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CARRERA DE INGENIERÍA EN ECOSISTEMAS

**DETECTABILIDAD DE ANUROS CON MONITOREO ACÚSTICO
PASIVO EN UN MOSAICO ANTROPIZADO DEL PAISAJE EN LA
CUENCA ALTA DEL RÍO TENA**

Proyecto de investigación previo a la obtención de Título de:

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AUTOR: Tatiana Salomé López Pabón

TUTOR: PhD. Hugo Mauricio Ortega Andrade

CO-TUTOR: Msc. Daniela Pareja Mejía

Napo - Ecuador

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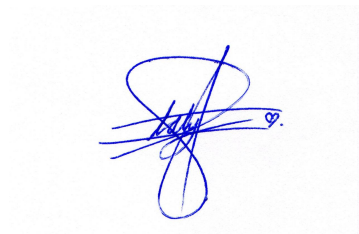
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PhD. Hugo Mauricio Ortega Andrade
C.I: 1717270811



Msc. Daniela Pareja Mejía
C.I: 1712615044

DEDICATORIA

A mis padres Katy Pabón y Roberto López quienes superaron cualquier obstáculo para apoyarme a seguir mis sueños. A ellos, quienes me inculcaron desde pequeña honestidad, respeto y justicia y quienes me enseñaron a ser auténtica, a no tener miedo de mostrar quién soy y a estar orgullosa de mis raíces. A mi hermana Sofía quien me ha impulsado a sobrellevar los problemas y para quien he tratado de ser siempre el mejor ejemplo a seguir.

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DETECTABILIDAD DE ANUROS CON MONITOREO ACÚSTICO PASIVO EN UN MOSAICO ANTROPIZADO DEL PAISAJE EN LA CUENCA ALTA DEL RÍO TENA

Tatiana Salomé López Pabón

RESUMEN

La comprensión de los procesos ecológicos y la gestión de los ecosistemas muchas veces se ven obstaculizadas debido a las limitaciones de los métodos tradicionales utilizados para investigar la biodiversidad. Estas limitaciones son especialmente relevantes en bosques tropicales, donde las actividades humanas suponen importantes amenazas para la biodiversidad y donde la estimación de ésta requiere una toma de datos exhaustiva y muchas veces costosa. Para hacer frente a éstos retos, nuevas herramientas tecnológicas han surgido para el estudio de la biodiversidad a gran escala. Una de ellas es el monitoreo acústico pasivo (PAM), el cual permite recopilar datos de alta resolución a largo plazo, minimizando las perturbaciones de los grupos muestreados, lo que es particularmente importante para el monitoreo de diversidad de anuros. Este estudio presenta la primera aplicación del PAM en paisajes fragmentados de la Amazonía ecuatoriana. Se estudió la relación entre la detectabilidad de anuros, la distribución de la cubierta vegetal y la variabilidad meteorológica. Los resultados mostraron que la detectabilidad de anuros varía en función de las variables analizadas indicando que una mayor cobertura vegetal y condiciones climáticas adecuadas se asocian a una mayor detectabilidad de anuros. Se detectaron nueve especies de anuros a partir de 18,065 minutos de grabación. Los resultados sugieren la importancia del PAM para el seguimiento de la biodiversidad en las selvas tropicales. Esta investigación amplía el conocimiento de la diversidad de anuros en la Amazonía ecuatoriana y subraya la necesidad de técnicas de monitoreo eficaces en la conservación de la biodiversidad.

Palabras clave: Monitoreo acústico, Anuros, Biodiversidad, Ecosistemas tropicales, Cobertura vegetal, Detectabilidad.

ABSTRACT

The understanding of ecological processes and the management of ecosystems are often hindered due to the limitations of traditional methods used to investigate biodiversity. These limitations are especially relevant in tropical forests, where human activities pose significant threats to biodiversity and where its estimation requires exhaustive and often costly data collection. To address these challenges, new technological tools have emerged for large-scale biodiversity studies. One of these is passive acoustic monitoring (PAM), which allows long-term, high-resolution data collection while minimizing disturbance to the sampled groups, which is particularly important for monitoring anuran diversity. This study presents the first application of PAM in fragmented landscapes of the Ecuadorian Amazon. The relationships between anuran detectability, vegetation cover distribution, and meteorological variability were studied. The results showed that anuran detectability varies as a function of the variables analyzed, indicating that greater vegetation cover and suitable climatic conditions are associated with greater anuran detectability. The study detected nine anuran species from 18,065 minutes of recording. The results suggest the importance of PAM for monitoring biodiversity in tropical rainforests. This research expands knowledge of anuran diversity in the Ecuadorian Amazon and underscores the need for effective monitoring techniques in biodiversity conservation.

Key words: Acoustic monitoring, Anurans, Biodiversity, Tropical ecosystems, vegetation cover, Detectability.

INTRODUCTION

Humans have altered tropical landscapes through activities such as agriculture, cattle raising, and urban expansion [1,2]. Within this context, fragmentation processes have led to a disconnection between habitats by creating numerous patches, consequently causing habitat fragmentation [1]. Environmental fluctuations in these fragmented landscapes result in substantial variances along an ecological gradient known as the "edge effect," wherein smaller patches along the forest margins tend to experience higher temperatures and lower humidity compared to the forest interior [3–5]. These modifications at the forest boundaries have implications for species' ecology, impacting population sizes, altering species distribution patterns, and influencing the genetic structure of populations [6].

All these pressures constitute a significant threat to amphibian diversity [7]. Habitat fragmentation influences the richness of amphibians, increases the chance of species loss for species with aquatic larvae, and contributes to amphibian diversity decline [1,8]. Amphibian species with a biphasic life cycle can be severely affected because their ecological niches shift drastically during their ontogenetic development (tadpoles live in aquatic sites, and post-metamorphic tadpoles live in terrestrial habitats) [1,9]. Consequently, landscapes with a high degree of habitat fragmentation show alterations in anurans community structure, and species with aquatic larvae show small proportions of individuals [1,8]. However, species with terrestrial development are less vulnerable to habitat splitting because they do not require a water site to complete their life cycle [1].

Ecuador is located within the world's richest biodiversity hotspot, the Tropical Andes [10,11]. This country has a wide diversity of amphibians, with 641 known species of anurans, of which 310 are endemic [12]; moreover, new species are constantly being discovered in Ecuador [12]. Regrettably, according to the Food and Agriculture Organization of the United Nations (FAO), Ecuador has maintained a significant deforestation rate for the last ten years, losing a net annual extension of forests of - 0.41% (2010-2020) [13]. The vegetation coverage loss occupied by humans and the fragmentation of terrestrial habitat and breeding sites could affect anuran communities, such as the Ecuadorian Amazon forests [1,8].

