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Evaluating the efficacy of visual encounter and automated acoustic survey methods in anuran assemblages of the Yungas Andean forests of Argentina

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ABSTRACT

Identifying adequate methods and tools for biodiversity monitoring is fundamental in ecology and conservation biology. Most of the standardised monitoring techniques involve the presence of the researchers at the survey sites meanwhile, passive acoustic monitoring (PAM) of the diversity of anuran species could be a valid alternative. In this study, we evaluated the effectiveness of the use of PAM as a method for anuran species survey and the use of acoustic indices as proxies for the species diversity and species' calling activity level in three species assemblages along the altitudinal gradient of the Yungas forests in NW Argentina. We collected bioacoustic data at three sites along an altitudinal gradient in the Parque Nacional Calilegua. Complementarily, monthly anuran surveys were carried out with the standard method of Visual Encounter Survey (VES). Our results showed that acoustic surveys using PAM could be a reliable tool to assess the anuran diversity in the complex environments of Andean forests. Also, available acoustic indices such as ACI, ADI, AEI, Bio, H and M, could be reliable tools to reflect the diversity of calling species in forest habitats with different levels of biophony in subtropical regions. Nevertheless, long-term monitoring programs must be coupled with VES to accurately reveal anuran diversity along the altitudinal gradient.

1. Introduction

One of the biggest challenges in ecology and conservation biology is to identify tools for biodiversity monitoring in a changing world (Magurran and Dornelas, 2010; Moreno, 2019). Amphibians have been highlighted as the group of vertebrates experiencing the higher rates of population declines and species extinctions (Stuart et al., 2004; Stuart, 2008; Lavilla and Heatwole, 2010). Anuran diversity surveys contribute to increasing our knowledge about population status and are usually stressed for the design of species conservation action plans (Heyer et al., 1994; Wren et al., 2015; Vaira et al., 2018). In the context of this global diversity crisis, it is imperative to have reliable survey methodologies that provide accurate and rapid information for assessment and monitoring of the conservation status of amphibian populations worldwide (IUCN, 2020; Vaira et al., 2018).

Traditional animal monitoring protocols may limit the ability of the researchers to understand patterns of diversity or population dynamics by producing potential biases in the detection of rare species or low-density populations (Fitzpatrick et al., 2009). Most of the standard monitoring methods used in anuran amphibians such as visual encounter surveys (VES) involve the presence of the researchers in the survey sites, so they are very time consuming, are expensive and there are substantial logistical limitations to obtain continuous records over prolonged periods (Heyer et al., 1994; Dodd et al., 2009). Also, the species identification by VES could be biased due to the observer's experience (Dodd et al., 2009).

Currently, the passive acoustic monitoring (PAM) employing autonomous recording units (ARUs) offers an efficient and alternative tool for long-term monitoring studies and biodiversity surveys (Obrist et al., 2010; Villanueva-Rivera et al., 2011; Sugai et al., 2019), and particularly for elusive and cryptic species or with highly asynchronous breeding individuals (Pérez-Granados et al., 2019; Ulloa et al., 2019; Willacy et al., 2015). Through the PAM method, animal populations can also be monitored over a long-continuous period, without the physical presence of researchers in the field, providing a large amount of information in different places simultaneously (Lammers et al., 2008; Brandes, 2008). Passive acoustic monitoring has been highlighted as an alternative methodology to undertake environmental impact assessments surveying and monitoring biodiversity across large spatial and temporal scales (Brandes, 2005; Ribeiro Jr. et al., 2017). ARUs can

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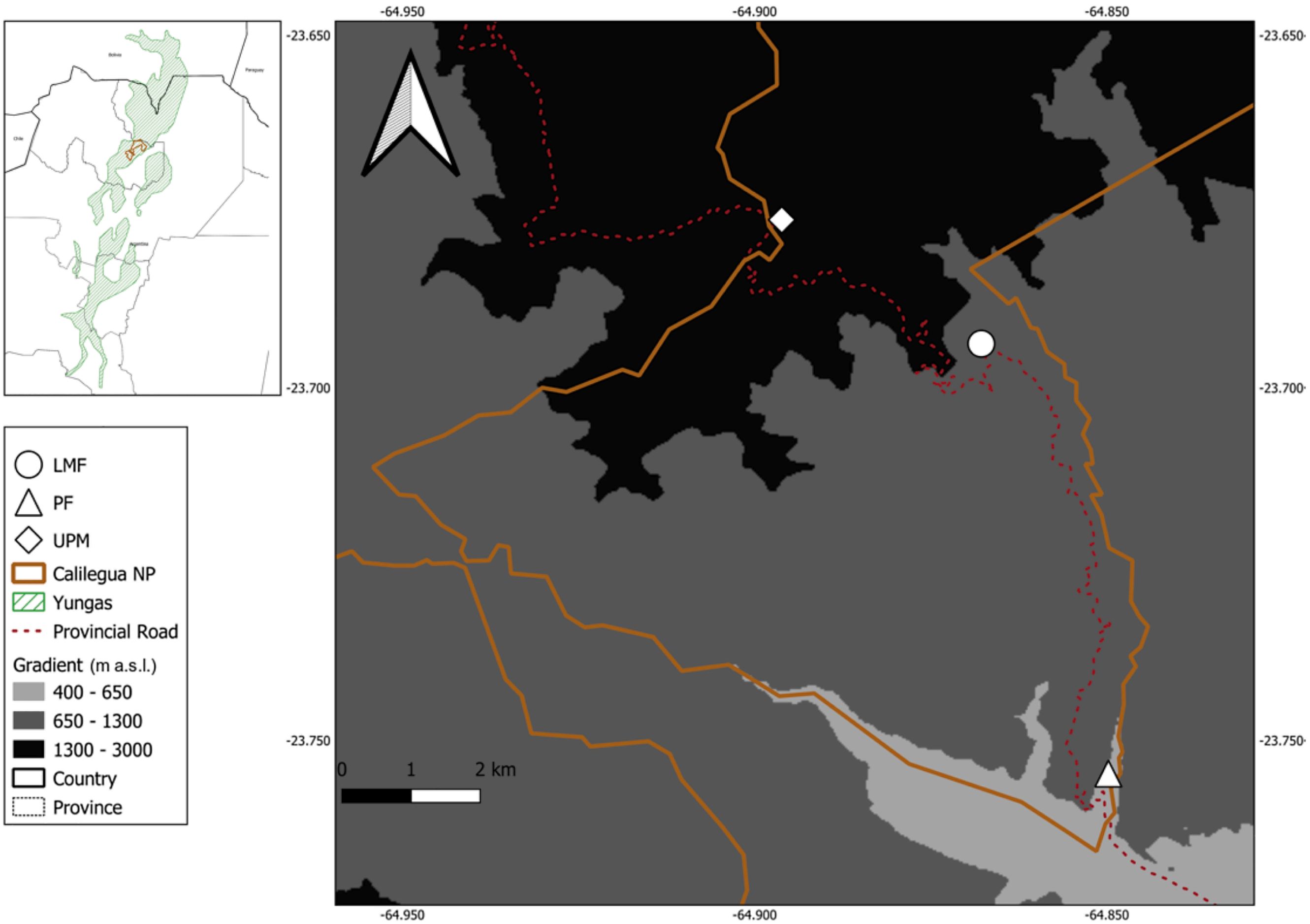

