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**Fenología de canto de** *Agalychnis lemur***: asociación de su actividad acústica con el ambiente abiótico**

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INTRODUCCIÓN

MARCO TEÓRICO

En las últimas décadas, se ha incrementado la rapidez en la que ocurre la pérdida de especies en el planeta tierra (Stuart et al, 2004; Mendelson et al, 2006; Wake & Vredenburg, 2008). Si bien las extinciones son un mecanismo evolutivo relevante, la disminución de individuos en poblaciones locales y cambios rápidos en una comunidad biológica pueden tener efectos en el funcionamiento de los ecosistemas a corto plazo (Dirzo et al, 2014). Esta época de defaunación se explica a partir de la sumatoria de factores de origen antropogénico y climático que inciden de forma directa en las comunidades biológicas (Redford, 1992). El cambio en los patrones climáticos junto con el cambio drástico en el uso de la tierra, generan una presión sobre la distribución de las especies (Pounds et al, 2006; Whitfield et al, 2007; González-de-Pliego et al, 2019; Sheldon, 2019).

Lo anterior conlleva a tres escenarios: El primero, que el rango de desplazamiento de un organismo se vea limitado producto de la interrupción de sus hábitats y por lo tanto se favorecen las extinciones locales (Pounds et al, 1999; Whitfield et al, 2007; Urbina-Cardona & Loyola, 2008). El segundo, que se den migraciones a nivel altitudinal hacia sitios donde se conserven las condiciones de hábitat óptimas para el desarrollo de una especie (Colwell et al, 2008; Campos-Cequeira & Aide, 2017). Y el tercero, que algunas especies logren adaptarse y tener una ventaja selectiva sobre otras especies más sensibles a los cambios ambientales en curso (Acosta-Chaves et al, 2019).

A pesar de que los bosques tropicales son los sitios que albergan el mayor porcentaje de biodiversidad del planeta, presentan el mayor número de especies deficientes de datos y especies crípticas (Howard & Bickford, 2014). Además, exhiben una mayor presión producto del cambio climático, la emergencia de enfermedades y la intervención humana (Young et al, 2016; Campos-Cequeira et al, 2017; Sheldon, 2019). Desde la década de los setentas, las poblaciones de diferentes grupos de vertebrados tropicales han disminuido drásticamente (Dirzo et al, 2014). Hay grupos que son más susceptibles al impacto antropogénico como lo son los anfibios, de los cuáles un 41% de las especies se consideran en categorías de amenaza (Urbina-Cardona & Loyola, 2008; Wake & Vredenburg, 2008; Dirzo et al, 2014) y 122 especies se han extinto desde la década de los ochentas (Mendelson et al, 2006).

Las especies de anfibios tropicales destacan por su alta vulnerabilidad (Pounds & Crump, 1994; Pounds, 2001; Mendelson et al, 2006). Esto se debe a que las especies ectotérmicas de los trópicos, han evolucionado en climas estables a diferencia de las especies que habitan en regiones templadas (Nowakowski et al, 2016; Sheldon et al, 2011; Sheldon et al, 2018). Diversos autores mencionan que por esta razón su capacidad adaptativa a distintos rangos de temperatura es limitada, por lo que se consideran especialistas térmicos sensibles a las fluctuaciones extremas en la temperatura ambiental (Janzen, 1967; Deutsch et al, 2008; Wake & Vredenburg, 2008; Nowakowski et al, 2016; Oyamaguchi et al, 2017).

Los datos sobre la sensibilidad y la respuesta de las especies de anuros ante las condiciones climáticas en curso son escasos (Bonnefond et al, 2019; Urbina-Cardona & Loyola, 2008). El monitoreo acústico en anfibios ha sido una herramienta que ha permitido el estudio de las especies. Muchos de estos estudios consistían en realizar muestreos visuales y auditivos por parte de los investigadores para registrar información. Sin embargo, en los últimos años, han surgido métodos de monitoreo acústico pasivo (PAM) que han permitido aumentar la resolución temporal y espacial para el estudio de las especies de anuros.

El monitoreo acústico pasivo (PAM) consiste en colocar grabadores en el hábitat de una especie y así registrar información acústica de manera continua, sistemática y no invasiva. De esta forma, es más posible el abordaje de estudios fenológicos con una amplia escala temporal, estudios poblacionales con una mayor escala espacial y estudios que permitan entender el cambio en la abundancia y distribución de las especies a lo largo del tiempo (Márquez et al, 2014; Sugai & Llusia, 2019). Sin embargo, al generar más cantidad de datos con el uso de grabadores acústicos, es necesario contar con herramientas computacionales capaces de procesar esta información de manera eficiente como lo son los reconocedores acústicos automáticos (Márquez et al, 2014).

