



Effects of a new highway on the microenvironmental factors and soundscape of the high-mountain frog assemblage in the buffer zone of Los Nevados Park (Colombia).



Preliminary report Rufford 44455-1

Effects of a new highway on the microenvironmental factors and soundscape of the high-mountain frog assemblage in the buffer zone of Los Nevados Park (Colombia).

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23 Nov, 2025

Summary

The Andean páramos harbor a high diversity of amphibians, many of them threatened and poorly studied, particularly regarding their ecology and vocal behavior. The buffer zone of Parque Nacional Natural (PNN) Los Nevados in Colombia is a key conservation area where passive acoustic monitoring can serve as an effective tool for long-term ecological assessment. As part of the project “*Effects of a new highway on the microenvironmental factors and soundscape of the high-mountain frog assemblage in the buffer zone of Los Nevados Park (Colombia)*,” we began field data collection in July 2025, following formal access permissions granted by PNN Los Nevados (4°54'39.6"N, 75°17'16.0"W). Here we present the preliminary analyses of this first month of recordings, aimed at exploring the basic pattern of vocal activity in *Pristimantis simoterus* (Figure 1) and its association with microenvironmental variables. Passive acoustic recorders (AudioMoth v1.2.0) and environmental loggers (Kestrel Drop-D2) were deployed at multiple sites, recording 2-minute intervals every 30 minutes. During July, analyses of the initial subset of recordings indicate that calling activity occurred both at night and during the day, with the majority of detections concentrated between 18:00 and 6:00 h. Calls were typically associated with low temperatures (2–10°C) and high relative humidity (>70%), and preliminary generalized linear models suggest that relative humidity exerts a positive influence on calling probability, whereas temperature shows a nonlinear effect consistent with a thermal performance curve. These early results provide an initial understanding of the environmental drivers of vocal activity in *P. simoterus*, and will serve as a baseline for comparison with subsequent months. Full analyses—including the assessment of highway impacts on microhabitat conditions and the acoustic landscape—will be completed once all field data (July–December) have been collected.



Figure 1 Male and female of *P. simoterus*

Introduction

The tropical Andean páramo ecosystems host exceptionally high levels of amphibian endemism, many of which are threatened, yet remain poorly understood in terms of their ecology, behaviour and physiological responses (Bernal & Lynch, 2008). These high-elevation habitats are characterised by steep climatic gradients, strong micro-environmental fluctuations and high vulnerability to land-use change and climate warming (Stuart et al., 2008). For example, infrastructure development, tourism and new transport corridors in the buffer zones of protected areas such as Parque Nacional Natural (PNN) Los Nevados in Colombia can alter microclimatic regimes and fragment otherwise continuous páramo patches, potentially affecting sensitive anuran assemblages (Mastrangelo et al., 2025). Moreover, a recent review by Saboyá Acosta & Urbina-Cardona (2023) indicates that although 142 amphibian species are currently recognised in páramo ecosystems, less than 20% of them possess ecological or behavioural information beyond their original species descriptions.

The genus *Pristimantis* is the most diverse among Neotropical anurans, comprising about 600 described species, representing nearly 8% of all known anurans (Frost, 2025). These frogs exhibit remarkable ecological and morphological diversification, particularly in high Andean ecosystems where humidity, vegetation cover, and thermal fluctuations shape their evolutionary adaptations (Lynch, 1980; Lynch et al., 1997). *Pristimantis simoterus* (Figure 2) is a nocturnal species that inhabits mainly high Andean forests and páramo ecosystems, recorded between 2.672 and 4.350 m a.s.l. (Acosta, 2025; Bernal & Lynch, 2008). Its presence has been documented in several departments along the Central Cordillera of Colombia, including Tolima, Caldas, Risaralda, and Quindío, where it occupies both conserved forest areas and, to a lesser extent, disturbed edges (Acosta, 2025).



Figure 2 Male of *Pristimantis simoterus* (A), and its diagnostic characters by Lynch 1997 (B).

According to the IUCN (2025), *P. simoterus* (Figure 2) is currently classified as *Near Threatened* (NT), due to the continuing decline in the extent and quality of its habitat caused by agricultural expansion, deforestation, and climate-driven shifts in vegetation zones. Despite being present in several protected areas, including PNN Los Nevados, its restricted

elevational range and microhabitat specialization make it especially sensitive to environmental disturbance (Stuart et al., 2008).

