

ENVIRONMENTAL INFLUENCES ON THE ACTIVITY PATTERNS OF THREE SPECIES
OF SEMI-FREE RANGING LEMURS AT THE LEMUR CONSERVATION
FOUNDATION'S MYAKKA CITY LEMUR RESERVE

by

Abby Richardson

A thesis submitted to the faculty of
The University of North Carolina at Charlotte
in partial fulfillment of the requirements
for the degree of Master of Arts in
Anthropology

Charlotte

2022

Approved by:

Dr. Lydia Light

Dr. Jonathan Marks

Dr. Tuan Cao

©2022

Abby Richardson

ALL RIGHTS RESERVED

ABSTRACT

ABBY RICHARDSON. Environmental Influences on the Activity Patterns of Three Species of Semi-Free Ranging Lemurs at the Lemur Conservation Foundation's Myakka City Lemur Reserve
(Under the direction of DR. LYDIA LIGHT)

Cathemerality is a unique and flexible activity pattern found mostly in lemurs. Cathemeral lemurs exhibit relatively evenly distributed activity across the 24-hour period. Although some species of lemur are broadly recognized as being cathemeral, other species have activity patterns that are still undetermined. The activity patterns of animals are largely influenced by environmental cues such as temperature, humidity, rainfall, and lunar illumination. However, the ways that animals respond to these cues are dependent on their own morphology and adaptations to a particular niche. This study examined the activity patterns of three species (*Eulemur mongoz*, *Lemur catta*, and *Varecia rubra*) of semi-free ranging lemurs living in the same 4-acre forest enclosure at the Lemur Conservation Foundation in Myakka City, Florida. Cross-species studies on captive and semi-free ranging populations can control for environmental variables and allow for inferences to be made about the driving forces of these activity patterns. I predicted that there would be a positive correlation between lunar illumination and activity patterns for all three species, and that there would be an inverse relationship between activity and meteorological factors such as temperature, humidity, and rainfall. I also predicted that overall, *E. mongoz* would exhibit the most evenly distributed activity pattern compared to the other two species, even in the absence of environmental differences experienced between species. Two individuals from each of the three species were fitted with accelerometers and activity was recorded in one-minute intervals for 45 days. Hourly temperature, hourly humidity, daily rainfall, and nightly illumination were also recorded. Behavioral observations were recorded and defined as "active" or "inactive" but were found to not accurately predict

accelerometer outputs. The mean daily activity divided by the mean nightly activity was obtained for each individual and used in GLMM, ANOVA, and ANCOVA analyses. Daily mean activity and nightly mean activity were also used for some analyses. Results suggest that daily temperature, rainfall, and humidity have little influence on semi-free ranging lemur activity. However, day length and the nocturnal illuminance index (NII) were a significant influence in some cases. Overall, mongoose lemurs were found to exhibit a more cathemeral activity pattern than ring-tailed lemurs and were found to exhibit more nocturnal activity than diurnal activity during the month of July. An understanding of the behavioral flexibility of lemurs and the evolutionary context of cathemeral behavior would allow us to make inferences about the impending impacts of climate change and other anthropogenic disturbances and provide us with insight on how we could mitigate or minimize these impacts on endangered lemur species. This knowledge also helps us to make informed decisions about relocations, breeding programs, animal welfare, and the release of a previously captive animal. Primates provide many ecosystem services to humans and are important members of their ecological communities. However, they face many threats and populations worldwide are continuing to decline despite current conservation efforts.

ACKNOWLEDGEMENTS

I would like to thank the Lemur Conservation Foundation, its staff, and review board for allowing me to conduct this research. I am very grateful for being allowed the opportunity to conduct my research at this unique and special facility. I especially thank Caitlin Kenney and the keepers for being so flexible and willing to provide daily support throughout the summer. I would also like to thank my advisor, Dr. Lydia Light, for her encouragement and continued support throughout this entire process. Thank you to my committee members, Dr. Tuan Cao and Dr. Jonathan Marks, for providing guidance and challenging me to reach my fullest potential as a master's student. Thank you to primatologists Dr. Erik Patel, Dr. Tim Eppley, and Dr. Marco Campera for dedicating time towards helping me with my proposal and developing research methods. Special thanks also go to the Department of Anthropology at the University of North Carolina at Charlotte for funding necessary data collection devices, Activinsights Customer Support for assisting me with the accelerometers, and Ken Glander for providing me with support and tools necessary for my research.

TABLE OF CONTENTS

LIST OF FIGURES	vii
LIST OF TABLES	viii
LIST OF ABBREVIATIONS	ix
INTRODUCTION	1
Animal Behavior: Circadian Rhythms and Zeitgebers	1
The Stable Adaptation Hypothesis	3
Thermoregulation and Food Availability	4
Predation and Competition	5
Lunar Illumination	5
Eye Morphology	6
Evolutionary Context	7
Semi-Free Ranging Lemurs in the United States	9
Hypotheses	10
MATERIALS AND METHODS	12
Study Site	12
Study Subjects	12
Procedures	13
Data Collection	14
Data Analyses	16
RESULTS	19
General Activity Patterns and Day-Night Ratios	19
Influence of Day Length and Lunar Illumination	20
Influence of Temperature, Humidity, and Rainfall	22
Species Comparison	23
DISCUSSION	25
Limitations	31
CONCLUSION	34
Significance Statement	34
Ethics Statement	35
REFERENCES	37
APPENDIX: ETHOGRAM OF LEMUR BEHAVIOR	40

LIST OF FIGURES

FIGURE 1. <i>Eulemur mongoz</i> DN ratios over time	19
FIGURE 2. <i>Lemur catta</i> DN ratios over time	20
FIGURE 3. <i>Varecia rubra</i> DN ratios over time	20
FIGURE 4. <i>Eulemur mongoz</i> DN ratios and NII	21
FIGURE 5. <i>Lemur catta</i> DN ratios and NII	22

LIST OF TABLES

TABLE 1. Ethogram of lemur behavior	41
-------------------------------------	----

LIST OF ABBREVIATIONS

LCF	Lemur Conservation Foundation
DN	Day-Night
NII	Nocturnal Illuminance Index
SVM	Sum of Vector Magnitudes
GLMM	Generalized Linear Mixed Model
ANOVA	Analysis of Variance
ANCOVA	Analysis of Covariance

INTRODUCTION

In the past four decades, researchers have been exploring a rare activity pattern seen in the family Lemuridae that was coined as “cathemeral” by Ian Tattersall in 1987 (Tattersall, 1987). While studying the common brown Mayotte lemur (*Eulemur fulvus*), it became evident that it did not exhibit an activity pattern that could be identified as diurnal, nocturnal, or crepuscular. Instead, the lemur was active during daylight and at night, with peaks around dusk and dawn. Based off these initial observations, cathemerality became defined as an evenly distributed sleep-wake cycle across the 24-hour period. Cathemeral behavior has also been observed to be a seasonal activity pattern, much like diurnality and nocturnality (see review in Tattersall, 2006). Since 1987, it has been observed that most members of the Lemuridae family are cathemeral either year-round or seasonally (see review in Curtis & Rasmussen 2002).

Animal Behavior: Circadian Rhythms and Zeitgebers

The activity patterns of all animals, plants, bacteria, and fungi are controlled by an internal, or endogenous, mechanism called the circadian rhythm that the scientific community became interested in around the 1950s (Golombek & Rosenstein, 2010). However, circadian rhythms typically follow a cycle that is slightly less than 24-hours, meaning that organisms would eventually desynchronize with their environment unless the circadian rhythm is adjusted by some other factor. External, or exogenous, factors such as temperature, predation, competition, phenology, and lunar illumination can entrain the circadian rhythm and cause the animal’s activity pattern to remain synchronized with the external environment. It is argued that the day-night illumination cycle is the most important exogenous factor entraining the circadian rhythm in mammals (Aschoff 1966; Aschoff et al. 1982; Gwinner 1986; Curtis & Rasmussen, 2002; Eppley, Ganzhorn, & Donati, 2015; Golombek & Rosenstein, 2010; Zordan *et al.*, 2001).

An environmental cue that entrains the circadian rhythm, such as the alternation of light to dark or day length, is referred to as a zeitgeber (Aschoff et al. 1982). Researchers have used special devices and captive manipulation studies to determine the true circadian rhythm of cathemeral primates. Some suggest that cathemerality itself is not considered to be a distinct endogenous rhythm (Curtis & Rasmussen, 2006; Erkert & Cramer, 2006; Rea et al., 2014), while others have found that, in the absence of environmental factors hypothesized to mask the true circadian rhythm, study subjects still exhibited cathemeral activity patterns (Bray, Samson, & Nunn, 2017). This could suggest that cathemerality is deeper rooted in evolutionary history than originally thought.

A zeitgeber is any environmental cue that resets an animal's endogenous circadian rhythm (Aschoff et al. 1982). The influence of a zeitgeber on an animal's circadian clock can be experimentally tested by removing the zeitgeber under controlled conditions. In animals where the zeitgeber being tested has a strong influence, their circadian rhythm will begin to "free-run" or shift away from the 24-hour period. By reintroducing the zeitgeber, it will entrain the internal clock and shift it back towards the 24-hour period. Experimental studies on cathemeral primates have suggested that the photoperiod, or the light-dark cycle, is the most influential zeitgeber on the primate's activity patterns (Erkert & Cramer, 2006; Razanaparany & Sato, 2020).

Interestingly, most lemur species have been found to be lunarphilic, unlike other mammals that are lunarphobic (Eppley, Ganzhorn, & Donati, 2015). Lunarphilic behavior is defined as nighttime activity levels that are positively correlated to lunar illumination. That is, as illumination increases, lunarphilic animals become more active. Most mammals are lunarphobic (Prugh & Golden, 2014), meaning that they decrease activity as illumination increases, usually to avoid predation. This means that the level of lunar illumination can have a strong masking effect

on the photoperiod-entrained endogenous circadian rhythm of lemurs (Donati and Borgognini-Tarli, 2006; Donati et al., 2009).

