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Lemur leaf frogs in Dark of the Moon: *Agalychnis lemur* call activity in relation to environmental factors focusing on lunar phase

Manuscript prepared for the journal of Herpetological Review

By

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Abstract

The presence of certain lunar phases is felt by many organisms and may affect biological rhythms within species such as the Critically Endangered lemur leaf frog (*Agalychnis lemur*). This study will focus on the effects of the full moon and other environmental variables, and how the nightly calls made by lemur leaf frogs may alter due to its presence. There are other species recorded to be affected by the presence of certain lunar phases. Activities performed by many species such as breeding, nesting, locomotion, and ovulation have shown synchronization or other changes due to the lunar phases. Especially amphibians, who are sensitive to their local conditions and are easily triggered by environmental changes as well as moon phases. Temperature and humidity are examples of variables that could change amphibians' patterns like nesting habits and breeding cycles. Environmental data was obtained from a local weather station near the study site in the Veragua Rainforest. This station provided hourly temperature, humidity, and cloud cover measures. Nightly call counts of *A. lemur* were obtained from a previous study and then compared to the lunar and environmental variables using general linear models. This analysis showed a significant increase in call counts when there was a full moon present. Call numbers in individual audio recorders were then compared separately with linear models to the same variables. Each audio recorder showed a significant difference in calls for different covariates. The first model claimed significant difference in temperature, the second claimed distance from water, and the third claimed humidity. This study concludes that the full moon does have a positive effect on calling activities in *A. lemur* and that further studies should be done on the effects of the moon and other environmental variables to further understand their influences.

Keywords

Lemur Leaf Frog, Lunar Phase, Calling Activity, *Agalychnis lemur*, Lunar Effects, Environmental Effects

1. Introduction

There are many anuran mysteries that happen with changes of the moon phase. The lunar phase has been shown to affect many species including but not limited to badgers (*Meles meles*), prairie rattlesnakes (*Crotalus Viridis Viridis*), salamanders (*Plethodon cinereus*), and a variety of anurans (Ralph 1957; Robertson 1978; Clarke et al. 1996; Dixon et al. 2006; Vignoli and Luiselli 2013; Underhill and Gerlinde 2018). The moon has been recorded to affect reproductive patterns, calling activity, and movement in amphibians (Ralph 1957; Church 1960 a,b; Underhill and Gerlinde 2018). The effects of environmental variables, like temperature, on anuran behaviors are generally recognized, but the influence of the moon has often been overlooked (Grant et al. 2013). It is expected that the moon would affect nocturnal amphibians with the light levels of the moon phase varying two degrees throughout the whole cycle, thus providing important environmental variations (Grant et al. 2013). The effects of the moon reach all the corners of the world, although it may affect all the locations differently due to the distinct times and angles the moonlight reaches them. The location focused on in this study is the Costa Rican Veragua rainforest. Costa Rica is very diverse in anuran species and must not be grouped geographically in aspects of lunar reactions because there are many species there and each one is different biologically and may be affected by the moon differently (IUCN 2020). Costa Rica is home to at least 148 identified anuran species, 77 of which are Threatened, including this study's focus the lemur leaf frog (IUCN 2020). The only other place the lemur leaf frog can be found is in Panama. The decline of this species is due to climate change, chytridiomycosis (*Batrachochytrium dendrobatidis*), and habitat loss. (Petchey 2014; Solis et al. 2008). Chytridiomycosis fungus is a highly dangerous fungus to many frog species and has been the cause of more than one species decrease and is seen to be the most substantial threat in undisturbed habitats (Leenders 2016).

It is crucial that this study is species specific because species have been shown to have differential sensitivity to changing environmental variables (Robertson 1978; Schalk and Saenz 2016). Amphibian populations show variations in the abiotic factors that drive their reproductive activities (Schalk and Saenz 2016). Such reproductive activities are shown to have both short-term and long-term effects from abiotic factors as well as individual species-specific responses (Schalk and Saenz 2016). Studies need to be done for individual species and not just based on assumptions that families are all affected the same. An example of species-specific reactions is

how *Rana temporaria* and *Rana esculenta* are active during different times of the day. Even though these species belong to the same genus, they have different activity patterns caused by reactions to light (Robertson 1978).

This study will drive the need for more lunar relevant research and how this research could be used to improve captive breeding programs by measuring the effect of lunar phases on lemur leaf frog calls. Studies like these are important for understanding species-specific breeding behaviors, which can be applied to manipulate environments of captive populations for optimal breeding success. If the appropriate moon phase can be replicated in captivity or if frogs can be held somewhere with access to natural moonlight, then the reproductive efforts of the lemur leaf frog could be maximized. This theory may instigate further research into lunar effects on reproductivity and may cause current breeding programs to move anurans where the lunar phases can be seen. Improving captive breeding is important for many Threatened species, but this particular Critically Endangered species has conservation programs in place already. This could mean change in those programs and benefits for the species may come easier since programs for the lemur leaf frog are already established. These breeding programs are important for reintroduction practices and are necessary to help increase population numbers that may have been devastated by anthropogenic causes, like habitat loss and spread of Chytrid fungus (*Batrachochytrium dendrobatidis*) (Solis et al. 2008).

Among the behavioral factors affected by the moon, the communicative calls of A. lemur are important for reproductive efforts. Calls are crucial in communication with conspecifics regarding territory boundaries, breeding availability, attracting mates, warnings, and other crucial messages (Gerhardt 1994; Jamieson 2003; Abrunhosa and Wogel 2004; Gomez et al. 2009; Dias et al. 2017). Anurans are capable of producing a variety of sound, each of which has a different length and frequency in order to portray a unique message (Gomez et al. 2009). Tropical frog choruses have been recorded to vary daily depending on their surrounding environment and have been reported to have significant correlations with temperature (Brooke et al. 2000), an environmental variable analyzed in this study. Anurans will decide on the best time to call by using social and environmental cues (Brooke et al. 2000). The main factors that affect the reproductive activity of anurans are determined by the amount of time in their breeding season (Oseen and Wassersug 2002). This is because depending on the length of an anuran's breeding period, the factors' effects may have more or less time to alter their activity (Oseen and

Wassersug 2002). Lemur leaf frog breeding season peaks during the months of April to August and the most common call during this time is their advertisement call (Abrunhosa and Wogel 2004; Leenders 2016). All the reasons above suggest that any variable that could affect such an important biological mechanism like reproduction, including calling activity, should be analyzed. Variables like moon phase, temperature, humidity, cloud cover, and rain have been studied and recorded to affect activities in many species, but there is still a need for more literature on these subjects (Clarke et al. 1996; Brooke et al. 2000; Dixon et al. 2006; Schalk and Saenz 2016).

Breeding and calling activity have been shown to be affected by the environment and moon phases in many anuran species (Ralph 1957; Robertson 1978; Clarke et al. 1996; Dixon et al. 2006; Vignoli and Luiselli 2013; Underhill and Gerlinde 2018). The full moon increased activity times in the toad *Bufo americanus* (Fitzgerald and Bider 1974) and an increase in mating activity for Javanese toad, *Bufo melanostictus* (Church 1960 a). There are many time-related rhythmic cues provided by the lunar cycle (Underhill and Gerlinde 2018). Nocturnal frogs, like *A. lemur*, may be affected by these cues from the phases of the moon not only due to its' light levels, but also from gravitational force, and geomagnetic activity (Grant et al. 2013; Underhill and Gerlinde 2018). Time important activities like migration and spawning could be synchronized to some sort of lunar cycle for many species (Underhill and Gerlinde 2018). The ovulation of many tropical anuran species is known to be synchronized with the lunar cycle as well (Church 1960 a, b). These lunar study reports gave cause for this study and helped hypothesize that lemur leaf frogs' (*A. lemur*) calling activity would be increased with the presence of the full moon.

