Environmental drivers of calling activity in the endangered species Lemur Leaf frog

Keywords: Causal modeling, Hylidae, Bayesian models, temperature, relative humidity, bioacoustics.

Abstract Tropical frog species are known to exhibit high sensitivity to weather regime alterations, which leaves them vulnerable to ongoing climate change. This challenge is exacerbated by limited knowledge of species-specific responses to environmental change. We integrated passive acoustic monitoring and automatic signal detection to investigate the calling activity of the Critically Endangered tree-frog, *Agalychnis lemur*. We combined template-based detection with machine learning mitigation of false positives to infer the calling activity of a lemur leaf frog population across 18 months. We used directed acyclic graphs (DAGs) to determine the covariates needed to infer causal relations between environmental variables and calling activity. Our findings revealed that daily temperature has a strong direct positive effect on calling activity, with additional indirect effects mediated by relative humidity. Moreover, higher activity of lemur leaf frog was triggered by increasing humidity independent of temperature, higher accumulated rainfall within the preceding 24 hours, and decreases in moonlight. This study provides unique insight into the complex interplay of environmental factors for determining calling activity in frogs. Our findings underscore the potential of passive acoustic monitoring for elucidating frog population activity and its responses to environmental changes, which can be valuable for understudied species in the context of climate change.

1. Introduction

The alarming trend of population declines and extinctions among amphibian species worldwide presents a significant challenge for their conservation efforts [1]. This challenge is exacerbated by limited knowledge of species-specific susceptibilities to environmental change. Nearly 60% of the world's threatened amphibian species are found in the Neotropics, where 92% face elevated levels of habitat loss and a third suffer from reduced rainfall and humidity [2]. Moreover, over 11% of all amphibian species are classified as data deficient, hindering effective conservation strategies [2]. As ectothermic organisms, amphibians rely on environmental cues to regulate most physiological processes and behaviours [3]. Short-term variations in weather or environmental factors can significantly impact reproductive success, rendering amphibians vulnerable to human-induced environmental changes that alter microclimates, with consequences for population dynamics [4–6]. Therefore, developing strategies to mitigate these impacts and implementing research tools enabling cost-efficient monitoring of critical species are crucial steps in addressing amphibian conservation challenges and safeguarding their ecosystems [7–9]. For animals whose communication and reproductive interactions largely rely on acoustic signals, as is the case of amphibians, passive acoustic monitoring (hereafter PAM) methods can be used to register vocal activity over extensive temporal and/or spatial scales [10,11]. These relatively novel technologies provide a non-invasive method that is particularly promising for generating key information on threatened, understudied anuran species. Such approaches are increasingly used for documenting population changes in frogs [12]. However, their analysis often rely on human observers which suffers from limited standardization, and make data curation time consuming and subjective [13]. Automated detection approaches of acoustic signals allow to solve many of these issues, as they are in principle verifiable, reproducible and can handle large amounts of acoustic data [12]. Thus, the combination of PAM and automated detection pipelines can offer a robust and efficient approach for the monitoring of highly vocal species [14]. Environmental factors can affect the vocal activity of frogs in diverse ways. First, rising evapotranspiration increases the likelihood of rainfall, which in turn increases relative humidity [15]. High relative humidity can support a higher rate of calling activity by reducing desiccation risk [5]. Second, higher ambient temperature decreases relative humidity, as warmer air holds more moisture. Calling activity can therefore be negatively affected by a rise in local temperature [16]. In contrast, temperature directly influences metabolic rates in ectotherms, potentially increasing vocalization rate and energy expenditure in frogs [3,17–19]. Therefore, temperature may also directly intensify calling activity [20]. In addition, rainfall can strongly influence calling activity and its effect can vary across temporal scales [8]. Preceding rainfall boosts relative humidity through evaporation, positively impacting calling activity [21]. Both daytime and nighttime rainfall contribute to increased humidity, further promoting calling behaviour. However, rainfall also lowers local temperatures, affecting frog calling activity through the metabolic processes described above. Moreover, it is known that heavy rains can dampen sound quality, potentially leading to decreased calling rates if frogs need to invest more energy for producing louder calls that can be detected by conspecifics [22]. Similarly, moonlight can affect calling activity because the lunar synodic cycle (i.e. the transition full from new moon to the next new moon) changes the brightness of lunar light that can be perceived by animals [23]. Most frog species are nocturnal and are at risk of visually oriented predators [24]. This predation risk is maximized during nights of high moon illumination that facilitate visual predators to locate their prey. In this work, we quantified the calling activity of the critically endangered lemur leaf frog (*Agalychnis lemur*) over an 18-month period aiming to understand the effect of weather and moonlight fluctuations on the calling behaviour of this species. We used graphical causal modeling in a directed acyclic graph (DAG) for depicting the array of potential causal pathways in the system [25]. This approach allowed us to explicitly represent our hypotheses of the causal relationships between environmental factors and calling activity and to derive the models to statistically evaluate these relationships [25,26]. Our results provide valuable insights about the climatic underpinnings of reproductive behaviour in an endangered and poorly known species and demonstrates the effectiveness of bioacoustics approaches for understanding key aspects of the species biology in a context of growing threats to biodiversity.

2. Materials and Methods

(a) Study Species

The lemur leaf frog is an arboreal nocturnal frog historically distributed in tropical rain forests of Costa Rica, Panama and Colombia from 440 to 1600 masl [27,28]. Currently restricted to few populations in the Talamanca Mountain Range in Costa Rica and the Caribbean slope of the Chiriqui province in Panama, considered critically endangered, with deforestation, chytridiomycosis infection, and habitat loss as its main threats [29–31]. In captivity, the lemur leaf frogs produce three types of vocalizations: an advertisement call, an encounter call, and a release call [32,33]. The advertisement call consists of a very short "tick" repeated every 25 s, used as a mating vocalization broadcasted during the breeding season as well as communicating territory occupancy among males [33,34]. We focused on the advertisement call because of its mating role, which makes it a proxy for breeding activity.

(b) Study site and weather data collection

We conducted the study at Veragua Rainforest (9.924819°N, -83.191206°W; 420 masl), a 13.7 km² private reserve in the Central Caribbean region of Costa Rica, situated on the northern edge of the Matama Mountains within the Talamanca Mountain Range. This reserve falls within the Basal Tropical Wet Forest life zone (200–600 m a.s.l.) as described by Holdridge [35]. The area features a mix of mature forest, secondary vegetation at various stages of regeneration, open areas, and dirt roads [36]. Located at the closest point of the Talamanca Mountain Range to the Caribbean Sea, it is part of the buffer zones of La Amistad International Park, a UNESCO World Heritage Site. Data was collected in an area with 5 experimental ponds established in 2012 (length 200 cm, width 150 cm, depth 50 cm) as a habitat restoration program that aimed to serve as in situ breeding sites for the different frog species inhabiting the area, and where the species was previously detected [36]. We obtained weather data from a station located 300 m from the study site. The station registered temperature (°C), relative air humidity (%), and rainfall (mm) every minute. From this data we estimated the average values for each 15 min recording interval (see the detailed description below) and the accumulated rainfall during the 48 and 24 hour previous to each sampling event. We obtained the moonlight intensity during those periods using the R package LunaR [37] which was used to estimate the percentage of lunar illumination per night based on the site's geographic location and the date of recording.

