some cases, complete vanishment of howler monkeys from ecosystems may coincide with declining dung beetle populations in the same ecosystems (Arroyo-Rodríguez et al., 2015). Population- and community-level seed dispersal is lower in highly disturbed habitats; therefore, the role that howler species play as seed dispersers in forest fragments is of utmost conservation concern (Arroyo-Rodríguez et al., 2015).

Howlers’ ability to cope in anthropogenically-altered habitats better than more frugivorous primates is credited to their leaf-heavy diet (Zunino et al., 2007; Amato et al., 2013), supporting claims of earlier studies that howler monkeys are preadapted for surviving in habitat fragments (see Jones, 1995). Primate group composition and dispersal patterns are also impacted by the pressures brought about by increased deforestation, climate change, and transmission of disease on account of selective logging operations (Zunino et al., 2007). Black and gold howlers display the extreme southernmost range of their genus, often in highly fragmented areas (Kowalewski & Zunino, 2005; Garber et al., 2015). In Argentina, black and gold howlers that inhabit continuous and less-impacted forests display the expected multimale/multifemale group composition, but those in fragmented forests live in uni-male groups (Kowalewski & Zunino, 2005; Collevatti et al., 2013; Oklander et al., 2014). Additionally, black and gold howler populations living in these fragmented forests show genetic differences. The data are indicative of reduced gene flow between the groups living in the fragments, including one case of incest, suggesting that dispersal becomes more costly to howlers as habitat fragmentation increases (Oklander et al., 2010).

Movement Ecology

A greater comprehension of how species, and which ones, persist in deteriorated habitats is of increasing concern in conservation efforts. As humans continue to move into wild spaces and alter habitats for their own use, partnered with the consequences of climate change, further research on the adaptations that facilitate the utilization of forest fragments becomes ever-pressing to previse which species will be under the most pressure in the future (Serio-Silva et al., 2019). Despite their typical arboreality, *A. fusca*, *A. caraya*, *A. seniculus*, *A. palliata*, and *A. pigra* have been observed locomoting terrestrially (see da Silva, 1981; Bicca-Marques & Calegaro-Marques, 1995; Racenis, 1951; Gilbert & Stouffer, 1989; Serio-Silva & Rico-Gray, 2000; Pozo-Montuy & Serio-Silva, 2007; Pozo-Montuy et al., 2011, respectively). The primary reason for howlers' terrestrial behavior is not only to obtain a more isolated tree or set of trees, but also for access to some water sources (Serio-Silva et al., 2019). The usage of more isolated trees increases howler groups' habitat use, which likely enables their survival in fragmented habitats. It is also hypothesized that larger groups need the space or simply range in a larger area in general.

As mentioned previously, howlers have an uncanny ability to cope in landscapes that are less suitable for many other species, but their continued success in degraded habitats is strongly dependent on the availability and conservation of native vegetation, which provides troops with access to food sources, water, and forest corridors between trees, thus eliminating the requirement to move to the ground and decrease their disease risk, exposure to ectoparasites, and a higher risk of predation (Serio-Silva et al., 2019). Fig trees (*Ficus* spp.) are among the most prevalent tree species in northern Argentina and have been observed as common sleeping and defecating sites for black and gold howlers. A major food source, *Ficus* yields year-round flowers and leaves for consumption, and is therefore the most crucial species for their diet (Kowalewski & Zunino, 2005). Whether the presence or absence of *Ficus* in fragments affects howlers remains unclear, but if this is the case, we can expect the howlers' diet and accordingly, their gut microbiome, to be affected as well.

Gut Microbiome

Recent work further indicates that forest fragmentation triggers responses at the microbial level, including microorganisms that make up the skin and gut microbiomes

(Barelli et al., 2020). The assemblage and genetic material of viruses, bacteria, archaea, fungi and protists that are found in the body make up microbiomes in all mammals (Clayton et al., 2015; Amato et al., 2016). Since mammals are therefore comprised not only of their own genetic material, but also that of their microbes, they can be defined as metagenomic (Ley et al., 2008). The functionality and composition of these microbes within the mammalian gut is crucial for a greater comprehension of mammalian nutrition and health (Amato et al., 2016). Moreover, it is becoming increasingly pertinent to understand the functionality and structure of communities in an ecosystem across all degrees of biological organization for establishing microbiomes in individuals and populations, beyond that of just humans (Cullen et al., 2020).

Microbial communities in the colon, which generally make up more than 70% of all microbes in the body (Sekirov et al., 2010, as cited in Amato, 2016), are dominated by high proportions of bacteria and also consist of various species of protozoa, bacteriophages, archaea, and anaerobic fungi. The three dominant bacterial phyla in human and primate gut microbial communities are Firmicutes, Proteobacteria, and Bacteroidetes (Amato & Righini, 2015). Additionally, Clostridia, Ruminococcaceae, and Lachnospiraceae are indicative of a leaf-heavy diet and are crucial for the successful digestion of complex plant parts like cellulose (Barelli et al., 2010). During periods of preferred and easy-to-digest food scarcity, all primates consume low-quality foods like mature leaves, woody plants and exudates that are hard on the digestive system because of defense mechanisms, deficient nutrient quality, and high amounts of indigestible contents like toxins (Lambert, 2011; Amato & Righini, 2015). Howler monkeys are heavily reliant on their gut microbiome to break down tough carbohydrates and cellulose in plants that they regularly consume (Amato & Righini, 2015).

It is important to note that simply labeling microbes as either “beneficial” or “pathogenic” is not entirely accurate, especially in the context of gut microbiomes and how they impact host disease resistance (Backhed et al., 2012; Amato, 2016). The microbiome of the gastrointestinal (GI) tract is responsible for sustaining the immune system (Benavidez et al., 2019), and the immune system in turn monitors the gut microbiota (Amato et al., 2016), so the uncontrolled growth of any microbe in the gut will lead to adverse health consequences (Backhed et al., 2012). Because a diverse gut microbial community is more difficult for an opportunistic microbe to invade, individuals with high gut microbial diversity should be more resistant to potential gastrointestinal pathogens. The gut microbiome is also critical in regulating a host’s digestive system; dysbiosis occurs when the gut microbiome is unbalanced and consequently, unhealthy.

Reductions in functionality and microbiota variance may cause the gut microbiome to be susceptible to habitat disturbance and more prone to pathogenic infection (Zunino et al., 2007; Amato et al., 2013; Clayton et al., 2015). For example, despite *Alouatta* spp. demonstrating the propensity to thrive even in less-than-ideal habitats, Mexican black howler monkeys (*A. pigra*) in degraded settings have lower microbial diversity in their guts compared to captive and protected forest counterparts, as well as fewer short-chain fatty acid-producers (Amato et al., 2013; Amato, 2016). The stability of howlers’ habitat appears to drive the composition of the gut microbiome, suggesting that although howler monkeys will adjust their diet depending on season and availability, dietary changes linked to anthropogenic disturbance will modify primate gut microbiomes and also have potentially adverse health effects. Additionally, black howlers living in fragmented habitats with limited access to high-quality foods and increased exposure to domesticated animals and human activity, such as logging, show higher levels of fecal cortical metabolites (Amato et al., 2013; Amato et al., 2014).

It is also notable that howler monkeys living in fragmented areas appear to have myriad parasites in their gut microbiome. In a population in northeastern Argentina, black and gold howlers occupying habitats with different degrees of human contact have varying rates of *Giardia*, with monkeys living closer to livestock having the highest rates. Although *Cryptosporidium* was prevalent in nearby human populations, it was not evidenced in the howlers (Kowalewski et al., 2010). Presumably, this could mean that Argentine howlers, even when living in areas with more intense land-use, are not exposed to *Cryptosporidium* although it is pathogenic (Kowalewski et al., 2010). This research supports the notion that howler monkeys are viable sentinels for the comprehensive health of ecosystems.

The findings of the above studies are consistent with a similar analysis performed by Barelli et al. (2015). Groups of Udzungwa red colobus monkeys (*Piliocolobus gordonorum*) living in different forests share several microbial families, although on average, bacterial richness and overall gut microbiota diversity was highest in colobus monkeys living in a continuous, intact habitat compared to individuals living in a fragmented habitat. This follows the general pattern that there tends to be higher intraspecific variation in the gut microbiome in fragmented environments (Amato et al., 2013). The lack of richness and diversity in the gut microbiota of the colobus living in the fragmented forest are likely the result of human disturbance in the area and may also be correlated with the contrasting food sources available between the two habitats. Consequently, assessments of gut microbiota composition in wild primates can be used as evidence to support that habitat destruction and forest fragmentation are causing issues at the microbial level (Barelli et al., 2015).

This is not to posit, however, that all primate species living in fragmented, suboptimal habitats all have less diverse or dysbiotic microbiomes. Mantled howler monkeys (*A. palliata*), for example, do not appear to be as influenced at the microbial level by anthropogenic activity (Amato et al., 2016). Therefore, microbial composition in the gut could be a determinant for the length of time a host species will persist in an ecosystem experiencing rapid change, which may further dictate a species' evolutionary success (Amato et al., 2016). The significance of Meso- and South America for global primate conservation in conjunction with the importance of understanding primate gut microbiota homeostasis cannot to be ignored. Argentina's forests are fragile environments, as evidenced by a recent record-year of fires that ravaged the South American continent between 2019 and 2020 (Heidt, 2020). Habitat degradation and anthropogenic activity have had increasingly detrimental effects on habitat quality and species richness of floral and faunal populations worldwide (Haddad et al., 2015). Because habitat loss has been shown to stimulate changes in gut microbiome composition of the world's primates, it may additionally shape individual health and increase the likelihood of local extinction events (Barelli et al., 2015).