The presence of the full moon is hypothesized to increase the amount of nightly calls from the lemur leaf frog. This paper will strengthen the theories of the moon phase affecting anuran calling by investigating whether the full moon affects nightly calling activity of lemur leaf frogs by use of call counts through linear models and statistical significance tests. Other variables, like temperature and humidity, were analyzed the same way for a more complete look at how calling activity could be affected by the surrounding environment. This topic is important because the scientific field needs more lunar studies and the lemur leaf frog (*A. lemur*) is a critically endangered species that will undoubtedly benefit from further understanding of environmental factors on behavioral/biological processes

2. Methods

2.1 Data Obtained

Call count data sets were obtained from a previous study done by Marcus Hogg and Dr. Tim Bray of Bristol Zoological Gardens. The data includes numeric hourly call counts from AudioMoths, an audio recording device. The three AudioMoths were placed throughout the study site in the Limon Province at the Veragua Rainforest (Fig. 1) at multiple locations up to ten meters distance from the same water body.



The audio files were analyzed in program R with the “getDetections” function to single out the calls of *A. lemur* and to then tally the total of calls per hour (Emmet et al. 2020; Rstudio Team 2020). The miniscule amount of calls that could have made it through their analysis would not change the results. The data from this set included eleven nights in July of 2019, but only nine viable nights were used. Two of the nights were missing hourly calls or had

been experimented with and were not suitable for use. Dates included in these nine nights are 5th-6th, and 17th-22nd of July 2019. Calls were separated by AudioMoth recorders and by hour and date. Hours recorded for calls was 18:00 to 5:00. This study focuses on the total nightly calls heard and compares them to environmental variables, particularly the lunar phase. The number of calls per night were seen as the response variable while the other variables included were noted as explanatory variables. These explanatory variables include humidity, illumination, temperature, cloud cover, visibility of moon, presence of full moon, lunar day, and distance from water. Measures of most of these environmental variables came from the meteoblue company Datasets from meteoblue were obtained as hourly logs for humidity, temperature, and cloud cover. Presence of the full moon and lunar day was found online with a moon phase calendar. The moon phase calendar showed the date, illumination percent, phase name, and lunar day ("Moon Phases July 2019").

To first analyze all the data an excel spreadsheet was compiled to concatenate all the data into a single workspace. Then by separating the days and other variable data, line graphs were made showing calls with corresponding lunar day (Fig. 2). The data was then uploaded to program R to run GLMs (general linear models) and ANOVA statistical tests (Appendix 4)(Rstudio Team 2020). General linear models were used to graph a regression between dependent and independent variables. Executing commands for the variables in R with interactions and dependencies noted in the linear model formula, the program would then graph these interactions to prepare for an ANOVA test (Rstudio Team 2020). The ANOVA tested the interactions for significance. ANOVA functioned by finding the degrees of freedom, sum of squares, mean of squares, f values, and Pr values. For analysis of explanatory variables significance was determined at the 5% level. These models were run more than once while replacing symbols that denote interactions with symbols that just state their presence in the study. This was done to ensure all perspectives or types of interactions were fully explored. Many times, the variables effects depend on another, so all possible interactions or noninteractions were attempted.

2.2 Testing for Lunar Correlations

Using the call count data obtained from Tim Bray and the moon phase calendar, the relationship between call numbers and full moon presence was tested using GLMs and ANOVA (Rstudio Team 2020). First the number of calls per night were graphed with lunar days corresponding to dates of the recorded data (Fig. 2). Example being

lunar day 15.32 which translates to July 17th, being a night with a full moon. Using lunar day on the graphs makes it easier for analysis and translation to moon phase associated with that day. Examples of phase names are full moon and new moon, which is determined by the percent of illumination. A lunar day is the day within the full lunar cycle, which is 29.19 days long. The new moon occurs on and around day 29.19 and the full moon occurs on and around day 14.39. Visibility of the moon was theorized to be the illuminated percent of the moon multiplied by the amount of sky that was not covered by clouds. For example, a full moon at 100% illumination on a 30% cloud cover night was calculated to have a 70% total visibility. General linear models were run on lunar related variables after nightly averages were calculated. ANOVA tests were then run on the moon model, the model with total nightly calls correlating with most variables (Appendix 3), to find Pr values (ANOVA, $df=1$, $p=.05$).

2.3 Testing for Environmental Correlations

Measured weather data was obtained from meteoblue for testing the current environmental variables. Call counts were obtained from the same dataset as before. These variables were looked at for the same hours the calls were recorded. An average for the night was calculated per variable and compared to number of nightly calls. Graphs were made for these comparisons on excel. These graphs were then analyzed for peaks and to see if calls increased on particular nights based on full moon presence or if increase correlated with other explanatory variables. Environmental variables investigated were temperature, humidity, and cloud cover. Trendlines and any obvious peaks in graphs were noted. Data was then compiled into a table for uploading and attaching in program R (Rstudio Team 2020). The data table was then uploaded into program R and attached. A linear model was then made for the response variable and the explanatory variables. ANOVA was run on the model for analysis of data (Rstudio Team 2020). Pr value was then noted for significance, which means it must be less than .05, to reject null hypothesis. Diagnostic plots were then made via program R for the models and analyzed for adequate representation (Rstudio Team 2020).

3. Results

3.1 Lunar Study

Although this dataset is only one that spans over nine days and could be considered small, this dataset still provides conclusions appropriate for a pilot study. This pilot study was useful due to the frog call patterns it recorded. Over the span of nine nights there was a minimum of 230 calls and a maximum of 1290 calls recorded per night. There were two nights considered a full moon, having 98% to 100% illumination. Cloud cover was anywhere between 11.98% to 100% with the moon visibility ranging from no visibility to 82.7% visible. The null hypothesis, which was the moon causing no effects on call counts, was rejected. There was a total of 5824 calls labelled as lemur leaf frog calls for the nine nights analyzed. There was a significant difference between presence of the full moon and total nightly calls (ANOVA, $p=0.05$, $df=1$; Table 1; Appendix 3). The Pr value was .002 when calls and full moon presence were run alone. Statistical difference was also found when all the explanatory variables were run against total nightly calls to find a Pr value of .044 (Table 1; Appendix 3). The diagnostic plots represented the data well and supported the use of the general linear model and ANOVA results. Since the Pr value was less than .05 for both full moon tests, the null hypothesis was rejected. The data supports that the presence of the full moon does influence calling activity of *A. lemur*. Total nightly calls were increased during nights with a full moon, even when there was high percent of cloud cover.

Table 1 Results of the ANOVA tests run on the GLMs labeled as moon models. The first section is a model with many variables while the second section is only one variable run at a time. P=0.05. Variables are listed in order which they were executed in linear model. Significance is denoted by the use of asterisks. See Appendix 4 for linear model formula used.

Analysis of Variance Table. Response: Calls

Variable	Degrees of Freedom	Sum Sq	Mean Sq	F value	Pr(>F)
FullMoon	1	980855	980855	21.0650	*0.04434*
Illumination	1	12958	12958	0.2783	0.65051
CloudCover	1	17089	17089	0.3670	0.60624
Humidity	1	130390	130390	2.8003	0.23622
Temp	1	1178	1178	0.0253	0.88825
LunarDay	1	16837	16837	0.3616	0.60870
FullMoon	1	980855	980855	25.282	*0.001517 *
Visible	1	146783	146783	0.9293	0.3672

The significance of a full moon is not reported in the individual Audiomoth models even though a peak is shown in the graphs (Fig. 3; Appendix 3). This may be due to the dataset for Audiomoths being about 70% smaller than dataset for total calls.