Fig. 1. Study map showing the study area in the Parque Nacional Calilegua, Jujuy, Argentina. PF = Premontane Forest; LMF = Lower Montane Forest; UPM = Upper Montane Forest.

provide useful and complementary information about diversity and occupancy estimates with the traditional surveys (human point-counts) in vocal species such as birds (Darras et al., 2018; Furnas and Callas, 2015) and anurans (Acevedo and Villanueva-Rivera, 2006). Furthermore, PAM can be implemented to evaluate human impacts on native vocal species (Alvarez-Berríos et al., 2016), as well as, changes of terrestrial communities along elevation gradients (Campos-Cerqueira and Aide, 2017). In the last decade, several designs of ARUs have been implemented in different monitoring programs and recorder prices are falling too (Hill et al., 2017). This methodology proved to be a valuable tool for the description of the daily and seasonal calling activity in cryptic and endemic frog species (Willacy et al., 2015). If only VES is used to monitor anurans, important reproductive events such as explosive breeding activity might be missed (Ulloa et al., 2019).

Acoustic indices are often used in PAM assessments as they provide a summary of the spectral and temporal information of sound recordings made in the field (Bradfer-Lawrence et al., 2019; Buxton et al., 2018; Sueur et al., 2008a, 2008b). The estimation of biodiversity through acoustic indices may provide valuable information about the conservation status of threatened areas such as aquatic ecosystems or protected areas, enabling the detection of diversity hotspots or regions with high species turnovers (Retamosa et al., 2018; Mammides et al., 2017; Linke and Deretic, 2020). However, there still exists strong biases in conducting studies aimed at exploring biophony relationships in birds, as well as in a few other taxonomic groups (Gasc et al., 2013; Ferreira et al., 2018). Recent evidence suggests using a combination of acoustic indices to distinguish among frog species and acoustic call features (Indraswari et al., 2018).

The soundscape comprises a combination of different sound sources: sounds produced by animals (biophony), sounds produced by climatic conditions (geophony), and sounds produced by humans (anthrophony) (Pijanowski et al., 2011a). Recent studies strongly recommend the use of multiple acoustic indices to cover the main acoustic features of a given soundscape and for quantifying bioacoustic activity (Buxton et al., 2018; Bradfer-Lawrence et al., 2019; Towsey et al., 2014).

Southern Andean Yungas ecoregion is the southernmost extension range of a Tropical Andes biodiversity hotspot (Myers et al., 2000) and one of the most biodiverse ecoregions of Argentina (Brown et al., 2006). This ecoregion represents less than 1% of the continental territory of Argentina and had suffered dramatic land-use change with the nearly complete loss of the premontane forest areas and vast alterations of montane forests (Brown et al., 2006, 2009). These changes have caused the formation of forest remnants that are severely threatened by human influence (Brown et al., 2006). Its remarkable anuran diversity and a high proportion of endemism make the Yungas an ecoregion with great significance for amphibian conservation in Argentina (Lavilla and Heatwole, 2010). Despite the conservation value of these Yungas forests, its anuran diversity is at greater risk compared to other ecoregions of the country (Vaira et al., 2017). This scenario compels researchers to test the efficiency of new techniques to monitor anuran species diversity, mainly in breeding areas with different species richness and/or vocal activity. In the Yungas ecoregion of Argentina, only a few studies on anuran species have been conducted with PAM, but in none of these studies, their methodology was used for the purpose of asses the amphibian diversity of one specific area (Akmentins et al., 2015; Pereyra et al., 2016; Boullhesen et al., 2019). Therefore, we highlight the need to test the efficiency of PAM as an adequate technique to reveal anuran species diversity, mainly in breeding areas with different species richness and/or vocal activity.

Our study aimed to compare and evaluate the efficiency of PAM versus VES to obtain anuran richness estimates in a species assemblage of the Yungas forests of NW Argentina. In addition, we tested the efficacy of eight acoustic indices as proxies of anuran richness and the calling activity level.

2. Materials and methods

2.1. Study area

The study was carried out in the Parque Nacional Calilegua, Jujuy province, Argentina. This national park which extends to 76,320 ha, is the most representative natural protected area belonging to the Yungas ecoregion in Argentina (Malizia et al., 2010). We selected three sites with representative reproductive habitats commonly used by the anuran assemblages in the altitudinal gradient of the phytogeographic strata of this protected area (Vaira, 2002). We employed the Yungas' phytogeographic strata classification proposed by Grau and Brown (2000): 1) The lowest site was in the Premontane Forest (PF), located at 23°45′16.84″ S; 64°50′59.35″ W and 650 m a.s.l. It is a permanent pond of an approximate area of 1114 m^2, surrounded by deciduous trees such as the "Cebil Rojo" (*Anadenanthera colubrina*) and the "Sauce criollo" (*Salix humboldtiana*); 2) The intermediate site was in the Lower Montane Forest (LMF), located at 23°41'36.84" S; 64°52'5.04" W 1125 m a.s.l., is a permanent stream dominated by an evergreen forest of "Nogal criollo" (*Juglans australis*), "Cedros" (*Cedrela balansae*) and "Pacará" (*Enterolobium contortisiliquum)*; 3) The highest site was in the Upper Montane Forest (UMF), located at 23°40 '28.56" S; 64°53'44.15" W and at 1650 m a. s. l., is a primary forest dominated by Myrtaceae trees (Fig. 1).