Species of *Pristimantis* exhibit a direct developmental mode, in which embryos develop completely inside the egg and hatch as miniature frogs, bypassing the free-living larval stage (Acosta, 2000). This reproductive strategy allows them to exploit terrestrial habitats but also makes them highly dependent on the microclimatic stability of their environment. For this reason, many *Pristimantis* species are considered sensitive to environmental changes, especially those associated with deforestation and climate variation at high elevations (Bernal & Lynch, 2008).

Acoustic communication plays a fundamental role in the reproductive biology of *Pristimantis*. Males produce advertisement calls to attract females and to defend calling territories from rival males. These calls are species-specific and constitute an essential taxonomic character for species identification, particularly in groups with cryptic morphology such as *Pristimantis* (Lynch et al., 1997). The species' vocalization has been described by Romero-Garcia et al. (2015), who recorded a calling male at 3.786 m a.s.l. near Ibagué, Tolima. The advertisement call consists of a single, non-pulsed note with amplitude modulation and three harmonics, emitted at a rate of approximately 12 calls per minute. Each note lasts about 60 ± 18 ms (range: 40–119 ms) with dominant frequencies between 1.72 and 2.41 kHz and a peak at ~ 2.09 kHz (Figure 3) These acoustic characteristics, coupled with its narrow thermal and humidity range, make *P. simoterus* an ideal model for studying how high-Andean frogs regulate their calling behaviour under fluctuating environmental conditions.

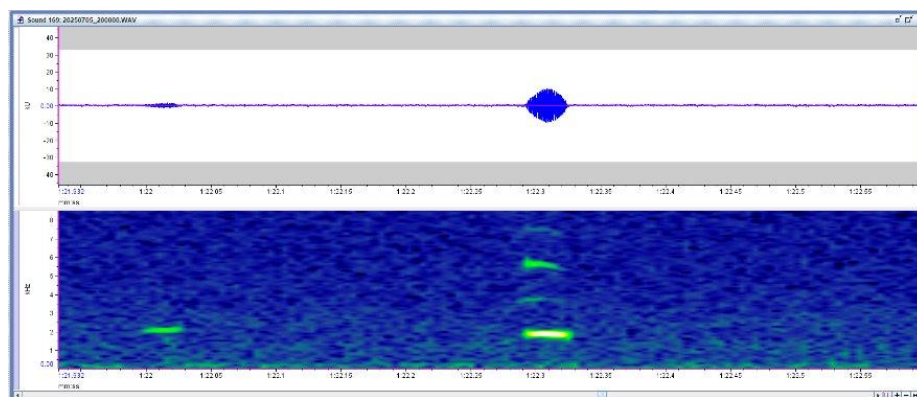


Figure 3 Oscillograms (top) and spectrograms (bottom) of the advertisement calls of *P. simoterus*.

Amphibians living in high-elevation tropical ecosystems exhibit physiological adaptations such as metabolic depression or hypometabolic states that allow them to cope with cold and resource scarcity (Ochoa-Ochoa et al., 2025). However, as highlighted by Ochoa-Ochoa et al. (2025), tropical montane amphibians are among the most vulnerable and least studied globally, particularly in the northern Andes. These species often operate near their physiological limits, and even minor temperature or humidity changes can disrupt their energy balance and behavioural patterns. Given this context, our aim was to evaluate how micro-environmental variation (temperature and relative humidity) influences the vocal

activity of *P. simoterus* in páramo habitats, through passive acoustic monitoring and fine-scale environmental data collection.

Project members

Ricardo Medina: Ric is a Master in Evolutionary Biology from Universidad Nacional Autónoma de México (México) and a Biologist from Universidad del Tolima. Ric is working with spatial ecology, population dynamics, and found new threatened frogs, likewise mentoring bioacoustics to Kata.

Katalina Gutiérrez Hernández: Kata is a Biologist from the University of Tolima (Colombia), holds a Master's degree in Biological Sciences from the same university, and is currently pursuing a Ph.D. in Biological Sciences there. Her research focuses on the physiology of early developmental stages in anurans, and she is currently learning about bioacoustic projects under the guidance of Richi.