(c) Acoustic monitoring and call recognition

We recorded the advertisement calls of the lemur leaf frog through passive acoustic monitoring using the SM4 digital sound recorder [38]. The sampling period spanned 18 months, from July 2019 to February 2021, . We set the recorder to collect acoustic data daily from 18:00 to 5:00 the following morning, coinciding with the expected reproductive activity of nocturnal species like the Lemur frog [34]. The equipment was programmed to record for 15 minutes every 20 minutes (with a 5-minute resting period), resulting in three 15-minute samples per hour. Recordings were stored in WAV stereo format at a sampling rate of 44.1 kHz and a 16-bit amplitude resolution. Afterwards we split each recording into five minute clips and resampled at 10 kHz to enhance computational performance during further analysis. We used a template detector model in the R package ohun [39] to automatically detect advertisement calls of the lemur leaf frog in our recordings. The automatic detector was trained with recordings used in this study and from others recorded at an additional site that hosts another population of the lemur leaf frog in order to improve detection performance. We selected a subset of recordings to be used as the training data set, using stratified random sampling for ensuring a balanced representation across months, weeks, hours and 4 hour sampling periods. Then, two recordings were randomly chosen from each level of a full factorial combination of these factors. The resulting training data set consisted of 467 recordings. We annotated all visually identifiable lemur leaf frog calls on the Fourier spectrograms of these recordings using the Raven software [40], noting the start and end times and frequency range of each call. These annotations were then imported into R [41] using the Rraven package [42] and processed in the package warbleR [43] to ensure precise time and frequency positioning of the signals. The template-based detection routine identifies sounds similar to template sounds using spectrographic cross-correlation. Therefore, detection performance usually varies among templates. We evaluated three acoustic templates in order to select the template with the best performance. We selected a set of templates that represented the structural diversity of calls. For this the warbleR package was used to measure 23 spectral features (related to the distribution of power in time and frequency) representing the acoustic structure of the manually annotated calls. We selected three candidate templates: two were calls with parameters closest to the mean duration and mean frequency respectively, and the third was the call closest to the mean of the first component from a Principal Component Analysis (PCA) on z-transformed acoustic features. We trained the template-based detection routine on the annotated recordings, serving as a reference dataset for evaluating its performance. We identified as signals of interest those sounds in which correlation with the template surpasses a predefined threshold. The three candidate templates were tested across 20 correlation thresholds ranging from 0.1 to 0.9. The template with the best performance was determined based on recall and precision metrics. Recall measures the ability to identify signals of interest, while precision indicates the routine's ability to exclude non-target sounds [44]. Hence, an efficient detection routine should maximize recall and precision, and this criterion is used to guide correlation threshold selection. However, as low precision can be mitigated in further analysis (see below) we favored a threshold that improved recall performance despite having a poor performance in precision. After applying the selected correlation threshold for defining detections we implemented a machine learning approach to mitigate incorrect detections [45]. For this secondary filter we trained a supervised random forest classification model (RF) [46] using the R package ranger [47]. The RF model identified the lemur leaf frog calls based on acoustic features, measuring the same spectral features used for selecting templates in addition to statistical descriptors of Mel-frequency cepstral coefficients (MFCCs) using warbleR. Cepstral coefficients represent perceptually relevant features as they are derived from spectral bands that resemble sound representation in animal auditory systems, and have been widely used in human speech recognition and increasingly in non-human animals [48–52]. We measured 25 cepstra on 10 warped spectral bands, analyzing the minimum, maximum, mean, median, skewness, kurtosis, and variance of each MFCC, along with the mean and variance of their first and second derivatives. The RF model was trained with 1000 trees and mtry (number of features considered for splitting trees) of 13 variables. The out-of-bag error (OOB) was used to evaluate classification performance.

(d) Causal modeling

Environmental factors which may influence the calling activity of the lemur leaf frog are part of a complex system, in which factors are also causally related to each other. Causal models can help to understand these complex systems by explicitly stating relationships among variables. Here, we used directed acyclic graphs (DAGs) to identify the set of variables that must and must not be taken into account to estimate causal effects of interest without bias (Fig. 1., [25]). We were interested in the effects of temperature, rainfall relative humidity and night illumination on frog calling activity. Our DAG hypothesizes that the effect of temperature can be direct, via effects on frog metabolism, or indirect by affecting relative humidity (Fig. 1). Rainfall was expected to affect calling activity through two time frames: "current rainfall" which refers to the cumulative rainfall during the hour before a sampling event and "prior rainfall" which represents the precipitation during previous days (Fig. 1). Current rainfall was expected to have a positive effect on frog calling activity by increasing relative humidity and a negative effect through a decrease in temperature (Fig. 1). Prior rainfall might increase activity by expanding breeding micro-habitats (e.g. ponds) and by increasing relative humidity (Fig. 1). Prior rainfall was represented by two variables that measured the 24 hour cumulative rainfall on two different periods: on the day prior to each sampling unit, and two days prior to each sampling unit. The two prior rainfall variables were modeled in separate analyses in order to determine the most relevant period for calling activity. We then estimated the direct effects of relative humidity and nocturnal light on frog calling activity. Our DAG depicts additional causal relationships that are not of particular interest in this study, but inform our variable selection process required for unbiased estimation of the causal effects. First, by controlling sunlight hours, the earth's rotation may influence calling activity both directly, and indirectly, via effects on temperature. Earth rotation also interacts with the Moon's rotation and thus influences nocturnal illumination. Earth rotation was modeled as the time of the day for statistical analyses. Similarly, evapotranspiration represents the total water loss to the atmosphere preceding cloud formation and eventual rainfall, and thus also influences relative humidity. In our DAG, evapotranspiration is an unmeasured (latent) variable that affects both current rainfall and prior rainfall.