Sugai & Llusia (2019) mencionan que el uso de PAM comenzó a partir de la década de 1990 y su uso ha ido incrementando de forma exponencial a través de los años. Los grupos que más se han estudiado con estos métodos son las aves y los murciélagos. En el caso de anfibios, no hay disponibles tantos estudios con esta metodología de trabajo a pesar de que la mayoría de las especies vocalizan y pueden ser registradas de forma acústica. La información disponible para anuros se ha generado principalmente en regiones templadas y de especies tropicales presentes en Latinoamérica se han reportado estudios de esta índole en Brasil. En el caso de Costa Rica, Hilje & Aide (2012) realizaron un estudio utilizando PAM con la especie *Diasporus diastema* para evaluar la actividad de canto según el tipo de hábitat.

En Costa Rica, debido a la alta fragmentación de los bosques se ha promovido la discontinuidad de hábitats para muchas especies. Además, una parte importante del suelo del país se ha destinado a plantaciones de monocultivos que involucran la pérdida de hábitat, el uso de agroquímicos y la intervención humana en sitios cercanos a puntos calientes de conservación como lo es la Cordillera de Talamanca. En el país, ya se ha reportado la pérdida y reducción en las poblaciones de muchas especies de anuros. Por lo tanto, el uso de PAM en Costa Rica nos podría permitir tener un mejor conocimiento de la abundancia, distribución y fenología de las especies, información que es de suma importancia para tomar decisiones de conservación informadas.

**JUSTIFICACIÓN**

La zona del Caribe Central de Costa Rica se ha catalogado como la región más biodiversa en anfibios anuros a nivel mesoamericano (Kubicki, 2008*;* Solís et al. 2008; Salazar-Zúñiga, et al*.* 2019). Sin embargo, de las 64 especies reportadas, un 11% se encuentran amenazadas (Solís et al. 2008). Un ejemplo es *Agalychnis lemur,* una especie endémica de Costa Rica y Panamá que actualmente se encuentra en peligro crítico de extinción (Salazar-Zúñiga et al, 2019).

Al igual que muchas especies de anfibios anuros de Costa Rica, las poblaciones de *A. lemur* declinaron drásticamente en la década de 1980 (Whitfield et al, 2007; Reid et al, 2019). En el presente, su distribución a nivel local se restringe a la Cordillera de Talamanca (Salazar-Zúñiga et al, 2019). En estudios previos se ha documentado la alta sensibilidad de la especie a la fragmentación de hábitat y al hongo *batracochitridium dendrobatidis* (BD) (Whitfield et al, 2017). La información disponible sobre la biología e historia natural de *A. lemur* es particularmente limitada. Los trabajos de Duellman (1970, 2001) sobre *A. lemur*  no aportan información robusta en cuanto al análisis bioacústico del canto y no detallan aspectos de la historia natural de la especie. Debido a que las poblaciones de *A. lemur* son cada vez más restringidas, tampoco hay estudios recientes sobre su fenología reproductiva y su eventual respuesta a los cambios ambientales en curso.

En esta investigación pretendo hacer uso de métodos de monitoreo acústico pasivo para registrar los períodos de actividad de *A. lemur.* La información acústica recopilada a partir de estos monitoreos, será procesada con herramientas computacionales para elaborar un reconocedor acústico automático del canto de *A. lemur.* Al diseñar un método que permita identificar las señales de *A. lemur*, espero en una etapa posterior, predecir su actividad acústica según distintas variables ambientales.

Los resultados de esta investigación tendrán aportes a nivel científico y de conservación. Este sería uno de los primeros estudios a largo plazo en aplicar métodos de detección pasiva que procesen la información bioacústica de una forma automatizada en especies de anuros amenazados en Costa Rica. Además, al programar un reconocedor acústico se podrá estandarizar un protocolo de trabajo y manejo de datos tanto para evaluar la vocalización de *A. lemur* como de otras especies del país.

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**OBJETIVOS**

**Objetivo general:**

* Determinar los patrones de actividad acústica a lo largo del año en *A. lemur* y su asociación con variables ambientales.