2.2. Anuran surveys techniques

2.2.1. Visual encounter surveys (VES)

Anuran count species were carried out in the three surveyed sites by 30 min' time-restricted random visual encounter surveys (Crump and Scott, 1994), in monthly intervals from September 2017 to September 2018 (totalling 13 surveys) by two observers (MB and MSA). The active searches were performed during night hours between 20:00 to 23:00. We recorded the total number of detected species. This data was then used for calculating anuran species richness using VES. The VES method assumes the same chance to detect all developmental stages and sexes of post-metamorphic amphibians, even non-vocal active anuran species (Crump and Scott, 1994; Dodd, 2009).

2.2.2. Passive acoustic monitoring (PAM)

In the same surveyed sites, three autonomous Song Meter SM4 recorders (Wildlife Acoustics, Inc., Concord, MA, USA) were installed (one recorder per site) at 1.5 m above the ground. ARUs recorded from September 2017 to September 2018 programmed to record 3-consecutive minutes per hour (72 min/day) on MONO channel (Shirose et al., 1997; Márquez et al., 2014). The sound recordings files were stored in. WAV format digital files of 16 bits' resolution with a frequency range of 16 kHz reducing the maximum recorded frequency to 8 kHz, to preserve exclusively the sounds emitted by the anuran assemblage of the surveyed sites. The recordings were listened to by experts in anuran call identification of the species assemblage of Yungas ecoregion (MB and MSA) in the laboratory using Raven Pro© 1.5 software (Center for Conservation Bioacoustics, 2014). Only adult males can be recorded by this method. From the full set of the data, we listened to one day per week: a total of 13,485 recordings resulting in 224.75 h in the three sites together (4488 recordings from PF, 4400 from LMF, and 4557 from UMF).

2.3. Acoustic indices analyses

We calculated eight of the most commonly used acoustic indices in ecoacoustic research (Bradfer-Lawrence et al., 2019; Fuller et al., 2015; Machado et al., 2017; Mammides et al., 2017; Moreno-Gómez et al., 2019) using an automated custom procedure in R ver 4.0.2 (R Core Team, 2020). Briefly, the Acoustic Complexity Index (ACI) was calculated as the sum of adjacent sound intensity intervals. This index was formerly created to estimate a direct quantification of bird vocal activity (Pieretti et al., 2011). The Acoustic Entropy index (H) is the product of temporal and spectral entropies of a recorded sound measured using the Shannon-Wiener diversity index (Sueur et al., 2008a, 2008b). This index value ranges between 0 and 1, with 1 corresponding to signals equally distributed across frequency bands (either noisy across bands or completely silent), and 0 results in a pure tone with all energy in one frequency band. The Acoustic Richness index (Depraetere et al., 2012), is similar to H but takes into account for the overall amplitude of a signal (M), are calculated as the rank of the product between M and Ht (temporal entropy) over the number of files recorded. AR varies between 0 (poor acoustic richness) and 1 (high acoustic richness). The Acoustic Diversity Index (ADI) uses the Shannon-Wiener index to estimate acoustic complexity (Pekin et al. 2012), calculating the proportion of sounds used in multiple frequency bands of the acoustic spectrum. This same information is used by the Acoustic Evenness Index (AEI), measured from the Gini coefficient, and therefore is inverse to the ADI (Villanueva-Rivera et al., 2011). The Bioacoustic Index (Bio) estimates the acoustic complexity of a sound by calculating the variations in the intensities values (dB) and the number of frequency bands used by the assemblage (Boelman et al., 2007).

Anuran calls occupy a significant space in the frequency spectrum, mainly between afternoons and nights (dusk choruses), resulting in very loud recordings. Thus, intensities indices such as ACI, M, H, and Bio would be expected to reflect a strong and positive relationship with the anuran richness and calling activity level recorded. Also, different anuran species produce a variety of calls particularly in the reproductive seasons, therefore, ADI and AR would be expected to correlate positively to the anuran richness and calling activity level in our study sites. AEI, as the inverse of ADI, would be expected to relate negatively to anuran vocalizations.

For ACI, ADI, AEI Bio, and H, indices calculation we used the *soundecology* package ver. 1.3.3 (Villanueva-Rivera et al., 2018). We adjusted the calculation of these indices to the frequency band range occupied by the species belonging to the anuran assemblages between 500 and 8000 Hz and to eliminate undesirable noise. The ACI was calculated using default parameters and J set to 5. ADI and AEI were calculated using 1000 steps and a decibel threshold of − 50. The AR, M, and Ht indices were calculated with the default parameters of the *seewave* package ver. 2.1.6 (Sueur et al., 2008a, 2008b).

2.4. Statistical analyses

2.4.1. Comparing methods

We calculated species richness (S) for each surveyed site to analyze differences in the composition of the amphibian community surveyed by each method. From data obtained by PAM, we also calculated the calling activity level for each species in the recordings according to the numerical classification proposed by Bridges and Dorcas (2000) as follows: 0 = no male vocalizing; 1 = one male vocalizing; 2 = multiple males vocalizing with the possibility of distinguishing occasionally single calls; 3 = multiple males vocalizing being unable to distinguish single calls. This data was used for calculating a calling activity for all species heard in each recording.