(e) Statistical analyses

For each predictor, we determined the set of variables (i.e. adjustment set) that allow for the asymptotic estimation of unbiased causal direct and total effects on call rate, using the R package daggity [53]. Direct effects represent the predictor's effect on the response that is not mediated by any other variable while total effects take into account both direct effects and those mediated by other variables. The difference between the two effects provides evidence of the mediated (indirect) effects. Variables in the adjustment sets were used as predictors in regression analyses. Regressions were fitted for each environmental variable (temperature, relative humidity, current rainfall, prior rainfall and moon illumination) using Bayesian generalized linear models in the R package brms [54]. The models accounted for temporal autocorrelation (i.e., the dependency among observations close to each other in time) using an Autoregressive Moving Average (ARMA) correlation structure, with time of the day (discretized

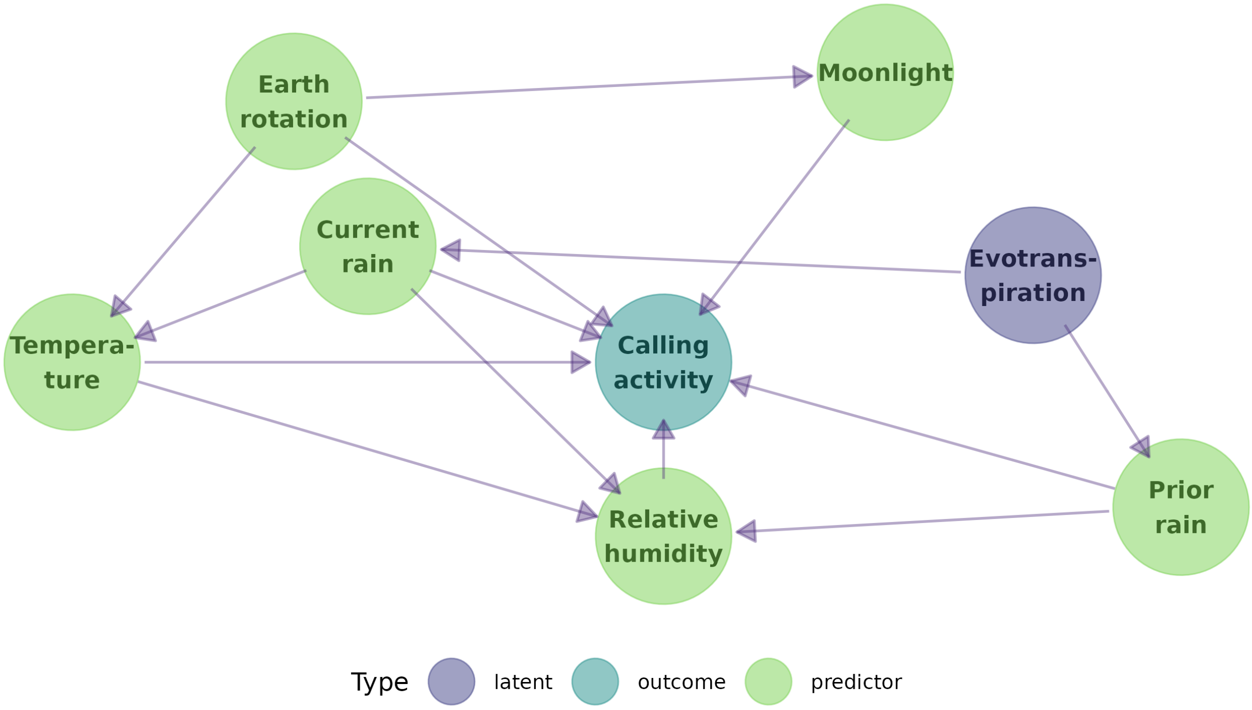

Figure 1. Directed acyclic graph (DAG) depicting the hypothesized causal pathways between environmental variables and the calling activity of lemur leaf frog. Our DAG includes two types of variables: observed variables which were measured in the field, such as "temperature" and "relative humidity" and latent variable, evapotranspiration, which was not measured. The stage of Earth's rotation is represented in our data as a continuous value between 0 and 24 hours.

in one hour periods) as the grouping variable. We modeled calling activity as a rate of calls per hour using a negative binomial distribution with a logarithmic link function. All predictor variables were zero-centered and transformed to unit variance. When several adjustment sets existed for modeling a predictor's effect, the posterior distribution of the correspondent models were averaged, weighted by their predictive performance using a stacking leave-one-out cross validation approach [55]. Effect size estimates were transformed into percentage of change to facilitate interpretation and are presented as medians of posterior distributions and 95% uncertainty intervals (UI). Parameters in which intervals did not include zero were regarded as affecting calling rate. Models were run for 10,000 iterations, following a warm-up of 10,000 iterations on four MCMC chains. For all parameters, the effective sample size was maintained at or above 4000. We plotted the trace and distribution of posterior estimates for all chains for visual evaluation of model performance. In order to assess the independence of the posterior samples, we also inspected the autocorrelation of successive sampled values and estimated the potential scale reduction factors for checking model convergence (kept below 1.01 for all parameter estimates).

3. Results

We examined a total of 370,718 calls of the lemur leaf frog in 1707 hours of recording. The vocalization consists of a single, non-frequency modulated call with amplitude modulation, emitted by males from the leaves and branches of vegetation surrounding swamps and other similar lentic environments. The call is tonal, with the amplitude peak typically located at the beginning of the call duration, depicting a triangular-shaped call in the spectrogram. The frequency bands exhibit no significant modulation throughout the call duration. Across the entire sampling period, we recorded an average of 27 vocalizations of lemur leaf frog per hour. The performance of the acoustic templates (mean peak frequency, mean duration and mean PCA1) for detecting the lemur leaf frog calls varied importantly according to the performance metrics recall and precision (Fig. 2). The mean PCA1 was chosen to be used as the template in subsequent analysis given its positive balance between the two performance metrics (70.4% recall and 3.7% precision with a 0.43 correlation threshold). After RF mitigation of false positives (OOB error rate of 0.88%) we obtained a 67% recall and 91% precision. The observed number of calls on the 5-min clips in the training data set and the number of calls found by our detection procedure were highly correlated (Pearson correlation score: 0.92; 0.89 after removing outlier).