The Stable Adaptation Hypothesis

Cathemeral behavior in primates has been debated to be a result of two non-mutually exclusive hypotheses: the Evolutionary Disequilibrium Hypothesis and the Stable Adaptation Hypothesis. According to the Evolutionary Disequilibrium Hypothesis, cathemeral lemurs were nocturnal until humans arrived in Madagascar about 2,300 years ago (Tofanelli et al., 2009). Due to anthropogenic forces, including the extinction of predators and large-bodied competitors, lemurs have been allowed to adapt to a new activity pattern (Curtis & Rasmussen, 2002). Although some studies support this hypothesis (Martin, 1972; Tattersall, 1982; van Schaik & Kappeler, 1996), most research today focuses on the Stable Adaptation Hypothesis. This hypothesis states that cathemerality is a stable evolutionary transition between nocturnality and diurnality and can be further broken down to describe the driving forces of the transition, such as thermoregulatory behavior, anti-predator behavior, a seasonal shift in food quality and availability, and competitor avoidance. Cathemerality has been observed in 14 out of the 19 orders within *Eutheria* (a group of mammals closely related to marsupials) and 1 out of 5 within *Metatheria* (marsupial mammals) (see review in Curtis & Rasmussen, 2002). The occurrence of cathemerality in this many orders suggests that a flexible activity pattern is a deep evolutionary adaptation for mammals, rather than a recent response to disturbance as suggested to have occurred in Madagascar. In one study, a model was used to provide insight into the debate between the abovementioned hypotheses. The results of this model suggest that cathemerality appeared in the lemur radiation about 20 million years ago and preceded diurnality during lemur evolution (Santini, Rojas & Donati, 2015). Furthermore, it is suggested that cathemerality was

the best strategy for surviving on transitioning continents and that it was replaced in areas with more stable conditions with a less flexible pattern. However, with Madagascar's unpredictable and relatively harsh environments (Dewar & Richard, 2007), cathemerality remained a stable strategy. Even during the late Pleistocene and prehuman Holocene, most of the lemur species of Madagascar survived major changes in climate and vegetation (Burney et al., 2004).

Thermoregulation and Food Availability

Thermoregulatory mechanisms have been observed to be correlated to cathemeral behavior in some species as a mechanism for avoiding heat stress or cold stress, especially in environments where temperatures fluctuate seasonally. Studies on various species of lemurs have found that nocturnal activity will increase when nighttime temperatures are at their lowest, although this shift in activity patterns could also be coordinated with a decrease in day length (Curtis & Rasmussen, 2002; Curtis, Zaramody, & Martin, 1999). *Lemur catta*, however, were found to do the opposite by increasing nighttime activity when temperatures were warmer and decreasing nighttime activity during cooler nights (LaFleur et al., 2014). *L. catta* have other important mechanisms for reducing thermoregulatory costs that may explain this behavior, such as huddling and sunning behaviors (Kelley et al., 2016). Seasonal changes in activity patterns can also be explained by differences in food availability and quality (Donati et al., 2009; Tarnaud, 2006; Eppley, 2016). The complexity and interrelatedness of the environmental factors that influence cathemeral behavior within species and the varying degrees of cathemeral behavior across species can be demonstrated in semi-free ranging populations. In a study on a semi-free ranging population of *Lemur catta* on Saint Catherine's Island in Georgia, USA, the lemurs are provisioned with high-quality food daily, yet they were still observed to range during the nighttime (Parga, 2011).

Predation and Competition

Parga (2011) suggested that the semi-free ranging lemurs on Saint Catherine's Island may display more night activity due to a lack of nocturnal predators on the island and to avoid diurnal predators such as hawks (Parga 2011). This relationship between cathemeral activity as an anti-predator behavior has been thoroughly researched and is thought to be one of the main driving forces of the unique activity pattern (Curtis & Rasmussen, 2002; LaFleur et al., 2014; Eppley et al., 2017; Curtis, Zaramody, & Martin, 1999; Parga, 2011). Primates in areas where cathemerality has been observed all deal with high levels of predation from birds of prey, snakes, and carnivores (Colquhoun, 2006). Survival of these primates depends on the ability to evade all three types of predators and be able to conserve enough energy for other necessary activities. The fossae on Madagascar are a huge threat to many lemur species and evidence shows that the flexible activity pattern of this animal may even suggest an evolutionary arms race between predator and prey (Colquhoun, 2006). Temporal niche separation means that species may also rely on a flexible activity pattern to avoid interspecific competition while foraging (Schoener, 1974). In lemurs, this type of niche separation could potentially be another explanation of cathemeral activity patterns. However, this hypothesis has not been widely studied or has not been supported in studies looking at competition (Donati et al., 2009). Although the relationship is unclear in lemurs, the owl monkey was found to use cathemeral activity patterns as a strategy to avoid the diurnal titi monkeys, capuchins, and spider monkeys (Wright, 1989).

Lunar Illumination

In addition to thermoregulation, food availability, anti-predator behavior, and interspecific competition, many studies have also made links between nocturnal behavior and lunar illumination (Colquhoun, 1998; Donati & Borgognini-Tarli, 2006; Erkert & Cramer, 2006;

Fernandez-Duque & Erkert, 2006; Donati et al., 2009; Donati et al., 2013; Eppley, Ganzhorn, & Donati, 2015; Razanaparany & Sato, 2020). As mentioned, lemurs are lunarphilic, meaning that on average they will increase nighttime activity with an increase in illumination (Eppley, Ganzhorn, & Donati, 2015). Other nocturnal or cathemeral species tend to be less active on brighter nights or during full moons because this makes them more vulnerable to nocturnal predation. Studies on collared brown lemurs suggest that their activity is influenced seasonally by photoperiod but variation within seasons is directly related to lunar illumination (Donati, 2006). A comparative study on brown lemurs in varying habitat types and quality found that cathemeral behavior in both locations was a result of two proximate causes, one of them being lunar illumination and the other being photoperiodic changes (Donati et al., 2009). In this case, the brown lemurs have an activity pattern that is almost nocturnal, but lunarphilic behavior meant that low lunar illumination nights shifted their activity into the daytime. *Eulemur macaco macaco*, *Haplemur meridionalis*, and *Lemur catta* have also been observed to be more active with group ranging and calling during brighter moon periods (Colquhoun, 1998; Eppley, Ganzhorn, & Donati, 2015; Eppley et al., 2017; Parga, 2011).

Eye Morphology

One reason why lemur species may exhibit varying degrees of lunarphilic behavior could be a result of differences in eye morphology. Two features have been found to have an influence on this behavior: the tapetum lucidum and the area centralis (Rohen & Castenholz, 1967; Pariente, 1979). The tapetum lucidum is a specialized layer of reflective cells and is a nocturnal adaptation, while the area centralis provides high visual acuity and is a diurnal adaptation. *Haplemur* and *Lemur* species have both the nocturnal adaptation of a tapetum lucidum and the diurnal adaptation of an area centralis. *Varecia* species have only an area centralis and no

tapetum lucidum. *Eulemur*, which is currently believed to be the only genus that is primarily cathemeral, lacks both adaptations (see review in Curtis & Rasmussen, 2002). It is believed that higher levels of lunarphilic behavior in *Eulemur* is associated with the idea that they cannot see as well at night without sufficient lunar illumination, making it more costly to venture out at a time when nocturnal predators could see them (Colquhoun, 1998; Razanaparany & Sato, 2020). Primates rely highly on visual cues, so those with a tapetum lucidum would rely less on lunar illumination for foraging at night, resulting in a weaker association between cathemeral behavior and illumination (Eppley, Ganzhorn, & Donati, 2015). However, the intermediate morphology of some lemur species may be a compromise between sensitivity to light and acuity (sharpness of vision), supporting the Stable Adaptation Hypothesis of cathemerality for animals that are exposed to varying light conditions (Donati et al., 2013). It is also important to note that the presence or absence of a tapetum lucidum does not explain lunarphilia alone (Razanaparany & Sato, 2020).

Evolutionary Context

Although the term cathemerality was coined because of primate behavioral research, the activity pattern is not entirely unique to primates. Studies on Przewalski horses (*Equus ferus przewalskii*: Berger et al., 1999), flying foxes (*Pteropus samoensis*: Brooke, 2001), and rodents (*Microtus* and *Clethrionomys*: Halle, 2006) have all found evidence of cathemeral behavior. Interestingly, the study on rodents found that lunar phase, not lunar illumination, was correlated to nighttime and daytime activity. Although lunar phase influences the level of lunar illumination, the study observed that clouding had no effect on activity. Cathemerality is not exclusively seen in primates, which may suggest a more deeply rooted evolutionary history of the activity pattern or provide an example of parallel evolution. The presence of cathemerality in

other non-primate mammal species may also demonstrate the influence of exogenous environmental factors on endogenous circadian rhythms. The environmental factors that were found to influence cathemeral behavior in horses, flying foxes, and rodents have also been suggested to be the driving forces of cathemeral activity in primates.

Outside of the family Lemuridae, the genus *Aotus* (owl monkeys) has also been found to exhibit a flexible activity pattern. Most owl monkeys are strictly nocturnal, but a few species have been observed regularly being active during the day (Fernandez-Duque & Erkert, 2006; Erkert & Cramer, 2006). Like many other cathemeral species, the activity patterns of Azara's night monkey seemed to shift with lunar and seasonal changes. On new moon nights, this species was observed to be less active at night and extended its activity period into the daytime. This could be because the species does not have the nocturnal adaptation of a tapetum lucidum, so it may rely more on lunar illumination for nighttime activity than other nocturnally adapted species. Another study on the same species suggested that these shifts in activity patterns could also be related to predatory avoidance and thermoregulation (Savagian & Fernandez-Duque, 2017).