To validate the comparisons of the performance for species inventory of each method (PAM versus VES), we calculated sample coverage curves for each site with the rarefaction method using individual-based data (Chao and Jost, 2012). This was computed with the package *iNEXT*

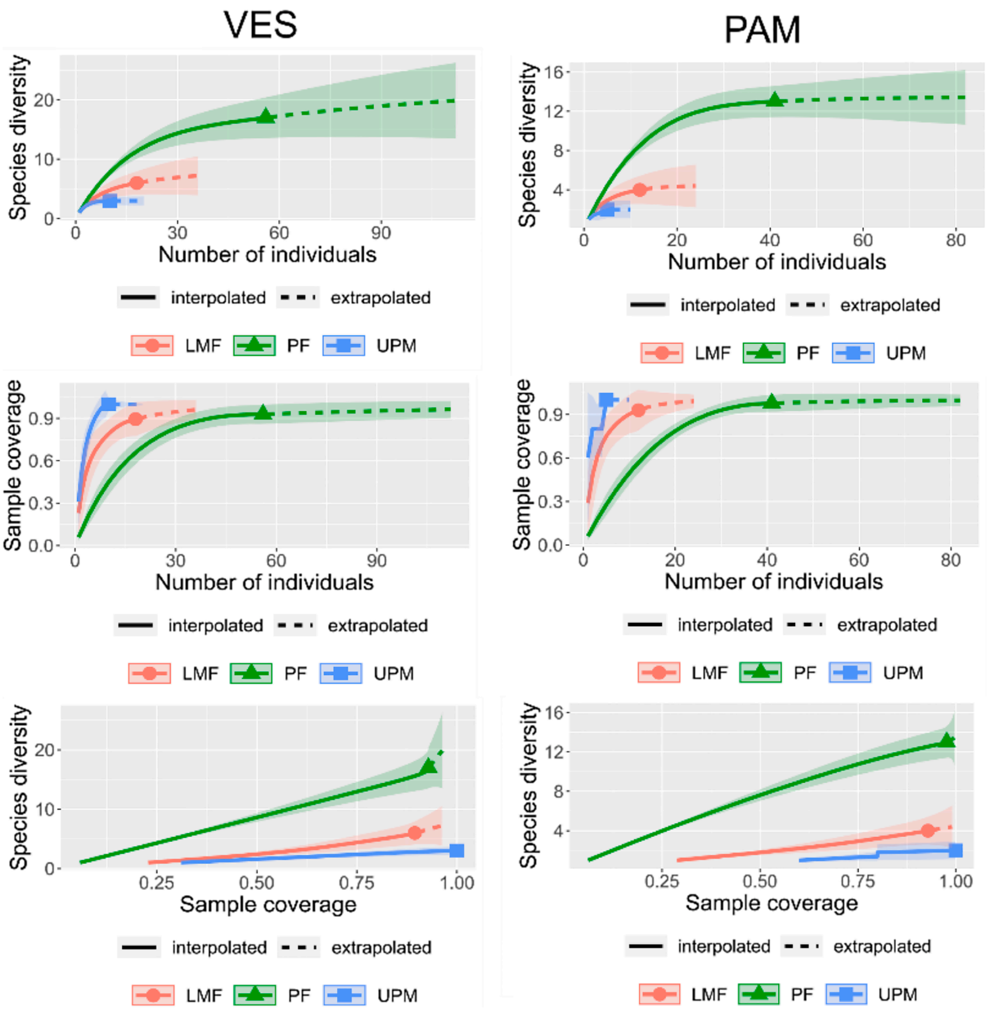

Fig. 2. Comparison of individual-based rarefaction curves for Hill numbers of order q = 0 of anuran species richness detected by visual encounter survey and passive acoustic monitoring methods in the three study sites in the Parque Nacional Calilegua, Jujuy, Argentina.

ver. 2.0.20 (Chao et al., 2016). To evaluate dissimilarities in results obtained between both methods (PAM versus VES) we used a complementarity analysis (Colwell and Coddington, 1994) using data recorded from each method in each site surveyed. This was calculated as:

$S_{jk} = S_j + S_k - V_{jk}$

$U_{jk} = S_j + S_k - 2V_{jk}$

$C_{jk} = U_{jk}/S_{jk}$

where: S_{jk} = total number of species recorded by both methods (PAM and VES); j = species number recorded by VES; k = species number recorded by PAM; V_{jk} = species number recorded in common; U_{jk} = unique species recorded by each method; C_{jk} = complementarity between both methods (VES and PAM). Analysis values range between 0 (full similarity) to 1 (full complementarity).

2.4.2. *Testing acoustic indices*

To test the effectiveness of the acoustic indices as proxies of anuran species richness and the calling activity level estimated by PAM, we ran linear mixed effect models (LMM) using the *lme4* package ver. 1.1 in R software (Bates et al., 2015). This analysis helps to control for possible temporal autocorrelation and nested data (Zuur et al., 2009). Before model fitting, we standardized the data to make acoustic indices values comparable using the normalize method with *vegan* package ver. 2.5–6 (Schielzeth, 2010; but see Bradfer-Lawrence et al., 2020; Fairbrass et al., 2017; Oksanen et al., 2019). Models were ran for each acoustic index (response variable), species richness (S), and the calling activity level calculated for each recording (CA) obtained from PAM were considered as fixed effects, dates, months and sites as random effects (predictors). For each acoustic index, we ran four models with a combination of the fixed and random effects. Models were constructed as follows:

M1: Index ~ Intercept + (1|site) + (1|dates) + (1|month)
M2: Index ~ Intercept + S + (1|site) + (1|dates) + (1|month)
M3: Index ~ Intercept + CA + (1|site) + (1|dates) + (1|month)
M4: Index ~ Intercept + S + CA + (1|site) + (1|dates) + (1|month)

For the model selection procedure, we used Akaike Information Criterion (AIC) by calculating AIC values and the differences between each candidate model and the model with the lowest AIC (Delta AIC). We then used the Akaike weight (wi) for each model (weight of the evidence of the model) to reach a final best model (Burnham and Anderson, 2002), using the *MuMIn* package ver.1.43.17 (Barton, 2020). For each model fitted, we checked the distribution of residuals to explore for significant outliers and deviations of the data using the *DAHRMa* package ver. 0.3.2.0 (Hartig, 2020).

All statistical analyses were performed in R version 4.0.2 (R Core Team, 2020).

Table 1
Complementarity analysis. PF = Premontane forest; LMF: Lower Montane Forest; UMF: Upper Montane Forest; Sjk = total numbers of species recorded by visual encounter survey and passive acoustic monitoring methods; Ujk = unique species recorded by both methods; Cjk = complementary between both methods in percentage.

