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# Analysis of Lepilemur edwardsi Seasonal Acoustic Activity

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#### Recommended Citation

Henry, Samantha, "Analysis of Lepilemur edwardsi Seasonal Acoustic Activity" (2023). Honors Undergraduate Theses. 1465. [https://stars.library.ucf.edu/honorstheses/1465](https://stars.library.ucf.edu/honorstheses/1465?utm_source=stars.library.ucf.edu%2Fhonorstheses%2F1465&utm_medium=PDF&utm_campaign=PDFCoverPages)

by

### SAMANTHA ABIGAIL HENRY

A thesis submitted in partial fulfillment of the requirements for the Honors in the Major Program in Biology in the College of Sciences and in the Burnett Honors College at the University of Central Florida Orlando, Florida

> Summer Term 2023

### ABSTRACT

Passive acoustic monitoring (PAM) has historically been used as a noninvasive technique to study biodiversity, activity patterns, distribution, and species dispersal. The use of acoustic data has expanded in ecological research; however, studies focusing on primates are underrepresented, especially for nocturnal species. This study seeks to increase the understanding of acoustic activity in the nocturnal Milne-Edwards' sportive lemur (*Lepilemur edwardsi*). This species is endangered and endemic to the lowland dry forests in northwest Madagascar. Specifically, this study aims to describe the daily and annual acoustic activity patterns of the *L. edwardsi*. The PAM survey was conducted using AudioMoth recorders in the Mariarano Forest at 20 sites, with a recording being taken one minute every hour for a year. *L. edwardsi* presence and absence data were obtained from the recordings by using semi-automated analysis tools from ARBIMON (Rainforest Connection®). The results show that the parametric coefficients, Month and Hour, have an effect on the vocal activity of *L. edwardsi* with the various locations of the sites being taken into account. The probability of recording a *L. edwardsi* presence increases as the year progresses. Conversely, the probability of a *L. edwardsi* presence being recorded increases as the night progresses until midnight and decreases until the early morning hours. These results suggest that acoustic communication is important in *L. edwardsi* offspring rearing and parental investment. In addition, this study demonstrates the great potential of PAM in monitoring endangered and elusive species.

### ACKNOWLEDGMENTS

I am extremely grateful to my thesis co-chair, Dr. Beatriz Otero Jiménez, who has been very patient and gracious with me from start to finish. Many thanks to my thesis co-chair, Dr. Patrick Bohlen, and my other committee members, Dr. David G. Jenkins and Frank Logiudice; my thesis would not have been what it is without their assistance and input. Lastly, I'd like to acknowledge the staff at the Burnett Honors College, my family, and my friends who have supported and encouraged me in various ways.

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#### INTRODUCTION

<span id="page-7-0"></span>Auditory-based behaviors are observed in many taxa, most notably in bats, birds, and marine mammals (Barrett-Lennard et al., 1996; Fleishman et al., 2023; Redondo, 1991; Schmidt et al., 1991; Tomasek et al., 2023; Zou et al., 2023). These behaviors are important drivers in the evolution of species (Laiolo, 2010). Intraspecific and interspecific communication has provided insight into ecological phenomena, such as reproductive cycles (Ehret & Schmid, 2009; Virant-Doberlet & Cokl, 2004), predators-prey dynamics (Alem et al., 2011; Barber & Conner, 2007; Remage-Healey et al., 2006), and food competition (Corcoran, 2022; Radford & Montgomery, 2016). Therefore, acoustic communication can provide valuable insights into wildlife behavior.

Acoustic monitoring was initially used as a tool to obtain population data, for example, using tape recordings of vocalizations to estimate population densities (Johnson et al., 1981) and later to study species behavior (Joseph M. Northrup et al., 2020). Current technological developments (Scheffer et al., 2023; Simpfendorfer et al., 2010) have allowed the field of acoustic research to expand into two main monitoring types: active and passive (Browning et al., 2017) Active acoustic monitoring (AAM) involves the reflected sound being picked up by one or more receivers and at least one receiver being placed next to the target organism or environment. This type of monitoring provides data on individual species' identity, location, and movement (Stein & Edson, 2016). Passive acoustic monitoring (PAM) involves the use of automated recorders that record environmental sounds over prolonged periods of time (Sugai et al., 2019). PAM can provide data on biodiversity, acoustic activity patterns, distribution, and dispersal. Passive acoustic monitoring has grown in recent years, mainly due to the technological capacity of programming recorders to collect data unattended for longer periods of time (Sugai et al.,

2019). In addition, tools for automating the processing of acoustic data have made PAM an attractive monitoring tool for researchers. Today, PAM has become a time-saving and costeffective form of monitoring (Markolf et al., 2022).