Cathemerality in lemurs is found mainly within the *Eulemur* genus. Colquhoun (1998) described their observations of *Eulemur macaco macacao* of Ambato Massif as providing "a textbook example of Tattersall's definition of 'cathemeral activity', exhibiting significant amounts of activity throughout the 24-hour cycle" (p. 31). Cathemeral behavior has also been studied extensively in mongoose lemurs (*Eulemur mongoz*: Curtis, Zaramody, & Martin, 1999), collared lemurs (*Eulemur collaris*: Donati et al., 2007; Donati et al., 2009), and bamboo lemurs (*Hapalemur meridionalis*: Eppley, Ganzhorn, & Donati, 2015). In the wild, ring-tailed lemurs have been observed displaying nighttime activity at various rates in Berenty, Bealoka, and

Tsimanampetsotsa National Park (LaFleur et al., 2014; Donati et al., 2013). Interestingly, evidence of cathemerality in *Varecia* is slim, but a captive study found that *Varecia* had activity patterns more similar to *Eulemur* than *Lemur catta* at the same site (Bray, Samson, & Nunn, 2017). In a study on *Avahi meridionalis* and *Lepilemur fleuretae*, Campera et al. (2019) found that the nocturnal primate *A. meridionalis* showed consistent activity during the day and increased night activity with an increase in lunar illumination while *L. fleuretae* decreased night activity with an increase in illumination, possibly to avoid competition.

Semi-Free Ranging Lemurs in the United States

In the eastern United States, there are three locations with semi-free ranging lemur species. These three locations are the Duke Lemur Center in Durham, NC, Saint Catherine's Island in Georgia, and the Lemur Conservation Foundation (LCF) in Myakka City, Florida. Studies have been conducted at each site and have all shown evidence of cathemeral behavior in resident *Eulemur mongoz*, *Lemur catta*, and *Varecia rubra*. In some cases, the activity patterns observed in these semi-free ranging environments vary from those observed in wild populations. However, this could also be a result of a lack of cathemerality-focused studies on wild populations. Parga (2011) studied ring-tailed lemurs on Saint Catherine's Island and found that moonlight had a positive effect on ranging behavior. It is suggested by the author that the lemurs on this island regularly range at night in response to diurnal predators such as hawks. Bray, Samson, & Nunn (2017) measured activity patterns in semi-free ranging lemurs at the Duke Lemur Center and found varying levels of daytime and nighttime activity between the species studied. Three of the subject species in this study were *Eulemur mongoz*, *Varecia rubra*, and *Lemur catta*. *V. rubra* was found to exhibit activity patterns more similar to *E. mongoz* than *L. catta*, which could be related to this species' lack of a tapetum lucidum and potentially stronger

lunarphilic behavior than *L. catta*. Rea et al. (2014) used accelerometers and light sensors on five species of lemur at the Duke Lemur Center to calculate a day-night (DN) ratio where lower values indicated more evenly distributed activity patterns. Interestingly, the red ruffed lemurs had one of the lowest DN ratios (1.90), followed by mongoose lemurs (1.96), and then ring-tailed lemurs (2.00).

Hypotheses

Many of the hypotheses for external factors that influence activity patterns were relatively controlled among the three lemur species living in the same forest enclosures at LCF. The three lemur species experienced the same levels of temperature, humidity, and rainfall, and all received adequate diets which minimized competition and poor diet quality. Since provisioning of the lemurs had a great impact on the hypotheses for competition and resource quality and availability, those two hypotheses were not tested in this study. Predation was also excluded from this study due to the structure of the forest enclosure. Although potential predators may live on the reserve, they do not have access to the lemurs inside the enclosures. Aerial raptors such as hawks and owls do have access to the forest, but it is unclear if they view the lemurs as prey or if the lemurs view them as predators. Instead, the focus was on the influence of lunar illumination and meteorological factors, as well as a comparison of species differences. It was hypothesized that there would be a positive correlation between illumination and activity for all species, but that *Eulemur mongoz* would be influenced the most by this variable due to their lack of a tapetum lucidum. It was also hypothesized that *E. mongoz* would decrease their activity the following day with an increase in illumination. Daily temperature, humidity, and rainfall were hypothesized to have an inverse relationship with activity of the three species. In other words, as temperature, humidity, and rainfall increased, the lemurs would

become less active during the day. Overall, it was hypothesized that there would be significant differences in the activity patterns of the three species despite all of them experiencing the same meteorological and lunar factors. The *Eulemur* genus has been widely studied in a variety of environmental contexts and is considered to be the most cathemeral lemur genus. Therefore, it was hypothesized that in the absence of environmental differences between the three species, *E. mongoz* would still exhibit the most evenly distributed activity pattern.

MATERIALS AND METHODS

Study Site

The Lemur Conservation Foundation's Myakka City Lemur Reserve is a 130-acre, Association of Zoos and Aquariums (AZA) Certified Related Facility located in Myakka City, Florida. The facility has both outdoor forest enclosures and indoor enclosures for the lemurs, usually connected by bridges. The enclosure of the study subjects is a 4-acre forest surrounded by an 8-foot chain link fence with 4 feet of electro-net at the top. The forest is adjacent to a 5-acre forest where other groups of the same species live. Each forest contains a mixture of native vegetation and planted food trees for foraging such as mango, passion fruit, guava, grapes, banana, persimmon, and bamboo. The facility is also home to alligators, bobcats, river otters, snakes, tortoises, and great horned owls, but measures are taken to keep terrestrial animals out of the lemur enclosures (The Lemur Conservation Foundation, 2020). According to the NOAA Online Weather Data, this area receives an average of 9.06, 9.38, and 11.56 inches of rainfall in June, July, and August, respectively. The average temperature during the summer months is 83 degrees Fahrenheit.

Study Subjects

Mongoose lemurs (*Eulemur mongoz*) are relatively small-bodied lemurs that live in family units (Curtis and Zaramody, 1999). These family units usually have one adult male, one adult female, and their offspring. They are classified as cathemeral lemurs because many studies have observed them to be active both during the day and at night (Curtis et al., 1999; Curtis and Rasmussen, 2006). For this study, two individuals from the same family unit and residing in the same forest enclosure were collared. One individual was a 25-year-old male (Felix), and the

other individual was a 20-year-old female (Kikeli). The two study subjects lived in the enclosure with one young female offspring (Consuela).

Ring-tailed lemurs (*Lemur catta*) live in multi-male multi-female groups with a dominant female. In the wild, these groups can range from 10 to 20 individuals (Sather et al., 1999), but at LCF the groups have three to five individuals. These lemurs are also considered to be diurnal but have been observed ranging at night (Parga, 2011; Donati et al., 2013; LaFleur et al., 2014; Rea et al., 2014; Bray, Samson, & Nunn, 2017). The two study subjects were an 18-year-old male (Molson) and a 10-year-old female (Sarsaparilla) living in a group with three other females. The three other females included a dominant 10-year-old (Sobe) and her two 5-year-old daughters (Indy and Elysian).

Red ruffed lemurs (*Varecia rubra*) live in fission-fusion multi-male multi-female groups (Vasey, 2000). These groups can range from 5 to 30 individuals in the wild but have three to five individuals in each group at LCF. These lemurs are considered diurnal in the wild but have been found ranging at night in other semi-free ranging environments (Bray, Samson, & Nunn, 2017). The study subjects for this species were a 1-year-old male (Frezy) and a 13-year-old male (Rivotra). However, the two males were in a group with an infant that was not allowed in the forest, so they spent the study period in a smaller indoor/outdoor enclosure adjacent to the forest. The two males only had access to the forest for a few days at the start of the study and were oftentimes kept inside during the night. As the infant got older, the entire group was given more access to the outside portion of the enclosure at night, but did not return to the large forest enclosure until after the study was over.

Procedures

All individuals were captured in their indoor enclosures using a net and hand-held for collar removal. An Activinsights GENEActiv accelerometer was then attached to the collar and the collar was re-fitted around the individual's neck. The accelerometers were a single device customized by Activinsights to not exceed weight restrictions suggested by LCF's head keeper and curator. The device itself weighs 16 grams, which is less than 5% of the lightest lemur's body weight (1.46 kg). All six individuals took a total of about one hour to capture, fit the accelerometers, and release into their enclosure to resume normal daily activities. This occurred on the morning of June 23, 2021, and the accelerometers began recording at 12:00 the following day. Raw activity data were collected from the accelerometers starting at 12:00 on June 24, 2021, and ended at various times throughout August, depending on when the accelerometer battery died. Data for analyses were extracted starting 70-minutes before sunrise on June 25, 2021, to allow the lemurs time to acclimate to the new devices.

Data Collection

The accelerometers were configured using Activinsights GENEActiv software prior to fitting them to the collars. They were set to collect data in three axes continuously at 10 Hz for 45 days. All accelerometers were placed in the same orientation on the back of the lemur's neck, so as not to interfere with sleeping, eating, drinking, or grooming. The pre-existing radio unit acted as a counterweight to keep the accelerometer in place. In most cases, this method of attachment was successful. However, it was observed that collars would sometimes rotate, and the device would end up on the side of the lemur's neck rather than the back. The devices also collected temperature and light exposure data, but this information was highly affected by the lemur's location and the obstruction of fur, so it was not included in the analyses. The six study subjects were caught and fitted by trained keepers at LCF.

Behavioral observations were recorded every five minutes using focal animal sampling (Altmann, 1974). An ethogram was used to describe behaviors and each behavior was classified as active or inactive. Observation sessions started at 6:00 and ended at 14:00 for five consecutive days for each individual. However, some sessions did not last the full eight hours due to extreme weather, long periods of out-of-sight observations, and routine forest maintenance. An out-of-sight observation period typically meant that a lemur had entered an “indoor” enclosure located within the forest. Ultimately, each individual had a total of 30-40 hours of direct behavioral observations.

Temperature and humidity data were collected every minute using a Govee Wireless Thermo-Hygrometer with WiFi Gateway that was placed just outside of a building located next to the forest enclosures. The data were downloaded twice, once in intervals of one hour and again in intervals of 15 minutes to account for four missing data points in the original download. To replace the four missing data points, the average temperature and humidity was taken from the time stamps 15 minutes before and 15 minutes after the missing hour. Daily maximum temperature, daily minimum temperature, and daily precipitation were also retrieved from the nearest NOAA Climate Data Online Myakka River State Park Station located 16.1 miles from LCF. Temperature data from this source were used to confirm the temperature readings from the Govee Thermo-Hygrometer. Nightly illumination was calculated using the Moon v2.0 program (Curtis et al., 1999) which used the exact geographic coordinates of the forest enclosure to record moon phase and transit time. The program then calculates a nocturnal illumination index (NII) using lunar phase, sunrise, sunset, moonrise, and moonset (Eppley, Ganzhorn, & Donati, 2015). This program partially minimizes the issue of general lunar phase not being equivalent to the

direct illumination that animals experience in a specific area, but it does not completely eliminate the issue due to the program not taking local cloud cover into account.