Aim and objectives

This project has the following six objectives:

- (1) identifying the temporal and spectral traits of the endemic and threatened frog call assemblage;
- (2) conducting six months of soundscape monitoring;
- (3) collecting and analysing microenvironmental variables;
- (4) evaluating landscape metrics for habitat changes;
- (5) assessing the highway's impact on vocal patterns through data associations; and
- (6) sharing the results with local stakeholders and decision-makers. Although no objective has been changed there were partial adjustments during the implementation of the project

Methodology

Data collection

During the first field trip, conducted in the municipality of Murillo, Tolima, Colombia, six AudioMoth recorders, six HOBO MX2201 loggers, and six Kestrel Drop D2 sensors were installed to record acoustic and microenvironmental data (Figure 4). Three of each device type (3 HOBO, 3 Kestrel, and 3 AudioMoth) (Figure 5) were deployed around three lagoons located within 200 m (straight-line distance) from the highway and separated from one another by at least 500 m to ensure data independence, thus allowing us to evaluate the potential effect of the highway on the measured variables. The remaining equipment was installed around three additional lagoons located far from the highway (straight-line distance > 300 m), also separated by a minimum of 500 m.

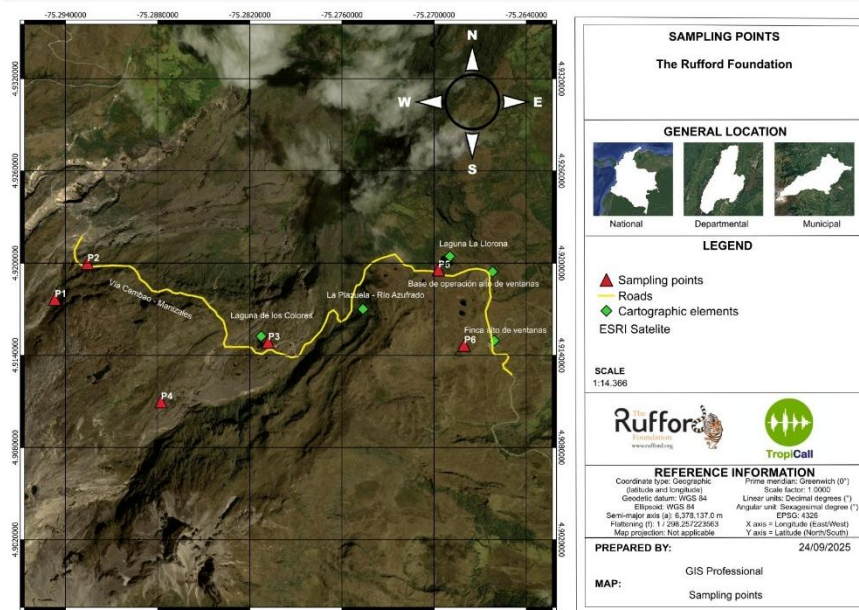


Figure 4 Geographic location of the six sampling points in Murillo, Tolima, Colombia.

We used passive acoustic monitoring with AudioMoth recorders to capture anuran vocalizations (Figure 5). Each device recorded for 2 minutes every 10 minutes (24/7 for six months) at a height of 1 m above the ground, using a sampling rate of 48 kHz, a gain of 20 dB, and a resolution of 16 bits. During each field trip, batteries and microSD cards were replaced. Four field campaigns were conducted between July and November 2025.

After each field trip, the calls of focal species were identified and their activity patterns analyzed. For the analysis of both focal calls and the anuran assemblage soundscape, we used Raven Pro (Cornell Bioacoustics Lab), as well as the R packages *Mice*, *Seewave* and *Soundecology*. Besides, we will use acoustic index, including the Acoustic Complexity Index (ACI), Acoustic Diversity Index (ADI), Acoustic Evenness Index (AEI), Acoustic Entropy (H), and Bioacoustic Index (BI), will be calculated using the same packages and Arbimon software. Spectral parameters were obtained using Fast Fourier Transformations under the Blackman algorithm with 724 samples per window and a 2048-sample frequency grid using the Discrete Fourier Transform (DFT) algorithm.