Further, in terrestrial primate species, like yellow (*Papio cynocephalus*) and Anubis baboons (*Papio anubis*), environmental drivers play an essential role in the bacterial composition of primate intestinal tracts. Grieneisen and colleagues (2019) suggest that soil properties could drive the composition of soil microbes that then are picked up by monkeys' fur or feet and colonize the gut microbiome. This has yet to be investigated in other terrestrial or arboreal species. Further, if Grieneisen et al. (2019) are correct that soil microbes can colonize primate gut microbiomes, then it is likely that terrestrial species are more exposed to those microbes than their arboreal counterparts and exploring what this means for arboreal species having to move on the ground becomes vital for the health and conservation of howlers.

Howler Monkey Conservation

The diminution and fragmentation of the world's tropical rainforests is caused largely by the burdens fostered by a rapidly growing human population and the global demand for food and trade goods (Estrada, 2006; Estrada, 2009; Estrada et al., 2017). By drastically changing the land around us with the establishment of crop plantations and livestock, humans have created agroecosystems, thereby displacing the native fauna and flora. The information presented here emphasizes howlers' ability to remain resilient even when faced with less-than-ideal habitats and resulting negative health outcomes; but despite their plasticity, howlers are not safe in agroecosystems and fragments long-term (Bicca-Marques, 2003; Estrada, 2006). Their populations are becoming increasingly vulnerable in forest fragments due to their relatively large body size, fruit specialization and seed dispersal, status as a delicacy among some groups, slow generation times, and an increased risk of hunting (Daily et al., 2003).

As aforementioned with the presented information, habitat fragmentation affects a multitude of factors in howler monkey conservation such as ranging, movement ecology, and diversity of preferred food sources. In addition, fragmentation has adverse effects on primate health, development, fitness, and gut microbiome function, and the consequences of these urgently need addressing in endangered primate groups (Barelli et al., 2015). Understanding the relationship between host microbiome and host habitat may prove beneficial for conservation efforts because changes in primate gut microbiomes have exhibited effects on host nutrition and health (Zunino et al., 2007). The mounting human population paired with increased human activity in primate habitats has consequently created increased possibilities for multi-directional pathogen swapping between humans, primates, and domesticated animals, which may cause tremendous issues for global health and conservation initiatives (Gillespie et al., 2008; Kowalewski et al., 2010).

Poverty rates continue to climb among indigenous groups of South America, where populations are experiencing physical and cultural displacement and acculturation by nonindigenous people. The indigenous populations face higher counts of unemployment, illiteracy, and disease; masses of these people live in remote villages where the government pays little to no regard to them (Estrada, 2009). Moreover, global corporations and big resident landowners of soy, palm oil, and many other internationally demanded products must be held responsible for generating the most profound impacts on wildlife and natural ecosystems. The role of researchers in conservation transcends more than just publications and articles in journals. Their role also includes building relationships with indigenous people who are also affected by habitat modification and urbanization, helping to establish integrative education programs for both researchers and local communities, as well as continuing to secure funding for research in developing countries (Kowalewski et al., 2010). Future conservation efforts must be shifted to not only include the health of wildlife, but the health of human communities, especially those that are poverty-stricken without access to adequate healthcare. It is these people who often suffer the most from the negative outcomes of extensive habitat alterations, although they are rarely the ones responsible for such drastic forest modification. By establishing relationships and long-term field sites, researchers can have a greater grasp on emerging infectious diseases and how to attempt to avert outbreaks in the future (Kowalewski et al., 2010).

However, some species are prioritized over others, creating a lack of consideration for other variables influencing distributions (Garber & Kowalewski, 2015). Comparative studies that examine the behaviors and health of different species in different gradations of fragmentation are needed in future studies. In addition, effective conservation of howlers should encompass more than just the monkeys themselves, but also the ways in which they are ecologically connected to other organisms, such as plants and dung beetles, all of which work together to maintain life in their habitats (Estrada et al., 1999). Consideration of cultural, economic, sociopolitical, and demographic facets of anthropogenic drivers causing massive deforestation in South America is also needed, not just for the well-being of the wildlife populations at stake, but also for preserving the livelihoods of indigenous peoples (Estrada, 2009).

STUDY OBJECTIVES

In the current investigation, I seek to examine how aspects of fragmentation (fragment size, ground movement, and overlapping home ranges) affected the overall diversity and richness of gut microbial communities black and gold howler monkeys at the Estación Biológica de Corrientes (EBCo) field site in northeastern Argentina. I posited that groups living in small forest fragments would have lower microbial diversity than groups living in a large forest fragment, where fragment sizes are large > 4 ha > small, and predicted significant differences in gut microbiome composition between howlers in fragments of different sizes. Additionally, I predicted that howler groups with increased ground movements would have a less diverse bacterial load than groups that do not move terrestrially. I further investigated how microbial diversity and richness were impacted by differences in geographic range of black and gold howler monkeys. I postulated that groups whose home ranges overlapped (up to four other groups) would have a more diverse bacterial load than groups whose home ranges have no overlap (between 0-1 groups). Due to the global COVID-19 pandemic and resulting inability to conduct fieldwork for 2020, data were acquired with permission from Dr. Katherine Amato at Northwestern University and Dr. Martin Kowalewski at the EBCo.

JOURNAL ARTICLE

CHAPTER III

Fragment Size, Terrestrial Movement, and Home Range Overlap Impact Argentine Black and Gold Howler Monkey (*Alouatta caraya*) Gut Microbiomes

JOURNAL-READY ARTICLE

FOR SUBMISSION TO:

International Journal of Primatology

Fragment Size, Terrestrial Movement, and Home Range Overlap Impact Argentine Black and Gold Howler Monkey (*Alouatta caraya*) Gut Microbiomes

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ACKNOWLEDGEMENTS

Funding was provided by Central Washington University, National Geographic, Northwestern University, and Goldberg Research Grant from the Nacey Maggioncalda Foundation. The authors thank the Estación Biológica de Corrientes (CONICET) team, the Parque Provincial de San Cayetano, the Dirección de Recursos Naturales, and the Dirección de Parques y Reservas de la Provincia de Corrientes for logistical support and permitting. The authors thank B. Glessner with Central Washington University Information Services for assistance with data analyses. The authors declare that they have no conflict of interest.

ABSTRACT

Habitat fragmentation has been shown to affect gut microbial communities in wild primate populations, which can potentially impact host physiology and health. Howler monkeys have been described as sentinels of ecosystem health; therefore, we must gain a greater understanding of what aspects are driving gut microbiome composition. Here, we examined how fragment size, terrestrial movement, and overlapping home ranges affected the overall diversity and richness of gut microbial communities in Argentine black and gold howler monkeys (*Alouatta caraya*). Howler gut microbiomes were characterized using 16S ribosomal RNA gene sequencing from 52 fecal samples collected across 14 groups, the results of which indicated that gut microbial richness and composition varied with fragment size, ground movement, and home range overlap. Howlers living in small forest fragments possessed more diverse gut microbes than those in large fragments. Howlers in groups that had no overlap also had distinct microbiomes compared to those that did. We also found significant differences in the microbiome composition between howlers that were observed coming to the ground and those that were strictly arboreal. These results encourage further inquiry into the adaptive behaviors of these primates that should be included in microbial research and conservation efforts.

Keywords: gut microbiome, fragmentation, *Alouatta*, black and gold howler monkeys

INTRODUCTION

Human-induced changes in land use within non-human primate habitats have presented newfound challenges for conservation, particularly pertaining to the ecological and anthropogenic implications of forest fragmentation on the primate gut microbiome. Fragmentation is the process by which habitats are broken up into smaller, more isolated forest pockets, disconnected by a matrix of anthropogenic human land cover (Haddad et al., 2015). The disturbance of wild animal populations due to habitat degradation may have downstream effects on the “microbiodiversity” of microbiomes that make up various body niches including the skin and gut (Barelli et al., 2020). The microbial communities that comprise these niches, specifically the vast majority (70%) that colonize the gut, are dominated by high proportions of bacteria and various anaerobic fungi, archaea, bacteriophages, and protozoa. Additionally, there is growing interest in the relationship between emerging pathogens and gut bacteria relative to ecological overlap in anthropogenic areas (Kuthyar et al., 2020), because gut bacteria may also play a role in host vulnerability to pathogenic infection and presentation of disease (Berrilli et al., 2012; Amato et al., 2016; Kuthyar et al., 2020). In this regard, monitoring primates with the ability to survive relatively undisturbed in fragmented habitats, such as howler monkeys (*Alouatta* spp.), will be continually important for comprehending the dynamics of disease transmission and to evaluate the capacity of primates to serve as sentinels, potentially providing early detections of viral outbreaks before human populations become infected (Holzmann et al., 2010; Kowalewski et al., 2010; Oklander et al., 2017; Kuthyar et al., 2020). Understanding how species persist in deteriorated habitats becomes increasingly critical to address in the face of climate change and as humans continue to alter habitats for their own use. Further research on the adaptations that facilitate the utilization of forest fragments becomes ever-pressing to previse which species will be under the most pressure in the future (Serio-Silva et al., 2019).