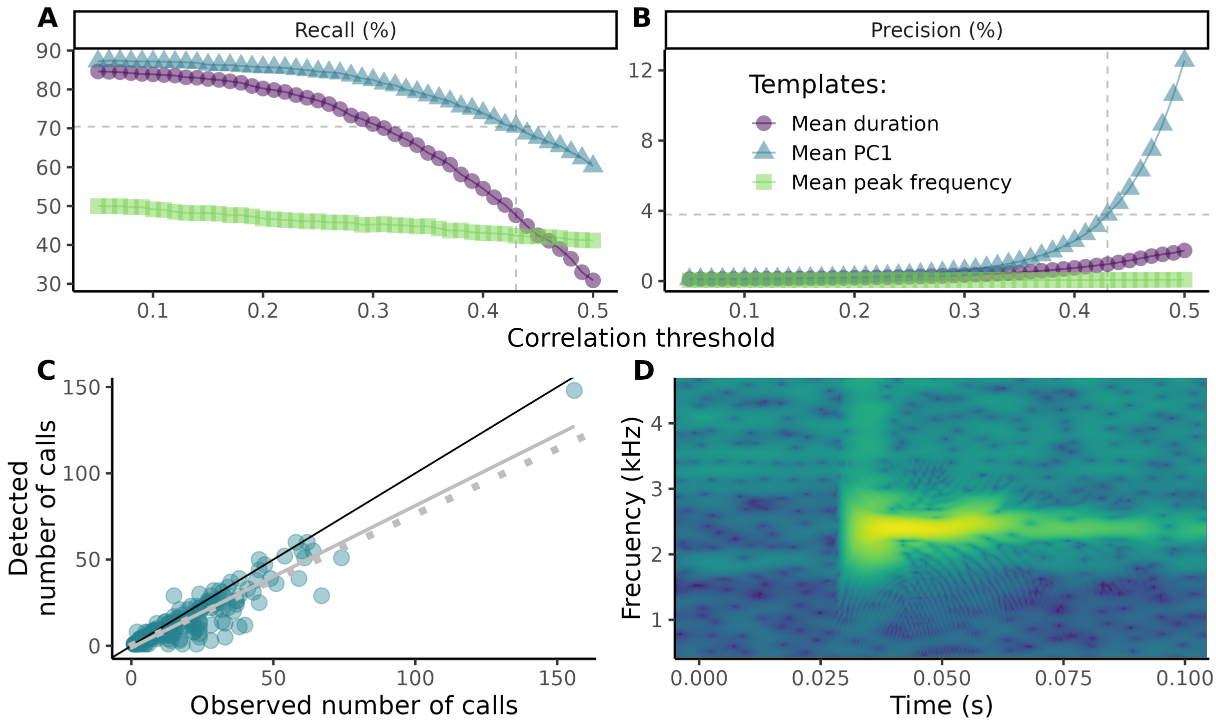

Figure 2. A) Recall and B) precision across increasing correlation threshold values for the three acoustic templates in the detection procedure. We selected template calls as those closer to the mean value of three acoustic features: duration, peak frequency and the first vector from a PCA summarizing several spectro-temporal features. Dashed gray lines show the selected correlation threshold (0.43) and correspondent recall (A) or precision (B) value. C) Scatterplot showing the number of calls observed on each 5-min clip used for training and those found by our detection procedure (template detector + Random Forest mitigation of false positives). The black line is the 1:1 diagonal. The solid gray line shows the best fit line from a linear regression on the entire data (slope: 0.82) while the dotted gray line shows the best fit line when excluding the outlier (slope: 0.76). D) Spectrogram of a Lemur frog call.

For the accumulated rainfall during the 24 hour prior to sampling we found a positive direct effect (effect size: 10% UI: 5.8% – 14.7%; Fig. 3) and total effect (effect size: 9.1% UI: 5.2% – 13.6%; Fig. 3), on calling activity, whereas accumulated rainfall during the day 48 hour before sampling did not have a detectable influence on calling (direct effect: 0.62% UI:-2.9% – 4.1%; total effect: 0.54% UI:-3.3% – 4.4%). Therefore, results hereafter are based on models using prior 24 hour accumulated rainfall whenever prior rainfall was included in the adjustment set for other effect estimates. Temperature has the largest positive direct effect on calling rate (effect size: 77% UI:65.1% - 92.5%; Fig. 3), followed by relative humidity (effect size: 37.0% (27.0% - 47.0%; Fig. 3), while moonlight showed a negative direct effect (effect size: -12.0% UI:-17.1% – -6.2%; Fig. 3). We did not found a detectable direct effect of current rainfall on calling activity (effect size: 3.6% UI:-0.0% – 7.9%). Total effects sizes were similar to direct effects sizes for all predictors (24 hour prior rainfall: 9.1% (Ui: 5.1% – 13.5%); 48 hour prior rainfall: 0.54% (-3.2% – 4.4%): moonlight: -9.6% (-15.6% – -3.9%); current rainfall: 3.6% (-3.3% – 3.5%); Fig. 3) except for temperature (36.1% (29.4% – 43.4%); Fig. 3).

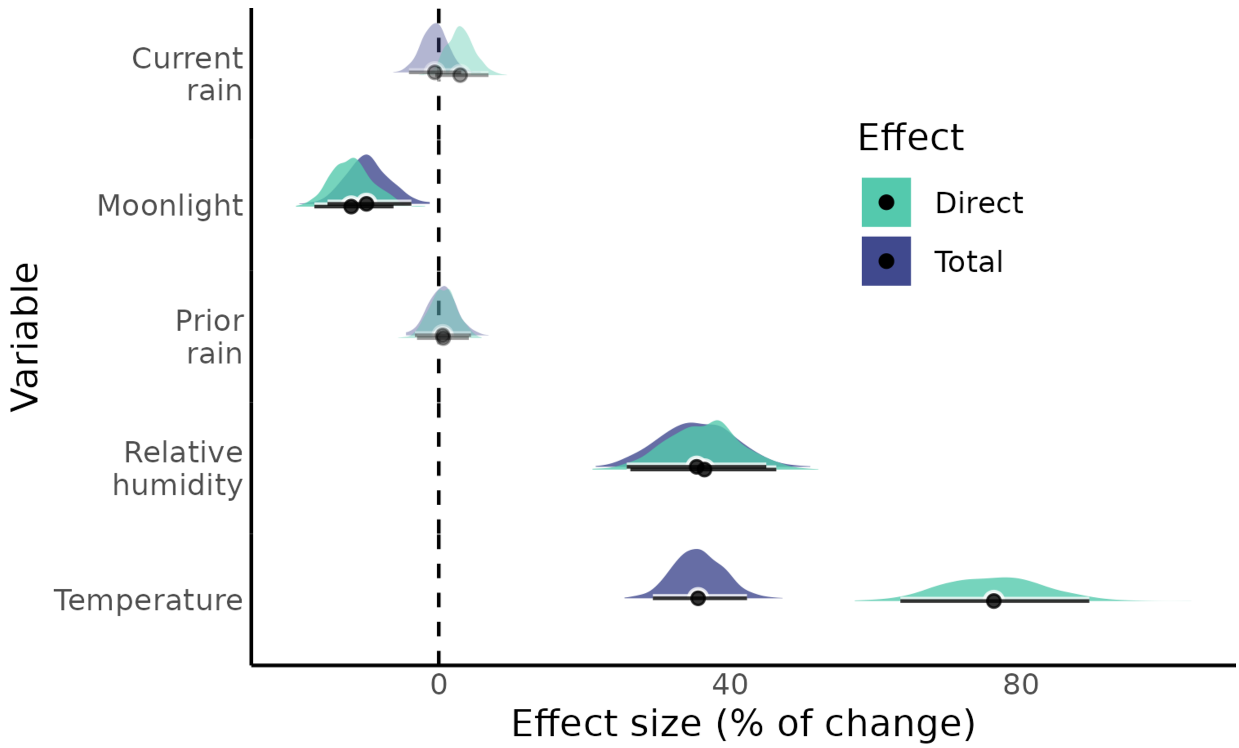

Figure 3. Posterior distribution of direct (green) and total (purple) effect sizes of environmental factors on the calling activity of lemur leaf frog. Posterior values were transformed into percentage change to facilitate interpretation. Dots and error bars show the median and 95% uncertainty intervals of the distributions. Solid color distributions correspond to effect sizes in which uncertainty intervals do not include zero. “Prior rain” accounts for the 48 hour period before sampling calling activity.