Nonetheless, anuran biodiversity has traditionally been investigated through conventional techniques such as active searches and visual encounters, ground pit traps, and water funnel nets [14]. These methods tend to be costly, and successful field implementation of monitoring protocols requires the direct involvement of experts [7]. Additionally, they demand prolonged sampling and data processing periods, while their application is intricate [15,16]. Consequently, these methods might constrain sampling endeavors in field biodiversity surveys, creating difficulties in obtaining standardized and consistent data on larger spatio-temporal scales [7,17].

Consequently, this approach results in limited comprehension of target areas, biased species detectability estimates, and hinders our ability to understand broader ecological processes and efficiently manage wildlife [7]. Moreover, an accurate estimate of species richness in diverse ecosystems, such as tropical forests, requires extensive field data collection to sample biodiversity correctly [18]. Information concerning species presence and abundance at wide spatio-temporal scales is indispensable for making conservation decisions and managing species in tropical forests [19]. Therefore, there is an urge to apply monitoring techniques that address research challenges while adapting to new technologies to improve biodiversity monitoring at a broader scale [17,20].

Autonomous recording units (ARUs) have been developed to enhance field data collection and increase biodiversity monitoring's spatio-temporal capabilities [17,21,22]. One notable technique among ARUs is passive acoustic monitoring (PAM), which can potentially monitor difficult-to-access ecosystems and provide long-term and high-resolution data over time and large sampling areas [23]. PAM is a non-invasive technology that enables data collection while minimizing disruption to the ecology and behavior of the sampled groups [24]. Furthermore, data collection automation, detailed long-term acoustical data collection, and permanent data storage improve our capacity to understand ecological linkages and responses to habitat degradation, providing valuable insights for conservation or restoration programs [7,16].

The development of bioacoustic research in herpetology has been significant in clarifying anurans' behavioral and communication mechanisms [25]. Generally, hearing the vocalizations emitted by anurans is more accessible than directly observing them [26]. Therefore, detecting their acoustic signals is a complementary technique for determining the presence of individuals in a specific area or monitoring species'

conservation [26,27]. In Ecuador, studies on analyzing anuran vocalizations predominantly emphasize taxonomy, ecology, and natural history [28–31]. This study marks the first application of PAM in the Ecuadorian Amazon region to monitor anuran detectability within a fragmented landscape in terrestrial ecosystems of the upper Tena River basin. Our approach emphasized the distribution of vegetation cover and weather variability to answer the following questions: (i) Does anuran detectability change according to the distribution of vegetation cover of the site where they inhabit? (ii) Does anuran detectability change concerning the meteorological variability of the sampling sites?

MATERIALS & METHODS

Study Area

Anuran vocalizations were obtained in terrestrial ecosystems of the evergreen piedmont forest of the north-central part of the Eastern Cordillera of the Andes, located northwest of Tena City, Napo, Ecuador (Figure 1). The study area is located in a Piedmont bioclimatic zone, 400 to 1,200 meters above sea level [32]. The landscape has a lower thermotropic climate characterized by year-round warm temperatures and a non-flood regime; it is predominantly mountainous and has a dense forest of 15 to 35 m in height with a complex structure of several strata [32]. The area has a mean annual temperature of 28.7 °C and a mean annual precipitation of 4,620 mm [33]. Urban and rural settlements surround the study area and are part of the buffer area of the Colonso Chalupas Biological Reserve, which is adjacent to the Sumaco Biosphere Reserve and is therefore considered a high-priority area for restoration [34].

Eight sampling sites were selected based on the Normalized Difference Vegetation Index (NDVI) calculated from multispectral Landsat 8 satellite pictures 2020 at 30 m resolution [35]. The characterization of sampling sites is detailed in supplementary material, table ST1. Figure 1 shows the gradient between sites, which ranges from a minimum of 0.29 to a maximum of 0.43. Each chosen location was given a unique value. Sampling sites were randomly selected, considering that an inter-point separation of 500 m to 2 km is necessary to ensure independence between locations and to prevent pseudoreplication [36,37]. Our sampling sites were established with a separation of at least 1 km to ensure these parameters. In addition, the sites were

located at a minimum distance of 200 m from the rivers to limit data collection to species associated with terrestrial ecosystems and to avoid river noise. Temperature and relative humidity values were continuously recorded using a Temlog 20H Elitech automatic recorder (temperature accuracy = $\pm 0.5^{\circ}\text{C}$; relative humidity accuracy = $\pm 0.5^{\circ}\text{C}$).

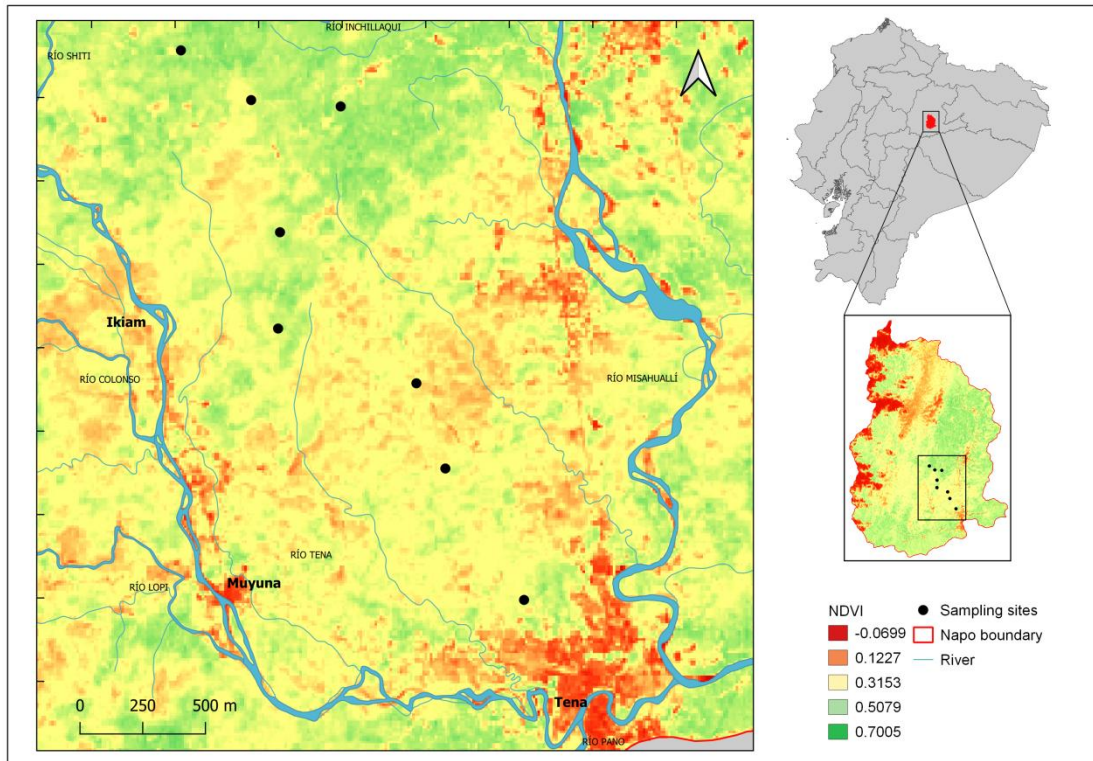


Figure 1. Study region in upper Tena river basin, Ecuador. Sampling sites and Normalized Difference Vegetation Index, (Landsat 8 images, 2020, 30 m resolution).