Northeastern Argentina presents a noteworthy locality for investigating forest fragmentation and how it influences the primate gut microbiome due to increased agricultural expansion and forest exploitation in the area, including the development of plantations and highways, selective logging practices, and presence of livestock (Zunino & Kowalewski, 2008; Holzmann et al., 2010; Kowalewski et al., 2010; Kuthyar et al., 2020). These anthropogenic changes also affect primate dispersal and resource availability, which can hinder genetic diversity and increase the likelihood of pathogenic swapping, ultimately leading to the risk of local extinction events (Oklander et al., 2017). Here, Argentine black and gold howler monkeys (*Alouatta caraya*) utilize a wide range of these altered settings that can expose them to humans and domesticated animals like dogs and cattle. In these disturbed habitats, howlers encounter humans and livestock when moving terrestrially between forest fragments (Kuthyar et al., 2020), which is a dangerous alternative to their usual arboreal movements through trees. Terrestrial locomotion by howler species (*A. guariba*, *A. caraya*, *A. seniculus*, *A. palliata*, and *A. pigra*) has previously been evidenced despite howlers' typical arboreality (see da Silva, 1981; Bicca-Marques & Calegaro-Marques, 1995; Racenis, 1951; Gilbert & Stouffer, 1989; Serio-Silva & Rico-Gray, 2000; Pozo-Montuy & Serio-Silva, 2007; Pozo-Montuy et al., 2011, respectively; Serio-Silva et al., 2019). This subjection to contact with humans and livestock and shared susceptibility to zoonoses increases their vulnerability to cross-species transmission of emerging infectious diseases, most notably yellow fever (Kowalewski & Gillespie, 2009; Garber et al., 2015).

Leaf-eating primate gut microbiomes are commonly characterized by microbes such as Clostridia, Ruminococcaceae, and Lachnospiraceae, which are associated with the digestion of complex plant parts like cellulose (Barelli et al., 2020). The composition of gastrointestinal microbiomes is linked to variation in how primates respond to habitat disturbance. For example, despite *Alouatta* spp. demonstrating the propensity to thrive in less-than-ideal habitats, Mexican black howler monkeys (*A. pigra*) in a fragmented, secondary forest have lower microbial diversity in their guts compared to their protected forest counterparts, as well as fewer short-chain fatty acid-producers (Amato et al., 2013; Amato, 2016). The stability of howlers' habitat appears to drive the composition of the gut microbiome, suggesting that although howler monkeys exhibit changes in their diet and microbiome depending on season and food availability, dietary changes linked to anthropogenic disturbance will negatively alter primate gut microbiomes and can also have potentially adverse health effects, including dysbiosis. Additionally, black howlers living in fragmented habitats with limited access to high-quality foods and increased exposure to domesticated animals and human activity, such as logging, show higher levels of fecal cortical metabolites (Amato et al., 2013; Amato et al., 2014).

Moreover, bridging the gap between existing gut microbial research and that of environmental microbiomes merits our efforts, especially in areas under continual human land production where primates are viable sentinels in maintaining overall ecosystem health. For example, in terrestrial yellow (*Papio cynocephalus*) and Anubis baboons (*Papio anubis*), soil composition impacts the bacterial composition of primate intestinal tracts (Grieneisen et al., 2019). If soil microbes can colonize primate gut microbiomes, then it is likely that terrestrial species are more exposed to those microbes than their arboreal counterparts. Exploring what this means for arboreal species adapting to terrestrial movement is vital for understanding the health and conservation of howlers.

The usage of more isolated trees increases howler groups' habitat use, which likely enables their survival in fragmented habitats. Howlers can occupy habitats that are less suitable for many other species, but their continued success in anthropogenic landscapes is strongly dependent on the availability and conservation of native vegetation, which provides troops with access to food sources, water, and forest corridors between trees, thus eliminating the requirement to move terrestrially and decrease their disease risk, exposure to ectoparasites, and a higher risk of predation (Serio-Silva et al., 2019).

The gut microbiomes of some primate taxa have been shown to be negatively influenced by increasing rates of forest fragmentation across the globe (Zunino et al., 2007; Amato et al., 2013; Clayton et al., 2015; Barelli et al., 2015). We seek to add to the overall understanding of what aspects of habitat are driving a sentinel species' health via the gut microbiome. We examine fragment size and the subsequent exigency of ground movement and overlapping home ranges to determine whether there is variation in the gut microbiome of Argentine black and gold howler monkeys due to these factors. We hypothesized that groups living in small forest fragments would have lower microbial diversity than groups living in large forest fragments, where fragment sizes were large > 4 ha > small, and predicted significant differences in the gut microbiomes due to these parameters. Additionally, we hypothesized that howler groups adopting terrestrial behavior would have less diverse bacterial loads than groups that do not come to the ground. In this study, ground use was noted if it was an observed behavior in the study groups, but we did not record how frequently the group used terrestrial locomotion. We further investigated how microbial diversity and richness were impacted by differences in the geographic range of black and gold howler monkeys. We postulated that groups whose home ranges overlapped (up to four other groups) would have a more diverse bacterial load than groups whose home ranges had no overlap (between 0-1 groups).

METHODS

Study Site & Subjects

The Estación Biológica de Corrientes (EBCo) is located in the northeast province of Corrientes, Argentina near San Cayetano (27°30'S, 58°41'W) and has been operating as a research institution through the Museo Argentino de Ciencias Naturales since 2001. The field station used to be the location of the Centro Argentino de Primates (CAPrim), which rendered primates for biomedical research and spearheaded primatological fieldwork in Argentina until the mid-1990s. Following its reestablishment, the EBCo now consists of one laboratory, classroom, library, kitchen and dining room, computer room, and five researcher houses. This infrastructure permits the establishment of long and short-term studies as well as field courses each year (Zunino & Kowalewski, 2008).

The area in and around the EBCo consists of nearly 80 hectares of protected flora and fauna. The landscape is characterized by rolling hills of savanna, semideciduous forest fragments up to 20 hectares in size, and flooded environments (Kowalewski et al., 2019). These diverse habitats host myriad populations of animal and plant species, including over 50 species of trees and terrestrial mammals as well as nearly 320 species of birds. Land around the field station is private property and primarily used for cattle, and the circumambient forests experience pressures from selective, clear cut logging (Zunino & Kowalewski, 2008).

Research on black and gold howler monkeys occurs at the EBCo, where 30-34 groups have been monitored since 1984 (see *Figure 1*). Thirty groups in the flooded forest on the island system have also been well-studied since 1997 (Zunino & Kowalewski, 2008). The ecological density of these monkeys ranges from one to three individuals per hectare, with higher densities on the island system than those at the mainland EBCo site (Kowalewski et al., 2019). In the flooded forest, groups' home ranges may overlap up to 80% due to more forest continuity when compared to groups on the mainland, where the costs of moving between forest fragments and groups are higher (Kowalewski et al., 2010; Oklander et al., 2010; Kowalewski et al., 2019). This group crowding at EBCo is a direct consequence of the clear-cutting practices (Kowalewski et al., 2019).

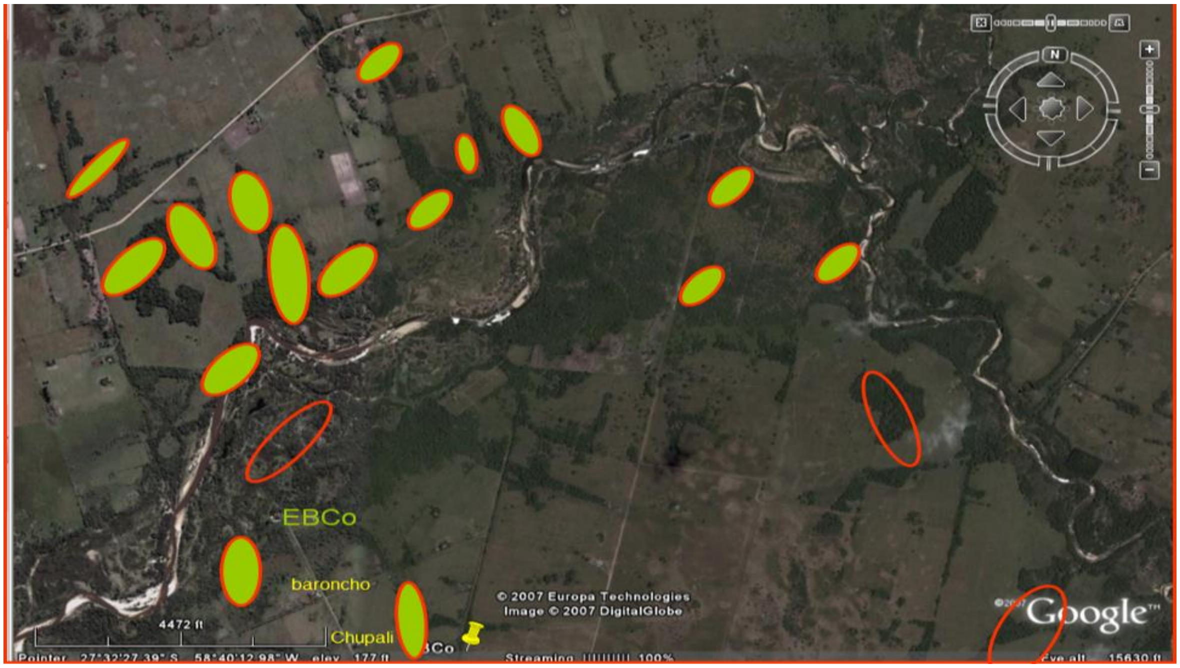

Figure 1. Aerial footage of approximate group ranges at the EBCo (M. Kowalewski, personal communication, March 4, 2020).

Data Collection

Data were collected during the 2017 Argentine winter season (June-July) and included fecal samples, GPS coordinates, and records of group composition and habitat occupation established by Kowalewski and contributing researchers (field collection by S. Kuthyar).

Fragments were considered large if their area totaled more than 4 hectares and small if their area totaled less than 4 hectares. We did not calculate how often the groups moved terrestrially; instead, we simply noted a group as using ground movement if it had previously been observed. Finally, one group typically occupied one fragment, but up to four groups have been found ranging in some fragments (Zunino et al., 2007; Kowalewski et al., 2019). For that reason, group overlap was defined as up to four groups and no overlap was defined as 0-1 groups.