Data Analyses

Raw accelerometer outputs were compressed and downloaded as Excel files using an epoch of 60, resulting in a summation of activity from the three axes into one data value every minute. To combine the three axes into one value, the sum of vector magnitudes (SVM) equation was used (see below) and 1g was subtracted from each value to remove the static acceleration (acceleration due to gravity).

$$SVM^9s = \sum | (x^2 + y^2 + z^2)^{1/2} - 1g |$$

Sample analyses on the correlation between behavioral observations and accelerometer readings revealed that behavioral observations were not precise enough to be used as a means of interpreting the accelerometer readings. Regressions between behavioral observations and accelerometer readings resulted in correlation coefficients ranging from 0.372 to 0.694. This is most likely due to discrepancies between human and accelerometer definitions of “activity”, as well as human precision when recording an observation being delayed when compared to accelerometer precision. The mean values of daytime and nighttime activity were found using the SVM values and a day-night (DN) ratio was calculated by dividing the mean daytime activity by the mean nighttime activity (Bray, Samsun, & Nunn, 2017). For each day from June 25, 2021, to August 9, 2021, the daytime mean was taken from values between 70 minutes before sunrise to 70 minutes after sunset, and the nighttime mean was taken from values between 71 minutes after sunset to 69 minutes before sunrise the following day. Positive DN values were interpreted as more daytime activity, negative DN values were interpreted as more nighttime activity, and values closer to 1.00 were interpreted as more evenly distributed activity across the 24-hour

period. “Daytime” and “nighttime” were defined using sunrise and sunset times from the Moon v2.0 program, with twilight (70 minutes before sunrise and after sunset) being counted as daytime (Eppley, Ganzhorn, & Donati, 2015).

The dependent variables of daily DN ratio, mean daytime activity, and mean nighttime activity were tested for normal distribution using Kolmogorov-Smirnov test and then log transformed to be used in the following parametric tests (Donati *et al.*, 2013; Eppley, Ganzhorn, & Donati, 2015). Five individuals were defined as “subjects” for GLMM analyses: Felix (*E. mongoz*), Kikeli (*E. mongoz*), Molson (*L. catta*), Sarsaparilla (*L. catta*), and Frezy (*V. rubra*). Each subject was tested independently as the factor of interest in a GLMM using DN ratio, daytime activity, and nighttime activity as separate dependent variables. Mean daily temperature, mean daily humidity, daily rainfall, nightly NII, and day length were fixed effects (Razanapary & Sato, 2020). For daytime activity, day length of the same day and NII from the previous night were used, while day length from the previous day and same night NII were used for nighttime activity.

For ANOVA and ANCOVA analyses, species (*Eulemur mongoz* and *Lemur catta*) were used for comparison instead of individual subjects. To evaluate differences between species while controlling for day length and NII, daytime activity was used in a one-way ANCOVA analysis with species as fixed factors and day length and previous night NII as covariates (Donati *et al.*, 2009; Donati *et al.*, 2013). The same procedure was repeated with night activity as the dependent variable and night length and NII as covariates. Lunar illumination was split into three relatively equal ordinal blocks (Donati *et al.*, 2009; Eppley, Ganzhorn, & Donati, 2015) where low NII was less than 0.036, moderate NII was greater than 0.036 but less than 0.191, and high NII was greater than 0.191. Other independent variables were split into three ordinal categories

with each category containing about 33% of the sample (Donati *et al.*, 2009). Rainfall was grouped into four categories containing about 25% of the sample to differentiate between no rain, light rain, moderate rain, and heavy rain. After splitting rainfall into four categories, no rain contained values of 0 inches, light rain contained values ranging from 0.01 to 0.03 inches, moderate rain contained values of 0.05 to 0.3 inches, and heavy rain contained values of 0.31 to 4.3 inches per day. Two-way ANOVA analyses with Scheffe's post hoc tests were used to analyze species differences in the effect of NII on nocturnal activity as well as day length on diurnal activity (Donati *et al.*, 2009; Donati *et al.*, 2013; Eppley, Ganzhorn, & Donati, 2015). For night activity, species and NII were between-subjects effects while species and day length were between-subjects effects for day activity. Finally, a two-way ANCOVA analysis was used to evaluate the influence of temperature, humidity, and rainfall on DN ratio while controlling for day length and NII as covariates (Eppley, Ganzhorn, & Donati, 2015).

Since the red ruffed lemurs in this study were housed in a different enclosure adjacent to the forest, and because one accelerometer failed after 15 days of data collection, this species was not used in the final ANOVA or ANCOVA analyses, although a DN ratio was obtained for Frezy and used in GLMM analyses.

RESULTS

General Activity Patterns and Day-Night Ratios

Daily DN ratios varied for each subject, but average DN ratios across the 45-day study period revealed that *E. mongoz* exhibited the most cathemeral behavior with an average overall DN ratio of 1.13 (1.07 for Felix and 1.18 for Kikeli). *L. catta* exhibited some cathemeral behavior with an overall DN ratio of 1.67 (1.94 for Molson and 1.40 for Sarsaparilla). For *V. rubra*, only Frezy's DN ratio of 4.18 was obtained. This DN ratio represents much higher diurnality than expected and may be influenced by the partially indoor enclosure that was used for this group during the study. Plotting the DN ratios over time shows a general shift from diurnal activity to more cathemeral or nocturnal activity throughout the study period. *E. mongoz* show a period of nocturnality while *L. catta* exhibit more evenly distributed activity but rarely more nocturnal activity than diurnal. *V. rubra*, which is only represented by one young individual (Frezy), shows high levels of diurnal behavior with activity distribution across the 24-hour period fluctuating frequently.

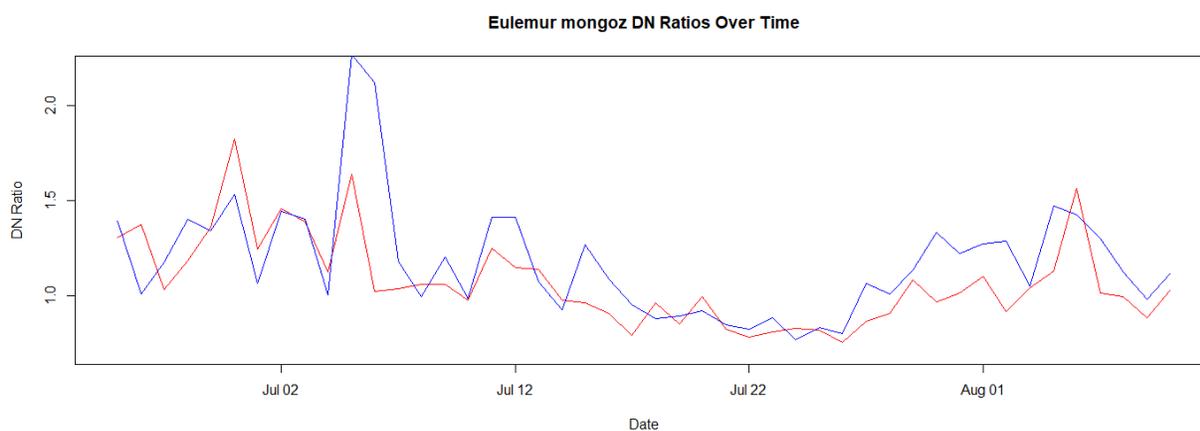


Figure 1. *Eulemur mongoz* DN ratios from June 25, 2021, to August 9, 2021. Red line represents the male lemur (Felix), and blue line represents the female lemur (Kikeli).

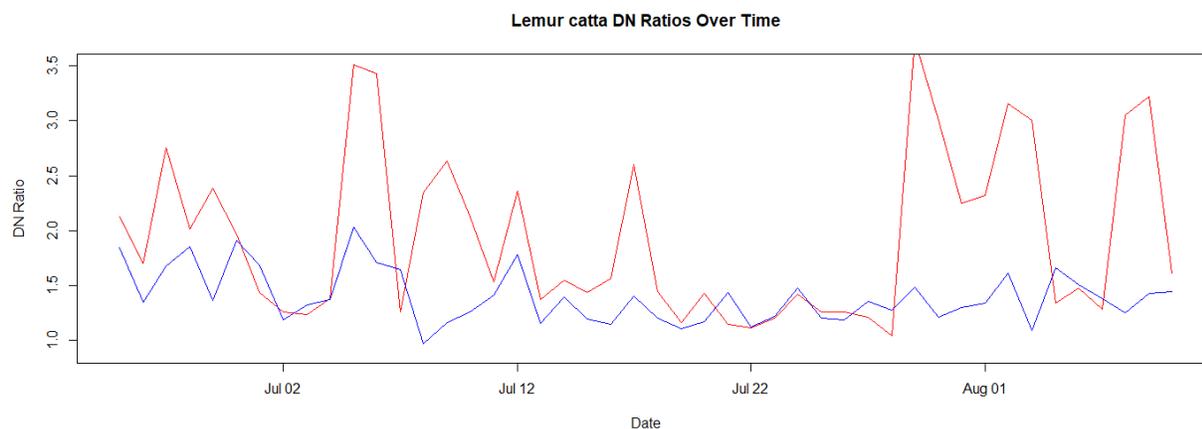


Figure 2. *Lemur catta* DN ratios from June 25, 2021, to August 9, 2021. Red line represents the male lemur (Molson), and blue line represents the female lemur (Sarsaparilla).