Site	Sjk	Ujk	Cjk
PF	17	1	6%
LMF	7	1	14%
UMF	3	1	33%

3. Results

3.1. Anurans surveys

We registered a total of 21 anuran species from 5 families on the three sites pooling both methodologies, including Bufonidae (4 species), Craugastoridae (2 species), Hylidae (5 species), Leptodactylidae (9 species), and Phyllomedusidae (1 species) (see supplementary material Table S1).

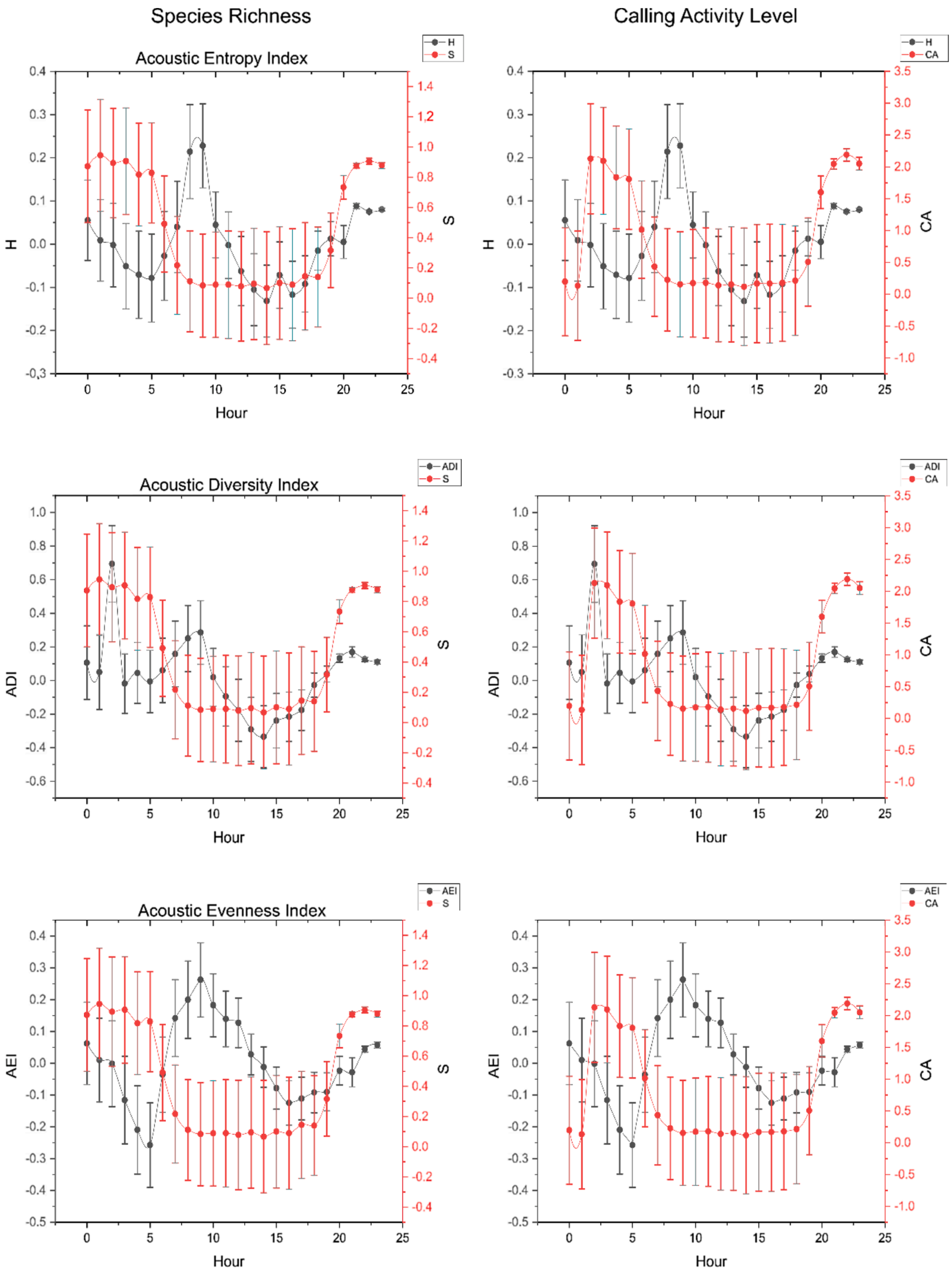

Fig. 3. Circadian pattern of normalized acoustic indices and anuran diversity detected in our study sites. X-axis = time of day; Y-axis = mean acoustic indices values, mean anuran richness (S) and mean calling activity level (CA); Error bars = standard deviation.