**Objetivos específicos:**

* Redescribir el canto de *A. lemur.*
* Desarrollar un método de detección automatizado del canto de *A. lemur.*
* Analizar la relación de las variables ambientales temperatura, humedad relativa, precipitación e intensidad lumínica sobre la actividad de canto de *A. lemur.*

**Hipótesis:**

* Al aumentar la temperatura, la humedad relativa tiende a disminuir en el ambiente, lo que implica un mayor riesgo de perder agua por evaporación y aumentar el riesgo de desecación en las ranas. Por lo tanto, tareas de alto costo energético como cantar (Wells, 2007) pueden estar comprometidas debido a fluctuaciones en la temperatura ambiental. Mi predicción es que en noches en las que la temperatura ambiental aumenta la humedad relativa disminuye, por lo que la tasa de canto de *A. lemur* tambiéndisminuye.
* Debido a su condición ectotérmica, los comportamientos de locomoción y reproducción tienen un rango de temperatura óptimo en anfibios. Por lo tanto, ante valores constantes de humedad relativa, la actividad vocal de *A. lemur* debe estar restringida por la temperatura ambiental. Mi predicción es que para un mismo nivel de humedad, a mayor temperatura ambiental la tasa de canto de *A. lemur* aumentará.
* La lluvia puede funcionar como un estímulo ambiental para la reproducción en ranas. Por un lado promueve la migración de los machos y las hembras a los sitios reproductivos (Oseen & Wassersug, 2000), aumentando su probabilidad de encuentro. Además, permite la formación de charcas y lagunas efímeras que aumentan la probabilidad de supervivencia de las larvas. Sin embargo, la lluvia también puede enmascarar las señales acústicas (Halfwerk et al. 2016). Por lo tanto, la actividad de *A. lemur* debe aumentar en noches poco lluviosas pero con lluvia previa (48, 24 y 12 horas). Mis predicciones son que en noches con días previos de lluvia habrá una mayor tasa de cantos de *A. lemur* que noches sin lluvia previa. Y en las noches lluviosas la tasa de canto de *A. lemur* disminuirá en comparación con noches menos lluviosas.
* Bajo una mayor luminosidad las ranas pueden ser más conspicuas para depredadores visuales. Por lo tanto, en noches de mayor intensidad lumínica la actividad vocal de *A. lemur* puede disminuir. Mi predicción es que en noches con mayor luminosidad (cercanas a la luna llena) la tasa de canto de *A. lemur* disminuirá.

**CAPÍTULO I: Calling description of *Agalychnis lemur* (Hylidae)**

(Formato para revista xxxx)

**Calling description of *Agalychnis lemur* (Anura: Hylidae)**

**Abstract:**

**Key words:**

Acoustic signals play a main role in aspects of behavior and reproduction for most animal groups. For terrestrial vertebrates, acoustic signals have predominantly a reproductive function (Wells, 2007; De Toledo & Haddad, 2009). The description of vocalization traits is important in species diagnoses, thus call descriptions can tell important information of the taxonomy, behavior and ecological traits within a group (Wells, 2007; Köhler et al, 2017; Röhr et al 2020).

Anurans have different types of calls, being the advertisement call the most common among species. This type of call is emitted by males to attract females and mediate aggressive interactions between males (Duellman & Trueb, 1994; Wells, 2007; Köhler et al, 2017; Guerra et al, 2017). It provides information about the species identity, sexual receptivity, position and size of a calling male (Wells & Schwartz, 2007; Guerra et al, 2017). For taxonomical and behavioral purposes, a detailed and verifiable bioacoustic description of this type of call is essential to characterize traits and their variation within a species (Köhler et al, 2017; Röhr et al 2020).

In the Tropics, are found the most biodiverse regions in terms of amphibians (xxx). However, tropical species are the least studied and the most threatened across the globe (González-del-Pliego et al, 2019). In order to gain a better understanding of the species composition in the tropics, a first step is to describe and make and inventory of the species (Acosta-Cháves et al, 2019).

*Agalychnis lemur* is a species of priority interest for conservation given its endemism for Costa Rica and Panamá and because of its risk of extinction. For this species, there is no updated information on its vocal repertoire. Previous studies of Duellman xxx, describe the acoustic parameters for its advertisement call. However, in his work, he does not report variation measures or the sample size he used to describe the *A. lemur* advertisement call. In this study, we aim to redescribe the advertisement call of *A. lemur* with a better understanding of the variation between different call traits among individuals.

MATERIALS AND METHODS

**Study site*:*** We conducted this study at Veragua Rainforest (9°55’30”N, 83°11’28”E; 420 msnm), a private reserve located in the province of Limón, in the Caribbean slope of Costa Rica. This reserve comprises 77 hectares of protected forest. The study site, Sukia comprises mature secondary forest and receives an annual precipitation between 3000 and 4000 mm, and has a mean annual temperature of 23 (Holdridge, 1967). In this site, we have five artificial ponds (dim) as a part of a restoration program for anuran populations. Many other species of anurans tend to visit this ponds for example *Diasporus diastema, Cruziohyla sylviae, Agalychnis callydrias* and *Litobathes warszewitschii.*

**Study species:** *A. lemur* is an arboreal nocturnal frog that occurs on the Atlantic slope from northwestern Costa Rica, to western Panamá. This species is endemic to Costa Rica and Panamá and can be found through middle elevation forests between 440 to 1600 msnm. The advertisement call has been described by Duellman xxx and Duellman & Trueb (1994). He described the call as a short “tlack” with a mean duration of 0.25 sec (range 0.20-20.29 sec), and an average fundamental frequency of 2396 Hz (range 2272-2520 Hz).