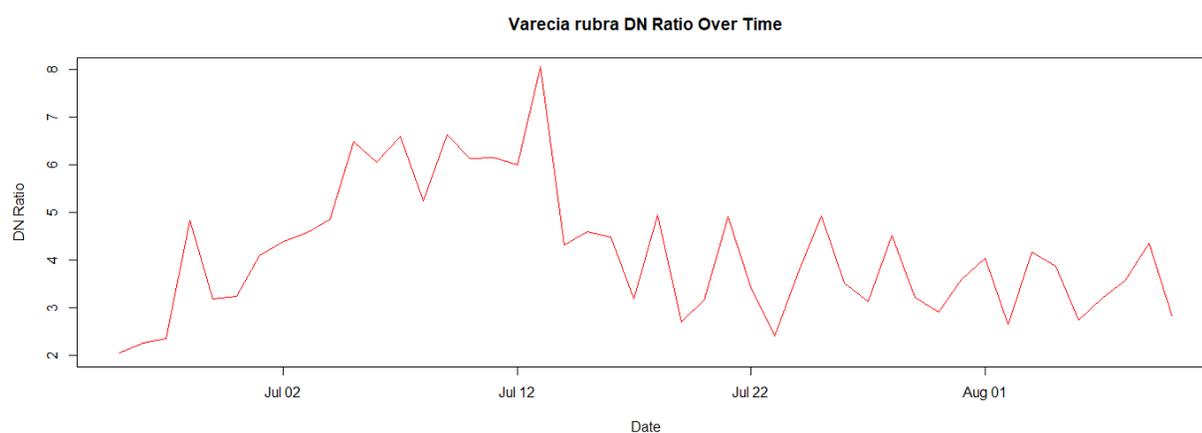


Figure 3. *Varecia rubra* DN ratios from June 25, 2021, to August 9, 2021. The DN ratio was only retrieved and calculated from one young lemur (Frezy) in an indoor enclosure with outdoor access.

Influence of Day Length and Lunar Illumination

GLMM tests found that day length had the greatest influence on the activity patterns of all individuals. Tests with daytime activity as the dependent variable showed significance for day length for Felix (GLMM, estimated coefficient = -0.084, $p = \mathbf{0.009}$), Kikeli (GLMM, estimated coefficient = -0.068, $p = \mathbf{0.034}$), Molson (GLMM, estimated coefficient = 0.054, $p = \mathbf{0.050}$), and Sarsaparilla (GLMM, estimated coefficient = 0.149, $p < \mathbf{0.001}$). The previous night NII approached a significant influence on the daytime activity of *E. mongoz* the following day (Felix:

GLMM, estimated coefficient = -0.081, $p = 0.071$, Kikeli: GLMM, estimated coefficient = -0.111, $p = \mathbf{0.016}$) but there was no significance for *L. catta* (Molson: GLMM, estimated coefficient = -0.039, $p = 0.307$, Sarsaparilla: GLMM, estimated coefficient = 0.005, $p = 0.912$). Day length and previous night NII had a significant influence on Frezy's daytime activity (day length: GLMM, estimated coefficient = 0.285, $p < \mathbf{0.001}$, previous NII: GLMM, estimated coefficient = -0.175, $p = \mathbf{0.031}$). The previous day length was significant for *E. mongoz* activity the following night (Felix: GLMM, estimated coefficient = -0.292, $p < \mathbf{0.001}$, Kikeli: GLMM, estimated coefficient = -0.195, $p = \mathbf{0.014}$) and approached significance for Sarsaparilla (GLMM, estimated coefficient = 0.105, $p = 0.063$) but was not significant for Molson (GLMM, estimated coefficient = 0.033, $p = 0.819$). Interestingly, NII was significant for Molson's nighttime activity (GLMM, estimated coefficient = 0.443, $p = \mathbf{0.041}$) but not for Sarsaparilla's (GLMM, estimated coefficient = -0.057, $p = 0.484$). Plots of DN ratios over time superimposed with NII over time show an inverse trend for both species. *Eulemur mongoz* displays a shift towards nocturnal behavior with an increase in NII (Figure 4), while *Lemur catta* displays a shift towards more evenly distributed daytime and nighttime activity with an increase in NII (Figure 5).

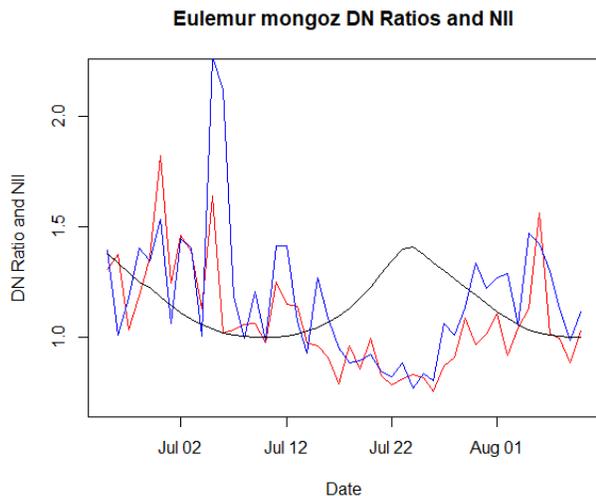


Figure 4. *E. mongoz* DN ratios and NII over time. Red line represents Felix and blue line represents Kikeli. The black line represents NII (plus 1 for visual and scaling purposes).

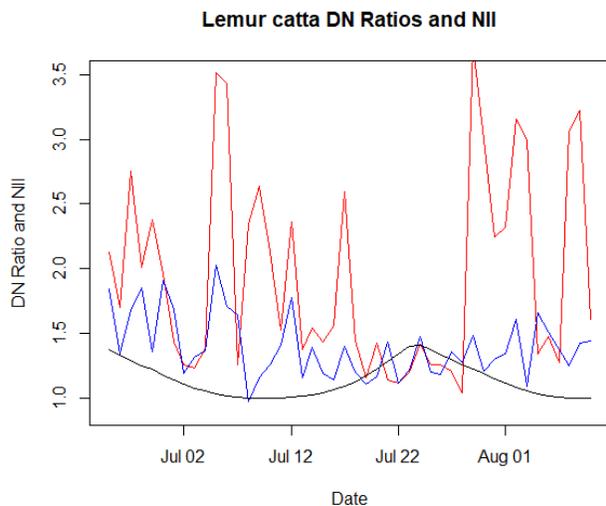


Figure 5. *L. catta* DN ratios and NII over time. Red line represents Molson and blue line represents Sarsaparilla. The black line represents NII (plus 1 for visual and scaling purposes).

Influence of Temperature, Humidity, and Rainfall

Temperature, humidity, and rainfall showed almost no significant influence on lemur activity patterns in GLMM analyses, although some variables had more of an influence than others. Temperature (GLMM, estimated coefficient = -0.008, $p = \mathbf{0.009}$) and humidity (GLMM, estimated coefficient = -0.004, $p = \mathbf{0.017}$) only significantly influenced Molson's daytime activity. From direct behavioral observations, it was observed that Molson would often be the only lemur in the forest to utilize the air-conditioned dome enclosure when temperatures were high. These observations were marked as "Out-of-Sight" but there were no auditory cues of activity within the enclosure. Although only temperature and humidity were significant for one lemur, some variables appear to have influenced the activity of other lemurs more than others, such as mean daily humidity (Felix: GLMM, estimated coefficient = 0.006, $p = 0.125$, Kikeli: GLMM, estimated coefficient = 0.004, $p = 0.388$) having more of an influence on *E. mongoz* DN ratios than temperature (Felix: GLMM, estimated coefficient = 0.004, $p = 0.553$, Kikeli: GLMM, estimated coefficient = 0.000, $p = 0.988$) and rainfall (Felix: GLMM, estimated coefficient =

0.005, $p = 0.793$, Kikeli: GLMM, estimated coefficient = 0.000, $p = 0.990$). For *L. catta*, rainfall (Molson: GLMM, estimated coefficient = -0.050, $p = 0.176$, Sarsaparilla: GLMM, estimated coefficient = 0.024, $p = 0.153$) influenced DN ratios more than temperature (Molson: GLMM, estimated coefficient = 0.005, $p = 0.735$, Sarsaparilla: GLMM, estimated coefficient = -0.002, $p = 0.805$) and humidity (Molson: GLMM, estimated coefficient = -0.001, $p = 0.891$, Sarsaparilla: GLMM, estimated coefficient = 0.001, $p = 0.891$). For Frezy, temperature (GLMM, estimated coefficient = -0.016, $p = 0.126$) was more influential on his DN ratio than rainfall (GLMM, estimated coefficient = -0.014, $p = 0.587$) and humidity (GLMM, estimated coefficient = -0.005, $p = 0.441$). Days with heavy rainfall such as 1.9 inches on June 30, 2021, 4.3 inches on July 7, 2021, and 2.1 inches on August 4, 2021, did cause shifts in activity. For all species, these dates show a positive spike in DN ratio, representing an increase in diurnal activity relative to nocturnal activity. However, it is known from direct behavioral observations that lemurs were kept inside during these times due to potential hurricanes. It is possible that the spike in daytime activity is a direct result of being kept inside overnight and therefore conclusions from these observations may not be made about direct correlations between rainfall and activity. Since analysis of the overall dataset revealed no significant correlations between daily rainfall and activity, these observation days were not removed. If some significant correlation had been present, further investigation would have been necessary to make inferences about the causes of this relationship.

Species Comparison

The two-way ANOVA model with night activity as the dependent variable showed that there was a significant difference ($F = 51.998$, $p < \mathbf{0.001}$) between species and that this variable accounted for 22.6% of the variation seen in night activity. The two-way ANOVA on daytime

activity found similar results, with species accounting for 7.6% of the variation seen in daytime activity ($F = 14.715$, $p < \mathbf{0.001}$). These ANOVA analyses also found that there was no significant difference between species in the effect of NII on nocturnal activity ($F = 0.184$, $p = 0.832$), but that there was a significant difference between species in the effect of day length on daytime activity ($F = 17.039$, $p < \mathbf{0.001}$). Descriptive statistics showed that *L. catta* and *E. mongoz* both increased their mean nighttime activity as NII went from “low” to “high.” However, *L. catta* decreased their mean daytime activity as day length went from “long” to “short” while *E. mongoz* increased their mean daytime activity as day length went from “long” to “short.”

One-way ANCOVA analyses with daytime activity as the dependent variable, species as fixed factors, and day length and NII as covariates revealed that there was still a significant difference between nighttime and daytime activity between species, even after the effects of day length and NII on activity were controlled for. The same is seen in the one-way ANCOVA analyses with nighttime activity as the dependent variable and night length and NII as covariates. Finally, the two-way ANCOVA analyses with DN ratio as the dependent variable found that when the effects of day length and NII on DN ratio were controlled for, there was still a significant difference between species ($F = 54.532$, $p < \mathbf{0.001}$), with species accounting for 27.5% of the variation seen in DN ratios. It was also revealed that temperature had some influence on DN ratio, although it was not significant ($F = 2.159$, $p = 0.119$). Humidity and rainfall still had no overall influence on DN ratio even after day length and NII were controlled for.