Environmental data were recorded continuously with Kestrel and HOBO data loggers (Figure 5), which measured underwater temperature, environmental temperature, relative humidity, heat index, and dew point every 10 minutes for six months. To model the probability of calling presence as a function of multiple environmental predictors (temperature, relative humidity, and hour), we fitted a generalized linear mixed model (GLMM) using a quadratic binomial logistic regression for temperature and including site as a random effect. The fitted model followed the formula: *Calling presence (yes/no) ~ temperature + temperature² + relative humidity + hour + (1 | site)*.



Figure 5 Equipment used to monitor environmental variables, with the black box and arrow indicating the specific devices employed.

Reasoning

The study area is located between 3.000 and 4.500 m a.s.l. (Figure 4), characterized by extreme environmental conditions such as near-freezing temperatures, strong winds, persistent fog, high soil moisture, and limited accessibility. These harsh conditions posed significant logistical challenges for conducting fieldwork in the páramo ecosystem. However, the construction of a new highway provided an opportunity to investigate the largely unknown amphibian fauna of this región (Saboyá Acosta & Urbina-Cardona, 2023).

Research in these environments required considerable effort and resources, including extensive field searches, long travel distances, and high operating costs to obtain reliable ecological and behavioral data. Passive acoustic monitoring and microenvironmental loggers proved to be cost-effective tools that enabled continuous (24/7) and spatially extensive data collection with minimal disturbance (Melo et al., 2021).

Furthermore, fine-scale microenvironmental temperature data for amphibians are scarce, and continuous monitoring is essential to understand how these species respond to short-term climatic fluctuations. Given that climate change is one of the main drivers of global amphibian declines (Luedtke et al., 2023), our data provide a valuable baseline for future studies assessing the response of high-Andean amphibian assemblages to ongoing warming trends.

Although this was a six-month project, it was not conceived as a rapid assessment. Instead, our objective was to collect integrated data on behavior, landscape metrics, and microenvironmental conditions for multiple anuran species, establishing a foundation for future long-term monitoring aimed at mitigating and managing the impacts of the new highway on páramo biodiversity.

Monitoring and dissemination

Two focal groups will be considered for the dissemination of project results. The first group included the academic community (researchers and universities) and local stakeholders (NGOs and residents). Findings will be prepared for publication in at least two peer-reviewed scientific articles describing the advertisement calls, vocal activity patterns, and ecological characteristics of high-Andean anurans in the buffer zone of PNN Los Nevados. All sound recordings and soundscape datasets will be archive in the repositories of the Instituto Humboldt and the Fonoteca Zoológica de Madrid.

A total of 500 stickers and 20 posters will be distribute across local schools, restaurants, and community establishments to promote environmental awareness. These outreach activities will be document through attendance records, photographs, and audio files, allowing evaluation of their reach and impact.

Additionally, a one-year extension of the acoustic and microenvironmental monitoring program will be plan to provide further insights into the species' thermal landscape and to assess potential effects of global warming on the high-mountain amphibian community.

The second focal group comprised decision-makers, including representatives of CORTOLIMA, local municipalities (Herveo, Murillo, Casabianca, and Villahermosa), the PNN authority, and the Ministry of Environment. Preliminary and final results will be presented to these stakeholders in separate meetings, and detailed written reports, including copies of scientific articles, will delivered to each institution. Attendance lists and photographic evidence will use to verify dissemination.

Outputs and results

These preliminary results correspond to acoustic and environmental data collected during the month of July 2025 across the five active monitoring sites in the páramos of PPN Los Nevados. We obtained a total of 6,440 acoustic records from the five functioning recorders; the unit installed at Site 6 did not produce usable data. Environmental monitoring produced 5,616 temperature and humidity records, from which approximately 13% were imputed using the mice package in R to complete missing values.

Figure 6 summarizes the proportion of calls detected at each of the five sites. The color scheme is consistent across all analyses and represents each site throughout the report. Calling activity varied notably among sites, with proportions ranging from 13.4% to 42.9% of all recordings, indicating spatial heterogeneity in the species' calling behavior.