PAM has been used as the primary tool in behavioral studies of birds (Abby L. J. Hensel et al., 2022; Szymański et al., 2021), fish (Desiderà et al., 2022; Simpfendorfer et al., 2010), and marine mammals (Todd et al., 2009; Van Parijs et al., 2009). Avian studies have used PAM to determine the annual vocal activity of a species (Abby L. J. Hensel et al., 2022; Szymański et al., 2021), which can be used to estimate populations and longevity (Abby L. J. Hensel et al., 2022). Many PAM studies concerning fish and marine mammals help researchers understand how these organisms interact with anthropogenic infrastructure (Simpfendorfer et al., 2010; Todd et al., 2009). More recently, PAM has been an important tool in the study of mammals. Early studies focused on marine mammals (Akamatsu et al., 2001; McDonald & Fox, 1999) and later expanded to include terrestrial mammals such as bats, elephants, and primates (Markolf et al., 2022; R. J. Thomas & Davison, 2022; Wrege et al., 2017). PAM has helped identify the effects of anthropogenic infrastructure on bat abundance and distribution (López-Bosch et al., 2022; Meramo et al., 2022) and allowed the monitoring of elusive and endangered species populations such as the African forest elephant (*Loxodonta cyclotis*), Pale Fork-marked Lemur (*Phaner pallescens*), and Vaquita (*Phocoena sinus*) (Markolf et al., 2022; Swider et al., 2022; L. Thomas et al., 2017; Wrege et al., 2017). However, acoustic research remains limited for many mammal groups, such as primates.

Early primate acoustic studies focused on diurnal species that are known for being vocal (Do Nascimento et al., 2021; Pérez-Granados & Schuchmann, 2021; Spillmann et al., 2015),

where other primate studies have focused on quantifying vocal activity while taking into account sexual dimorphism and group dynamics (Crunchant et al., 2021; Do Nascimento et al., 2021; Pérez-Granados & Schuchmann, 2021). Due to the limited number of acoustic studies of primates, there is a lack of automation in this group's acoustic analysis methods and an underrepresentation of nocturnal surveys (Clink et al., 2020; Do Nascimento et al., 2021; Zhong et al., 2021). Many primates vocalize at night (Piel, 2018) even when the species is well known for its diurnal activity, which in turn increases our understanding of primate behavior. Acoustic studies concerning nocturnal primates are important for understanding life cycle events, mate selection, species differentiation, and group dynamics (Braune et al., 2005; Schneiderová et al., 2020; Seiler et al., 2015). Due to the lack of nocturnal studies concerning primates, more research is necessary to better understand the role of acoustic communication in this group.

This study aims to examine the acoustic ecology of the Milne-Edwards'sportive lemur (*Lepilemur edwardsi*). This species is an endangered nocturnal lemur endemic to the island of Madagascar (Hokan et al., 2017; Méndez-Cárdenas & Zimmermann, 2009). Daily and seasonal acoustic activity patterns of the *L. edwardsi* were evaluated using presence and absence data obtained from PAM using semi-automated analysis. More specifically, this study evaluates the incidence of a call type used by both males and females. Looking at the acoustic activity of the *L. edwardsi* will expand the knowledge about their vocal behavior, all while supporting conservation efforts concerning the ecosystem of Northwest Madagascar.