DISCUSSION

Overall, day length had the most influence on activity patterns in all species, which has also been found in other studies (Curtis, Zaramody, and Martin, 1999; Donati & Borgognini-Tarli, 2006; Razanaparany & Sato, 2020; Donati *et al.*, 2009) but was not a primary hypothesis for this study. Most research looking at the influence of environmental variables on activity patterns has suggested that photoperiod is the primary zeitgeber for the entrainment of the circadian rhythm in mammals (Curtis *et al.*, 1999; Donati & Borgognini-Tarli 2006; Fernandez-Duque & Erkert 2006; Aschoff *et al.*, 1982; Kappeler & Erkert, 2003). In this study, day length had a significant influence on the daytime activity of all individuals. Interestingly, the previous day length had less of an influence on the following night activity for *L. catta*, but significantly influenced nighttime activity for *E. mongoz*. Within *L. catta*, previous day length had more of an influence on Sarsaparilla's nighttime activity than Molson's, while Molson's nighttime activity was more influenced by NII. The differences found between Sarsaparilla and Molson may be attributed to age and sex differences, however, it has been found in previous studies that despite individual differences in activity patterns between groups, males and females within the same group showed similar patterns (Parga, 2011). The differences seen between Sarsaparilla's and Molson's activity patterns may also be due to differences in eye morphology. Although all ring-tailed lemurs possess the nocturnal adaptation of a tapetum lucidum, studies on other primates have found age-related changes in activity due to a loss of responsiveness to light (Gomez et al., 2012; Goncalves et al., 2020). This may imply that Molson was more reliant on NII for nocturnal activity, or that his circadian rhythm was not as synchronized with environmental light-dark cycles.

Despite day length having the greatest influence on both species, there was a significant difference between species when looking at the effect of day length on daytime activity. *L. catta* was found to decrease their mean daytime activity as daylength went from “long” to “short”, while *E. mongoz* increased their mean daytime activity as day length went from “long” to “short.” These results are similar to a study on brown lemurs, which found that the lemurs decreased their daytime activity and increased their nighttime activity on long days (Razanaparany & Sato, 2020). Curtis, Zaramody, and Martin (1999) found opposite results in their study on wild mongoose lemurs, which showed that daily activity increased as daylength increased between mid-June and mid-December. The patterns seen in *E. mongoz* may be related to other covariates that were not revealed in the statistical analyses. For example, day length tends to have a positive linear relationship with average temperature. Mongoose lemurs may have decreased activity with an increase in day length not in response to day length itself, but rather in response to longer, hotter afternoons. These environmental changes may not have influenced *L. catta* as much due to their thermoregulatory strategies (Kelley *et al.*, 2016). The results between this study and wild studies may be influenced by other meteorological covariates that vary between Florida and Madagascar, as well as the duration of the study influencing the scale of analysis. For example, this study was conducted within one summer season, so analyses were done on a daily scale. In wild studies that look at many seasons, analyses are typically done on a monthly scale.

Similar to other studies on *Eulemur spp.* and consistent with this study’s hypotheses, this study revealed that NII the previous night had an influence on *E. mongoz* daytime activity the following day (Razanaparany & Sato, 2020). It has been suggested that a reliance on high NII for nighttime activity, which is related to lunarphilic behavior, results in a decrease in activity the

following day in an attempt to balance activity budgets. *L. catta* and *E. mongoz* both increased their mean nighttime activity as NII went from “low” to “high”, which resulted in no significant difference between the two species for the effect of NII on nocturnal activity. This is interesting due to differences in eye morphology and hypotheses about lunarphilic behavior. However, other studies have also found that ring-tailed lemurs will increase their night activity with an increase in NII (Parga, 2011). The influence of daylength and NII on activity is very consistent with other studies and supports the Stable Adaptation Hypothesis. The Stable Adaptation Hypothesis states that a flexible activity pattern such as cathemerality is a stable evolutionary advantage for primate species living in highly variable environments. Since this study only looked at local NII calculated from sunrise, sunset, lunar phase, moonrise, and moonset, variables such as cloud cover were not taken into consideration. Although there may have been some relationship between experienced NII and theoretical NII (without cloud cover), there were still patterns found between lemur activity and NII. This suggests that lemurs may be entrained to moon phase, rather than reacting to micro-level environmental changes that would influence the actual level of illuminance experienced in the forest. This is consistent with other studies on mammals, where it was found that lunar phase, or NII, and not actual light conditions were influencing activity (Halle, 2006; Eppley, Ganzhorn, & Donati, 2015).

The Lemur Conservation Foundation and related facilities are unique in that they provide a middle ground between wild environments and captive zoo environments. Due to increased human interaction, provisioning, access to indoor environments, and control of social groups, many variables that have been found to influence activity patterns in the wild are controlled in semi-free ranging environments. This may explain why only a slight influence of temperature on DN ratio was found after day length and NII were controlled for. In the wild, temperature and

rainfall have been observed to have a more significant impact on lemur activity patterns (Curtis, Zaramody, & Martin, 1999; Donati & Borgognini-Tarli, 2006; Razanaparany & Sato, 2020). However, some of these studies have speculated that observed activity patterns in response to temperature and rainfall were more related to cloud cover as an unobserved covariate. The lemurs in this study were provided with temperate shelter to resort to during extreme weather conditions. They were also provisioned with high quality diets, so activity patterns did not need to take into account balancing weather conditions with foraging to conserve energy and maintain thermoregulation. Differences in responses to environmental variables such as temperature, humidity, and rainfall may be more due to individual variation and preference rather than a reflection of group, species, or genus behavior. During behavioral observations, it was often observed that some individuals within the same group would find shelter to rest in during a rainstorm while others would continue with normal activities. Results from this study show that temperature and humidity only significantly influenced the activity patterns of one individual – a large, adult male ring-tailed lemur. This lemur is the only male in the group and therefore the lowest on the hierarchical structure due to female dominance in ring-tailed lemurs. Through behavioral observations, it was found that this lemur would often spend most of the afternoon inside the air-conditioned dome enclosure, sometimes with the mongoose lemur group. During this afternoon period, the females in the group would often be lightly foraging or resting elsewhere in the forest. There were also periods throughout the study where all lemurs were kept inside overnight when hurricanes were expected. Being kept inside overnight was often followed by an increase in activity the following day, as shown by the spikes in diurnal activity seen in figures 1 and 2. It is unclear how these environmental variables may have influenced behavior if the structures within their semi-free ranging environment were not available. Literature on the

influence of similar variables on wild animals is highly ambiguous and dependent on weather, forest type, and the relationships between variables, making it difficult to make inferences.

At LCF, *E. mongoz* was found to have the most evenly distributed activity pattern, followed by *L. catta* and *V. rubra*. This varies from studies conducted in another semi-free ranging enclosure at the Duke Lemur Center, where *V. rubra* had the lowest overall DN ratio, followed by *E. mongoz* and *L. catta* (Rea et al., 2014). As previously mentioned, the high DN ratio of 4.18 seen in *V. rubra* may not be attributed to environmental variables at all. The red ruffed lemur group in this study had an infant present, so the group spent most of the summer in their controlled, indoor environment. This enclosure features an indoor room with a door leading to a completely enclosed outdoor area. The presence of an infant, the increased interaction and attention from caretakers, and the new pattern of being kept inside overnight may have temporarily altered the overall activity pattern of the group. However, it is also possible that the relatively high DN ratio in *V. rubra* is only reflective of Frezy's activity patterns, who was a one-year-old juvenile at the time of the study. Wild studies have found that the activity budgets, activity rhythms, and ranging behavior of red ruffed lemurs vary between sexes and across seasons due to the fission-fusion dynamic and the reproductive strategies found within red ruffed lemur groups (Vasey, 2005a; Vasey, 2005b). However, a study on captive lemurs found that although younger lemurs were more active at night than older individuals, there was no significant difference between activity ratios (Bray, Samson, & Nunn, 2017).

The overall DN ratio of *E. mongoz* is slightly lower than what has been found in other studies (Rea et al., 2014) but may be influenced by the duration of the study. Since *E. mongoz* exhibited a period of nocturnality during the month of July, some of their DN ratios were less than 1.00 which would have shifted the overall DN ratio throughout the study period. In other

studies that lasted only a few weeks, this nocturnal behavior may not have been captured, resulting in a higher DN ratio. Curtis, Zaramody, and Martin (1999) found in their 10-month long study that there was significant variability within seasons, but that overall, the mongoose lemurs exhibited more daytime activity in the wet season and more nighttime activity in the dry season. Although DN ratios are seen as a universal variable that can be used for cross-studies comparisons, we must remain aware of the study length and time period in which the DN ratio was calculated. This is especially important for *Eulemur* species, which are known to shift their activity patterns seasonally (Curtis, Zaramody, & Martin, 1999; Curtis and Rasmussen, 2006; Curtis, 2007). Similar to *E. mongoz*, the *L. catta* group displayed a period of more evenly distributed activity throughout the month of July, compared to higher fluctuations in daytime activity during June and August. This behavior will have a similar effect on the overall DN ratio for the study period, resulting in a ratio that is slightly closer to 1.00 than what has been found in other studies (Rea et al., 2014). Parga (2011) found that *L. catta* groups on Saint Catherine's Island steadily decreased their nighttime travel throughout the study period, but a similar relationship was not found for daytime travel. This resulted in fluctuating ratios between overall daytime and nighttime activity, similar to what is seen in this study.