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Passive Acoustic Monitoring

Passive acoustic monitoring was used to gather bioacoustic data for the study. The period of data collection was from June through October 2022. The recordings were made using AudioMoth standalone recorders that were set up to record for one minute every 30 minutes from six o'clock in the evening to six in the morning at a sample rate of 48 kHz and 16-bit resolution. Approximately 3000 min of recording was recorded at each site, applying a similar sampling effort at all sites. For each sampling site, one recorder and datalogger were deployed at a height of 1.5 meters in handmade enclosures (Figure 2). The analysis of the call recordings was done using Automated Remote Biodiversity Monitoring Network (ARBIMON), a combination of hardware (Audiomoth recorders) and software (ARBIMON online application) to automate data acquisition and management and species identification from audio recordings [7].



Figure 2. AudioMoth recorders set up: a) Installation of equipment 1.5 m above the ground, b and c) Deployed recorder and datalogger handmade enclosure.
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Bioacoustic Analyses and Preprocessing

The training audio files of the species related to the sample area were downloaded from the BLOWEB portal of the Pontificia Universidad Católica del Ecuador [12]. We used a 30,000 m radius (centered at the coordinates -1.084214275172169, -77.73450079765628) from the study area into the tool “advanced search” to filter and select species that have been recorded and used them to make a referenced database for bioacoustic analysis (Supplementary Material, ST2). Before using the sampled recordings from the field, they were reproduced on a PC and recorded with Audiomoth recorders at a distance of 4 m to standardize recording parameters such as sampling rate and resolution.

Field audio recordings also underwent a preprocessing stage using the Ocenaudio program [38], where they were normalized to -3 dB, and the noise was removed with a reduction factor of 6 dB. Finally, a pattern-matching model was used to compare and validate the presence of the calls from the species bioacoustic database. Pattern matching analysis uses a minimum confidence value for detection based on a simple

correlation score; the parameters used in ARBIMON [7] were threshold =0.2, matches/recording = 1, and matches/site = no limit.

Statistical Analyses

We explored the variation in nocturnal patterns of acoustic activity among anuran species to analyze the differences between the activity hours of each species and their Detection frequency (df) [26]. Alpha diversity metrics, including Richness, Shannon Wiener index, and Simpson index, were employed to determine anuran presence variation related to the vegetation coverage among habitats; analyses were performed with the Vegan package [39]. We also estimated the biserial correlation between the presence of anurans and NDVI for all species and sites with the Psych package [40].

Principal component analysis (PCA) was made using the Vegan package [39] to find the best summary of the data in the Principal Components (PCs). The number of PCs to keep was determined using extracted eigenvalues (>1) and the cumulative variance captured by the components [36,41]. To determine which variables are the most relevant to explain the variability of the presence of anurans, we used analyses of squared cosines (Cos2) and contributions.

We fitted a generalized linear model (GLM) with four predictor variables (temperature, humidity, NDVI, and altitude) for each anuran species to test if anuran presence varies concerning the meteorological variability of the sampling sites [17,42]. The vocalizations of species were classified using a binary approach, where presence was denoted as 1 and absence as 0 [42]. A binomial distribution with a logit-link function was used to model the presence of vocalizations. Considering that the temperature and humidity variables showed a moderate correlation (Pearson's $r = -0.438$), the model included both variables. Finally, Akaike's information criterion (AIC) was used to choose the best-fitting model by comparing AIC results [42].

Finally, the effect of each environmental variable on each species' probability of occurrence was used to interpret the GLMs as the transformation of the odds ratio of every variable. Odds ratios were transformed to define positive and negative effects. Odds values higher than 1 were transformed as *odds ratio* - 1, indicating a relatively positive effect on the probability of occurrence. Odds values lower than 1 were transformed as $1/\text{odds ratio} - 1$, indicating a relatively negative effect on the probability

of occurrence. All statistical analyses in this study were conducted using R software [36].

RESULTS

A total of nine anuran species (30% of the species registered in the region [18]) were identified in 18,065 minutes of audio recordings with PAM, with a varying number of species across sites from one to five species (Table 1). The complete database is detailed in Supplementary Material, Appendix SM1. Seven types of vocalizations were identified at the species taxonomic level, and two were classified at the genus level, corresponding to the genera *Adenomera* and *Hyloscirtus* (Table 1). *Adenomera cf. andreae* was detected at every site, making it the most frequently encountered species (higher in Site 4, $df=0.233$), followed by *Boana lanciformis* (higher in site 5, $df=0.065$) which was found in five sites (Table 1). *Boana boans*, *Hyloxalus yasuni*, and *Osteocephalus verruciger* were detected at two sites each, while the remaining species were only found at one site (Table 1).

Table 1. Identified species detection frequency (df) value per site in the upper Tena river basin, Napo, Ecuador.

Species	Site 1	Site 2	Site 4	Site 5	Site 7	Site 8
<i>Adenomera cf. andreae</i>	0.044	0.051	0.233	0.139	0.076	0.122
<i>Pristimantis altamazonicus</i>	0	0	0	0	0	0.025
<i>Boana lanciformis</i>	0	0.055	0.003	0.065	0.001	0.001
<i>Boana boans</i>	0	0.006	0	0	0.001	0
<i>Dendropsophus parviceps</i>	0	0	0.087	0	0	0
<i>Hyloxalus yasuni</i>	0	0.002	0	0	0.003	0
<i>Hyloscirtus cf. phyllognatus</i>	0	0	0	0	0	0.013
<i>Osteocephalus verruciger</i>	0	0	0	0.001	0	0.003
<i>Pristimantis omeviridis</i>	0	0	0	0.001	0	0

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Various anuran species' nocturnal acoustic activity patterns exhibited distinct differences (see Figure 3). Specifically, *Adenomera cf. andreae*, vocalizes throughout the night, most frequently between 7:00 p.m. and 11:00 p.m. *Boana lanciformis* and *Pristimantis altamazonicus* exhibited heightened call activity during the evening hours (19-22 h) and (19-20h) respectively. *Dendropsophus parviceps* demonstrated an irregular pattern of calling activity, with an increase observed in the early morning. The

remaining species displayed a less frequent but consistent calling pattern throughout the night.