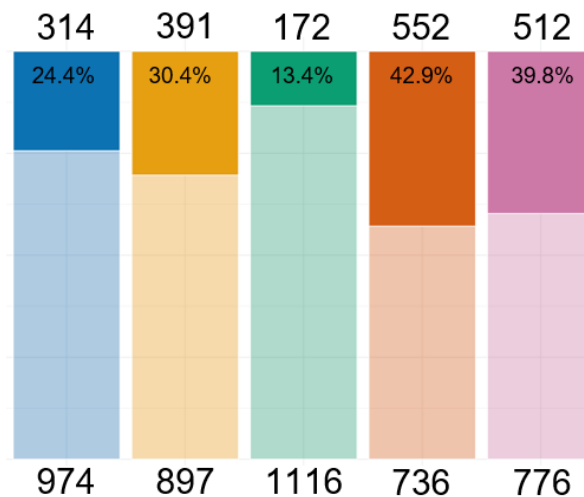


Figure 6 Total number and percentage of *P. simoterus* calls detected at each monitoring site in July - 2025.

Figure 7 shows the hourly call density across the entire July sampling period for each site. Black dots represent mean call values for each time interval, colored bars correspond to 30-minute acoustic windows, and the shaded background highlights nighttime hours (18:00–06:00). As expected, calling activity peaked during the night. However, contrary to previous assumptions for *P. simoterus*, the species also exhibited consistent low-level daytime calling, a pattern that was repeated across all five sites.

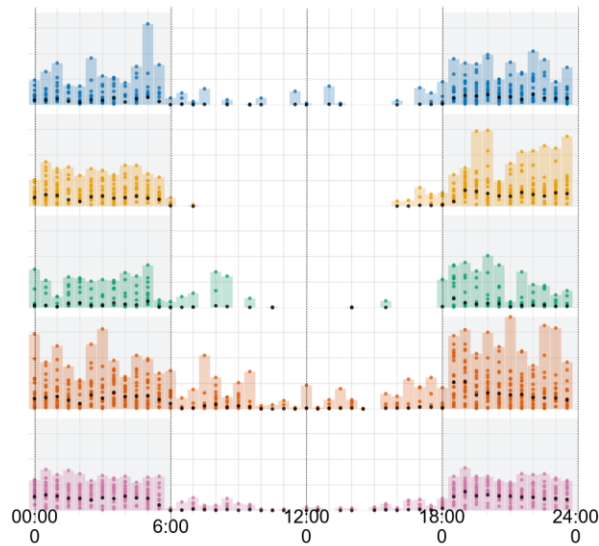


Figure 7 Temporal distribution of calling activity across sites during July.

Temperature records showed a clear and consistent daily thermal cycle at all sites throughout July, with warm peaks during the day and sharp declines during the night. Vocal activity (black points) tended to occur during the coldest moments of each 24-hour cycle, suggesting a strong preference for calling under low-temperature conditions (Figure 8A). This pattern was stable across the entire month and across all sites, as shown by the close clustering of calling events around nighttime and early-morning temperature minima. When all days are summarized into a single daily cycle, the association becomes even more evident: calling events consistently coincide with the coolest hours, particularly during nocturnal and early-morning periods (Figure 8B). Despite site-level differences in absolute temperature, the species' thermal calling preference is remarkably consistent across the landscape.