Sample Collection. Fifty-two individuals were sampled at the EBCo. All fecal samples were collected non-invasively and without direct contact with the howler monkeys, under permit approval, and following prior protocols (Gillespie, 2006). Collections occurred opportunistically and sex, social group, age class (juveniles, subadult, adult), and reproductive stage (i.e., lactating) were recorded; most individuals were only sampled once. While freezing fecal samples for the preservation of bacterial DNA is ideal, it usually proves challenging to achieve under field conditions. Therefore, one gram of fecal matter was taken per sample, preserved in vials with 95% ethanol, shipped at 4°C, and stored at 20°C.

Ethical Note. Research procedures were approved by Central Washington University in Washington and Northwestern University in Illinois (Northwestern IACUC Field Research 2019-001) and complied with Argentine law.

Microbiome Analyses. DNA was extracted from the fecal samples using the Qiagen Powersoil Kit per the manufacturer’s instructions. The V4-5 region of the 16S ribosomal RNA gene was amplified utilizing a modified Earth Microbiome Project protocol (Thompson et al., 2017; Mallot & Amato, 2018) and the 515a/926R primer set

(Walters et al., 2016). Sequencing included extraction as well as negative controls, and all amplicons were barcoded and pooled in equimolar concentrations for sequencing via the Illumina MiSeq V2 platform at the DNA Services Facility (University of Illinois at Chicago, Chicago, Illinois). The DADA2 plug-in for QIIME2 (v2020.11, Boylen et al., 2019) was used to join and quality filter paired-end sequences. Quality filtering revealed 798,926 reads with 14,016 average reads per sample. Clustering of amplicon sequence variants (ASVs) was performed with the DADA2 algorithm. QIIME2 was used to calculate alpha diversity (Faith's phylogenetic distance, Shannon diversity index, and number of observed ASVs) and beta diversity (unweighted and weighted UniFrac distance matrices) metrics.

Statistical Analyses. The microbiome data were analyzed in R (*p*-value cutoff set at .05). To test for significant differences in bacterial community composition across fragment size, home ranges, and whether individuals came to the ground, permutational analyses of variance (PERMANOVA) was performed using the *adonis* function (R Core Team, 2014) in the *vegan* package (Oksanen et al., 2008) for both unweighted and weighted UniFrac distances. To test for the effects of these factors on alpha diversity, we fit regression models and also tested for effects on the relative abundances of individual microbial genera using the same models, correcting p-values using the *fdrtool* package (Strimmer, 2008).

RESULTS

The dominant bacterial phyla across all groups were Bacteroidetes, Firmicutes, and Proteobacteria. Specifically, howler monkey gut microbiomes were colonized by microbes Clostridia, Lachnospiraceae, Prevotellaceae, Ruminococcaceae, and Spirochaetaceae, which represented the top five most abundant families.

Fragment Size and Gut Bacteria

A total of 29 fragments were sampled ranging from .08 to 23 ha. Bacterial community composition varied across fragment size (unweighted UniFrac: PERMANOVA $F_{1,1}$ = 1.31, $R^2 = 0.02608$, p-value < .05, weighted UniFrac: PERMANOVA $F_{1,1}$ = 2.45, $R^2 = 0.04763$, p-value < .05). There were no differences in diversity when accounting for phylogenetic diversity of the microbes (Shannon's p-value = 0.033). On average, groups in smaller fragments (< 4 ha) displayed more diverse gut microbiomes (Shannon's H = 6.334) than larger fragments (> 4 ha; Shannon's H = 5.765).

Terrestrial Movement by Howlers and Gut Bacteria

There were significant differences in the bacterial composition between groups that moved terrestrially and those that did not (*Figure 2*; unweighted UniFrac: PERMANOVA $F_{1,1}$ = 3.09, $R^2 = 0.0593$, p-value < 0.05, weighted UniFrac: PERMANOVA F_1 = 3.93, $R^2 = 0.07426$, p-value < 0.05). There were no differences in diversity between howlers that did versus did not move terrestrially. Only one group was not observed utilizing the ground (n = 5). A linear regression model tested for the effect of ground movement on the microbiome and showed that more microbes vary in abundance significantly in response to terrestrial movement by howlers (q-value < 0.05). Further, *Methanosphaera* was significantly abundant (p = 0.001) with ground movement.

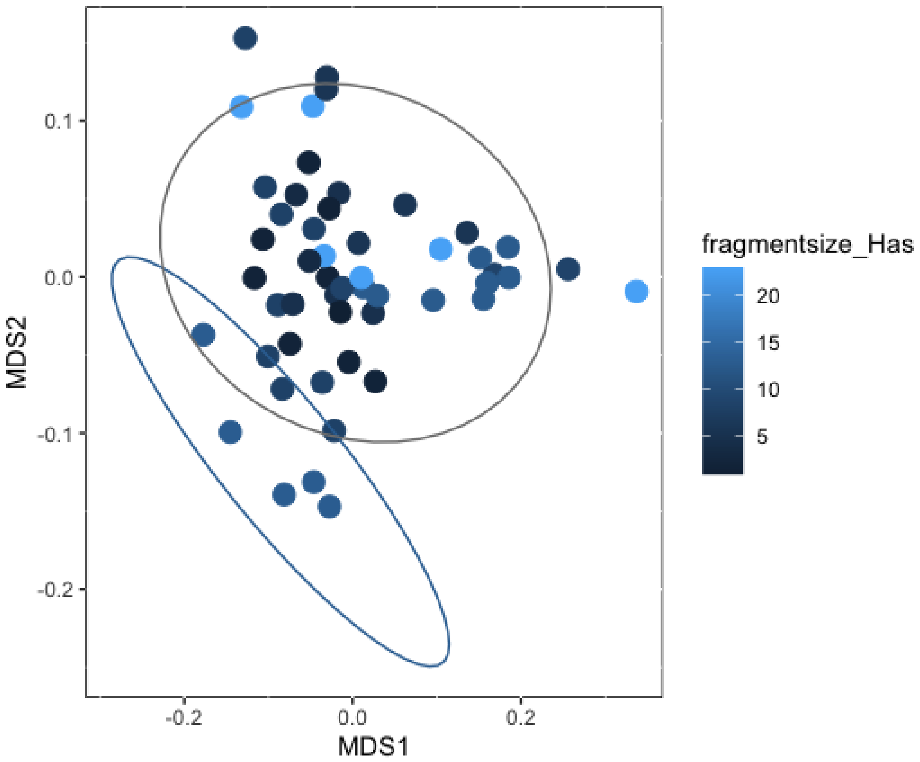

Figure 2. Beta diversity based on weighted UniFrac distance matrix. Groups are plotted by fragment size and clusters are shown for terrestrial movement. No ground movement observed is represented by the ellipses below, left; ground movement is represented by the center ellipses.

Home Ranges and Gut Bacteria

Differences in microbiome composition were also observed between groups with overlapping home ranges and those without (unweighted UniFrac: PERMANOVA $F_{1,1} = 2.1899$, $R^2 = 0.0427$, p-value < 0.05, weighted UniFrac: PERMANOVA $F_{1,1} = 10.102$, $R^2 = 0.17092$, p-value < 0.05). Groups with no overlap in their ranges had higher diversity on average than groups that did overlap (Shannon's $H = 6.143$ for no overlap, Shannon's $H = 5.376$ for overlap, $p < 0.05$). Several bacterial taxa including *Prevotella*, *Clostridia*, *Sphaerochaeta*, *Paraprevotella*, and *Monoglobus* varied significantly in abundance (q-value < 0.05) in response to differences in the overlap of howler home ranges.

DISCUSSION

This study examined the implications of forest fragmentation on the gut microbiome of black and gold howler monkeys at a highly fragmented site in northeastern Argentina. Our results demonstrate that the gastrointestinal tract of the monkeys sampled possessed the three typical dominant phyla Bacteroides, Firmicutes, and Proteobacteria, and the top five most abundant families were characterized by high proportions of Clostridia, Lachnospiraceae, Prevotellaceae, Ruminococcaceae, and Spirochaetaceae. The gut microbiota and host immune system sustain each other bidirectionally. This was evidenced by several genera associated with short-chain-fatty-acid production and anti-inflammatory properties that were found in high abundances, including *Roseburia*, *Faecalibacterium*, *Ruminococcus*, which are necessary for the digestion of cellulose and plant polysaccharides. This relationship may additionally increase host immunity response to pathogens (Amato, 2016). Overall, the bacterial composition in these howlers is reflective of a folivorous diet, as previously found in leaf-eating primates and other ruminants (Barelli et al., 2020).

As hypothesized, there were significant differences in gut microbiome composition in howler monkeys based on fragment size. However, our initial prediction was not supported because groups in larger fragments displayed less diverse bacterial loads than groups in smaller fragments. This may be due to monkeys in smaller fragments ranging in a smaller area with more dependable food resources than those in large fragments, where preferred vegetation may be more sparsely distributed. Food availability has previously been determined to be impacted by forest patch and home range size, as well as the vegetation structure (Arroyo-Rodríguez & Dias, 2010). Fig trees

(*Ficus* spp.) are among the most prevalent tree species in northern Argentina and have been observed as common sleeping and defecating sites for black and gold howlers. A major food source, *Ficus* yields year-round flowers and leaves for consumption, and is therefore the most crucial species for their diet (Kowalewski & Zunino, 2005). *Ficus* was present in every fragment and remains a prevalent and preferred food source despite severe fragmentation at Corrientes. Variation in fragment size may further be related to terrestrial movement or overlap with other groups, and future statistical revisions will include models to test the effect of variables on each other.