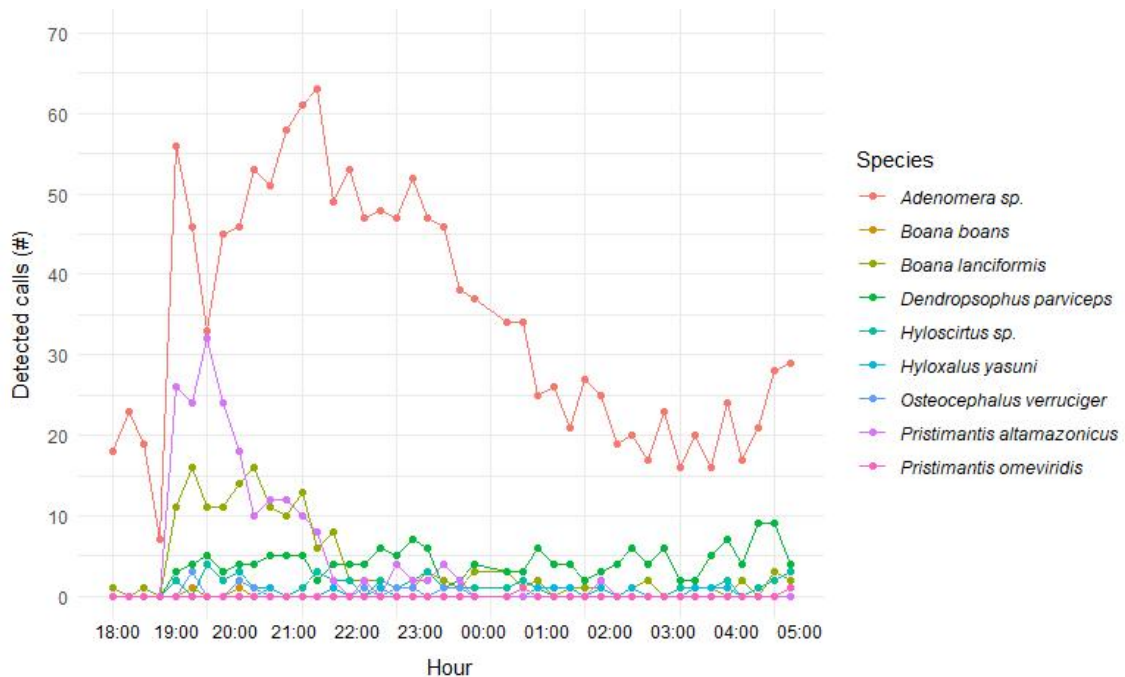


Figure 3. Variation of the nocturnal patterns of acoustic activity of nine species of anurans detected in the study area.

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Site 8, with the lowest NDVI value (0.294), exhibited the highest alpha diversity indexes, with five species detected. Sites 2, 5, and 7, with higher NDVI, showed four species, and sites 1 and 4, with the highest values in NDVI, showed one and three species, respectively. The complete set of biodiversity alpha metrics can be found in Table 2.

Table 2. Biodiversity alpha metrics and species detected in the study sites.

Site	NDVI	Richness	Shannon	Simpson	Species
8	0.2941057	5	1.609	0.80	<i>Adenomera cf. andreae</i> , <i>Boana lanciformis</i> , <i>Hyloscirtus cf. phyllonotus</i> , <i>Osteocephalus verruciger</i> , <i>Pristimantis altamazonicus</i>
5	0.3323767	4	1.386	0.75	<i>Adenomera cf. andreae</i> , <i>Boana lanciformis</i> , <i>Osteocephalus verruciger</i> , <i>Pristimantis omeviridis</i>
7	0.3593912	4	1.386	0.75	<i>Adenomera cf. andreae</i> , <i>Boana boans</i> , <i>Boana lanciformis</i> , <i>Hyloxalus yasuni</i>
2	0.3701269	4	1.386	0.75	<i>Adenomera cf. andreae</i> , <i>Boana boans</i> , <i>Boana lanciformis</i> , <i>Hyloxalus yasuni</i>
1	0.3732838	2	0	0	<i>Adenomera cf. andreae</i>
4	0.4253271	3	1.099	0.67	<i>Adenomera cf. andreae</i> , <i>Boana lanciformis</i> , <i>Dendropsophus parviceps</i>

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Two components with eigenvalues > 1.0 were extracted from the PCA. Both PCs account for 78.76%. PC1 is primarily influenced by NDVI and Humidity (47.15 % variance), and PC2 is primarily influenced by Temperature and Altitude (31.61 % variance). Figure 4 shows the PCA biplot where closer vectors indicate higher correlations and vectors in opposite quadrants suggest negative correlations.

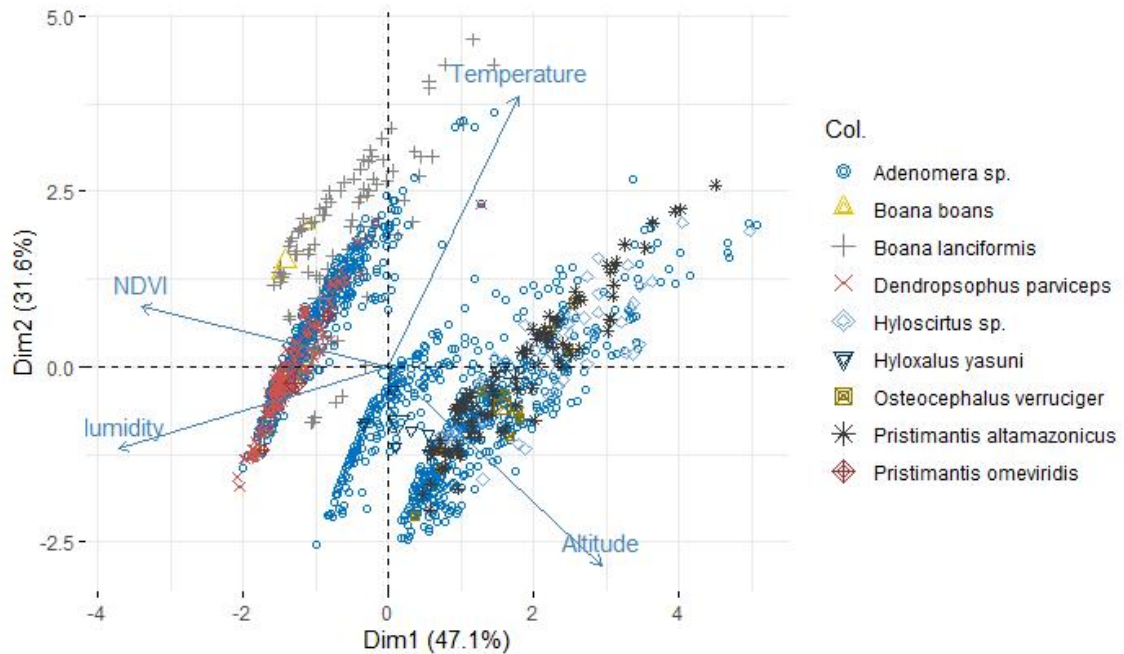


Figure 4. PCA biplot showing the relationships between the indices and the principal components (PCs), and the distribution of data for species presence.