4. Discussion

In this study, we aimed to untangle the causal effects of several environmental variables on the calling activity of the lemur leaf frog, using passive acoustic monitoring. We provide quantitative evidence of the way in which environmental variation shapes calling activity. We found that calling activity is mainly triggered by the increase in temperature and relative humidity, as well as by the rainfall accumulated during the day before, although this effect is only moderate. Contrary, the calling rate of this species falls during luminous nights but in general is not affected by current rainfall. More broadly, our results demonstrate the potential of monitoring efforts that passively collect information in a continuous and systematic manner, to gain insights into the ecological drivers of reproductive behaviour in natural populations. The precision of our automated detection procedure allowed us to accurately infer the variation in call activity. Despite having a moderate performance for detecting individual calls (67% recall), our approach succeeded in predicting call activity in 5 min intervals with high accuracy (Fig. 2-C). The applied routine for call detection is relatively simple compared to recently developed deep learning approaches. However, Random Forest, the algorithm used for mitigating incorrect detections, stands as a highly robust machine learning classification method [56]. Overall our results demonstrate that traditional bioacoustic methods can be boosted by combining them with classification approaches that are already familiar to ecologists in order to drastically improve automated detection in PAM studies. The lemur leaf frog, like other species of Leaf Frogs (subfamily Phyllomedusinae), has been classified as a prolonged breeder [57]. This strategy is characterized by linking their energy allocation to advertisement calling, based on local environmental cues [7,58,59]. Our results indicate that the calling activity of lemur leaf frogs respond to environmental conditions in a small time scale, especially temperature and humidity, whereas prior environmental conditions such as those of cumulative rainfall from previous days are less relevant.

Temperature had a direct positive effect on calling activity (Fig. 3), as expected if calling activity responds to a general increase in metabolic rate [18]. Temperature was also expected to have a negative effect on calling activity, mediated by a reduction in relative humidity. The total effect of temperature on calling activity was substantially smaller than its direct effect estimate, suggesting a negative indirect effect through relative humidity (Fig. 1). Thus, while our data supports this dual mechanism, the net effect of temperature on calling activity was positive. The skin of arboreal Leaf frogs is particularly resistant to evaporative water loss [60–62], which may allow them to tolerate relatively dry conditions, compared to other frogs, and exhibit an overall positive response to high temperatures [20]. It is plausible, however, that in face of climate change, frogs will be exposed to a wider range of temperatures, in which the relationship between temperature and calling rate is unlikely to remain unaltered [18,63]. Thus, the indirect negative effect of temperature may be amplified by global warming while the direct positive effect on calling rate may not [21,64]. While in the current temperature ranges warmer conditions seem to favor reproductive activity, our finding of two opposing effects of temperature on calling activity suggests a possible mechanism for the effect of total temperature to be reduced or even change direction with climate change [65]. Our findings that temperature and relative humidity have the largest effects on the calling activity of the lemur leaf frog are also consistent with previous studies in other tropical frogs [8,21,63,66–68]. Frog vocal activity is likely maximized at a temperature that is high enough to promote calling, but that does not reduce humidity to the point of risking desiccation, or where a large input of water, such as heavy rainfall during day time, is available to maintain a humid environment [18,69]. In our study site, the relative humidity was 90% on average, which likely enables a relatively continuous year-round calling activity of the lemur leaf frog [68]. As expected, rainfall impacts the acoustic activity of lemur leaf frogs, but interestingly, these effects seem to vary across temporal scales. A rainy period of a few days or even hours can elicit migration to reproductive areas, in species that depend on temporary ponds to reproduce, particularly for explosive breeders [57]. Lemur leaf frogs reproduce in both temporary and permanent pools v [70]. The artificial ponds where the present study was conducted do not dry up and are therefore a constant breeding resource. In addition, adult frogs are often seen resting during daytime in close proximity to this permanent water resource (F. C. personal observation), suggesting that movements towards the reproductive sites can be accomplished at a short notice. Consistently, we found that rainfall accumulated during the prior 24 hour influenced the calling activity of the lemur leaf frog, but effects from 48 hour prior rainfall were undetectable. Alternatively, the total effect of prior rainfall could be owed to an increase in relative humidity in the coming night. However, the similarity between total and direct effects for prior rainfall suggest little indirect effects through mediator variables. Our results are therefore consistent with a direct but short-term effect of prior rainfall on calling activity. Calling activity is thought to be influenced by lunar light in some species, but not others [23,71,72]. The lemur leaf frog seems to exhibit lunar phobia, with activity peaking during the darker evenings. Other species such as *Engystomops pustulosus*, *Boana albopunctata* and *Rana dalmatina* present greater calling activity on illuminated nights [24,72,73]. This behaviour is associated with species that rely on visual communication to reproduce and to identify potential predators [23]. Future research assessing the Moon's influence using other more direct measures of reproductive activity, such as the occurrence of amplexus and the number of egg masses laid per night [73] could help disentangle the influence of environmental light on amphibian reproductive behaviour. Our analyses support with robust quantitative evidence the widely assumed but rarely tested relationship between weather variables and frog calling activity [59,74]. Current computational tools and monitoring methods allowed us to confirm and expand previous evidence on the role of environmental factors that influence the advertisement calling behaviour of anurans [32]. These approaches also enable studies at a higher temporal resolution and over longer time periods of uninterrupted sampling, which can contribute to building a more nuanced understanding of the effects of the abiotic environment on calling activity. This detailed perspective can also be used to predict the potential response of frog populations under plausible scenarios of climatic change and its associated implications in conservation. Protecting habitats with suitable environmental conditions is essential for the remaining populations of the lemur leaf frog. Given that weather variables impact the calling behaviour and associated reproductive activity in this species, we recommend the implementation of conservation strategies considering monitoring of microclimatic factors, including humidity, temperature, and regular rainfall to optimize their effectiveness. Conservation efforts attempting to recreate climatic conditions at small spatial scales might help to mitigate the impact of climate change by providing suitable environmental conditions and therefore prevent local extinctions [75]. In this regard, passive acoustic protocols emerge as key tools to generate behaviourally data to inform such conservation efforts, but also to evaluate the response of managed populations in a timely, objective, and low-cost manner. With our protocol, we provide a baseline for phenological studies that aim for greater temporal precision and thus can document population dynamics in a context of climate uncertainty.