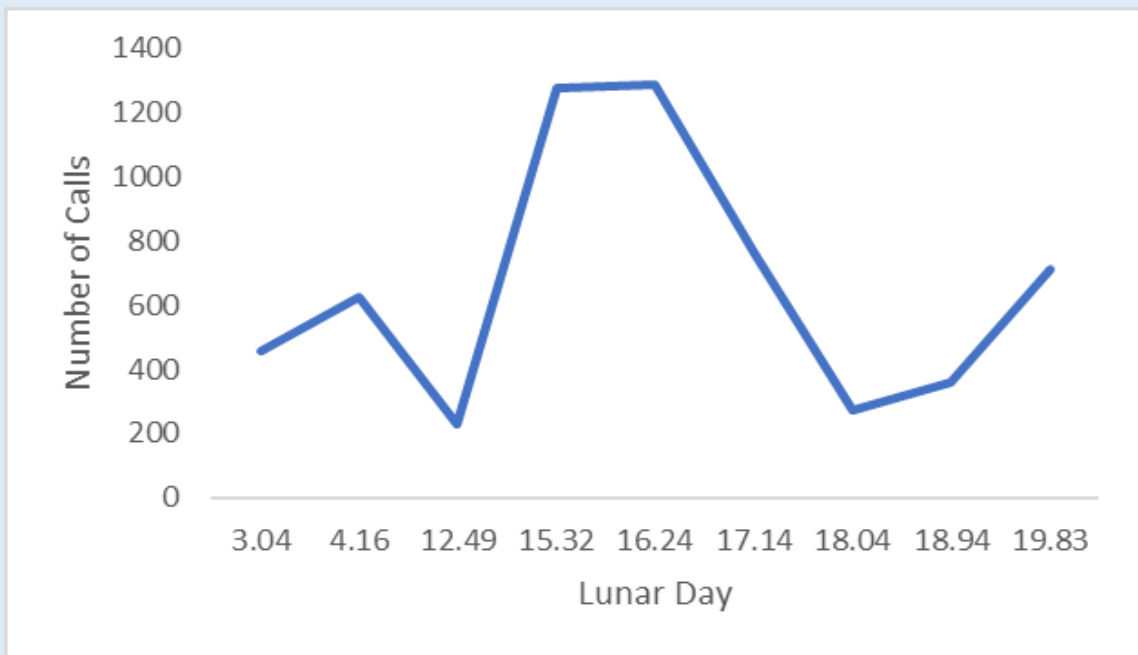


Fig. 2 A comparison of nightly calls with the lunar day they were recorded.

There is a peak on lunar day 15.32 and 16.24 (Fig.2). Both of these dates are nights with a full moon. Full moon nights are categorized as 98%to 100% illumination of the moon.

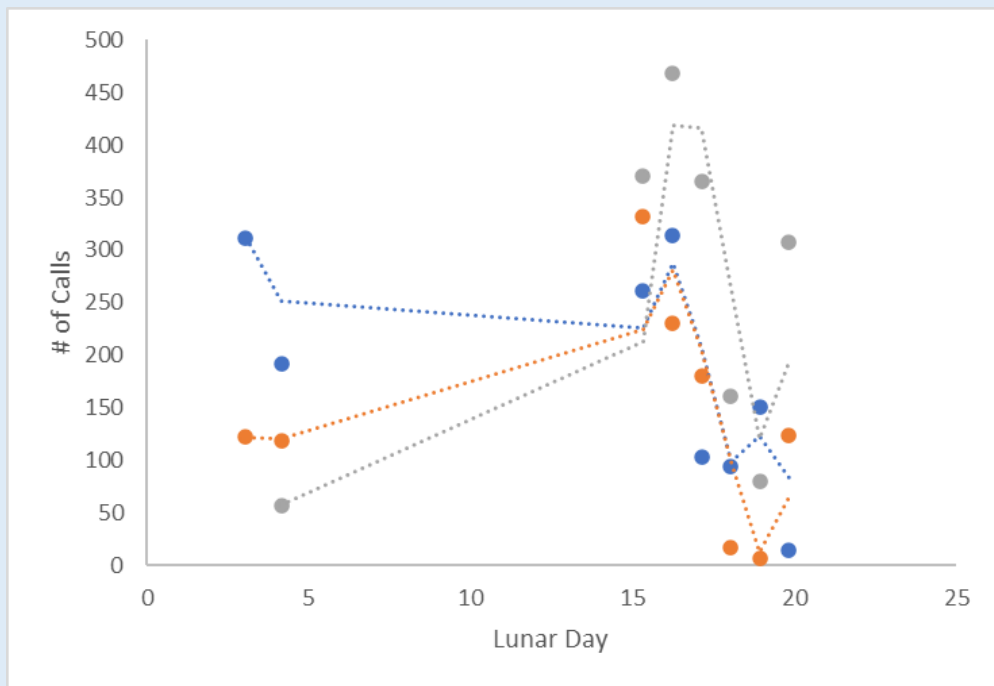
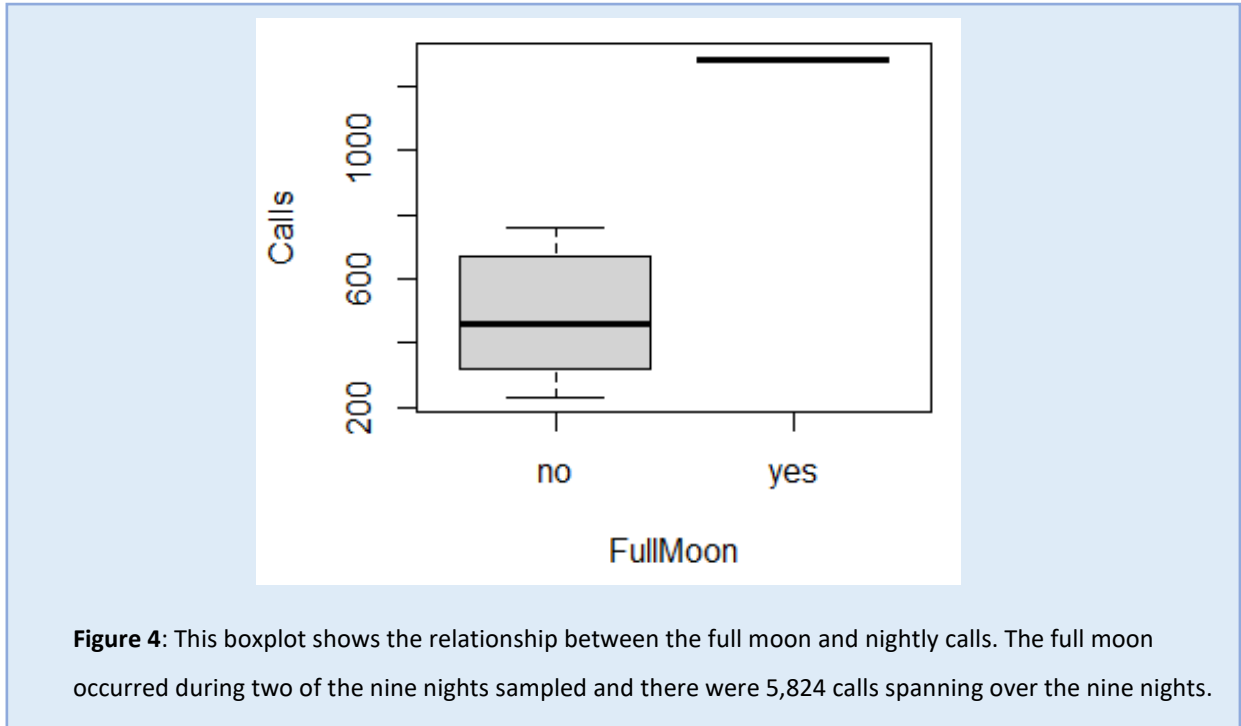


Fig. 3 This graph shows each Audiomoths nightly call count compared to the corresponding lunar day. The dotted lines are averages for each Audiomoth and make peaks easier to locate.

Lunar days 15.32 and 16.24 have the highest number of nightly calls per Audiomoth recorder (Fig. 3). Like stated earlier, these lunar days are days when a full moon is present.



There are more calls on nights with full moon than nights without (Fig. 4). This supports the significance shown in the ANOVA tests and supports the rejection of the null hypothesis.

3.2 Environmental Study

Audiomoths 1 through 3 showed significant difference ($p < .05$) for separate variables.

Table 2 Significant results found in singular linear models between different AudioMoths. Models listed in order of Audiomoth number. Statistical significance is denoted by the use of asterisks. (See Appendix 3 for complete stat sets)

Analysis of Variance Table. Response: Calls

Variable	Degrees of Freedom	Sum Sq	Mean Sq	F value	Pr(>F)
Moth 1					
Temperature	1	64664	64664	7.2564	*0.03587*
Moth 2					
Distance	1	56704	56704	8.0171	*0.0299*
Moth 3					
Humidity	1	181567	181567	12.034	*0.01787*

4. Discussion

4.1 Difference Found in Calling Activity

These results were expected with the full moon and it showed statistical difference with nightly calls, even with only nine nights worth of data. The results found in this dataset may not reflect on other months with full moons, so further research should take place to ensure these nights' recordings also represent a wider sample and the study should be replicated for a longer period to assure the effect of the full moon. There is a probability of error to hold in mind while considering effects and calling counts. Mr. Hogg's study (that data was obtained from) counted the calls has a percentage of error and there is a chance that a few tree frog (*A. callidryas*) were included in the count due to similar calling frequencies (Emmet et al. 2020). Although the amount included are not enough to influence the results of this study, it could be considered that tree frogs may be affected by the full moon in a similar way. This means tree frogs could of increased calls on a number of nights. Variables that affect calling activity negatively could also be in place and should not be forgotten. This study would preferably been done in a controlled environment, but that was not achievable for this study.