**Field work:** Field work began on January 21 and lasted until May of 2022. We recorded calling males of *A. lemur* using a digital recorder Zoom H4n and a unidirectional microphone Sennheiser ME 66 positioned 0.5 – 1 m from the calling individuals. For every calling male we register: Hour, body temperature of the calling male, snout-vent length (SVL), calling site and additional information such as level of activity of other males and weather conditions.

**Data processing:** For the SVL data we took pictures of each calling male in a dorsoventral position. Photographs were titled according to location, date, time and individual number and then we analyzed each picture using ImageJ (Rasband, 2012). Using Image J, a selected image must initially be calibrated using the “set to scale” function. A 20 cm ruler was included in each image along the same plane as the photographed individual for calibration. A 10 cm straight line was added to the image using the “straight line” function. The “set to scale” function was utilized to define the length of the straight line allowing calibrated measurements. SVL was measured using the “straight line” function of Image J, which allows length measurements for straight lines.

We analyzed each spectrogram and oscillogram of the recording males in Raven Pro 1.6.1 (Cornell Laboratory of Ornithology) using the following settings: window type Hann, window size of 512 samples, 70% overlap (averiguar que otros presets). The selection tables generated in Raven were then analyzed in Rstudio (R Core Team, 2021). We exported the files using the package Rraven (Araya-Salas, 2020) and then we created catalogs of the spectrograms to conduct visual inspections of the call of *A. lemur* using WarbleR (Araya-Salas & Smith Vidaurre, 2017), tuneR (Ligges et al, 2018) and Seewave (Sueur et al, 2008).

Agregar sample rate y resolucion (44 kHz y 16 bit ?)

In order to describe properly the advertisement call of *A. lemur* we follow the protocol of Köhler et al., 2017. The type of calling of A. lemur can be referred as a tonal – not frequency modulated call. For each call, we quantified their duration, dominant frequency, duration, bandwidth, call rate and intervalos entre llamados. We analyzed an average of xxx calls. To estimate the variability of acoustic parameters we calculated the coefficient of variation (CV), an standardized measure of dispersion obtained by the mean (X̄w) and standard deviation (SDw) of each call parameter evaluated at an intra-population level.

RESULTS

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**CAPÍTULO II: Case study of an automatic detection approach in the calling activity of** *Agalychnis lemur*

(Formato para revista xxxx)

**Case study of an automatic detection approach in the calling activity of** *Agalychnis lemur*

**Abstract:**

**Key words:**

Species are declining worldwide and the necessity to document, report and monitor populations is becoming urgent (Wilson, 2017; Gibb et al, 2018). For many years, scientists had limited resources to properly conduct long-term analysis. In the present, passive acoustic methods (PAM) are becoming a powerful tool to collect biological information in a broader temporal and special scale (Gibb et al, 2018; Sugai & Llusia, 2019; Bateman et al, 2021). Automatic acoustic recorders offer the opportunity to collect acoustic data of species that present vocal communication in a non-invasive, continuous and systematic manner (Sueur & Farina, 2015; Willacy et al, 2015; Sugai & Llusia, 2019). Thanks to these passive acoustic sensors, it has been possible to identify cryptic species, study acoustic landscapes, identify species phenology and distribution patterns (Obrist et al, 2010; Sugai & Llusia, 2019).

Although these methods are key for monitoring fauna, they have critical challenges. Monitoring procedures are non-standarized, also the identification process and data curation can be time consuming and subjective (Gibb et al, 2018; Sugai et al, 2019). It has been proposed that bioacoustic automatic detection of species signals is a powerful solution for handling large amounts of acoustic data (Márquez et al, 2014; Sugai et al, 2019). Automated systems therefore can scale up PAM studies and with machine learning approaches the identification process can become species-specific (Aide et al, 2013; Gibb et al, 2018).

Amphibians are the most threatened group in terms of extinction. Most species of frogs vocalize mainly for reproductive purposes (Wells, 2007). Thus, their calling activity can be used as a proxi of reproductive activity and therefore with PAM and automatic detection we can develop greater insights of reproductive phenology in anurans, information that is valuable for conservation efforts (Hilje & Aide, 2012; Sugai et al, 2021).