5. Acknowledgements

We are grateful to the Veragua Foundation for providing us with research funding to conduct this study as part of our efforts to increase research related to tropical endangered species. We also express our thanks to Leo, a local indigenous person who helped gather the bioacoustic data from Cerro Chimú. Without Leo, it would have been impossible to collect this data. Special thanks to the University of Costa Rica team, Jimena Víquez and Elena Cantero, for their essential contributions during the annotation phase. Both dedicated countless hours of hard work to analyze the 467 recordings. We also thank Professor Gerardo Ávalos and to Adrián García Rodríguez for his manuscript recommendations. WCA thanks ANPCYT (PICT 346/2019, 59/2021), CONICET (PIP2800), FAPESP (2018/15425-0, 2021/10639-5), and the Mohamed Bin Zayed Species Conservation Fund (Project 242534066).

References

1. Luedtke JA, Chanson J, Neam K, Hobin L, Maciel AO, Catenazzi A, Borzée A, Hamidy A, Aowphol A, Jean A, Ángel Sosa-Bartuano, G AF, de Silva A, Fouquet A, Angulo A, Kidov AA, Saravia AM, Diesmos AC, Tominaga A, Shrestha B, Gratwicke B, Tjaturadi B, Rivera CCM, Almazán CRV, Señaris C, Chandramouli SR, Strüssmann C, Fernández CFC, Azat C, Hoskin CJ, Hilton-Taylor C, Whyte DL, Gower DJ, Olson DH, Cisneros-Heredia DF, Santana DJ, Nagombi E, Najafi-Majd E, Quah ES, Bolaños F, Xie F, Brusquetti F, Álvarez FS, Andreone F, Glaw F, Castañeda FE, Kraus F, Parra-Olea G, Chaves G, Medina-Rangel GF, González-Durán G, Ortega-Andrade HM, Machado IF, Das I, Dias IR, Urbina-Cardona JN, Crnobrnja-Isailović J, Yang JH, Jianping J, Wangyal JT, Rowley JJ, Measey J, Vasudevan K, Chan KO, Gururaja KV, Ovaska K, Warr LC, Canseco-Márquez L, Toledo LF, Díaz LM, Khan MMH, Meegaskumbura M, Acevedo ME, Napoli MF, Ponce MA, Vaira M, Lampo M, Yánez-Muñoz MH, Scherz MD, Rödel MO, Matsui M, Fildor M, Kusrini MD, Ahmed MF, Rais M, Kouamé NG, García N, Gonwouo NL, Burrowes PA, Imbun PY, Wagner P, Kok PJ, Joglar RL, Auguste RJ, Brandão RA, Ibáñez R, von May R, Hedges SB, Biju SD, Ganesh SR, Wren S, Das S, Flechas SV, Ashpole SL, Robleto-Hernández SJ, Loader SP, Incháustegui SJ, Garg S, Phimmachak S, Richards SJ, Slimani T, Osborne-Naikatini T, Abreu-Jardim TP, Condez TH, Carvalho TRD, Cutajar TP, Pierson TW, Nguyen TQ, Kaya U, Yuan Z, Long B, Langhammer P, Stuart SN. 2023 Ongoing declines for the world's amphibians in the face of emerging threats. *Nature* **622**, 308–314. (10.1038/s41586-023-06578-4)
2. Synchronicity Earth ISASGR. 2023 State of the World's Amphibians: The Second Global Amphibian Assessment. .
3. Taylor EN, Diele-Viegas LM, Gangloff EJ, Hall JM, Halpern B, Massey MD, Rödder D, Rollinson N, Spears S, jun Sun B, Telemeco RS. 2021 The thermal ecology and physiology of reptiles and amphibians: A user's guide. *J. Exp. Zool. Part A* **335**, 13–44. (10.1002/JEZ.2396)
4. Saenz D, Fitzgerald LA, Baum KA, Conner RN. 2006 Abiotic correlates of anuran calling phenology: the importance of rain, temperature, and season. *Herpetol. Monogr.* **20**, 64. (10.1655/0733-1347(2007)20)
5. Caldart VM, Iop S, Lingnau R, Cechin SZ. 2016 Calling Activity of a Stream-Breeding Frog from the Austral Neotropics: Temporal Patterns of Activity and the Role of Environmental Factors. *Herpetologica* **72**, 90–97. (10.1655/HERPETOLOGICA-D-15-00029)
6. Cayuela H, Arsovski D, Thirion JM, Bonnaire E, Pichenot J, Boitaud S, Miaud C, Joly P, Besnard A. 2016 Demographic responses to weather fluctuations are context dependent in a long-lived amphibian. *Glob. Chang. Biol.* **22**, 2676–2687. (10.1111/GCB.13290)
7. Llusia D, Márquez R, Beltrán JF, Benítez M, do Amaral JP. 2013 Calling behaviour under climate change: Geographical and seasonal variation of calling temperatures in ectotherms. *Glob. Change Biol.* **19**, 2655–2674. (10.1111/GCB.12267)
8. Pérez-Granados C, Schuchmann KL, Ramoni-Perazzi P, Marques MI. 2020 Calling behaviour of Elachistocleis matogrosso (Anura, Microhylidae) is associated with habitat temperature and rainfall. *Bioacoustics* **29**, 670–683. (10.1080/09524622.2019.1658642)
9. Larsen AS, Schmidt JH, Stapleton H, Kristenson H, Betchkal D, McKenna MF. 2021 Monitoring the phenology of the wood frog breeding season using bioacoustic methods. *Ecol. Indic.* **131**. (10.1016/J.ECOLIND.2021.108142)
10. Figueroa-Huitrón R, Ochoa-Ochoa LM, Sánchez-Ochoa D. 2020 Calling phenology of four anurans in tropical Mexico. *Rev. Mex. Biodivers.* **91**, e912945–e912945. (10.22201/IB.20078706E.2020.91.2945)
11. Melo I, Llusia D, Bastos RP, Signorelli L. 2021 Active or passive acoustic monitoring? Assessing methods to track anuran communities in tropical savanna wetlands. *Ecol. Indic.* **132**, 108305. (10.1016/J.ECOLIND.2021.108305)
12. Gibb R, Browning E, Glover-Kapfer P, Jones KE. 2019 Emerging opportunities and challenges for passive acoustics in ecological assessment and monitoring. *Methods. Ecol. Evol.* **10**, 169–185. (10.1111/2041-210X.13101)
13. Sugai LSM, Llusia D. 2019 Bioacoustic time capsules: Using acoustic monitoring to document biodiversity. *Ecol. Indic.* **99**,

149–152. (10.1016/J.ECOLIND.2018.12.021)