Among the abundant families, the genus *Methanosphaera* was significantly abundant (p = 0.001) with ground movement only but was not significant when tested across fragment size and overlapping home ranges. Presumably, this could mean that the howlers moving terrestrially are increasingly exposed to methane in the soils or from encountering cattle in their ranges. This finding aligns with previous work indicating that soil properties can drive gut microbiota in baboons (Grieneisen et al., 2019). As stated previously, the frequencies of ground use have not been calculated for this howler populations at the time of this writing. Therefore, what was deemed a “large” fragment was the total area of several small fragments clustered together, so groups in large fragments with less diverse microbiomes that still come to the ground may really be a function of using small fragments that they travel terrestrially between. In support, it is especially notable that the Corrientes site has since lost an estimated 90% of the park due to fire-fueled land clearing practices, exacerbated by climate change and a dry spring. It is likely that the surviving howler populations may utilize the ground much more to access resources in what is left of the remaining forest, likely in fragments even more disconnected from each other than before. However, we acknowledge that terrestriality might be a potential limitation of the current study, since only one group (n = 5) from the dataset retained arboreal behavior. Therefore, the small sample size likely had a strong influence on the statistical analyses that were conducted. It will be pertinent to monitor the gut microbiome, ecosystem microbiota, and the behavioral adaptations of movement, in this regard.

Changes in habitat due to fragmentation (i.e., decreases in patch size, increases in patch isolation) are tied to landscape supplementation, which occurs when howlers utilize multiple patches with supplementary resources clustered together (Dunning et al., 1992; Arroyo-Rodríguez & Dias, 2010) to meet their dietary requirements. This is typical of the howlers at Corrientes (Zunino et al., 2007; M. Kowalewski, personal communication, April 28, 2021). The current results highlight the continued ability of howler monkeys to both utilize small forest fragments and move between fragments, lending support to earlier studies that howlers are preadapted and have the behavioral flexibility to survive in fragmented forests (Jones, 1995; Bicca-Marques, 2003; Arroyo-Rodríguez & Dias, 2010).

Finally, overlap with other groups also revealed significant differences in gut microbial communities, but the initial prediction about the relationship of bacterial diversity and group overlap was not supported. Shannon diversity was higher on average for groups that had no overlap (between 0-1 groups) than groups that did (up to four other groups). This may be a result of transmission, because within group variation tends to be higher than between group variation. At Corrientes, the remaining howler troops may overlap in range more often with each other, so future investigations to measure the effects of fragmentation on group dynamics, dispersal, and ranging behavior are needed.

It is becoming more accepted that healthy environmental microbiomes dictate the health of human microbiomes (Lloyd-Price et al., 2016 as cited in Cullen et al., 2020). The same could be said in the context of primate gut microbiomes, especially for species that are important to tropical ecosystems. As a result, broadening research to include microbiome analyses on different parts of ecosystems (i.e., soil) becomes increasingly pertinent to our understanding of host-environment-microbe interactions. While results from this study are indicative that aspects of fragmentation drive changes at the microbial level of the tropics' primates, further research is necessary to investigate whether the type and prevalence of soil microbes that colonize howler monkey microbiomes are associated with higher or lower rates of howler terrestrial behavior. We also suggest testing whether monkey microbiomes are similar to those of the cattle encountered in their ranges. The information presented here echoes the findings of Kuthyar and colleagues (2020) that understanding the role of black and gold howler monkeys as a sentinel species in fragments is continually crucial for monitoring wildlife and the broader ecological health. Howler exploitation of anthropogenic habitats speaks to their resiliency, but the data support previous claims that howler monkeys' plasticity will not always be virtually unimpacted. The understudied movement ecology of these monkeys in relation to microbiome composition, and the greater impacts between host microbes, pathogens, and anthropogenic disturbance may ultimately lead to negative health outcomes and local extinction events. Other areas of our existing information, such as the role that environmental microbiota play in the gut microbiome of primates, remain fragmentary, at best. Once again, uniting our current knowledge of the gut microbiome with our anthropogenic studies will be important for studying the health of wild animals and implementing effective conservation strategies.

Reference List

Amato, K.R. (2016). Incorporating the gut microbiota into models of human and non-human primate ecology and evolution. *Yearbook of Physical Anthropology, 159,* 196-2015. https://doi.org/10.1002/ajpa.22908.

Amato, K.R., Martinez-Mota, R., Righini, N., Raguet-Schofield, M., Corcione, F.P., Marini, E., Humphrey, G., Gogul, G., Gaffney, J., Lovelace, E., Williams, L., Luong, A., Dominguez-Bello, M.G., Stumpf, R.M., White, B., Nelson, K.E., Knight, R., & Leigh, S.R. (2016). Phylogenetic and ecological factors impact the gut microbiota of two Neotropical primate species. *Oecologia, 180,* 717-733. https://doi.org/10.1007/s00442-015-3507-z.

Amato, K.R. & Righini, N. (2015). The howler monkey as a model for exploring host-gut microbiota interactions in primates. In M.M. Kowalewski, P.A. Garber, L. Cortés-Ortiz, B. Urbani, B., & D. Youlatos (Eds.), *Howler Monkeys, Developments in Primatology: Progress and Prospect* (pp. 229-258). New York, NY: Springer-Verlag.

Amato, K.R., Leigh, S.R., Kent, A., Mackie, R.I., Yeoman, C.J., Stumpf, R.M., Wilson, B.A., Nelson, K.E., White, B.A., & Garber, P.A. (2014). The gut microbiota appears to compensate for seasonal diet variation in the wild black howler monkey (*Alouatta pigra*). *Microbial Ecology, 69,* 434-443. https://doi.org/10.1007/s00248-014-0554-7.

Amato, K.R., Yeoman, C.J., Kent, A., Righini, N., Carbonero, F., Estrada, A., Gaskins, H.R., Stumpf, R.M., Yildirim, S., Torralba, M., Gillis, M., Wilson, B.A., Nelson, K.E., White, B.A., & Leigh, S.R. (2013). Habitat degradation impacts black howler monkey (*Alouatta pigra*) gastrointestinal microbes. *International Society for Microbial Ecology, 7*, 1344-1353. https://doi.org/10.1038/ismej.2013.16

Arroyo-Rodríguez, V. & Dias, P.A.D. (2010). Effects of habitat fragmentation and disturbance on howler monkeys: A review. *American Journal of Primatology, 72,* 1-16. https://doi.org/10.1002/ajp.20753.

Barelli, C., Albanese, D., Stumpf, R.M., Asangba, A., Donati, C., Rovero, F., & Hauffe, H.C. (2020). The gut microbiota communities of wild arboreal and ground-feeding tropical primates are affected differently by habitat disturbance. *American Society for Microbiology, 5,* 1-18. https://doi.org/10.1128/mSystems.00061-20

Berrilli, F., Di Cave, D., Cavallero, S., & D'Amelio, S. (2012). Interactions between parasites and microbial communities in the human gut. *Frontiers in Cellular and Infection Microbiology, 2*, 1-6. https://doi.org/10.3389/fcimb.2012.00141.

Bicca-Marques, J.C. & Calegaro-Marques, C. (1995). Locomotion of black howlers in a habitat with discontinuous canopy. *Folia Primatologica, 64,* 55-61. https://doi.org/10.1159/000156833.

Boylen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., … Caporaso, J.G., (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology, 37*, 852-857. https://doi.org/0.1038/s41587-019-0209-9.

Clayton, J.B., Gomez, A., Amato, K.R., Knights, D., Travis, D.A., Blekhman, R., Knight, R., Leigh, S.R., Stumpf, R.M., Wolf, T., Glander, K.E., Cabana, F., & Johsnon, T.J. (2018). The gut microbiome of nonhuman primates: Lessons in ecology and evolution. *American Journal of Primatology, 80*, 1-27. https://doi.org/10.1002/ajp.22867.

da Silva, E.C. (1981). A preliminary survey of brown howler monkeys (*Alouatta fusca*) at the Cantareira Reserve (Sao Paulo, Brazil). *Revista Brasileira de Biologia, 41*, 897-909.

Garber, P.A., Righini, N., & Kowalewski, M.M. (2015). Evidence of alternative dietary syndromes and nutritional goals in the Genus *Alouatta*. In M.M. Kowalewski, P.A. Garber, L. Cortés-Ortiz, B. Urbani, B., & D. Youlatos (Eds.), *Howler Monkeys, Developments in Primatology: Progress and Prospects* (pp. 85-109). New York, NY: Springer-Verlag.

Gilbert, K.A. & Stouffer, P.C. (1989). Use of a ground water source by mantled howler monkeys (*Alouatta palliata*). *Biotropica, 21*, 380.

Gillespie, T.R. (2006). Noninvasive assessment of gastrointestinal parasite infections in free-ranging primates. *International Journal of Primatology, 27*, 1129-1143. https://doi.org/10.1007/s10764-006-9064-x.

Grieneisen, L.E., Charpentier, M.J.E., Alberts, S.C., Blekhman, R., Bradburg, G., Tun, J., & Archie, E.A. (2019). Genes, geology and germs: gut microbiota across a primate hybrid zone are explained by site soil properties, not host species. *Proceedings of the Royal Society B, 286*, 1-9. https://doi.org/10.1098/rspb.2019.0431.

Haddad, N.M., Brudvig, L.A., Clobert, J., Davies, K.F., Gonzalez, A., Holt, R.D., Lovejoy, T.E., Sexton, J.O., Austin, M.P., Collins, C.D., Cook, W.M., Damschen, E.I., Ewers, R.M., Foster, B.L., Jenkins, C.N., King, A.J., Laurance, W.F., Levey, D.J., Margules, C.R.,… Townshend, J.R. (2015). Habitat fragmentation and its lasting impact on Earth's ecosystems. *Applied Ecology, 1*, 1-9. https://doi.org/10.1126/sciadv.1500052.

Holzmann, I., Agostini, I., Areta, J.I., Ferreyra, H., Beldomenico, P., & Di Bitetti, M.S. (2010). Impact of yellow fever outbreaks on two howler monkey species (*Alouatta guariba clamitans* and *A. caraya*) in Misiones, Argentina. *American Journal of Primatology, 72*, 475-480. https://doi.org/10.1002/ajp.20796.