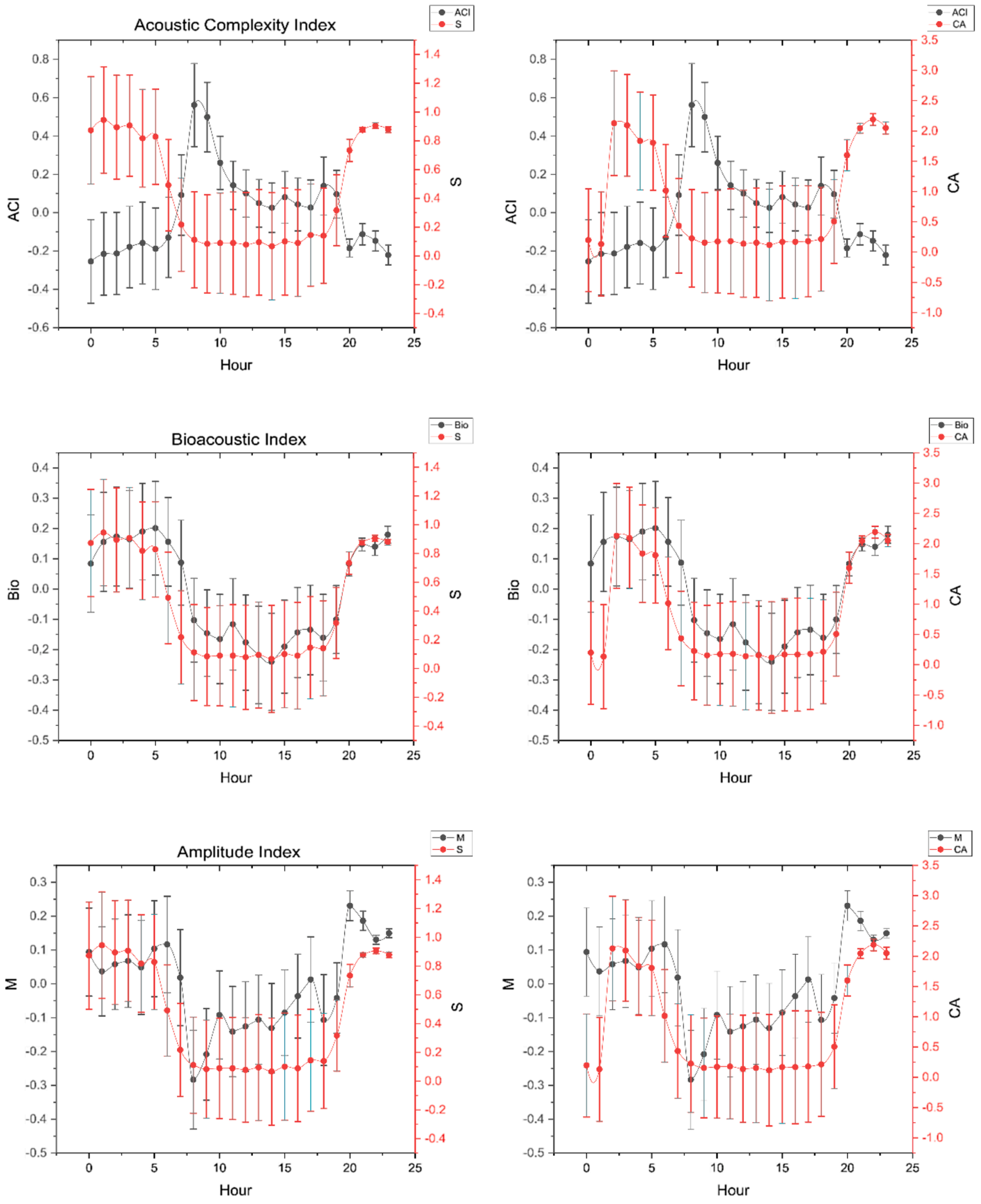

Fig. 3. (*continued*).

3.2. *PAM versus VES*

Individual-based rarefaction curves for the VES method showed a sample coverage of 92% in the PF site, 89% in the LMF site, and 100% in the UMF site, respectively (Fig. 2). Meanwhile, the sample coverage for PAM technique was nearly 100% in the three surveyed sites (Fig. 2). These results indicated adequate sampling effort in the study area using both methods. Overall by the VES method, there was a trend to detect more species (19) than with the PAM method (18) in all sites pooled (Table S1). The PF showed the highest species richness detected by both methods, with a total of 17 species by VES and 16 species by PAM (Table S1). In the LMF we registered 6 species by VES and 7 species by PAM (Table S1). In the UMF were registered 3 species by VES and 2 species by PAM, respectively (Table S1).

The complementarity analysis between methods showed consistent results among the sites surveyed. In the PF we found 0.5% of complementarity levels between both monitoring techniques. In the LMF we found 0.14% of complementarity between methods and 33% in the UMF (Table 1). Suggesting a similar species detection level between both techniques.

3.3. *Acoustic Index as proxies of anuran diversity*

Hourly mean values of acoustic indices and anuran diversity over the monitored sites revealed the overall patterns recorded (Fig. 3). The anuran richness and calling activity levels showed similar patterns, with high values slightly decreasing along the night-time 00:00 to 06:00 h., decreasing through day-time (06:00 to 18:00 h.), and steadily increasing after evening (19:00 to 23:00 h., time-period corresponding to the dusk choruses). Acoustic Entropy (H) and Evenness indices showed similar patterns decreasing along the night (00:00 to 05:00 h.), rising between 05:00 to 08:00 h. reaching its peak (time-period corresponding to the

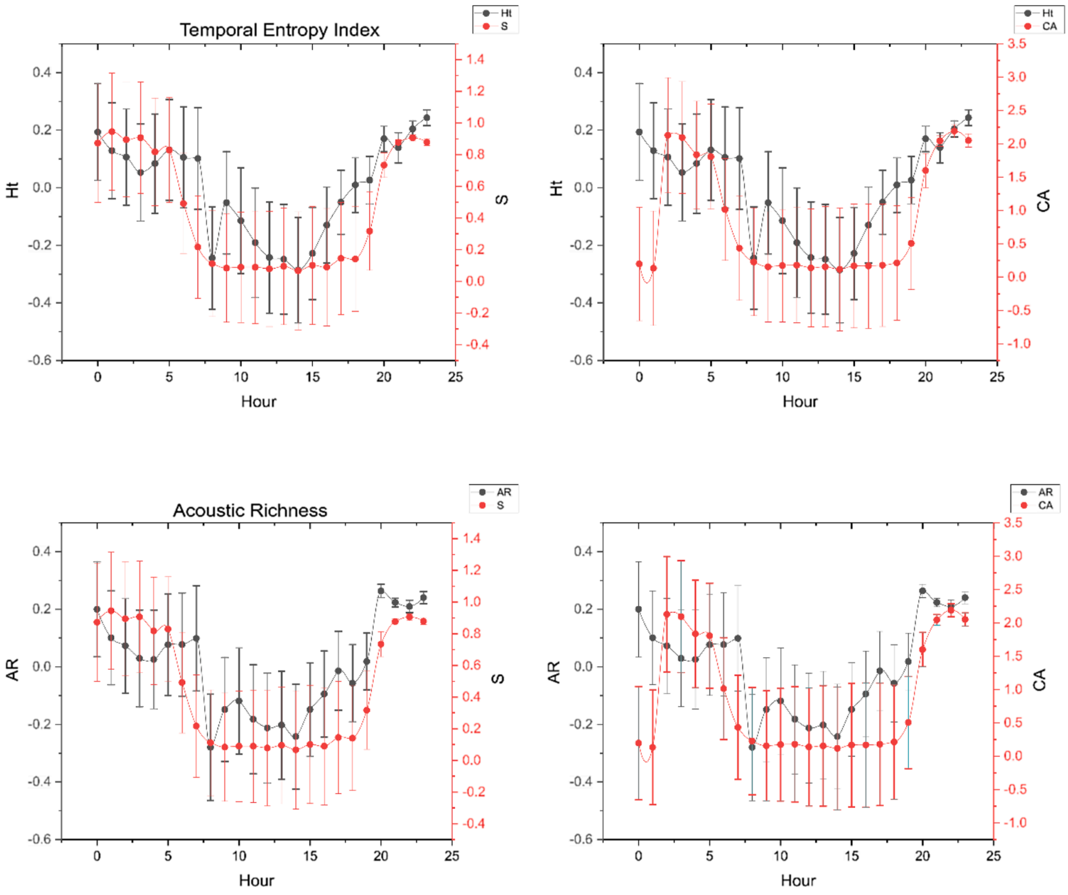

Fig. 3. (*continued*).