The purpose of this study was to look at the differences in activity patterns between three species of semi-free ranging lemur and how these differences are influenced by environmental variables. The three species in this study are also members of three different genera – *Eulemur*, *Lemur*, and *Varecia*. This is important because many cathemerality studies will form hypotheses based on similar species within the same genus as their subject species, or they will make comparisons in their discussion to species within the same genus. The *Eulemur* genus is often described as the genus containing mostly cathemeral species, with other genera being broken

down by individual species. We often focus on the species or the genera of a focal group for comparative studies due to morphological and behavioral similarities. However, there are many variable factors within each genus that may influence activity patterns. For example, not all species within the same genus will have the same eye morphology or the same group structure. We can also find this variation within species, such as age and sex differences influencing activity patterns. Although this study found significant differences between species, and genera, when looking at activity patterns, it is important to note that there were also individual differences within species. This suggests that studies on cathemerality may benefit from shifting the aim away from defining activity patterns within species or genera. Rather, future directions should focus more on individual and group variation to expand the literature and further our understanding of activity patterns and circadian entrainment in primates.

Limitations

Due to the semi-free ranging nature of the subject species in this study, it may be difficult to make true comparisons between the six individual lemurs and their wild counterparts. However, this study still reveals important information about the influence of different variables on activity patterns. This study presented a few limitations that should be considered for future studies. Behavioral observations during this study included recording the presence of predators, anti-predator behavior, and intraspecific alarm calls. However, these observations were not included in the analyses because it is unclear how lemurs perceive predation in a semi-free ranging environment. On a few occasions, a large raptor would be observed circling above the forest enclosure and this would be followed by vocalizations from all groups. On the other hand, this same vocalization would sometimes follow the observation of an airplane overhead, or it would not follow the observation of a large raptor inside the forest enclosure. The enclosure also

has a light canopy, making it difficult to judge whether a lemur's location under the canopy was a direct form of anti-predator behavior.

The placement and movement of the accelerometers also presents another limitation. The accelerometers were placed on the back of each lemur's neck with the existing radio unit acting as a counterweight. The collars are loosely fitted on the lemurs so as not to interfere with breathing or eating, so they would sometimes rotate. Tri-axial accelerometers are extremely sensitive devices that combine measurements of static (acceleration due to gravity) and dynamic (acceleration due to movement) acceleration. This means that any shifting or movement of the collar could have been recorded as animal movement. Many researchers will apply a smoothing function during data analysis to minimize these recordings, but when looking at fine-scale movements, it can be unclear what was movement of the head and what may have been movement of the collar. The sensitivity of tri-axial accelerometers also means that movement of the substrate may be recorded, resulting in a resting lemur on a swaying branch being recognized as movement.

Cathemerality studies appear to have many methodological limitations. Tri-axial accelerometers are extremely sensitive and may be more complex than what is needed for studies looking at overall activity patterns. On the other hand, studies that rely only on observations will often assign 24-hour shifts or multiple collaborators splitting up the time. This method can introduce observer error, whether a single observer becomes tired throughout their observation period or multiple observers define "active" and "inactive" differently. Activity switches can also be used for these studies, but every device is calibrated differently and may result in different interpretations of the data. These limitations bring up the question of what do we define as movement? When looking at cathemerality and other activity-based studies, what is activity

and what is movement? If a lemur is sitting or in another position defined as rest, but is alert and moving their head, researchers may choose to define this as movement and not activity.

However, their device may record this as activity and cause discrepancies during data analysis.

Future studies may benefit from a standardization of these definitions, especially if we continue to compare studies using different data collection methodologies.

CONCLUSION

The results of this study are highly variable when compared to the results of studies on the same species in different environments – captive, semi-free ranging, disturbed forest, and “natural” habitats have all found different results when looking at cathemerality in lemurs. In conclusion, this research adds to the wealth of knowledge that the scientific community is gaining on the complexity of activity patterns. Other studies looking at multiple species within the same forest enclosure have discovered similar patterns between species as well as unusual patterns when comparing individuals of the same species to their wild counterparts. There is a wide range of literature showing that cathemerality is not straightforward and is dependent on many exogenous and endogenous variables. This study further supports the idea that lemurs are capable of adjusting their behavior in response to their environment and the influences of controlled human interaction. As the literature expands and we continue to study cathemerality, it may be beneficial to do more comparative studies between landscapes rather than between species. By focusing on the individual or group level and comparing these subjects in varying environments, we may gain a better understanding of how primate morphology and behavior interacts with regular photoperiodic changes as well as micro-level environmental changes.

Significance Statement

This study looked at the environmental influences on activity patterns of lemurs, specifically in the context of cathemerality, in a multi-acre forest enclosure. The three subject species in this project were held in the same forest environment in southern Florida, meaning that the environmental factors hypothesized to influence cathemeral behavior were relatively controlled among the three species. Studies on captive and semi-free ranging populations can control for environmental variables and allow us to make inferences about the driving forces of

activity patterns in the Lemuridae family (Bray, Samson, & Nunn, 2017). This research can then be used to advance our knowledge on the activity patterns of wild lemur species. The presence of cathemeral activity patterns in lemurs demonstrates a uniquely high level of behavioral flexibility because this often means that the lemur will alter activity throughout the 24-hour cycle in response to changing conditions. Understanding the behavioral flexibility of lemurs and the evolutionary context of cathemeral behavior will allow us to make predictions about the impending impacts of climate change and anthropogenic disturbances, and potentially how we could mitigate or minimize these impacts on endangered lemur species. This knowledge may also help us to make informed decisions about relocations, breeding programs, animal welfare, and the release of a previously captive animal. Primates provide many ecosystem services to humans and are important members of their ecological communities. However, they face many threats and populations worldwide are declining.

Ethics Statement

I strongly believe in the conservation efforts of wild animals, and especially believe that primates are an important group of animals to conserve. Primates provide ecosystem services, have important ecological roles, and have unique characteristics that can provide scientific insight into the study of other wild animals. Having said that, I am a United States citizen and have never lived in an area where primates are native or wild, nor have I experienced the benefits or challenges of coexisting with wild primates. I understand that for some people, living with primates can be difficult. I also understand that for others, primates may be a primary source of protein. I want to acknowledge the impacts that conservation research can have on the people and communities who live in the same area as wild primates. For some, conservation efforts and initiatives mean that conflicts with crop-raiding primates may increase, or that they may lose

access to the forest that they once used as a food resource. I believe that humans should work towards conserving endangered species, but that this should not be done at the risk of harming other people. This project aims to understand the influence of different environmental variables on the activity patterns of primates and how primates respond to varying conditions. The implications of this project are for conservation purposes, but any applications of conservation research towards applied methods and initiatives should be done carefully to ensure that no harm is being done to those who live near or with endangered species.

Capture of the lemurs by the Lemur Conservation Foundation staff was included in this project. Only two individuals per species were fitted with accelerometers to minimize stress and handling for both the staff and the resident lemurs. This research was reviewed and accepted by the Lemur Conservation Foundation's review board for IACUC approval.

REFERENCES

- Altmann, J. (1974). Observational Study of Behavior: Sampling Methods. *Behaviour*, 49, 227-267.
- Aschoff, J. (1966). Circadian Activity Pattern with Two Peaks. *Ecology*, 47(4), 657-662.
- Aschoff, J., Daan, S. & Groos, G. A. (1982). Vertebrate Circadian Systems. Berlin: Springer-Verlag.
- Berger, A., Scheibe, K. M., Eichhorn, K., Scheibe, A., & Streich, J. (1999). Diurnal and ultradian rhythms of behaviour in a mare group of Przewalski horse (*Equus ferus przewalskii*), measured through one year under semi-reserve conditions. *Applied Animal Behaviour Science*, 64, 1-17.
- Bray, J., Samson, D. R., & Nunn, C. L. (2017). Activity patterns in seven captive lemur species: Evidence of cathemerality in *Varecia* and *Lemur catta*? *American Journal of Primatology*, 79(6), e22648-n/a. doi:10.1002/ajp.22648
- Brooke, A. P. (2001). Population status and behaviors of the Samoan flying fox (*Pteropus samoensis*) on Tutuila Island, American Samoa. *Journal of Zoology*, 254, 309-319.
- Burney, D. A., Burney, L. P., Godfrey, L. R., Jungers, W. L., Goodman, S. M., Wright, H. T., & Jull, A.J. T. (2004). A chronology for late prehistoric Madagascar. *Journal of Human Evolution*, 47, 25-63.
- Campera, M., Balestri, M., Chimienti, M., Nijman, V., Nekaris, K. A. I., & Donati, G. (2019). Temporal niche separation between the two ecologically similar nocturnal primates *Avahi meridionalis* and *Lepilemur fleuretae*. *Behavioral Ecology and Sociobiology*, 73(5). doi:10.1007/s00265-019-2664-1
- Colquhoun, I. C. (1998). Cathemeral Behavior of *Eulemur macaco macaco* at Ambato Massif, Madagascar. *Folia Primatologica*, 69(1), 22-34.
- Colquhoun, I. C. (2006). Predation and cathemerality. Comparing the impact of predators on the activity patterns of lemurids and ceboids. *Folia Primatol (Basel)*, 77(1-2), 143-165. doi:10.1159/000089701
- Curtis, D. J., & Rasmussen, M. A. (2002). Cathemerality in lemurs. *Evolutionary Anthropology: Issues, News, and Reviews*, 11(S1), 83-86. doi:10.1002/evan.10064
- Curtis, D. J., & Rasmussen, M. A. (2006). The evolution of cathemerality in primates and other mammals: a comparative and chronoecological approach. *Folia Primatologica*, 77(1-2), 178-193. doi:10.1159/000089703
- Curtis, D. J., Zaramody, A., & Martin, R. D. (1999). Cathemerality in the mongoose lemur, *Eulemur mongoz*. *American Journal of Primatology*, 47, 279-298.
- Dewar, R. E. & Richard, A. F. (2007). Evolution in the hypervariable environment of Madagascar. *PNAS*, 104(34), 13723-13727.
- Donati, G., Baldi, N., Morelli, V., Ganzhorn, J. U., & Borgognini-Tarli, S. M. (2009). Proximate and ultimate determinants of cathemeral activity in brown lemurs. *Animal Behaviour*, 77(2), 317-325. doi:10.1016/j.anbehav.2008.09.033
- Donati, G., Bollen, A., Borgognini-Tarli, S. M., & Ganzhorn, J. U. (2007). Feeding over the 24-h cycle: dietary flexibility of cathemeral collared lemurs (*Eulemur collaris*). *Behavioral Ecology and Sociobiology*, 61(8), 1237-1251. doi:10.1007/s00265-007-0354-x
- Donati, G., & Borgognini-Tarli, S. M. (2006). Influence of abiotic factors on cathemeral activity: the case of *Eulemur fulvus collaris* in the littoral forest of Madagascar. *Folia Primatol (Basel)*, 77(1-2), 104-122. doi:10.1159/000089698