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### METHODOLOGY

#### Study Site

<span id="page-10-1"></span><span id="page-10-0"></span>The study site is known as the Mariarano Forest (Fig. 1). It is located in north-western Madagascar, about 44.9 km from Mahajanga City (Palfrey et al., 2019). The mean annual temperature in the region ranges from 25.5°C to 28.9°C (Ramilison et al., 2021). The wet season extends from December to February, and monthly rainfall is highly variable (1 mm- 360 mm) (Ramilison et al., 2021). The dry season in the region extends from July to September (Palfrey et al., 2019). The elevation reaches a maximum of about 80 meters above sea level (Palfrey et al., 2019). The Mariarano Forest vegetation is dominated by low-lying dry deciduous forest intermixed with areas of wetlands, grasslands, scrub habitats, and agricultural land (Palfrey et al., 2019). The varying vegetation allows for a diversity of organisms to inhabit the forest (Koechlin, 1972).

<span id="page-11-1"></span>Figure 1. Maps of the Study Site



*Note.* Aerial map of Madagascar with a pinpoint at the Mariarano Forest (Left) and map of the Mariarano Forest with pins where each recorder is located (Right).

#### Study Organism

<span id="page-11-0"></span>*Lepilemur edwardsi,* also known as the Milne-Edwards' sportive lemur, inhabits the lowland dry deciduous forest in northwest Madagascar (Fig. 2). The species is classified as an endangered nocturnal lemur by the IUCN Red List (Louis et al., 2020). It is an arboreal folivorous primate with a diet consisting mostly of leaves (Méndez-Cárdenas & Zimmermann, 2009). They live in dispersed male-female pairs as the smallest social unit (Rasoloharijaona et al., 2006). Pair partners share a home range that includes suitable feeding and sleeping sites throughout most of the year (Rasoloharijaona et al., 2006). *L. edwardsi* reproduces seasonally,

with their mating period occurring from May-June, gestation from July-October, and rearing from November-April (Méndez-Cárdenas & Zimmermann, 2009).

<span id="page-12-0"></span>

Figure 2. Map of the *Lepilemur edwardsi* Range with a Headshot of the *L. edwardsi*

*Note.* (Louis et al., 2020; Vassen, 2009)

Few studies have explored *L. edwardsi* vocalizations. A previous study revealed nine major call types: five male-specific, three female-specific, and a call produced by both sexes (Fig. 3) (Rasoloharijaona et al., 2006). Acoustic studies revealed that pairs regularly participate in duetting or coordinated vocalizations (Méndez-Cárdenas & Zimmermann, 2009) and that vocalizations can be used to identify individuals based on call type and duration (Rasoloharijaona et al., 2006).

<span id="page-13-1"></span>Figure 3. A Typical *Lepilemur edwardsi* Call



*Note.* The call was recorded in the Mariarano Forest. The spectrogram includes two high-pitched calls at 44.5 and 46.5 seconds.

#### Data Collection

<span id="page-13-0"></span>Acoustic data was collected using passive acoustic monitoring (PAM) with AudioMoth recorders (version 1.0.0) (Hill et al., 2019)(https://www.openacousticdevices.info/). 20 recorders were placed across five different trails within the Mariarano Forest (Fig. 1). The recorders were placed at least 400m apart to prevent acoustic data overlap and to maintain data integrity. Recorders were set to record one minute of each hour continuously for a year from May 2018 to April 2019. Every month the recorder batteries were changed during their inactive state to ensure continuous data collection. Over 150,000 recordings were collected. The recordings were then uploaded as .wav files to the Automated Remote Biodiversity Monitoring Network (ARBIMON) cloud-based analytical tool for data acquisition, management, and species identification (Aide et al., 2013) (https://arbimon.rfcx.org/).

#### Acoustic Data Analysis

<span id="page-14-0"></span>To evaluate the annual acoustic patterns of activity for *L. edwardsi,* the study focused on the *L. edwardsi* high-pitch call (Fig. 3). The high-pitched vocalization is produced by both the males and the females, as contrasted with the sex-specific calls (Rasoloharijaona et al., 2006), allowing for the exploration of *L. edwardsi* activity.