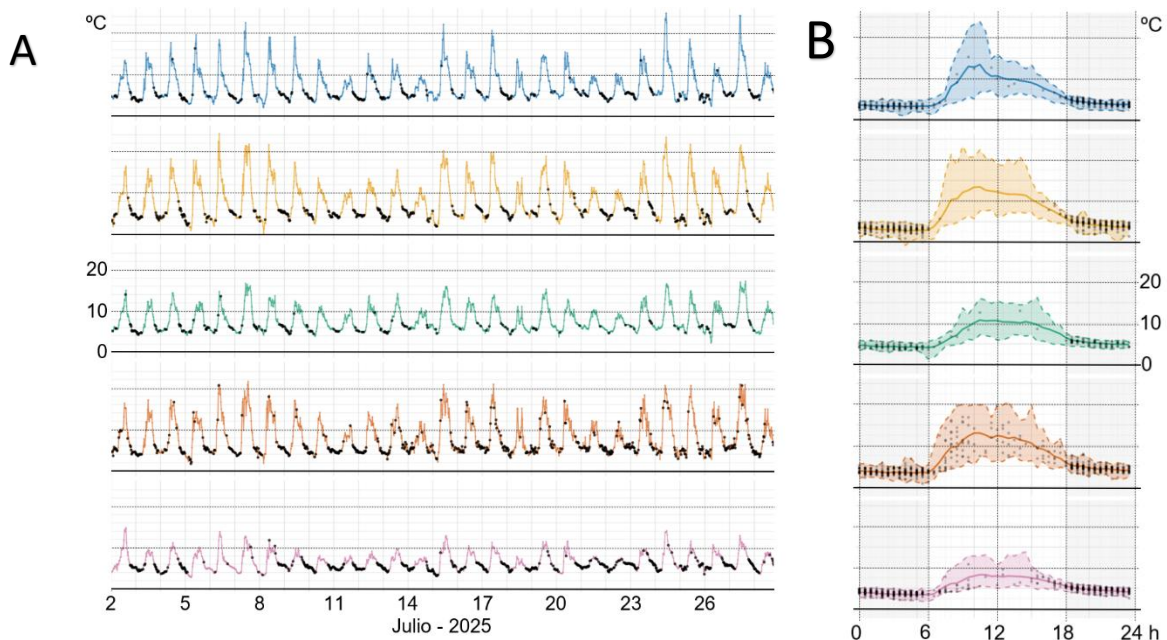


Figure 8 Monthly and daily temperature patterns associated with vocal activity of *P. simoterus*. (A) Temperature variation throughout July with calling detections. (B) Aggregated 24-hour temperature cycle with calling events.

Relative humidity showed a stable daily pattern across all monitoring sites, with high nocturnal humidity followed by a sharp decrease during daytime hours. Vocal activity closely tracked these fluctuations: calling events (black points) were consistently concentrated during periods of higher humidity (Figure 9A). This relationship remained highly consistent throughout the entire month of July, indicating that the species tends to vocalize during the most humid moments of the daily cycle.

When all days are aggregated into a single 24-hour pattern, the association becomes even clearer. Calling activity aligns almost exclusively with nighttime and early-morning hours, precisely when relative humidity peaks at each site (Figure 9B). Despite differences among sites in absolute humidity values, the pattern of increased calling under higher humidity conditions is remarkably conserved.

Together, these findings suggest that *P. simoterus* vocal activity is strongly favored by cool and highly humid environments, which is consistent with the ecology of many high-Andean, direct-developing amphibians.