The development of automatic detection analysis in frogs can be a more effective process than in other animals. That is because acoustic signals in anurans are conserved at the phylogenetic level. Also, their calling patterns are assumed to have limited variation among individuals and populations (Obrist et al, 2010; Köhler et al. 2017). Therefore, there is a greater possibility to standardize monitoring protocols using PAM and posterior automatic processes. Studies using PAM in frog populations has become recently popular in species of temperate zones. Nonetheless, species of the tropics have a lower number of this studies, despite being the region with the greatest interest in conservation.

In this study we aim to describe our procedure using PAM and developing an automatic detection method for *Agalychnis lemur.* This species is endemic to Costa Rica and Panamá and is currently at risk of extinction (xxx). The information recopilated in this study will support the conservation efforts of a tropical species and also will contribute to the sampling and developing methodology for the tropical fauna, the region with greater biodiversity and least studied.

**MATERIALS AND METHODS**

**Study site*:*** We conducted this study in two locations in the Central Caribbean part of Costa Rica. The first site (Sukia) is located in the private reserve Veragua Rainforest (9°55’30”N, 83°11’28”E; 420 msnm). This reserve comprises 77 hectares of protected forest. The data was collected from three artificial ponds (agregar mediciones) surrounded by mature secondary forest. The second site (Chimú) is located in Cerro Chimú (9°52’48”N, 83°14’13”E; 741 msnm). The data was collected from a natural pond (agregar mediciones) surrounded of primary forest. Both sites are tropical wet forests characterized by high levels of potential evotranspiration ratios and annual precipitation (Holdridge, 1967).

**Study species:** *A. lemur* is an arboreal nocturnal frog that occurs on the Atlantic slope from northwestern Costa Rica, to western Panamá (xxx). This species is endemic to Costa Rica and Panamá and can be found through middle elevation forests between 440 to 1600 msnm. *A. lemur* is critically endangered according to the UICN (xxx). In captivity *A. lemur* produces three types of vocalizations, an advertisement call, an encounter call and a release call. For our purposes, we will focus only in the advertisement call characterized by being no-stereotype and composed of a single note (Emmrich et al, 2020; Jungfer, 1994).

**Study design:**  Calling activity of the two sampling populations was monitored for a 2 year period. The study began in July of 2019 and ended in February of 2021. We register the advertisement calls of *A. lemur* through passive acoustic monitoring using automated digital sound recorders SM4 of Wildlife Acoustics. We put one recorder in each site, tied to a tree 1.5-2 m above the ponds. The two recorders were set to register the activity every 15 min and 5 min each hour beginning from 17:00 pm at the afternoon until 5:00 am in the morning. This time comprises the expected reproductive day hour activity of *A. lemur.* Recording settings were adjusted to produce sounds in WAV stereo format at a sampling rate of 44.1 kHz and a 16 bit resolution.

**Sound analysis:** We conducted this analysis following the protocol proposed by Araya-Salas (2021) that consists in 5 steps: (1) Obtain the acoustic recordings from the sound recorders, (2) Define the signal acoustic structure to be quantified, (3) Generate a stratified random sample of acoustic files to be manually annotated for the detector configuration, (4) Create an automatic acoustic detector for the advertisement call of *A. lemur* using different tunning parameters, (5) Apply the automatic recognizer to all acoustic data (Fig.X).

1. *Obtain acoustic recordings*

Se obtuvieron los archivos de audio del período de análisis comprendido y se almacenaron en un disco duro externo.

1. *Generate a stratified random simple of acoustic files to be manually annotated*

We generate a stratified sampling method in R (R Core team, 2021) of the recordings with the purpose of creating a random sample of files capable of representing the variation of our data set not only through the night but also through different seasons of the year. The first stratum corresponds to the month (January-December), each one represented by a randomly selected week. The second stratum corresponds to subsets of 4 hours of the sampling periods. The first subset is from 17:00 to 20:20, the second is from 21:00 to 00:20 and the third one is from 1:00 to 4:20. We randomly select two recordings for each subset, week and month. We obtained 467 audio files that were cut to a 5 minute period in R using the Seewave package (Seur et al, 2008) and then manually annotated in Raven Pro 1.6 (Center for Conservation Bioacustics, 2019).

For each recording we register: (1) Begin Time, (2) End Time, (3) Low frequency, (4) High frequency, (5) Begin file, (7) File Offset, (8) Begin path. The information of each sound file was saved as a selection table and then imported to R Studio (R Core Team, 2021) using the package Rraven (Araya-Salas 2020). As a first step, we create catalogs of the spectrograms from the manual annotated sound files*.* The spectrograms were created with a window length of 512 amplitude values, a “hanning” window function and a 70% overlap.