14. Aide TM, Corrada-Bravo C, Campos-Cerqueira M, Milan C, Vega G, Alvarez R. 2013 Real-time bioacoustics monitoring and automated species identification. *PeerJ* **2013**, e103. (10.7717/PEERJ.103/FIG-4)
15. Jones RH, Westra S, Sharma A. 2010 Observed relationships between extreme sub-daily precipitation, surface temperature, and relative humidity. *Geophys. Res. Lett.* **37**. (10.1029/2010GL045081)
16. Jiang J, Yuan Y. 2013 Relationship of moisture content with temperature and relative humidity in concrete. *Mag. Concrete Res.* **65**, 685–692. (10.1680/MACR.12.00190)
17. Navas CA. 1996 The Effect of Temperature on the Vocal Activity of Tropical Anurans: A Comparison of High and Low-Elevation Species. *J. Herpetol.* **30**, 488. (10.2307/1565691)
18. Navas CA, Gomes FR, Carvalho JE. 2008 Thermal relationships and exercise physiology in anuran amphibians: Integration and evolutionary implications. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **151**, 344–362. (10.1016/j.cbpa.2007.07.003)
19. Hilje B, Aide TM. 2012 Calling activity of the common tink frog (Diasporus diastema) (Eleutherodactylidae) in secondary forests of the Caribbean of Costa Rica. *Trop. Conserv. Sci.* **5**, 25–37. (10.1177/194008291200500104)
20. Navas C, Bevier C. 2001 Thermal dependency of calling performance in the eurythermic frog Colostethus subpunctatus. *Herpetologica* **57**, 384–395.
21. Bonnefond A, Courtois EA, Sueur J, Sugai LSM, Llusia D. 2020 Climatic breadth of calling behaviour in two widespread Neotropical frogs: Insights from humidity extremes. *Glob. Chang. Biol.* **26**, 5431–5446. (10.1111/GCB.15266)
22. Halfwerk W, Ryan MJ, Wilson PS. 2016 Wind- and rain-induced vibrations impose different selection pressures on multimodal signaling. *Am. Nat.* **188**, 279–288. (10.1086/687519)
23. Underhill VA, Höbel G. 2018 Moonlighting? - Consequences of lunar cues on anuran reproductive activity. *Acta Oecol.* **87**, 20–28. (10.1016/J.ACTAO.2018.02.001)
24. Bonachea LA, Ryan MJ. 2011 Simulated Predation Risk Influences Female Choice in Túngara Frogs, Physalaemus pustulosus. *Ethology* **117**, 400–407. (10.1111/J.1439-0310.2011.01889.X)
25. Arif S, MacNeil MA. 2022 Predictive models aren't for causal inference. *Ecol. Lett.* **25**, 1741–1745. (10.1111/ELE.14033)
26. Glymour C, Zhang K, Spirtes P. 2019 Review of causal discovery methods based on graphical models. *Front. Genet.* **10**, 418407. (10.3389/FGENE.2019.00524/BIBTEX)
27. Duellman WE. 1970 *The hylid frogs of Middle America*. University of Kansas. (10.5962/bhl.title.2835)
28. Stuart S, Hoffmann M, Chanson J, Cox N, Berridge R, Ramani P, (eds.) Young B. 2008 *Threatened Amphibians of the World*. Lynx Edicions.
29. Group ISAS. 2020 Agalychnis lemur. The IUCN Red List of Threatened Species 2020: e.T55855A3033153. (https://dx.doi.org/10.2305/IUCN.UK.2020-3.RLTS.T55855A3033153.en)
30. Hertz A, Lotzkat S, Carrizo A, Ponce M, Köhler G, Streit B. 2012 Field notes on findings of threatened amphibian species in the central mountain range of western Panama. *Amphib. Reptile Conserv.* **6**, 9–30.
31. Lotzkat S, Wehrenberg G, Haas M, Köhler G. 2021 Amphibians (Amphibia, anura and caudata) and reptiles (reptilia, squamata) from the headwaters of río guázaro on the caribbean versant of veraguas province, western Panama. *Check List* **17**, 1541–1556. (10.15560/17.6.1541)
32. Duellman WE, Trueb L. 1994 *Biology of amphibians*. Johns Hopkins University Press.
33. Jungfer KH, Weygoldt P. 1994 The reproductive biology of the leaf frog Phyllomedusa lemur Boulenger, 1882, and a comparison with other members of the Phyllomedusinae. *Revue française d'aquariologie* **21**, 57–64.
34. Savage JM. 2002 *The Amphibians and Reptiles of Costa Rica:a herpetofauna between two continents, between two seas*. University of Chicago Press.

35. Holdridge L. 1967 *Life zone ecology*. Tropical Science Center rev. ed edition.
36. Salazar-Zúñiga J, Chaves-Acuña W, Chaves G, Acuña A, Abarca-Odio J, Lobón-Rovira J, Méndez EG, Gutiérrez-Vannucchi AC, Bolaños F. 2019 The most frog-diverse place in Middle America, with notes on the conservation status of eight threatened species of amphibians. *Amphib. Reptile Conserv.* **13**, 304–322.
37. Lazaridis E. 2022 lunar: Lunar Phase Distance, Seasons and Other Environmental Factors. (Version 0.2-01).
38. Inc. WA. 2019 SM4 Song Meter [Audio recording device]. .
39. Araya-Salas M, Smith-Vidaurre G, Chaverri G, Brenes JC, Chirino F, Elizondo-Calvo J, Rico-Guevara A. 2023 ohun: An R package for diagnosing and optimizing automatic sound event detection. *Methods. Ecol. Evol.* **14**, 2259–2271. (10.1111/2041-210X.14170)
40. for Conservation Bioacoustics at the Cornell Lab of Ornithology LYC Raven Pro: Interactive Sound Analysis Software. .
41. Team RC. 2023 R: A language and environment for statistical Computing. .
42. Araya-Salas M. 2017 Rraven: connecting R and Raven bioacoustic software. (Version 1).
43. Araya-Salas M, Smith-Vidaurre G. 2017 warbleR: an r package to streamline analysis of animal acoustic signals. *Methods in Ecology and Evolution* **8**, 184–191. (10.1111/2041-210X.12624)
44. M H, M.N S. 2015 A Review on Evaluation Metrics for Data Classification Evaluations. *Int. J. Data Min. Knowl. Manag.* **5**, 01–11. (10.5121/IJDKP.2015.5201)
45. Balantic CM, Donovan TM. 2020 Statistical learning mitigation of false positives from template-detected data in automated acoustic wildlife monitoring. *Bioacoustics* **29**, 296–321. (10.1080/09524622.2019.1605309)
46. Breiman L. 2001 Random Forests. *Mach. Learn.* **45**, 5–32. (10.1023/A:1010950718922)
47. Wright MN, Ziegler A. 2017 ranger: A Fast Implementation of Random Forests for High Dimensional Data in C++ and R. *J. Stat. Softw.* **77**, 1–17. (10.18637/jss.v077.i01)
48. Lee CH, Han CC, Chuang CC. 2008 Automatic classification of bird species from their sounds using two-dimensional cepstral coefficients. *IEEE T. Audio Speech* **16**, 1541–1550. (10.1109/TASL.2008.2005345)
49. Prat Y, Taub M, Yovel Y. 2016 Everyday bat vocalizations contain information about emitter, addressee, context, and behavior. *Sci. Rep-UK* **6**, 1–10. (10.1038/srep39419)
50. Clemins PJ, Johnson MT, Leong KM, Savage A. 2005 Automatic classification and speaker identification of African elephant (Loxodonta africana) vocalizations. *J. Acoust. Soc. Am.* **117**, 956–963. (10.1121/1.1847850)
51. Roch MA, Soldevilla MS, Burtenshaw JC, Henderson EE, Hildebrand JA. 2007 Gaussian mixture model classification of odontocetes in the Southern California Bight and the Gulf of California. *J. Acoust. Soc. Am.* **121**, 1737–1748. (10.1121/1.2400663)
52. Araya-Salas M, Hernández-Pinsón HA, Rojas N, Chaverri G *Anim. Behav.*(10.1016/J.ANBEHAV.2020.05.018)
53. Textor J, van der Zander B, Gilthorpe MS, Liśkiewicz M, Ellison GT. 2016 Robust causal inference using directed acyclic graphs: the R package 'dagitty'. *Int. J. Epidemiol.* **45**, 1887–1894. (10.1093/IJE/DYW341)
54. Bürkner PC. 2017 brms: An R Package for Bayesian Multilevel Models Using Stan. *J. Stat. Softw.* **80**, 1–28. (10.18637/JSS.V080.I01)
55. Vehtari A, Gelman A, Gabry J. 2015 Practical Bayesian model evaluation using leave-one-out cross-validation and WAIC. *Stat. Comput.* **27**, 1413–1432. (10.1007/s11222-016-9696-4)
56. Cutler DR, Edwards TC, Beard KH, Cutler A, Hess KT, Gibson J, Lawler JJ. 2007 Random forests for classification in ecology. *Ecology* **88**, 2783–2792. (10.1890/07-0539.1)
57. Donnelly MA, Guyer C. 1994 Patterns of reproduction and habitat use in an assemblage of Neotropical hylid frogs. *Oecologia* **98**, 291–302. (10.1007/BF00324217/METRICS)
58. Bevier CR. 1997 Breeding Activity and Chorus Tenure of Two Neotropical Hylid Frogs. *Herpetologica* **53**, 297–311.