4.2 Vocalization Rate Affected by Lunar Phase

With the calling peaks shown in excel graphs, boxplots, and statistical tests, it can be seen that the full moon does have an effect on calling activity of the lemur leaf frog. Diagnostic plots for the linear models were adequate at representing the data and the “Residual vs Leverage” showed residuals within the cook’s distance line. Points outside cook’s distance lines are marked as influencers, which is needed to confirm statistical difference. Therefore, the correlation of increased calls with the presence of the full moon was supported. Since visibility showed no clear relationship with number of calls in the linear model with total nightly calls it was not tested against individual Audiomoths. Since no statistical difference was reported, accessibility to moonlight may not be important for captive lemur leaf frogs. It seems just the presence of the moon is enough. Another study should be done on moonlight accessibility verses cover to confirm these theories. The individual peaks on Audiomoth graphs may not of been large enough to show statistical difference or it may be because the data set was smaller and did not fit model adequately. The graphs seem to contradict the ANOVA tests, but still the Pr value must be noted. With the Audiomoth diagnostic plots (Appendix 2) not supporting the ANOVA results, one may wish to disregard differences found in the test. In future studies one may consider using a larger dataset or transforming the data to fix this problem.

4.3 Vocalization Rate Affected by Environmental Factors

The other explanatory variables studied that were more weather based did not show any obvious trends or peaks in the excel graphs. When looking at the correlation graphs made in excel it was easy to see that there was no obvious relation between the data collected and weather variables. After running the linear models and Anova on the moon model, the lack of correlations was confirmed. This was surprising considering the large amount of literature available on environmental variables affecting anurans and their calling activity. The environment may vary well affect lemur leaf frogs, but perhaps the data was collected over to short of a period to see any smaller influences that may be present. It may also be that lemur leaf frogs are not affected by their surrounding environment like how western toads (*Bufo boreas*) and Cascades frogs (*Rana cascadae*) are unaffected by climate change (Blaustein et al. 2001), but there was no species-specific literature found on the subject.

When all the variables were run in a singular Audiomoth linear model together an ANOVA would not properly run. Fixing this problem would be an improvement for future studies. Each variable had to be tested singularly with an AudioMoths average nightly calls. This could be because of the smaller dataset or because of the different distances they were placed. It could be from the different combinations of other non-included variables for each individual Audiomoth recorder. Each Audiomoth was placed at different distances from the water and could of recorded some of the same calls, but be affected differently by the variable combinations. The differences in results between the Audiomoth variable and call correlations was unexpected. Analyzing the linear model plots gave more insight on why the Audiomoth models were different though. The diagnostic plots for all the Audiomoths were inadequate representations of data (Kim 2015). The “Residual vs. Fitted” graph and the “Scale-Location” graph had mostly sporadic residuals (Kim 2015)(Appendix 2). Points outside cook’s distance lines are marked as influencers, which is usually needed to support statistical difference (Kim 2015). Audiomoth 1 model plots for temperature looked semi-adequate, but without the support of statistical difference for temperature in other models, it could just be a coincidence and not an explanatory variable for the reported higher call counts (Appendix 2). The variable that showed statistical difference in the Audiomoth 2 ANOVA fell within cook’s distance lines, therefore disproving the variable’s significance (Kim 2015). The Audiomoth 3 linear models look inadequate as well. When looking at the “Residual vs. Leverage” plot for humidity it shows a residual point outside cook’s distance lines, but looking at the other plots for humidity contradicts the leverage plot since they do not represent data well (Appendix 2). In order to avoid these challenges, future studies should consider transforming their data to fix use of models and/or gather call data over a longer period of time.

This pilot study laid important foundations for lunar studies and for *A. lemur* specific studies. There are a few reports on how the lunar cycle may affect important activities for wild populations like breeding, but even fewer studies, if any, have been done on captive populations. Recording *A. lemur* calls is important because anurans only mate after advertisement calls (Jamieson 2003). This means most of the calls recorded for this study served a purpose for breeding and the rate of vocalization could alter mating success (Gerhardt 1994; Toledo et al. 2015) This study should support the need for future studies on captive lemur leaf frog populations and other species too. Critically Endangered species need scientists to balance the scales after the decrease in frog numbers from anthropogenic causes. Though breeding captive frogs cannot fix habitat loss, it may help return the

population numbers to what it was before their habitat was lost. This study has the results to support lunar influences on *A. lemur* and has addressed problems to fix in future studies.

5. Conclusion

Overall, a significant difference was found between calling activity and full moon presence. The moon model GLMs and ANOVA reported significant values for full moon presence and how it affects the frogs' nightly calls. Other variables were shown to have statistical difference but were ruled out as false positives due to the diagnostic plots not supporting the data models. More data is needed for a larger confirmation of this study's results. More information is needed on this species and other Threatened species and how they are affected by the presence of the full moon. With an increase in calling activity, lemur leaf frog's reproductive success ratio could increase as well. This study should be evaluated further to see if it can be replicated in captivity or if the full moon presence is already felt by captive lemur leaf frog breeding populations. Either way, more research is needed on this subject and the next step should be observations on captive populations during the lunar days.

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Literature Cited

Abrunhosa, Patrícia A. and Henrique Vogel. 2004. Breeding behavior of the leaf-frog *Phyllomedusa Burmeisteri* (Anura: Hylidae). *Amphibia-Reptilia*. 25(2):125–35.

Blaustein, Andrew R. et al. 2001. Amphibian Breeding and Climate Change. *Conservation Biology* 15(6):1804–9.

Brooke, P. N., R. A. Alford, and L. Schwarzkopf. 2000. Environmental and social factors influence chorusing behaviour in a tropical frog: examining various temporal and spatial scales. *Behavioral Ecology and Sociobiology* 49(1):79.

Chakraborty, Ujjwal. 2020. Effects of different phases of the lunar month on living organisms. *Biological Rhythm Research*. 51(2):254–82.

Church, Gilbert. 1960 a. Annual and lunar periodicity in the sexual cycle of the Javanese toad *Bufo melanostictus* (Schneider). *Zoologica* 45: 181-188.

Church, Gilbert. 1960 b. The effects of seasonal and lunar changes on the breeding pattern of the edible Javanese frog *Rana cancrivora* (Gravenhorst). *Treubia* 25: 215-233.

Clarke, Jennifer A., Joseph T. Chopko, and Stephen P. Mackessy. 1996. The Effect of Moonlight on Activity Patterns of Adult and Juvenile Prairie Rattlesnakes (*Crotalus Viridis Viridis*). *Journal of Herpetology*. 30(2):192–97

Dias, Tailise Marques, Cynthia P. A .Prado, and Rogério Pereira Bastos. 2017. Nightly Calling Patterns in a Neotropical Gladiator Frog. *Acta Ethologica* 20(3):207–14.

Dixon, D. R., L. R. Dixon, J. D. Bishop, and R. A. Pettifor. 2006. Lunar-related reproductive behaviour in the badger (*Meles meles*). *Acta Ethologica* 9(2):59–63.

Emmett, C., D. Hending, A. Davis, G. McCabe, T.C. Bray. 2020. Hearing Ghosts: Acoustic Survey Protocol and Vocalization Characterization for Lemur Leaf Frogs. *Herpetological Review* 51(1):24–29.

FitzGerald, G. J. and J. R. Bider 1974. Influence of Moon Phase and Weather Factors on Locomotory Activity in *Bufo americanus*. *Oikos* 25(3):338–40.