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Indices with longer vectors signify increased influence on that particular PC. An increase in Dim.1 is associated with a decrease in NDVI and Humidity and an increase in Temperature and Altitude. An increase in Dim.2 is associated with an increase in NDVI and Humidity and a decrease in Temperature and Altitude.

According to the Cos2 analyses and contributions, the global importance of the environmental factors in explaining the data variability follows this order: Temperature is the most relevant and best-represented factor, followed by Altitude, Humidity, and lastly, NDVI (as shown in Table 3). Higher Cos2 values, closer to 1, indicate a better representation in the principal components. The Contributions values demonstrate the relative importance of each variable in explaining the variance in the data for each principal component.

Table 3. Variables' Global importance expressed by Cos2 and contributions.

	Cos2	Contributions
NDVI	0.622744	19.7665
Temperature	0.913459	28.9941
Altitude	0.844460	26.8040
Humidity	0.769835	24.4353

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It is worth noting that each species exhibits a different relationship with the analyzed variables. For example, *Pristimantis omeviridis* is most influenced by NDVI and humidity, *Boana lanciformis* by NDVI and temperature, and *Pristimantis altamazonicus* by altitude and temperature (Figure 5). The selected landscape metrics influenced the probability of each species' presence in different magnitudes (high, medium, and low), including NDVI, temperature, humidity, and altitude (Figure 5).

NDVI showed a significant positive association with the presence of all anuran species, suggesting that anuran presence tends to be higher in areas with more vegetation cover. Temperature and humidity had a slight negative effect on most species, indicating that increasing humidity and temperature affect the probability of individual species' presence. However, three species, *Boana boans*, *Boana lanciformis*, and *Pristimantis omeviridis*, responded positively to the increment of temperature, although a single species, *Pristimantis omeviridis*, had a positive response to the increasing humidity. Finally, the altitude had a slight negative effect; no species responded positively to the increment of altitude.

According to the AIC values, the GLM models fit well with the data. However, it is crucial to note that the amount of data and the sampling sites may limit the generalizability of these results. Furthermore, it is essential to consider the correlation (Pearson's $r = -0.438$) between temperature and humidity, even though this correlation level does not generally cause significant information bias or present significant multicollinearity problems.

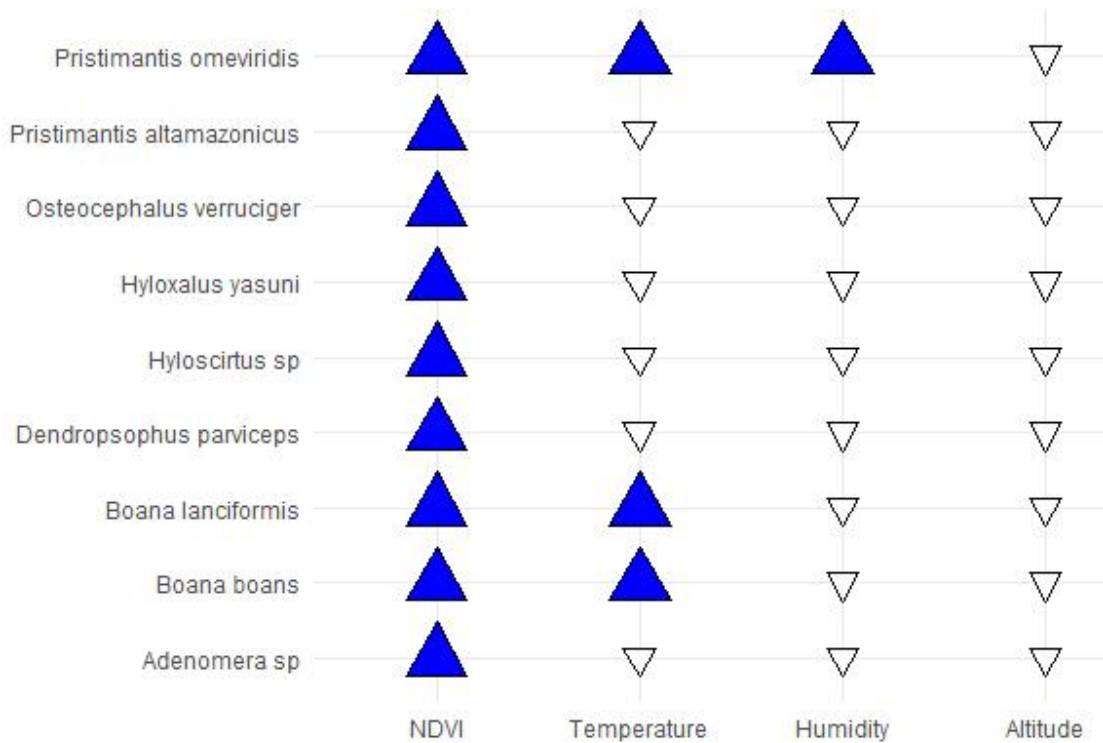


Figure 5. Effects of predictor variables on a species' likelihood of occurring, as expressed by transformed odds ratio. Figures symbolize positive and negative effects.

Made by: López, 2023.

DISCUSSION

Our study determined the relationship between anuran communities and the gradient of vegetative coverage. Our results reveal, in general terms, a positive and significant association between the presence of anuran vocal activity and vegetation cover (NDVI); this result is consistent with previous findings (e.g., [18,37,43]). We analyzed anthropogenic pressures and urban density using vegetation cover as a variable, determining that it can change anuran community composition. Previous reports have indicated that plant communities determine the physical structure of various habitats [44]. Consequently, they significantly affect the distribution and interactions of animal species, highlighting that plant communities are one of the leading environmental factors influencing anuran communities [43,44].

Our study area is in an anthropogenic landscape mosaic where human-induced pressures impact community diversity, structure, and species-calling activity. Previous studies have demonstrated a negative relationship between urban density and species calling activity and showed that landscape-scale factors, such as temperature, humidity,

altitude, and vegetation coverage, significantly drive differences in species abundance along urban-rural gradients [37].

We detected 30% of the species registered in the region because the anuran community analyzed is composed of frogs associated with disturbed and ecotone environments. These species are not exclusive to primary forests; they can adapt to disturbed environmental conditions and have relatively good conservation status [45–53]. Therefore, the presence of these species increases slightly at sites with lower NDVI where other sensible species could not survive. The diversity indices were calculated based on the prevalence of the most abundant species within the defined area and during the specific sampling period. It is important to note that these indices provide insights into the diversity of dominant species but do not directly compare with the overall diversity of the entire study area.