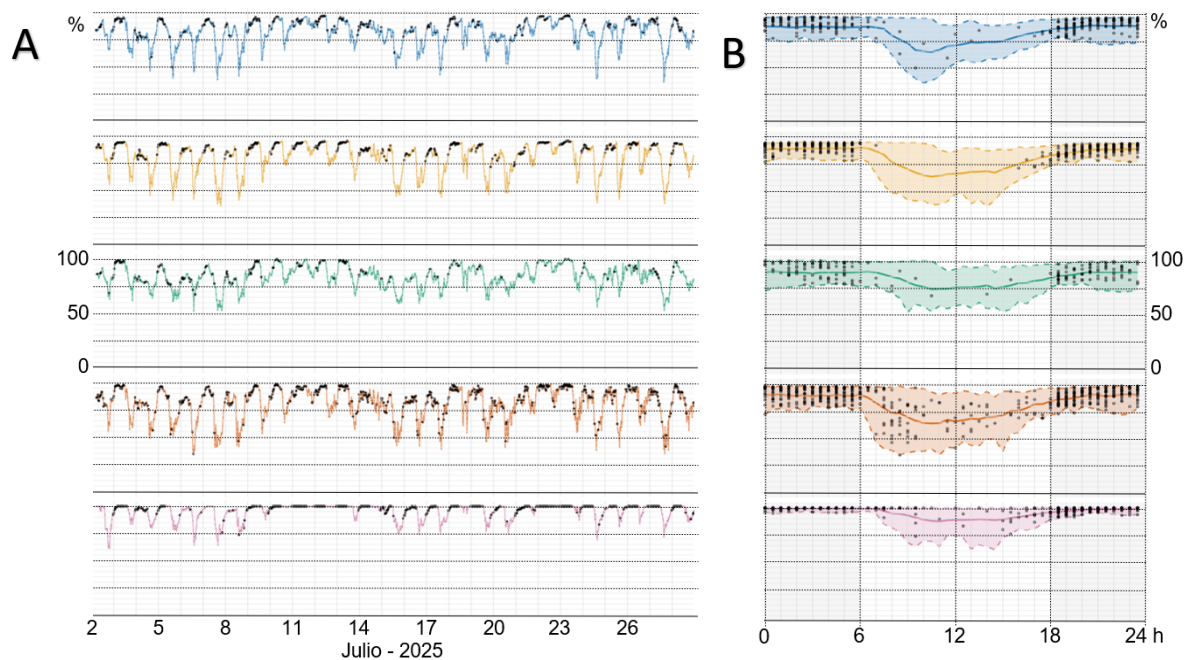


Figure 9 Relative humidity dynamics and vocal activity of *P. simoterus* across five monitoring sites during July. **(A)** Monthly humidity patterns with corresponding calling events. **(B)** Daily humidity cycle showing hourly calling activity.

The model GLMM showed a good overall fit with no signs of substantial overfitting. The random effect of site indicated considerable spatial variability in calling probabilities, suggesting differences among locations but consistent overall trends across the study area.

The intercept was highly significant and negative, meaning that at theoretical values of 0 °C and 0% relative humidity, the probability of detecting calling activity approaches zero—an expected biological outcome.

Thermal response

Temperature had a strong and significant effect on calling activity. The significant quadratic term indicated a unimodal thermal response, with calling probability increasing with temperature up to a peak and then declining at higher temperatures. As shown in Figure 10, the blue curve represents the mean observed calling probability across the temperature gradient, with grey shading indicating the 95% confidence interval. The red dashed lines represent the predicted thermal optimum (T_{opt}) estimated through 5.000 bootstrap iterations, resulting in a mean optimum of 5.23 °C with 95% confidence intervals between 4.8 and 5.9 °C. This pattern reflects a clear thermal optimum for calling behavior.

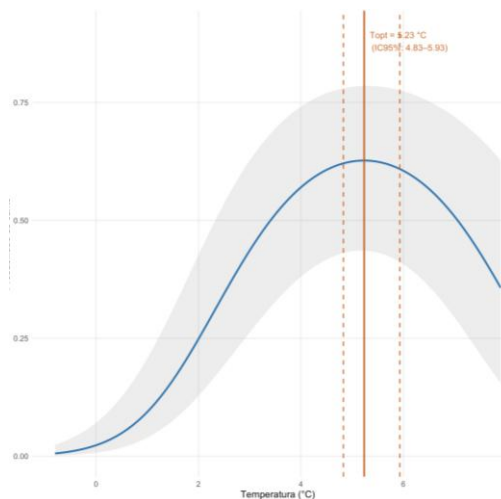


Figure 10 Thermal response curve of calling activity (quadratic binomial GLMM).

Relative humidity response

In contrast, relative humidity showed a strong and strictly positive linear effect on calling activity. As illustrated in Figure 11, calling probability increased consistently as relative humidity rose, a result supported by the Wald test ($\beta = 0.13 \pm 0.008$, $z = 15.9$, $P < 0.001$). This indicates that higher humidity strongly promotes calling behavior, with no evidence of a threshold or saturation within the observed range.

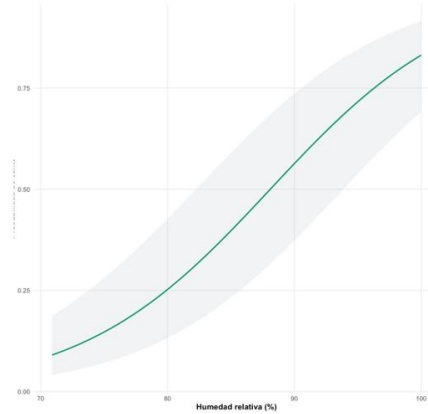


Figure 11 Relative humidity response curve of calling activity.