- Gerhardt, H. C. 1994. "The Evolution of Vocalization in Frogs and Toads." *Annual Review of Ecology and Systematics* 25(1):293–324.
- Gomez, Doris et al. 2009. The Role of Nocturnal Vision in Mate Choice: Females Prefer Conspicuous Males in the European Tree Frog (*Hyla arborea*). *Proceedings: Biological Sciences* 276(1666):2351–58.
- Grant, Rachel A., Elizabeth A. Chadwick, and Tim Halliday. 2009. The lunar cycle: a cue for amphibian reproductive phenology?. *Animal Behaviour* 78(2):349–57.
- Grant, Rachel, Tim Halliday, and Elizabeth Chadwick. 2013. Amphibians' response to the lunar synodic cycle-a review of current knowledge, recommendations, and implications for conservation. *Behavioral Ecology* 24(1):53–62.
- Jamieson, Barrie G. M. 2003. *Reproductive Biology and Phylogeny of Anura*. Enfield, NH: Science. pp. 226.
- Kim, Bommae. 2015. *Understanding Diagnostic Plots for Linear Regression Analysis*. University of Virginia Library. <https://data.library.virginia.edu/diagnostic-plots/>
- Leenders, Twan. 2016. *Amphibians of Costa Rica : A Field Guide*. Cornell University Press, Ithaca. pp. 346-347 .
- IUCN 2020. The IUCN Red List of Threatened Species. Version 2020-2. <https://www.iucnredlist.org>
- Moon Phases July 2019. c 2011-2020. [accessed 2020 June 16]. https://www.calendar12.com/moon_calendar/2019/july
- Oseen, Kerri L. and Richard J. Wassersug. 2002. Environmental Factors Influencing Calling in Sympatric Anurans." *Oecologia* 133(4):616–25.
- Petchey, A. et al. 2014. Characterisation of 9 polymorphic microsatellite markers for the Critically Endangered lemur leaf frog *Agalychnis lemur*. *Conservation Genetics Resources* 6, 971–973.
- Ralph, Charles L. 1957. A Diurnal Activity Rhythm in *Plethodon cinereus* and Its Modification by an Influence Having a Lunar Frequency. *Biological Bulletin* 113(1):188–97.

Robertson, Douglas R. 1978. The Light-Dark Cycle and a Nonlinear Analysis of Lunar Perturbations and Barometric Pressure Associated with the Annual Locomotor Activity of the Frog, *Rana pipiens*. *Biological Bulletin* 154(2):302–21.

RStudio Team. 2020. RStudio: Integrated Development for R. RStudio. <http://www.rstudio.com/>.

Schalk, Christopher M. and Daniel Saenz. 2016. Environmental drivers of anuran calling phenology in a seasonal Neotropical ecosystem. *Austral Ecology*. 41(1):16–27.

Skov, Martin W. et al. 2005. Marching to a Different Drummer: Crabs Synchronize Reproduction to a 14-Month Lunar-Tidal Cycle. *Ecology* 86(5):1164–71.

Solís F., Ibáñez, R., Savage, J., Jaramillo, C., Fuenmayor, Q., Kubicki, B., Pounds, J., Chaves, G., Jungfer, K. & Lips, K. 2008. *Agalychnis lemur*. The IUCN Red List of Threatened Species 2008.

Toledo, Luís F., Itamar A. Martins, Daniel P. Bruschi, Michel A. Passos, César Alexandre, and Célio F. B. Haddad. 2015. The anuran calling repertoire in the light of social context. *Acta Ethologica* 18(2):87–99.

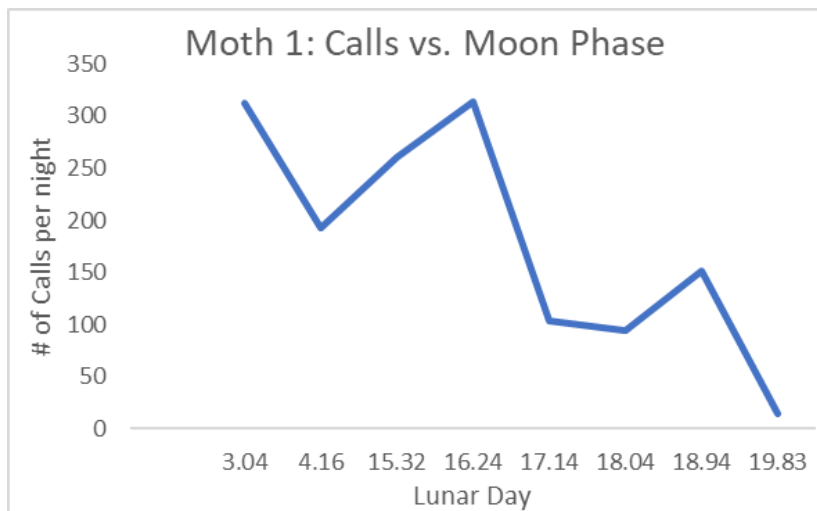
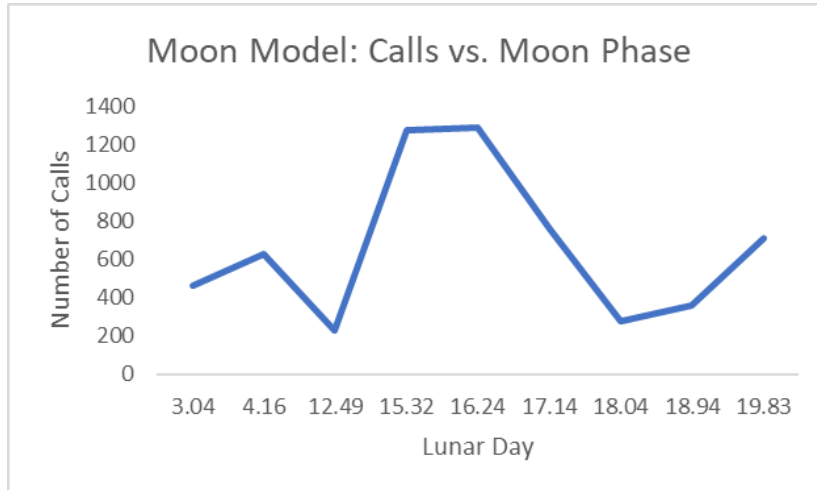
Underhill, Victoria A. and Höbel Gerlinde. 2018. “Moonlighting? - Consequences of Lunar Cues on Anuran Reproductive Activity. *Acta Oecologica* 87:20–28.

Vignoli, L. and L. Luiselli. 2013. Better in the Dark: Two Mediterranean Amphibians Synchronize Reproduction with Moonlit Nights. *Web Ecology* 13(1):1–11.

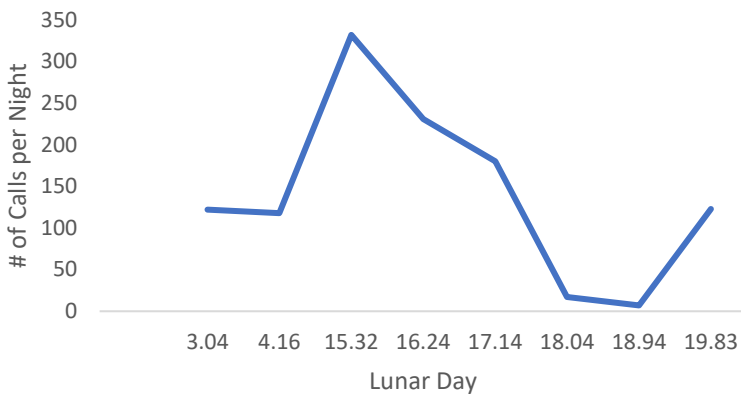
Appendices

1. Graphs

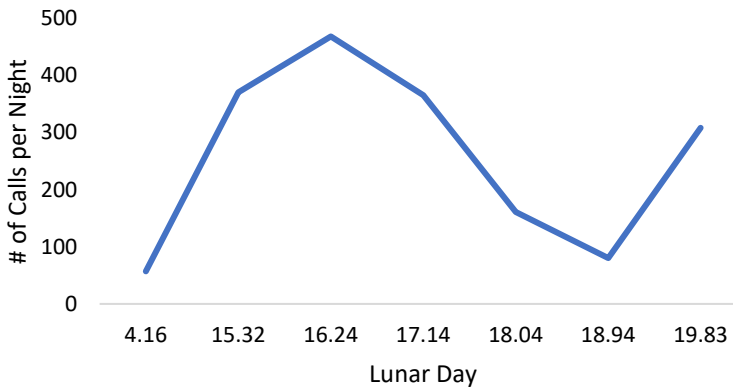
Compilation of graphs made to visualize any correlations between explanatory variables and nightly calling activity. Each graph is individually labeled with title and labeled variables.