1. *Define the signal acoustic structure to be quantified*

The acoustic features of each annotation were then inspected using the R package WarbleR (Araya-Salas & Smith Vidaurre 2017). We measure the following frequency range parameters: duration, mean frequency, standard deviation, freq.median, freq.Q25, freq.Q75, freq.IQR, time median, time.Q25, timeQ75, timeIQR, skew, kurtosis, spatial entropy, time entropy, entropy, sfm, mean dominant frequency, minimum dominant frequency, maximum dominant frequency, df range, modindx, startdom, enddom, df slope and mean peak frequency.

1. *Program the automatic acoustic recognizer*

We programmed the acoustic automatic recognizer using the R package Ohun (Araya-Salas 2021, R Core Team, 2021) based on a template-based detection method. This type of detection routine uses a correlation method that generates a correlation coefficient between the sound template and the automatically detected signal (Khanna et al.1997). For instance, the process of training a template-based detector can be summarize in 3 steps: choosing the right template, estimating the cross-correlation scores of templates along sound files and detecting the signals once we set an optimal correlation threshold (Araya-Salas, 2021).

In order to have a proper recognition method, the detected sounds must have a high similarity with the templates. For this reason, the templates more similar with the mean acoustic structure of the signal were expected to have the best performance. We identify the annotations closest to the mean duration and mean peak frequency. We also did a Principal Component Analysis to summarize the acoustic structure in one single variable. The purpose was to find the annotation closest to the mean of all the acoustic parameters to be taken into account as another template.

The package Ohun (Araya-Salas, 2021) uses a correlation threshold to distinguish the detected signals from the background noise. For each template, we used the manual annotations as a reference to defined the optimal value for the correlation threshold. The best threshold is the one that is capable of detecting most of the signals (high sensitivity) and decrease the number of the not detected signals (high specificity) in the annotations. With a high sensibility the number of detected sounds increase but also the number of false positives (signals that are not of interest) increase, that means that the specificity decreases. On the other hand, a greater specificity produces a high number of true positives (signals of interest), but the number of signals detected may decrease due to a high detection threshold. For each template we optimize the correlation threshold according to the best combination of specificity and sensitivity. The optimization process of the acoustic recognizer consists in finding a good balance between the sensitivity and specificity. Due to the above, we tested how the specificity and sensitivity varied according to different thresholds.

The detection routine also deals with classifying the identified signals by the recognizer in 7 categories: (1) True positives, signals correctly identified as “signal”, (2) False positives, background noise incorrectly identified as signal, (3) False negatives, signals incorrectly identified as background noise, (4) True negatives, background noise correctly identified as background noise, (5) split positives, target signals overlapped by more than one detection, (6) Merged positives, number of cases in which 2 or more target signals overlapped by the same detection, (7) Proportional overlap true positives, ratio of the time overlap of true positives with its reference table (Araya-Salas, 2021).

1. *Machine learning approach*

The performance of the detection routine can be also optimized through classification algorithms once the recognizer detects and classify the signals as true positives or false positives. In our case, these algorithms use acoustic parameters to discriminate the target signal from the background noise. This second filter in the detection is useful when the sensibility of the recognizer is high but the specificity is low.

This analysis was based on a *randomForest* classification model (Breiman, 2000) through the ranger package in R (Liaw & Weiner, 2002; R Core team, 2021). This model uses binary partitioning to split predictor variables (in our case acoustic parameters), replicated through boot-strapped trees. This model is nonparametric and is independent of previous assumptions. For instance, *randomForest* models allow the study of nonlinear relationships.

Each *randomForest* tree is built from a randomized subset of data and “out-of-bag error” (OOB) that is set by assessing each tree against the accuracy of predictions for the excluded data set. Each tree gives an OOB error of self-assessment classification accuracy and each node within a decision tree is in accordance to a random sample of predictor variables. Trees within a forest are then compared and aggregated to make a final classification.