The acoustic data analysis can be split into five steps: call identification, pattern matching, call validation, model training and optimization, and classification. First, a highquality example of a high-pitch call was found in the recordings (i.e., call identification). Next, using this example, the Pattern Matching (PM) model was run in ARBIMON (Threshold  $= 0.2$ , Matches/Recording  $= 1$ , Matches/Site  $=$  no limit) to search for calls with a similar structure based on a correlation score (LeBien et al., 2020). The correlation score is based on how similar detection events are to the template, also known as the user-defined region of interest (ROI). Next, the results were manually validated (i.e., call validation). If a detection event located a *L. edwardsi* high-pitch call then the recording was marked present, and if the detection event falsely identified a high-pitch call then the recording was marked absent. These validations were then used to train the PM model for the call of interest. Model training and optimization consisted of running the Random Forest Model (RFM) on validated recordings. The RFM is a more extensive form of decision tree that can predict future examples using multiple classifiers to reach the initial prediction's accuracy and precision (Shaik  $&$  Srinivasan, 2019). In the RFM, the outputs include the Precision [true positives/ (true positives + false positives)] and Accuracy [(true positives + true negatives) / total] (Aide et al., 2013). Model training and optimization were done repeatedly, adding more manual validations until the desired precision and accuracy were

obtained ( $\geq 0.9$ ). Once the model had satisfactory accuracy (0.895) and precision (0.899) values, the classification model (RFM) was run on all night-time recordings (6 pm to 6 am) and obtained the presence–absence data for the *L. edwardsi* high-pitched call. Due to the number of recordings, the classification model needed to run multiple times, with the grouping based on months of the year (e.g., July, August, and September). From there, the subsets of data were combined into one Excel file (.xlsx).

#### Statistical Analysis

<span id="page-15-0"></span>To make sure the comparison between the sites were even, only sites containing >10 months of recordings were counted in the analysis. From there, the presence-absence data was exported in the form of a .xlsx file to R v.4.3.0 (R Core Team, 2023). Then a Generalized Linear Mixed Effects Model (GLMM) was used to analyze the data in R. For the mixed model, the dependent variable was the amount of acoustic activity (presences). The explanatory variables (i.e., independent variables and fixed effects) were months of the year and hours of the night (6 pm to 6 am), and the random effects were the sites where the recorders were placed. The GLMM was first run with the dependent variable and the random effects. Then another GLMM was run with the dependent variable, fixed effects, and random effects. From there, a chi-squared test was used to compare the two models and then select which model to use. The results of the chisquare revealed that the latter GLMM was adequate to proceed with so that GLMM was used to predict the probability of presence for months of the year and hours of the day.

#### RESULTS

<span id="page-16-0"></span>During a year-long sampling period, the recorders  $(n = 20)$  recorded a total of 83,838 minute-long recording intervals. Out of 83,838 total recordings, 3,107 presences of *L. edwardsi* were identified (Table 3). For the statistical analysis, data were only used from sites with recordings for >10 months to ensure the representation of activity throughout the year (Table 1). This data subset included 13 recording sites with a total of 64,110 recordings with a total of 2,218 presences were found, representing 3.5% of all recordings in the subset. The highest amount of presence was observed in November with 11.8% (Fig. 5) and during the night hour 5 (11 PM) with 4.6% (Fig. 5).

Results from our generalized linear mixed effect model (GLMM) identified both months  $(p = 8.15E-10)$  and night hours  $(p = 0.000486)$  as significant variables explaining patterns of *L*. *edwardsi* acoustic presence (Table 2). Although both variables were significant, Month had a stronger effect on the presence of *L. edwardsi* (Table 2). Model results from predicted presence showed November as the month with the highest presence (4.5%) (Fig. 5). Results from the model show that the predicted presence of *L. edwardsi* is highest during night hours 0 (6 PM) and 1 (7 PM), 3.5% and 3.4% respectively (Fig. 6).

The variability of the random effects can be used to infer variability with other sites that were not in the subset and possibly even other locations in the *L. edwardsi*'s range (Fig. 7).

		Percentage of			
<b>Site</b>	<b>Total recordings</b>	presences			
1	4897	1.980804574			
9	4984	3.631621188			
11	5027	3.819375373			
12	5019	0.318788603			
13	4808	3.202995008			
16	4847	0.598308232			
17	4411	0.36272954			
18	4982	0.240867122			
20	5163	4.338562851			
23	4973	6.153227428			
24	4785	6.938349007			
25	5278	2.671466465			
26	4936	10.49432739			
All sites	64110	3.459678677			

<span id="page-17-0"></span>Table 1. Data Subset Used for GLMM Analysis

*Note.* Total number of recordings and the percentage of total presences per site (i.e., recorder).