Moth 2: Calls vs. Moon Phase

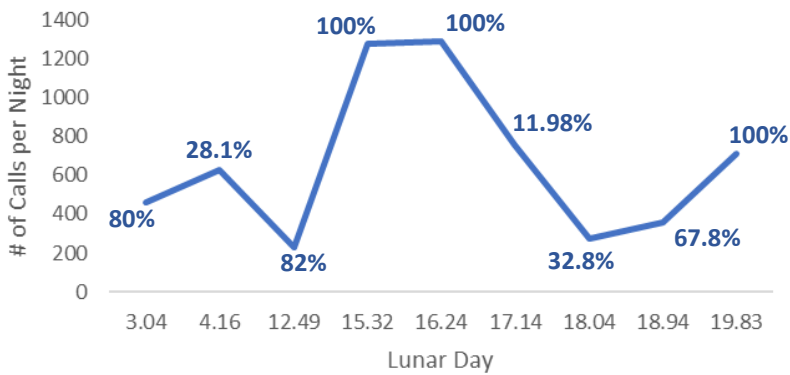


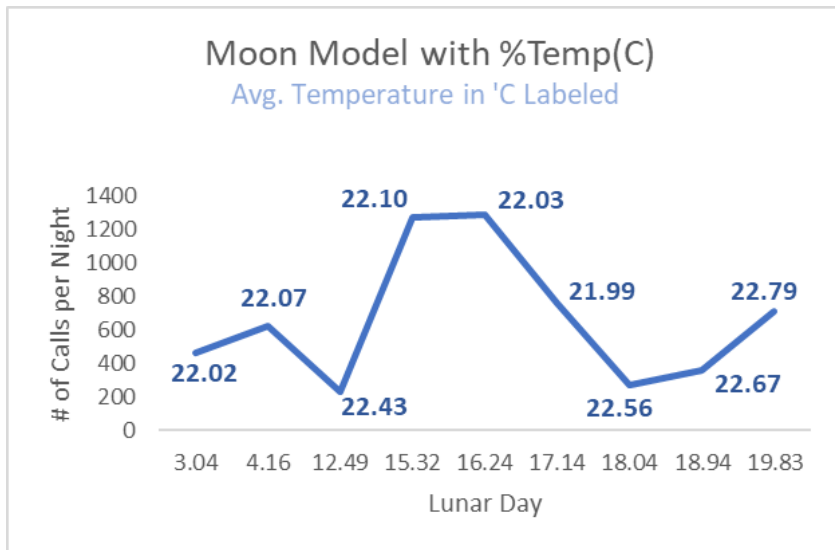
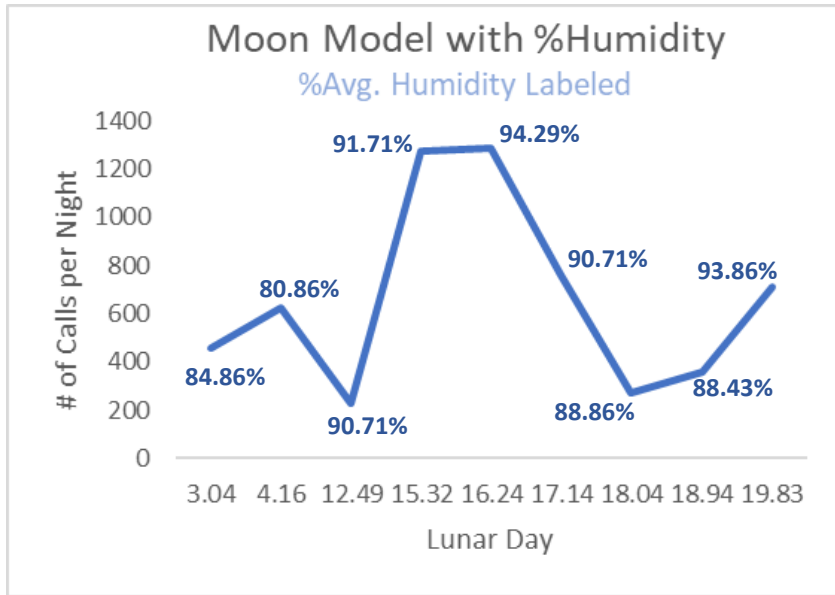
Moth 3: Calls vs. Moon Phase



Moon Model with %Cloud Cover

%Avg. Cloud Cover Labeled

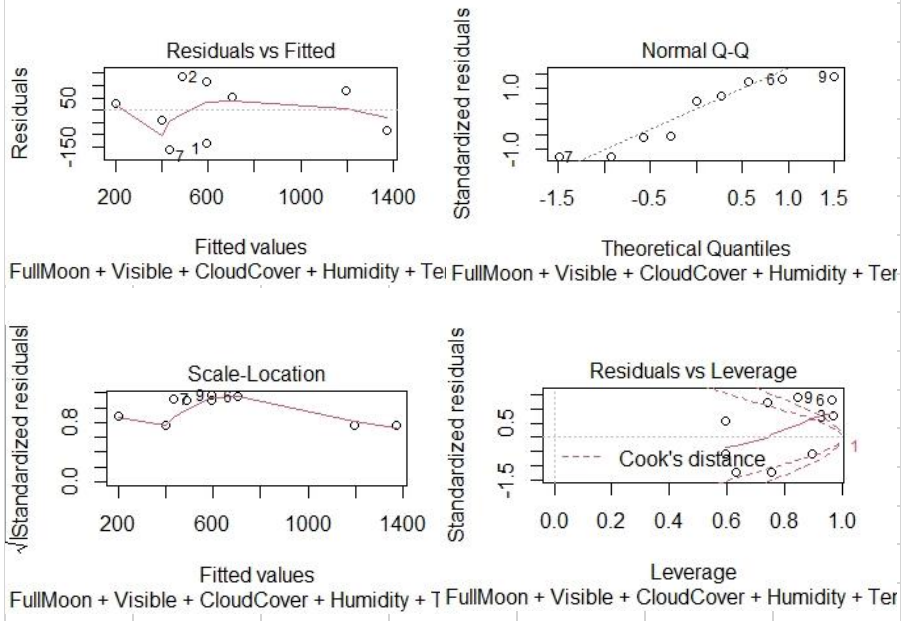




2. Diagnostic Plots

Compilation of diagnostic plots for moon model and individual Audiomoth models. Each set of plots is labeled with the model name and variables.

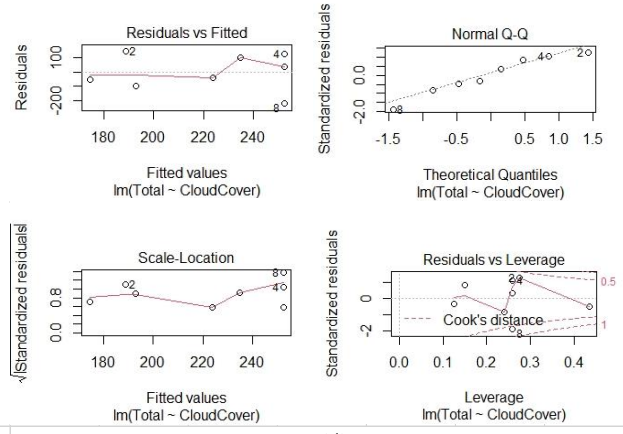
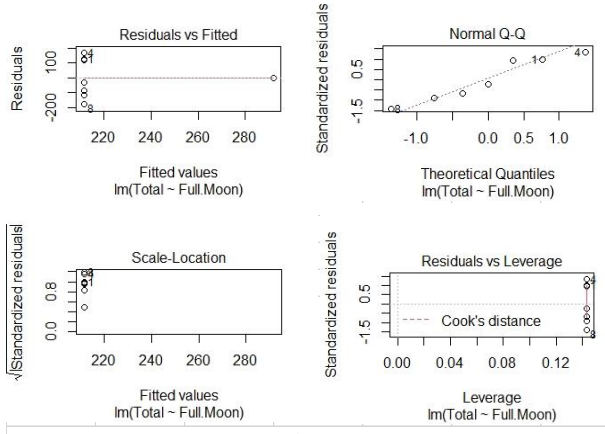
Moon Plots