- Donati, G., Santini, L., Razafindramanana, J., Boitani, L., & Borgognini-Tarli, S. (2013). (Un-) expected nocturnal activity in "Diurnal" Lemur *catta* supports cathemerality as one of the key adaptations of the lemurid radiation. *American Journal of Physical Anthropology*, *150*(1), 99-106. doi:10.1002/ajpa.22180
- Eppley, T. M., Ganzhorn, J. U., & Donati, G. (2015). Cathemerality in a small, folivorous primate: proximate control of diel activity in *Hapalemur meridionalis*. *Behavioral Ecology and Sociobiology*, *69*(6), 991-1002. doi:10.1007/s00265-015-1911-3
- Eppley, T. M., Watzek, J., Ganzhorn, J. U., & Donati, G. (2017). Predator avoidance and dietary fibre predict diurnality in the cathemeral folivore *Hapalemur meridionalis*. *Behavioral ecology and sociobiology*, *71*(1). doi:10.1007/s00265-016-2247-3
- Erkert, H. G., & Cramer, B. (2006). Chronobiological background to cathemerality: circadian rhythms in *Eulemur fulvus albifrons* (Prosimii) and *Aotus azarai boliviensis* (Anthropoidea). *Folia Primatologica (Basel)*, *77*(1-2), 87-103. doi:10.1159/000089697
- Fernandez-Duque, E., & Erkert, H. G. (2006). Cathemerality and lunar periodicity of activity rhythms in owl monkeys of the Argentinian Chaco. *Folia Primatologica*, *77*(1-2), 123-138. doi:10.1159/000089699
- "Florida." The Lemur Conservation Foundation, Lemur Conservation Foundation, 2 Oct. 2020, www.lemurreserve.org/about-lcf/myakka-city-lemur-reserve/.
- Gwinner, E. (1986). Circannual rhythms. Berlin: Springer.
- Halle, S. (2006). Polyphasic activity patterns in small mammals. *Folia Primatologica (Basel)*, *77*(1-2), 15-26. doi:10.1159/000089693
- Kappeler, P. M., & Erkert, H. G. (2003). On the move around the clock: correlates and determinants of cathemeral activity in wild red-fronted lemurs (*Eulemur fulvus rufus*). *Behavioral Ecology and Sociobiology*, *54*(4), 359-369. doi:10.1007/s00265-003-0652-x
- Kelley, E. A., Jablonski, N. G., Chaplin, G., Sussman, R. W., & Kamilar, J. M. (2016). Behavioral thermoregulation in *Lemur catta*: The significance of sunning and huddling behaviors. *American Journal of Primatology*, *78*(7), 745-754. doi:10.1002/ajp.22538
- LaFleur, M., Sauther, M., Cuozzo, F., Yamashita, N., Jacky Youssouf, I. A., & Bender, R. (2014). Cathemerality in wild ring-tailed lemurs (*Lemur catta*) in the spiny forest of Tsimanampetsotsa National Park: camera trap data and preliminary behavioral observations. *Primates*, *55*(2), 207-217. doi:10.1007/s10329-013-0391-1
- Martin, R. D. (1972). Review Lecture: Adaptive Radiation and Behaviour of the Malagasy Lemurs. *Philosophical Transactions of the Royal Society of London*, *264*(862), 295-352.
- Parga, J. A. (2011). Nocturnal ranging by a diurnal primate: are ring-tailed lemurs (*Lemur catta*) cathemeral? *Primates*, *52*(3), 201-205. doi:10.1007/s10329-011-0257-3
- Pariente, G. F. (1979). The role of vision in prosimian behavior. In: Doyle GA, Martin RD (eds) *The study of prosimian behavior*. Academic, New York, pp 411-459.
- Prugh, L. R., Golden, C. D. (2014). Does moonlight increase predation risk? Meta-analysis reveals divergent responses of nocturnal mammals to lunar cycles. *Journal of Animal Ecology*, *83*:504-514.
- Razanaparany, P. T., & Sato, H. (2020). Abiotic factors affecting the cathemeral activity of *Eulemur fulvus* in the dry deciduous forest of north-western Madagascar. *Folia Primatologica (Basel)*, *91*(5), 463-480. doi:10.1159/000506128
- Rea, M. S., Figueiro, M. G., Jones, G. E., & Glander, K. E. (2014). Daily activity and light exposure levels for five species of lemurs at the Duke Lemur Center. *American Journal of Physical Anthropology*, *153*(1), 68-77. doi:10.1002/ajpa.22409

- Santini, L., Rojas, D., & Donati, G. (2015). Evolving through day and night: origin and diversification of activity pattern in modern primates. *Behavioral Ecology*, 26(3), 789-796. doi:10.1093/beheco/arv012
- Savagian, A., & Fernandez-Duque, E. (2017). Do predators and thermoregulation influence choice of sleeping sites and sleeping behavior in Azara's owl monkeys (*Aotus azarae azarae*) in Northern Argentina? *International Journal of Primatology*, 38(1), 80-99. doi:10.1007/s10764-016-9946-5
- Schoener, T. W. (1974). Resource partitioning in ecological communities. *Science*, 185(4145), 27-39.
- Tarnaud, L. (2006). Cathemerality in the Mayotte brown lemur (*Eulemur fulvus*): seasonality and food quality. *Folia Primatologica (Basel)*, 77(1-2), 166-177. doi:10.1159/000089702
- Tattersall, I. (1982). *The primates of Madagascar*. New York: Columbia University Press.
- Tattersall, I. (1987). Cathemeral activity in primates: A definition. *Folia Primatologica* 49:200-202.
- Tattersall, I. (2006). The concept of cathemerality: history and definition. *Folia Primatologica*, 77(1-2), 7-14. doi:10.1159/000089692
- Tofanelli, S., Bertoncini, S., Castri, L., Luiselli, D., Calafell, F., Donati, G., & Paoli, G. (2009). On the origins and admixture of Malagasy: new evidence from high-resolution analyses of paternal and maternal lineages. *Molecular Biology and Evolution*, 26(9), 2109-2124. doi:10.1093/molbev/msp120
- van Schaik, C. P., and Kappeler, P. M. (1996). The social systems of gregarious lemurs: lack of convergence with anthropoids due to evolutionary disequilibrium? *Ethology* 102:915-941.
- Vasey, N. (2000). Niche separation in *Varecia variegata rubra* and *Eulemur fulvus albifrons*: I. Interspecific patterns. *American Journal of Physical Anthropology*, 112: 411-431
- Vasey, N. (2005a). Activity budgets and activity rhythms in red ruffed lemurs (*Varecia rubra*) on the Masoala Peninsula, Madagascar: seasonality and reproductive energetics. *Am J Primatol*, 66(1), 23-44. doi:10.1002/ajp.20126
- Vasey, N. (2005b). New developments in the behavioral ecology and conservation of ruffed lemurs (*Varecia*). *Am J Primatol*, 66(1), 1-6. doi:10.1002/ajp.20124
- Wright, P. C. (1989). The nocturnal primate niche in the New World. *Journal of Human Evolution*, 18, 635-658.

APPENDIX: ETHOGRAM OF LEMUR BEHAVIOR

Table 1. Ethogram of lemur behavior created at the Lemur Conservation Foundation in March 2020.

Category	Behavior	Description
Active		
Locomotion	Running (Ru)	Fast quadrupedal movement with tail held upright or elevated above spine.
	Hopping (Hop)	Using hind legs only to move quickly through forest and over objects.
	Walking (Wa)	Slow quadrupedal movement with tail held upright or elevated about spine
	Climbing (Cl)	Moving on substrate other than ground including branches and fence.
	Jumping (Ju)	Moving between objects suspended in air such as ropes and branches. Lemur is temporarily out of contact with objects during movement.
Social	Attempting to mate (At)	Grabbing waist, attempting to mount, and sniffing genitals of female.
	Auto-grooming (Gr1)	Picking at fur with teeth or hands; long, repetitive licks across fur.
	Allo-grooming (Gr2)	Picking at fur of other lemur with teeth or hands; long, repetitive licks across fur.
	Mutual grooming (Gr3)	Sitting opposite of partner and simultaneously grooming each other (see allo-grooming).
Other	Scent Marking (SM)	Rubbing genitals against object with tail held upright, usually followed by excretion of liquid.
	Drinking (Dr)	Consumption of water (observed by licking bottle in dome that released water).
	Eating (Ea)	Movement of jaw to chew food and swallowing.
	Foraging (Fo)	Scavenging through bush while examining and processing leaves and bark.
Inactive		
Postural	Sitting (Si)	Stationary posture with hindquarters on ground and legs stretched or bent in front of body. Arms are held in relaxed state or bearing weight.
	Standing (St)	Stationary quadrupedal position with tail held upright and head above shoulders.
	Basking (Ba)	Stationary posture with hindquarters on ground and legs bent in front of body. Arms are held to side and body is facing sun.
	Laying (La)	Resting on ground with legs extended or curled under body. No limbs are bearing weight.

	Perching (Pe)	Hindquarters on branch with hands and feet grasping branch.
	Leaning (Le)	Bearing weight on hind legs while pressing shoulder or hands to fence.
	Hanging (Ha)	Stationary position where hands or feet are grasping an object above and there is no contact with substrate below.
Vocalizations	Grunting (Gr)	Short, deep pitch, throaty vocalizations with mouth closed.
	Chirping (Ch)	Short, high pitch vocalizations where mouth opens briefly.
	Howling (Ho)	Loud and deep vocalizations usually followed by a response from other group members.
Other	Urinating (Ur)	Excreting urine, usually while stationary.
	Defecating (De)	Excreting feces.
	Scratching (Sc)	Quickly and repetitively moving hands or feet across body.
	Licking (Li)	Moving tongue in quick, dragging movements across body.
N/A		
Other	Out of Site (OS)	Lemur not visible to observer.