Two of the observed species were identified at the genus level. *Adenomera cf. andreae*, could not be identified because the *Adenomera* species in the Amazonian Ecuadorian rainforests (*Adenomera adreae* and *Adenomera hylaedactyla*) have identification problems. Several individuals have been recorded as *Adenomera andreae* in Jatún Sacha (Napo, Ecuador) [54,55]; however, the records were made at night in a secondary forest so they may have been confused with *Adenomera hylaedactyla* [45]. In the case of *Hyloscirtus cf. phyllognatus*, identification was impossible because this species was detected only at site 8, with a shallow detection frequency. For this reason, it was impossible to make an adequate comparison to determine which *Hyloscirtus* species it corresponds to (*Hyloscirtus phyllogatus* or *Hyloscirtus albopunctulatus*)

Meteorological variability was also analyzed, and the three environmental factors showed importance in explaining the variability of the data. As described previously in other studies, temperature, altitude, and humidity are, in general terms, essential factors that influence anurans' detectability, abundance, and activity [56]. Even though it is important to note that meteorological variables can influence each species differently, some species could respond positively to the increment of the variables. In contrast, others could have a slight negative effect, and this could be due to several factors ranging from the specific characteristics of each species, its ecology and reproductive patterns, and endogenous factors, such as hormones, to seasonal changes and variations in climate [57].

The analysis of nocturnal acoustic activity showed no global pattern for the acoustic activity of the nine anuran species detected, even with the variability of meteorological factors and vegetation cover. In contrast with other studies where the species maintained very similar circadian patterns of acoustic activity in variable thermal environments [26], the species could not be detected during sampling despite being present at the site [56]. Some species are easily detectable, such as *Adenomera cf. andreae*, with a constant pattern of vocalizations and a peak of activity in the evening. Moreover, on the other hand, we can find less detectable species because of their irregular or less frequent calling patterns, such as *Pristimantis omeviridis*.

In highly biodiverse ecosystems like Ecuador's Amazon forests, characterized by disrupted forest coverage and limited resources, techniques such as PAM can provide an effective means of data collection. Applying this approach in a complementary way in conjunction with traditional methods such as visual encounters, pit traps, and funnel nets can improve the study of diversity, especially in tropical regions [58]. Other research findings indicate that combining visual (VES) and acoustic (AES) encounter surveys can provide an adequate approximation to real scenarios; these two techniques combined can reveal a higher number of species [18]. However, this is only applicable if only species richness is considered because if measures of relative abundance are included, these methods may be insufficient. However, it should be noted that the differences in efficiency between acoustic sampling and any other sampling method can vary between taxa [18].

Previous studies demonstrated that PAM permits continuous data collection without human influence on animal behavior and allows monitoring of multiple sites simultaneously over extended periods, which is impossible with conventional techniques [58–60]. Furthermore, new emerging tools and artificial intelligence recognition algorithms such as ARBIMON, R, and Python [58,61] will facilitate detecting and identifying vocally active individuals. These tools, coupled with the integration of passive acoustic studies and the development of machine learning technologies (e.g., [62]), will allow this type of research to identify changes in vulnerable populations and species diversity [23].

Our results also support the importance of ARUs in biodiversity conservation and management. Large-scale, non-invasive monitoring techniques such as PAM are increasingly used for various ecological and conservation management purposes,

helping to guide conservation efforts and providing crucial data for informed decision-making in tropical forest biodiversity management [16,23,63,64]. Currently, ecoacoustics research is exploring the viability of developing a reliable standard acoustic monitoring procedure for long-term international conservation programs [23]. Moreover, many PAM initiatives aim to contribute the collected recordings to global efforts to create ecological baselines for future conservation assessment and management [23].

Our research provides a first breakthrough in monitoring anuran detectability in the Ecuadorian Amazon by using passive acoustic monitoring (PAM) over a gradient of vegetation cover. Conducting a large-scale study with similar objectives would be impossible with active monitoring by one researcher or even a group of researchers. However, the automatic data collection allows for improved monitoring and facilitates the detectability of patterns of presence and frequency of species at sampling times and sites.

Other publications involving acoustic monitoring that have been published in Ecuador have mainly included taxonomic analysis and analysis of the acoustic characteristics of sound signals (intra- and interspecific comparisons) (e.g., [28–31,65,66]). However, our emphasis on passive acoustic monitoring offers a complementary perspective to these studies, contributing significantly to understanding the factors influencing anuran detectability in this region. This innovative approach also has important implications for the conservation and adaptive management of these species by using a technique that is efficient in terms of time and resources yet highly effective in obtaining information.

However, PAM has challenges, such as determining genuine absences, estimating species abundance, and disentangling individual vocalizations. Also, the sample of sites analyzed in this research was limited, and the sample of anuran species studied was relatively small. It also requires a significant amount of practical work, which restricts its general use because it creates vast amounts of data that can be difficult to store, analyze, and preserve [23]. These constraints may impair the representativeness of the total diversity in Ecuador's Amazon region, and they should be considered when interpreting our results.

CONCLUSIONS

The main objective of this study was to monitor the diversity of anurans in terrestrial ecosystems of the upper Tena River basin using passive bioacoustics. Anuran presence showed changes according to the sampling sites' temperature, humidity, and altitude. However, the effect of the variables depends on the species, with no global effect for all species. These findings emphasize the significance of ARUs, notably PAM, in biodiversity conservation and management. This method could contribute to a fuller understanding of anuran diversity in Ecuador's Amazon region and provide critical data for informed decision-making and conservation efforts in tropical forest biodiversity management. However, Considering the limitations of this research, it is suggested that further research broaden the technique by increasing the sample size, including species associated with watery environments, widening sampling sites and times, and combining sampling methods.

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SUPPLEMENTARY MATERIAL

ST1: Characterization of sampling sites

Site	Latitude	Longitude	NDVI	Land use
1	-0.9794	-77.821520	0.37328380	Secondary forest, deforestation
2	-0.9507	-77.847710	0.37012690	Agroforestry system
4	-0.9396	-77.847597	0.42532712	Guayusa agroforestry system
5	-0.9564	-77.832770	0.33237675	Pasture
7	-0.9256	-77.850655	0.35939118	Cocoa plantation
8	-0.9198	-77.858255	0.29410574	Secondary forest

Made by: López, 2023.