Jones, C.B. (1995). Howler monkeys appear to be preadapted to cope with habitat fragmentation. *Endangered Species Update, 12*, 9-10.

Kowalewski, M.M. & Gillespie, T.R. (2009). Ecological and anthropogenic influences on patterns of parasitism in free-ranging primates: A meta-analysis of the Genus *Alouatta*. In P.A. Garber, A. Estrada, J.C. Bicca-Marques, E.W. Heymann, & K.B. Strier (Eds.), *South American Primates, Developments in Primatology: Progress and Prospects* (pp. 433-461). New York, NY: Springer-Verlag.

Kowalewski, M.M., Pavé, R., Fernández, V.A., Raño, & Zunino, G.E. (2019). Life-history traits and group dynamic in black and gold howler monkeys in flooded forests of northern Argentina. In K. Nowak, A.A. Barnett, & I. Matsuda (Eds.), *Primates in Freshwater Flooded Forests* (pp. 263-269). Cambridge, U.K.: Cambridge University Press.

Kowalewski, M.M., Salzer, J.S., Deutsh, J.C., Raño, M., Kuhlenschmidt, M.S., Gillespie, T.R. (2010). Black and gold howler monkeys (*Alouatta caraya*) as sentinels of ecosystem health: Patterns of zoonotic protozoa infection relative to degree of human-primate contact. *American Journal of Primatology, 73,* 75-83. https://doi.org/10.1002/ajp.20803.

Kuthyar, S., Kowalewski, M.M., Roellig, D.M., Mallot, E.K., Zeng, Y. Gillespie, T.R., & Amato, K.R. (2020). Effects of anthropogenic habitat disturbance and *Giardia duodenalis* infection on a sentinel species' gut bacteria. *Ecology and Evolution, 11*, 1-13. https://doi.org/10.1002/ece3.6910.

Lloyd-Price, J., Abu-Ali, G., & Huttenhower, C. (2016). The healthy human microbiome. *Genome Medicine*, 8, 1-11. https://doi.org/10.1186/s13073-016-0307-y.

Mallot, E.K. & Amato, K.R. (2018). The microbial reproductive ecology of white-faced capuchins (*Cebus capucinus*). *American Journal of Primatology, 80,* 1-11. https://doi.org/10.1002/ajp.22896.

Oklander, L.I., Kowalewski, M.M., & Corach, D. (2010). Genetic consequences of habitat fragmentation in black-and-gold howler (*Alouatta caraya*) populations from northern Argentina. *International Journal of Primatology, 31,* 813-832. https://doi.org/10.1007/s10764-010-9430-6.

Oklander, L.I., Mino, C.I., Fernandez, G., Caputo, M., & Corach, D. (2017). Genetic structure in the southernmost populations of black-and-gold howler monkeys

(*Alouatta caraya*) and its conservation implications. *PLoS One, 23,* 1-20. https://doi.org/10.1371/journal.pone.0185867.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Simpson, G.L., Stevens, M.H.H., & Wagner, H. (2008). *The vegan package: community ecology package, version 1.13-1*.

Pozo-Montuy, G. & Serio-Silva, J.C. (2007). Movement and resource use by a group of *Alouatta pigra* in a forest fragment in Balancan, Mexico. *Primates, 48,* 102-107. https://doi.org/10.1007/s10329-006-0026-x.

Pozo-Montuy, G., Serio-Silva, J.C., Bonilla-Sánchez, Y.M. (2011). Influence of the landscape matrix on the abundance of arboreal primates in fragmented landscapes. *Primates*, *52*, 139-147. https://doi.org/10.1007/s10329-010-0231-5.

R Core Team (2014). R: A language and environment for statistical computing. R Foundation for statistical computing. http://www.R-project.org/

Racenis, J. (1951). Some observations on the red howling monkey (*Alouatta seniculus*) in Venezuela. *Journal of Mammalogy, 33,* 114-115. https://doi.org/10.1093/jmammal/33.1.114-a.

Serio-Silva, J.C. & Rico-Gray, V. (2000). Use of a stream by Mexican howler monkeys. *The Southwestern Naturalist, 45*, 332-333. https://doi.org/10.2307/3672837.

Serio-Silva, J.C., Ramírez-Julián, R., Eppley, T.M., & Chapman, C.A. (2019). Terrestrial locomotion and other adaptive behaviors in howler monkeys (*Alouatta pigra*) living in forest fragments. In R. Reyna-Hurtado & C.A. Chapman (Eds.), *Movement Ecology of Neotropical Forest Mammals, Focus on Social Animals*. Springer Nature Switzerland.

Strimmer, K. (2008). fdrtool: a versatile R package for estimating local and tail area-based false discovery rates. *Bioinformatics*, *24*, 1461–1462. https://doi.org/10.1093/bioinformatics/btn209.

Thompson, L.R., Sanders, J.G., McDonald, D., Amir, A., Ladau, J., Locey, K.J., Prill, R.J., Tripathi, A., Gibbons, S.M., Ackermann, G., Navas-Molina, J.A., Jansenn, S., Kopylova, E., Vázquez-Baeza, Y., González, A., Morton, J.T., Mirarab, S., Zech Xu, Z., Jiang, L., …K Knight, R. (2017). A communal catalogue reveals Earth's multiscale microbial diversity. *Nature, 551,* 457-463. https://doi.org/10.1038/nature24621.

Walters, W., Hyde, E.R., Berg-Lyons, D., Ackermann, G., Humphrey, G., Parada, A., Gilbert, J.A., Jansson, J.K., Caporaso, J.G., Fuhrman, J.A., Apprill, A., & Knight, R. (2016). Improved bacterial 16S rRNA gene (V4 and V4-5) and fungal internal transcribed spacer gene primers for microbial community surveys. *mSystems*, *1,* 4-5. https://doi.org/10.1128/mSystems.00009-15.

Zunino, G.E. & Kowalewski, M.M. (2008). Primate research and conservation in northern Argentina: the field station Corrientes (Estación Biológica de Usos Múltiples - EBCo). *Tropical Conservation Science, 1,* 140-150. https://doi.org/10.1177/194008290800100206.

Zunino, G.E., Kowalewski, M.M., Oklander, L.I., & González, V. (2007). Habitat fragmentation and population size of the black and gold howler monkey (*Alouatta caraya*) in a semideciduous forest in northern Argentina. *American Journal of Primatology, 69,* 966-975. https://doi.org/10.1002/ajp.20389.

CHAPTER IV

CONCLUSIONS

The future of native plant and animal diversity in the tropics is strongly dependent upon continued conservation efforts in anthropogenic environments (Daily et al., 2013), including forest fragments. Therefore, it is important to understand the effects these altered landscapes have on the health of ecologically important species. Much of the existing literature has been aimed at studying howler monkeys in fragmented areas. Howlers are crucial to the ecosystems they inhabit and can survive in habitats that vary in anthropogenic disturbance, which has previously been shown to have effects on the gut microbiome (Amato et al, 2013; Amato et al., 2014; Barelli et al., 2015). Thus, the knowledge we have for howlers provides a baseline model for addressing questions regarding primate response to habitat fragmentation (Arroyo-Rodríguez & Dias, 2010). Increasing pressures for land use and the widening demand for global food and trade goods have been identified as the primary cause for rainforest and biodiversity loss worldwide. Unsettlingly, places where forest loss is greatest also happen to be where large numbers of primate taxa are endemic (Estrada, 2009).

Black and gold howler monkeys, in particular, present as a unique study species for investigating the implications of forest fragmentation on primate health via the gut microbiome since they make up the southernmost distribution of the genus *Alouatta* and are prone to zoonotic diseases such as yellow fever (Garber et al., 2015), and also because gut function and host health are linked to the diversity and composition of the gut microbiota. It is becoming more critical to assess gut microbiomes across all communities and levels of biological organization for a greater understanding of how microbes interact with hosts and their environments, and further fill pertinent gaps in our existing knowledge of nonhuman primate ecology, evolution, and conservation. Howlers' exploitation of anthropogenic habitats speaks to their resiliency, but the data support previous claims that howler monkeys' plasticity will not always be virtually unimpacted. The understudied movement ecology of these monkeys in relation to microbiome composition, and the greater impacts between host microbes, pathogens, and anthropogenic disturbance may ultimately lead to negative health outcomes and local extinction events. Other areas of our existing information, such as the role that environmental microbiota play in the gut microbiome of primates, remain fragmentary, at best. Once again, uniting our current knowledge of the gut microbiome with our anthropogenic studies will be important for studying the health of wild animals and implementing effective conservation strategies.

COMPREHENSIVE REFERENCES

Amato, K.R. (2016). Incorporating the gut microbiota into models of human and non-human primate ecology and evolution. *Yearbook of Physical Anthropology, 159*(61), 196-2015.

Amato, K.R. & Garber, P.A. (2014). Nutrition and foraging strategies of the black howler monkey (*Alouatta pigra*) in Palenque National Park, Mexico. *American Journal of Primatology*, *76*(8), 774-787.

Amato, K.R., Martinez-Mota, R., Righini, N., Raguet-Schofield, M., Corcione, F.P., Marini, E., Humphrey, G., Gogul, G., Gaffney, J., Lovelace, E., Williams, L., Luong, A., Dominguez-Bello, M.G., Stumpf, R.M., White, B., Nelson, K.E., Knight, R., & Leigh, S.R. (2016). Phylogenetic and ecological factors impact the gut microbiota of two Neotropical primate species. *Oecologia, 180*, 717-733.

Amato, K.R. & Righini, N. (2015). The howler monkey as a model for exploring host-gut microbiota interactions in primates. In M.M. Kowalewski, P.A. Garber, L. Cortés-Ortiz, B. Urbani, B., & D. Youlatos (Eds.), *Howler Monkeys, Developments in Primatology: Progress and Prospect* (pp. 229-258). New York, NY: Springer-Verlag.