dawn choruses), decreasing along day-time (08:00 to 14:00 h.) and rising after 18:00 to 20:00 h. Acoustic Diversity Index patterns were similar to H and AEI but showed peak values at night between 1:00 and 02:00 h., alongside high anuran calling activity level values. Acoustic Complexity Index showed increasing values over the night (0:00 to 05:00 h.) and registered a steep increase after 06:00 h. (dawn chorus), decreased over day-time 08:00 to 18:00 h., and registered a slight increase between 19:00 and 20:00 h. The Bioacoustic Index showed similar patterns to anuran diversity, increasing values along nights with a peak at 06:00 h., decreasing values along day-time (6:00 to 14 h.), and steep rise from 18:00 to 21:00 h. Temporal Entropy, Amplitude, and Acoustic Richness Indices showed similar patterns with abrupt decreases after 06:00 h., slightly increases during day-time (14:00 to 18:00 h.), and steadily rises to 20:00 h. (Fig. 3).

Fitted LMMs showed most of the acoustic indices were associated with either anuran species richness or the calling activity level recorded by PAM in the study sites. The anuran calling activity level was positively associated with the ACI, Bio, and M indices meanwhile, Ht and AR were negatively associated. Anuran richness was positively associated with H, Bio, and ADI indices and negatively with AEI. AIC model selection showed a stronger fit (higher weight) in the anuran species richness (S) with H, AEI, ADI, and Bio indices (Table 2). Meanwhile, ACI, M, Ht, and AR were better fitted with the calling activity level recorded (Table 2).

4. Discussion

4.1. PAM versus VES

We recorded similar species richness of anuran amphibians employing active and passive survey techniques along the altitudinal gradient represented by the phytogeographic strata of the Yungas forests of the Parque Nacional Calilegua in NW Argentina. Based on species richness estimates derived from PAM, this method could be a viable technique for estimating anuran species richness in Yungas Andean forests assemblages.

The use of PAM was also tested in the Cerrado forests of Brazil, where no significant differences in species richness between traditional counts and recording units were detected (Alquezar and Machado, 2015). The relative better performance of the VES technique for species inventory than the PAM differs from other results previously reported for anurans in tropical regions (Acevedo and Villanueva-Rivera, 2006), and in grassland ecosystems (Madalozzo et al., 2017). These differences detected in the survey methods could be explained by the landscape heterogeneity linked to the Yungas forests (Grau and Brown, 2000), influencing the detection probability of anuran survey methods. Nevertheless, considering the complementarity values between methods, we found a small number of unique species detected by VES instead of the PAM technique. This situation could be explained by the limited effective detection range of ARUs for anuran calls, which highly decays after a distance of about 250 m for sounds within the range of 1 to 5 kHz (MacLaren et al., 2018). Conversely, with VES it was possible to detect vagrant individuals of cryptic anuran species that use reproductive habitats further from the surveyed site and even voiceless anuran juveniles and females (*pers. obs.*).

Our complementarity analysis showed an overall similarity of the anuran species detection capabilities between techniques. This result, in contrast with other findings in more homogeneous landscapes (Acevedo and Villanueva-Rivera, 2006; Madalozzo et al., 2017), suggests that PAM should be employed as an alternative technique to the traditional VES in highly heterogeneous environments as Yungas Andean forest.

Table 2
Model selection table to evaluate the effect of each acoustic indices on anuran species richness (S) and calling activity level (CA) recorded by PAM. AIC = model selection; ΔAIC = difference between AIC values; w_i = Probability of the model; H = Acoustic Entropy Index; ADI = Acoustic Diversity Index; AEI = Acoustic Evenness; Bio = Bioacoustic Index; ACI; Acoustic Complexity Index; M = Amplitude Index; Ht = Temporal Entropy Index; AR = Acoustic Richness.

Index	Model	Intercept	S	CA	df	logLik	AIC	Δ AIC	weight (w_i)
H	M1	−0.459			6	−1106.32	2224.6	107.24	0
	M2	−0.5465	0.182		7	−1051.7	2117.4	0	0.748
	M3	−0.4652		0.064	7	−1053.67	2121.3	3.94	0.104
	M4	−0.5113	0.102	0.031	8	−1053.32	2120.7	3.24	0.148
ADI	M1	−0.232			6	−1347.63	2707.3	44.52	0
	M2	−0.326	0.157		7	−1324.37	2662.7	0	0.973
	M3	−0.259		0.052	7	−1329	2672	9.27	0.009
	M4	−0.317	0.134	0.009	8	−1327.37	2670.8	8.01	0.018
AEI	M1	0.24			6	−1220.33	2452.7	40.2	0
	M2	0.324	−0.134		7	−1199.23	2412.5	0	0.98
	M3	0.267		−0.04	7	−1207.1	2428.2	15.73	0
	M4	0.336	−0.167	0.012	8	−1202.15	2420.3	7.83	0.02
ACI	M1	−0.216			6	−1614.8	3241.16	116.61	0
	M2	−0.409	0.235		7	−1576.33	3166.7	41.69	0
	M3	−0.354		0.104	7	−1555.71	3125.4	0.43	0.44
	M4	−0.291	−0.15	0.152	8	−1554.49	3125	0	0.554
Bio	M1	0.045			6	−964.15	1940.3	0.82	0.29
	M2	0.002	0.043		7	−962.74	1939.5	0	0.43
	M3	0.014		0.015	7	−963.25	1940.5	1.01	0.264
	M4	0.011	0.009	0.012	8	−965.68	1947.4	7.88	0.009
M	M1	−0.018			6	−1240.33	2492.7	9.49	0.006
	M2	−0.057	0.059		7	−1238.61	2491.2	8.04	0.012
	M3	−0.043		0.029	7	−1234.59	2483.2	0	0.69
	M4	−0.007	−0.097	0.06	8	−1234.45	2484.9	1.72	0.292
Ht	M1	0.39			6	−1466.34	2944.7	94.97	0
	M2	0.512	−0.158		7	−1447.12	2908.2	58.53	0
	M3	0.48		−0.079	7	−1424.78	2863.6	13.86	0.001
	M4	0.386	0.238	−0.155	8	−1416.58	2849.7	0	0.99
AR	M1	0.31			6	−1132.33	2276.7	7.86	0.014
	M2	0.344	−0.052		7	−1131.33	2276.7	7.86	0.014
	M3	0.335		−0.027	7	−1127.4	2268.8	0	0.69
	M4	0.303	0.089	−0.055	8	−1127.31	2270.6	1.82	0.279