<span id="page-17-1"></span>Table 2. Summary of the GLMM



*Note.* The results explain the relationship between *L. edwardsi* vocal activity throughout the year and month and night hour.

<span id="page-18-0"></span>Figure 4. Raw Data from ARBIMON



*Note.* Percentage of *L. edwardsi* acoustic presence per month (Top) and night hour (Bottom). In the x axis, hours are presented as hours since sunset  $(0 =$  sunset at 6 pm).



<span id="page-19-0"></span>Figure 5. Presence Probabilities by the Month

*Note.* A plot showing box plots for each month of the year (2018-2019) and its probability of having recorded a *L. edwardsi* call.



<span id="page-19-1"></span>Figure 6. Presence Probabilities by the Hour

*Note.* Effects of recording time (night hours) on the calling activity of *L*. *edwardsi*. The box plot represents the mean predicted presence from the GLMM per night hour.

<span id="page-20-0"></span>Figure 7. Random Effects for Each Site



*Note.* The sites listed are the ones in the subset of data (Table 1).

#### DISCUSSION

<span id="page-21-0"></span>This study focused on understanding the acoustic activity of *Lepilemur edwardsi* across nighttime hours and months throughout one year based on the analysis of their high-pitched call. Based on previous research cataloging *L. edwardsi* activity, they are assumed to be active from 6 pm to 6 am (Méndez-Cárdenas & Zimmermann, 2009; Warren & Crompton, 1997). The results in this study confirm that activity increases at around 6 pm and decreases by 6 am ( $p =$ 0.000486), forming an inverse parabola (Fig. 4).

The results show that the highest acoustic activity occurred in October, November, and December, which coincides with the end of the gestation (July-October) and beginning of the rearing periods (November-April) (Méndez-Cárdenas & Zimmermann, 2009) These results contradict previous studies that deduced that the *L. edwardsi* would increase their acoustic activity during the mating period (May-June) to locate mates (Méndez-Cárdenas & Zimmermann, 2009). Rather, these results suggest that the high-pitch call is related to parental investment and offspring care. These findings are supported by a previous study on *L. edwardsi* vocalizations, which found that the highest amount of vocalization occurs during the offspring care period (November - April) (Méndez-Cárdenas & Zimmermann, 2009). Studies on other lemur species have found vocalizations linked to offspring care (De Gregorio et al., 2022; Seiler et al., 2015). In *Lepilemur edwardsi,* one of the female-specific calls (i.e., bark) is used to warn offspring of potential predators (Seiler et al., 2015). In the diurnal lemur species, Indri (*Indri indri*), parents' solo vocalization has been shown to be instrumental in regulating and motivating offspring to vocalize (De Gregorio et al., 2022). These findings are not surprising since biparental care is more prevalent in primates than in any other mammal group (Storey & Ziegler,

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2016). An increase in parental care could be a sign of how the species is compensating due to the decrease in population (Badyaev & Ghalambor, 2001; Louis et al., 2020). However, due to the nature of this study, a direct link between vocalization and behavior of the high-pitched call in *L. edwardsi* cannot be made.

The observed monthly acoustic activity could be linked to resource availability. In the Mariarano Forest, food resources are scarce during the dry season, extending from July to September. The results show increased vocal activity at the beginning of the wet season (December-February) (Randrianambinina et al., 2007). If vocalizations were used to protect scarce resources, the results would show higher activity during the dry season. However, our results suggest that acoustic activity could be related to the abundance of foliage. For instance, chimpanzees have been shown to produce calls to draw other members of their group to a food source, and calls were more frequently made by estrous females (Kalan & Boesch, 2015). *L. edwardsi* could be using the high pitch vocalizations in a similar way. The results in this study confirm that activity increases at around 6 pm and sustains near that level until about 4 am when it starts to decrease to a low point by 6 am (Fig. 4). The results from previous studies show that the *L. edwardsi* traveled the most from 5 pm to 7 pm, which is around the time the *L. edwardsi* are shown to vocalize the most (Warren & Crompton, 1997). The variability in the number of presences per site, without the effects of night hour and month (Fig. 7), contributes to the idea that the *L. edwardsi* are using high-pitched vocalizations to draw other conspecifics to food sources as they find an abundance of food. Further analysis could support the hypothesis that both parental investment and food availability could be playing a role in the observed activity patterns.