Amato, K.R., Leigh, S.R., Kent, A., Mackie, R.I., Yeoman, C.J., Stumpf, R.M., Wilson, B.A., Nelson, K.E., White, B.A., & Garber, P.A. (2014). The gut microbiota appears to compensate for seasonal diet variation in the wild black howler monkey (*Alouatta pigra*). *Microbial Ecology*, *69*(2), 434-443.

Amato, K.R., Yeoman, C.J., Kent, A., Righini, N., Carbonero, F., Estrada, A., Gaskins, H.R., Stumpf, R.M., Yildirim, S., Torralba, M., Gillis, M., Wilson, B.A., Nelson, K.E., White, B.A., & Leigh, S.R. (2013). Habitat degradation impacts black howler monkey (*Alouatta pigra*) gastrointestinal microbes. *International Society for Microbial Ecology, 7*(7), 1344-1353.

Arroyo-Rodríguez, V., Andresen, E., Bravo, S.P., & Stevenson, P.R. (2015). Seed dispersal by howler monkeys: Current knowledge, conservation implications, and future directions. In M.M. Kowalewski, P.A. Garber, L. Cortés-Ortiz, B. Urbani, B., & D. Youlatos (Eds.), *Howler Monkeys, Developments in Primatology: Progress and Prospect* (pp. 111-139). New York, NY: Springer-Verlag.

Arroyo-Rodríguez, V. & Dias, P.E.D. (2010). Effects of habitat fragmentation and disturbance of howler monkeys: A review. *American Journal of Primatology, 72*(1), 1-16.

Backhed, F., Fraser, C.M., Ringel, Y., Sanders, M.E., Sartor, R.B., Sherman, P.M., Versalovic, J., Young, V., & Finlay, B.B. (2012). Defining a healthy human gut microbiome: Current concepts, future directions, and clinical applications. *Cell Host Microbe, 12*(5), 611-622.

Barelli, C., Albanese, D., Donati, C., Pindo, M., Dallago, C., Rovero, F., Cavalieri, D., Tuohy, K.M., Hauffe, H.C., & De Filippo, C. (2015). Habitat fragmentation is associated to gut microbiota diversity of an endangered primate: implications for conservation. *Nature, Scientific Reports, 5*, 1-12.

Barelli, C., Albanese, D., Stumpf, R.M., Asangba, A., Donati, C., Rovero, F., & Hauffe, H.C. (2020). The gut microbiota communities of wild arboreal and ground-feeding tropical primates are affected differently by habitat disturbance. *American Society for Microbiology, 5*(3), 1-18.

Berrilli, F., Di Cave, D., Cavallero, S., & D'Amelio, S. (2012). Interactions between parasites and microbial communities in the human gut. *Frontiers in Cellular and Infection Microbiology, 2*(141), 1-6.

Benavidez, K.M., Iruri-Tucker, A., Steiniche, T., & Wasserman, M.D. (2019). Primate microbial endocrinology: An uncharted frontier. *American Journal of Primatology, 81*(10-11), 1-5.

Bicca-Marques, J.C., (2003). How do howler monkeys cope with habitat fragmentation? In L.K. Marsh (Ed.), *Primates in Fragments* (pp. 282-303). Boston, MA: Springer.

Bicca-Marques, J.C. & Calegaro-Marques, C. (1995). Locomotion of black howlers in a habitat with discontinuous canopy. *Folia Primatologica, 64*(1-2), 55-61.

Boylen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.m>, Chase, J., … Caporaso, J.G., (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology, 37*(8), 852-857.

Clayton, J.B., Gomez, A., Amato, K.R., Knights, D., Travis, D.A., Blekhman, R., Knight, R., Leigh, S.R., Stumpf, R.M., Wolf, T., Glander, K.E., Cabana, F., & Johsnon, T.J. (2018). The gut microbiome of nonhuman primates: Lessons in ecology and evolution. *American Journal of Primatology, 80*(6), 1-27.

Collevatti, R.G., Souza-Neto, A.C., Silva-Jr., N.J., & Telles, M.P.C. (2013). Kin structure and parallel dispersal in the black and gold howler monkey *Alouatta caraya* (Platyrrhini, Atelidae). *Genetics and Molecular Research, 12*(4), 6018-6031.

Crockett, C.M. & Eisenberg, J.F. (1987). Howlers: variations in group size and demography. In B.B. Smuts, D.L. Cheney, R.M. Seyfarth, R.W. Wrangham, & T.T. Struhsaker (Eds.), *Primate Societies*. (pp. 54-68). Chicago, IL: University of Chicago Press.

Cullen, C.M., Aneja, K.K., Beyhan, S., Cho, C.E., Woloszynek, S., Convertino, M. McCoy, S.J., Zhang, Y., Anderson, M.Z., Alvarez-Ponce, D., Smirnova, E., Karstens, L., Dorrestein, P.C., Li, H., Sen Gupta, A., Cheung, K., Powers, J.G., Zhao, Z., & Rosen, G.L. (2020). Emerging priorities for microbiome research. *Frontiers in Microbiology, 11*(136), 1-27.

Daily, G.C., Ceballos, G., Pacheco, J., Suzán, G., & Sánchez-Azofiefa, A. (2003). Countryside biogeography of neotropical mammals: Conservation opportunities in agricultural landscapes of Costa Rica. *Conservation Biology, 17*(6), 1814-1826.

de Guinea, M., Estrada, A., Nekaris, K.A.I., Van Belle, S. (2019). Arboreal route navigation in a neotropical mammal: energetic implications associated with tree monitoring and landscape attributes. *Movement Ecology, 7*(39), 1-12.

da Silva, E.C. (1981). A preliminary survey of brown howler monkeys (*Alouatta fusca*) at the Cantareira Reserve (Sao Paulo, Brazil). *Revista Brasileira de Biologia, 41*, 897-909.

Dew, J.L. (2011). Feeding ecology, frugivory, and seed dispersal. In J.M. Setchell & D.J. Curtis (Eds.), *Field and Laboratory Methods in Primatology: A Practical Guide* (pp. 225-233). Cambridge, U.K.: Cambridge University Press.

Estrada, A. (2006). Human and non-human primate co-existence in the neotropics: A preliminary view of some agricultural practices as a complement for primate conservation. *Ecological and Environmental Anthropology, 2*(2), 17-29.

Estrada, A. (2009). Primate conservation in South America: The human and ecological dimensions of the problem. In P.A. Garber, A. Estrada, J.C. Bicca-Marques, E.W. Heymann, & K.B. Strier (Eds.), *South American Primates, Developments in Primatology: Progress and Prospects* (pp. 463-505). New York, NY: Springer-Verlag.

Estrada, A., A. Anzures D., & Coates-Estrada, R. (1999). Tropical rain forest fragmentation, howler monkeys (*Alouatta palliata*), and dung beetles at Los Tuxtlas, Mexico. *American Journal of Primatology, 48*(4), 253-262.

Estrada, A., Garber, P.A., Rylands, A.B., Roos, C., Fernandez-Duque, E., Di Fiore, A., Nekaris, K.A.I., Nijman, V., Heymann, E.W., Lambert, J.E., Rovero, F., Barelli, C., Setchell, J.M., Gillespie, T.R., Mittermeier, R.A., Arregoitia, L.V., de Guinea, M., Gouveia, S., Dobrovolski, R., … Li, B. (2017). Impending extinction crisis of the world’s primates: why primates matter. *Science Advances, 3*(1), 1-16.

Fleagle, J.G. (2013). *Primate Adaptation and Evolution* (3rd ed.). San Diego, CA: Academic Press.

Fortes, V.B., Bicca-Marques, J.C., Urbani, B., Fernández, V.A., & da Silva Pereira, T. (2015). Ranging behavior and spatial cognition of howler monkeys. In M.M. Kowalewski, P.A. Garber, L. Cortés-Ortiz, B. Urbani, B., & D. Youlatos (Eds.), *Howler Monkeys, Developments in Primatology: Progress and Prospects* (pp. 219-255). New York, NY: Springer-Verlag.

Garber, P.A. & Kowalewski, M.M. (2015). New challenges in the study of howler monkey behavioral ecology and conservation: Where we are and where we need to go. In M.M. Kowalewski, P.A. Garber, L. Cortés-Ortiz, B. Urbani, B., & D. Youlatos (Eds.), *Howler Monkeys, Developments in Primatology: Progress and Prospects* (pp. 413-428). New York, NY: Springer-Verlag.

Garber, P.A., Righini, N., & Kowalewski, M.M. (2015). Evidence of alternative dietary syndromes and nutritional goals in the Genus *Alouatta*. In M.M. Kowalewski, P.A. Garber, L. Cortés-Ortiz, B. Urbani, B., & D. Youlatos (Eds.), *Howler Monkeys, Developments in Primatology: Progress and Prospects* (pp. 85-109). New York, NY: Springer-Verlag.

Ganzhorn, J.U., Rakotondranary, S.J., & Ratovonamana, Y.R. (2011). Habitat description and phenology. In J.M. Setchell & D.J. Curtis (Eds.), *Field and Laboratory Methods in Primatology: A Practical Guide* (225-233). Cambridge, U.K.: Cambridge University Press.

Gilbert, K.A. & Stouffer, P.C. (1989). Use of a ground water source by mantled howler monkeys (*Alouatta palliata*). *Biotropica, 2*(4)1, 380.

Gillespie, T.R. (2006). Noninvasive assessment of gastrointestinal parasite infections in free-ranging primates. *International Journal of Primatology, 27*(4), 1129-1143.

Gillespie, T.R., Nunn, C.L., & Leendertz, F.H. (2008). Integrative approaches to the study of primate infectious disease: Implications for biodiversity conservation and global health. *American Journal of Physical Anthropology, 137*(47), 53-69.