4.2. Acoustic indices for anuran monitoring

The use of acoustic indices as proxies of direct measurements of biodiversity is increasing when conducting rapid assessments using PAM techniques (Ferreira et al., 2018; Mammides et al., 2017; Retamosa et al., 2018; Towsey et al., 2014). Acoustic indices can also be used for environmental monitoring and management by detecting the impact of anthrophony in natural habitats (Pavan et al., 2015). In this study, we found that several existing acoustic indices can be reliably associated with estimates of anuran species richness and its calling activity level. According to the landscape characteristics of our surveyed sites (a permanent pond, a permanent mountain stream, and primary forests) which harbours a high anuran diversity, the use of acoustic indices proved to be good proxies of the species richness and the vocal activity levels of the local anuran assemblages, despite the high level of soundscape heterogeneity detected (*personal obs.*). These results agree with those reported for other freshwater ecosystems (Linke and Deretic, 2020; Desjonquères et al., 2019).

Previous studies have found the use of the Acoustic Complexity Index as a good proxy of the diversity of species in different ecosystems (Desjonquères et al., 2015; Harris et al., 2016; Towsey et al., 2014). Pieretti et al. (2011) detected a high correlation between the number of vocalizations of 13 bird species and the ACI in a protected area of northern Italy. Likewise, the calling activity level of anuran males recorded in our study was positively related to the ACI values. The Acoustic Entropy (H) and Acoustic Diversity (ADI) indices showed positive relationships with the anuran diversity (species richness and calling activity level) recorded. These findings are in agreement with the results obtained with the same taxonomic group in the Cerrado tropical dry forest of Brazil (Ferreira et al., 2018). Another study also found positive relationships between the ADI index and the species richness detected (Jorge et al., 2018). However, recently, negative relationships were detected between bird's richness and the ADI in both tropical and temperate forests, showing inconsistencies in the use of such acoustic entropy index (Eldridge et al., 2018). The Acoustic Richness (AR) and Temporal Entropy Index (Ht) were negatively associated with the anuran calling activity level. AR is sensitive to species identity and could decrease with the addition of species (Gasc et al., 2015). In addition, the geophony of the soundscapes recorded could impact on AR variation, biasing the index values (Depraetere et al., 2012). The Ht index was the most promising one to reflect calling activity in simulated bird assemblages (Gasc et al., 2015). However, our soundscape recordings may have been too noisy (i. e. high levels of background sounds) to link this index values with anuran vocalizations. Also, the high level of species calling simultaneously (mainly in the PF site) may have been overpassing the thresholds for the index precision (Gasc et al., 2015).

Mean daily Bioacoustic Index values reflected a clear positive relationship with the anuran richness and calling activity level recorded in our study sites. Also, the model selection procedure revealed a positive relationship of Bio to anuran richness recorded. Bio index was found to relate positively with avian abundance in Hawaiian sub-montane forests (Boelman et al., 2007). Also, another study found Bio to correlate with bird species in the presence of researchers (Jorge et al., 2018). However, there is evidence of no relationships between the vocal activity of the anuran assemblage and the Bio index in the Brazilian Cerrado (Ferreira et al., 2018). Acoustic indices may be sensitive to geophony and by anthrophony in noisy soundscapes (Bradfer-Lawrence et al., 2019; Ferreira et al., 2018). This highlights the importance of the adequate preprocessing of the recordings aiming to obtain accurate information of the soundscape by an acoustic index (Gasc et al., 2015).

5. Conclusions

Our results highlight the implementation of PAM as an alternative technique to VES in anuran monitoring programs in areas encompassing spatial and structural heterogeneity as an altitudinal gradient and forest composition. The information gathered by the ARUs may improve the sampling precision, mainly for species with cryptic life traits and explosive breeding. Nevertheless, VES is still a precise technique that provides great survey completeness. This technique is necessary in the case of implementing a long-term monitoring program, enabling the detection of species that are not vocally active. However, the VES methods to survey anuran diversity in unsurveyed areas still require highly trained researchers in the recognition of morphologically cryptic species, who can otherwise be easily recognized by the differences in their calls structures obtained from analysing sound recordings provided by personnel not specifically trained in species recognition but who can easily install PAM equipment in the field.

With the results reported in this study, we conclude the use of several available acoustic indices could be considered as good proxies of anuran richness and calling activity level in Andean forests areas with relatively high levels of species diversity. These results have direct implications in the conservation of cryptic and endangered species, enabling researchers to undertake rapid anuran assessments using acoustic indices. However, the use of such indices should be tested in soundscapes with different levels of acoustic complexity (Pijanowski et al., 2011b) and through extended periods, mainly in subtropical mountain regions, such as the Yungas Andean forests where seasonality is a key factor that triggers vertebrate reproduction (Brown and Malizia, 2004).

6. Author contribution statement

MB and MSA conceived the idea; MB, MSA, MV, and RMB designed the methodology; MB and MSA undertook the PAM and VES; MB analysed the data; MB led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2021.107750. These data include Google maps of the most important areas described in this article.

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