To evaluate whether the model-predicted thermal optimum matched the environmental conditions that favored calling activity during the day, we examined the joint distribution of temperature and relative humidity at the moments when calls were detected across the five study sites (Figure 12). The data show that Site 5 (fuchsia) aligns very closely with the model prediction, with most calling events occurring near the temperature–humidity combination expected from the estimated optimum. In contrast, the remaining sites exhibit greater variability, with calls recorded across a wider range of environmental conditions. This suggests that although the predicted optimum captures well the calling behavior in Site 5, local microclimatic differences across sites influence the realized conditions of calling activity. In this plot, the dashed line and grey confidence band depict the temperature–humidity relationship predicted by the GLMM around the estimated thermal optimum.

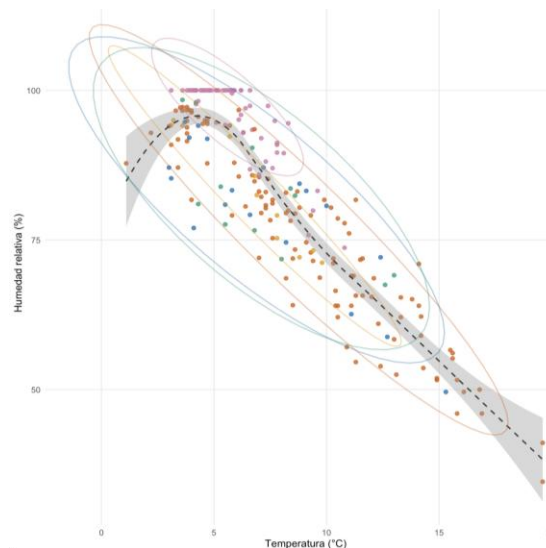


Figure 12 Environmental conditions during calling activity across sites (temperature–humidity scatterplot with 95% confidence ellipses). Each point represents a calling event, and colors indicate the site of origin (blue = Site 1, yellow = Site 2, green = Site 3, red = Site 4, fuchsia = Site 5).

The results show that calling activity exhibits a clear nonlinear relationship with temperature, following the typical shape of a thermal performance curve in which behavior reaches a maximum within an optimal range before declining at both lower and higher temperatures (Angilletta, 2009). This trend, representing the predicted probability of calling according to the quadratic binomial GLMM, suggests that males adjust their vocal activity based on thermal conditions that enhance both physiological efficiency and effective communication. Cooler temperatures appear to limit calling frequency, possibly due to metabolic constraints or reduced muscular capacity, whereas excessively high temperatures may increase physiological costs and disrupt the motor coordination required for sound production (Gerhardt & Huber, 2002). Together, these patterns indicate that temperature acts as a key modulator of reproductive behavior, likely influencing both call quality and the willingness of individuals to vocalize, with important implications for understanding how present and future environmental conditions, particularly under climate-change scenarios, may affect the reproductive dynamics and acoustic ecology of the species.

Although the analyses presented here offer valuable preliminary insights into the environmental drivers of calling behavior, the central question of the project—*the effects of a new highway on the microenvironmental conditions and the soundscape of the high-mountain frog assemblage in the buffer zone of Los Nevados National Park*—remains unanswered. This is because field data collection is still ongoing. We were only able to begin acoustic recordings in July, once the required access arrangements and permits were formally approved by PNN Los Nevados, and recordings were authorized from July to December. As a result, we are still actively gathering environmental and acoustic data across all study sites. Once fieldwork is completed, we will integrate and analyze the full dataset to evaluate how the new highway is modifying microhabitat conditions and the acoustic landscape of the frog community. These analyses will allow us to address the main research question and produce the final report for the project.

Conclusion

We can therefore conclude that our hypotheses and predictions were partially correct, and that *P. simoterus* calls both during the day and at night. However, daytime calling appears to occur only when environmental conditions closely resemble those of the night, suggesting that call activity may depend more on temperature and humidity than on the hour itself. At night, individuals show an optimal calling performance around 5.2 °C, with activity dropping to zero as temperatures approach 0 °C and also declining at higher temperatures, although calling remains possible at relatively warm conditions near 17 °C. Likewise, high relative humidity substantially increases the probability of calling, a pattern that is consistent across both day and night.



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