ST2: Referential list of anuran species and vocalizations collected for bioacoustic analysis

Species	Database	Province	Copyright	Bioweb description
<i>Adenomera andreae</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, río Tiputini, vía Pompeya, km. 33, Yasuní.
<i>Adenomera hylaedactyla</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, orilla del río Napo al oeste de Pompeya Sur. QCAZ 8266, 8269, 8270, 8271. Canto mostrado en la figura.
<i>Adenomera cf. andreae</i>	Project recordings	Napo	Tatiana López	Ecuador, Provincia Napo, Bosque Universidad Regional Amazónica Ikiam.
<i>Agalychnis hulli</i>	Bioweb	Orellana	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia de Orellana, Parque Nacional Yasuní, vía Pompeya Sur - Iro, Pozo Capirón 1 QCAZA10658
<i>Allobates femoralis</i>	Bioweb	Pastaza	©Morley Read	Ecuador, Provincia de Pastaza, Boanamo, Sendero Puerco, 29 de septiembre de 2012.
<i>Allobates insperatus</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia de Orellana, Boanamo, cerca al campamento, 23 de septiembre de 2012.
<i>Allobates zaparo</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, Pindo. Canto mostrado en la figura.
<i>Ameerega bilinguis</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, Pindo. Canto mostrado en la figura.
<i>Ameerega hahneli</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia de Orellana, Boanamo, Sendero Puerco, 27 de septiembre de 2012.
<i>Ameerega parvula</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, Boanamo, Sendero Araña, 22 de septiembre del 2012.
<i>Boana alfaroi</i>	Bioweb	Orellana	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia de Orellana, Estación Científica Yasuní (PUCE), en NPF km 1, 20 de junio de 2009, 24H00 hs, al borde de un riachuelo, perchando a 50 cm del suelo. Grabado por Marcel A. Caminer y Edwin Carrillo Ponce. Canto mostrado en la figura.
<i>Boana appendiculata</i>	Bioweb	Orellana	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia Orellana, vía Pompeya, km. 73, Yasuní. Canto mostrado en la figura.

<i>Boana boans</i>	Bioweb	Orellana	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia Orellana, vía Pompeya Sur, km. 66, río Boyopare, Yasuní. Canto mostrado en la figura.
<i>Boana calcarata</i>	Bioweb	Napo	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia de Napo, cerca a Comunidad Santa Rosa, a 30 minutos en carro desde Tena, por la vía Tena-Jatun Sacha, 1 de marzo de 2009, 23H00 hs, al borde de un riachuelo, perchando a 50 cm del suelo. Grabado por Marcel A. Caminer y Edwin Carrillo Ponce.
<i>Boana cinerascens</i>	Bioweb	Pastaza	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia de Pastaza, alrededores de Destacamento Militar Lorocachi, banco norte del Río Curaray, 25 de junio de 2013, 23H18 hs, coro cantando junto a un estero. Grabado por Santiago Ron.
<i>Boana lanciformis</i>	Bioweb	Pastaza	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia de Pastaza, Destacamento Militar Lorocachi, Río Curaray, 16 de junio de 2013, 21H09 hs, cantando desde pastizal. Grabado por Gabriela Galarza.
<i>Boana maculateralis</i>	Bioweb	Napo	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia de Napo, 640 m al SE de la Comunidad Santa Rosa, vía al Tena, 28 de febrero de 2009, 2H00 hs, en el borde de la carretera, perchando a 2 m del suelo. Grabado por Marcel A. Caminer y Edwin Carrillo Ponce.
<i>Boana punctata</i>	Bioweb	Orellana	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia de Orellana, Tambococha, Parque Nacional Yasuní, 13 de marzo de 2013, 21H00 hs, cantando desde pastizal. Grabado por Andrea Varela. ©Pontificia Universidad Católica del Ecuador.
<i>Boana tetete</i>	Bioweb	Napo	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia de Napo, Jatun Sacha, 31 de marzo de 2010, 21H30 hs, en pantano inundado con Mauritia flexuosa. Grabado por Santiago R. Ron.
<i>Chiasmocleis anatis</i>	Bioweb	Napo	©Morley Read	Ecuador, Provincia Napo, Estación Jatun Sacha. QCAZ 51042. Canto mostrado en la figura.
<i>Chimerella mariaelenae</i>	Bioweb	Pastaza	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia de Pastaza, Parque Nacional Llanganates, Comunidad Zarentza QCAZA59365 ©Pontificia Universidad Católica del Ecuador.
<i>Cruziohyla craspedopus</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, Pozo Hormiguero-1, Pindo. Canto mostrado en la figura.
<i>Dendropsophus bifurcus</i>	Bioweb	Orellana	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia Orellana, Yasuní, vía Pompeya-Iro.
<i>Dendropsophus bokermanni</i>	Bioweb	Pastaza	©Morley Read	Ecuador, Provincia Pastaza, Palora, Estación INIAP cerca del río Pastaza.
<i>Dendropsophus brevifrons</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, Yasuní, km. 80. QCAZ23890. Canto mostrado en la figura.
<i>Dendropsophus marmoratus</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, Pompeya Sur, km. 1.7, Yasuní. Canto mostrado en

				la figura.
<i>Dendropsophus minutus</i>	Bioweb	Zamora Chinchipe	©Pontificia Universidad Católica del Ecuador	Ecuador, Zamora Chinchipe, Paquisha, 3 de febrero de 2014, 20H30 hs, cantando a los márgenes del pozas en pastizales. Grabado por David Velalcázar.
<i>Dendropsophus parviceps</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, vía Pompeya Sur, km. 38, río Piraña, Yasuní. Canto mostrado en la figura.
<i>Dendropsophus reticulatus</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, río Tiputini, Yasuní. Canto mostrado en la figura.
<i>Dendropsophus rhodopeplus</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, vía Pompeya, km. 75, río Rosa, Yasuní. Canto mostrado en la figura.
<i>Dendropsophus sarayacuensis</i>	Bioweb	Napo	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia de Napo, Río Azuela, 10 de Agosto de 2000, 23h45 hs. Grabado por Juan Manuel Guayasamín y Luis Coloma.
<i>Dendropsophus shiwarum</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, vía Pompeya, km. 101, Yasuní. QCAZ22752. Canto mostrado en la figura.
<i>Dendropsophus triangulum</i>	Bioweb	Orellana	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia Orellana, río Napo banco sur, Chiroisla. QCAZ44290. Grabado por Santiago Ron, Eduardo Toral e Italo Tapia. Canto mostrado en la figura.
<i>Edalorhina perezii</i>	Bioweb	Orellana	©Pontificia Universidad Católica del Ecuador	Canto de anuncio. Ecuador, Provincia Orellana, vía Pompeya, km. 66, río Boyopare, Yasuní. Grabado por Francisco Sornoza. Canto mostrado en la figura.
<i>Engystomops petersi</i>	Bioweb	Pastaza	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia Pastaza, vía Puyo - Tena, km. 5, Hostería Safari. QCAZ26260. Canto mostrado en la figura.
<i>Gastrotheca testudinea</i>	Bioweb	Morona Santiago	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia de Morona Santiago, Bosque Protector del Río Abanico, vía Nueve de Octubre-Macas QCAZA77403
<i>I. albopunctulatus</i>	Bioweb	Morona Santiago	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia de Morona Santiago, alrededores de la comunidad Shaime, cerca al Mirador de la Virgen, 23 de julio de 2012, 22H23 hs. Grabado por Teresa Camacho Badani.
<i>Hyloscirtus phyllognathus</i>	Bioweb	Tungurahua	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia Tungurahua, Machay, a 5 km. de río Verde, carretera hacia Puyo. SC38765. Grabado por Mario Yáñez, Fernando Ayala y Santiago Ron. Canto mostrado en la figura.
<i>Hyloxalus bocagei</i>	Bioweb	Napo	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia Napo, Parque Nacional Sumaco. QCAZCC186. Canto mostrado en la figura.
<i>Hyloxalus maculosus</i>	Bioweb	Pastaza	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia de Pastaza, Parque Nacional Llanganates, Comunidad Zarentza QCAZA59725.
<i>Hyloxalus sauli</i>	Bioweb	Orellana	©Pontificia Universidad	Ecuador, Provincia Orellana, Parque Nacional Yasuní, NPF, 25 de septiembre