*RandomForest* models are controlled by two adjustable parameters: forest size and the number of variables considered at each node within forest trees. For our purpose we used the acoustic parameters as predictor variables and a number of 10000 trees. (falta explicar lo que hicimos nosotros en detalle …)

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**CAPITULO III: Calling phenology of** *Agalychnis lemur*

(Formato para la revista xxx)

**Calling phenology of** *A. lemur*

**Abstract:**

**Key words:**

Climate affects and regulates many physiological aspects in species (Wells, 2007). In amphibians, water availability and temperature are two basic climatic components that regulate almost every aspect of their life cycle (Wells, 2007; Akmentins et al, 2014; Caldart et al, 2016; Pérez-Granados et al, 2019). Because of their ectothermic condition, their ability to perform basic physiological tasks (like oviposit and vocalize) are temperature dependent (Navas, 1996; Brooke et al, 2000; Sáenz et al, 2006; Llusia et al, 2013; Köhler et al. 2017). Also, rainfall and humidity decrease the probability of suffering a high rate of evaporative water loss in individuals, and can correlate seasonal timing of activities such as growth and reproduction (Yoo & Jang, 2012; Dostine et al. 2013; Lemckery, 2013; Sáenz et al. 2013; Caldart et al, 2016). Some species can show a response with other environmental cues such as moon phases (Saenz et al. 2006; Vignoli & Luiselli, 2013, Lima et al. 2021). Probably, the moon is associated to hormonal cycles, visibility to predators and it may provide a rhythmic temporal cue for species that migrate to their breeding sites (FitzGerald & Bider, 1974; Grant et al. 2009; Grant et al. 2012; Kronfeld-Schor et al. 2013).

Many anuran species vocalize with the purpose of attract females and mediate male interactions (Wells, 2007; Yoo & Jang, 2012; Wang et al, 2012; Köhler et al. 2017). Vocalizing is one of the tasks with the highest energetic and survival cost for males, since it implies the movement of the trunk muscles (mechanism that is temperature dependent) and it increases the probability of predation (Well, 2007; Llusia et al, 2013). For these reasons, it would be expected that the movement, timing and duration of the vocal activity in males has been optimized through natural selection (Guerra et al, 2020; Huitrón et al, 2020). Thus, it is possible that the best time of displays may be influenced by the climatic conditions. For example, periods of rainfall may increase the activity and the breeding sites available in their habitat and nights of less lighting would be favorable for prolonged breeding activity (Osseen & Wassersug, 2002; Lemckert et al, 2001; Bonnefond et al, 2019).

Depending on the species, their reproductive activity can be highly correlated to a specific environmental condition such as temperature (Donnelly & Crump, 1994; Duellman and Trueb 1994; Ficetola et al, 2016; von May et al. 2017; Pintanel et al. 2018). Whether in some cases, species response can be more generalized or associated to a specific combination of climatic factors (Cui et al, 2011; Lemckert et al, 2013; Bonnefond et al, 2019). In explosive spawners males may be more environmentally sensitive when they call because they focused their activity in certain periods of the year, whereas is more important the efficiency of the call than the speed (Oseen & Wassersug, 2002; Ulloa et al, 2019). On the other hand, prolonged breeders may respond to a set of daily environmental cues that correlate with nights of more calling intensity like the possible effect of accumulated rainfall (Sáenz et al, 2006; Schalk & Saenz, 2016).

Amphibians are highly sensitive to environmental fluctuations (Stuart et al. 2004; Mendelson et al. 2006; Llusia et al. 2013; Bonnefond et al, 2019; Gonzáles-del-Pliego et al. 2019). In order to understand their response to the current global conditions of drastic climatic changes, studies that focus in which climatic variables have a greater impact on reproduction aspects of the species can become a useful tool to improve conservation efforts (Donnelly & Crump, 1998; Brooke et al. 2000; Sáenz et al. 2006; Llusia et al, 2013; Pérez-Granados et al. 2019). In this study we aim to analyze the association between the vocal activity of *A. lemur* with different climatic factors. *A. lemur* is endemic to the tropics, so we expect that daily variation in climatic conditions would be a strong predictor for the species activity.

MATERIALS AND METHODS

**Study site*:*** We conducted this study in two locations in the Central Caribbean part of Costa Rica. The first site (Sukia) is located in the private reserve Veragua Rainforest (9°55’30”N, 83°11’28”E; 420 msnm). This reserve comprises 77 hectares of protected forest. The data was collected from five artificial ponds (agregar mediciones) surrounded by mature secondary forest. The second site (Chimú) is located in Cerro Chimú (9°52’48”N, 83°14’13”E; 741 msnm). The data was collected from a natural pond (agregar mediciones) surrounded of primary forest. Both sites are tropical wet forests characterized by high levels of potential evo-transpiration ratios and annual precipitation (Holdridge, 1967).

**Studied species:** *A. lemur* is an arboreal nocturnal frog that occurs on the Atlantic slope from northwestern Costa Rica, to western Panamá (xxx). This species is endemic to Costa Rica and Panamá and can be found through middle elevation forests between 440 to 1600 msnm. *A. lemur* is sensitive to habitat fragmentation and epidemiological diseases such as BD fungi. Nonetheless, there is no much information of *A. lemur* biology and response to these disturbances that probably are affecting their populations (xxx). *A. lemur* advertisement call is characterized by being non-stereotyped and composed of a single note (Emmrich et al, 2020; Jungfer, 1994).