This study demonstrates the utility of PAM techniques in studying nocturnal species. Results were able to identify areas in the Mariarano Forest where *L. edwardsi* is present. Results of differential activity throughout the year demonstrate that environmental and behavioral factors influence vocalization activity. This study provides the groundwork to explore the acoustic phenology of *L. edwardsi*. The plan is to continue this research in the future by analyzing sexspecific calls (Rasoloharijaona et al., 2006). For instance, knowing the sex could provide a better estimate of the population's demographics. Future research that combines PAM techniques with field-based behavioral observations is something to hope for, as that information will explain the role that each call type plays in species communication and behavior.

# <span id="page-24-0"></span>APPENDIX

<span id="page-25-0"></span>

Figure 8. Presence Probabilities by the Month for Each Recorder

*Note.* A plot showing box plots for each month of the year (2018-2019) and its probability of having a recorded *Lepilemur edwardsi* call for each site individually.

<span id="page-26-0"></span>

	<b>Recordings per month</b>											<b>Total Recordings</b>	
<b>Site</b>	January	February	March	April	May	June	July	August	September	October	November	December	per site
1	391	353	393	379	388	375	461	801	771	226	$\overline{0}$	359	4897
$\,8\,$	45	$\mathbf{0}$	$\boldsymbol{0}$	69	390	379	535	779	579	392	411	341	3920
9	394	361	417	376	389	381	511	801	765	224	$\overline{0}$	365	4984
10	9	$\mathbf{0}$	$\boldsymbol{0}$	29	256	$\mathbf{0}$	226	784	761	228	$\mathbf{0}$	281	2574
11	394	352	391	375	384	376	506	738	379	351	381	400	5027
12	391	351	393	383	424	410	533	782	753	226	$\mathbf{0}$	373	5019
13	269	356	388	353	391	375	546	799	767	224	$\mathbf{0}$	340	4808
15	276	27	$\overline{0}$	$\overline{0}$	$\overline{0}$	$\mathbf{0}$	394	403	390	226	$\overline{0}$	371	2087
16	390	348	381	384	398	376	550	531	380	348	381	380	4847
17	396	358	396	362	392	378	545	661	376	186	$\mathbf{0}$	361	4411
18	394	348	395	383	392	379	555	783	761	221	$\mathbf{0}$	371	4982
19	323	$\mathbf{0}$	$\boldsymbol{0}$	265	395	380	522	785	757	228	$\mathbf{0}$	359	4014
20	400	392	393	336	389	374	524	781	430	370	390	384	5163
23	391	355	388	375	403	392	536	782	767	227	$\mathbf{0}$	357	4973
24	387	354	387	365	337	321	486	782	775	229	$\boldsymbol{0}$	362	4785
25	403	364	403	388	394	72	352	1177	1130	225	$\overline{0}$	370	5278
26	391	352	390	379	391	372	534	784	764	222	$\mathbf{0}$	357	4936
28	392	356	396	313	$\overline{0}$	$\mathbf{0}$	$\mathbf{0}$	$\mathbf{0}$	$\mathbf{0}$	$\mathbf{0}$	$\mathbf{0}$	357	1814
29	388	352	385	310	$\overline{0}$	$\mathbf{0}$	109	393	381	$\boldsymbol{0}$	$\mathbf{0}$	361	2679
30	341	352	395	310	$\mathbf{0}$	$\mathbf{0}$	108	392	382	$\theta$	$\theta$	360	2640
<b>All sites</b>	6765	5731	6291	6134	6113	5340	8533	13738	12068	4353	1563	7209	83838

Table 3. All the Recordings Analyzed by the RFM

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