			Católica del Ecuador	de 1999.
<i>Hyloxalus yasuni</i>	Bioweb	Orellana	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia Orellana, Parque Nacional Yasuní, NPF, 21 de septiembre de 1999, 16H00 hs. Grabado por Diana Prado.
<i>Leptodactylus discodactylus</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, Estación Científica Yasuní. Canto mostrado en la figura.
<i>Leptodactylus knudseni</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, vía Pompeya, km. 57. Canto mostrado en la figura.
<i>Leptodactylus mystaceus</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, vía Pompeya, km. 5, cerca del río Indillama. Canto mostrado en la figura.
<i>Leptodactylus pentadactylus</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, vía Pompeya, km. 107, 4 km. al oeste de la carretera. Canto mostrado en la figura.
<i>Leptodactylus rhodomystax</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, vía Pompeya, km. 93, Yasuní. Canto mostrado en la figura.
<i>Leptodactylus wagneri</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, vía Pompeya, km. 38, al borde de la carretera. ©Morley Read. Canto mostrado en la figura.
<i>Lithodytes lineatus</i>	Bioweb	Sucumbíos	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia de Sucumbíos, Cabañas Neotropic, Reserva de Producción Faunística Cuyabeno, 3 de febrero de 2002, cantando sobre una colonia de hormigas podadoras. Grabado por Ítalo Tapia. QCAZ18865. Católica del Ecuador.
<i>Nyctimantis rugiceps</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, Vía Pompeya. Canto mostrado en la figura.
<i>Nymphargus siren</i>	Bioweb	Sucumbíos	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia Sucumbíos, vía Santa Bárbara - La Bonita. QCAZ46663. Grabado por Elicio Tapia y Samael Padilla. Canto mostrado en la figura.
<i>Osteocephalus buckleyi</i>	Bioweb	Napo	©Morley Read	Ecuador, Provincia Napo, Librarius Study Center, Jatun Sacha.
<i>Osteocephalus deridens</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, río Tiputini, camino a Pozo Hormiguero. QCAZ14847. Canto mostrado en la figura
<i>Osteocephalus fuscifacies</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, vía Pompeya, km. 57, Yasuní. QCAZ30626. Canto mostrado en la figura.
<i>Osteocephalus mutabor</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, Reserva Río Bigal. QCAZ485223. Canto mostrado en la figura.
<i>Osteocephalus planiceps</i>	Bioweb	Napo	©Morley Read	Ecuador, Provincia Napo, Reserva Biológica Jatun Sacha.
<i>Osteocephalus verruciger</i>	Bioweb	Napo	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia de Napo, Cascada de San Rafael, entre el río Gradas y el mirador de la cascada, 10 de agosto de 2000, 23H45 hs. Grabado por Juan Manuel Guayasamín y Luis Coloma. QCAZ32036-32033-32034-32035.
<i>Phyllomedusa</i>	Bioweb	Napo	©Morley	Ecuador, Provincia Napo, Reserva Río

<i>tarsius</i>			Read	Bigal. Canto mostrado en la figura.
<i>Phyllomedusa tomopterna</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, vía Pompeya, km. 57, Yasuní. Canto mostrado en la figura.
<i>Phyllomedusa vaillantii</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, vía Pompeya, km. 39, cerca del río Piraña, Yasuní. Canto mostrado en la figura.
<i>Pristimantis altamazonicus</i>	Bioweb	Napo	Mauricio Ortega	Grabado por Mauricio Ortega
<i>Pristimantis conspicillatus</i>	Bioweb	Orellana	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia Orellana, Estación Científica Yasuní. SC37073. Grabado por Teresa Camacho.
<i>Pristimantis diadematus</i>	Bioweb	Morona Santiago	©Diego Batallas R	Ecuador, Morona Santiago, Parque Nacional Sangay, Sardinayacu. Cantando en el tercer refugio mientras vocalizaba cautivo y en amplexus. Grabado por Diego Batallas R.
<i>Pristimantis lacrimosus</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, Boanamo, Sendero Venado, 23 de septiembre de 2012.
<i>Pristimantis omeviridis</i>	Bioweb	Orellana	©Pontificia Universidad Católica del Ecuador	Ecuador. Provincia Orellana. Estación Científica Yasuní. Grabación por Samael Padilla.
<i>Rana palmipes</i>	Bioweb	Orellana	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia de Orellana, Loreto, Parroquia San José de Dahuano, Cotapino, 8 de marzo de 2000.
<i>Rhinella margaritifera</i>	Bioweb	Napo	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia de Napo, cinco minutos antes de llegar a El Chaco, Paradero de Don Vaca, 16 de marzo de 2013, 19H10 hs, en piscina para tilapias. Grabado por Juan Jiménez.
<i>Rhinella marina</i>	Bioweb	Napo	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia de Napo, cinco minutos antes de llegar a El Chaco, Paradero de Don Vaca, 16 de marzo de 2013, 19H10 hs, en piscina para tilapias. Grabado por Juan Jiménez.
<i>Rhinella proboscidea</i>	Arbimon	Brasil	Anderson Saldanha Bueno	Arbimon, Birds of Balbina (Proyect)
<i>Scinax cruentomma</i>	Bioweb	Orellana	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia de Orellana, Vía Pompeya Sur - Iro, km 100,9 QCAZA23892
<i>Scinax garbei</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, vía Pompeya, km. 80, Yasuní. QCAZ23889. Canto mostrado en la figura.
<i>Scinax ruber</i>	Bioweb	Orellana	©Morley Read	Ecuador, Provincia Orellana, vía Pompeya, km. 57, Yasuní.
<i>Teratohyla midas</i>	Bioweb	Pastaza	©Pontificia Universidad Católica del Ecuador	Ecuador, Provincia Pastaza, Reserva Sanja Arajuno. QCAZ48742. Grabado por Santiago Ron y Lucas Bustamante. Canto mostrado en la figura.

<i>Trachycephalus macrotis</i>	Bioweb	Napo	©Morley Read	Ecuador, Provincia Napo, Librarius Study Center, Jatun Sacha. Canto mostrado en la figura.
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Made by: López, 2023.

SM1: Arbimon database which include data on presence/absence of species, NDVI, Temperature and Humidity.

<https://arbimon.rfcx.org/project/bioacustica-anuros-tena>