**Acoustic monitoring and analysis:** We placed one Song Meter SM4 recorder (Wildlife Acoustics) in both sites tied to a tree 1.5-2 m above the ponds. The study began in Sukia site in July 2019 until February 2021 and in Chimú site in XXx until February 2021. Each recorder was programmed to record sounds for a 15 min period and 5 min period each hour from 17:00 h to 5:00 h every day. This time comprises the expected reproductive day hour activity of *A. lemur.* We adjusted the recording settings to produce sounds in WAV stereo format at a sampling rate of 44.1 kHz and a 16 bit resolution.

In order to standardize and process the acoustic information efficiently, we developed an automatic detector programmed for the advertisement call of *A. lemur.* This method is based on a spectrogram cross-correlation between sound templates of the signal of interest and the acoustic recordings. To accomplish this task, we follow the protocol proposed by Araya-Salas (2021) that consists in 5 steps: (1) Obtain the acoustic recordings from the sound recorders, (2) Generate a stratified random sample of acoustic files to be manually annotated for the detector configuration, (3) Define the signal acoustic structure to be quantified, (4) Create an automatic acoustic detector for the advertisement call of *A. lemur* using different tunning parameters, (5) Apply the automatic recognizer to all acoustic data.

Once we obtained the sound files we started generating a random sample of acoustic files. The purpose of the above was to have a sub-sample representative of the sampling period comprised. We used R Studio (R Core Team, 2022) and the package Seewave (Seur et al, 2008) to cut the selected recordings to a 5 min period. We obtained 467 randomized acoustic files and then we started with the next step of annotating each recording to find the acoustic structure of *A. lemur* using the program Raven Pro 1.6 (Center for Conservation Bioacustics, 2019). We register the signal of *A. lemur* and create a selection table for each recording to be later imported to R Studio using the package Rraven (xxx). With the package WarbleR (Xxx) we inspected the annotations, find the acoustic structure and create catalogs of the spectrograms of the signal. This spectrograms were created with a window length of 512 amplitude values, a “hanning” window function and a 70% overlap.

We measured the following frequency range parameters: duration, mean frequency, standard deviation, freq.median, freq.Q25, freq.Q75, freq.IQR, time median, time.Q25, timeQ75, timeIQR, skew, kurtosis, spatial entropy, time entropy, entropy, sfm, mean dominant frequency, minimum dominant frequency, maximum dominant frequency, df range, modindx, startdom, enddom, df slope and mean peak frequency. This parameters help us find the variables that best characterize the signal of *A. lemur* to create the sound templates that were used to automate the detection routine.

The acoustic recognizer configuration was performed using the R package Ohun (Araya-Salas, 2021). As mentioned above, this process is based on the template-based detection method that generates a correlation coefficient between the sound template and the automatically detected signal (Khanna et al.1997). For instance, the process of training a template-based detector can be summarize in 3 steps: choosing the right template, estimating the cross-correlation scores of templates along sound files and detecting the signals once we set an optimal correlation threshold (Araya-Salas, 2021).

To choose the best template, we identify the annotations closest to the mean duration and mean peak frequency. We also did a Principal Component Analysis to summarize the acoustic structure in one single variable. The purpose was to find the annotation closest to the mean of all the acoustic parameters to be taken into account as another template. To distinguish the detected signals from the background noise the package Ohun uses a correlation threshold that we can adjust using the manual annotations as a reference to evaluate the best performance of each template. The optimal threshold is the one capable of detecting most of the signals (high sensitivity) and decrease the number of the not detected signals (high specificity) in the annotations. For each template we optimize the correlation threshold trying to find a right balance between the specificity and sensitivity.

For a better optimization process we used a second filter based on a *RandomForest* model (Breiman, 2000) with the R package randomForest (Liaw & Weiner, 2002; R Core team, 2021). This model uses binary partitioning to split predictor variables (in our case acoustic parameters), replicated through boot-strapped trees. This model is nonparametric and is independent of previous assumptions.

**Environmental variables:** We obtained the climatic data from a weather station located in the study area at a distance of xxxx from the Sukia site. From this station we recovered the temperature (C), relative air humidity (%), and rainfall (mm) that was registered every minute each day. We processed this data using R Studio (R Core Team), finding the mean of each variable per hour (from 17:00 h to 5:00 h). We incorporated the accumulated rainfall of the previous xxx.We recovered the moon data from the R package LunaR (Xxx) and obtained the percentage of illumination per night.

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