Grieneisen, L.E., Charpentier, M.J.E., Alberts, S.C., Blekhman, R., Bradburg, G., Tun, J., & Archie, E.A. (2019). Genes, geology and germs: gut microbiota across a primate hybrid zone are explained by site soil properties, not host species. *Proceedings of the Royal Society B, 286*, 1-9.

Haddad, N.M., Brudvig, L.A., Clobert, J., Davies, K.F., Gonzalez, A., Holt, R.D., Lovejoy, T.E., Sexton, J.O., Austin, M.P., Collins, C.D., Cook, W.M., Damschen, E.I., Ewers, R.M., Foster, B.L., Jenkins, C.N., King, A.J., Laurance, W.F., Levey, D.J., Margules, C.R.,… Townshend, J.R. (2015). Habitat fragmentation and its lasting impact on Earth's ecosystems. *Applied Ecology, 1*(2), 1-9.

Heidt, A. (2020, October 15). Argentinian field site devastated by fire. The Scientist. https://www.the-scientist.com/news-opinion/argentinian-field-site-devestated-by-fire-68049

Holzmann, I., Agostini, I., Areta, J.I., Ferreyra, H., Beldomenico, P., & Di Bitetti, M.S. (2010). Impact of yellow fever outbreaks on two howler monkey species (*Alouatta guariba clamitans* and *A. caraya*) in Misiones, Argentina. *American Journal of Primatology, 72*(6), 475-480.

Jones, C.B. (1995). Howler monkeys appear to be preadapted to cope with habitat fragmentation. *Endangered Species Update*, *12*, 9-10.

Julliot, C., (1996). Fruit choice by red howler monkeys (*Alouatta seniculus*) in a tropical rain forest. *American Journal of Primatology, 40*(3), 261-282.

Kowalewski, M.M., Garber, P.A., Cortés-Ortiz, L., Urbani, B., & Youlatos, D., (2015). Why is it important to continue studying the behavioral ecology and conservation management of howler monkeys? In M.M. Kowalewski, P.A. Garber, L. Cortés-Ortiz, B. Urbani, B., & D. Youlatos (Eds.), *Howler Monkeys, Developments in Primatology: Progress and Prospects* (pp. 3-17). New York, NY: Springer-Verlag.

Kowalewski, M.M. & Gillespie, T.R. (2009). Ecological and anthropogenic influences on patterns of parasitism in free-ranging primates: A meta-analysis of the Genus *Alouatta*. In P.A. Garber, A. Estrada, J.C. Bicca-Marques, E.W. Heymann, & K.B. Strier (Eds.), *South American Primates, Developments in Primatology: Progress and Prospects* (pp. 433-461). New York, NY: Springer-Verlag.

Kowalewski, M.M., Pavé, R., Fernández, V.A., Raño, & Zunino, G.E. (2019). Life-history traits and group dynamic in black and gold howler monkeys in flooded forests of northern Argentina. In K. Nowak, A.A. Barnett, & I. Matsuda (Eds.), *Primates in Freshwater Flooded Forests* (pp. 263-269). Cambridge, U.K.: Cambridge University Press.

Kowalewski, M.M., Salzer, J.S., Deutsh, J.C., Raño, M., Kuhlenschmidt, M.S., Gillespie, T.R. (2010). Black and gold howler monkeys (*Alouatta caraya*) as sentinels of ecosystem health: Patterns of zoonotic protozoa infection relative to degree of human-primate contact. *American Journal of Primatology, 73*(1), 75-83.

Kowalewski, M.M. & Zunino, G.E. (2005). The parasite behavior hypothesis and the use of sleeping sites by black howler monkeys (*Alouatta caraya*) in a discontinuous forest. *Neotropical Primates, 13*(1), 22-26.

Kuthyar, S., Kowalewski, M.M., Roellig, D.M., Mallot, E.K., Zeng, Y. Gillespie, T.R., & Amato, K.R. (2020). Effects of anthropogenic habitat disturbance and *Giardia duodenalis* infection on a sentinel species' gut bacteria. *Ecology and Evolution, 11*(1), 1-13.

Lambert, J.A. (2011). Primate nutritional ecology: Feeding biology and diet at ecological and evolutionary scales. In C. Campbell, A. Fuentes, K.C. MacKinnon, M. Panger, S.K. Panger (Eds.), *Primates in Perspective, 2nd ed.* Oxford University, New York.

Ley, R.E., Hamady, M., Lozupone, C. Turnbaugh, P. Ramey, R.R., Bircher, S., Schlegel, M.L., Tucker, T.A., Schrenzel, M.D., Knight, R., & Gordon, J.I. (2008). Evolution of mammals and their gut microbes. *Science, 320*, 1647-1651.

Lloyd-Price, J., Abu-Ali, G., & Huttenhower, C. (2016). The healthy human microbiome. *Genome Medicine, 8*(51), 1-11. doi: 10.1186/s13073-016-0307-y.

Mallot, E.K. & Amato, K.R. (2018). The microbial reproductive ecology of white-faced capuchins (*Cebus capucinus*). *American Journal of Primatology, 80*(4), 1-11.

Martínez-Mota, R., Garber, P.A., Palme, R., Gillespie, T.R. (2017). The relative effects of reproductive condition, stress, and seasonality on patterns of parasitism in wild female black howler monkeys (*Alouatta pigra*). *American Journal of Primatology, 79*(8), 1-12.

Milton, K. (1979). Factors influencing leaf choice by howler monkeys: A test of some hypotheses of food selection by generalist herbivores. *The American Naturalist, 114*(3), 362-378.

Oklander, L.I., Kowalewski, M.M., & Corach, D. (2010). Genetic consequences of habitat fragmentation in black-and-gold howler (*Alouatta caraya*) populations from northern Argentina. *International Journal of Primatology, 31*(5), 813-832.

Oklander, L.I., Kowalewski, M.M., & Corach, D. (2014). Male reproductive strategies in black and gold howler monkeys (*Alouatta caraya*). *American Journal of Primatology, 76*(1), 43-55.

Oklander, L.I., Mino, C.I., Fernandez, G., Caputo, M., & Corach, D. (2017). Genetic structure in the southernmost populations of black-and-gold howler monkeys (*Alouatta caraya*) and its conservation implications. *PLoS One, 23*(10), 1-20.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Simpson, G.L., Stevens, M.H.H., & Wagner, H. (2008). *The vegan package: community ecology package, version 1.13-1*.

Ostro, L.E.T., Silver, S.C., Koontz, F.W., Young, T.P., & Horwich, R.H. (1999). Ranging behavior of translocated and established groups of black howler monkeys *Alouatta pigra* in Belize, Central America. *Biological Conservation, 87*(2), 181-190.

Pozo-Montuy, G. & Serio-Silva, J.C. (2007). Movement and resource use by a group of *Alouatta pigra* in a forest fragment in Balancan, Mexico. *Primates, 48*(2), 102-107.

Pozo-Montuy, G., Serio-Silva, J.C., Bonilla-Sanchez, Y.M. (2011). Influence of the landscape matrix on the abundance of arboreal primates in fragmented landscapes. *Primates, 52*(2), 139-147.

R Core Team (2014). R: A language and environment for statistical computing. R Foundation for statistical computing.

Racenis, J. (1951). Some observations on the red howling monkey (*Alouatta seniculus*) in Venezuela. *Journal of Mammalogy, 33*(1), 114-115.

Sekirov, I., Russel, S.I., Antunes, C.M., Finlay, B.B. (2010). Gut microbiota in health and disease. *Physiological Reviews, 90*(3), 859-904.

Serio-Silva, J.C. & Rico-Gray, V. (2000). Use of a stream by Mexican howler monkeys. *The Southwestern Naturalist, 45*(3), 332-333.

Serio-Silva, J.C., Ramírez-Julián, R., Eppley, T.M., & Chapman, C.A. (2019). Terrestrial locomotion and other adaptive behaviors in howler monkeys (*Alouatta pigra*) living in forest fragments. In R. Reyna-Hurtado & C.A. Chapman (Eds.), *Movement Ecology of Neotropical Forest Mammals, Focus on Social Animals*. Springer Nature Switzerland.

Strimmer, K. (2008). fdrtool: a versatile R package for estimating local and tail area-based false discovery rates. *Bioinformatics, 24*, 1461–1462. https://doi.org/10.1093/bioinformatics/btn209.

Thompson, L.R., Sanders, J.G., McDonald, D., Amir, A., Ladau, J., Locey, K.J., Prill, R.J., Tripathi, A., Gibbons, S.M., Ackermann, G., Navas-Molina, J.A., Jansenn, S., Kopylova, E., Vázquez-Baeza, Y., González, A., Morton, J.T., Mirarab, S., Zech Xu, Z., Jiang, L., …K Knight, R. (2017). A communal catalogue reveals Earth's multiscale microbial diversity. *Nature, 551,* 457-463.

Walters, W., Hyde, E.R., Berg-Lyons, D., Ackermann, G., Humphrey, G., Parada, A., Gilbert, J.A., Jansson, J.K., Caporaso, J.G., Fuhrman, J.A., Apprill, A., & Knight, R. (2016). Improved bacterial 16S rRNA gene (V4 and V4-5) and fungal internal transcribed spacer gene primers for microbial community surveys. *mSystems, 1*(1), 4-5.

Zunino, G.E. & Kowalewski, M.M. (2008). Primate research and conservation in northern Argentina: the field station Corrientes (Estación Biológica de Usos Múltiples - EBCo). *Tropical Conservation Science, 1*(2), 140-150.

Zunino, G.E., Kowalewski, M.M., Oklander, L.I., & González, V. (2007). Habitat fragmentation and population size of the black and gold howler monkey (*Alouatta* *caraya*) in a semideciduous forest in northern Argentina. *American Journal of Primatology*, *69*(9), 966-975.