Moth 1

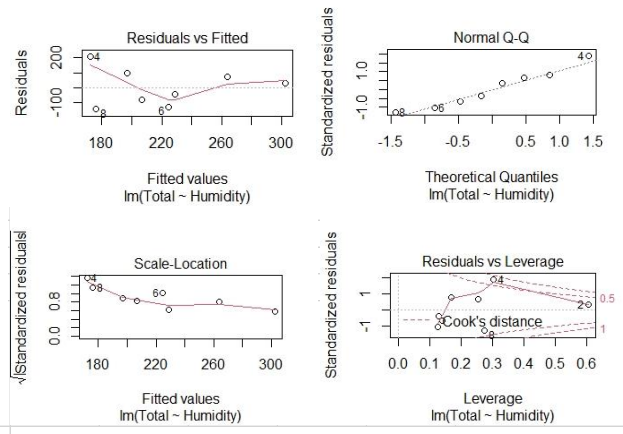
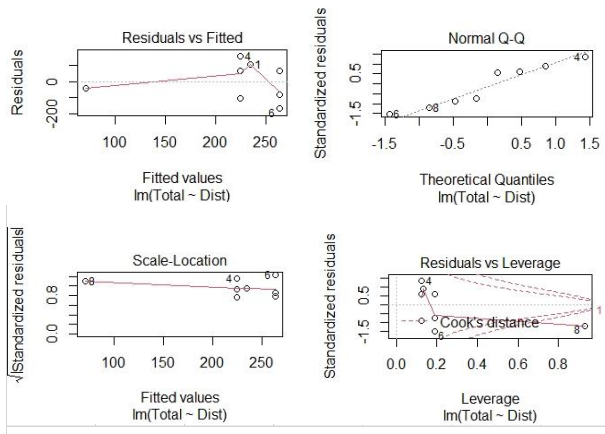
Full Moon

Cloud Cover



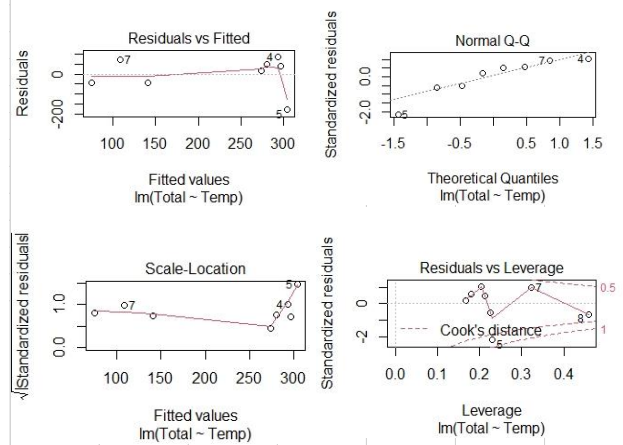
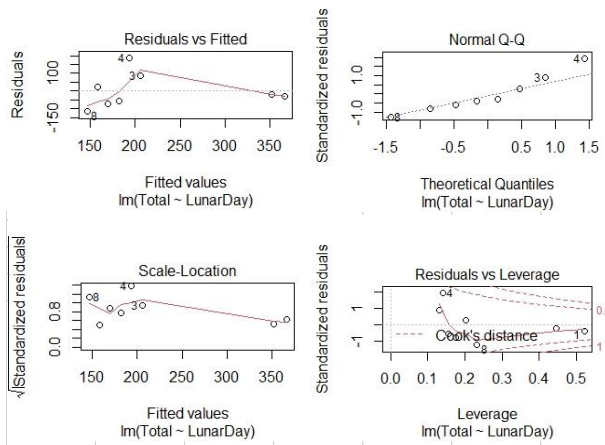
Distance