59. Gibbs JP, Breisch AR. 2001 Climate warming and calling phenology of frogs near Ithaca, New York, 1900-1999. *Conserv. Biol.* **15**, 1175–1178. (10.1046/J.1523-1739.2001.0150041175.X)

60. Farrell MP, MacMahon JA. 1969 An Eco-Physiological Study of Water Economy in Eight Species of Tree Frogs (Hylidae). *Herpetologica* **25**, 279–294.

61. Young JE, Christian KA, Donnellan S, Tracy CR, Parry D. 2005 Comparative analysis of cutaneous evaporative water loss in frogs demonstrates correlation with ecological habits. *Physiol. Biochem. Zool.* **78**, 847–856. (10.1086/432152)

62. Faivovich J, Haddad CF, Baêta D, Jungfer KH, Álvares GF, Brandão RA, Sheil C, Barrientos LS, Barrio-Amorós CL, Cruz CA, Wheeler WC. 2010 The phylogenetic relationships of the charismatic poster frogs, Phyllomedusinae (Anura, Hylidae). *Cladistics* **26**, 227–261. (10.1111/J.1096-0031.2009.00287.X)

63. Ospina OE, Villanueva-Rivera LJ, Corrada-Bravo CJ, Aide TM. 2013 Variable response of anuran calling activity to daily precipitation and temperature: implications for climate change. *Ecosphere* **4**, 1–12. (10.1890/ES12-00258.1)

64. Gunawardhana LN, Al-Rawas GA, Kazama S. 2017 An alternative method for predicting relative humidity for climate change studies. *Meteorol. Appl.* **24**, 551–559. (10.1002/MET.1641)

65. von May R, Catenazzi A, Santa-Cruz R, Gutierrez AS, Moritz C, Rabosky DL. 2019 Thermal physiological traits in tropical lowland amphibians: Vulnerability to climate warming and cooling. *PLOS ONE* **14**, e0219759. (10.1371/JOURNAL.PONE.0219759)

66. Bellis ED. 1962 The Influence of Humidity on Wood Frog Activity. *Am. Midl. Nat.* **68**, 139–148. (10.2307/2422640)

67. Cui J, Song X, Fang G, Xu F, Brauth SE, Tang Y. 2011 Circadian rhythm of calling behavior in the Emei music frog (Babina daunchina) is associated with habitat temperature and relative humidity. *Asian Herpetol. Res.* **2**, 149–154. (10.3724/SP.J.1245.2011.00149)

68. da Silva FR, Almeida-Neto M, do Prado VHM, Haddad CFB, de Cerqueira Rossa-Feres D. 2012 Humidity levels drive reproductive modes and phylogenetic diversity of amphibians in the Brazilian Atlantic Forest. *J. Biogeogr.* **39**, 1720–1732. (10.1111/J.1365-2699.2012.02726.X)

69. Ficetola GF, Maiorano L. 2016 Contrasting effects of temperature and precipitation change on amphibian phenology, abundance and performance. *Oecologia* **181**, 683–693. (10.1007/S00442-016-3610-9/METRICS)

70. Duellman WE. 2001 *Hylid Frogs of Middle America*. Society for the Study of Amphibians and Reptiles second edition edition.

71. Onorati M, Vignoli L. 2017 The darker the night, the brighter the stars: Consequences of nocturnal brightness on amphibian reproduction. *Biol. J. Linn. Soc.* **120**, 961–976. (10.1093/BIOLINNEAN/BLW007)

72. Lima MS, Pederassi J, Caramaschi U, Sousa KDS, Souza CAS, Lima MS. 2021 Frog vocalization is influenced by moon phases: Brazilian frogs tend to prefer low-albedo phases. *Web Ecol.* **21**. (10.5194/WE-21-1-2021)

73. Grant RA, Chadwick EA, Halliday T. 2009 The lunar cycle: a cue for amphibian reproductive phenology?. *Anim. Behav.* **78**, 349–357. (10.1016/J.ANBEHAV.2009.05.007)

74. Brooke PN, Alford RA, Schwarzkopf L. 2000 Environmental and social factors influence chorusing behaviour in a tropical frog: Examining various temporal and spatial scales. *Behav. Ecol. Sociobiol.* **49**, 79–87. (10.1007/S002650000256/METRICS)

75. Lemckert F. 2001 The influence of micrometeorological factors on the calling activity of the frog Crinia signifera (Anura: Myobatrachidae). *Aust. Zool.* **31**, 625–631. (10.7882/AZ.2001.009)