Humidity

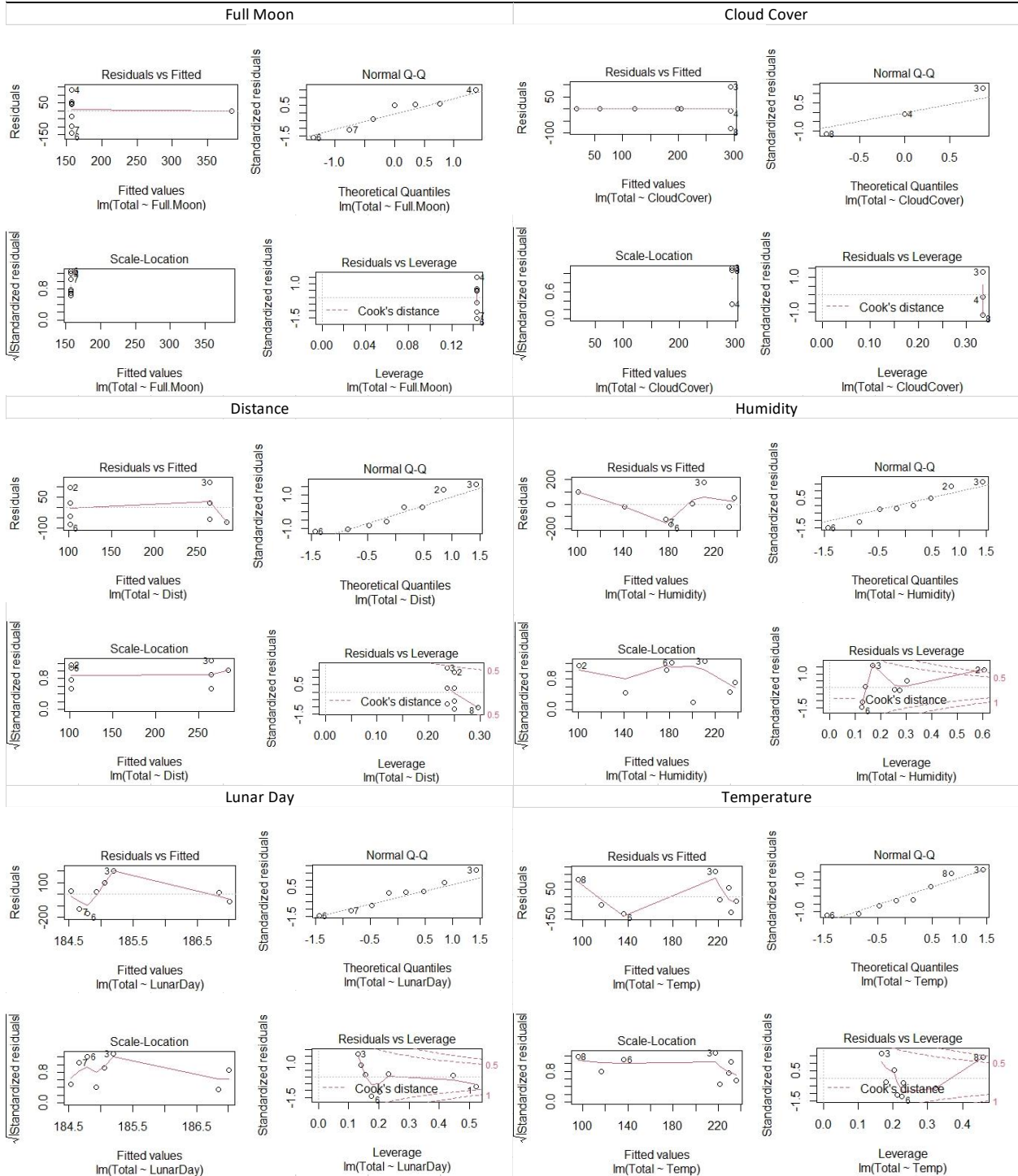


Lunar Day

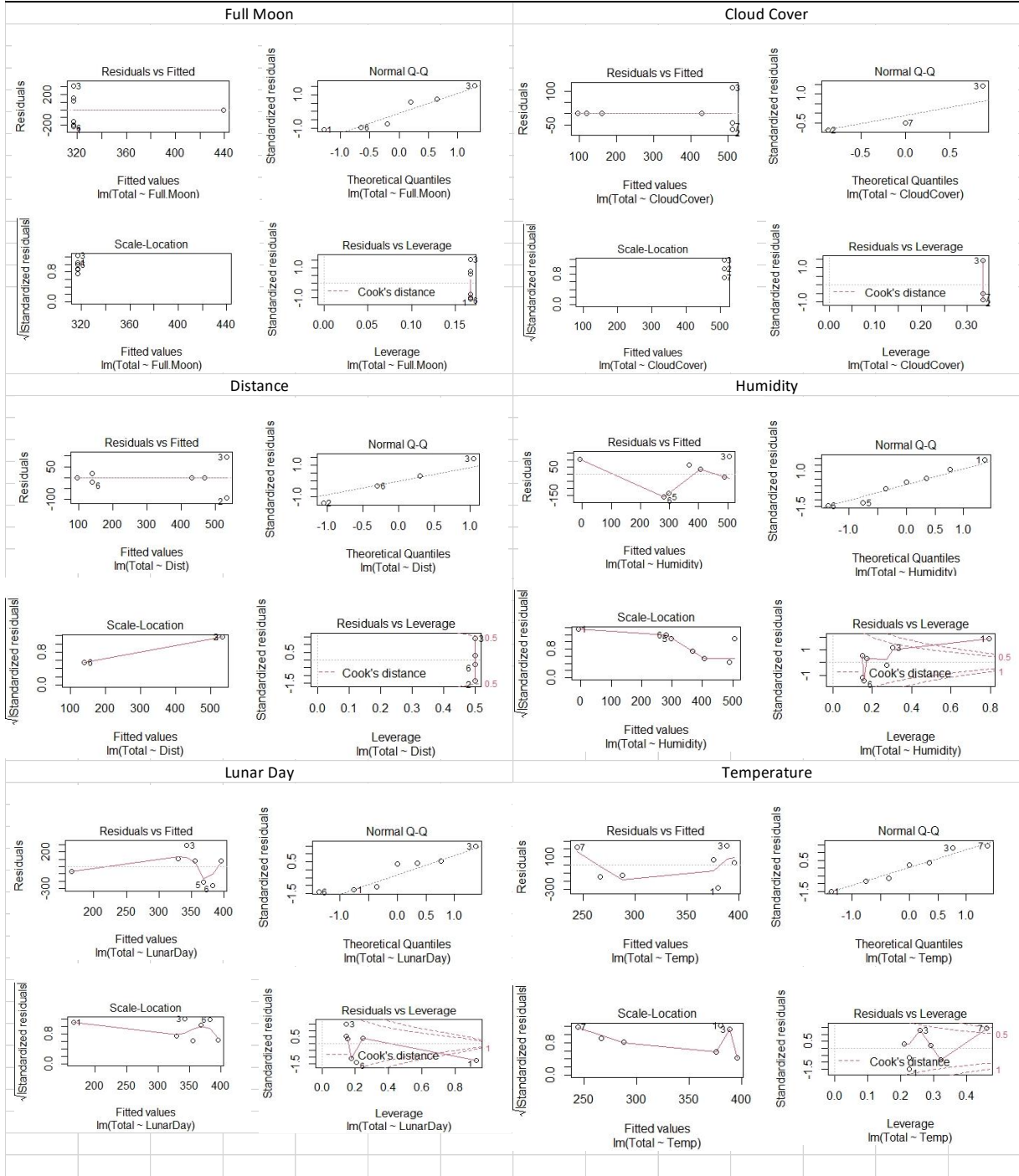
Temperature



Moth 2



Moth 3



3. Statistics Tables

Compilation of ANOVA results for each model run. Moon model was run with total nightly calls. Empty rows represent separation between variables run in linear models.

Moon Model					
Variable	DF	Sum of Sq	Mean Sq.	F Value	Pr(>F)
FullMoon	1	980855	980855	21.065	*0.04434*
Visible	1	12958	12958	0.2783	0.65051
CloudCover	1	17089	17089	0.367	0.60624
Humidity	1	130390	130390	2.8003	0.23622
Temp	1	1178	1178	0.0253	0.88825
LunarDay	1	16837	16837	0.3616	0.6087
FullMoon	1	980855	980855	25.282	*0.001517 *
Visible	1	146783	146783	0.9293	0.3672

Moth 1					
Variable	DF	Sum of Sq.	Mean Sq.	F Value	Pr(>F)
Full Moon	1	5680	5680.3	0.3031	0.6018
Humidity	1	13632	13632	0.7827	0.4104
Distance	1	28493	28493	1.9072	0.2165
Temp	1	64664	64664	7.2564	0.03587*
LunarDay	1	52683	52683	4.8297	0.07033
CloudCover	1	7168	7168.5	0.3876	0.5565

Moth 2					
Variable	DF	Sum of Sq.	Mean Sq.	F Value	Pr(>F)
Full Moon	1	45088	45088	5.0048	0.06661
Humidity	1	15008	15008	1.0703	0.3408
Distance	1	56704	56704	8.0171	0.0299*
Temp	1	24074	24074	1.9242	0.2147
LunarDay	1	7	6.7	0.0004	0.9846
CloudCover	5	84089	16817.8	2.2345	0.3375

Moth 3					
Variable	DF	Sum of Sq.	Mean Sq.	F Value	Pr(>F)
Full Moon	1	12828	12828	0.2627	0.6301
Humidity	1	181567	181567	12.034	0.01787 *
Distance	4	238638	59660	6.4966	0.1378
Temp	1	25636	25636	0.554	0.4902
LunarDay	1	36171	36171	0.819	0.407
CloudCover	4	236832	59208	5.8701	0.1508

4. Script

Script for Program R that was used for the completion of this study. This is the organized version with hashtags denoting what the following script is for.

<pre>#Moon Model #Attach data > Acoustic.Moon.Data <- read.csv("~/Acoustic Moon Data.csv", stringsAsFactors=TRUE) > View(Acoustic.Moon.Data) > attach(Acoustic.Moon.Data) #Calls- Response Var. #LunarDay, FullMoon, etc.- Explanatory Var. #Run anova on total nightly calls > MoonModel<- lm(formula = Calls~FullMoon+Visible+CloudCover+Humidity+Temp+ LunarDay, data=Acoustic.Moon.Data) > anova(MoonModel) #Run anova on significant variable > anova(lm(formula=Calls~FullMoon, data=Acoustic.Moon.Data)) > boxplot(Calls~FullMoon) #Get diagnostic plot > plot(MoonModel) Hit <Return> to see next plot : #AudioMoth Models #Attach data > Moth.1 <- read.csv("~/Moth 1.csv", stringsAsFactors=TRUE) > View(Moth.1) > Moth.2 <- read.csv("~/Moth.2.csv", stringsAsFactors=TRUE) > View(Moth.2) > Moth3 <- read.csv("~/Moth3.csv", stringsAsFactors=TRUE) > View(Moth3) > attach(Moth.1) > attach(Moth.2) > attach(Moth3)</pre>	<pre>#Run anova on single variable GLMs > anova(lm(formula=Total~Full.Moon, data=Moth.2)) > anova(lm(formula=Total~Humidity, data=Moth.2)) > anova(lm(formula=Total~Dist, data=Moth.2)) > anova(lm(formula=Total~Temp, data=Moth.2)) > anova(lm(formula=Total~LunarDay, data=Moth.2)) > anova(lm(formula=Total~CloudCover, data=Moth.2)) > anova(lm(formula=Total~Humidity, data=Moth3)) > anova(lm(formula=Total~Temp, data=Moth3)) > anova(lm(formula=Total~Full.Moon, data=Moth3)) > anova(lm(formula=Total~LunarDay, data=Moth3)) > anova(lm(formula=Total~CloudCover, data=Moth3)) > anova(lm(formula=Total~Dist, data=Moth3)) > anova(lm(formula=Total~Full.Moon, data=Moth.1)) > anova(lm(formula=Total~LunarDay, data=Moth.1)) > anova(lm(formula=Total~Temp, data=Moth.1)) > anova(lm(formula=Total~Humidity, data=Moth.1)) > anova(lm(formula=Total~CloudCover, data=Moth.1)) > anova(lm(formula=Total~Dist, data=Moth.1)) #Repeat Diagnostic plot for each GLM > plot(lm(formula=Total~Humidity, data=Moth3))</pre>
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